

Investigating Christianson syndrome neuronal development in induced pluripotent stem cells

Abbie M. Maguire

B.A. Colby College, 2009

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Biology and Medicine as satisfying the dissertation requirement
for the degree of Doctor of Philosophy.

Date: _____

Eric M. Morrow, MD, PhD, Advisor

Recommended to the Graduate Council

Date: _____

Richard N. Freiman, PhD, Committee Chair

Date: _____

Erica N. Larschan, PhD, Committee Member

Date: _____

Elena Oancea, PhD, Committee Member

Date: _____

Stormy J. Chamberlain, PhD, Outside Reader

Date: _____

Andrew G. Campbell, PhD, Dean of the
Graduate School

ABBIE M. MAGUIRE
Email abbie_maguire@brown.edu

EDUCATION

Brown University, Providence, RI

Fall 2011- present

PhD Candidate: Department of Molecular Biology, Cell Biology, and Biochemistry
Morrow Laboratory

Colby College, Waterville, ME

B.A. 2009 with honors

Chemistry-concentration in Cell and Molecular Biology/Biochemistry; Biology

RESEARCH EXPERIENCE

Graduate Thesis Research, 2012- present

Brown University, Dr. Eric Morrow

- PhD Thesis: Using induced pluripotent stem cells to study the molecular mechanisms of Christianson syndrome
- Analyzed runs of homozygosity in autism to discover autosomal recessive mutations

Research Associate, 2009- 2011

Broad Institute, Cancer Program, Dr. Matthew Meyerson

- Worked in collaboration with the Broad RNAi consortium to perform genome-wide RNAi screens in small cell lung cancer and liposarcoma and confirm candidate genes
- Validated mutations from whole exome sequencing in breast cancer

Honors undergraduate thesis in chemistry, 2007- 2009

Colby College Chemistry Department, Dr. Kevin Rice

- Honors Thesis: Involvement of DNA Polymerase Beta in the cellular response to Cloretazine
- Demonstrated that Cloretazine inhibits the nucleotidyl transferase activity of DNA Polymerase Beta but does not inhibit the lyase activity at clinically relevant doses

Internship in Biomedical Research at the National Institutes of Health, 2008

National Human Genome Research Institute, NIH, Dr. David Bodine

- Mapped regions of open chromatin in *Kcnn4*, a gene implicated in sickle cell disease encoding the Gardos channel, using a DNase I hypersensitivity assay

PUBLICATIONS

Investigation of Christianson syndrome neurodevelopment in induced pluripotent stem cells. *In preparation*

Gamsiz, ED*, Sciarra, LN*, **Maguire, AM**, Pescosolido, MF, van Dyck, LI, and Morrow, EM (2015). Discovery of rare mutations in autism: Elucidating neurodevelopmental mechanisms. *Neurotherapeutics* 12: 553-571

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- **Maguire, A.**, Lizarraga, S., Van Dyck, L., and Morrow, E.M. (2015). "Investigating Christianson Syndrome in Induced Pluripotent Stem Cells." Brown University Department of Molecular Biology, Cell Biology, and Biochemistry Graduate Program Retreat, Bristol, RI. August 2015. – (speaker)
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- **Frederick, AM**, Davis, ML, Langenborg, KM, Praggastis, VA, Bushee, JL, Rice, KP, Shyam, K, and Sartorelli, AC. "The consequences of carbamoylating activity from anticancer sulfonylhydrazines on the enzymes of DNA base excision repair". National Meeting of the American Association for Cancer Research, Denver, CO. April 2009 – (poster)
- **Frederick, AM**, Davis, ML, Rice, KP. "Inhibition of DNA polymerase beta by the anticancer prodrug Cloretazine". INBRE/MDIBL Symposium, Salisbury Cove, ME, August 2007 – (speaker)

AWARDS AND FELLOWSHIPS

- Poster Award: New York Stem Cell Foundation Translational Stem Cell Research Conference, 2017
- Robin Chemers Neustein Fellowship in Brain Science, 2016
- Poster Award: Dept. of Molecular Biology, Cell Biology, and Biochemistry Graduate Program Retreat. Bristol, RI, 2014
- Brown Institute for Brain Science Graduate Research Award, 2014
- Brown University graduate student trainee, NIH Grant T32-GM007601: Training in Molecular and Cell Biology and Biochemistry, 2012- 2014
- National Science Foundation Graduate Research Fellowship: Honorable Mention, 2012, 2013
- Colby College Sophomore Chemistry Award, 2007
- Maine INBRE Summer Research Fellowship, 2007

TEACHING, SERVICE, OTHER ACTIVITIES

- Trainee, iPS Cells to Neural Progenitors Training Course: Reprogramming, Maintenance, and Differentiation, Vancouver, CA. StemCell Technologies, 2015.
- MCB Journal Club Organizer, Brown University, 2013- 2014
- Teaching Assistant, Brown University, Molecular Genetics, 2013

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CHAPTER 1: Introduction

Christianson syndrome: human genetics and disease

Christianson syndrome (CS) is an X-linked, neurological disorder caused by mutations in *SLC9A6*, the gene encoding Na⁺/H⁺ exchanger 6 (NHE6). CS was first reported in a five-generation family with X-linked intellectual disability (XLID) and linkage analysis mapped the disease locus to between Xq24-Xq27.3 (Christianson et al., 1999). In another pedigree presenting with similar symptoms, sequencing of genes under the linkage interval revealed a mutation in *SLC9A6* that segregated with the disease (Gilfillan et al., 2008). CS has since been reported in numerous pedigrees with inherited and *de novo* putative loss of function mutations in *SLC9A6* (Bosemani et al., 2014; Garbern et al., 2010; Masurel-Paulet et al., 2016; Mignot et al., 2013; Pescosolido et al., 2014; Riess et al., 2013; Schroer et al., 2010; Takahashi et al., 2011; Zanni et al., 2014).

Although almost 90 genes are associated with XLID (Stevenson and Schwartz, 2009), mutations in *SLC9A6* may be one of the most common causes of XLID. Sequencing of X-chromosome exons in 208 probands with XLID revealed truncating mutations in *SLC9A6* in two families (Tarpey et al., 2009). Additionally, an exome sequencing screen of 19 families with ID revealed one family with a nonsense mutation in *SLC9A6* (Schuurs-Hoeijmakers et al., 2013). These unbiased screens reveal that the prevalence of CS may be higher than previously anticipated. The increased use of genetic diagnostics in the clinic will likely reveal increasing numbers of CS patients.

The core symptoms of CS include intellectual disability, microcephaly, epilepsy, ataxia, non-verbal status, and hyperkinesia (Pescosolido et al., 2014). Although CS shares some symptoms with Angelman syndrome and can be misdiagnosed (Gilfillan et al., 2008), several criteria distinguish the syndromes. CS patients may show decreases in weight and height and may have a progressive cerebellar atrophy not observed in Angelman syndrome. Also, some CS patients have autistic features, eye movement

disorders, gastroesophageal reflux disease, and hypotonia (Pescosolido et al., 2014).

How these symptoms which affect multiple body systems and show both developmental and progressive deficits relate to NHE6 mutation is not fully understood.

Endosome biology and the role of NHEs in the developing neuron

Na⁺/H⁺ exchangers and disease

NHE6 belongs to a family of Na⁺/H⁺ exchangers (NHEs) that serve critical roles in ion homeostasis. Almost every organism uses Na⁺/H⁺ exchange to regulate ion concentration and pH. NHEs were first discovered in *Escherichia coli* (West and Mitchell, 1974) where two exchangers, NhaA and NhaB, export protons in order to regulate intracellular pH and maintain ion gradients (Padan et al., 2001). The NHE in *Saccharomyces cerevisiae*, Nhx1, has been shown to have a role in salt tolerance and vesicle trafficking (Bowers et al., 2000), while NHEs in *Caenorhabditis elegans* and *Arabidopsis thaliana* function mainly to control salt tolerance and have diversified to include seven and nine NHEs, respectively (Nehrke and Melvin, 2002; Yokoi et al., 2002).

In mammals, there are nine NHE family members with diverse tissue and subcellular distributions. NHEs have 12 predicted transmembrane domains which function in ion transport and a divergent C-terminal tail which responds to signaling cues (Wakabayashi et al., 1992). NHE1, NHE6, NHE7, NHE8, and NHE9 are ubiquitously expressed while NHE2, NHE3, and NHE4 are expressed in the gastrointestinal tract and kidney and NHE5 is expressed in the brain (Donowitz et al., 2013). Moreover, NHE1-5 localize to the plasma membrane while NHE6-9 localize to organelles. Specifically, NHE7 is localized to the trans-Golgi network, NHE8 to the mid to trans-Golgi network,

NHE6 to early and recycling endosomes, and NHE9 to late recycling endosomes (Nakamura et al., 2005).

In addition to the mutations in NHE6 causing Christianson syndrome, other NHEs are implicated in human disease. Loss function mutations in NHE1 cause Lichtenstein-Knorr syndrome, a disorder characterized by cerebellar ataxia and deafness, possibly due to a loss of pH regulation in the inner ear (Guissart et al., 2015). Also, NHE1 overactivity has been associated with cardiac ischemia as a result of an increase in intracellular Na^+ levels which in turn overactivates $\text{Na}^+/\text{Ca}^{2+}$ exchangers and leads to cell death (Allen and Xiao, 2003). Mutations in NHE3 cause congenital sodium diarrhea due to decreased absorption of Na^+ and water in intestinal brush-border cells (Janecke et al., 2015). Mutations in NHE9 have been reported in cases of attention deficit hyperactivity disorder (ADHD) (de Silva et al., 2003) and GWAS studies in ADHD identified NHE9 as being one of the most significantly associated genes (Mick et al., 2010). Homozygosity mapping revealed a large deletion directly upstream of the NHE9 gene in a patient with autism and epilepsy (Morrow et al., 2008). Furthermore, a nonsense mutation and an exonic deletion in the NHE9 gene have been reported in comorbid autism and epilepsy (Cardon et al., 2016; Morrow et al., 2008). These extensive disorders underline the importance of NHE function in human health and development.

NHE6 Structure and Function

NHE6 is ubiquitously expressed with the highest levels in brain, skeletal muscle, and heart (Numata et al., 1998). In the mouse brain, NHE6 is expressed prenatally in growing axon tracts in the cortical plate, striatum, and thalamus and postnatally in the corpus callosum, anterior commissure, and hippocampus (Ouyang et al., 2013). Furthermore, in mouse hippocampal neurons, NHE6 localizes to perinuclear regions

and branchpoints and is enriched at the presynapse (Ouyang et al., 2013). In the human brain, RNA sequencing of brain regions across developmental timepoints revealed that NHE6 is expressed prenatally and in infancy. Expression decreases in early childhood followed by an increase that is sustained through adulthood (Brainspan, 2011). Studies of subcellular localization in mammalian cell lines and in mouse primary hippocampal cultures have discovered NHE6 localization in early, recycling, and late endosomes and transiently at the plasma membrane (Brett et al., 2002; Nakamura et al., 2005; Ouyang et al., 2013).

NHE6 was first isolated as a partial cDNA (RefSeq NM_001177651) and the complete transcript containing an additional 5' coding segment was later characterized (RefSeq NM_006359) (Numata et al., 1998). An additional isoform (RefSeq NM_001042537) has been reported that contains an added 96-bp in exon 2. This added segment extends the second hydrophilic loop and contains a glycosylation site (Miyazaki et al., 2001).

All NHEs are predicted to contain 12 transmembrane domains and a cytoplasmic C-terminal tail (Donowitz et al., 2013). Mutagenesis studies of NHE1 have shown that the transmembrane domains are required for ion transport while the C-terminal tail responds to signaling cues to modulate transport function (Wakabayashi et al., 1992). Although crystal structures of mammalian NHEs have not been produced, protein folding models can be predicted based on the crystal structure of the bacterial exchanger, NhaA (Hunte et al., 2005). Based on the NhaA crystal structure, the mechanism of NHE transport was suggested to begin with a pH-induced conformational change in transmembrane domain (TM) IX that reorients TMIV and TMXI to expose the Na⁺ binding site. Binding of Na⁺ causes a change in structure to the inward open conformation allowing Na⁺ to enter. Aspartate residues are then protonated causing a shift back the

outward open conformation (Hunte et al., 2005). Although the primary sequences of NhaA and the mammalian NHEs do not closely align, protein folding may be similar and NhaA structure may inform NHE modeling (Slepko et al., 2007).

The C-terminal tail of mammalian NHEs is not conserved between family members and is a highly unstructured domain (Hendus-Altenburger et al., 2014). The lack of conservation between NHE family members reveals that this region may contribute to the varied localization and functions of the NHEs. The C-terminal tail is proposed to act as a scaffold to mediate interactions with regulators of trafficking and ion transport. Studies in NHE1 have found numerous interactions of the C-terminal tails with calcium binding proteins, kinases, and phosphatases that can activate or inhibit exchanger activity (Sardet et al., 1990). Also, interactions between NHE1 and cytoskeletal proteins may be important to regulate cell size and stabilize regions of plasma membrane (Denker et al., 2000). For NHE6, two binding partners have so far been determined. First, the C-terminal tail of NHE6 was shown to interact with receptor for activated C kinase 1 (RACK1). Knockdown of RACK1 resulted in decreased NHE6 localization to the plasma membrane and increased endosomal pH indicating a role for RACK1 as a regulator of NHE6 trafficking (Ohgaki et al., 2008). Second, angiotensin II receptor subtype 2 (AT2) was shown to interact with the C-terminal tail of NHE6 and this interaction is proposed to increase NHE6 activity (Pulakat et al., 2005).

Dimerization of NHE6 may be important to promote ion exchange. NHE1 and NHE6 have been shown to form homodimers directed by interactions between transmembrane domains and between C-terminal tails (Fafournoux et al., 1994; Hisamitsu et al., 2004; Ilie et al., 2016). Although NHE1 monomers have been shown to be sufficient for Na^+/H^+ exchange (Fafournoux et al., 1994), exchange activity of the monomer is proposed to occur primarily at acidic pH and at a decreased rate at neutral

pH (Hisamitsu et al., 2006). Dimerization of NHE1 with a transport-deficient mutant inhibited ion exchange at physiological pH but not at pH 5.6. This data suggests that that dimerization may enhance NHE6 function because NHE6 localizes to early and recycling endosomes where the pH is 6.0-6.3 (Yamashiro and Maxfield, 1987).

The role of NHE6 in endosomes

Endocytosis is the process by which cells internalize receptors, fluids, membrane components, and solutes into vesicles through deformation of the plasma membrane. Through endocytosis, cells sample and respond to the extracellular environment. In fact, it is estimated that every hour mammalian cells internalize between 50% and 190% of their surface area and 3% to 26% of their volume (Steinman et al., 1976). Methods of endocytosis are broadly categorized as either phagocytosis or pinocytosis. Phagocytosis is performed by immune cells to destroy pathogens or degrade apoptotic cells (Conner and Schmid, 2003). Pinocytosis is more widely used and can be classified into four mechanisms: clathrin mediated endocytosis, caveolae mediated endocytosis, macropinocytosis, or clathrin and caveolae independent endocytosis. Clathrin mediated endocytosis is critical to nutrient uptake, signal transduction, and synapse vesicle fusion. Clathrin nucleates an assembly of proteins into a pit in the plasma membrane. After a receptor binds to adaptor proteins in the pit, the clathrin-coated membrane domain pinches off, the coat is removed, and the newly formed endosome fuses with other new endosomes or with early endosomes (Maxfield and McGraw, 2004). In caveolae mediated endocytosis, endosomes form through the invagination of membrane coated with caveolin protein. Through binding with cholesterol, caveolin directs the formation of plasma membrane domains enriched for cholesterol and sphingolipids in order to concentrate signaling molecules (Maxfield and McGraw, 2004). Macropinocytosis occurs

in response to growth factor stimulation whereby fusion of membrane ruffling forms large endocytic vesicles. Lastly, clathrin and caveolae independent endocytosis is not as well understood as other mechanisms but functions to internalize regions of the plasma membrane containing lipid rafts enriched for specific membrane proteins and glycolipids.

After internalization, endosomes undergo a process of maturation by which newly formed endosomes fuse with early endosomes, are transported along microtubules, and the endosomal lumen is acidified (Fig. 1.1). The early endosome reaches a weakly acidic pH of 6.0-6.3 which mediates the dissociation of ligands from receptors (Yamashiro and Maxfield, 1987). Early endosomes can transport cargo to the plasma membrane, to the recycling endosome, or to late endosomes. Ubiquitination of cargo protein directs targeting to the late endosome (Hu et al., 2015). Proteins targeted for degradation may be transported to the late endosome through vesicle budding or can be retained in the early endosomes and targeted to the late endosome through the process of endosome maturation (Maxfield and McGraw, 2004). The maturation of early endosomes to late endosomes is directed by a switch in the composition of Rab GTPases in endosomal membranes where Rab5 is replaced by Rab7. By recruiting specific motor proteins, vesicle tethering factors, and signaling effectors, Rabs define specific endosomal compartments (Huotari and Helenius, 2011). Late endosomes fuse with lysosomes forming the endolysosome where digestion of cargo by acid hydrolases takes place (Hu et al., 2015).

Progressive acidification of endosomes is required for proper regulation of endosome maturation, cargo sorting and trafficking, ligand and receptor interaction, and activation of lysosomal hydrolases. The pH decreases from pH 6.0-6.3 in early endosomes, to pH 5.0-6.0 in late endosomes, to pH 4.5-5.0 in lysosomes (Ohkuma and Poole, 1978; Schmid et al., 1989; Yamashiro and Maxfield, 1987). Endosomal pH is

maintained through the concerted function of several transporters (Fig. 1.2). The vacuolar (V-) ATPase acidifies endosomes by pumping H^+ into endosomes. The hydrolysis of ATP in the V_1 domain of the V-ATPase causes rotation of the V_0 domain to allow for transport of H^+ into the endosome (Forgac, 2007). To compensate for changes in the electric potential due to increased H^+ in the endosome, Cl^- channels and Cl^-/H^+ exchangers transport Cl^- into endosomes. When Cl^- channels or Cl^-/H^+ exchangers are disrupted, endosome acidification is attenuated indicating their importance in generating a proton gradient (Hara-Chikuma et al., 2005). Overacidification of endosomes is likely prevented through the action of NHEs. Mutation of the yeast NHE, Nhx1, results in overacidification of endosomes and trafficking defects (Brett et al., 2005). Several studies in mammalian cells support the role of the endosomal NHEs in pH regulation. Knockdown of NHE6 or NHE9 in mammalian cell lines had been found to increase endosome acidification while overexpression alkalinized endosomes (Ohgaki et al., 2010; Prasad and Rao, 2015; Xinhan et al., 2011). However, this effect can be variable as one study showed that overexpression of NHE6 in a mammalian cell line did not change endosomal pH (Nakamura et al., 2005). Another study found that endosomes became acidified when NHE6 and NHE9 were knocked down together but not individually suggesting that these proteins may have similar roles in pH regulation (Roxrud et al., 2009). In a NHE6 null mouse model, endosomes were shown to be overacidified and acidic endosomes were more often located along growing neurites than close to the soma as compared to controls (Ouyang et al., 2013). The combined actions of the V-ATPase, Cl^- channels and Cl^-/H^+ exchangers, and endosomal NHEs regulate intraendosomal pH.

The mechanisms that control the precise changes in pH during endosome maturation are unknown. Regulation of V-ATPase activity is proposed to establish the

specific pH of different types of endosomes. V-ATPase activity can be influenced by the rate of assembly and dissociation of the V_0 and V_1 V-ATPase complexes, modulation in the density of V-ATPases on membrane, and changes in the efficiency of transport due to the incorporation of subunit isoforms (Forgacs, 2007). In addition, varied NHE localization, expression level, and rate of exchange in different endosome compartments may establish the specific pH of an endosome compartment.

Specialized functions of neuronal endosomes and the role of NHE6

In order to meet the unique spatial demands of the highly polarized neuron, endosomes function in long range trafficking and localized signaling. Endosomes mediate the retrograde trafficking of neurotrophins that are released by postsynaptic cells and endocytosed in the presynaptic axon through binding to tropomyosin receptor kinase (Trk) receptors (Grimes et al., 1996). These signaling endosomes contain downstream signaling components allowing for signaling transduction through the Ras, PI3-kinase, and PLC- γ 1 pathways (Reichardt, 2006). The long range transport of neurotrophin signaling endosomes to the soma induces transcriptional changes that promote cell survival and neurite outgrowth (Cosker et al., 2008).

Activation of TrkB by the neurotrophin, BDNF, can also act locally by causing an increase in cyclic AMP (cAMP) and protein kinase A (PKA) activity that in turn promotes cytoskeletal changes to drive axon formation (Harrington and Ginty, 2013). In addition, increased cAMP and PKA activity triggers BDNF secretion and increases anterograde transport of TrkB receptors to axons working in an autocrine loop to enhance axon growth (Cheng et al., 2011). Endosomes are also required to direct dendritic growth and branching. In response to BDNF, recycling endosomes accumulate in dendrites and

recycle TrkB receptors to the surface to increase neurotrophin signaling thereby promoting dendritic growth (Lazo et al., 2013).

NHE6 plays a critical role in endosomal function in neurons. In a NHE6 null mouse model, the endosomes of hippocampal neurons are overacidified (Ouyang et al., 2013). Axon and dendrite branching, synapse number, and BDNF signaling through TrkB were reduced as compared to control. Functional connectivity was attenuated in the NHE6 null mouse due to reduced action potentials in presynaptic axons. Additionally, defects in neurite branching in the NHE6 null mouse could be rescued in cultured hippocampal neurons through treatment with BDNF (Ouyang et al., 2013). In other work, transfection of mutant NHE6 into primary hippocampal neurons decreased dendritic arborization and transferrin endocytosis while increasing cell death by apoptosis (Ilie et al., 2016).

Additional findings in NHE6 null mice have revealed phenotypes similar to that of lysosomal storage disease. In NHE6 null mice, GM2 ganglioside and unesterified cholesterol were found to accumulate in late endosome and lysosomes in neurons of the amygdala and hippocampus (Stromme et al., 2011). Staining for lysosomal markers suggested an expansion of lysosomes in NHE6 null mice as compared to control. In addition, there was a progressive loss of Purkinje cells in the cerebellum of NHE6 null mice indicating a role for NHE6 in Purkinje cell survival (Stromme et al., 2011).

Studies have also suggested a role for NHE6 at the excitatory synapse. NHE6 was shown in primary mouse hippocampal neurons to accumulate in dendritic spines and colocalize with markers of early and recycling endosomes as well as a subunit of the AMPA receptor (Deane et al., 2013). During induction of long-term potentiation (LTP), the trafficking of vesicles containing NHE6 to dendritic spines increased (Deane et al.,

2013). These results indicate that NHE6 may contribute to the endosomal recycling of AMPA receptors at the postsynapse in order to establish LTP.

Accumulating evidence points to a role for NHE6 in endosomal pH regulation in the mediation of axon and dendrite branching, trafficking of signaling receptors, and possibly synaptic vesicle recycling. However, the function of NHE6 in a model of human neuronal development has yet to be tested. Also, there is need for an improved understanding of the potential therapeutic interventions for the treatment of Christianson syndrome.

Using iPSCs to model neurological disease

The development of iPSC technology

Developing models that accurately represent human neurological disease is key to identifying causative cellular mechanisms and opportunities for treatment. Mouse models are often poor predictors of clinical success due to the increased complexity of the human brain and the inability of mouse model to represent the variability between patients (Dragunow, 2008). Brain tissue can be obtained from patients postmortem, but causality of disease mechanisms is difficult to infer. It is possible to culture human neural stem cells from patient biopsy samples or from postmortem tissues, but neural stem cells have limited potential for self-renewal and are difficult to obtain (Sterneckert et al., 2014). Human embryonic stem cells (hESCs) with pluripotent capacity can be derived from the inner cell mass of pre-implantation embryos that have undergone screening for genetic disease. These cells can be differentiated to neuronal fate to model disease, but the difficulty and ethical concerns in attaining hESCs are challenging to disease modeling (Sterneckert et al., 2014). Therefore, induced pluripotent stem cells (iPSCs)

provide an invaluable preclinical model to study the cellular and molecular mechanisms of human disease and to develop therapies.

The discovery of methods to produce iPSCs has revolutionized the stem cell field and disease modeling. The ability to induce pluripotency in somatic cells was observed in 1962 by John Gordon through somatic cell nuclear transfer (SCNT). Gurdon observed in *Xenopus laevis* that transplantation of the nucleus from a cell of the intestinal epithelium into an enucleated egg, could give rise to tadpoles (Gurdon, 1962). These results suggested that the specification of a somatic cell is reversible and oocytes contain factors that can induce pluripotency in a somatic cell nucleus. SCNT was later replicated in human cells through the fusion of a human fibroblast cell and an enucleated oocyte. Blastocysts developed from which pluripotent hESCs could be derived from the inner cell mass thereby generating patient specific hESCs (Tachibana et al., 2013). Also key to the development of iPSCs was the discovery that the ectopic expression of master transcription factors can change the fate of a cell. This was first demonstrated when the expression of the muscle specific transcription factor, MyoD, was shown to convert fibroblasts to myoblasts (Davis et al., 1987).

Building on this work, Shinya Yamanaka's group discovered a combination of transcription factors that can induce pluripotency when expressed in somatic cells (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). By screening a pool of 24 candidate transcription factors for the ability to turn on the expression of an ESC-specific gene when retrovirally transduced into fibroblasts, a set of four necessary transcription factors were identified: Oct3/4, Sox2, c-Myc, and Klf4 (Takahashi and Yamanaka, 2006). Expression of these four factors in fibroblasts generated colonies that had similar gene expression patterns and pluripotency characteristics to hESCs and were termed iPSCs.

Improved methods to reprogram somatic cells to iPSCs have since been developed. Lentiviral expression of a polycistronic vector expressing the four Yamanaka transcription factors from a single promoter improved transduction efficiency (Carey et al., 2009). However, virus integration may have mutagenic effects and although integrated genes are silenced when pluripotency is achieved, re-activation is possible (Takahashi and Yamanaka, 2006). Therefore, non-integrating methods of reprogramming have been developed. Sendai virus, an RNA virus, can be used to express the Yamanaka factors and is diluted out from cells after several passages (Fusaki et al., 2009). The transfection of *in vitro* transcribed mRNAs of the Yamanaka factors has been used to reprogram fibroblasts although daily transfections are required (Warren et al., 2010). A single transfection of an Epstein-Barr virus based self-replicating episome expressing nine different genes can be used to reprogram somatic cells but with low efficiency (Yu et al., 2009). The choice of reprogramming method depends on the type of somatic cell being reprogrammed as efficiency varies based on the starting cell type. Also, the potential application for clinical use may favor the use of non-integrating methods. After careful testing for pluripotency and genome integrity, iPSC lines provide a renewable source of pluripotent cells. The potential to easily obtain patient tissue for reprogramming and the capacity of iPSCs to differentiate to any tissue in the body make iPSCs an invaluable tool for disease modeling.

Differentiation of iPSCs to neuronal fate

Protocols to differentiate iPSCs to neuronal fate follow the principles of developmental biology in order to recapitulate early development (Fig. 1.3). During *in vivo* neural induction, the suppression of bone morphogenic protein (BMP) signaling by the BMP antagonists, noggin and chordin, in cells of the neural plate specifies the

formation of neural ectoderm (Liu and Niswander, 2005). Folding of the neural plate forms the neural tube and a rapid division of neuroepithelial stem cells along the neural tube walls follows. A dorsal-ventral axis is specified in the neural tube by Wnt signaling dorsally and expression of sonic hedgehog (SHH) ventrally (Wilson and Rubenstein, 2000). Posterior regionalization is directed through retinoic acid (RA) and fibroblast growth factor (FGF) signaling to develop the areas that will become the midbrain and spinal cord (Mason, 2007; Rhinn and Dolle, 2012). Cells in the anterior maintain anterior fate by expressing antagonists of caudalizing signaling such as the Wnt inhibitor, Dickkopf-related protein 1 (DKK1). The anterior region of the neural tube expands to form the forebrain containing the telencephalon, which will give rise to the cerebral cortex and basal telencephalon, and the diencephalon.

To differentiate iPSCs or ESC to a specific neuronal fate, cultures can be treated with activators or inhibitors of the factors that direct regional patterning during *in vivo* brain development thereby restricting cell fate to the desired cell type. For example, addition of SHH, RA, and FGF to human pluripotent stem cells (hPSCs) produces neurons with characteristics of posterior spinal motor neurons or midbrain neurons (Elkabetz et al., 2008). To direct hPSCs to ventral anterior fate, inhibition of Wnt and activation of SHH can be used to differentiate to cortical interneurons (Maroof et al., 2013). Treatment of hPSCs with inhibitors of BMP signaling through dual SMAD inhibition generates cells with features of excitatory neurons of the neocortex (Shi et al., 2012b).

The differentiation of iPSCs to cortical fate allows researchers to study a large number of neurological diseases with compromised cortical function including epilepsy, autism, and neurodegenerative diseases such as Alzheimer's (Pasca et al., 2014). In CS, patient symptoms including intellectual disability, autistic features, epilepsy,

microcephaly, and non-verbal status may result from deficiencies of the cortex. The human cortex is critical to cognition, sensory perception, language, and motor function and has expanded in size and complexity during human evolution (Franco and Muller, 2013). The neocortex is composed of approximately 80% excitatory projection neurons and 20% inhibitory interneurons (Molyneaux et al., 2007). Cortical projection neurons develop from progenitor cells in the dorsal telencephalon that line the ventricles to form the ventricular zone (VZ). Neuroepithelial stem cells in the VZ give rise to radial glia (RG) neural stem cells. RG extend processes from the VZ to the pial surface providing a scaffold for the migration of projection neurons. RG divide asymmetrically to produce another RG stem cell and either an early-born neuron or an intermediate progenitor cell (IPC). IPCs migrate from the VZ to the subventricular zone (SVZ) and are transit-amplifying cells that undergo symmetric divisions to produce either more IPCs or a pair of projection neurons (Molyneaux et al., 2007).

Another class of progenitors cells located in the outer SVZ (oSVZ) called outer radial glia (oRG) has recently been identified that can self-renew and produce neurons (Hansen et al., 2010). The increase in size of the human brain is largely due to an increase in the size of the cerebral cortex. A longer period of neurogenesis in humans allows for increased division of progenitor cells and an expansion particularly of the cells of the oSVZ (Lui et al., 2011). The neocortex is composed of organized layers formed by the migrations of projection neurons. The earliest born neurons migrate from the VZ to form the preplate. The formation of the cortical plate splits the preplate to give rise to the marginal zone, layer I of the cortex, and the subplate. The marginal zone consists of Cajal-Retzius that secrete Reelin to direct the migration of projection neurons during development. The subplate is a region of axonal targeting and receives afferents from the thalamus (Lui et al., 2011). During neurogenesis, neurons migrate from proliferative

zones to the cortical plate. Later born neurons migrate past earlier born neurons in an inside-out fashion to form an organized six layered structure. The neurons within each layer project in a layer-specific manner across the corpus collosum (layers II/III, IV, V, and VI), to the thalamus (layer VI), or to subcerebral targets (layer V) (Molyneaux et al., 2007). Transcription factor networks direct cortical layer identity and projection neurons can be classified based in their location and the expression of transcription factor layer markers (Qian et al., 1998). Inhibitory interneurons migrate into the cortex from the medial and caudal ganglionic eminences of the ventral telencephalon. Interneurons make local connections within the layers of the cortex and serve to modulate the activity of the excitatory projection neurons (Franco and Muller, 2013). The development of the neocortex is critical to establishing the neuronal circuitry needed for complex cognitive function.

Protocols to differentiate iPSCs to generate cells with features of excitatory cortical neurons recapitulate *in vivo* development. The first methods described for neuronal induction from hPSCs used an embryoid body (EB) aggregate method (Zhang et al., 2001). When hPSCs are grown in the absence of mitogens, inhibitory factors, or feeder layers, they spontaneously differentiate forming floating spherical aggregates called EBs. EBs were differentiated to produce mixed cultures of glutamatergic and GABAergic neurons and glia (Zhang et al., 2001). To differentiate EBs specifically to cortical neuronal fate, EBs can be treated with DKK1, a Wnt inhibitor, and LeftyA, an inhibitor of Nodal signaling (Eiraku et al., 2008). EB aggregate methods generate neuronal cells expressing features of deep layer cortical neurons but not upper layer cortical neurons possibly due to a lack of neuronal progenitor cell diversity in cultures (Eiraku et al., 2008).

A second approach to differentiate iPSCs to excitatory cortical neuronal fate is through an adherent monolayer method. In this approach, iPSCs are plated in a monolayer and treated with retinoic acid (RA) and two inhibitors of SMAD signaling, dorsomorphin and SB431542, to generate neuronal progenitors (Shi et al., 2012a). RA may serve a similar function in iPSC neuronal differentiation as has been observed in the mouse cortex *in vivo*. RA has been shown to play a role in the radial expansion of the mouse cortex by promoting the transition from VZ progenitor expansion to the onset of neurogenesis (Siegenthaler et al., 2009). The use of dual SMAD inhibition for hPSC differentiation to neuronal fate was first described in hESCs (Chambers et al., 2009). Dorsomorphin inhibits the phosphorylation of receptor SMADs (SMAD1/5/8) by BMP receptor type I (Yu et al., 2008). During *in vivo* neural induction, BMP inhibition is needed in the formation of the neural plate (Liu and Niswander, 2005). SB431542 blocks TGF- β and activin signaling by inhibiting the TGF- β type I receptors: ALK4, ALK5, and ALK7, thereby blocking phosphorylation of SMAD2 (Inman et al., 2002). Inhibition of this signaling pathway blocks the formation of mesoderm and promotes neural induction (Hemmati-Brivanlou and Melton, 1994).

In contrast to the EB aggregate protocols, the dual SMAD inhibition protocol generates diverse populations of neuronal progenitor cells including radial glia, intermediate progenitors, and oRGs (Shi et al., 2012b). Addition of FGF2 to the cultures allows for expansion of neuronal progenitors and removal promotes the onset of neurogenesis (Mason, 2007). In comparison to the EB aggregate methods which generate only deep layer neurons, the dual SMAD inhibition method produces neurons with features of deep and upper layer cortical neurons possibly as a result of having more diverse classes of neuronal progenitors (Shi et al., 2012b). Formation of mature neurons follows the sequential production of cortical projection neurons *in vivo* with cells

expressing markers of deep layer cortical neurons being formed two to three weeks after neural induction and cells expressing markers of upper layer neurons being generated subsequently for up to 90 days after neuronal induction (Shi et al., 2012b). These protocols make it possible to generate patient specific iPSC-derived neurons to model neurological diseases affecting the cortex.

The potential for iPSCs in disease modeling and therapeutic development

The differentiation of patient derived iPSCs to neuronal fate provides a preclinical model to study disease phenotypes and inform therapeutic interventions (Fig. 1.4). Modeling neurological disease with patient iPSCs differentiated to neuronal fate allows for the study of a disease relevant cell type typically inaccessible to researchers. Furthermore, the ability to study an individual patient's cells allows for the influence of genetic background to be assessed (Marchetto et al., 2010). This is particularly important in diseases with complex genetics, such as autism spectrum disorder, to model the contributions of genetic variants at several loci (Sterneckert et al., 2014).

iPSC-derived models of neurological disease enable the discovery and testing of potential drug therapies. After identifying a disease relevant phenotype that can be monitored and robustly quantified, candidate therapies and drug libraries can be screened for phenotype reversal (Pasca et al., 2011). Also, new druggable targets can be identified by testing if overexpressing or reducing the expression of candidate targets in iPSC-derived neurons changes the disease phenotype (Sterneckert et al., 2014).

Patient iPSCs have been used to model an increasing number of neurological diseases including Rett syndrome, Timothy syndrome, Phelan-McDermid syndrome, Fragile X syndrome, Prader-Willi and Angelman syndromes, Alzheimer's disease and autism spectrum disorder (Chamberlain et al., 2010; Marchetto et al., 2010; Mariani et

al., 2015; Muratore et al., 2014; Pasca et al., 2011; Shcheglovitov et al., 2013; Sheridan et al., 2011). High throughput drug screening has begun to identify potential therapies for these diseases. For example, a 50,000 compound library was used to screen neuronal cultures derived from iPSCs of patients with fragile X syndrome. Researchers identified drugs that allowed for re-expression of *FMR1* which is silenced in Fragile X syndrome due to repeat expansion induced hypermethylation of *FMR1* (Kaufmann et al., 2015).

Testing of candidate drugs in iPSC-derived neuronal cultures can also be used to identify potential therapies. In Rett syndrome, IGF1 was identified as a candidate drug in mouse models (Tropea et al., 2009). When IGF1 treatment was tested in iPSC-derived neuronal cultures from Rett syndrome patients, IGF1 restored deficits in the number of excitatory synapses (Marchetto et al., 2010). In another study, Phelan-McDermid syndrome patient iPSCs differentiated to neuronal fate demonstrated deficits in excitatory synapse formation. The testing of a few candidate drugs for the ability to restore synaptic defects identified IGF1 as a potential therapy (Shcheglovitov et al., 2013). Clinical trials for the treatment of Rett syndrome and Phelan-McDermid syndrome with IGF1 have shown promise for alleviating patient symptoms (Khwaja et al., 2014; Kolevzon et al., 2014). These discoveries highlight the potential for iPSCs as preclinical models of neurological disorders. Through establishing iPSCs from patients with Christianson syndrome and differentiating to neuronal fate, we aim to gain insight into disease mechanisms and to provide a platform for testing drug therapies.

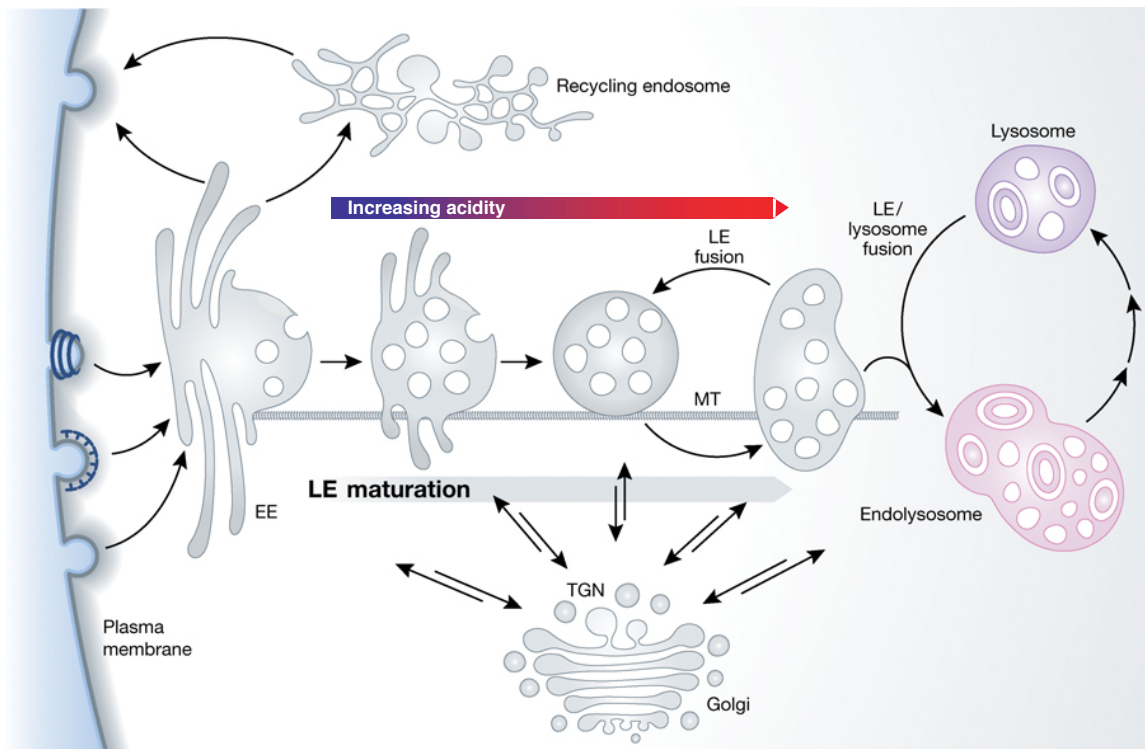


Figure 1.1 Endosomal trafficking and maturation. Endocytic vesicles from the plasma membrane fuse with early endosomes (EE). Cargo can be sorted to the recycling endosomes for return to the plasma membrane or can be trafficked to late endosomes (LE) and lysosomes for degradation. As endosomes mature moving along microtubules (MT) toward the perinuclear space, the endosomal pH becomes more acidic. The LE fuses with lysosomes to form the endolysosome where cargo is degraded by acid hydrolases. Finally, the endolysosome matures to form the lysosome. Throughout this process, endosomes send and receive protein cargo from the *trans*-Golgi network (TGN). (Adapted from Huotari, J. and Helenius, A. (2011) Endosome Maturation. *The EMBO Journal*, 30 (17) 3481-3500. with permission from Wiley Company.)

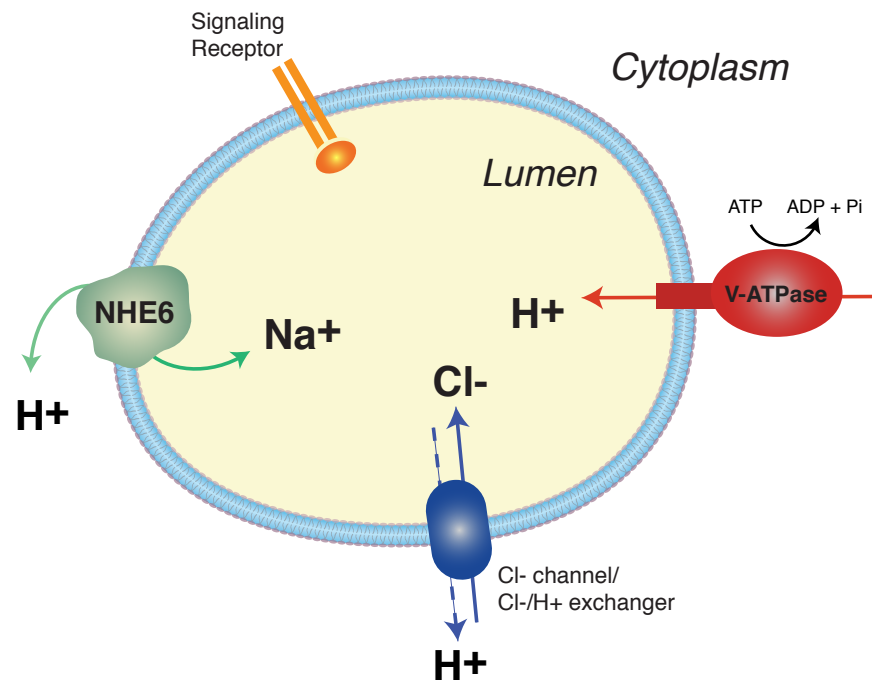


Figure 1.2 Control of endosome acidification. The vacuolar ATPase (V-ATPase) pumps H^+ into the endosome in an ATP dependent manner. To compensate for changes in the electric potential, Cl^- channels and Cl^-/H^+ exchangers import Cl^- into the endosome. NHE6 transports Na^+ into the endosome in exchange for H^+ to maintain pH homeostasis. pH maintenance is critical to the trafficking of cargo such as signaling receptors.

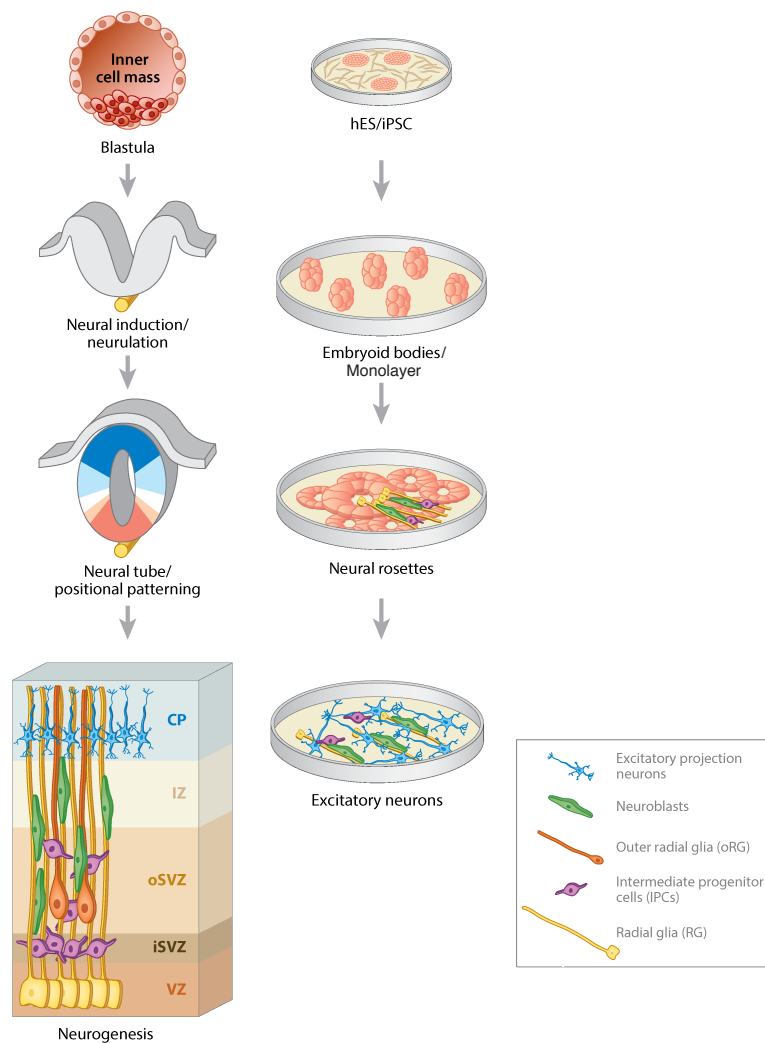


Figure 1.3 Differentiation of iPSCs to neuronal fate recapitulates *in vivo* development. Human embryonic stem cells (hES) and induced pluripotent stem cells (iPSC) share features of pluripotency with the inner cell mass of the blastula. The formation of embryoid bodies or formation of a monolayer of neuroepithelial cells mirrors the beginning stages of neural induction *in vivo*. Neural rosettes are composed of neural progenitor cells and reflect formation of the neural tube. Excitatory neurons formed in culture have features of projection neurons generated *in vivo*. VZ, ventricular zone; iSVZ, inner subventricular zone; oSVZ, outer subventricular zone; IZ, intermediate zone; CP, cortical plate. (Adapted from Pasca, S., Panagiotakos, G., and Dolmetsch, R. (2014) Generating Human Neurons and Using them to Understand Neuropsychiatric Disease. *Annual Review of Neuroscience*. 37:479-501. with permission.)

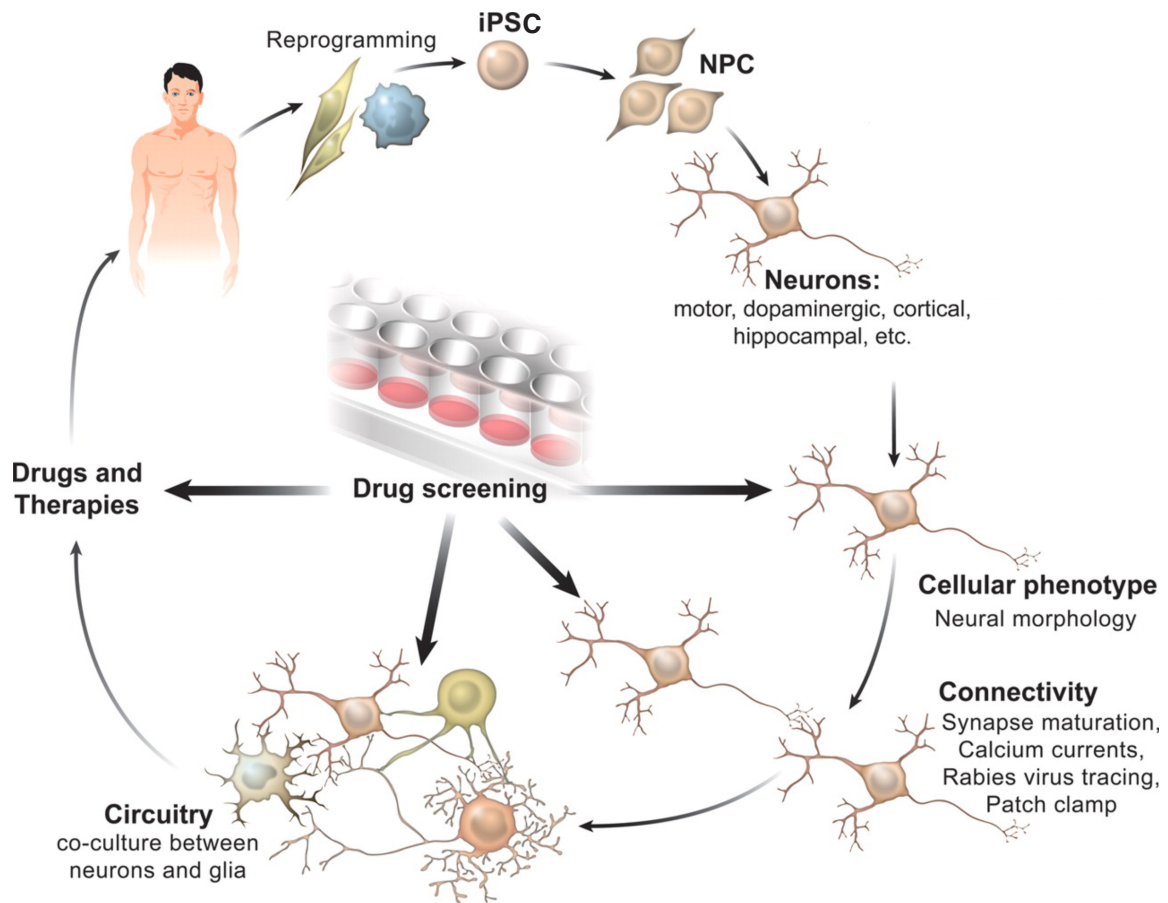


Figure 1.4 Using iPSCs to model neurological disease. Somatic cells from patients with neurological disease and controls can be reprogrammed to generate iPSCs. Differentiation of iPSCs to neural progenitor cells (NPC) and neurons allows researchers to assess diverse cellular phenotypes including neuronal morphology, connectivity, and circuitry. Importantly, iPSC-derived neurons provide a platform for testing the cellular response to candidate drug therapies in an individualized manner. (Adapted from Marchetto, M., Winner, B., and Gage, F. (2010) Pluripotent stem cells in neurodegenerative and neurodevelopmental diseases. *Hum Mol Gen*, 19 (R1) R71-6. by permission of Oxford University Press.)

CHAPTER 2:

Establishment of a model of Christianson syndrome using iPSCs

The iPSC lines used in this study were generated and characterized for pluripotency by the Cincinnati Children's Hospital Medical Center Pluripotent Stem Cell Facility. The laboratory of Dr. Avner Schlessinger performed the structural modeling in Fig. 2.14 – Fig. 2.16.

INTRODUCTION

Christianson syndrome (CS) is an X-linked, monogenic, neurological disorder associated with mutations in the gene encoding Na⁺/H⁺ exchanger 6 (NHE6), *SLC9A6*. Symptoms of CS include intellectual disability, autistic features, epilepsy, ataxia, microcephaly, non-verbal status, and hyperkinesia. Currently there are no treatments for CS apart from management of disease symptoms. CS is one of the most common forms of X-linked intellectual disability (Tarpey et al., 2009); however, the cellular mechanisms driving CS are not fully understood. Improved understanding of disease mechanisms will inform the development of drugs to treat CS. Further, cell models closely linked to human disease may be critical for therapeutic development.

Studies of the NHE6 null mouse have revealed defects in neuronal circuitry and endosomal signaling (Ouyang et al., 2013). However, the specific mechanism by which gene mutation alters protein production in CS remains to be carefully defined to assess the genetic construct validity of the NHE6 null mouse for human disease. Furthermore, the human brain differs from that of the mouse in many respects. Specifically, the human cortex, which is responsible for cognitive and sensory processing, is greatly expanded in size and complexity largely due to an increase in progenitor cells (Lui et al., 2011). Cortical development is longer in humans allowing for an expansion of upper layer neurons and an increase in the diversity of cell types (Hill and Walsh, 2005). Additionally, unlike the inbred mouse model of CS, the human condition occurs in highly variable genetic backgrounds. A true model of the human disease should incorporate an opportunity to explore the nature of this variability in order to understand how such variability may impact the efficacy of treatments. An improved understanding of NHE6 mutation in a model of human neuronal development will provide insight into the neurological deficits seen in CS patients.

Advances in stem cell technology have made it possible to examine the effects of NHE6 mutations in human derived neuronally fated cells. The discovery of methods to reprogram human somatic cells to produce pluripotent stem cells has revolutionized the stem cell field (Takahashi et al., 2007). These induced pluripotent stem cells (iPSCs) have the potential to differentiate to any cell in the body. Protocols have been developed to direct the differentiation of iPSCs to many cell fates including cardiomyocytes, skeletal muscle, pancreatic β -cells, motor neurons, astrocytes, and cortical neurons with the addition of small molecules, growth factors, and culture media supplements (Shi et al., 2017).

Patient-derived iPSCs provide a valuable model for human neurological disease enabling researchers to study mechanisms of disease in the genetic background of an individual patient. For example, iPSCs derived from patients with neurological diseases including Timothy syndrome, Phelan-McDermid syndrome, and idiopathic autism have been differentiated to neuronal fate to reveal insights into disease pathology and provide platforms for personalized drug screening (Marchetto et al., 2016; Pasca et al., 2011; Shcheglovitov et al., 2013). Another unique advantage of iPSCs for disease modeling is that differentiation of iPSCs to cortical neuronal fate follows the timing of human development *in vivo* allowing researchers to interrogate cellular dysfunction occurring early in human development (Shi et al., 2012b).

In this study, we generated multiple iPSC lines from peripheral blood mononuclear cells (PBMCs) donated by CS patients and their unaffected siblings as controls. The NHE6 mutations in these CS patients were all distinct *de novo* mutations. The CS patients displayed intellectual disability, seizures, microcephaly, autistic features, ataxia, non-verbal status, and hyperkinesia (Pescosolido et al., 2014). The iPSCs were tested for pluripotency by marker expression and the ability to differentiate

to tissues derived from all three germ layers. We found that NHE6 expression was lost or decreased in CS patients at both the mRNA and protein level. Any remaining NHE6 protein was predicted to be misfolded and nonfunctional. These results demonstrate that in CS, mutations of NHE6 likely lead to complete loss of protein and thereby mutations are full null mutations.

RESULTS

Reprogramming somatic cells from CS patients and controls

To determine the cellular and molecular mechanisms underlying CS pathophysiology in humans, we derived iPSCs from CS patients with distinct mutations in NHE6. iPSCs were also derived from each proband's unaffected male sibling as a paired control. The proband mutations based on NHE6 transcript NM_001042537 are: Family 1) a single base pair duplication leading to a frameshift and premature stop codon in exon 11 corresponding to a loop region between predicted transmembrane domains 10 and 11 (c.1414dupA, p.R472fsX4), Family 2) a nonsense mutation in exon 12 located in predicted transmembrane domain 12 (c.1568G>A, p.W523X), and Family 3) a missense mutation occurring at the first base of exon 9 in predicted transmembrane domain eight (c.1148 G>A, p.G383D) (Fig. 2. 1 and Fig. 2.2) (Pescosolido et al., 2014; Yates et al., 2016). Transmembrane domains were predicted by the transmembrane hidden Markov model (TMHMM) for Ensembl transcript ENST00000370695 (Krogh et al., 2001; Yates et al., 2016). iPSCs were derived from peripheral blood mononuclear cells (PBMC) donated by the patients and controls. PBMCs were transduced with a multicistronic lentivirus encoding OCT4, KLF4, SOX2 and C-MYC (Kunisato et al., 2011; Warlich et al., 2011). Mutations in NHE6 did not inhibit the reprogramming potential of the PBMCs. Thus, we were able to generate multiple subclonal iPSC lines from most donors with the exception of the CS patient carrying the R472fsX4 mutation, where only one subclonal line was obtained (Table 2).

Characterization of CS and control iPSCs

To assess the efficiency of reprogramming, iPSC sub-clonal lines for each CS patient and control donor were analyzed for growth characteristics, genomic stability,

and pluripotency (Table 2). All sub-clonal lines shared similar morphological features with human embryonic stem cells (hESC) when grown on a feeder free substrate (Fig. 2.3). iPSC colonies were multicellular and composed of tightly packed cells with a large nucleus to cytoplasm ratio and visible nucleoli. Minimal spontaneous differentiation was observed. Karyotypes of representative sub-clonal lines were normal demonstrating that reprogramming had not caused any large chromosomal aberrations (Fig. 2.4).

Several measures were used to evaluate iPSC pluripotency. First, alkaline phosphatase activity, which has been shown to be increased in pluripotent cells as compared to differentiated cells, was measured (O'Connor et al., 2008). Representative iPSC sub-clones stained positive for alkaline phosphatase activity indicating successful reprogramming (Fig. 2.5). Second, immunohistochemistry of iPSC subclones showed nuclear expression of OCT4 and NANOG, transcription factors required for maintenance of pluripotency (Fig. 2.6). Third, the cell surface pluripotency markers, Tra-1-60 and SSEA, were highly expressed in iPSC subclones as analyzed by flow cytometry (Fig. 2.7). Fourth, the expression of pluripotency markers, DNMT3B, GABRB3, NANOG, POU5F1, TDGF1, and ZFP42, was measured by qRT-PCR. iPSC subclones expressed these markers to similar levels as undifferentiated H1 and H9 hESCs (Fig. 2.8). In the absence of leukemia inhibitory factor or feeder layers, hESC and iPSCs differentiate spontaneously to form spherical aggregates called embryoid bodies (EBs) which contain tissue from all three germ layers. iPSC subclones express pluripotency markers to higher levels than H1 and H9 hESC-derived EBs (Fig. 2.8).

Fifth, sub-clonal lines were characterized for their *in vitro* differentiation potential by differentiating iPSCs to EBs and measuring germ layer markers by qRT-PCR. EBs derived from iPSC sub-clones show decreased expression of pluripotency markers and increased expression of endoderm, ectoderm, and mesoderm markers as compared to

undifferentiated iPSCs (Fig. 2.9). Lastly, iPSCs subcutaneously injected into NOD/SCID GAMMA C^{-/-} mice formed teratomas containing tissues of endoderm, mesoderm and ectoderm demonstrating *in vivo* differentiation potential (Fig 2.10). In summary, the iPSC morphology, normal karyotypes, expression of pluripotency markers, and ability to differentiate to tissues derived from all three germ layers both *in vitro* and *in vivo* demonstrate the successful reprogramming of CS and control PBMCs to iPSCs.

NHE6 mRNA expression in patient derived iPSCs

To determine the effect of the patient mutations on NHE6 expression, we conducted northern and western blot analyses in representative sub-clonal lines for each mutation. Northern blotting revealed that expression of *SLC9A6* mRNA is lost or reduced in CS iPSCs as compared to controls. *SLC9A6* mRNA was absent in the CS patients carrying the c.1414dupA/p.R472fsX4 and c.1568G>A/p.W523X mutations while 58% $\pm$ 18.4% of the transcript remained in the CS patient carrying the c.1148G>A/p.G383D mutation (Fig. 2.11).

To test if mRNA is degraded by nonsense-mediated decay (NMD), we treated iPSC with cycloheximide (CHX). CHX prevents NMD by inhibiting translation thereby interfering with the recognition of premature stop codons. The *SLC9A6* transcript was recovered to control levels after treatment of iPSCs with CHX indicating that the degradation of *SLC9A6* mRNA in CS is mediated by NMD. Although this result may be expected for the truncating mutations which generate an early stop codon, R472fsX4 and W523X, the missense mutation, G383D, also shows recovery of transcript after CHX treatment. The c.1148G>A/p.G383D mutation is located at the first base of exon 9 in *SLC9A6* in the region of the 3' splice site (Fig. 2.2). This mutation may disrupt splicing causing skipping of exon 9 and splicing of exon 8 to exon 10 resulting in a frameshift.

This alternative transcript would generate a premature stop codon two codons downstream of the exon 8 and exon 10 junction. Therefore, an alternative transcript that skips exon 9 would be degraded by NMD and could be recovered by CHX treatment. This transcript would be 89 base pairs smaller than the full-length transcript which may not be resolved by northern blot.

NHE6 protein expression in patient derived iPSCs

We next determined the relative amount of NHE6 protein expressed in CS and control iPSCs. A mouse anti-NHE6 antibody generated in the Morrow laboratory showed loss of NHE6 protein in a NHE6 null mouse model (Ouyang et al., 2013). The C-terminal epitope for this antibody shows 100% identity with human NHE6, however the epitope also overlaps with NHE7 (Fig. 2.12 A). Therefore, immunoprecipitation (IP) of NHE6 followed by western blot was performed with the hypothesis that binding to antibody conjugated beads and withstanding subsequent washes would require stronger protein-antibody interactions than western blotting. In this case, the higher degree of overlap between the antibody epitope and NHE6 as compared to NHE7 would allow for increased detection of NHE6 and decreased detection of background NHE7 bands. IP of NHE6 followed by western blotting for NHE6 demonstrated that NHE6 protein was absent in the CS lines carrying the c.1414dupA/p.R472fsX4 and c.1568G>A/p.W523X mutations (Fig. 2.12 B). However, as compared to control, $0.82\% \pm 0.34\%$ of NHE6 monomer and $7.33\% \pm 2.90\%$ of NHE6 dimer remained in CS lines with the c.1148G>A/p.G383D mutation.

Although a small percentage of full length NHE6 protein is expressed in the CS lines carrying the missense mutation, the protein may not be functional. Structural modeling of the c.1148G>A/p.G383D mutation was performed based on the crystal

structure of the *E.coli* Na⁺/H⁺ exchanger, NhaA (Hunte et al., 2005). The G383 residue is highly conserved across human, yeast, and bacterial Na⁺/H⁺ exchanger homologs (Fig 2.13). Additionally, Consurf was used to calculate the rate of evolution at each residue and map the level of conservation onto the protein surface (Ashkenazy et al., 2016). Consurf shows a high conservation score for the G383 residue (Fig. 2.14). Mapping of hydrophobicity onto the NHE6 model provides a test of the model accuracy. In the NHE6 model, hydrophobic residues are located in the transmembrane domains while polar residues are located in loops (Fig. 2.15).

The NHE6 model predicts that mutation to D383 would disrupt the helix packing between transmembrane domain 8 and transmembrane domain 2 due creation of a hydrogen bond between D383 and N166 and loss of an interaction between D383 and E133. This would cause the inward open NHE6 conformation to be stabilized (Fig. 2.16). Also, in the G383D model the R500 residue on transmembrane domain 11 may form a salt bridge which may negatively affect ion transport (Fig. 2.16). Therefore, NHE6 protein expressed with the missense mutation may be misfolded leading to disrupted protein function.

DISCUSSION

Mutations in *SLC9A6* cause the severe neurological disorder, Christianson syndrome, as previously reported in numerous pedigrees (Christianson et al., 1999; Garbern et al., 2010; Gilfillan et al., 2008; Pescosolido et al., 2014; Schroer et al., 2010). Further, sequencing of exons on the X-chromosome in patients with X-linked intellectual disability (XLID) identified *SLC9A6* among the most commonly mutated genes in XLID (Tarpey et al., 2009). Despite these reports, much remains to be understood concerning the development and treatment of CS. Establishing a model of CS in human iPSCs provides a valuable system to investigate disease mechanisms and provides a platform for therapeutic screening.

We established iPSC lines derived from three CS patients and their unaffected siblings as controls. PBMCs donated from these individuals were reprogrammed to generate multiple iPSC subclones. iPSCs had normal karyotypes ensuring that major chromosomal aberrations had not occurred as a result of reprogramming. To confirm effective reprogramming, iPSCs were extensively tested for pluripotency. iPSCs expressed markers of pluripotency and demonstrated the ability to differentiate to tissues from all three germ layers *in vitro* and *in vivo*. Therefore, these results indicate the successful development of a human iPSC model for CS.

To test the level of NHE6 expression in the CS iPSC lines, we first assessed mRNA levels by northern blot. Although *SLC9A6* mRNA was absent in the R472fsX4 and W523X CS lines, transcript remained in the G383D CS line. Upon cycloheximide treatment, *SLC9A6* transcript was recovered in all three lines indicating recovery of transcripts otherwise degraded by NMD. Recovery of the *SLC9A6* transcript was anticipated in the R472fsX2 and W523X CS lines which contain early stop codons. However, for the G383D CS line an increase in *SLC9A6* transcript levels would not be

hypothesized for a missense mutation. On closer inspection, this missense mutation may also function to alter splicing and generate a premature stop codon.

The c.1148G>A, p.G383D mutation occurs at the first base of exon 9. Past analysis of the 3' splice site consensus sequence has shown that at most intron-exon boundaries, there is an invariant intronic AG dinucleotide followed most commonly by G as the first exonic base (Zhang, 1998). The G>A mutation at the first base of exon 9 may disrupt splicing by interfering with interactions between the 3' splice site and components of the spliceosome. For example, the small subunit of U2AF binds to the 3' splice site and is required for the recruitment of the U2 small nuclear ribonucleoprotein (snRNP) to the branch point in order to initiate splicing (Wu et al., 1999). It is possible that mutation from guanine at G383 which 1) is the base most commonly present at the first base of an exon and 2) is evolutionarily conserved in *SLC9A6*, may disrupt the interaction of *SLC9A6* pre-mRNA with U2AF.

Splice acceptor site mutations can result in either exon skipping, where the splice acceptor site of the next downstream exon is used, or in the use of a downstream cryptic splice site (Sibley et al., 2016). Disruption of splicing at exon 9 may result in exon skipping causing exon 8 to be spliced to exon 10. Because exons 8 and 10 are out of frame, a premature stop codon would be generated two codons after the exon 8 and exon 10 junction. Due to this premature stop codon, this alternative transcript would be degraded by NMD and therefore would be recoverable by CHX treatment. Alternatively, a cryptic splice site downstream of the intron-exon 9 boundary could be used as the splice acceptor site, but this site would need to fit the specifications of having the 3' splice site sequence YAG (Y is a pyrimidine) and be preceded by both a polypyrimidine tract and the branch point consensus sequence (Sibley et al., 2016).

To summarize, in iPSCs from the c.1148G>A, p.G383D CS line, $58\% \pm 18.4\%$ of mRNA from is still expressed. The mRNA seen by Northern blot in untreated cells may represent full length *SLC9A6* containing the c.1148G>A mutation. The mRNA level is decreased possibly due to a defect in splicing that leads to a frameshift and premature stop codon. Recovery of an alternative transcript 89 base pairs smaller than full length *SLC9A6* may account for transcript recovery with CHX treatment.

Next, we found that NHE6 protein was absent in the R472fsX2 and W523X CS lines and reduced in the G383D line. Although full length *SLC9A6* mRNA containing the c.1148G>A/p.G383D mutation was found to be produced at 58% of control levels, the protein product was present at comparatively lower levels with $0.82\% \pm 0.34\%$ of NHE6 monomer and $7.33\% \pm 2.90\%$ of NHE6 dimer remaining. This indicates a post-translational mechanism resulting in decreased protein levels. In cell lines transfected with another reported patient mutation, a 6 base pair inframe deletion (E287_S88del), mutated NHE6 was shown to be degraded primarily through endoplasmic reticulum-associated degradation (ERAD) (Ilie et al., 2016; Roxrud et al., 2009). Additionally, a fraction of mutant NHE6 was shown to be trafficked through the endosomal system and ubiquitinated for degradation in lysosomes (Ilie et al., 2016; Roxrud et al., 2009). NHE6 G383D mutant protein may also be processed by the proteasome and lysosome for degradation.

Structural modeling of NHE6 with the G383D mutation predicted protein misfolding due to the disruption of the helix packing between transmembrane domains. Therefore, any remaining NHE6 protein in the G383D line may not function properly. The G383 residue is located in predicted transmembrane domain 8 which has been proposed to function in the regulation of NHE ion transport (Hendus-Altenburger et al., 2014). Examination of bacterial NhaA and human NHE1 suggests that aromatic residues in

several transmembrane domains including transmembrane domain 8 may function as gatekeepers for ion translocation. Protonation of these aromatic residues may cause a conformational change that opens the ion translocation center to enable the exchange of ions (Hendus-Altenburger et al., 2014). Disruption of this process due to structural changes in this domain caused by the G383D mutation may prevent ion transport by NHE6.

In summary, we have established iPSC lines derived from CS patients and controls. These lines are genetically stable and have passed rigorous pluripotency testing. Our finding that NHE6 expression is lost or reduced in CS patients has revealed that mutations in NHE6 in CS are loss of function. Differentiating these CS patient-derived iPSCs to neuronal fate will provide an invaluable system for the investigation of neurological dysfunction in CS.

METHODS

Generation of iPSCs and maintenance

Peripheral blood donated from CS patients and controls was mixed with heparin (AAP Pharmaceuticals) to a final concentration of 23 U/mL heparin and transported overnight at room temperature or on ice. The iPSC lines used in this study were generated by the Cincinnati Children's Hospital Medical Center Pluripotent Stem Cell Facility. The protocol for generation of iPSCs from PBMCs was adapted from previously published methods (Kunisato et al., 2011). Peripheral blood mononuclear cells (PBMC) were enriched by layering blood diluted 1:1 with PBS + 2% FBS over Ficoll-Paque PLUS (StemCell Technologies). After centrifugation at 400 x g for 30 min, the upper plasma layer was discarded and the mononuclear cell layer was removed. PBMCs were washed in PBS + 2% FBS and plated at 5×10^6 cells per well of a 6-well ultra low attachment plate in media containing: 1x Ex-Vivo 15 media (Lonza), 10% dialyzed fetal calf serum (dFCS) (Hyclone), 100 ng/mL human stem cell factor (SCF) (Peprotech), 100 ng/mL human thrombopoietin (TPO) (Peprotech), 100 ng/mL interleukin 3 (IL-3) (Peprotech), 20 ng/mL interleukin 6 (IL-6) (Peprotech), 100 ng/mL Fms-related tyrosine kinase 3 ligand (Flt3L) (Peprotech), 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peprotech), and 50 ng/mL macrophage colony-stimulating factor (M-CSF) (Peprotech). Two days later, adherent and suspension PBMCs were collected and transduced with VSV-G pseudotyped lentivirus (pRRRL.PPT.SF.hOct3/4.hKlf4.hSox2.hMyc.i2dTomato.pre) expressing *SOX2*, *OCT4*, *KLF-4*, and *C-MYC* (Warlich et al., 2011) at a multiplicity of infection of 25. Cells were incubated with 1000x polybrene and lentivirus for 3 hr at 37°C in one well of a 24-well dish and then replated into one well of a 6-well ultralow attachment dish. Two days after transduction, cells were replated onto mouse embryonic fibroblasts (plated 1 to 2 days

prior) in media containing 1x Ex-Vivo 15 media (Lonza), 10% dFCS (Hyclone), 100 ng/mL SCF (Peprotech), 100 ng/mL TPO (Peprotech), 100 ng/mL IL3 (Peprotech), 20 ng/mL IL6 (Peprotech), 100 ng/mL Flt3L (Peprotech). A half change of media was performed six days after transfection followed by a full change on day 6 to hESC media containing: 1x DMEM/F12 (Sigma), 20% Knock-out Serum Replacement (Invitrogen), 4 ng/mL human basic fibroblast growth factor (bFGF), 50 U/mL penicillin-streptomycin, 2 mM L-glutamine, 0.1 mM β -mercaptoethanol, and 100 μ M nonessential amino acids. Cultures were fed daily with hESC media and were monitored for the development of iPSC colonies. Colonies were expanded 20-30 days after transduction by manual dissection. iPSCs clonal lines were cultured in feeder-free conditions on Matrigel (Corning)-coated plates and maintained in mTeSR media (StemCell Technologies). Enzyme-free passaging was performed using ReleSR (StemCell Technologies).

Alkaline phosphatase activity

Alkaline phosphatase activity was assessed using the alkaline phosphatase staining kit (Millipore; SCR004) as specified by the manufacturer. Briefly, putative iPSC cultures were fixed with a 3.7% formaldehyde (FA) solution in PBS for 2 minutes and permeabilized in TBS-T buffer containing 20mM Tris-HCl, pH 7.4, 0.15M NaCl, and 0.05% Tween-20. Alkaline phosphatase activity was detected following incubation in Naphthol:Fast Red Violet solution in H₂O (mixed at a ratio of 2:1:1) for 20 minutes in the dark at room temperature. Substrate was replaced with TBS-T for imaging and identification of alkaline phosphatase-positive colonies.

Karyotype analysis

Standard G-banded metaphase spreads were prepared and interpreted by a cytogeneticist in the Cincinnati Children's Hospital cytogenetics lab as previously described (McCracken et al., 2014).

Immunofluorescence

For detection of pluripotency marker expression by immunocytochemistry, cells were fixed directly in cell culture dishes with 3.7% paraformaldehyde for 10 minutes at room temperature and permeabilized for 10 minutes in IF permeabilization buffer (PBS containing 0.5% Triton X-100). Non-specific binding was blocked by incubation with blocking buffer (IF permeabilization buffer + 5% normal donkey serum) for 30 minutes, and cells were incubated overnight at 4°C with mouse anti-Oct4 IgG (Santa Cruz sc-5279; 1:250) and rabbit anti-Nanog IgG (Abcam ab21624; 1:100) primary antibodies diluted in blocking buffer. Following washing with PBS, cells were incubated for 30 mins at room temperature with donkey anti-mouse-IgG-alexaFluor488 (Life Technologies, A21202) and donkey anti-rabbit-IgG-alexaFluor546 (Life Technologies, A10040) diluted 1:500 in blocking buffer containing 0.3 μ M DAPI. Following washing with PBS, cells co-expressing Oct4 and Nanog were visualized by fluorescent microscopy on a Leica DM IRB inverted fluorescent microscope.

FACS analysis

Antibodies against Tra-1-60 and SSEA4 were used for flow cytometric analysis of pluripotency marker expression. Briefly, iPSCs were dissociated to single cells with Accutase (Life Technologies) and $0.5 - 1.0 \times 10^6$ cells were incubated on ice for 30 minutes with FITC mouse anti-human TRA-1-60 (BD Pharmingen 560876; 62.5

µg/reaction) or PE mouse anti-human SSEA4 (BD Pharmingen 560128; 7.5 µg/reaction) antibodies diluted in FACS wash buffer (PBS containing 0.2% BSA and 0.05% sodium azide). To exclude dead cells, 7AAD was included at a concentration of 2.5 µg/mL. Unstained cells and cells incubated with isotype control antibodies FITC mouse IgM (BD Pharmingen 553474) and PE mouse IgG3 (BD Pharmingen 559926) were used to define positive Tra-1-60 and SSEA4 populations.

Embryoid body differentiation

For embryoid body formation, iPSCs were pre-differentiated for 3 days in EB media consisting of DMEM containing 20% FCS and 1X non-essential amino acids. Pre-differentiated cultures were then incubated with 1mg/mL dispase for ~5 minutes and washed 3x with DMEM/F12 50:50 media. Following addition of EB media, a pulled glass pipet was used to score colonies. Colony pieces were then lifted using a cell scraper, transferred into a 6 well ultra-low attachment dish in EB media, and incubated overnight at 37°C with gentle rotation. The following day, EBs were transferred to a 15mL Falcon tube, gently triturated to remove debris from the edges of the EBs, and replated in an ultra-low attachment dish in EB media. After 4 days in suspension culture, EBs were plated in EB media on 0.1% gelatin-coated cell culture plates for an additional 10 days until harvest and analysis. EB media was changed every 2 days.

qRT-PCR

Total RNA was harvested from undifferentiated and EB differentiated hPSCs using Trizol (Life Technologies) and DNA was eliminated using the DNase-free kit (Life Technologies). RNA was reverse transcribed to cDNA using the High-capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies) following

manufacturer's recommendations. Gene expression levels were determined using 96 well Fast Custom TaqMan Array Plates containing Taqman assays for detection of pluripotency markers (Pou5F1, Nanog, GABRB3, DNMT3B, TDGF1, and ZPF42) or germ layer-specific markers (endoderm: AFP, ALB, FABP2; mesoderm: TNNT2, HBZ, MEOX1; ectoderm: PAX6, TH, KRT1). Taqman reactions were performed following the manufacturer's protocols in a StepOne Plus real time PCR device and consisted of 20 ng of each cDNA in 1x Fast Universal PCR Master Mix in a final volume of 10 μ L. Thermal cycling conditions were 50°C for 2 minutes, 95°C for 30 seconds followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. Relative expression of each gene was calculated for each sample using the $\Delta\Delta C_t$ method. For pluripotency gene expression analysis (Fig. 2.8), mean expression (+/- standard deviation from 2 replicates) of each gene is shown relative to the expression of the same gene in H1 hESCs differentiated *in vitro* using the embryoid body (EB) method. For Taqman analysis of differentiation (Fig. 2.9), for a given EB-differentiated iPSC line, the expression of each gene is calculated relative to the expression of that gene in the undifferentiated sample for the same iPSC line. GAPDH was used for normalizing TaqMan data.

***In vivo* pluripotency characterization**

Teratoma formation in immunodeficient mice, harvest, and analysis was performed as previously described (McCracken et al., 2014). Briefly, iPSCs from 3 wells of a 6-well plate were collected with dispase in 50 μ L of DMEM/F12 media. Before injection, 30 μ L of Matrigel was added to the sample. Cells were injected subcutaneously in the rear flank of a NOD/SCID GAMMA C-/- mouse. Teratomas were removed 6-12 weeks post injection, fixed, sectioned, and evaluated histologically by haematoxylin and eosin staining.

Northern Blotting

iPSCs were treated with 100 µg/mL cycloheximide (Sigma) or 1:1000 DMSO for three hours prior to RNA collection. Total RNA was isolated from iPSCs using the RNeasy Mini kit (Qiagen) followed by mRNA collection using the PolyATtract mRNA Isolation System (Promega) according to the suppliers' instructions. Northern blot analysis was performed using the NorthernMax-Gly kit (Ambion). 200 ng of mRNA for each condition was run on a 1% glyoxyl/DMSO agarose gel and transferred onto a nylon membrane. Blots were probed with a *SLC9A6* or *GAPDH* probe labeled with α -³²P UTP (Perkin Elmer) by T7 *in vitro* transcription of PCR amplified cDNA. *SLC9A6* PCR primers were forward: 5'-caggcttgcgtcagaagtt -3' and reverse: 5'-taatacgactcactatagggagagccacgaaagggtacaacat - 3'. *GAPDH* PCR primers were forward: 5'-ctgagaacgggaagcttgc - 3' and reverse: 5'-taatacgactcactatagggagaggtgctaagcagttggtgg - 3'. Radioactivity was measured using phosphorimaging (Molecular Dynamics) and quantified in ImageJ.

Immunoprecipitation and Western Blotting

iPSCs were lysed in buffer containing Tris pH 7.5, 1% TritonX-100 (Sigma), 150 mM NaCl, 20 mM β -glycerophosphate, 2 mM sodium orthovanadate, and 1x Complete Protease Inhibitor Cocktail (Roche). Protein concentration was measured by BCA assay (Pierce). For immunoprecipitation, 10 µg rabbit anti-NHE6 (C-terminal epitope, Covance) antibody was conjugated per 1.5 mg Dynabeads M-270 Epoxy overnight at 37°C. After washing the beads, 500 µg protein lysate was incubated with beads for 2 hours at 4°C. Samples were washed with buffer containing 20 mM Tris pH7.4 and 120 mM NaCl and eluted with buffer containing 20 mM Hepes pH 8, 9 M urea, 1 mM sodium

orthovanadate, 2.5 mM sodium pyrophosphate, and 1 mM β -glycerophosphate. Samples were run on a NuPAGE 4-12% Bis-Tris gel (Novex), transferred to a nitrocellulose membrane (Novex), membranes were blocked, and proteins were detected with antibodies for rabbit anti-NHE6 and mouse anti-tubulin (Sigma).

Structural Modeling

NHE6 models were constructed using MODELLER-9v14 (Fiser and Sali, 2003) based on the crystal structures of the *E. coli* Na⁺/H⁺ exchanger, NHaA (PDB identifiers 1ZCD (Hunte et al., 2005) and 4AU5 (Lee et al., 2014)). For each template structure, 100 models were built and these models were assessed with Z-DOPE, a normalized atomic distance-dependent statistical potential based on known protein structures (Shen and Sali, 2006). The alignment between NHE6 and NHaA was generated based on a previously published alignment between NHaA and NHE9, a close homolog of NHE6 (Kondapalli et al., 2013). Models constructed based on 1ZCD have six segments that are variable in sequence and were thus, excluded from modeling. They included the loops between transmembrane helices 1 and 2 (TM1-2), TM3-4, TM5-TM6, TM6-TM7, and the N- and C- termini. This model covered 53% of the NHE6 sequence. Models built based on 4AU5 contained the loops between transmembrane region, while the N- and C- termini were not modeled. This model covered 65% of NHE6 sequence. The models were visualized using PyMOL (Schrödinger, LLC).

Residue conservation and hydrophobicity

The ConSurf server (<http://consurf.tau.ac.il/>) was used to calculate evolutionary conservation (default parameters) (Glaser et al., 2003). To allow for a more complete analysis of NHE6 conservation, a model that contains loops was used (4AU5 template).

The computed conservation values were mapped onto the NHE6 model with UCSF Chimera (Pettersen et al., 2004), using the scripts provided by the ConSurf server. The hydrophobicity profile was computed using Chimera with the Wolfenden hydrophobicity scale (Wolfenden, 2007). Hydrophobicity values were mapped onto the NHE6 model's surface, and the residues were colored in orange for the most hydrophobic, to white, and to blue for the most hydrophilic.

Modeling of the G383D variant

The G383D sidechain was modeled with PyMol on a fixed backbone of the NHE6 WT model from MODELLER. The initial G383D model predicted a hydrogen bond between N166 and G383D that may stabilize the inward open conformation and negatively affect transport. Furthermore, in the WT model N166 interacts with E377, which is also lost in the G383D model. The mutated model was further refined with Molecular dynamics (MD) simulations, where short MD simulations in implicit membrane were used as a control to test whether significant sidechain rearrangements occur in our model. GROMACS4.5 was run on the G383D models with varying orientations of the G383D aspartate rotamer as described previously (Pronk et al., 2013; Schlessinger et al., 2014; Schlessinger et al., 2012). Each model was subjected to a short 10 ns simulation with 501 conjugate gradient minimizations. Interestingly, the MD simulation of the NHE6 G383D model show that the mutated residue might favor a formation of a salt bridge with R500 on TM11 which may also affect transport negatively.

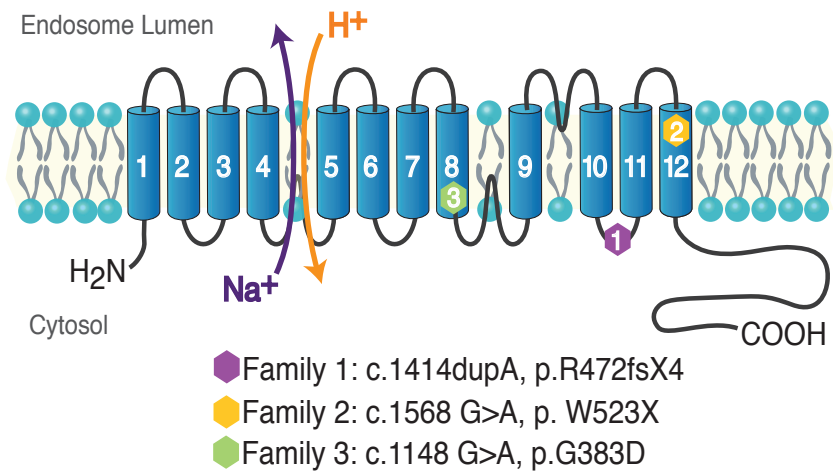


Figure 2.1 Generation of iPSCs from CS patients with mutations in NHE6. iPSCs were derived from three CS patients and from their unaffected siblings as controls. For each patient, the mutation in NHE6 cDNA (c.) and protein (p.) sequences are shown and mapped onto a diagram of NHE6 protein. NHE6 has 12 predicted transmembrane domains and a cytosolic C-terminal tail. NHE6 transmembrane domains were predicted by TMHMM based using Ensembl transcript ENST00000370695. (Modified from Pescosolido and Stein, et al. 2014)

| Exon 1

1 ATGGCTCGGCGGGCTGGCGGGGGCACCCCTCCGCCGTGGCGTCGGCAGCAGTCCCCGA 60
1 -M--A--R--R--G--W--R--R--A--P--L--R--R--G--V--G--S--S--P--R- 20

61 GCCCGCAGGCTCATGCGGCCCTTTGGTTGCTCCTCGCAGTGGGCGTCTTTGACTGGGCA 120
21 -A--R--R--L--M--R--P--L--W--L--L--L--A--V--G--V--F--D--W--A- 40

121 GGGGCTTCGGACGGCGGGCGGAGAGGCTAGAGCCATGGACGAGGAGATCGTGTCCGAG 180
41 -G--A--S--D--G--G--G--G--E--A--R--A--M--D--E--E--I--V--S--E- 60

181 AAGCAAGCCGAGGAGAGCCACCGGCAGGACAGCGCCAACCTGCTCATCTTCATCCTGCTG 240
61 -K--Q--A--E--E--S--H--R--Q--D--S--A--N--L--L--I--F--I--L--L- 80

241 CTCACCCTCACCATTCTCACAATCTGGCTCTTCAAGCACCGCCGGGCGCTTCCTGCAC 300
81 -L--T--L--T--I--L--T--I--W--L--F--K--H--R--R--A--R--F--L--H- 100

| Exon 2

301 GAAACCGGCCTGGCTATGATTTATGGTCTTTTGGTGGGCCTTGTGCTTCGGTATGGCATT 360
101 -E--T--G--L--A--M--I--Y--G--L--L--V--G--L--V--L--R--Y--G--I- 120

361 CATGTTCCGAGTGATGTAAATAATGTGACCCTGAGCTGTGAAGTGCAGTCAAGTCCAAC 420
121 -H--V--P--S--D--V--N--N--V--T--L--S--C--E--V--Q--S--S--P--T- 140

421 ACCTTACTGGTAAATGTTAGTGGAAATTTTATGAGTATATGCTGAAAGGAGAGATTAGT 480
141 -T--L--L--V--N--V--S--G--K--F--Y--E--Y--M--L--K--G--E--I--S- 160

| Exon 3

481 TCACATGAACCAATAATGTTCAAGATAATGAAATGCTTAGAAAGTTACTTTTGATCCA 540
161 -S--H--E--L--N--N--V--Q--D--N--E--M--L--R--K--V--T--F--D--P- 180

541 GAAGTATTTTTCAACATATTACTTCCTCCTATCATATTTTATGCAGGTTATAGCCTGAAA 600
181 -E--V--F--F--N--I--L--L--P--P--I--I--F--Y--A--G--Y--S--L--K- 200

| Exon 4

601 AGGAGACATTTTTTCGAAATCTTGGGTCTATCCTAGCATAACGCTTTTCTTGAACAGCA 660
201 -R--R--H--F--F--R--N--L--G--S--I--L--A--Y--A--F--L--G--T--A- 220

| Exon 5

661 ATTTCTTGTTTCGTTATTGGGTCAATAATGTATGGCTGTGTAACGCTGATGAAGGTAACG 720
221 -I--S--C--F--V--I--G--S--I--M--Y--G--C--V--T--L--M--K--V--T- 240

721 GGACAACTTGCAGGAGATTTTTACTTTACAGATTGCCTACTGTTTGGTGCCATTGTATCA 780
241 -G--Q--L--A--G--D--F--Y--F--T--D--C--L--L--F--G--A--I--V--S- 260

| Exon 6

781 GCAACTGATCCAGTGAAGTCTTCTTGCTATATTCCACGAGCTTCAAGTTGATGTTGAAC 840
261 -A--T--D--P--V--T--V--L--A--I--F--H--E--L--Q--V--D--V--E--L- 280

| Exon 7

841 TATGCACTTCTTTTTGGTGAAAGTGTCTCAATGATGCTGTTGCCATAGTGCTGTCCTCC 900
281 -Y--A--L--L--F--G--E--S--V--L--N--D--A--V--A--I--V--L--S--S- 300

901 TCAATAGTGGCATAACCAGCCAGCTGGAGACAACAGTCACACCTTTGATGTCACAGCGATG 960
301 -S--I--V--A--Y--Q--P--A--G--D--N--S--H--T--F--D--V--T--A--M- 320

961 TTCAAGTCTATTGGGATCTTCCTTGGAATCTTCAGTGGATCTTTTGCAATGGGTGCTGCT 1020
321 -F--K--S--I--G--I--F--L--G--I--F--S--G--S--F--A--M--G--A--A- 340

| Exon 8

1021 ACTGGAGTGGTGACAGCTTTAGTGACAAAGTTACCAAATTACGGGAGTTCCAGTTGTTG 1080
341 -T--G--V--V--T--A--L--V--T--K--F--T--K--L--R--E--F--Q--L--L- 360

1081 GAGACAGGCCTGTTCTTCTTGATGTCCTGGAGTACCTTCCTCTTGGCTGAAGCATGGGGC 1140
361 -E--T--G--L--F--F--L--M--S--W--S--T--F--L--L--A--E--A--W--G- 380

| **Exon 9**
Family 3: c.1148G>A, p.G383D

1141 TTCACAGGTTGTAGTTGCAGTATTGTTTTGTGGCATCACACAAGCACATTATACGTATAAT 1200
381 -F--T--G--V--V--A--V--L--F--C--G--I--T--Q--A--H--Y--T--Y--N- 400

| **Exon 10**

1201 AATTTGTCCACGGAGTCTCAGCATAGAACTAAACAGTTGTTTGAGCTTCTCAATTTCTTG 1260
401 -N--L--S--T--E--S--Q--H--R--T--K--Q--L--F--E--L--L--N--F--L- 420

1261 GCAGAGAATTTTCATCTTCTCCTACATGGGGCTGACACTGTTTACCTTCCAGAACCATGTC 1320
421 -A--E--N--F--I--F--S--Y--M--G--L--T--L--F--T--F--Q--N--H--V- 440

| **Exon 11**

1321 TTTAACCCAACATTTGTAGTAGGAGCATTTGTTGCTATTTTCTTGGAAGAGCTGCCAAT 1380
441 -F--N--P--T--F--V--V--G--A--F--V--A--I--F--L--G--R--A--A--N- 460

Family 1: c.1414dupA, p.R472fsX3

1381 ATTTACCCCTTGTCCCTCTTACTTAATTTGGGTGAAGAAGTAAGATTGGATCAAATTTT 1440
461 -I--Y--P--L--S--L--L--L--N--L--G--R--R--S--K--I--G--S--N--F- 480

| **Exon 12**

1441 CAACACATGATGATGTTTGCTGCGCTTCGTGGTGCAATGGCATTTCGCTTGGCCATTCTGA 1500
481 -Q--H--M--M--M--F--A--G--L--R--G--A--M--A--F--A--L--A--I--R- 500

1501 GATACTGCCACTTATGCACGGCAAATGATGTTTACGACCACGCTTCTGATTGTGTTTTTTT 1560
501 -D--T--A--T--Y--A--R--Q--M--M--F--S--T--T--L--L--I--V--F--F- 520

Family 2: c.1568G>A, p.W523X

| **Exon 13**

1561 ACCGTGTGGGTATTTGGTGGTGGCACCCTGCAATGCTGTCATGCTTGCATATCAGGGTT 1620
521 -T--V--W--V--F--G--G--G--T--T--A--M--L--S--C--L--H--I--R--V- 540

| **Exon 14**

1621 GGTGTTGATTACAGACCAAGAACAACACTTGGGTGTTCTCGAAAATGAAAGGAGAACTACCAA 1680
541 -G--V--D--S--D--Q--E--H--L--G--V--P--E--N--E--R--R--T--T--K- 560

| **Exon 15**

1681 GCAGAGAGTGCTTGGCTTTTCCGGATGTGGTACAACCTTTGATCATAACTATCTGAAGCCT 1740
561 -A--E--S--A--W--L--F--R--M--W--Y--N--F--D--H--N--Y--L--K--P- 580

1741 CTGCTGACCCACAGCGGGCCTCCGCTGACAACAACACTCCCTGCCTGCTGTGGACCCATC 1800
581 -L--L--T--H--S--G--P--P--L--T--T--L--P--A--C--C--G--P--I- 600

| **Exon 16**

1801 GCCAGGTGCCTCACCAGCCCCAGGCTTACGAAAACAGGAACAGTTGAAAGATGATGAT 1860
601 -A--R--C--L--T--S--P--Q--A--Y--E--N--Q--E--Q--L--K--D--D--D- 620

1861 TCTGATCTTATTCTCAATGATGGTGACATCAGTTTGACATATGGAGATTCTACTGTGAAC 1920
621 -S--D--L--I--L--N--D--G--D--I--S--L--T--Y--G--D--S--T--V--N- 640

1921 ACTGAACCGGCCACATCCAGCGCCCCAAGGAGATTTATGGGAAACAGTTCTGAAGATGCC 1980
641 -T--E--P--A--T--S--S--A--P--R--R--F--M--G--N--S--S--E--D--A- 660

1981 TTGGATCGGGAGCTTGCATTTGGGGACCATGAACTGGTCATTTCGAGGAACACGCCTGGTT 2040
661 -L--D--R--E--L--A--F--G--D--H--E--L--V--I--R--G--T--R--L--V- 680

2041 CTTCCAATGGATGATTCTGAACCCCCGCTAAATTTGTTAGATAATACGAGACATGGTCCA 2100
681 -L--P--M--D--D--S--E--P--P--L--N--L--L--D--N--T--R--H--G--P- 700

2101 GCCTAA 2106
701 -A--*- 701

Figure 2.2 Mutations in *SLC9A6* cDNA from three CS probands. Ensembl *SLC9A6* transcript ENST00000370695 is shown with alternating exons in black and blue. The corresponding protein sequence is labeled below. The locations of the three proband mutations are labeled in red.

Family	Subclone	Genotype	Karyotype	Alkaline Phosphatase	IHC Pluripotency	FACS Pluripotency	iPSC RT-PCR	Embryoid Body RT-PCR	Teratoma Induction
1	6	Y/+	Normal	+	+	+	+	+	+
1	4	Y/+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
1	1	c.1414dupA, p.R472fsX4	Normal	+	+	+	+	+	+
2	5	Y/+	Normal	+	+	N.A.	+	+	+
2	10	Y/+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2	1	c.1568 G<A, p.W523X	Normal	+	+	N.A.	+	+	+
2	7	c.1568 G<A, p.W523X	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2	11	c.1568 G<A, p.W523X	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3	12	Y/+	Normal	+	+	+	+	+	+
3	10	Y/+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3	7	Y/+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3	1	c.1148 G<A, p.G383D	Normal	+	+	+	+	+	+
3	2	c.1148 G<A, p.G383D	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3	3	c.1148 G<A, p.G383D	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Table 2. Characterization of PBMC-derived iPSC lines. Subclonal lines from CS and control pairs were assessed for normal karyotype and pluripotency. “+” indicates that the subclonal line passed the indicated test.

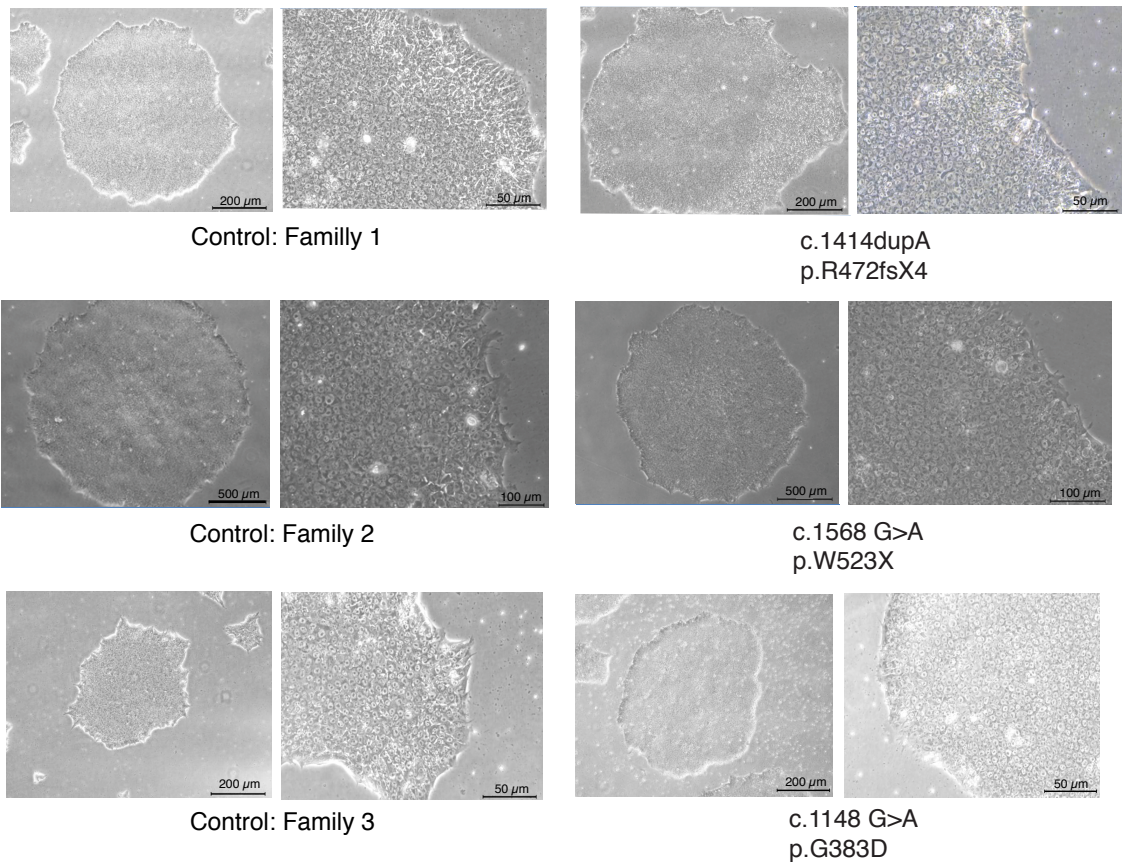


Figure 2.3 Morphological characterization of CS and control iPSCs. iPSCs grew in multicellular colonies composed of tightly packed cells. A large nucleus to cytoplasm ratio and large nucleoli were observed. Subclonal iPSC lines from each donor were grown in feeder-free conditions on a Matrigel coated surface. Images of a single colony and a magnified view are shown. Scale bars, 500 μm , 200 μm , 100 μm , or 50 μm as labeled.

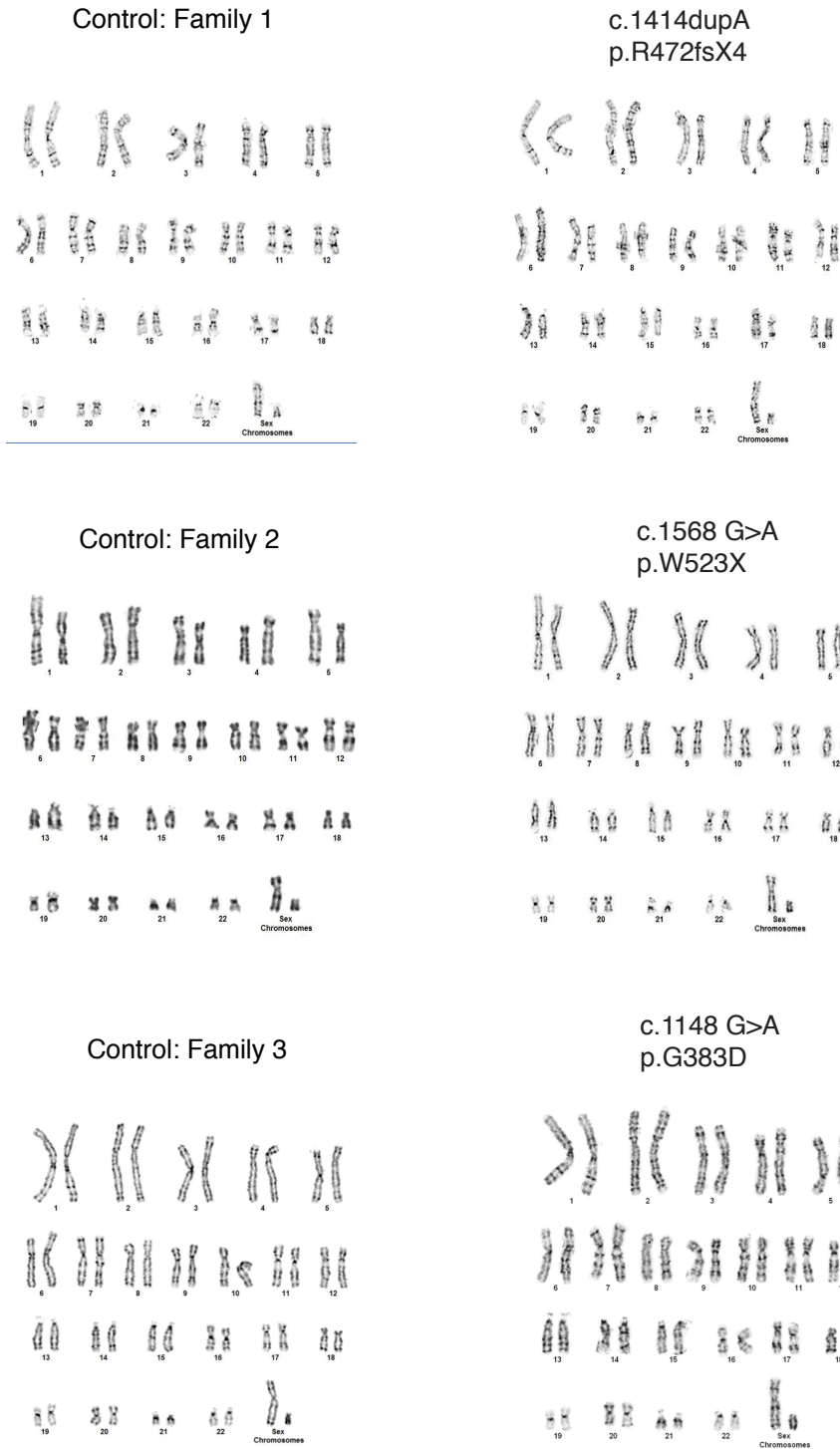


Figure 2.4 Karyotype analysis of CS and paired control iPSC lines. Example karyotypes from CS and control iPSC lines showed normal male karyotypes as demonstrated by G-banding pattern indicating that genomic stability was maintained after reprogramming.

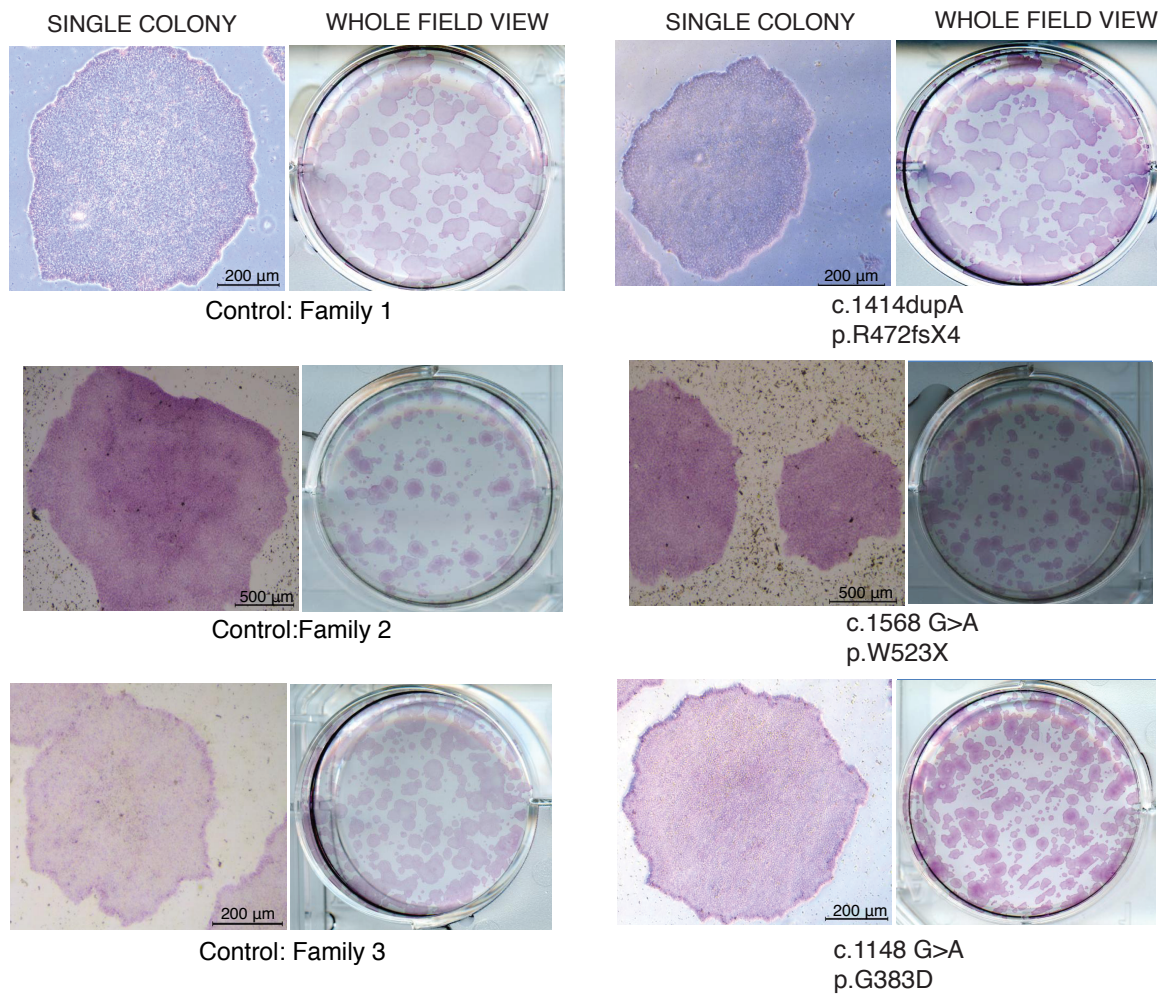


Figure 2.5 Alkaline phosphatase testing of CS and paired control iPSC lines. iPSCs from CS and control subclonal lines stained positively for alkaline phosphatase activity, a marker of pluripotency. A single colony (scale bar, 200 μm or 500 μm) and a whole field view (one well of a six-well plate) are shown.

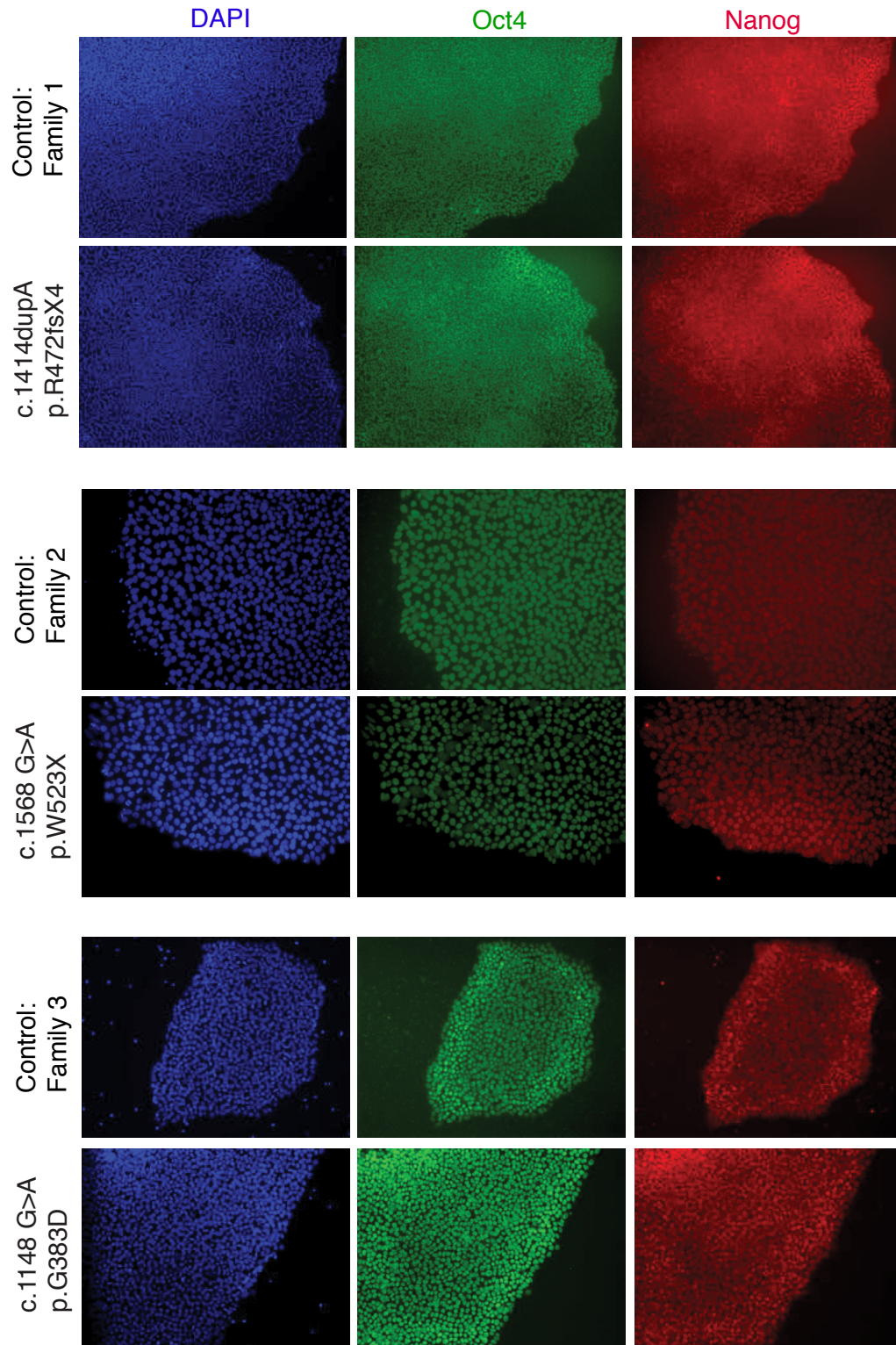


Figure 2.6 Immunostaining of CS and control iPSC colonies for pluripotency markers. Example iPSC subclones from CS and control donors show positive nuclear staining of the pluripotency markers, OCT4 (green) and NANOG (red). DAPI (blue) was used to mark nuclei. Images were taken at 100x magnification.

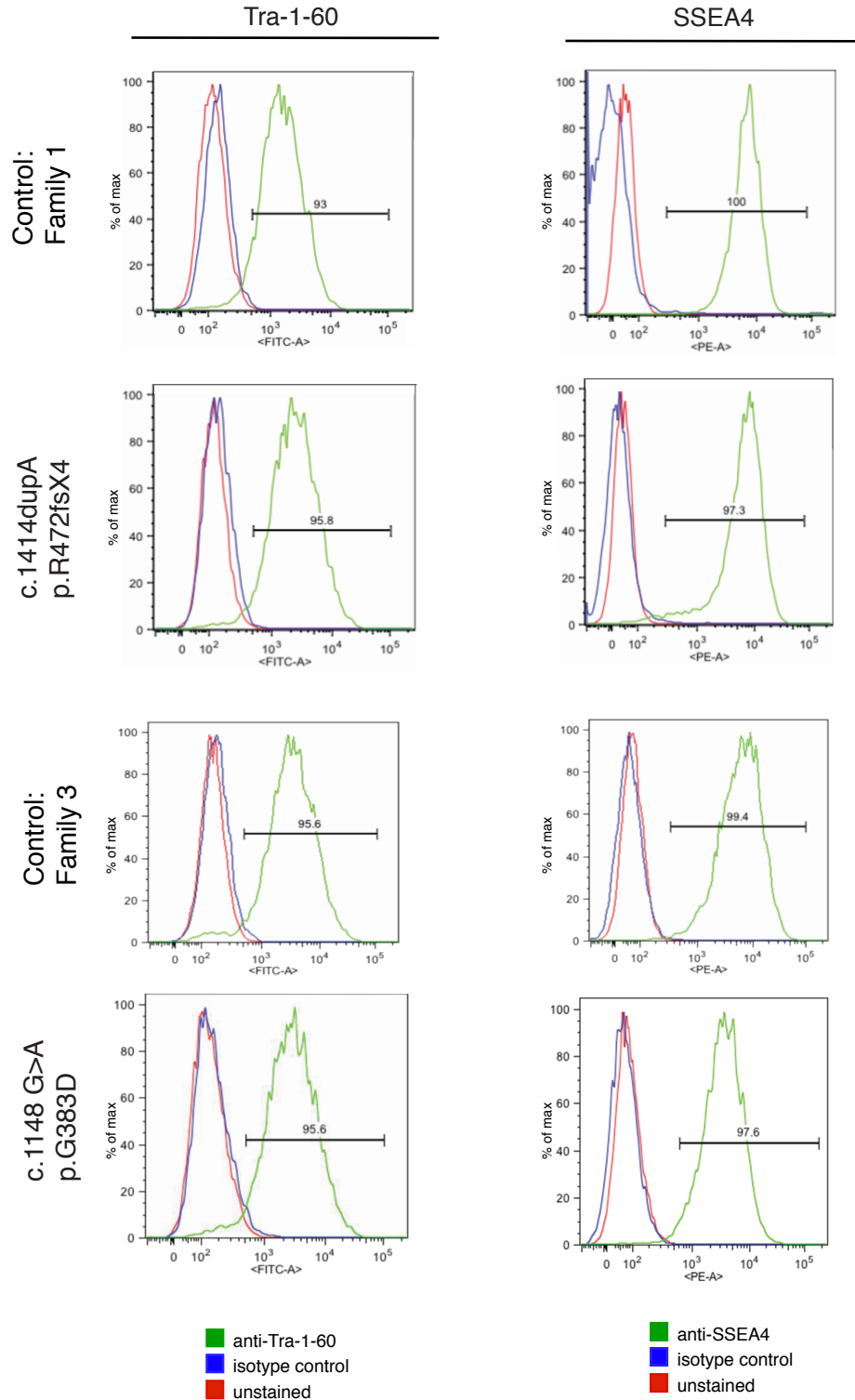


Figure 2.7 FACS analysis of stem cell specific markers. Cell surface pluripotency markers, TRA-1-60 (left column) and SSEA4 (right column), were highly expressed in CS and control iPSCs. Unstained and isotype controls for each experiment are shown. The percentage of cells positively staining for each marker is indicated.

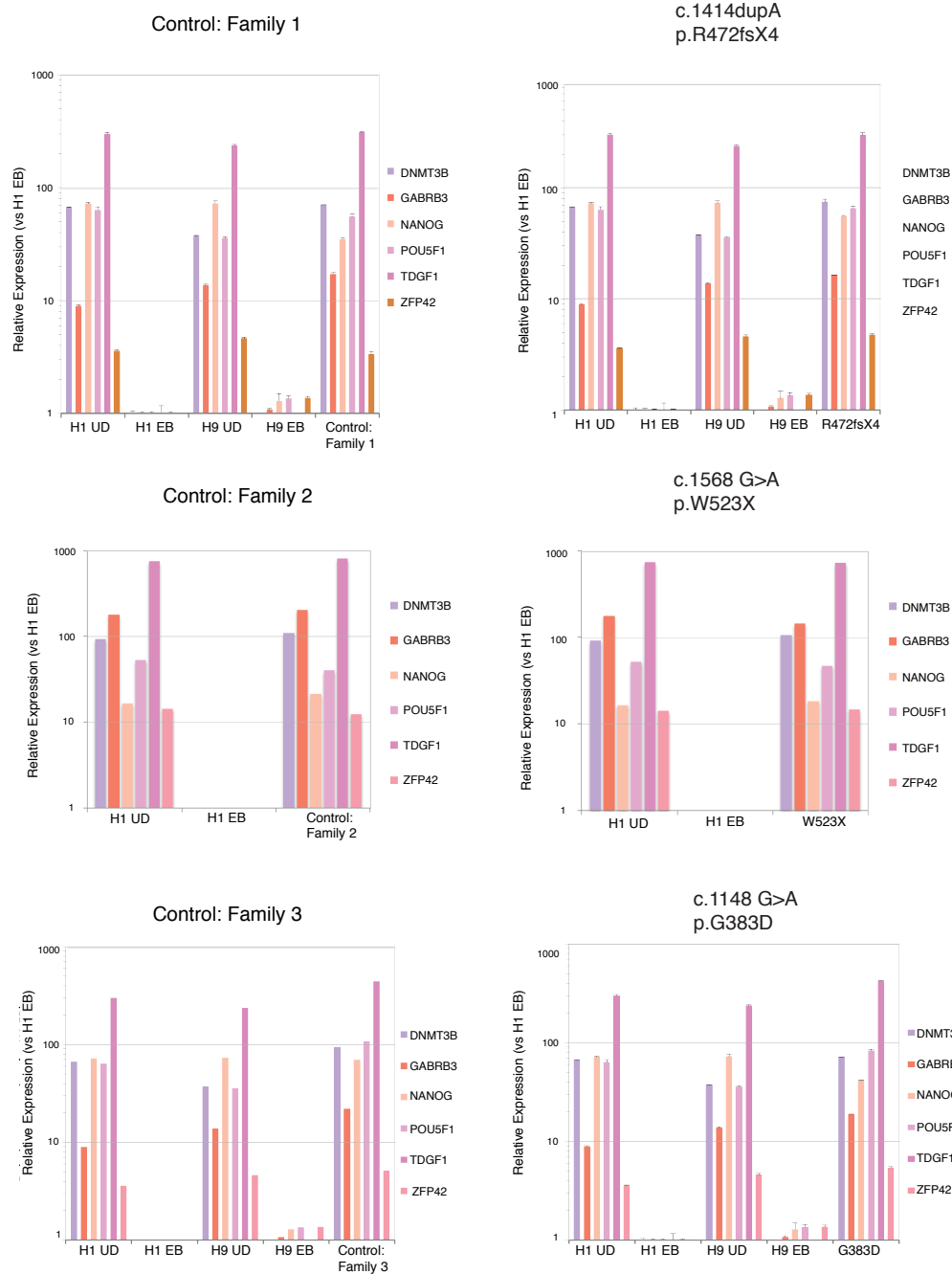


Figure 2.8 qRT-PCR analysis of pluripotency markers in undifferentiated iPSCs (UD) as compared to hESC-derived embryoid bodies (EB). Pluripotency marker expression was similar between iPSC lines derived in this study and the undifferentiated hESC lines, H1 and H9. The relative expression of each gene was calculated using the $\Delta\Delta C_t$ method. Expression of each gene is plotted relative to the expression of the same gene in H1 EBs. Error bars, sd from two replicate qRT-PCR wells.

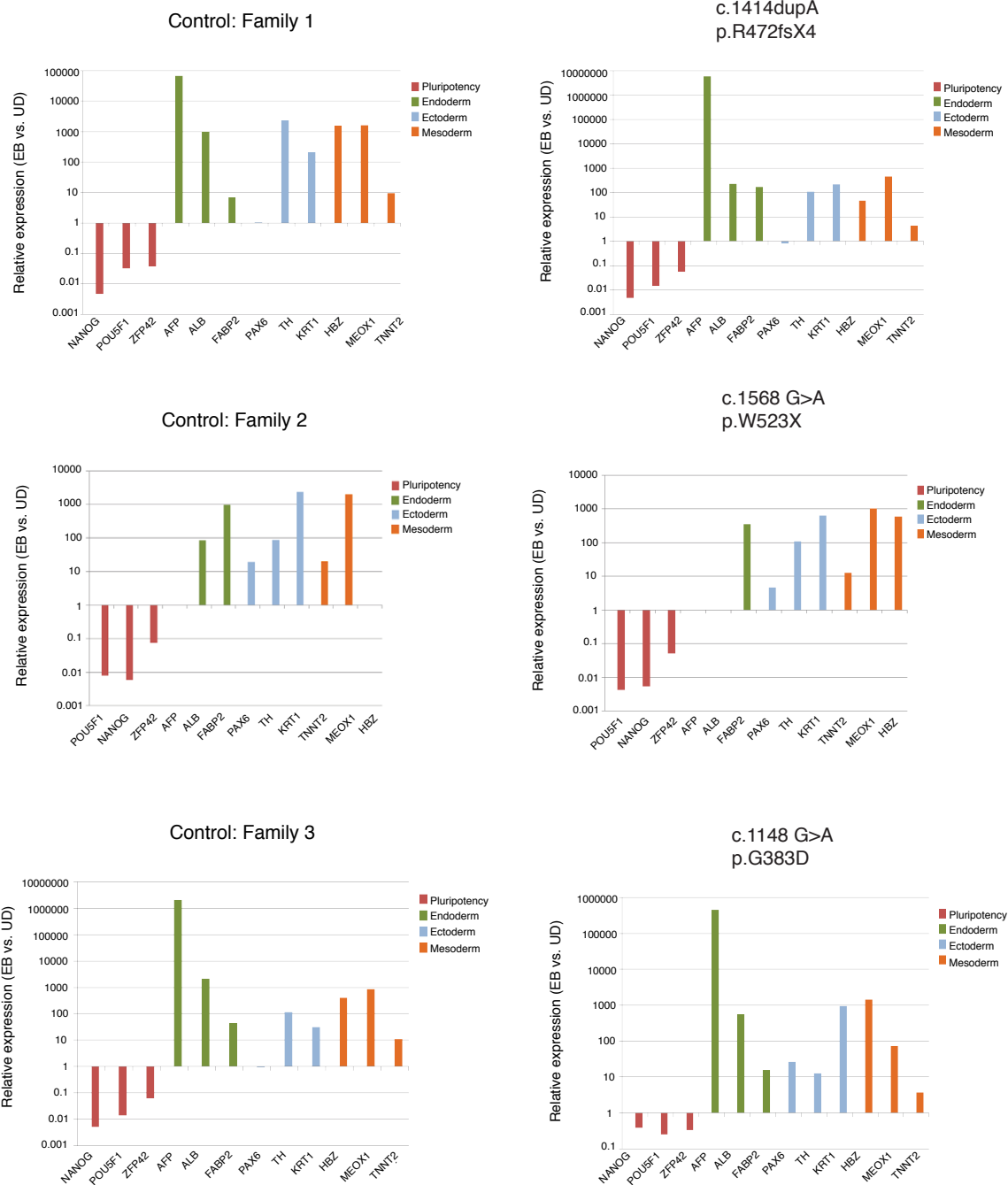


Figure 2.9 iPSC differentiation potential *in vitro*. To test iPSC differentiation potential, iPSCs were differentiated to embryoid bodies. The expression of germ layer markers was measured by qRT-PCR in undifferentiated iPSCs (UD) and iPSCs differentiated to embryoid bodies (EB). The relative expression of each gene was calculated using the $\Delta\Delta C_t$ method. Expression of each gene is plotted relative to the expression of the same gene in the undifferentiated sample for each iPSC line.

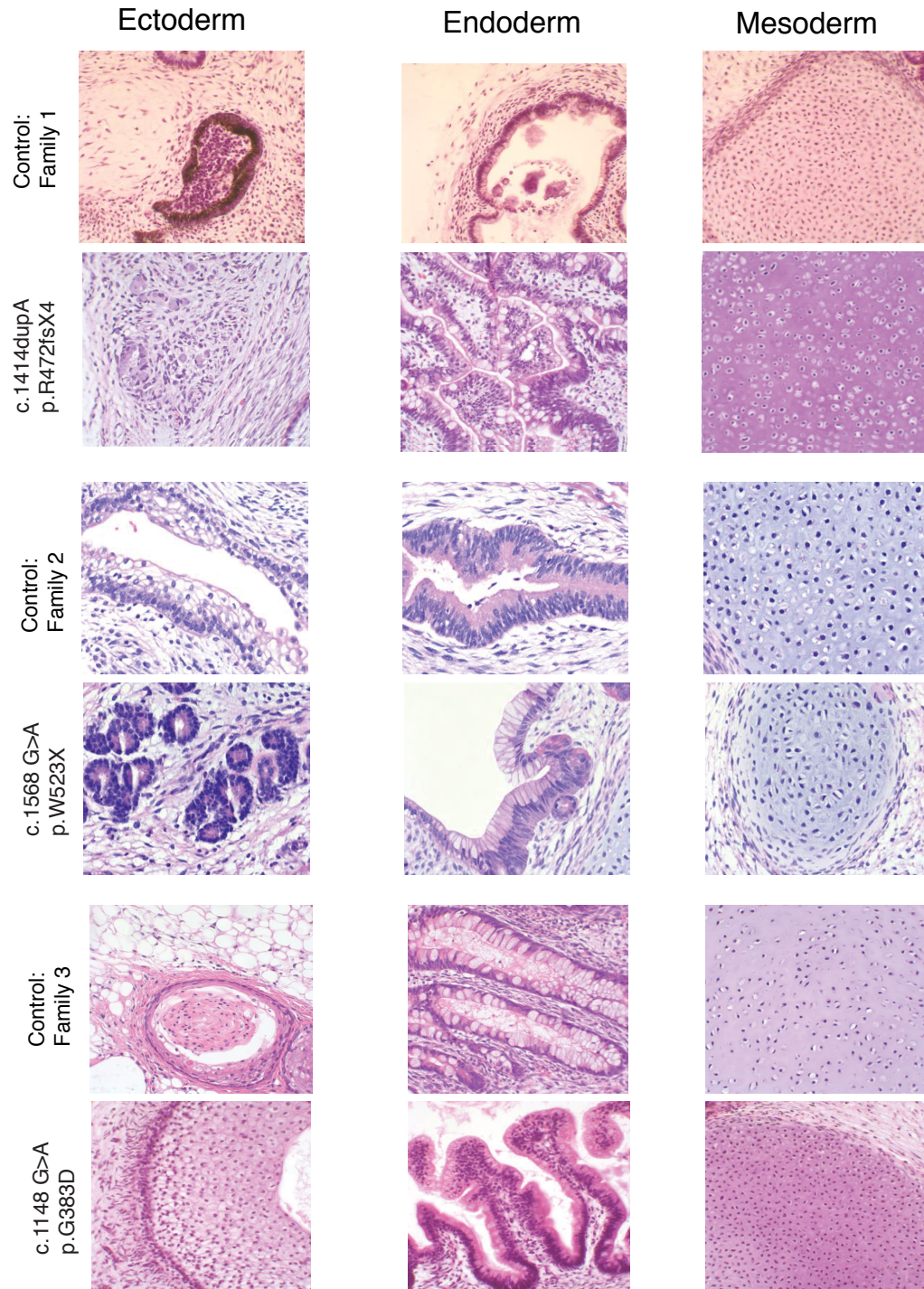


Figure 2.10 iPSC differentiation potential *in vivo*. Hematoxylin and eosin staining of teratomas from immunodeficient mice injected with iPSCs show formation of tissues derived from all three germ layers. Images were taken at 400x magnification.

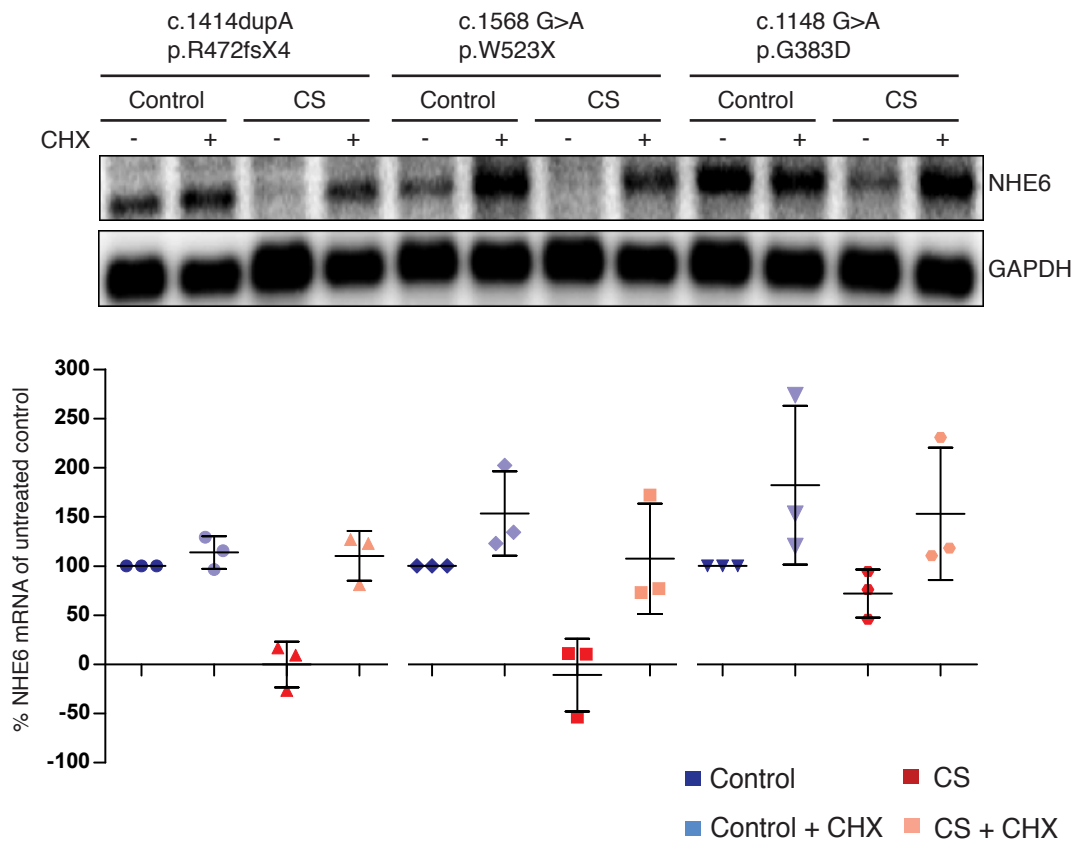


Figure 2.11 NHE6 mRNA quantification in CS iPSCs. Northern blotting was performed in CS and control iPSCs treated (+) or untreated (-) with cycloheximide. Expression of *SLC9A6* mRNA was normalized to *GAPDH* and plotted as a percentage of untreated control cells for each mutation. $n = 3$ experiments with subsequent iPSC passages, mean $\pm$ sem.

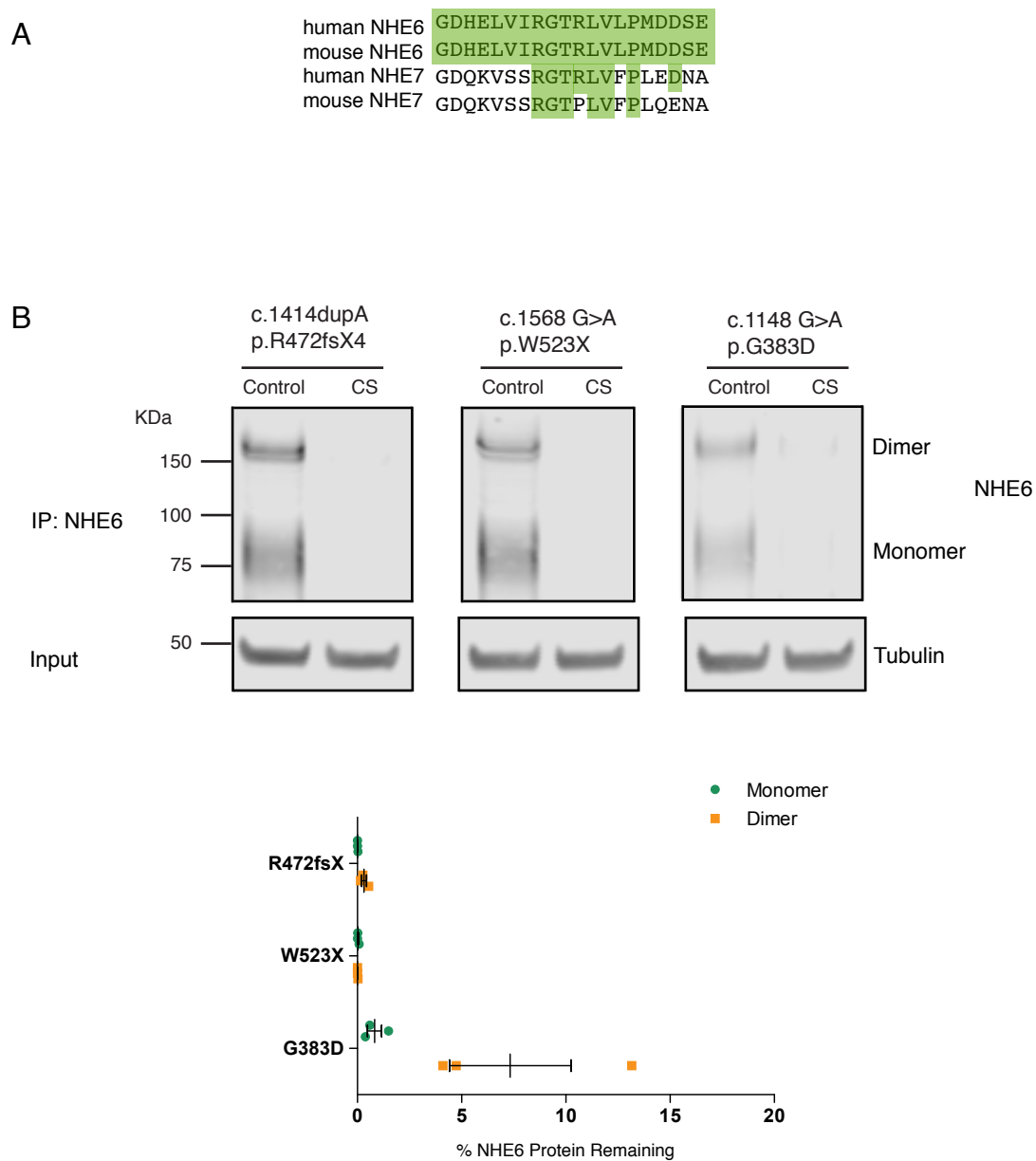


Figure 2.12 NHE6 protein quantification in CS iPSCs. A) NHE6 antibody epitope (highlighted in green) matches the mouse and human NHE6 protein sequence with 100% identity. The epitope also has similarity to human NHE7 and to a lesser extent to mouse NHE7. B) Immunoprecipitation of NHE6 followed by western blotting for NHE6 in CS and control iPSCs. Expression of NHE6 dimer and monomer is plotted as a percentage of control. n = 3 experiments with subsequent iPSC passages, mean $\pm$ sem.

NHE6	MARRGWRAPLRRRGVSSPRARRLMRPLWLLLAAGV-----FDWA	40
NHX1	-----MLSKVLNLNIAFKVLLTTAKRAVPDPPDDDELLSPSLDP	37
NhaA	-----	0
NHE6	GASDGGGGGEARAMDEEIVSEKQAEESHQRDSANLLIFILLTLTILTIWLFKHRRARFLH	100
NHX1	GSDDPIAGD----PDVDLNPTV---EEMFSSSWALFIMLLLLISALWSSYYLTQKRIRAVH	90
NhaA	-----MKHLHRFFSSDA-----	12
	 *	
NHE6	ETGLAMIYGLVLGLVLRGIHVPSDVNNVTLSCEVQSSTPTLLVNVSCKFYEYMLKG---	157
NHX1	ETVLSIFYGMVIGLIIRMSPG-----	111
NhaA	SGGIILITAAIILAMIMANS-GA-----TSGWYHDPLETPVQ	47
	. : : . . : : :	
NHE6	-EISSHELNNVQDNEMLRKVTFDPVEVFNNILLPPIIFYAGYSKKRHHFRNLGSLILAYAF	216
NHX1	-----HYIQDTVTTFNSSYFPNVLLPPIILNSGYELNQVNFNNMLSLILFAI	158
NhaA	LRVGSLEINKN--MLL-----WINDALMAVFLLVGLEVKRELMQGSLASLRQAAF	96
	: : : * : : * : : : : . : * :	
NHE6	LGTA-ISC FVIGSIMYGCVTLMKVTG-----QLAGDFYFTDCLLFGAIVSATDPVTTL	269
NHX1	POTF-ISAVVIGIIILIWT---PLG-----LESIDISPADAMS VATLSATDPVTLLS	207
NhaA	PVIAAIGGMIVPALLYLAFNYADPITREGWAIPAATDIAFAL--GVLALLGSRVPLALK-	153
	* . : : : * : : * : : * : : *	
NHE6	IFHELQVDVELYALLFGESVLNDAVAIVLSSSI---VAYQPAGDNSHT-----	314
NHX1	IFNAYKVDPKLYTIIFGESLLND AISIVMFETC---QKFHG---QP-----	247
NhaA	-----IFLMALAI IDDLGAI IIALFYTNDLSMASLGVA AVAIAVLAVNLCCG	201
	: : . : : : * : : :	
NHE6	FDTV TAMFSIGIFLGI--FS---GSFAMGAATGVV TALVTKFTKLREFQLLETGLFPLMS	369
NHX1	ATFSSVPEGAGFLMT--FS---VSLLIGVLIGILVALLKHTHIRRYQPIESCILLIIA	302
NhaA	ARRTGVIYLGVVLTAVLKSGVHATLAGVIGFFFPLKEKHGRSPA---KRLEHVLHP	257
	: : : * : * : . : * . * * . : : *	
NHE6	WSTFL LAEAWGF-----TCVVAVLFCGITQAHYTYNNLSTESQHRTKQLFELL	417
NHX1	YESYFFSNGCHM-----SCIVSLLFCGITLKH YAYYNNMSRRSQITIKYIFQLL	350
NhaA	WWAYILPL LPAFANAGVSLQGVTLDCLTSLPLGIIA-----	294
	: : : : : * : : : *	
NHE6	NFLAENFI FSYMG LTLTFQNH-----VFNP TFV V GA-----FVAIFLGRAAN	460
NHX1	ARLS ENFI FYI VLGLELFTEVEL-----VYKPLLIVA-----AISICVARCA	393
NhaA	----GLLIGKPLGISLFCWLALRLKLAHLPEGTTYQQIMVVGILCGIGFTMSIFIASLAF	350
	: : * : * : * : : : : : : *	
NHE6	IYPLSLLLNLG-----RRSKIGSNFQHMMMFAGLRGAMAFALAIRD T	502
NHX1	VFPLSQFVNWIYRKVTIRMSGITGENISVPDEIPYNQMMTFWAGLRGAVGVALALGIQ	453
NhaA	GSVDP ELINWAKLGILV GSI-----SSA VIGYSWLRVRL-----RPSV-----	388
	: : * : : : : * : : *	
NHE6	ATYARQMFMSTLLIVFFT VWVFGGGTTAML SCLHIRGVSDSQEH LGVPENERRTTKAE	562
NHX1	GEYKFT-LLATLVVVVLTVI IFGGTTAGMLEVLNKITGCISEEDTS DDEFDIE-APR--	509
NhaA	-----	388
NHE6	SAWLFRMWYN-FDHN YLKP LLTHSGPPLTTLPACCGPIARCLTSPQAYENQEQLKDDDS	621
NHX1	---AINLLNGSSIQTDLGPYSDNNSPDISIDIQA-----VSSKNL PN-----	549
NhaA	-----	388
NHE6	DLILNDGD ISLT YGD--STVN--TEPATSSAPRRFMGNSS EDALDRELA-FGDHEL VIRG	676
NHX1	--NISTTGGN--TFGGLNETENTSPNPARSSMDKRNRLDKLGTIFN SDSQWFQNF-----D	601
NhaA	-----	388
NHE6	TRLVLP--MDDSEPPLNLLDNTRHGA-----	701
NHX1	EQVLKPVFLDNVSP--SLQDSATQSPADFSSQN	633
NhaA	-----	388

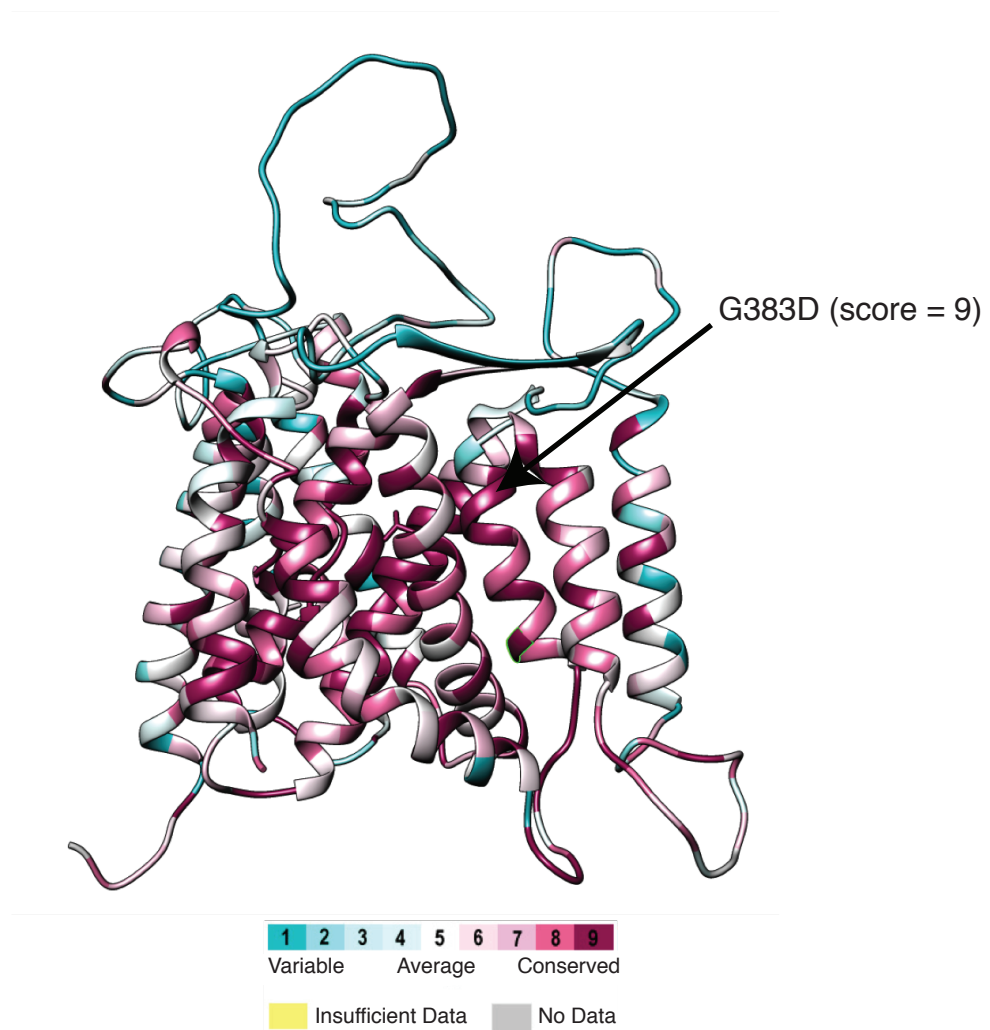


Figure 2.14 Consurf conservation of NHE6. Variable and conserved regions (turquoise through maroon) are mapped into the NHE6 structural model. The G383D residue has the highest possible conservation score.

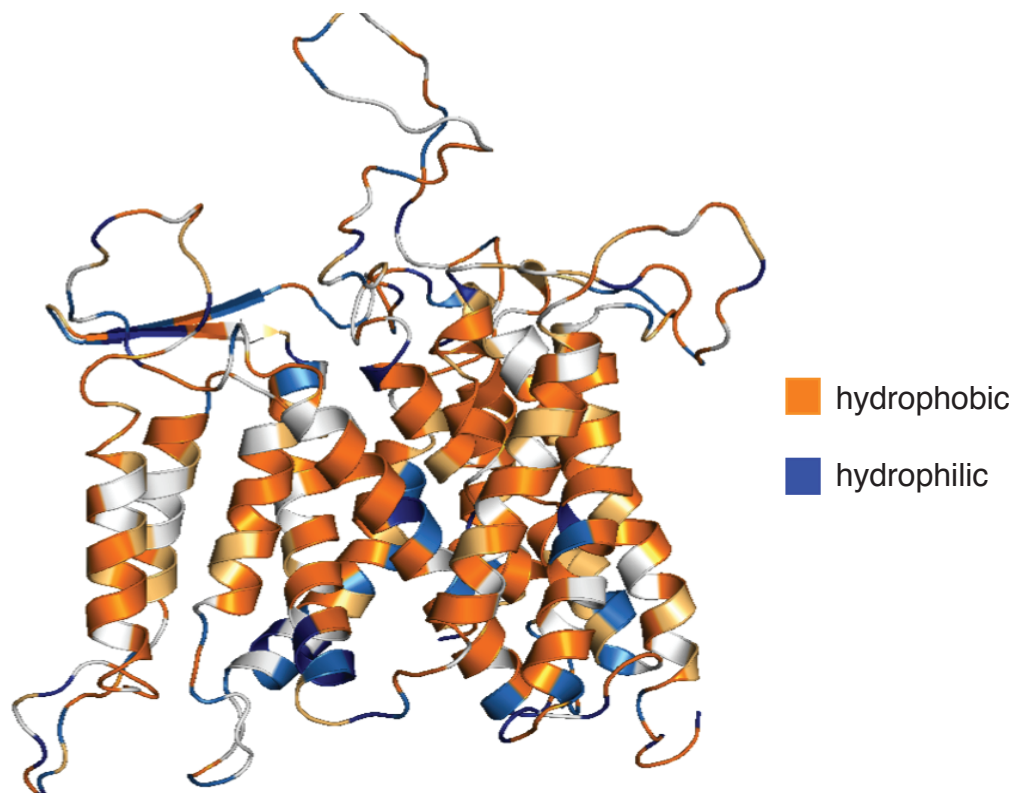


Figure 2.15 Hydrophobicity analysis of the NHE6 structural model. Hydrophobic (orange) residues are located in transmembrane regions while hydrophilic (blue) residues are located in loop regions.

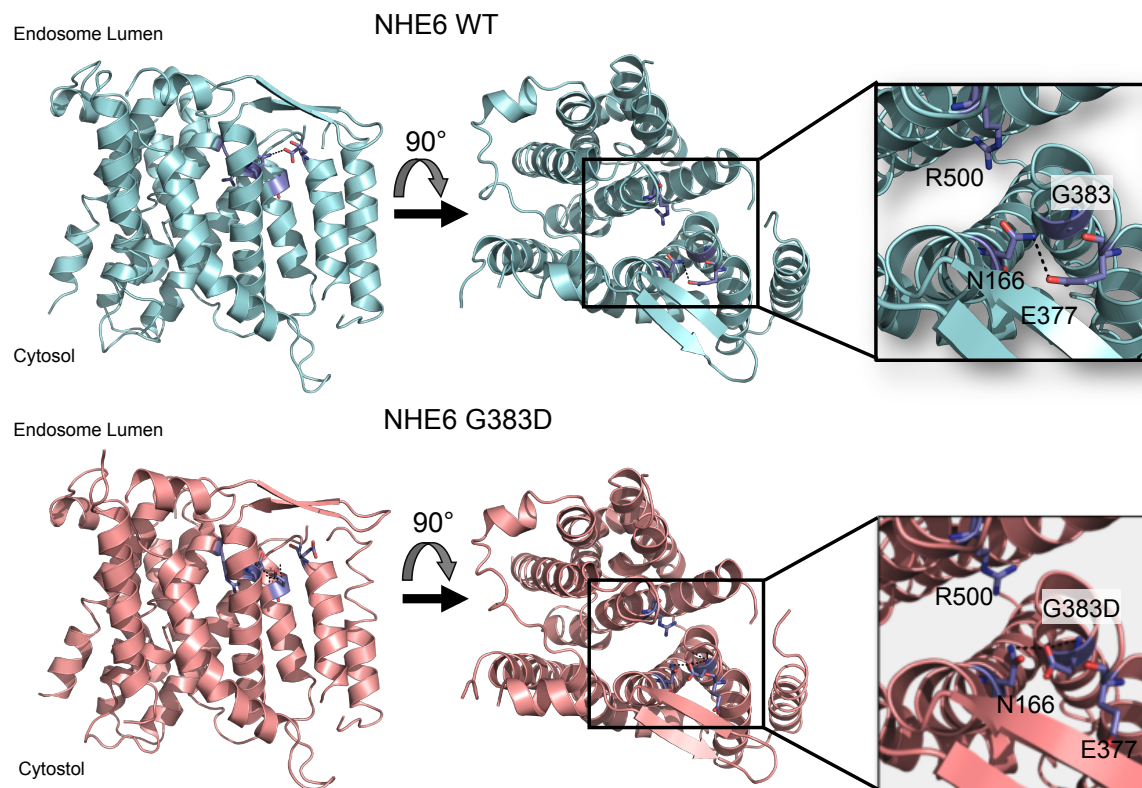


Figure 2.16 Structural modeling of the G383D mutation. The NHE6 structural model predicts that the G383D mutation disrupts helix packing between TM8 and TM2 due to loss of an interaction between N166 and E377 and creation of a hydrogen bond between G383D and N166.

CHAPTER 3:

Neuronal differentiation and gene expression in

a Christianson syndrome iPSC model

INTRODUCTION

The potential to differentiate patient iPSCs to any cell type in the body enables researchers to produce disease specific model systems. Differentiation of human iPSCs to cells with features of cerebral cortical neurons provides a model for neurodevelopmental diseases in a relevant developmental time and tissue typically inaccessible to researchers. The human cortex plays a major role in cognition, motor function, language, and learning and has expanded greatly during human evolution (Franco and Muller, 2013). During development, the neocortex is formed in an inside-out fashion, with later born neurons migrating past earlier born neurons to form an organized, six layered structure. Excitatory neurons in the neocortex project either across the corpus callosum, to the thalamus, or to other subcerebral targets (Molyneaux et al., 2007). The development and axonal outgrowth of cortical projection neurons are critical to forming the neural circuits necessary for complex cognitive function. The neurological phenotypes observed in CS including intellectual disability, autistic features, epilepsy, microcephaly, and non-verbal status (Christianson et al., 1999; Gilfillan et al., 2008; Pescosolido et al., 2014) may result from deficiencies of cortical development and establishment of neuronal circuitry.

Several studies have pointed to dysfunction of the cortex as underlying neurological disorders. Gene expression microarray analysis of post-mortem brain showed that in the cortex of individuals with autism spectrum disorder (ASD), genes related to synaptic function were downregulated and genes related to immune response were upregulated. The cortex was the only one of the three brain regions examined that had significant changes in gene expression (Voineagu et al., 2011). Further analysis of this data set revealed that NHE6 was downregulated in the cortex of individuals with autism indicating a possible role for NHE6 in cortical function (Schwede et al., 2014).

Additionally, co-expression networks of genes recurrently mutated in autism have been shown to converge with the gene expression networks of deep layer cortical projection neurons during midfetal development (Willsey et al., 2013). Therefore, we focused our studies on early born, deep layer cortical neurons.

The development of methods to differentiate neural cultures from iPSCs has provided new models to investigate neurological disorders. The neural differentiation of stem cells was first described by Zhang *et al.* in hESCs using an embryoid body (EB) protocol where neural precursor aggregates were differentiated to produce neurons, astrocytes, and oligodendrocytes (Zhang et al., 2001). The EB protocol was later adapted to generate cortically fated cells by treatment with the Wnt inhibitor, DKK1, and BMP inhibitor, LeftyA (Eiraku et al., 2008). Chambers *et al.* developed an adherent monolayer protocol to differentiate hESCs and hiPSCs to neuronal precursors by treatment with two inhibitors of SMAD signaling (Chambers et al., 2009). Building on these studies, Shi *et al.* used dual SMAD inhibition with the addition of retinoids to differentiate hESCs and hiPSCs to cerebral cortical stem and progenitor cells (Shi et al., 2012a). The withdrawal of FGF2 allowed for terminal differentiation to cortical neuronal fate (Shi et al., 2012a). The formation of glutamatergic projection neurons in these cultures reflects the temporal order of human development *in utero*, with the formation of deep layer cortical neurons beginning three weeks after neuronal induction and upper layer neurons formed subsequently for up to three months (Shi et al., 2012b).

In this study, the dual SMAD inhibition protocol was used to differentiate iPSCs derived from CS patients to cortical-like forebrain neurons thereby establishing a new disease model for the study of CS. The proliferative capacity and extent of cell death was also compared between CS and control neuronal cultures. To assess the ability of

CS iPSCs to differentiate to cortical fate, gene expression was analyzed before and after differentiation.

As described above, gene expression changes in autism cortex may provide insight into disease mechanisms. Furthermore, previous studies of post-mortem autism cortex found a negative correlation between the expression of *SLC9A6* and *SLC9A9*, a member of the NHE family that also localizes to endosomal membranes (Schwede et al., 2014). To test for gene expression changes in CS iPSCs and iPSC-derived neurons as compared to controls, a targeted approach was used to examine the expression of a set of genes hypothesized to be involved in CS disease mechanisms and pathways. To this end, Nanostring nCounter technology was used to determine the expression levels of a set of 358 genes related to neurodevelopment, cell fate, endosomal signaling, endosome biology, autophagy, lysosome biogenesis, lysosomal storage disease, neurodegeneration, the V-ATPase, and NHE family members. Investigation of CS iPSCs differentiated to cortical fate provides insight into early neurodevelopment in patients.

RESULTS

Differentiation of iPSCs to cortical-like forebrain neurons

CS and control iPSCs were differentiated to cortical neuronal fate using a modified monolayer protocol (Fig. 3.1) (Shi et al., 2012a). On Day 0, iPSCs were dissociated to single cells and replated in the presence two small molecule SMAD inhibitors, dorsomorphin and SB431542. Formation of a neuroepithelial sheet characterized by a uniform layer of cells with nuclei smaller than that of iPSCs occurred on Days 10-12. On Day 10-12, the neuroepithelial sheet was expanded and maintained in media without SMAD inhibitors. Neural progenitor cells were observed in rosette structures and were expanded by the addition of FGF2 for 4 days. From Days 25 to 34, cultures were passaged and cryopreserved. During this time, the ratio of post-mitotic neurons to progenitors increased.

Gene expression analysis of iPSCs vs iPSC-derived neurons

In order to understand gene expression changes after neuronal induction, we designed a custom panel of Nanostring probes to measure the expression of 358 genes (Table 3.1). For each gene, a biotinylated capture probe and a reporter probe containing a unique sequence of fluorophores were generated. The panel was chosen to include genes related to neurodevelopment, cell fate, endosomal signaling, endosome biology, autophagy, lysosome biogenesis, lysosomal storage disease, neurodegeneration, the V-ATPase, and NHE family members. RNA was collected from: 1) four separate wells of iPSCs for each CS and control line and 2) four independent neuronal inductions for each CS and control pair 38 days after neuronal induction (Fig 3.2). RNA was hybridized to the custom capture probe and reporter probe library, probe-RNA complexes were bound to a streptavidin coated slide, and complexes were imaged using the nCounter Digital

Analyzer. Gene expression was first normalized to control sequences to account for variation in hybridization between samples. Next, to account for variation in sample input, gene expression was normalized to a set of five housekeeping genes: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, and *RPL27*. Changes in gene expression between iPSCs and iPSC-derived neurons were tested using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR correction.

When gene expression in iPSCs was compared to iPSC-derived neurons, significant changes in gene expression (FDR adjusted p-value < 0.05) were observed for 280 genes. A volcano plot showing the p-value for each gene versus the fold change highlights genes with the highest significance and greatest fold changes in the upper corners of the plot (Fig. 3.3). The genes most highly upregulated after differentiation included neuronal progenitor markers (*PAX6*, *SOX1*, *HES5*), cortical layer markers (*TBR1*, *FEZF2*, *FOXP2*, *POU3F2/BRN1*, *POU3F3/BRN2*), neurogenesis markers (*DCX*, *NCAM*), a dendritic marker (*MAP2*), and an axonal marker (*MAPT*) (Table 3.2). The genes most highly downregulated after differentiation included pluripotency markers (*POU5F1*, *NANOG*) (Table 3.3). Moreover, hierarchical clustering revealed that changes in gene expression discriminated neurons from iPSCs (Fig. 3.4). This shift in gene expression after neuronal differentiation supports the validity of the neuronal induction protocol. Additionally, with one exception replicate iPSC samples from each donor clustered together while replicate neuronal samples did not cluster together (Fig. 3.4). This result indicates that there is increased variability in neuronal cultures likely due to differences in differentiation efficiency between neuronal inductions.

Measurement of proliferation and cell death in iPSC-derived neurons

To measure the proliferative capacity of the neuronal cultures, immunocytochemistry was used to stain cultures with the proliferative marker, Ki67. The cultures were assayed 35 days after induction to neuronal fate. At this stage, the cultures are composed of mitotically active neuronal progenitors and terminally differentiated neurons putatively reflecting deep layer excitatory neuronal cell fate (Shi et al., 2012b). The number of mitotically cycling cells, positive for Ki67, was not statistically different ($p=0.075$, two-tailed Welch's t-test) between CS (26.67% of cells ± 3.7) and control cultures (31.18% of cells ± 1.68) (Fig 3.5).

To measure the extent of cell death in the differentiated cultures, cells were stained with a marker of apoptosis, Caspase 3, 35 days after neuronal induction. Caspase-3 functions in a proteolytic caspase cascade to initiate apoptosis. The number of cells positive for caspase-3 was unchanged ($p=0.0354$, two-tailed Welch's t-test) between CS (16.43% of cells ± 2.13) and control cultures (16.25% of cells ± 4.94) (Fig. 3.5). Taken together, these data show that the proliferative capacity and amount of cell death is similar in CS and control cultures. This indicates that the interpretation of our experiments is not dependent on the rate of neurogenesis or apoptosis in the cultures but is based on other changes caused by loss of NHE6. In addition, these results support the clinical finding of postnatal microcephaly in CS (Pescosolido et al., 2014) which is often caused by deficits in arborization, myelination, or synaptogenesis rather than cell proliferation (van Dyck and Morrow, 2017).

Cell fate analysis of neuronal cultures

To determine the fate of cells differentiated to become cortical-like forebrain neurons, we examined gene expression by immunocytochemistry and Nanostring

analysis. Immunocytochemistry was performed after 38 days of neuronal induction for neuronal progenitor, general neuronal, and deep layer cortical markers. Immunostaining showed that the percentage of cells expressing the neuronal progenitor marker, PAX6, and the neuron-specific marker, MAP2, was similar in CS and control neuronal cultures (PAX6, $p = 0.487$; MAP2, $p = 0.830$; two-tailed Welch's t-test) (Fig 3.6). Furthermore, neuronal cultures derived from CS patients and controls showed comparable percentages of cells expressing TBR1, a marker of layer VI corticothalamic projection neurons, and CTIP2, a marker of layer V corticospinal, corticotectal, and corticopontine projection neurons (TBR1, $p = 0.914$; CTIP2, $p = 0.666$; two-tailed Welch's t-test) (Fig. 3.6).

Additionally, Nanostring analysis of RNA expression showed comparable expression levels of neuronal markers within each control/CS pair. Markers of neuronal progenitors (*NES*, *PAX6*, *SOX1*, *SOX2*), neurons (*DCX*, *MAP2*, *MAPT*, *NCAM*, *TUJ1*), cortical progenitors (*FOXP2*), deep layer cortical neurons (*FOXP2*, *TBR1*, *CTIP2*), and upper layer cortical neurons (*CUX1*, *SATB2*) were similar in CS and control iPSC-derived neurons (p-values not significant by two-tailed, Welch's t-test and Benjamini-Yekutieli FDR adjustment) (Fig 3.7). On the other hand, markers of pluripotency (*KLF4*, *NANOG*), mesodermal or endodermal fate (*AFP*, *MYOD1*, *TWIST1*), glia (*GFAP*, *OLIG2*), and the midbrain (*TH*) all had low expression levels in both CS and control neuronal cultures (Fig. 3.7). Immunostaining and Nanostring gene expression analyses indicate that our induction directed CS and control iPSCs to cortical neuronal fate. Cells of both genotypes expressed neuronal progenitor, neuronal, and cortical markers, but not mesodermal, endodermal, glial, or midbrain markers.

Synaptic markers (*PSD95*, *SHANK3*, *SYP*, *SYN1*) were expressed to similar levels in CS and control neuronal cultures (p-values not significant by two-tailed, Welch's

t-test and Benjamini-Yekutieli FDR adjustment) (Fig. 3.7). However, at the time point we assessed, synaptic markers were generally expressed at low levels. As the cultures mature to form functional synapses at approximately Day 50 using this differentiation protocol (Shi et al., 2012b), synaptic marker expression may increase. Additional analysis of more mature cultures may reveal differences in synaptic markers when marker expression is more robust.

Differential gene expression in CS cells relative to control cells

In order to assess changes in gene expression changes between CS and controls, Nanostring expression data was compared between genotypes first in iPSCs and then in neuronal cultures. Differences in expression were examined within each control/CS family. RNA was collected from four separate wells of iPSCs for each CS and control line and four independent neuronal inductions for each CS and control pair 38 days after neuronal induction (Fig. 3.2). RNA was analyzed with our custom Nanostring gene panel. After normalizing to hybridization controls, gene expression in iPSCs was normalized to a set of seven housekeeping genes: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, *HPRT1*, *RPL13A*, and *RPL27*. Gene expression in neuronal cultures was normalized to hybridization controls and to a set of four housekeeping genes: *GUSB*, *HPRT1*, *RPL13a*, and *POLR2A*. *B2M*, *GAPDH*, and *RPL27* were excluded as housekeepers due to having a high % coefficient of variation among samples. Changes in gene expression between CS and control were tested using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR correction.

Gene Expression differences in iPSCs

First, gene expression between CS iPSCs and control iPSCs was compared for each family. For Family 1 iPSCs, 12 genes were significantly (FDR adjusted p-value <

0.05) upregulated in R472fsX4 CS as compared to control (Table 3.4) and 18 genes were significantly downregulated (Table 3.5) (Fig. 3.8). Interestingly, six subunits of the V-ATPase were downregulated in R472fsX4 iPSCs with fold changes ranging from -1.25 to -1.72 (Fig. 3.9 and Table 3.5). In Family 1 neuronal cultures, there was a trend in the downregulation of the *ATPVOA2* subunit in CS, but significance did not withstand FDR adjustment. In the other families, significant changes in the expression of the V-ATPase were not seen in iPSCs or neuronal cultures (Fig 3.9). The V-ATPase acts in opposition to NHE6 to transport H⁺ into endosomes. The downregulation of the V-ATPase in R472fsX4 lines may represent a compensatory mechanism to prevent further endosome acidification when NHE6 is lost.

For Family 2 iPSCs, fold changes in gene expression in W523X iPSCs as compared to control were generally less than two-fold with the exception of *SLC9A6* (Table 3.6 and Table 3.7). Changes in gene expression in W523X iPSCs were not significant after FDR adjustment.

For Family 3 iPSCs, two genes were significantly misexpressed in G383D iPSCs when compared to control (Table 3.8 and Table 3.9). Cathepsin F (*CTSF*) was upregulated 3.41-fold in G383D iPSCs (p= 0.0087). In neuronal cultures from Family 3, *CTSF* was also upregulated 4.09-fold in CS, but this trend was not significant after FDR adjustment. In other families, *CTSF* expression trended down in Family 2 W523X iPSCs (-1.12 fold change) and W523X neuronal cultures (-2.12 fold change), down in Family 1 R472fsX4 iPSCs (-1.16 fold change), and up in R472fsX4 neuronal cultures (5.47 fold change). *CTSF* encodes a lysosomal cysteine protease that is activated at acidic pH (Wang et al., 1998). Upregulation of *CTSF* may result in increased substrate cleavage in G383D CS cells.

The second gene significantly misexpressed in Family 3 G383D iPSCs was vimentin (*VIM*) with a -1.39-fold decrease ($p=0.000378$). *VIM* expression was also significantly decreased in Family 1 R472fsX4 iPSCs (-1.65 fold change, $p=0.000296$). In Family 2 W523X iPSCs and in neuronal cultures from all three families, *VIM* expression trended down but changes were not significant after FDR adjustment. *VIM* encodes an intermediate filament protein expressed in neuronal precursors and is replaced by neurofilament in post-mitotic neurons (Cochard and Paulin, 1984). *VIM* plays an important role in the initiation of neurite outgrowth (Shea et al., 1993). Therefore, decreased *VIM* expression in CS may point to a mechanism for decreased neurite growth.

Gene Expression differences in iPSC-derived neurons

Next, changes in gene expression between CS and control iPSC derived neurons were compared. Significant changes in gene expression tested using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR correction were not observed when CS neuronal cultures were compared to control neuronal cultures for each family (Tables 3.10-3.15). Variation in the efficiency of neuronal induction may generate cultures with varied numbers of cells of other lineages. Differences in the ratio of progenitors to neurons may also account for why few significant changes were seen. Additionally, genes not included in our targeted panel may be misexpressed in CS.

NHE family member expression in iPSCs and iPSC-derived neurons

To evaluate changes in expression of the Na^+/H^+ exchanger (NHE) family members in CS as compared to controls, Nanostring expression data was analyzed. Expression of the nine NHEs was compared between CS and paired controls in iPSCs and in neuronal cultures. *SLC9A6* was downregulated in all CS iPSCs and neuronal

cultures when compared to paired controls (Fig. 3.10). Other NHE family members did not have significant changes in gene expression. There was a trend toward overexpression for *SLC9A8* in all iPSC and neuronal cultures with fold changes between 1.09-1.48. Therefore, gene expression analysis of NHE family members indicates that *SLC9A6* is the only family member significantly misregulated in CS.

DISCUSSION

The generation of cortical-like forebrain neurons from CS patient-derived iPSCs presents a new opportunity to study neurodevelopmental phenotypes in a relevant developmental time and tissue. Upon differentiating iPSCs to neuronal fate, analysis of mRNA expression using a custom panel of 358 genes showed a major shift in gene expression. Pluripotency genes were downregulated while neurodevelopmental genes were upregulated providing evidence of effective neuronal differentiation following the double SMAD inhibition protocol.

The amount of proliferative progenitor cells was compared between CS and control neuronal cultures. CS and control neuronal cultures had similar numbers of Ki67+ cells demonstrating that equivalent numbers of cycling progenitor cells were present. These cells may represent progenitor cells of several types. One advantage of the Shi *et al.* protocol over embryoid aggregate protocols is that iPSCs are directed to generate the diverse classes of stem and progenitor cells seen *in vivo*. Radial glia represent the major class of stem and progenitor cells produced by this method, but intermediate progenitors and outer radial glia are also present (Shi et al., 2012b). By replicating this diversity in cell types, an improved representation of the cortex is produced for disease modeling.

The levels of cell death were also compared between CS and control cultures. The number of cells expressing caspase-3, a marker of apoptosis, was unchanged in CS as compared to control cells. Taken together, the equal numbers of proliferative and apoptotic cells observed in CS and control cultures indicates that cell number may not contribute to the microcephaly observed in CS patients. While prenatal microcephaly is often caused by an impairment of neuronal progenitor expansion, postnatal microcephaly can be caused by defects in cell migration or synapse development

(Barkovich et al., 2012). Our results therefore are consistent with the observation of postnatal microcephaly in CS (Pescosolido et al., 2014).

iPSCs differentiated using the monolayer double SMAD inhibition protocol took on a cortical forebrain fate. CS and control neuronal cultures analyzed 38 days after neuronal induction expressed markers of neuronal progenitors, neurons, and cortex as measured by immunohistochemistry and Nanostring analysis. Differentiation of iPSCs to cortical neuronal fate mirrors the development of the human cortex. *In vivo*, glutamatergic projection neurons develop in an inside-out manner with later born neurons migrating past earlier born neurons to form an organized six-layered structure. Our expression analyses showed expression of deep layer markers at day 38 while expression of upper layer markers would be expected at later timepoints. This is consistent with previous analysis of this induction protocol showing that production of cells expressing the layer VI and V markers, TBR1 and CTIP2, peaks after 30 to 40 days of differentiation followed by production of cells expressing upper layer markers from days 45 to 60 (Shi et al., 2012b). Our analysis of cell fate shows that CS and control iPSCs have equivalent capacities for neuronal differentiation. Subsequent experiments using CS iPSC-derived neurons were performed at days 32-40 post neuronal induction. The results above indicate that the cells being assayed in our experiments are deep layer cortical-like neurons.

Comparison of gene expression in CS and controls cells revealed several interesting candidates. In iPSCs from the Family 1 R472fsX4 line, several subunits of the V-ATPase were significantly downregulated. The V-ATPase is a multisubunit complex that functions in an ATP dependent manner to transport protons into organelles or across the plasma membrane. In this manner, the V-ATPase works in opposition to NHE6 to acidify endosomes (Forgac, 2007). In the R472fsX4 line, 6 of the 13 V-ATPase

subunits were significantly downregulated. Downregulation of V-ATPase subunits may disrupt assembly of the multisubunit complex. These results suggest a compensatory mechanism in which the V-ATPase is downregulated as a means to decrease endosome acidification induced by the loss of NHE6.

Furthermore, inhibition of the V-ATPase presents a route for therapeutic intervention. To prevent further endosome acidification when NHE6 is lost, blocking proton transport through the V-ATPase could help restore pH homeostasis. Several naturally occurring V-ATPase inhibitors including, bafilomycin, concanamycin, and archazolid have been studied for their potential as cancer treatments, but none have been used clinically (Holliday, 2014). Our gene expression analysis highlights the variability between CS patients and emphasizes that some therapies may not be effective for all CS patients. For example, although the V-ATPase may be a druggable target for CS, drugs that would downregulate the V-ATPase may not be effective in CS patients with the R472fsx4 mutation as in other patients because the V-ATPase is already downregulated in this patient. iPSC-derived neurons from patients will provide an important model for testing potential therapies in a personalized manner.

The misexpression of genes in CS may point to disease mechanisms. The lysosomal protease, cathepsin F (CTSF), was upregulated in Family 2 G383D iPSCs as compared to control. The substrates of CTSF are mostly unknown, but recently scavenger receptor class B, member 2 (SCARB2) was identified as a substrate (Peters et al., 2015). SCARB2 is a lysosomal membrane protein involved in the regulation of endosome maturation (Kuronita et al., 2002). Loss of function mutations in SCARB2 cause action myoclonus-renal failure syndrome (AMRF), a neurological and renal disease that presents with abnormal accumulations of material in the brain indicating a role for SCARB2 in lysosomal trafficking (Berkovic et al., 2008). In CS, the increased

expression of CTSF observed in G383D cells may lead to increased cleavage of SCARB2 and misregulation of endosome maturation or lysosome trafficking. A selective inhibitor of human CTSF has been identified but not developed for clinical use (Reis et al., 2007). Additionally, because CTSF is activated at acidic pH (Wang et al., 1998), alkalinizing the endosome compartment presents another method to decrease CTSF activity. The FDA-approved lysomotrophic weak bases, amantadine and chloroquine, alkalinize endosomes by diffusing across membranes and becoming protonated. Amantadine has been used clinically for the treatment of Parkinson disease and as an antiviral drug (Aoki and Sitar, 1988) and chloroquine has been used as an antimalarial drug (Ducharme and Farinotti, 1996). Amantadine and chloroquine could possibly be used to alkalinize endosomes in CS.

Another gene misexpressed in CS, vimentin (VIM), may contribute to CS disease mechanisms. The fold change for VIM was significant but small (-1.39 fold change) in Family 2 G383D iPSCs and showed a similar but insignificant trend toward downregulation CS iPSCs and neurons from the other two CS patients. VIM is an important component of the cytoskeleton expressed in neuronal progenitors and has been shown to play a role in neurite outgrowth. Inhibition of VIM function in rat hippocampal neurons treated with VIM antisense oligonucleotides prevented the initiation of neurite growth (Boyne et al., 1996). Conversely, overexpression of VIM in a neuroblastoma cell line treated with factors to promote neuronal differentiation resulted in an increase in neurite length (Dubey et al., 2004). In CS, downregulation of VIM could result in decreased initiation of neurite outgrowth.

NHE6 is part of a conserved family of Na^+/H^+ exchangers (NHE) that play important roles in ion and pH homeostasis. The relationship between loss of NHE6 and the expression of NHE family members informs both endosomal biology and the

development of therapeutic targets for CS treatment. The varied tissue and cellular distribution of the NHEs leads to diverse cellular roles. While NHE1, NHE6, NHE7, NHE8, NHE9 are ubiquitously expressed, NHE2, NHE3, and NHE4 expression is highest in the gastrointestinal tract and kidney (Donowitz et al., 2013). Furthermore, NHE1-5 localize to the plasma membrane while NHE6-9 localize to organelles. NHE6 and NHE9 both localize to endosomes with NHE6 in early and recycling endosomes and NHE9 in late recycling endosomes (Nakamura et al., 2005). NHE9 may have overlapping roles with NHE6 in the maintenance of endosomal pH in these compartments. Overexpression of NHE9 has been shown to alkalinize endosomes while NHE9 knockdown acidifies endosomes in mouse astrocytes (Kondapalli et al., 2013). In our gene expression study, NHE6 was downregulated in CS iPSCs and neuronal cultures. Expression of other NHEs was not significantly changed. In the NHE6 null setting in CS, alkalinization of endosomes by activating or upregulating NHE9 may provide a strategy for therapeutic intervention. Although NHE inhibitors have been studied, for example to block NHE1 hyperactivation during cardiac ischemia (Masereel et al., 2003), pharmaceutical activators of NHEs have not been reported.

Gene expression analysis revealed potential CS disease mechanisms and drug targets. However, changes in gene expression occurring in only one CS line may represent pathology specific to that NHE6 mutation or to the genetic background of the individual. Consideration of patient specific gene expression will be important in designing strategies to treat CS. Additionally, changes in gene expression in CS often had small fold changes (< 2) and in some CS lines few or no significant changes in gene expression were observed. This may indicate that loss of NHE6 instead results in changes in protein post-translational modifications or substrate availability. For example, loss of NHE6 could weaken signaling transduction from signaling endosomes. Signaling

endosomes transport activated receptors endocytosed at axon terminals retrogradely to the soma to initiate downstream signaling events. For example, neurotrophin signaling, which is critical to neuronal growth and development, occurs through signaling endosomes. Studies in a NHE6 null mouse model have shown attenuation of neurotrophin signaling as compared to control (Ouyang et al., 2013). Endosome overacidification in the absence of NHE6 may decrease the rate of recycling of signaling receptors to the plasma membrane or may increase the rate of ligand-receptor dissociation. These mechanisms would influence endosome signaling without direct changes in gene expression.

In summary, we differentiated cortical neuronal cultures from iPSCs derived from patients with CS. The neuronal differentiation capacity, proportion of proliferative cells, and amount of apoptotic cells were similar in CS and control cultures. Gene expression analysis revealed potential candidates for therapeutic intervention and highlighted the need to examine the variability between patients to develop effective drugs. The iPSC-derived neuronal cultures we generated provide a platform to interrogate mechanistic phenotypes and test possible therapeutics.

METHODS

Neuronal Induction monolayer protocol

Human neurons were generated using the previously described double SMAD inhibition monolayer protocol with some modifications (Shi et al., 2012a). iPSC lines with less than 30 passages were used for neuronal differentiation. In brief, iPSC colonies were treated with Gentle Cell Dissociation Reagent (StemCell Technologies) for 10 minutes at 37°C and 1×10^6 cells were plated onto one well of a Matrigel coated 12-well plate in Neural Maintenance Media (NMM) (1:1 DMEM/F-12 GlutaMAX (Gibco):Neurobasal (Gibco)/1x N2 supplement (Gibco)/1x B27 supplement (Gibco)/5 µg per mL insulin (Sigma)/1 mM L-glutamine (Gibco)/500 µM sodium pyruvate (Sigma)/100 µM nonessential amino acids solution (Gibco)/100 µM β-mercaptoethanol (Gibco)/50 U per mL penicillin-streptomycin (Gibco)) in the presence of 10 µM Y-27632 dihydrochloride (Tocris). After one day, if the cells had reached confluency, the media was changed to NMM + 1 µM Dorsomorphin (Stemgent) + 10 µM SB431542 (StemCell Technologies). Cells were fed daily for 10 to 12 days, after which they were passaged using Gentle Cell Dissociation Reagent, plated at 1:1 in one well of a 6-well plate, and fed with NMM. Cultures were inspected daily for formation of neuronal rosettes and were treated with 20 ng/mL FGF2 (PeproTech) for 4 days to expand neural stem cells. Neurons were expanded and frozen at days 22, 26, 30, and 34 after neural induction.

Immunofluorescence

For immunofluorescence experiments, iPSC colonies or neuronal cultures were grown on 8-well glass chamber slides (Nunc Lab-Tek) or on autoclaved glass 12mm round coverslips (Ted Pella) coated with poly-L-ornithine (Sigma) followed by laminin (Sigma). Cells were fixed with 4% paraformaldehyde at room temperature for 10 min,

permeabilized with 0.25% TritonX-100 (Sigma) in PBS for 10 min, and blocked with 10% goat serum (Jackson laboratories)/0.01% TritonX-100/1xPBS for 1 hour. Primary antibodies were diluted in 2% goat serum/0.01% TritonX-100/1xPBS and incubated for either 2 hr at room temperature or overnight at 4°C. The primary antibodies used were: mouse anti-Ki67 (BD Biosciences), rabbit anti-NeuN (Abcam), rabbit anti-cleaved-Caspase 3 (CellSignaling), and mouse anti-NeuN (Millipore). After three, 5 min washes with 1xPBS, secondary antibodies were diluted to 1:1000 in 2% goat serum/0.01%TritonX-100/1xPBS + 1:1000 Hoechst (Invitrogen) and incubated for 1h at room temperature. After PBS washes, cells were mounted with Fluoromont-G (SouthernBiotech) and imaged on a Zeiss LSM 710 confocal laser scanning microscope.

Nanostring analysis of iPSCs and neuronal cultures

For each CS and paired control line, total RNA was collected from iPSCs and iPSC-derived neurons at 38 days post-induction (Fig. 3.2) using the RNeasy Mini kit (Qiagen). RNA was tested for quality using a Bioanalyzer (Agilent) and all samples had a RIN of 9 or above. RNA was analyzed using a custom designed Nanostring platform containing probes for 358 genes related to neurodevelopment, endosome signaling, autophagy, lysosomal storage disease, neurodegeneration, endosomes, lysosome biogenesis, the V-ATPase, and NHEs. For each sample, 100 ng RNA was hybridized to a capture probe and reporter probe library for 12 hours at 65°C. Target-probe hybrids were immobilized onto a streptavidin coated slide and imaged using the nCounter Digital Analyzer. Nanostring data was analyzed with nSolver software. First, RNA counts were normalized to internal control sequences to account for variation in hybridization between samples. The geometric mean of the positive controls within each lane was calculated. Next, the average of the geomeans across lanes was calculated. Dividing the average across

lanes by the geomean within each lane gives a lane-specific scaling factor. Counts within each lane were multiplied by the lane specific scaling factor. Second, to account for variation in sample input, RNA counts were normalized to a set of housekeeping genes including: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, *HPRT1*, *RPL13a*, and *RPL27*. A lane specific scaling factor was then calculated as above and counts within each lane were multiplied by its scaling factor.

Hierarchical clustering

Gene expression was measured for 358 genes using the custom designed Nanostring panel in iPSCs and iPSC-derived neurons. Gene expression was z-score transformed and used to perform agglomerative unsupervised hierarchical clustering using the Euclidean distance metric and average linkage criteria. Clustering was visualized using Java Tree View.

Statistics

Nanostring gene expression differences were tested for significance with a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. Fold changes with an adjusted p-value < 0.05 were considered significant.

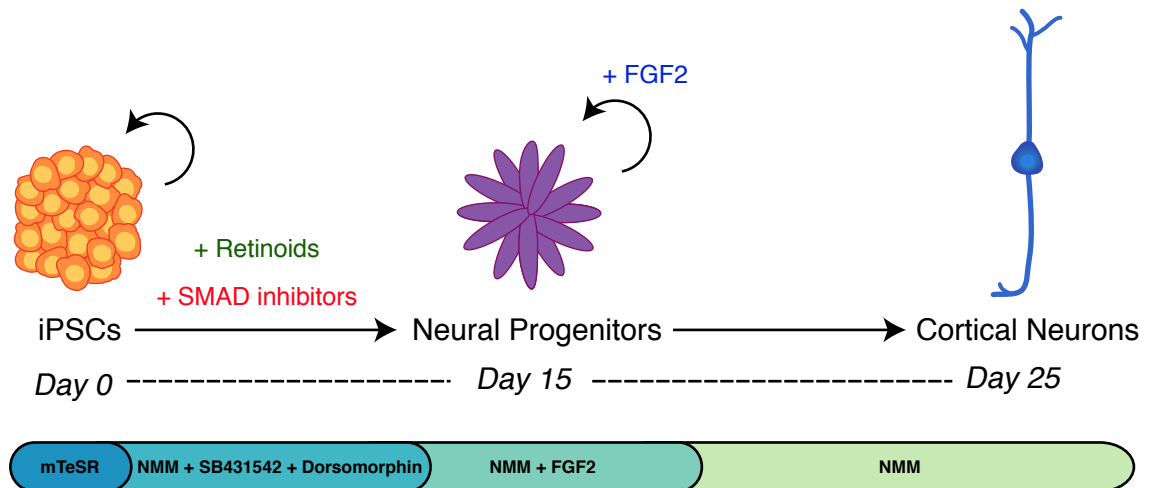


Figure 3.1 iPSC neuronal differentiation. iPSCs were induced to cortical neuronal fate by dual SMAD inhibition in the presence of retinoids as detailed in the Methods. FGF2 allowed for expansion of neural progenitors and was removed to permit the differentiation of post-mitotic cortically fated neurons. The protocol was adapted from Shi *et al*, 2012a.

Table 3.1 Nanostring custom designed probe set. Probes were designed for 358 genes related to neurodevelopment, endosomal signaling, autophagy, lysosomal storage disease, neurodegeneration, endosomes, lysosome biogenesis, the V-ATPase, and NHEs. The probe target sequence and category annotation is listed for each gene.

Gene	Accession	Position	Target Sequence	Category
ADAM10	NM_001110.2	1931-2030	AACTGAAACCTGGGAAACAGTGCAGTCCAAGTCAAGGTCCTTGTGTACAGCACA GTGTGCAATTCAGTCAAAGTCTGAGAAGTGTGCGGATGATTGAGA	Neurodegeneration
ADAM19	NM_023038.3	1691-1790	GAGAAGGTGAATGTGGCAGGAGACACCTTTGGAACTGTGGAAGGACATGAAT GGTGAACACAGGAAGTGAACATGAGAGATGCGAAGTGTGGGAAGA	Neurodegeneration
ADAM9	NM_001005845.1	696-795	TGTCTTGCCACAGACCCGGTATGTGGAGTGTTCATTGTGCTAGACAAGGAAAGG TATGACATGATGGGAAGAATCAGACTGTGTGAGAGAAGAGATG	Neurodegeneration
AFP	NM_001134.1	396-495	AGTGAAGGGAAGACATAACTGTTTTCTTGCACAAAAAGCCACTCCAGCAT CGATCCCACTTTTCCAAAGTTCAGAACCTGTGCACAAAGTGTGAAG	Cell Fate/Neurodevelopment
AGA	NM_001171988.1	465-564	GGTGTGGCAGCGAAGTACTGGAAACATACAACACACACTTTTAGTAGGAGAGT CAGCCACCACTTTGTCTCAAAGTATGGGGTTTATCAATGAAGACT	Lysosomal Storage Disorder
AKT1	NM_001014432.1	1276-1375	GGACGGGCACATTAAAGATCACAGACTTCGGGCTGTGCAAGGAGGGGATCAAGGA CGGTGCCACCATGAAGACCTTTTGGCCACACCTGAGTACCTGGCC	Signaling, Autophagy
AKT1S1	NM_032375.3	1851-1950	TAGTCGTGGACAGCTCTTTTGTGTTGGCTCAAATCTGTAAAGGCTTGACCA GTCTCTACATAGTACCCTCGGCTTTCTCTGAGTTCTCCCTCCC	Autophagy
AKT2	NM_001626.4	700-799	AGGAGATGGAAGTGGCGTGCAGCAAGGCACGGGCTAAAGTGACCATGAATGACT TCGACTATCTCAAACCTCTTGGCAAGGGAACCTTTGGCAAGTGCAT	Signaling
AKT3	NM_005465.4	288-387	GCCAGTTAATGAACAGAACGACCAAGCCAAACACATTATATCAGATGTCTC CAGTGGACTACTGTTATAGAGAGACATTTCATGTAGATACTCC	Signaling
AMBRA1	NM_017749.2	561-660	CTTAGCCTCCACCATGTGAACCAATAATCTATTACGGAGGTGAAGACTGGC AAGTGTGTTCTATTCCTGTGATGGACACCGCCGCTACTCCATGGTGT	Autophagy
AMY	NM_006334.3	635-734	CACAGCAGACCATGTGTTCACGGGATGCCGCACAAAAAGCTGAGGCGAGTAC TGGAGAAGGTGCAGAAACATGTCCTCAATCCATAGAGGTCTTGACAG	Neurodegeneration
AP1S2	NM_003916.3	729-828	CCACGTAGTGTCTTGAAGAAATGGACTGACATAACTCTCTCCCTGTTGTATGA CTTCTTGGGATTTCACACACTGTAGATGGTCACTCCCTTCAT	Endosome
APBA1	NM_001163.3	4861-4960	CATCAGCCAGTTTACTTCTCTGCCAATAAGAGTGGATAGAACAGGAATGAAG TGTTACTGGTGTATTTGAGGCATCAGGAGCCTTGGAAAAATCATC	Neurodegeneration
APBA2	NM_001130414.1	1186-1285	AAGCAGCAGCGCTCTGATCTCAATGGACCTGTTGACATAACAACATCCAGAGA CAAGAAGGTGGCATCATTTCCAAAGTTTGTGGCTGTCCAGGGC	Neurodegeneration
APBA3	XM_006722950.2	1572-1671	GCTGCGTGCCAGGCGCTGTCCGCGAGACGAAGTCGACAGCTCGGTGACACT CAGCATCGTCACTGCCCTCCCGTACCACCGCCATCATCCACCGGC	Neurodegeneration
APBB1	NM_001164.2	1590-1689	AGATCTGCTCTAAGATCATGGCCGAACGGCGTAATGCCGCTGCTTGGTAAATGG ACTCTCCCTGAGACCACTCTAACTTGTGGATGTCCTTTTCCAAGT	Neurodegeneration
APBB3	NM_133172.2	1931-2030	CCTTCTCTGCTGTCCCTCCGATCTGTGTCCACCGTCTATTATTGCCCAATTTATT GTTTATACGGATGACTGGGAGGCACTGCACCACAACGTAGGAC	Neurodegeneration
aph1Avar3	NM_001243771.1	485-584	CGCTTCGCGTTATCATCTCGTGTGCGAGGGAAGGCAGATGAGGGGTTAGCATCGC TGAGTGAGGACGGAAGATCACCCATCTCCATCCGCAGATGGCCTA	Neurodegeneration
aph1Avar4	NM_001243772.1	484-583	CCGCTTCGCGTTATCATCTCGTGTGCGAGGGAAGGTGTTCCGCTTGGCTACTACA GCTGCTTAATTTCTGGTCTCTCTCTCGGTATCATCAGTGGTGTCT	Neurodegeneration
aph1Bvar1	NM_031301.3	383-482	TATAAACCCAGGTGAGACAGACCCCTCTATGCGACTGCTGGCCTATGTTTCTGGC TTGGCTTTTGAATTCAGATGAGTGAGTATTTTCTTTGTGAATACC	Neurodegeneration
aph1Bvar2	NM_001145646.1	401-500	AGCACCTCTATGCGACTGCTGGCTATGCTTTTACGACGCTGGTCAATTATCTTG CTGATGATTTCTGGGGCACTTGATTTTGGTGGCTGTGAGAAG	Neurodegeneration
APLP1	NM_005166.3	2341-2440	GCTCTTTAAGGTGACCGCCACCTTGGTCTAGTGTCTATTCCCTGGAATTCACCT CTCATGTTTCCCTACTAACATCCCAATAAAGTCTCTTCCCTAC	Neurodegeneration
APLP2	NM_001243299.1	2856-2955	GAAAGCAGTGAAGAGACCCCTCGCAAAATGATGTGAAGAGGACAGTTCCTTGAAGTC CAGTGTTCACAGGCTGGATACCTGTGTGTCTCCATAAAAGTCTCT	Neurodegeneration
APOE	NM_000041.2	97-196	GGGCTGCGTGTGCTGTCACATTCTGGCAGGATGCCAGGCCAAGGTGGAGCAAG CGGTGGAGACAGAGCCGGAGCCGAGCTGCGCCAGCAGACCGAGTG	Neurodegeneration
APOER2	NM_033300.2	1591-1690	ATCTCTCCACTGACTTCTGAGCCACCCCTTTGGGATAGCTGTGTTTGAAGACA AGGTGTTCTGGACAGACCTGGAGAACGAGGCCATTTTCAAGTGCAA	Neurodegeneration
APP-695	NM_001136129.2	870-969	ACAGAGTCTGTGGAAGAGGTGTTTCAGTTCCTACAACAGCAGCCAGTACCCCT GATGCCCTTGACAAGTATCTCGAGACACCTGGGGATGAGAATGAAC	Neurodegeneration
APP-770	NM_001136130.2	1016-1115	GAAACAATTTGACACAGAAGACTGTCATGGCCGTGTGTGGCAGCGCCATGTC CCAAAGTTTACTCAAGACTACCCAGGAACCTCTTCCCGAGATCCT	Neurodegeneration
APP-Ail	NM_000484.3	1726-1825	TAAAGCATTTGAGCATGTGCGCATGGTGGATCCCAAGAAAGCCGCTCAGATCCG GTCCAGGTTATGACACACCTCCGTGTGATTATGAGCGCATGAA	Neurodegeneration
APP-KPI	NM_201413.2	1164-1263	GGCGGATGTGGCGCAACCGGAACAACCTTTGACACAGAAGAGTACTGCATGGCC GTGTGTGGCAGCGCATTCCTACAACAGCAGCCAGTACCCCTGATG	Neurodegeneration
APPBP2	NM_006380.2	1996-2095	GTGGTGCAGTCTCTCGATTTCTCAGAATGTGAGGGACCGAGCTGCTGAGGG AGGACCTCAGTTAACCAATTACCTTTTCCGGATTCCAGGGAATTC	Neurodegeneration
APPL1	NM_012096.1	751-850	AAGCGTTCAAGATGTTTCGAGGGAAATGGACAGTGAATAGAGACCATGCAACAG ACAATAGAGGATTGGGAAGTAGCCAGTATCCCTTATATGTGCCCT	Endosome
ARF6	NM_001663.3	1363-1462	TGGCTTTCGTTAGGATGCTCTGATCTGCATTTGACATGAACACAAAGTTGCTAG ATGCTCTGTTGACTTCCAGCAGATGGGATGGGGAAACACAGC	Endosome
ARSA	NM_000487.5	1055-1154	TTCTTCTGACTATGCTCTCACCACCCACTACCTCAGTTCACTGGGCAGA GCTTTGCAGAGCTGTGAGCCGCGGGCCATTTGGGAGTCCCTGA	Lysosomal Storage Disorder
ARSB	NM_000046.3	2079-2178	CACATGACAGGAATGGTGTCCCTTATGGATGAAGCAGTAGGAATGTCACTGCAG CTTTAAAGAGCAGTGGGCTCTGGAAACAACCGGTGTCACTCTTTT	Lysosomal Storage Disorder
ASAH1	NM_177924.3	1276-1375	GACCGTTGGAACATCCCTTCTTCTTGATGATGCGAGAAGCCCTGCAAGATGT GTCTGAACCGCACCGCAAGAGAAATATCTCATTTGAAACCATGT	Lysosomal/TFEB
ATG10	NM_001131028.1	986-1085	TCAACTATATCATCATGCTGAGCATTGTAGGGCCAGTTGTTGGGCTGAATCT ACCTCTGATTATGCCAAAGCAACGTCTCAGGATGAACGAAATGT	Autophagy
ATG101	NM_021934.4	940-1039	CGTGGAGGTGATGAATCGGCATGAGTACTTGCCCAAGATGCCACACAGTCGGA GGTGGATAACGTGTTTGACACAGGCTTGCGGGACGTGACGCCCTAC	Autophagy
ATG12	NM_004707.2	26-125	CCGCTCCTCTCCGAGGCTGTAGTCGCGGGAAGAACATGTTGCGTTACTAACG TTCAGAGGCTCGCAGAGCTTCGATTTGAATGACTAGCCGGGAAC	Autophagy
ATG14	NM_014924.4	771-870	CCTCTCAGGACGATGGGCTGTGACGATCACAACGGAGACACCAGCATTAGCATT ACAGGGCTTGGATTAGCCTCCCTACAATGGGGACTACTCTGCC	Autophagy
ATG16L1	NM_017974.3	2406-2505	GTCTTCCAAAGTCGGTTCTGGCCTAACGCATGTCCCAACACCTTGGGTTCAATT GCCCGGTGAACCTCACTTTAAGCATTTGATTAAACGGAAACCTCCGA	Autophagy
ATG16L2	NM_033388.1	1587-1686	ACCTGCTCAGTGTTCGCCAGACAACACACTCAAGGTCAATGACCTGCGTGTGAG CAACATCCGCGCAGGTGTCAGGGCCGATGGCTCAAGTGTGGTTC	Autophagy
ATG2A	NM_015104.2	3575-3674	CTCAGGTTATCCTCTGACTGCTCCGCTGTACCTGTCCGACAAGTGTGAGGTGG AGACCCTGGACCTGCGCGAGATTATGCTGTGTTTGGATGTTG	Autophagy
ATG2B	NM_018036.5	1723-1822	CTGTGCTTCAGCCCACTTGGGGAGAGTTCCTTGATCATATAAAGAACGCCAGT AAGAGGGTCAACATTTCCATCCAACTAGTTCAACCAACACCTTT	Autophagy
ATG3	NM_022488.3	594-693	GAGAGTGGATGTTGGAACAGATGAGGCTACCTAGATACAAGGAAATAGTAG AAGCTGTAAAGCCAAACTGATGCTGGCGGTGAAGATGCTATT	Autophagy
ATG4A	NM_052936.3	1291-1390	TTGACTCTACTGAGCACTGGAGGAGTTTGTATCTGGAGGAAGATTTGAGATTCT GAGTGTGATGATCCTGGGAACCTCAACTGAAGGTGTGCTTCCA	Autophagy

ATG4B	NM_013325.4	163-262	TGAGACCTCAGAGCCCGTTTGGATACTGGGTAGAAAAATACAGCATTTTCACAGAA AAGGACGAGATCTTGTCTGATGTGGCATCTAGACTTTGGTTTACA	Autophagy
ATG4C	NM_178221.2	842-941	AGAATATGGAAGAAGTCTGGGAAAAAGCAGGAGATTGGTATGGACCAGCTGTG GTTGCTCACATTTTAAGAAAAAGCAGTTGAAGAAAGCAAGGCATCCT	Autophagy
ATG4D	NM_001281504.1	515-614	GTTAAAGCGCCGACAGCCTTTAGCAAGATCTCCAGCATCCACCTCTGTGGCCGCC GCTACCGCTTTCGAGGGCGAGGGTGACATACAGCGTTTCCAGCGGG	Autophagy
ATG5	NM_004849.3	351-450	AGAATGACAGATGACAAAGATGTGCTCGAGATGTGTGGTTTGGACGAATTCCTAA CTTGTTCACGCTATATCAGGATGAGATAACTGAAAGGGAAGCAG	Autophagy
ATG7	NM_001136031.2	811-910	TATGATCCCTGTAACCTAGCCAGTACCCTGGATGGCCTTTGAGGAATTTTTTGGT CCTAGCAGCCACAGATGGAGTAGCAGTTTCCAGTCTGTTGAAG	Autophagy
ATG9A	NM_001077198.1	436-535	GTCACCTGCGTGGACTATGACATCTTATTTGCCAACAAGATGGTGAACCACAGTC TTCACCTACTGAACCCGTCAAGGTCACTCTGCCAGACGCCCTTT	Autophagy
ATG9B	NM_173681.5	2021-2120	GCCCTGGAGATTATGCACTTTTTCATCACTTCACTGTGGATGTGGCTGGGGTTG GGGACATCTGTTCCCTTGGCCCTTATGGATGTGAAGCGCCACCGAC	Autophagy
ATO1H1	NM_005172.1	846-945	GGGAGGTTACTCGGTGACGCTGGACGCTCGCACTTCTCGACTTTCGAGGACAG CGCCCTGACAGCGATGATGGCGCAAAAGAAATTTGTCTCCTTCTCTC	Cell Fate/Neurodevelopment
ATP13A2	NM_001141974.1	926-1025	TGGCTGGCTGACCACTACTGCTGTACGCCCTGTGCATCTTCTCATTTCTCCAT TCCATCTGCCTGTCTGCTGTACAAAGACCAGAAAGCAAGCCAGA	Lysosomal Storage Disorder
ATP6V0A1	NM_001130021.1	655-754	GCACTCTTTAAGACTTGGCTGTGGCTGGTGTCTTAAACGGGAGCGCATCCC TACTTTTGAGCGCATGCTTTGGCGGGTATGCCGGGGAATGTGTT	vATPase, Lysosomal/TFEB
ATP6V0A2	NM_012463.3	4527-4626	GAGCTGAACAGCGTTGACACAGCGCTGACTTACAATTAACCCCTTCAATTACAGT ACAGATTCTGTGTGCTACTTGTATGAGATGTTACCAAGGCCAGT	vATPase
ATP6V0A4	NM_130840.2	566-665	ACCTCCTGAAGAAACCCAAAGACTTTTGGAGCGGAAACCAATTTAGCTGATGAT TTCTTTACTGAGGACACTTCTGGCCCTCTGGAGTTGAAAGCAGT	vATPase
ATP6V0B	NM_004047.3	717-816	GATGGGTGACTAGATGATATGTGTGGGTGGGGCCGTGCCTCATTTTATTTATG CTGGTTTCTCGGGACAGCTGGAGCTGTGCTCCCTAGCCCTTTCAG	vATPase, Lysosomal/TFEB
ATP6V0C	NM_001198569.1	187-286	GGTACCGGCAATGGCGCCATGTCTGTCATGCGCCGGAGCAGATCATGAAGTCC ATCATCCAGTGGTCAATGGCTGCATCATCGCCATCTACGGCCTGG	vATPase, Lysosomal/TFEB
ATP6V0D1	NM_004691.4	971-1070	GAGGACCGATTCTTGAGCAGAGGTTAAAGCTGAACAAGTTGGCCCTCTGAACC AGTTCCACTTTGGTGTCTTCTATGCTTCTGTAAGCTCAAGGAGC	vATPase, Lysosomal/TFEB
ATP6V0D2	NM_152565.1	481-580	ATGGAAGCTGCAACACTTGAAGAGACACCTTCAGATCTCTTAAAGCCATTCTGAT CGAAACGCCATTAGCTCCATTCTTCCAAGACTGCATGCTGAAA	vATPase, Lysosomal/TFEB
ATP6V0E1	NM_003945.3	355-454	GACATGCTCTACAGTCTCAGCTCTTGAAGTCAAGAGAGAATGCTTCTAGA TGCAAAACCACTCCAAACCAAGACCACTTTCTTGACTTGCTGT	vATPase, Lysosomal/TFEB
ATP6V0E2	NR_110612.1	2510-2609	ACGACAGCCTCACTTCTGTATGAGTCTGATGTGGCTGCCATCTGTTTCAGCG GGCATTTGCTTTGGAGCGCAGAGAGAAATAGGATGCCTCTCACTGA	vATPase
ATP6V1A	NM_001690.2	1321-1420	GATCCAGTTACATCTGCCACTCTTGGTATCGTTCAAGTGTCTGGGGCTTAGATAA GAAACTAGCTCAACGTAAGCAATTCCTCCTCTGTCAATTGGCTCA	vATPase, Lysosomal/TFEB
ATP6V1B1	NM_001692.3	736-835	CATGACGACAACCTTCGCCATGCTCTTTCAGCCATGGGGGTGAACATGGAGACA GCCAGATTCTTCAAGTCTGACTTTGAGCAGAAATGGAACCATGGGGA	vATPase
ATP6V1B2	NM_001693.3	406-505	GTTTCCCGAGTTATGCTGAATTTGTCCATTTGACCTTACCGGATGGCAACAAAGAGA AGTGGGCAAGTTCTGGAAGTTAGTGGTTCCAAGGCAGTAGTTTCAG	vATPase, Lysosomal/TFEB
ATP6V1C1	NM_001695.4	5540-5639	AGATAATGCAAGAGAAAAAGCTTTCAACAAATCAGAAGGCAGTCAACAAACA GAAAGGGGGAACATTCTCCTGGCAGTTACTCAAACTGAAAT	vATPase, Lysosomal/TFEB
ATP6V1D	NM_015994.2	391-490	CCAAGTTCACAGCAGGTGACTTCAGCACTACAGTTATCCAAATGTCAATAAAGC GCAAGTGAAGATTTCGAGCGAAGAAAGATAATGTAGCAGGTGTTAC	vATPase, Lysosomal/TFEB
ATP6V1E1	NM_001039367.1	1104-1203	TTTGGTTTGGGCCCTGCAGTCCCACTCTTGAGGCTTAAGGCGCATGTGGCACA CCATCTCTCCAGCAGTAGTCGCTTACTGTTACCTGTTTAGGCC	vATPase, Lysosomal/TFEB
ATP6V1E2	XM_005264633.1	1037-1136	ACAGGAGTCCATCTGACTGAATCCCTAATTTTGGCCTGATTCTTCTCCCTCTGT GAACCATCTCTTAGAAGTCACTGAACCAAGAGTGCCAGAAAAAC	vATPase
ATP6V1F	NM_001198909.1	674-773	AGGTGAGGCGCTTCTAGGTTGCTGGGGCTCTGCTGGTTAAGGAACAGGAAGCCT GACCATCTCCCTCCACTACCTCTTCCCTGTGCTGTTACACAGTGTCT	vATPase
ATP6V1G1	NM_004888.3	1364-1463	TCTAGAGATCCTTGTCTGGGACAGCCTGGGGCAAGTTATTAAAGCATATTTCCCTC CTGGTGGGCTAGATGATGTGATTATACCTGTAGTACCTTCTATA	vATPase, Lysosomal/TFEB
ATP6V1G2	NM_130463.2	921-1020	GAAACATCCTGACTCAGTGTCTGGCCGATGTGGGTCCCTCTTGACCCCTGTCT ACTTGTGGCTGTGAAACCAAGGACAAGCTACTTAATTTGGTAGCC	vATPase
ATP6V1G3	NM_133262.2	73-172	TTTCACAGAGCCACTTGTCTGGAGCAGACTACCATGACAAGCCAGTCTCAGGGG ATCCACCGCTTCTTCAGGCAGAAAAACGGCCCAAGGACAAAGCTA	vATPase
ATP6V1H	NM_213620.1	491-590	CTATGCAAGATGTAGCAAGAACACTGCTGGCCCTACTTTCTGCCAATGTTGAAT GCCAGGATCCCTTCACTGTTCTATATGGCAGCAAGAAATATTGCC	vATPase, Lysosomal/TFEB
B2M	NM_004048.2	26-125	CGGGCATTCTGTAAGCTGACAGCACTTGGGCCGAGATGTCTCGCTCCGTGGCCT TAGCTGTGCTCGCGCTACTCTCTCTTCTGGCCTGGAGGCTATCCA	Housekeeping
B3GAT1	NM_018644.3	2389-2488	GAGGGAGGCCTGAGCAGCACTGCTTTGGAATTAATCTAAACACAAAAAGGAAA GAAATGTTATTTCTCCCTAAGTCAGGAGCATGCGAGAGTAGGCC	Cell Fate/Neurodevelopment
BACE1	NM_012104.3	2401-2500	GCTGGCCAAAGTCAGTAGGAGAGGATGCACAGTTTGTCTATTGCTTTAGAGACAG GACTGTATAAACAAGCCCTAACATTGGTGCAAGAAATGCTCTTG	Neurodegeneration, Endosome
BACE2	NM_012105.3	2276-2375	GGCTCTATGGGTTCTGTTATGCCAAAGTGTCTACATGTGCCACCAATAAAAACA AAACAAGCCTTGGCTCGTCTCTCTCTCTCTCAATCTCTGGA	Neurodegeneration, Endosome
BCL11B	NM_022898.1	3421-3520	GAGATGTAGCACTCATGCTCGCCGAGTCAGCGCCCTTTCTGTGTTGATTTCG GCTTTCATATTACATAAGGAAACCTTGAGTGGTGGTGTGGGGG	Cell Fate/Neurodevelopment
BDNF	NM_170732.4	731-830	CAGGGTGATGCTCAGTAGTCAAGTGCCCTTTGAGGCCTCCTCTCTCTTTCTGCTG GAGGAATACAAAAATACCTAGATGCTGCAACATGTCCATGAGG	Signaling
BECN1	NM_003766.2	811-910	AGCTCAGTATCAGAGAGAATAACAGTGAATTTAAACGACAGCAGCTGGAGCTGGAT GATGAGCTGAAGAGTGTGAAACACAGATGCGTTATGCCAGAGC	Autophagy
BLOC1S1	NM_001487.3	383-482	TGGAGAAGTGGGCTCGGAGCATCGAGCTGGACATGCGCAACATTGCCACTGCACT TGGAATATGTCTACAAAGGGCAGCTGCACTGTGCCCTTCTTACGCC	Lysosomal/TFEB
BLOC1S3	XM_011526952.1	361-460	ACTGAGGCCAGCGGGGAGCAGCTGACACCAAGTCGTTAAGAGAATCAGCGAA GGGGCTGGGAATCCAGGACCTGCGCCTTTTACCACCCGCGGCGCCG	Lysosomal/TFEB
C1ORF85	NM_001256605.1	165-264	GTGCTTGAGTTTACAGCAGCAACCTGTCCGATACGGCAGCAAGCCCTTGGGAA GACCATATCCTCCATACTCTTGGCCGATTCTCTTGGAAACACA	Lysosomal/TFEB
CALB1	NM_004929.2	911-1010	TGGAGGGAAGCTGTACCGAAGGATCTTGCTCTTATTCTCTGTGCTGGGATAAC TAGAGTTGGTGGCCGCAACCACTTGCTAGTGATACACTGTATCTA	Cell Fate/Neurodevelopment
CALB2	NM_001740.2	498-597	CGAACCGCCGCTACGATGAGCCCAAGCTCCAGGAATACACCCAAACCATACTAC GGATGTTTGACTTGAACCGGGATGGCAATTTGGGCCTCTCAGAGAT	Cell Fate/Neurodevelopment
CKK	NM_001174138.1	614-713	AAAATGTGTCTGAAGATTGTCCAGTGCAACACACACCTCACCAGAATTTGTGCA ATGGAAGACAAAATGTTTCTCTCATCTGTGACTCTGGTGTGAA	Cell Fate/Neurodevelopment
CCND1	NM_053056.2	691-790	TTGAACACTTCTCTCCAAAAATGCCAGAGGGGGAGGAGAAACACAGATCATCCG CAACACGGCAGACCTTGTGTGCCCTCTGTGCCACAGATGTGAA	Cell Fate/Neurodevelopment
CD63	NM_001040034.1	351-450	GTCACTATCGCAGTGGGTGCTTCTCTCTTCTGGTGGCTTTTGGGGCTGCTCG GGGCGTCAAGAGGAGCACTATTGCTCTATGATCAGCTTGGCATCT	Lysosomal/TFEB
CD74	NM_001025159.1	965-1064	TTGACCCGCCAGCCCTCCCCATCTCCCAACCTGTACCTCATCCATGAGACCC TGTGCTGCTGCTTCTTCTGACCTTTGGACAAGACAACCAAGTC	Neurodegeneration
CDK5	NM_004935.2	736-835	GCAGTGGCCCTCTATGACCAAGCTGCCAGACTATAAGCCCTATCCGATGTACCCG GCCACAACATCCCTGGTGAACGTCGTGCCAAACTCAATGCCACA	Neurodegeneration
CDK5R1	NM_003885.2	1211-1310	TTTGTGTACAGTATGTGTACGAAAGCCACCAAGGGCCTCACTTTCCACAGT CTCTCCCTGGGTTTTTTTTCATCCCTGCCAAGAACTCTGGGCACT	Neurodegeneration
CHAT	NM_020549.4	1106-1205	TCATTAAATTCGCCGCTCTCAGTGAGGGGGATCTGTTCACTCAGTTGAGAAAGAT AGTCAAATGGCTTCCAACAGAGCAGGCTTTTGCTTCCAATTGG	Cell Fate/Neurodevelopment
CLCN3	NM_001243372.1	1056-1155	GGTACAACCCAGCAAGTCTGTTTGGGGATCTAATGAACAAACATTTGAAGAGAG GGATAAATGTCCACAGTGGAAACATGGGCAGAAATTAATCATAGG	Endosome
CLCN7	NM_001114331.1	996-1095	AACCAGTCTCGACCTGAGGATCTCTTTGCTTCCATGATCTCCACGTTCCACCT GAATTTTGTCTGAGCATTACCACGGGAACATGTGGACCTGT	Endosome

GNS	NM_002076.3	1341-1440	ATGGATGGGATGTCTCTATTGCCCATTTTGAAGGTCGCCAGTAACCTGACCTGGC GATCAGATGCTCGTGGTGAATACCAAGGAGAAGGCCGTAACGCTCA	Lysosomal/TFEB, Lysosomal Storage Disorder
GRIN3A	NM_133445.2	3168-3267	ATGCTGACTGCAAACTTCTCAGTGTGGGGAAGCCATTGCCATAGAAGGATACGG CATTGGCCTCCCAACCACTCTCCATTGACCGCCACATATCCGA	Cell Fate/Neurodevelopment
GRIPAP1	NM_207672.1	86-185	CCTGGAACCTCGGACAACAACTACCAGCTTTTCAGATGAACACGCAAGATGGT GTTGAATCACCAGTCTTCGACAGAAGGTGCGCTACTTGGATAAG	Endosome
GSAP	NM_017439.3	969-1068	ACAGCCCCAAGTGTGCCCTTTGGGGACAATACATATTCAAGTGTTCATCTCAT AAAGGACACAGCAAGACCTTCAACCATCTCTCTGAGAATGTTGG	Neurodegeneration
GSK3A	NM_019884.2	481-580	ACATCAAAAGTATTGGCAATGGCTCATTTGGGGTCGTGTACCAGGCACGGCTGG CAGAGACCAGGAACTAGTGCCATCAAGAAGTTCTCCAGGACAA	Neurodegeneration
GSK3B	NM_002093.2	926-1025	ACTGATTATACCTCTAGTATAGATGTATGGTCTGCTGGCTGTGTGTGGCTGAGCT GTTACTAGGACAACTAATTTCCAGGGGATAGTGGTGTGGATC	Neurodegeneration
GUSB	NM_000181.1	1351-1450	CGGTGCTGATGTGGTCTGTGGCCAACGAGCCTGGTCCCACCTAGAATCTGTCTG GCTACTACTTGAAGATGGTGTATCGCTCACACCAAACTCCTTGGAGCC	Lysosomal/TFEB, Lysosomal Storage Disorder
HES5	NM_001010926.3	1161-1260	CTCCACTATGATCCTTAAAGGATTCCTCTGTGTGGGTGGATGCGTGTGGGCACGA CTTTGACTACAGAAATGAACCTCTCAGTCAGTGGGAAGCCACGGG	Cell Fate/Neurodevelopment
HEXA	NM_000520.4	703-802	CGCTTTCTCCACGGGGCTTGTGTTGGATACATCTGCCATTACCTGCCACTCT CTAGCATCTCGGACACTCTGGATGTCATGGCGTACAATAAATGA	Lysosomal/TFEB, Lysosomal Storage Disorder
HEXB	NM_000521.3	951-1050	CAGATTACGAGGAATTCGAGTCTGCCAGAATTTGATACCCCTGGGCATACACTA CTTTGGGGAAGAGTCAGAAAGACCTCCTGACTCCATGTTACAGT	Lysosomal/TFEB, Lysosomal Storage Disorder
HGS	NM_004712.4	631-730	TGCCACCGCTGCAGGTGCAGTTCGGGGTGTGACCCGTAAGCACCACTGCCGG CGGTGTGGGCAGATATTCTGTGGAAGATGTTCTTCCAAGTACTCCA	Endosome
HGSNAT	NM_152419.2	1255-1354	TGGCTGGGCTTGACATTCCTCCTGCCAGTCCCTGGGTGCCCTACTGTTATCTTG TGCTGGGGCATTGGAAGATTTGGCAAGTATCCAAATTCGACTG	Lysosomal Storage Disorder
HIF1A	NM_001530.2	1986-2085	ATGGATGATGACTTCCAGTTACGTTCTTCGATCAGTTGTCCACATTAGAAAGCAG TTCCGCAAGCCCTGAAAGCGCAAGTCTCAAGCAGACAGTTACAG	Cell Fate
HOXA1	NM_005522.4	1256-1355	CAGATAATTCTGGACACAGACTTGGTGGGGGTTAACACCTTTCATCCGATTGG GTGCCAGCATACATTTCTGGTGGGCTTAACTCCCTCCTGCTT	Cell Fate/Neurodevelopment
HOXA2	NM_006735.3	1081-1180	CCCAAGTTTCCAGCTCTCGCCTTTAACAGCAATGAGAAAATCTGAAACATTTT CAGCACCACTCACCACTGTTCCCACTGCTGTGCAACATGGG	Cell Fate/Neurodevelopment
HOXB1	NM_002144.3	439-538	GGGAACGAGCAGACCGGAGCTTTGCAACGGCCTATGCTGATCTCCTCTCGAG GACAAGGAACACCTGCCCTTCAGAACCTAACACCCACGCGCC	Cell Fate/Neurodevelopment
HOXB13	NM_006361.5	1221-1320	CCACCAGGTTCCCAAGAACCTGGCCCACTCATATCATTCATCTGACAGTGG CAATAATCACGATAACCAAGTACTAGCTGCCATGATCGTTAGCCTC	Cell Fate/Neurodevelopment
HoxB4	NM_024015.4	1892-1991	CCTTTCTCTTCTGACATTCCAAACCGGCCCTTCTACCTCTGGGCTGCTT GAGTCTAGAACCTCTCGTATGTGGAATATCTGTGTGCTGTACA	Cell Fate/Neurodevelopment
HOXB6	NM_018952.4	904-1003	GTTCCAGAACCAGCGATGAAGTGGAAAAGGAGAGCAAACTGCTCAGCGCTC TCAGCTCAGTCCGAGGAGGAGGGAAGAAAACAGGCCGAGTGAAGG	Cell Fate/Neurodevelopment
HPRT1	NM_000194.1	241-340	TGTGATGAAGGAGATGGGAGGOCATCACATTGTAGCCCTCTGTGTGCTCAAGGG GGGCTATAAATTTCTTGTGACCTGCTGGATTACATCAAAAGCACTG	Housekeeping
HPS1	NM_182639.2	894-993	GCACTCCAAGCTGCTGGCATTCTACTCTAGCCACAGTCCGAGCTCCCTGCGCCC GGCCGAGCTGTTGCCCTATCCTCCTGGTTCAGGACCTCTACCCC	Lysosomal/TFEB
HPS3	NM_032383.3	885-984	GATGATTTTGTATCTGCCAGAAGCCCTGGAACTTCTTGGTGA AAAAGTGAAC AGTCTGGATTATCTGTTACACTGGAGTCTACGGGATTAGCTGATG	Lysosomal/TFEB
HPSS	NM_181507.1	981-1080	CTGATATATTGTGCTCGCCAGGCTTAGGATGTGGGAAGTGAACTTTGTAGGAG AAGTTATAAGTACACATCAGTTCAAGAACTCCTCTCGTTGCCAC	Lysosomal/TFEB
HYAL1	NM_153282.1	1511-1610	AGACTGGAATAGTGGCATAGGAGTTAGAACACAGCAGACCACTTATCCAT GTCCATATGCATCTACTTGGCAAGGTATAGACAAATTCCTCCAGA	Lysosomal Storage Disorder
Iba1	NM_032955.1	186-285	ACCTAGAGCTAAAGAAATTAATTGGAGAGGTGTCAGTGGCTCCGGGGAGACGTT CAGCTACCCCTGACTTTCTCAGGATGATGCTGGGCAAGAGATCTGC	Cell Fate/Neurodevelopment
IDS	NM_000202.6	1123-1222	CTCTGTGTCTATTTTGAATACACAGGTGCGGCCCTCTTGAAGTCTTTGACGAT CTTCAGCTGGCCAAACAGCACCATTTGCAATTTACCTCGGATCAT	Lysosomal Storage Disorder
IDUA	NM_000203.3	1781-1880	GTTCTGGTCTGGTGGGATGAACAGTGGGCTCCAAAGTGCCTGTGGACATACGAG ATCCAGTTCTCTCAGGACGGTAAAGCGCTACACCCGCTCAGCAGGA	Lysosomal Storage Disorder
IGF1	NM_000618.3	492-591	CGTGGATGAGTGTCTGCTCCGGAGCTGTGATCTAAGGAGGCTGGAGATGTATTG CGCACCCCTCAAGCTGCCCAAGTCAGCTCGCTCTGTCCGTGCCAG	Signaling
IGF1R	NM_000875.3	10811-10910	TTGTCTACTCTGGGTATCCCTTTGTCTGGGATAAAAAATCAAAACAGAAGCGG GGATGGAAATGGATGCACCGCAAATATGCAATTTCTGAGTTTTTC	Signaling
IGF2	NM_001127598.1	2418-2517	ACACACACTCATCGCAGCACATACATGAACACAGCTCAGCACACAAACACGC AGCACACAGTTTGCACAGCAAGCAACCCACCTGCACACACACATG	Signaling
IGF2R	NM_000876.1	2606-2705	TGCCGGCTGCAACCGATATGATCGGCTTGGCAGATGAAGTATGAAAAGATCA GGGCTCCTTCACTGAAGTGGTTTCCATCAGTAACCTGGGAATGGC	Endosome, Lysosomal/TFEB
Isl1	NM_002202.2	1376-1475	CTTACAGGCTAACCCAGTGAAGATGACAAAGTACCAGCCACCTTGGAAAAGTCTG AGCGACTTCGCCCTTGCAGAGTGCATAGATCAGCCTGCTTTTCAG	Cell Fate/Neurodevelopment
KIAA0226	NM_001145642.2	6521-6620	GAGCGAACCACTTCACTTCTCGGGTTTCTGGTGCATGGTGTAACTGTCACTTAA ATGAGGTGTTCTCCTCGCCGCAACATCTGACTTGCTTATAGTGC	Autophagy
KLF4	NM_004235.4	1981-2080	CGAGCATTTTCCAGTTCGGACCACTCGCCTTACACATGAAGAGGCATTTTAA TCCAGACAGATGGATATGACCCCACTGCCAAGAAGAAATTCAGT	Cell Fate/Neurodevelopment
KMT2D	NM_003482.3	6071-6170	CCAACATTAATTTTCTTAATCTAAGCAAGACTACCCAGACTGGTCAAGCGTTGC AAACAATCATGAAGCTCTGGAGAAAGTTCCAGCAGCTGACAA	Lysosomal Storage Disorder
LAMP1	NM_005561.3	2071-2170	ATTTAAGCCTTACAAAGGGAAGCCTCTGGCGCTCACAGTAGGACGATGAAGG TCACCTGTTGGTGAAGGCTGACATGCTCACACATTAACACAGTAGAG	Lysosomal/TFEB
LAMP2	NM_002294.2	381-480	AGACCATTGGCATGTGACATATAATGGAAGCATTTGTGGGGATGATCAGAATGGT CCCAAAATAGCAGTGCAGTTCGGACCTGGCTTTTCTGGAATGCG	Lysosomal Storage Disorder
LHX6	NM_001242333.1	291-390	AAGCCTTTGACTGGGAAGCAGTGCCCAAGAAATTTTTCATTACAACAGAGTTAT CCTCCACATACAAATGTGAAGGATGCTTGTGCCACTGACAT	Cell Fate/Neurodevelopment
LIPA	NM_000235.3	1221-1320	GAGAGCTGCGCGAGCCTGGACCCCTCAAGCCCTTATCATTTTCTCCAACGCC CTCCACATACAAATGTGAAGGATGCTTGTGCCACTGACAT	Lysosomal Storage Disorder
LRP1	NM_002332.2	4241-4340	GGAGGATTTGGGCAGAAGCTGAGGTTTTGCCATCCACAATCCCTCTACAGGGCC TGGCTCACAAAAAGAGTGCAACAAATGCTCTATTCCATAGCTAC	Neurodegeneration
LRP10	NM_014045.3	3101-3200	GCCAGGAGGCTTCCGCTCTGTGGCTCCGCCATCCCACCTGCTTCATCTCTG CCCGGACCATGCTGGAATTTTCTCACTCAGAGGCTCCAGCTC	Endosome
LRP3	NM_002333.3	431-530	ATAGGGCTGTGAAACAGAGGCTTTTGATTTGTCTTGTGACATCAGAAGTTACAA TAGGCATATGGGCAAAATGGTGTAGCAGGCTCACTGGCGTTTG	Endosome, Lysosomal/TFEB, Endosome
M6PR	NM_001207024.1	1079-1178	ACCACACTGTGATGACCATGGGCTCGGACTTCCAATATGAGAATGCCAACATGTG GTTCAAGAACCTTGACAAAGCTCATCCGGCTGGTAAATGCGCAGCA	Lysosomal Storage Disorder
MAN2B1	NM_000528.3	1005-1104	CAAGCCTGTGCCGGGGTTCCTGGAATCTGTGCCAGGACTGTGTTTATGCCCTT CACCTCTCAGCTTTAGCAGGACATGACCAAGTTATAACAAAGATGG	Lysosomal Storage Disorder
MANBA	NM_005908.3	2940-3039	ACCAGCAGCATGGTGAAGTGTGCCAGGCCATCGCGACATCTACGAGCAGG AGAAAGACGAGACGGCTTCTCTATATGGTCTACGCTCCAGGA	Autophagy
MAP1LC3A	NM_032514.2	405-504	AGAGCCGTACGCTCTTACAGATACTAATGTCAAGAGTTAAACCTCCTCAGGTTCA ACCTGTGATAAAAGACTAGTCTCCAGTACTTGCATGGGGTT	Autophagy
MAP2	NM_031845.2	5171-5270	TACTCTGTATGCTGGGATCCGAGGTTCCAAACACACTGTTACAAATCTGTGGGG GTTTCTTCTCTGATAATTTCTAGAGCCTGTTACCATAGAAAGGC	Cell Fate/Neurodevelopment
MAP4	NM_030884.3	1884-1983	AGAAGTCAAGAAAGTACTGCAAGTCTGTACCAGCTGACTTGAGTCGCCCAAG AGCACCTCCACCTTTCATGAAGAAACCACTCTCAGTGGG	Neurodegeneration
MAPK1	NM_138957.2	431-530	ACTGCCAGAGAACCTGAGGGAGATAAAATCTTACTGCGCTTCAGACATGAGAA CATCATTGGAATCAATGACATTTATTCGAGCACCACCATCGAGCA	Signaling
MAPK3	NM_001040056.1	581-680	AACGTGCTCCACGAGATCTAAAGCCCTCAACCTGCTCATCAACACCCTGCG ACCTTAAGATTGTGATTTGCGCCTGGCCGGATTGCGGATCCTG	Signaling

MAPT	NM_016841.4	1511-1610	ACTTAACCTGCTTTGTGCTACTGGGCTTTGGCTCGGGACTTCAAATCAGTGATGG GAGTAAGAGCAAATTTTCATCTTTTCCAAATTTGATGGTGGGCTAGT	Cell Fate/Neurodevelopment, Neurodegeneration
MARK1	NM_018650.2	1871-1970	GTGAACAGAAAGAGGAGTGGGACAAAGATGTGGCTCGAAACTTGGCAGCACAA CAGTTGGATCAAAAGCGAGATGACTGCAAGCCCTCTGTAGGGCC	Neurodegeneration
MARK2	NM_017490.2	496-595	ATTTTGCCAAAGGTGAAGTTGGCCCGACACATCCTGACTGGGAAAGAGGTAGCTGT GAAGATCATTGACAAAGCTCAACTGAATCCTCCAGCCTCCAGAA	Neurodegeneration
MARK3	NM_002376.4	2071-2170	TCCTAGTAGTAACACAGCATCTGGTGGAAATGACACGACGAAATCTATGTTTGC AGTGAGAGAACTACAGCTGATAGACACTCAGTGATTGAGAATGG	Neurodegeneration
MBP	NM_002385.2	133-232	GTACCTGGCCACAGCAAGTACCATTGGACCATGCCAGGCATGGCTTCCTCCCAAG GCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGGC	Cell Fate/Neurodevelopment
MCOLN1	NM_020533.2	1486-1585	CCCTATCATGTGAAGTTCGCTCACTCTCCATGGTGTCTGAGTGCCTGTCTCGC TCATCAATGGGACGACATGTTTGTGACGTTGCGCGCCATGCAAG	Lysosomal/TFEB, Lysosomal Storage Disorder
MFSD8	NM_152778.2	1569-1668	CTATGTTTCATCAGCCAGTGTATGCTCACTGGGACCCAGATGGGCATTCAGCCT GGTGTGTGAATAATAGTGCTCACCATGACCCCTCTGGGAGTGGT	Lysosomal Storage Disorder
MLST8	NM_001199173.1	811-910	GGCTATCCACATCTGGGACTTGAAACAGACCCACAACGAGCAGCTGATCCCTGAG CCCGAGGTCTCCATCAGCTCCGCCACATCGATCCCGACGCCAGC	Signaling, Autophagy
MNX1	NM_005515.3	1681-1780	CCTGGGCGCTTCCCTTTAAGCAAGGGCGCCTCAGCTGCTCTTCAAGAAACAGCG AGAGGGAGACCCAGGGGGCTGAAGACTTGAACCTCTGGTCTTTTAA	Cell Fate/Neurodevelopment
MTOR	NM_004958.3	1866-1965	TCACCTTTCGCTCCGAAGCTTGGCAGCTTTGAATTTGAAGGCCACTCTCTGAC CCAATTTGTTGCGCACTGTGCGGATCATTTCTCGAACAGTGAGCA	Signaling
MYOD1	NM_002478.4	1636-1735	TGTAATCTATTCTGTAAATGAAGTTGCTTTGCCAGCAGGAGCCCTGGGGC TGATTTATCTCTGAGGCTGTTGTGTGGTGTCTACAGGGAATTTG	Cell Fate/Neurodevelopment
NAE1	NM_001018159.1	849-948	GGTATAGTGAACAAATGGACGAATACCTAAACGATATAAGAAAGAGGAGCTT CAGAGATTTGATTAGACAGGAATCTAAAAATGAAGTGGGGC	Neurodegeneration
NAGA	NM_000262.2	2246-2345	AAAGGAGACCTTTGGCTCCCTCAGGTCACCAATAAACCTGTTCTTTAATCAAGTGT TGTAATATGGACCTGGGGTACCTTTAGTTAGGGAACCTGGTGGC	Lysosomal Storage Disorder
NAGLU	NM_000263.3	697-796	GGAGCTGACCGAGGCCACGCCAACAGGTACCGCTATTACCAAGATGTGTCAC GCAAAGCTACTCTTTCGTGTGGTGGGACTGGGCCCGCTGGGAGCGA	Lysosomal/TFEB, Lysosomal Storage Disorder
NAGPA	NM_016256.3	829-928	TGGGAAATGGCGGAGTTCTCCTGCTGAACAGGACGTGGTCAACGCCATCAACCTG GATGGGGGTGGCTCTGCCACCTTTGTGCTCAACGGGACCTTGGCCA	Lysosomal/TFEB
nanog	NM_024865.2	1101-1200	CTACTCCATGAACATGACAACTGAAGACGTGAAGATGAGTGAAGTGAATTA CTCAATTTCAAGCTGGACACTGGCTGAATCCTTCTCTCCCTCC	Cell Fate/Neurodevelopment
NBR1	NM_031858.2	1547-1646	CCTGTACCTCGTTATGCAATGCTCAGTGCAGCATTTGTGGATGAGAATTTGCCT GATGGGACTCACCTTCAGCCAGGAACCAAGTTTATCAACACTG	Autophagy
NCAM	NM_000615.5	1621-1720	GGTATTTGCCATTCACAGTGCCAGATCTCATGGTTTCGGGATGGCAGCTGCTG CCAAGCTCCAATTACAGCAATATCAAGATCTACAACACCCCTCT	Cell Fate/Neurodevelopment
NCSTN	NM_015331.2	541-640	GTGCCCAATGATGGGTTTGGTGTTTACTCCAATTCCTATGGGCCAGAGTTTGCT CACTGCAGAGAAATCAGTGAATTCGCTGGGCAATGGTTGGCT	Neurodegeneration
NECAP2	NM_018090.4	991-1090	CTCTCTCTCTCCTTGTCTGGCTGTTGACAAACCGGGCATGTTTGGCAGTAA ATTGGACCGGTGTCACACTGTTTCTCGGGATTCAGATATGCAACC	Endosome
NEDD4	NM_006154.1	751-850	CCCTCAGGACAACTAACAGATGCTGAGAATGGCAACATTCACCTGCAAGCACA CGTGCAATTTACCAACAGCGCGAGATATCCGAGGAAACAGAAAGT	Endosome
NES	NM_006617.1	2346-2445	CAGAGATCACAAATCAGTGAGGTCTTGAAGAACAGGACCAAGAGACATTGAG AACTCTTGAAGAAAGAGACTCAACAGCGACGGAGGTCTCTAGGGGA	Cell Fate/Neurodevelopment
NEU1	NM_000434.3	1509-1608	CTTTAGACTCCAGCCTTGGCAAAATCACCTTCCCTTTACAGGGAAATCACTTCT TTAGGACTGAAGCTAGGCGCTCTCTCCCAAAAAAAGTCTCGT	Lysosomal/TFEB, Lysosomal Storage Disorder
NGF	NM_002506.2	635-734	GAGGTGATGGTGTGGGAGAGGTGAACATTAAACACAGTGATTCAACACAGTACT TTTTTGAGACCAAGTCCGGGACCCAAATCCCGTTGACAGCGGT	Signaling
NGFR	NM_002507.3	2731-2830	TGAAGAAAGTGGGCCAGTGTGGGAATCGGCCAAGAAAGGAATGACTTCGACTG TGACCTTGGGGATTCTCCAGCTCTAGACAACTGCAAAAGGAC	Signaling
Nkx2-1	NM_003317.3	2012-2111	GCGCCATAGGCTCAGCGGGACCGCCCTCCGCGAAATAGTTTGTATATGTGAA CTTGAGCTGTAAACCGCTGTCAAAGTTGGACTAAATGCCTAGT	Cell Fate/Neurodevelopment
NMDA NR1	NM_057175.3	4146-4245	GTGGAAACAGAGGTGCAAGCCAGAGGCCAATGAATATGCTGTGAAGGCTAGTGCA GATGGGAGCTTTTGAAGGGGCTAAGTCTGCTGTGTGTCAGGAAAT	Cell Fate/Neurodevelopment
NPC1	NM_000271.3	3301-3400	GACCTCTGCTGACTTTATTGACGCTTGAAGAAAGCCGACTTATAGCCAGTAA GTCACCGAAACCTAGGCGCTTAAACGGCATAGTGCCTACCGAGTATT	Lysosomal Storage Disorder
NPC2	NM_006432.3	194-293	TGTGGATGGAGTATAAAGGAAGTGAATGTGAGCCCATGCCACCCACCCCTGCG CAGCTGAGCAAAAGGACATCTAACGGCTCAATGTACACCTTCACG	Lysosomal Storage Disorder
NPY	NM_000905.2	271-370	AGAGATATGGAACAGTCCAGCCAGAGCACTGATTTCAGACCTCTTGATGAG AGAAAGCACAGAAATGTTCCAGAACTCGGCTGAAGACCCCTGC	Cell Fate/Neurodevelopment
NRBF2	NM_030759.3	1236-1335	CTTGGGAAACAGTCACTGTGAATGCGCTGCGTATCTCATTCACCTCACTCAGCTA ATGATTCGAGCTTGGCAGACGCTAAACTCATGGAGGTTGCGTTT	Autophagy
NTF3	NM_002527.4	152-251	AAGGAGTTTGCCAGAAGACTCGCTCAATTCCTCATTTAAGCTGATCCAGGCA GATATTTGAAAAAACAGCTCTCCAAAGCAGATGGTGGACGTTAAG	Signaling
NTF4	NM_006179.4	900-999	CTCAATCTCGAGGATGGGTGAACCCAGTGGGGTTTGGAGGTTGAATAGGAG TTCTCTGGAGCAACTTGAGGGTAATATGATGATGATATAATAA	Signaling
NTRK1	NM_001012331.1	1366-1465	GACGGAGAAACAAGTTTGGGATCAACCGCCCGGCTGTGCTGGCTCCAGAGGATG GGCTGGCCATGTCCCTGCAATTCATGACATTGGGTGGCAGCTCCCT	Signaling
NTRK2	NM_001007097.1	1606-1705	TCACATGAACATGGGGACTACACTCTAATAGCCAAGATGAGTATGGGAAGGAT GAGAAACAGATTTCTGCTCACTTCATGGGCTGGCCTGGAATTGAC	Signaling
NTRK3	NM_001007156.1	553-652	TATATAACCTGTCAAGTAACCGGCTCACCACTCTCTGGCAGCTCTTCCAGA CGCTGAGCTTCGGGAATTGCAAGTTGGAGCAGAACTTTTCAACT	Signaling
OLIG2	NM_005806.2	1691-1790	TAGTTGGAAGCCGGCTTCGGTATCAGAAGCGCTGATGGTCATATCCAATCTCAA TATCTGGGTCAATCCACCCCTCTAGAAGTGTGGCCGTTCTCTCC	Cell Fate/Neurodevelopment
OTX2	NM_172337.1	1086-1185	GGCTGGACATTCAGTTTATGCCAGGCAATGGTTAAAGAGTTAGATGGGATGAT GCTCAGACTCATGTCAAGAGTCCGAGAGGCATAGAAGGAAAA	Cell Fate/Neurodevelopment
Pax6	NM_000280.3	1174-1273	TTGGACTGTGATATAAACAAGTTTACCGATCTTGACCTGCAGCATGAGCTAAAGA AACTGGCGGGTTTGGCTAGAGATTTTACAGTACTTGTGGATC	Cell Fate/Neurodevelopment
PEX1	NM_000466.2	2365-2464	ATAGTCTTATAGTTATGGATATAATGGGCTGGGCTACAACCGCTCCGTGTAGA TGATCTTCCACCAGTAGATTTGTTGACGAACTGAAGAAAGCAG	Lysosomal Storage Disorder
PEX13	NM_002618.3	361-460	CAGTTACCTGCTGGCAGGTGATTCGCCGATTCGCACGAGCTGTGAGAGCTGGT GTACTCTGCTCTAACCTGCTTGTGCTGCTCAATGACGGGATCCTA	Lysosomal Storage Disorder
PEX16	NM_004813.2	408-507	CTCTTGCTTCAGACAGACCATCTGAAATCTCTAAAGGAAAGGGCTTTTGATC TAATCAAAAGAGTTGAAGAGAGGCGCTAGGATTCCTCTCTC	Lysosomal Storage Disorder
PEX19	NM_001193644.1	1376-1475	AGAACGGGACGAGCGGCGCTTGTGCTGGTCTCTGGGCCAGGTGACGAAGAAAC AGTTTCTGGTGAAGAGCTCCCTCAACCCCTAGTCAGCCACACCCCT	Lysosomal Storage Disorder
PEX3	NM_003630.2	151-250	TGGCAGCTGTGCGGCTGGACCTCCTCCTGACCTGATGTGAGTGTGGCTT GGGAGTCTTTTCAACCTGAGTGGGAGTATGACAAAGGCGTGGAA	Lysosomal Storage Disorder
PEX5	NM_001131024.1	1397-1496	CGGAAGGCAATTCCTGCCATTTGCCAGTGGCCATGGCCACTACATTGACGTGA AGGGCACCAGTCAAGAAACATCGAAGGAAGTTGTAGGAATAG	Lysosomal Storage Disorder
PHYH	NM_001037537.1	1016-1115	TTGCCAACTCCACCTAGCAAGTATGATCTGATGACTTGGATTCTCTTTAGCC AACCTTGTGGCAATCTTGGCATCGGAAATGGAACCACTAAGAA	Neurodegeneration
PIK3R4	NM_014602.1	3621-3720	GGGAAGACCACCACTACAGATCTATTCTTACATACAGCCGAATTTGGAGGACGAG TCAAGACGCTCACATTCGCCAAGGCTCCCACTATTTAGCCATAG	Autophagy
PLBD2	NM_173542.3	1305-1404	GCCAGCTACACATACCGTCTCTGAGACTGTGTTCAATGCCAGTGGGCTGCAGG CCCTAGTGGCCAGTATGGGGAAGTGGTTTCTTATGACGGGAGCC	Autophagy
POLR2A	NM_000937.2	3776-3875	TTCCAAGAGCCAAAGACTCTTCCGCTTACTGTCTTCTGTTGGGCCAGTCCGCT CGAGATGCTGAGAGAGCCAAAGGATATCTGTGCGCTCTGGAGCAT	Housekeeping
POU3F2	NM_005604.2	3491-3590	GGGCACGGAGCTGCTCGGGTGCATCAGCTGCTGCTTCTGAGGTATGGGAAC TGGCCTTATGTAAGCTATCCAGAGCAGGCAAAATGCCACTGGTA	Cell Fate/Neurodevelopment

SLC9A7	NM_032591.1	380-479	TGGCCGTGACAACTCACTCAGCTGCACTCAGGAAGACAGGGCCTTCAGTACCTTA TTAGTGAATGTCAGCGGAAGTCTTCGAAATACACTCTGAAAGGA	Endosome
SLC9A8	NM_015266.1	2526-2625	GTCCCTTACCTGATTGGCACTTCGCAGTCTATCTCCCTGGGTAGCAGACGGCTGC TGCCCTTCTCTGGGCATGTTCTGAATGTTTACACTGGTACCTTCT	Endosome
SLC9A9	NM_173653.3	1103-1202	GTGACCAAATTTACCAAGCTGTGTAGTTCGCCAGTGTGGAACCGGCTGTTT TTCTGCTTTCTGGAGTGCCCTTCGTCTGCCGAGGCTGCCGGC	Endosome
SMPD1	NM_000543.4	543-642	AAGCTGTGCAATCTGCTGAAGATAGCACCACCTGCCGTGTGCCAATCCATTGTCC ACCTCTTTGAGGATGACATGGTGGAGGTGTGGAGACGCTCAGTGC	Lysosomal Storage Disorder
SNCA	NM_000345.2	141-240	AGACAAAAGAGGGTGTCTCTATGTAGGCTCCAAAACCAAGGAGGGAGTGGTGC ATGGTGTGGCAACAGTGGCTGAGAAGACCAAGAGCAAGTACAAA	Neurodegeneration
SORL1	NM_003105.5	4799-4898	AAGTACAGAATCTTCAGTGGACAGCTGACTTCTCTGGGGATGTGACTTTGACCTG GATGAGGCCCAAAAAATGCCCTCTGCTTCTGTGTATATAATGT	Endosome
SORT1	NM_002959.5	381-480	CAGTATCCTTGTCTGGGTTGGAGATAGCACTGGGGTCACTTCTAGCTTGACTAC CTTCCATGTACCAGTGGTAATTATGACTTTTGGACAGTCCAAGCT	Endosome
Sox1	NM_005986.2	1496-1595	TATTTACACCTACGGAGGAAGCGGAAGCGTTTTCTTTGCTCGAGGGGACAAA AAGTCAAAACAGAGCGAGAGGGCGAAGCCCACTTTTGTAATACCGGC	Cell Fate/Neurodevelopment
Sox10	NM_006941.3	2160-2259	GGCCGTGTCTCCCACTCAGGGGCTGAGAGTAGCTTTTGGAGAGCTCATTGGGGA GTGGGGGGTTCGAGGGACTTAGTGGAGTTCTCATCCCTTCAATGCC	Cell Fate/Neurodevelopment
Sox2	NM_003106.2	152-251	CTTAAGCCTTTCCAAAAAATAATAAACAATCATCGGCGGGCGGACAGTCCGCC AGAGGAGGAGGGAAGCGCTTTTTTGTAGCTCTGATTCCAGTTTGCC	Cell Fate/Neurodevelopment
SQSTM1	NM_001142298.1	215-314	ATTAATCTCTCATGGCCGCTGCACAAGAACCTGGCTTTTAGCTGAACTAAGGAGA AAGTCTCTACACAGTTTGGCGTGCACATAGGGGCTTGAGAAAGGA	Autophagy
STH	NM_001007532.2	93-192	GCCGGCCCTCATTTGAATGCGGGTTAATTTAACTCAGCCTCTGTGTGAGTGGATG ATTGAGTTGCCAGAGACAGAACCTCAGCTAGCATGGGAAGTA	Neurodegeneration
STK4	NM_006282.2	611-710	TTACAGATACCATGGCCAAAGCGGAATACAGTGATAGGAACACCATTTTGGATGGC TCCAGAAGTGATTTCAGGAATTTGGATACAACGTGTGTAGCAGACAT	Signaling
STX13	NM_177424.2	946-1045	AAACGAAGTGATTGCCTCCGATGTTCTCCCGCTGAGCTGTTTCAAGGGCAAGT GCTTGTGTAAGTCTTGCCAGAACCAACTGATCACAAGAAAGACAGC	Endosome
STX6	NM_001286210.1	769-868	GAACAGGACGATTATGTTGGAAGATTCTCTCAGCAATTGGAGAGCACTCAGTCCC GGCTGGCAATGTGATGAAGAAGTCTGCAAAAGTATCTCATATGA	Endosome
SUMF1	NM_001164674.1	799-898	GCTGTGTATGCTCTCCCTCCCAATGGTTATGGCTTATACACATAGTGGGGAAC GCATGGGAATGGAGCTCAGACCTGGTGGACTGTTCACTATTCTGTT	Lysosomal/TFEB, Lysosomal Storage Disorder
SYN1	NM_006950.3	566-665	CCGAATCTCTGATCTCAACCTTGTGGCCATGGCAATGGTGGATTCTCTGTGGA TATGGAAGTCTTCGGAATGGGTGAAGGTCGTGGCGTCTCTGAA	Cell Fate/Neurodevelopment
YYP	NM_003179.2	2266-2365	TAGTCCCTGTGATCGTGTGGTTCGCTTTGTCTGGCTGTGGCCCTCTCTCTCCC CTCCAGACCCCTACCCCTTTCCCAACCCCTCGGTATTGTTCAAAG	Cell Fate/Neurodevelopment
TACE/ADAM17	NM_003183.4	1596-1695	AGAAGCGAGCAATAAAGTTTGTGGGAAGCTGAGGGTGGATGAAGGAGAAGAGTG TGATCTGGCATCATGTATCTGAACACGACACCTGCTGCAACAGC	Neurodegeneration
Tau_3R	NM_016835.4	2051-2150	ATCGGCTCCACTGAGAAGCTGAAGCACCAGCGGGGAGGGGGAAGGTGCAGATA ATTAATAAGAAAGTGGATCTTAGCAACGTCCAGTCCAAGTGTGGCT	Neurodegeneration
Tau_4R	NM_016841.4	921-1020	CCAGATCGGGTCCACTGAGAAGCTGAAGCACCAGCGGGGAGGGGGAAGGTG CAAAATAGTCTACAAACCAAGTTCGACCTGAGCAAGGTGACCTCCAAGTG	Neurodegeneration
Tbr1	NM_006593.2	2476-2575	GCCGTCTGCAGCGAATAAGTGCAGGTCTCCGAGCGTGATTTTAACTTTTTTGCA CAGCAGTCTGTGCAATTAGCTCACCAGACCTTCAACTTTGCTGTAA	Cell Fate/Neurodevelopment
TCF4	NM_001083962.1	3136-3235	TTAGGGGAAGCTCGGCTGCCCTAGTAACAAAACAGCAACCTCTGATGACAAC GAAGTGATGACATTAGCCATTCTTAGGGTAGGAGGAACAGATGG	Lysosomal Storage Disorder
TFE3	NM_006521.3	2936-3035	GGTATTAGTGGGGGACTGAGTTGAGTTTACCAGAACAGTACCTCAGTATCTTT TTCAACATGTAGGGCAAGAGGATGAAGGAAGGGGCTATCTGGGA	TFEB
TFEB	NM_007162.2	2027-2126	GGGCCCTGGTGAGATTCCCTGTCTTCTGAGCCCGAGCCCTCATTACAGTGAA GGACATGCTGAGGGGTTTCGGGAAGTCTCCTCATCTGAGGCAACTGG	Lysosomal/TFEB
TFRC	NM_003234.1	1221-1320	CAGTTTCCACCATCTCGGTCTCAGGATTGCCTAATATACCTGTCCAGACAATCTC CAGAGCTGCTGCAGAAAAGCTGTTTGGGAATATGGAAGGAGACT	Endosome
TH	NM_000360.3	1307-1406	GTCTACTCTGCTGTGAGAGCTTCAGTGACGCCAAGGACAAAGCTCAGGAGCTATG CCTCAGCATCGCAGAACGCCCTTCTCCGTGAAGTTCCAGCCGTACA	Cell Fate/Neurodevelopment
TJP1	NM_003257.3	6278-6377	CACATTTTCTTAGGGAAGGATACAAAAGCATGTGAGACTGGTTCATGGCCCTCTT CAGATCTCTAATCTACCATATTACACAGACATACTAACCAGC	Cell Fate/Neurodevelopment
TMEM55B	NM_144568.2	1189-1288	TTTCTCCAAAGGGTGTCTGTGTCTAGGGTGAAGTAGGCAAAACGTTGCCCTTAA ACTGGGCCCTGAAGACGGTTCCAGCCTTGTCTTCTGTGTGCTC	Lysosomal
TMEM74	NM_153015.1	1925-2024	TGGTGTGCTTCCAGGTAAACATCTTGTATTGCTGTTTGGGGAACACCAATAGA ATTGCAGCTAGACTTTTGGGAGCTGTGCCCTCCAGGCTGTATCA	Autophagy
TP73	NM_005427.2	2397-2496	GTCTTCCAAAGGAAGGATCTCTTTGCTGTGAGTGGACTGCCAAAAGTATTTGCA GACATCTTTTGGTTCTGGATAGTAGTGAGGAGCCCAAGTGACTGTG	Cell Fate/Neurodevelopment
TPP1	NM_000391.3	747-846	AGTTCTGGAGCAGTATTTCCATGACTCAGACCTGGCTCAGTTTCATGCGCCTCTT CGGTGGCACTTTGCACATCAGGCATCAGTAGCCCGTGTGGTTGG	Lysosomal Storage Disorder
TTBK1	NM_032538.1	2297-2396	ATGGCCTCCACAGAGCTGTGCCCTGAGTCTGCCCTACAGGACTTCAAAGAGA CCTCTCCGATTACCGAAGACGGGCGGGTGTCTCAACAGGGTCCG	Neurodegeneration
TTBK2	NM_173500.2	2601-2700	TTGCCAATGCTACTCCCATCCCTGGAGACTTGTCTCGAGAAAATACAGATGAGGT ATTTCCAGATGAACAGCTTAGCGATTGGAAGAAATGGCATCCCTGT	Neurodegeneration
TUBB3	NM_006086.2	1538-1637	GCGGCCCTCTGACAGTATTATGGCTCTGCTCCCTCCACCTAGGCCACGTGTGAG CTGCTCTGTCTGTCTTATTGACAGCTCCAGGCTGACGTTTTTA	Cell Fate/Neurodevelopment
TWIST1	NM_000474.3	394-493	GACAGCCTGAGCAACAGCGAGGAAGAGCCAGACCGGCAGCAGCCGCCGAGCGG CAAGCGCGGGGAGCGAAGCGCGCAGCAGCAGCGCAGCGCGGGCG	Cell Fate/Neurodevelopment
ULK1	NM_003565.1	466-565	CCTGAAGGAATGAAACATGAAACATCGTGGCCCTGTACGACTTCCAGGAAATG GCTAATCTGTCTACCTGGTTATGAGTACTGCAACGCGTGGGGAC	Autophagy
ULK2	NM_014683.2	2341-2440	AAGCATCTTCCAACTGTGAGCTTGTGCTTACTGCTATGGGCTGCTGAAGAACAA GTCCGAAAGATGGGAATGAGCCACGGGAATGTGCCATTGCTCTT	Autophagy
ULK3	NM_001099436.1	196-295	GGCCTACGCCAAGAGGACACTCGTGAAGTGGTAGCCATAAGTGTGTAGCCAA GAAAGTCTGAACAAGGCATCGGTGGAGAACCTCTCACGGAGATT	Autophagy
ULK4	NM_017886.2	2625-2724	TCACATTGTGCAGGAGCTGCCAGCAATCTGGGTGACATCTTAACTCCTTGGCT AATGTTTCTGGAGCTAAACACCATCAACAGTTCAAGTGAACAG	Autophagy
USP10	NM_005153.2	1921-2020	CCAGCGGATTTTGTTCAGACTCCAATCACCAGGCTTTTGGTGGACACATCAGG TCTGTGGTTACACGAGAGTTCAAAAGAACTGCCACTTTCAG	Autophagy
USP13	NM_003940.1	2451-2550	CAAACTATTCTGAGGCCAAGCCGGAAGGACCTAGAGTCAAGGTGGATCTGG AACATATGAGCTATTGCTTATCATGCTCAGATGGGAACATCCAC	Autophagy
UVRAG	NM_003369.3	1259-1358	TTTGATGCGGTGTCAAGTTGCCTAATCTGAGGACTTCCAAAGCAAAAGATGATG GAAGCATTTGCTTGGCCCTGGTTATACGTGCACATCTGGTCTCCA	Endosome
VAMP1	NM_199245.1	2731-2830	TCCTGGGTTCTGTCCCTCTGAGGCTCACCAGGTGTAGTTGGCCTTGTCTTTGG GGGTATTAGCCCTTACTTCTTCTTCTCACCCACCCCTGTACCCCTC	Endosome
VIM	NM_003380.2	695-794	GAGGAGATGCTTCAGAGAGGAAGCGGAACACCCCTGCAATCTTTCAGACAG GATGTTGACAATGCGTCTTGGCAGCTTGGACCTTGAACGCAAGG	Cell Fate/Neurodevelopment
VLDLR	NM_003383.3	2911-3010	GAAAACCACTGAAGAGGACCTCTCCATAGACATTGGTAGACACAGTGCTTCTGT GGACACACGTACCCAGCAATATCAGTTGTAAGCACAGATGATGAT	Neurodegeneration
VPS11	NM_001290185.1	2239-2338	TTTCCCTGCTGAAGAGTGGTGGCTTCTGCGAGCTCTTTCGAAAGGCCCTGGTCT GTGCCAGATGACAGACTTCCAGGATGGTGTCTTACCTTTATGA	Endosome
VPS18	NM_020857.2	2671-2770	GCTAGCTGCCCTTGTCTCAAGATTGAGGATGTGCTGCCCTTCTTCTGATTTCGT CACCATCGACACTTCAAGGAGGCGATCTGCAGCTCACTTAAGG	Endosome
VPS26A	NM_004896.3	2419-2518	TGGCACTTTGATAAAATGGTCAGGAACCACTTTACTGGCAAAAGGGTCCATGTA CCACCATGTGCTGGAGCATCTGTTCTACATGTGGATATCTATGAA	Endosome
VPS33A	NM_022916.4	1746-1845	ACTCTGATATTTTCTTGGGGGCGTAACCTCTGCTGAAATGCTGCCCTGCGATT TCTCTCCAGTTTGAAGATGGAGGTACAGAAATATGTCATTGCCA	Endosome
VPS35	NM_018206.4	1601-1700	AGGACCCCTGACCAGCAGTACTTGAATTTGAACACAGCAGGAAACATTTGGAGC TGGTGGAAATCAGCGGATTCGCTTCACTGCCCACCTTTGGTATT	Endosome

VPS8	NM_001009921.2	2689-2788	CGGCAGCTTGCAAAGCCTGACAACACCTTGTGGTAAACAGAACACTTTTGTATCA GGTCCTTGAATTCCTTTGTAGTCCTGACGATGACTCCCGACACT	Endosome
WDFY2	NM_052950.3	859-958	TGACCACTCAGGCCAAGTAACAATCCTCAAACCTGGAGCAAGAAACTGCACCCCTG GTCACAACATTCAGAGGACACACAGGTGGGGTGACCGCTCTCTGT	Endosome
WDFY3	NM_178583.2	2146-2245	GCTGGGAGAAAGTGAACCAAGTCAAGTGTGGTAACTTCTTCACACTGTGTTCTG CACGTTGACTGCAGCAATGCGCTATGAGCCAGCCAACTCTCATTT	Autophagy
WDR45	NM_007075.3	1391-1490	TCCAAGAACGTCAACTCTGTCAATTGCCATCTGCGTAGATGGGACCTTCCACAAAT ATGTCCTTCACTCCTGATGGAAACTGCAACAGAGAGGCTTTCGACG	Autophagy
WDR45L	NM_019613.3	1331-1430	CTGCCCAGGGACCTTGGTCTCGAAGCCATACGTGGTTGTCTGCTTTCCTAAGGAC TCCATTTCAGTATTAAAGAGAGAATCATCAAGGCACCGTA	Autophagy
WIPI1	NM_017983.5	1326-1425	CTGAACATGAGTTTGGCAGCGGACCAAGTGTGCTTGATGATGAGAATGAGTTTCC TCCTATAATCTTGTGCCGTGGAAATCAGAAAGGGCAAAACGAAGCA	Autophagy
WIPI2	NM_001033520.1	3216-3315	GCTAGGGTTTCCTGTCAACAAAGAGTGAGCTGCATTTCCAGTTCTGCGTTAAAGT ATCGTTTGTCTGGTGCCTGCCTGTGGTGGTGATTGGAGACTCA	Autophagy

Nanostring Gene Expression Study Design

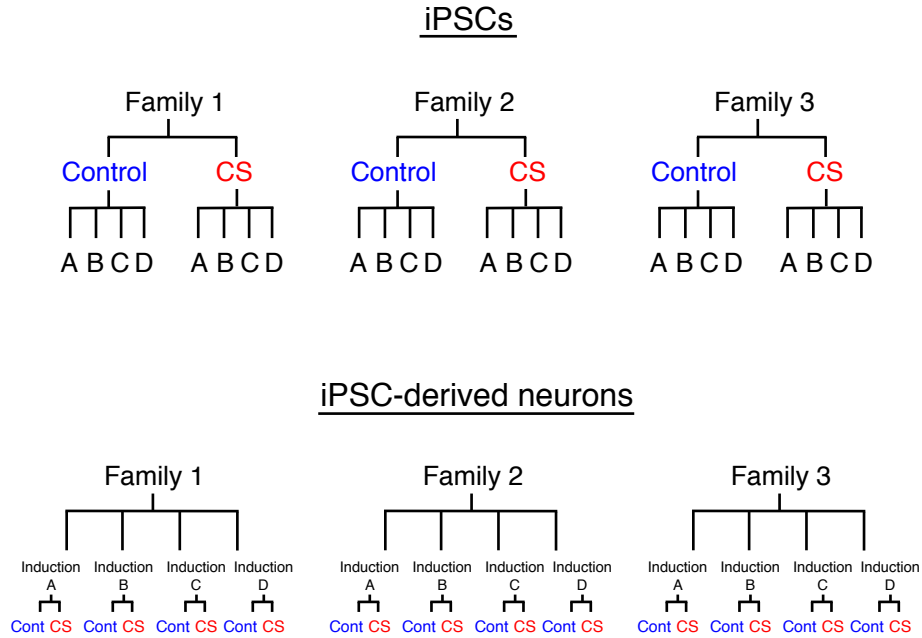


Figure 3.2 Nanostring gene expression analysis study design. For iPSCs, RNA was collected from four replicate wells of cells for each individual. For iPSC-derived neurons, RNA was collected from four paired independent neuronal inductions for each CS and control pair 38 days after neuronal induction.

Table 3.2 Genes upregulated in iPSCs vs neurons.

Table 3.3 Genes downregulated in iPSCs vs neurons.

The expression of 358 genes was measured using Nanostring in iPSCs and in neuronal cultures differentiated for 38 days. Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, and *RPL27*. The fold change in gene expression was compared between iPSCs and neuronal cultures using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. n = 24 iPSC RNA samples collected from: 12 control samples (4 replicate wells from each of 3 control lines) and 12 CS samples (4 replicate wells from each of 3 CS lines), and n = 24 neuronal RNA samples collected from: 12 control samples (4 neuronal inductions from each of 3 control lines) and 12 CS samples (4 neuronal inductions from each of 3 CS lines).

Table 3.2 Genes upregulated in iPSCs vs neurons.

Gene	iPSC (Counts)	StDev of iPSC	Neuron (Counts)	StDev of Neuron	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
MAP2	31.65	20.68	16461.74	6392.06	520.08	9.02	9.00E-41	6.78E-38	Neurodevelopment
Pax6	8.13	4.68	3878.61	3289.88	476.87	8.9	1.44E-31	1.71E-29	Neurodevelopment
Sox1	3.05	2.17	988.92	558.69	323.84	8.34	5.88E-35	1.39E-32	Neurodevelopment
NTRK2	7.62	3.27	2278.8	1640.29	299.01	8.22	3.34E-33	6.28E-31	Signaling
HES5	13.28	3.48	3849.12	2129.71	289.75	8.18	3.34E-42	3.77E-39	Neurodevelopment
DCX	27.87	6.71	5800.76	3820.65	208.14	7.7	5.83E-36	1.65E-33	Neurodevelopment
POU3F3	5.63	1.79	1005.56	361.3	178.74	7.48	6.00E-44	1.36E-40	Neurodevelopment
Tbr1	5.16	1.59	867.08	2262.05	168.17	7.39	8.40E-22	2.43E-20	Neurodevelopment
Tau_4R	24.83	6.86	3572.4	2691.97	143.88	7.17	1.12E-32	1.80E-30	Neurodegeneration
NCAM	55.06	12.72	7328.7	3488.74	133.1	7.06	7.48E-40	3.84E-37	Neurodevelopment
MAPT	38.65	9.31	4888.38	3629.08	126.47	6.98	1.41E-32	2.00E-30	Neurodevelopment
DLX1	9.58	2.15	1177.63	1667.22	122.87	6.94	3.17E-21	8.63E-20	Neurodevelopment
RELN	5.48	3.01	502.38	408.38	91.75	6.52	1.02E-26	5.76E-25	Neurodegeneration
Fzf2	3.76	2.18	333	773.37	88.68	6.47	9.07E-20	2.11E-18	Neurodevelopment
Isl1	5.35	3.47	407.75	714.49	76.21	6.25	5.98E-20	1.44E-18	Neurodevelopment
APLP1	165.72	16.95	10559.67	5276.15	63.72	5.99	7.56E-37	2.44E-34	Neurodegeneration
GAD2	6.6	2.82	381.22	640.57	57.79	5.85	4.68E-18	8.82E-17	Neurodevelopment
POU3F2	8	3.35	453.41	251.31	56.67	5.82	1.04E-32	1.80E-30	Neurodevelopment
GAD1	9.14	3.41	482.95	619.86	52.82	5.72	4.28E-20	1.06E-18	Lysosomal
FOXP2	8.81	3.11	454.45	562.18	51.6	5.69	1.76E-25	8.13E-24	Neurodevelopment
FoxG1	10.07	3.72	478.47	243.26	47.51	5.57	1.29E-32	1.95E-30	Neurodevelopment
APBA1	14.56	4.21	607.26	427.21	41.71	5.38	1.25E-28	9.77E-27	Neurodegeneration
SLC12A5	12.06	3.79	500.42	562.1	41.49	5.37	1.17E-22	4.00E-21	Neurodevelopment
CNTN2	15.49	4.13	633.98	514.78	40.92	5.35	2.29E-29	1.92E-27	Neurodegeneration
BCL11B	52.36	15.39	2030.97	2856.04	38.79	5.28	3.80E-21	1.02E-19	Neurodevelopment
CDK5R1	121.41	28	4151.39	2949.32	34.19	5.1	2.18E-28	1.54E-26	Neurodegeneration
ATP6V1G2	32.3	9	1016.08	572.29	31.46	4.98	2.06E-31	2.33E-29	vATPase
SYP	15.9	4.24	436.93	295.77	27.48	4.78	1.54E-26	8.51E-25	Neurodevelopment
NTRK3	11.86	4.09	254.55	148.21	21.47	4.42	2.04E-28	1.49E-26	Signaling
SLC32A1	6.45	2.55	138.52	218.58	21.47	4.42	8.99E-15	1.32E-13	Neurodevelopment
TUBB3	249.54	107.32	5342.99	1735.99	21.41	4.42	4.43E-31	4.77E-29	Neurodevelopment
HGSNAT	21.37	7.61	448.23	167.82	20.98	4.39	4.85E-32	6.44E-30	LSD
NPY	9.43	5.53	177.28	567.96	18.8	4.23	1.81E-10	2.10E-09	Neurodevelopment
SMPD1	6.09	2.45	96.86	45.16	15.91	3.99	1.02E-25	4.79E-24	LSD
VIM	1248.41	607.21	18382.9	4128.34	14.73	3.88	1.61E-28	1.21E-26	Neurodevelopment
GRIN3A	10.86	3.68	151.15	188.19	13.91	3.8	8.56E-20	2.02E-18	Lysosomal
APBB1	214.07	29.54	2954.86	1265.05	13.8	3.79	1.10E-31	1.38E-29	Neurodegeneration
OLIG2	12.56	3.18	167.46	343.71	13.33	3.74	6.07E-18	1.09E-16	Neurodevelopment
SLC17A6	22.79	7.78	284	669.94	12.46	3.64	1.22E-13	1.66E-12	Neurodevelopment
ATP6V0E2	48.34	11.4	572.14	275.77	11.84	3.57	5.54E-27	3.39E-25	vATPase
DLG4	228.51	34	2686.88	1593.47	11.76	3.56	7.22E-25	3.02E-23	Neurodevelopment
CNTN4	14.1	6.14	160.12	70.6	11.35	3.5	1.23E-23	4.57E-22	Neurodegeneration
TTBK1	9.23	3.9	102.38	52.51	11.1	3.47	3.46E-22	1.03E-20	Neurodegeneration
Nkx2-1	4.96	1.93	52.85	759.17	10.66	3.41	3.78E-07	3.58E-06	Neurodevelopment
MAP1LC3A	82	14.89	852.74	718.04	10.4	3.38	9.23E-20	2.13E-18	Autophagy
APBA2	53.4	9.93	539.5	185.53	10.1	3.34	5.22E-31	5.37E-29	Neurodegeneration
STX13	172.06	18.99	1636.2	777.71	9.51	3.25	2.27E-26	1.16E-24	Endosome
IDS	216.65	54.11	1984.75	965.56	9.16	3.2	3.32E-24	1.29E-22	LSD
ULK2	52.71	12.56	458.72	158.09	8.7	3.12	3.79E-29	3.06E-27	Autophagy
APBB3	33.66	8.63	289.53	156	8.6	3.1	1.10E-22	3.83E-21	Neurodegeneration
TH	10.3	3	87.57	152.74	8.5	3.09	4.91E-11	5.84E-10	Neurodevelopment
AKT3	422.79	109.87	3582.95	1104.84	8.47	3.08	6.11E-30	5.53E-28	Signaling
SYN1	41.92	10	322.95	221.05	7.7	2.95	4.94E-19	1.04E-17	Neurodevelopment
CALB2	31.91	6.92	240.18	283.18	7.53	2.91	8.03E-14	1.11E-12	Neurodevelopment
BACE1	113.91	14.13	856.57	382.86	7.52	2.91	9.06E-26	4.36E-24	Neurodegeneration
LHX6	5.15	2.21	38.74	137.82	7.52	2.91	5.78E-08	5.86E-07	Neurodevelopment
B3GAT1	87.22	28.74	601.77	230.84	6.9	2.79	6.54E-25	2.79E-23	Neurodevelopment
DNAJC5	62.97	14.15	433.6	209.94	6.89	2.78	2.30E-23	8.39E-22	LSD
APBP2	214.24	33.71	1412.47	363.65	6.59	2.72	2.14E-33	4.40E-31	Neurodegeneration
ATP6V0A1	340.75	54.11	2065.72	1179.4	6.06	2.6	1.08E-19	2.47E-18	vATPase
S100b	9.35	4.82	51.51	69.98	5.51	2.46	1.26E-11	1.54E-10	Neurodevelopment
CUX2	67.41	9.54	368.32	127.28	5.46	2.45	9.13E-27	5.43E-25	Neurodevelopment
EOMES	10.02	4.51	52.54	89.92	5.24	2.39	5.13E-08	5.23E-07	Neurodevelopment
SHANK3	38.72	7.09	200.5	98.74	5.18	2.37	1.08E-21	3.09E-20	Neurodevelopment
ATG4A	31.64	6.14	156.39	70.07	4.94	2.31	3.26E-20	8.20E-19	Autophagy
MARK1	292.7	47.65	1440.01	562.23	4.92	2.3	2.36E-22	7.52E-21	Neurodegeneration
SLC9A6	141.73	125.89	696.52	1011.72	4.91	2.3	5.87E-07	5.42E-06	Endosome
HOXA1	2.57	1.55	12.57	39.95	4.89	2.29	2.57E-07	2.45E-06	Neurodevelopment
IDUA	37.4	11.19	182.66	99	4.88	2.29	1.62E-16	2.67E-15	LSD
AKT1	169.83	31.42	827.79	203.95	4.87	2.29	1.56E-29	1.36E-27	Signaling
STH	6.96	2.39	33.2	44.89	4.77	2.25	8.26E-11	9.73E-10	Neurodegeneration
GSK3B	764.59	73.22	3400.13	988.09	4.45	2.15	2.38E-27	1.63E-25	Neurodegeneration

KIAA0226	32.83	8.67	145.32	83.11	4.43	2.15	3.40E-16	5.53E-15	Autophagy
NES	918.8	110.77	4041.52	1791.06	4.4	2.14	1.92E-24	7.76E-23	Neurodevelopment
SLC9A6 TV 1 2	59.54	53.86	258.1	388.73	4.33	2.12	3.91E-06	3.54E-05	Endosome
VPS11	70.01	8.63	299.61	120.65	4.28	2.1	8.93E-23	3.16E-21	Endosome
GABARAPL2	1131.1	106.7	4797.21	1524.87	4.24	2.08	5.27E-25	2.29E-23	Autophagy
LRP3	138.5	21.89	582.87	153.25	4.21	2.07	9.92E-27	5.75E-25	Endosome
GABARAP	1948.4	171.47	8155.04	1411.41	4.19	2.07	6.15E-35	1.39E-32	Autophagy
DAB1	80.02	24.4	333.05	135.53	4.16	2.06	5.73E-18	1.04E-16	Neurodegeneration
ATP6V0D1	762.57	99.2	3122.56	1221.72	4.09	2.03	1.42E-21	4.02E-20	vATPase
NTRK1	3.16	1.86	12.87	61.97	4.08	2.03	1.11E-05	9.77E-05	Signaling
SHH	9.34	2.93	38.1	89.75	4.08	2.03	3.27E-09	3.56E-08	Neurodevelopment
MAP1LC3B	605.67	45.89	2399.72	780	3.96	1.99	4.91E-25	2.18E-23	Autophagy
CDK5	197.44	37.02	776.81	304.62	3.93	1.98	5.59E-20	1.36E-18	Neurodegeneration
CLCN3	109.52	14.89	395.7	139.53	3.61	1.85	2.86E-22	8.61E-21	Endosome
GRIPAP1	236.38	36.54	852.39	383.71	3.61	1.85	2.44E-17	4.18E-16	Endosome
ULK3	339.51	58.36	1213.42	600.74	3.57	1.84	3.26E-16	5.34E-15	Autophagy
AP1S2	678.76	134.54	2367.32	888.01	3.49	1.8	9.79E-19	2.01E-17	Endosome
CTSB	280.86	40.59	970.86	220.49	3.46	1.79	4.71E-27	2.96E-25	Lysosomal
AMY	235.51	39.53	809.15	343.85	3.44	1.78	1.43E-18	2.83E-17	Neurodegeneration
ATP6V0C	1478.62	160.78	5079.2	2223.93	3.44	1.78	4.93E-18	9.15E-17	vATPase
MCOLN1	67.52	11.61	230.9	112.6	3.42	1.77	1.17E-15	1.82E-14	Lysosomal
TTBK2	169.5	23.91	579.66	170.6	3.42	1.77	1.54E-22	5.16E-21	Neurodegeneration
ATP6V1G1	20.1	4.99	68.17	41.76	3.39	1.76	5.92E-14	8.26E-13	vATPase
EHD1	93.28	21.81	313.99	74.29	3.37	1.75	2.78E-22	8.48E-21	Endosome
TMEM74	42.2	10.96	142.18	69.75	3.37	1.75	1.31E-14	1.92E-13	Autophagy
MFS08	137.94	23.03	452.75	195.4	3.28	1.71	5.32E-18	9.71E-17	LSD
AMBRA1	224.78	30.72	733.26	229.51	3.26	1.71	2.12E-21	5.84E-20	Autophagy
ARSA	36.72	7.99	119.84	56.23	3.26	1.71	1.72E-15	2.66E-14	LSD
WDFY3	235.16	37.33	766.05	283.53	3.26	1.7	2.30E-19	5.06E-18	Autophagy
SLC9A8	33.95	6.99	110.46	40.26	3.25	1.7	1.41E-18	2.81E-17	Endosome
GGA1	107.41	15.24	343.29	123.63	3.2	1.68	2.23E-19	4.93E-18	Endosome
VPS35	645.27	51.87	2034.26	374.81	3.15	1.66	2.06E-30	2.03E-28	Endosome
LRP1	235.54	51.33	740.19	349.93	3.14	1.65	7.46E-15	1.10E-13	Neurodegeneration
SLC9A6 TV 1 3	8.53	3.96	26.79	25.42	3.14	1.65	1.54E-07	1.49E-06	Endosome
NAGPA	31.17	9.05	96.78	61.71	3.1	1.63	2.15E-12	2.68E-11	Lysosomal
TMEM55B	93.25	9.37	286.92	95.01	3.08	1.62	7.73E-21	2.01E-19	Lysosomal
ULK1	203.66	28.03	624.62	214.88	3.07	1.62	3.23E-19	6.88E-18	Autophagy
ATG16L2	61.26	15.23	186.46	104.81	3.04	1.61	2.11E-12	2.65E-11	Autophagy
SQSTM1	168.63	19.56	512.5	177.37	3.04	1.6	2.40E-19	5.22E-18	Autophagy
PICALM	496.77	88.06	1498.35	423.65	3.02	1.59	1.01E-20	2.59E-19	Neurodegeneration
SNCA	47.39	11.01	143.25	98.13	3.02	1.6	1.52E-10	1.77E-09	Neurodegeneration
UVRAG	213.83	50.16	642.36	290.15	3	1.59	1.64E-14	2.37E-13	Endosome
Cux1	314.07	32.15	932.52	217.03	2.97	1.57	6.11E-26	3.06E-24	Neurodevelopment
RRAGC	209.34	21.83	617.17	174.08	2.95	1.56	3.66E-22	1.07E-20	Lysosomal
CHAT	6.17	2.71	18.11	42.27	2.93	1.55	1.08E-05	9.54E-05	Neurodevelopment
NBR1	522.48	46.46	1525.05	478.63	2.92	1.55	4.04E-21	1.07E-19	Autophagy
ATP6V1F	1073.75	144.24	3068.58	1071.99	2.86	1.51	1.03E-18	2.09E-17	vATPase
Rab35	892.76	126.71	2542.48	766.61	2.85	1.51	6.72E-20	1.60E-18	Endosome
ATG2B	152.97	20.52	429.52	154.38	2.81	1.49	3.16E-18	6.21E-17	Autophagy
SLC36A1	69.98	28.44	196.33	87.21	2.81	1.49	1.16E-11	1.41E-10	Lysosomal
GSK3A	838.27	120.98	2346	851.8	2.8	1.48	1.04E-17	1.86E-16	Neurodegeneration
HGS	46.45	7.41	128.21	41.89	2.76	1.46	3.68E-18	7.11E-17	Endosome
MAPK3	493	47.48	1358.47	352.42	2.76	1.46	1.55E-22	5.16E-21	Signaling
SORT1	1052.68	176.54	2907.95	868.96	2.76	1.47	4.94E-18	9.15E-17	Endosome
ATP6V1B1	2.9	1.95	7.93	7.1	2.74	1.45	2.34E-06	2.13E-05	vATPase
ATP6V1H	501.26	39.92	1370.19	614.66	2.73	1.45	3.57E-14	5.04E-13	vATPase
ATP6V1B2	797.98	66.46	2171.72	709.04	2.72	1.44	3.15E-19	6.77E-18	vATPase
GALC	31.42	5.84	85.24	36.01	2.71	1.44	1.33E-14	1.92E-13	LSD
GBA	173.49	19.89	467.72	161.07	2.7	1.43	5.14E-18	9.45E-17	Lysosomal
FUCA1	180.91	40.41	474.49	257.58	2.62	1.39	1.03E-11	1.27E-10	LSD
HEXA	236.23	19.44	619.23	211.77	2.62	1.39	4.58E-18	8.70E-17	Lysosomal
PTEN	478.37	53.49	1240.6	410.44	2.59	1.37	8.56E-19	1.79E-17	Signaling
CLN5	87.31	15.56	224.53	43.5	2.57	1.36	1.62E-22	5.32E-21	LSD
EN1	8.46	3.58	21.78	69.05	2.57	1.36	0.00088	0.00726	Neurodevelopment
PEX13	293.03	42.93	751.82	167.89	2.57	1.36	1.69E-22	5.45E-21	LSD
GNS	222.88	26.83	570.54	97.87	2.56	1.36	2.18E-26	1.15E-24	Lysosomal
SLC9A9	14.14	3.58	36	39.42	2.55	1.35	6.32E-08	6.38E-07	Endosome
ATP6V0B	534.59	61.86	1356.65	613.65	2.54	1.34	3.76E-13	5.03E-12	vATPase
RPTOR	101.81	17.97	258.98	72.7	2.54	1.35	9.95E-19	2.03E-17	Signaling
MARK3	355.85	39.34	899.49	255.88	2.53	1.34	1.22E-19	2.75E-18	Neurodegeneration
CSNK1D	727	73.24	1829.92	501.35	2.52	1.33	7.16E-21	1.88E-19	Neurodegeneration
ATP13A2	263.54	25.22	655.08	266.59	2.49	1.31	5.96E-15	8.86E-14	LSD
BLOC1S3	14.21	3.33	35.3	16.67	2.48	1.31	4.69E-11	5.61E-10	Lysosomal
SLC12A2	143.21	32.97	355.79	111.3	2.48	1.31	1.49E-16	2.50E-15	Neurodevelopment
VPS26A	445.79	60.15	1095.57	198.45	2.46	1.3	5.22E-24	1.97E-22	Endosome

SLC17A7	12.22	3.99	29.84	54.54	2.44	1.29	4.15E-05	0.000359	Neurodevelopment
SLC9A2	11.56	3.71	28.08	36.27	2.43	1.28	4.32E-05	0.000373	Endosome
ATP6V1D	394.51	36.8	949.71	263.63	2.41	1.27	2.03E-19	4.55E-18	vATPase
ARSB	283.81	48.79	680.68	178.39	2.4	1.26	1.34E-17	2.37E-16	LSD
TACE/ADAM17	331.57	44.09	795.82	126.79	2.4	1.26	2.52E-25	1.14E-23	Neurodegeneration
ASAH1	638.51	118.28	1527.25	556.61	2.39	1.26	3.23E-14	4.59E-13	Lysosomal
RABGEF1	317.79	44.69	753.15	271.06	2.37	1.24	3.71E-15	5.59E-14	Endosome
ATP6V1A	1548.17	168.44	3656.07	2096.15	2.36	1.24	7.50E-10	8.47E-09	vATPase
ADAM9	153.78	29.19	361.47	123.54	2.35	1.23	1.77E-15	2.72E-14	Neurodegeneration
HOXA2	4.71	2.01	10.91	35.47	2.32	1.21	0.00328	0.026	Neurodevelopment
MAPK1	872.81	65.64	2015.05	539.5	2.31	1.21	4.97E-20	1.22E-18	Signaling
CREB1	370.23	75.8	844.97	208.93	2.28	1.19	2.33E-17	4.02E-16	Signaling
CLN3	64.21	12.13	145.61	50.3	2.27	1.18	1.78E-14	2.54E-13	LSD
NCSTN	445.85	63.11	1000.17	174.09	2.24	1.17	2.67E-22	8.28E-21	Neurodegeneration
ATP6V1C1	214.98	67.7	478.55	233.94	2.23	1.15	4.70E-09	5.03E-08	vATPase
SLC9A4	8.06	2.89	17.88	38.58	2.22	1.15	0.000928	0.00763	Endosome
PEX5	361.48	45.25	800.57	247.38	2.21	1.15	5.32E-16	8.46E-15	LSD
AKT2	625.43	99.58	1356.14	316.34	2.17	1.12	3.89E-18	7.46E-17	Signaling
CLCN7	320.28	37.18	693.52	212.87	2.17	1.11	2.12E-15	3.23E-14	Endosome
ATG4D	237.66	21.51	510.24	146.96	2.15	1.1	4.39E-16	7.03E-15	Autophagy
GALNS	92.98	18.47	198.84	52.08	2.14	1.1	4.26E-16	6.88E-15	Lysosomal
ATG16L1	311.1	87.72	663.87	305.75	2.13	1.09	2.36E-09	2.60E-08	Autophagy
WDR45	127.73	17.25	272.2	104.21	2.13	1.09	4.73E-12	5.84E-11	Autophagy
WIPI2	205.25	28.99	435.29	176.85	2.12	1.08	1.45E-12	1.86E-11	Autophagy
CTSK	46.54	11.96	97.82	78.76	2.1	1.07	5.74E-07	5.34E-06	Lysosomal
MNX1	6.21	3.51	12.8	20.76	2.06	1.04	0.00289	0.0231	Neurodevelopment
PRKAA1	213.99	34	440.21	146.07	2.06	1.04	3.45E-13	4.64E-12	Autophagy
Tau_3R	13.05	3.48	26.86	13.53	2.06	1.04	1.38E-07	1.34E-06	Neurodegeneration
GNPTG	190.99	13.37	388.07	110.07	2.03	1.02	3.10E-17	5.27E-16	Lysosomal
ATG2A	72.44	10.54	145.96	44.87	2.02	1.01	1.20E-13	1.64E-12	Autophagy
TCF4	279.52	45.02	564.09	190.97	2.02	1.01	1.08E-12	1.41E-11	LSD
RICTOR	231.04	48.82	462.87	156.33	2	1	3.08E-11	3.71E-10	Signaling
PSEN1	526.76	68.07	1046.85	194.92	1.99	0.991	8.79E-19	1.82E-17	Neurodegeneration
VP58	338.35	30.62	669.65	232.36	1.98	0.985	2.07E-12	2.62E-11	Endosome
APPL1	773.77	93.8	1523.87	343.85	1.97	0.978	1.96E-17	3.43E-16	Endosome
ATP6V0A2	165.11	36.78	318.91	80.06	1.93	0.95	1.11E-13	1.53E-12	vATPase
SGSH	39.05	11.21	74.47	32.48	1.91	0.932	1.16E-08	1.21E-07	LSD
BLOC1S1	926.03	58.93	1759.53	391.72	1.9	0.926	1.97E-17	3.43E-16	Lysosomal
MARK2	364.21	64.34	691.98	197.74	1.9	0.926	1.16E-12	1.49E-11	Neurodegeneration
KMT2D	77.47	15.1	146.45	66.07	1.89	0.919	7.44E-09	7.86E-08	LSD
MYOD1	10.35	2.6	19.59	38.05	1.89	0.921	0.00247	0.0198	Neurodevelopment
PRKAG2	349.67	46.91	658.02	358.48	1.88	0.912	3.98E-07	3.73E-06	Signaling
RB1CC1	479.16	91.25	897.07	283.68	1.87	0.905	6.81E-11	8.06E-10	Autophagy
ATG9A	454.92	47.76	848.11	269.65	1.86	0.899	1.52E-12	1.95E-11	Autophagy
ATG9B	12.67	3.46	23.18	38.71	1.83	0.872	0.0025	0.02	Autophagy
RAB7A	1668.63	211.52	3021.9	963.13	1.81	0.857	2.37E-11	2.86E-10	Lysosomal
AFF	6.59	2.19	11.85	23.59	1.8	0.846	0.0036	0.0285	Neurodevelopment
EEA1	340.48	76.34	611.64	172.25	1.8	0.845	2.13E-10	2.45E-09	Endosome
STX6	826.23	119.82	1464.07	359.91	1.77	0.825	8.24E-13	1.08E-11	Endosome
ATG14	69.25	9.67	119.81	50.42	1.73	0.791	8.87E-08	8.80E-07	Autophagy
SLC9A7	320.2	68.64	553.15	205.3	1.73	0.789	3.63E-08	3.73E-07	Endosome
ATG7	147.52	18.2	254.21	49.81	1.72	0.785	9.34E-16	1.47E-14	Autophagy
LAMP2	907.09	99.73	1557.86	252.35	1.72	0.78	3.61E-18	7.03E-17	LSD
PRKAA2	241.14	45.71	414.99	127.51	1.72	0.783	3.06E-09	3.34E-08	Autophagy
VP518	202.44	28.1	348.64	113.81	1.72	0.784	4.40E-10	5.03E-09	Endosome
ATOH1	7.22	2.52	12.24	37.42	1.7	0.762	0.0274	0.203	Neurodevelopment
PEX3	228.21	24.06	388.28	125.24	1.7	0.767	3.09E-10	3.54E-09	LSD
SATB2	22.18	6.2	37.68	13.21	1.7	0.764	2.13E-07	2.04E-06	Neurodevelopment
AKT1S1	269.33	43.85	453.76	93.21	1.68	0.753	3.06E-13	4.15E-12	Autophagy
ATG4B	1342.18	136.61	2250.24	552	1.68	0.746	7.18E-13	9.49E-12	Autophagy
HOXB6	4.98	2.87	8.31	19.91	1.67	0.738	0.048	0.348	Neurodevelopment
APOER2	270.35	53.01	442.25	139.35	1.64	0.71	2.22E-08	2.32E-07	Neurodegeneration
MAP4	1335.36	213.15	2194.19	558.53	1.64	0.716	1.29E-10	1.51E-09	Neurodegeneration
PEX16	143.08	17.6	233.66	88.61	1.63	0.708	8.23E-08	8.23E-07	LSD
aph1Avar3	608.81	48.46	984.58	171.7	1.62	0.694	1.47E-16	2.48E-15	Neurodegeneration
GGA3	585.68	78.72	949.14	303.78	1.62	0.697	8.86E-09	9.31E-08	Endosome
HOXB1	9.95	3.44	16.1	24.58	1.62	0.695	0.0124	0.094	Neurodevelopment
IGF2	34.29	14.21	55.3	41.01	1.61	0.69	0.00175	0.0144	Signaling
HoxB4	8.99	3.96	14.3	30.88	1.59	0.67	0.0453	0.33	Neurodevelopment
APP-All	5247.42	549.3	8186.96	2911.45	1.56	0.642	4.45E-07	4.16E-06	Neurodegeneration
ADAM19	93.75	28.34	145.18	50.2	1.55	0.631	1.35E-05	0.000118	Neurodegeneration
IGF1R	332.75	75.09	514.61	148.44	1.55	0.629	2.53E-08	2.61E-07	Signaling
MTOR	465.51	51.06	720.48	196.77	1.55	0.63	1.85E-09	2.06E-08	Signaling
Sox2	1521.12	108.34	2361.38	495.58	1.55	0.634	5.06E-13	6.72E-12	Neurodevelopment
Rab10	810.87	65.55	1252.78	341.59	1.54	0.628	6.03E-10	6.85E-09	Endosome

GAA	171.75	31.08	260.94	104.14	1.52	0.603	1.21E-05	0.000106	Lysosomal
PLBD2	111.93	17.66	169.9	49.92	1.52	0.602	1.26E-07	1.24E-06	Autophagy
PEX1	402.51	33.64	607.11	223.61	1.51	0.593	1.66E-06	1.51E-05	LSD
APP-695	5662.78	687.92	8449.12	3155.62	1.49	0.577	9.83E-06	8.78E-05	Neurodegeneration
PSAP	3476.07	269.42	5186.45	1560.83	1.49	0.577	1.76E-07	1.70E-06	Lysosomal
CTNS	66.84	12.88	99.2	20.33	1.48	0.57	5.29E-09	5.64E-08	Lysosomal
HOXB13	10.38	3.66	15.13	35.05	1.46	0.544	0.0801	0.566	Neurodevelopment
STK4	482.45	88.99	697.27	120.75	1.45	0.531	3.89E-09	4.19E-08	Signaling
APBA3	52.89	7.9	76.09	42.03	1.44	0.525	0.000726	0.00603	Neurodegeneration
aph1Avar4	315.37	33.39	455.3	177.31	1.44	0.53	0.000619	0.00518	Neurodegeneration
BECN1	770.95	108.92	1111.73	292.44	1.44	0.528	2.06E-07	1.98E-06	Autophagy
TFEB	15.3	3.6	21.43	33.26	1.4	0.485	0.0743	0.528	Lysosomal
ATP6V1E1	1439.77	150.86	2006.39	711.05	1.39	0.479	5.18E-05	0.000445	vATPase
GNPTAB	514.01	150.91	716.84	415.28	1.39	0.48	0.0127	0.0964	LSD
Sox10	8.25	2.78	11.41	32.05	1.38	0.467	0.191	1	Neurodevelopment
ATG12	72.6	11.24	99.37	76.07	1.37	0.453	0.00888	0.0683	Autophagy
VAMP1	47.43	8.45	65.2	47.13	1.37	0.459	0.0104	0.0798	Endosome
HYAL1	57.19	11.96	77.84	65.82	1.36	0.445	0.0192	0.143	LSD
PSENEN	771.83	105.29	1037.75	191.97	1.34	0.427	8.29E-08	8.25E-07	Neurodegeneration
Satb1	521.15	129.27	698.52	211.21	1.34	0.423	0.000287	0.00241	Neurodevelopment
SLC9A5	25.23	4.44	33.9	33.64	1.34	0.426	0.0672	0.479	Endosome
NPC1	308.73	57.25	406.27	65.89	1.32	0.396	1.21E-06	1.10E-05	LSD
NECAP2	390.07	51.97	510.5	82.54	1.31	0.388	6.37E-08	6.40E-07	Endosome
OTX2	194.95	39.97	255.69	125.28	1.31	0.391	0.00792	0.0611	Neurodevelopment
TWIST1	7.01	2.71	9.13	7.19	1.3	0.381	0.0913	0.643	Neurodevelopment
ULK4	81.22	31.99	105.28	30.13	1.3	0.374	0.00757	0.0586	Autophagy
IGF2R	242	44.35	309.8	76.44	1.28	0.356	0.000274	0.00231	Lysosomal
AGA	147.34	21.54	182.38	43.76	1.24	0.308	7.00E-04	0.00584	LSD
NEDD4	38.88	10.87	48.37	14.84	1.24	0.315	0.0159	0.12	Endosome
RAB5A	1321.88	125.44	1634.93	515.12	1.24	0.307	0.00215	0.0173	Endosome
GFAP	7.26	2.94	8.93	5.35	1.23	0.299	0.121	0.847	Neurodevelopment
HPS1	48.38	9.52	59.52	22.28	1.23	0.299	0.0224	0.166	Lysosomal
MLST8	311.13	41.64	383.71	66.36	1.23	0.302	1.00E-05	8.93E-05	Signaling
PEX19	431.28	47.88	531.03	74.38	1.23	0.3	3.91E-07	3.68E-06	LSD
ATP6V1E2	101.84	13.13	123.32	40.77	1.21	0.276	0.00442	0.0347	vATPase
HP55	255.89	25.69	308.47	92.24	1.21	0.27	0.00309	0.0246	Lysosomal
Rab4	555.91	83.14	674.04	98.26	1.21	0.278	3.59E-05	0.000312	Endosome
VPS33A	306.15	24.67	371.34	106.91	1.21	0.279	0.00199	0.0162	Endosome
ADAM10	951.99	129.4	1129.52	178.14	1.19	0.247	0.000138	0.00118	Neurodegeneration
GABARAPL1	871.72	93.14	1033.68	389.97	1.19	0.246	0.0364	0.267	Autophagy
USP10	1619.5	158.93	1912.84	350.44	1.18	0.24	0.00014	0.00119	Autophagy
WDR45L	888.8	115.24	1044.55	256.76	1.18	0.233	0.00586	0.0457	Autophagy
CTSF	74.8	131.95	87.66	174.07	1.17	0.229	0.66	1	Lysosomal
TPP1	334.52	48.14	391.59	91.76	1.17	0.227	0.00468	0.0366	LSD
APLP2	2746.98	302.46	3174.36	744.21	1.16	0.209	0.00732	0.0569	Neurodegeneration
HIF1A	3000.74	561.3	3490.79	641.81	1.16	0.218	0.00403	0.0317	Neurodevelopment
SLC9A1	200.51	35.9	233.38	73.81	1.16	0.219	0.0356	0.262	Endosome
ATG3	1090.3	157.51	1253.01	279.36	1.15	0.201	0.011	0.0844	Autophagy
CTSA	390.73	55.96	443.86	121.28	1.14	0.184	0.0624	0.447	Lysosomal
aph1Bvar1	329.66	61.21	371.24	84.45	1.13	0.171	0.0495	0.358	Neurodegeneration
LAMP1	1970.85	212.89	2217.68	460.03	1.13	0.17	0.0174	0.131	Lysosomal
NEU1	153.45	22.36	173.25	44.98	1.13	0.175	0.0476	0.346	Lysosomal
ARF6	1468.06	174.94	1640.86	296.13	1.12	0.161	0.0111	0.0847	Endosome
PPP2R4	1312.56	129.74	1465.81	296	1.12	0.159	0.0175	0.131	Neurodegeneration
TFE3	48.39	10.67	53.89	15.19	1.11	0.155	0.134	0.931	Lysosomal
NRBF2	832.94	99.22	915.41	285.33	1.1	0.136	0.153	1	Autophagy
CALB1	354.53	82	384.75	350.21	1.09	0.118	0.599	1	Neurodevelopment
LRP10	189.85	48.8	207.03	68.89	1.09	0.125	0.244	1	Neurodegeneration
USP13	362.38	39.53	392.6	63.43	1.08	0.116	0.041	0.3	Autophagy
SUMF1	202.22	29.98	216.6	47.83	1.07	0.0991	0.181	1	Lysosomal
MAN2B1	381.75	34.01	404.58	71.01	1.06	0.0838	0.158	1	LSD
NAGA	76.32	11	80.74	14.16	1.06	0.0812	0.213	1	LSD
TFRC	1256.63	343.34	1294.12	244.26	1.03	0.0424	0.644	1	Endosome
NGF	6.99	3.36	7.09	11.56	1.02	0.0216	0.946	1	Signaling

Table 3.3 Genes downregulated in iPSCs vs neurons.

Gene	iPSC (Counts)	StDev of iPSC	Neuron (Counts)	StDev of Neuron	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
POU5F1	7943.04	791.31	12.65	19.39	-627.96	-9.29	1.87E-37	7.03E-35	Neurodevelopment
APOE	3038.1	255.92	40.06	20.21	-75.84	-6.24	8.50E-40	3.84E-37	Neurodegeneration
nanog	1781.84	296.72	24.5	78.02	-72.74	-6.18	5.85E-23	2.10E-21	Neurodevelopment
CD74	298.44	49.73	16.33	25.61	-18.27	-4.19	1.14E-20	2.90E-19	Neurodegeneration
APP-770	3675.72	409.39	266.58	145.28	-13.79	-3.79	3.45E-30	3.25E-28	Neurodegeneration
CTSC	3872.27	403.43	432.96	250.33	-8.94	-3.16	3.00E-24	1.19E-22	LSD
Iba1	126.13	15.61	15.05	24.75	-8.38	-3.07	3.40E-15	5.16E-14	Neurodevelopment
EHD4	343.39	50.61	48.43	20.59	-7.09	-2.83	3.27E-27	2.17E-25	Endosome
C1ORF85	425.72	39.35	86.49	29.12	-4.92	-2.3	6.23E-26	3.06E-24	Lysosomal
SCPEP1	516.44	70.64	130.83	53.63	-3.95	-1.98	1.89E-21	5.28E-20	LSD
NTF4	62.92	11.09	17.63	28.65	-3.57	-1.84	1.51E-09	1.69E-08	Signaling
MBP	100.63	23.82	32.16	40.18	-3.13	-1.65	7.05E-09	7.48E-08	Neurodevelopment
BDNF	122.16	13.97	40.46	65.08	-3.02	-1.59	9.97E-08	9.84E-07	Signaling
WIP1	100.6	28.33	34.07	9.94	-2.95	-1.56	5.43E-16	8.58E-15	Autophagy
GM2A	475.75	55.39	164.75	41.38	-2.89	-1.53	4.06E-24	1.56E-22	LSD
PVALB	25.93	8.26	9	6.91	-2.88	-1.53	2.23E-09	2.47E-08	Neurodevelopment
HP53	948.91	105.17	337.77	66.33	-2.81	-1.49	4.35E-27	2.81E-25	Lysosomal
NPC2	3640.53	369.69	1318.71	258.94	-2.76	-1.47	1.64E-26	8.82E-25	LSD
aph1Bvar2	78.7	12.21	29.21	9.05	-2.69	-1.43	5.03E-15	7.53E-14	Neurodegeneration
APP-KPI	406.82	50.64	154.92	37.09	-2.63	-1.39	2.54E-22	7.98E-21	Neurodegeneration
SORL1	399.44	48.11	156.88	81.32	-2.55	-1.35	1.53E-12	1.95E-11	Endosome
HEXB	846.2	68.97	342.08	66.76	-2.47	-1.31	9.73E-25	4.00E-23	Lysosomal
Rab11	253.03	39.53	110.47	41.11	-2.29	-1.2	3.35E-12	4.16E-11	Endosome
NGFR	83.9	19.55	43.24	49.2	-1.94	-0.956	0.000219	0.00185	Signaling
DRAM1	96.97	23.8	50.22	19.97	-1.93	-0.949	3.80E-09	4.11E-08	Autophagy
PHYH	51.25	7.57	26.58	12.3	-1.93	-0.947	2.44E-09	2.67E-08	LSD
TJP1	2152.35	247.45	1266.31	214.32	-1.7	-0.765	1.52E-16	2.52E-15	Neurodevelopment
LIPA	840.78	283.51	520.4	135.4	-1.62	-0.692	3.63E-07	3.45E-06	LSD
CLN6	338.35	36.27	215.5	44.88	-1.57	-0.651	9.35E-13	1.22E-11	LSD
MANBA	120.57	20.9	78.21	30.98	-1.54	-0.624	7.70E-06	6.90E-05	LSD
CLN8	271.71	41.48	186.86	44.75	-1.45	-0.54	2.43E-08	2.52E-07	LSD
PPT1	2556.36	222.3	1809.4	248.28	-1.41	-0.499	4.92E-14	6.91E-13	Lysosomal
ATP6V0E1	1844.44	251.2	1377.11	196.48	-1.34	-0.422	1.24E-09	1.40E-08	vATPase
NAGLU	118.03	10.96	88.56	24.24	-1.33	-0.414	4.86E-06	4.37E-05	Lysosomal
BACE2	112.03	20.63	86.03	32.03	-1.3	-0.381	0.00205	0.0167	Neurodegeneration
CCK	34.79	5.55	26.89	29.98	-1.29	-0.372	0.0786	0.557	Neurodevelopment
ATG5	451.63	37.28	351.38	61.56	-1.29	-0.362	4.72E-08	4.83E-07	Autophagy
NMDA NR1	598.16	70.06	477.38	101.42	-1.25	-0.325	1.80E-05	0.000157	Neurodevelopment
RAB9A	669.54	79.35	538.36	79.55	-1.24	-0.315	1.20E-06	1.10E-05	Endosome
M6PR	580.36	44.33	472.5	72.08	-1.23	-0.297	1.11E-07	1.09E-06	Lysosomal
VLDLR	841.3	175.6	694.21	440.63	-1.21	-0.277	0.157	1	Neurodegeneration
CTSD	1060.97	95.62	879.23	212.67	-1.21	-0.271	8.00E-04	0.00663	Lysosomal
ATG4C	582.91	63.05	488.11	50.18	-1.19	-0.256	5.84E-07	5.41E-06	Autophagy
GLA	722.19	81.92	609.85	148.16	-1.18	-0.244	0.00209	0.0169	Lysosomal
SLC17A5	218.1	20.75	185.82	62.1	-1.17	-0.231	0.0273	0.202	LSD
CD63	3535.25	338.43	3025.15	433.48	-1.17	-0.225	5.54E-05	0.000475	Lysosomal
IGF1	19.38	5.39	16.62	10.85	-1.17	-0.221	0.157	1	Signaling
SLC9A3	5.42	2.05	4.76	5.35	-1.14	-0.187	0.492	1	Endosome
SIRT1	1179.38	184.67	1046.22	275.44	-1.13	-0.173	0.0534	0.384	Autophagy
TP73	45.72	9.48	40.64	32.91	-1.12	-0.17	0.334	1	Neurodevelopment
CCND1	947.59	121.44	847.33	640.91	-1.12	-0.161	0.449	1	Neurodevelopment
ATP6V0A4	14.82	5.37	13.34	9.72	-1.11	-0.152	0.445	1	vATPase
ATG101	367.79	38.14	331.98	82.85	-1.11	-0.148	0.0566	0.406	Autophagy
GSAP	14.64	4.3	13.23	15.15	-1.11	-0.145	0.565	1	Neurodegeneration
PSEN2	216.68	41.41	196.61	47.93	-1.1	-0.14	0.123	0.863	Neurodegeneration
ATG10	270.95	52.32	250.32	64.81	-1.08	-0.114	0.23	1	Autophagy
PIK3R4	461.15	104.75	427.76	83.13	-1.08	-0.108	0.202	1	Autophagy
KLF4	45.18	11.52	41.99	28.94	-1.08	-0.106	0.565	1	Neurodevelopment
WDFY2	193.92	33.19	180.15	36.89	-1.08	-0.106	0.17	1	Endosome
NTF3	14.27	4.14	13.32	14.22	-1.07	-0.0994	0.696	1	Signaling
ATP6V0D2	8.28	3.21	7.78	9.97	-1.06	-0.0898	0.758	1	vATPase
NAE1	2612.7	300.35	2569.63	289.45	-1.02	-0.024	0.616	1	Neurodegeneration
ATP6V1G3	6.32	2.5	6.24	9.2	-1.01	-0.0179	0.948	1	vATPase
GLB1	299.84	33.55	296.89	58.45	-1.01	-0.0143	0.827	1	Lysosomal
GGA2	834.43	154.97	828.09	130.35	-1.01	-0.011	0.883	1	Endosome

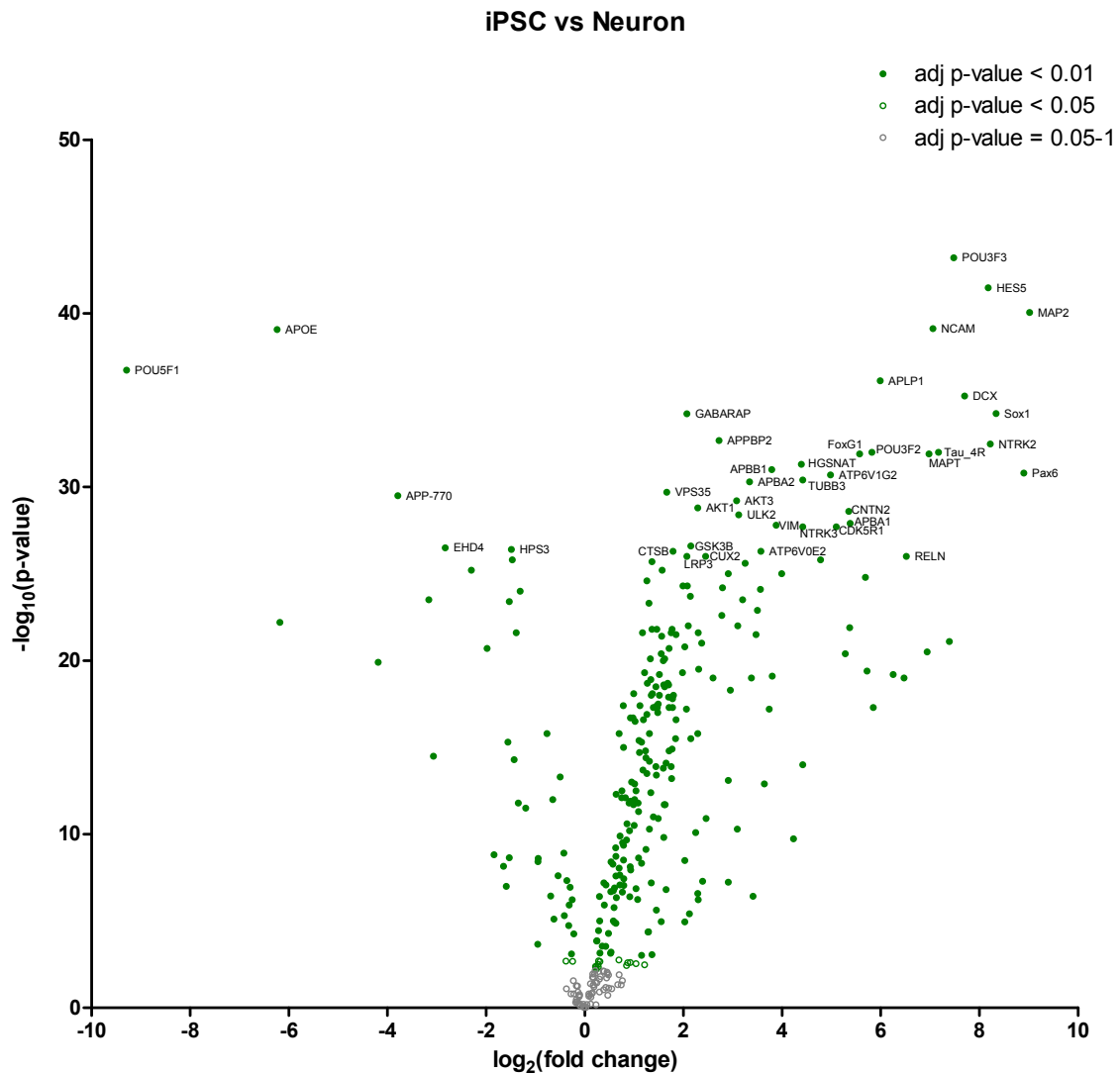


Figure 3.3 Volcano plot of gene expression changes in iPSCs as compared to iPSC-derived neurons. The expression of 358 genes was measured using Nanostring in iPSCs and in neuronal cultures differentiated for 38 days. Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, and *RPL27*. The mean fold change in gene expression was compared between iPSCs and neuronal cultures using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. The $-\log_{10}(\text{p-value})$ for each gene was plotted versus the $\log_2(\text{fold change})$. Significance after Benjamini-Yekutieli FDR adjustment is indicated by circle color. The 40 genes with the most highly significant fold changes are labeled. n= 24 iPSC RNA samples collected from: 12 control samples (4 replicate wells from each of 3 control lines) and 12 CS samples (4 replicate wells from each of 3 CS lines), and n= 24 neuronal RNA samples collected from: 12 control samples (4 neuronal inductions from each of 3 control lines) and 12 CS samples (4 neuronal inductions from each of 3 CS lines).

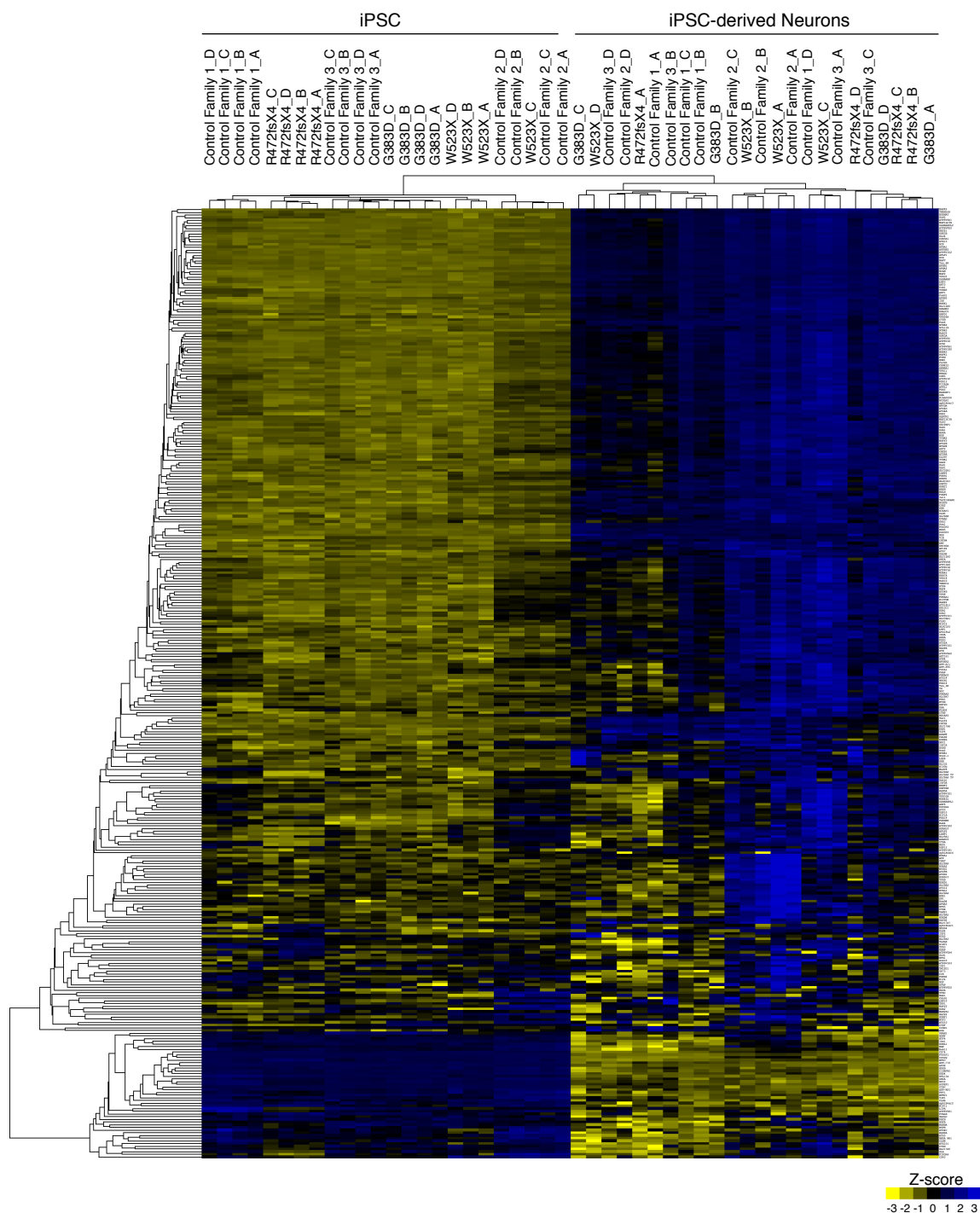


Figure 3.4 Unsupervised hierarchical clustering of iPSCs and iPSC-derived neurons by gene expression. The expression of 358 genes was measured in iPSCs and in neuronal cultures differentiated for 38 days using Nanostring. Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *B2M*, *GUSB*, *POLR2A*, *GAPDH*, and *RPL27*. Gene expression levels were transformed to Z-scores and used to perform agglomerative unsupervised hierarchical clustering using the Euclidean distance metric and average linkage criteria. Columns represent the individual iPSC line or iPSC-derived neuronal line and rows define the z-score for each gene. n= 24 iPSC RNA samples collected from: 12 control samples (4 replicate wells from each of 3 control lines) and 12 CS samples (4 replicate wells from each of 3 CS lines), and n= 24 neuronal RNA samples collected from: 12 control samples (4 neuronal inductions from each of 3 control lines) and 12 CS samples (4 neuronal inductions from each of 3 CS lines).

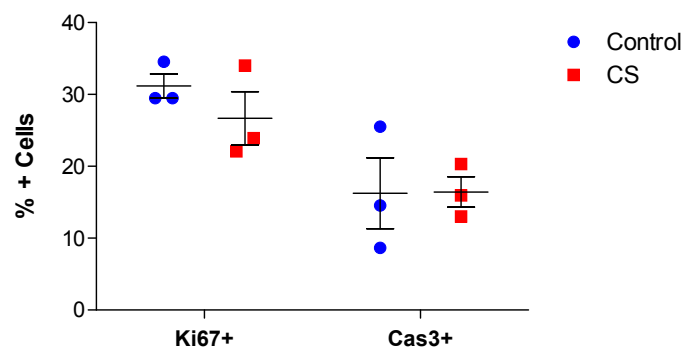
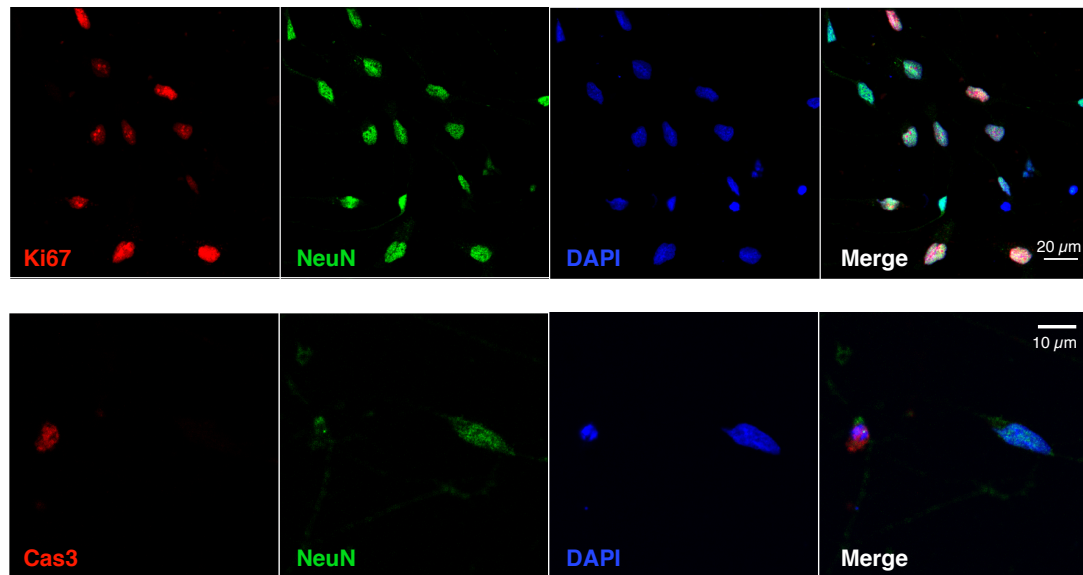


Figure 3.5 Measurement of proliferation and cell death in neuronal cultures. Immunohistochemistry was performed to stain proliferative cells, Ki67+, and apoptotic cells, Cas3+, in iPSC-derived neurons 35 days after neuronal induction. The percentage of cells that positively stained for Ki67 or Cas3 cells is quantified below. $n = 3$ independent neuronal inductions of iPSCs derived from the c.1568 G>A/p.W523X mutant line and the paired control line. For each staining, 200-300 cells per induction were counted for each genotype, mean $\pm$ sem.

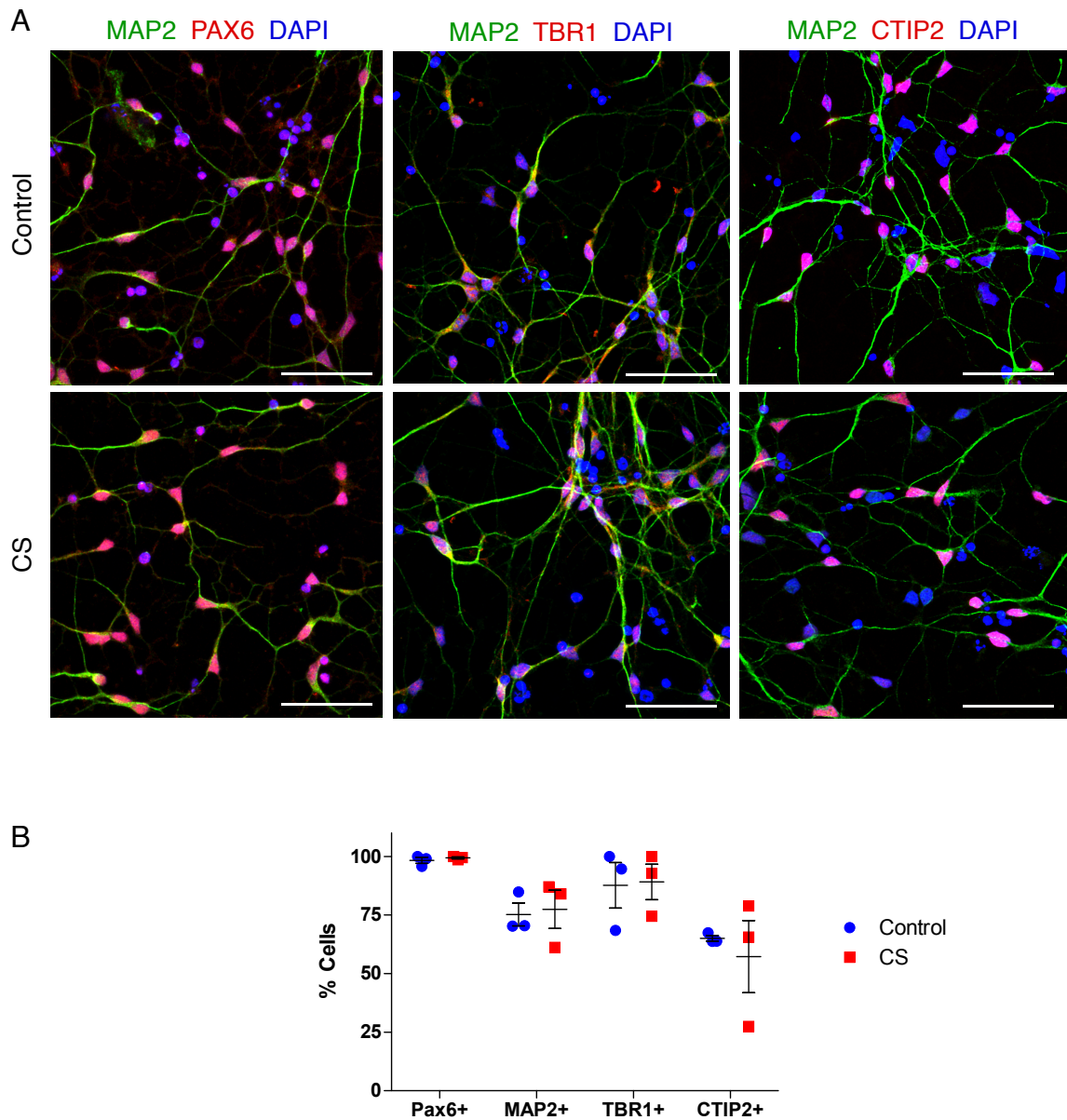


Figure 3.6 Neuronal and cortical marker expression in iPSC-derived neurons. (A) Immunohistochemistry was performed on CS and control iPSC-derived neurons 38 days after neuronal induction. Representative images are shown of cells stained for PAX6, CTIP2, TBR1, and MAP2. Scale bars, 50 μ m. (B) Quantification of the number of cells with positive staining for each marker is shown. $n = 3$ independent neuronal inductions of derived from the R472fsX4 mutant line and the paired control line. For PAX6, CTIP2, and TBR1, 100-200 cells were counted in each induction for each genotype. For MAP2, 300-600 cells were counted in each induction for each genotype, mean $\pm$ sem.

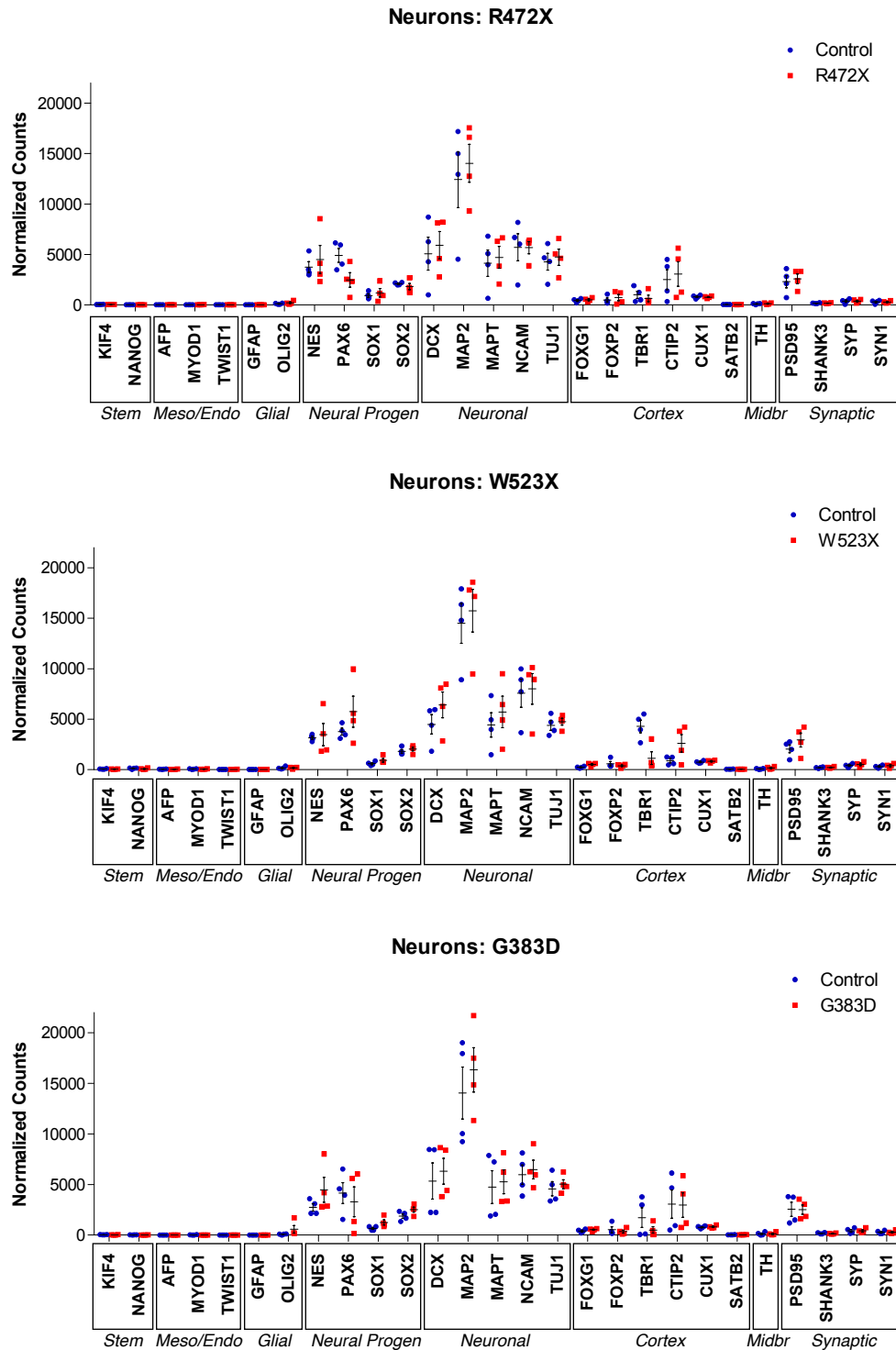


Figure 3.7 Gene expression of cell fate markers in iPSC-derived neurons. The expression of stem, mesoderm, endoderm, glial, neuronal, cortical, midbrain, and synaptic markers were measured using Nanostring in neuronal cultures differentiated for 38 days. Data was normalized to hybridization controls and to a set of housekeeping genes: *GUSB*, *HPRT1*, *RPL13A*, and *POLR2A*. Gene expression was compared between CS and control neuronal cultures using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. No significant changes in gene expression (adjusted p-value < 0.05) were seen. For each family, n= 4 control RNAs and four CS RNAs from paired neuronal inductions collected 38 days after neuronal induction, mean $\pm$ sem.

R472X iPSCs vs Control

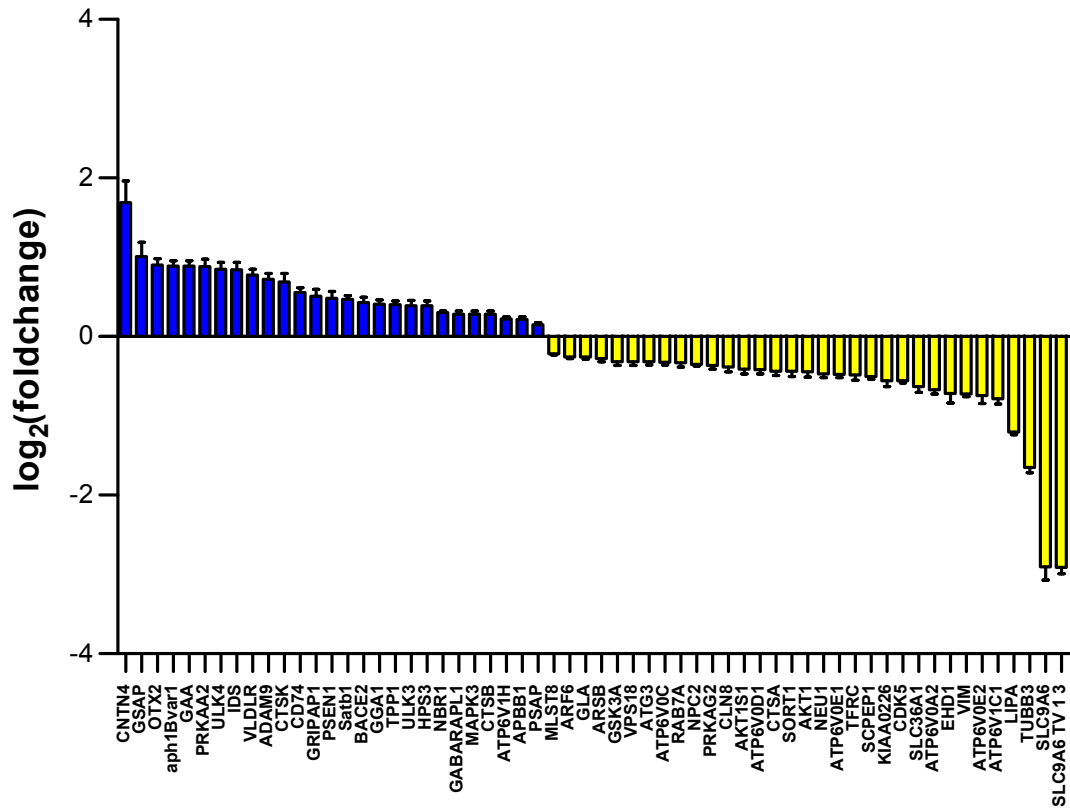


Figure 3.8 Changes in gene expression in R472fsX4 iPSCs. The expression of 358 genes was measured using Nanostring. Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *B2M*, *GUSB*, *GAPDH*, *HPRT1*, *POLR2A*, *RPL13A*, and *RPL27*. Changes in gene expression were compared between CS and control iPSCs using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. The log₂(foldchange) for genes with adjusted p-values < 0.05 is plotted showing standard error. n = 4 control RNAs collected from replicate wells of Family 1 Control iPSCs, and n = 4 CS RNAs collected from replicate wells of R472fsX4 CS iPSCs.

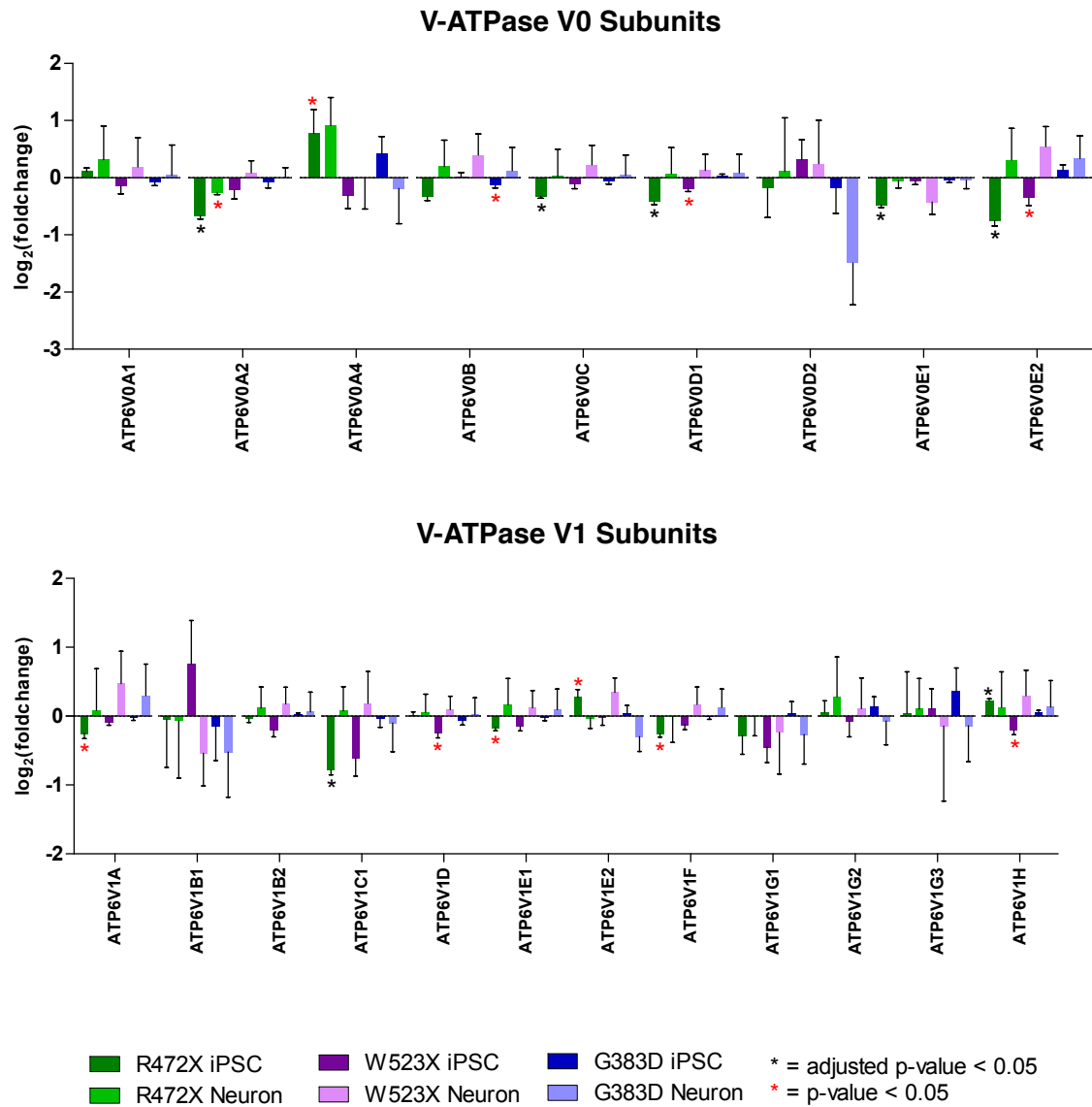


Figure 3.9 V-ATPase RNA expression levels in CS and control iPSCs and iPSC-derived neurons. The expression of the V0 and V1 subunits of the V-ATPase were measured using Nanostring in iPSCs and in neuronal cultures differentiated for 38 days. Data was normalized to hybridization controls and to a set of housekeeping genes. Gene expression was compared between each CS line and paired control in 1) iPSCs and 2) neuronal cultures using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. The log₂(foldchange) for each gene is plotted showing standard error. Adjusted p-values < 0.05 are indicated by a black asterisk and p-values < 0.05 before adjustment are indicated with a red asterisk. For each of the three CS individuals and the three control individuals, n = 4 iPSC RNA samples collected from replicates wells of iPSCs and n = 4 neuronal RNA samples collected from independent neuronal inductions.

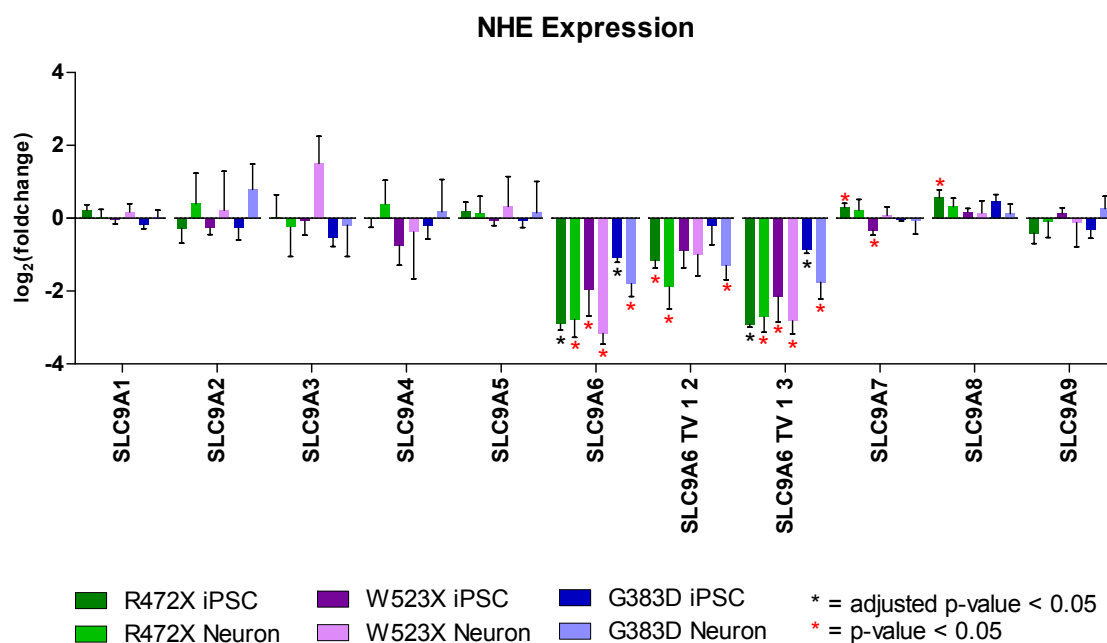


Figure 3.10 Expression of NHE family members in CS and control iPSCs and iPSC-derived neurons. The expression of NHEs was measured using Nanostring in iPSCs and in neuronal cultures differentiated for 38 days. Data was normalized to hybridization controls and to a set of housekeeping genes. Gene expression was compared between each CS line and paired control in 1) iPSCs and 2) neuronal cultures using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. *SLC9A6* has three probes with the probes labeled "TV 1 2" and "TV 1 3" recognizing transcript variants 1 (NM_001042537) and 2 (NM_006359) or 1 (NM_001042537) and 3 (NM_001177651), respectively. The "SLC9A6" probe recognizes all three transcript variants. The $\log_2(\text{foldchange})$ for each gene is plotted showing standard error. Adjusted p-values < 0.05 are indicated by a black asterisk and p-values < 0.05 before adjustment are indicated with a red asterisk. For each of the three CS individuals and the three control individuals, $n = 4$ iPSC RNA samples collected from replicates wells of iPSCs and $n = 4$ neuronal RNA samples collected from independent neuronal inductions.

Table 3.4 Genes upregulated in R472fsX4 iPSCs vs control iPSCs

Table 3.5 Genes downregulated in R472fsX4 iPSCs vs control iPSCs

Table 3.6 Genes upregulated in W523X iPSCs vs control iPSCs

Table 3.7 Genes downregulated in W523X iPSCs vs control iPSCs

Table 3.8 Genes upregulated in G383D iPSCs vs control iPSCs

Table 3.9 Genes downregulated in G383D iPSCs vs control iPSCs

The expression of 358 genes was measured using Nanostring in CS and control iPSCs.

Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *B2M*, *GUSB*, *GAPDH*, *HPRT1*, *POLR2A*, *RPL13A*, and *RPL27*. Changes in gene expression were compared between CS and control using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. For each Control/CS pair: n = 4 control RNAs collected from replicate wells of the paired control iPSCs, and n = 4 CS RNAs collected from replicate wells of CS iPSCs.

Table 3.4 Genes upregulated in R472fsX4 iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	R472X (Counts)	StDev of R472X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
NBR1	528.77	12.58	652.6	10.31	1.23	0.304	5.90E-06	0.00148	Autophagy
aph1Bvar1	268.38	19.19	497.47	29.38	1.85	0.89	1.14E-05	0.00235	Neurodegeneration
GAA	138.45	11.5	256.28	11.59	1.85	0.888	1.45E-05	0.00272	Lysosomal
Satb1	458.14	20.78	635.99	22.76	1.39	0.473	2.92E-05	0.00403	Neurodevelopment
OTX2	142.23	11.6	265.87	20.77	1.87	0.902	3.51E-05	0.00418	Neurodevelopment
VLDLR	782.88	64.12	1339.88	93.4	1.71	0.775	5.22E-05	0.00562	Neurodegeneration
CD74	294.17	21.22	433.74	11.69	1.47	0.56	6.36E-05	0.00653	Neurodegeneration
ADAM9	121.75	10.36	200.82	11.55	1.65	0.722	6.73E-05	0.00662	Neurodegeneration
PRKAA2	184.86	17.8	341.38	31.44	1.85	0.885	8.03E-05	0.00698	Autophagy
ULK4	79.31	6.15	142.72	13.65	1.8	0.848	7.88E-05	0.00698	Autophagy
IDS	136.08	6.13	244.65	28.79	1.8	0.846	8.71E-05	0.00729	LSD
ATP6V1H	513.33	9.87	600.22	17.66	1.17	0.226	0.000113	0.00878	vATPase
TPP1	283.4	12.54	373.97	22.15	1.32	0.4	0.000255	0.016	LSD
GGA1	106.91	5.98	141.72	7.54	1.33	0.407	0.000314	0.0187	Endosome
GABARAPL1	967.22	54.81	1176.32	12.1	1.22	0.282	0.000439	0.0242	Autophagy
MAPK3	484.76	12.02	589.36	30.19	1.22	0.282	0.000487	0.0253	Signaling
APBB1	219.34	6.33	254.8	8.34	1.16	0.216	0.000484	0.0253	Neurodegeneration
PSAP	3628.11	84.31	4027.52	81.79	1.11	0.151	0.000522	0.0262	Lysosomal
HPS3	1003.39	77.45	1315.39	35.6	1.31	0.391	0.000555	0.0267	Lysosomal
BACE2	98.39	6.62	132.8	8.42	1.35	0.433	0.000628	0.0296	Neurodegeneration
CTSK	37.37	5.41	60.25	3.27	1.61	0.689	0.000662	0.0299	Lysosomal
CTSB	321.87	9.86	390.85	20.05	1.21	0.28	0.000657	0.0299	Lysosomal
CNTN4	7.77	2.41	25.06	3.49	3.23	1.69	0.000811	0.034	Neurodegeneration
ULK3	311.75	26.75	409.28	13.99	1.31	0.393	0.00103	0.0415	Autophagy
GRIPAP1	226.01	15.96	321.53	31.53	1.42	0.509	0.00113	0.0442	Endosome
PSEN1	463.68	46.61	649.23	37.86	1.4	0.486	0.00112	0.0442	Neurodegeneration
GSAP	11.27	0.84	22.74	5.09	2.02	1.01	0.0013	0.049	Neurodegeneration
SUMF1	190.83	6.85	225.22	10.56	1.18	0.239	0.00146	0.0534	Lysosomal
ASAH1	581.03	40.73	713.19	25.21	1.23	0.296	0.00183	0.0649	Lysosomal
HGSNAT	23.14	2.88	38.96	7.39	1.68	0.752	0.00365	0.11	LSD
ATG16L2	49.43	8.06	81.14	11.85	1.64	0.715	0.00359	0.11	Autophagy
TACE/ADAM17	306.23	29.07	390.41	15.2	1.27	0.35	0.00364	0.11	Neurodegeneration
MAP2	20.58	4.65	32.27	1.25	1.57	0.649	0.00568	0.161	Neurodevelopment
ULK2	61.95	8.39	85.64	6.16	1.38	0.467	0.00593	0.163	Autophagy
UVRAG	174.59	15.45	218.42	15.01	1.25	0.323	0.00615	0.168	Endosome
TP73	37.98	8.48	59.53	2.34	1.57	0.648	0.00715	0.188	Neurodevelopment
CLN5	79.93	3.2	104.3	13.32	1.3	0.384	0.00709	0.188	LSD
HEXA	246.7	6.74	274.68	13.21	1.11	0.155	0.00799	0.205	Lysosomal
NCAM	40.67	6.35	58.14	4.98	1.43	0.516	0.00817	0.207	Neurodevelopment
HPS1	47.41	7.41	69.62	9.32	1.47	0.554	0.00885	0.222	Lysosomal
PEX19	473.18	41.34	573.63	27.09	1.21	0.278	0.00904	0.224	LSD
NGFR	69.97	6.64	87.64	7.23	1.25	0.325	0.0102	0.246	Signaling
TTBK2	153.22	30.24	221.09	4.46	1.44	0.529	0.0104	0.247	Neurodegeneration
SQSTM1	174.23	16.99	210.3	6.42	1.21	0.271	0.0105	0.247	Autophagy
LAMP2	920.88	72.21	1068.27	32.82	1.16	0.214	0.0125	0.287	LSD
PVALB	25.52	6.96	44.35	7.13	1.74	0.797	0.0142	0.315	Neurodevelopment
WDR45L	1038.64	60.73	1170.79	44.38	1.13	0.173	0.0148	0.325	Autophagy
PHYH	56.13	6.34	69.07	3.55	1.23	0.299	0.0156	0.333	LSD
GLB1	325.92	37.35	401	19.9	1.23	0.299	0.0164	0.347	Lysosomal
WIP1	129.36	11.47	155.92	11.22	1.21	0.269	0.0169	0.351	Autophagy
WDFY2	176.48	16.36	211.83	14.78	1.2	0.263	0.0182	0.37	Endosome
WIP12	217.18	19.63	253.85	8.9	1.17	0.225	0.0183	0.37	Autophagy
AGA	156.04	9.73	189.61	20.04	1.22	0.281	0.02	0.401	LSD
APBB3	25.28	7.95	41.09	6.31	1.63	0.701	0.0213	0.419	Neurodegeneration
BDNF	132.19	13.77	157.66	6.8	1.19	0.254	0.0235	0.449	Signaling
VPS8	344.15	19.61	389.43	26.04	1.13	0.178	0.0294	0.543	Endosome
NAE1	2799.97	149.92	3033.87	51.78	1.08	0.116	0.0296	0.543	Neurodegeneration
LRP1	193.02	72.18	316.29	41.26	1.64	0.712	0.0337	0.59	Neurodegeneration
TFEB	16.48	3.88	23.58	2.94	1.43	0.517	0.0333	0.59	Lysosomal
Iba1	144.47	14.64	169.43	9.17	1.17	0.23	0.0359	0.619	Neurodevelopment
SLC9A8	29.15	7.75	43.01	7.62	1.48	0.561	0.0403	0.684	Endosome
PEX1	424.53	39.52	499.06	40.18	1.18	0.233	0.04	0.684	LSD
HPS5	250.9	25.44	290.47	17.23	1.16	0.211	0.0409	0.684	Lysosomal
ATP6V1E2	103.15	14.87	124.89	7.64	1.21	0.276	0.0447	0.733	vATPase
SIRT1	1516.36	73.74	1676.23	102.79	1.11	0.145	0.0453	0.736	Autophagy
CTSC	3911.98	69.53	4066.76	103.89	1.04	0.056	0.0474	0.765	LSD
SLC9A7	274.72	41.06	336.65	26.79	1.23	0.293	0.049	0.779	Endosome
Rab10	862.45	50.62	932.45	22.48	1.08	0.113	0.0495	0.782	Endosome
NAGLU	125.78	12.35	144.24	8.37	1.15	0.198	0.0538	0.827	Lysosomal
PEX5	395.66	36.59	442.7	13.06	1.12	0.162	0.0537	0.827	LSD
TJP1	2217.7	223.73	2528.54	113.12	1.14	0.189	0.0547	0.835	Neurodevelopment
NTF4	60.33	15.15	82.42	8.89	1.37	0.45	0.0605	0.906	Signaling
ULK1	195.95	18.7	226.07	19.89	1.15	0.206	0.0625	0.928	Autophagy

HEXB	869.16	50.56	951.25	51.22	1.09	0.13	0.0649	0.952	Lysosomal
ATP6V0A4	13.37	7.97	22.9	7.22	1.71	0.776	0.113	1	vATPase
HOXA1	1.77	0.91	2.93	2.05	1.66	0.73	0.298	1	Neurodevelopment
MNX1	4.2	4.41	6.65	6.06	1.58	0.662	0.493	1	Neurodevelopment
Sox10	6.47	3.77	9.83	2.48	1.52	0.604	0.276	1	Neurodevelopment
NEDD4	39.32	15.43	58.28	8.31	1.48	0.568	0.102	1	Endosome
NTRK1	3.14	1.88	4.49	1.89	1.43	0.515	0.427	1	Signaling
NTRK3	12.27	4.45	16.68	3.66	1.36	0.443	0.186	1	Signaling
KMT2D	70.01	28.71	91.19	6.3	1.3	0.381	0.175	1	LSD
FOXP2	9.4	2.75	11.71	3.94	1.25	0.317	0.376	1	Neurodevelopment
RELN	5.7	2.44	7.1	2.4	1.25	0.317	0.42	1	Neurodegeneration
VAMP1	52	9.41	65	6.34	1.25	0.322	0.0778	1	Endosome
SLC17A6	19.29	6.05	23.5	6.16	1.22	0.285	0.349	1	Neurodevelopment
ATG2B	165.18	25.86	199.8	16.35	1.21	0.275	0.0863	1	Autophagy
SORL1	380.77	60.06	451.47	19.8	1.19	0.246	0.0706	1	Endosome
STX13	178.74	27.1	212.72	8.13	1.19	0.251	0.0735	1	Endosome
WDFY3	240.2	44.85	286.51	19.84	1.19	0.254	0.116	1	Autophagy
ARSA	35.85	3.44	42.48	6.38	1.18	0.245	0.111	1	LSD
PLBD2	108.04	25.34	127.23	21.05	1.18	0.236	0.28	1	Autophagy
NGF	9.45	4.51	11.08	2.76	1.17	0.229	0.541	1	Signaling
TCF4	283.27	37.56	331.08	33.82	1.17	0.225	0.115	1	LSD
GAD1	10.16	4.12	11.77	4.46	1.16	0.212	0.627	1	Lysosomal
RICTOR	234.24	33.93	271.32	7.14	1.16	0.212	0.0846	1	Signaling
SLC9A1	192.89	24.72	223.82	37.43	1.16	0.215	0.211	1	Endosome
GAD2	6.58	4.71	7.56	2.08	1.15	0.2	0.697	1	Neurodevelopment
SLC9A5	27.05	8.62	31.04	3.39	1.15	0.199	0.446	1	Endosome
NAGA	79.68	5.71	90.52	8.83	1.14	0.184	0.0843	1	LSD
CLCN3	115.33	3.89	129.97	16.24	1.13	0.172	0.101	1	Endosome
HOXB1	11.12	6.51	12.59	4.15	1.13	0.179	0.77	1	Neurodevelopment
MARK1	267.43	39.41	302.29	16.75	1.13	0.177	0.163	1	Neurodegeneration
OLIG2	14.02	3.87	15.85	2.85	1.13	0.177	0.458	1	Neurodevelopment
NTF3	12.87	5.18	14.47	3.12	1.12	0.17	0.663	1	Signaling
USP13	396.84	60.35	442.73	26.42	1.12	0.158	0.22	1	Autophagy
APBA2	59.5	4.27	66.02	7.3	1.11	0.15	0.18	1	Neurodegeneration
CUX2	75.76	10.29	84.07	4.68	1.11	0.15	0.206	1	Neurodevelopment
DLG4	219.34	21.58	244.45	25.28	1.11	0.156	0.184	1	Neurodevelopment
HGS	48.6	4.51	53.76	1.07	1.11	0.146	0.0792	1	Endosome
SGSH	33	11.31	36.59	3.64	1.11	0.149	0.558	1	LSD
TFE3	64.07	11.98	71.01	3.8	1.11	0.148	0.329	1	Lysosomal
FUCA1	206.68	10.11	227.25	25.45	1.1	0.137	0.172	1	LSD
IGF2R	217.95	26.98	240.56	23.01	1.1	0.142	0.246	1	Lysosomal
TMEM55B	102.8	13.16	112.84	7.79	1.1	0.134	0.249	1	Lysosomal
ATG7	165.44	18.14	180.88	3.07	1.09	0.129	0.145	1	Autophagy
VPS26A	435.53	29.81	475.81	32.63	1.09	0.128	0.121	1	Endosome
ATG4B	1425.33	104.33	1536.89	36.07	1.08	0.109	0.0974	1	Autophagy
ATP6V0A1	321.08	15.01	346.79	24.64	1.08	0.111	0.119	1	vATPase
BLOC1S1	989.18	89.6	1064.01	27.36	1.08	0.105	0.173	1	Lysosomal
GALN5	90.51	2.3	97.53	9.05	1.08	0.108	0.165	1	Lysosomal
IDUA	55.68	8.21	59.98	5.96	1.08	0.107	0.458	1	LSD
VPS35	660.98	35.32	711.48	33.23	1.08	0.106	0.0818	1	Endosome
CLCN7	313.8	30.9	334.36	9.03	1.07	0.0916	0.271	1	Endosome
STH	6.78	2.96	7.23	2.37	1.07	0.0942	0.823	1	Neurodegeneration
CALB2	39.76	10.02	42.1	3.54	1.06	0.0828	0.705	1	Neurodevelopment
GALC	30.63	4.95	32.39	5.98	1.06	0.0804	0.665	1	LSD
MTOR	499.87	96.86	528.96	17.15	1.06	0.0816	0.582	1	Signaling
NAGPA	28.85	9.75	30.66	3.87	1.06	0.0879	0.72	1	Lysosomal
STX6	871.14	75.13	919.77	20.86	1.06	0.0784	0.269	1	Endosome
APLP1	195.01	13.35	204.23	9.49	1.05	0.0666	0.308	1	Neurodegeneration
APP-770	4095.74	379.69	4297.63	224.9	1.05	0.0694	0.398	1	Neurodegeneration
Cux1	321.84	47.9	338.7	21.62	1.05	0.0737	0.55	1	Neurodevelopment
ATG4D	274.28	11.86	285.75	10.72	1.04	0.0591	0.202	1	Autophagy
ATP6V1G2	37.37	5.05	38.89	6.92	1.04	0.0574	0.737	1	vATPase
CNTN2	13.09	3.61	13.63	1.8	1.04	0.0591	0.801	1	Neurodegeneration
LRP10	181.46	3.25	188.97	9.42	1.04	0.0585	0.173	1	Neurodegeneration
MAN2B1	384.18	43.32	399.62	10.9	1.04	0.0569	0.502	1	LSD
NCSTN	524.84	40.46	548.23	29.72	1.04	0.0629	0.401	1	Neurodegeneration
PTEN	497.39	19.41	518.53	59.72	1.04	0.06	0.516	1	Signaling
RAB5A	1574.06	55.27	1638.56	99.21	1.04	0.0579	0.299	1	Endosome
Sox2	1789.64	165.26	1855.51	30.73	1.04	0.0521	0.469	1	Neurodevelopment
SYN1	40.97	2.91	42.74	2.68	1.04	0.061	0.412	1	Neurodevelopment
aph1Bvar2	92.52	2.47	95.5	14.26	1.03	0.0458	0.693	1	Neurodegeneration
ATP6V1G3	5.58	4.38	5.74	3.15	1.03	0.0422	0.946	1	vATPase
CREB1	359.38	21.81	370.43	18.84	1.03	0.0437	0.479	1	Signaling
DRAM1	98.43	11.03	101.01	9.5	1.03	0.0374	0.74	1	Autophagy
HIF1A	3266	197.79	3347.83	127.76	1.03	0.0357	0.514	1	Neurodevelopment
MAP1LC3B	669.64	66.22	688.8	23.22	1.03	0.0407	0.618	1	Autophagy
RB1CC1	451	36.29	462.34	14.87	1.03	0.0358	0.581	1	Autophagy

AKT3	395.05	30.25	403.93	13.94	1.02	0.0321	0.627	1	Signaling
MFSD8	142.07	14.99	144.41	8.68	1.02	0.0235	0.803	1	LSD
PEX13	338.68	31.87	344.46	25.6	1.02	0.0244	0.786	1	LSD
RPTOR	113.92	9.11	115.89	10.09	1.02	0.0247	0.788	1	Signaling
USP10	1806.81	110.92	1851.85	41.68	1.02	0.0355	0.478	1	Autophagy
APPBP2	235.98	12.56	237.74	13.9	1.01	0.0107	0.857	1	Neurodegeneration
APPL1	833.64	63.5	842.96	47.13	1.01	0.016	0.823	1	Endosome
ATG5	501.3	36.38	504.79	16.18	1.01	0.00999	0.868	1	Autophagy
ATP6V1D	424.43	27.08	426.81	15.42	1.01	0.00807	0.884	1	vATPase
CDK5R1	119.77	24.93	120.98	14	1.01	0.0145	0.938	1	Neurodegeneration
CSNK1D	818.75	34.76	828.56	28.45	1.01	0.0172	0.681	1	Neurodegeneration
GGA2	868.57	80.41	874.2	22.82	1.01	0.00933	0.897	1	Endosome
GSK3B	816.8	79.99	821.51	34.38	1.01	0.0083	0.918	1	Neurodegeneration
MAPK1	929.13	63.92	935.95	25.88	1.01	0.0105	0.849	1	Signaling
MARK3	392.8	18.7	396.3	18.56	1.01	0.0128	0.798	1	Neurodegeneration
SLC12A5	12.58	1.13	12.68	4.03	1.01	0.0114	0.966	1	Neurodevelopment
SLC9A3	6.58	1.81	6.65	4.37	1.01	0.0153	0.981	1	Endosome
APLP2	2820.89	174.11	2829.73	70.55	1	0.00452	0.928	1	Neurodegeneration
APP-KPI	449.11	40.55	451.14	16.48	1	0.00652	0.929	1	Neurodegeneration
Fezf2	4.33	1.84	4.35	5.48	1	0.00516	0.995	1	Neurodevelopment
NECAP2	403.58	41.9	403.63	19.37	1	0.000165	0.998	1	Endosome
PRKAA1	224	20.86	224.13	17.19	1	0.000803	0.993	1	Autophagy
WDR45	139.52	12.92	139.88	12.59	1	0.0038	0.969	1	Autophagy

Table 3.5 Genes downregulated in R472fsX4 iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	R472X (Counts)	StDev of R472X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
SLC9A6 TV 1 3	295.8	17.55	39.3	3.74	-7.53	-2.91	3.01E-08	6.80E-05	Endosome
LIPA	1493.01	62.18	647.93	22.2	-2.3	-1.2	7.86E-08	8.88E-05	LSD
TUBB3	484.69	24.26	154.06	12.1	-3.15	-1.65	2.98E-07	0.000224	Neurodevelopment
VIM	1091.08	3.65	661.05	29.5	-1.65	-0.723	5.23E-07	0.000296	Neurodevelopment
NPC2	4481.79	99.73	3516.93	36.38	-1.27	-0.35	1.08E-06	0.000487	LSD
CDK5	289.68	8.37	196.98	6.07	-1.47	-0.556	1.69E-06	0.000637	Neurodegeneration
MLST8	417.63	3.65	358.97	5.44	-1.16	-0.218	2.41E-06	0.000764	Signaling
SLC9A6	127.2	12.74	17.03	4.03	-7.47	-2.9	2.70E-06	0.000764	Endosome
SCPEP1	681.77	27.89	481.78	14.55	-1.42	-0.501	1.05E-05	0.00235	LSD
ATP6V0A2	253.27	16.44	159.48	7.38	-1.59	-0.667	2.15E-05	0.0035	vATPase
ATP6V1C1	271.37	24.43	157.37	2.89	-1.72	-0.786	2.17E-05	0.0035	vATPase
GLA	803.91	19.98	671.97	12.65	-1.2	-0.259	2.70E-05	0.00403	Lysosomal
ATP6V0E1	2458	128.74	1768.12	48.33	-1.39	-0.475	3.03E-05	0.00403	vATPase
ARF6	1754.95	42.96	1471.61	30.74	-1.19	-0.254	3.45E-05	0.00418	Endosome
NEU1	195.59	9.02	141.23	6.1	-1.38	-0.47	4.71E-05	0.00532	Lysosomal
ATG3	1400.39	53.12	1121.49	30.34	-1.25	-0.32	7.57E-05	0.00698	Autophagy
SLC36A1	105.9	6.87	68.37	5.01	-1.55	-0.631	0.000102	0.00826	Lysosomal
ATP6V0C	1768.82	86.96	1416.03	23.45	-1.25	-0.321	0.000126	0.00952	vATPase
ATP6V0D1	996.12	62.15	744.79	27.16	-1.34	-0.419	0.000183	0.0134	vATPase
ARSB	324.68	14.77	267.33	5.88	-1.21	-0.28	0.000232	0.0159	LSD
PRKAG2	453.45	22.69	352.48	14.58	-1.29	-0.363	0.000229	0.0159	Signaling
CTSA	474.55	33.09	351.21	12.52	-1.35	-0.434	0.000241	0.016	Lysosomal
ATP6V0E2	74.28	6.29	44.27	4.72	-1.68	-0.746	0.000254	0.016	vATPase
KIAA0226	43.44	1.88	29.59	2.7	-1.47	-0.554	0.00029	0.0177	Autophagy
TFRC	1598.29	115.9	1142.81	70.48	-1.4	-0.484	0.000393	0.0228	Endosome
GSK3A	1081.23	60.06	869.34	24.55	-1.24	-0.315	0.000426	0.0241	Neurodegeneration
AKT1S1	342.64	25.45	257.58	8.8	-1.33	-0.412	0.000493	0.0253	Autophagy
AKT1	242.32	11.46	178.39	14.25	-1.36	-0.442	0.00054	0.0265	Signaling
CLN8	333.7	22.51	255.71	12.54	-1.3	-0.384	0.000708	0.0314	LSD
RAB7A	1996.38	125.25	1585.41	67.42	-1.26	-0.333	0.000801	0.034	Lysosomal
SORT1	1239.23	73.39	916.1	71.22	-1.35	-0.436	0.000801	0.034	Endosome
VPS18	241.92	4.18	194.41	13.38	-1.24	-0.315	0.000856	0.0352	Endosome
EHD1	126.16	14.98	76.87	9.16	-1.64	-0.715	0.00121	0.0462	Endosome
ATP6V1F	1456.32	78.13	1217.92	43.8	-1.2	-0.258	0.00144	0.0534	vATPase
PPP2R4	1532.75	72.13	1334.62	31.81	-1.15	-0.2	0.00184	0.0649	Neurodegeneration
SLC9A6 TV 1 2	14.47	4.27	6.51	0.96	-2.22	-1.15	0.00191	0.0665	Endosome
BECN1	1040.46	38.74	883.99	45.65	-1.18	-0.235	0.00223	0.0763	Autophagy
ATG16L1	357.15	26.75	280.67	18.57	-1.27	-0.348	0.00301	0.102	Autophagy
DNAJC5	91.42	8.59	71.24	3.96	-1.28	-0.36	0.00318	0.106	LSD
ATP6V0B	656.9	59.14	522.7	17.87	-1.26	-0.33	0.00326	0.107	vATPase
MARK2	439.51	18.62	363.33	25.13	-1.21	-0.275	0.00353	0.11	Neurodegeneration
STK4	574.99	48.25	455.72	24.34	-1.26	-0.335	0.00341	0.11	Signaling
GNPTAB	730.43	83.78	550.39	26.61	-1.33	-0.408	0.00366	0.11	LSD
S100b	18.76	5.16	8.99	1.14	-2.09	-1.06	0.0039	0.116	Neurodevelopment
MCOLN1	83.1	11.15	61.08	3.55	-1.36	-0.444	0.00432	0.127	Lysosomal
GFAP	10.1	1.55	6.32	0.87	-1.6	-0.676	0.00467	0.135	Neurodevelopment
POU5F1	9555.47	435.12	8642.34	61.57	-1.11	-0.145	0.0048	0.137	Neurodevelopment
APOE	3410.46	113.25	3174.58	27.05	-1.07	-0.103	0.00585	0.163	Neurodegeneration
C1ORF85	481.66	11.52	432.06	21.38	-1.11	-0.157	0.00702	0.188	Lysosomal
APOER2	341.66	34.17	252.33	29.86	-1.35	-0.437	0.00763	0.198	Neurodegeneration
ATP6V1E1	1798.27	102.6	1600.9	36.72	-1.12	-0.168	0.00921	0.226	vATPase
CLN6	398.71	23.97	345.56	16.31	-1.15	-0.206	0.0099	0.241	LSD
SNCA	66.8	7.19	44.74	9.36	-1.49	-0.578	0.0123	0.287	Neurodegeneration
EHD4	394.81	28.5	341.78	12.63	-1.16	-0.208	0.0127	0.291	Endosome
HYAL1	69.25	12.76	45.46	7.1	-1.52	-0.607	0.0129	0.291	LSD
B3GAT1	94.09	3.79	68.49	11.88	-1.37	-0.458	0.0136	0.305	Neurodevelopment
ATP6V1A	1977.25	194.66	1661.95	54.85	-1.19	-0.251	0.0154	0.333	vATPase
PIK3R4	471.63	40.29	395.39	23.65	-1.19	-0.254	0.0155	0.333	Autophagy
Rab4	660.4	16.36	615.2	21.87	-1.07	-0.102	0.0166	0.348	Endosome
PSENEN	1013.94	84.2	876.4	32.19	-1.16	-0.21	0.0183	0.37	Neurodegeneration
NPY	20.28	4.91	11.83	3.29	-1.71	-0.778	0.0205	0.406	Neurodevelopment
ATP13A2	297.22	8.04	268.81	16.13	-1.11	-0.145	0.0225	0.435	LSD
SATB2	33.12	5.16	24.26	3.33	-1.37	-0.449	0.0224	0.435	Neurodevelopment
NRBF2	1029.08	53.74	934	34.97	-1.1	-0.14	0.0241	0.459	Autophagy
ATG2A	82.82	4.19	67.72	8.81	-1.22	-0.29	0.0272	0.511	Autophagy
Tau_3R	18.88	1.87	13.22	2.88	-1.43	-0.514	0.0289	0.54	Neurodegeneration
RABGEF1	387.83	25.76	350.63	9.57	-1.11	-0.145	0.03	0.543	Endosome
MANBA	126.32	12.48	106.24	7.41	-1.19	-0.25	0.0298	0.543	LSD
KIF4	70.66	6.41	50.68	10.79	-1.39	-0.479	0.0329	0.59	Neurodevelopment
FoxG1	14.92	5.76	8.54	1.76	-1.75	-0.804	0.0334	0.59	Neurodevelopment
BLOC1S3	19.43	2.95	15.57	0.96	-1.25	-0.319	0.034	0.591	Lysosomal
SLC17A5	243.72	11.8	218.7	15.3	-1.11	-0.156	0.0405	0.684	LSD
GABARAPL2	1279.6	46.69	1207.33	33.11	-1.06	-0.0839	0.0436	0.721	Autophagy

CCND1	1116.78	102.67	988.28	28.52	-1.13	-0.176	0.0437	0.721	Neurodevelopment
AKT2	688.05	45.52	633.17	10.51	-1.09	-0.12	0.0485	0.777	Signaling
BCL11B	70.43	4.35	56.65	9.78	-1.24	-0.314	0.052	0.816	Neurodevelopment
CTNS	71.69	6.3	63.38	3.53	-1.13	-0.178	0.0525	0.819	Lysosomal
MBP	105.49	10.5	92.51	4.72	-1.14	-0.19	0.0568	0.862	Neurodevelopment
GBA	200.17	4.02	183.53	13.47	-1.09	-0.125	0.0603	0.906	Lysosomal
LAMP1	2049.86	131.83	1890	56.32	-1.08	-0.117	0.0628	0.928	Lysosomal
VPS33A	347.18	26.87	346.68	28.75	-1	-0.0021	0.981	1	Endosome
ADAM19	84.94	4.88	84.08	4.07	-1.01	-0.0147	0.797	1	Neurodegeneration
DCX	30.43	7.95	30.18	9.5	-1.01	-0.0116	0.968	1	Neurodevelopment
GNPTG	214.06	13.02	211.67	13.55	-1.01	-0.0163	0.807	1	Lysosomal
PSEN2	267.36	19.38	263.83	24.69	-1.01	-0.0192	0.83	1	Neurodegeneration
SLC9A4	9.8	2	9.67	2.42	-1.01	-0.0195	0.935	1	Endosome
AFP	7.32	0.91	7.15	2.86	-1.02	-0.0337	0.908	1	Neurodevelopment
AMY	229.15	28.49	224.32	7.06	-1.02	-0.0308	0.759	1	Neurodegeneration
aph1Avar4	355.08	20.65	348.11	12.64	-1.02	-0.0286	0.582	1	Neurodegeneration
ATG10	295.96	31.84	290.85	14.61	-1.02	-0.0251	0.78	1	Autophagy
CALB1	416.74	24.46	407.96	28.7	-1.02	-0.0308	0.661	1	Neurodevelopment
LHX6	6.36	1.93	6.25	2.61	-1.02	-0.0272	0.947	1	Neurodevelopment
M6PR	617.88	25.36	606	22.13	-1.02	-0.028	0.509	1	Lysosomal
MAP1LC3A	111.89	17.04	109.62	4.74	-1.02	-0.0295	0.804	1	Autophagy
RRAGC	250.49	13.39	245.74	10.95	-1.02	-0.0276	0.603	1	Lysosomal
VPS11	83.9	4.16	82.61	7.85	-1.02	-0.0224	0.774	1	Endosome
APBA3	68.47	5.75	66.59	8.91	-1.03	-0.0401	0.733	1	Neurodegeneration
ATP6V1B2	892.66	60.45	869.61	29.25	-1.03	-0.0377	0.517	1	vATPase
nanog	2002.23	123.14	1942.44	79.85	-1.03	-0.0437	0.442	1	Neurodevelopment
NES	1002.07	145.24	968.66	55.81	-1.03	-0.0489	0.663	1	Neurodevelopment
PEX16	180.47	12.81	175.59	21.12	-1.03	-0.0396	0.701	1	LSD
PPT1	2700.03	97.04	2630.63	105.19	-1.03	-0.0376	0.37	1	Lysosomal
SYP	16.38	5.4	15.84	5.14	-1.03	-0.0482	0.889	1	Neurodevelopment
Tau_4R	36.19	6.11	35.05	5.34	-1.03	-0.0462	0.799	1	Neurodegeneration
ATP6V1B1	2.97	0.88	2.87	3.58	-1.04	-0.0504	0.944	1	vATPase
CTSD	1102.5	55.94	1060.7	43.4	-1.04	-0.0558	0.284	1	Lysosomal
NMDA NR1	701.76	54.21	676.05	10.58	-1.04	-0.0538	0.378	1	Neurodevelopment
PEX3	261.54	16.44	251.29	19.1	-1.04	-0.0577	0.452	1	LSD
RAB9A	741.03	50.06	713.6	24.31	-1.04	-0.0544	0.346	1	Endosome
AP1S2	633.09	70.03	604.08	19.39	-1.05	-0.0677	0.427	1	Endosome
APP-All	5956.09	371.77	5652.85	158.64	-1.05	-0.0754	0.176	1	Neurodegeneration
ATG9A	485.91	41.02	463.3	26.88	-1.05	-0.0687	0.389	1	Autophagy
CCK	42.46	6.9	40.52	4.81	-1.05	-0.0674	0.668	1	Neurodevelopment
DLX1	10.97	1.31	10.49	3.88	-1.05	-0.0646	0.837	1	Neurodevelopment
GM2A	511.81	45.56	488.24	4.33	-1.05	-0.068	0.329	1	LSD
MAP4	1450.4	133.4	1379.4	52.52	-1.05	-0.0724	0.337	1	Neurodegeneration
NPC1	328.08	42.37	312.48	14.68	-1.05	-0.0703	0.51	1	LSD
Rab11	301.3	62.33	286.26	32.38	-1.05	-0.0739	0.662	1	Endosome
aph1Avar3	691.71	25.47	654.13	26.09	-1.06	-0.0806	0.0872	1	Neurodegeneration
BACE1	144.09	10.66	135.54	3.47	-1.06	-0.0882	0.158	1	Neurodegeneration
CD63	3813.27	225.38	3600.47	97.95	-1.06	-0.0828	0.128	1	Lysosomal
GABARAP	2203.86	198.32	2077.44	54.17	-1.06	-0.0852	0.246	1	Autophagy
IGF1R	334.27	77.09	315.88	26.54	-1.06	-0.0816	0.647	1	Signaling
ATG4C	698.73	51.38	652.51	28.22	-1.07	-0.0987	0.153	1	Autophagy
EEA1	353.44	47.63	330.18	18.7	-1.07	-0.0982	0.389	1	Endosome
GNS	229.85	19.49	214.3	9.99	-1.07	-0.101	0.196	1	Lysosomal
PICALM	564.44	52.91	528.85	29.63	-1.07	-0.094	0.271	1	Neurodegeneration
ATG101	433.74	24.93	402.66	21.21	-1.08	-0.107	0.107	1	Autophagy
ATG14	83.66	4.75	77.2	11.38	-1.08	-0.116	0.334	1	Autophagy
Tbr1	6.03	2.27	5.59	1.67	-1.08	-0.108	0.754	1	Neurodevelopment
ADAM10	1031.32	93.43	949.92	26.21	-1.09	-0.119	0.127	1	Neurodegeneration
GGA3	654.22	53.97	600.27	24.01	-1.09	-0.124	0.111	1	Endosome
Rab35	1050.2	78.45	963.65	27.44	-1.09	-0.124	0.0723	1	Endosome
SLC12A2	135.49	17.76	124.34	7.82	-1.09	-0.124	0.303	1	Neurodevelopment
APP-695	6404.79	608.89	5829.74	276.66	-1.1	-0.136	0.134	1	Neurodegeneration
MAPT	53.39	11.04	48.67	8.61	-1.1	-0.134	0.508	1	Neurodevelopment
DAB1	107	6.61	96.29	9.49	-1.11	-0.152	0.13	1	Neurodegeneration
CLN3	69.53	14.95	62.07	3.93	-1.12	-0.164	0.332	1	LSD
LRP3	148.91	23.41	132.97	7.65	-1.12	-0.163	0.246	1	Endosome
POU3F3	6.36	1.33	5.69	3.39	-1.12	-0.162	0.703	1	Neurodevelopment
TH	14.47	1.86	12.86	4.36	-1.12	-0.17	0.55	1	Neurodevelopment
ATG12	86.7	9.23	76.62	5.85	-1.13	-0.178	0.112	1	Autophagy
ATP6V0D2	8.38	2.04	7.38	4.88	-1.13	-0.183	0.733	1	vATPase
MYOD1	12.23	1.6	10.8	4.06	-1.13	-0.179	0.543	1	Neurodevelopment
ATG9B	14.86	2.71	13.04	3.52	-1.14	-0.188	0.454	1	Autophagy
IGF2	63.34	10.54	55.43	6.12	-1.14	-0.192	0.211	1	Signaling
TMEM74	45.59	7.23	39.73	8.13	-1.15	-0.198	0.319	1	Autophagy
AMBRA1	257.53	26.61	221.3	20.29	-1.16	-0.219	0.0781	1	Autophagy
CTSF	20.41	5.73	17.61	1.85	-1.16	-0.213	0.361	1	Lysosomal
SLC17A7	13.99	6.68	11.96	5.42	-1.17	-0.227	0.66	1	Neurodevelopment

SLC32A1	8.41	3.56	7.2	3.31	-1.17	-0.225	0.628	1	Neurodevelopment
SMPD1	8.27	1.45	7.09	4.89	-1.17	-0.223	0.739	1	LSD
SHANK3	45.83	7	38.75	8	-1.18	-0.242	0.242	1	Neurodevelopment
ATP6V1G1	23.89	6.87	19.67	5.59	-1.21	-0.281	0.343	1	vATPase
SLC9A2	12.42	4.9	10.23	3.8	-1.21	-0.28	0.51	1	Endosome
ATG4A	39.39	5.95	32.21	3.31	-1.22	-0.29	0.0792	1	Autophagy
APBA1	17.92	7.96	14.38	3.04	-1.25	-0.317	0.444	1	Neurodegeneration
Nkx2-1	6.73	3.03	5.34	0.68	-1.26	-0.333	0.401	1	Neurodevelopment
ATOH1	11	3.01	8.65	3.66	-1.27	-0.347	0.382	1	Neurodevelopment
HOXA2	7.79	2.23	6.15	2.38	-1.27	-0.343	0.327	1	Neurodevelopment
EN1	12.57	4.63	9.59	3.11	-1.31	-0.39	0.287	1	Neurodevelopment
HES5	20.13	2.51	15.33	3.45	-1.31	-0.393	0.0974	1	Neurodevelopment
TTBK1	13.21	3.79	10.07	2.63	-1.31	-0.392	0.202	1	Neurodegeneration
HoxB4	12.9	3.39	9.71	2.84	-1.33	-0.409	0.18	1	Neurodevelopment
SHH	10.74	5.49	8.09	2.14	-1.33	-0.41	0.349	1	Neurodevelopment
SLC9A9	18.49	5.63	13.94	3.57	-1.33	-0.408	0.207	1	Endosome
EOMES	13.9	5.64	10.34	1.16	-1.34	-0.427	0.194	1	Neurodevelopment
Sox1	4.2	2.61	3.14	4.48	-1.34	-0.419	0.631	1	Neurodevelopment
TWIST1	7.7	3.51	5.76	1.06	-1.34	-0.419	0.214	1	Neurodevelopment
IGF1	24.87	6.48	18.45	2.12	-1.35	-0.431	0.0798	1	Signaling
NTRK2	10.65	5.5	7.65	1.9	-1.39	-0.478	0.253	1	Signaling
POU3F2	11.15	5.25	7.99	2.63	-1.4	-0.481	0.296	1	Neurodevelopment
GRIN3A	11.82	3.49	7.91	4.96	-1.49	-0.58	0.273	1	Lysosomal
HOXB13	14.33	4.5	9.41	5.43	-1.52	-0.608	0.228	1	Neurodevelopment
CHAT	7.77	2.6	4.97	4.52	-1.56	-0.644	0.296	1	Neurodevelopment
HOXB6	6.96	4.07	4.44	4.01	-1.57	-0.65	0.459	1	Neurodevelopment
Pax6	11.64	6.5	6.54	3.09	-1.78	-0.831	0.187	1	Neurodevelopment
Isl1	6.84	2.29	2.98	2.7	-2.3	-1.2	0.107	1	Neurodevelopment

Table 3.6 Genes upregulated in W523X iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	W523X (Counts)	StDev of W523X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
CD74	315.32	5.6	403.84	55.19	1.28	0.357	0.0123	1	Neurodegeneration
CCND1	895.05	71.11	1134.87	124.69	1.27	0.342	0.0141	1	Neurodevelopment
FoxG1	10.71	2.02	15.61	2.02	1.46	0.543	0.0155	1	Neurodevelopment
IDUA	31.14	3.29	42.43	7.52	1.36	0.447	0.0212	1	LSD
PHYH	52.49	5.18	64.4	5.49	1.23	0.295	0.0212	1	LSD
SIRT1	1192.76	67.39	1309.64	57.35	1.1	0.135	0.0412	1	Autophagy
FUCA1	187.51	13.13	277.88	80.96	1.48	0.568	0.0427	1	LSD
UVRAG	304.2	19.32	332.35	12.12	1.09	0.128	0.0555	1	Endosome
CLN6	406.02	16.42	441.02	26.22	1.09	0.119	0.0608	1	LSD
GAA	186.39	12.44	218.94	32.21	1.17	0.232	0.0785	1	Lysosomal
CTSB	289.29	8.5	355.5	70.64	1.23	0.297	0.0801	1	Lysosomal
Sox10	9.72	3.55	14.09	1.56	1.45	0.534	0.0993	1	Neurodevelopment
APOE	3299.09	104.46	3681.56	426.89	1.12	0.158	0.113	1	Neurodegeneration
GNPTG	219.17	11.17	239.95	20.26	1.09	0.131	0.123	1	Lysosomal
MAN2B1	446.43	13.27	482.7	42.44	1.08	0.113	0.132	1	LSD
GLB1	332.39	16.33	369.52	44.67	1.11	0.153	0.15	1	Lysosomal
NTRK3	13.23	4.39	18.43	6.2	1.39	0.479	0.167	1	Signaling
NGFR	84.96	5.02	102.74	23.56	1.21	0.274	0.167	1	Signaling
TH	10.24	3.86	13.7	2.25	1.34	0.42	0.175	1	Neurodevelopment
RELN	8.16	1.65	10.68	3.42	1.31	0.389	0.186	1	Neurodegeneration
SLC9A8	41.61	5.68	46.57	3.79	1.12	0.162	0.195	1	Endosome
PSAP	4216.25	193.46	4551.96	461.59	1.08	0.111	0.203	1	Lysosomal
GAD1	8.79	0.72	11.43	4.54	1.3	0.379	0.204	1	Lysosomal
Iba1	132.14	15.76	146.84	13.09	1.11	0.152	0.204	1	Neurodevelopment
DLX1	9.88	2.32	12.84	3.43	1.3	0.377	0.207	1	Neurodevelopment
TFE3	51.52	6.96	57.3	3.25	1.11	0.154	0.211	1	LSD
BLOC1S1	1112.54	27.92	1148.19	43.46	1.03	0.0455	0.218	1	Lysosomal
VLDLR	977.99	36.58	1091.83	206.3	1.12	0.159	0.26	1	Neurodegeneration
ATP6V1B1	2.13	1.74	3.6	2.59	1.69	0.76	0.272	1	vATPase
NAGLU	132.87	15.34	145.42	15.41	1.09	0.13	0.283	1	Lysosomal
GAD2	5.77	2.34	7.71	2.32	1.34	0.418	0.287	1	Neurodevelopment
TFEB	15.27	4.26	18.64	4.18	1.22	0.288	0.307	1	Lysosomal
BACE1	120.74	14.88	132.65	16.77	1.1	0.136	0.318	1	Neurodegeneration
TTBK1	8.62	5.69	11.9	2.29	1.38	0.465	0.32	1	Neurodegeneration
POU3F3	6.27	2.39	7.62	1.32	1.21	0.28	0.377	1	Neurodevelopment
ATP6V0D2	8.77	4.62	10.94	0.69	1.25	0.319	0.394	1	vATPase
LRP1	269.66	40.33	303.32	67.42	1.12	0.17	0.401	1	Neurodegeneration
STH	7.18	1.76	8.76	3.96	1.22	0.288	0.416	1	Neurodegeneration
DNAJC5	76.05	3.83	80.94	11.1	1.06	0.09	0.419	1	LSD
PVALB	21.55	5.96	25.96	8.59	1.2	0.269	0.43	1	Neurodevelopment
NCAM	77.21	9.23	83.5	12.46	1.08	0.113	0.434	1	Neurodevelopment
HOXA2	4.14	1.44	5.25	2.69	1.27	0.34	0.442	1	Neurodevelopment
SLC9A9	15.27	1.68	16.66	3.02	1.09	0.126	0.451	1	Endosome
Nkx2-1	4.98	0.95	6.03	2.39	1.21	0.278	0.455	1	Neurodevelopment
ATG9B	14.98	2	16.99	5.36	1.13	0.182	0.471	1	Autophagy
CNTN2	19.68	6.83	22.17	1.63	1.13	0.172	0.49	1	Neurodegeneration
MANBA	133.85	8.93	141.19	19.65	1.05	0.077	0.511	1	LSD
CNTN4	13.1	5.38	14.99	2.3	1.14	0.194	0.534	1	Neurodegeneration
HOXB13	10.71	1.83	12.14	4.01	1.13	0.18	0.548	1	Neurodevelopment
CD63	4519.17	114.49	4638	374.21	1.03	0.0374	0.554	1	Lysosomal
HOXB1	10.5	3.65	12.22	4.03	1.16	0.219	0.567	1	Neurodevelopment
HES5	14.23	4.5	15.66	2.53	1.1	0.138	0.573	1	Neurodevelopment
NAGA	93.98	16.87	99.73	10.01	1.06	0.0856	0.59	1	LSD
SLC12A5	17.29	4.15	18.7	2.72	1.08	0.113	0.601	1	Neurodevelopment
CTSD	1249.03	68.6	1288.84	143.31	1.03	0.0453	0.617	1	Lysosomal
APBB3	37.9	3.17	41.33	15.02	1.09	0.125	0.647	1	Neurodegeneration
NTRK1	3.48	0.45	3.94	2.32	1.13	0.177	0.67	1	Signaling
Tbr1	4.93	2.59	5.74	2.04	1.16	0.219	0.672	1	Neurodevelopment
TPP1	425.12	47.15	440.53	59.04	1.04	0.0514	0.694	1	LSD
ARSB	399.97	7.61	404.37	22.16	1.01	0.0158	0.719	1	LSD
Fezf2	4.77	2.43	5.39	1.48	1.13	0.177	0.728	1	Neurodevelopment
ATP6V1G3	8.18	3.24	8.78	2.1	1.07	0.103	0.738	1	vATPase
BCL11B	73.29	2.78	76.01	16.91	1.04	0.0526	0.739	1	Neurodevelopment
APBA3	59.14	10.67	60.89	2.43	1.03	0.0421	0.745	1	Neurodegeneration
MAP1LC3A	90.52	6.49	92.43	10.17	1.02	0.0301	0.763	1	Autophagy
CTSC	5071.83	143.81	5149.42	518.95	1.02	0.0219	0.777	1	LSD
GALNS	131.22	13.84	135.04	24.77	1.03	0.0414	0.791	1	Lysosomal
S100b	9.61	1.83	10.08	2.64	1.05	0.0693	0.794	1	Neurodevelopment
Sox2	1700.55	71.72	1712.15	66	1.01	0.00981	0.821	1	Neurodevelopment
CHAT	7.18	3.61	7.8	4.84	1.09	0.12	0.827	1	Neurodevelopment
HPS1	61.52	8.34	62.77	8.18	1.02	0.029	0.831	1	Lysosomal
PRKAA2	314	13.9	317.23	28.95	1.01	0.0148	0.851	1	Autophagy
ULK2	53.19	9.92	54.87	15.05	1.03	0.0449	0.864	1	Autophagy

GFAP	8.87	3.3	9.26	4.33	1.04	0.0623	0.871	1	Neurodevelopment
Sox1	3.41	2.96	3.66	0.66	1.08	0.105	0.878	1	Neurodevelopment
HOXB6	5.7	2.13	6.06	4.66	1.06	0.0886	0.881	1	Neurodevelopment
ATP6V0B	651.57	47.22	657.1	59.32	1.01	0.0122	0.887	1	vATPase
aph1Bvar1	411.33	16.1	414.85	46.41	1.01	0.0123	0.89	1	Neurodegeneration
GALC	40.95	1.73	41.8	12.94	1.02	0.0298	0.898	1	LSD
HYAL1	68.62	4.53	69.41	12.36	1.01	0.0165	0.903	1	LSD
CALB2	31.49	4.4	32.08	10.17	1.02	0.0268	0.928	1	Neurodevelopment
CCK	38.96	1.12	39.27	6.84	1.01	0.0117	0.931	1	Neurodevelopment
SCPEP1	627.72	13.09	629.21	55.34	1	0.00341	0.96	1	LSD
NGF	7.32	2.42	7.42	5.08	1.01	0.0201	0.972	1	Signaling
MCOLN1	88.69	6.31	88.74	10.56	1	0.000887	0.993	1	Lysosomal

Table 3.7 Genes downregulated in W523X iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	W523X (Counts)	StDev of W523X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
PEX1	503.43	3.65	463.6	11.7	-1.09	-0.119	0.000776	1	LSD
ATG4D	302.71	12.95	266.96	7.24	-1.13	-0.181	0.00274	1	Autophagy
RPTOR	150.97	11.15	117.69	9.33	-1.28	-0.359	0.00367	1	Signaling
NTF4	87.57	7.86	67.58	4.83	-1.3	-0.374	0.00391	1	Signaling
HoxB4	16.01	1.91	10.45	1.89	-1.53	-0.616	0.00655	1	Neurodevelopment
Rab10	1069.61	30.36	943.16	52.78	-1.13	-0.182	0.00673	1	Endosome
ATG12	102.32	6.98	81.42	7.55	-1.26	-0.33	0.00685	1	Autophagy
PRKAG2	452.54	3.5	387.01	34.22	-1.17	-0.226	0.0107	1	Signaling
AMY	336.13	11.68	251.98	40.24	-1.33	-0.416	0.011	1	Neurodegeneration
STX13	225.49	13.28	197.71	9.03	-1.14	-0.19	0.013	1	Endosome
ATP6V1D	517.34	33.06	436.43	32.12	-1.19	-0.245	0.0131	1	vATPase
ATP6V0D1	953.26	16.01	838.86	61.38	-1.14	-0.184	0.014	1	vATPase
CLN8	363.83	26.05	305.3	22.99	-1.19	-0.253	0.0146	1	LSD
NAE1	3554.11	118.71	2698.41	481.89	-1.32	-0.397	0.0164	1	Neurodegeneration
AKT1	225.8	10.96	186.62	19.88	-1.21	-0.275	0.0168	1	Signaling
ATG10	392.4	3.66	293.9	57.6	-1.34	-0.417	0.0177	1	Autophagy
GSK3A	1132.81	26.27	957.11	102.4	-1.18	-0.243	0.0179	1	Neurodegeneration
ATP6V1H	603.88	27	522.87	41.32	-1.15	-0.208	0.0182	1	vATPase
STK4	715.55	22.73	508.63	114.4	-1.41	-0.492	0.0183	1	Signaling
Rab35	1257.15	59.03	957.78	170.59	-1.31	-0.392	0.0186	1	Endosome
GGA2	1226.6	28.36	781.06	263.81	-1.57	-0.651	0.0202	1	Endosome
MARK2	545.49	7.09	417.42	77.36	-1.31	-0.386	0.021	1	Neurodegeneration
RAB7A	2203.6	33.93	1823.43	225.78	-1.21	-0.273	0.021	1	Lysosomal
GABARAPL1	1074.53	19.22	990.73	47.78	-1.08	-0.117	0.0213	1	Autophagy
VPS11	88.71	1.69	71.44	9.96	-1.24	-0.312	0.0213	1	Endosome
PRKAA1	316.18	17.94	264.32	27.91	-1.2	-0.258	0.0218	1	Autophagy
ATG5	585.02	15.85	529.41	31.98	-1.11	-0.144	0.0232	1	Autophagy
BECN1	935.01	50.21	774.66	92.05	-1.21	-0.271	0.0236	1	Autophagy
SLC9A6 TV 1 3	360.09	20.05	81.87	159.69	-4.4	-2.14	0.0236	1	Endosome
Satb1	878.38	37.4	616.46	162.87	-1.42	-0.511	0.0245	1	Neurodevelopment
AKT3	732.89	11.35	537.76	120.38	-1.36	-0.447	0.0265	1	Signaling
VIM	1849.66	117.11	1228.37	391.39	-1.51	-0.591	0.0272	1	Neurodevelopment
AMBRA1	308.99	18.19	248.98	34.55	-1.24	-0.312	0.028	1	Autophagy
MAP1LC3B	763.29	31.73	675.63	48.35	-1.13	-0.176	0.0281	1	Autophagy
NRBF2	1061.49	37.1	880.36	117.23	-1.21	-0.27	0.0286	1	Autophagy
MAP4	1971.77	81.28	1516.49	301.44	-1.3	-0.379	0.0297	1	Neurodegeneration
ATG4C	736.36	52.36	605.92	74.96	-1.22	-0.281	0.0299	1	Autophagy
Cux1	416.27	16.36	380.53	19.51	-1.09	-0.13	0.0312	1	Neurodevelopment
ULK1	284.64	17.5	226.03	35.94	-1.26	-0.333	0.0317	1	Autophagy
GSK3B	1009.03	20.69	854.43	105.86	-1.18	-0.24	0.032	1	Neurodegeneration
GNPTAB	845.21	48.85	604.38	148.14	-1.4	-0.484	0.0321	1	LSD
SLC9A6	144.73	17.33	37.03	73.12	-3.91	-1.97	0.0329	1	Endosome
ARF6	1965.26	22.17	1634.95	228.64	-1.2	-0.265	0.033	1	Endosome
CDK5R1	190.1	12.22	138.59	34.19	-1.37	-0.456	0.0333	1	Neurodegeneration
USP10	2151.16	21.9	1816.11	237.64	-1.18	-0.244	0.0333	1	Autophagy
MBP	166.13	12.61	130.85	21.94	-1.27	-0.344	0.0339	1	Neurodevelopment
ATG101	469.99	11.23	391.33	54.16	-1.2	-0.264	0.0345	1	Autophagy
STX6	1157.71	26.47	831.44	230.2	-1.39	-0.478	0.0347	1	Endosome
ATP6V0E2	60.24	2.3	47.21	9.03	-1.28	-0.352	0.0354	1	vATPase
CALB1	561.18	44.56	363.46	134.68	-1.54	-0.627	0.0358	1	Neurodevelopment
HIF1A	4611.46	171.24	3325.61	914.66	-1.39	-0.472	0.0362	1	Neurodevelopment
POU5F1	10269.05	331.73	9302.04	628.79	-1.1	-0.143	0.0364	1	Neurodevelopment
TFRC	2079.81	98.86	1381.2	467.77	-1.51	-0.591	0.0367	1	Endosome
Tau_3R	19.16	4.16	12.84	2.4	-1.49	-0.577	0.0371	1	Neurodegeneration
GABARAPL2	1491.09	58.9	1274.82	145.08	-1.17	-0.226	0.0374	1	Autophagy
VPS18	266.22	26.46	214.45	28.61	-1.24	-0.312	0.0382	1	Endosome
EHD4	491.48	9.39	402.9	63.58	-1.22	-0.287	0.0383	1	Endosome
NBR1	676.31	13.7	606.95	50.1	-1.11	-0.156	0.0384	1	Autophagy
WDR45L	1133.7	35.74	956.43	126.89	-1.19	-0.245	0.0388	1	Autophagy
RAB9A	910.45	27.94	725.68	131.86	-1.25	-0.327	0.0389	1	Endosome
WDFY2	287.82	11.78	232.39	39.85	-1.24	-0.309	0.0394	1	Endosome
aph1Avar3	783.33	12.11	697.57	62.55	-1.12	-0.167	0.0402	1	Neurodegeneration
SYP	21.57	2.37	15.09	3.8	-1.43	-0.516	0.0411	1	Neurodevelopment
TJP1	2934.02	111.47	2417.29	376.91	-1.21	-0.279	0.0415	1	Neurodevelopment
Pax6	13.86	3.49	8.43	2.9	-1.64	-0.717	0.0439	1	Neurodevelopment
LRP10	321.85	20.07	270.64	34.07	-1.19	-0.25	0.0468	1	Neurodegeneration
SLC9A7	512.55	18	407.9	75.07	-1.26	-0.329	0.0478	1	Endosome
Rab11	341.25	23.49	280.57	43.98	-1.22	-0.282	0.0511	1	Endosome
ATG4B	1790.59	81.7	1558.8	169.64	-1.15	-0.2	0.0516	1	Autophagy
NMDA NR1	793.59	45.21	656.27	104.07	-1.21	-0.274	0.0525	1	Neurodevelopment
DLG4	312.13	29.47	251.65	38.73	-1.24	-0.311	0.0526	1	Neurodevelopment
APBB1	300.15	16.71	234.35	51.53	-1.28	-0.357	0.0527	1	Neurodegeneration
AKT2	908.28	74.81	751.37	109.4	-1.21	-0.274	0.0544	1	Signaling
CLN5	128.22	8.16	104.93	16.35	-1.22	-0.289	0.0546	1	LSD
SLC36A1	130.11	15.99	73.43	42.12	-1.77	-0.825	0.0549	1	Lysosomal

VPS26A	557.47	19.53	457.41	78.76	-1.22	-0.285	0.0549	1	Endosome
GGA3	797.56	42.61	631.03	130.17	-1.26	-0.338	0.0553	1	Endosome
GABARAP	2483.77	45.14	2192.29	229.54	-1.13	-0.18	0.0556	1	Autophagy
ATG14	92.33	7.22	77.99	9.62	-1.18	-0.243	0.0561	1	Autophagy
ATG3	1456.61	83.14	1197.48	203.17	-1.22	-0.283	0.0568	1	Autophagy
PICALM	741.23	30.54	607.99	108.05	-1.22	-0.286	0.0572	1	Neurodegeneration
KIAA0226	52.46	5.03	40.11	9.27	-1.31	-0.387	0.0575	1	Autophagy
ATP6V1C1	365.61	14.24	239.67	105.85	-1.53	-0.609	0.0581	1	vATPase
TP73	66.76	5.28	49.48	13.7	-1.35	-0.432	0.0583	1	Neurodevelopment
CREB1	585.43	22.95	457.23	106	-1.28	-0.357	0.0586	1	Signaling
NECAP2	550.69	11.48	484.72	51.93	-1.14	-0.184	0.06	1	Endosome
MARK3	474.38	35.52	399.53	55.24	-1.19	-0.248	0.0612	1	Neurodegeneration
aph1Avar4	417.62	27.37	358.44	43.4	-1.17	-0.22	0.0616	1	Neurodegeneration
MAP2	79.75	6.74	36.59	35.98	-2.18	-1.12	0.0631	1	Neurodevelopment
B3GAT1	158.8	13.25	113.7	36.75	-1.4	-0.482	0.0638	1	Neurodevelopment
CSNK1D	952.64	38.99	808.65	120.47	-1.18	-0.236	0.0638	1	Neurodegeneration
MYO1D	14.01	1.53	11.69	1.3	-1.2	-0.261	0.0644	1	Neurodevelopment
TMEM74	69.51	2.55	53.24	12.49	-1.31	-0.385	0.0646	1	Autophagy
EEA1	547.59	17.1	424.9	95.01	-1.29	-0.366	0.0655	1	Endosome
nanog	2627.8	63.97	2191.06	375.34	-1.2	-0.262	0.0681	1	Neurodevelopment
IGF1R	532.74	24.3	439.19	74.5	-1.21	-0.279	0.0687	1	Signaling
APOER2	394.79	15.48	316.08	62.8	-1.25	-0.321	0.0709	1	Neurodegeneration
ATP6V1B2	1008.5	27.47	874.41	116.48	-1.15	-0.206	0.071	1	vATPase
RABGEF1	427.87	29.83	366.24	48.22	-1.17	-0.224	0.0731	1	Endosome
WDFY3	329.74	26.62	270.13	44.14	-1.22	-0.288	0.0775	1	Autophagy
GBA	233.81	10.94	201.72	26.17	-1.16	-0.213	0.0779	1	Lysosomal
ATP6V1E1	1792.53	65.86	1619.23	142.51	-1.11	-0.147	0.0789	1	vATPase
ULK4	79.8	6.12	66.46	10.08	-1.2	-0.264	0.0794	1	Autophagy
VPS35	824.66	25.42	734.86	81.16	-1.12	-0.166	0.0814	1	Endosome
ATP6V1G1	26.24	5.83	19.17	4.16	-1.37	-0.453	0.0843	1	vATPase
TWIST1	11.34	2.26	8.05	2.19	-1.41	-0.493	0.0868	1	Neurodevelopment
DAB1	129.35	12.08	86.92	40.53	-1.49	-0.573	0.0898	1	Neurodegeneration
PIK3R4	734.91	11.03	569.04	148.66	-1.29	-0.369	0.0923	1	Autophagy
MARK1	423.47	15.95	356.81	61	-1.19	-0.247	0.0935	1	Neurodegeneration
LIPA	869.39	25.18	768.21	95.11	-1.13	-0.179	0.095	1	LSD
LRP3	190.66	18.9	157.95	26.2	-1.21	-0.272	0.0951	1	Endosome
NEDD4	56.59	8.45	47.6	4.75	-1.19	-0.25	0.0969	1	Endosome
AP1S2	918.33	75.52	750.97	149.15	-1.22	-0.29	0.097	1	Endosome
PEX5	476.84	31.73	385.89	87.13	-1.24	-0.305	0.1	1	LSD
APP1	1051.52	31.42	901.97	152.34	-1.17	-0.221	0.101	1	Endosome
Isl1	12.66	3.84	8.89	1.4	-1.42	-0.51	0.101	1	Neurodevelopment
ADAM19	166.85	7.81	131.3	32.49	-1.27	-0.346	0.102	1	Neurodegeneration
DRAM1	100.97	2.95	85.31	14.7	-1.18	-0.243	0.102	1	Autophagy
MAPK3	588.1	40.76	522.19	55.49	-1.13	-0.171	0.103	1	Signaling
HOXA1	4.45	0.83	1.9	2.77	-2.34	-1.23	0.104	1	Neurodevelopment
PEX13	400.28	34.79	331.48	63.08	-1.21	-0.272	0.104	1	LSD
ATP6V1A	1853.41	44.81	1745.23	103.9	-1.06	-0.0868	0.106	1	vATPase
PPP2R4	1684.5	53.77	1523.93	159.97	-1.11	-0.145	0.106	1	Neurodegeneration
APPBP2	308.32	17.14	268.31	37.55	-1.15	-0.201	0.107	1	Neurodegeneration
MAPT	48.39	8.47	40.5	2.4	-1.2	-0.257	0.107	1	Neurodevelopment
aph1Bvar2	102.84	4.91	92.15	9.71	-1.12	-0.158	0.108	1	Neurodegeneration
PLBD2	125.33	7.06	118.76	1.22	-1.06	-0.0778	0.108	1	Autophagy
RICTOR	355.72	6.37	286.09	72.91	-1.24	-0.314	0.108	1	Signaling
MAPK1	1106.85	33.85	1012.48	93.23	-1.09	-0.129	0.109	1	Signaling
ATOX1	9.49	1.5	7.09	2.21	-1.34	-0.421	0.11	1	Neurodevelopment
EHD1	133.46	5.17	107.15	25.99	-1.25	-0.317	0.11	1	Endosome
SLC12A2	222.99	12.53	189.67	31.07	-1.18	-0.233	0.111	1	Neurodevelopment
APLP1	204.55	9.59	182.93	21.29	-1.12	-0.161	0.112	1	Neurodegeneration
ATP6V1F	1266.34	50.07	1160.53	102.61	-1.09	-0.126	0.114	1	vATPase
CDK5	229.15	9.93	196.21	33.84	-1.17	-0.224	0.114	1	Neurodegeneration
SLC9A6 TV 1 2	12.76	1.27	6.9	4.4	-1.85	-0.886	0.114	1	Endosome
RB1CC1	729.2	43.53	617.83	105.6	-1.18	-0.239	0.117	1	Autophagy
TCF4	400.14	27.88	344.18	53.02	-1.16	-0.217	0.117	1	LSD
KMT2D	106.39	16.78	86.49	14.32	-1.23	-0.299	0.119	1	LSD
OTX2	235.16	15.66	203.22	28.33	-1.16	-0.211	0.12	1	Neurodevelopment
AFP	9.92	1.91	6.67	3.33	-1.49	-0.572	0.124	1	Neurodevelopment
M6PR	734.18	14.09	679.57	57.86	-1.08	-0.112	0.124	1	Lysosomal
TUBB3	361.67	50.35	286.76	68.69	-1.26	-0.335	0.124	1	Neurodevelopment
LAMP2	1235.59	33.3	1127.67	112.73	-1.1	-0.132	0.126	1	LSD
HPS5	338.59	23.1	307.26	27.32	-1.1	-0.14	0.127	1	Lysosomal
ATG16L1	524.56	46.35	419.97	110.6	-1.25	-0.321	0.128	1	Autophagy
Rab4	772.93	71.69	709.53	26.59	-1.09	-0.123	0.128	1	Endosome
SQSTM1	216	15.77	190.61	24.2	-1.13	-0.18	0.129	1	Autophagy
GM2A	652.68	7.62	583.68	77.3	-1.12	-0.161	0.146	1	LSD
PEX16	161.47	18.78	145.93	7.1	-1.11	-0.146	0.15	1	LSD
ATG2A	96.89	6.48	83.21	14.11	-1.16	-0.22	0.151	1	Autophagy
NEU1	196.18	17.84	180.13	9.57	-1.09	-0.123	0.151	1	Lysosomal
AKT1S1	344.03	31.58	290.85	57.07	-1.18	-0.242	0.153	1	Autophagy

ATG9A	604.97	41.22	551	53.22	-1.1	-0.135	0.156	1	Autophagy
ASAH1	961.35	25.3	876.01	98.58	-1.1	-0.134	0.157	1	Lysosomal
APBA2	75.11	7.39	63.04	12.18	-1.19	-0.253	0.161	1	Neurodegeneration
BLOC1S3	18.47	3.11	15.1	2.62	-1.22	-0.291	0.171	1	Lysosomal
ATP6V0C	1873.1	110.76	1730.48	144.97	-1.08	-0.114	0.172	1	vATPase
VPS33A	376.8	18.29	350.06	28.18	-1.08	-0.106	0.172	1	Endosome
ULK3	495.21	24.16	425.93	82.52	-1.16	-0.217	0.176	1	Autophagy
SORT1	1491.32	45.86	1336.49	188.02	-1.12	-0.158	0.179	1	Endosome
PTEN	640.98	12.35	588.35	66.46	-1.09	-0.124	0.186	1	Signaling
IGF2R	327.69	25.93	271.68	67.93	-1.21	-0.27	0.203	1	Lysosomal
SATB2	27.89	7.64	20.94	5.67	-1.33	-0.413	0.203	1	Neurodevelopment
SYN1	61.19	13.86	45.29	17.18	-1.35	-0.434	0.203	1	Neurodevelopment
ATG7	192.62	10.46	172.52	25.19	-1.12	-0.159	0.206	1	Autophagy
ATP6V0A2	203.79	11.4	175.19	36.72	-1.16	-0.218	0.211	1	vATPase
SLC9A4	9.33	2.5	5.57	3.91	-1.67	-0.744	0.223	1	Endosome
SNCA	65.73	4.04	56.87	11.95	-1.16	-0.209	0.226	1	Neurodegeneration
HGS	63.25	7.31	55.24	9.69	-1.15	-0.195	0.227	1	Endosome
ATP6V0A4	20.29	2.88	16.38	4.32	-1.24	-0.309	0.228	1	vATPase
SLC9A2	17.81	3.08	14.92	2.96	-1.19	-0.255	0.235	1	Endosome
CLCN3	145.97	10.28	126.77	26.85	-1.15	-0.203	0.236	1	Endosome
CTSA	510.45	39.28	464.93	54.32	-1.1	-0.135	0.236	1	Lysosomal
VPS8	433.39	20.22	392.73	60.23	-1.1	-0.142	0.236	1	Endosome
TACE/ADAM17	453.4	31.85	412.86	51.35	-1.1	-0.135	0.238	1	Neurodegeneration
ADAM10	1322.58	48.5	1203.21	168.19	-1.1	-0.136	0.245	1	Neurodegeneration
TMEM55B	108.68	4.28	99.27	13.65	-1.09	-0.131	0.247	1	Lysosomal
USP13	458.53	27.7	425.9	43.19	-1.08	-0.106	0.251	1	Autophagy
SMPD1	7.91	3.37	5.98	1.59	-1.32	-0.402	0.252	1	LSD
CTSF	151.8	9.65	135.88	22.42	-1.12	-0.16	0.259	1	Lysosomal
GLA	899.83	43.3	841.32	80.93	-1.07	-0.097	0.265	1	Lysosomal
GNS	291.17	11.49	265.43	37.27	-1.1	-0.134	0.266	1	Lysosomal
SLC17A6	37.56	3.36	31.15	10.38	-1.21	-0.27	0.272	1	Neurodevelopment
ATP13A2	341.31	27	320.42	21.82	-1.07	-0.0911	0.275	1	LSD
MNX1	9.38	3.57	6.6	2.76	-1.42	-0.508	0.275	1	Neurodevelopment
KIF4	56.03	7.23	48.91	9.37	-1.15	-0.196	0.279	1	Neurodevelopment
GGA1	136.22	19.36	120.73	19.05	-1.13	-0.174	0.283	1	Endosome
APP-695	7624.43	235.43	7018.6	1009.9	-1.09	-0.119	0.301	1	Neurodegeneration
SORL1	524.58	37.25	475.36	78.21	-1.1	-0.142	0.312	1	Endosome
TTBK2	222.96	21.32	210.39	9.39	-1.06	-0.0837	0.315	1	Neurodegeneration
APP-KPI	552.83	10.32	507.18	85.68	-1.09	-0.124	0.327	1	Neurodegeneration
NPC1	447.73	41.36	394.29	88.04	-1.14	-0.183	0.33	1	LSD
WIP1	138.19	14.48	128.69	11.78	-1.07	-0.103	0.346	1	Autophagy
ATP6V0A1	481.05	40.41	436.01	79.67	-1.1	-0.142	0.351	1	vATPase
EN1	11.71	4.49	8.78	3.38	-1.33	-0.415	0.352	1	Neurodevelopment
CLN3	91.52	12.61	84.61	5.68	-1.08	-0.113	0.353	1	LSD
IDS	313.97	14.6	288.46	49.02	-1.09	-0.122	0.365	1	LSD
PEX3	285.97	40.72	266.42	9.84	-1.07	-0.102	0.377	1	LSD
VAMP1	60.97	6.12	55.35	9.96	-1.1	-0.14	0.377	1	Endosome
PSEN2	301.06	29.31	276.71	38.56	-1.09	-0.122	0.383	1	Neurodegeneration
WDR45	172.02	20.48	157.66	23.37	-1.09	-0.126	0.386	1	Autophagy
SHH	12.01	1.54	10.58	2.52	-1.14	-0.184	0.388	1	Neurodevelopment
AGA	194.62	6.74	182.25	25.97	-1.07	-0.0948	0.396	1	LSD
NPY	10.48	4.95	7.91	4.04	-1.33	-0.406	0.403	1	Neurodevelopment
ATG2B	191.91	18.06	179.8	19.33	-1.07	-0.0941	0.406	1	Autophagy
Tau_4R	28.65	5.55	24.78	7.06	-1.16	-0.209	0.424	1	Neurodegeneration
NES	1202.51	124.65	1134.11	115.05	-1.06	-0.0845	0.436	1	Neurodevelopment
APP-All	6879.77	132.6	6524.64	834.69	-1.05	-0.0765	0.44	1	Neurodegeneration
ATP6V0E1	2233.04	104.9	2151.19	171.46	-1.04	-0.0539	0.441	1	vATPase
BACE2	163.5	11.01	156.85	12.15	-1.04	-0.0599	0.446	1	Neurodegeneration
GRIPAP1	309.59	25.88	292.49	32.65	-1.06	-0.0819	0.446	1	Endosome
NAGPA	43.74	9.15	35.97	14.35	-1.22	-0.282	0.45	1	Lysosomal
LAMP1	2544.25	88.33	2438.21	247.95	-1.04	-0.0614	0.455	1	Lysosomal
SLC32A1	7.54	2.43	5.98	3.06	-1.26	-0.332	0.459	1	Neurodevelopment
OLIG2	16.78	1.57	14.77	4.44	-1.14	-0.184	0.46	1	Neurodevelopment
NPC2	4415.65	69.85	4298.09	292.51	-1.03	-0.0389	0.468	1	LSD
RRAGC	240.69	14.23	224.1	38.74	-1.07	-0.103	0.473	1	Lysosomal
C1ORF85	524.3	32.81	501.16	56.52	-1.05	-0.0651	0.503	1	Lysosomal
DCX	40.03	6.08	37.27	5.05	-1.07	-0.103	0.511	1	Neurodevelopment
PPT1	3296.64	160.94	3170.43	345.2	-1.04	-0.0563	0.535	1	Lysosomal
SHANK3	49.37	8.67	44.91	10.34	-1.1	-0.137	0.535	1	Neurodevelopment
FOXP2	12.02	3.88	10.29	4.67	-1.17	-0.224	0.551	1	Neurodevelopment
MTOR	580.04	46.53	558.72	55.83	-1.04	-0.054	0.579	1	Signaling
APLP2	3626.67	131.57	3493.3	451.62	-1.04	-0.0541	0.59	1	Neurodegeneration
CUX2	83.3	18.41	77.31	12.7	-1.08	-0.108	0.608	1	Neurodevelopment
HGSNAT	23.83	9.73	20.77	6.8	-1.15	-0.198	0.608	1	LSD
PEX19	523.05	33.19	511.83	29.64	-1.02	-0.0313	0.629	1	LSD
GRIN3A	14.29	0.91	13.64	2.26	-1.05	-0.0679	0.638	1	Lysosomal
NTRK2	10.69	4.25	9.37	2.83	-1.14	-0.19	0.642	1	Signaling
APBA1	17.7	2.84	16.75	2.56	-1.06	-0.0788	0.645	1	Neurodegeneration

SLC9A5	31.47	5.17	30.12	3.39	-1.05	-0.0636	0.67	1	Endosome
WIP1	275.75	18.5	271.5	14.7	-1.02	-0.0224	0.731	1	Autophagy
ADAM9	207.56	9.95	201.44	34.96	-1.03	-0.0431	0.732	1	Neurodegeneration
BDNF	141.92	9.66	138.53	16.44	-1.02	-0.0349	0.74	1	Signaling
NCSTN	576.34	38.38	565.99	47.82	-1.02	-0.0262	0.751	1	Neurodegeneration
ATP6V1G2	48.36	10.48	45.94	9.7	-1.05	-0.0741	0.753	1	vATPase
IGF1	22.25	2.62	20.9	7.24	-1.06	-0.0901	0.753	1	Signaling
SLC9A1	282.52	10.61	275.83	47.08	-1.02	-0.0346	0.781	1	Endosome
SGSH	58.33	7.02	55.45	16.6	-1.05	-0.0731	0.789	1	LSD
PSENEN	947.72	20.93	941.32	51.39	-1.01	-0.00977	0.824	1	Neurodegeneration
SUMF1	282.36	22.7	279.02	16.69	-1.01	-0.0172	0.824	1	Lysosomal
HEXB	1060.88	49.07	1045.42	135.34	-1.01	-0.0212	0.838	1	Lysosomal
PSEN1	691.69	19.08	682.64	85.02	-1.01	-0.019	0.838	1	Neurodegeneration
NTF3	21.5	6.93	20.83	1.63	-1.03	-0.0461	0.851	1	Signaling
LHX6	7.16	2.3	6.78	3.3	-1.05	-0.0771	0.861	1	Neurodevelopment
ATG4A	37.02	6.7	36.21	6.14	-1.02	-0.032	0.863	1	Autophagy
SLC9A3	6.09	1.9	5.81	2.63	-1.05	-0.069	0.868	1	Endosome
CTNS	90.96	10.09	88.96	23.11	-1.02	-0.0322	0.884	1	Lysosomal
HPS3	1103.88	40.41	1098.88	56.49	-1	-0.00655	0.889	1	Lysosomal
IGF2	34.16	5.82	33.53	6.16	-1.02	-0.027	0.889	1	Signaling
EOMES	11.15	5.78	10.78	3.64	-1.03	-0.0495	0.906	1	Neurodevelopment
SLC17A7	14.14	4.96	13.83	2.58	-1.02	-0.0319	0.911	1	Neurodevelopment
ATP6V1E2	130.12	10.13	129.09	19.29	-1.01	-0.0115	0.927	1	vATPase
POU3F2	9.74	5	9.46	2.92	-1.03	-0.0417	0.929	1	Neurodevelopment
GSAP	15.3	3.61	15.13	2.02	-1.01	-0.0164	0.931	1	Neurodegeneration
ATG16L2	80.24	9.3	79.53	17.29	-1.01	-0.0128	0.943	1	Autophagy
CLCN7	401.81	9.02	400.07	60.56	-1	-0.00628	0.954	1	Endosome
HEXA	277.56	20.16	276.42	30.8	-1	-0.00593	0.954	1	Lysosomal
MFSD8	188.41	16.6	187.37	33.75	-1.01	-0.00797	0.956	1	LSD
RAB5A	1545.61	105.68	1543.86	37.55	-1	-0.00163	0.976	1	Endosome
SLC17A5	274.45	13.49	274.06	28.02	-1	-0.00205	0.981	1	LSD
CTSK	65.81	8.06	65.62	20.03	-1	-0.00415	0.985	1	Lysosomal
MLST8	337.12	19.33	337.03	41.38	-1	-0.000379	0.997	1	Signaling
APP-770	4684.69	120.73	4683.71	737.52	-1	-0.000305	0.998	1	Neurodegeneration
ARSA	50.98	2.69	50.97	11.9	-1	-0.000306	0.999	1	LSD

Table 3.8 Genes upregulated in G383D iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	G383D (Counts)	StDev of G383D	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
CTSF	124.59	21.25	425.48	14.15	3.41	1.77	7.67E-06	0.00867	Lysosomal
ATG10	240.56	23.78	372.01	20.72	1.55	0.629	0.000229	0.104	Autophagy
UVRAG	196.89	16.66	282.75	29.32	1.44	0.522	0.00161	0.403	Endosome
CDK5R1	118.38	7.15	159.1	16.62	1.34	0.426	0.00253	0.519	Neurodegeneration
BLOC1S1	980.22	11.04	1074.46	39.18	1.1	0.132	0.00298	0.55	Lysosomal
WDR45L	867.21	25.94	968.1	38.69	1.12	0.159	0.00439	0.586	Autophagy
GSK3B	856.64	13.8	905.3	20.34	1.06	0.0797	0.00687	0.755	Neurodegeneration
HoxB4	5.57	8.23	10.14	2.4	1.82	0.866	0.352	1	Neurodevelopment
Sox1	2.37	2.88	4.21	1.6	1.78	0.83	0.286	1	Neurodevelopment
POU3F2	7.25	3.25	9.89	2.68	1.36	0.448	0.301	1	Neurodevelopment
SLC9A8	32.2	8.56	43.95	3.2	1.36	0.449	0.0658	1	Endosome
S100b	8.24	4.06	11.12	5.88	1.35	0.432	0.448	1	Neurodevelopment
ATP6V0A4	13.23	5.37	17.69	4.46	1.34	0.42	0.214	1	vATPase
ATG16L2	59.58	22.72	77.84	18.36	1.31	0.386	0.244	1	Autophagy
ATP6V1G3	6.97	2.76	8.91	1.51	1.28	0.355	0.342	1	vATPase
PHYH	49.67	6.69	62.51	9.22	1.26	0.332	0.0556	1	LSD
Nkx2-1	4.97	3.49	6.19	1.62	1.25	0.317	0.586	1	Neurodevelopment
POU3F3	5.76	2.12	7.15	2.19	1.24	0.312	0.373	1	Neurodevelopment
Tau_4R	21.69	1.37	26.93	5.84	1.24	0.312	0.0835	1	Neurodegeneration
NTRK2	6.62	4.27	8.15	1.36	1.23	0.3	0.633	1	Signaling
SNCA	43.07	4.45	52.87	5.25	1.23	0.296	0.0293	1	Neurodegeneration
CHAT	6.85	1.07	8.37	1.5	1.22	0.289	0.152	1	Neurodevelopment
Tbr1	6.01	1.25	7.36	1.14	1.22	0.292	0.162	1	Neurodevelopment
CD74	286.32	28.36	341.06	30.71	1.19	0.252	0.0409	1	Neurodegeneration
CLN5	86.22	8.5	102.87	15.35	1.19	0.255	0.0919	1	LSD
CLN8	282.81	30.25	337.07	32.92	1.19	0.253	0.0576	1	LSD
GAD1	9.57	2.45	11.41	6.22	1.19	0.254	0.513	1	Lysosomal
HOXB13	11.64	3.57	13.77	4.52	1.18	0.242	0.473	1	Neurodevelopment
MAPT	35.25	8.2	41.71	9.05	1.18	0.243	0.354	1	Neurodevelopment
VPS11	72.09	5.46	83.46	7.66	1.16	0.211	0.0487	1	Endosome
CALB2	34.92	4.05	40.16	7.2	1.15	0.202	0.235	1	Neurodevelopment
APBB3	41.91	8.87	47.9	7.39	1.14	0.193	0.328	1	Neurodegeneration
NPY	8.35	2.86	9.51	6.24	1.14	0.187	0.684	1	Neurodevelopment
NTF4	64.57	13.02	73.3	3.68	1.14	0.183	0.289	1	Signaling
APBA1	15.77	6.92	17.75	2.29	1.13	0.17	0.567	1	Neurodegeneration
BACE1	118.2	13.48	132.69	14.25	1.12	0.167	0.192	1	Neurodegeneration
GAD2	8.66	5.15	9.67	1.11	1.12	0.16	0.772	1	Neurodevelopment
TP73	49.62	2.76	55.36	6.29	1.12	0.158	0.129	1	Neurodevelopment
GSAP	18.35	2.86	20.3	4.55	1.11	0.146	0.482	1	Neurodegeneration
Iba1	130.93	14.92	145.36	11.48	1.11	0.151	0.182	1	Neurodevelopment
NAGA	76.88	11.24	85.39	14.53	1.11	0.151	0.382	1	LSD
SGSH	42.86	11.02	47.53	9.15	1.11	0.149	0.53	1	LSD
AKT2	640.32	72.95	705.99	56.01	1.1	0.141	0.206	1	Signaling
APBA2	50.54	8.95	55.42	10.78	1.1	0.133	0.485	1	Neurodegeneration
ATP6V0E2	52.82	2.89	57.98	6.62	1.1	0.134	0.201	1	vATPase
ATP6V1G2	26.76	2.94	29.51	4.56	1.1	0.141	0.352	1	vATPase
FUCA1	170.95	12.49	188.17	12.59	1.1	0.138	0.103	1	LSD
MTOR	492.68	45.87	541.22	44.92	1.1	0.136	0.182	1	Signaling
NAGPA	36.63	14.65	40.43	4.93	1.1	0.143	0.596	1	Lysosomal
PEX1	419.08	13.61	460.84	21.41	1.1	0.137	0.015	1	LSD
CTSB	279.67	19.3	304.61	7.38	1.09	0.123	0.0599	1	Lysosomal
Cux1	337.92	13.07	369.37	13.38	1.09	0.128	0.0147	1	Neurodevelopment
HPS3	968.55	65.42	1057.19	52.61	1.09	0.126	0.0824	1	Lysosomal
MCOLN1	69.9	5.83	76.21	8.48	1.09	0.125	0.246	1	Lysosomal
NAGLU	126.19	16.16	137.22	7.52	1.09	0.121	0.263	1	Lysosomal
Satb1	512.63	21.66	556.23	39.6	1.09	0.118	0.101	1	Neurodevelopment
VAMP1	45.39	10.12	49.38	2.95	1.09	0.122	0.498	1	Endosome
AGA	142.19	21.74	153.96	17.79	1.08	0.115	0.433	1	LSD
CCK	37.43	10.76	40.43	3.34	1.08	0.111	0.629	1	Neurodevelopment
CTSK	46.64	7.15	50.29	8.09	1.08	0.109	0.531	1	Lysosomal
Fezf2	3.44	1.33	3.7	0.89	1.08	0.105	0.767	1	Neurodevelopment
GALC	34.26	5.27	37.14	2.5	1.08	0.117	0.361	1	LSD
HPS5	278.06	8.93	299.01	9.37	1.08	0.105	0.0183	1	Lysosomal
ATG4B	1416.09	135.59	1516.15	84.25	1.07	0.0985	0.252	1	Autophagy
CNTN4	20.78	3.19	22.24	6.48	1.07	0.0981	0.666	1	Neurodegeneration
KIAA0226	31.26	7.5	33.29	2.8	1.07	0.0909	0.644	1	Autophagy
PEX19	430.97	28.47	461.43	22.42	1.07	0.0985	0.145	1	LSD
Rab4	526.85	35.7	564.57	22	1.07	0.0998	0.132	1	Endosome
TPP1	384.08	26.79	412.77	44.74	1.07	0.104	0.29	1	LSD
APBPB2	210.51	19.17	222.26	25.32	1.06	0.0784	0.486	1	Neurodegeneration
ARSB	281.25	24.16	297.23	15.87	1.06	0.0797	0.319	1	LSD
CDK5	221.19	11.39	234.45	10.74	1.06	0.084	0.144	1	Neurodegeneration
CLCN3	114.8	16.56	121.66	6.18	1.06	0.0838	0.483	1	Endosome

CNTN2	19.38	4.16	20.59	3.24	1.06	0.0869	0.65	1	Neurodegeneration
KMT2D	88.2	10.34	93.68	12.1	1.06	0.087	0.513	1	LSD
NPC2	4064.54	141.91	4299.37	161	1.06	0.081	0.0707	1	LSD
OTX2	253.99	14.39	268.54	15.66	1.06	0.0804	0.223	1	Neurodevelopment
B3GAT1	89.8	4.11	94.62	13.49	1.05	0.0754	0.516	1	Neurodevelopment
C1ORF85	481.53	36.36	507.68	26.96	1.05	0.0763	0.3	1	Lysosomal
CTNS	71.87	7.37	75.77	11.42	1.05	0.0764	0.568	1	Lysosomal
GGA3	661.81	55.74	692.88	40.05	1.05	0.0662	0.407	1	Endosome
MAPK1	984.83	42.32	1034.37	24.24	1.05	0.0708	0.0888	1	Signaling
MAPK3	591.44	48.69	621.28	10.95	1.05	0.071	0.28	1	Signaling
MLST8	337.09	42.64	354.76	24.51	1.05	0.0737	0.498	1	Signaling
NECAP2	415.46	14.78	437.84	23.21	1.05	0.0757	0.155	1	Endosome
SLC36A1	60.27	8.54	63.27	7	1.05	0.07	0.606	1	Lysosomal
STK4	532.81	60.65	557.27	38.01	1.05	0.0648	0.528	1	Signaling
AMBRA1	252.44	24.89	261.69	12.44	1.04	0.0519	0.526	1	Autophagy
CCND1	1178.55	62.82	1230.4	41.67	1.04	0.0621	0.222	1	Neurodevelopment
GABARAP	2175.38	45.19	2265.3	69.9	1.04	0.0584	0.071	1	Autophagy
GM2A	513.05	18.65	533.08	16.46	1.04	0.0552	0.158	1	LSD
LRP3	160.26	23.17	165.89	15.14	1.04	0.0498	0.704	1	Endosome
MAP1LC3A	80.13	4.27	83.01	5.68	1.04	0.051	0.452	1	Autophagy
PPP2R4	1452.37	36.13	1505.55	85.72	1.04	0.0519	0.292	1	Neurodegeneration
SMPD1	6.29	1.55	6.53	3.19	1.04	0.0533	0.893	1	LSD
VPS8	376.86	11.39	391.93	11.01	1.04	0.0566	0.105	1	Endosome
APBA3	53.89	6.51	55.58	3.24	1.03	0.0447	0.66	1	Neurodegeneration
APP-KPI	417.93	32.18	428.72	22.39	1.03	0.0368	0.597	1	Neurodegeneration
ATP6V1E2	106.01	15.86	109.22	7.62	1.03	0.0431	0.713	1	vATPase
ATP6V1H	595.4	19.84	614.97	24.4	1.03	0.0466	0.256	1	vATPase
DRAM1	145.21	12.26	150.09	16.6	1.03	0.0477	0.64	1	Autophagy
EHD1	101.51	14.46	105.05	15.05	1.03	0.0495	0.745	1	Endosome
GGA1	116.01	21.64	119.25	12.22	1.03	0.0398	0.808	1	Endosome
GNPTG	210.62	18.77	217.15	8.52	1.03	0.0441	0.542	1	Lysosomal
HGS	49.04	10	50.38	10.43	1.03	0.039	0.851	1	Endosome
PICALM	493.59	28.67	508.26	19.72	1.03	0.0423	0.437	1	Neurodegeneration
TTBK2	180.19	13.83	185.95	22.22	1.03	0.0454	0.672	1	Neurodegeneration
aph1Avar4	341.98	19.7	349.13	23.95	1.02	0.0298	0.663	1	Neurodegeneration
ATP6V0D1	855.93	31.55	869.55	40.82	1.02	0.0228	0.615	1	vATPase
ATP6V1G1	24.74	1.16	25.32	6.62	1.02	0.033	0.86	1	vATPase
CSNK1D	790.25	58.67	802.75	46.66	1.02	0.0226	0.755	1	Neurodegeneration
CTSC	4224.58	238.43	4312.77	94.06	1.02	0.0298	0.523	1	LSD
CUX2	70.91	3.37	72.42	6.71	1.02	0.0303	0.709	1	Neurodevelopment
HEXA	271.38	33.83	276.56	33.65	1.02	0.0273	0.835	1	Lysosomal
MARK3	388.68	27.76	397.59	31.73	1.02	0.0327	0.682	1	Neurodegeneration
PSENEN	765.86	29.01	781.9	25.82	1.02	0.0299	0.441	1	Neurodegeneration
RABGEF1	325.82	29.92	333.14	47.86	1.02	0.032	0.792	1	Endosome
SLC32A1	7.61	2.71	7.79	3.13	1.02	0.0325	0.931	1	Neurodevelopment
Sox2	1677.3	25.34	1715.23	14.27	1.02	0.0323	0.0416	1	Neurodevelopment
VPS18	237.19	17.24	242.7	24.08	1.02	0.0331	0.722	1	Endosome
VPS35	746.41	26.05	761.98	23.23	1.02	0.0298	0.411	1	Endosome
APLP1	175.41	12.82	177.95	13.03	1.01	0.0207	0.795	1	Neurodegeneration
APP-695	6057.53	278.65	6117.63	106.24	1.01	0.0142	0.701	1	Neurodegeneration
APP-All	5536.37	436.62	5602.78	123.21	1.01	0.0172	0.783	1	Neurodegeneration
ATG16L1	302.13	35.88	305.1	23.58	1.01	0.0141	0.893	1	Autophagy
ATG2A	84.39	5.68	85.01	10.04	1.01	0.0106	0.914	1	Autophagy
ATP6V1B2	914.66	25.31	925.97	24.74	1.01	0.0177	0.547	1	vATPase
GABARAPL2	1259.4	35.07	1271.45	22.41	1.01	0.0137	0.585	1	Autophagy
GGA2	1013.54	74.68	1028.07	33.78	1.01	0.0205	0.73	1	Endosome
GSK3A	869.63	50.66	879.15	36.22	1.01	0.0157	0.769	1	Neurodegeneration
HIF1A	3115.9	111.81	3137.7	77.52	1.01	0.0101	0.761	1	Neurodevelopment
HPS1	47.37	2.51	47.69	3.37	1.01	0.00979	0.881	1	Lysosomal
NMDA NR1	640.45	15.22	649.95	35.87	1.01	0.0212	0.643	1	Neurodevelopment
NTF3	14.98	1.85	15.19	1.94	1.01	0.0196	0.887	1	Signaling
STX6	956.12	11.17	968.71	11.73	1.01	0.0189	0.172	1	Endosome
SYP	20.67	3.73	20.8	5.9	1.01	0.00897	0.973	1	Neurodevelopment
USP13	382.31	22.98	387.66	33.93	1.01	0.02	0.799	1	Autophagy
VPS26A	573.68	32.95	581.38	40.41	1.01	0.0192	0.777	1	Endosome
WIP1	201.1	6.45	202.24	16.73	1.01	0.00811	0.902	1	Autophagy
aph1Avar3	678.44	12	681.68	23.76	1	0.00688	0.815	1	Neurodegeneration
DCX	27.68	3.84	27.74	6.32	1	0.00306	0.988	1	Neurodevelopment
MARK1	339.68	31.93	340.35	25.89	1	0.00282	0.975	1	Neurodegeneration
PPT1	2900.98	70.47	2909.92	42.77	1	0.00444	0.836	1	Lysosomal
PSAP	3756.4	168.99	3760.35	79.25	1	0.00152	0.968	1	Lysosomal
SUMF1	212.51	16.68	213.4	13.93	1	0.00605	0.937	1	Lysosomal
VPS33A	340.6	22.21	341.9	19.93	1	0.00547	0.935	1	Endosome

Table 3.9 Genes downregulated in G383D iPSCs vs control iPSCs

Gene	Control (Counts)	StDev of Control	G383D (Counts)	StDev of G383D	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
VIM	2688.25	60.92	1929.67	17.36	-1.39	-0.478	1.67E-07	0.000378	Neurodevelopment
SLC9A6 TV 1 3	311.82	32.7	170.36	12.89	-1.83	-0.872	8.79E-05	0.0503	Endosome
SLC9A6	135.52	16.53	63.61	6.8	-2.13	-1.09	8.91E-05	0.0503	Endosome
BCL11B	64.45	12.57	32.29	1.71	-2	-0.997	0.000514	0.181	Neurodevelopment
ULK4	149.06	21.32	72.01	12.68	-2.07	-1.05	0.000561	0.181	Autophagy
ATG3	1208.89	21.85	1139.08	13.68	-1.06	-0.0858	0.00155	0.403	Autophagy
ASAH1	716.76	37.39	608.32	19.96	-1.18	-0.237	0.00179	0.404	Lysosomal
aph1Bvar1	379.1	15.78	333.7	11.32	-1.14	-0.184	0.00317	0.55	Neurodegeneration
RAB5A	1495.46	52.72	1301.68	65.6	-1.15	-0.2	0.0038	0.586	Neurodegeneration
WDFY2	237.3	14.94	201.21	7.8	-1.18	-0.238	0.0042	0.586	Endosome
SQSTM1	205.36	9.84	166.66	13.46	-1.23	-0.301	0.00441	0.586	Autophagy
SLC12A2	188.73	17.28	144.04	11.74	-1.31	-0.39	0.00466	0.586	Neurodevelopment
BDNF	152.36	9.33	119.54	11.36	-1.27	-0.35	0.00506	0.602	Signaling
USP10	1814.08	31.07	1708.49	41.82	-1.06	-0.0865	0.00702	0.755	Autophagy
ADAM9	193.19	24.46	150.35	9.73	-1.28	-0.362	0.0106	1	Neurodegeneration
SLC17A6	31.59	4.8	18.79	5.49	-1.68	-0.749	0.0138	1	Neurodevelopment
APLP2	3279.64	226.08	2909	71.56	-1.13	-0.173	0.0169	1	Neurodegeneration
TUBB3	294.14	31.74	238.03	20.72	-1.24	-0.305	0.0192	1	Neurodevelopment
IGF1R	372.66	20.7	331.1	18.33	-1.13	-0.171	0.0246	1	Signaling
GAA	209.98	12.47	189.54	7.3	-1.11	-0.148	0.0264	1	Lysosomal
ATP6V0B	624.94	12.95	570.5	33.94	-1.1	-0.131	0.027	1	vATPase
NEDD4	40.71	2.59	30.86	5.48	-1.32	-0.4	0.0274	1	Endosome
WIP1	94.38	11.2	68.76	15.26	-1.37	-0.457	0.0372	1	Autophagy
APOE	3882.17	261.74	3458.82	195.14	-1.12	-0.167	0.0383	1	Neurodegeneration
GALNS	102.17	8.57	90.76	3.4	-1.13	-0.171	0.0397	1	Lysosomal
NCSTN	464.58	46.71	404.51	12.27	-1.15	-0.2	0.0426	1	Neurodegeneration
MXN1	11.22	3.19	6.61	1.94	-1.7	-0.765	0.043	1	Neurodevelopment
EEA1	390.61	39.64	329.87	31.07	-1.18	-0.244	0.0463	1	Endosome
Sox10	11.17	0.53	7.27	2.6	-1.53	-0.618	0.0511	1	Neurodevelopment
TTBK1	14.08	5.8	7.2	2.57	-1.95	-0.966	0.0535	1	Neurodegeneration
HGSNAT	25.44	3.9	18.83	4.14	-1.35	-0.434	0.054	1	LSD
MYOD1	13.69	4.34	9.32	1.72	-1.47	-0.554	0.0619	1	Neurodevelopment
AKT3	476.86	45.79	427.68	8.84	-1.12	-0.157	0.0688	1	Signaling
APOER2	306.43	27	265.98	25.5	-1.15	-0.204	0.0688	1	Neurodegeneration
SLC9A3	7.29	1.25	5.06	1.43	-1.44	-0.528	0.075	1	Endosome
ULK2	60.7	6	51.22	5.96	-1.19	-0.245	0.0762	1	Autophagy
CD63	3989.62	116.19	3818.2	115.29	-1.04	-0.0634	0.0809	1	Lysosomal
PTEN	553.72	45.32	499.61	28.91	-1.11	-0.148	0.0822	1	Signaling
TCF4	302.79	23.43	273.85	15.74	-1.11	-0.145	0.0825	1	LSD
CALB1	381.01	18.33	337.47	35.46	-1.13	-0.175	0.0829	1	Neurodevelopment
Rab35	997.54	55.15	935.28	28.6	-1.07	-0.093	0.0835	1	Endosome
HES5	14.94	1.7	12.01	2.14	-1.24	-0.315	0.0877	1	Neurodevelopment
MANBA	174.84	11.28	155.58	14.81	-1.12	-0.168	0.0882	1	LSD
HYAL1	78.86	8.57	66.42	8.72	-1.19	-0.248	0.0912	1	LSD
ATG12	83.36	10.08	71.27	6.62	-1.17	-0.226	0.0916	1	Autophagy
GNPTAB	478.64	53.57	421.33	25.05	-1.14	-0.184	0.0944	1	LSD
SIRT1	1276.12	52.21	1192.61	64.71	-1.07	-0.0976	0.0964	1	Autophagy
WDR45	141.22	8.53	130.44	7.33	-1.08	-0.115	0.101	1	Autophagy
AMY	308.9	20.61	284.93	14.66	-1.08	-0.117	0.103	1	Neurodegeneration
NGFR	131.48	17.69	111.29	12.24	-1.18	-0.241	0.107	1	Signaling
Pax6	11.23	7.18	6.51	2.48	-1.72	-0.786	0.109	1	Neurodevelopment
PIK3R4	546.05	39.45	507.14	14.64	-1.08	-0.107	0.111	1	Autophagy
WDFY3	268.57	29.56	230.67	27.89	-1.16	-0.219	0.114	1	Autophagy
TFRC	1421.59	292.48	1185.42	38.78	-1.2	-0.262	0.117	1	Endosome
SCPEP1	585.97	19.62	560.4	20.23	-1.05	-0.0644	0.119	1	LSD
AP1S2	958.34	79.94	868.77	61.1	-1.1	-0.142	0.121	1	Endosome
APPL1	889.76	79.24	812.7	37.11	-1.09	-0.131	0.122	1	Endosome
BECN1	860	36.74	822.92	19.94	-1.05	-0.0636	0.124	1	Autophagy
HOXB1	12.65	0.87	9.57	3.33	-1.32	-0.403	0.132	1	Neurodevelopment
DNAJC5	67.39	9.43	51.8	13.1	-1.3	-0.38	0.133	1	LSD
GRIPAP1	258.85	28.73	230	19.84	-1.13	-0.17	0.136	1	Endosome
HOXA2	5.76	0.77	4.09	1.49	-1.41	-0.493	0.137	1	Neurodevelopment
LIPA	1143.05	78.05	1074.15	24.61	-1.06	-0.0897	0.138	1	LSD
APP-770	3869.49	149.14	3705.81	121.75	-1.04	-0.0624	0.141	1	Neurodegeneration
NPC1	349.99	44.35	306.97	29.1	-1.14	-0.189	0.144	1	LSD
SATB2	27.1	4.94	21.04	5.08	-1.29	-0.365	0.146	1	Neurodevelopment
CTSD	1345.23	84.8	1264.72	48.1	-1.06	-0.089	0.149	1	Lysosomal
RRAGC	249.59	8.3	228.08	24.73	-1.09	-0.13	0.16	1	Lysosomal
Rab11	275.82	15.76	258.74	15.73	-1.07	-0.0922	0.175	1	Endosome
RAB9A	777.26	31.35	744.91	28.26	-1.04	-0.0613	0.176	1	Endosome
FoxG1	12.15	2.25	9.05	3.72	-1.34	-0.424	0.18	1	Neurodevelopment
IGF2R	327.81	35.87	294.75	26.98	-1.11	-0.153	0.183	1	Lysosomal
ARSA	41.58	10.13	33.46	4.42	-1.24	-0.313	0.191	1	LSD
GBA	194.73	17.62	181.49	7.37	-1.07	-0.102	0.193	1	Lysosomal
NES	1047.96	91.54	973.81	48.51	-1.08	-0.106	0.197	1	Neurodevelopment

EOMES	14.18	8.21	9.28	0.96	-1.53	-0.612	0.198	1	Neurodevelopment
SLC9A1	221.1	25.77	196.18	22.09	-1.13	-0.173	0.198	1	Endosome
ATP6V0C	1739.25	96.11	1661.89	57.67	-1.05	-0.0656	0.207	1	vATPase
RAB7A	1980.25	62.96	1924.65	47.44	-1.03	-0.0411	0.208	1	Lysosomal
SLC9A9	18.48	2.82	14.8	4.74	-1.25	-0.321	0.209	1	Endosome
NAE1	2999.89	30.84	2922.53	106.9	-1.03	-0.0377	0.218	1	Neurodegeneration
MAP2	37.7	5.28	30.91	8.56	-1.22	-0.287	0.219	1	Neurodevelopment
SLC12A5	13.55	5.42	9.89	2.36	-1.37	-0.454	0.22	1	Neurodevelopment
IDS	292.04	36.97	263.72	23.2	-1.11	-0.147	0.222	1	LSD
ATG4D	260.87	13.25	244.91	19.69	-1.07	-0.0911	0.233	1	Autophagy
nanog	1837.27	106.81	1746.83	87.16	-1.05	-0.0728	0.235	1	Neurodevelopment
Isl1	5.35	0.87	4.13	1.37	-1.29	-0.372	0.238	1	Neurodevelopment
NBR1	578.53	22.74	557.41	23.84	-1.04	-0.0537	0.243	1	Autophagy
ARF6	1669.01	72.35	1623.09	20.85	-1.03	-0.0403	0.267	1	Endosome
NTRK3	12.79	3.16	9.78	3.68	-1.31	-0.388	0.278	1	Signaling
TJP1	2415.59	122.4	2323.81	91.93	-1.04	-0.0559	0.278	1	Neurodevelopment
OLIG2	14.1	5.7	11.33	1.77	-1.24	-0.316	0.281	1	Neurodevelopment
AFP	8.31	2.8	6.4	2.25	-1.3	-0.376	0.285	1	Neurodevelopment
SORL1	476.41	30.84	446.6	39.56	-1.07	-0.0932	0.285	1	Endosome
BACE2	119.31	7.11	111.05	11.88	-1.07	-0.103	0.289	1	Neurodegeneration
aph1Bvar2	86.42	18.99	73.75	12.21	-1.17	-0.229	0.291	1	Neurodegeneration
CTSA	463.43	50.3	435.89	11.19	-1.06	-0.0884	0.297	1	Lysosomal
GFAP	10.05	1.85	6.22	5.8	-1.62	-0.693	0.3	1	Neurodevelopment
TH	11.42	1.22	8.99	3.59	-1.27	-0.345	0.316	1	Neurodevelopment
RPTOR	110.24	21.74	96.73	13.09	-1.14	-0.189	0.318	1	Signaling
RELN	5.54	4.13	2.99	3.69	-1.85	-0.89	0.328	1	Neurodegeneration
ATP6V0E1	2087.67	74.94	2028.49	82.67	-1.03	-0.0415	0.329	1	vATPase
ATP6V0A1	397.6	35.64	378.57	10.67	-1.05	-0.0708	0.33	1	vATPase
ATG9B	15.54	3.84	12.12	5.6	-1.28	-0.359	0.344	1	Autophagy
CLN6	382.63	46.7	356.87	24.76	-1.07	-0.101	0.349	1	LSD
TFE3	48.61	6.16	44.18	6.22	-1.1	-0.138	0.353	1	Lysosomal
BLOC1S3	16.46	4.22	13.29	4.46	-1.24	-0.308	0.356	1	Lysosomal
ATG2B	164.62	14.58	153.46	16.53	-1.07	-0.101	0.361	1	Autophagy
ATG9A	528.05	43.84	502.29	27.78	-1.05	-0.0721	0.362	1	Autophagy
ATP6V1D	464.97	16.65	445.14	37.06	-1.04	-0.0629	0.373	1	vATPase
ADAM10	1058.42	81.17	1013.01	49.31	-1.04	-0.0633	0.378	1	Neurodegeneration
M6PR	691.4	45.82	664.25	36.69	-1.04	-0.0578	0.391	1	Lysosomal
NRBF2	940.42	96.26	887.77	63.23	-1.06	-0.0831	0.409	1	Autophagy
PSEN2	202.67	12.05	195.11	12.44	-1.04	-0.0548	0.409	1	Neurodegeneration
PRKAA1	232.99	10.49	220.54	25.83	-1.06	-0.0792	0.416	1	Autophagy
HOXA1	4.25	1.13	3.11	1.94	-1.37	-0.452	0.417	1	Neurodevelopment
STX13	190.44	19.17	180.5	13.49	-1.06	-0.0773	0.417	1	Endosome
LAMP2	965.62	35.02	945.08	32.95	-1.02	-0.031	0.42	1	LSD
TMEM55B	111.33	9.73	105.96	7.96	-1.05	-0.0714	0.425	1	Lysosomal
ATG101	423.41	28.84	408.67	20.8	-1.04	-0.0511	0.435	1	Autophagy
ATP13A2	299.12	21.8	287.84	15.93	-1.04	-0.0554	0.436	1	LSD
PSEN1	590.99	26.56	571.04	38.66	-1.03	-0.0495	0.441	1	Neurodegeneration
LAMP1	2381.87	185.52	2298.83	82.19	-1.04	-0.0512	0.445	1	Lysosomal
SLC9A2	13.82	4.03	11.47	4.61	-1.2	-0.268	0.45	1	Endosome
Rab10	894.34	22.82	879.74	29.49	-1.02	-0.0237	0.461	1	Endosome
ULK1	243	17.13	231.37	23.4	-1.05	-0.0707	0.466	1	Autophagy
SLC17A7	16.27	5.58	14.04	1.98	-1.16	-0.212	0.479	1	Neurodevelopment
MAN2B1	468.58	51.25	448.3	22.16	-1.05	-0.0638	0.481	1	LSD
CLCN7	391.74	50.12	366.84	44.92	-1.07	-0.0947	0.488	1	Endosome
TMEM74	45.9	2.06	41.21	13.33	-1.11	-0.156	0.488	1	Autophagy
ADAM19	101.2	11.14	96.29	6.95	-1.05	-0.0717	0.49	1	Neurodegeneration
PEX16	162.46	9.61	158.84	3.39	-1.02	-0.0326	0.49	1	LSD
LRP1	292.49	58.89	262.55	47.65	-1.11	-0.156	0.491	1	Neurodegeneration
DLX1	11.33	2.33	10.52	0.46	-1.08	-0.108	0.497	1	Neurodevelopment
RB1CC1	550.75	31.11	525.31	60.56	-1.05	-0.0682	0.506	1	Autophagy
LHX6	5.35	3.06	4.06	2.33	-1.32	-0.397	0.512	1	Neurodevelopment
MAP4	1481.08	96.93	1436.95	85.49	-1.03	-0.0436	0.515	1	Neurodegeneration
ATP6V0A2	182.16	20.35	173.17	16.79	-1.05	-0.073	0.517	1	vATPase
ATP6V1E1	1561.2	52.53	1533.26	63.8	-1.02	-0.0261	0.522	1	vATPase
IGF2	31.63	3.92	28.67	7.36	-1.1	-0.142	0.522	1	Signaling
NEU1	178.28	17.01	168.47	22.66	-1.06	-0.0817	0.522	1	Lysosomal
RICTOR	237.45	36.12	222.38	25.6	-1.07	-0.0946	0.522	1	Signaling
LRP10	191.77	24.55	183.55	6.92	-1.04	-0.0632	0.529	1	Neurodegeneration
ATG14	74.86	6.09	71.15	9.7	-1.05	-0.0735	0.533	1	Autophagy
PVALB	34.25	3.85	31.46	6.75	-1.09	-0.123	0.535	1	Neurodevelopment
PLBD2	150.86	18.75	143.02	16.33	-1.05	-0.077	0.538	1	Autophagy
SYN1	52.02	4.8	48.25	10.65	-1.08	-0.109	0.542	1	Neurodevelopment
APBB1	238.36	15	230.61	18.55	-1.03	-0.0477	0.552	1	Neurodegeneration
GNS	273.26	17.29	264.63	21.51	-1.03	-0.0463	0.553	1	Lysosomal
TACE/ADAM17	373.04	44.84	357.42	24.81	-1.04	-0.0617	0.563	1	Neurodegeneration
NGF	7.64	3.82	6.01	3.93	-1.27	-0.345	0.574	1	Signaling
GLA	896.01	69.12	869.39	64.56	-1.03	-0.0435	0.596	1	Lysosomal
VLDLR	852.66	61.95	833.65	37.64	-1.02	-0.0325	0.61	1	Neurodegeneration

NCAM	65.39	5.54	62.78	8.8	-1.04	-0.0589	0.627	1	Neurodevelopment
SLC9A7	357.09	24.4	350.54	10.44	-1.02	-0.0267	0.634	1	Endosome
MARK2	382.58	20.49	376.76	11.99	-1.02	-0.0221	0.635	1	Neurodegeneration
SLC9A4	11.95	2.4	10.5	4.52	-1.14	-0.188	0.64	1	Endosome
MAP1LC3B	686.46	27.19	678.88	18.8	-1.01	-0.016	0.66	1	Autophagy
ATG4A	37.77	11.17	35.06	5.93	-1.08	-0.108	0.662	1	Autophagy
PRKAA2	268.96	26.42	261.12	24.67	-1.03	-0.0427	0.682	1	Autophagy
MBP	105.91	5.37	103.77	8.65	-1.02	-0.0294	0.686	1	Neurodevelopment
SHANK3	44.87	6.42	42.85	7.54	-1.05	-0.0666	0.696	1	Neurodevelopment
SLC9A6 TV 1 2	10.99	2	9.52	5.6	-1.15	-0.207	0.705	1	Endosome
ATP6V0D2	11.72	2.58	10.39	6.09	-1.13	-0.173	0.715	1	vATPase
SLC17A5	247.45	10.6	243.6	17.59	-1.02	-0.0226	0.722	1	LSD
SLC9A5	27.62	2.48	26.34	6.49	-1.05	-0.0686	0.727	1	Endosome
GLB1	321.68	32.82	316.02	9.54	-1.02	-0.0256	0.749	1	Lysosomal
ATG4C	662.07	44.71	654.08	18.81	-1.01	-0.0175	0.751	1	Autophagy
PRKAG2	386.57	26.65	379.33	34.26	-1.02	-0.0273	0.751	1	Signaling
CLN3	69.9	11	67.39	10.73	-1.04	-0.0527	0.753	1	LSD
IDUA	37.82	4.75	36.85	3.5	-1.03	-0.0374	0.757	1	LSD
ATP6V1C1	246.68	25.43	240.15	34.08	-1.03	-0.0387	0.764	1	vATPase
KIF4	44.98	6.35	43.29	8.51	-1.04	-0.0553	0.764	1	Neurodevelopment
HEXB	952.56	44.98	943.89	39.26	-1.01	-0.0132	0.782	1	Lysosomal
ATP6V1B1	4.63	1.41	4.2	2.86	-1.1	-0.139	0.792	1	vATPase
NTRK1	3.65	3.2	3.14	3.48	-1.16	-0.215	0.792	1	Signaling
STH	9.41	3.26	8.82	2.34	-1.07	-0.0931	0.796	1	Neurodegeneration
ATP6V1A	1713.16	110.49	1697.95	26.96	-1.01	-0.0129	0.797	1	vATPase
PEX3	254.06	15.15	249.31	32.23	-1.02	-0.0272	0.8	1	LSD
AKT1S1	314.98	54.05	308.1	19.41	-1.02	-0.0319	0.806	1	Autophagy
CREB1	405.13	27.67	400.57	23.47	-1.01	-0.0163	0.811	1	Signaling
FOXP2	9.05	2.65	8.5	3.59	-1.06	-0.0904	0.811	1	Neurodevelopment
SHH	11.91	4.62	11.36	0.77	-1.05	-0.0681	0.813	1	Neurodevelopment
POU5F1	8479.02	196.69	8431.36	341.94	-1.01	-0.00813	0.817	1	Neurodevelopment
Tau_3R	13.6	1.55	13.29	2.22	-1.02	-0.0332	0.824	1	Neurodegeneration
PEX5	395.21	15.89	393.29	8.58	-1	-0.00704	0.838	1	LSD
IGF1	24.18	8.3	23.06	7.06	-1.05	-0.0679	0.839	1	Signaling
DAB1	72.17	16.01	70.54	8.42	-1.02	-0.0329	0.85	1	Neurodegeneration
ATP6V1F	1151.38	58.65	1146.65	36.47	-1	-0.00594	0.895	1	vATPase
SORT1	1164.89	75.05	1159.26	42.67	-1	-0.00698	0.9	1	Endosome
GABARAPL1	903.19	16.73	900.67	34.73	-1	-0.00402	0.901	1	Autophagy
EN1	8.31	3.87	7.99	3.64	-1.04	-0.0555	0.902	1	Neurodevelopment
TFEB	16.27	1.96	16.04	2.99	-1.01	-0.0207	0.906	1	Lysosomal
HOXB6	5.72	0.97	5.44	4.66	-1.05	-0.072	0.909	1	Neurodevelopment
DLG4	276.65	44.11	274.38	21.69	-1.01	-0.0119	0.927	1	Neurodevelopment
MFSB8	146.72	21.63	145.55	14.87	-1.01	-0.0115	0.932	1	LSD
ULK3	358.16	35.68	356.79	17.96	-1	-0.00552	0.947	1	Autophagy
ATG5	493.68	12.74	492.5	33.56	-1	-0.00345	0.95	1	Autophagy
EHD4	371.53	18.13	370.85	10.36	-1	-0.00264	0.951	1	Endosome
AKT1	172.05	4.93	171.91	10.96	-1	-0.00114	0.983	1	Signaling
PEX13	303.19	24.41	302.93	22.99	-1	-0.00125	0.988	1	LSD
TWIST1	8.13	3.36	8.09	3.84	-1	-0.0065	0.988	1	Neurodevelopment
ATG7	152.62	10.84	152.51	13.48	-1	-0.00111	0.99	1	Autophagy
ATOH1	7.05	1.65	7.04	1.84	-1	-0.00333	0.99	1	Neurodevelopment
GRIN3A	14.25	3.88	14.21	6.52	-1	-0.00395	0.991	1	Lysosomal

Table 3.10 Genes upregulated in R472fsX4 iPSC-derived neurons vs control

Table 3.11 Genes downregulated in R472fsX4 iPSC-derived neurons vs control

Table 3.12 Genes upregulated in W523X iPSC-derived neurons vs control

Table 3.13 Genes downregulated in W523X iPSC-derived neurons vs control

Table 3.14 Genes upregulated in G383D iPSC-derived neurons vs control

Table 3.15 Genes downregulated in G383D iPSC-derived neurons vs control

The expression of 358 genes was measured using Nanostring in CS and control iPSC-derived neurons. Gene expression was normalized to hybridization controls and to a set of housekeeping genes: *GUSB*, *HPRT1*, *POLR2A*, and *RPL13A*. Changes in gene expression were compared between CS and control using a two-tailed Welch's t-test followed by Benjamini-Yekutieli FDR adjustment. For each Control/CS pair: n = 4 control RNAs collected independent neuronal inductions, and n = 4 CS RNAs collected from independent neuronal inductions.

Table 3.10 Genes upregulated in R472fsX4 iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	R472X (Counts)	StDev of R472X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
ULK4	127.47	17.32	87.81	10.68	1.45	0.538	0.00616	1	Autophagy
WIP1	46.58	9.01	26.1	5.67	1.79	0.836	0.0097	1	Autophagy
GALNS	210.26	10.3	162.97	23.05	1.29	0.368	0.0136	1	Lysosomal
ARSA	175.69	58	94.87	32	1.85	0.889	0.0293	1	LSD
CTSF	57.92	48.69	10.59	19.57	5.47	2.45	0.0365	1	Lysosomal
OTX2	390.48	63.26	255.08	81.14	1.53	0.614	0.0485	1	Neurodevelopment
KMT2D	161.19	9.39	124.9	31.72	1.29	0.368	0.0833	1	LSD
ATP6V0A4	15.89	4.01	8.43	5.28	1.88	0.914	0.109	1	vATPase
TFRC	1490.43	388.71	1172.85	97.63	1.27	0.346	0.116	1	Endosome
MAPK3	1587.57	308.69	1202.55	331.95	1.32	0.401	0.147	1	Signaling
MANBA	91.17	17.61	67.63	21.48	1.35	0.431	0.152	1	LSD
MFSD8	498.06	52.56	404.69	92.04	1.23	0.3	0.156	1	LSD
aph1Bvar2	36.13	5.09	29.3	6.98	1.23	0.302	0.167	1	Neurodegeneration
PRKAA2	458.2	90.55	378.81	55.58	1.21	0.275	0.169	1	Autophagy
CNTN4	172.91	92.1	103.43	45.43	1.67	0.741	0.174	1	Neurodegeneration
HYAL1	79.75	34.2	58.15	11.27	1.37	0.456	0.194	1	LSD
HOXB1	12.31	3.71	7.31	4.23	1.68	0.751	0.198	1	Neurodevelopment
Nkx2-1	119.54	1124.37	18.68	65.21	6.4	2.68	0.203	1	Neurodevelopment
PEX1	716.45	170.75	568.48	139.9	1.26	0.334	0.217	1	LSD
SLC9A8	116.14	26.05	93.36	21.81	1.24	0.315	0.227	1	Endosome
HoxB4	9.09	3.22	6.74	2.2	1.35	0.432	0.234	1	Neurodevelopment
Iba1	12.3	6.33	8.43	3.43	1.46	0.545	0.237	1	Neurodevelopment
NAGA	84.09	20.39	71.27	8.79	1.18	0.239	0.241	1	LSD
ULK3	1508.73	452.79	1064.5	483.85	1.42	0.503	0.259	1	Autophagy
aph1Bvar1	418.69	126.81	340.56	54.6	1.23	0.298	0.267	1	Neurodegeneration
LRP1	792.46	136.01	622.41	215.29	1.27	0.348	0.268	1	Neurodegeneration
PRKAA1	493.29	53.78	420.35	98.29	1.17	0.231	0.273	1	Autophagy
AP1S2	2568.06	229.59	1858.12	888.27	1.38	0.467	0.283	1	Endosome
LHX6	55.64	97.7	16.18	58.28	3.44	1.78	0.299	1	Neurodevelopment
WDFY3	782.11	91.92	676.94	142.51	1.16	0.208	0.305	1	Autophagy
Isl1	715.58	994.3	187.7	585.66	3.81	1.93	0.312	1	Neurodevelopment
CALB1	361.04	153.6	236.2	152.87	1.53	0.612	0.318	1	Neurodevelopment
ULK1	673.59	135.67	554.29	165.75	1.22	0.281	0.328	1	Autophagy
ATG4B	2389.8	389.4	2086.34	408.51	1.15	0.196	0.331	1	Autophagy
SGSH	79.93	63.19	58.29	13.31	1.37	0.455	0.338	1	LSD
NEDD4	52.17	18.17	44.04	5.92	1.18	0.244	0.339	1	Endosome
IGF2R	337.69	50.16	296.92	58.41	1.14	0.186	0.342	1	Lysosomal
MAP4	2413.74	470.81	2018.23	589.16	1.2	0.258	0.344	1	Neurodegeneration
APBB3	355.66	91.42	268.28	120.72	1.33	0.407	0.346	1	Neurodegeneration
ATG4A	169.19	29.57	125.52	67.33	1.35	0.431	0.347	1	Autophagy
TACE/ADAM17	845.36	130.68	779.55	65.29	1.08	0.117	0.368	1	Neurodegeneration
OLIG2	200.56	208.04	124.7	72.32	1.61	0.686	0.369	1	Neurodevelopment
APOER2	460.24	28.28	409.78	95.44	1.12	0.168	0.372	1	Neurodegeneration
Satb1	799.24	245.03	636.35	229.04	1.26	0.329	0.373	1	Neurodevelopment
ATG16L2	237.5	108.19	186.62	66.54	1.27	0.348	0.375	1	Autophagy
HGSNAT	494.7	96.85	408.25	143.95	1.21	0.277	0.38	1	LSD
PSEN1	1061.09	148.97	941.31	194.14	1.13	0.173	0.382	1	Neurodegeneration
IDUA	249.53	87.71	193.14	90.08	1.29	0.37	0.388	1	LSD
WDR45	282.27	42.2	226.08	89.69	1.25	0.32	0.395	1	Autophagy
EEA1	642.43	130.82	564.89	112.99	1.14	0.186	0.404	1	Endosome
AKT2	1469.51	244.19	1299.47	288.41	1.13	0.177	0.405	1	Signaling
POU3F3	1296.13	562.3	1027.48	336.31	1.26	0.335	0.408	1	Neurodevelopment
PHYH	35.22	14.08	29.16	3.99	1.21	0.272	0.417	1	LSD
CLN8	175.48	9.81	168.74	12.3	1.04	0.0566	0.42	1	LSD
ADAM19	159.05	29.7	136.07	38.49	1.17	0.225	0.421	1	Neurodegeneration
WDFY2	199.27	29.55	181.65	30.54	1.1	0.134	0.446	1	Endosome
HPS5	321.1	68.28	285.99	57.72	1.12	0.167	0.451	1	Lysosomal
NTRK3	305.08	151.93	237.08	104.35	1.29	0.364	0.456	1	Signaling
GRIPAP1	947.56	224.81	760.12	383.99	1.25	0.318	0.464	1	Endosome
HGS	129.1	27.62	112.63	30.39	1.15	0.197	0.464	1	Endosome
GGA1	366.4	90.72	310.42	102.54	1.18	0.239	0.465	1	Endosome
DLX1	1923.08	1619.09	779.4	2112.04	2.47	1.3	0.468	1	Neurodevelopment
CTSK	94.37	54.23	74.71	20.62	1.26	0.337	0.471	1	Lysosomal
TMEM74	147.39	72.96	113.18	65.06	1.3	0.381	0.474	1	Autophagy
APBA2	582.02	198.22	469.32	194.26	1.24	0.31	0.474	1	Neurodegeneration
IGF2	74.54	33.06	62.18	15.32	1.2	0.262	0.48	1	Signaling
POU3F2	579.2	411.57	429.91	272.7	1.35	0.43	0.483	1	Neurodevelopment
SLC9A7	532.74	136.44	457.65	140.45	1.16	0.219	0.49	1	Endosome
VPS8	750.15	136.51	639.26	239.52	1.17	0.231	0.494	1	Endosome
TTBK2	593.11	129.66	523.33	130.7	1.13	0.181	0.499	1	Neurodegeneration
APBA3	69.26	22.22	61.18	13.92	1.13	0.179	0.516	1	Neurodegeneration
RICTOR	478.85	95.29	422.16	128.21	1.13	0.182	0.517	1	Signaling
LAMP2	1574.84	108.98	1502.36	171.73	1.05	0.068	0.517	1	LSD
GAD1	658.33	392.08	377.68	686.11	1.74	0.802	0.524	1	Lysosomal
Rab11	133.5	21.27	118.41	34	1.13	0.173	0.531	1	Endosome

GAD2	520.69	292	246.93	639.72	2.11	1.08	0.535	1	Neurodevelopment
HOXA1	7.64	2.54	6.66	1.25	1.15	0.199	0.536	1	Neurodevelopment
ATG9B	17.28	4.96	15.31	3.15	1.13	0.174	0.537	1	Autophagy
nanog	12.57	3.86	11.23	1.93	1.12	0.163	0.537	1	Neurodevelopment
CREB1	904.5	176.85	800.18	261.03	1.13	0.177	0.538	1	Signaling
TMEM55B	308.47	64.1	275.8	70.29	1.12	0.161	0.545	1	Lysosomal
TH	121.32	99.94	74.93	78.65	1.62	0.695	0.546	1	Neurodevelopment
ATG9A	869.78	164.46	776.88	234.85	1.12	0.163	0.55	1	Autophagy
PSEN2	187.12	33.86	173.73	25.19	1.08	0.107	0.55	1	Neurodegeneration
STH	27.4	13.76	21.21	13.14	1.29	0.369	0.553	1	Neurodegeneration
ATG12	89.35	23.09	79.23	20.24	1.13	0.173	0.558	1	Autophagy
HEXA	665.46	159.28	590.27	184.37	1.13	0.173	0.561	1	Lysosomal
CLN3	146.12	36	130.82	35.22	1.12	0.16	0.561	1	LSD
MAP2	16357.39	4574.33	13315.7	6883.46	1.23	0.297	0.563	1	Neurodevelopment
KIAA0226	149.19	33.61	125.67	59.28	1.19	0.247	0.568	1	Autophagy
APP-All	8581.7	1968.6	7305.29	3084.94	1.17	0.232	0.569	1	Neurodegeneration
WIP1	463.05	118.74	399.74	162.57	1.16	0.212	0.57	1	Autophagy
SLC9A4	14.06	12.08	10.73	2.72	1.31	0.39	0.571	1	Endosome
TWIST1	7.64	2.57	6.63	2.35	1.15	0.205	0.571	1	Neurodevelopment
DCX	6475.54	3310.62	4697.16	4224.67	1.38	0.463	0.576	1	Neurodevelopment
BECN1	1191.52	146.41	1085.98	302.43	1.1	0.134	0.578	1	Autophagy
VLDLR	823.83	382.84	634.83	489.55	1.3	0.376	0.58	1	Neurodegeneration
ATP13A2	653.42	175.43	567.69	219.3	1.15	0.203	0.584	1	LSD
RELN	477.91	360.52	379.8	180.48	1.26	0.331	0.589	1	Neurodegeneration
ATP6V0E2	596.59	244.9	480.67	270.57	1.24	0.312	0.593	1	vATPase
NEU1	178.9	15.88	164.43	44.73	1.09	0.122	0.593	1	Lysosomal
PSENEN	1009.07	188.11	946.09	119.52	1.07	0.093	0.598	1	Neurodegeneration
MAPT	5121.01	2671.03	3691.04	3445.25	1.39	0.472	0.603	1	Neurodevelopment
FoxG1	573.77	218.49	481.17	228.23	1.19	0.254	0.604	1	Neurodevelopment
ATP6V0A1	2146.7	685.44	1720.92	1140.72	1.25	0.319	0.607	1	vATPase
Tau_4R	3676.29	1889.34	2672.92	2555.76	1.38	0.46	0.612	1	Neurodegeneration
IDS	2059.6	447.31	1766.83	853.47	1.17	0.221	0.627	1	LSD
SHH	39.84	93.78	28.1	14.15	1.42	0.504	0.629	1	Neurodevelopment
NPC1	407.91	49.96	389.1	54.45	1.05	0.0681	0.632	1	LSD
BLOC1S3	34.3	6.18	28.88	19.37	1.19	0.248	0.637	1	Lysosomal
PSAP	5023.43	980.54	4495.98	1693.88	1.12	0.16	0.639	1	Lysosomal
ATP6V1G2	929.99	230.02	764.62	518.75	1.22	0.282	0.642	1	vATPase
DLG4	2908.75	1158.4	2384.06	1517.89	1.22	0.287	0.646	1	Neurodevelopment
NAGLU	86.94	22.13	81.38	5.16	1.07	0.0953	0.646	1	Lysosomal
APP-KPI	168.21	44.11	157.15	22.08	1.07	0.0981	0.647	1	Neurodegeneration
UVRAG	600.65	187.37	523.37	230.27	1.15	0.199	0.648	1	Endosome
GABARAPL1	1014.55	251.18	887.98	400.86	1.14	0.192	0.655	1	Autophagy
SLC9A2	23.49	11.82	17.88	17.79	1.31	0.394	0.657	1	Endosome
Tau_3R	28.05	6	23.75	13.41	1.18	0.24	0.657	1	Neurodegeneration
ATG4D	537.2	135.3	487.63	155.16	1.1	0.14	0.667	1	Autophagy
AMBRA1	760.39	172.07	692.01	232.32	1.1	0.136	0.668	1	Autophagy
SHANK3	202.84	49.92	186.45	51.35	1.09	0.121	0.668	1	Neurodevelopment
SLC32A1	161.13	139.74	98.77	230.93	1.63	0.706	0.669	1	Neurodevelopment
MAP1LC3B	2494.47	768.52	2232.21	831.48	1.12	0.16	0.673	1	Autophagy
APLP2	3195.38	250.32	3007.3	727.51	1.06	0.0875	0.674	1	Neurodegeneration
ATP6V1E1	1920.56	477.75	1711.42	708.11	1.12	0.166	0.677	1	vATPase
APP-695	8551.49	1685.33	7721.72	2948.59	1.11	0.147	0.681	1	Neurodegeneration
ATG16L1	657.51	142.08	587.28	261.91	1.12	0.163	0.682	1	Autophagy
ATP6V0B	1343.43	481.45	1173.63	515.75	1.14	0.195	0.688	1	vATPase
TUBB3	5403.98	1955.39	4762.47	2051.52	1.13	0.182	0.689	1	Neurodevelopment
PEX3	363.99	45.37	346.39	65.77	1.05	0.0715	0.689	1	LSD
NTRK1	12.22	143.54	7.39	3	1.65	0.726	0.69	1	Signaling
GSK3B	3455.54	806.41	3181.3	928.12	1.09	0.119	0.693	1	Neurodegeneration
ATG2B	413.32	37.47	392.59	89.19	1.05	0.0742	0.694	1	Autophagy
IGF1	13.95	2.93	13.19	2.17	1.06	0.0809	0.695	1	Signaling
APLP1	11125.85	4946.58	9311.77	6281.41	1.19	0.257	0.698	1	Neurodegeneration
VAMP1	65.72	45.78	57.78	23.9	1.14	0.186	0.704	1	Endosome
PEX16	244.72	56.19	220.9	97.65	1.11	0.148	0.704	1	LSD
ATP6V1B2	2196.04	382.44	2020.79	694.05	1.09	0.12	0.705	1	vATPase
PEX13	722.83	58.19	699.44	102.61	1.03	0.0475	0.707	1	LSD
SYP	414.54	203.98	331.73	301.79	1.25	0.321	0.713	1	Neurodevelopment
PPP2R4	1463.67	228.01	1389.14	318.56	1.05	0.0754	0.717	1	Neurodegeneration
MARK2	717.93	130.94	670.78	201.78	1.07	0.098	0.721	1	Neurodegeneration
RAB9A	520.21	29.8	504.51	74.66	1.03	0.0442	0.724	1	Endosome
STX13	1624.22	616.43	1403.76	844.66	1.16	0.21	0.726	1	Endosome
Rab4	670.21	47.36	648.29	100.23	1.03	0.048	0.726	1	Endosome
VPS35	2007.95	331.3	1911.98	375.15	1.05	0.0707	0.73	1	Endosome
BCL11B	2658.96	3116.73	2010.12	2631.47	1.32	0.404	0.731	1	Neurodevelopment
GABARAPL2	4564.72	1434.86	4156.39	1476.32	1.1	0.135	0.735	1	Autophagy
CLCN7	699.29	97.69	658.25	194.05	1.06	0.0872	0.738	1	Endosome
CSNK1D	1927.58	354.62	1820.39	514.75	1.06	0.0825	0.74	1	Neurodegeneration
LRP3	610.43	151.88	569.08	163.65	1.07	0.101	0.749	1	Endosome
CDK5	760.85	258.48	683.36	334.35	1.11	0.155	0.75	1	Neurodegeneration

GABARAP	8169.01	1756.86	7753.51	1636.78	1.05	0.0753	0.751	1	Autophagy
RB1CC1	908.91	207.08	849.49	255.8	1.07	0.0975	0.753	1	Autophagy
MBP	23.69	10.05	20.78	12.71	1.14	0.189	0.767	1	Neurodevelopment
SLC9A5	31.81	8.52	28.82	18.21	1.1	0.142	0.769	1	Endosome
SLC12A5	511.55	178.29	422.84	492.68	1.21	0.275	0.774	1	Neurodevelopment
MARK1	1452.83	268.93	1333.55	599.83	1.09	0.124	0.775	1	Neurodegeneration
PICALM	1493.23	372.32	1406.46	421.15	1.06	0.0864	0.784	1	Neurodegeneration
NCAM	6676.03	1453.48	6065.78	3314.01	1.1	0.138	0.788	1	Neurodevelopment
MTOR	736.87	106.37	711.41	158.61	1.04	0.0507	0.791	1	Signaling
STX6	1536.39	306.3	1467.47	377.24	1.05	0.0662	0.793	1	Endosome
NES	4772.34	3421.36	4388.11	1309.06	1.09	0.121	0.795	1	Neurodevelopment
NTF4	12.2	6.31	11.33	4.05	1.08	0.107	0.795	1	Signaling
APBB1	2842.23	817.57	2617.22	1268.73	1.09	0.119	0.802	1	Neurodegeneration
CLCN3	383.56	68.55	362.14	129.09	1.06	0.0829	0.804	1	Endosome
GAA	266.43	64.45	255.66	54.25	1.04	0.0595	0.805	1	Lysosomal
RAB5A	1623.94	321.83	1541.43	516.89	1.05	0.0752	0.807	1	Neurodevelopment
GSK3A	2336.21	582.91	2202.08	805.45	1.06	0.0853	0.808	1	Neurodegeneration
SMPD1	93.59	24.51	85.65	46.41	1.09	0.128	0.809	1	LSD
SYN1	315.04	130.1	276.63	222.16	1.14	0.188	0.81	1	Neurodevelopment
ATP6V1H	1316.58	547.15	1203.59	581.87	1.09	0.129	0.81	1	vATPase
APBA1	580.12	229.38	509.74	407.46	1.14	0.187	0.812	1	Neurodegeneration
ATG10	216.72	40.48	211.32	13.94	1.03	0.0364	0.812	1	Autophagy
AKT3	3661.46	857.58	3447.93	1310.5	1.06	0.0867	0.813	1	Signaling
Sox1	1170.11	1071.62	1056.84	423.3	1.11	0.147	0.822	1	Neurodevelopment
GBA	447.6	71.91	426.78	150.27	1.05	0.0687	0.824	1	Lysosomal
ATP6V1G3	4.3	0.73	4.01	2.97	1.07	0.101	0.828	1	vATPase
ATP6V1C1	483.74	93.04	458.13	181.82	1.06	0.0785	0.831	1	vATPase
AKT1	849.83	164.85	819.86	206.37	1.04	0.0518	0.831	1	Signaling
NRBF2	925.1	211.42	890.66	228.46	1.04	0.0547	0.833	1	Autophagy
ATP6V1D	917.06	193.92	882.46	234.79	1.04	0.0555	0.839	1	vATPase
RABGEF1	725.57	124.11	698.86	194.57	1.04	0.0541	0.839	1	Endosome
ATG7	235.61	11.74	232.39	28.04	1.01	0.0198	0.84	1	Autophagy
Rab10	1188.76	251.18	1149.64	268.67	1.03	0.0483	0.842	1	Endosome
SIRT1	1054.18	205.01	1017.39	286.01	1.04	0.0513	0.843	1	Autophagy
SORT1	2928.41	592.06	2794.89	960.55	1.05	0.0673	0.845	1	Endosome
APOE	46.4	39.85	42.99	15.48	1.08	0.11	0.846	1	Neurodegeneration
CDK5R1	4139.35	1726.58	3734.92	3160.14	1.11	0.148	0.848	1	Neurodegeneration
BACE1	831.65	243.81	781.92	361.66	1.06	0.089	0.848	1	Neurodegeneration
CTSD	836.3	129.69	806.61	253.81	1.04	0.0521	0.848	1	Lysosomal
GGA3	998.34	229.37	963.25	268.44	1.04	0.0516	0.852	1	Endosome
ADAM9	386.25	207.47	369.42	76.51	1.05	0.0643	0.855	1	Neurodegeneration
WDR45L	1038.73	155.67	1011.71	234.56	1.03	0.038	0.861	1	Autophagy
ASAH1	1509.27	567.87	1442.29	442.83	1.05	0.0655	0.864	1	Lysosomal
MARK3	889.37	138.18	866.96	202.49	1.03	0.0368	0.867	1	Neurodegeneration
PEX5	740.1	123.62	718.61	209.81	1.03	0.0425	0.868	1	LSD
FOX2	537.91	839.5	472.46	499.2	1.14	0.187	0.87	1	Neurodevelopment
ATP6V0D1	2987.03	1003.81	2836.73	1338.28	1.05	0.0745	0.875	1	vATPase
PEX19	524.08	52.12	519.08	37.34	1.01	0.0138	0.881	1	LSD
LAMP1	2195.41	176.61	2153.65	475.32	1.02	0.0277	0.883	1	Lysosomal
ATP6V1A	3398.62	1290.94	3201.15	2060.75	1.06	0.0864	0.891	1	vATPase
IGF1R	494.89	35.82	491.4	35.01	1.01	0.0102	0.893	1	Signaling
ULK2	449.59	63.23	441.04	98.06	1.02	0.0277	0.895	1	Autophagy
ATP6V0D2	4.66	4.38	4.31	2.89	1.08	0.116	0.905	1	vATPase
RRAGC	611.63	132.81	596.45	192.34	1.03	0.0363	0.905	1	Lysosomal
FUCA1	442.59	191.76	425.99	232.72	1.04	0.0551	0.918	1	LSD
SLC17A5	171.33	47.07	168.07	46.45	1.02	0.0277	0.928	1	LSD
ATG14	120.43	28.95	118.51	28.99	1.02	0.0231	0.928	1	Autophagy
DNAJC5	433.32	126.09	422.38	187.9	1.03	0.0369	0.932	1	LSD
USP10	1924.27	286.41	1903.4	388.3	1.01	0.0157	0.932	1	Autophagy
GNPTAB	702.98	308.94	679.28	389.96	1.03	0.0495	0.933	1	LSD
HPS3	336.01	34.07	333.54	44.94	1.01	0.0106	0.934	1	Lysosomal
NAGPA	89.66	17.92	88.24	27.85	1.02	0.023	0.939	1	Lysosomal
EHD1	294.68	44.18	291	84.99	1.01	0.0181	0.94	1	Endosome
MYOD1	11.53	8.67	11.28	1.08	1.02	0.0309	0.941	1	Neurodevelopment
SLC9A1	224.92	26.69	222.34	65.49	1.01	0.0166	0.944	1	Endosome
ATP6V0C	4731.04	1743.01	4652.03	2170.38	1.02	0.0243	0.961	1	vATPase
MCOLN1	218.18	61.04	214.52	114.28	1.02	0.0244	0.962	1	Lysosomal
GNS	534.44	53.87	532.77	57.42	1	0.00451	0.966	1	Lysosomal
PVALB	6.14	3.28	6.09	2.04	1.01	0.012	0.978	1	Neurodevelopment
VPS18	333.37	53.76	332.23	97.96	1	0.00496	0.985	1	Endosome
ATG5	331.02	22.86	330.69	29.03	1	0.00144	0.987	1	Autophagy
Rab35	2445.23	530.14	2436.91	892.14	1	0.00492	0.989	1	Endosome
HOXA2	5.4	3.65	5.36	4.84	1.01	0.0104	0.99	1	Neurodevelopment
VPS26A	1048.4	167.1	1047.92	135.16	1	0.000671	0.996	1	Endosome
SUMF1	196.55	17.66	196.52	18.54	1	0.000257	0.998	1	Lysosomal

Table 3.11 Genes downregulated in R472fsX4 iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	R472X (Counts)	StDev of R472X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
SLC9A6 TV 1 3	231.65	58.65	1514.95	705.92	-6.54	-2.71	0.000644	1	Endosome
ATP6V0A2	299.47	9.51	356.34	17.4	-1.19	-0.251	0.00106	1	vATPase
SLC9A6	86.56	32.11	592.96	292.06	-6.85	-2.78	0.00133	1	Endosome
SLC9A6 TV 1 2	10.57	8.38	38.8	19.6	-3.67	-1.88	0.0215	1	Endosome
AGA	145.48	44.5	219.33	48.05	-1.51	-0.592	0.0755	1	LSD
Pax6	2499.94	1828.05	5717.87	1628.87	-2.29	-1.19	0.0812	1	Neurodevelopment
SATB2	26.22	9.53	39.76	11.37	-1.52	-0.601	0.0971	1	Neurodevelopment
SLC17A6	137.36	111.32	334.64	378.18	-2.44	-1.28	0.117	1	Neurodevelopment
LIPA	404.94	125.34	581.1	196.58	-1.44	-0.521	0.133	1	LSD
EHD4	40.03	18.9	61.66	12.58	-1.54	-0.623	0.149	1	Endosome
Fezf2	183.64	425.9	632.01	996.62	-3.44	-1.78	0.155	1	Neurodevelopment
MNX1	5.07	4.39	10.59	6.17	-2.09	-1.06	0.18	1	Neurodevelopment
EOMES	22.55	56.38	67.88	76.36	-3.01	-1.59	0.208	1	Neurodevelopment
ARSB	586.37	136.49	752.26	180.82	-1.28	-0.359	0.219	1	LSD
HOXB13	7.74	0.81	10.59	5.92	-1.37	-0.454	0.23	1	Neurodevelopment
DAB1	274.53	126.39	375.96	89.96	-1.37	-0.454	0.245	1	Neurodegeneration
MAN2B1	394.84	62.26	440.81	31.06	-1.12	-0.159	0.249	1	LSD
PPT1	1730.77	202.95	1860.4	39.15	-1.07	-0.104	0.258	1	Lysosomal
GLB1	262.39	54	300.14	23.9	-1.14	-0.194	0.264	1	Lysosomal
CTSA	411.82	58.16	465.45	71.57	-1.13	-0.177	0.295	1	Lysosomal
GM2A	136.1	37.35	167.03	45.18	-1.23	-0.295	0.304	1	LSD
Sox2	2096.78	746.79	2512.64	150.49	-1.2	-0.261	0.307	1	Neurodevelopment
CNTN2	395.21	250.36	577.79	229.39	-1.46	-0.548	0.315	1	Neurodegeneration
S100b	32.94	31.14	57.11	62.68	-1.73	-0.794	0.318	1	Neurodevelopment
AMY	614.58	123.26	769.19	308.98	-1.25	-0.324	0.322	1	Neurodegeneration
TCF4	497.32	183.55	609.2	117.28	-1.22	-0.293	0.324	1	LSD
HES5	3153.2	2340.43	4443.44	899.36	-1.41	-0.495	0.329	1	Neurodevelopment
NCSTN	919.39	140.51	1007.13	99.79	-1.1	-0.131	0.335	1	Neurodegeneration
CCND1	950.05	465.09	1368.92	906.68	-1.44	-0.527	0.344	1	Neurodevelopment
NTRK2	1501.05	1435.76	2672.73	1781.15	-1.78	-0.832	0.345	1	Signaling
ATOH1	5.4	2.88	7.54	4.16	-1.4	-0.482	0.348	1	Neurodevelopment
CHAT	11.57	2.71	14.5	5.78	-1.25	-0.326	0.36	1	Neurodevelopment
NTF3	6.74	10.76	11.81	8.96	-1.75	-0.809	0.362	1	Signaling
GLA	566.03	74.59	623.62	92.73	-1.1	-0.14	0.373	1	Lysosomal
M6PR	454.6	56.29	498.62	78.93	-1.1	-0.133	0.375	1	Lysosomal
SORL1	114.11	17.92	137.62	47.11	-1.21	-0.27	0.39	1	Endosome
BLOC1S1	1542.31	315.37	1797.8	436.41	-1.17	-0.221	0.397	1	Lysosomal
HEXB	337.14	81.2	376.08	42.82	-1.12	-0.158	0.399	1	Lysosomal
CUX2	316.96	103.77	383.67	110.48	-1.21	-0.276	0.401	1	Neurodevelopment
C1ORF85	75.35	33.58	90.88	14.73	-1.21	-0.27	0.415	1	Lysosomal
Tbr1	561.61	814.62	948.61	912.03	-1.69	-0.756	0.416	1	Neurodevelopment
B3GAT1	477.43	73.25	550.25	148.39	-1.15	-0.205	0.43	1	Neurodevelopment
aph1Avar4	458.12	81.01	503.42	83	-1.1	-0.136	0.452	1	Neurodegeneration
SCPEP1	118.08	61.69	150.48	75.29	-1.27	-0.35	0.463	1	LSD
NGF	4.17	3	5.76	4.01	-1.38	-0.466	0.509	1	Signaling
CLN5	216.54	16.73	228.76	30.37	-1.06	-0.0792	0.519	1	LSD
CALB2	173.91	87.92	244.98	211.24	-1.41	-0.494	0.519	1	Neurodevelopment
CTSC	466.86	379.2	598.6	292.16	-1.28	-0.359	0.539	1	LSD
SLC12A2	316.95	30.14	332.19	35.58	-1.05	-0.0677	0.544	1	Neurodevelopment
GRIN3A	94.04	34.17	127.78	91.89	-1.36	-0.442	0.546	1	Lysosomal
HOXB6	4.11	5.59	5.9	4.99	-1.44	-0.524	0.546	1	Neurodevelopment
PIK3R4	401.02	37.73	420.69	49.37	-1.05	-0.0691	0.553	1	Autophagy
NGFR	22.06	9.18	32.34	81.58	-1.47	-0.552	0.553	1	Signaling
CD63	2797.75	381.06	2987.34	513.9	-1.07	-0.0946	0.559	1	Lysosomal
GSAP	8.5	4.52	10.15	4.18	-1.19	-0.255	0.565	1	Neurodegeneration
BDNF	24.09	12.76	30.4	27.21	-1.26	-0.336	0.58	1	Signaling
MLST8	381.76	46.96	397.73	31.64	-1.04	-0.0591	0.593	1	Signaling
ATG101	328.17	35.97	349.24	66.53	-1.06	-0.0898	0.6	1	Autophagy
CCK	17.54	4.37	19.34	5.05	-1.1	-0.141	0.612	1	Neurodevelopment
Sox10	5.37	4.37	8.7	51.15	-1.62	-0.696	0.613	1	Neurodevelopment
TJP1	1251.43	305.74	1353.34	258.85	-1.08	-0.113	0.615	1	Neurodevelopment
TPP1	365.23	100.81	396.6	62.24	-1.09	-0.119	0.615	1	LSD
MAPK1	1846.79	252.92	1982.02	429.68	-1.07	-0.102	0.616	1	Signaling
VPS33A	327.11	79	360.37	96.59	-1.1	-0.14	0.623	1	Endosome
CLN6	196.03	43.32	212.03	50.79	-1.08	-0.113	0.63	1	LSD
HPS1	55.38	22.05	61.81	5.98	-1.12	-0.159	0.642	1	Lysosomal
aph1Avar3	937.38	104.9	985.68	171.94	-1.05	-0.0725	0.655	1	Neurodegeneration
DRAM1	44.21	17.27	49.56	15.28	-1.12	-0.165	0.659	1	Autophagy
NECAP2	494.95	100.49	518.51	28.28	-1.05	-0.0671	0.673	1	Endosome
TP73	25.91	4.81	29.51	21	-1.14	-0.188	0.676	1	Neurodevelopment
NAE1	2665.5	152.21	2736.17	302.74	-1.03	-0.0378	0.682	1	Neurodegeneration
ATP6V0E1	1306.84	167.35	1354.8	175.35	-1.04	-0.052	0.694	1	vATPase
AKT1S1	427.49	34.5	442.63	70.93	-1.04	-0.0502	0.701	1	Autophagy
USP13	392.64	4.47	399.81	35.49	-1.02	-0.0261	0.707	1	Autophagy
SLC36A1	184.04	58.15	200.91	66.7	-1.09	-0.127	0.717	1	Lysosomal

CD74	11.05	5.36	12.86	7.02	-1.16	-0.219	0.72	1	Neurodegeneration
GGA2	828.31	142.23	859.18	93.76	-1.04	-0.0528	0.727	1	Endosome
STK4	696.67	62.72	716.03	89.2	-1.03	-0.0395	0.73	1	Signaling
SLC17A7	17.04	23.57	20.43	16.53	-1.2	-0.262	0.744	1	Neurodevelopment
LRP10	199.17	97.22	213.24	29.53	-1.07	-0.0985	0.754	1	Neurodegeneration
EN1	8.75	3.59	10.4	18.81	-1.19	-0.249	0.758	1	Neurodevelopment
GNPTG	367.28	37.22	384.09	93.07	-1.05	-0.0646	0.765	1	Lysosomal
SLC9A3	3.59	2.95	4.25	2.88	-1.19	-0.245	0.772	1	Endosome
NPC2	1361.5	313.03	1419.01	239.29	-1.04	-0.0597	0.778	1	LSD
CTNS	94.86	16.35	97.95	13.99	-1.03	-0.0462	0.789	1	Lysosomal
AFP	6.76	1.63	7.12	2.06	-1.05	-0.0758	0.798	1	Neurodevelopment
PTEN	1133.53	168.05	1179.58	303.91	-1.04	-0.0575	0.812	1	Signaling
Cux1	916.2	125.41	943.2	185.62	-1.03	-0.0419	0.821	1	Neurodevelopment
ATG4C	487.38	44.54	494.3	45.72	-1.01	-0.0204	0.832	1	Autophagy
RAB7A	2843.34	742.89	2979.72	1023.94	-1.05	-0.0676	0.846	1	Lysosomal
ADAM10	1123.31	96.59	1139.4	135.5	-1.01	-0.0205	0.852	1	Neurodegeneration
SLC9A9	22.88	4.17	24.28	12.05	-1.06	-0.0859	0.852	1	Endosome
ATP6V1E2	109.25	19.25	111.45	15.03	-1.02	-0.0287	0.854	1	vATPase
ARF6	1622.91	218.1	1656.2	282.98	-1.02	-0.0293	0.856	1	Endosome
APP-770	317.34	324.75	340.9	124.55	-1.07	-0.103	0.858	1	Neurodegeneration
CTSB	922.49	163.45	947.54	217.88	-1.03	-0.0387	0.866	1	Lysosomal
VIM	19908.88	5332.27	20352.42	1974.91	-1.02	-0.0318	0.87	1	Neurodevelopment
NMDA NR1	479.18	59.22	486.54	69	-1.02	-0.022	0.872	1	Neurodevelopment
PLBD2	190.16	16.97	194.09	43.74	-1.02	-0.0296	0.874	1	Autophagy
GALC	75.9	19.83	78.63	24.03	-1.04	-0.051	0.875	1	LSD
POU5F1	8.27	2.02	8.73	8.23	-1.05	-0.0766	0.883	1	Neurodevelopment
PRKAG2	638.91	178.53	667.11	293.01	-1.04	-0.0623	0.884	1	Signaling
SNCA	118.91	90.72	127.23	62.91	-1.07	-0.0976	0.884	1	Neurodegeneration
TFEB	12.16	8.44	12.97	6.33	-1.07	-0.0926	0.887	1	Lysosomal
ATG3	1212.84	197.67	1236.88	244.57	-1.02	-0.0283	0.888	1	Autophagy
NPY	185.54	381.47	221.14	464.67	-1.19	-0.253	0.892	1	Neurodevelopment
MAP1LC3A	793.14	560.93	844.59	402.9	-1.06	-0.0907	0.893	1	Autophagy
KIF4	44.03	12.28	45.71	24.03	-1.04	-0.054	0.898	1	Neurodevelopment
GFAP	6.29	2.8	6.52	1.78	-1.04	-0.0509	0.9	1	Neurodevelopment
BACE2	70.05	39.58	72.47	21.1	-1.03	-0.0489	0.902	1	Neurodegeneration
VPS11	277.66	51.53	285.47	117.8	-1.03	-0.04	0.909	1	Endosome
TFE3	49.88	9.56	50.85	14.19	-1.02	-0.0279	0.91	1	Lysosomal
SQSTM1	493.25	103.16	501.27	93.13	-1.02	-0.0232	0.914	1	Autophagy
APPL1	1482.42	211.31	1499.02	227.4	-1.01	-0.0161	0.92	1	Endosome
ATP6V1B1	5.95	5.44	6.23	1.89	-1.05	-0.0664	0.939	1	vATPase
TTBK1	99.86	38.02	102.19	50.39	-1.02	-0.0333	0.946	1	Neurodegeneration
NBR1	1444.93	325.97	1466.25	513.54	-1.01	-0.0211	0.949	1	Autophagy
HIF1A	3653.55	339.46	3672.54	546.25	-1.01	-0.00748	0.956	1	Neurodevelopment
ATG2A	138.84	20.88	139.69	29.06	-1.01	-0.0088	0.964	1	Autophagy
APPBP2	1362.21	130.94	1369.32	305.85	-1.01	-0.0075	0.966	1	Neurodegeneration
RPTOR	251.36	34.38	251.97	73.44	-1	-0.00348	0.988	1	Signaling
ATP6V1F	2877.93	878.67	2887.87	1077.05	-1	-0.00497	0.99	1	vATPase
ATP6V1G1	56.38	18.68	56.44	8.25	-1	-0.00151	0.996	1	vATPase

Table 3.12 Genes upregulated in W523X iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	W523X (Counts)	StDev of W523X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
FoxG1	170.88	66.68	364.13	127.66	2.13	1.09	0.0334	1	Neurodevelopment
POU3F3	466.87	78.38	630.36	102.7	1.35	0.433	0.0447	1	Neurodevelopment
SHH	13.38	8.51	33.3	10.83	2.49	1.32	0.0458	1	Neurodevelopment
WIP12	254.17	55.7	374.27	81.7	1.47	0.558	0.0488	1	Autophagy
UVRAG	416	93.54	600.68	117.47	1.44	0.53	0.0513	1	Endosome
TFRC	734.94	73.46	843.46	67.09	1.15	0.199	0.0774	1	Endosome
DLX1	346.77	558.3	1225.16	578.64	3.53	1.82	0.0847	1	Neurodevelopment
SLC9A3	1.94	2.65	5.5	3.82	2.84	1.5	0.0913	1	Endosome
CLN5	147.79	8.48	162.31	12.98	1.1	0.135	0.0975	1	LSD
Isl1	147.06	137.82	317.99	102.46	2.16	1.11	0.108	1	Neurodevelopment
Sox1	427.9	158.03	666.33	281.89	1.56	0.639	0.12	1	Neurodevelopment
aph1Avar3	568.99	43.99	621.99	40.96	1.09	0.128	0.126	1	Neurodegeneration
NAGA	44.99	7.38	55.71	9.92	1.24	0.308	0.133	1	LSD
MLST8	227.01	23.47	256.68	26.78	1.13	0.177	0.137	1	Signaling
VPS26A	620.33	96.02	709.34	45.93	1.14	0.193	0.146	1	Endosome
ATP6V1E2	70.49	9.07	89.57	23.77	1.27	0.346	0.15	1	vATPase
BCL11B	606.44	320.53	1477.27	1433.49	2.44	1.28	0.167	1	Neurodevelopment
GSAP	5.41	8.78	16.58	10.8	3.06	1.62	0.173	1	Neurodegeneration
ATP6V0E2	290.37	110.48	423.77	147.16	1.46	0.545	0.174	1	vATPase
SLC32A1	51.32	61.38	159.7	144.78	3.11	1.64	0.176	1	Neurodevelopment
GAD2	125.67	185.26	424.62	419.11	3.38	1.76	0.179	1	Neurodevelopment
APPBP2	843.12	176.93	1000.9	132.59	1.19	0.247	0.198	1	Neurodegeneration
PPP2R4	828.45	141.06	939.93	64.5	1.13	0.182	0.199	1	Neurodegeneration
GNPTG	225.46	52.29	281.1	61	1.25	0.318	0.2	1	Lysosomal
ATG4B	1346.09	212.68	1575.25	238.32	1.17	0.227	0.215	1	Autophagy
FUCA1	269.82	106.58	392.23	165.99	1.45	0.54	0.227	1	LSD
APP-695	4543.59	2273.43	6472.47	1256.8	1.42	0.51	0.237	1	Neurodegeneration
PHYH	11.36	4.46	17.81	12	1.57	0.649	0.249	1	LSD
NPY	56.7	47.33	183.86	372.62	3.24	1.7	0.258	1	Neurodevelopment
MARK2	385.23	83.03	467.82	96.3	1.21	0.28	0.258	1	Neurodegeneration
CREB1	490.34	74.58	545.46	38.36	1.11	0.154	0.259	1	Signaling
ARF6	919.27	153.17	1039.09	126.82	1.13	0.177	0.263	1	Endosome
RAB5A	911.71	239.53	1114.6	233.04	1.22	0.29	0.264	1	Endosome
Nkx2-1	25.35	49.14	83.27	139.12	3.28	1.72	0.265	1	Neurodevelopment
aph1Avar4	169.63	179.8	306.36	102.25	1.81	0.853	0.268	1	Neurodegeneration
TH	36.91	36.41	86.7	104.57	2.35	1.23	0.275	1	Neurodevelopment
VPS33A	231.28	56.24	271.6	35.04	1.17	0.232	0.275	1	Endosome
HES5	1639.63	838.13	2565.39	2226.55	1.56	0.646	0.276	1	Neurodevelopment
WIP1	17.92	6.71	23.5	5.99	1.31	0.39	0.279	1	Autophagy
GSK3A	1301.13	377.91	1648.81	475.67	1.27	0.342	0.285	1	Neurodegeneration
NBR1	902.94	180.84	1067.75	221.24	1.18	0.242	0.285	1	Autophagy
AKT1	472.86	50.73	530.94	91.95	1.12	0.167	0.293	1	Signaling
APP-All	4860.24	1594.83	6019.78	1232.31	1.24	0.309	0.298	1	Neurodegeneration
Pax6	2817.78	503.33	3906.6	2394.71	1.39	0.471	0.3	1	Neurodevelopment
USP10	1156.77	129.33	1249.2	103.93	1.08	0.111	0.3	1	Autophagy
Rab4	397.76	47.41	429.97	27.98	1.08	0.112	0.303	1	Endosome
Rab10	735.24	168.66	851.62	133.88	1.16	0.212	0.304	1	Endosome
GLB1	178.55	30.83	200.07	25.93	1.12	0.164	0.315	1	Lysosomal
ATP6V0B	762.93	282.75	1005.52	335.86	1.32	0.398	0.317	1	vATPase
CLN3	95.84	25.91	115.43	21.69	1.2	0.268	0.331	1	LSD
LRP3	338.42	73.23	388.31	60.62	1.15	0.198	0.333	1	Endosome
PTEN	757.23	107.24	887.41	235.08	1.17	0.229	0.337	1	Signaling
aph1Bvar2	12.65	10.12	19.02	3.78	1.5	0.588	0.339	1	Neurodegeneration
GLA	369.64	43.79	421.63	98.9	1.14	0.19	0.345	1	Lysosomal
EEA1	374.82	82.06	431	54.98	1.15	0.202	0.351	1	Endosome
VLDLR	355.48	215.2	513.21	162.49	1.44	0.53	0.355	1	Neurodegeneration
ATP6V1A	2134.62	987.52	2959.31	1324.07	1.39	0.471	0.356	1	vATPase
PEX1	339.63	100.51	418.15	107.26	1.23	0.3	0.357	1	LSD
GAD1	204.39	299.72	476.93	436.62	2.33	1.22	0.36	1	Lysosomal
DCX	3084.19	1507.8	4479.21	1986.86	1.45	0.538	0.36	1	Neurodevelopment
ATP13A2	391.47	131.16	490.91	155.43	1.25	0.327	0.368	1	LSD
Tau_3R	13.22	8.21	18.61	6.07	1.41	0.494	0.37	1	Neurodegeneration
BECN1	639.2	116.91	723.69	131.66	1.13	0.179	0.387	1	Autophagy
TFE3	34.1	8.44	39.38	5.48	1.15	0.208	0.391	1	Lysosomal
MTOR	450.72	75.83	502.54	74.64	1.11	0.157	0.391	1	Signaling
APBA2	315.56	79.23	360.03	52.61	1.14	0.19	0.392	1	Neurodegeneration
MARK1	790.82	285.43	1023.35	344.89	1.29	0.372	0.397	1	Neurodegeneration
NGFR	40.75	21.66	53.38	17.72	1.31	0.389	0.4	1	Signaling
Cux1	560.49	91.85	619.1	90.17	1.1	0.143	0.401	1	Neurodevelopment
GGA3	553.13	122.5	642.31	148.21	1.16	0.216	0.409	1	Endosome
PICALM	882.03	226.16	994.14	108.88	1.13	0.173	0.411	1	Neurodegeneration
ATG16L1	388.65	120.16	499.53	195	1.29	0.362	0.413	1	Autophagy
LHX6	26.3	22.93	45.21	41.31	1.72	0.782	0.419	1	Neurodevelopment
PRKAG2	356.73	145.61	485.21	237.41	1.36	0.444	0.424	1	Signaling
CDK5	443.09	107.39	520.38	151.14	1.17	0.232	0.426	1	Neurodegeneration

PIK3R4	269.76	37.77	293.41	43.52	1.09	0.121	0.428	1	Autophagy
Sox2	1351.72	275.87	1519.31	284.77	1.12	0.169	0.429	1	Neurodevelopment
ASAH1	886.39	217.48	1018.09	222.06	1.15	0.2	0.438	1	Lysosomal
DLG4	1446.5	612.42	1985.69	1073.62	1.37	0.457	0.439	1	Neurodevelopment
VPS8	409.27	96.2	469.05	97.84	1.15	0.197	0.446	1	Endosome
HGSNAT	262.91	86.55	320.2	87	1.22	0.284	0.447	1	LSD
CDK5R1	2146.58	995.39	3142.71	2221.25	1.46	0.55	0.449	1	Neurodegeneration
ATP6V1H	831.2	331.7	1020.82	308.47	1.23	0.296	0.454	1	vATPase
STX13	968.54	342.73	1191.04	433.27	1.23	0.298	0.466	1	Endosome
BLOC1S1	1111.3	203.04	1200.93	101.14	1.08	0.112	0.467	1	Lysosomal
MARK3	542.57	120.16	614.97	126.29	1.13	0.181	0.471	1	Neurodegeneration
BACE1	520.83	204.02	641.05	209.52	1.23	0.3	0.475	1	Neurodegeneration
PRKAA1	255.43	73.94	295.12	67.75	1.16	0.208	0.483	1	Autophagy
ATP6V1B2	1333.49	309.94	1508.63	336.2	1.13	0.178	0.488	1	vATPase
SLC9A1	159.66	40.73	179.31	31.34	1.12	0.167	0.492	1	Endosome
POU3F2	262.68	105.21	325.29	148.81	1.24	0.308	0.494	1	Neurodevelopment
VPS18	216.29	57.98	247.13	60.57	1.14	0.192	0.494	1	Endosome
VPS35	1226.38	213.3	1312.49	128.09	1.07	0.0979	0.498	1	Endosome
ATG16L2	102.16	55.45	139.06	78.01	1.36	0.445	0.499	1	Autophagy
TTBK1	56.71	36.53	77.47	27.93	1.37	0.45	0.502	1	Neurodegeneration
NEDD4	28	12.13	32.71	5.38	1.17	0.224	0.507	1	Endosome
MAPK3	736.05	114.73	796.55	136.27	1.08	0.114	0.509	1	Signaling
SIRT1	633.71	158.91	700.77	95.07	1.11	0.145	0.51	1	Autophagy
ULK3	712.31	262.79	880.65	338.54	1.24	0.306	0.511	1	Autophagy
GRIPAP1	511.78	208.44	630.55	212.2	1.23	0.301	0.529	1	Endosome
ATP6V1F	1804.05	285.71	2026.96	685.99	1.12	0.168	0.529	1	vATPase
CLCN3	237.22	68.54	271.08	89.02	1.14	0.193	0.531	1	Endosome
ATP6V0C	3175.21	898.87	3715.01	1364.72	1.17	0.227	0.532	1	vATPase
USP13	231.97	24.98	246.42	35.73	1.06	0.0872	0.535	1	Autophagy
TUBB3	3269.67	735.32	3566.04	511.88	1.09	0.125	0.538	1	Neurodevelopment
CSNK1D	1073.33	217.05	1173.91	246.07	1.09	0.129	0.542	1	Neurodegeneration
SLC12A5	303.37	275.42	488.1	410.65	1.61	0.686	0.543	1	Neurodevelopment
ATG3	725.33	154.71	793.1	146.39	1.09	0.129	0.544	1	Autophagy
APP-KPI	96.81	13.05	108.36	38.31	1.12	0.163	0.545	1	Neurodegeneration
HGS	81.22	20.72	91.08	19.47	1.12	0.165	0.546	1	Endosome
TWIST1	6.1	5.9	9.25	5.66	1.52	0.602	0.548	1	Neurodevelopment
Tau_4R	2095.17	1364.66	2840.19	1808.14	1.36	0.439	0.548	1	Neurodegeneration
ULK1	377.39	120.94	443.1	137.11	1.17	0.232	0.55	1	Autophagy
ATG9A	529.98	83.6	588.32	156.4	1.11	0.151	0.554	1	Autophagy
PEX16	130.67	32.51	148.61	49.28	1.14	0.186	0.556	1	LSD
RAB7A	1821.59	431.97	2025.52	545.29	1.11	0.153	0.556	1	Lysosomal
CTSD	548.68	115.94	585.51	61.26	1.07	0.0937	0.571	1	Lysosomal
OTX2	116.71	5.84	145.38	168.25	1.25	0.317	0.576	1	Neurodevelopment
DNAJC5	277.3	81.13	324.14	129.56	1.17	0.225	0.578	1	LSD
KIAA0226	87.01	45.89	113.87	66.75	1.31	0.388	0.579	1	Autophagy
GABARAPL2	2986.92	412.88	3181.67	544.51	1.07	0.0911	0.581	1	Autophagy
RPTOR	163.06	25.14	174.34	30.45	1.07	0.0966	0.585	1	Signaling
AKT3	1992.35	277.69	2154.86	464.28	1.08	0.113	0.587	1	Signaling
APBB3	153.81	70.95	191.06	99.96	1.24	0.313	0.589	1	Neurodegeneration
MAPT	2895.84	1909.82	3765.24	2448.54	1.3	0.379	0.599	1	Neurodevelopment
ATG4D	311.31	51.76	334.61	60.52	1.07	0.104	0.607	1	Autophagy
RRAGC	378.33	73.43	405.56	73.42	1.07	0.1	0.613	1	Lysosomal
TMEM55B	163.45	33.84	182.17	63.08	1.11	0.156	0.62	1	Lysosomal
RB1CC1	550.3	103.54	599.01	143.31	1.09	0.122	0.622	1	Autophagy
EN1	35.62	45	54.33	73.52	1.53	0.609	0.623	1	Neurodevelopment
SYN1	205.64	105.42	256.88	161.68	1.25	0.321	0.63	1	Neurodevelopment
SORL1	120.55	45.37	138.67	43.67	1.15	0.202	0.63	1	Endosome
APBB1	1851.72	532.39	2100.22	827.77	1.13	0.182	0.63	1	Neurodegeneration
APLP2	2044.88	423.53	2185.59	325.94	1.07	0.096	0.63	1	Neurodegeneration
ATG14	76	30.71	88.7	30.29	1.17	0.223	0.631	1	Autophagy
APBA1	388.01	254.03	493.42	307.3	1.27	0.347	0.635	1	Neurodegeneration
ATP6V1E1	1247.55	284.54	1358.51	365.67	1.09	0.123	0.639	1	vATPase
CTSC	233.22	170.35	278.83	110.63	1.2	0.258	0.65	1	LSD
SYP	290.35	135.93	346.98	182.07	1.2	0.257	0.65	1	Neurodevelopment
ATP6V1D	584.53	108.95	623.73	129.68	1.07	0.0937	0.65	1	vATPase
SHANK3	133.96	55.16	154.44	67.72	1.15	0.205	0.655	1	Neurodevelopment
GAA	169.05	61.85	189.81	56.32	1.12	0.167	0.668	1	Lysosomal
APLP1	6599.02	2045.79	7424.59	2601.44	1.13	0.17	0.669	1	Neurodegeneration
ATP6V0D1	1978.1	585.06	2160.37	660.96	1.09	0.127	0.67	1	vATPase
VPS11	199.38	40.41	216.9	64.02	1.09	0.121	0.67	1	Endosome
ATG2B	285.44	62.93	315.26	112.82	1.1	0.143	0.672	1	Autophagy
ATG101	209.02	38.7	221.5	42.72	1.06	0.0837	0.672	1	Autophagy
MAPK1	1255.73	253.05	1342.2	315.71	1.07	0.0961	0.674	1	Signaling
SLC9A8	71.57	20.16	79.07	27.82	1.1	0.144	0.68	1	Endosome
LRP1	508.21	211.82	578.26	240.12	1.14	0.186	0.683	1	Neurodegeneration
MAP1LC3A	538.23	298.6	669.78	527.87	1.24	0.315	0.688	1	Autophagy
Rab11	66.9	28.8	74.82	11.59	1.12	0.161	0.69	1	Endosome
HEXA	405.93	108.93	438.07	93.49	1.08	0.11	0.696	1	Lysosomal

ATP6V0A2	210.14	44.79	223.09	44.35	1.06	0.0863	0.699	1	vATPase
ATP6V1C1	319.58	136.44	363.33	146.87	1.14	0.185	0.702	1	vATPase
PEX19	326.13	55.03	343.35	65.96	1.05	0.0742	0.702	1	LSD
PSAP	3424.28	582.45	3579.73	547	1.05	0.064	0.708	1	Lysosomal
SLC9A5	25.3	17.25	31.62	23.47	1.25	0.321	0.709	1	Endosome
MCOLN1	152.78	56.77	172.26	72.49	1.13	0.173	0.712	1	Lysosomal
GNPTAB	453.36	205.06	527.86	280.98	1.16	0.22	0.716	1	LSD
ATP6V0A1	1360.23	524.61	1551.17	751.73	1.14	0.19	0.724	1	vATPase
MAP2	10621.22	3015.85	11536.48	3232.06	1.09	0.119	0.724	1	Neurodevelopment
NTRK2	1643.2	329.11	1826.07	938.01	1.11	0.152	0.725	1	Signaling
WDR45L	641.7	141.56	673.85	108.31	1.05	0.0705	0.725	1	Autophagy
AMBRA1	461.75	106.78	489.91	93.51	1.06	0.0854	0.729	1	Autophagy
MFSB8	299.84	87.76	329.12	130.7	1.1	0.134	0.731	1	LSD
GGA1	221.62	62.24	240.3	72.28	1.08	0.117	0.731	1	Endosome
SMPD1	63.23	26.68	70.56	28.27	1.12	0.158	0.739	1	LSD
SLC9A7	378.69	56.3	399.3	111.41	1.05	0.0764	0.755	1	Endosome
STK4	432.01	45.69	441.62	37.06	1.02	0.0318	0.755	1	Signaling
Rab35	1593.86	297.12	1677.53	417.88	1.05	0.0738	0.756	1	Endosome
TFEB	25.65	22.71	31.08	24.88	1.21	0.277	0.758	1	Lysosomal
ATP6V0D2	6.58	2.47	7.79	9.42	1.18	0.243	0.76	1	vATPase
RABGEF1	483.39	120.85	516.08	151.72	1.07	0.0944	0.76	1	Endosome
APPL1	917.68	58.23	944.52	150.37	1.03	0.0416	0.76	1	Endosome
NTRK3	136.07	26.71	147.19	73.74	1.08	0.113	0.767	1	Signaling
AKT2	855.94	139.76	885.97	148.53	1.04	0.0497	0.781	1	Signaling
IGF2R	199.04	36.03	205.42	24.8	1.03	0.0455	0.784	1	Lysosomal
PEX13	487.99	43.95	500.22	69.84	1.03	0.0357	0.785	1	LSD
ATG2A	97.34	20.81	102.87	29.05	1.06	0.0798	0.789	1	Autophagy
MAP1LC3B	1454.41	411.17	1522.53	350.92	1.05	0.066	0.792	1	Autophagy
CLN8	118.13	21.78	124.1	38.02	1.05	0.0712	0.799	1	LSD
NMDA NR1	303.32	14.3	310.71	52.2	1.02	0.0347	0.799	1	Neurodevelopment
ARSA	78.06	26.58	82.64	25.35	1.06	0.0823	0.802	1	LSD
KMT2D	104.07	46.72	112.4	46.47	1.08	0.111	0.805	1	LSD
ATP6V1G2	768.28	263.79	830.75	423.84	1.08	0.113	0.807	1	vATPase
HIF1A	2079.02	474.94	2141.48	255.18	1.03	0.0427	0.809	1	Neurodevelopment
WDR45	173.93	47.35	184.76	65.24	1.06	0.0871	0.814	1	Autophagy
PRKAA2	259.64	68.15	271.29	56.55	1.04	0.0633	0.815	1	Autophagy
GALC	51.69	17.06	55.87	28.52	1.08	0.112	0.819	1	LSD
CLCN7	457.5	98.34	477.85	119.65	1.04	0.0628	0.819	1	Endosome
NAGPA	75.23	34.27	82.14	49.32	1.09	0.127	0.83	1	Lysosomal
GBA	309.46	89.54	324.2	92.47	1.05	0.0672	0.843	1	Lysosomal
SLC9A2	24.31	29.74	28.28	30.46	1.16	0.218	0.845	1	Endosome
SNCA	99.61	33.59	106.41	61.72	1.07	0.0952	0.848	1	Neurodegeneration
PEX5	533.62	63.44	549.74	144.91	1.03	0.0429	0.849	1	LSD
HPS3	214.31	38.12	218.86	21.82	1.02	0.0303	0.849	1	Lysosomal
M6PR	294.93	39.47	301.63	59.2	1.02	0.0324	0.857	1	Lysosomal
STH	35.29	30.88	39.51	35.44	1.12	0.163	0.859	1	Neurodegeneration
IDS	1261.36	489.61	1341.28	665.39	1.06	0.0886	0.863	1	LSD
PSEN1	672.66	82.73	680.46	31.96	1.01	0.0166	0.866	1	Neurodegeneration
GRIN3A	141.06	113.64	155.02	176.18	1.1	0.136	0.867	1	Lysosomal
NTRK1	14	8.08	15.17	16.14	1.08	0.116	0.87	1	Signaling
SORT1	1756.8	745.73	1819.86	289.2	1.04	0.0509	0.877	1	Endosome
HPS5	209.08	59.84	215.46	49.66	1.03	0.0434	0.877	1	Lysosomal
GABARAPL1	685.11	276.03	710.64	207.13	1.04	0.0528	0.885	1	Autophagy
GSK3B	2199.09	373.57	2245.5	469.59	1.02	0.0301	0.89	1	Neurodegeneration
IGF2	31.68	17.04	33.54	34.35	1.06	0.082	0.892	1	Signaling
NCAM	5364.73	2140.56	5609.58	2345.35	1.05	0.0644	0.899	1	Neurodevelopment
PEX3	272.66	62.48	279.89	78.41	1.03	0.0377	0.901	1	LSD
Iba1	19.92	16.4	21.33	19.1	1.07	0.0982	0.911	1	Neurodevelopment
Sox10	11.76	9.5	12.49	9.81	1.06	0.0864	0.912	1	Neurodevelopment
RELN	515.5	188.87	528.09	174.88	1.02	0.0348	0.923	1	Neurodegeneration
ATG4A	107.91	39.11	111.21	43.88	1.03	0.0435	0.926	1	Autophagy
SLC36A1	130.78	43.34	134.55	52.1	1.03	0.041	0.926	1	Lysosomal
RICTOR	294.02	68.28	299.49	82.96	1.02	0.0266	0.93	1	Signaling
NRBF2	602.87	127.57	610.61	157.93	1.01	0.0184	0.942	1	Autophagy
PPT1	1161.96	183.13	1171.85	181.4	1.01	0.0122	0.942	1	Lysosomal
MAN2B1	251.71	33.71	253.59	40.02	1.01	0.0108	0.944	1	LSD
ATG4C	285.86	61.64	288.04	17.27	1.01	0.011	0.945	1	Autophagy
aph1Bvar1	207.13	34.6	209	38.77	1.01	0.0129	0.947	1	Neurodegeneration
PLBD2	95.13	24.6	95.59	22.04	1	0.00698	0.979	1	Autophagy
OLIG2	104.55	104.35	105.92	71.14	1.01	0.0188	0.982	1	Neurodevelopment
LAMP2	984.56	145.38	985.18	72.74	1	0.000918	0.994	1	LSD
SLC12A2	278.73	72.33	279.08	62.93	1	0.00183	0.995	1	Neurodevelopment
BLOC1S3	24.64	7.51	24.68	11.8	1	0.00249	0.996	1	Lysosomal
B3GAT1	433.87	48.74	434.02	148.87	1	0.000524	0.999	1	Neurodevelopment

Table 3.13 Genes downregulated in W523X iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	W523X (Counts)	StDev of W523X	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
SLC9A6	417.28	128.75	46.78	11.82	-8.92	-3.16	3.84E-05	0.0868	Endosome
SLC9A6 TV 1 3	1066.86	326.88	151.57	58	-7.04	-2.82	0.000225	0.254	Endosome
HEXB	246.29	17.74	181.06	20.28	-1.36	-0.444	0.00356	1	Lysosomal
ATG10	215.65	24.73	145.43	20.28	-1.48	-0.568	0.00443	1	Autophagy
Tbr1	3125.51	961.92	580.64	1018.38	-5.38	-2.43	0.0157	1	Neurodevelopment
Fezf2	693.19	492.16	178.74	114.67	-3.88	-1.96	0.0158	1	Neurodevelopment
CNTN2	942.56	335.73	474.15	162.82	-1.99	-0.991	0.0232	1	Neurodegeneration
TPP1	289.81	41.6	226.67	24.72	-1.28	-0.355	0.0322	1	LSD
TCF4	469.81	99.13	309.72	110.18	-1.52	-0.601	0.0642	1	LSD
LRP10	163.01	40.63	112.4	27.81	-1.45	-0.536	0.0691	1	Neurodegeneration
CNTN4	138.55	49.77	93.02	8.2	-1.49	-0.575	0.0692	1	Neurodegeneration
GGA2	562.59	46.76	480.11	59.3	-1.17	-0.229	0.0764	1	Endosome
SLC17A6	813.92	568.62	251.57	303.95	-3.24	-1.69	0.0809	1	Neurodevelopment
ATP6V0E1	1030.85	222.35	766.27	132.83	-1.35	-0.428	0.0902	1	vATPase
EOMES	102.82	37.05	40.29	73.97	-2.55	-1.35	0.119	1	Neurodevelopment
CTSF	101.22	38.55	47.69	45.89	-2.12	-1.09	0.129	1	Lysosomal
PVALB	11.38	4.7	5.03	3.86	-2.26	-1.18	0.13	1	Neurodevelopment
SLC9A6 TV 1 2	25.89	13.14	12.96	9.45	-2	-0.998	0.138	1	Endosome
RAB9A	365.27	50.88	317.69	27.44	-1.15	-0.201	0.14	1	Endosome
C1ORF85	71.34	10.01	56.72	13.02	-1.26	-0.331	0.161	1	Lysosomal
ADAM9	263.42	86.75	192.72	44.17	-1.37	-0.451	0.167	1	Neurodegeneration
BACE2	75.67	22.86	52.52	19.93	-1.44	-0.527	0.188	1	Neurodegeneration
GNS	429.11	64.78	368.83	46.61	-1.16	-0.218	0.188	1	Lysosomal
S100b	48.04	28.34	29.54	11.56	-1.63	-0.702	0.192	1	Neurodevelopment
NPC2	912.54	301.89	640.35	264.25	-1.43	-0.511	0.208	1	LSD
SCPEP1	109.83	45	70.63	42.49	-1.56	-0.637	0.209	1	LSD
EHD4	42.65	16.49	29.92	9.37	-1.43	-0.512	0.216	1	Endosome
NTF3	20.47	6.81	12.09	9.85	-1.69	-0.759	0.235	1	Signaling
CD63	2179.37	412.52	1868.18	243.18	-1.17	-0.222	0.246	1	Lysosomal
ATP6V1B1	8.98	6.23	6.18	0.96	-1.45	-0.54	0.296	1	vATPase
SLC17A7	43.97	28.05	25.12	25.16	-1.75	-0.808	0.304	1	Neurodevelopment
APP-770	208.29	73.79	150.86	85.78	-1.38	-0.465	0.328	1	Neurodegeneration
Satb1	482.4	73.18	393.3	124.32	-1.23	-0.295	0.329	1	Neurodevelopment
NPC1	268.78	23.97	244.21	38.6	-1.1	-0.138	0.334	1	LSD
GM2A	122.18	30.01	95.6	40.7	-1.28	-0.354	0.338	1	LSD
NAGLU	70.32	12.93	60.09	15.75	-1.17	-0.227	0.343	1	Lysosomal
BDNF	68.7	68.73	37.26	30.38	-1.84	-0.883	0.346	1	Signaling
HOXB13	32.54	32.38	16.16	15.82	-2.01	-1.01	0.349	1	Neurodevelopment
NEU1	113.6	13.31	101.12	19.84	-1.12	-0.168	0.36	1	Lysosomal
TACE/ADAM17	569.61	45.89	522.64	86.55	-1.09	-0.124	0.384	1	Neurodegeneration
CALB2	340.69	171.31	234.85	131.61	-1.45	-0.537	0.394	1	Neurodevelopment
CTSA	331.26	45.96	282.66	85.07	-1.17	-0.229	0.406	1	Lysosomal
FOXP2	376.9	333.88	259.67	128.19	-1.45	-0.538	0.41	1	Neurodevelopment
MYOD1	37.65	27.12	20.82	27.79	-1.81	-0.855	0.416	1	Neurodevelopment
DRAM1	43.96	15.61	34.49	13.76	-1.27	-0.35	0.423	1	Autophagy
ATG7	186.31	18.09	173.02	24.3	-1.08	-0.107	0.429	1	Autophagy
AP1S2	1819.89	1031.65	1442.58	347.04	-1.26	-0.335	0.436	1	Endosome
SUMF1	167.35	11.62	148.66	40.05	-1.13	-0.171	0.437	1	Lysosomal
POU5F1	18.83	14.61	11.24	16.02	-1.68	-0.745	0.453	1	Neurodevelopment
CTNS	70.39	9.87	64.57	10.6	-1.09	-0.124	0.46	1	Lysosomal
SATB2	30.89	12.24	26.02	5.58	-1.19	-0.248	0.466	1	Neurodevelopment
STX6	911.88	74.95	842.27	152	-1.08	-0.115	0.477	1	Endosome
NGF	10.25	8.35	5.79	10.07	-1.77	-0.825	0.478	1	Signaling
HOXA2	27.08	23.05	14.7	31.54	-1.84	-0.882	0.488	1	Neurodevelopment
CD74	23.91	19.77	14.55	20.97	-1.64	-0.717	0.497	1	Neurodegeneration
SLC17A5	136.06	27.67	120.91	30.45	-1.13	-0.17	0.502	1	LSD
ADAM19	104.95	43.66	86.52	24.72	-1.21	-0.279	0.509	1	Neurodegeneration
NECAP2	344.39	26.97	326.49	42.6	-1.05	-0.077	0.511	1	Endosome
TP73	45.46	11.52	36.43	29.15	-1.25	-0.319	0.512	1	Neurodevelopment
WDFY2	123.34	26.92	109.54	32.04	-1.13	-0.171	0.522	1	Endosome
NTF4	29.1	22.28	20.36	18.69	-1.43	-0.516	0.525	1	Signaling
AKT1S1	313.2	31.39	293.38	48.12	-1.07	-0.0943	0.53	1	Autophagy
TJP1	861.07	119.06	756.21	311.67	-1.14	-0.187	0.536	1	Neurodevelopment
GFAP	7.95	4.1	6.2	4.49	-1.28	-0.359	0.538	1	Neurodevelopment
AFP	18.97	16.66	12.23	21.16	-1.55	-0.632	0.548	1	Neurodevelopment
CCND1	696.65	192.48	500.14	669.53	-1.39	-0.478	0.548	1	Neurodevelopment
LIPA	376.03	96.35	327.88	126	-1.15	-0.198	0.552	1	LSD
KIF4	37.61	24.58	28.06	21.35	-1.34	-0.422	0.561	1	Neurodevelopment
HOXB1	22.79	15.17	16.13	22.32	-1.41	-0.498	0.591	1	Neurodevelopment
nanog	57.16	63.3	34.47	59.52	-1.66	-0.729	0.592	1	Neurodevelopment
IGF1R	397.99	118.04	361.04	79.49	-1.1	-0.141	0.603	1	Signaling
PSEN2	146.28	27.26	137.92	16.03	-1.06	-0.0849	0.605	1	Neurodegeneration
VAMP1	59.13	38.88	46.67	28.76	-1.27	-0.342	0.608	1	Endosome
MAP4	1417	383.83	1318.83	103.32	-1.07	-0.104	0.612	1	Neurodegeneration
CALB1	412.61	380.51	330.41	112.51	-1.25	-0.321	0.624	1	Neurodevelopment
MNX1	19.44	16.37	14.13	13.3	-1.38	-0.46	0.629	1	Neurodevelopment

HOXA1	28.03	34.72	17.89	27.25	-1.57	-0.647	0.644	1	Neurodevelopment
CUX2	298.34	77.04	273.14	76.23	-1.09	-0.127	0.648	1	Neurodevelopment
AMY	662.15	226.39	582.23	276.36	-1.14	-0.186	0.659	1	Neurodegeneration
HOXB6	15.39	13.02	11.28	17.57	-1.36	-0.448	0.677	1	Neurodevelopment
CHAT	33.45	31.73	25.01	32.09	-1.34	-0.419	0.681	1	Neurodevelopment
NCSTN	698.6	69.18	674.24	89.07	-1.04	-0.0512	0.683	1	Neurodegeneration
ATOH1	25.98	32.57	18.8	25.32	-1.38	-0.467	0.7	1	Neurodevelopment
HoxB4	22.69	24.18	16.11	25.74	-1.41	-0.494	0.702	1	Neurodevelopment
TMEM74	109.36	41.21	99.13	29.45	-1.1	-0.142	0.708	1	Autophagy
ATP6V1G1	57.74	27.9	49.04	32.91	-1.18	-0.236	0.711	1	vATPase
GALNS	146.93	23.93	139.74	30.13	-1.05	-0.0724	0.72	1	Lysosomal
LAMP1	1543.38	290.09	1491.06	99.83	-1.04	-0.0498	0.737	1	Lysosomal
VIM	10002.27	3283.31	9163.1	4092.68	-1.09	-0.126	0.75	1	Neurodevelopment
CTSB	672.61	93.76	652.3	92.15	-1.03	-0.0442	0.762	1	Lysosomal
DAB1	265.87	81.04	253.22	39.54	-1.05	-0.0703	0.771	1	Neurodegeneration
PSENEN	678.08	76.53	661.97	79.02	-1.02	-0.0347	0.775	1	Neurodegeneration
SQSTM1	350.92	34.93	330.97	108.67	-1.06	-0.0844	0.775	1	Autophagy
SLC9A4	23.53	24.02	18.14	41	-1.3	-0.375	0.781	1	Endosome
AGA	129.96	15.83	122.12	54.24	-1.06	-0.0897	0.788	1	LSD
GABARAP	4982.9	930.05	4849.08	406.93	-1.03	-0.0393	0.796	1	Autophagy
CTSK	91.95	65.01	80.54	61.65	-1.14	-0.191	0.799	1	Lysosomal
WDFY3	570.74	142.25	535.45	212.91	-1.07	-0.0921	0.799	1	Autophagy
ATG12	90.11	68.94	79.86	49.99	-1.13	-0.174	0.811	1	Autophagy
SGSH	57	16.02	53.93	20.46	-1.06	-0.0798	0.826	1	LSD
ARSB	458.15	45.69	450.39	63.74	-1.02	-0.0246	0.852	1	LSD
SLC9A9	41.36	23.01	37.99	32.76	-1.09	-0.122	0.859	1	Endosome
CLN6	146.49	29.29	141.39	49.05	-1.04	-0.0511	0.863	1	LSD
MBP	36.14	49.93	32.55	12.52	-1.11	-0.151	0.871	1	Neurodevelopment
IGF1	14.2	12.27	13.32	2.58	-1.07	-0.0926	0.873	1	Signaling
CCK	25.36	26.09	22.95	24.26	-1.11	-0.144	0.881	1	Neurodevelopment
MANBA	49.99	26.72	47.73	12.45	-1.05	-0.0668	0.888	1	LSD
ULK2	304.72	91.59	293.51	114.8	-1.04	-0.0541	0.891	1	Autophagy
TTBK2	399.17	75.2	391.02	90.98	-1.02	-0.0298	0.9	1	Neurodegeneration
ATP6V1G3	7.13	8.01	6.46	6.97	-1.1	-0.141	0.901	1	vATPase
NES	2377.4	237.69	2286.2	1709.42	-1.04	-0.0564	0.901	1	Neurodevelopment
ULK4	67.5	23.71	66.01	11.88	-1.02	-0.0322	0.918	1	Autophagy
IDUA	106.03	44.64	101.4	66.74	-1.05	-0.0645	0.925	1	LSD
ATG9B	28.47	30.3	26.6	31.47	-1.07	-0.0983	0.927	1	Autophagy
NAE1	1540.98	397.32	1518.33	266.13	-1.01	-0.0214	0.927	1	Neurodegeneration
ADAM10	721.54	72.15	716.69	76.45	-1.01	-0.00972	0.929	1	Neurodegeneration
APBA3	61.34	29.58	59.64	28.31	-1.03	-0.0404	0.939	1	Neurodegeneration
HPS1	42.72	6.53	42.08	16.46	-1.02	-0.0219	0.947	1	Lysosomal
ATG5	234.42	20.78	233.43	34.11	-1	-0.00611	0.962	1	Autophagy
APOE	25.49	16.64	25.13	8.24	-1.01	-0.02	0.969	1	Neurodegeneration
EHD1	205.89	48.78	205.33	17.32	-1	-0.00386	0.985	1	Endosome
HYAL1	68.56	61.94	68.04	40.86	-1.01	-0.011	0.989	1	LSD
APOER2	310.91	93.72	310.5	80.49	-1	-0.00188	0.995	1	Neurodegeneration
ATP6V0A4	9.38	6.64	9.35	5.58	-1	-0.00388	0.995	1	vATPase

Table 3.14 Genes upregulated in G383D iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	G383D (Counts)	StDev of G383D	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
CTSF	102.14	31.56	417.41	29.27	4.09	2.03	9.35E-05	0.211	Lysosomal
ATG10	180.17	23.12	264.7	23.73	1.47	0.555	0.00246	1	Autophagy
Sox1	627.08	204.18	1177.74	511.52	1.88	0.909	0.042	1	Neurodevelopment
OLIG2	67.41	49.26	328.79	798.92	4.88	2.29	0.0687	1	Neurodevelopment
Nkx2-1	8.73	36.98	150.54	1695.01	17.25	4.11	0.0928	1	Neurodevelopment
Sox2	1816.45	448.23	2456.07	501.98	1.35	0.435	0.115	1	Neurodevelopment
NEDD4	33.03	12.78	51.31	13.26	1.55	0.636	0.119	1	Endosome
SHH	22.89	17.83	74.61	205.75	3.26	1.7	0.126	1	Neurodevelopment
IM6PR	373.06	15.43	411.87	44.65	1.1	0.143	0.135	1	Lysosomal
GALC	67.55	24.53	91.72	18.51	1.36	0.441	0.145	1	LSD
NTRK1	3.8	3.06	16.83	93.48	4.44	2.15	0.154	1	Signaling
DLX1	496.46	1606.92	2348.81	917.81	4.73	2.24	0.156	1	Neurodevelopment
UVRAG	415.87	184.53	598.74	114.68	1.44	0.526	0.17	1	Endosome
POU3F2	261.7	119.28	434.43	251.32	1.66	0.731	0.172	1	Neurodevelopment
LHX6	12.18	28.5	72.2	315.96	5.93	2.57	0.182	1	Neurodevelopment
NES	2652.68	713.89	4018.32	2473.86	1.51	0.599	0.185	1	Neurodevelopment
TFRC	1025.27	53.19	1177.16	244.78	1.15	0.199	0.23	1	Endosome
Satb1	528.15	73.6	634.57	162.61	1.2	0.265	0.231	1	Neurodevelopment
IGF1R	361.4	36.81	409.44	64.6	1.13	0.18	0.241	1	Signaling
POU3F3	753.68	135.86	974.43	372.72	1.29	0.371	0.247	1	Neurodevelopment
AP1S2	1696.45	758.79	2305.8	497.8	1.36	0.443	0.25	1	Endosome
FoxG1	372.85	171.91	521.51	122.85	1.4	0.484	0.254	1	Neurodevelopment
OTX2	170.67	155.32	269.71	115.62	1.58	0.66	0.27	1	Neurodevelopment
GAD1	227.39	589.78	640.97	307.88	2.82	1.5	0.274	1	Lysosomal
CNTN4	126.89	54.29	160.41	26.8	1.26	0.338	0.298	1	Neurodegeneration
GAD2	209.95	709.09	593.21	365.61	2.83	1.5	0.3	1	Neurodevelopment
SLC9A2	16.53	16.66	28.59	9.75	1.73	0.79	0.3	1	Endosome
TJP1	927.15	281.37	1122.76	177.53	1.21	0.276	0.301	1	Neurodevelopment
HES5	3280.47	521.9	4683.22	3097.51	1.43	0.514	0.304	1	Neurodevelopment
IGF2	28.29	16.72	57.13	73.06	2.02	1.01	0.305	1	Signaling
CLN8	155.2	23.14	175.92	33.13	1.13	0.181	0.306	1	LSD
aph1Bvar1	315.25	60.9	374.52	91.37	1.19	0.249	0.308	1	Neurodegeneration
Isl1	314.76	553.86	647.23	813.32	2.06	1.04	0.353	1	Neurodevelopment
WIP1	26.47	7.81	32.11	6.4	1.21	0.279	0.362	1	Autophagy
TWIST1	6.32	4.5	9.05	3.83	1.43	0.516	0.407	1	Neurodevelopment
ATP6V0E2	467.91	240.09	591.49	160.69	1.26	0.338	0.426	1	vATPase
TTBK2	429.59	140.24	507.48	122.07	1.18	0.24	0.441	1	Neurodegeneration
TACE/ADAM17	583.62	21.59	617.67	85.22	1.06	0.0818	0.45	1	Neurodegeneration
PIK3R4	345.3	47.02	369.89	37.08	1.07	0.0992	0.452	1	Autophagy
SLC32A1	86.31	229.78	174.69	129.81	2.02	1.02	0.458	1	Neurodevelopment
LRP3	464.98	95.99	514.34	77.98	1.11	0.146	0.458	1	Endosome
CUX2	239.53	106.55	292.82	77.28	1.22	0.29	0.462	1	Neurodevelopment
SLC9A9	24.08	9.34	28.83	7.59	1.2	0.26	0.476	1	Endosome
ATG2A	111.35	22.69	124.51	24.53	1.12	0.161	0.476	1	Autophagy
ATG4C	421.49	38.95	450.34	65.76	1.07	0.0955	0.479	1	Autophagy
SORT1	2341.43	576.43	2747.71	974.66	1.17	0.231	0.481	1	Endosome
MAP2	13199.81	5150.89	15726.99	4361.03	1.19	0.253	0.482	1	Neurodevelopment
ARF6	1416.66	129.71	1493.97	177.42	1.05	0.0767	0.494	1	Endosome
GSK3B	2643.57	757.47	2997.71	581.67	1.13	0.181	0.496	1	Neurodegeneration
APBP2	1126.89	249.99	1246.34	214.65	1.11	0.145	0.498	1	Neurodegeneration
VPS33A	300.49	62.49	328.09	49.25	1.09	0.127	0.506	1	Endosome
ATG3	1075.9	176.34	1160.52	163.25	1.08	0.109	0.506	1	Autophagy
MARK1	1163.59	437.7	1360.69	347.34	1.17	0.226	0.509	1	Neurodegeneration
DCX	4317.43	3718.04	5846.74	2575.4	1.35	0.437	0.514	1	Neurodevelopment
GABARAP	7123.59	937.47	7511.6	676.82	1.05	0.0765	0.515	1	Autophagy
Rab11	71.85	46.63	89.01	15.65	1.24	0.309	0.518	1	Endosome
TUBB3	4378.94	1412.99	4929.29	878.28	1.13	0.171	0.52	1	Neurodevelopment
HPS3	271.38	27.28	289.83	49.4	1.07	0.0949	0.522	1	Lysosomal
NPY	85.35	596.64	236.71	708.86	2.77	1.47	0.524	1	Neurodevelopment
MAPK3	1203.35	226.62	1299.07	147.16	1.08	0.11	0.527	1	Signaling
SIRT1	841.71	186.1	910.73	100.47	1.08	0.114	0.533	1	Autophagy
PEX19	443.24	49.08	462.38	29.67	1.04	0.061	0.533	1	LSD
NAGA	68.86	9.84	74.56	14.74	1.08	0.115	0.534	1	LSD
HIF1A	2848.47	194.44	2960.48	289.06	1.04	0.0556	0.537	1	Neurodevelopment
PICALM	1233.27	375.64	1387.14	268.7	1.12	0.17	0.538	1	Neurodegeneration
MAP4	1757.42	404.88	1941.96	367.45	1.11	0.144	0.541	1	Neurodegeneration
BECN1	895.25	211.73	980.88	143.31	1.1	0.132	0.543	1	Autophagy
ATP6V1A	2808.23	1745.96	3445.24	1124.59	1.23	0.295	0.545	1	vATPase
EN1	10.88	10.76	15.54	15.92	1.43	0.514	0.557	1	Neurodevelopment
RAB5A	1376.21	403.36	1518.88	258.98	1.1	0.142	0.58	1	Endosome
SLC12A5	307.35	567.38	444.14	275.93	1.45	0.531	0.598	1	Neurodevelopment
CLN5	168.15	28.37	178.56	23.37	1.06	0.0867	0.604	1	LSD
NAE1	2119.16	288.15	2216.44	222.24	1.05	0.0647	0.604	1	Neurodegeneration

GABARAPL2	4078.51	1722.77	4614.31	1079.91	1.13	0.178	0.612	1	Autophagy
MAPT	3812.84	3364.04	4834.53	2366.11	1.27	0.343	0.616	1	Neurodevelopment
BACE1	662.1	325.78	765	223.33	1.16	0.208	0.616	1	Neurodegeneration
EEA1	471.37	167.18	527.48	120.33	1.12	0.162	0.621	1	Endosome
Tau_4R	2798.01	2460.17	3536.99	1838.8	1.26	0.338	0.624	1	Neurodegeneration
CLCN3	329.78	104.18	362.86	88.44	1.1	0.138	0.63	1	Endosome
CDK5	673	296.89	758.21	163.81	1.13	0.172	0.631	1	Neurodegeneration
Rab35	2091.22	497.84	2257.12	441.3	1.08	0.11	0.632	1	Endosome
APBA1	450.4	327.28	547.54	263.36	1.22	0.282	0.634	1	Neurodegeneration
PTEN	1010.05	331.31	1118.47	326.38	1.11	0.147	0.634	1	Signaling
ADAM10	919.84	92.33	949.68	79.45	1.03	0.046	0.638	1	Neurodegeneration
IGF1	12.09	6.59	13.95	3.03	1.15	0.206	0.642	1	Signaling
PEX3	301.07	94.05	329.3	60.1	1.09	0.129	0.642	1	LSD
ATP6V1F	2645.62	890.7	2890.88	514.02	1.09	0.128	0.649	1	vATPase
SLC9A8	86.99	21.66	94.75	27.07	1.09	0.123	0.659	1	Endosome
Rab4	581.56	63.92	604.17	78.05	1.04	0.055	0.667	1	Endosome
STX13	1336.85	698.65	1519.3	449.95	1.14	0.185	0.67	1	Endosome
MARK2	576.79	115.97	610.61	102.86	1.06	0.0822	0.672	1	Neurodegeneration
RB1CC1	720.32	252.91	791.43	207.46	1.1	0.136	0.674	1	Autophagy
CDK5R1	3410.18	2467.17	4006.86	1548.4	1.17	0.233	0.679	1	Neurodegeneration
PSEN1	883.16	96.89	907.52	47.31	1.03	0.0393	0.68	1	Neurodegeneration
NCAM	5680.47	1928.37	6231.33	1823.05	1.1	0.134	0.683	1	Neurodevelopment
MBP	21.58	12.5	24.65	11.85	1.14	0.192	0.688	1	Neurodevelopment
VPS35	1755.21	231.32	1819.01	196.55	1.04	0.0515	0.691	1	Endosome
AMBRA1	579.63	187.76	627.2	134.47	1.08	0.114	0.695	1	Autophagy
NBR1	1267.47	377.89	1352.92	204.78	1.07	0.0941	0.712	1	Autophagy
AKT1	699.63	144.42	731.96	86.96	1.05	0.0652	0.717	1	Signaling
LAMP2	1288.35	200.88	1346.01	229.12	1.04	0.0632	0.717	1	LSD
Tau_3R	23.73	12.29	26.87	13.35	1.13	0.179	0.718	1	Neurodegeneration
aph1Avar3	870.22	93.21	891.28	60.29	1.02	0.0345	0.724	1	Neurodegeneration
POU5F1	8.27	2.86	9.03	3.26	1.09	0.127	0.726	1	Neurodevelopment
RAB7A	2533.67	619.4	2676.86	486.6	1.06	0.0793	0.73	1	Lysosomal
RABGEF1	608.08	260.57	660.57	165.15	1.09	0.119	0.734	1	Endosome
TH	54.27	178.6	79.54	157.4	1.47	0.552	0.736	1	Neurodevelopment
TMEM55B	239.92	81.02	255.57	35.64	1.07	0.0912	0.736	1	Lysosomal
aph1Bvar2	25.13	3.98	26.99	10.95	1.07	0.103	0.739	1	Neurodegeneration
aph1Avar4	447.75	73.65	462.18	37.82	1.03	0.0458	0.741	1	Neurodegeneration
APPL1	1310.76	316.03	1380.92	258.99	1.05	0.0752	0.742	1	Endosome
ATP6V1H	1118.9	508.94	1224.64	420.33	1.09	0.13	0.747	1	vATPase
PEX13	624.51	138.39	652.31	98.94	1.04	0.0628	0.749	1	LSD
ATP6V1E1	1746.78	606.06	1866.54	489.49	1.07	0.0957	0.76	1	vATPase
ATG4D	413	119.99	435.94	80.33	1.06	0.078	0.761	1	Autophagy
GRIN3A	105.21	65.48	120.1	91.72	1.14	0.191	0.764	1	Lysosomal
APBA2	455.71	177.22	485.38	81.3	1.07	0.091	0.768	1	Neurodegeneration
Rab10	1094.23	275.87	1149.46	255.28	1.05	0.071	0.769	1	Endosome
BLOC1S1	1466.54	262.1	1522.31	269.72	1.04	0.0538	0.771	1	Lysosomal
ATP6V0B	1147.46	583.18	1250.61	424.13	1.09	0.124	0.772	1	vATPase
PSENEN	885.39	188.64	916.46	72.97	1.04	0.0498	0.776	1	Neurodegeneration
ATG4A	121.14	58.65	131.91	40.94	1.09	0.123	0.777	1	Autophagy
PEX1	472.64	227.52	509.93	122.87	1.08	0.11	0.778	1	LSD
RICTOR	378.64	154.86	406.24	101.46	1.07	0.102	0.779	1	Signaling
APP-AII	6558.36	2149.34	6969.06	1949.35	1.06	0.0876	0.783	1	Neurodegeneration
GSK3A	1996.82	683.03	2106.09	403.53	1.05	0.0769	0.789	1	Neurodegeneration
Cux1	769.32	111.37	793.6	139.79	1.03	0.0448	0.791	1	Neurodevelopment
PEX5	670.39	175.73	700.78	138.57	1.05	0.064	0.795	1	LSD
AGA	139.06	45.89	145.49	11.27	1.05	0.0653	0.799	1	LSD
ATP6V0D1	2592.32	1041.58	2749.34	601.75	1.06	0.0848	0.804	1	vATPase
SYN1	249.58	162.88	274.94	158.62	1.1	0.14	0.805	1	Neurodevelopment
APLP1	8364.16	4912.34	9091.7	3236.58	1.09	0.12	0.807	1	Neurodegeneration
CREB1	711.44	153.48	735.74	106.32	1.03	0.0484	0.808	1	Signaling
CSNK1D	1513.74	353.45	1562.02	190.18	1.03	0.0453	0.826	1	Neurodegeneration
MAP1LC3B	2036.31	649.91	2117.59	332.43	1.04	0.0565	0.83	1	Autophagy
WDR45L	878.68	186.51	905.67	148.24	1.03	0.0437	0.83	1	Autophagy
VLDLR	541.11	511.85	603.6	220.63	1.12	0.158	0.831	1	Neurodegeneration
ATP6V1B2	1771.22	602.55	1847.16	448.44	1.04	0.0606	0.841	1	vATPase
SLC9A1	174.93	36.99	179.62	20.34	1.03	0.0381	0.841	1	Endosome
SLC9A4	11.18	11.36	12.63	15.16	1.13	0.176	0.85	1	Endosome
CCND1	446.46	628.56	509.33	581.05	1.14	0.19	0.858	1	Neurodevelopment
PPP2R4	1311.35	151.65	1330.11	155.1	1.01	0.0205	0.867	1	Neurodegeneration
SLC9A5	22.81	38.55	25.27	14.46	1.11	0.148	0.869	1	Endosome
SYP	361.11	271.45	387.21	213.1	1.07	0.101	0.869	1	Neurodevelopment
DLG4	2224.84	1435.48	2363.86	931.56	1.06	0.0874	0.87	1	Neurodevelopment
BCL11B	1897.41	3041.54	2150.65	2570.34	1.13	0.181	0.877	1	Neurodevelopment
USP10	1598.82	144.2	1611.72	92.02	1.01	0.0116	0.883	1	Autophagy
AKT3	3251.44	989.59	3342.35	623.62	1.03	0.0398	0.884	1	Signaling
NRBF2	726.67	184.36	743.26	131.36	1.02	0.0326	0.887	1	Autophagy
NTRK2	1719.16	1275.96	1883.87	1506.12	1.1	0.132	0.888	1	Signaling

HGSNAT	358.7	129.07	370.24	107.36	1.03	0.0457	0.894	1	LSD
ATG12	71.27	34.54	73.81	23.22	1.04	0.0504	0.901	1	Autophagy
SLC36A1	153.63	87.23	159.52	49.33	1.04	0.0543	0.906	1	Lysosomal
ATP6V0C	4226.46	1760.2	4356.57	1361.93	1.03	0.0437	0.906	1	vATPase
GRIPAP1	667.06	338.67	688.72	167.24	1.03	0.0461	0.908	1	Endosome
NPC2	1127.65	353.79	1150.09	264.58	1.02	0.0284	0.921	1	LSD
ATP6V1D	824.91	226.98	839.32	178.03	1.02	0.025	0.921	1	vATPase
ATG9B	14.27	15.54	15.11	12.08	1.06	0.0827	0.924	1	Autophagy
VIM	16869.28	5948.61	17150.16	1861.51	1.02	0.0238	0.926	1	Neurodevelopment
APP-695	7152.16	2179.84	7292.72	2104.85	1.02	0.0281	0.927	1	Neurodegeneration
ATG5	294.33	57.83	297.21	31.16	1.01	0.014	0.93	1	Autophagy
FUCA1	393.93	198.88	404.19	128.27	1.03	0.0371	0.935	1	LSD
ATP6V0A1	1641.32	1092.73	1690.89	828.75	1.03	0.0429	0.938	1	vATPase
GABARAPL1	873.76	258.37	886.21	170.07	1.01	0.0204	0.939	1	Autophagy
LRP1	555.95	227.42	568.19	244.87	1.02	0.0314	0.944	1	Neurodegeneration
GGA3	766.28	262.27	777.45	176.07	1.01	0.0209	0.945	1	Endosome
APOE	29.56	23.59	30.46	15.14	1.03	0.0436	0.946	1	Neurodegeneration
GNPTAB	567.53	353.51	582.48	279.96	1.03	0.0375	0.946	1	LSD
STX6	1253.03	306.87	1263.67	187.96	1.01	0.0122	0.955	1	Endosome
APP-KPI	116.91	63.66	118.73	38.92	1.02	0.0223	0.956	1	Neurodegeneration
APBB1	2516.24	992.12	2545.76	606.7	1.01	0.0168	0.96	1	Neurodegeneration
SMPD1	79.82	32.87	80.55	24.84	1.01	0.013	0.971	1	LSD
WDFY3	587.45	194.39	591.93	191.63	1.01	0.011	0.976	1	Autophagy
PSAP	4410.19	1539.2	4438.19	767.93	1.01	0.00913	0.977	1	Lysosomal
IDS	1656.35	831.16	1668.9	741.68	1.01	0.0109	0.981	1	LSD
VPS26A	997.15	107.61	998.69	62.02	1	0.00221	0.981	1	Endosome
CHAT	8.32	7.45	8.41	1.61	1.01	0.014	0.983	1	Neurodevelopment
MAPK1	1736.23	271.37	1739.99	387.24	1	0.00312	0.987	1	Signaling
ATG7	206.2	19.66	206.42	30.83	1	0.00159	0.991	1	Autophagy
ATP6V0A2	241.84	49.67	242.08	35.55	1	0.00143	0.994	1	vATPase
VPS11	242.33	112.1	242.46	47.82	1	0.000756	0.998	1	Endosome

Table 3.15 Genes downregulated in G383D iPSC-derived neurons vs control

Gene	Control (Counts)	StDev of Control	G383D (Counts)	StDev of G383D	Fold Change	Log2 Fold Change	p-value	Adj p-value	Gene Category
SLC9A6	535.5	259.69	153.41	21.15	-3.49	-1.8	0.00205	1	Endosome
ULK4	115.85	19.93	65.62	12.27	-1.77	-0.82	0.00397	1	Autophagy
SLC9A6 TV 1 3	1377.67	822.38	406.63	120.35	-3.39	-1.76	0.00823	1	Endosome
GALNS	170.85	6.58	143.15	12.88	-1.19	-0.255	0.0106	1	Lysosomal
SLC9A6 TV 1 2	42.97	20.5	17.39	5.37	-2.47	-1.3	0.0153	1	Endosome
MXN1	13.64	6.78	6.07	1.33	-2.25	-1.17	0.0204	1	Neurodevelopment
NAGLU	75.75	10.91	59.31	6.41	-1.28	-0.353	0.0399	1	Lysosomal
NCSTN	838.1	33.41	762.44	46.73	-1.1	-0.137	0.0426	1	Neurodegeneration
NTF4	13.6	5.03	7.5	3	-1.81	-0.859	0.056	1	Signaling
APBA3	73.96	21.81	47.12	14.19	-1.57	-0.651	0.0762	1	Neurodegeneration
MAN2B1	354.93	33.13	309.66	30.28	-1.15	-0.197	0.0825	1	LSD
GGA2	748.15	111.28	624.25	58.88	-1.2	-0.261	0.085	1	Endosome
ATP6V0D2	12.97	5.05	4.62	3.45	-2.81	-1.49	0.0879	1	vATPase
SUMF1	189.27	20.14	158.08	22.66	-1.2	-0.26	0.0945	1	Lysosomal
PSEN2	172.01	27.87	142.56	14.85	-1.21	-0.271	0.103	1	Neurodegeneration
CLN6	205.25	51.75	157.82	18.87	-1.3	-0.379	0.106	1	LSD
GLB1	281.8	44.64	240.51	24.06	-1.17	-0.229	0.128	1	Lysosomal
RELN	490.93	329.26	184.5	263.35	-2.66	-1.41	0.129	1	Neurodegeneration
NTF3	12.43	7.08	6.36	3.14	-1.95	-0.966	0.13	1	Signaling
HoxB4	12.41	5.58	8.56	1.8	-1.45	-0.536	0.133	1	Neurodevelopment
CNTN2	590.65	514.01	299.89	64.94	-1.97	-0.978	0.133	1	Neurodegeneration
GAA	239.93	80.55	167.6	53.26	-1.43	-0.518	0.155	1	Lysosomal
AFP	11.55	3.53	5.98	5.13	-1.93	-0.95	0.155	1	Neurodevelopment
SQSTM1	498.42	142.52	352.46	123.89	-1.41	-0.5	0.165	1	Autophagy
HOXA1	8.8	6.72	4.9	2.63	-1.8	-0.846	0.167	1	Neurodevelopment
C1ORF85	77.45	31.89	54.3	15.02	-1.43	-0.512	0.172	1	Lysosomal
NECAP2	442.64	69.26	385.8	41.14	-1.15	-0.198	0.189	1	Endosome
HEXB	316.41	87.39	249.37	50.55	-1.27	-0.343	0.189	1	Lysosomal
CLN3	121.07	25.62	103.65	8.33	-1.17	-0.224	0.194	1	LSD
MLST8	330.4	36.05	303.99	9.08	-1.09	-0.12	0.207	1	Signaling
CTNS	84.29	12.63	74.55	7.28	-1.13	-0.177	0.215	1	Lysosomal
HOXB6	6.57	3.6	3.38	2.36	-1.95	-0.961	0.222	1	Neurodevelopment
ATP6V1E2	125.01	30.4	101.88	18.28	-1.23	-0.295	0.225	1	vATPase
NTRK3	291.15	142.31	179.58	115.36	-1.62	-0.697	0.23	1	Signaling
DAB1	293.79	164.29	186.12	73.02	-1.58	-0.659	0.232	1	Neurodegeneration
WDFY2	150.7	32.24	129.14	7.44	-1.17	-0.223	0.235	1	Endosome
APOER2	363.72	54	314.55	57.36	-1.16	-0.21	0.246	1	Neurodegeneration
PVALB	10.54	7.32	6.58	2.8	-1.6	-0.68	0.25	1	Neurodevelopment
CTSA	388.05	48.3	305.68	115.56	-1.27	-0.344	0.256	1	Lysosomal
NGF	7.22	4.89	3.72	3.68	-1.94	-0.957	0.267	1	Signaling
KIF4	32.26	14.83	22.28	10.45	-1.45	-0.534	0.269	1	Neurodevelopment
IDUA	168.33	77.4	118.57	55.86	-1.42	-0.506	0.296	1	LSD
GNPTG	346.21	52.21	307.23	46.63	-1.13	-0.172	0.306	1	Lysosomal
BACE2	83.56	27.64	66.93	17.62	-1.25	-0.32	0.373	1	Neurodegeneration
HOXB1	13.63	8.76	10.75	0.7	-1.27	-0.342	0.382	1	Neurodevelopment
CLCN7	594.77	136.53	517.54	112	-1.15	-0.201	0.391	1	Endosome
SLC17A7	31.65	99.79	16.14	3.65	-1.96	-0.972	0.393	1	Neurodevelopment
SGSH	61.5	5.44	53.93	15.85	-1.14	-0.189	0.405	1	LSD
Pax6	3637.81	2108.61	1655.9	3471.35	-2.2	-1.14	0.41	1	Neurodevelopment
B3GAT1	570.99	188.07	468.04	166.2	-1.22	-0.287	0.415	1	Neurodevelopment
CCK	26.34	12.43	20.23	8.42	-1.3	-0.381	0.417	1	Neurodevelopment
SLC17A5	168.5	36.59	143.59	43.23	-1.17	-0.231	0.427	1	LSD
NAGPA	77.25	31.76	60.57	26.27	-1.28	-0.351	0.428	1	Lysosomal
VAMP1	51.34	24.46	39.76	13.73	-1.29	-0.369	0.428	1	Endosome
CTSB	813.82	107.42	747	115.89	-1.09	-0.124	0.429	1	Lysosomal
AMY	728.98	183.25	588.13	282.33	-1.24	-0.31	0.433	1	Neurodegeneration
HEXA	509.39	144.91	441.12	87.29	-1.15	-0.208	0.434	1	Lysosomal
ATP6V1B1	6.95	7.19	4.84	1.73	-1.44	-0.523	0.455	1	vATPase
Fezf2	266.64	1132.72	99.01	295.1	-2.69	-1.43	0.473	1	Neurodevelopment
SHANK3	167.9	73.01	137.69	51.19	-1.22	-0.286	0.495	1	Neurodevelopment
DNAJC5	369.84	180.96	304.11	86.26	-1.22	-0.282	0.496	1	LSD
MYOD1	14.61	16.66	10.57	2.95	-1.38	-0.467	0.498	1	Neurodevelopment
NEU1	157.3	35.77	141.65	30.82	-1.11	-0.151	0.507	1	Lysosomal
NMDA NR1	397.78	45.88	377.02	38.83	-1.06	-0.0773	0.51	1	Neurodevelopment
ATOH1	7.76	6.55	6.07	2.08	-1.28	-0.354	0.521	1	Neurodevelopment
AKT1S1	384.74	52.18	363.28	43.74	-1.06	-0.0828	0.541	1	Autophagy
ARSA	97.62	34.73	78.59	48.75	-1.24	-0.313	0.546	1	LSD
MANBA	70.66	24.63	61.32	16.33	-1.15	-0.205	0.549	1	LSD
ATP6V1G1	61.14	28.11	50.7	16.01	-1.21	-0.27	0.55	1	vATPase
SORL1	137.74	38.28	114.76	76.11	-1.2	-0.263	0.557	1	Endosome
Tbr1	524.69	2352.45	233.22	703.64	-2.25	-1.17	0.56	1	Neurodevelopment
CALB2	156.65	301.61	100.07	137.94	-1.57	-0.647	0.568	1	Neurodevelopment
LAMP1	1794.01	192.65	1696.65	257.77	-1.06	-0.0805	0.569	1	Lysosomal

ULK3	1032.56	544.42	854.29	360.07	-1.21	-0.273	0.569	1	Autophagy
ADAM19	122.21	35.98	108.54	29.5	-1.13	-0.171	0.573	1	Neurodegeneration
NPC1	358.44	40.29	335.27	63.83	-1.07	-0.0964	0.58	1	LSD
Iba1	9.88	9.24	7.42	2.99	-1.33	-0.412	0.585	1	Neurodevelopment
ADAM9	318.26	197.19	269.39	90.58	-1.18	-0.241	0.588	1	Neurodegeneration
TFE3	46.26	6.76	43.28	7.62	-1.07	-0.0959	0.59	1	Lysosomal
AKT2	1118.96	158.99	1068.79	103.64	-1.05	-0.0662	0.602	1	Signaling
S100b	56.29	120.27	34.62	59.46	-1.63	-0.701	0.607	1	Neurodevelopment
GGA1	288.25	87.77	261	59.3	-1.1	-0.143	0.61	1	Endosome
RAB9A	485.98	59.42	457.98	88.55	-1.06	-0.0856	0.612	1	Endosome
HGS	113.37	30.38	101.39	31.65	-1.12	-0.161	0.623	1	Endosome
SLC12A2	271.93	25.96	253.59	69.35	-1.07	-0.101	0.628	1	Neurodevelopment
ARSB	573.77	72.33	530.24	151.1	-1.08	-0.114	0.633	1	LSD
nanog	16.15	9.23	13.38	5.56	-1.21	-0.271	0.634	1	Neurodevelopment
CTSC	360.38	349.45	280.03	176.46	-1.29	-0.364	0.641	1	LSD
FOXp2	366.48	588.96	269.04	306.3	-1.36	-0.446	0.641	1	Neurodevelopment
HPS5	248.57	58.48	233.24	36.32	-1.07	-0.0918	0.648	1	Lysosomal
SATB2	32.03	7.75	29.67	7.02	-1.08	-0.11	0.661	1	Neurodevelopment
LIPA	456.4	168.62	417.62	70.21	-1.09	-0.128	0.663	1	LSD
GBA	403.95	96.48	376.5	82.09	-1.07	-0.102	0.667	1	Lysosomal
EOMES	38.37	94.79	24.08	63.96	-1.59	-0.672	0.668	1	Neurodevelopment
ASAH1	1415.11	564.49	1270.94	361.02	-1.11	-0.155	0.672	1	Lysosomal
TPP1	338.99	154.04	311.2	36.04	-1.09	-0.123	0.675	1	LSD
TMEM74	120.2	46.97	105.79	44.53	-1.14	-0.184	0.677	1	Autophagy
CALB1	317.58	330.43	244.63	200.73	-1.3	-0.377	0.677	1	Neurodevelopment
ATG16L2	145.13	67.55	128.05	49.2	-1.13	-0.181	0.681	1	Autophagy
TCF4	480.97	316.7	423.04	43.74	-1.14	-0.185	0.693	1	LSD
SCPEP1	107.46	55.38	94.26	40.66	-1.14	-0.189	0.705	1	LSD
CTSD	789.26	162.01	751.94	105.34	-1.05	-0.0699	0.709	1	Lysosomal
PEX16	216.15	79.6	199.68	45.47	-1.08	-0.114	0.718	1	LSD
APP-770	184.5	138.35	159.31	98.35	-1.16	-0.212	0.727	1	Neurodegeneration
CD74	11.28	7.31	9.48	8.42	-1.19	-0.25	0.728	1	Neurodegeneration
HPS1	47.15	13.89	42.64	17.96	-1.11	-0.145	0.735	1	Lysosomal
CD63	2550.12	328.71	2459	385.37	-1.04	-0.0525	0.738	1	Lysosomal
MFSD8	364.2	185.17	331	95.69	-1.1	-0.138	0.744	1	LSD
EHD1	275.23	36.81	267.38	30.92	-1.03	-0.0418	0.757	1	Endosome
PLBD2	145.83	36.61	136.81	44.41	-1.07	-0.0922	0.761	1	Autophagy
HYAL1	55.38	22.39	51.71	12.23	-1.07	-0.099	0.763	1	LSD
RRAGC	536.85	127.06	511.98	106.05	-1.05	-0.0684	0.769	1	Lysosomal
ATP6V0E1	1183.19	210.08	1147.51	111.65	-1.03	-0.0442	0.773	1	vATPase
ATP6V0A4	12.33	11.87	10.84	1.71	-1.14	-0.186	0.773	1	vATPase
ATG14	96	42.24	89.15	28.82	-1.08	-0.107	0.783	1	Autophagy
HOXB13	9.42	9.54	8.23	4.69	-1.14	-0.195	0.783	1	Neurodevelopment
MCOLN1	192.74	85.06	177.72	79.85	-1.08	-0.117	0.792	1	Lysosomal
DRAM1	38.35	16.39	35.04	17.48	-1.09	-0.131	0.793	1	Autophagy
STH	23.03	23.14	19.98	12.93	-1.15	-0.205	0.793	1	Neurodegeneration
TFEB	15.42	12.24	13.89	6.5	-1.11	-0.151	0.797	1	Lysosomal
WIP1	334.2	85.82	321.84	43.29	-1.04	-0.0544	0.801	1	Autophagy
ATP6V1G3	4.81	3.59	4.37	1.7	-1.1	-0.137	0.803	1	vATPase
VPS18	297.28	86.78	284.11	57.04	-1.05	-0.0654	0.806	1	Endosome
TTBK1	87.35	42.98	81.84	19.87	-1.07	-0.094	0.813	1	Neurodegeneration
ATP6V1C1	372.92	190.06	348.46	135.01	-1.07	-0.0979	0.822	1	vATPase
Sox10	10.49	46.06	8.51	10.57	-1.23	-0.301	0.822	1	Neurodevelopment
APBB3	251.61	164.69	232.46	87.22	-1.08	-0.114	0.83	1	Neurodegeneration
STK4	585.73	45.02	573.96	94.59	-1.02	-0.0293	0.832	1	Signaling
SLC9A7	478.05	184.79	452.54	149.27	-1.06	-0.0791	0.833	1	Endosome
SLC9A3	4.81	4.34	4.22	5.04	-1.14	-0.189	0.833	1	Endosome
ATG4B	1869.7	337.16	1828.6	225.2	-1.02	-0.0321	0.846	1	Autophagy
IGF2R	249.5	55.28	240.65	66.1	-1.04	-0.0521	0.851	1	Lysosomal
VPS8	533.54	192.76	511.14	140.32	-1.04	-0.0619	0.851	1	Endosome
ATP6V1G2	840.91	333.18	802.77	309.45	-1.05	-0.067	0.854	1	vATPase
TP73	32.06	10.44	30.91	5.65	-1.04	-0.0528	0.855	1	Neurodevelopment
NGFR	35.67	32.73	32.88	19.14	-1.09	-0.118	0.857	1	Signaling
ATG9A	712.16	179.56	690.55	152.89	-1.03	-0.0445	0.858	1	Autophagy
EH04	34.38	7.76	33.19	12.35	-1.04	-0.051	0.858	1	Endosome
GSAP	14.43	11.23	13.58	4.3	-1.06	-0.0872	0.859	1	Neurodegeneration
ATG101	268.06	45.51	262.03	58.1	-1.02	-0.0328	0.87	1	Autophagy
GNS	465.57	87.22	457.7	44.42	-1.02	-0.0246	0.871	1	Lysosomal
HOXA2	6.83	12.63	6.07	4.7	-1.12	-0.169	0.884	1	Neurodevelopment
BDNF	26.46	17.51	24.63	27.15	-1.07	-0.103	0.888	1	Signaling
PRKAA1	365.22	130.27	354.91	89.64	-1.03	-0.0413	0.895	1	Autophagy
ATG2B	350.73	112.47	341.34	95.67	-1.03	-0.0392	0.903	1	Autophagy
PHYH	22.05	6.13	21.43	6.6	-1.03	-0.0413	0.903	1	LSD
KIAA0226	120.98	50.14	116.66	54.74	-1.04	-0.0524	0.904	1	Autophagy
ATP13A2	562.32	165.93	548.85	191.32	-1.02	-0.035	0.915	1	LSD
MAP1LC3A	687.15	680.91	645.11	659.42	-1.07	-0.0911	0.917	1	Autophagy
MARK3	763.57	176.43	750.64	179.15	-1.02	-0.0246	0.919	1	Neurodegeneration

USP13	338.85	19.99	335.29	70.81	-1.01	-0.0152	0.92	1	Autophagy
ATG16L1	571.82	262.2	555.97	178.45	-1.03	-0.0405	0.92	1	Autophagy
WDR45	237.71	91.3	232.32	59.22	-1.02	-0.0331	0.921	1	Autophagy
GLA	513.43	110.22	507.05	81.44	-1.01	-0.018	0.923	1	Lysosomal
LRP10	170.32	128.02	165.4	44.74	-1.03	-0.0423	0.925	1	Neurodegeneration
SNCA	125.33	88.55	119.69	100.48	-1.05	-0.0664	0.927	1	Neurodegeneration
KMT2D	109.19	26.6	106.82	50.9	-1.02	-0.0316	0.936	1	LSD
ULK2	384.54	109.59	380.37	100.18	-1.01	-0.0157	0.955	1	Autophagy
ULK1	516.87	145.84	512.34	93.12	-1.01	-0.0127	0.959	1	Autophagy
GM2A	145.5	59.96	144.1	23.34	-1.01	-0.0139	0.961	1	LSD
SLC17A6	127.12	255.95	122.11	216.03	-1.04	-0.058	0.964	1	Neurodevelopment
PRKAG2	557.7	340.81	549.31	294.37	-1.02	-0.0219	0.969	1	Signaling
BLOC1S3	30.06	12.91	29.76	11.96	-1.01	-0.0146	0.97	1	Lysosomal
MTOR	574.03	129.91	571.35	146.49	-1	-0.00674	0.979	1	Signaling
PPT1	1494.01	104.85	1491.52	208.42	-1	-0.0024	0.983	1	Lysosomal
PRKAA2	341.83	114.26	340.25	95.88	-1	-0.0067	0.984	1	Autophagy
CTSK	68.54	30.01	68.15	18.54	-1.01	-0.00821	0.984	1	Lysosomal
RPTOR	216.22	49.58	215.76	33.56	-1	-0.00309	0.988	1	Signaling
APLP2	2576.43	408.02	2574.34	294.64	-1	-0.00117	0.994	1	Neurodegeneration
GFAP	8.36	1.22	8.35	3.56	-1	-0.00206	0.995	1	Neurodevelopment

CHAPTER 4:

Determining the neural circuitry deficits associated with Christianson syndrome and methods of rescue

Sofia Lizarraga performed the experiments in Fig. 4.1 and Fig. 4.2 in collaboration with laboratory of Diane Hoffman-Kim. Sofia Lizarraga, Laura van Dyck, and Dipal Nagda performed experiments in Fig 4.7. Sofia Lizarraga performed experiments and Rich Jones performed statistical analysis in Fig 4.8.

INTRODUCTION

The establishment of neural circuitry in the neocortex is critical to cognition, sensory perception, language, and motor function. In the developing cortex *in utero*, cortical projection neurons generated from progenitor cells in the ventricular and subventricular zones migrate along radial glia to reach their final position in the layered cortex. Neurons then must extend axons and dendrites to form synaptic connections either within the cortex or to subcortical targets (Tau and Peterson, 2010). In neurological disorders such as ASD, connectivity within the cortex can be either decreased or overactivated depending on the patient and the brain region (Geschwind and Levitt, 2007). Deficits in synaptic connectivity have been observed in iPSC-derived neurons from patients with Rett syndrome or Phelan-McDermid syndrome (Marchetto et al., 2010; Shcheglovitov et al., 2013). iPSCs differentiated to cortical neuronal fate allow researchers to interrogate prenatal circuit establishment in neurological disease.

To establish neuronal circuitry, neurons must extend axons and dendrites to reach their synaptic targets. The growth of neurites is controlled by the coordination of extracellular guidance cues, intracellular signaling, cytoskeletal factors, and extracellular matrix proteins (Tojima and Kamiguchi, 2015). Endosomes play a critical role in neurite growth by controlling the signaling events that direct neuronal growth, migration, and survival. Endosomal control of signaling can occur through several mechanisms. Endocytosis of receptors from the plasma membrane can attenuate signaling by reducing the number of receptors available for ligand binding. The trafficking of internalized receptors and ligands either to the lysosome for degradation or to the plasma membrane through recycling endosomes can also modulate signaling from the plasma membrane (Sorkin and von Zastrow, 2009).

In addition, transduction of signaling pathways can occur through endosomes to promote signaling. This mechanism is especially important in the polarized neuron to transmit signals over long distances. For example, neurotrophins that are released by postsynaptic cells and endocytosed in presynaptic axons are retrogradely transported through signaling endosomes from the distal axon to the cell body (Grimes et al., 1996). Neurotrophins promote axonal and dendritic growth, cell migration, and neuronal survival through downstream transcriptional changes in the presynaptic neuron (Cosker et al., 2008). Signaling endosomes can contain components of downstream signaling pathways thereby transducing the signaling event from the endosomal compartment (Reichardt, 2006).

Regulation of endosomal pH is critical to endosomal signaling. The affinity of a ligand for its receptor and the rate of ligand recycling is influenced by endosomal pH (French et al., 1995). The pH of endosomes had been shown to decrease from the axon tip to the soma (Overly and Hollenbeck, 1996) and the maintenance of acidification may be particularly important to the long range retrograde transport of neurotrophin signaling endosomes. For instance, in NHE6 null mice which have overacidified endosomes, the neurotrophin receptor, TrkB, is degraded and signaling through the ligand, BDNF, is decreased (Ouyang et al., 2013).

In CS, we hypothesize that an overacidification of endosomes will lead to defects in neuronal circuitry. In this study, deficits in neurite growth rates and morphology were compared in CS in patient iPSC-derived neurons. iPSCs provide a valuable model to test therapies that may restore or reduce aberrant neuronal circuitry in neurological disease. Restoration of neurite arborization was tested by re-expressing NHE6 and by treating cells with exogenous growth factors. Further, the connection between endosomal pH and neural circuitry defects in CS was examined. Our investigation of neuronal circuitry

and the efficacy of cell autonomous and nonautonomous means of rescue revealed both overriding paradigms in CS and pointed to variation between patients.

RESULTS

Neurite outgrowth dynamics in CS iPSC derived neurons

To compare neurite outgrowth dynamics in CS and control cells, time-lapse imaging of iPSC-derived neurons was performed. Neurons were plated on imaging slides patterned with laminin stripes (Chelli et al., 2014). The neurons aligned along the laminin stripes and extended and retracted their neurites. Twelve hours after plating, images were acquired every 5 minutes for up to 12 hours (Fig. 4.1). There was a significant decrease in the elongation rates in W523X and G383D CS lines as compared to their respective controls (Fig 4.2 A). Additionally, the retraction rate was decreased in R472fsX4 and W523X CS lines as compared to controls (Fig. 4.2 B). Diminished neurite dynamics in CS may indicate an inability of neurons to migrate to their proper place in the cortex or to search and find synaptic targets.

Cell autonomous re-expression of NHE6 ameliorates arborization deficits

To further understand the ability of CS neurons to grow and form arbors, Sholl analysis was performed. Cells were transfected with GFP or GFP and full-length NHE6 tagged with HA and imaged using confocal microscopy 5 days after transfection. Punctate NHE6-HA expression was seen along neurites and in perinuclear regions while GFP expression delineated neuronal morphology (Fig. 4.3). After tracing neurites and marking branches in a manner blind to genotype using Neurolucida software, Sholl analysis was performed using 5 μ m radii (Fig. 4.4). For each CS and control pair, the average number of times neurites intersected each Sholl radius and the neurite length within each radius were plotted as a function of distance from the soma and compared using a two-way ANOVA with Bonferroni multiple comparisons test. In CS cells at distances close to the cell body, there was a decrease in the number of times neurites

intersected with Sholl radii as compared to control. Furthermore, the neurite length within each shell was decreased at distances close to the soma in CS cells as compared to control (Fig. 4.5). In R472fsX4 and W523X CS cells, cell-autonomous re-expression of NHE6 increased both the number of intersections and neurite length (Fig. 4.5 A, B). However, in G383D CS cells, re-expression of NHE6 was unable to restore deficits in arbor complexity to control levels (Fig 4.5 C). The Sholl analysis results indicate that there is a decrease in the complexity of neuronal arborization in CS. Additionally, differences in the extent to which re-expression of NHE6 rescues deficits in neuronal arborization demonstrates variation between CS patients. NHE6 re-expression fails to increase arborization in the G383D CS cells. G383D may behave as a dominant negative by forming heterodimers with newly expressed NHE6.

Next, the role of NHE6 in neuronal morphology was further examined from the Neurolucida neurite reconstruction analysis. The number of primary branches originating at the cell soma, the total number of branches, and the number of branchpoints were decreased in all three CS patient lines (Fig. 4.6). Re-expression of NHE6 increased the number of primary branches in the R472fsX4 CS line (Fig. 4.6 B). Also, neurite length per cell was decreased in all CS lines as compared to their respective controls. Neurite length was increased upon re-expression of NHE6 in the W523X CS line (Fig. 4.6 A). Conversely, re-expression of NHE6 in the G383D CS line decreased the number of primary branches, total branches, and branchpoints (Fig 4.6 C). These results provide evidence of diminished neuronal arborization in CS. The decrease in branching observed when NHE6 is re-expressed in G383D CS cells further supports the model of G383D acting as dominant negative mutation.

Neurotrophin and growth factor treatment restore neurite growth defects

Next, the ability of neurotrophin or growth factor treatment to restore the capacity to grow and form arbors was tested in CS iPSC-derived neurons. Cells were treated for three days with exogenous addition of either BDNF or IGF-1. The cells were stained for MAP2 and TAU followed by neurite reconstruction using Neurolucida software (Fig 4.7 A). Treatment with BDNF or IGF increased the mean length per neurite in all CS lines as compared to untreated CS cells (Fig 4.7 B). In addition, BDNF or IGF treatment increased the number of branchpoints per neurite in all CS lines as compared untreated CS cells (Fig 4.7 B). Therefore, neurite arborization defects in CS cultures may be restored by treatment with BDNF or IGF-1.

Endosomal pH in CS iPSC-derived neurons

In order to determine if endosomal pH regulation is impaired in CS patient cells, fluorescent ratiometric measurements of endosomal pH were conducted in iPSC-derived neurons. Following incubation with Tf-fluorescein conjugate (pH sensitive) and Tf-Alexa 546 conjugate (pH insensitive), cells were live imaged. A standard curve for endosomal pH was generated by incubating cells in a range of pH buffered solutions. The ratio of fluorescein to 546 fluorescence was used to calculate intraendosomal pH in CS and control cells (Fig. 4.8 A). In two of the CS lines, R472fsX4 and G383D, the endosomal pH in CS cells was more acidic as compared to control cells (Control family 1 pH = 5.94 ± 0.06 vs R472fsX4 pH = 5.48 ± 0.04 , $p < 0.001$; Control family 3 pH = 6.35 ± 0.07 vs W523X pH = 6.27 ± 0.06 , $p < 0.001$) (Fig. 4.8 B). However, in the W523X CS line, endosomal pH was more alkaline than control (Control family 2 pH = 5.87 ± 0.04 vs W523X pH = 5.96 ± 0.03 , $p = 0.025$) (Fig 4.8 B). These results indicate that pH

homeostasis may be disrupted in CS, however the phenotype shows variable expressivity.

DISCUSSION

In this study, the level of neural circuitry deficits in CS and several routes of intervention to restore these deficits were examined. Analysis of neurite growth dynamics revealed slower rates of neurite extension and retraction in iPSC-derived neurons as compared to the paired control lines. Additionally, arborization complexity was decreased in CS iPSC-derived neurons as measured by Sholl analysis. The number of primary branches, total number of branches, and neurite length were all decreased in CS iPSC-derived neurons. These deficits in neuronal growth and arborization indicate a mechanism by which neurons may fail to reach synaptic targets during development. In addition, postnatal microcephaly has been reported in 92% of CS patients (Pescosolido et al., 2014) and a failure in axonal and dendritic arborization and outgrowth may contribute to a decrease in white matter in the developing brain.

Re-expression of NHE6 in CS iPSC-derived neurons restored arborization complexity as analyzed by Sholl in R472fsX4 and W523X CS cells but not in G383D CS cells (Fig. 4.5). Restoration of arbor complexity in R472fsX4 and W523X CS cells reveals a cell-autonomous mechanism of rescue. However, the failure of NHE6 re-expression to rescue arbor complexity in G383D CS cells may indicate that the missense NHE6 protein could act as a dominant negative. NHE proteins have been shown to form homodimers mediated by interactions between transmembrane domains and between the cytoplasmic tails (Fafournoux et al., 1994; Hisamitsu et al., 2004; Ilie et al., 2016). Studies of NHE1 have shown that dimerization with dominant negative mutants inhibits ion exchange activity (Hisamitsu et al., 2006). Re-expression of NHE6 in G383D mutant cells may result in the formation of heterodimers between newly expressed NHE6 and G383D mutant NHE6. These heterodimers may have impaired exchange activity and an inability to regulate intra-endosomal pH.

In addition to cell autonomous re-expression of NHE6, cell-nonautonomous methods of rescue were tested. Treatment of neuronal cultures with the growth factors, BDNF or IGF-1, increased neurite length and branching (Fig. 4.7). BDNF is a neurotrophin that binds to its receptor, TrkB, to promote cell survival and neurite growth. Neurotrophins released by post-synaptic cells initiate endosomal retrograde signaling in pre-synaptic cells thereby enhancing the survival of neurons that have reached their synaptic target (Cosker et al., 2013; Oppenheim, 1989). In addition, BDNF promotes neurite growth and arborization by activating endosomal signaling through the Ras, PI3-kinase, and PLC- γ 1 pathways (Reichardt, 2006). Rescue of neuronal arborization and growth in CS neuronal cultures through the addition of exogenous BDNF, may result from upregulation of neurotrophin signaling pathways.

IGF-1 also improved neurite length and branching in CS iPSC-derived neurons. IGF-1 promotes cell survival and growth through binding to the IGF-1 receptor and signaling through the PI3-kinase and Ras pathways (Bondy and Cheng, 2004). IGF-1 has been shown to be a potential therapy for the autism-associated syndromes, Rett syndrome and Phelan-McDermid syndrome. Preclinical studies in Rett syndrome showed that treatment of iPSC-derived neurons with IGF-1 increased levels of glutamatergic synapses (Marchetto et al., 2010). Further, treatment of a Rett syndrome mouse model with IGF-1 rescued brain size, synapse density, and excitatory synapse transmission to control levels (Tropea et al., 2009). In a phase I clinical trial for IGF-1 in Rett syndrome, patients showed improved symptoms of anxiety and breathing difficulty (Khawaja et al., 2014). In addition, IGF-1 treatment of iPSC-derived neurons from Phelan-McDermid syndrome patients improved deficits in mature excitatory synapses as compared to control levels (Shcheglovitov et al., 2013). A preliminary clinical trial of IGF-1 in Phelan-McDermid syndrome showed improvement of social withdrawal and

restricted behaviors (Kolevzon et al., 2014). Because IGF-1 has been shown to be effective in other neurological disorders, IGF-1 presents a promising treatment for CS. However, more research is needed in both CS mouse models and iPSC-derived neurons to study the effect of IGF-1 on synapse formation, synaptic transmission, brain growth, and behavior.

To test if neuronal circuitry deficits in CS cells were associated with abnormal endosome acidification, endosomal pH was measured in iPSC-derived neurons. Endosomal pH was more acidic in R472fsX4 and G383D CS iPSC-derived neurons than in controls. This result indicates that loss of NHE6 impairs endosomal pH regulation in these patient cells leading to overacidification. An overacidified endosome compartment may impact endosomal signaling by increasing signaling receptor degradation, decreasing receptor-ligand affinity, or decreasing receptor recycling to the cell surface. On the other hand, W523X CS iPSC-derived neurons showed an alkalinization of endosomes as compared to control cells. The severity of decreased branching and neurite length reduction in this patient is similar to the phenotypes observed in the R472fsX4 and G383D cells which have overacidified endosomes. In the W523X patient it is possible that loss of the interactions mediated by the C-terminal tail of NHE6 result in disease rather than loss of ion translocation through NHE6.

Investigation of neural circuitry defects in iPSC-derived neurons revealed decreased arbor complexity, branching, and neurite growth in CS. In addition pH homeostasis was shown to be disrupted in CS. The ability of NHE6 re-expression and growth factor treatment to restore neuronal circuitry was tested. These treatments rescued neural circuitry defects to varying extents in cells derived from different patients. Therefore, iPSC-derived neurons present an important preclinical model for testing potential therapies in individual patients.

METHODS

Time-lapse imaging of neurite outgrowth dynamics

iPSC-derived neurons were dissociated using Gentle Cell Dissociation Reagent or Accutase (StemCell Technologies) 32 days after neuronal induction. 30,000 cells were plated on 15mm coverslips stamped with laminin stripes as previously described (Chelli et al., 2014). Cells were cultured in Neural Maintenance Media (NMM) (1:1 DMEM/F-12 GlutaMAX (Gibco):Neurobasal (Gibco)/1x N2 supplement (Gibco)/1x B27 supplement (Gibco)/5 µg per mL insulin (Sigma)/1 mM L-glutamine (Gibco)/500 µM sodium pyruvate (Sigma)/100 µM nonessential amino acids solution (Gibco)/100 µM β-mercaptoethanol (Gibco)/50 U per mL penicillin-streptomycin (Gibco)) and a half media change was performed with fresh NMM after 12 hr. Cells were then incubated in an inverted Nikon Eclipse TE2000-S microscope equipped with an enclosed temperature and CO₂ controlled chamber to maintain 37°C and 5% CO₂. Volocity software was used to acquire time-lapse series using a using a Hamamatsu Orca-ER camera and orbit shutter controller. For each sample, 12 z-stack planes were acquired at 4 different XY positions every 5 min for 12 hr. Positions were selected based on the number of cells in the plane. Planes with too many cells crowded together were not used as it was important to be able to measure dynamics of individual neurites. Images were analyzed by quantifying neurite retraction and extension using the neurite outgrowth plugin in ImageJ. Two independent neuronal inductions for each mutation and corresponding control were analyzed.

NHE6 re-expression and neuronal arborization measurement

iPSC-derived neurons grown to 32 days after neuronal induction were transfected with plasmids expressing GFP or human NHE6 (NM_006359) with a C-terminal HA tag

cloned into a pReceiver-M07 mammalian expression vector using Lipofectamine 3000 (ThermoFisher). Five days after transfection, cells were fixed and stained with rabbit anti-HA (Cell Signaling) as described in Chap. 3 methods. Z-stack images were acquired using a Zeiss LSM 710 confocal laser scanning microscope and traced for morphological analysis and Sholl analysis using Neurolucida software.

Neuronal arborization and small molecule treatments

iPSC-derived neurons were plated on coverslips and cultured in NMM. After 24 hr, media was replaced NMM containing vehicle, IGF-1 (20ng/ml) (PeproTech), or BDNF (10ng/ml) (PeproTech). A half change of the respective media was performed the following day and cells were fixed for staining 72 hours after the addition of small molecules.

Immunocytochemistry and microscopy was performed as described in Chap. 3 methods with chicken anti-MAP2 (Millipore) and mouse anti-TAU1 (gift of Dr. Peter Dans). Neurite length and branching was quantified by tracing imaged cells using Neurolucida software.

Endosomal acidification analysis

iPSC-derived neurons plated on glass bottom imaging dishes (Nunc) were incubated in NMM without B27 and N2 supplements for 30 min. The media was then replaced with complete NMM containing 66 µg/mL transferrin-fluorescein conjugate (ThermoFisher) and 33 µg/mL transferrin-Alexa 546 conjugate (ThermoFisher). After 10 min, the cells were washed with PBS and the media was replaced with NMM without phenol red. Cells were imaged live using a Zeiss LSM 710 confocal laser scanning microscope. To calculate a standard curve, control iPSC-derived neurons were incubated and imaged in buffers of pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5. Fluorescence intensity of endosomes

was measured in ImageJ and pH was calculated by measuring the ratio of fluorescein fluorescence to 546 fluorescence in endosomes.

Statistics

Analysis of Sholl data was performed using a two-way ANOVA with Bonferroni multiple comparisons test. Neurite length and branching was compared using two-tailed Welch's t-tests. pH measurements were analyzed using a mixed effect generalized linear model with control/CS pair and individual person treated as random effects.

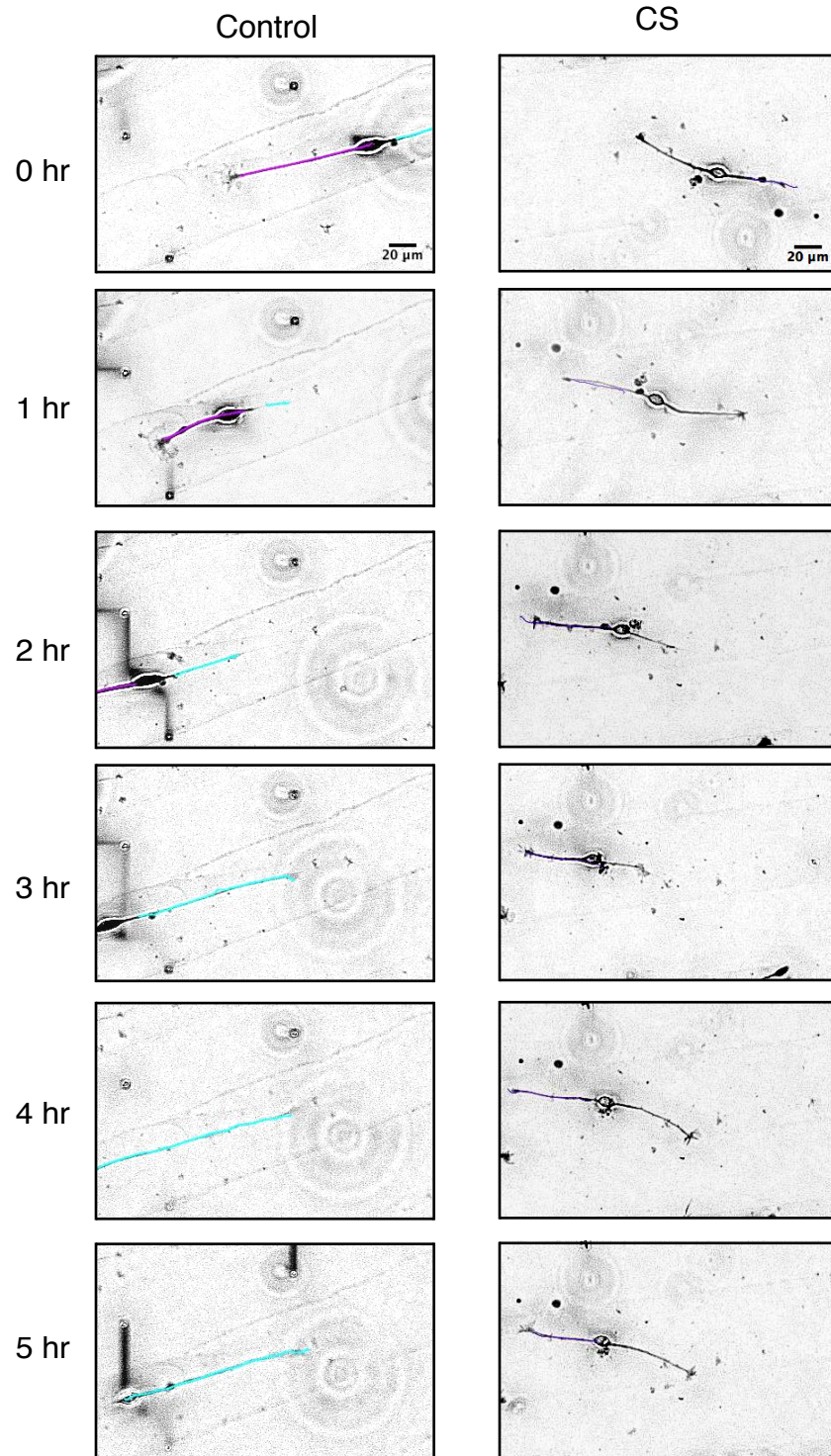


Figure 4.1 Neurite outgrowth dynamics in iPSC-derived neurons. Time-lapse images of iPSC-derived neurons plated on laminin striped coverslips. Images were acquired every 5 minutes for 12 hours. Representative cells and tracings are shown from the c.1148 G>A/p.G383D mutant line and paired control line. Scale bars, 20 μ m.

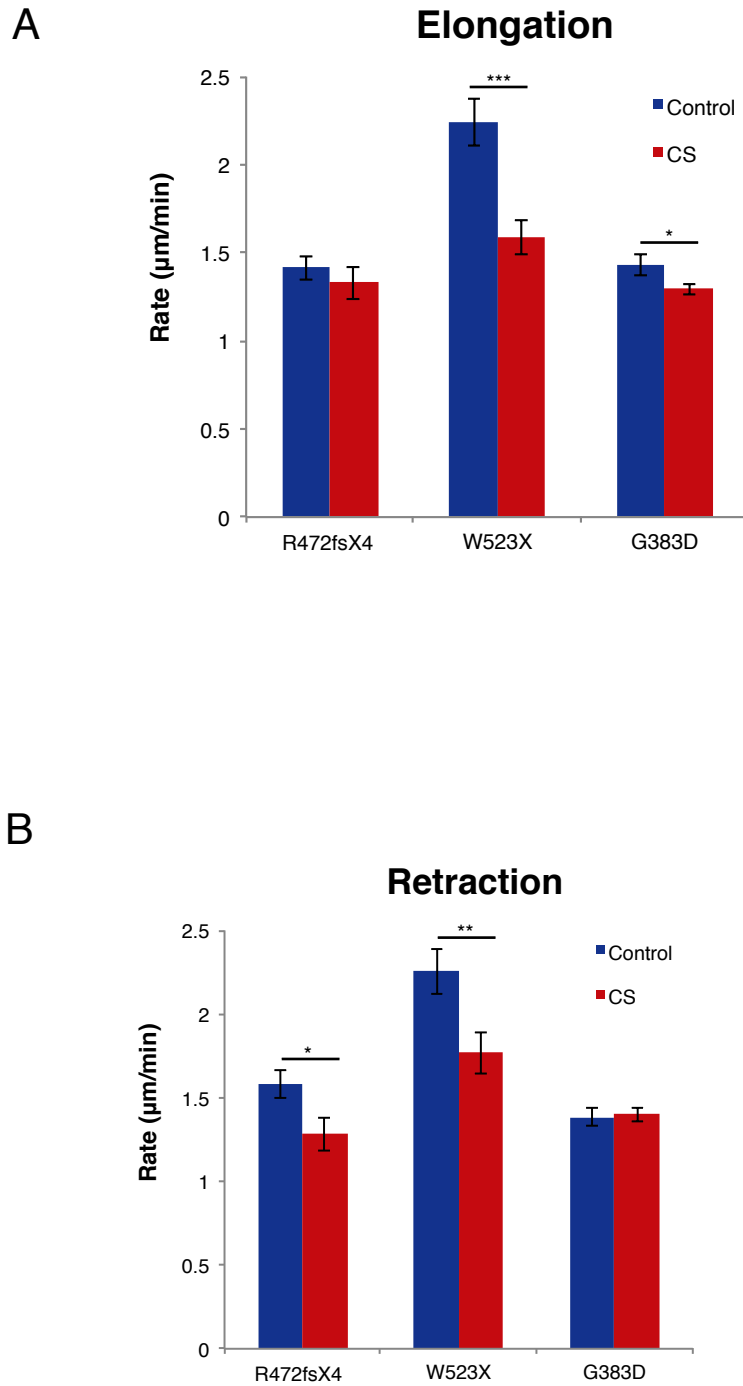


Figure 4.2 Neurite elongation and retraction rates. The rates of neurite elongation (A) and retraction (B) of iPSC-derived neurons plated on laminin striped coverslips were measured by live-imaging using Velocity software. Four XY positions per sample were imaged every 5 min for 12 hr. Imaging was performed on two independent neuronal inductions for each CS/control pair. n= 20-30 cells per cell line, mean $\pm$ sem. Two tailed, Welch's t-test: * $p < 0.045$, ** $p < 0.008$, *** $p < 0.00008$.

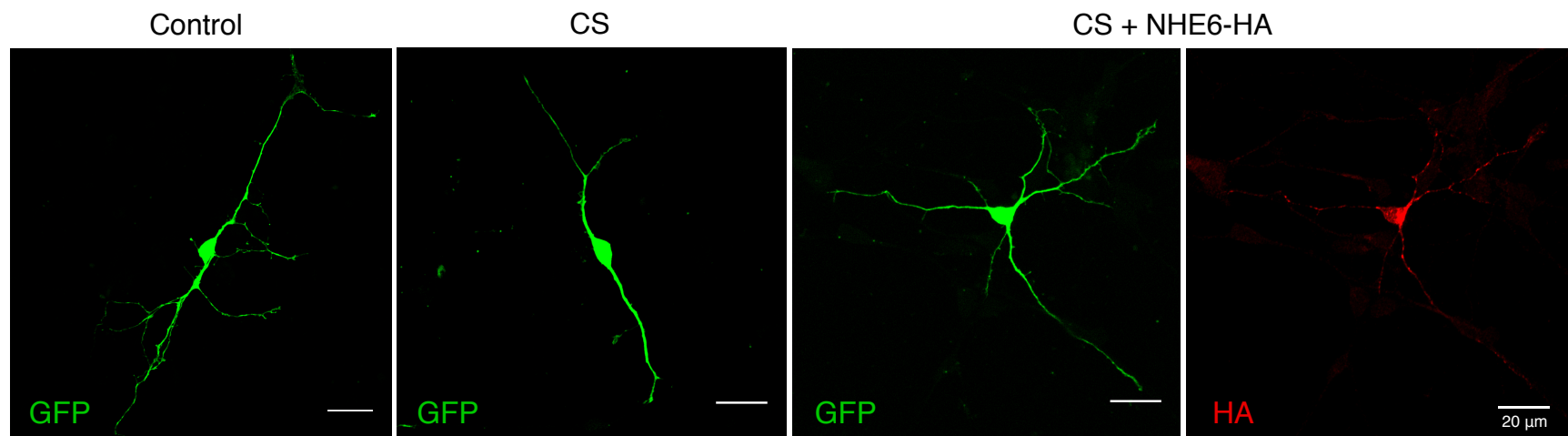


Figure 4.3 Re-expression of NHE6 in CS neurons. iPSC-derived neurons were transfected with GFP or GFP + full-length NHE6 tagged with HA and analyzed 5 days after transfection. Example images of each condition are shown. Scale bars, 20 μ m.

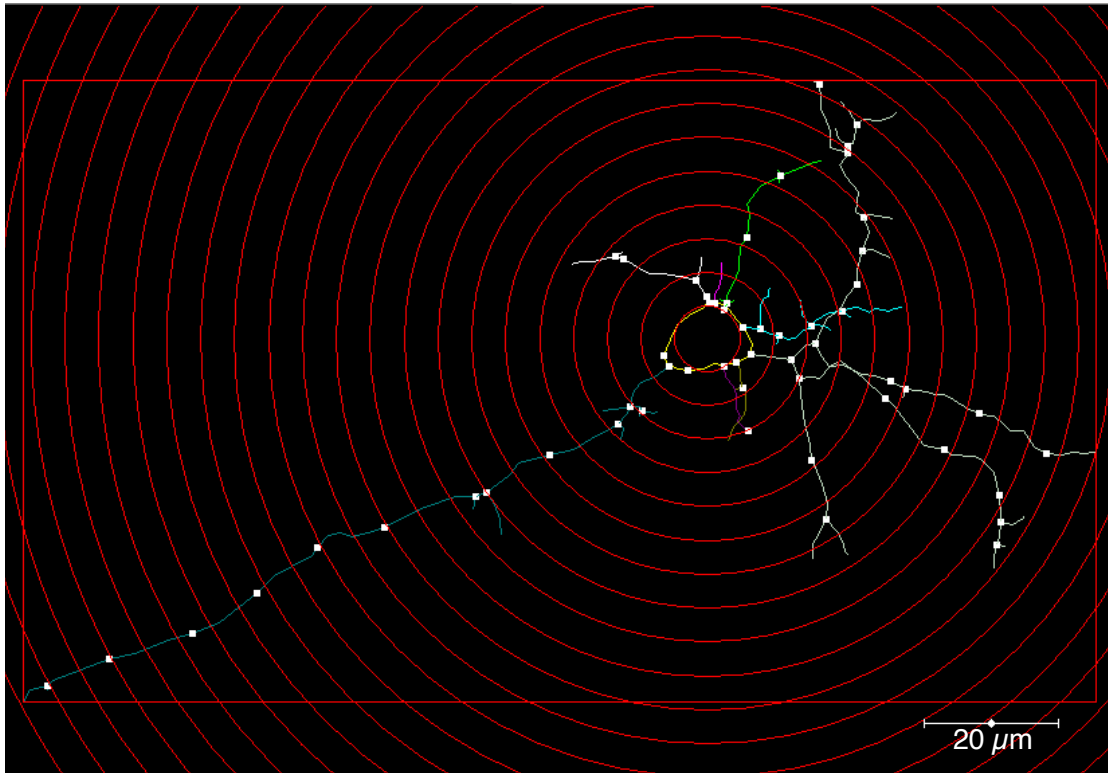


Figure 4.4 Sholl analysis of CS and control iPSC-derived neurons. After neurite reconstruction in Neurolucida, concentric circles with 5 μm radii were centered at the cell body. An example cell is shown with the cell body outlined in yellow, each primary neurite traced in a different color, and branch points labeled with white squares. Scale bar, 20 μm .

A

R472fsX4

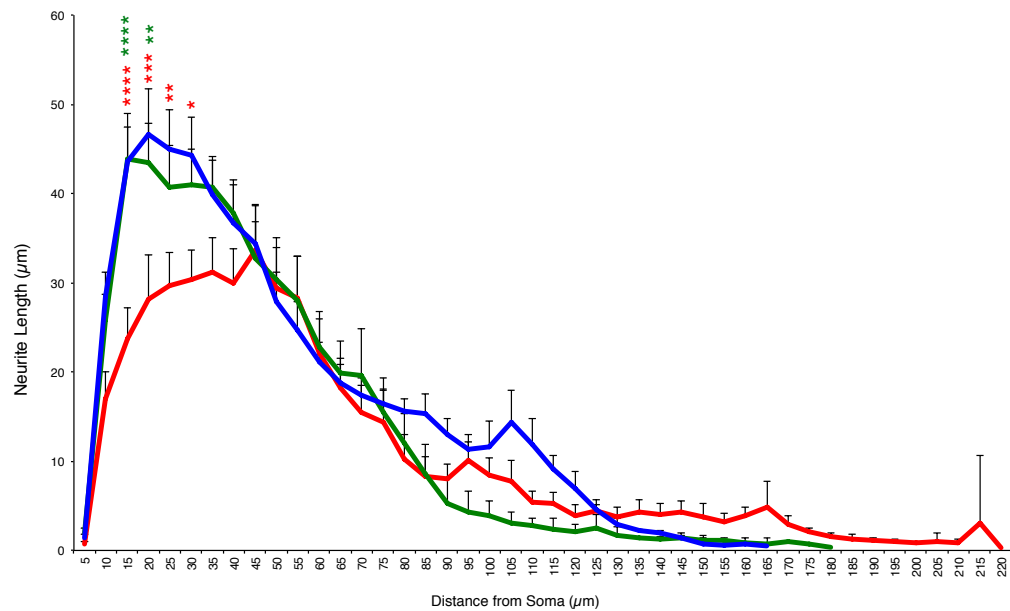
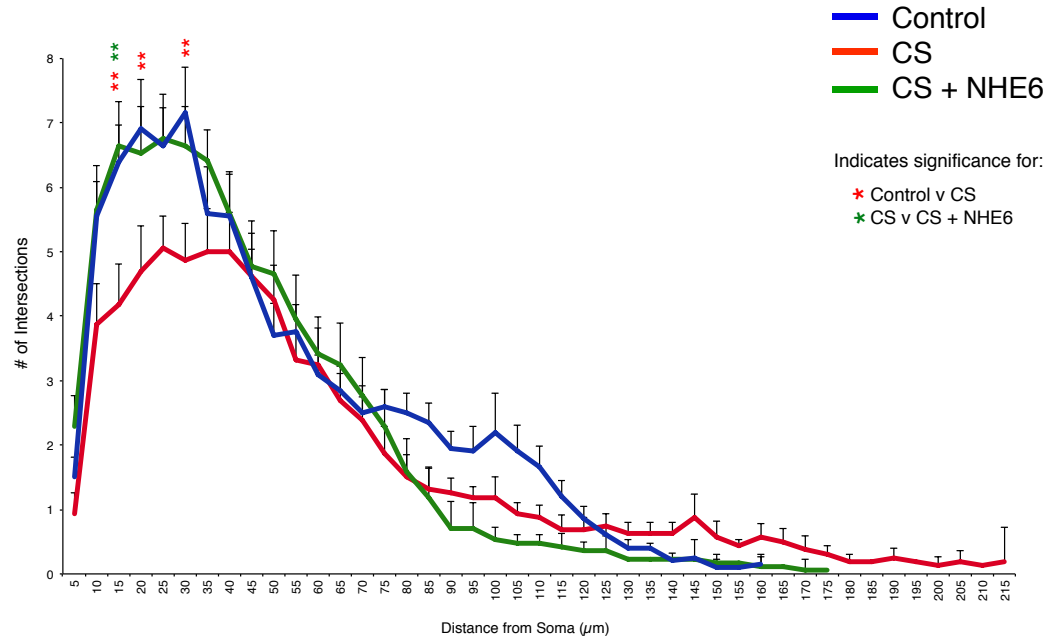
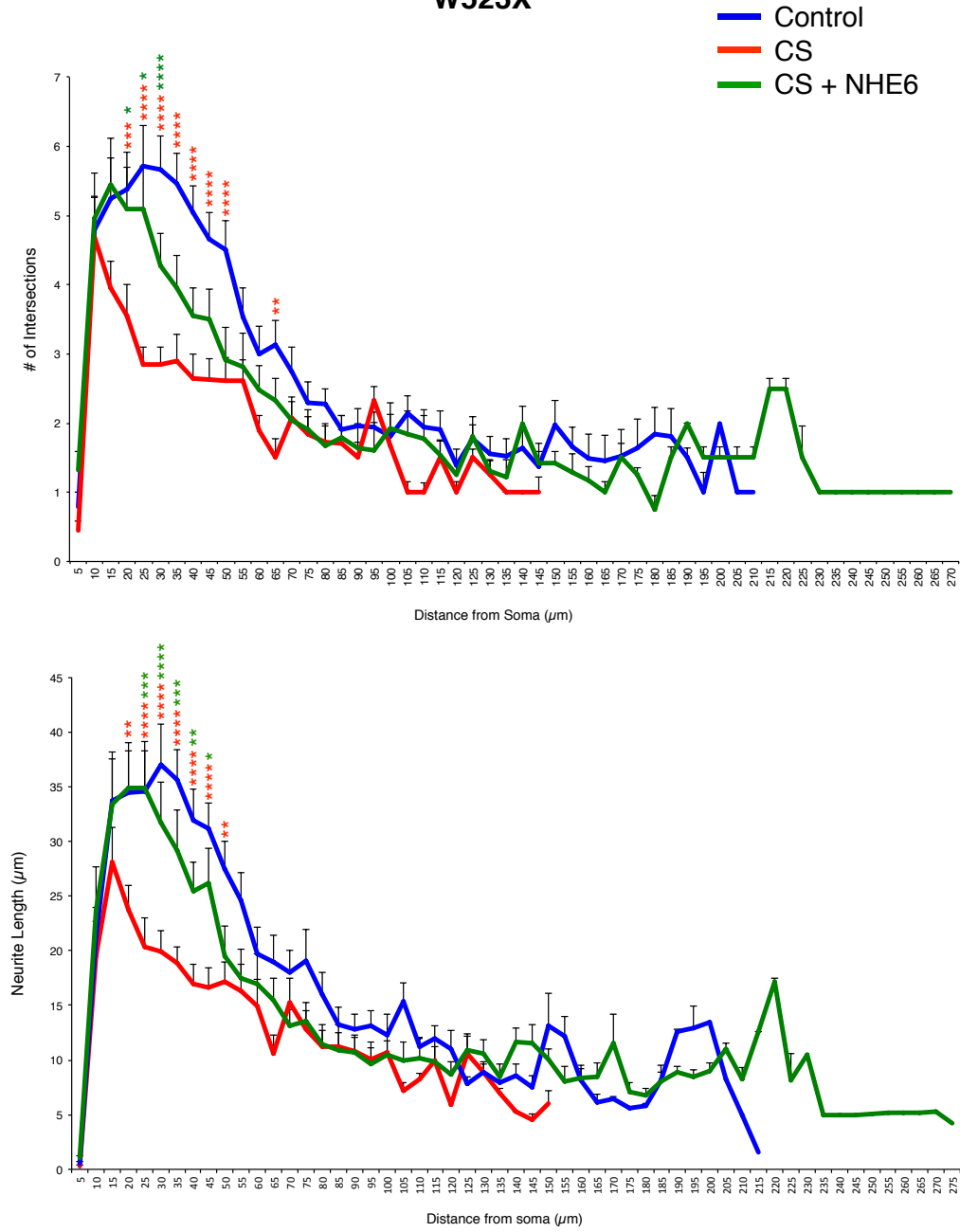


Figure 4.5 Deficits in neuronal arborization complexity can be rescued by re-expression of NHE6. The average number of times neurites intersect each Sholl radius and the neurite length within each radius was plotted as a function of distance from the soma for each CS and control pair: (A) R472fsX4 (B) W523X (C) G383D. $n = 20$ -25 cells per line per condition. Error bars, sem. Two-way ANOVA with Bonferroni multiple comparisons test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, **** $p < 0.00001$. Red asterisks denote significance when comparing CS to control cells. Green asterisks denote significance when comparing CS to CS cells transfected with NHE6.

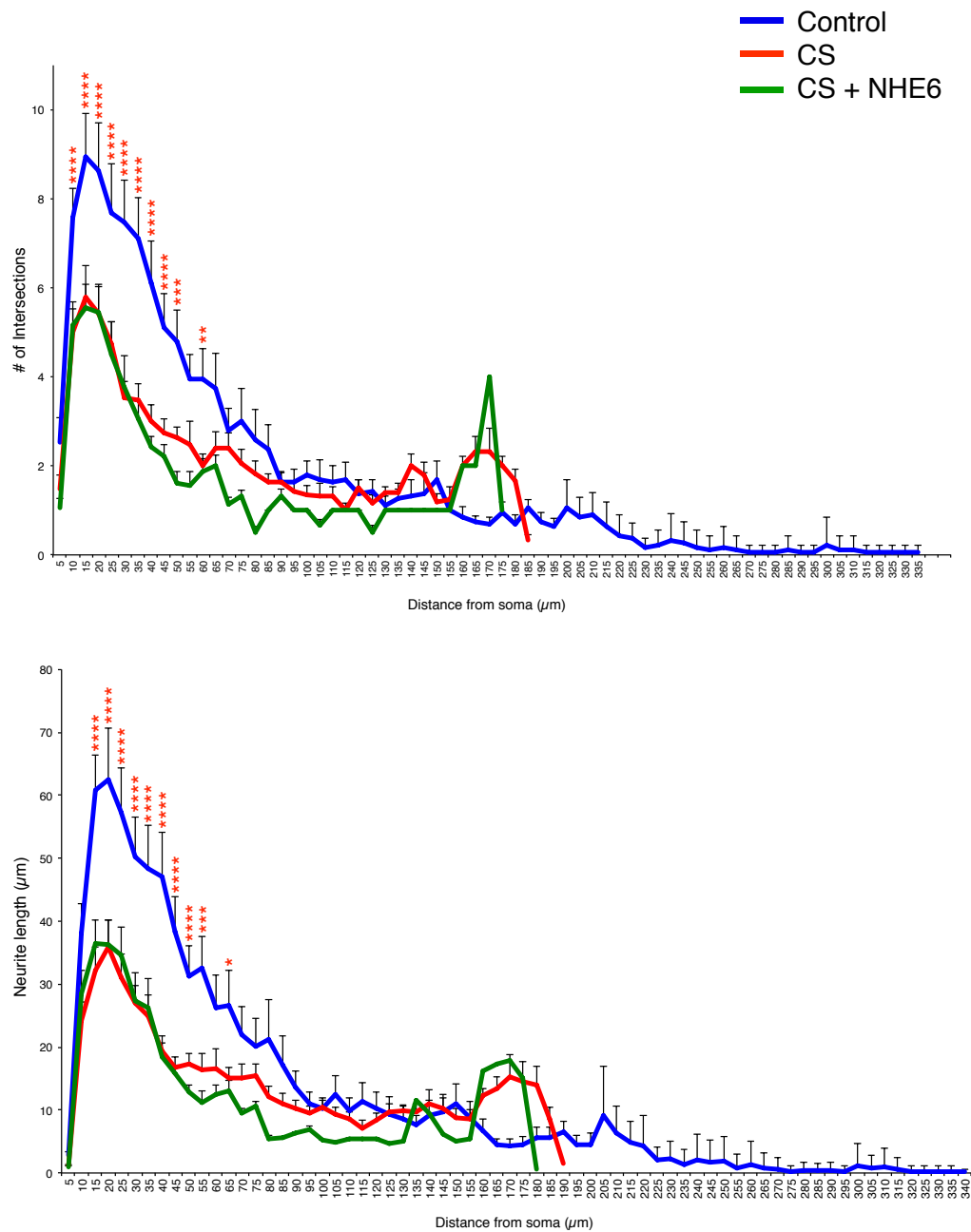
B

W523X



C

G383D



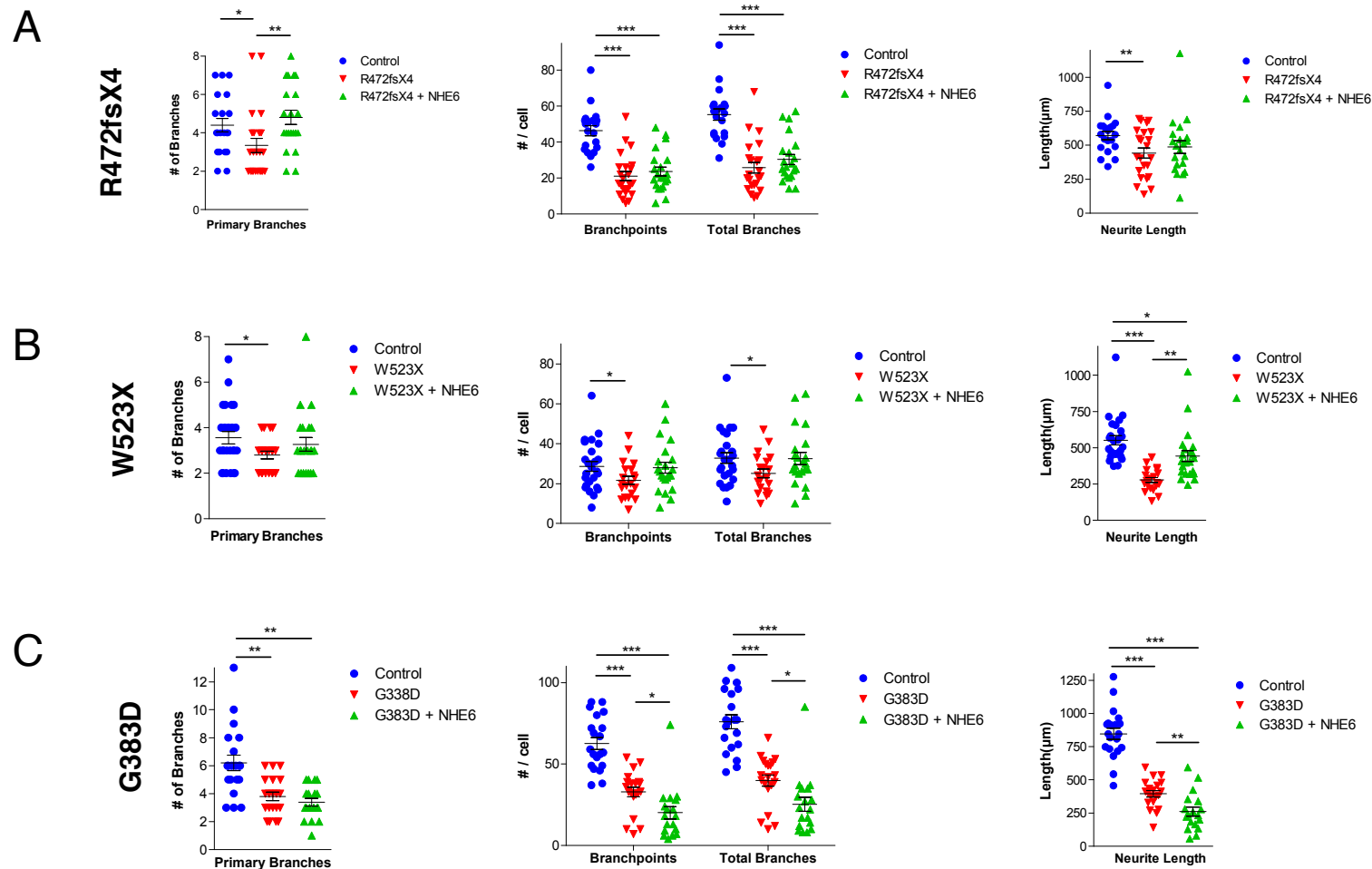


Figure 4.6 Neurite morphology and length analysis of CS and control neurons. iPSC-derived neurons were transfected with GFP or GFP + full-length NHE6 tagged with HA and analyzed 5 days after transfection. The number of primary branches, branchpoints, total number of branches, and neurite length were quantified for each CS line and paired control: (A) R472fsX4 (B) W523X (C) G383D. Cells were imaged by confocal microscopy and traced using Neurolucida software. n= 20-25 cells per line per condition. Two-tailed Welch's t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 1 \times 10^{-6}$.

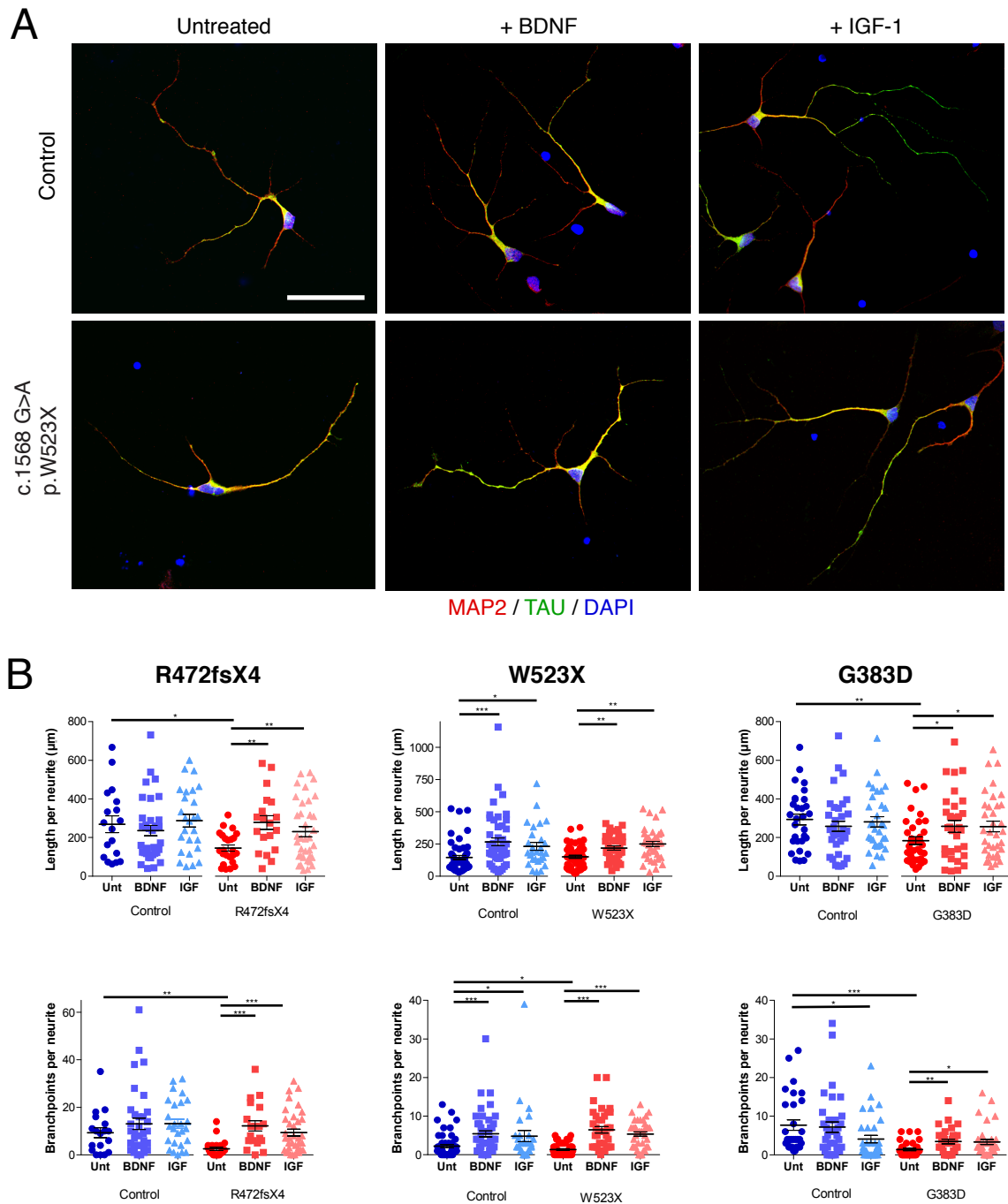


Figure 4.7 BDNF and IGF treatment rescues deficiencies in arborization. (A) Immunostaining for MAP2 and TAU in iPSC-derived neurons treated with BDNF and IGF. Representative images are shown from the W523X CS line and the paired control line. Scale bar, 50 μm. (B) Average length per neurite and the number of branchpoints per neurite were measured using Neurolucida tracing software. R472fsX4: n= 24(UNT), 19(BDNF), 36(IGF-1) neurites. Control Family 1: n= 18(UNT), 35(BDNF), 26(IGF-1) neurites. W523X: n= 53(UNT), 37(BDNF), 37(IGF-1) neurites. Control Family 2: n= 54(UNT), 48(BDNF), 28(IGF-1) neurites. G383D: n= 32(UNT), 31(BDNF), 35(IGF-1) neurites. Control Family 3: n= 30(UNT), 35(BDNF), 32(IGF-1) neurites. mean ± sem; Two-tailed Welch's t-test: * p < 0.05, ** p < 0.005, *** p < 0.0005.

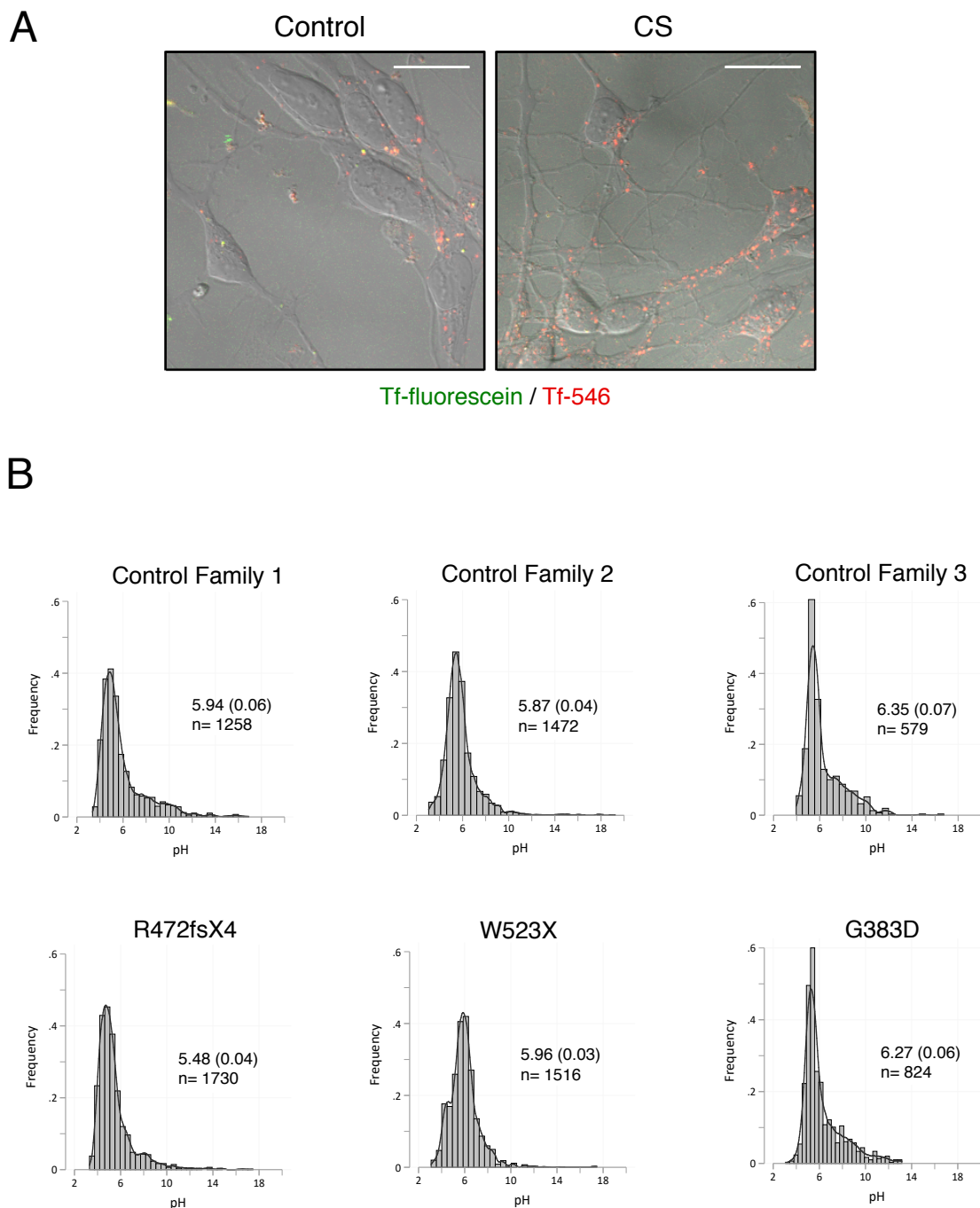


Figure 4.8 Endosome pH in CS iPSC-derived neurons. (A) Live-cell imaging of iPSC-derived neurons incubated with a pH-sensitive transferrin (Tf-fluorescein) and a pH-insensitive transferrin (Tf-546). Representative images are shown. Scale bars, 20 μ m. (B) The ratio of 488 to 546 fluorescence was used to calculate pH after generating a standard curve by incubating cultures with buffers of known pH. The mean pH value is listed with sem in parenthesis. n = the number of endosomes counted as indicated (data was collected from 3 – 5 independent neuronal inductions for each CS/Control pair)

CHAPTER 5: Discussion

Christianson syndrome is one of the most common causes of XLID and patients often present with severe symptoms including epilepsy, microcephaly, the inability to speak, and ataxia. Despite increasing reports of CS diagnosis in the clinic, treatment options only address the management of symptoms. There is a need for increased understanding of the biological mechanisms driving CS that will in turn inform the development of targeted treatments. In this study, the development of CS patient-derived iPSCs differentiated to cortical neuronal fate has pointed to key cellular mechanisms disrupted in CS and has provided a platform for testing potential therapeutics.

Loss of function mutations in CS patients

We derived iPSCs from three patients with CS and from their unaffected siblings as controls. In Family 1, the proband has a single base pair duplication in *SLC9A6* which causes a frameshift leading to a premature stop codon four codons downstream of the duplication (c.1414dupA, p.R472fsX4). The proband in Family 2 has a nonsense mutation in *SLC9A6* (c.1568G>A, p.W523X). In Family 3, the proband has a single base pair substitution at the first base of exon 9 which leads to missense mutation and may also function as a splicing mutation (c.1148 G>A, p.G383D).

Since the initial discovery of mutations in *NHE6* in neurological disease (Christianson et al., 1999), clinical reports of *NHE6* mutations in CS patients have been accumulating (Bosemani et al., 2014; Garbern et al., 2010; Gilfillan et al., 2008; Masurel-Paulet et al., 2016; Mignot et al., 2013; Pescosolido et al., 2014; Riess et al., 2013; Schroer et al., 2010; Takahashi et al., 2011; Zanni et al., 2014). Mutations in *NHE6* are predicted to be loss of function, but the level of *NHE6* expression in patients has not been well characterized. When we examined the expression of *NHE6* at the mRNA and protein level in iPSCs from CS patients, we found that for the two probands with

truncating mutations, Family 1 (R472fsX4) and Family 2 (W523X), NHE6 was undetectable at the mRNA and protein level. In iPSCs from the proband in Family 3 with a missense mutation (G383D), mRNA was expressed at 58% of control levels and NHE6 protein dimers were expressed at 7% of control levels. Structural modeling showed that the G383D mutation may lead to protein misfolding and likely to disruption of ion translocation. The reduced expression and predicted functional disruption of NHE6 containing the missense mutation suggest that the G383D mutation is a hypomorphic mutation. Our analysis of NHE6 expression in patient lines has revealed that mutations in NHE6 in CS are loss of function.

One other study has examined the expression of NHE6 in patient cells. Gilfillan *et al.* found that in a CS patient with an inframe deletion in *SLC9A6*, mRNA was expressed to similar levels as in unrelated controls (Gilfillan et al., 2008). However, transfection of NHE6 containing this deletion into mammalian cell lines showed decreased protein expression as compared to transfection of full-length NHE6 (Ilie et al., 2016). These findings are consistent with our evidence that NHE6 mutations are loss of function.

Modeling early neuronal development in CS

Differentiation of CS patient iPSCs to cortical fate reflected *in vivo* development of the human cortex. At 38 days after the induction of differentiation, cultures shifted gene expression downregulating stem cell markers and upregulating markers of neuronal identity. iPSC-derived neurons from CS patients and from controls expressed markers of deep layer cortical neurons and not markers of other brain regions or cell types suggesting that loss of NHE6 does not disrupt the neurogenesis of early born cortical neurons.

In addition, the levels of apoptotic and proliferative cells did not change in CS as compared to controls. Where prenatal microcephaly can be caused by decreased expansion of progenitor populations or impairment of neuronal migration, postnatal microcephaly can be caused by deficits in arborization, myelination, or synaptogenesis (van Dyck and Morrow, 2017). We did not observe changes in cell death or proliferation in neuronal cultures that can lead to prenatal microcephaly. This is consistent with the observation that the majority of CS patients present with postnatal microcephaly (Pescosolido et al., 2014). Other work has shown that transfection of mutant NHE6 into mouse primary hippocampal neurons derived from P0 mice and grown for 12 days *in vitro* results in an increase in cell death (Ilie et al., 2016). This work however investigated a postnatal timepoint as compared to our analysis of a prenatal timepoint. Our work differentiating iPSCs to cortical neuronal fate recapitulated *in vivo* development and serves as a model to investigate early development in CS.

Gene expression changes in CS

In this study, we performed for the first time a large-scale gene expression analysis in CS. Through a targeted approach examining the mRNA expression of 358 candidate genes, we identified changes in gene expression that (1) uncover possible biological disease mechanisms, (2) inform drug design, and (3) reveal differences between individual CS patients. Gene expression analysis identified patient specific misexpression in several genes. In R472fsX4 CS iPSCs, V-ATPase subunits were downregulated suggesting a compensatory mechanism to prevent endosome acidification when NHE6 is lost. The downregulation of vimentin in R472fsX4 and G383D iPSCs suggests that reduced levels of vimentin may contribute to the decreased

induction of neurite outgrowth in CS. In G383D iPSCs, cathepsin F was upregulated which may lead to increased cleavage of lysosomal substrates.

These changes in gene expression highlight important considerations for the development of treatments for CS. For example, alkalinization of endosomes presents a possible treatment for CS. One way to alkalinize endosomes is through the inhibition of the V-ATPase with specific inhibitors such as bafilomycin, concanomycin, and archazolid (Holliday, 2014). However, in patients where the V-ATPase is downregulated such as the R472fsX4 CS patient, V-ATPase inhibitors may not be effective. In these cases, lysomotropic weak bases such as amantadine and chloroquine may be better used to alkalinize endosomes. Furthermore, alkalinization of the endosome compartment may be an important approach for treatment in patients who have upregulated hydrolases. For example, upregulation of cathepsin F in the G383D CS patient may be counteracted by alkalinization. Our study of gene expression in CS revealed the importance of assessing an individual patient's gene expression in order to understand biological mechanisms and inform the selection of potential therapies.

Other studies of gene expression have examined the expression patterns of NHE6 in the brains of idiopathic autism patients (Schwede et al., 2014) from re-analysis of microarray data (Voineagu et al., 2011). NHE6 was found to be significantly downregulated in autism (Schwede et al., 2014), which is consistent with our finding of downregulation of NHE6 in CS and suggests that NHE6 may be widely important in neurological disease. In addition, in the analysis of autism brain by Schwede *et al*, NHE9 expression was upregulated and the expression of NHE6 and NHE9 were negatively correlated (Schwede et al., 2014). In our gene expression analysis, NHE9 was not significantly changed and we did not see a negative correlation between the expression of NHE6 and NHE9. We may not have replicated this finding because NHE9 is

expressed at low levels in the prenatal brain while expression increases after birth (Brainspan, 2011). Our study assessed NHE9 levels during a prenatal timepoint while Schwede *et al.* examined NHE9 expression in patients with a median age of 27 (Voineagu et al., 2011). Because NHE6 and NHE9 both localize to endosomes (Nakamura et al., 2005), they may have overlapping roles in pH maintenance. Strategies to upregulate or activate NHE9 in order to restore pH homeostasis in endosomes present another therapeutic approach for CS.

Failures in neuronal circuitry establishment in CS

Our study of neurite growth and arborization revealed a failure in the establishment of neuronal circuitry in CS. Although the effect was sometimes variable between individuals, we observed a decrease in neurite growth and retraction rates, neurite arborization, and neurite length. These results suggest a mechanism for the postnatal microcephaly observed in CS patients. At birth, a newborn's brain is 36% the size of the adult brain and undergoes a rapid period of expansion during the first two years of life (Knickmeyer et al., 2008). Despite an initial period of neurogenesis in the months after birth, the number of new neurons in the brain does not dramatically increase in infancy (Shankle et al., 1999). Instead, postnatal brain growth results from an increase in neuropil consisting of axon terminals, dendrites, and glial processes (Knickmeyer et al., 2008). In the postnatal brain, dendrite arborization and spine density increases (Petanjek et al., 2008) and the number of synapses rapidly increases followed by a period of pruning in adolescence through adulthood (Huttenlocher, 1979). Our observation of decreased arborization and neurite growth in CS suggests that NHE6 has a critical role in the expansion of neuropil in the developing brain. Although our model

system interrogates a prenatal timepoint, failures in neuronal circuitry establishment prenatally may be exacerbated postnatally.

Other studies of neurite circuitry in CS have also found decreased arborization when NHE6 function is lost. Consistent with our study, axonal and dendritic branching was reduced in an NHE6 null mouse model as compared to control (Ouyang et al., 2013). In addition, the synaptic density, spine maturity, and circuit activity were decreased in the NHE6 null mouse as compared to control (Ouyang et al., 2013). The investigation of synapse development in mature cultures of CS iPSC-derived neurons may reveal similar phenotypes. Also in line with our findings, the transfection of mutant NHE6 in mouse primary hippocampal neurons reduced dendrite length and branching (Ilie et al., 2016).

Endosomal pH in CS iPSC-derived neurons

To determine if endosomal pH regulation is impaired in CS patient cells, we measured the intraendosomal pH of iPSC-derived neurons using fluorescent ratiometric imaging of fluorophore tagged transferrin. Endosomes were found to be overacidified in CS as compared to control in R472Xfs4 and G383D cells while W523X cells showed alkalinization. In other studies, the effect of NHE6 loss has been variable. Endosomes were found to be overacidified in an NHE6 null mouse model (Ouyang et al., 2013). *In vitro* knockdown or overexpression of NHE6 acidified or alkalinized pH, respectively (Ohgaki et al., 2010; Prasad and Rao, 2015; Xinhan et al., 2011). Conversely, one study showed that overexpression of NHE6 did not change endosome pH (Nakamura et al., 2005). Another study showed that endosomes become overacidified only when NHE6 and NHE9 are both knocked down but not individually (Roxrud et al., 2009).

The overacidification of endosomal pH in some CS patients supports a model where endosomal signaling is disrupted leading to deficits in neuronal circuitry. Overacidification of endosomes can disrupt ligand-receptor binding in endocytosed vesicles, increase trafficking of receptors to the late endosome and lysosome, or decrease the recycling of signaling receptors to the plasma membrane. The retrograde trafficking of neurotrophins by endosomes is critical for cell survival and the growth and arborization of neurites (Cheng et al., 2011; Cosker et al., 2013; Harrington and Ginty, 2013; Lazo et al., 2013). Disruption of neurotrophin signaling through BDNF and its receptor, TrkB, was attenuated in a mouse model of NHE6 loss indicating the importance of NHE6 in mediating endosomal signaling (Ouyang et al., 2013). The overacidification of endosomes in CS may impair neuronal circuitry establishment through attenuating neurotrophin signaling and possibly through decreasing synaptic vesicle recycling although this remains to be tested.

Overacidification of endosomes in CS shows variable expressivity in our experiments. Endosomes in the W523X CS patient were alkalinized, yet this patient exhibits similar deficits in arborization and neuronal growth as in patients with overacidified endosomes. It is possible that disease manifestation in this patient is due to the loss of NHE6 protein interactions that occur on the C-terminal tail rather than due to loss of ion translocation. The C-terminal tail of NHE proteins are thought to serve as a scaffold to bind regulatory proteins and contain multiple phosphorylation sites, lipid binding domains, and protein interaction domains (Hendus-Altenburger et al., 2014). NHE6 has been shown to interact with the G protein coupled receptor, angiotension II subtype AT2 (Pulakat et al., 2005). Activation of AT2 receptor induces signaling through protein kinase α , p21^{Ras}, Rap1/B-Raf, and cGMP to promote neurite outgrowth (Guimond and Gallo-Payet, 2012). Although AT2 receptor is not endocytosed upon activation (Hein

et al., 1997), the interaction between NHE6 and AT2 receptor possibly at the plasma membrane could be important for AT2 receptor signaling. Disruption of this interaction could lead to diminished neurite outgrowth. A second NHE6 interacting protein has been identified. NHE6 has been shown to interact with RACK1 (Ohgaki et al., 2008). RACK1 serves as a scaffolding protein for signaling molecules and is involved in diverse cellular functions including regulation of NMDA activation, GABA receptor function, cell migration, and apoptosis (Adams et al., 2011). Interaction between NHE6 and RACK1 could be important to localize RACK1 to endosomes.

Restoring neuronal circuitry in CS

In this study, the extent to which the re-expression of NHE6 or the addition of growth factors could rescue neuronal circuitry deficits was tested. Re-expression of NHE6 restored arborization complexity as measured by Sholl analysis in the NHE6 null lines, R472fsX4 and W523X, but failed to rescue in the G383D line possibly due to a dominant negative effect of the missense NHE6 protein. Re-expression of NHE6 increased neurite length in W523X CS cells and increased primary branches in R472fsX4 CS cells. Because the measurement of arborization by Sholl analysis provides more complex information taking into account neurite branching and growth as a function of distance from the soma, more precise changes can be observed than in the endpoint measurements of length and total branchpoints. Restoration of neuronal circuitry by re-expression of NHE6 shows a cell-autonomous rescue. Because transfection efficiency was low in our experiments, only the phenotype of the cell re-expressing NHE6 is likely to be impacted. Rescue of branching after re-expression of NHE6 is consistent with data from the NHE6 null mouse model. Transfection of NHE6 in primary hippocampal neurons null for NHE6 increases neurite branching (Ouyang et al., 2013).

Treating CS neuronal cultures with the growth factors, BDNF or IGF-1, increased neurite length and branching in all three CS lines. The neurotrophin, BDNF, promotes cell survival and neurite outgrowth through endosome mediated retrograde signaling (Cosker et al., 2008). The increase in neurite growth and arborization we observed after treating CS neuronal cultures with BDNF may result from increased neurotrophin endosomal signaling. Consistent with our findings, branching defects seen in the NHE6 null mouse can be rescued with exogenous BDNF (Ouyang et al., 2013). Transitioning BDNF treatment to clinical use has not been successful. Clinical trials using BDNF to treat amyotrophic lateral sclerosis have not shown benefit to the patient (BDNFStudyGroup, 1999). There are conflicting studies concerning the ability of BDNF to cross the blood-brain barrier (Pan et al., 1998; Pardridge et al., 1994). Additionally, BDNF has a short half-life in the blood (Lu et al., 2013). The inability of BDNF to reach the target tissue may explain why clinical trials have failed. Approaches to deliver BDNF to the brain using nanoparticles or conjugation to carrier molecules as well as methods to stabilize BDNF in the blood are being studied to improve drug delivery (Lu et al., 2013).

In addition to BDNF, IGF-1 treatment also increased branching and neurite length in CS neuronal cultures. To increase the supply of nutrients to the brain, IGF-1 signals through the PI3-kinase and Ras pathways to promote the translocation of glucose and glycogen synthesis (Bondy and Cheng, 2004). IGF-1 null mice have decreased brain size due in part to a reduction in neuropil indicating the importance of IGF-1 in branching and neurite growth (Cheng et al., 2003). Early successes in clinical trials administering IGF-1 to treat Rett syndrome and Phelan-McDermid syndrome (Khwaja et al., 2014; Kolevzon et al., 2014) suggest the possibility of treating CS patients with IGF-1. Before moving to clinical trials, for both Rett syndrome and Phelan-McDermid syndrome, IGF-1

treatment was studied in patient derived iPSCs and in mouse models (Bozdagi et al., 2013; Marchetto et al., 2010; Shcheglovitov et al., 2013; Tropea et al., 2009). The ability of IGF-1 to reverse neurological and behavioral phenotypes was tested for both disease models. The mouse provided a systems-level model for drug testing as compared to *in vitro* iPSC-derived neurons and allowed researchers to determine an effective treatment dose. Before transitioning to clinical trials of IGF-1 in CS, further testing of IGF-1 treatment in the NHE6 null mouse would be needed. Also, it will be important to consider the non-neuronal effects of IGF-1 as IGF-1 receptors are widely expressed in the body (Wilcox, 2005). IGF-1 is FDA approved to increase height in Laron syndrome, a disease characterized by short stature (Laron, 2001), and treatment of neurological disorders with IGF-1 will likely have effects throughout the patient's body.

Variability in iPSC-derived neurons: Pros and cons

In our experiments, we observed variability in phenotypes between individual donors and between cultured lines. The ability to quantify the variable expressivity in patient phenotypes is a key advantage to studying patient-derived tissues. For example, in the CS patient carrying the W523X mutation we did not observe an overacidification of endosomes. It is possible that the genome of this individual has genetic modifiers that suppress this phenotype. Although we did observe any candidate modifiers in our gene expression analysis for this patient, for instance a downregulation of the V-ATPase could suppress an overacidification phenotype, a genome-wide analysis may reveal variants specific to this patient. In addition, treatments that work to alkalinize endosomes may not be effective in this patient. The ability to assess variations in cellular phenotypes and drug response in individual patients using iPSCs is a crucial advantage over modeling disease in the mouse.

On the other hand, the variability in the iPSC reprogramming and neuronal differentiation process can be disadvantageous. Reprogramming of somatic cells to iPSCs can result in aneuploidy or the generation of CNVs (Taapken et al., 2011). Also, clonal expansion of genetic variants derived from the source cells or from variants generated through passaging may lead to variability in iPSC lines. Mistakes in DNA methylation during reprogramming or retention of the epigenetic identity of the source cell due to incomplete reprogramming can occur (Liang and Zhang, 2013). These genetic and epigenetic variations can impair iPSC differentiation potential. Therefore, iPSCs must be extensively characterized for chromosomal abnormalities, pluripotency markers, and the ability to differentiate to all germ layers.

Moreover, upon differentiation, incomplete conversion of iPSCs to neuronal cells can result in heterogeneity in culture composition. To purify cultures, methods have been developed using fluorescence activated cell sorting (FACS) to isolate neuronal progenitor and neuronal cells (Yuan et al., 2011). Also, protocols have been developed to select cells after conversion to neuronal fate thereby improving culture heterogeneity. For example, transduction of neurogenin-2 (NGN2) in iPSCs has been shown to induce neuronal fate and co-expression with puromycin allows for selection of NGN2 expressing cells (Zhang et al., 2013). However, this type of direct conversion protocol may bypass developmental processes that would more accurately model disease. In summary, inter-individual variation in iPSCs can reveal interesting biological differences in patient phenotypes and inform drug therapy while variation due to iPSC reprogramming and incomplete neuronal conversion present challenges to disease modeling.

Future applications

Our work has examined the role of NHE6 in the projection neurons of the developing cortex. However, NHE6 is widely expressed in the body and NHE6 loss of function in CS may impact other tissues. For example, progressive cerebellar atrophy is commonly seen in CS patients and loss of Purkinje cells is observed in a NHE6 null mouse model (Pescosolido et al., 2014; Stromme et al., 2011). Modeling the cerebellum using iPSCs could provide mechanistic insights into the pathology of this brain region. Protocols to differentiate iPSCs to Purkinje cells are being established although the complexity of Purkinje cell morphology and development has been difficult recapitulate (Watson et al., 2015).

Moreover, NHE6 expression in glia may function in the establishment of neuronal circuitry and brain growth. The number of glia increase during postnatal development to represent half of the cells in the nervous system and function to regulate synapse formation and plasticity (Zuchero and Barres, 2015). In particular, the number of oligodendrocytes increases rapidly and myelination of axons contributes to an increase in white matter in the postnatal brain (Lenroot and Giedd, 2006; O'Kusky and Colonnier, 1982). NHE6 has been shown to be expressed in glia (Kondapalli et al., 2013; Ouyang et al., 2013) and could mediate the glial contribution to neurodevelopment. For example, glia are needed at the synapse to mediate the clearance of neurotransmitters. When NHE9 expression is increased in mouse astrocytes, the uptake of glutamate is increased suggesting a role for endosomal NHEs in glial neurotransmitter clearance (Kondapalli et al., 2013). Differentiation of CS iPSCs to glia could allow for the contribution of glia to neurodevelopment in CS to be studied. Protocols to generate astrocytes from iPSCs have been established (Krencik and Zhang, 2011) although the differentiation protocols for oligodendrocytes are inefficient and protocols for microglia differentiation have not

been developed due to a lack of understanding of *in vivo* microglial development (Mertens et al., 2016).

Another area of future research may examine the influence of NHE6 on neuronal circuitry establishment through a role at the excitatory synapse. In primary mouse hippocampal neurons, NHE6 accumulates in dendritic spines and colocalizes with markers of early endosomes, recycling endosomes, and a subunit of the AMPA receptor. Recent studies have suggested a role for NHE6 in long-term potentiation (LTP). In the establishment of LTP, AMPA receptors open upon binding to glutamate allowing an influx of Na⁺ and depolarization of the postsynaptic cell. During LTP establishment, AMPA receptors traffic from recycling endosomes to the surface of dendritic spines (Park et al., 2006). The increase in AMPA receptors at the synapse allows for a greater postsynaptic response to glutamate and furthermore promotes dendritic spine formation (Park et al., 2006). Upon induction of LTP, vesicles containing NHE6 accumulate in dendritic spines (Deane et al., 2013). These data suggest that NHE6 could play a role in the endosomal recycling of AMPA receptors and the establishment of LTP.

Furthermore, NHE6 may function at the presynapse. NHE6 colocalizes with the presynaptic marker, SV2, more frequently than with the postsynaptic marker PSD-95 (Ouyang et al., 2013). A role for NHE activity in the loading of the excitatory neurotransmitter, glutamate, into synaptic vesicles had been proposed. Glutamate requires a pH gradient for loading and the need for a cation/H⁺ exchanger to establish a favorable membrane potential for glutamate uptake has been shown (Goh et al., 2011). It is possible that NHE6 could serve this role in presynaptic vesicles to promote glutamate loading. The investigation of NHE6 at the presynapse and postsynapse in neuronal cultures differentiated from CS iPSCs could provide insight into the contribution of NHE6 in synaptic development and transmission.

Our model of human cortex examines the development of a single neuronal cell type cultured in a monolayer. However, *in vivo* cortical development integrates morphogen gradients, extracellular signals, and contacts between different cell types to form a highly structured tissue. Three-dimensional cultures systems have been developed to differentiate human pluripotent stem cells into polarized cerebral organoids that have loosely structured cortical layers and regions of progenitor cells (Lancaster et al., 2013; Mariani et al., 2012). Depending on the protocol used, other structured regions can be generated within the organoid including tissues expressing markers of the retina, hippocampus, and choroid plexus (Lancaster et al., 2013). Also, the ability to generate both glutamatergic and GABAergic neurons in organoids allows the balance of excitatory and inhibitory synapses to be evaluated in disease (Mariani et al., 2015). The use of three dimensional culture models in CS could enhance investigations of neuronal circuitry allowing for characterization of long-range connections, interactions between different cell types, and synapse formation.

In addition, iPSCs may be used to assess the phenotypes of female carriers of *SLC9A6* mutations. Some female carriers have been reported to have mild to moderate intellectual disability, behavioral problems, speech and language delays, and progressive cognitive decline (Christianson et al., 1999; Pescosolido et al., 2014; Schroer et al., 2010; Sinajon et al., 2016). Studies of female mice heterozygous for loss of *SLC9A6* revealed behavioral and neuropathological deficits as compared to wild type females (Sikora et al., 2016). Deficits in female heterozygotes however were less severe than in hemizygous male mice (Sikora et al., 2016). Using human iPSC-derived neurons to evaluate the phenotypes of female carriers of *SLC9A6* mutations would provide further understanding of the biological mechanisms underlying disease. Also, due to the mosaicism of X chromosome inactivation (XCI) in female tissues, the study of iPSCs

derived from female carriers may provide understanding of the effect of *SLC9A6* gene dosage on disease manifestation.

There are conflicting reports concerning the XCI status of iPSCs. Some studies have reported that XCI is maintained during reprogramming leading to expression of a single X chromosome in iPSCs (Tchieu et al., 2010). In this case, screening of iPSC clones derived from female carriers for expression of the X chromosome containing the *SLC9A6* mutation will be necessary for disease modeling (Dandulakis et al., 2016). Other studies have reported that X chromosomes undergo reactivation during reprogramming and that XCI is reset during differentiation (Marchetto et al., 2010). In the case of X chromosome reactivation, clonal populations of cells expressing the mutant X chromosome must be selected after differentiation. Alternatively, a mixed population of cells either expressing or silencing the mutated X after differentiation could more closely recapitulate mutant *SLC9A6* expression *in vivo* (Dandulakis et al., 2016). Future studies using iPSCs to model the phenotypes of female carriers of the *SLC9A6* mutation will need to carefully consider the XCI status of iPSCs.

Conclusion

In summary, we have established a model of human cortical development in CS using iPSCs derived from patients and differentiated to cortical forebrain fate. We have determined that NHE6 mutations in CS are loss of function. Through studies of gene expression, we have identified patient-specific disease mechanisms and gene expression signatures that may predict drug response. We found that neuronal arborization was impaired in CS and have implicated endosome acidification in this process although with variable expressivity. Lastly, we tested the extent of arborization rescue through cell autonomous and non-autonomous methods. Future studies could

use CS iPSCs to evaluate molecular pathology at the synapse, in glia, or in the cerebellum. iPSC-derived neurons provide a personalized disease model that can be used to screen potential therapies through a candidate drug approach, as we have done here, or using high throughput screening. In closing, the establishment of an iPSC model for CS has identified critical disease mechanisms and potential therapies while providing a platform for future drug screens.

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