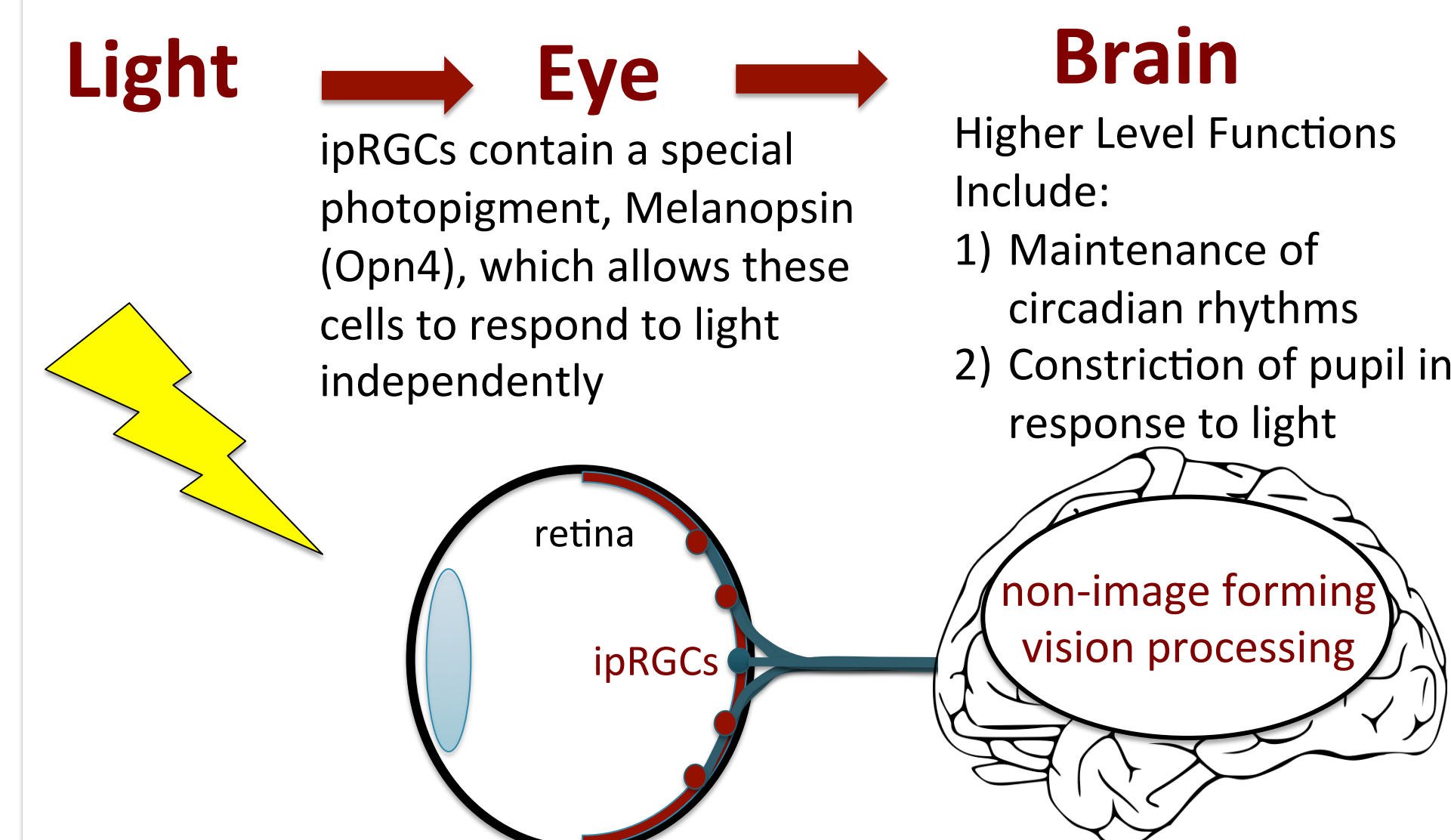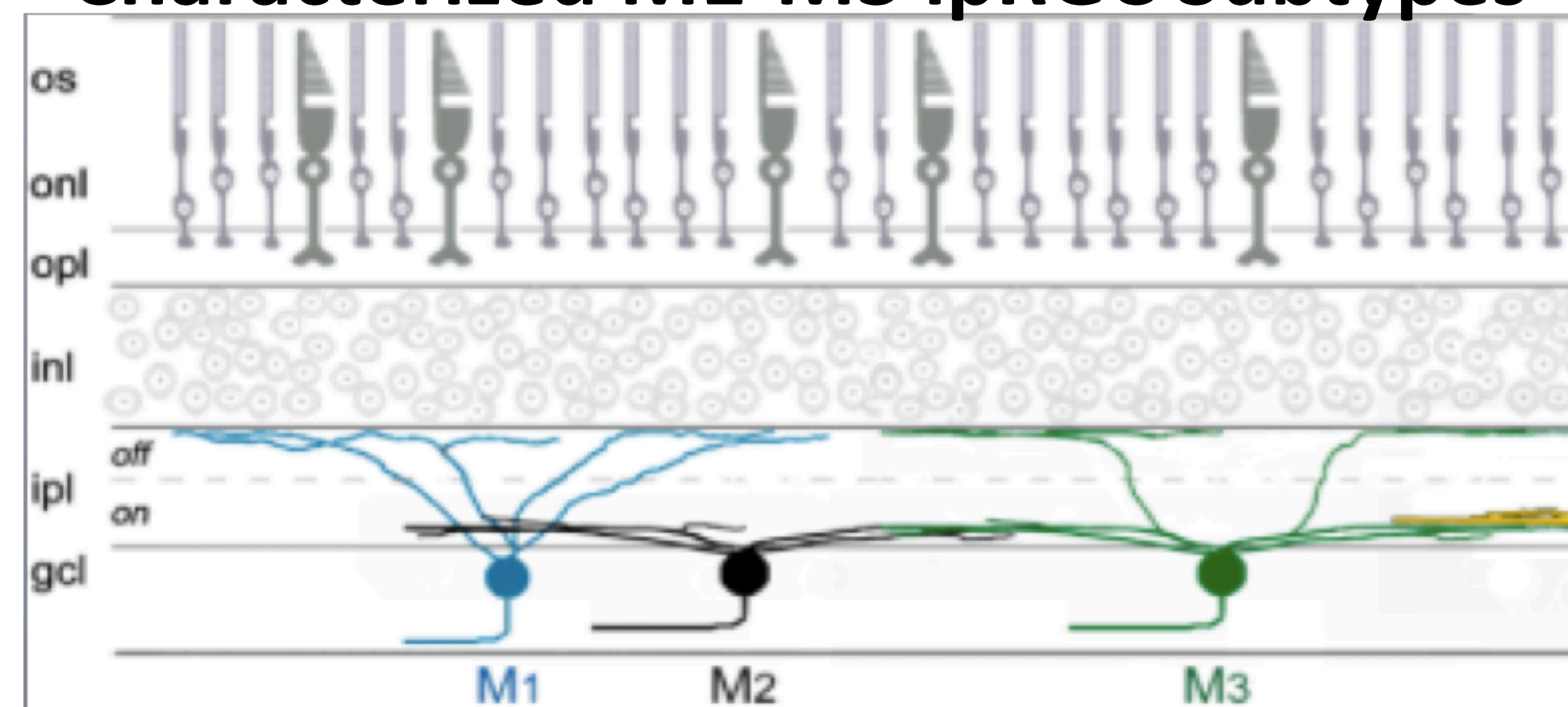# Diversity Demystified
Tbx20 sheds light on the molecular program of melanopsin-expressing retinal ganglion cells

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## Intrinsically Photosensitive Retinal Ganglion Cells

Light ➡ **Eye** ➡ **Brain**

ipRGCs contain a special photopigment, Melanopsin (Opn4), which allows these cells to respond to light independently

Higher Level Functions Include:
1) Maintenance of circadian rhythms
2) Constriction of pupil in response to light

retina
ipRGCs
non-image forming vision processing

Characterized M1-M3 ipRGC Subtypes

os
onl
opl
inl
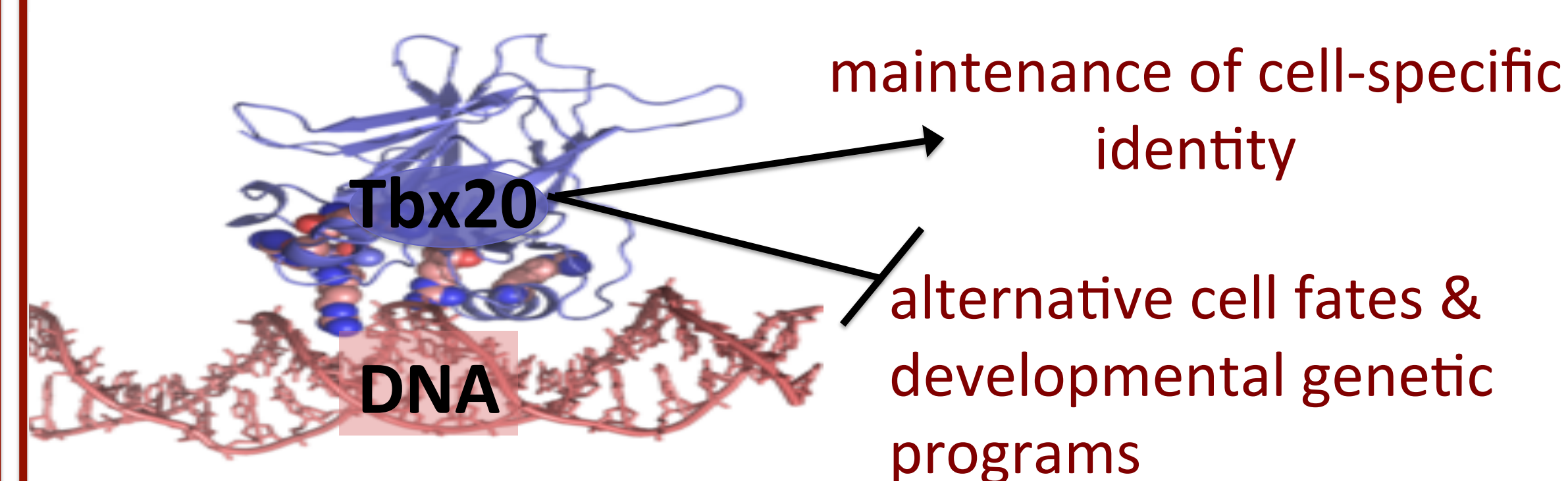off
ipl on
gcl

M1   M2   M3

Project Aims

1. Identify which genes are specifically expressed in ipRGCs
2. Investigate selectively expressed genes for potential ipRGC subtype-specific functions

Unique Molecular Features of ipRGCs

- ipRGC (GFP+)
- generic RGC (GFP-)

Opn4   Trpc7   Adcyap1   Tbr2   Tbx20
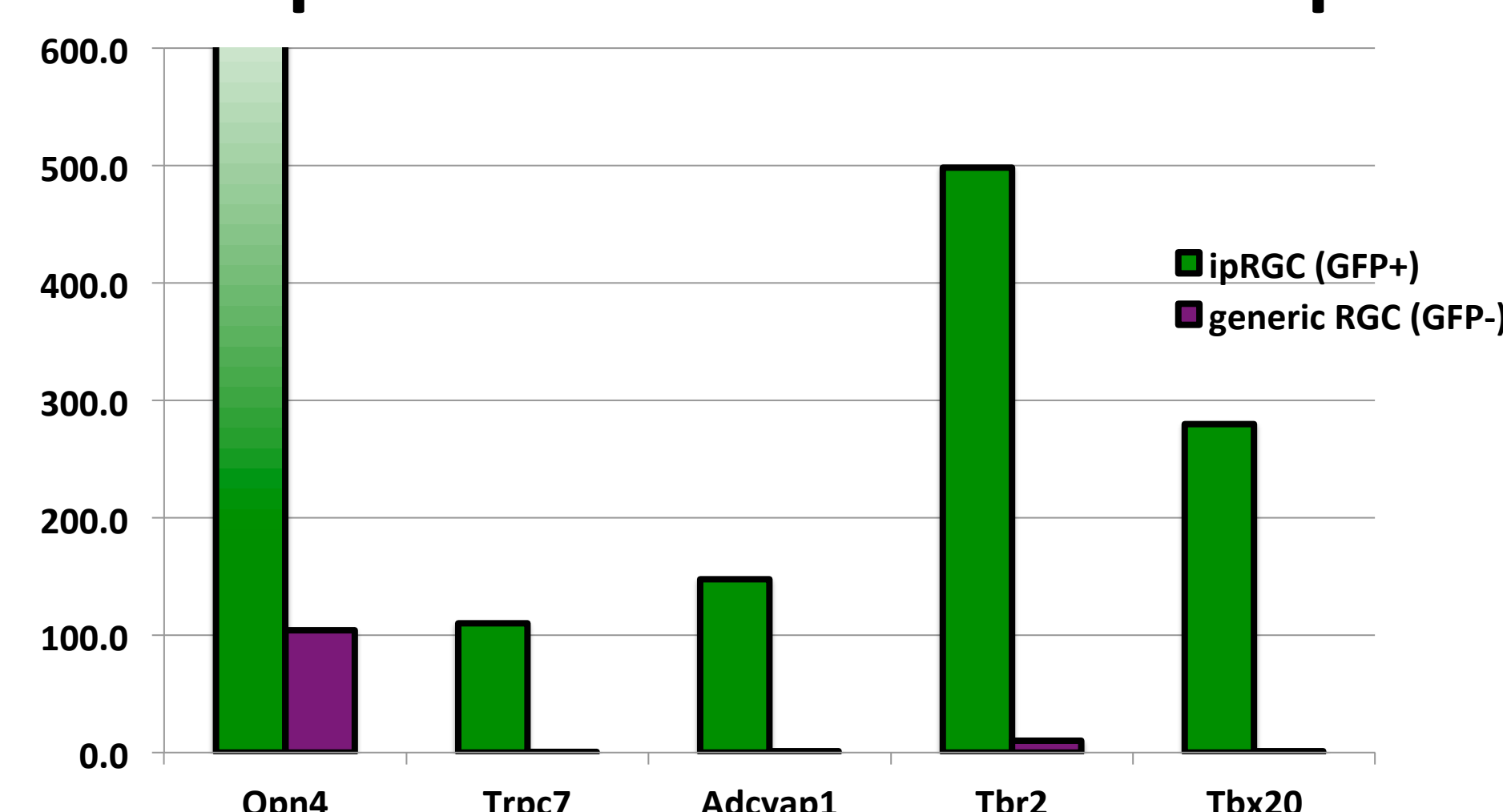
- Conducted differential expression analysis of M1-M3 ipRGCS against all other retinal ganglion cells (RGCs) by first enriching for RGCs and then isolating ipRGCS in a BAC-transgenic *Opn4:GFP* reporter
- Tbx20 is suggested to be selectively expressed in ipRGCS

Known Function of Tbx20 (in adult cardiomyocytes)

Tbx20

DNA

maintenance of cell-specific identity

alternative cell fates & developmental genetic programs

Implications of Tbx20 Investigation in ipRGCs

- Reveal previously unknown features of the ipRGC molecular program
- Clarify ipRGC subtype classification
- Provide insights into how ipRGC neuronal identity is maintained through adulthood

Experimental Plan

- *Immunoflourescence*- Test for colocalization of Tbx20 and ipRGC subtypes
- Cell counting- Quantify which ipRGC subtype(s) express Tbx20

Tbx20 is Expressed in a Subset of ipRGCs
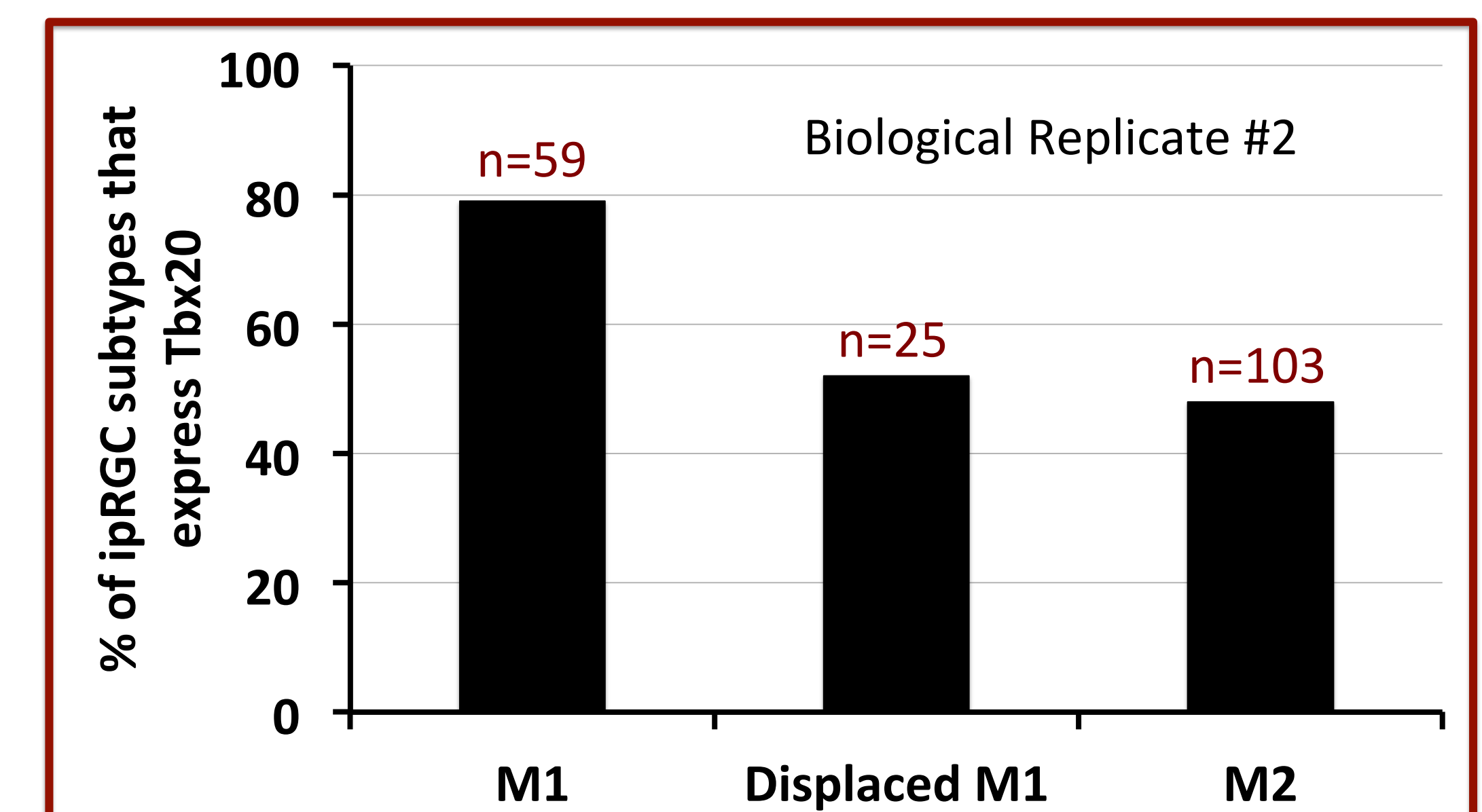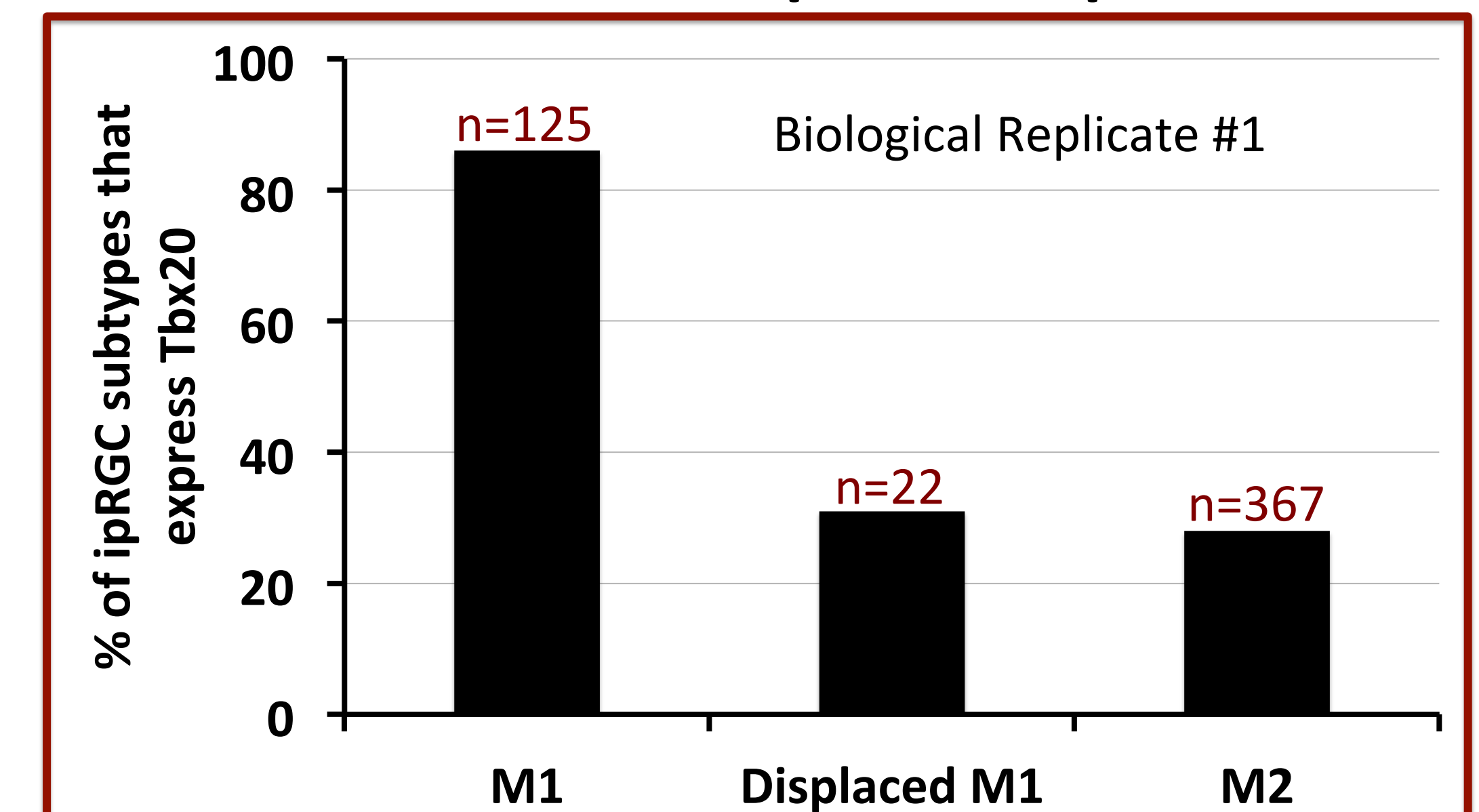
Immunofluorescence in mouse retinas
- Tbx20-specific antibody used to determine which cells in the retina express Tbx20
- In order to differentiate M1 versus M2 subtypes, we used a melanopsin-specific antibody, providing morphological characterization
- To prevent bias, expression of melanopsin and Tbx20 were independently determined before co-expression analysis

Melanopsin   Tbx20   Merged
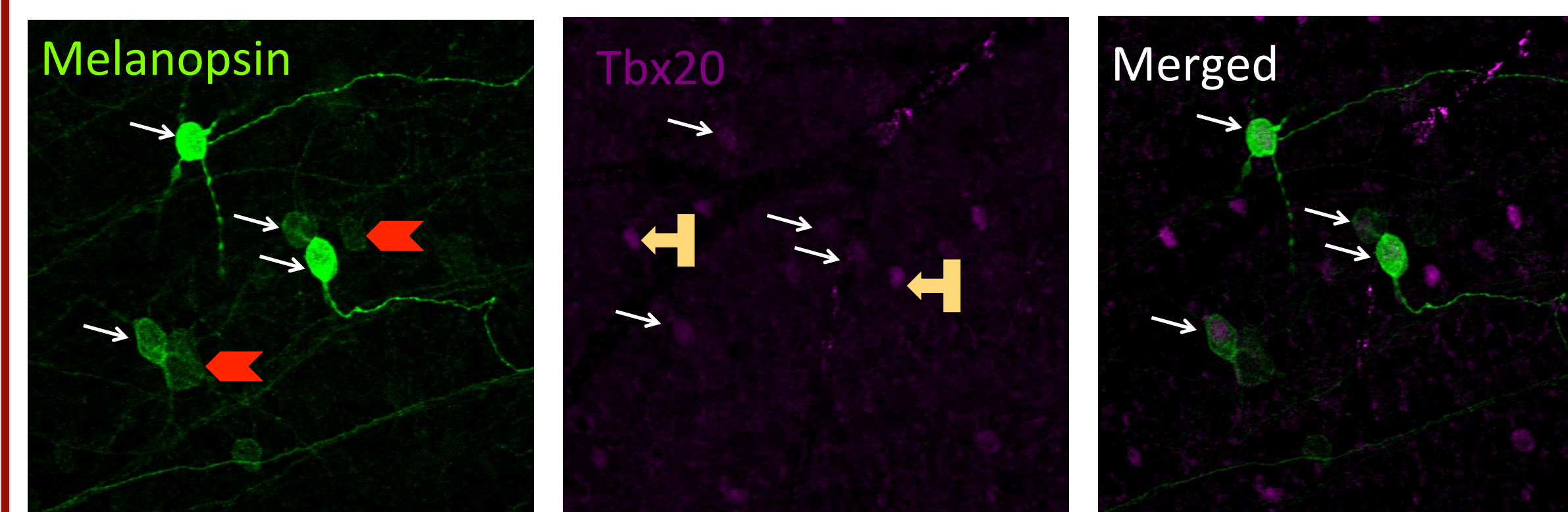
→ indicates ipRGCs expressing Tbx20
◄ indicates an ipRGC that does not express Tbx20
⊢ indicates Tbx20 expressed in a generic RGC

Conclusion: Tbx20 is expressed in a subset of ipRGCs

A Subset of M1 & M2 ipRGCs Express Tbx20

Biological Replicate #1

% of ipRGC subtypes that express Tbx20

n=125
n=22
n=367

M1   Displaced M1   M2

Biological Replicate #2

% of ipRGC subtypes that express Tbx20

n=59
n=25
n=103

M1   Displaced M1   M2

- **M1**: 79-86% of M1's express Tbx20
- **Displaced M1**: 32-53% of Displaced M1's express Tbx20
- **M2**: 28-48% of M2's express Tbx20
- Only ~55 % of RGCs expressing Tbx20 are melanopsin-immunopositive

Future Directions

- More biological replicates are needed to determine the variability of Tbx20 expression in ipRGC subtypes
- Explore Tbx20 expression in other ipRGC subtypes using known reporters
- Explore the other ganglion cell types Tbx20 is labeling
- Determine the spatial distribution of Tbx20-expressing ipRGCs

Acknowledgements