

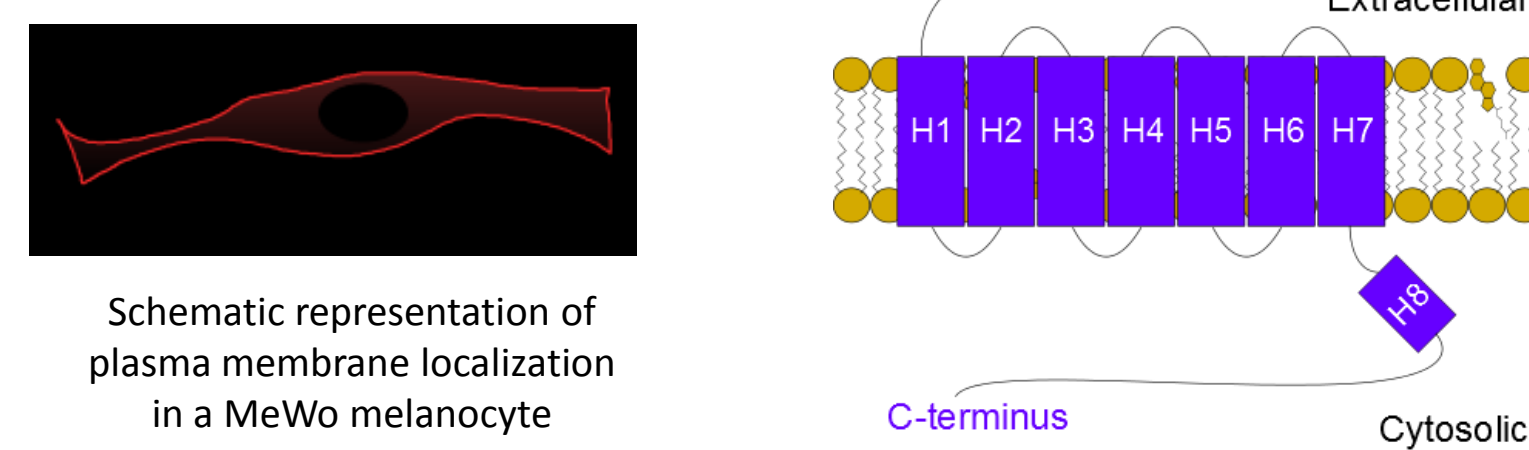
# Localization of human opsin 3 (OPN3) in human epidermal melanocytes

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## BACKGROUND

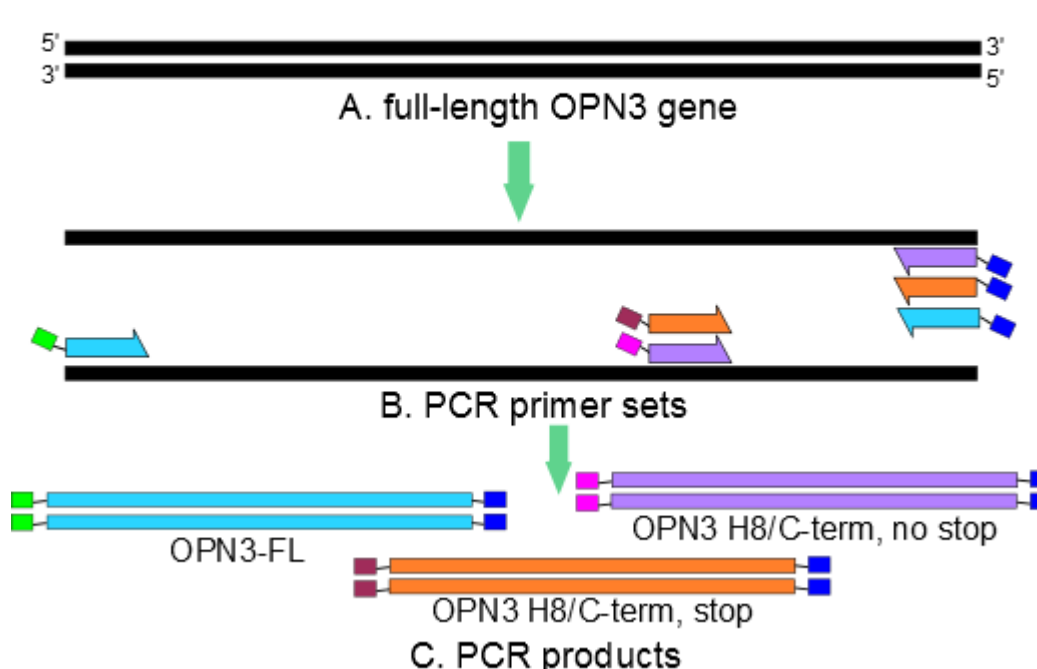
- Opsins are light-sensitive G-protein coupled receptors (GPCRs).
- OPN3 has elongated C-terminus relative to other opsins.
- The C-terminal region does not have homology to any other opsin or GPCR.
- Similar to other opsins, OPN3 has a C-terminal helix (H8).
- OPN3 is expressed in human epidermal melanocytes<sup>2</sup>
- GPCRs have seven transmembrane domains and most of them localize to plasma membrane.



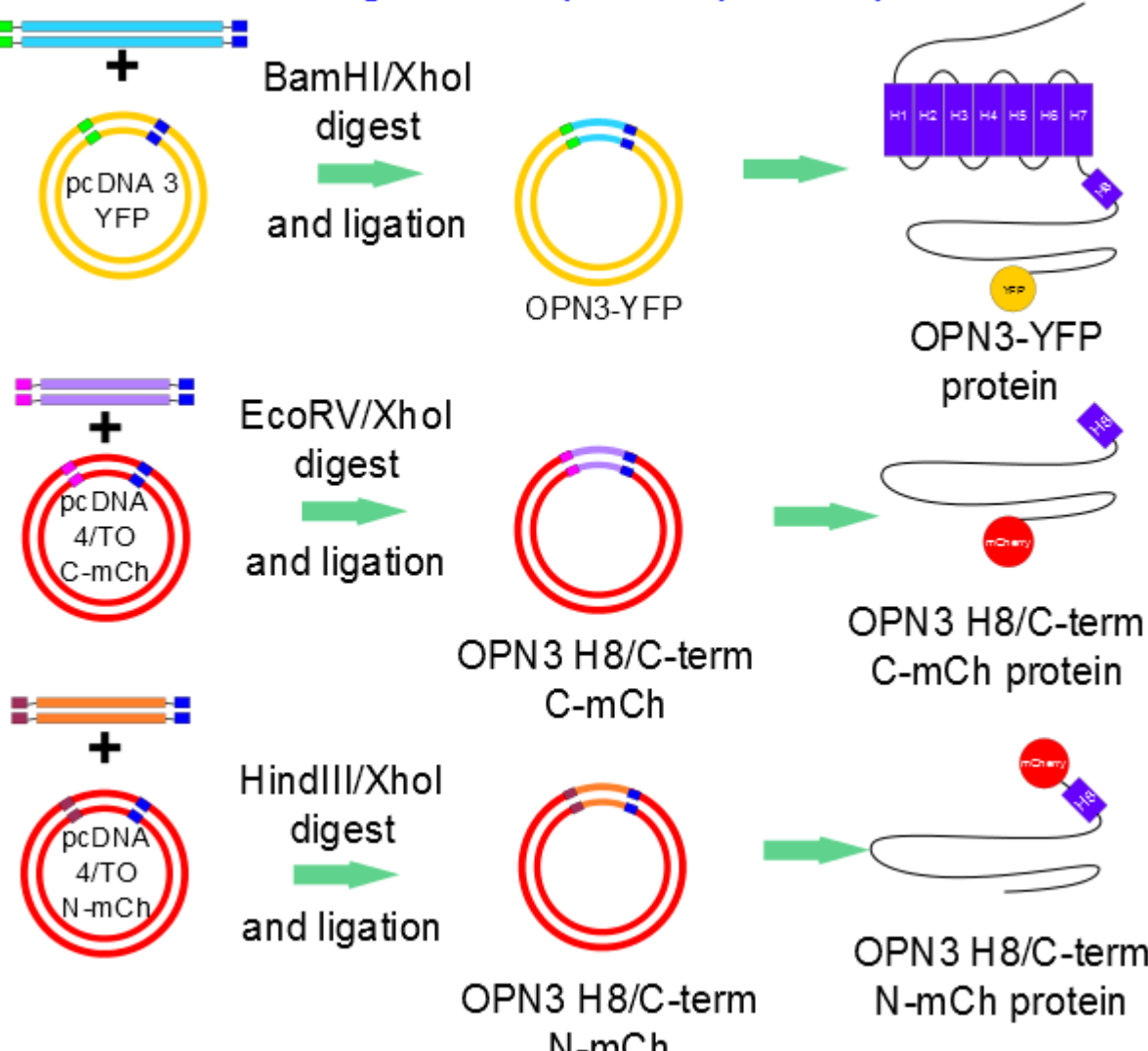
- OPN3 function and activation mechanism are not known.
- The cellular localization of OPN3, a determining factor in its function, is not known.

## METHODS

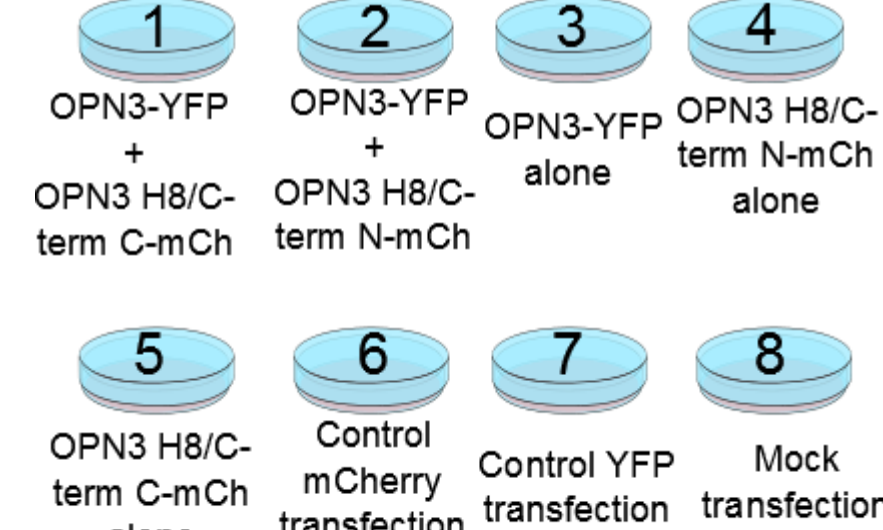
1. PCR with primers designed to select desired region and add restriction sites for cloning



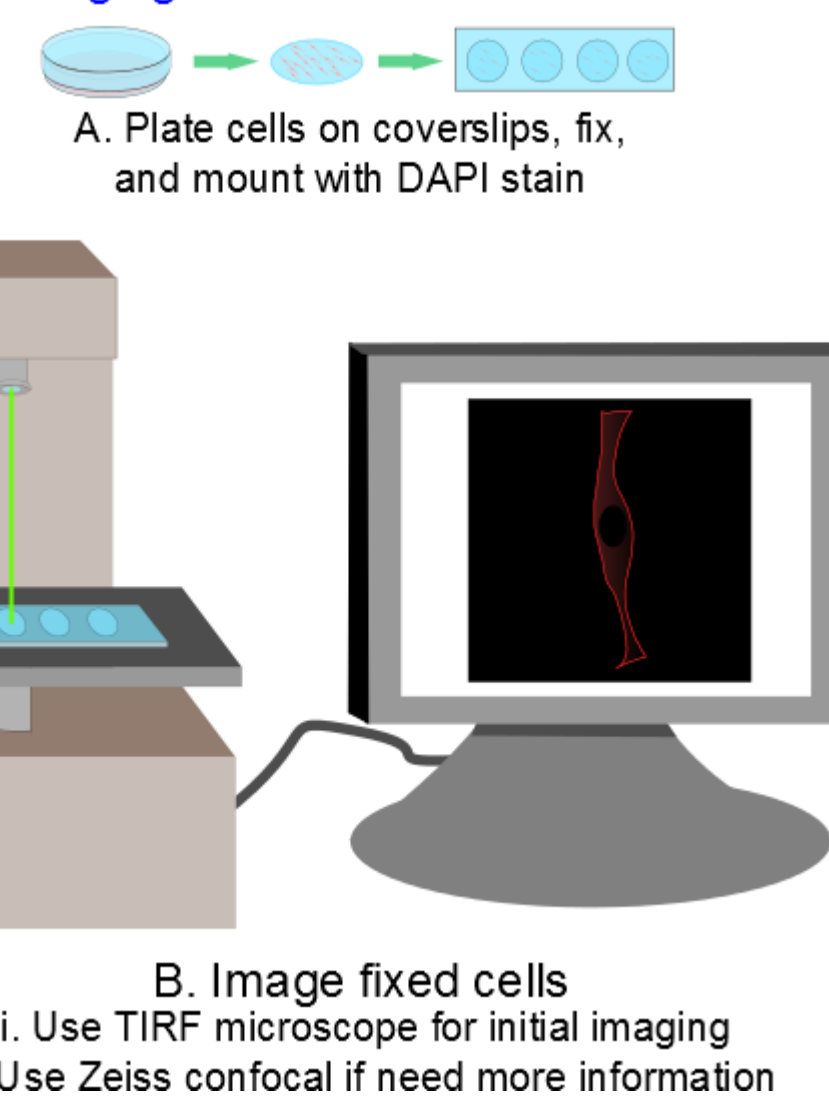
2. Cloning of inserts into vectors containing desired fluorescent tags and expected protein products



3. Transfection of MeWo cells with OPN3 constructs



4. Imaging of transfected MeWo cells



## RESULTS

Fig. 1. Localization of OPN3-YFP (A) and of OPN3 C-terminal constructs containing helix 8 and tagged with mCherry at the C-terminus (B) or N-terminus (C) in MeWo melanocyte cell line.

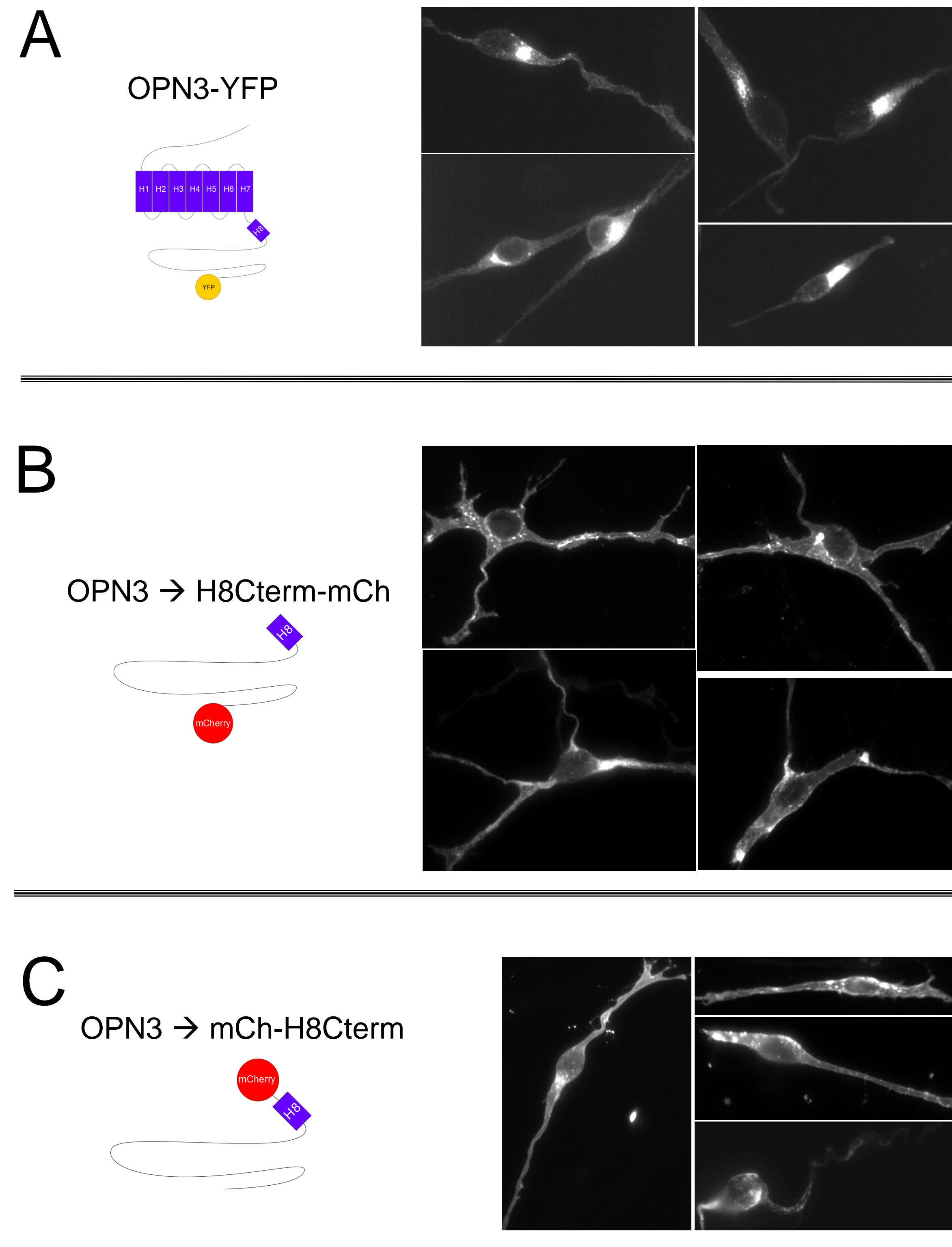
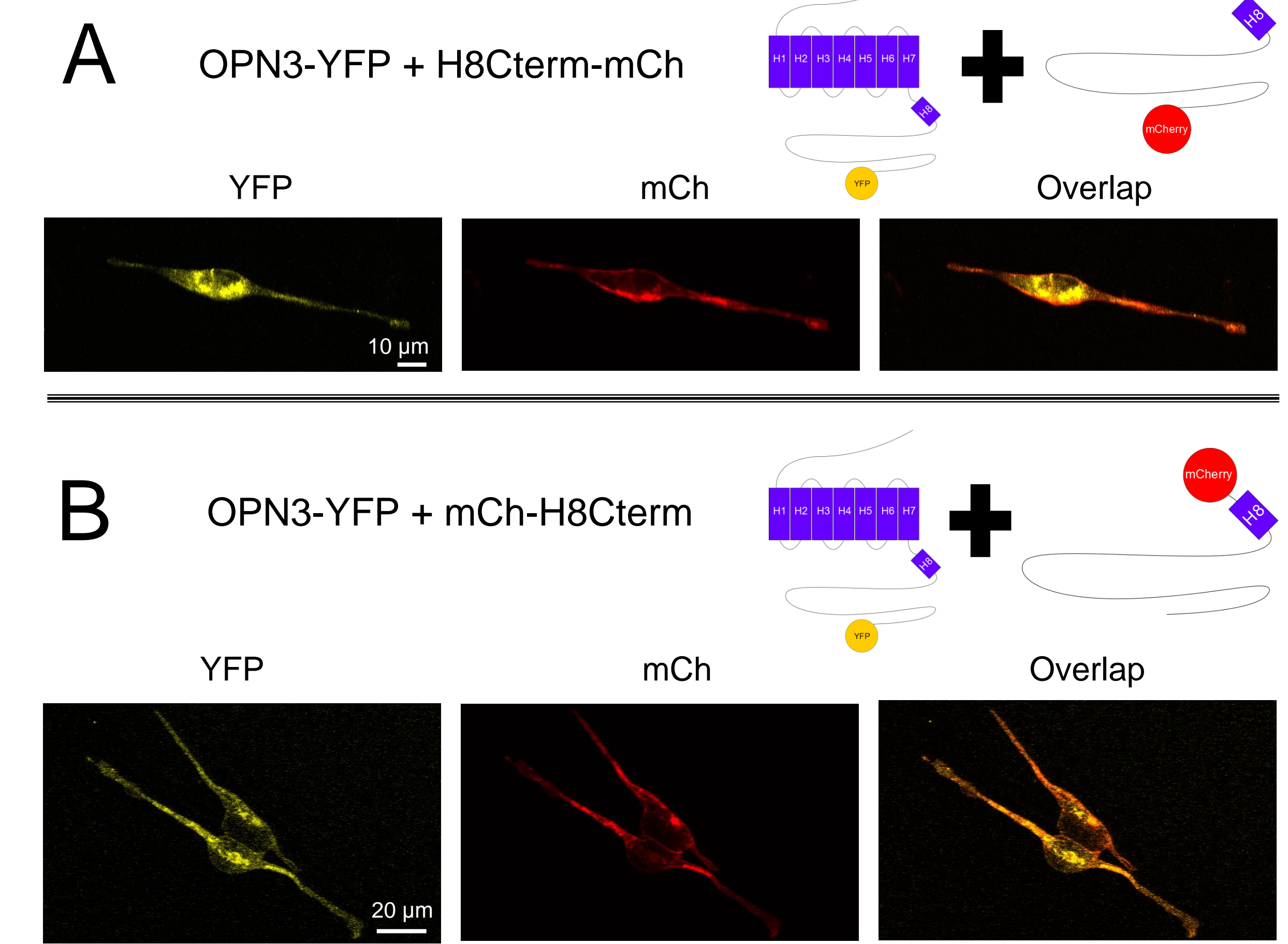


Fig. 2. Colocalization of OPN3-YFP with OPN3 C-terminal constructs containing helix 8 and tagged with mCherry at the C-terminus (A) or N-terminus (B) in MeWo melanocyte cell line.



## CONCLUSIONS

- Full-length OPN3 does not exhibit exclusive plasma membrane localization (Figs. 1A, 2A, 2B).
- OPN3-YFP is primarily intracellular and it is enriched in the perinuclear region resembling Golgi apparatus.
- Unlike full length OPN3, the helix 8 C-terminus tagged with mCherry at either end exhibit both plasma membrane and intracellular vesicular localization (Figs. 1B, 1C, 2A, 2B)
- There was minimal colocalization of OPN3 full-length and its helix8 C-terminus (Figs 2A, 2B), suggesting that the N-terminal of OPN3, containing the first seven putative transmembrane domains (H1 – H7) determines the intracellular localization of the protein.

## REFERENCES

1. Blackshaw S, and Snyder SH (1999). Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain. *J Neurosci.* 1999 May 15;19(10):3681-90.
2. Ozdeslik, R.N., Haltaufderhyde, K., Wicks, N.L., Najera, J.A. and Oancea, E. (2014) Opsin expression in human epidermal skin. *Photochemistry and Photobiology* (in review)

## ACKNOWLEDGEMENTS

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