Using single cell pseudotime lineage tracing to identify co-regulated genes in olfactory neuron development

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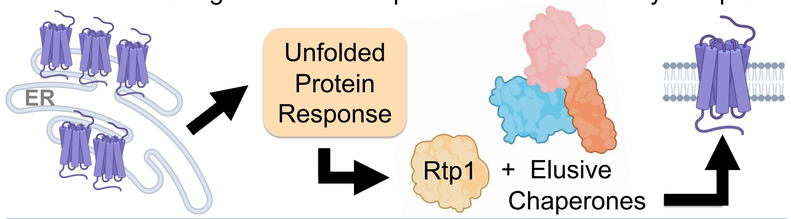
Introduction

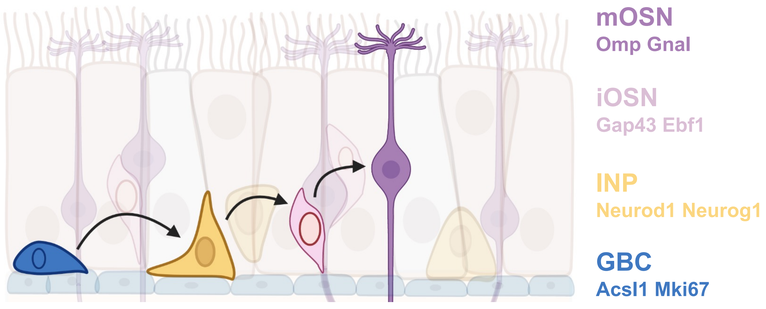
Odor detection in mammals is mediated by olfactory receptors the largest family of G protein-coupled receptors expressed in the olfactory sensory neurons deep in the nasal cavity[1]. However, our understanding in odor ligand and olfactory receptor (OR) interactions are poorly understood. This is due to the limitation that most olfactory receptor show little to no cell surface expression in non-olfactory cell types[2]. For ORs to express on the cell surface, the OR first needs to be translated and expressed in the endoplasmic reticulum (ER) which then induces the unfolded protein response (UPR) pathway that triggers a negative feedback loop necessary for ORs to be trafficked to the surface[3]. Our lab previously identified Receptor transporting protein 1 (Rtp1) as a chaperone, which enhances the cell surface expression of many but not all ORs in heterologous cells[2]. We hypothesize that there are additional elusive chaperones that are co-expressed with RTP1 that's important for relieving the UPR in ER in order to increase cell surface expression of all ORs.

Here we reanalyzed whole olfactory epithelium single cell sequencing data[3] in order to identify the elusive chaperones co-expressed with RTP1 in increasing cell surface expression of the olfactory receptors.



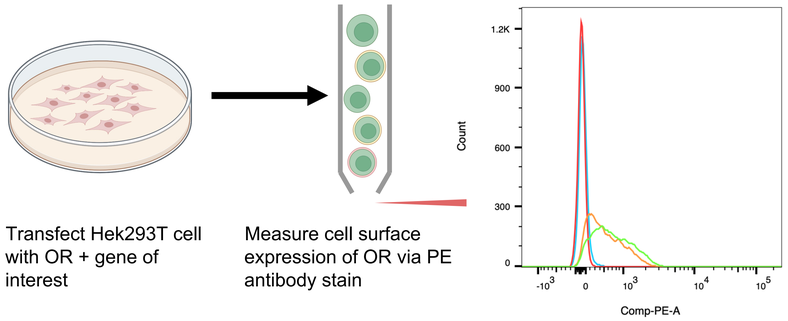
Objective

- Identify elusive genes co-expressed with Rtp1
- Show increase in cell surface expression of ORs when transfected with target gene



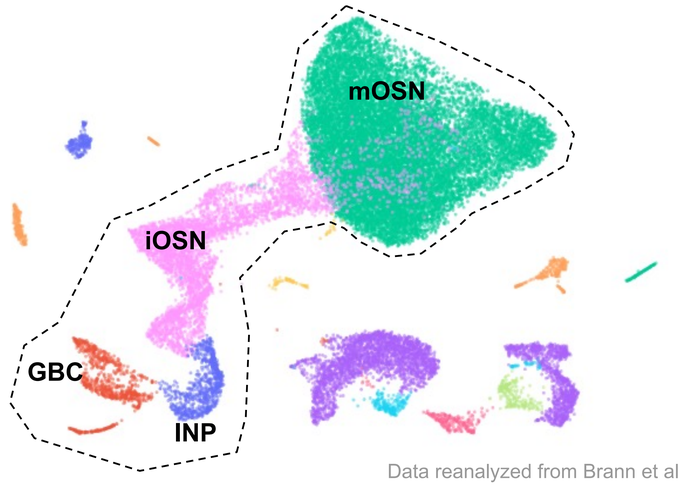
Method

Evaluate Cell Surface Expression via Flow Cytometry

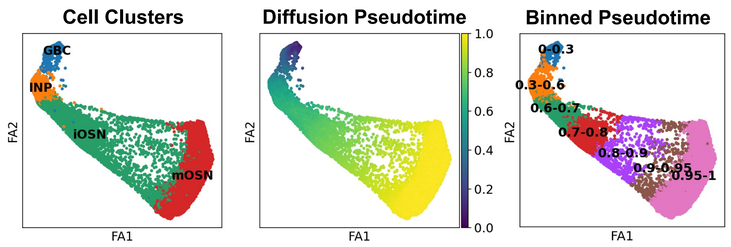


Results

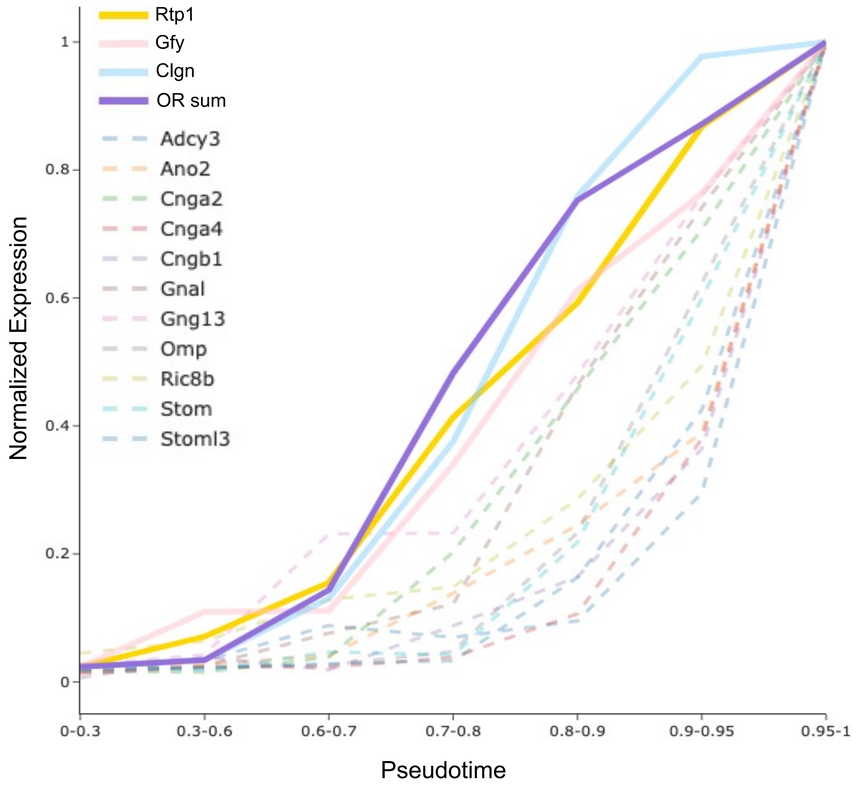
Isolation of Olfactory Sensory Neuron cell lineage from whole olfactory epithelium single cell sequencing data



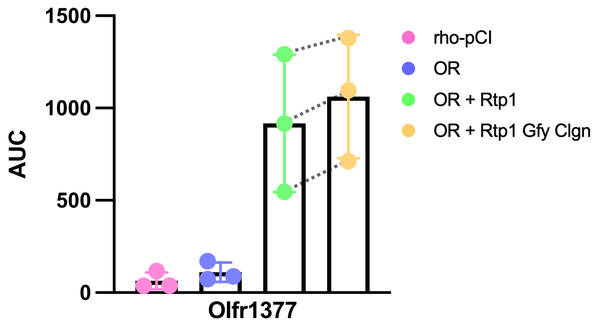
Data reanalyzed from Brann et al.



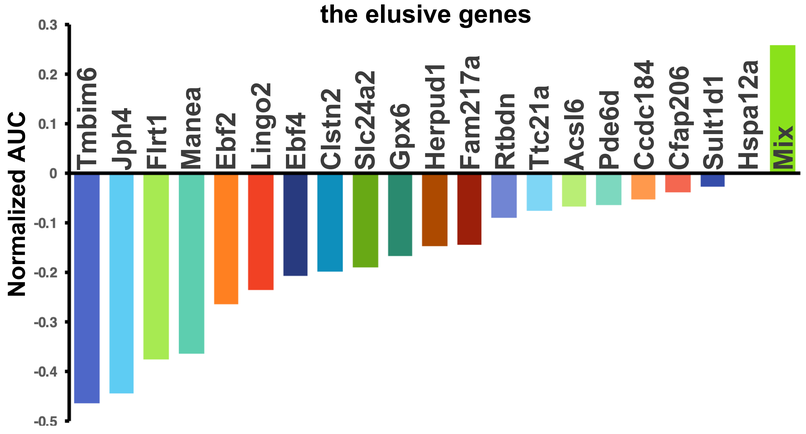
Canonical mOSN markers does not follow Rtp1 expression pattern



Combination of Rtp1, Gfy and Clgn further enhances cell surface expression of Olfr1377

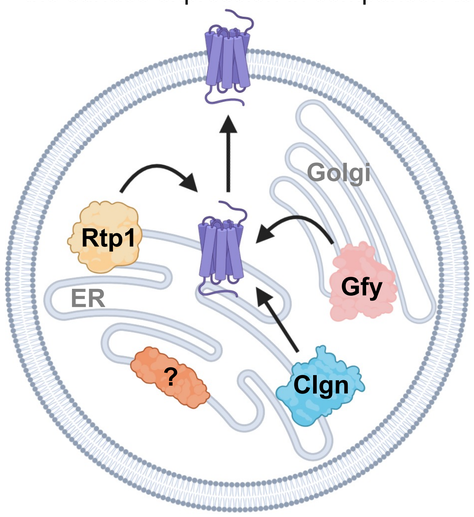


Cell surface expression of OR requires specific combination of the elusive genes



Conclusion

From the single cell sequencing data, we have obtained a shortlist of genes to be co-expressed with Rtp1. So far, we have successfully cloned and screened 21 of the candidates in flow cytometry for enhancing cell surface expression. From the preliminary data collected, we've identified Gfy and Clgn to be potential proteins facilitating cell surface expression of ORs when co-expressed with RTP1. The master mixture of all the 21 genes even further increased the surface expression in comparison to Rtp1, Gfy and Clgn.



Future directions include continuing effort in identifying specific proteins in enhancing OR cell surface expression. Success in the study may directly contribute to OR structure elucidation and open the possibilities for large-scale screening on OR ligand pairs.