Investigating the role of *Vsx1* in bipolar cell differentiation of the mouse retina

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BACKGROUND

- The retina contains multiple **bipolar cell (BPC)** subtypes that relay signals from photoreceptors to ganglion cells and contribute to parallel visual processing pathways.

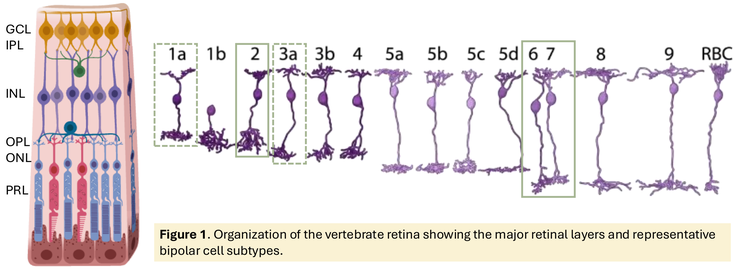

Figure 1. Organization of the vertebrate retina showing the major retinal layers and representative bipolar cell subtypes.

- Homeobox transcription factor ***Vsx1*** is expressed in specific BPC subtypes.
- Genetic loss of *Vsx1* does not change the total number of bipolar cells in the retina (Chow *et al.*, 2004).
- Previous studies have examined subtype-specific molecular markers that correspond to distinct bipolar cell populations.
- By comparing the expression of these markers in wild type and *Vsx1* knockout mice, researchers were able to identify which BPC subtypes show disrupted gene expression when *Vsx1* is absent (Chow *et al.*, 2004; Shi *et al.*, 2011; Star *et al.*, 2012).
- Sox6 has been identified as a BPC type 5a marker (Shekar *et al.*, 2016)

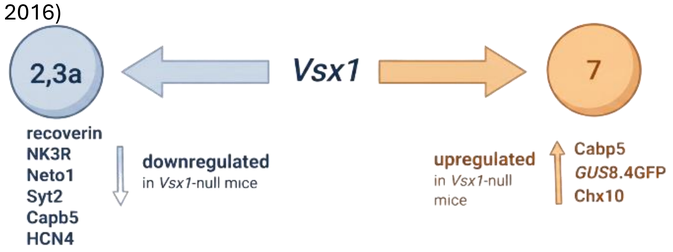

Figure 2. Summary of transcriptional changes observed in bipolar cell subtypes following loss of *Vsx1*. Previous studies show that markers associated with BC2 and BC3a are downregulated in Vsx1-null mice, while markers associated with BC7 cells are upregulated.

HYPOTHESES & OBJECTIVES

Hypothesis
Understanding how *Vsx1* regulates bipolar cell subtype specification will provide insight into the molecular mechanisms controlling retinal circuit development.

Research Question
If the total number of bipolar cells remains unchanged following Vsx1 loss, do these cells undergo transcriptional respecification to adopt different bipolar subtype identities?

Objective
To identify subtype-specific transcriptional markers associated with *Vsx1*-dependent bipolar cells and determine whether their expression is altered in *Vsx1* knockout retinas.

METHODS

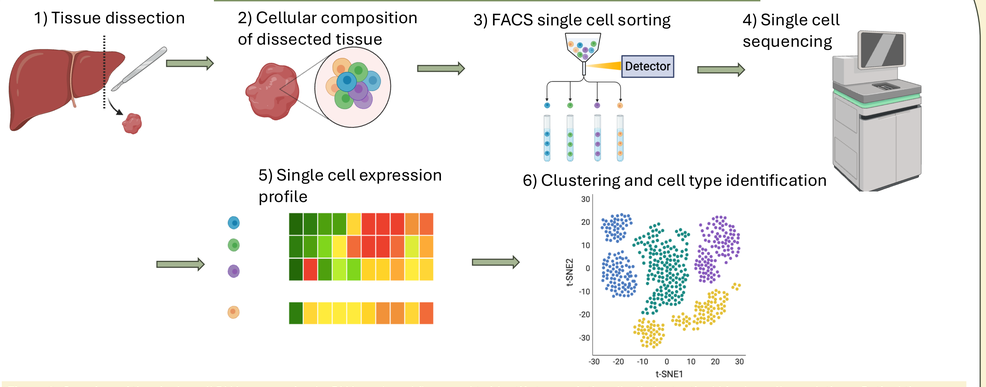

Figure 3. Overview of the single-cell RNA sequencing (scRNA-seq) workflow used to identify transcriptionally distinct retinal bipolar cell populations. Retinal tissue was dissociated into single cells, sorted using fluorescence-activated cell sorting (FACS), and processed for single-cell RNA sequencing. Gene expression profiles were analyzed to generate heat maps and tSNE clustering plots for identification of bipolar cell subtypes.

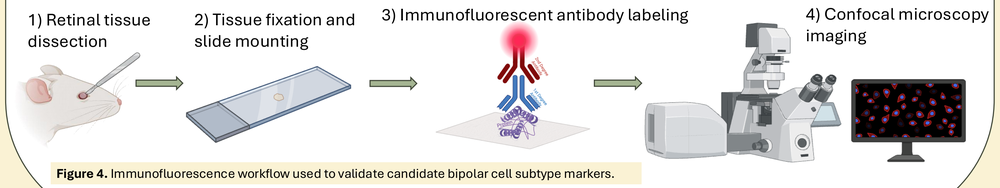

Figure 4. Immunofluorescence workflow used to validate candidate bipolar cell subtype markers.

RESULTS

Analysis of single cell RNA sequencing (scRNAseq) datasets using tSNE clustering and heat map visualization indicates that BPC subtypes 2, 6, and 7 are significantly affected by loss of *Vsx1*, while subtypes 1a and 3a are weakly affected.

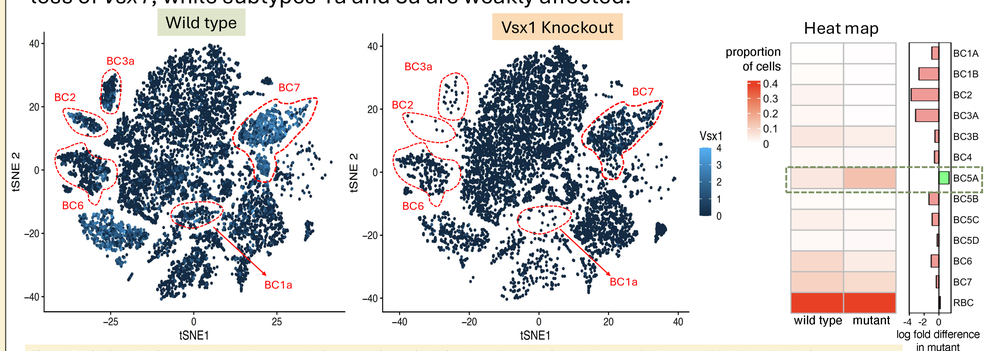

Figure 5. tSNE clustering plot and heat map analysis comparing retinal bipolar cell populations between wild-type and Vsx1 knockout mice

Immunofluorescent labeling of *Sox6* in prenatal mouse brain tissue used as a positive control to validate antibody specificity. As expected, *Sox6*-positive neurons were detected in the medial prefrontal cortex (mPFC) (Christodoulou *et al.*, 2022), while minimal signal was observed in the negative control.

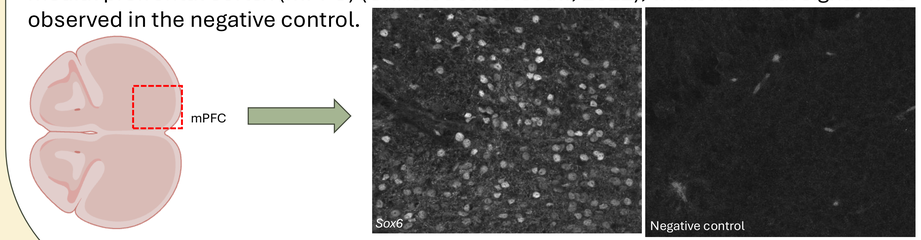

Figure 6. Immunofluorescent labeling of Sox6 in prenatal mouse brain tissue imaged by confocal microscopy on the mPFC region

CONCLUSIONS

- *Vsx1* plays an important role in regulating transcriptional identity in specific retinal bipolar cell subtypes.
- Loss of *Vsx1* alters gene expression patterns in multiple bipolar cell populations, including BC1a, BC2, BC3a, BC6, and BC7.
- Increased expression of markers associated with Type 5a bipolar cells suggests potential transcriptional respecification following *Vsx1* loss.

NEXT STEPS

Working model:

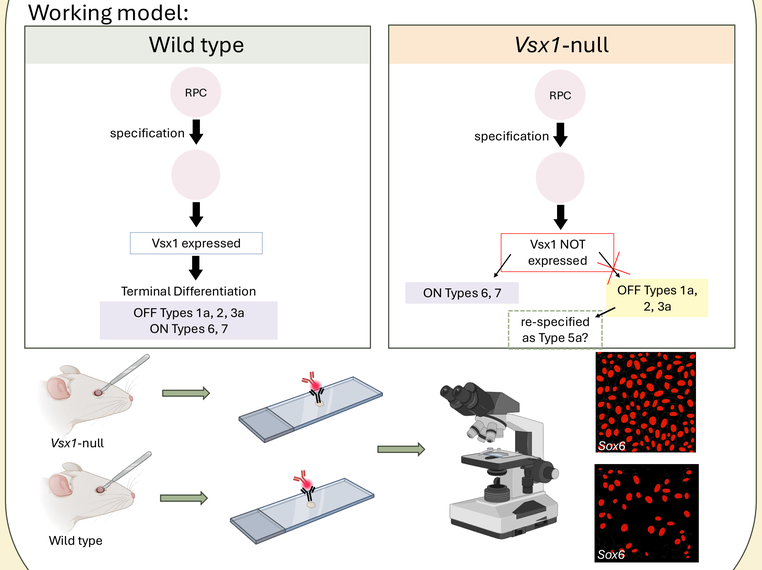

Figure 7. Proposed experimental approach to test the working model of bipolar cell subtype respecification.

REFERENCES

Chow, R. L., B. Volgyi, R. K. Szilard, D. Ng, C. McKerlie, S. A. Bloomfield, D. G. Birch, and R. R. McInnes. 2004. Control of late off-center cone bipolar cell differentiation and visual signaling by the homeobox gene Vsx1. *Proceedings of the National Academy of Sciences - PNAS* **101**:1754-1759.
Christodoulou O., Maragkos I., Antonakou V., Denaxa M. 2022. The development of MGE-derived cortical interneurons: An Lhx6 tale. *Int. J. Dev. Biol.* **66**:43-49.
Shekhar, K., S. W. Lapan, I. E. Whitney, N. M. Tran, E. Z. Macosko, M. Kowalczyk, X. Adiconis, J. Z. Levin, J. Nemesh, M. Goldman, S. A. McCarroll, C. L. Cepko, A. Regev, and J. R. Sanes. 2016. Comprehensive Classification of Retinal Bipolar Neurons by Single-Cell Transcriptomics. *Cell* **166**:1308-1323.e1330.
Shi, Z., S. Trenholm, M. Zhu, S. Buddingh, E. N. Star, G. B. Awatramani, and R. L. Chow. 2011. Vsx1 Regulates Terminal Differentiation of Type 7 ON Bipolar Cells. *The Journal of neuroscience* **31**:13118-13127.
Star, E. N., M. Zhu, Z. Shi, H. Liu, M. Pashmforoush, Y. Sauve, B. G. Bruneau, and R. L. Chow. 2012. Regulation of retinal interneuron subtype identity by the Iroquois homeobox gene Irx6. *Development (Cambridge)* **139**:4644-4655.

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