Pten regulates the timing of photoreceptor differentiation by altering glycolysis and pHi

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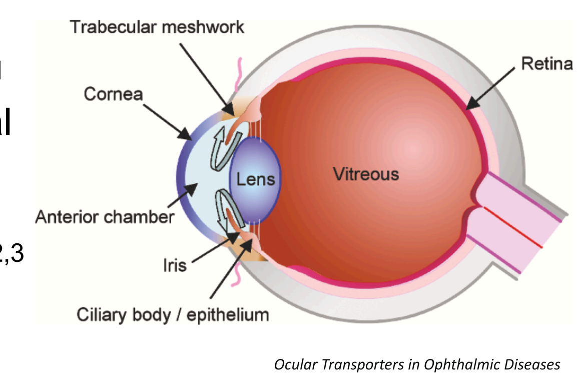
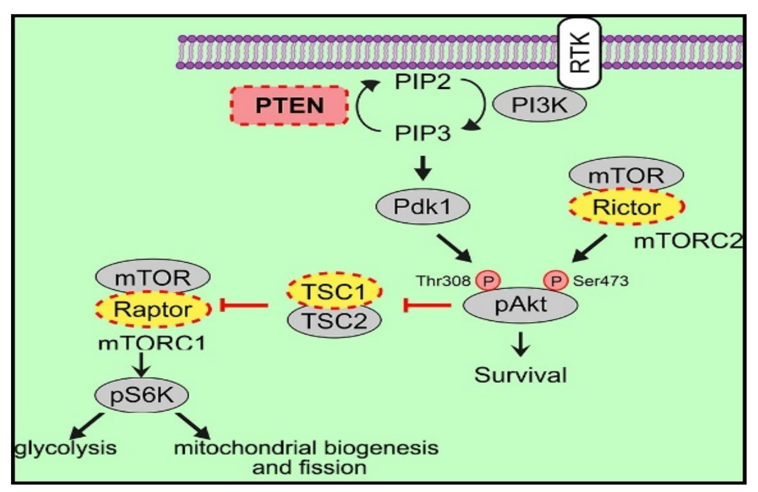
Introduction

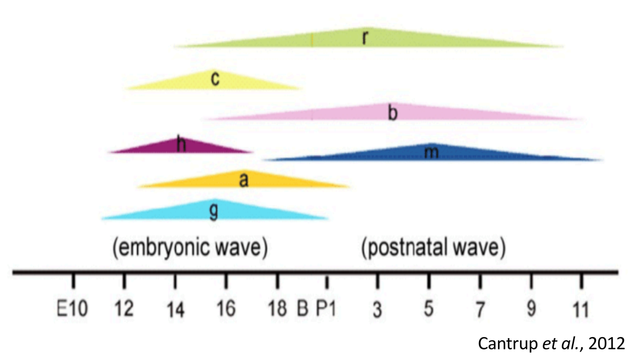
- Visual impairment is devastating, affecting 2 billion individuals worldwide[1]
- A notable cause of vision loss in several blinding eye diseases, such as age-related macular degeneration, is the death or dysfunction of photoreceptors[2,3]
- Designing novel therapies requires a deep understanding of the factors affecting photoreceptor differentiation





- *Phosphatase and tensin homolog* (*Pten*) encodes a lipid and protein phosphatase that plays a critical role in nervous system development[4]
- PTEN negatively regulates PI3k/Akt/mTor signaling
- PTEN is expressed in retinal progenitor cells (RPCs) throughout retinal development

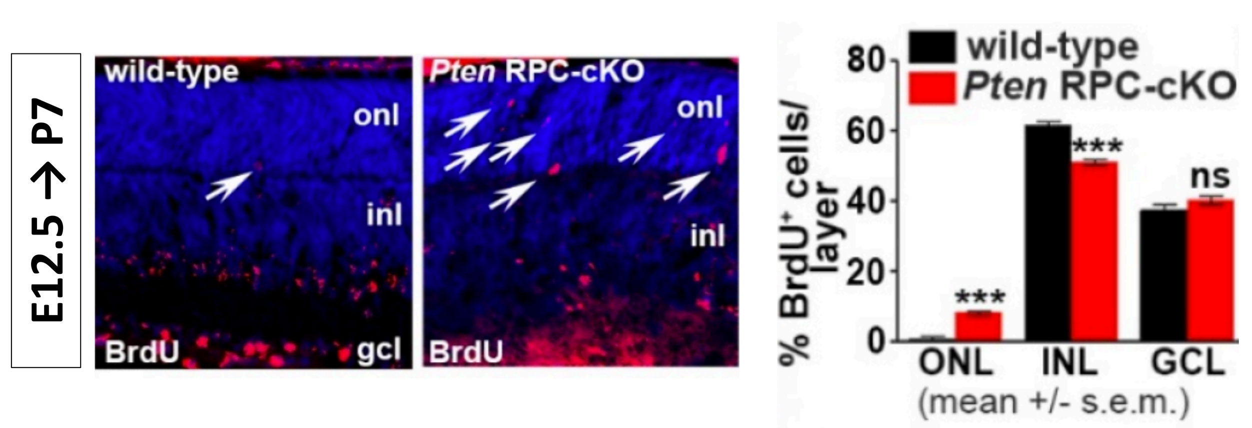
Seven retinal cell types are generated from a multipotent pool of retinal progenitor cells (RPCs) in a defined order during development.



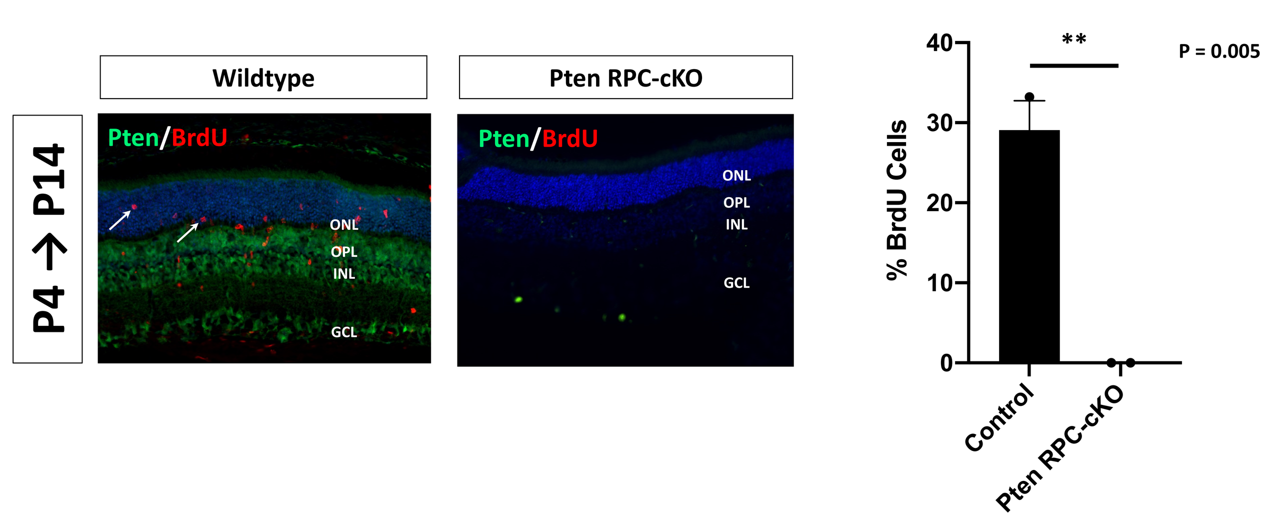
G, ganglion cell; a, amacrine cell; b, bipolar cell; c, cone photoreceptor; h, horizontal cell; r, rod photoreceptor; m, Muller glia

- Herein, we investigated the role of *Pten* in photoreceptor development by conditional deletion in retinal progenitor cells (RPCs), using a *Pax6::Cre* driver and *Pten*^{fl} allele to generate *Pten* cKO mice

Timing of rod differentiation is disrupted in *Pten* RPC-cKOs

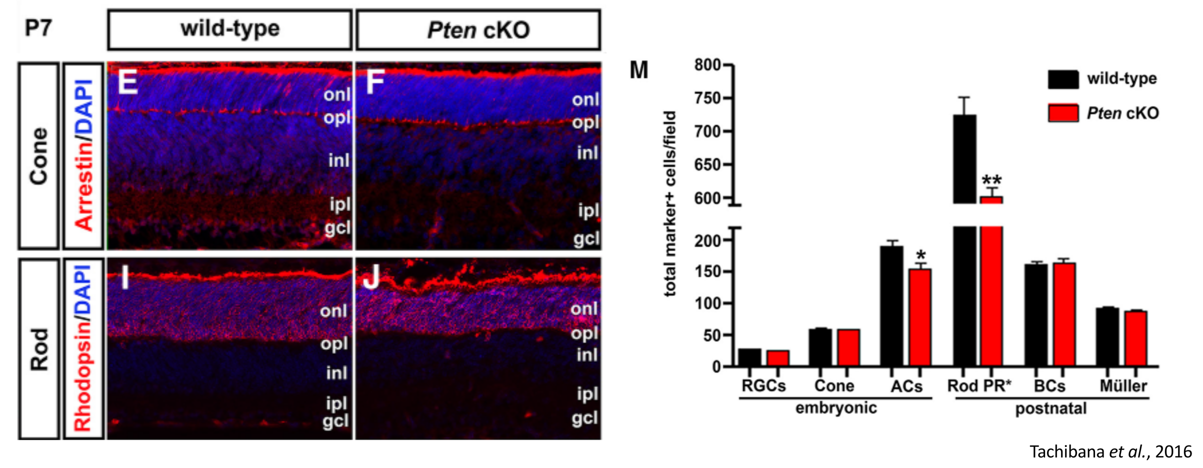


- At E12.5, more RPCs give rise to rod photoreceptors in Pten RPC-cKOs



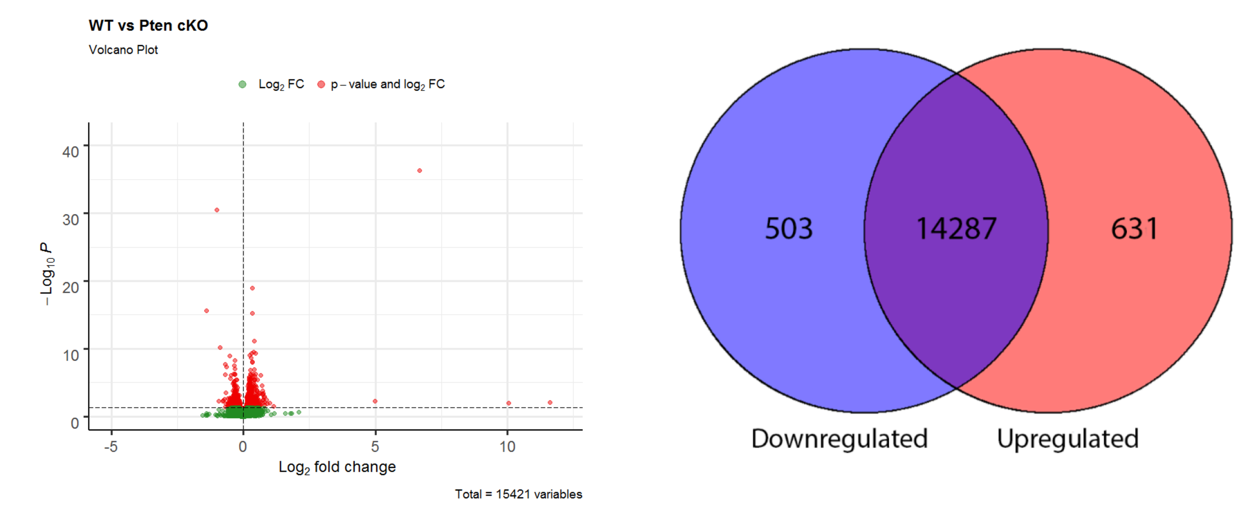
- At P4, fewer RPCs give rise to rod photoreceptors in Pten RPC-cKOs

Fewer rods are present in *Pten* RPC-cKOs from P7

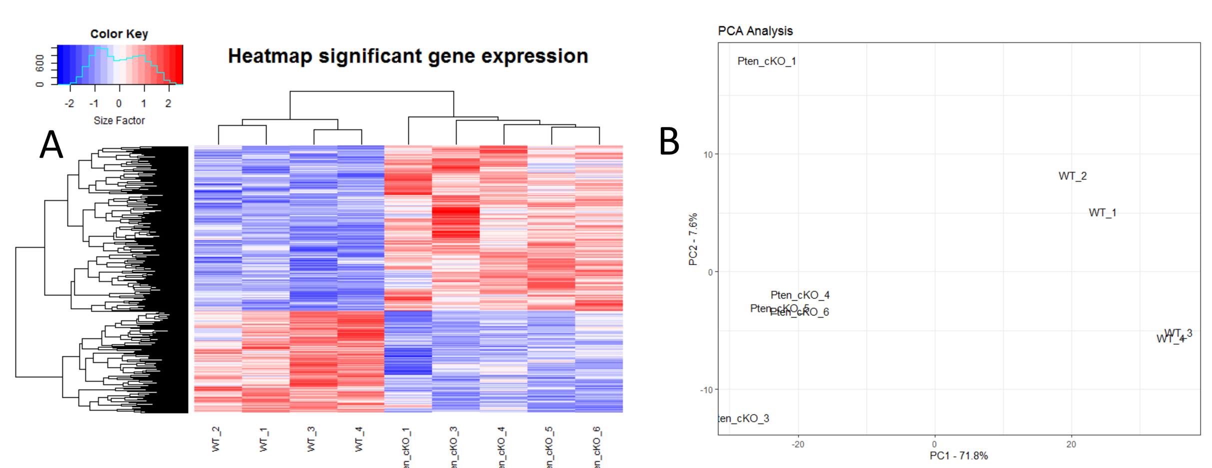


- Altered timing of rod differentiation culminates in Pten RPC-cKOs having significantly less rod photoreceptors by P7

Transcriptomic differences in P0 *Pten* RPC-cKOs

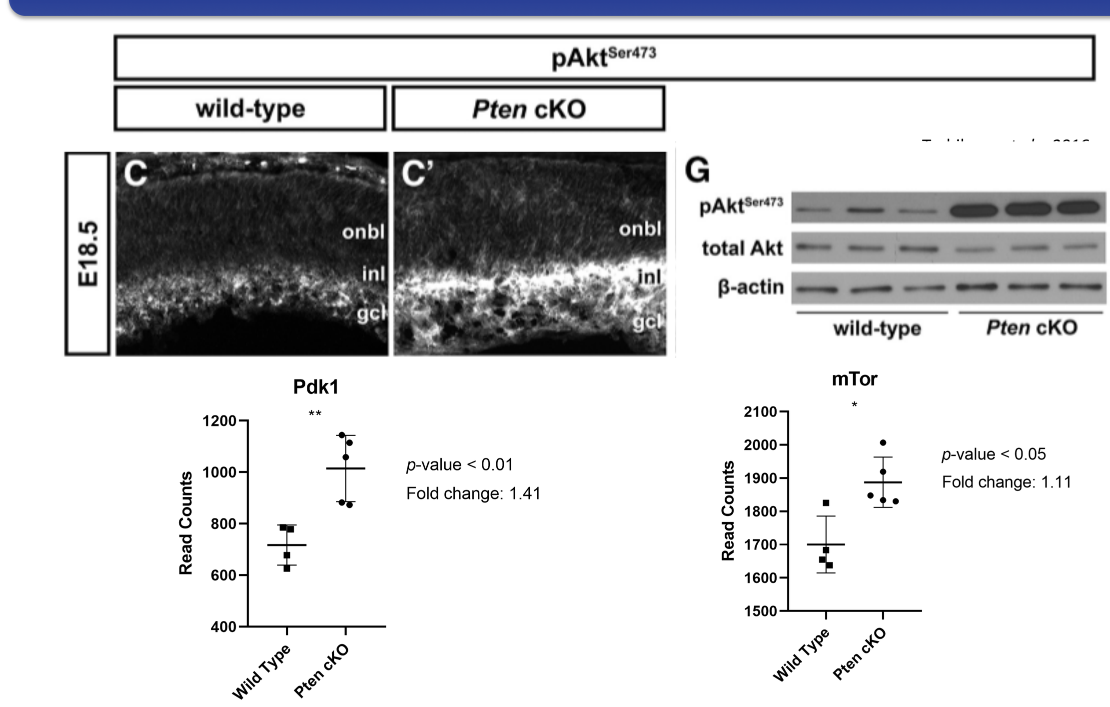


- Volcano plot (A) with plotted log2 fold change in the x-axis, and the –log10 p value in the y-axis. Venn diagram (B) showing a total of 1075 dysregulated genes in *Pten* cKO retinas. Blue circle = downregulated genes, Red circle = upregulated genes, Purple = non-significant genes.



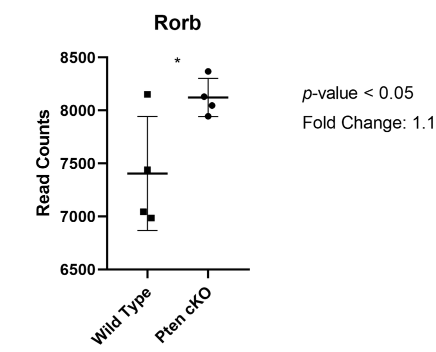
- Heatmap (A) showing expression of individual genes in both groups
- Principal component analysis (PCA) showing separation between Pten RPC-cKO and controls RNA seq data analysis

Increased PI3K signaling in *Pten* RPC-cKOs



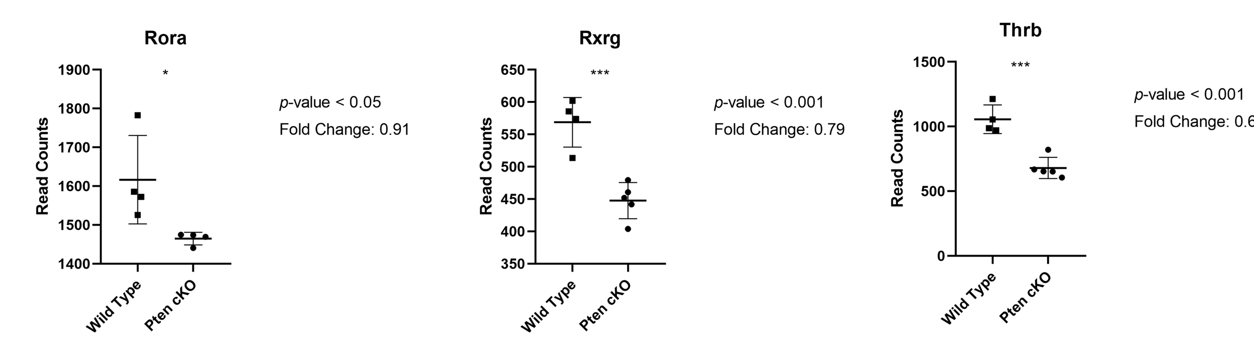
- Pdk1 and mTor downstream of Pten were significantly upregulated in Pten RPC-cKO

Rod photoreceptor gene expression higher in P0 *Pten* RPC-cKOs



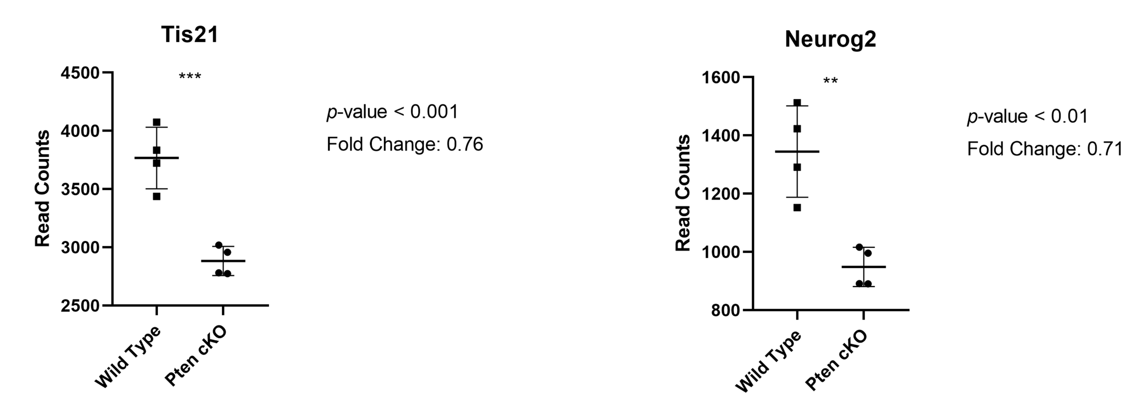
- Rod photoreceptors are still differentiating in excess at this stage
- Rorb (rod differentiation) transcripts were significantly increased in P0 Pten RPC-cKOs

Cone photoreceptor gene expression lower in P0 *Pten* RPC-cKOs



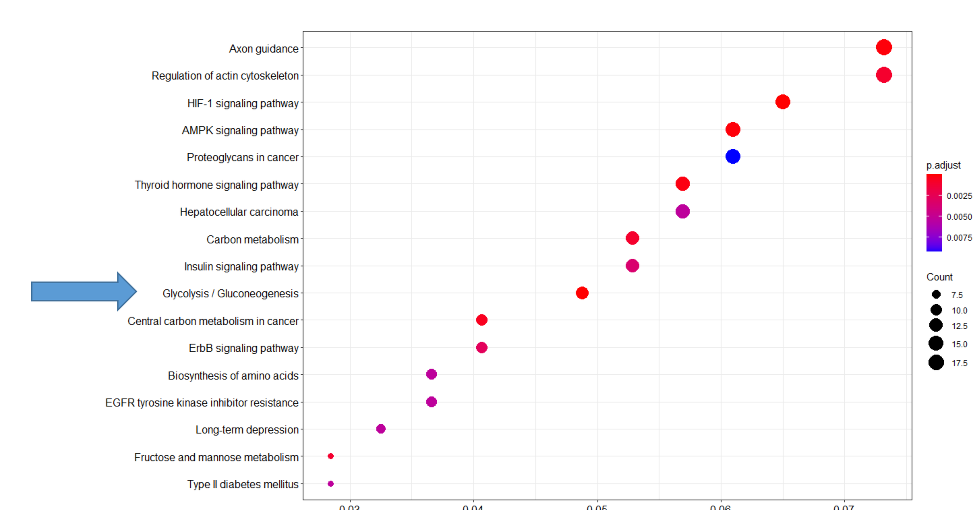
- Cone photoreceptor precursor transcripts Rora, Rxrg and Thrb were significantly reduced in Pten RPC-cKO mice compared to controls

Genes that commit RPCs to differentiation expressed at lower levels in P0 *Pten* RPC-cKOs



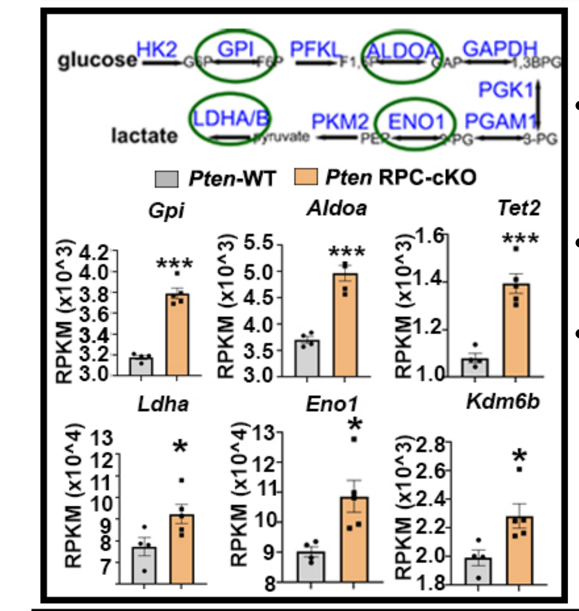
- Expression of *Tis21*, a marker of commitment to differentiate, and *Neurog2*, a proneural gene, were significantly reduced in *Pten RPC-cKO*

KEGG pathway enrichment in P0 *Pten* RPC-cKOs

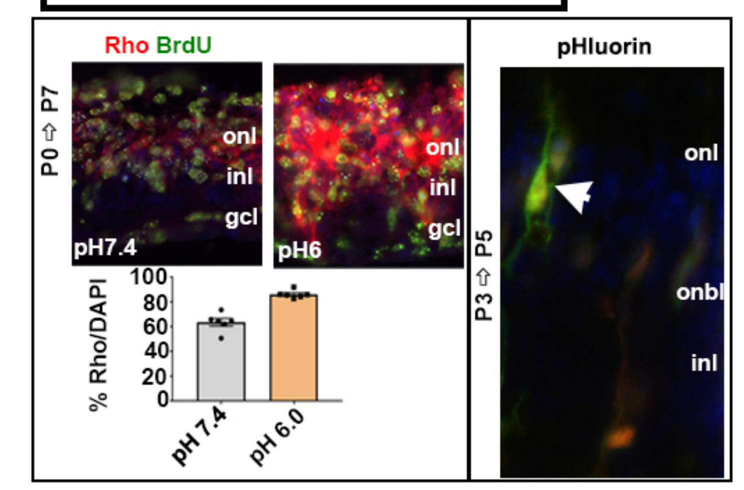


- Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showing upregulation of glycolysis genes in P0 Pten RPC-cKOs. Glycolysis genes were up, prompting additional studies

Upregulation of glycolysis genes



- Glycolytic pathway showing key enzymes involved in converting glucose to lactate
- Glycolytic genes are upregulated in P0 *Pten* RPC-cKOs
- Tet2 is a DNA demethylase and Kdm6b is a H3K27me3 demethylase, both upregulated in Pten RPC-cKOs, which could correlate with chromatin opening



Increased lactate should decrease intracellular pH (pHi)

- Increased Rho⁺BrdU⁺ rods in P0 explants cultured at low pH with BrdU in media.
- Electroporation of pHluorin ratiometric sensor in P3 retinas cultured 2 days in vitro. 488 nm excitation gave highest emission in rod-shaped cells in the onl vs RPCs in the outer neuroblast layer (onbl).
- higher 488 nm excitation means lower pHi

Conclusions

- *Pten RPC-cKO* mice showed early rod photoreceptor differentiation followed by a decline at postnatal stages
- Overall Rod photoreceptor numbers were significantly reduced at P7
- Rod and cone differentiation genes and generic neural differentiation genes are down in P0 *Pten* RPC-cKOs
- Glycolytic pathway genes are up in P0 *Pten* RPC-cKOs, which should increase lactate production and reduce pHi
- Reducing pHi elevates rod differentiation in retinal explants

References

1. Ding X, Patel M, Chan C-C. Molecular pathology of age-related macular degeneration. *Prog Retin Eye Res*. 2009;28(1):1-18. doi:10.1016/j.preteyeres.2008.10.001.

2. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol*. 2004;122(4):564-572. doi:10.1001/archopht.122.4.564.

3. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2(2):e106-e116. doi:10.1016/S2214-109X(13)70145-1.

4. Stambolic V, Suzuki A, la Pompa de JL, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*. 1998;95(1):29-39. doi:10.1016/s0092-8674(00)81780-8

Cantrup, R. et al. Cell-type specific roles for PTEN in establishing a functional retinal architecture. PLoSOne 7, e32795, doi:10.1371/journal.pone.0032795 (2012).

Tachibana, N. et al. Pten Regulates Retinal Amacrine Cell Number by Modulating Akt, Tgfbeta, and Erk Signaling. J Neurosci 36, 9454-9471, doi:10.1523/JNEUROSCI.0936-16.2016 (2016).

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