Pten Regulates the Stereotyped Morphology of Starburst Amacrine Cells

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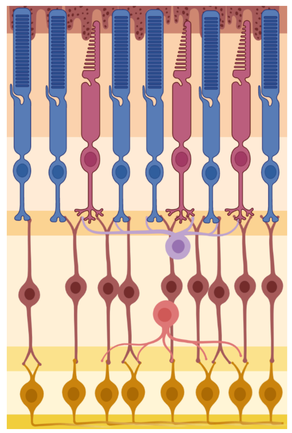
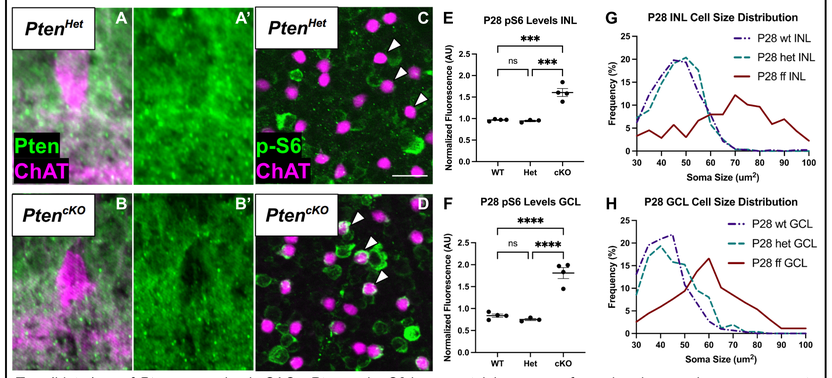
Abstract

Starburst amacrine cells (SACs) have a highly stereotyped dendritic architecture which is critical for their computation in the direction-selective circuit. SACs laminate in the S2 and S4 sublamina of the IPL by extending a dendritic process which expands to form their planar, radially symmetric arbor. To achieve their characteristic morphology across all members of their subtypes, SACs must carefully regulate the growth and refinement of their dendritic arbors during development. Phosphatase and tensin homolog (Pten) serves as the primary negative regulator in the PI3K-Akt pathway, which is critical for the growth and survival of developing cells. In neurons, Pten is particularly important for the regulated growth of dendrites and axons to form proper synaptic connections during development. Previous work has shown that pan-retinal deletion of *Pten* via targeting of retinal progenitor cells leads to widespread disruptions in the cell density, migration, and lamination of multiple neuronal subtypes[1,2]. In order to examine the role of Pten specifically in regulating dendritic development, we use a *ChAT*^{Cre} driver line to delete *Pten* selectively in SACs, beginning at postnatal day 1 (P1). Preliminary results indicate that SACs lacking *Pten* exhibit normal migration and mosaic spacing, but their dendrites show hypertrophic branching and disrupted lamination in the IPL. Ultimately, this work will elucidate how Pten functions in the development of neurons with highly stereotyped dendritic arbors.

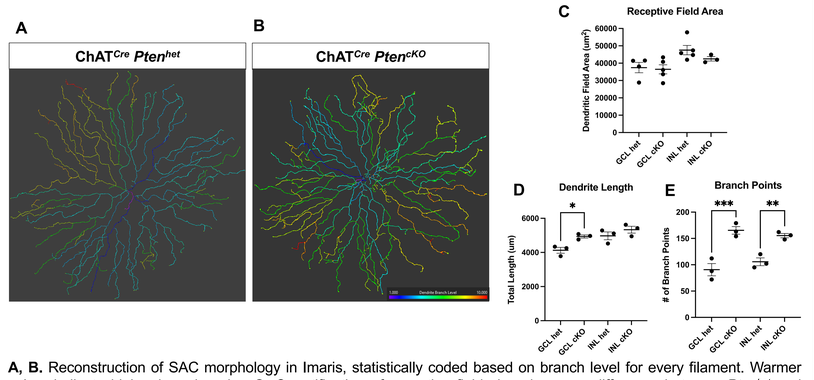


Validation of *Pten* Deletion



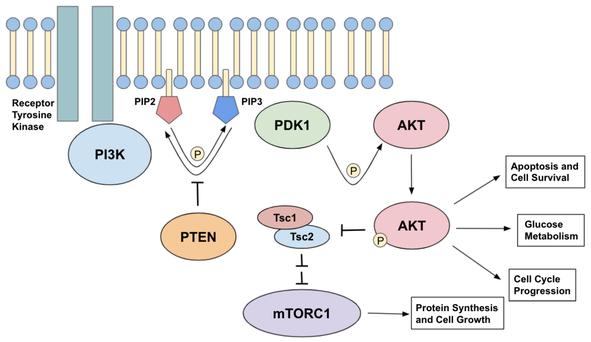
To validate loss of *Pten* expression in SACs, Pten and p-S6 immunostaining was performed and soma size measurements were taken. **A, B.** Pten immunostaining reveals gaps in *Pten*^{cKO} SACs at P21. **C, D.** p-S6 immunostaining reveals increased levels in *Pten*^{cKO} SACs at P28. Scalebar = 25 um. **E, F.** Quantification via ANOVA of p-S6 staining shows an increase in p-S6 signal in *Pten*^{cKO} SACs. **G, H.** Quantification of soma sizes reveals somal hypertrophy in *Pten*^{cKO} SACs at P28.

Analysis in Imaris reveals differences in dendritic branching



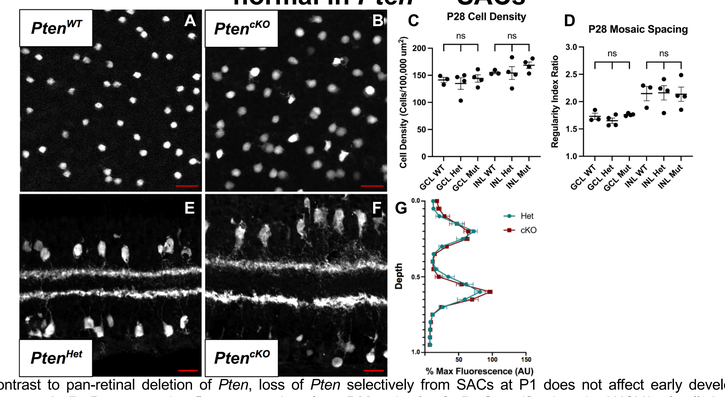
A, B. Reconstruction of SAC morphology in Imaris, statistically coded based on branch level for every filament. Warmer colors indicate higher branch order. **C.** Quantification of receptive field size shows no difference between *Pten*^{het} and *Pten*^{cKO} SACs. **D.** SACs in the GCL show a significant increase in total dendritic length in *Pten*^{cKO} SACs. **E.** *Pten*^{cKO} SACs in both the GCL and INL show close to a 50% increase in the number of branch points in their dendrites compared the *Pten*^{het} SACs. Statistics were calculated via student's t-test.

Schematic of the PI3K-Akt-mTOR Pathway



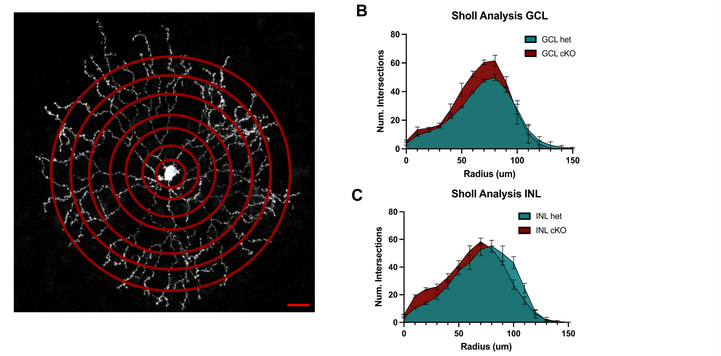
Extracellular signals are received by a receptor tyrosine kinase which triggers PI3K to phosphorylate PIP2 to generate PIP3. PTEN functions in direct competition to PI3K by dephosphorylating PIP3. PDK1 binds to PIP3 enabling it to phosphorylate and activate AKT, which then regulates many downstream pathways inside the cell. Notably, p-Akt inhibits Tsc1 and Tsc2, leading to the activation of mTORC1 and resulting in cell growth. Pan-retinal disruption of Pten in progenitor cells results in defects in migration, cell specification, and dendrite lamination in SACs.

Cell density, mosaic spacing and stratification remain grossly normal in *Pten*^{cKO} SACs



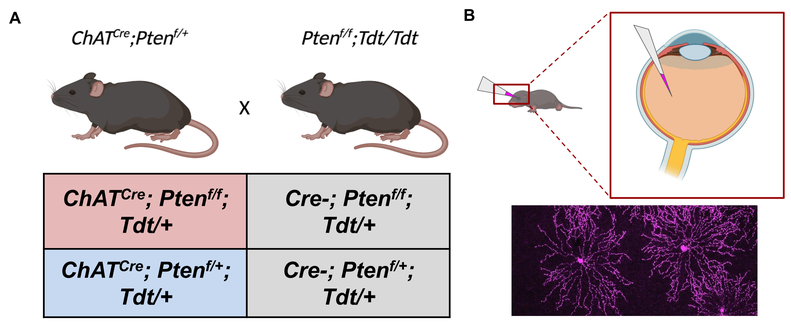
In contrast to pan-retinal deletion of *Pten*, loss of *Pten* selectively from SACs at P1 does not affect early developmental processes. **A, B.** Representative flatmounts taken from P28 animals. **C, D.** Quantification via ANOVA of cell density and mosaic spacing reveal no changes in SACs within both the INL and GCL. **E, F.** SACs in *Pten*^{cKO} retinas show abnormal somal shape and stray dendritic projections, particularly between the INL and S2. **G.** Quantification with IPLaminator shows that overall SAC stratification in S2/S4 the IPL remains grossly unchanged in Pten^{cKO} retinas. Scalebars = 25um

Sholl Analysis reveals increased density in proximal dendrites



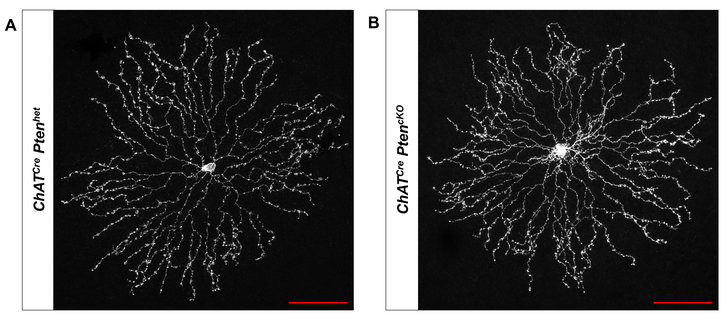
A. Example image describing how Sholl analysis is performed. Concentric circles are drawn from the soma, and the number of intersections with dendritic processes are quantified for each circle. **B, C.** Sholl analysis shows an increase of dendritic density in the first 75% of the arbor for *Pten*^{cKO} SACs in both the GCL and INL. This corresponds to the SAC dendritic compartment which receives input from upstream bipolar cells.

Mouse Line and Methodology



A. To focus on the changes in dendritic morphology while minimizing disruptions in migration and cell specification, we use a *ChAT*^{Cre} line to delete *Pten* specifically in SACs starting at P1. We will use the *ChAT*^{cre}*;Pten*$^{f/f}$*;Tdt/+* animals (Red) as *Pten*^{cKO} animals. The *ChAt*^{cre}*;Pten*$^{f/f}$*;Tdt/+* (Blue) will function as *Pten*^{het} animals for comparison. **B.** To sparsely label SACs, we will use a viral injection approach. Pups will be injected at P2 and then taken at P21 to visualize mature SACs. Schematics made with Biorender.com

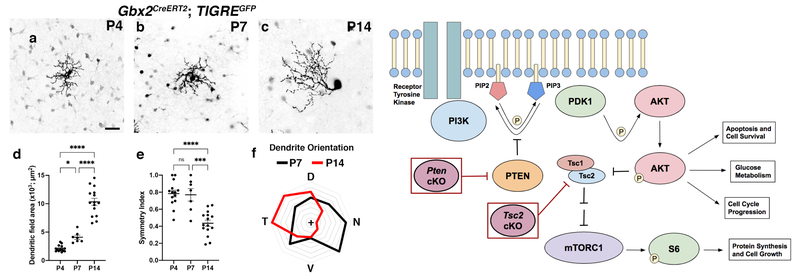
Single cell labeling reveals aberrant morphology in *Pten*^{cKO} SACs



Cells were sparsely labeled via *AAV-FLeX-TdTomato* injections at P2, and then imaged at P21. Both images are from SACs in the INL. **A.** *Pten*^{het} SACs show normal, canonical SAC morphology. **B.** *Pten*^{cKO} SACs show a clear increase in branching and dendritic density, as well as increased dendrite crossing. Scalebars = 50um.

Future Directions

- Perform single cell morphology analysis at other time points (P7, 14, 60)
- Further investigate the regulation of the PI3K Pathway in SACs (Tsc2 cKO)
- Investigate changes in synaptic density (PSD-95 labeling)
- Extend analysis to other amacrine cells (Gbx2+ AC)



Funding:
NEI R01EY032057 (KW)
NIH/NINDS T32NS007466 (NGP)
NEI T325T32EY023211 (CEI)
NEI F32EY029974 (PK)
ARCS Scholarship

References:
1 Sakagami et al., Molecular and Cellular Neuroscience, 49 (2012)
2 Cantrup et al., PLoS One, 7 (2012)