Plagl1 is required to sustain murine Müller glial cell quiescence and retinal homeostasis

Yacine Touahri[1,2,3,4], Luke Ajay David[1,5], Yaroslav Ilnytskyy[6], Edwin van Oosten[1,2], Joseph Hanna[1,5], Nobuhiko Tachibana[1,3], Lata Adnani[1,3], Jiayi Zhao[1,2], Mary Hoffman[3], Rajiv Dixit[1,2,3,4], Laurent Journot[7], Yves Sauve[8], Igor Kovalchuk[6], Isabelle Aubert[1,5], Jeffrey Biernaskie[4], Carol Schuurmans[1,2,3,5]

1Sunnybrook Research Institute, 2075 Bayview Ave, Toronto, ON, Canada, M4N 3M5.
2Department of Biochemistry, University of Toronto, Toronto, ON, Canada
3Department of Biochemistry and Molecular Biology, Alberta Children's Hospital Research Institute, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada
4Department of Comparative Biology and Experimental Medicine, Alberta Children's Hospital Research Institute, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada
5Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada
6Dept of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada.
7Institut de Génomique Fonctionnelle, Montpellier, France
8Department of Ophthalmology and Visual Sciences and Department of Physiology, University of Alberta, Edmonton, Alberta, Canada

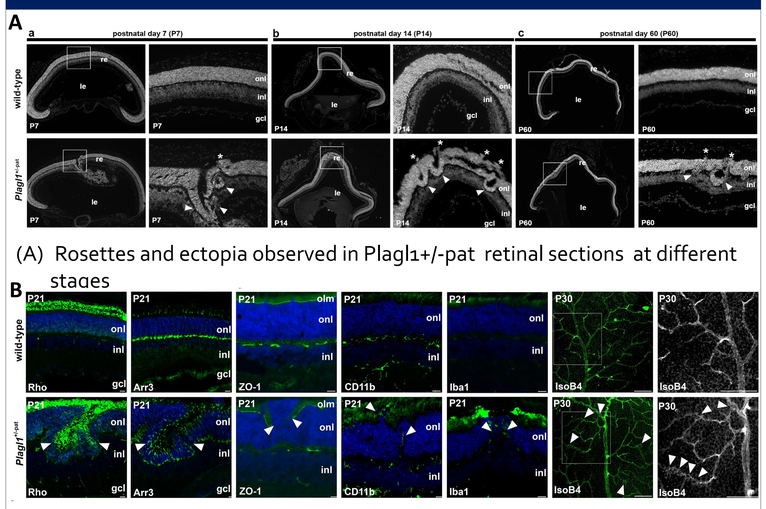
1- Abstract

In the retina, Müller glia are 'activated' by injury but only in certain species, such as fish and frogs, do they de-differentiate into proliferative progenitor cells to replace lost retinal cells. This regenerative response is latent in mammals. Mining single cell and bulk RNA-seq datasets revealed that transcripts for Plagl1, a maternally imprinted gene, are enriched and dynamically regulated in reactive Muller glia. At perinatal stages, Plagl1+/-pat null mutants develop defects in retinal architecture and visual signal processing that correlate with a reactive gliotic phenotype. Plagl1+/-pat Müller glia proliferate ectopically and contribute to an extended neurogenic period. Transcriptomic and ATAC-seq profiles of Plagl1+/-pat retinas revealed similarities to neurodegenerative and injury models, including an upregulation of pro-gliogenic and pro-proliferative pathways, such as Notch, not observed in wild-type retinas post-insult. Plagl1 is thus an essential component of the transcriptional regulatory networks that retain mammalian Müller glia in quiescence and sustain retinal homeostasis.

HYPOTHESIS

***Plagl1* is plays an essential role in healthy and diseased Müller glia .**

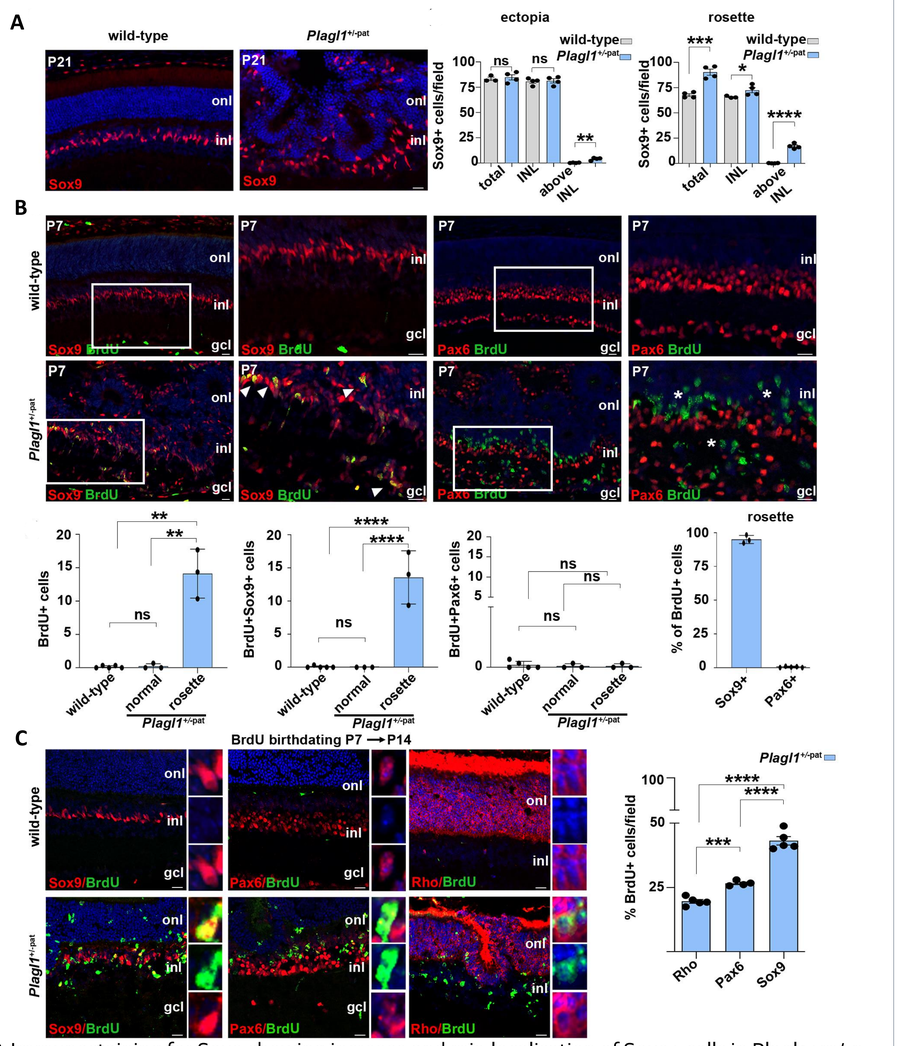
4-*Plagl1* is required to maintain retinal integrity



(A) Rosettes and ectopia observed in Plagl1+/-pat retinal sections at different stages

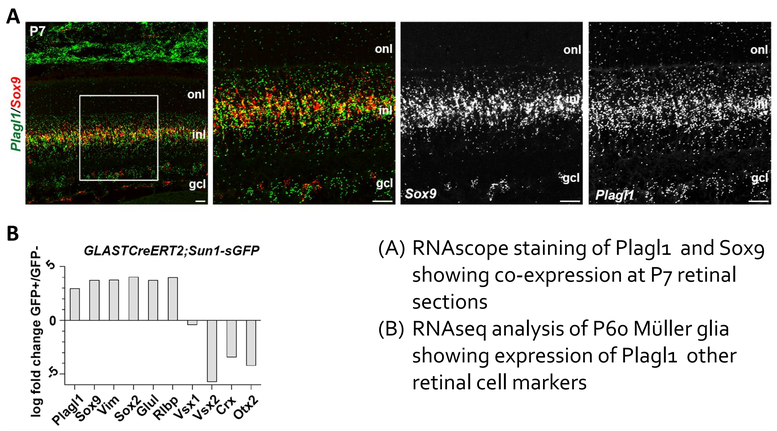
(B) Photoreceptors, OLM (zo1), macrophages (Iba1,Cd11b) and vasculature defects observed at P21 in Plagl1+/-pat retinal sections

7- Plagl1 mutant Müller glia proliferate ectopically give rise to new Müller glia , Rods and Amacrine cells



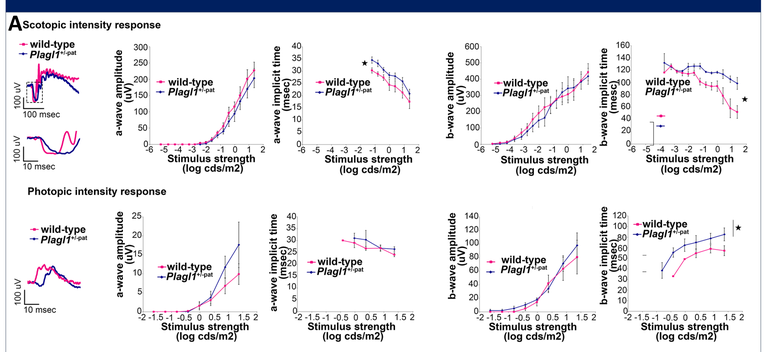
(A) Immunostaining for Sox9 showing increase and mis-localisation of Sox9+ cells in Plagl1+m/-p
(B) Immunostaining for Sox9, Pax6, and BrdU on P7 showing proliferating Sox9+/BrdU+ cells
(C) BrdU birthdating (P7 BrdU to P14 dissection) in vivo, followed by BrdU co-immunostaining with Sox9, Pax6, and Rhodopsin

2-*Plagl1* is expressed in Müller glial



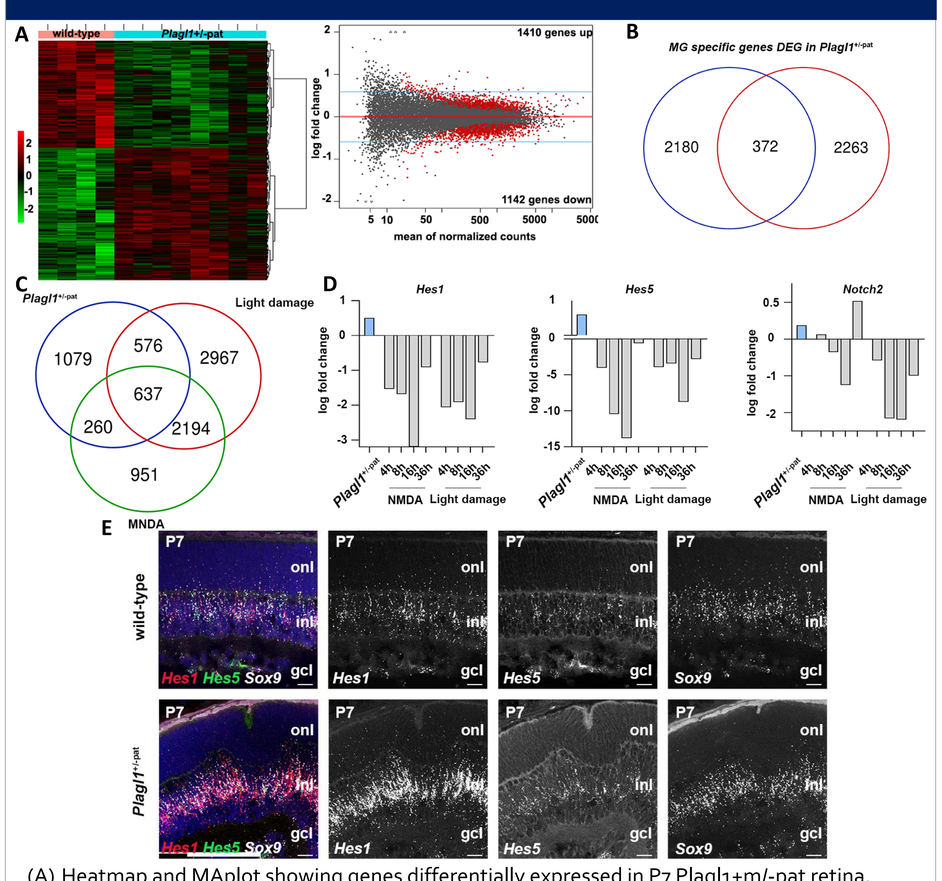
(A) RNAscope staining of Plagl1 and Sox9 showing co-expression at P7 retinal sections
(B) RNAseq analysis of P60 Müller glia showing expression of Plagl1 other retinal cell markers

5- *Plagl1* loss of function affects proper visual function



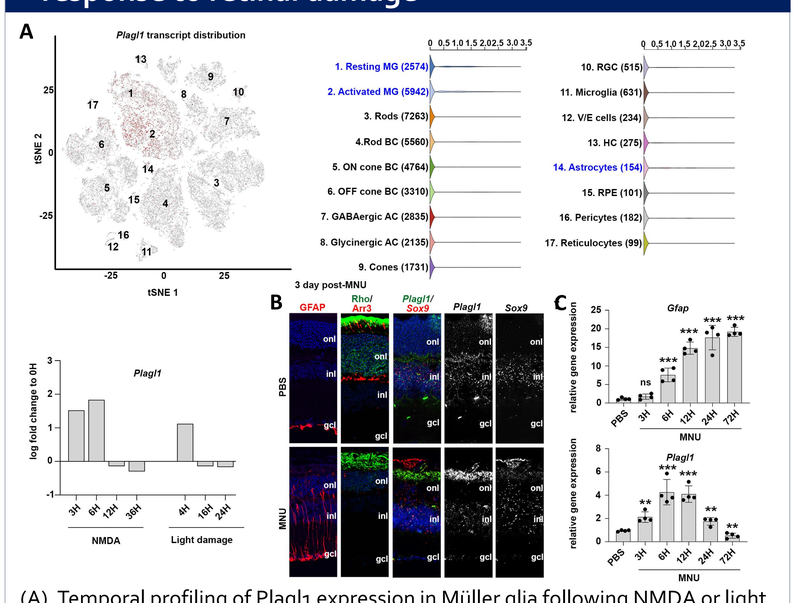
(A) Full field ERG recordings of P30 wild-type and Plagl1+/-pat mice showing normal amplitudes but prolonged implicit time

8-Plagl1 acts via Notch signalling pathway in Müller glia



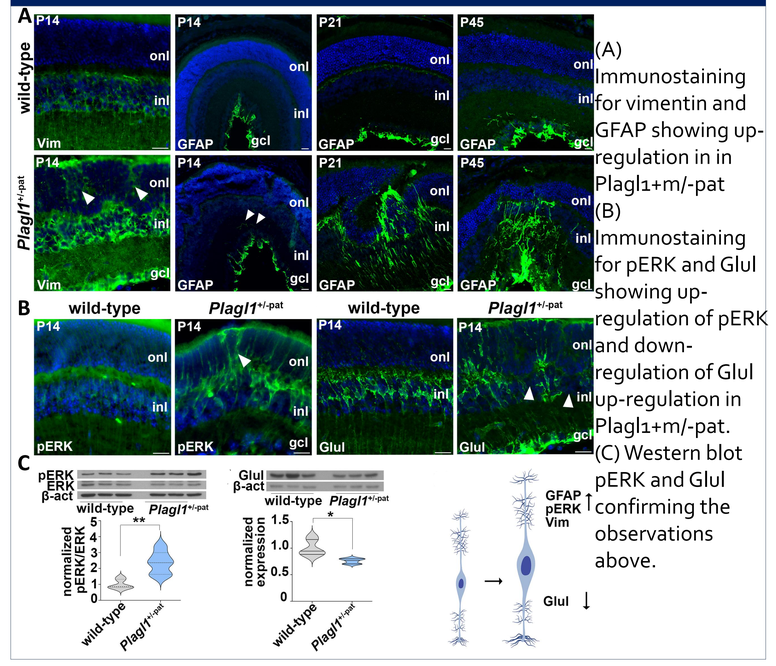
(A) Heatmap and MAplot showing genes differentially expressed in P7 Plagl1+m/-pat retina.
(B,C) Venn diagrams identifying Müller glia specific genes that are differentially expressed in Plagl1+m/-pat and shared with NMDA and Light damaged Müller glia.
(D) Comparison of Notch transcript levels in Plagl1+m/-pat retinas to NMDA/LD Müller glia.
(E) RNAscope probe labelling of Hes1, Hes5, and Sox9 showing Hes1 and Hes5 up-regulation is colocalized with Sox9 staining.

3-*Plagl1* expression is dynamically regulated in response to retinal damage



(A) Temporal profiling of Plagl1 expression in Müller glia following NMDA or light damage
(B) Immunolabeling of GFAP, Rho, and Arr3 and RNAscope ISH for Plagl1 and Sox9 3 days post MNU treatment.
(C) Gfap and Plagl1 expression quantified by RTqPCR

6-Plagl1 mutant Müller glia undergo insult-independent reactive gliosis



(A) Immunostaining for vimentin and GFAP showing up-regulation in in Plagl1+m/-pat
(B) Immunostaining for pERK and Glul showing up-regulation of pERK and down-regulation of Glul up-regulation in Plagl1+m/-pat.
(C) Western blot pERK and Glul confirming the observations above.

Conclusions and future directions

- Plagl1 is expressed in Müller glia and its expression is finely regulated following injury
- Loss of Plagl1 affetcs retinal integrity and induces spontaneous reactive gliosis
- Plagl1 mutant Müller glia undergo ectopic proliferation and generate new Müller glia and neurons.
- Plagl1 plays an important role in preventing Müller glia proliferation probably by maintaining a low level of Notch signalling pathway