

PLD3 contributions to vascular dysfunction in the context of AD

Elizabeth S. Fisher^{1#}, Kate Tubbesing^{1#}, Alexis Guevara¹, Dylan Murphy¹, Katie Stevens¹, Steve Lotz¹, Jay Hayes^{2*}, Jessica Suiter², Thomas R. Kiehl¹, Celeste M. Karch³, Sally Temple¹, Taylor Bertucci¹

1 - Neural Stem Cell Institute, 150 New Scotland Ave, Albany, NY 12208

2 - Rensselaer Polytechnic Institute, 110 8th St, Troy, NY 12180

3 - Dept. of Psychiatry, Washington University School of Medicine, 4370 Duncan Ave, St. Louis, MO 63110

- co-first author, * -deceased



Background

Phospholipase D3 (PLD3) is a non-canonical phospholipase, which has been shown to be important in lysosomal function and DNA degradation. A SNP in *PLD3* P4442A (V232M) is overrepresented in Late-Onset Alzheimer's Disease (LOAD) patients, and carriers also demonstrate a trend towards faster cognitive decline. Recent work has demonstrated defective lysosomal processing may impact APP processing and inflammation in a variety of contexts, though work has primarily focused on neurons. Vascular cells also express APP, and their role in the development of LOAD is still incompletely understood. Here, we have focused on 2D and 3D models to determine the role of PLD3 in vascular cells.

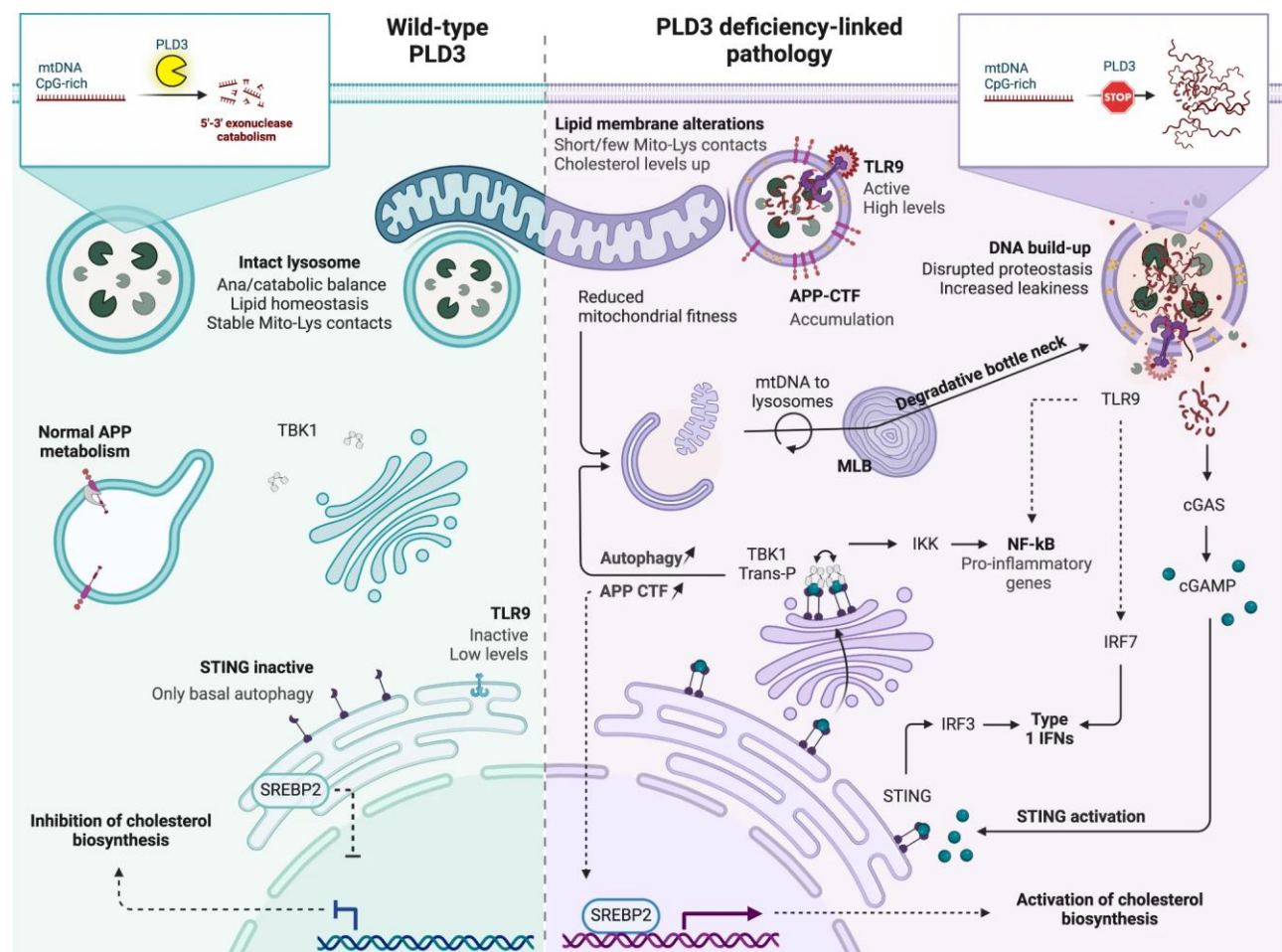


Figure 1. *PLD3* pathology is linked to altered APP processing and activation of immune signaling pathways because of defective lysosomal processing, which increases DNA build up in cells. Figure from: Van Acker, Z.P., Perdok, A., Hellemans, R. et al. Phospholipase D3 degrades mitochondrial DNA to regulate nucleotide signaling and APP metabolism. Nat Commun 14, 2847 (2023).

APP/PS1xPLD3^{KO} mice demonstrate strong alterations in vascular-associated pathways in the cortex

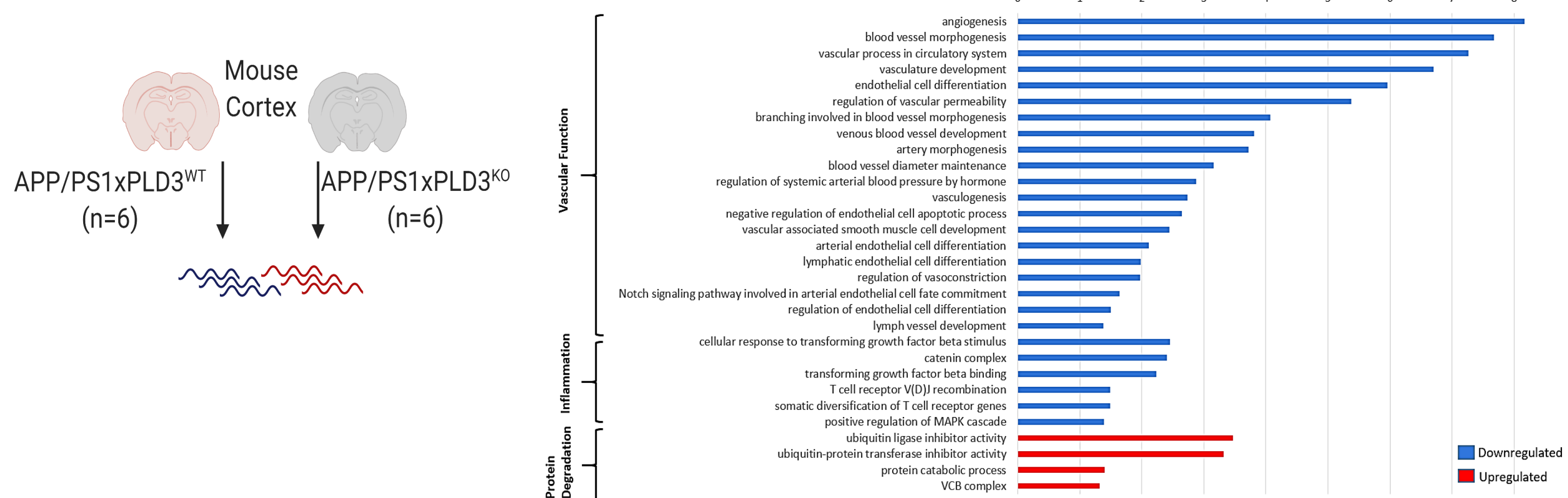


Figure 5. Bulk RNA sequencing of mouse cortex was performed from animals carrying either the APP/PS1 mutation alone or crossed to *PLD3*^{KO}. Differential gene expression analysis was performed followed by pathway enrichment analysis. Results demonstrate significant changes in pathways almost exclusively associated with the vasculature and inflammation.

APP/PS1 mice exhibit reduced vascular networks by 8 months

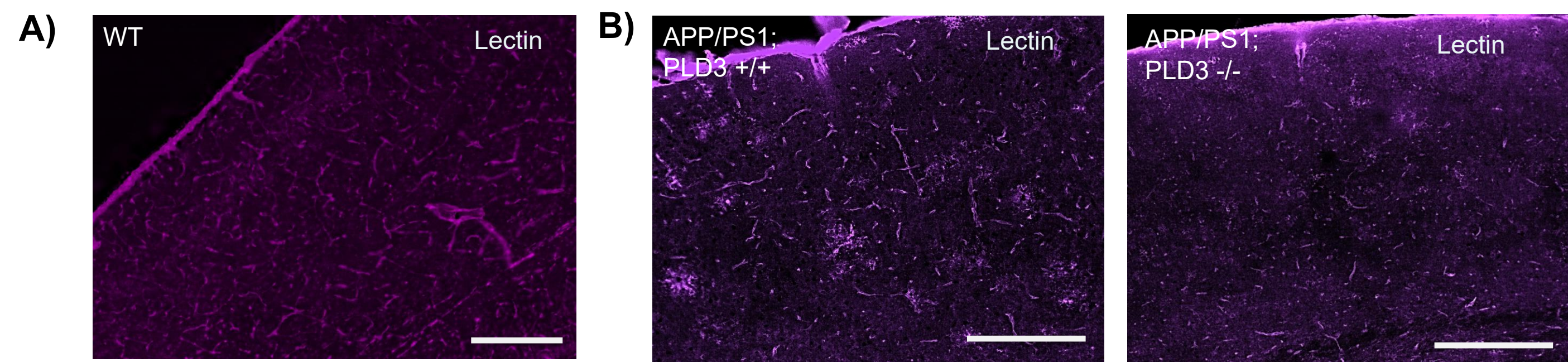


Figure 6. Mouse cortices were stained with isolectin direct conjugate, which detects blood vessels and microglia. A) 2-month-old Wild type mice show robust vascular networks throughout the cortex. B) 8-month-old APP/PS1 mice, both with and without *PLD3*, show distinct loss of overall vasculature. Scale bar = 200µm.

PLD3 KD ECs have altered inflammation, cell division, and metabolism-related pathways

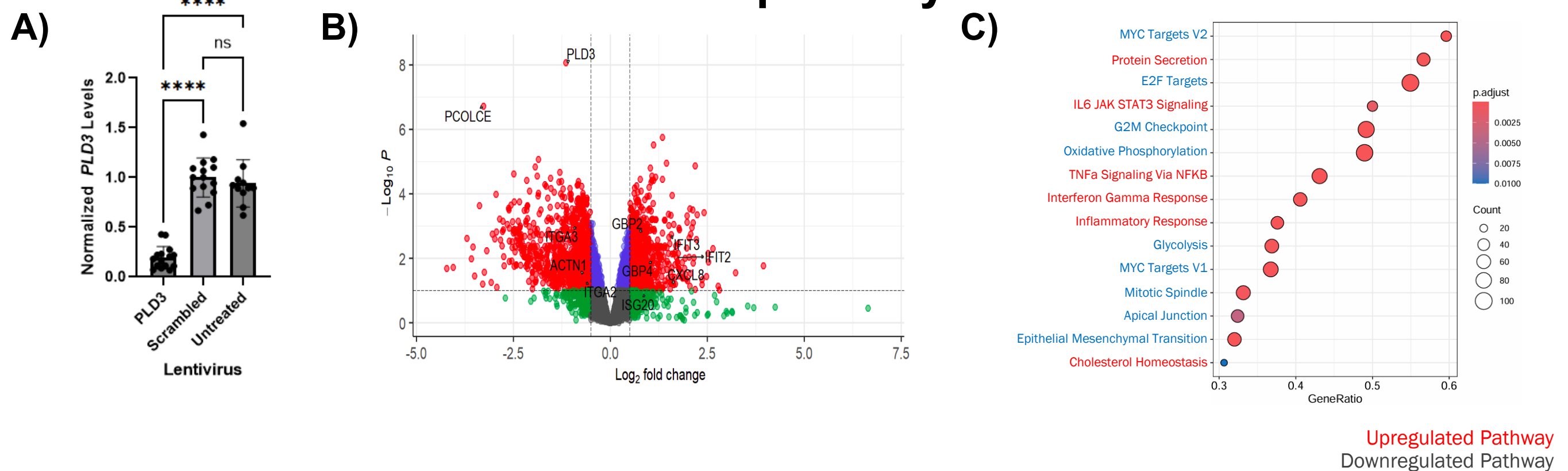


Figure 7. A) *PLD3* KD was validated using qPCR, and shows robust reduction compared to scrambled and untreated controls. B) Bulk-RNA seq was performed on purified ECs and a volcano plot shows differentially expressed genes (DEGs) between scrambled and *PLD3* KD groups. Genes highlighted include genes involved in the inflammatory response and junctional stability. C) Pathway enrichment of DEGs using gene set enrichment analysis (GSEA).

KD of *PLD3* in ECs impairs barrier function of the monolayer

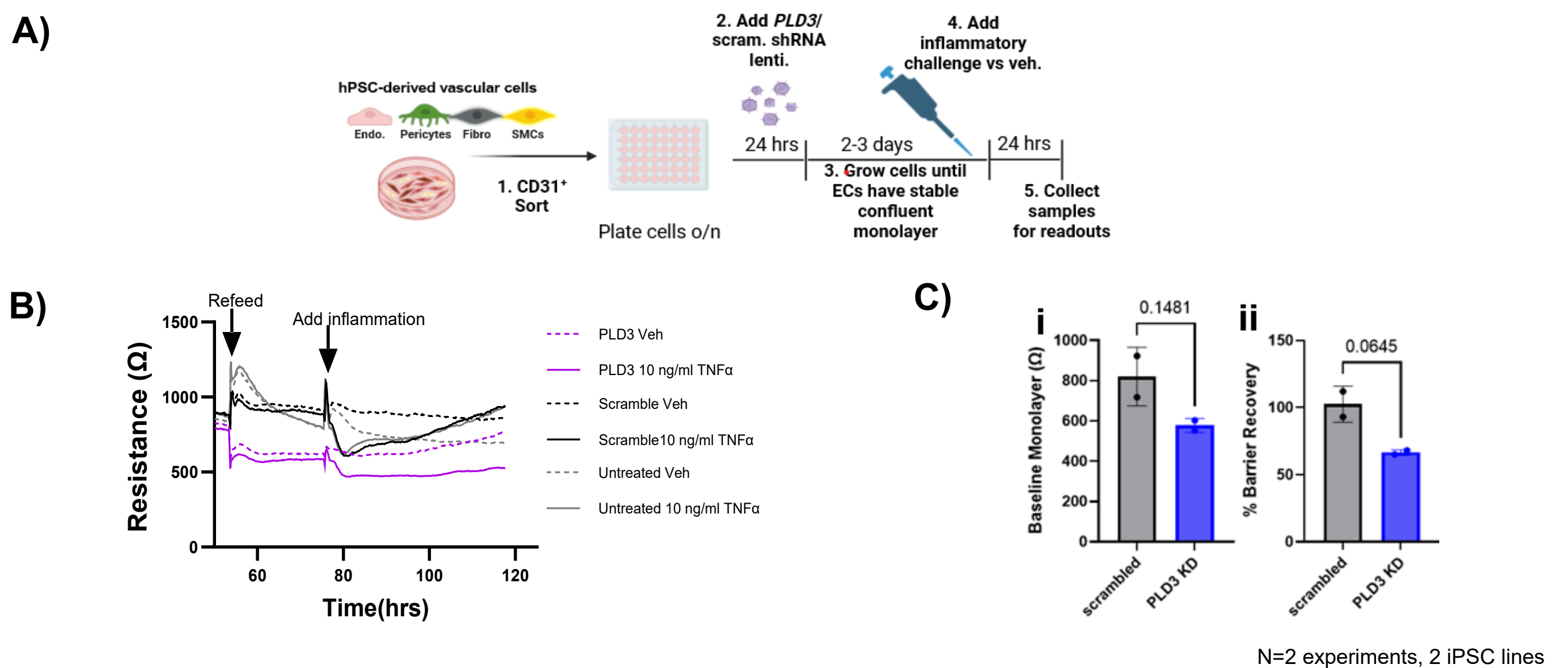


Figure 8. A) Experimental Timeline. B) *PLD3* KD ECs were read continuously via Electric Cell substrate Impedance Sensing (ECIS), a measure of barrier strength. Once a stable monolayer formed, ECs were challenged with 50 ng/ml TNFα and allowed to recover for 48 hours. C) Quantification of baseline monolayer resistance and percent recovery at the end timepoint.

PLD3 KD alters APP processing gene expression in iPSC-ECs

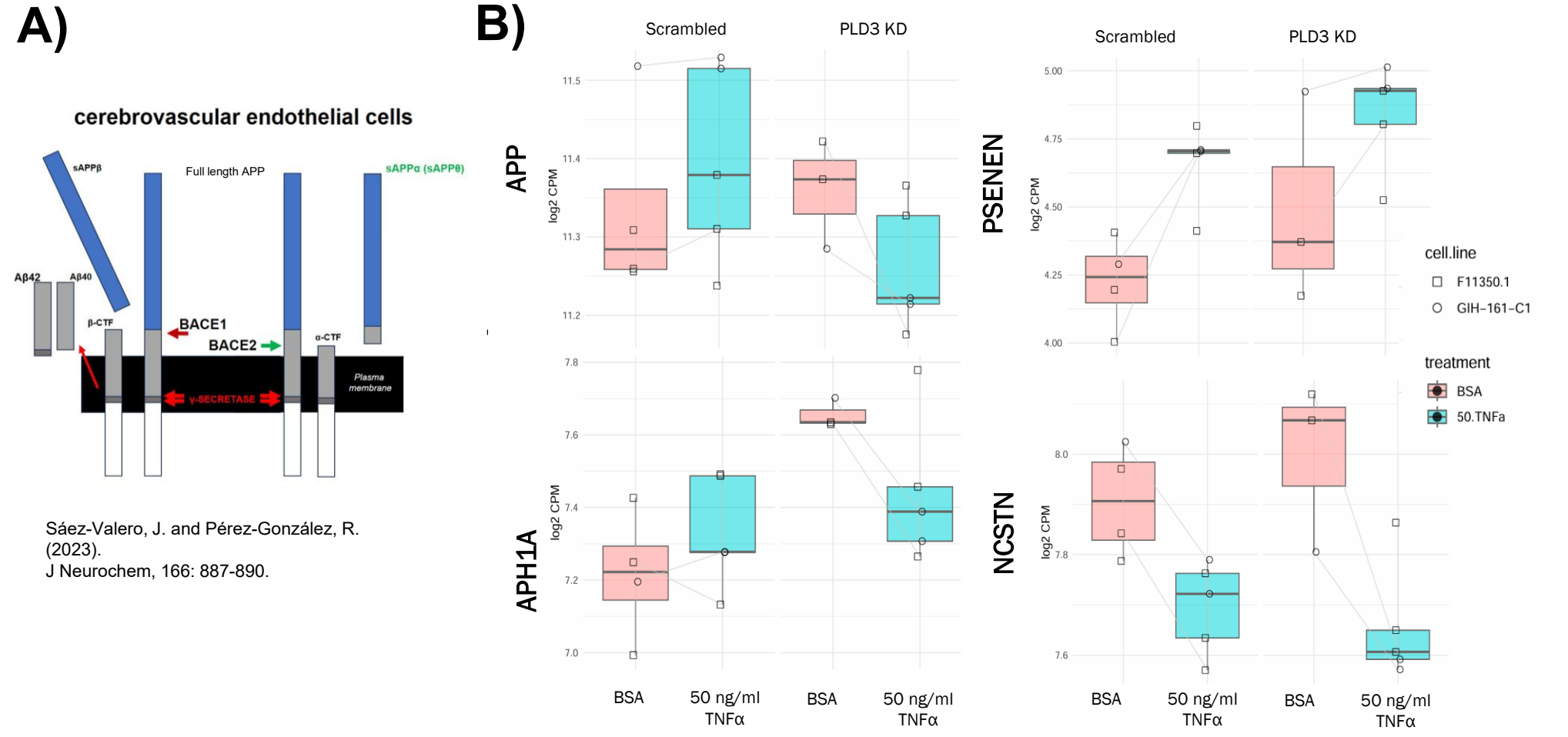


Figure 9. A) Simplified schematic of APP processing in brain endothelial cells. B) *PLD3* KD ECs show alterations in several genes involved in γ-secretase activity.

PLD3 KD iPSC-ECs show increased inflammation at rest & following inflammatory challenge

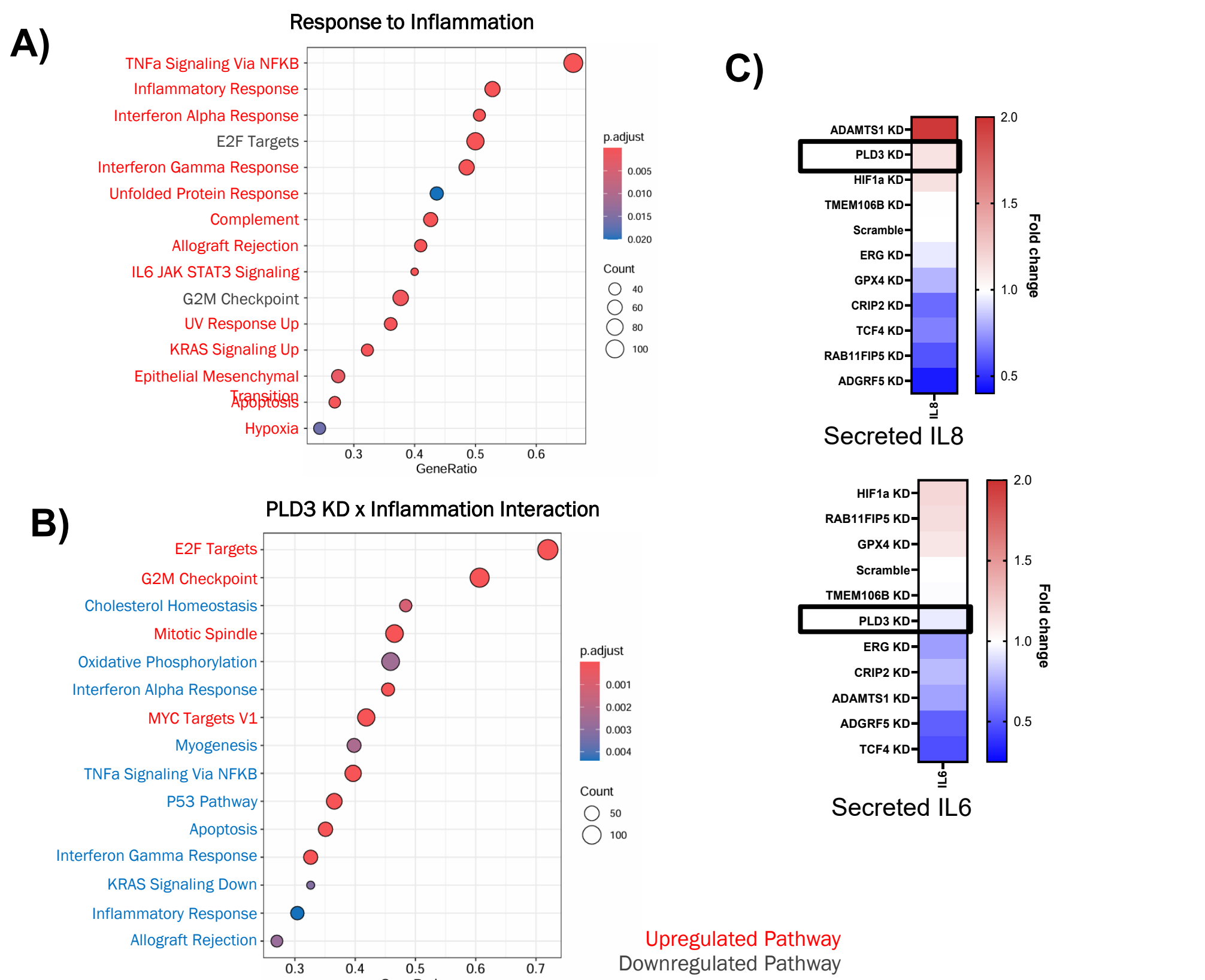


Figure 11. A) Pathway enrichment analysis of DEGs between ECs at rest and 24 hours following 50 ng/ml TNFα treatment using GSEA. B) GSEA for genes that are differentially regulated in *PLD3* KD ECs over controls upon treatment with inflammation. C) *PLD3* KD ECs show higher secretion of IL6 and IL8 at baseline into the conditioned medium.

Conclusions & Future Directions

- *PLD3* plays an important role in APP processing and inflammatory signaling
- In patients with the P4442A variant, *PLD3* splicing is altered
- APP/PS1 mice with *PLD3* KO show vascular pathway alterations in the cerebral cortex, along with reduced vascular networks in APP/PS1 mice compared to controls
- *PLD3* KD ECs have altered cell division, metabolism, and inflammation related pathways at rest and following inflammation
- *PLD3* KD results in loss of barrier in iPSC-EC monolayers
- Future studies will investigate barrier morphology in EC monolayers following *PLD3* KD, and additional aspects of vasculature will be analyzed in APP/PS1 x *PLD3* KO mice

References

- *Van Acker, Z.P., Perdok, A., Hellemans, R. et al. (2023). Phospholipase D3 degrades mitochondrial DNA to regulate nucleotide signaling and APP metabolism. Nat Commun 14, 2847.
- *Cruchaga C, Karch CM, Jin S, et al. (2014). Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature.
- *Rosene, MJ, Hsu, S, You, SF, et al. (2022). Phospholipase D3 contributes to Alzheimer's disease risk via disruption of AB clearance and microglia response to amyloid plaques. Medrxiv.
- *Bertucci, T., Kakarla, S., Kim, D., Dai, G. (2022). Differentiating Human Pluripotent Stem Cells to Vascular Endothelial Cells for Regenerative Medicine, Tissue Engineering, and Disease Modeling. In: Zhao, F., Leong, K.W. (eds) Vascular Tissue Engineering. Methods in Molecular Biology, vol 2375. Humana, New York, NY.
- *Bertucci T, Kakarla S, Winkelman MA, Lane K, Stevens K, Lotz S, Grath A, James D, Temple S, Dai G. (2023) Direct differentiation of human pluripotent stem cells into vascular network along with supporting mural cells. APL Bioeng.
- *Yang, A.C., Vest, R.T., Kern, F. et al. (2022) A human brain vascular atlas reveals diverse mediators of Alzheimer's risk. Nature 603, 885–892

Acknowledgements

- We would like to thank the Temple Lab and all of the PIs at the NSCI for useful discussion and contributions to this project. We would also like to thank NeuraCell for their support in all things iPSC related.
- This research was supported by AARF Grant 22-971644 to ESF, and NIH grant 5U01AG072464-04 to ST and CMK.
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