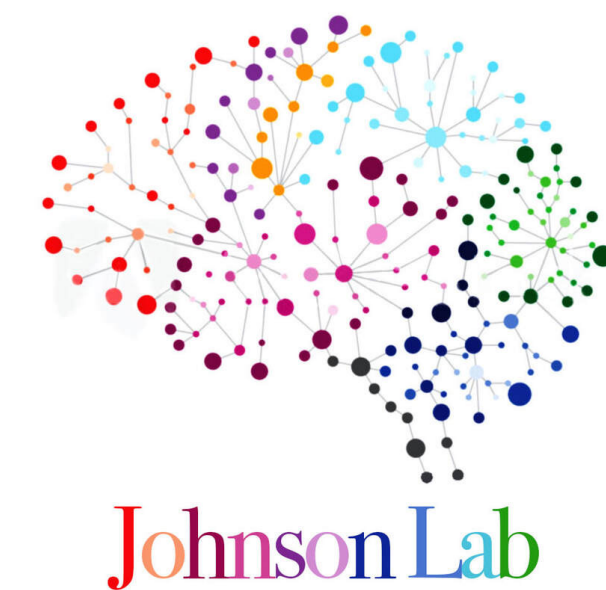




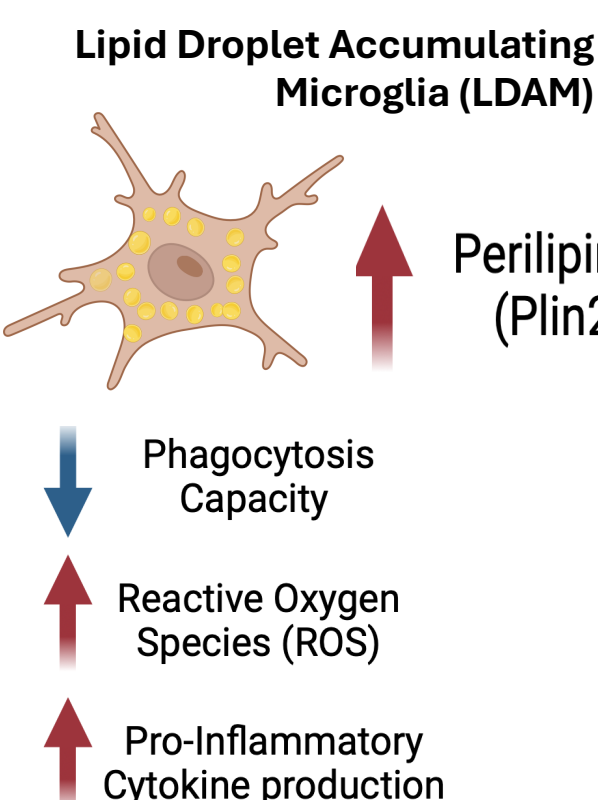
Lipid Droplets as Immunometabolic Hubs in Alzheimer's Disease: Roles for APOE4 and PLIN2 in Microglia

Isaiah O Stephens^{1,2}, Cassi Friday^{1,2}, Katy Smith^{1,2}, Nicholas Devanney^{1,2}, Diksha Satish¹, Scott Gordon^{1,3}, Lance Johnson^{2,4}
¹ Department of Physiology, ² Sanders Brown Center on Aging, ³ Saha Cardiovascular Research Center, ⁴ Department of Neuroscience, University of Kentucky, Lexington, Kentucky



Introduction

Lipid droplets (LDs) are bona fide organelles composed of a neutral lipid core of triglycerides and sterol esters surrounded by a phospholipid monolayer. Beyond lipid storage, LDs contain a unique proteome and are increasingly recognized as regulators of brain health and neurodegeneration.

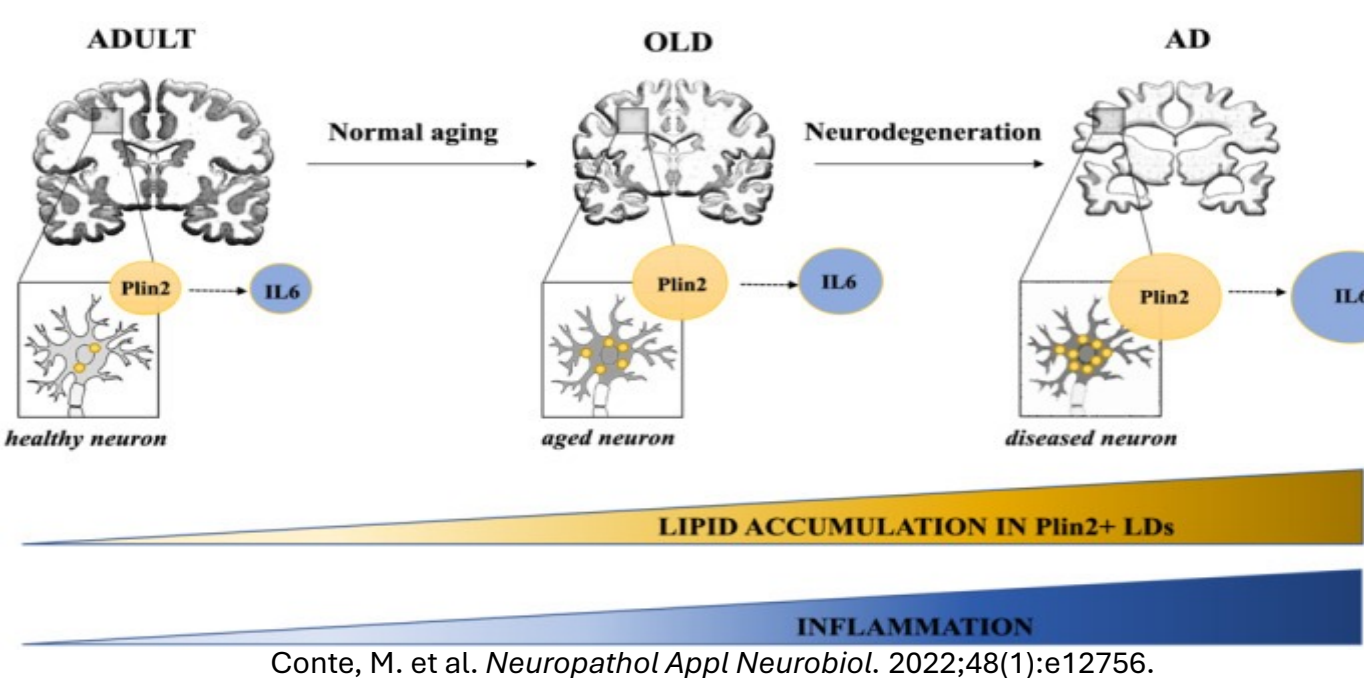
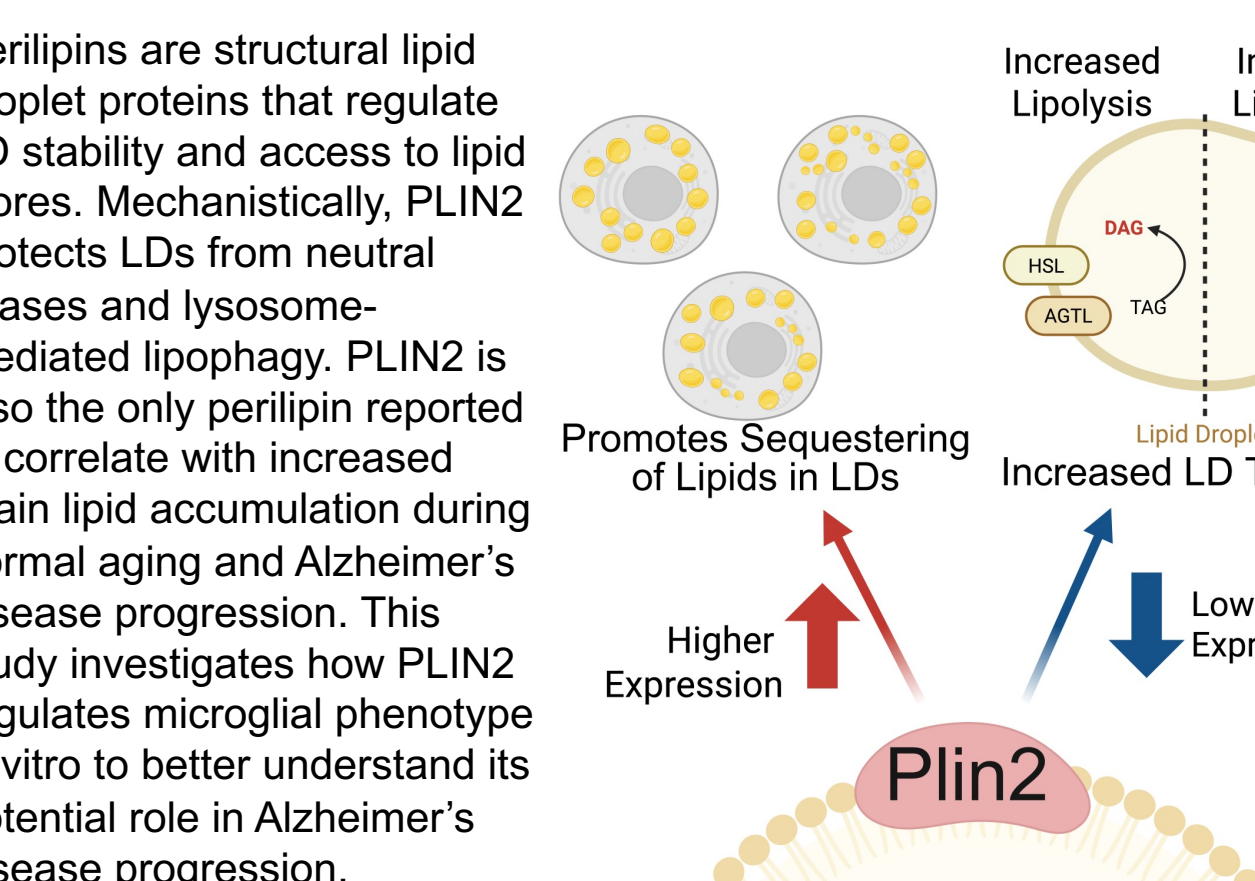


Alois Alzheimer originally noted lipid-like "adipose saccules" in glial cells alongside plaques and tangles. Recent work has identified lipid droplet-accumulating microglia in AD mouse models and human AD tissue, where this phenotype is linked to disease progression, impaired microglial function, and elevated Perilipin-2 (Plin2).

APOE4 drives accumulation of Plin2+ LDs in Microglia

APOE4, the strongest genetic risk factor for late-onset Alzheimer's disease, is closely linked to disrupted lipid metabolism and neuroinflammation. Across APP/PS1 mice, cuprizone-induced demyelination, and APOE4/4 human iPSC-derived microglia, APOE4 is associated with increased PLIN2-positive lipid droplet accumulation.

Because PLIN2 regulates lipid droplet stability and turnover, APOE4-driven PLIN2 accumulation may represent a convergent link between lipid stress and microglial dysfunction in AD.



Methods

APOE LD-Enriched Fraction Proteomics: APOE3/3 and APOE4/4 mice were injected with saline or LPS. Twenty-four hours later, mice were perfused with ice-cold saline, and liver tissue was collected, homogenized, and processed for lipid droplet enrichment by density gradient centrifugation. The buoyant LD-enriched fraction was collected for quantitative proteomic analysis.

APOE Primary Microglia Lipid Droplet Assay: Primary microglia were isolated from APOE3/3 and APOE4/4 targeted replacement mouse pups and plated on poly-L-lysine-coated coverslips. Cells were treated for 24 hours with control media, oleic acid, LPS, dead N2a cells, or combined lipid/inflammatory stimuli to induce lipid droplet accumulation. Cells were stained for neutral lipids, counterstained with DAPI, imaged, and analyzed to compare APOE genotype-dependent LD accumulation.

BV2 PLIN2 KO Cell Culture and Treatments: CRISPR/Cas9-generated Plin2 knockout BV2 microglia and matched wild-type controls were cultured in DMEM/F-12 + GlutaMAX supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C and 5% CO₂. Plates and chamber slides were coated with poly-L-lysine prior to seeding. Cells were treated for 24 hours with Alzheimer's disease-relevant stimuli, including oleic acid, amyloid- β , myelin debris, or differentiated neuron-like N2a cells at a 5:1 dN2a:BV2 ratio.

Lipid Droplet Imaging and Phagocytosis: For lipid droplet analysis, cells were incubated with 0.15 μ M LipiGreen in OptiMEM for 35 minutes, fixed with 4% PFA, counterstained with DAPI, and imaged by confocal microscopy. Lipid droplet burden was quantified by LD area, LD number, and/or average LD size per cell. Phagocytosis was assessed by incubating BV2 cells with pHrodo-labeled zymosan particles for 2 hours, followed by fixation, DAPI mounting, imaging, and quantification of internalized pHrodo signal per cell.

BV2 Lipidomics and Transcriptomics: For lipidomics, BV2 cells were plated on poly-L-lysine-coated 6-well plates, treated as indicated, washed with DPBS, and scraped into 50:50 methanol:butanol containing 10 mM ammonium formate. Samples were stored at -80°C prior to LC-MS analysis. For RNA-seq, total RNA was extracted using the Qiagen RNeasy Mini Kit, and samples with RIN ≥ 8 were submitted for poly(A)-enriched paired-end sequencing. Differential expression analysis was performed in R using DESeq2, with downstream PCA, clustering, and Gene Ontology enrichment.

'LD enriched' fractions; PLIN2 is increased in E4 and following LPS

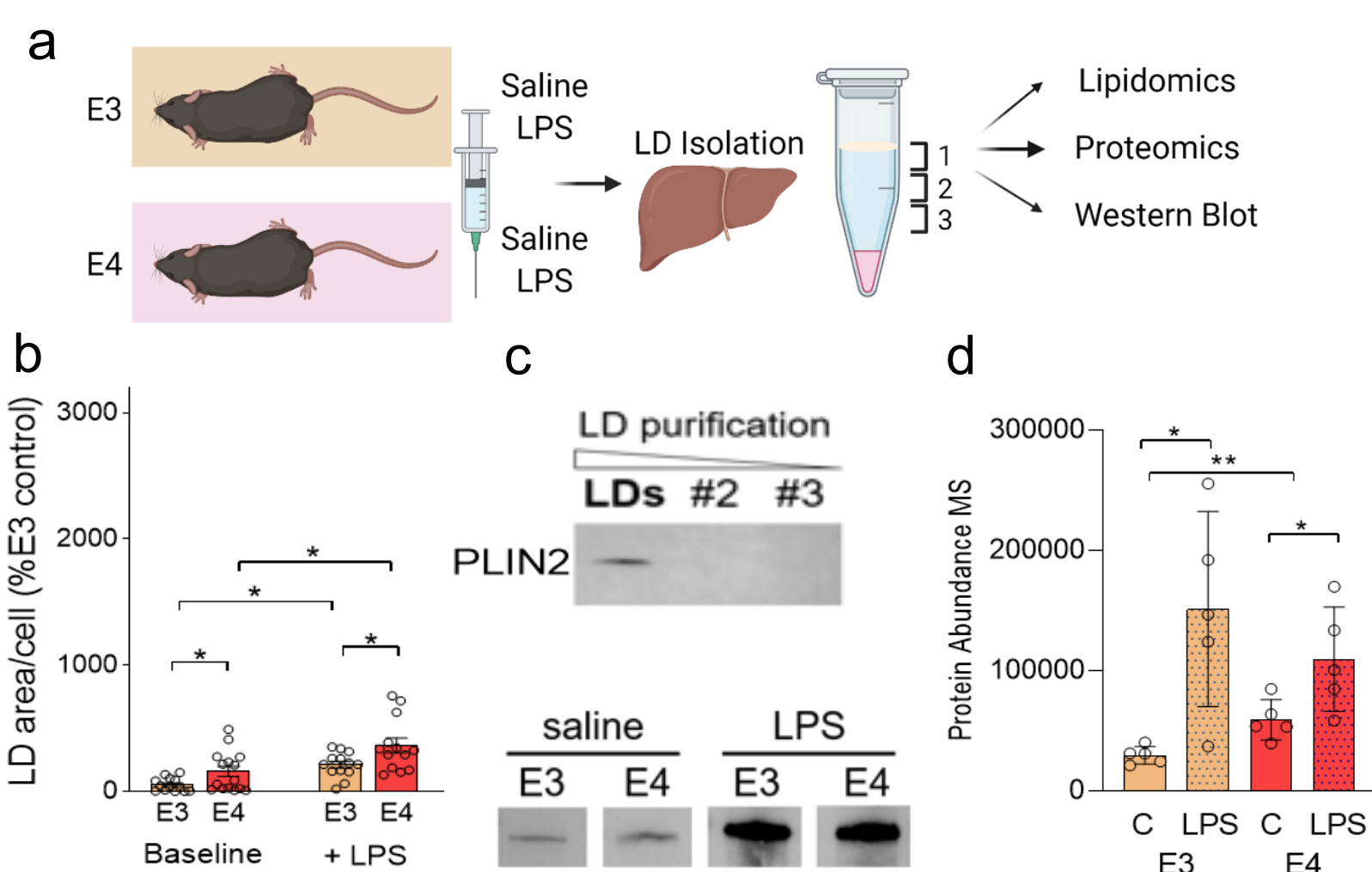


Figure 1. (a) Schematic displaying the harvesting and isolation of lipid droplets from APOE-TR mouse livers for downstream analysis. (b) Primary microglia from E3 and E4 mice stimulated with LPS for 24 hours compared to baseline. (c) LD enriched fractions (top section after gradient centrifugation) are positive for PLIN2. The #2 and #3 (same volume fractions down the gradient) contained no detectable PLIN2. (Bottom) PLIN2 western blots of lipid droplet enriched fractions from each genotype and condition. (d) LD enriched fractions from E4 livers express more relative abundance of PLIN2 at baseline. LPS increased abundance of PLIN2, suggesting an increased amount of lipid droplets in the inflamed condition.

Plin2 KO reduces LD accumulation and enhances phagocytosis in microglia

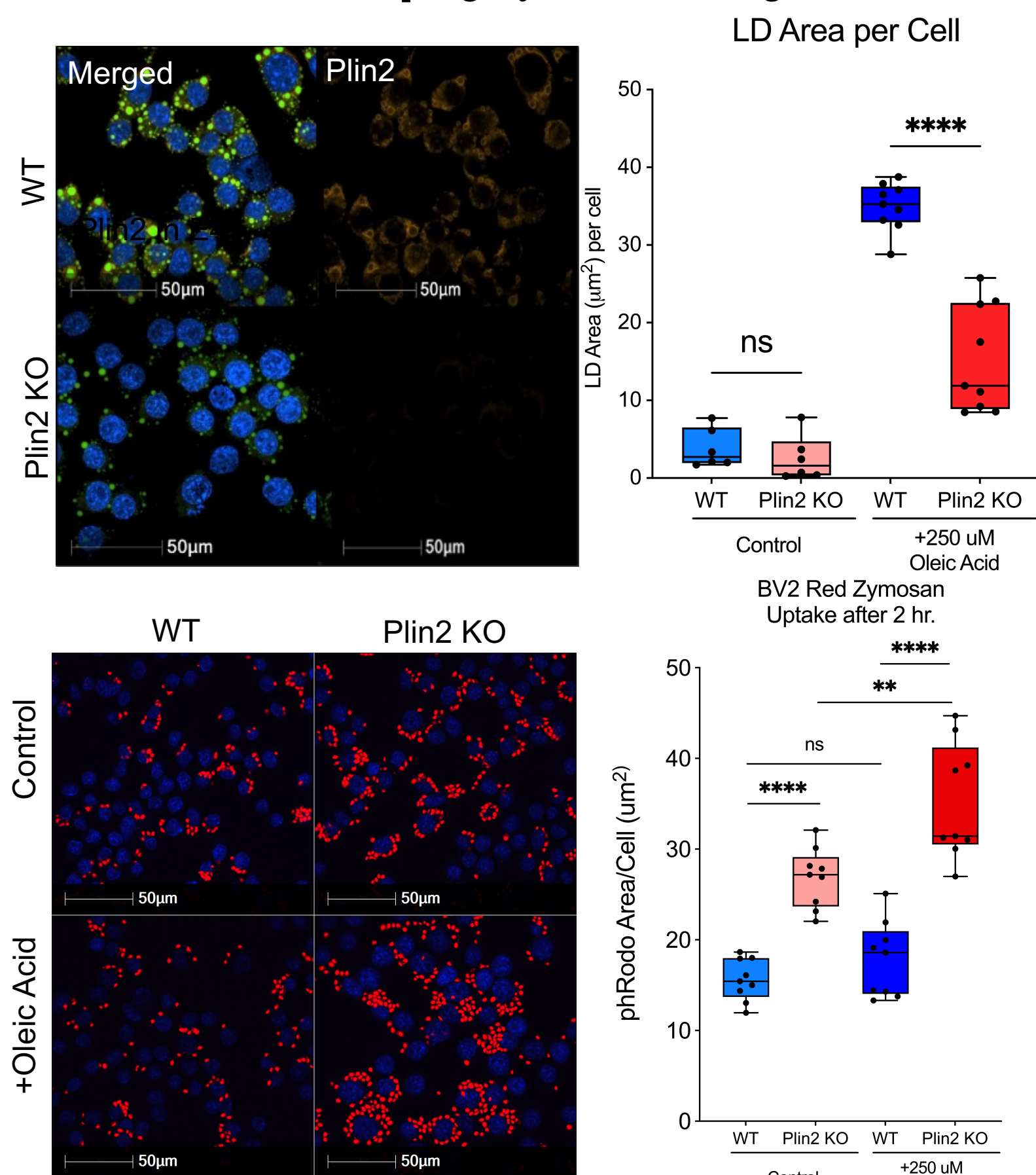


Figure 4. PLIN2 knockout reduces oleic acid-induced lipid droplet accumulation and enhances phagocytosis in BV2 microglia. WT and Plin2 KO BV2 cells were treated with control media or 250 μ M oleic acid for 24 hours. (a) Representative images and quantification show that Plin2 KO cells have reduced lipid droplet accumulation following oleic acid treatment compared to WT cells. Lipid droplets are shown in green, PLIN2 in orange, and nuclei in blue. (b) Representative images and quantification of pHrodo-labeled zymosan uptake show increased phagocytic signal in Plin2 KO cells under control and oleic acid-treated conditions. Data are presented as individual wells with mean $\pm$ SEM. Statistical comparisons were performed using one-way ANOVA with Sidak post hoc testing: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Plin2 KO significantly changes the microglial lipidome

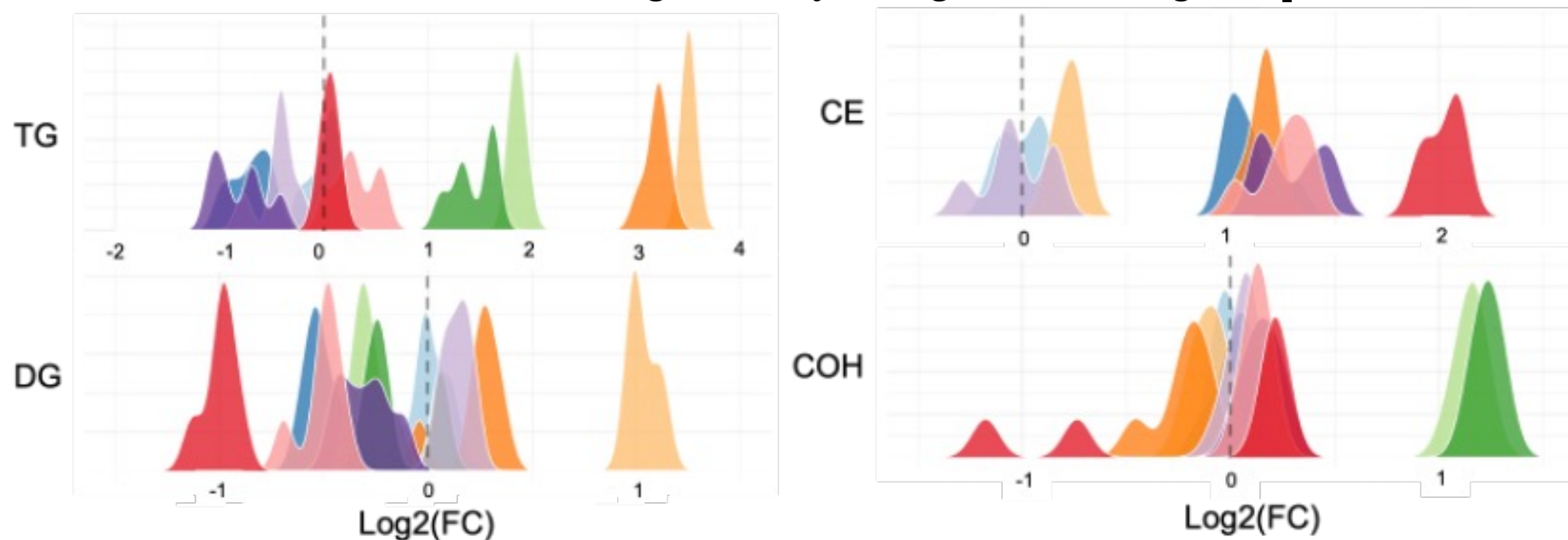


Figure 6. PLIN2 knockout alters neutral lipid subclass distribution in a treatment-dependent manner. Targeted lipidomic profiling was performed on WT and Plin2 knockout BV2 microglia under control conditions or following 24-hour exposure to oleic acid, myelin debris, amyloid- β , or damaged neuron-like N2a cells. Ridge plots show the distribution of log₂ fold changes for lipid species within four major lipid subclasses: triglycerides (TG), diacylglycerols (DG), cholesteryl esters (CE), and free cholesterol (COH). Each ridge represents a genotype and treatment group, with the dashed vertical line at log₂ fold change = 0 indicating the WT average for each lipid species. PLIN2 loss produced treatment-specific shifts in neutral lipid profiles, supporting a role for PLIN2 in regulating microglial lipid storage and lipid remodeling in response to AD-relevant stimuli.

Acknowledgments



Johnson Lab: Cecily Wood, Chloe Lucido, Colton Lysaker, Gabriela Hernandez, Grace Yeric, Jessica Funnell, Lilly Smith, Mahmood Ateyeh, Omar Hamid

Funding Sources:

NIA R01 AG081421
COBRE P20GM148326



Results

Proteins enriched on E4 LDs implicate immunity/protein transport; E3-enriched LD proteins are associated with fatty acid beta-oxidation

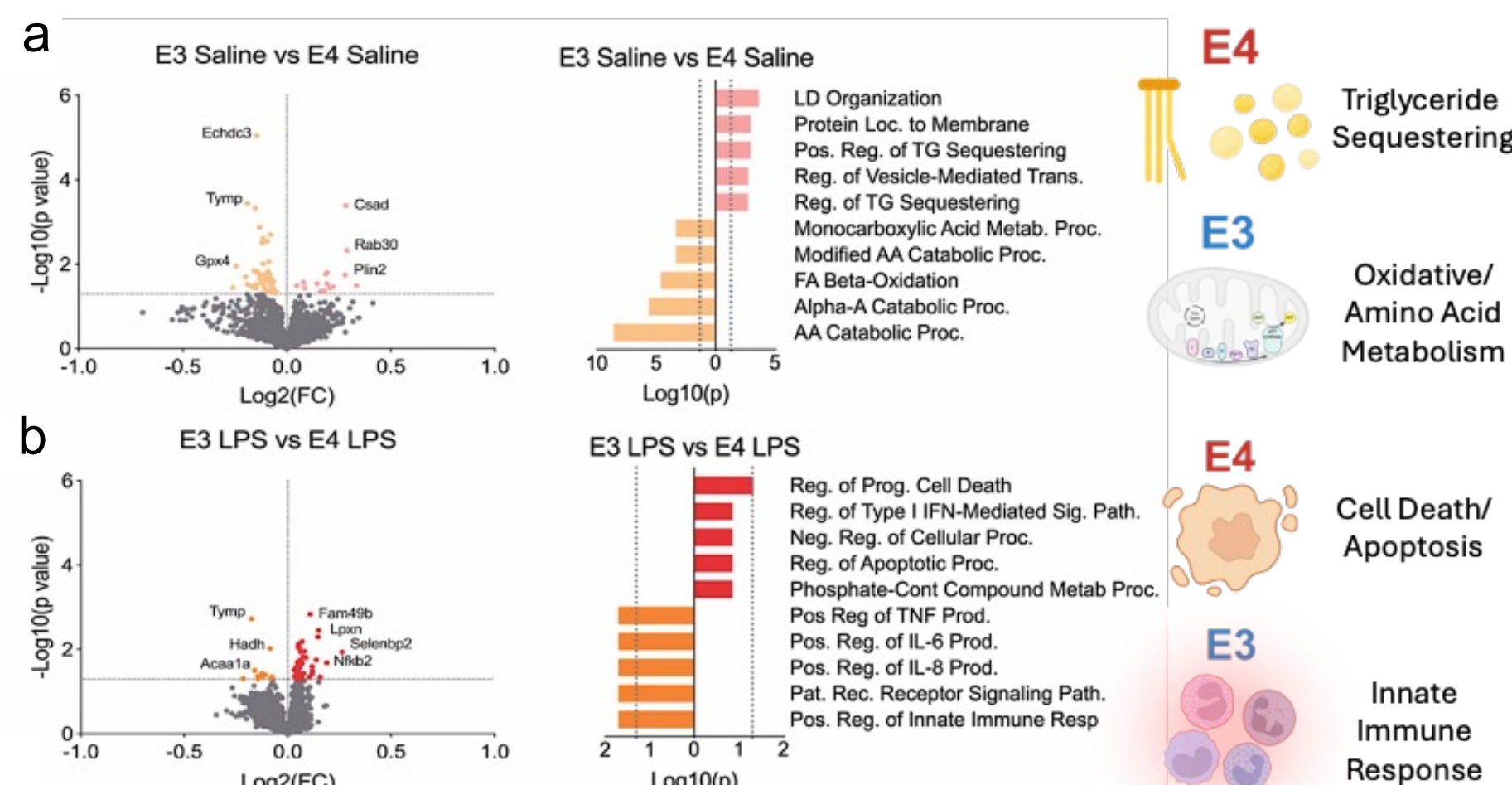


Figure 2. APOE genotype remodels the lipid droplet-associated proteome. Quantitative proteomics of lipid droplet-enriched fractions from APOE3/3 and APOE4/4 mice revealed genotype-specific differences at baseline and following LPS stimulation. (a) Under saline conditions, APOE4 droplets were associated with lipid droplet organization, membrane trafficking, and triglyceride sequestration, whereas APOE3 droplets were linked to fatty acid β -oxidation and amino acid metabolism. (b) Following LPS, APOE4 droplets were associated with cell death/apoptosis and type I interferon signaling, while APOE3 droplets retained stronger associations with innate immune response pathways. Volcano plots display genotype-dependent protein abundance, and bar plots summarize enriched biological pathways.

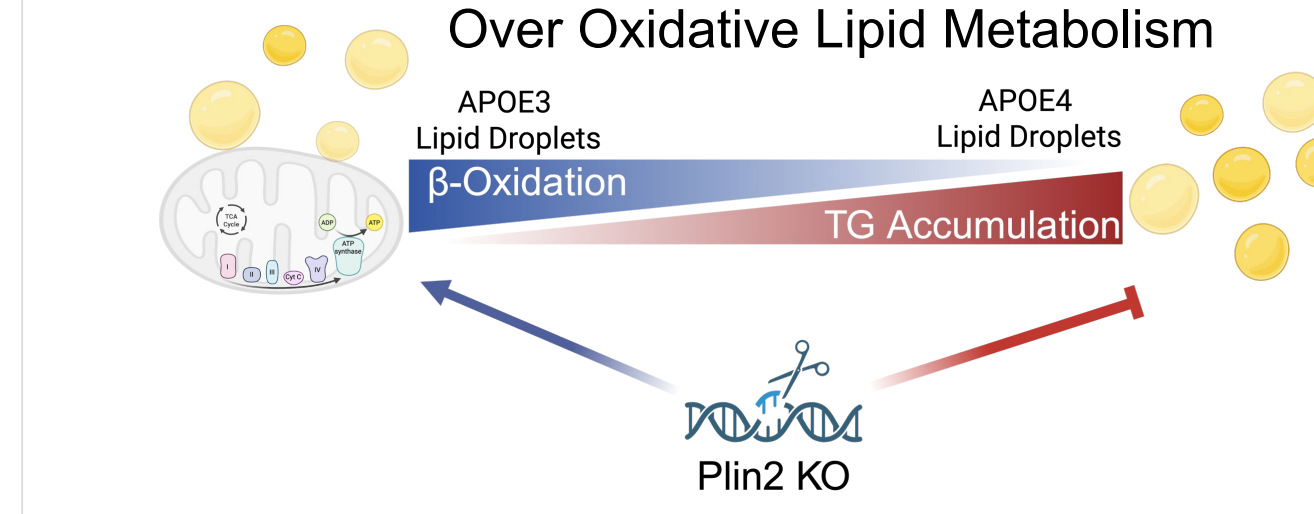
Plin2 KO significantly impacts the transcriptomic response of microglia to AD relevant stimuli



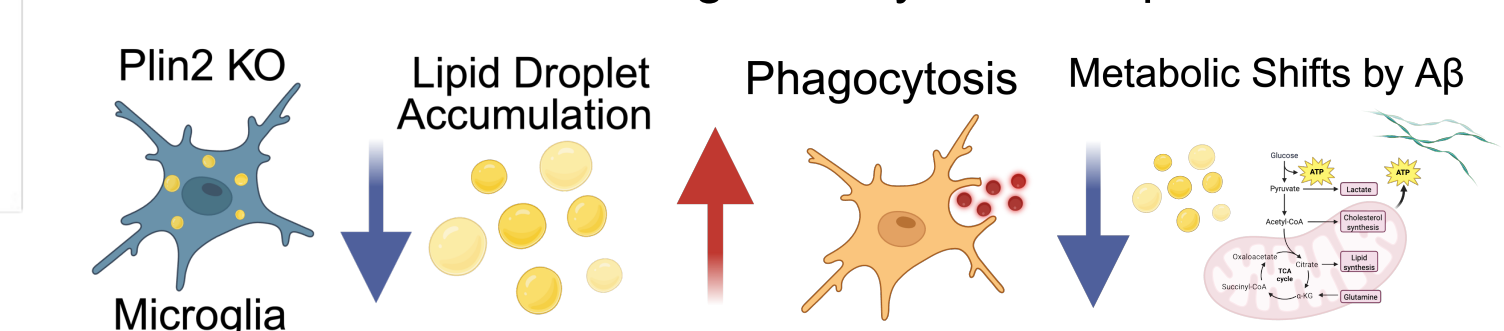
Figure 5. PLIN2 knockout reshapes BV2 microglial transcriptional responses to AD-relevant stimuli. WT and Plin2 KO BV2 microglia were treated with control media, oleic acid, damaged N2a cells, myelin debris, or amyloid- β for 24 hours prior to bulk RNA-seq. PCA revealed distinct transcriptional clustering by treatment and genotype. Gene Ontology analysis showed that WT cells were associated with sterol/cholesterol biosynthetic programs across several conditions, while KO cells displayed stimulus-specific changes in immune migration, mitochondrial respiration, oxidative phosphorylation, and RNA processing pathways. In the amyloid- β condition, KO cells exhibited a unique transcriptional response enriched for mitochondrial respiratory chain assembly, NADH dehydrogenase complex assembly, oxidative phosphorylation, and RNA splicing. These findings indicate that PLIN2 loss alters both basal and stimulus-induced microglial transcriptional states.

Summary & Works in Progress

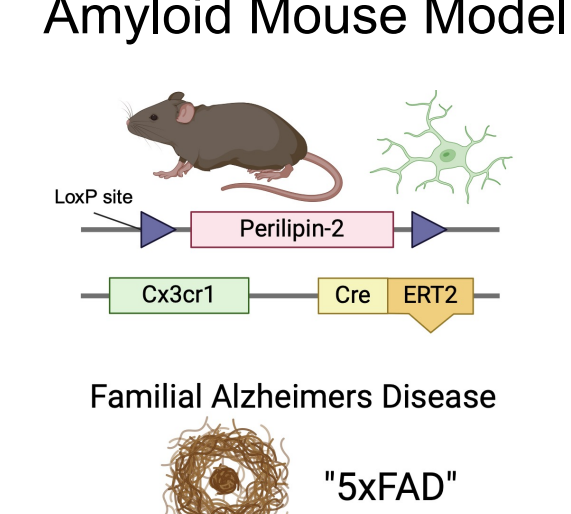
APOE4 Favors Triglyceride Accumulation Over Oxidative Lipid Metabolism



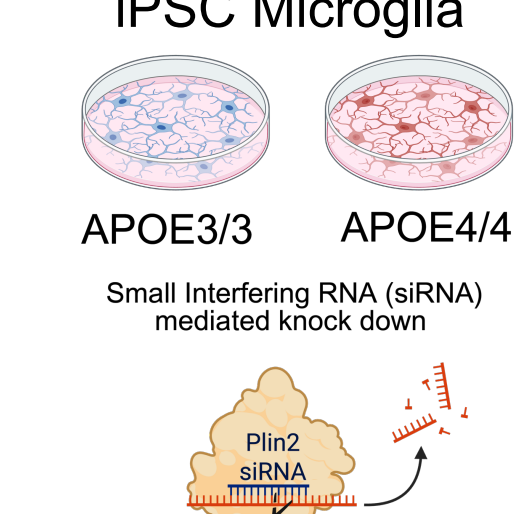
Plin2 Knockout Shifts Microglia Away from a Lipid Laden State



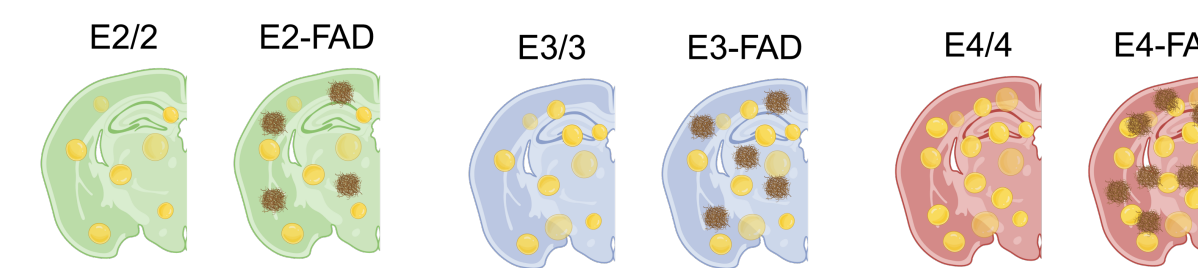
Microglia Plin2 KO in Amyloid Mouse Model



Lowering Plin2 in E4 iPSC Microglia



APOE Mouse Brain Lipid Droplet Atlas



References

- Alzheimer, A. An English translation of Alzheimer's 1907 paper. "Über eine eigenartige Erkrankung der Hirnrinde." Clin Anat. 1995;8(6):429-431.
- Cohen, S. Lipid droplets as organelles. Int Rev Cell Mol Biol. 2018;337:83-110.
- Olzmann, J. A., & Carvalho, P. Dynamics and functions of lipid droplets. Nat Rev Mol Cell Biol. 2019;20:137-155.
- Farmer, B. C., Walsh, A. E., Klumper, J. C., & Johnson, L. A. Lipid droplets in neurodegenerative disorders. Front Neurosci. 2020;14:742.
- Marshallinger, J. et al. Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. Nat Neurosci. 2020;23(2):194-208.
- Conte, M. et al. Expression pattern of perilipins in human brain during aging and in Alzheimer's disease. Neuropathol Appl Neurobiol. 2022;48(1):e12756.
- Itabe, H. et al. Perilipins: a diversity of intracellular lipid droplet proteins. Lipids Health Dis. 2017;16:83.
- Liu, C. C. et al. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol. 2013;9(2):106-118.
- Farmer, B. C., Klumper, J. C., & Johnson, L. A. Apolipoprotein E4 alters astrocyte fatty acid metabolism and lipid droplet formation. Cells. 2019;8(2).