

# Reduced LACTB expression in myeloid cells is associated with elevated succinylcarnitine and reduced Alzheimer's disease risk

**Alison Goate**

[alison.goate@mssm.edu](mailto:alison.goate@mssm.edu)

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-0576-2472>

**Carmen Romero-Molina**

Icahn School of Medicine at Mount Sinai

**Ruben Gomez-Gutierrez**

The Belfer Neurodegeneration Consortium

**Wen Yi See**

Icahn School of Medicine at Mount Sinai

**Tulsi Patel**

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-2797-1443>

**Hayk Davtyan**

University of California Irvine <https://orcid.org/0000-0001-8554-6456>

**Jiacheng Ma**

The Belfer Neurodegeneration Consortium

**Quanyun Xu**

University of Texas MD Anderson Cancer Center

**Michael Sewell**

<https://orcid.org/0000-0003-0113-6551>

**Kendra Allton**

The University of Texas M. D. Anderson Cancer Center <https://orcid.org/0000-0002-4204-0807>

**Morgan McReynolds**

The Belfer Neurodegeneration Consortium

**Olivia Calderon**

The Belfer Neurodegeneration Consortium

**Yaima Lightfoot**

The Belfer Neurodegeneration Consortium

**Guido Bommer**

de Duve Institute, UCLouvain <https://orcid.org/0000-0001-6898-4884>

**Carlos Cruchaga**

Washington University School of Medicine <https://orcid.org/0000-0002-0276-2899>

**Mathew Blurton-Jones**

University of California, Irvine <https://orcid.org/0000-0002-7770-7157>

**William Ray**

The University of Texas MD Anderson Cancer Center <https://orcid.org/0000-0002-4491-167X>

**Edoardo Marcora**

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-3829-4927>

---

## Article

**Keywords:** LACTB, microglia, Alzheimer's Disease, immunometabolism

**Posted Date:** July 13th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10216155/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

**Additional Declarations:** **Yes** there is potential Competing Interest. W.J.R. consults for Cerevance, OrbiMed, SealRock, NeuroVanda, and Reservoir. A.M.G. serves on the SRB for Genentech and for Muna Therapeutics, she has served as a consultant for Merck and Arbor-bio. M.B.-J. is a co-founder and consultant for Savanna Biotherapeutics.

---

## Abstract

Lactamase  $\beta$  (LACTB) is a serine  $\beta$ -lactamase-like mitochondrial enzyme genetically associated with obesity, kidney disease, and hyperlipidemia. *LACTB* is located in an Alzheimer's Disease (AD) risk locus and its expression in the brain has been genetically associated with AD susceptibility. The aim of this study was to investigate LACTB function and genetic link to AD in myeloid cells, due to their central role in modulating AD risk. Our Mendelian randomization analyses revealed that lower *LACTB* expression in myeloid cells is genetically associated with reduced disease susceptibility and increased succinylcarnitine, a metabolite independently associated with AD risk. We identified LACTB as a primary enzyme responsible for succinylcarnitine hydrolysis. In human macrophages and microglia, LACTB loss promoted enhanced oxidative phosphorylation, reduced protein synthesis and altered lipid homeostasis. *LACTB* expression was upregulated following interferon or TNF stimulation, and LACTB loss modified efferocytosis-related functions under inflammatory conditions. *In vivo*, xenotransplanted human LACTB knockout microglia showed enhanced association with amyloid plaques in the mouse brain. Together, these findings experimentally validated the genetic association between reduced LACTB expression and elevated succinylcarnitine and identified LACTB as an inflammation-responsive regulator of myeloid cell metabolism and function that may contribute to its protective genetic association with AD. Given its druggability and potential to use succinylcarnitine as a genetically-validated endophenotype and target engagement biomarker, LACTB represents a promising therapeutic target for AD.

## Background

Lactamase  $\beta$  (LACTB) is a  $\beta$ -lactamase-like protein that forms filamentous structures in the mitochondrial intermembrane <sup>1</sup>. Structural and biochemical analyses demonstrated that LACTB contains a conserved catalytic triad characteristic of serine proteases <sup>2</sup> and harbors a D-aspartyl endopeptidase (DAEP) activity that preferentially cleaves at D-Asp residues, which accumulate in long-lived proteins during aging and are present in Alzheimer's disease (AD)-relevant proteins such as A $\beta$  and tau <sup>3–6</sup>.

LACTB has been implicated in controlling cell proliferation and differentiation, with emerging roles in regulating tumor progression and metabolic homeostasis <sup>7,8</sup>. LACTB has been nominated as an obesity risk gene <sup>9</sup>, and more specifically, to be part of a macrophage-enriched metabolic network causally tied to metabolic syndrome traits <sup>9–11</sup>. Mice overexpressing LACTB have shown increased fat-mass-to-lean-mass ratio <sup>10</sup>, and impaired glucose tolerance and insulin resistance <sup>12</sup>.

Genes in this macrophage-enriched metabolic network, in which LACTB is a key node, are significantly enriched within a immune/microglia brain module identified in late-onset AD, in which the TREM2 signaling adaptor TYROBP is a master regulator <sup>13</sup>. *LACTB* is located in a well-established AD risk locus (15q.22) <sup>14–16</sup>, often labeled as “APH1B locus” due to the fact that this gene, located in close proximity to *LACTB*, encodes one of the subunits of the  $\gamma$ -secretase complex that cleaves APP and thus is highly relevant to AD pathogenesis. Importantly, LACTB has been associated with AD in a proteome-wide association study using brain protein abundance quantitative trait loci (pQTLs) <sup>17</sup> and nominated as an AD target by Agora <sup>18,19</sup>. The human genome contains one other  $\beta$ -lactamase-like gene (on chromosome 8) named LACTB2, which has also been associated with AD risk by a transcriptome-wide association study integrating myeloid expression quantitative trait loci (eQTLs) with GWAS data <sup>20</sup>.

AD, the most common type of dementia, is a neurodegenerative disorder associated with the abnormal accumulation of amyloid plaques and neurofibrillary tangles in the brain, coupled with neurodegeneration, gliosis, lipidosis, and vascular alterations. Genetic studies have consistently implicated myeloid cells of the innate immune system in the

etiology of AD <sup>21–23</sup>. Microglia are the resident macrophages of the central nervous system (CNS), responsible for maintaining brain health and responding to injury or infection, mainly through efferocytosis <sup>24,25</sup>. Microglial activation and/or dysfunction are hallmarks of AD and other neurodegenerative disorders. While microglia are the major CNS-resident macrophages, other macrophage populations—including perivascular, meningeal, and choroid plexus macrophages—also contribute to neuroinflammation and disease <sup>26</sup>.

LACTB loss-of-function has been associated with increased succinylcarnitine levels both in mice <sup>27</sup> and human <sup>28–30</sup> metabolite GWAS. Importantly, higher succinylcarnitine levels in cerebrospinal fluid (CSF) and brain have also been associated with lower AD risk <sup>31</sup>. Together these data suggest that lower LACTB expression leads to higher succinylcarnitine levels and may protect against AD. LACTB has a hydrolase activity <sup>3</sup>, but it is still unknown whether it can alter succinylcarnitine levels by direct cleavage.

The aim of this study is to better understand the relationship between reduced expression of LACTB in myeloid cells and i) succinylcarnitine levels, ii) AD risk, and iii) disease-relevant phenotypes *in vitro* and *in vivo*.

## Results

### Lower LACTB expression in myeloid cells leads to higher succinylcarnitine levels and lower Alzheimer's disease risk

To evaluate the potential contribution of LACTB (and APH1B) expression and succinylcarnitine levels to AD risk within the chromosome 15q GWAS locus, we performed Mendelian Randomization (MR) analyses integrating AD GWAS associations<sup>14</sup> with myeloid gene expression<sup>32</sup> and CSF metabolite abundance<sup>31</sup> quantitative trait loci (e/mQTLs). As shown in Fig. 1A, our pairwise MR analyses suggest that lower LACTB mRNA expression in myeloid cells and higher succinylcarnitine levels in the CSF are causally associated with lower AD risk. Of the 440 CSF metabolites in the mQTL dataset<sup>31</sup>, we only tested the association with succinylcarnitine because it is the only metabolite with statistically significant associations at this locus, consistent with previous plasma metabolome-wide association studies<sup>31</sup>. Moreover, we did not find a statistically significant association between genetically encoded APH1B expression in myeloid cells and either succinylcarnitine levels in the CSF or AD risk (Additional file 1). This does not exclude the possibility that APH1B may play a causal role in modulating AD risk in other cell types.

Although LACTB is expressed in several cell types within the brain, it is more highly expressed in immune cells compared to other cell populations, in contrast to the expression of other mitochondrial proteins such as SDHA<sup>33</sup> (Fig. 1B). In addition, we tested whether LACTB expression is upregulated when human progenitor cells are differentiated into macrophages or microglia *in vitro*. Upon differentiation of THP1 monocytes into macrophages, or induced-pluripotent stem cells (iPSCs) into microglia (iMGLs), we observed an increase in LACTB expression at both mRNA and protein levels (Fig. 1C, D). Consistent with this, the Abud et al., 2017 dataset <sup>34</sup>, interrogated via Stemformatics website <sup>35</sup>, confirmed increased LACTB expression in myeloid cells compared to hematopoietic progenitor cells (HPCs) or to iPSC (Supplementary Fig. 1A).

Next, to investigate the impact of reduced LACTB levels, we generated LACTB knock-down (KD) THP1 macrophages (~ 50% reduction at the protein level), and LACTB knock-out (KO) WTC11 iMGLs (Supplementary Fig. 1B-F). In both models, we observed a significant increase in succinylcarnitine levels in cell lysates upon LACTB KD/KO (Fig. 1E), experimentally validating the causal predictions of our MR analyses. No other metabolites in our panel were statistically significantly altered (Supplementary Fig. 1G, H). We also observed a concordant increase in succinylcarnitine levels in the cell culture media from LACTB KD THP1 macrophages and LACTB KO WTC11 iMGLs compared to scrambled (SCR) and wild-type (WT) controls, respectively (Fig. 1F).

We further validated the causal link between reduced LACTB expression and increased succinylcarnitine levels *in vivo*. First, we used a mixed background (C57BL/6 x SJL) mouse model, where the SJL background includes a mutation that reduces LACTB protein levels (unpublished work <sup>36</sup>). We observed that there are mice with high or low LACTB levels in the brain lysate within the same litter, which was inversely correlated with succinylcarnitine levels (Fig. 1G). Second, we generated a mouse model with a S162I mutation, inactivating the key residue within the LACTB catalytic pocket <sup>2,37</sup>, thereby leading to a LACTB enzymatically-dead (ED) mouse model. This mutation barely alters LACTB mRNA expression in total brain lysate (hippocampus), isolated microglia or isolated astrocytes (Supplementary Fig. 1G). However, using an antibody directed against an epitope located in the active site, we observed a mutation-dependent decrease in LACTB immunodetection when one or both alleles were mutated (Supplementary Fig. 1H). Succinylcarnitine levels in liver, brain lysate (posterior cortex), isolated microglia and isolated astrocytes were increased upon LACTB inactivation in a dose dependent manner (wild-type (WT) LACTB<sup>+/+</sup> < Het LACTB<sup>ED/+</sup> < Hom LACTB<sup>ED/ED</sup> mice) (Fig. 1H, n = 2 mice per group), corroborating the hypothesis that LACTB expression is a major determinant of succinylcarnitine levels *in vivo*.

As aging is the primary risk factor for AD, we measured the levels of LACTB and succinylcarnitine in aged mice. We observed a mild increase in LACTB levels (Fig. 1I), accompanied by a significant decrease in succinylcarnitine (Fig. 1J) in the brains of aged (12 months old) compared to young (2 months old) WT mice. Therefore, inhibition of LACTB may restore age-related declines in succinylcarnitine levels.

In summary, we showed that lower LACTB expression in myeloid cells and higher succinylcarnitine levels in the CSF are associated with lower AD risk. In addition, we experimentally demonstrated that lower expression of LACTB in myeloid cells or inactivation of LACTB enzymatic activity lead to higher levels of succinylcarnitine in mouse and human models.

## LACTB directly cleaves succinylcarnitine, and succinylation inhibits LACTB activity

To explain how reduced LACTB expression leads to increased succinylcarnitine levels, we investigated whether LACTB can directly cleave succinylcarnitine. We performed a cell-free assay using in-house generated recombinant LACTB and a custom <sup>13</sup>C<sub>7</sub>-labeled-succinylcarnitine substrate (all carbons labeled, TRC-S102985, LGC). This tracer allows for direct quantification of the cleavage product. Incubation of recombinant LACTB (100 nM) with increased concentrations of <sup>13</sup>C<sub>7</sub>-labeled-succinylcarnitine for 2 hours yielded a dose-dependent increase in the cleavage product <sup>13</sup>C<sub>3</sub>-carnitine (Fig. 2A), confirming direct LACTB enzymatic cleavage of succinylcarnitine. Importantly, this cleavage was abolished when recombinant LACTB protein was preincubated with a LACTB inhibitor (Ac-IEPD-CHO), further demonstrating that succinylcarnitine cleavage is dependent on LACTB activity.

To confirm these findings in a biological system, we conducted isotope tracing experiments in WT and LACTB KO iMGLs with commercially available <sup>13</sup>C<sub>3</sub>-succinylcarnitine (TRC-S688832, LGC). As in the recombinant LACTB assay, we hypothesized that, after succinylcarnitine cleavage, there would be an increase in labeled carnitine (<sup>13</sup>C<sub>3</sub>-carnitine). Consistent with our cell-free results, we observed a progressive increase in the pool of labeled carnitine after 6 and 24 hours in WT iMGLs (Fig. 2B). At both time points, there was a significant reduction in the abundance of labeled carnitine in LACTB KO iMGLs compared to control iMGLs (n = 2 clones per genotype).

Finally, we utilized the <sup>13</sup>C<sub>7</sub>-labeled-succinylcarnitine tracer to track the metabolic fate of both the carnitine and succinyl moieties following succinylcarnitine cleavage. After 24 hours of treatment in WTC11 iMGLs, we detected the

expected labeling of carnitine, alongside modest enrichment in acetyl-carnitine, myristoyl-carnitine, palmitoyl-carnitine, and succinate (Fig. 2C, n = 2 clones).

Protein succinylation is a recently described reversible post-translational modification (PTM) where a succinyl group is covalently attached to a lysine residue driven, for example, by increased succinate levels. By adding bulk and changing the lysine's charge, this PTM can drastically alter protein structure and function. Prior work has shown that LACTB activity is reduced when LACTB is succinylated in cancer cells<sup>38</sup>. Therefore, LACTB succinylation may be part of a negative feedback loop regulating LACTB cleavage of succinylcarnitine into succinate and carnitine. To explore LACTB succinylation, we transfected HEK cells with empty, LACTB WT or LACTB ED (S164I) vectors and treated them with diethyl succinate (2 mM). Upon treatment with diethyl succinate for 4 hours, we observed substantial succinylation of LACTB in HEK cells overexpressing LACTB WT protein. When LACTB protein was pulled-down by immunoprecipitation (IP) using the LACTB antibody (top 2 blots (IP:LACTB), Fig. 2D), only cells expressing the WT version of LACTB showed succinylation of LACTB (IB: SuK blot, Fig. 2D) after the treatment. In contrast, cells expressing enzymatic-dead LACTB (S164I mutant) did not show succinylation, suggesting that LACTB succinylation is modulated by its own activity. We confirmed this finding in a follow up immunoprecipitation using the succinyllysine antibody (bottom 2 blots (IP:SuK), Fig. 2D). By blotting this SuK-IP fraction, we found that LACTB was the main succinylated protein in these cells (IB: LACTB blot, Fig. 2D). To investigate how post-translational modification further influences LACTB enzymatic function, we measured LACTB activity using the previously reported succinyl-D-aspartate fluorescent assay<sup>3</sup>, and levels of succinylcarnitine. LACTB succinylation reduced the cleavage of the succinyl-D-aspartate substrate and increased succinylcarnitine levels, indicating inhibition of LACTB activity (Figs. 2E and F). Similarly, we observed that treatment with diethyl succinate led to LACTB succinylation in THP1 macrophages (Fig. 2G) and to a corresponding reduction of LACTB activity, measured by succinyl-D-aspartate cleavage and succinylcarnitine levels (Figs. 2H and 2I).

Taken together, experiments with purified protein and isotopic tracing demonstrate that LACTB not only cleaves succinylcarnitine under cell-free biochemical conditions but is also a major determinant of its catabolism in cells. In addition, we showed that succinylation is a post-translational modification that inhibits LACTB activity, suggesting a regulatory negative feedback loop.

### **LACTB KD/KO in myeloid cells alters expression of genes in pathways related to oxidative phosphorylation, protein translation, cell proliferation and immune response**

To investigate the transcriptional consequences of LACTB KD/KO, and the consequent increase in succinylcarnitine, in myeloid cells, we performed bulk RNA-seq in our cell models. In THP1 macrophages (n = 4), LACTB KD led to the upregulation of 82 genes (logFC > 1, p.value < 0.05) and downregulation of 66 genes (logFC < -1, p.value < 0.05) (Table 2). We performed Gene Set Enrichment Analysis (GSEA) with the "Biological Process" subset of Gene Ontology genesets, and found a positive enrichment of pathways related to the response to interferon (IFN) and to the tumor necrosis factor (TNF) ("GOBP\_response\_to\_virus"), oxidative phosphorylation, and protein localization to the endoplasmic reticulum, in LACTB KD macrophages compared to macrophages treated with scrambled siRNA (SCR) as controls (Fig. 3A and Table 2). We also found a negative enrichment of proliferation-related pathways (Fig. 3A and Table 2).

Next, we performed bulk RNA-seq on WTC11 LACTB KO and WT iMGLs (n = 3 clones per genotype, 2 differentiations per clone). Similar to the observations found in THP1 macrophages, there was a positive enrichment of pathways related to the immune response, oxidative phosphorylation, and protein translation in LACTB KO iMGLs compared to isogenic controls (Fig. 3B and Table 2). In contrast, we found that complete loss of expression of LACTB in microglia

led to a positive enrichment of cell proliferation-related pathways (“GOBP\_DNA\_replication”), compared to the negative enrichment observed in LACTB KD macrophages. Also, complete loss of LACTB led to a negative enrichment of cell adhesion and cell signaling pathways (Fig. 3B and Table 2).

To examine the relevance of these findings based on macrophage/microglia-like cells *in vitro* to microglial cells *in vivo* under disease conditions, we interrogated and re-analysed six publicly available single-cell RNAseq microglial human AD datasets<sup>39–44</sup>, and performed correlation analyses to identify genes whose expression is associated with LACTB levels across microglial states. Our analysis focused on determining associations with leading-edge genes from significantly enriched pathways identified by GSEA of our bulk RNA-seq data derived from THP1 KD and iMGL KO systems. Strikingly, LACTB expression was most often strongly positively associated with genes implicated in pro-inflammatory pathways identified in both THP1 and iMGL leading-edge analyses, including *IL1B*, *IL6*, *CCL3*, and *CCL4* (Fig. 3C, Supplementary Fig. 2A), which encode pro-inflammatory cytokines<sup>45</sup>. These findings further support the idea that LACTB acts as a modulator of immune responses in myeloid cells. Genes consistently negatively correlated with LACTB expression included the AD risk gene *ABCA7*, identified in GSEA leading-edge analyses for both THP1s and iMGLs (Supplementary Fig. 2A), and implicated in pathways such as lipid metabolism and efferocytosis<sup>46–48</sup>. Additional negatively correlated genes included those involved in cell cycle regulation identified in our THP1 and iMGL leading-edge analyses, such as *CDKN2C*<sup>49</sup>, *MCM2*<sup>50</sup>, and *PCNT*<sup>51</sup>, implicating a role for LACTB in cell-cycle regulation in an *in vivo* context.

Single-cell RNAseq on WT and LACTB KO microglia (3 clones per genotype, 1–2 differentiations per clone) also implicated LACTB in similar pathways. Across the 12 clusters identified (Fig. 3D and Table 3), we found that LACTB was most highly expressed in clusters enriched for cytokine response (iMG3) and MHC/antigen presenting (iMG10) processes (Fig. 3E and Table 3). In agreement with our bulk RNAseq results in microglia, our single-cell data showed alterations in the cell proportion for clusters enriched for ribosomal processing and translation functions (iMG7), and related to the immune response (iMG10, iMG12) (Fig. 3F and Table 3).

Finally, we explored the *in vivo* effects of loss of LACTB enzymatic activity by performing bulk RNA sequencing and GSEA analysis of mouse microglia isolated from LACTB ED (homozygous) and control mice (3 months old, n = 3) (Fig. 3G and Table 2). In line with the *in vitro* results, we observed a positive enrichment of pathways related to IFN/TNF response and antigen-presentation. Negatively enriched pathways included proliferation, cell signaling and vesicle fusion.

In short, at the transcription level, we observed that LACTB is involved in pathways related to immune response, cell proliferation, translation and mitochondrial metabolism.

## **LACTB KD/KO in myeloid cells increases oxidative phosphorylation, and reduces protein synthesis and triacylglycerides**

Previous reports have linked LACTB to differentiation and proliferation processes in cancer models<sup>7</sup>. Also, our data showed an increase in LACTB gene and protein expression after differentiation from THP1 monocytes to macrophages and iPSC to iMGLs (Fig. 1C, D). To determine whether the observed transcriptional differences were due to alterations in the differentiation process, we examined the expression of microglia/macrophage markers. As shown in Supplementary Fig. 2B, LACTB KD/KO did not induce changes in the mRNA expression of microglia/macrophage markers (*CSF1R*, *P2RY12*, *CX3CR1*, *TGFBR1*) in either THP1-derived macrophages or WTC11 iMGLs, measured at the end of the differentiation process. In addition, as proliferation pathways were

negatively/positively enriched in LACTB KD/KO macrophages/microglia, respectively, we analyzed cell division using a DNA intercalating agent (EdU). A statistically significant reduction in proliferation was observed in LACTB KD THP1 macrophages compared to SCR (Supplementary Fig. 2C, left panel), as predicted by the transcriptional analysis (Fig. 3A). However, no statistically significant changes were observed between LACTB KO and WT WTC11 iMGLs (Supplementary Fig. 2C, right panel).

Next, we validated the positive enrichment in the oxidative phosphorylation pathway at the transcriptional level observed upon LACTB KD/KO in myeloid cells through Seahorse assays. Importantly, we observed that LACTB depletion increased basal and maximal respiration in human myeloid cells (Fig. 4A). Given that LACTB forms filaments in the mitochondrial intermembrane, we also examined other mitochondrial features. LACTB KD/KO myeloid cells showed a slight reduction in mitochondrial membrane potential compared to controls (Supplementary Fig. 3A). No major qualitative morphological alterations in mitochondria were observed in LACTB KO microglia compared to WT, by electron microscopy (Supplementary Fig. 3B). Our results suggest that LACTB loss enhances bioenergetic capacity without causing gross alterations to mitochondrial structure.

Changes in cellular metabolism can directly impact protein synthesis, as energy and nutrient availability regulate translation initiation<sup>52</sup>. Consistent with this, our bulk RNAseq analysis of LACTB KD/KO myeloid cells revealed enrichment of pathways related to translation (“GOBP\_protein\_localization\_to\_endoplasmic\_reticulum” and “GOBP\_cytoplasmic\_translation”, Fig. 3A and 3B). Supporting this relationship, we observed that, in our iMGL single-cell RNAseq analysis, the genes co-expressed with LACTB were most enriched in cluster iMG7 (Supplementary Fig. 3C and Table 3), which is characterized by ribosomal biogenesis and translation-related processes (Fig. 3D, using ARACNe<sup>53</sup>). At the functional level, using a methionine analog to measure nascent protein synthesis, we observed a significant reduction in protein synthesis in LACTB KD/KO myeloid cells compared to controls (Fig. 4B).

To complete our metabolic characterization, we evaluated the impact of LACTB on the lipidomic profile, as LACTB has been nominated as an obesity gene in adipocytes<sup>9</sup> and the LACTB locus shows genome-wide significant associations with several plasma lipid/lipoprotein traits (phospholipids, cholesterol, cholesteryl esters, triglycerides, apolipoprotein A) in GWAS studies<sup>54</sup>. Additionally, our single-cell study has shown a mild increase in the proportion of cells in iMG6 (a cluster related to lipid transport) in LACTB KO microglia compared to WT (Fig. 3F and Table 3). Compared to SCR, LACTB KD macrophages exhibited increased levels of ceramides, including dihydroceramides (dhCer), ceramides (Cer), and lactosylceramides (LacCer) (Fig. 4C1). Alterations in ceramide abundance may reflect changes in lysosomal function<sup>55</sup>. In contrast, ceramide levels were not significantly changed in LACTB KO iMGLs compared to WT (Fig. 4C2).

In addition, we observed a decrease in cholesterol ester (CE) and in tri-acylglycerides (TG) in LACTB KD/KO myeloid cells compared to SCR/WT (Fig. 4C). To further explore this finding, we measured the content of neutral lipids using BODIPY fluorescent dye (Supplementary Fig. 3D). While no significant differences were found between LACTB KD and SCR macrophages, LACTB KO microglia exhibited a mild reduction in BODIPY fluorescence compared to WT. We also assayed cholesterol efflux, measuring HDL-associated labeled-cholesterol in the supernatant relative to cellular content upon treatment with HDL as cholesterol acceptor. We observed a significant increase in cholesterol efflux in LACTB KD THP1 macrophages compared to SCR, while no differences across genotypes in iMGLs (Supplementary Fig. 3E). Together, these data suggest that reduction in LACTB expression may contribute to lower TGs levels, potentially through enhanced cholesterol efflux or reduced neutral lipid accumulation, in a cell-type-dependent manner.

In conclusion, we have observed that LACTB KD/KO increases oxidative phosphorylation, reduces protein synthesis and modifies lipid metabolism in myeloid cells.

## **LACTB KD/KO in myeloid cells alters efferocytosis-related pathways after immune stimulation**

Because pathways related to the IFN and TNF response emerged as some of the most consistently affected pathways in LACTB KD/KO/ED myeloid cells across model systems, we tested whether LACTB expression is modulated by these cytokines. Upon stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$ , we detected an increase in LACTB both at the mRNA and protein levels (Fig. 5A and Supplementary Fig. 4A). In line with this induction of LACTB following immune activation, succinylcarnitine levels decreased in cells treated with these cytokines (Fig. 5B).

To assess whether LACTB KD/KO would impair the transcriptional response to IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$  treatment, we quantified the expression of IFN/TNF-responsive genes after 6–24 hours of stimulation with these cytokines (qPCRs, Supplementary Fig. 4B). Overall, LACTB loss did not substantially alter the transcriptional response to cytokine stimulation relative to baseline in either macrophages or microglia. However, LACTB KD THP1 macrophages showed increased induction of IL1B, STAT1, and TNF- $\alpha$  following IFNs treatment, accompanied by reduced expression of CLEC7A and HLA-DQ1 compared to controls. LACTB KO iMGLs showed an increase in TNF- $\alpha$  and HLA-DQA1 compared to WT iMGLs. In addition, we analyzed cytokine secretion in cell supernatants (Supplementary Fig. 4C). While LACTB reduction/loss did not broadly alter cytokine release in response to stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$ , THP1 LACTB KD macrophages secreted higher levels of IL-8, and MIP-1 $\alpha$  (also known as CCL3, a cytokine found to be co-expressed with LACTB, Fig. 3C), whereas LACTB KO iMGLs released lower levels of IL-6, IL-10, IP-10, and TNF $\alpha$  compared to control cells.

As pro-inflammatory microglia activation has been proposed to be required to clear myelin<sup>56,57</sup>, we sought to investigate the efferocytic activity of myeloid cells after stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$ . Efferocytosis refers to the ability of macrophages to find, phagocytose, degrade, and dispose of lipid-rich cellular debris. In the brain parenchyma, efferocytosis is mainly performed by microglia and it is critically involved in AD pathogenesis<sup>24</sup>. We examined whether LACTB KD/KO myeloid cells exhibited altered efferocytosis of myelin debris following stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$ . In THP1 macrophages we observed a trend towards a reduction in myelin phagocytosis and a significant decrease in lysosomal acidification (lysosensor marker) in LACTB KD macrophages compared to SCR after stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$  (Fig. 5D). In LACTB KO iMGLs, we observed a decrease in the lysosomal mass (lysotracker marker) and lysosomal proteolytic capacity (DQBSA processing) in LACTB KO iMGLs compared to WT after stimulation with these cytokines.

In both cell systems, LACTB KD/KO affected some steps of the efferocytosis pathway. These results are in line with the reduction in phagocytic and lysosomal capacity observed in DLAM THP1 macrophages<sup>58</sup> and PU1 KD BV2 cells<sup>59</sup>, both proposed to be associated with AD protection.

## **LACTB KO human microglia show increased clustering around amyloid plaques in a mouse model of amyloid deposition**

Finally, to address whether LACTB KO in human microglial cells would modify their function related to amyloid pathology *in vivo*, we xenotransplanted WT and isogenic LACTB KO (2 clones per genotype) microglia precursors into the brain of newborn hCSF1-5xFAD female mice<sup>60,61</sup>, which differentiated into microglia. Mice were sacrificed at 6 months old (Fig. 6A) and we confirmed engraftment of human microglia in the mouse brain, and did not detect

significant differences between LACTB KO vs WT xenotransplanted human microglia (Fig. 6B). As shown in Fig. 6B, isolated LACTB KO human microglia showed alterations in the metabolic panel compared to WT human microglia, including a significant increase in acetyl-CoA and a mild decrease in  $\alpha$ -ketoglutarate, choline and serine. Due to the low sample input, we could not detect succinylcarnitine levels. However, we observed a decrease in carnitine, acetyl-carnitine, palmitoyl-carnitine and succinate in LACTB KO versus control microglia, which may be a result of the expected reduction in succinylcarnitine cleavage (as shown in Fig. 2C and D, *in vitro*).

To characterize the *in vivo* consequences of LACTB KO in microglia, we performed immunohistochemistry followed by quantitative image analysis. We did not observe significant differences in the area covered by microglia, intensity, or average size of microglia (IBA1+, Supplementary Fig. 5A). However, we found a significant increase in the number of LACTB KO human microglia (IBA1 + KU80+) around amyloid plaques compared to WT (Fig. 6E). We also found a mild decrease in the amyloid plaque area, average size, and plaque number measured by A $\beta$ 42 immunostaining (Fig. 6E and Supplementary Fig. 5B). When classifying amyloid plaques by size, we observed a slight reduction in the number of large plaques (> 40  $\mu$ m) in mice xenotransplanted with LACTB KO vs WT human microglia (Supplementary Fig. 5B). Similar effects were observed using another amyloid marker (MOAB, Supplementary Fig. 5C). We also double-stained the amyloid plaques with a diffuse amyloid marker (MOAB) and a dense core marker (ThioS) and observed a mild increase in plaque compaction (higher ThioS/MOAB ratio) in hCSF1-5XFAD mice xenotransplanted with LACTB KO compared to mice xenotransplanted with WT human microglia (Fig. 6F).

Finally, as we observed that LACTB is involved in the immune response *in vitro*, we immunostained with microglia activation makers (LGALS3 and CD9). We observed a subtle increase in CD9 and LGALS3 markers (area covered in cortex plus hippocampal regions) in mice xenotransplanted with LACTB KO human microglia compared to WT (Fig. 6G, H). Because we observed that LACTB KO led to altered transcription of genes with roles in antigen-presentation (Fig. 3F: iMG10, iMG12 and Fig. 3G), we also immunostained for the HLA-DRB1 marker. We observed an increase in HLA microglia (area covered in the subiculum region) in mice xenotransplanted with LACTB KO human microglia (Fig. 6I).

In short, LACTB KO altered the metabolism of human microglia *in vivo* and promoted mild changes in their activation profile and higher association with amyloid plaques.

## Discussion

Lactamase  $\beta$  (*LACTB*) is a mitochondrial enzyme encoded by a gene that resides within an AD risk locus on chromosome 15; however, its functional contribution to disease pathogenesis has not been investigated. Although broadly expressed, its expression is enriched in myeloid and other immune cells and our MR analysis using AD GWAS and monocyte-derived macrophage eQTL data indicates that lower LACTB expression in myeloid cells is associated with reduced AD risk. Importantly, in a study using pQTL data, lower LACTB protein levels in the brain have also been associated with decreased AD risk<sup>17</sup>. Together with LACTB2, they are the only two  $\beta$ -lactamase genes in the human genome, and lower LACTB2 expression in monocytes has also been associated with reduced AD risk by TWAS in an independent study<sup>20</sup>.

LACTB loss-of-function mutations in mice and humans have also been genetically linked to higher levels of a single, poorly characterized metabolite: succinylcarnitine<sup>29,36</sup>. Here, we experimentally validated these observational findings showing that interventions leading to partial or total loss of LACTB protein, or loss of its enzymatic activity, result in higher succinylcarnitine levels across multiple *in vitro* and *in vivo* models. We also showed that LACTB hydrolyzes succinylcarnitine into carnitine and succinate, two critical metabolites at the intersection of glycolysis and

lipid metabolism. Higher succinate availability may lead to an increase in APP and tau succinylation, which have been shown to promote A $\beta$  accumulation and plaque formation, and tau aggregation to tangles<sup>62</sup>. Hydrolysis of succinylcarnitine by LACTB may also explain, at least in part, why its loss leads to higher succinylcarnitine levels. Remarkably, higher succinylcarnitine levels in human CSF and brain are associated with lower AD risk<sup>31</sup>, thereby suggesting a causal path from genetically encoded lower LACTB expression in myeloid cells to lower AD risk, possibly but not necessarily mediated by elevated succinylcarnitine levels. In addition, the oxoglutarate carrier SLC25A11, which is located in the inner mitochondrial membrane and can exchange succinate, has been nominated as an AD risk gene in an African American admixture GWAS<sup>63</sup>. On the other hand, succinylation of LACTB reduces its enzymatic activity<sup>(12,38 and our data)</sup>, and this is particularly relevant given that SUCLG2—which represses LACTB succinylation at the K288 residue<sup>64</sup>—is genetically linked to AD risk, cognitive decline and succinylcarnitine levels<sup>65</sup>. SUCLG2 is predominantly expressed in microglial cells in postmortem brains of both AD patients and age-matched controls<sup>65</sup>.

LACTB is a mammalian homolog of bacterial  $\beta$ -lactamases; however, the physiological relevance of its DAEP activity in eukaryotic cells remains poorly understood. Age-related studies have documented an accumulation of D-Asp residues in long-lived proteins<sup>4</sup>, and DAEP activity may prevent the accumulation of racemized proteins in aging<sup>66</sup>. More specifically, racemization of L-aspartate residues at position 7 and/or 23 of A $\beta$  to D-Asp alters aggregation kinetics of amyloid fibrils<sup>5</sup>; and an accumulation of D-Asp residues has been detected in tau proteins forming paired helical filaments<sup>6</sup>. In addition, tau can be cleaved at D421 by caspases, promoting aggregation<sup>67</sup>, and the recessive AD-associated APP mutation A673V shifts the preferential  $\beta$ -cleavage site of APP by BACE1 from the glutamate residue at position 11 of A $\beta$  to the aspartate residue at position 1 (D672 of APP), resulting in higher C99 generation (and subsequent A $\beta$  production)<sup>68</sup>. These functionally relevant aspartate sites in AD-relevant proteins like APP, A $\beta$  and tau may also represent potential targets of LACTB serine hydrolase activity. In fact, LACTB has been identified as the first mammalian protein with intrinsic DAEP activity<sup>(3 and our data)</sup> and is able to directly cleave a 10-residue peptide corresponding to A $\beta$ 1–10 containing D-Asp at position 7<sup>3</sup>. Further studies are required to explore the ability of LACTB to modify APP processing or A $\beta$  and tau aggregation *in vitro* and *in vivo*.

Through transcriptomics and functional assays, we showed that LACTB alters the metabolism of myeloid cells, leading to an increase in oxidative phosphorylation and a reduction in triacylglycerides. Boosting TREM2 signaling (known to be protective against AD) with an activating antibody shows a similar phenotype in iPSC-derived microglia<sup>69</sup>. In addition, we observed that LACTB KD/KO leads to a reduction of protein synthesis in myeloid cells. Notably, genes within the LACTB co-expression module were enriched in a microglial subpopulation characterized by elevated expression of ribosome biogenesis and protein synthesis-related genes (cluster 7 in our single-cell RNAseq results). Interestingly, a repression in translation has been described upon microglia LPS-activation *in vitro*<sup>70</sup>. In this regard, LACTB KO human xenotransplanted microglia showed an increase in activation markers compared to WT. By shifting energy away from bulk protein synthesis and lipid storage, LACTB reduction may promote a bioenergetically efficient state—characterized by high OXPHOS—that supports microglial function and counters the metabolic exhaustion seen in AD<sup>71</sup>.

IFN and TNF response-related clusters have been identified in several mouse and human single-cell microglia studies<sup>72–75</sup>. Our data showed increased LACTB levels and decreased succinylcarnitine levels in THP1 macrophages and iPSC-derived microglia stimulated with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$  *in vitro*. In addition, LACTB KD/KO resulted in altered expression of genes involved in the IFN/TNF response. After IFN/TNF stimulation, LACTB KD/KO myeloid cells reduced myelin phagocytosis and lysosomal activity. Similarly, lower PU1 levels, associated with AD protection,

reduced myelin phagocytosis<sup>59</sup> and THP1 macrophages polarized towards a lipid disease-associated state also showed lower phagocytic and lysosomal activity<sup>58</sup>. Lower microglia efferocytosis activity in pro-inflammatory conditions could prevent microglial cell exhaustion and excessive synaptic pruning.

Even though LACTB KD in THP1 macrophages and LACTB KO in WTC11 iMGLs shared a similar phenotype, functional alterations in cell proliferation, ceramide levels or lipid droplet accumulation were only detected in LACTB KD macrophages. Further studies are required to determine whether these differences reflect the distinct nature and duration of the genetic perturbations, including potential compensatory mechanisms in constitutive KO cells, or instead arise from cell type-specific functions of LACTB in macrophages and microglia.

Finally, in a mouse model of amyloid deposition, LACTB KO in human microglia did not markedly alter amyloid plaque density, similar to what has been observed in experiments targeting other myeloid AD risk genes such as TREM2 and PLCG2<sup>76,77</sup>. However, LACTB KO in human microglia led to increased microglia-plaque association, and activation measured by an antigen presentation marker. In this regard, the P522R gain-of-function and protective variant of PLCG2 has also been shown to promote the expression of antigen presentation genes by human microglia in 5XFAD-hCSF1 xenotransplanted mice<sup>76</sup>. Overall, these effects are directionally consistent with human genetics and functional genomics evidence linking TREM2 loss-of-function with increased AD risk, and PLCG2 gain-of-function or LACTB loss-of-function with decreased AD risk. In the future, we will also investigate the effect of LACTB KO in mouse models of tauopathy, encouraged by the fact that succinylcarnitine levels have been shown to correlate with phospho-Tau (181P) in CSF<sup>78</sup>.

Together these data implicate a novel gene (the mitochondrial serine hydrolase LACTB) and metabolite (succinylcarnitine) pair, in myeloid cells like microglia and macrophages, in the pathogenesis of AD. In contrast to many AD risk genes, LACTB is structurally and enzymatically characterized, and small-molecule inhibitors of its bacterial orthologs are already in clinical use to treat  $\beta$ -lactam-resistant infections, highlighting its potential druggability. In addition, LACTB DAEP activity can be quantified using a scalable fluorometric assay, enabling high-throughput screening of small molecule inhibitors. Importantly, succinylcarnitine represents a proximal, mechanistically-linked readout of LACTB activity that can be measured in cell culture, mouse brain, and human biofluids (blood, CSF, or urine). The availability of a readily quantifiable causal endophenotype and pharmacodynamic biomarker of target engagement substantially strengthens the translational tractability of LACTB inhibition.

## Methods

| REAGENT or RESOURCE                                    | SOURCE                   | IDENTIFIER |
|--------------------------------------------------------|--------------------------|------------|
| <b>Antibodies</b>                                      |                          |            |
| Anti-rabbit-HRP                                        | Vector Laboratories      | AB_2336198 |
| Anti-mouse-HRP                                         | Vector Laboratories      | AB_2336177 |
| Anti-ACTIN                                             | Sigma-Aldrich            | AB_476744  |
| Anti-GAPDH                                             | Abcam                    | AB_2630358 |
| Anti-LACTB                                             | Abcam                    | AB_131171  |
| Anti-LACTB (immunoprecipitation, (IP))                 | ProteinTech              | 18195-1-AP |
| Anti-Succinyllysine (SuK) (IP)                         | PTM Bio                  | PTM-419    |
| Anti-Succinyllysine (SuK)                              | Thermo                   | PA5-120740 |
| Anti-Iba1 (western-blot)                               | FUJIFILM Wako            | 016-20001  |
| Anti-GFAP                                              | Cell Signaling           | 3670S      |
| Anti- $\beta$ -Amyloid clone D54D2 Alexa Fluor 488     | Cell Signaling           | 51374      |
| Anti-MOAB                                              | Biosensis                | M-1586-100 |
| Anti-KU80                                              | Cell Signaling           | 2180S      |
| Anti-IBA1 (immunostaining)                             | Abcam                    | AB5076     |
| ThioS                                                  | Sigma-Aldrich            | T1892      |
| Anti-LGALS3                                            | R&D Systems              | AF1197     |
| Anti-HLA-DRB1 (LN3)                                    | Invitrogen               | 14-9956-82 |
| Anti-CD9                                               | Biolegend                | 312102     |
| <b>Chemicals, peptides, and recombinant proteins</b>   |                          |            |
| Gibco™ RPMI 1640 Medium                                | Thermo Fisher Scientific | 11875093   |
| Gibco™ Fetal Bovine Serum, qualified, heat inactivated | Thermo Fisher Scientific | 16-140-07  |
| Gibco™ Penicillin-Streptomycin (10,000 U/mL)           | Thermo Fisher Scientific | 15140-122  |

| REAGENT or RESOURCE                                                       | SOURCE                   | IDENTIFIER |
|---------------------------------------------------------------------------|--------------------------|------------|
| <b>Antibodies</b>                                                         |                          |            |
| Gibco™ HEPES (1M)                                                         | Thermo Fisher Scientific | 15630080   |
| Gibco™ DMEM/F-12, HEPES                                                   | Thermo Fisher Scientific | 11330057   |
| Gibco™ GlutaMAX™ Supplement                                               | Thermo Fisher Scientific | 35050061   |
| Gibco™ MEM Non-Essential Amino Acids Solution (100X)                      | Thermo Fisher Scientific | 11140050   |
| Gibco™ B-27™ Supplement (50X) with vitamin A, serum free                  | Thermo Fisher Scientific | 17504044   |
| Gibco™ N-2 Supplement (100X)                                              | Thermo Fisher Scientific | 17502048   |
| 1-Thioglycerol                                                            | Sigma -Aldrich           | M1753      |
| Gibco™ Insulin-Transferrin-Selenium) (100X)                               | Thermo Fisher Scientific | 41400045   |
| Insulin Solution Human                                                    | Sigma-Aldrich            | I9278      |
| Gibco™ StemFlex™ Medium                                                   | Thermo Fisher Scientific | A3349401   |
| STEMdiff™ Hematopoietic Kit                                               | STEMCELL Technologies    | 05310      |
| Gibco™ DMEM/F-12, GlutaMAX™ supplement                                    | Thermo Fisher Scientific | 10565018   |
| Heparin Solution                                                          | STEMCELL Technologies    | 07980      |
| Epidermal Growth Factor, human                                            | Sigma-Aldrich            | E9644      |
| Stemfactor FGF-basic, Human Recombinant                                   | amsbio                   | 03 – 0002  |
| Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, LDEV-free | Corning                  | 354230     |
| Fibronectin                                                               | STEMCELL Technologies    | 07159      |
| Phorbol 12-myristate 13-acetate (PMA)                                     | Calbiochem (Millipore)   | 524400     |

| REAGENT or RESOURCE                                  | SOURCE                   | IDENTIFIER |
|------------------------------------------------------|--------------------------|------------|
| <b>Antibodies</b>                                    |                          |            |
| Lipofectamine RNAiMAX Transfection                   | Thermo Fisher Scientific | 13778150   |
| Transforming growth factor beta (TGFβ)               | Peprotech                | 100 – 21   |
| Macrophage colony-stimulating factor (MCSF)          | Peprotech                | 300 – 25   |
| Interleukin 34 (IL34)                                | Peprotech                | 200 – 34   |
| Recombinant Human CD200 (C-His)                      | Bon Opus Biosciences     | BP004-500  |
| Recombinant Human Fractalkine (CX3CL1)               | Peprotech                | 300 – 31   |
| Recombinant Human Interferon-gamma (IFNγ)            | R&D Systems              | 285-IF     |
| Recombinant Human Interferon-beta (IFNβ)             | R&D Systems              | 8499-IF    |
| Recombinant Human Tumor Necrosis Factor-alpha (TNFα) | R&D Systems              | 210-TA     |
| Click iT AHA (L Azidohomoalanine)                    | Thermo Fisher Scientific | C10102     |
| Tetramethylrhodamine (TAMRA)                         | Thermo Fisher Scientific | T10183     |
| Methanol                                             | Sigma Aldrich            | 34860      |
| Chloroform                                           | Sigma Aldrich            | C2432      |
| <b>Commercial assays</b>                             |                          |            |
| Cholesterol Efflux Fluorometric Assay kit            | Biovision                | K582-100   |
| Edu Flow Cytometry Kit                               | Sigma-Aldrich            | BCK-FC647  |
| Click iT Protein Reaction Buffer Kit                 | Thermo Fisher Scientific | C10276     |
| EZQ Protein Quantification Kit                       | Thermo Fisher Scientific | R33200     |
| BODIPY 493/503                                       | Thermo Fisher Scientific | D3922      |
| MitoProbe DiIC1(5) Assay Kit for Flow                | Molecular Probes         | M34151     |

| REAGENT or RESOURCE                                               | SOURCE                              | IDENTIFIER                                                                                                                                                      |
|-------------------------------------------------------------------|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Antibodies</b>                                                 |                                     |                                                                                                                                                                 |
| LysoTracker Red DND99                                             | Thermo Fisher Scientific            | L7528                                                                                                                                                           |
| LysoSensor Green DND-189                                          | Thermo Fisher Scientific            | L7535                                                                                                                                                           |
| DQ Red BSA                                                        | Thermo Fisher Scientific            | D12051                                                                                                                                                          |
| Succinylcarnitine-13C3 Chloride                                   | LGC                                 | TRC-S688832                                                                                                                                                     |
| Live/Dead Fixable Violet Dead Cell Stain kit for 405nm excitation | Invitrogen                          | L34955                                                                                                                                                          |
| MILLIPLEX Human Immunology Configurable Multiplex Assay           | Millipore Sigma                     | HCYTA-60K-10                                                                                                                                                    |
| PureLink RNA Mini Kit                                             | Invitrogen                          | 12183025                                                                                                                                                        |
| RNeasy Mini Kit                                                   | Qiagen                              | 74106                                                                                                                                                           |
| <b>Deposited data</b>                                             |                                     |                                                                                                                                                                 |
| Bulk RNAseq                                                       | This paper                          | THP1 macrophages: GSE323383<br>WTC11 iMGLs: GSE323384<br>Mouse isolated microglia: GSE324607                                                                    |
| scRNAseq                                                          | This paper                          | GSE324494                                                                                                                                                       |
| Experimental models: Cell lines                                   |                                     |                                                                                                                                                                 |
| THP1 human monocytic leukemia line                                | ATCC                                | TIB-202 RRID:CVCL_0006                                                                                                                                          |
| WTC11 human iPSC line                                             | Coriell Institute                   | GM25256                                                                                                                                                         |
| HEK cells                                                         | ATCC                                | CRL-1573                                                                                                                                                        |
| Software and algorithms                                           |                                     |                                                                                                                                                                 |
| R version 4.3.2                                                   | The Comprehensive R Archive Network | <a href="https://cran.r-project.org/">https://cran.r-project.org/</a>                                                                                           |
| pipseeker v3.3.0                                                  | Fluent Biosciences                  | <a href="https://www.fluentbio.com/products/pipseeker-software-for-data-analysis/">https://www.fluentbio.com/products/pipseeker-software-for-data-analysis/</a> |
| Seurat R package v5.0.2,                                          | Butler et al., 2018 <sup>79</sup>   | <a href="https://satijalab.org/seurat/get_started.html">https://satijalab.org/seurat/get_started.html</a><br>RRID:SCR_016341                                    |

| REAGENT or RESOURCE                                   | SOURCE                                          | IDENTIFIER                                                                                                                                                                |
|-------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Antibodies</b>                                     |                                                 |                                                                                                                                                                           |
| V4.3.0 for re-analysis of publicly available datasets |                                                 |                                                                                                                                                                           |
| harmony R package v1.2.0                              | Korsunsky et al., 2019 <sup>80</sup>            | <a href="https://portals.broadinstitute.org/harmony/">https://portals.broadinstitute.org/harmony/</a>                                                                     |
| edgeR R package v3.40.2                               | Robinson et al., 2010 <sup>81</sup>             | <a href="https://bioconductor.org/packages/release/bioc/html/edgeR.html">https://bioconductor.org/packages/release/bioc/html/edgeR.html</a>                               |
| variancePartition R package v 1.23.1                  | Hoffmann and Roussos et al., 2021 <sup>82</sup> | <a href="https://rdrr.io/github/GabrielHoffman/variancePartition/man/dream-method.html">https://rdrr.io/github/GabrielHoffman/variancePartition/man/dream-method.html</a> |
| MSigDb Molecular Gene Signature collection v7.5.1     | Broad Institute                                 | <a href="https://www.gsea-msigdb.org/gsea/msigdb">https://www.gsea-msigdb.org/gsea/msigdb</a>                                                                             |
| ARACNe                                                | Margolin et al., 2006 <sup>53</sup>             | <a href="https://github.com/califano-lab/ARACNe-AP">https://github.com/califano-lab/ARACNe-AP</a>                                                                         |
| scDbIFinder v1.16.0                                   | Germain et al., 2022 <sup>83</sup>              | <a href="https://github.com/plger/scDbIFinder">https://github.com/plger/scDbIFinder</a>                                                                                   |
| CellBender python package v0.3.1                      | Flemin et al., 2023 <sup>84</sup>               | <a href="https://github.com/broadinstitute/CellBender">https://github.com/broadinstitute/CellBender</a>                                                                   |
| SCTransform R package v0.4.1                          | Hafemeister et al., 2019 <sup>85</sup>          | <a href="https://github.com/satijalab/sctransform">https://github.com/satijalab/sctransform</a>                                                                           |
| clustree R package v0.5.1                             | Zapia et al., 2018 <sup>86</sup>                | <a href="https://github.com/lazappi/clustree">https://github.com/lazappi/clustree</a>                                                                                     |
| ComplexHeatmap Bioconductor package v2.18.0           | Gu et al., 2016 <sup>87</sup>                   | <a href="https://github.com/jokergoo/ComplexHeatmap">https://github.com/jokergoo/ComplexHeatmap</a>                                                                       |
| TwoSampleMR package v0.6.22                           | Hemani et al., 2018 <sup>88</sup>               | <a href="https://mrcieu.github.io/TwoSampleMR/">https://mrcieu.github.io/TwoSampleMR/</a>                                                                                 |
| Lipidr package v2.18.0                                | Mohamed et al., 2020 <sup>89</sup>              | <a href="https://bioconductor.org/packages/lipidr">https://bioconductor.org/packages/lipidr</a>                                                                           |

## Mendelian randomization

Two-sample Mendelian randomization (MR) analyses were performed using the TwoSampleMR R package <sup>90</sup>. Summary statistics from cis-eQTL (STARNET, <sup>32</sup>), cis-mQTL <sup>31</sup>, and AD GWAS datasets <sup>14</sup> were harmonized to evaluate the causal relationships between LACTB mRNA levels, CSF succinylcarnitine levels, and AD risk. Independent genetic instruments were selected by linkage disequilibrium clumping using a European 1000 Genomes reference panel <sup>91</sup>. Exposure and outcome datasets were harmonized to align effect alleles and remove ambiguous palindromic variants. Causal effects were primarily estimated using the inverse-variance weighted method. Sensitivity analyses included MR-Egger, weighted median, simple mode, and weighted mode approaches. Heterogeneity was

assessed using Cochran's Q statistic, horizontal pleiotropy using the MR-Egger intercept test, and robustness using leave-one-out analyses. All analyses were conducted in R, and the complete reproducible analysis script is available in Additional file 1.

## Mouse models

Wild-type (WT) mice from the C57BL/6 x SJL mixed background (Jackson #034840) were used to assess the levels of LACTB protein and of succinylcarnitine in 2-month-old mice. WT mice (Jackson #006554) were used to assess the brain levels of LACTB protein and of succinylcarnitine in 2-month-old and 12-month-old mice. LACTB ED mice were generated by Ozgene. A targeting vector was constructed for the generation of a conditional knock-in of S162I into exon 3 of the *Lactb* gene (Additional file 2) and electroporated into C57BL/6J embryonic stem cells. Transfected embryonic stem cells were selected in the presence of geneticin and screened for correct targeting to the *Lactb* locus by loss of allele qPCR. Chimeric animals were generated by injection of targeted embryonic stem cell clones into goGermline™ blastocysts and the selection cassette was excised by breeding chimeras to flp expressing females to generate heterozygous conditional knock-in F1 animals. WT and LACTB ED mice were sacrificed at 3 months old to measure LACTB and succinylcarnitine levels.

5XFAD immune-deficient mice (hCSF1-5XFAD) capable of accepting human cells were utilized for xenotransplantation experiments. hCSF1 mice (available as JAX #017708) express human CSF1 on a Rag2/Il2rg deficient background, significantly increasing their ability to engraft and maintain human hematopoietic cells. hCSF1-5XFAD mice overexpress co-integrated APP and PSEN1 transgenes containing five familial AD mutations (the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations in APP, and the M146L and L286V mutations in PSEN1) and recapitulate aspects of human AD, including age-dependent cognitive deficits, amyloid accumulation and neuroinflammation<sup>92</sup>. The offspring were backcrossed to hCSF1 mice to return the 3 hCSF1 mutations to homozygosity and maintain the 5XFAD transgene in the hemizygous state<sup>60</sup>. All mice were age-matched and group-housed on a 12-h/12-h light/dark cycle with food and water. Mice were housed with ambient temperature and humidity, and cages and bedding were changed every week. All procedures involving experimentation on animal subjects were done in accordance with the National Research Council's guide for the care and use of laboratory animals and under an approved protocol.

Induced pluripotent stem cells (iPSC) donor lines and Hematopoietic Progenitor Cells (HPC) generation.

The WTC-11 human iPSC line (Coriell Institute for Medical Research, GM25256) was tested for genomic integrity at passage 36 using SNP-array technology (Global Diversity Array v1.0 BeadChip, Illumina). No CNV larger than 1.5 Mb or AOH larger than 3 Mb were detected on autosomal chromosomes. The known WTC-11 deletion of 2.9 Mb on Yp11.2 was detected. We used CRISPR-mediated Non-Homologous End Joining to generate isogenic control (WT) and LACTB KO clonal lines (3 independent clones per genotype), at the Stem Cell Core of the Icahn School of Medicine at Mount Sinai. All clones were validated by next-generation sequencing of both alleles to confirm indels resulting in frameshift mutations or premature stop codons. The use of independent clones is essential to attribute any effect to genotype and rule out that the differences observed are due to random clone to clone variability. Normal karyotype was confirmed after editing. iPSC lines were thawed into matrigel-coated 6-well plates and maintained by feeding every other day with Stemflex supplemented media (Thermo Fisher Scientific) at 37°C, 5% CO<sub>2</sub>. When 70–80% confluence was reached, iPSCs were passed using ReLeSR dissociation reagent (STEMCELL Technologies, 05872). Hematopoietic stem cells (HPCs) were differentiated from iPSCs using the STEMdiff Hematopoietic kit (STEMCELL Technologies, 05310) according to a previously published protocol<sup>93</sup>. HPCs were collected after a

minimum of ten days in culture. If viability was > 75%, HPCs were frozen in Bambanker media (Wako Chemicals) or directly differentiated into microglial cells.

iPSC-derived microglia differentiation.

Hematopoietic stem cells were differentiated to induced microglial-like cells (iMGLs) using a previously published protocol<sup>93</sup>. iMGLs were maintained and fed with a microglial medium supplemented with three factors (100 ng/ml IL34, 50 ng/ml TGFβ, 25 ng/ml MCSF) for 25 days. On day 25, iMGLs were additionally supplemented with two factors (CX3CL1 and CD200, 100 ng/ml) for an additional three days. Mature iMGLs (day 28) were collected for transcriptomics, lipidomics, metabolomics or RNA/protein quantification or used for functional assays.

## THP1 differentiation and siRNA treatment

We used the monocytic human immortalized cell line THP1 (ATCC, TIB-202 RRID:CVCL\_0006), which we treated with phorbol 12-myristate 13-acetate (PMA, 25 ng/ml) for 72 hours to differentiate into macrophages. THP1 cells were cultured in RPMI medium supplemented with 10% FBS, 1x Penicillin Streptomycin and 10 mM HEPES. We used 40 nM small-interfering RNA (siRNA, ON-TARGETplus SMARTpool, L-021008-01-0005) for 6 days (before and after PMA addition) to reduce LACTB expression. Additional treatments were added during the last 24 hours.

## HEK cells culture and transfection

Stable HEK293 cell lines overexpressing wild-type (WT) or mutant (S164I) LACTB were generated by using the pCDH-CMV-EF1a-puro lentiviral system. HEK cells were selected because of their low endogenous LACTB levels (data not shown) and their feasibility for transfection. HEK cells (ATCC, CRL-1573) were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% Penicillin/Streptomycin. Cells seeded at  $3.5 \times 10^5$ /well (6-well plate) were transduced at multiplicities of infection (MOI) of 1, 2, 5, and 10, followed by a 48-hour incubation. Selection for stably integrated clones was performed using puromycin (2 µg/mL) for 4 days, after which viability was assessed, and overexpression was confirmed via Western Blot and qPCR. Following transduction at varied MOIs and 4-day puromycin selection, clones were expanded and cryopreserved, with MOI 1 selected for LACTB WT overexpressing cells and MOI 5 for LACTB S164I/Vector control overexpressing cells to ensure comparable expression.

## Isolation of microglia and astrocytes from mouse brain

Microglia were isolated from freshly dissected mouse brains using enzymatic dissociation followed by CD11b-based magnetic separation. Brains were sectioned into sagittal slices on ice and dissociated using the Adult Brain Dissociation Kit (Miltenyi Biotec) according to the manufacturer's instructions with the gentleMACS Octo Dissociator with Heaters. The resulting cell suspension was filtered through a 100 µm cell strainer and centrifuged at 300×g for 10 min at 4°C. Myelin and debris were removed using Debris Removal Solution (Miltenyi Biotec) with density gradient centrifugation (300×g, 10 min, 4°C). The cell pellet was washed and resuspended in MojoSort buffer (BioLegend) and incubated with CD11b MicroBeads (Miltenyi Biotec) for 15 min at 4°C in the dark. Cells were washed and applied to MS columns placed in an OctoMACS separator (Miltenyi Biotec). After washing, magnetically labeled CD11b<sup>+</sup> cells were eluted, representing the enriched microglial fraction. Isolated microglia were pelleted and stored at – 80°C for downstream analyses.

Astrocytes were isolated using magnetic-activated cell sorting (MACS) from the cell pellet remaining after microglia isolation. The pellet was carefully resuspended in 160 µL of cold 1X MojoSort buffer (BioLegend, Cat# 480113) and incubated with 20 µL of FcR Blocking Reagent (Miltenyi Biotec, Cat# 120-097-678) for 10 min at 4°C. Following

blocking, cells were magnetically labeled with 20  $\mu$ L of Anti-ACSA-2 MicroBeads (Miltenyi Biotec, Cat# 130-097-678) for 15 min at 4°C. The cells were then washed with 1 mL of MojoSort buffer, centrifuged at 300xg for 10 min at 4°C, and resuspended in 500  $\mu$ L of buffer. Magnetic separation was performed using an MS column (Miltenyi Biotec, Cat# 130-042-201) placed in an OctoMACS Separator (Miltenyi Biotec, Cat# 130-042-109). After applying the cell suspension and washing the column three times with 500  $\mu$ L of buffer, the column was removed from the magnetic field. The positive astrocyte fraction was eluted by flushing the column with 1 mL of MojoSort buffer using a plunger. The resulting suspension was centrifuged at 2500xg for 5 min at 4°C. The pellet was washed with cold 1X PBS before being stored at -80°C.

#### Recombinant Human LACTB (63–547) protein purification.

In-house recombinant human LACTB was generated by expressing LACTB (residues 63–547) as an N-terminal GST-tagged fusion protein containing an HRV 3C protease cleavage site from a pET-29b (+) vector in *Escherichia coli* C41 (DE3) cells. Cells were cultivated in 36 L of Terrific Broth at 37°C, induced with 0.1 mM IPTG upon reaching an optical density of 0.9–1.0 at 600 nm, and incubated overnight at 37°C before harvesting. The resulting cell pellet was resuspended in ice-cold lysis buffer (50 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.1 mM EDTA, 1 mM PMSF, 5% v/v glycerol, 5 mM DTT, 0.1% v/v Triton X-100, protease inhibitors, DNase-I, and lysozyme), lysed via sonication, and clarified by centrifugation at 13,000  $\times$  rpm. The supernatant was filtered through a 0.8  $\mu$ m filter and loaded onto 5 mL GSTrap columns (Cytiva), washed, and eluted with an isocratic gradient of reduced glutathione. To prevent protein fibrils precipitation, the pooled target protein fractions were adjusted to pH 8.5. The GST tag was cleaved overnight at 4°C via dialysis against a pH 8.5 buffer containing 6 mg of HRV 3C protease. Free GST and uncut protein were removed by passing the sample back over the GSTrap columns. The flow-through containing untagged LACTB was concentrated using a liquid nitrogen pressure system and further purified by size exclusion chromatography on a Superose® 6 16/60 column (Cytiva) equilibrated in SEC buffer (50 mM HEPES pH 8.5, 1 M NaCl, 5% v/v glycerol, and 5 mM DTT). High-molecular-weight fractions corresponding to the target macromolecular assembly were pooled and concentrated to a final yield of 3.75 mg at 5.6 mg/mL, with sample homogeneity and stability validated by SDS-PAGE, dynamic light scattering, and differential scanning fluorimetry.

#### Succinylcarnitine recovery assay.

In-house generated recombinant human LACTB (IACSp-1689-07c) was used to determine kinetic parameters for succinylcarnitine cleavage. Reactions were performed in an assay buffer containing 20 mM Tris-HCl (pH 8.5) and 1 mM TCEP (Tris(2-carboxyethyl)phosphine). LACTB was used at a final concentration of 100 nM. Where indicated, the inhibitor Ac-IEPD-CHO (10  $\mu$ M final concentration, Abcam, ab142030) or vehicle (DMSO) was pre-incubated with the enzyme for 15 min prior to substrate addition. Ac-IEPD-CHO inhibitor is similar to Z-AAD-CMK inhibitor 2; both are Granzyme B (a serine protease similar to LACTB) inhibitors with the difference that the aldehyde group (-CHO) makes the IEPD-CHO an reversible inhibitor compared to the -CMK being irreversible. Reactions (200  $\mu$ L total volume) were initiated by adding succinylcarnitine- $^{13}\text{C}_7$  chloride (TRC-S102985) at varying concentrations and incubated for 2 h at room temperature with shaking (300 rpm). Reactions were quenched by addition of 800  $\mu$ L of 80% methanol, followed by incubation at -80°C for 24 h to precipitate protein. Samples were centrifuged at 21,000xg for 15 min, and supernatants were collected for metabolite quantification. Substrate (succinylcarnitine- $^{13}\text{C}_7$  chloride) and product (carnitine- $^{13}\text{C}_3$ ) were measured by targeted mass spectrometry.

## Immunoprecipitation

Immunoprecipitation (IP) was performed using the Pierce™ Crosslink Magnetic IP/Co-IP Kit (Thermo Fisher Scientific, 88805) according to the manufacturer's protocol, with the following modifications. Briefly, 750  $\mu$ g of total cell lysate

was used per immunoprecipitation. Lysates were incubated with 3.75 µg anti-LACTB antibody (Proteintech, 18195-1-AP, rabbit IgG), anti-SuK antibody (PTM Biolabs, Cat. No. PTM-419, mouse IgG), or the corresponding control IgG (rabbit IgG, I-1000; mouse IgG, I-2000). Antibody–antigen complexes were captured using 30 µL of magnetic Protein A/G beads and incubated for 1.5 h with gentle rotation at 4°C. Beads were washed according to the kit protocol using IP lysis buffer, followed by a final wash with ultrapure water. Bound proteins were eluted in 35 µL of the elution buffer, and eluates were immediately neutralized with the provided neutralization buffer. The entire eluted immunoprecipitated material was resolved by SDS-PAGE and transferred to membranes. Membranes were immunoblotted with anti-LACTB antibody (Abcam, ab131171) or anti-SuK antibody (Thermo Fisher Scientific, PA5-120740), as indicated. Blots were developed using Clean-Blot™ HRP-conjugated secondary antibody according to the manufacturer's instructions.

## Suc-D-asp assay

To measure LACTB cleavage of Suc-D-Asp substrate, cells were washed with DPBS and incubated with an assay buffer consisting of 10 mM Tris-HCl (pH 8.5), 200 mM NaCl, and 3 mM MnCl<sub>2</sub> (for THP1 macrophages) or in PBS (for HEK cells). The fluorogenic substrate Suc-D-Asp (7-methoxycoumarin-4-acetyl-L-succinyl-D-aspartyl-L-lysine, #3222-v, Peptide) was added to each well at a final concentration of 5 or 20 µM. Kinetic fluorescence measurements ( $\lambda_{\text{ex}}$  = 380 nm,  $\lambda_{\text{em}}$  = 460 nm) were performed immediately using a Varioskan or a BioTek Synergy Neo2 plate reader at 37°C, with 30–60 readings taken at 2-min intervals with shaking before each read (300 rpm). Following the kinetic run, cells were washed with PBS and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors for 30 minutes on ice. Total protein concentration was determined via BCA assay, and fluorescence slopes were normalized to total protein content to account for variation in cell density.

## Western-blotting

Cells were lysed in RIPA buffer (Thermo Scientific, 89900) supplemented with Protease/Phosphatase Inhibitor Cocktail (Cell Signaling, 5872) following manufacturer's instructions. Protein concentration was measured using BCA kit (Thermo Fisher Scientific, 23225) and equal quantities were used to prepare samples for western blotting. Samples were resolved by electrophoresis with Bolt 4–12% Bis-Tris Plus Gels (Invitrogen) in Bolt MES SDS running buffer (Invitrogen, B0002) and transferred using iBlot 2 nitrocellulose transfer stacks (Invitrogen). Membranes were blocked for 1 h and probed with primary antibodies in 5% BSA in PBS/0.1% Tween-20 buffer overnight at 4°C. Secondary antibody at 1:10,000 dilution was applied for 1 h at RT, visualized using WesternBright ECL HRP Substrate Kit (Advansta, K-12045), and measured using iBright imaging system (Applied Bioscience). Images were analyzed using ImageJ (NIH). Uncropped western blot images are provided in Additional file 3.

## Metabolomic assays

For THP1 macrophage collection (700,000 cells per sample), media was aspirated and cells were quickly washed with ice-cold 0.85% (w/v) ammonium bicarbonate in water. 1 mL of buffer was added per well, and cells were gently detached using a cell scraper. Cell suspensions were transferred to tubes and centrifuged at 500 × g for 3 min. Supernatants were aspirated, and pellets were snap-frozen on dry ice and stored at -80°C.

For the collection of semi-adherent WTC-11 iMGLs (500,000 cells per sample), media was collected, and 1 mL of ice-cold DPBS was added to each well. Cells were gently detached using a cell scraper and pooled with their respective media. Samples were centrifuged at 300×g for 5 minutes, and supernatants were aspirated. Pellets were resuspended in 1 mL of ice-cold DPBS, centrifuged again at 300×g for 5 min. The final supernatants were aspirated, and the pellets were snap-frozen on dry ice and stored at -80°C.

For ion chromatography-mass spectrometry (ICMS) analysis, metabolites were extracted using ice-cold 50/30/20 (v/v/v) methanol/acetonitrile/water. Extracts were left on ice for 10 mins, centrifuged at 17,000 g for 5 min at 4°C, and supernatants were transferred to autosampler vials, and 10 µL was injected. IC mobile phase A (MPA; weak) was water, and mobile phase B (MPB; strong) was water containing 100 mM KOH. A Thermo Scientific Dionex ICS-6000 + system included a Thermo IonPac AS11 column (4 µm particle size, 250 x 2 mm) with column compartment kept at 35°C. The autosampler tray was chilled to 4°C. The mobile phase flow rate was 360 µL/min, and the gradient elution program was: 0–2 min, 1% MPB; 2–25 min, 1–40% MPB; 25–39 min, 40–100% MPB; 39–50 min, 100% MPB; 50–50.5, 100–1% MPB. The total run time was 55 min. To assist the desolvation for better sensitivity, methanol was delivered by an external pump and combined with the eluent via a low dead volume mixing tee. Data were acquired using a Thermo Orbitrap IQ-X Tribrid Mass Spectrometer under ESI negative ionization mode at a resolution of 240,000.

For Hydrophilic Interaction Liquid Chromatography analysis, the same samples were injected by liquid chromatography (LC)-MS. LC mobile phase (MP) A was 95/5 (v/v) water/acetonitrile containing 20 mM ammonium acetate and 20 mM ammonium hydroxide (pH ~ 9), and MPB was acetonitrile. Thermo Vanquish LC system included a Xbridge BEH Amide column (3.5 µm particle size, 100 x 4.6 mm) with column compartment kept at 30°C. The autosampler tray was chilled to 4°C. The mobile phase flow rate was 350 µL/min, and the gradient elution program was: 0–1 min, 85% MPB; 1–16 min, 85 – 5% MPB; 16–20 min, 5% MPB; 20–21 min, 5–85% MPB; 21–25 min, 85% MPB. The total run time was 25 min and data were acquired using a Thermo Orbitrap Exploris 240 Mass Spectrometer under ESI positive/negative ionization (polarity switching) mode at a resolution of 240,000.

<sup>13</sup>C<sub>7</sub>-succinylcarnitine and <sup>13</sup>C<sub>3</sub>-carnitine were quantitatively analyzed by using an LC-MS/MS method. Acetyl d<sub>3</sub>-carnitine was used as an internal standard. An Agilent Technologies 1290 Infinite liquid chromatograph consisting of a binary pump, a multisampler with a 10-µL loop, and a column heater was used. The stationary phase was a Waters X-Bridge amide column (100 x 4.6 mm, 3.5 µm particle size). The column temperature was set at 40°C. MPA was 5% acetonitrile in water containing 20 mM ammonium acetate and 20 mM ammonium hydroxide and MPB acetonitrile. The MP was delivered at 0.3 mL/min in a gradient mode at 85% B from 0 to 3 minutes, linearly decreased to 30% B at 10 min, then to 2% B at 20 min, and equilibrated at 85% B from 20.2 to 25 min. The injection volume was 5 µL. Under these conditions, <sup>13</sup>C<sub>7</sub>-succinyl L-carnitine, <sup>13</sup>C<sub>3</sub>-carnitine, and acetyl d<sub>3</sub>-carnitine had retention times of 11.1, 11.0, 11.6, and 10.9 minutes, respectively. An Agilent Technologies 6460 Triple Quad mass spectrometer was operated in the positive electrospray ionization mode. The following operating conditions were set: desolvation gas temperature 300°C; nebulizer gas flow 11 L/min, nebulizer gas pressure 35 psi; sheath gas flow 11 L/min, sheath gas temperature 400°C; capillary voltage 4 kV. <sup>13</sup>C<sub>7</sub>-succinyl L-carnitine, <sup>13</sup>C<sub>3</sub>-carnitine, and acetyl d<sub>3</sub>-carnitine were monitored in MRM mode at mass transitions: 262.1 > 85.1, 270.2 > 86.0, 165.2 > 63.1, and 207.1 > 85.1, respectively. Stock solutions of <sup>13</sup>C<sub>7</sub>-succinyl L-carnitine, <sup>13</sup>C<sub>3</sub>-carnitine were prepared in water and its working solution was prepared by diluting the stock solution with a mixture of methanol/water (80: 20, v/v). Calibration curves were constructed in the concentration ranges from 0.078 to 20 ng/mL or 0.78 to 200 ng/mL. An aliquot of 50 µL of samples, standards, and QC samples was diluted with 150 µL of the internal standard solution containing acetyl d<sub>3</sub>-carnitine at 5 ng/mL in acetonitrile before the analysis by LC-MS/MS method.

All the raw files were imported to Skyline Daily for final analysis. For the labeled metabolites analysis, the fractional abundance of each isotopologue is calculated by the peak area of the corresponding isotopology normalized by the sum of all isotopology areas.

## Bulk RNAseq and qPCR analyses

RNA from human THP1 macrophages and WTC11 iMGLs was extracted using the RNeasy Plus Mini kit (Qiagen, 74106) following manufacturer's instructions. RNA was extracted from microglia isolated from LACTB ED mice using PureLink RNA Mini Kit (Invitrogen, 12183025) following the manufacturer's instructions. mRNA quantity was measured using Nanodrop 8000 (Thermo Fisher Scientific).

For both bulk and single-cell RNAseq, RNA was submitted to Azenta (New Jersey, NJ, USA) for QC, library preparation, and next-generation sequencing. Samples passed quality control with Qubit and BioAnalyzer showing RIN > 9.0. Libraries were prepared using TruSeq RNA Sample Prep Kit v2 and paired-end sequenced using HiSeq2500 at a read length of 150bp to obtain 20-30M mapped fragments per sample. Sequenced reads were assessed for quality (FastQC v0.11.8), trimmed for adapter contamination (Cutadapt v2.6), and aligned to the human genome hg38 (STAR v2.5.3a). Differential gene expression analysis (DGEA) was performed using a linear mixed model implemented in DREAM (Differential expression for repeated measures, variancePartition R package <sup>94</sup>). To identify pathways, we used Gene Set Enrichment Analysis (GSEA) <sup>95</sup>. Briefly, ranked lists were generated from differential gene expression analyses by ordering genes according to the signed Z statistic (Table 2). Our preranked lists were tested for enrichment against genesets from the Molecular Signatures Database (MSigDB v7.5.1, Broad Institute). Pathways displayed were selected among the most significant pathways, the full list is provided in Table 2. Enrichment scores were normalized by geneset size to generate normalized enrichment scores (NES) according to the standard protocol <sup>95</sup>.

For qPCR analysis, reverse transcription reaction was performed with 1000 ng of RNA using High-Capacity RNA-to-cDNA kit (Thermo Fisher Scientific, cat. 4387406). 10 ng cDNA was used in the qPCR reaction with Power SYBR Green Master Mix (Applied Biosystems, cat. 4368706) run using QuantStudio 7 Flex Real-Time PCR System (Thermo Fisher Scientific). Primers were designed using the IDT software and are listed in Table 5. Ct values were averaged from two technical replicates for each gene, GAPDH Ct values were used for normalization. Gene expression levels were quantified using the 2-ddCt method.

## Analysis of publicly available data

Fastq files from publicly available single-cell RNA sequencing datasets were downloaded from the Gene Expression Omnibus for Mancuso et al. (GSE216999), Tuddenham et al. (GSE204702), and Smith et al. (GSE160936), and from the Synapse platform for Olah et al. (syn21438358), Prater et al. (syn51272688, accessed via data use agreement), and Lee et al. (syn52795287). Raw sequencing data were processed using Cell Ranger (v9.0, 10x Genomics). Doublet detection and removal were performed using scDblFinder (v1.16.0). Ambient RNA correction and batch integration were conducted using CellBender (v3.0.1) and Harmony (v1.2.0), respectively, where appropriate. All downstream analyses were performed using Seurat (v4.3.0), with normalization carried out using either the NormalizeData() function or in conjunction with SCTransform (v0.4.1). Clustering for each dataset was determined through differential expression analysis in Seurat and resolution assessment using the clustree package (v0.5.1). Pearson correlation analysis with the cor() function in R was used to assess correlations across clusters between aggregated expression for *LACTB* and leading-edge genes contributing to significantly enriched terms identified by GSEA in LACTB THP1 knockdown and iMGL knockout datasets. Correlation data were then visualized using the ComplexHeatmap() package.

## Single-cell RNAseq analyses

Paired-end FASTQ files were processed using Pipseeker v3.3.0 following the recommended pipeline. Reads were trimmed, aligned to the GRCh38 reference genome, and quantified to generate feature-barcode count matrices at multiple sensitivity levels; sensitivity level 3 was selected for downstream analyses. Count matrices were imported

into Seurat v5.0.2 for quality control and analysis. Cells expressing fewer than 100 genes, less than 500 UMIs and greater than 95% of total UMIs, or with > 10% reads mapped to the mitochondrial genome were excluded. Doublets were identified and removed using scDblFinder v1.20.2. Raw counts were normalized using SCTransform v0.4.1, with regression of mitochondrial mapping percentage. Principal component analysis was performed for dimensionality reduction, and samples were integrated using Harmony v1.2.0 to account for batch effects. Unsupervised clustering was conducted in Seurat and clusters were visualized using UMAP. Marker genes were identified using FindMarkers. Cluster annotation was based on (i) expression of established marker genes from the literature, (ii) enrichment of published myeloid gene signatures calculated with hypergeometric overlap, and (iii) biological pathway enrichment analysis using GSEA analysis to determine functional relevance.

For pseudobulk differential gene expression analysis (DGEA) between genotypes, cells were aggregated per sample to obtain average expression for each gene (N = 3 independent samples per condition) using Seurat AggregateExpression. Pseudobulk DGEA was performed using the DREAM package in R<sup>94</sup>, with adjustment for the experimental batch to account for technical variability. Geneset enrichment across clusters was calculated using the Seurat AddModuleScore function, which computes a per-cell module score to assess relative enrichment of gene signatures. Differential cell-type abundance between genotypes was assessed per cluster using CrumblR (under review<sup>96</sup>). Pseudobulk cell-type proportions were calculated per sample and differential abundance testing was performed using generalized linear modeling while accounting for batch.

## LACTB co-expression module generation

A normalized scRNA-seq gene-by-cell matrix was used to compute pairwise Pearson correlations, which were used to construct k-nearest neighbor graphs. Metacells were generated by aggregating counts from neighboring cells using the PISCES MetaCell framework (unpublished<sup>97</sup>) prior to construction of the LACTB co-expression module using ARACNe<sup>53</sup>. Genes with non-zero counts in at least 75% of metacells were retained for downstream analysis. ARACNe was applied with default parameters to reconstruct a gene co-expression module centered on LACTB, using a mutual information threshold of  $p=1 \times 10^{-8}$  and 100 bootstraps. Only significant interactions (FDR-adjusted  $P < 0.05$ ) were retained to define the LACTB co-expression module. Enrichment of genes co-expressed with LACTB across clusters was assessed using Seurat AddModuleScore, and enrichment in external published datasets was evaluated by hypergeometric overlap with cluster marker genes.

## Proliferation assays

We used the Edu Flow Cytometry Kit 647 (Sigma-Aldrich, BCK-FC647-50). Cells were stained with EdU at 10  $\mu$ M for 2 hours and manufacturer's instructions were followed. Briefly, detached cells were collected and washed with 1% BSA in DPBS. Cells were centrifuged 300xg 5 min and resuspended in the Fixative solution. Cells were washed again with 1% BSA in DPBS, centrifuged 300xg 5min and pellets were resuspended in saponin 1X buffer (20 min RT). The assay cocktail mix was added for 30 min RT. Cells were centrifuged 300xg 5 min and washed with saponin 1X buffer. Cells were centrifuged 300g 5min and resuspended in saponin 1X buffer for flow analysis (using a low-rate flow, as indicated in the manufacturer's instructions). Cells were gated based on forward scatter and singlet discrimination. Non-EdU-treated cells processed in parallel were used to define the negative gate, accounting for the background signal generated by the Click-iT reaction.

## Seahorse assays

THP1 monocytes were plated at a density of 50,000 cells per well in a Seahorse 96 well-plate; and WTC11 iMGLs at a density of 100,000 cells per well in a fibronectin-coated Seahorse 96 well-plate. Agilent Seahorse Mito Stress Test Kit (103015-100) was used to assess mitochondrial respiration. DMEM XF Base Media was supplemented with 1 mM

pyruvate, 2 mM glutamine and 10 mM glucose. Cell media was replaced with 180  $\mu$ l of this media and cells were incubated for 1 h at 37°C in a non-CO<sub>2</sub> incubator. Cells were stimulated with 1  $\mu$ M oligomycin, 0.5  $\mu$ M FCCP, 0.5  $\mu$ M Rot/AA.

## Protein synthesis assay

THP1 monocytes and WTC-11 iMGLs were plated in 6-well plates at densities of 700,000 and 500,000 cells per well, respectively. On the day of collection for THP1 macrophages, culture media was aspirated, and cells were washed once with methionine-free THP1 media (methionine-free RPMI supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 1% HEPES). Wells were replenished with 1 mL of methionine-free media and incubated for 1 hour to deplete endogenous methionine. For iMGLs, methionine-free incubation was omitted. Cells were then treated with 50  $\mu$ M of Click-iT AHA (L-Azidohomoalanine), a methionine analog used for nascent protein labeling. Thapsigargin, a known inducer of the integrated stress response that suppresses protein translation, was used as a positive control at a final concentration of 250 nM for THP1 macrophages and 500 nM for iMGLs. Cells were treated for 2 h (THP1) or 3 h (iMGLs).

Following treatment, cells were collected, washed in DPBS, and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors Cell Signaling, 5872S). Lysates were incubated on ice for 30 minutes with vortexing every 10 min, followed by sonication. Samples were then centrifuged at 13,000 rpm for 12 min, and the supernatants were collected as protein lysates. Click-iT protein labeling reactions and subsequent protein quantification using the EZQ Protein Quantification Kit were performed according to the manufacturer's instructions.

## Lipidomic profiling and analysis

For THP1 macrophages, we used 3 million cells per sample, pooling 3 independent siRNA-treated wells for each. We performed 4 independent macrophage differentiations. For iMGLs, we used 2 million cells per sample, pooling cells from 6 wells of a 6-well plate.

Cell pellets were shipped to Columbia Biomarker Core (NY, US, <sup>98</sup>), where a standard lipid panel was performed. Lipidomics profiling was performed using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry <sup>99</sup>. Lipid extracts were prepared from cell lysates spiked with appropriate internal standards using a modified Bligh and Dyer method and analyzed on a platform comprising Agilent 1260 Infinity HPLC integrated to Agilent 6490A QQQ mass spectrometer controlled by Masshunter v 7.0 (Agilent Tecmethod and Santa Clara, CA). Glycerophospholipids and sphingolipids were separated with normal-phase HPLC as described before <sup>100</sup>, with a few modifications. An Agilent Zorbax Rx-Sil column (2.1 x 100 mm, 1.8  $\mu$ m) maintained at 25°C was used under the following conditions: mobile phase A (chloroform: methanol: ammonium hydroxide, 89.9:10:0.1, v/v) and mobile phase B (chloroform: methanol: water: ammonium hydroxide, 55:39:5.9:0.1, v/v); 95% A for 2 min, decreased linearly to 30% A over 18 min and further decreased to 25% A over 3 min, before returning to 95% over 2 min and held for 6 min. Separation of sterols and glycerolipids was carried out on a reverse phase Agilent Zorbax Eclipse XDB-C18 column (4.6 x 100 mm, 3.5  $\mu$ m) using an isocratic mobile phase, chloroform, methanol, 0.1 M ammonium acetate (25:25:1) at a flow rate of 300  $\mu$ l/min. Quantification of lipid species was accomplished using multiple reaction monitoring transitions <sup>100</sup> under both positive and negative ionization modes in conjunction with referencing of appropriate internal standards: PA 14:0/14:0, PC 14:0/14:0, PE 14:0/14:0, PG 15:0/15:0, PI 17:0/20:4, PS 14:0/14:0, BMP 14:0/14:0, APG 14:0/14:0, LPC 17:0, LPE 14:0, LPI 13:0, Cer d18:1/17:0, SM d18:1/12:0, dhSM d18:0/12:0, GalCer d18:1/12:0, GluCer d18:1/12:0, LacCer d18:1/12:0, D7-cholesterol, CE 17:0, MG 17:0, 4ME 16:0 diether DG, D5-TG 16:0/18:0/16:0 (Avanti Polar Lipids, Alabaster, AL). Lipid levels for each sample were calculated by summing the total number of moles of all lipid species

measured by all three LC-MS methodologies and then normalizing that total to mol %. Results were analyzed using the lipidr package v2.18.0 <sup>89</sup>.

## Electron microscopy

WTC11 iMGLs (2 clones per genotype) were seeded at 700,000 cells per well in a 6-well plate, combining 6 wells for each collection. After collection, cells were fixed with 2.5% glutaraldehyde in 100mM cacodylate buffer pH 7.4 + 60 mM sucrose and stored at 4°C. Samples were analyzed at Mayo Microscopy and Cell Analysis Core (Rochester, MN, US, <sup>101</sup>). Briefly, samples were rinsed three times for 5 min with a rinse buffer (100 mM cacodylate, 200 mM sucrose) at RT. Secondary fixation was performed for 60 min using 50 mM cacodylate, 100 mM sucrose, and 1% OsO<sub>4</sub>, followed by 3 washes in dH<sub>2</sub>O. A tertiary fixation step was carried out in 1% uranyl acetate (UAc) in dH<sub>2</sub>O for 60 min at RT or overnight at 4°C. Dehydration was performed through a graded ethanol series (25%, 50%, 70%, 95%, and 2 changes of 100% ethanol), followed by final embedding. Qualitative assessment was performed based on size, morphology, quantity and distribution.

## Neutral lipid and mitochondrial potential assays

Lipid droplet (LD) and mitochondrial potential were quantified using FACS. Cells were collected and incubated with 3.7 μM BODIPY (D3922) or 50 nM DiIC<sub>1</sub>(5) (M34151) for 30 minutes at room temperature (RT) or 37°C 5% CO<sub>2</sub>, respectively, protected from light. For FACS, single-cell data were acquired using an Attune flow cytometer (Thermo Fisher Scientific) and analyzed using FCS Express 7 (De Novo Software). Cells were gated based on forward scatter and singlet discrimination. Non-treated cells processed in parallel were used to define the negative gate.

## Cholesterol efflux assay

Cholesterol efflux was measured using a Cholesterol Efflux Fluorometric Assay kit (Biovision, K582-100) following the manufacturer's instructions. Cells were seeded in a 96-well plate at 50,000 cells/well. Cells were labeled with a Labeling Reagent for 1 h at 37°C followed by loading cells with Equilibration Buffer. After overnight incubation, media containing Equilibration Buffer was aspirated and replaced with media containing a cholesterol acceptor human HDL (40 μg/ml) for 5 h at 37°C. Following incubation, supernatants were transferred to flat bottom clear 96-well white polystyrene microplates (Greiner Bio-one, 655095). Adherent cell monolayers were lysed with Cell Lysis Buffer and incubated for 30 min at RT with gentle agitation followed by pipetting to disintegrate cells. Cell lysates were transferred into flat bottom clear 96-well white polystyrene microplates. Fluorescence intensity (Ex/Em = 485/523nm) of supernatants and cell lysates was measured using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, VL0000D0). Percentage of cholesterol effluxed was quantified: % cholesterol efflux = Fluorescence intensity of supernatant / fluorescence intensity of supernatant plus fluorescence intensity of cell lysate x 100.

## Cytokine measurements

Media from THP1 macrophages or WTC11 iMGLs were collected and centrifuged at 300xg for 5 min to eliminate floating cells. The supernatant was transferred to a clean tube and stored at -80°C until use. A custom 10-plex MILLIPLEX Human kit (Millipore; HCYTA-60K-10) was used to determine the levels of IL-6, IL-8, IL-10, IP-10, MCP-1, MDC, MIP-1α, MIP-1β, RANTES, and TNF-α.

## Efferocytosis assays (myelin, lysosensor, lysotracker and DQBSA)

Myelin fragments were isolated from human brain tissue (corpus callosum) as described in <sup>102</sup>. Isolated myelin fragments were labeled with pHrodo dye (Thermo Fisher Scientific, cat. P36600) in PBS for 30 min in the dark at RT, followed by two washes in PBS. Cell confluence and red fluorescence signal were quantified after 15 h of treatment

(pH-rodo myelin 20 µg/ml) using the Incucyte S3 live imaging system. Total integrated density was calculated as mean red fluorescence intensity multiplied by surface area of masked object [RCU x µm<sup>2</sup>] and normalized by cell confluence (phase channel).

THP1 macrophages and WTC11 iMGLs were incubated with 75 nM LysoTracker-Red (Thermo Fisher Scientific, L7525) for 20 min at 37°C followed by 1 µM LysoSensor-Green (Thermo Fisher Scientific, L7535) for 1 min at 37°C. To characterize hydrolytic capacity of lysosomes, cells were incubated with 1µg/ml DQ Red BSA (Thermo Fisher Scientific) for 24h at 37°C. After collecting the cells, single-cell data were acquired using an Attune flow cytometer (Thermo Fisher Scientific) and analyzed using FCS Express 7 (De Novo Software).

## Xenotransplantation

iPSC-derived hematopoietic stem cells (HPCs) from WT and LACTB KO WTC11 line (two clones per genotype) were transplanted into 5xFAD-hCSF1 (n = 16 WT, n = 17 LACTB KO HPCs) mice in accordance with previous publications<sup>60,61,76</sup>. P2 pups from the transplantation-competent 5xFAD-hCSF1 mouse strain were placed on ice to induce hypothermic anesthesia. The site of injection was disinfected. In between donor HPCs, needles were cleaned with consecutive washes of PBS, 70% ethanol, followed by PBS. Tools were kept sterile. Free-hand transplantation was performed using a 30-gauge needle affixed to a 10 µl Hamilton syringe. Mice received 1 µl of HPCs suspended in 1x DBPS at 62.5K cells/µl at each of 8 injection sites, totaling 500k cells/pup. Bilateral injections were performed at 2/5th of the distance from the lambda suture to each eye, injecting into the lateral ventricles at 3 mm and into the overlying anterior cortex at 1 mm, and into the posterior cortex in line with the forebrain injection sites, and perpendicular to lambda at a 45° angle. Transplanted pups were then returned to their home cages and weaned at P21.

Brain tissue dissociation and human microglia isolation from xenotransplanted mouse brain.

Six and half months after HPC transplantation, mice were anesthetized and intracardially perfused with 1X DPBS, half brains were dissected, the cerebellum was removed, and tissue was stored in RPMI 1640 until subsequent perfusions were completed. All steps were performed on ice or at 4°C with ice-cold reagents and all centrifuge steps were performed for 10 min at 400xg with full brake and acceleration unless otherwise stated. Brains were manually homogenized using a 7 mL Dounce homogenizer by adding 4 mL of RPMI 1640 and performing 10 strokes with the “loose” pestle (A) followed by 10 strokes with the “tight” pestle (B). Samples were then run through a pre-soaked 70 µM filter then the filter was washed with 10mL of RPMI 1640. The sample was pelleted by centrifugation, and myelin was removed by resuspension in 8mL of 30% Percoll overlaid with 2 mL of 1X DPBS centrifuged at 400xg for 20 min with acceleration set to 0 and brake set to 4. The myelin band and supernatant were discarded, and cell pellets were resuspended in 70 µL MACS buffer (0.5% bovine serum albumin in 1X DPBS) + 30 µL Mouse Cell Removal beads (Miltenyi) and incubated at 4°C for 15 min. Magnetically labeled mouse cells were separated using LS columns and the MidiMACs separator (Miltenyi) while the unlabeled human cells were collected in the flow through. Human cells were then pelleted by centrifugation and transferred to -80°C for metabolomic analysis.

## Immunohistochemistry (IHC)

Six-month-old mice were perfused with PBS and a hemibrain was fixed in PFA 4%, cryoprotected in sucrose solution and stored at -80°C. Hemibrains were coronally sectioned in a cryostat (30 µm) and stored as free-floating sections in a cryoprotective solution at -20°C.

For IHC, 4 sections per mouse were selected and placed on a 24-well plate. Tissue sections were washed three times in TBS-Tween (TBST) buffer for 5 min each. Only for Ku80/IBA1 staining, a 20-minute incubation in pretreatment buffer (0.6% H<sub>2</sub>O<sub>2</sub>, 0.1% Triton-X in TBST) and antigen retrieval (incubation at 80°C for 30 min in a heat block in sodium citrate buffer pH = 6) steps were performed. Next, sections were washed three times in TBS-Tween, and incubated in blocking solution (5% normal donkey serum in TBST + 0.3% Triton X100) for 2 h. Sections were incubated in primary antibodies overnight at 4°C. Specifically for the Ku80/IBA1, sections were in blocking buffer overnight and the incubation with primary antibodies was at 37°C for 2 h on a thermoblock. After three washes with TBST buffer, sections were incubated for 1.5 hours at room temperature with secondary antibodies (Thermo Scientific 1:200) diluted in TBST. For additional staining for amyloid pathology, sections were washed again in TBST buffer three times and incubated with the antibody Aβ42 (β-Amyloid clone D54D2 Alexa Fluor 488 Conjugate, Cell Signaling Technologies #51374S, 1:250) overnight; or with Thioflavin S (Sigma-Aldrich #T1892, 1:10,000) for 2 min. In all cases, sections were washed again and stained with DAPI (ThermoFisher #D1306, 300 μM) for 5 min at room temperature. Sections were washed 3 times and mounted using PBS onto superfrost slides with TrueBlack mounting media (Biotium).

## Image analysis

A Keyence microscope was used to image full brain sections (single-plane 20x stitched images), except for microglia-plaque association and plaque-compaction analyses, where a Zeiss LSM900 confocal microscope was used to acquire z-stack 20 slides 20X images 2x3 tiles. A custom ImageJ Fiji <sup>103</sup> macro plugin was generated for each analysis. To calculate engraftment efficiency, the number of human microglia (Ku80<sup>+</sup> cells) was divided by the total microglia number (IBA1<sup>+</sup> cells).

To study the number of plaque-associated microglia Imaris software was used for 3D reconstruction. Volume masks were generated for microglia and plaques using a Gaussian filter and specific thresholds. The number of microglia associated with amyloid plaques was manually counted and divided by the corresponding plaque volume. We analyzed 3–4 brain sections per mouse.

## Statistical analysis

Data were visualized and analyzed using R <sup>104</sup>. Differences in marginal means between treatment groups were estimated and tested using the emmeans v1.11.2-8 R package <sup>105</sup> with appropriate linear mixed-effects regression models conditioned on the data using the lmer R package <sup>106</sup>. Batch, iPSC clone, and mouse identifiers were added as covariates in statistical models when appropriate; the model used for each analysis is provided in Table 1.

## Abbreviations

AD - Alzheimer's disease

AUC - area under the curve

CE - cholesterol ester

Cer - ceramides

CNS - central nervous system

CSF - cerebrospinal fluid

DAEP - D-aspartyl endopeptidase activity

DEGA - differential gene expression analysis

DMSO - dimethyl Sulfoxide

DREAM - differential expression analysis for repeated measures

dhCer - dihydroceramides

ED - enzymatically-dead

GSEA - gene set enrichment analysis

GWAS - gene wide association study

HPCs hematopoietic progenitor cells

ICMS - ion chromatography-mass spectrometry

IFN - interferon

IHC - immunohistochemistry

IP - immunoprecipitation

iPSC - induced pluripotent stem cell

iMGLs - iPSC-derived microglia

KD - knock-down

KO - knock-out

LACTB - lactamase B

LACTB2 - lactamase B2

LacCer - lactosylceramide

LPS - lipopolysaccharide

MCSF - macrophage colony-stimulating factor

MG/DG/TG - mono/di/tri-acylglycerides

MP - mobile phase

MR - mendelian randomization

NES - normalized enrichment score

OXPHOS - oxidative phosphorylation

PMA - Phorbol 12-myristate 13-acetate

QTL - quantitative trait locus

RT - room temperature

SCR - scrambled

TNF - tumor necrosis factor

TWAS - transcriptome wide association studies

WT - wild-type

## Declarations

**Ethics approval and consent to participate** - Not applicable

**Consent for publication** - Not applicable

**Availability of data and materials** -

The data generated in this manuscript has been deposited in GEO: bulk RNAseq in THP1 macrophages (GSE323383), WTC11 iMGLs (GSE323384), mouse isolated microglia (GSE324607), and single-cell RNAseq in WTC11 iMGLs (GSE324494). Fastq files of external datasets analyzed in this manuscript are available to download from NCBI GEO for Mancuso et al., (GSE216999), Tuddenham et al., (GSE204702) and Smith et al., (GSE160936), and from the Synapse portal for Olah et al., (syn21438358), Prater et al., (syn51272688 through a data access request) and Lee et al., (syn52795287).

**Competing interests** - W.J.R. consults for Cerevance, OrbiMed, SealRock, NeuroVanda, and Reservoir. A.M.G. serves on the SRB for Genentech and for Muna Therapeutics, she has served as a consultant for Merck and Arbor-bio. M.B.-J. is a co-founder and consultant for Savanna Biotherapeutics.

**Funding** - This work was funded by grants from The Belfer Neurodegeneration Consortium (A.M.G., E.M., C.R-M.) and Freedom Together Foundation. C.R-M. acknowledges support from the Alfonso Martin Escudero Postdoctoral Fellowship and the Alzheimer's Association (24AARF-1191936). Xenotransplantation work was supported by U19 AG069701 (A.M.G, M.B.-J.).

**Authors' contributions** - Conceptualization, study design, and methodology: C.R-M., A.M.G., E.M., R.G-G, J.R; data collection, analysis, and visualization: C.R-M., R.G-G., W.Y.S.; MR analysis: E.M.; bioinformatic support: T.P., M.S., immunoprecipitation experiments: K.A., mouse brain cell isolation: O.C., mouse work support: M.R., Y.L.L., succinylcarnitine measurements: Q.X., xenotransplantation of human cells into the mouse brain: H.D, M.B-J.; enzymatic activity advice: G.B., succinylcarnitine dataset: C.C., writing of original draft: C.R-M., revising: R.G-G., J.R., A.M.G., E.M. All authors read and approved of the final manuscript.

**Acknowledgements** - We would like to acknowledge the Stem Cell Core at Mount Sinai (NY, USA) for the generation of the WTC11 LACTB CRISPR-edited iPSC lines (directed by Samuelle Marro). We also would like to acknowledge Tiago Olivera (University of Minho, Portugal) for his advice in interpreting lipidomic data, Isabelle Gerin for her advice on LACTB DAEP activity (UCLouvain University, Belgium) and Ciyang Wang (Washington University, USA) for support regarding succinylcarnitine data in humans. Metabolomic data were generated in part through the use of the MDACC

Metabolomics Core Facility, which received partial support from the National Cancer Institute under grant P30CA016672 to MD Anderson Cancer Center. The research reported in this publication was not directly funded through the P30CA016672 grant to MD Anderson Cancer Center and is not within the scope of such grant.

This work was supported in part through the computational and data resources and staff expertise provided by Scientific Computing and Data at the Icahn School of Medicine at Mount Sinai and supported by the Clinical and Translational Science Awards (CTSA) grant UL1TR004419 from the National Center for Advancing Translational Sciences. Research reported in this publication was also supported by the Office of Research Infrastructure of the National Institutes of Health under award number S10OD026880 and S10OD030463. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## References

1. Polianskyte Z et al (2009) LACTB is a filament-forming protein localized in mitochondria. PNAS 106:18960–18965
2. Zhang M et al (2022) Structural basis for the catalytic activity of filamentous human serine beta-lactamase-like protein LACTB. Structure 30:685–696e5
3. Ito G, Utsunomiya-Tate N (2025) Discovery and biochemical characterization of the D-aspartyl endopeptidase activity of the serine protease LACTB. J Biol Chem 0
4. Man EH, Sandhouse ME, Burg J, Fisher GH (1983) Accumulation of D-Aspartic Acid with Age in the Human Brain. Science 220:1407–1408
5. Hsiao L-C et al (2025) Impacts of D-aspartate on the Aggregation Kinetics and Structural Polymorphism of Amyloid  $\beta$  Peptide 1–42. J Mol Biol 437:169092
6. Kenessey A, Yen SH, Liu WK, Yang XR, Dunlop DS (1995) Detection of D-aspartate in tau proteins associated with Alzheimer paired helical filaments. Brain Res 675:183–189
7. Cascone A, Lalowski M, Lindholm D, Eriksson O (2022) Unveiling the Function of the Mitochondrial Filament-Forming Protein LACTB in Lipid Metabolism and Cancer. Cells 11:1703
8. Keckesova Z et al (2017) LACTB is a tumour suppressor that modulates lipid metabolism and cell state. Nature 543:681–686
9. Pan DZ et al (2018) Integration of human adipocyte chromosomal interactions with adipose gene expression prioritizes obesity-related genes from GWAS. Nat Commun 9:1512
10. Chen Y et al (2008) Variations in DNA elucidate molecular networks that cause disease. Nature 452:429–435
11. Yang X et al (2009) Validation of candidate causal genes for obesity that affect shared metabolic pathways and networks. Nat Genet 41:415–423
12. Shi W et al The depletion of serine beta-lactamase-like protein (LACTB) ameliorates metabolic dysfunction–associated steatotic liver disease by reducing ubiquitin-mediated degradation of carnitine palmitoyltransferase 2. *Diabetes, Obesity and Metabolism* n/a
13. Zhang B et al (2013) Integrated Systems Approach Identifies Genetic Nodes and Networks in Late-Onset Alzheimer’s Disease. Cell 153:707–720
14. Bellenguez C et al (2022) New insights into the genetic etiology of Alzheimer’s disease and related dementias. Nat Genet 54:412–436
15. Jansen IE et al (2019) Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer’s disease risk. Nat Genet 51:404–413

16. Wightman DP et al (2021) A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nat Genet* 53:1276–1282
17. Wingo AP et al (2021) Integrating human brain proteomes with genome-wide association data implicates new proteins in Alzheimer's disease pathogenesis. *Nat Genet* 53:143–146
18. Cary GA et al (2024) Genetic and multi-omic risk assessment of Alzheimer's disease implicates core associated biological domains. *Alzheimer's Dementia: Translational Res Clin Interventions* 10:e12461
19. Agora AD (2026) portal. <https://agora.adknowledgeportal.org/genes/nominated-targets>. Accessed 22 January
20. Harwood JC et al (2021) Defining functional variants associated with Alzheimer's disease in the induced immune response. *Brain Commun* 3:fcab083
21. Kosoy R et al (2022) Genetics of the human microglia regulome refines Alzheimer's disease risk loci. *Nat Genet* 54:1145–1154
22. Novikova G et al (2021) Integration of Alzheimer's disease genetics and myeloid genomics identifies disease risk regulatory elements and genes. *Nat Commun* 12:1610
23. Nott A et al (2019) Brain cell type–specific enhancer–promoter interactome maps and disease-risk association. *Science* 366:1134–1139
24. Romero-Molina C, Garretti F, Andrews SJ, Marcora E, Goate AM (2022) Microglial efferocytosis: Diving into the Alzheimer's disease gene pool. *Neuron* 110
25. Andrews SJ et al (2023) The complex genetic architecture of Alzheimer's disease: novel insights and future directions. *eBioMedicine* 90:104511
26. Sun R, Jiang H (2024) Border-associated macrophages in the central nervous system. *J Neuroinflammation* 21:67
27. Ghazalpour A et al (2014) Genetic regulation of mouse liver metabolite levels. *Mol Syst Biol* 10:730
28. Suhre K et al (2011) Human metabolic individuality in biomedical and pharmaceutical research. *Nature* 477:54–60
29. Bomba L et al (2022) Whole-exome sequencing identifies rare genetic variants associated with human plasma metabolites. *Am J Hum Genet* 109:1038–1054
30. Tokolyi A et al (2025) The contribution of genetic determinants of blood gene expression and splicing to molecular phenotypes and health outcomes. *Nat Genet* 57:616–625
31. Wang C et al (2024) Genetic architecture of cerebrospinal fluid and brain metabolite levels and the genetic colocalization of metabolites with human traits. *Nat Genet* 56:2685–2695
32. Franzén O et al (2016) Cardiometabolic risk loci share downstream cis- and trans-gene regulation across tissues and diseases. *Science* 353:827–830
33. Brain Map. <https://knowledge.brain-map.org/> Accessed 19 December 2023
34. Abud EM et al (2017) iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases. *Neuron* 94:278–293e9
35. Stemformatics (2026) website. <https://www.stemformatics.org/> Accessed January
36. Arslan A et al (2020) High Throughput Computational Mouse Genetic Analysis. 09.01.278465 Preprint at <https://doi.org/10.1101/2020.09.01.278465> (2020)
37. Bennett JA, Steward LR, Rudolph J, Voss AP, Aydin H (2022) Architecture and Assembly Mechanism of Human LACTB. 04.21.489104 Preprint at <https://doi.org/10.1101/2022.04.21.489104> (2022)

38. Ma W et al (2024) OXCT1 functions as a succinyltransferase, contributing to hepatocellular carcinoma via succinylating LACTB. *Mol Cell* 84:538–551e7
39. Mancuso R et al (2024) Xenografted human microglia display diverse transcriptomic states in response to Alzheimer's disease-related amyloid- $\beta$  pathology. *Nat Neurosci* 27:886–900
40. Olah M et al (2020) Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun* 11:6129
41. Prater KE et al (2023) Human microglia show unique transcriptional changes in Alzheimer's disease. *Nat Aging* 3:894–907
42. Tuddenham JF et al (2024) A cross-disease resource of living human microglia identifies disease-enriched subsets and tool compounds recapitulating microglial states. *Nat Neurosci* 27:2521–2537
43. Smith AM et al (2022) Diverse human astrocyte and microglial transcriptional responses to Alzheimer's pathology. *Acta Neuropathol* 143:75–91
44. Lee D et al (2024) Plasticity of Human Microglia and Brain Perivascular Macrophages in Aging and Alzheimer's Disease. *medRxiv* 2023.10.25.23297558 10.1101/2023.10.25.23297558
45. Wang C, Wang J, Zhu Z, Hu J, Lin Y (2024) Spotlight on pro-inflammatory chemokines: regulators of cellular communication in cognitive impairment. *Front Immunol* 15:1421076
46. Ma X et al (2025) Alzheimer's disease risk ABCA7 p.A696S variant disturbs the microglial response to amyloid pathology in mice. *Neurobiol Dis* 206:106813
47. von Maydell D et al (2025) ABCA7 variants impact phosphatidylcholine and mitochondria in neurons. *Nature* 647:462–471
48. Fu Y, Hsiao J-HT, Paxinos G, Halliday GM, Kim WS (2016) ABCA7 Mediates Phagocytic Clearance of Amyloid- $\beta$  in the Brain. *J Alzheimers Dis* 54:569–584
49. Pellarin I et al (2025) Cyclin-dependent protein kinases and cell cycle regulation in biology and disease. *Sig Transduct Target Ther* 10:11
50. Sun Y, Cheng Z, Liu S (2022) MCM2 in human cancer: functions, mechanisms, and clinical significance. *Mol Med* 28:128
51. Delaval B, Doxsey SJ (2010) Pericentrin in cellular function and disease. *J Cell Biol* 188:181–190
52. Saxton RA, Sabatini DM (2017) mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168:960–976
53. Margolin AA et al (2006) ARACNE: An Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context. *BMC Bioinformatics* 7:S7
54. GWAS Catalog <https://www.ebi.ac.uk/gwas/genes/LACTB>. Accessed 18 January 2026
55. Paciotti S, Albi E, Parnetti L, Beccari T (2020) Lysosomal Ceramide Metabolism Disorders: Implications in Parkinson's Disease. *J Clin Med* 9:594
56. Cunha MI et al Pro-inflammatory activation following demyelination is required for myelin clearance and oligodendrogenesis. <https://doi.org/10.1084/jem.20191390> doi:10.1084/jem.20191390
57. Kocur M et al (2015) IFN $\beta$  secreted by microglia mediates clearance of myelin debris in CNS autoimmunity. *Acta Neuropathol Commun* 3:20
58. Podleśny-Drabiniok A et al (2025) Cytokine-induced reprogramming of human macrophages toward Alzheimer's disease-relevant molecular and cellular phenotypes in vitro. *Cell Rep* 44
59. Pimenova AA et al (2021) Alzheimer's-associated PU.1 expression levels regulate microglial inflammatory response. *Neurobiol Dis* 148:105217

60. Hasselmann J et al (2019) Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. *Neuron* 103:1016–1033e10
61. Romero-Molina C et al (2024) Autosomal Dominant Alzheimer's Disease Mutations in Human Microglia Are Not Sufficient to Trigger Amyloid Pathology in WT Mice but Might Affect Pathology in 5XFAD Mice. *Int J Mol Sci* 25:2565
62. Yang Y et al (2022) Altered succinylation of mitochondrial proteins, APP and tau in Alzheimer's disease. *Nat Commun* 13:159
63. Rajabli F et al (2023) Admixture mapping identifies novel Alzheimer's disease risk regions in African Americans. *Alzheimer's Dement* 19:2538–2548
64. Zhang X et al (2023) Metabolic enzyme *Suc1g2* maintains tolerogenicity of regulatory dendritic cells *diffDCs* by suppressing *Lactb* succinylation. *J Autoimmun* 138:103048
65. Ramirez A et al (2014) *SUCLG2* identified as both a determinant of CSF A $\beta$ 1–42 levels and an attenuator of cognitive decline in Alzheimer's disease. *Hum Mol Genet* 23:6644–6658
66. Kinouchi T et al (2007) Isolation and characterization of mammalian D-aspartyl endopeptidase. *Amino Acids* 32:79–85
67. Rissman RA et al (2004) Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest* 114:121–130
68. Zhang S et al (2017) BACE1 Cleavage Site Selection Critical for Amyloidogenesis and Alzheimer's Pathogenesis. *J Neurosci* 37:6915–6925
69. van Lengerich B et al (2023) A TREM2-activating antibody with a blood–brain barrier transport vehicle enhances microglial metabolism in Alzheimer's disease models. *Nat Neurosci* 26:416–429
70. Boutej H et al (2017) Diverging mRNA and Protein Networks in Activated Microglia Reveal SRSF3 Suppresses Translation of Highly Upregulated Innate Immune Transcripts. *Cell Rep* 21:3220–3233
71. Minhas PS et al (2021) Restoring metabolism of myeloid cells reverses cognitive decline in ageing. *Nature* 590:122–128
72. Hammond TR et al (2018) Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* <https://doi.org/10.1016/j.immuni.2018.11.004>  
doi:10.1016/j.immuni.2018.11.004
73. Dolan M-J et al (2023) Exposure of iPSC-derived human microglia to brain substrates enables the generation and manipulation of diverse transcriptional states in vitro. *Nat Immunol* 24:1382–1390
74. Olah M et al (2018) A transcriptomic atlas of aged human microglia. *Nat Commun* 9:539
75. Sun N et al (2023) Human microglial state dynamics in Alzheimer's disease progression. *Cell* 186:4386–4403e29
76. Claes C et al (2022) The P522R protective variant of *PLCG2* promotes the expression of antigen presentation genes by human microglia in an Alzheimer's disease mouse model. *Alzheimers Dement* 18:1765–1778
77. Ulrich JD et al (2014) Altered microglial response to A $\beta$  plaques in APPPS1-21 mice heterozygous for TREM2. *Mol Neurodegener* 9:20
78. Panyard DJ et al (2023) Large-scale proteome and metabolome analysis of CSF implicates altered glucose and carbon metabolism and succinylcarnitine in Alzheimer's disease. *Alzheimers Dement*.  
<https://doi.org/10.1002/alz.13130>
79. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R (2018) Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* 36:411–420

80. Korsunsky I et al (2019) Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods* 16:1289–1296
81. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139–140
82. Hoffman GE, Roussos P (2021) Dream: powerful differential expression analysis for repeated measures designs. *Bioinformatics* 37:192–201
83. Germain P-L, Lun A, Garcia Meixide C, Macnair W, Robinson MD (2022) Doublet identification in single-cell sequencing data using scDblFinder. *F1000Res* 10, 979
84. Fleming SJ et al (2023) Unsupervised removal of systematic background noise from droplet-based single-cell experiments using CellBender. *Nat Methods* 20:1323–1335
85. Hafemeister C, Satija R (2019) Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. *Genome Biol* 20:296
86. Zappia L, Oshlack A (2018) Clustering trees: a visualization for evaluating clusterings at multiple resolutions. *Gigascience* 7:giy083
87. Gu Z, Eils R, Schlesner M (2016) Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32:2847–2849
88. Hemani G et al (2018) The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 7:e34408
89. Mohamed A, Molendijk J, Hill MM (2020) lipidr: A Software Tool for Data Mining and Analysis of Lipidomics Datasets. *J Proteome Res* 19:2890–2897
90. Hemani G, Tilling K, Smith GD (2017) Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet* 13:e1007081
91. Auton A et al (2015) A global reference for human genetic variation. *Nature* 526:68–74
92. Oakley H et al (2006) Intraneuronal  $\beta$ -Amyloid Aggregates, Neurodegeneration, and Neuron Loss in Transgenic Mice with Five Familial Alzheimer's Disease Mutations: Potential Factors in Amyloid Plaque Formation. *J Neurosci* 26:10129–10140
93. McQuade A et al (2020) Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. *Nat Commun* 11:1–17
94. Hoffman GE, Roussos P (2021) Dream: powerful differential expression analysis for repeated measures designs. *Bioinformatics* 37:192–201
95. Subramanian A et al (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 102:15545–15550
96. Hoffman G, Roussos P, Fast (2025) flexible analysis of differences in cellular composition with crumblr. Preprint at. <https://doi.org/10.21203/rs.3.rs-5921338/v1>
97. Vlahos L et al (2021) Systematic, Protein Activity-based Characterization of Single Cell State. 05.20.445002 Preprint at <https://doi.org/10.1101/2021.05.20.445002> (2023)
98. Columbia Biomarker Core (2026) <https://www.irvinginstitute.columbia.edu/about-us/resources-and-cores/biomarkers-core-laboratory-bcl> Accessed January
99. Area-Gomez E et al (2021) Lipidomics study of plasma from patients suggest that ALS and PLS are part of a continuum of motor neuron disorders. *Sci Rep* 11:13562
100. Chan RB et al (2012) Comparative Lipidomic Analysis of Mouse and Human Brain with Alzheimer Disease\*. *J Biol Chem* 287:2678–2688

101. Electron Microscopy Core (2026) <https://www.mayo.edu/research/core-resources/microscopy-cell-analysis-core/overview> Accessed January
102. Machlovi SI et al (2022) APOE4 confers transcriptomic and functional alterations to primary mouse microglia. *Neurobiol Dis* 164:105615
103. Schindelin J et al (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682
104. R Core Team (2026) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/> Accessed February 2026
105. Lenth R, Piaskowski J (2026) emmeans: Estimated Marginal Means, aka Least-Squares Means. doi:10.32614/CRAN.package.emmeans <https://doi.org/10.32614/CRAN.package.emmeans> Accessed March 2026
106. Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw* 67:1–48

## Figures

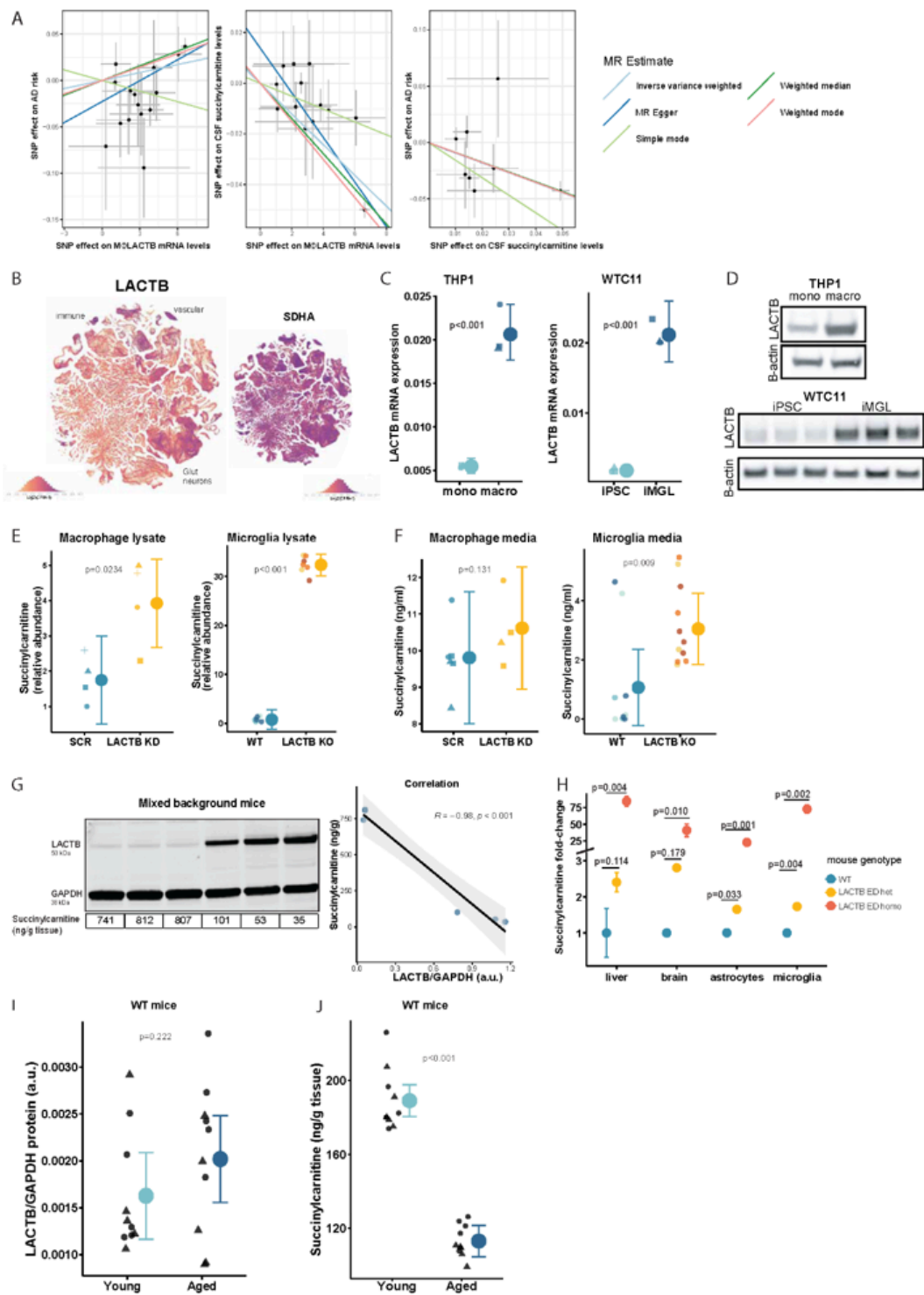


Figure 1

**Lower LACTB expression in myeloid cells leads to higher succinylcarnitine levels and lower AD risk.** A) Genetically predicted lower LACTB expression—instrumented using macrophage cis-eQTLs at the LACTB locus—is associated with lower AD risk (left panel). Genetically predicted lower LACTB expression is also associated with higher succinylcarnitine levels in the CSF (middle panel). Furthermore, genetically predicted higher succinylcarnitine levels are associated with lower AD risk (right panel). B) LACTB is expressed higher in the immune cluster compared to other brain cells, in contrast to another mitochondrial protein (SDHA), data from Brain Atlas <sup>33</sup>. C, D) LACTB mRNA (C)

and protein levels (D) are increased upon differentiation (THP1 macrophages vs monocytes and WTC11 iMGLs vs iPSC). E) Succinylcarnitine levels are increased in the lysate of LACTB KD/KO myeloid cells compared to SCR/WT. F) Succinylcarnitine is increased in the cell culture media of LACTB KD/KO myeloid cells compared to SCR/WT. G) Higher succinylcarnitine levels in mice correlate with lower LACTB expression (C57B6 x SLJ background, 2 months old). H) Higher succinylcarnitine levels in the liver, brain, isolated microglia and astrocytes of LACTB enzymatically-dead (ED) mice compared to WT (n=2 mice per genotype, 3 months old). I) LACTB protein levels increase with age in WT mice (12 vs 2 months old). J) Succinylcarnitine levels decrease with age in WT mice (12 vs 2 months old). Graphs display individual data points (left) alongside estimated marginal means with 95% confidence intervals (right). For the raw data, dot shapes represent independent differentiations (for macrophages) or different sex (for mouse data); dot colors indicate distinct microglia clones. Statistical details are provided in Table 1.

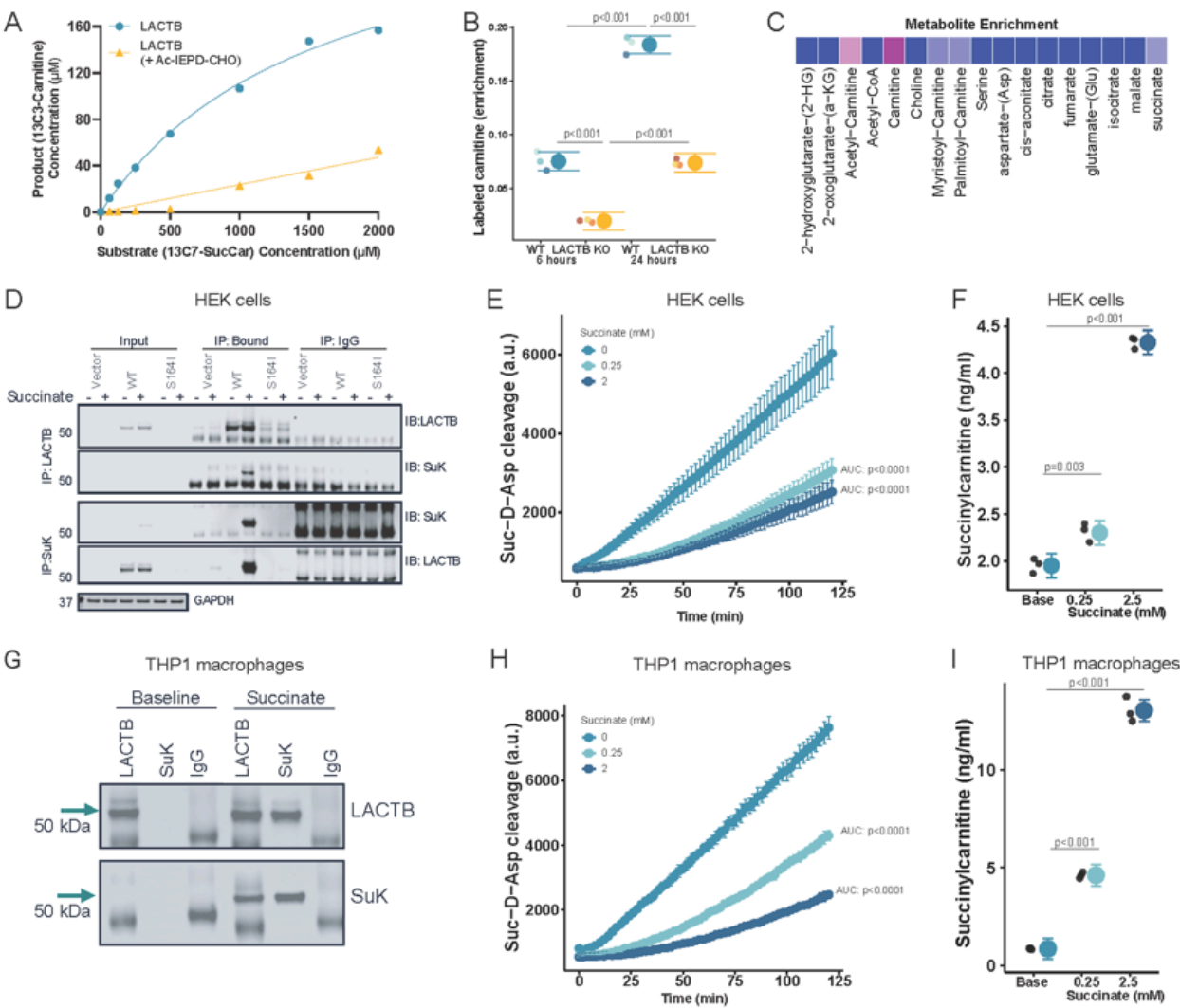


**Figure 2**

**LACTB directly cleaves succinylcarnitine and succinylation inhibits LACTB.** A)  $^{13}\text{C}_3$ -carnitine generation after substrate ( $^{13}\text{C}_7$ -succinylcarnitine) incubation with LACTB recombinant protein in the absence or presence of LACTB inhibitor (Ac-IEPD-CHO) in a cell-free assay. B) Reduced label incorporation into carnitine in LACTB KO iMGLs after incubation with  $^{13}\text{C}_3$ -succinylcarnitine compared to WT iMGLs (n=3 clones per genotype). C) Representative heatmap

showing label incorporation from  $^{13}\text{C}_7$ -succinylcarnitine into the indicated metabolites in WT iMGLs. D, G) Immunoprecipitation (IP) analysis from HEK cell (D) or THP1 cell (G) lysates harvested 4 h post-treatment with 2 mM diethyl succinate. Lysates were subjected to immunoprecipitation using an anti-LACTB antibody (IP: LACTB) or a pan-succinyllysine antibody (IP: SuK). Non-specific IgG control antibodies (IP: IgG) were used as controls. Precipitated proteins (IP: Bound) and initial cell lysates (Input) were resolved by SDS-PAGE and analyzed by western blot (WB) using specific antibodies against LACTB (IB: LACTB) or succinyllysine (IB: SuK). Representative blots are shown from (n=2) independent biological replicates. E, H) Succinylation of LACTB is associated with a reduced rate of succinyl-D-Asp cleavage in HEK cells (n=2) (E) and THP1 macrophages (n=2) (H). AUC was calculated and used as outcome variable in statistical analyses. F, I) Succinylation of LACTB is associated with higher succinylcarnitine levels in HEK cells (n=3) (F) and THP1 macrophages (n=3) (I). Graphs display individual data points (left) alongside estimated marginal means with 95% confidence intervals (right). For the raw data, dot shapes represent independent macrophage differentiations, and dot colors indicate distinct microglia clones. Statistical details are provided in Table 1.

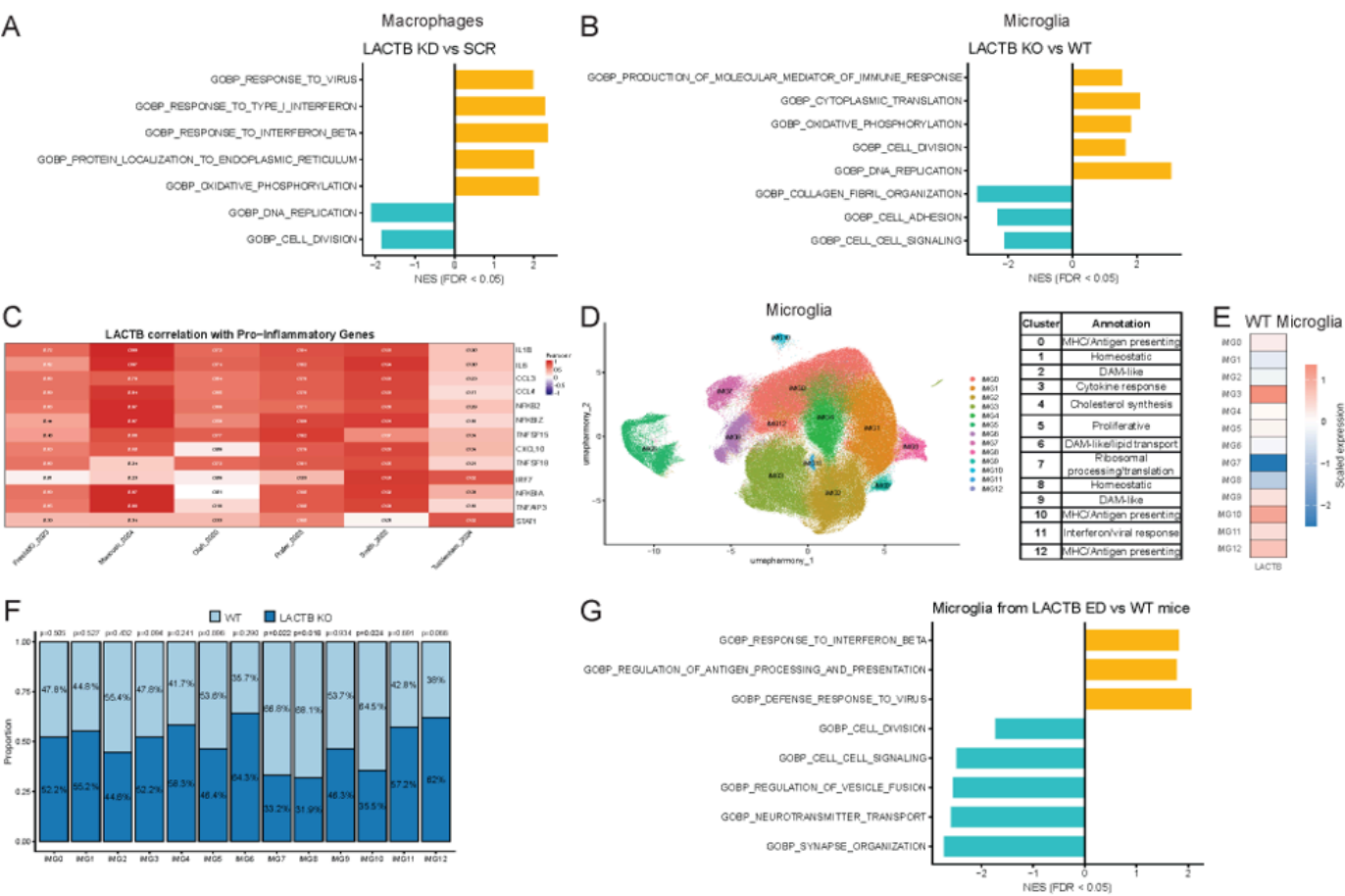


**Figure 3**

**LACTB KD/KO in myeloid cells alters expression of genes in pathways related to oxidative phosphorylation, protein translation, cell cycle and immune response.** A) Pathways enriched in GSEA analysis of bulk RNAseq data from LACTB KD THP1 macrophages compared to SCR (n=5 independent differentiations). B) Pathways enriched in GSEA analysis of bulk RNAseq data from LACTB KO iMGLs compared to WT (3 clones per genotype, 2 differentiations per clone). C) Heatmap of Pearson correlation coefficients between LACTB expression and genes encoding pro-

inflammatory cytokines across microglial states in publicly available human single-cell microglia datasets<sup>39–44</sup>. D) Single-cell clusters and corresponding annotations of WT and LACTB KO iMGLs (3 clones per genotype, 1-2 differentiations per clone). E) *LACTB* expression across clusters in WT iMGLs. F) Cluster proportions in WT and LACTB KO iMGLs. Cluster proportions were estimated using crumbly and compared by genotype using a generalized linear model with batch as a covariate (see Methods for further details). G) Pathways enriched in GSEA analysis of bulk RNAseq data from mouse microglia isolated from the brains of LACTB ED mice compared to controls (3 months old, n=3). Complete lists of genes and pathways are provided in Tables 2 and 3. NES = Normalized Enrichment Score.

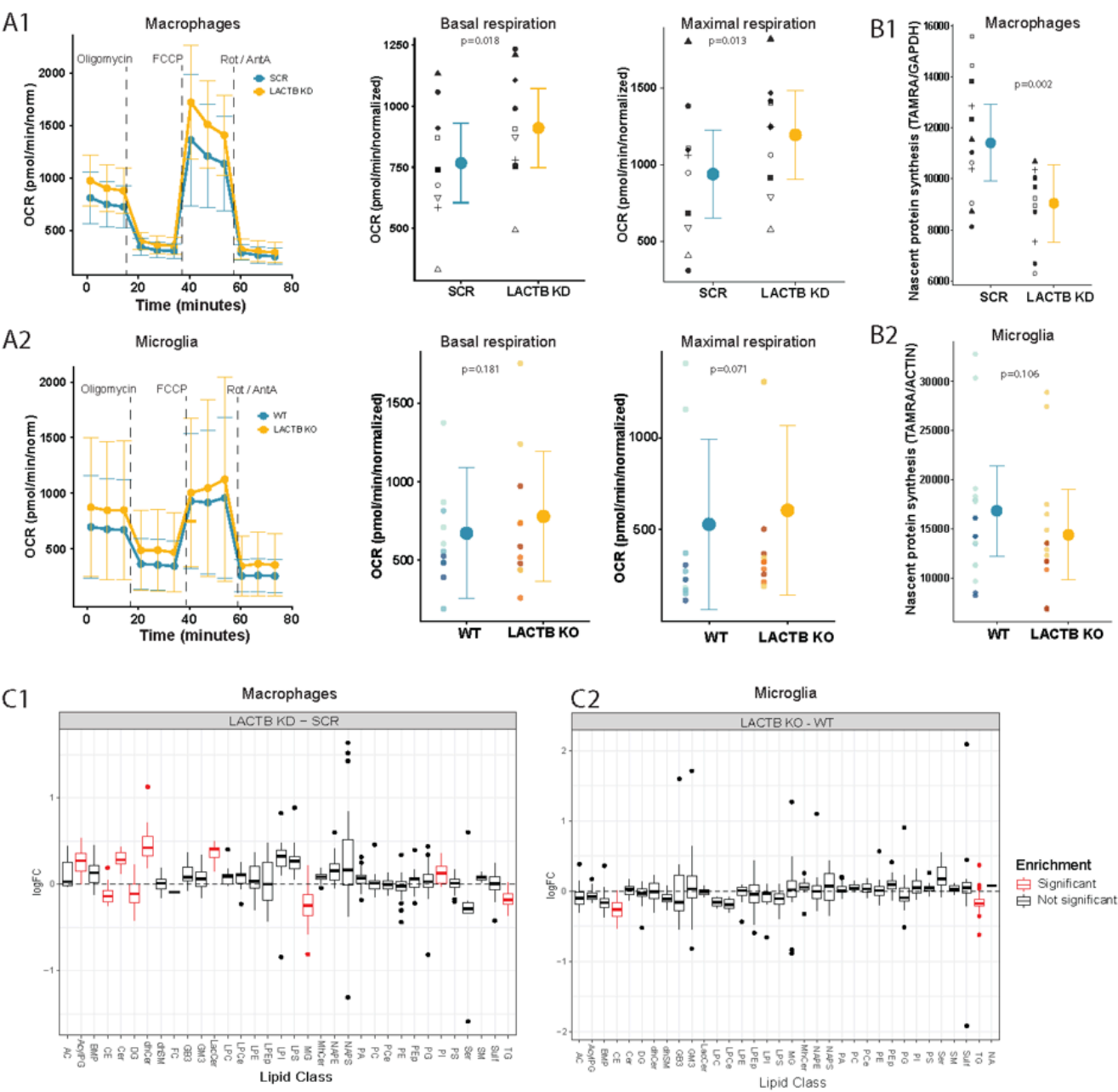


**Figure 4**

**LACTB KD/KO in myeloid cells increases oxidative phosphorylation and reduces protein synthesis, cholesteryl esters, and triacylglycerides.** A) Increase in OXPHOS (measured by mitostress seahorse assays) in LACTB KD THP1 macrophages (10 differentiations, 3-6 technical replicates each), and WTC11 LACTB KO iMGLs (3 clones per

genotype, 3 differentiations per clone, 3-6 technical replicates each) compared to controls. B) Reduction in nascent protein synthesis (measured with a methionine analog) in LACTB KD/KO myeloid cells compared to controls. Graphs display individual data points (left) alongside estimated marginal means with 95% confidence intervals (right). For the raw data, dot shapes represent independent macrophage differentiations, and dot colors indicate distinct microglia clones. Statistical details are provided in Table 1. C) Lipidomics alterations in LACTB KD vs SCR THP1 macrophages (n=3) and WTC11 LACTB KO vs WT iMGLs (n=3 clones, 1-2 differentiations each), showing a consistent reduction in CE and TG across the two myeloid cell types. Statistical details are provided in Table 4.

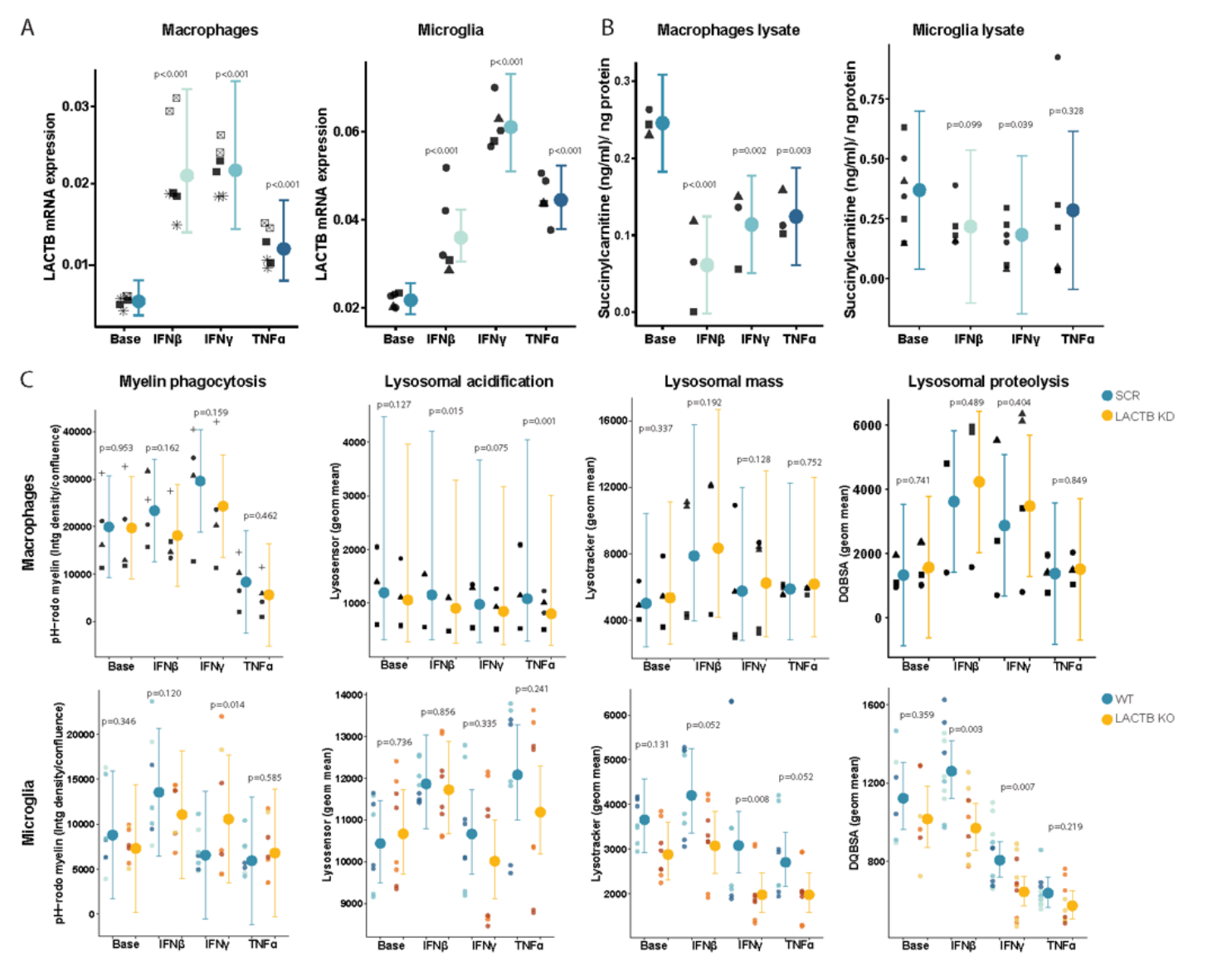


Figure 5

**LACTB KD/KO in myeloid cells alters efferocytosis-related pathways after immune stimulation.** A) LACTB mRNA expression increases after stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$  in THP1 macrophages (6 hours treatment) or WTC11 iMGLs (24 hours treatment). B) Succinylcarnitine levels decrease after stimulation with IFN- $\beta$ , IFN- $\gamma$ , or TNF- $\alpha$  in THP1 macrophages (6 hours treatment) or WTC11 iMGLs (24 hours treatment). C) Efferocytosis-related assays (myelin phagocytosis and lysosomal acidification, mass and proteolytic capacity) in LACTB KD/KO myeloid cells compared to SCR/WT. Graphs display individual data points (left) alongside estimated marginal means with 95%

confidence intervals (right). For the raw data, dot shapes represent independent differentiations, and dot colors indicate distinct microglia clones. Statistical details are provided in Table 1.

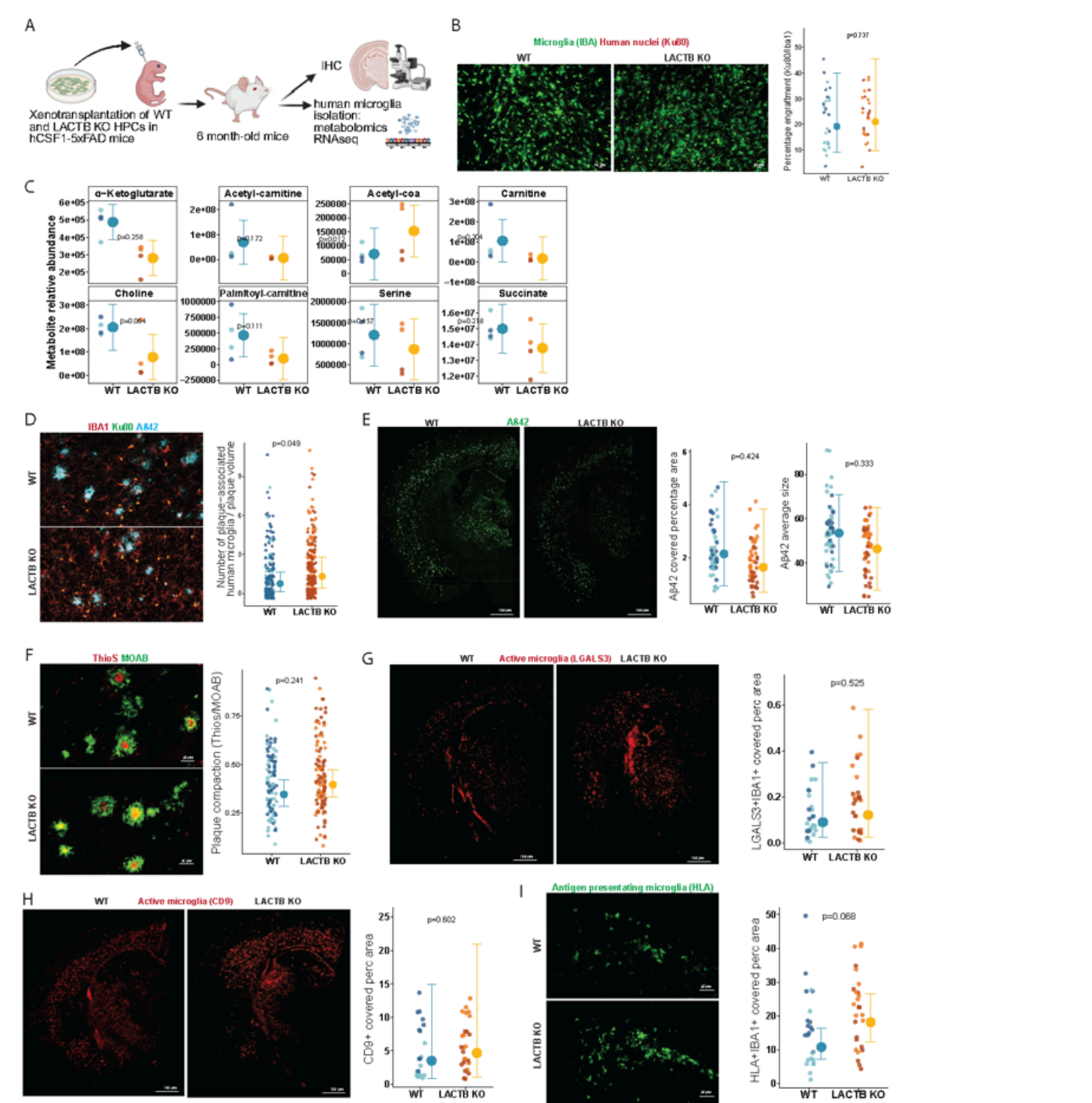


Figure 6

**LACTB KO human microglia show increased clustering around amyloid plaques in a mouse model of amyloid deposition.** A) Diagram summarizing the experimental design for xenotransplantation of human microglia precursors in mice. B) No differences in engraftment between human LACTB KO and WT microglia in hCSF1-5xHAD mice. C)

Metabolomics in human LACTB KO or WT microglia isolated from the brains of hCSF1-5xFAD mice (n=2 clones per genotype, 2 mice injected per clone). D-I) Immunostaining analysis of the number of human microglia (Ku80+Iba1+ cells) associated per plaque (A $\beta$ 42) (D), the area covered by plaques and the average plaque size (A $\beta$ 42) (E), the plaque compaction (ThioS/MOAB ratio) (F), and the area covered by active (LGALS3 (G), CD9 (H) markers) or antigen-presenting microglia (HLA marker (I)) microglia in hCSF1-5xFAD mice xenotransplanted with LACTB KO or WT human microglia (n=2 clones per genotype, 4-5 mice injected per clone). Graphs display individual data points (left) alongside estimated marginal means with 95% confidence intervals (right). For the raw data dot colors indicate distinct xenotransplanted microglia clones. Statistical details are provided in Table 1.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tablesandadditionalfiles.zip](#)
- [SupplementaryFigures.docx](#)