Microglia mitochondrial complex I deficiency during development induces glial dysfunction and early lethality

Bella Mora-Romero[1,2,3,*], Nicolas Capelo-Carrasco[1,3,4,*], Juan J. Pérez-Moreno[1,2,3,*,†], María I. Alvarez-Vergara[1,2,3,5], Laura Trujillo-Estrada[3,6], Carmen Romero-Molina[1,3,4,7], Emilio Martinez-Marquez[1,3,8], Noelia Morano-Catalan[1,3], Marisa Vizuete[1,3,4], Jose Lopez-Barneo[1,3,8], Jose L. Nieto-Gonzalez[1,3,8], Pablo, Garcia-Junco-Clemente[1,3,8], Javier Vitorica[1,3,4], Antonia Gutierrez[3,6], David Macias[1,8], Alicia E. Rosales-Nieves[1,3,4], and Alberto Pascual[1,3,†]

[1]Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain. [2]Department of Biología Celular, Facultad de Biología, Universidad de Sevilla, Seville, Spain. [3]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain. [4]Department of Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, Seville, Spain. [5]Present address: Institute for Neurovascular Cell Biology, University Hospital Bonn, Bonn, Germany. [6]Department of Biología Celular, Genética y Fisiología, Facultad de Ciencias, Instituto de Investigacion Biomedica de Malaga (IBIMA), Universidad de Málaga, Málaga, Spain. [7]Present address: Ronald M. Loeb Center for Alzheimer's Disease - Icahn School of Medicine at Mount Sinai, New York, NY, USA. [8]Departamento de Fisiología Médica y Biofísica, Universidad de Sevilla, Seville, Spain.

***These authors contributed equally.**

[†]**Corresponding author. E-mail:** jpmoreno@us.es (J.J.P.-M.); apascual-ibis@us.es (A.P.)

Abstract

Primary mitochondrial diseases (PMDs) associate with pediatric neurological disorders and are traditionally related to oxidative phosphorylation system (OXPHOS) defects in neurons. Interestingly, both PMD mouse models and patients also show gliosis, and pharmacologic depletion of microglia, the brain resident innate immunity, ameliorates the multiple signs of a mouse model. Since microglia activation correlates with the expression of OXPHOS genes, we studied whether OXPHOS deficits in microglia may contribute to PMDs. We first observed that the metabolic rewiring associated to microglia stimulation *in vitro* (IL-33 or TAU treatment), was partially changed by complex I (CI) inhibition (rotenone treatment). *In vivo*, we generated a mouse model deficient for CI activity in microglia (MGcCI). MGcCI microglia showed metabolic rewiring and gradual transcriptional activation, which leads to hypertrophy and dysfunction in juvenile (1-month-old) and adult (3-month-old) stages, respectively. MGcCI mice presented widespread reactive astrocytes, a decrease of synaptic markers accompanied by increased number of parvalbumin neurons, a behavioral deficit characterized by prolonged periods of immobility, loss of weight, and premature death that was partially rescued by pharmacologic depletion of microglia.

Our data demonstrate that microglia development depends on mitochondrial Complex I and suggest a direct microglial contribution to PMDs.

Introductory paragraph

Microglia are the most abundant brain macrophages, and among other functions, phagocyte unconnected synapses, dying neurons, and detrimental products (i.e., β-Amyloid plaques). This activity involves morphologic changes, including cytoplasmic protrusions, and the production of signaling molecules (i.e., cytokines), which require biosynthetic activity with a demanding energy cost[1,2]. Microglia contribute to neurological disorder progression either by reduced[3,4] or increased activity[5], highlighting the interest in understanding the regulation of microglial states.

Main text

Previous work has shown that microglia upregulate glycolysis and OXPHOS during phagocytosis, in a process driven by IL-33[6], and in Aß and TAU accumulating mouse models[2]. To understand the metabolic reorganization induced by either physiologic (IL-33) or pathologic (TAU) stimulation, we first performed targeted metabolomics in mouse primary microglial cell cultures (Extended Data Fig. 1a,b and Supplementary Table 1). IL-33 and TAU treatments increased, among others, amino acid, ATP, and GSH levels (Extended Data Fig. 1a,b), suggesting augmented metabolic activity. To define the role of OXPHOS in the stimuli-induced metabolic rewiring, we focused on CI activity, known to have a key role in neurological disorders[7]. To this end, we exposed mouse primary cell cultures to rotenone, a well described CI inhibitor. Rotenone treatment (24 h) modified several metabolites (Extended Data Fig. 1a,b), including accumulation of NADH (in IL-33; not detected in TAU), previously observed in other cells lacking CI activity[8,9], and a decrease in ATP, UTP, GTP,

and GSH levels (Extended Data Fig. 1a,b), suggesting that energy and antioxidant production are OXPHOS-dependent in microglia. On stimuli-induced microglia, rotenone treatment reduced the levels of the glycolytic intermediate fructose 1,6-bisphosphate, and the anaplerotic providers of the tricarboxylic acid cycle, aspartate and glutamate. In addition, rotenone induced accumulation of fumarate and alpha-ketoglutarate (when combined with IL-33) or malate (when combined with TAU) (Extended Data Fig. 1a,b), suggesting the inversion of the mitochondrial malate dehydrogenase. This adaptation has already been described in OXPHOS deficient cells to partially compensate the accumulation of NADH[10]. Overall, our data indicate that both physiologic or pathologic stimuli activate microglial metabolism, and that CI inhibition modifies this metabolic rewiring, impairing the production of energy and antioxidants. This supports an important role of CI in microglial function.

To evaluate the *in vivo* role of CI in microglia, we deleted the essential CI subunit *Ndufs2*[8] with the *Cx3cr1-Cre* mouse line, generating a microglial conditional CI mouse model (MGcCI; Fig. 1a). The *Ndufs2* gene encodes a 49-kDa protein that forms the CI ubiquinone-binding site (quinone cage) together with the mitochondrially encoded subunits ND1 and ND3[11]. This site is involved in the last transfer of electrons from CI to ubiquinone during the NADH/ubiquinone oxidoreductase reaction and is where the rotenone binds CI[12]. As expected by its relevant position in the CI catalytic core, we have previously shown that ubiquitous deficiency of NDUFS2 disturbs CI assembly and function in kidney[8].

As the *Cx3cr1-Cre* line has shown some leakiness in astrocytes and neurons when combined with a ROSA26 fluorescent Cre reporter[13], we first verified if the

deletion of the *Ndufs2* gene was taking place specifically in microglia. Using FACS-isolated microglia and astrocytes from MGcCI animals (Extended Data Fig. 2a–e), we observed decreased mRNA levels of *Ndufs2* in 1- and 3-month-old microglia (Fig. 1b) but not in 3-month-old astrocytes (Extended Data Fig. 2f). To discard Cre-mediated recombination in neurons, we measured the NDUFS2 mRNA and protein levels in total RNA from cortex, hippocampus, and striatum, showing no differences in 1- or 3-month-old mice (Extended Data Fig. 2g and h). Moreover, MGcCI primary cell cultures showed reduced *Ndufs2* mRNA and NDUFS2 protein levels in microglia (Fig. 1c) but not in astrocytes (Extended Data Fig. 2i); and increased NADH and decreased aspartate in microglia (Fig. 1d). These data indicate a successful and specific recombination of the *Ndufs2* locus in MGcCI microglia.

To determine the effect of CI deficiency on mitochondrial respiration, we measured the oxygen consumption rate. MGcCI microglia showed dramatically reduced mitochondrial basal and maximal respiration (Fig. 1e), although CI-dependent activity was still observed, suggesting that, as previously described, long-lasting OXPHOS proteins require a long time to be replaced[14]. Similar to other CI-deficient cells[9], MGcCI microglia did upregulate fermentative glycolysis (glucose to lactate), as estimated by the measurement of the extracellular acidification rate (Fig. 1f). Finally, we studied the mitochondria of MGcCI microglia at the ultrastructural level, showing a non-significant trend to increase in the area and load of mitochondria (Extended Data Fig. 3a), similar to what we previously observed in carotid body cells deficient in *Ndufs2*[8].

Transcriptomic analysis of both isolated juvenile (1-month-old) and adult (3-month-old) microglia showed reduced *Ndufs2* mRNA levels (Supplementary

Table 2) and enrichment of the OXPHOS, glycolysis (a trend in juvenile microglia), and ribosome gene sets (Fig. 1g, Extended Data Fig. 3b, and Supplementary Table 3), suggesting changes in microglial metabolism. Adult but not juvenile MGcCI microglia showed simultaneous upregulation of the methionine cycle, the cytosolic one-carbon by folate, and the pentose phosphate pathways (Fig. 1h, Extended Data Fig. 4a,b, Supplementary Table 3, and Supplementary Table 4). This metabolic rewiring was not observed in phagocytic microglia during development or in adult microglia from AD mouse models (Extended Data Fig. 4c,d,e and Supplementary Table 4), and is similar to the previously reported in LPS-treated monocyte-derived cells[15]. In terms of effectors and regulators of microglial function, MGcCI juvenile and adult microglia showed an enrichment of the disease-associated microglia (DAM) signature[16] with partial repression of the homeostatic signature (Fig. 1i, Extended Data Fig. 4f, and Supplementary Table 3), similar to the immune responses of microglia during development (Extended Data Fig. 4g and Supplementary Table 3) and in models of chronic neurodegenerative diseases[6,17]. Interestingly, the AKT/mTOR pathway and the TNFα signaling pathway *via* NFκB (Fig. 1i and Supplementary Table 3) were downregulated in juvenile and enriched in adult MGcCI microglia, suggesting differential and progressive microglial adaptations to CI deficiency.

To further investigate the progressive effect of CI deficiency in microglia, we characterized their morphology and function in both juvenile and adult microglia in different brain areas. To quantify morphology, we stained brain slices with ionized calcium-binding adapter molecule 1 (IBA1, a marker of tissue-resident macrophages that is not upregulated in NDUFS2 deficient microglia, Supplementary Table 2) and performed computational segmentation of high-

resolution images[18]. Juvenile MGcCI cortical and striatal microglia displayed increased total cell volume and area (a trend in the cortex) and a trend to increase filament length and filament area (Fig. 2a and Extended Data Fig. 5a), suggesting hypertrophy of juvenile MGcCI microglia. By contrast, adult MGcCI cortical microglia showed reduced total cell volume, area, filament length, and filament area (Fig. 2b). Striatal adult MGcCI microglia showed a similar non-significant trend (Extended Data Fig. 5b), characteristic of dystrophic microglia[19]. Since microglial dystrophy may correlate with increased proliferation[20], we estimated the division rate and cell density. No dividing cell stained with the Ki67 proliferative marker was observed in juvenile microglia (n = 4 control and MGcCI mice) and, correspondingly, a decrease was observed in the transcriptomic G2M checkpoint gene set related with cell proliferation (Extended Data Fig. 5c and Supplementary Table 3). Adult MGcCI microglia showed an increased density of Ki67-positive cells, while the total microglial density was unaffected (Extended Data Fig. 5d), suggesting an augmented cellular turnover. In agreement, transcriptomic analysis showed the G2M checkpoint gene set as enriched in adult microglia (Extended Data Fig. 5e and Supplementary Table 3).

To assess the functionality of MGcCI microglia, we estimated their ability to phagocyte dying cells by surrounding them with cytoplasmic projections[21]. We measured the number of dying cells (cleaved CASPASE-3 immunoreactive) and the phagocytic index of MGcCI (in the dentate gyrus for juvenile and in the whole brain for adult mice). Compared with controls, the juvenile MGcCI brain presented a significant decrease in the number of dying cells and a trend to increase the phagocytic index (Fig. 2c), suggesting a not decreased or even a higher performance in recognizing and clearing these cells. On the contrary, adult

MGcCI brains showed accumulation of dying cells and decreased phagocytic index (Fig. 2d), reflecting a deterioration of the phagocytic response concomitant with the long-term reduction in CI function. The reduction of phagocytosis in MGcCI mice was similar in different brain areas (cortex, hippocampus, and striatum –a trend–) (Extended Data Fig. 5f). To investigate whether phagocytosis of non-physiological cell death was also altered in adult MGcCI, we treated adult control and MGcCI mice with sub-epileptogenic doses of kainate[22], which induces cell death in the hippocampus. As physiologic phagocytosis, adult MGcCI microglia showed a non-significant trend to decrease the phagocytic index (Fig. 2e). Although a rare finding, we found dying (cCASPASE-3 immunoreactive) and dividing microglia (two nuclei, one pyknotic; Fig. 2f), suggesting that, as described[23], division and cell death are coupled in these cells. Altogether, our data indicate that MGcCI microglia are characterized by altered morphology and progressive weakening of housekeeping phagocytosis.

The MGcCI model is independent of injury, infection, or disease, making it ideal to understand the crosstalk between microglia and other cell types, and the physiological impact of long-term microglial dysfunction. We studied the response of astrocytes, a cell type that is altered together with microglia in many disorders, including PMDs[24,25]. Glial fibrillary acidic protein (GFAP) immunostaining, a marker of reactive astrocytes, showed an extensive and enhanced signal all over the brain of MGcCI mice, particularly clear in cortical areas, where it is not normally expressed (Fig. 3a). We detected an increase in both the number of GFAP immunoreactive cells and the GFAP load (Fig. 3b and Extended Data Fig. 6a). This GFAP upregulation was observed in different brain regions for both juvenile and adult MGcCI mice (Fig. 3a), as confirmed by qRT-PCR (Extended

Data Fig. 6b) and western blot (cortex, Extended Data Fig. 6c). To further study the effect of CI-deficient microglia in astrocytes, we isolated astrocytes by FACS and performed global transcriptomic profiling analysis (Supplementary Table 2). Interestingly, GSEA revealed enriched gene sets of inflammation as the TNFα signaling via NFκB and the interferon alpha-related response (Fig. 3c and Supplementary Table 3), characteristics of reactive astrocytes in neurodegeneration[26]. To confirm that microglia were inducing the reactive astrocytic phenotype, we first excluded an immune peripheral contribution by generating the *Lyz2-Cre/+*; *Ndufs2*$^{Flox/Flox}$ (LYcCI) mouse model, with low recombination in microglia but high in other immune derived-cells[27], and did not observed any changes in microglial morphology or in astrocytic reactivity (Extended Data Fig. 6d), in agreement with a recent report showing that viability was not compromised in mice with *Lyz2-Cre*- or *Cd11c-Cre*-mediated *Tfam* deficiency[28]. Second, we treated MGcCI mice with the CSF1 receptor inhibitor PLX3397 at two different postnatal times, at weaning (P24, early) and at the postnatal day 45 (P45, late). We validated that PLX treatment produced microglia depletion (Fig. 3d) and observed a strong reduction in the number of GFAP immunoreactive astrocytes in the cortex after either early or late PLX treatment (Fig. 3d), supporting that microglial signals induce astrocytes reactivity in MGcCI mice.

As microglia are involved in brain postnatal maturation[29] and changes in microglia and astrocytes during postnatal development have an impact on brain circuits[30], we studied pre- and post-synaptic markers in juvenile and adult MGcCI brains. Juvenile synaptic markers did not reveal any substantial changes (Extended Data Fig. 7a). However, adult MGcCI mice presented a significant

220 reduction in the levels of the presynaptic markers VGAT and VGLUT in the cortex
221 (Fig. 4a), and a decrease in the mRNA levels of the *Pvalb* but not *Sst*, markers
222 of inhibitory interneurons, which are particularly vulnerable in brain disorders[31]
223 (Extended Data Fig. 7b). Finally, we measured the number of parvalbumin (PV)
224 immunoreactive cells in juvenile and adult MGcCI mice. Interestingly, the density
225 in the adult cortex (Fig. 4b) but not in the hippocampus (Extended Data Fig. 7c)
226 was increased, suggesting a reorganization in the cortical circuitry.

227 To interrogate if the observed changes alter brain function, we studied the
228 behavior of MGcCI mice. Adult but not juvenile MGcCI mice were characterized
229 by prolonged periods of absolute immobility, up to 30 seconds (Supplementary
230 Videos 1 and 2), as revealed by the inactivity time (percentage of total) in a
231 chamber with electrical detection of movement (Fig. 4c). We confirm this
232 behavioral phenotype by studying locomotor activity of adult MGcCI, observing
233 reduced ambulation and increased freezing events (Extended Data Fig. 7d)
234 without alterations in the track length or average velocity (Extended Data Fig. 7e).
235 This phenotype was followed by a sharp decrease in weight (Extended Data Fig.
236 7f,g), spasticity, and premature death between 3-4 months of age (Fig. 4d).

237 To validate that the described defects were specifically due to the loss of
238 NDUFS2 in microglia, we used the LYcCI mouse model, which did not present
239 weight, behavioral, or life span alterations (Extended Data Fig. 7h–j). In addition,
240 we checked if pharmacologic depletion of microglia could prevent the premature
241 death of MGcCI mice. Interestingly, postnatal treatment of 45-day-old mice did
242 not delay the endpoint (20% weight loss), but earlier treatment (at weaning)
243 significantly postponed the death of the mice (Fig. 4e), suggesting that abnormal

244 microglial activity during early postnatal development is involved in the death of
245 MGcCI mice.

246 Microglia activity depends on high glucose consumption[32–35], and recent
247 reports link mitochondrial metabolic activity with phagocytosis in microglia[33,36,37].
248 Here, we show that CI activity is required in microglia during development to
249 prevent dysfunction of these cells. Paradoxically, a recent article showed that CI
250 activity sustains neuroinflammation in a multiple sclerosis mouse model through
251 reverse activity of CI[38]. However, the increased expression of the DAM and
252 inflammatory signatures observed in the adult MGcCI model cannot be due to
253 reverse activity of CI, as, in the absence of *Ndfus2*, the soluble N and Q CI
254 modules are not formed[8], avoiding CI direct or reverse activity. Therefore, our
255 data strongly argue that, at least during development, CI activity in microglia
256 prevents neuroinflammation. Interestingly, developmental alteration of the TGF-
257 ß signaling pathway disrupts the acquisition of the surveillance state of adult
258 microglia and causes a progressive behavioral phenotype[30] similar to the
259 observed in the MGcCI mouse model, where overactivation of microglia during
260 the perinatal period associates with long-lasting changes in neuronal cells,
261 behavior defects, and premature death, which cannot be rescued by depletion of
262 microglia after this critical period. These results suggest a short time window for
263 therapeutic interventions.

264 The absence of CI activity in microglia led to limited levels of ATP, which might
265 explain the exhausted functionality and dystrophic features observed in adult
266 MGcCI microglia. However, this energetic debt does not clearly explains the
267 inability of microglia to reprogram to a surveillance state (less energy-demanding)
268 after postnatal development[19]. There are different known routes by which CI

could regulate the microglia state. CI deficiency produces NADH accumulation, which in agreement with our data (Supplementary Table 2), correlates with increased expression of *Gdf15* mRNA levels[39]. Mitochondrial NAD^+ depletion inhibits several enzymes in the TCA cycle and leads to the generation of some TCA components (i.e. alpha-ketoglutarate) by anaplerotic mechanisms, modulating the immune response[40]. In addition, the lack of NAD^+ avoids the completion of the mitochondrial one-carbon by folate pathway, involved in the expression of inflammatory genes, a mechanism previously reported to drive inflammatory macrophages[15]. Further work is needed to discriminate the main cause of the dysfunction observed in MGcCI microglia. A suitable approach would be to impair CIII activity in microglia during development, which will directly affect ATP production but not the NADH/NAD^+ ratio. Additional strategies could be to study the rescue by ectopic expression of enzymes that restore NAD^+ (i.e., NDI1[41] or LbNOX[42]), or the effect of accumulating mitochondrial NADH by expressing mitoEcSTH[39].

The fact that behavioral and viability phenotypes appear only from 2-3 months of age, indicates that these defects are due to the sustained state of microgliosis. Long-term microglia recruitment might impact the ability to properly perform their physiological roles, as suggested by our phagocytic ability data. However, microglia depletion in mice requires a longer time to produce subtle behavioral or cognitive abnormalities[43]. Microglia can also induce a neurotoxic phenotype in astrocytes by secreting proinflammatory cytokines, similar to the one observed in AD or PD patients[24]. This might relate to the upregulation of proinflammatory cytokines in microglia and the accumulation of reactive astrocytes in MGcCI mice. Also, since hypothalamic microglia are known regulators of metabolic

physiology[44], MGcCI microglia might affect feeding behavior and cause the observed weight loss and ultimately premature death. Finally, we hypothesize that high uptake of glucose and other metabolites by MGcCI microglia, also observed in AD mouse models and human patients[32], might be leading to brain hypometabolism, compromising the function of microglia and other cells in the CNS, including neurons.

PMDs are associated with multiple pathologies of the CNS[25], and mutations affecting CI-related genes in the mtDNA are found in most of these diseases (https://www.mitomap.org). In comparison to MGcCI mice, a neuronal model of CI deficiency (brain dopaminergic cells) required a similar time to alter neuronal function and to produce behavioral deterioration[9], supporting a key contribution for microglial mitochondrial dysfunction to PMD. Moreover, microglial dysfunction[20] or depletion[3,4] are associated with neurodevelopmental disorders.

A parallel work shows that adult depletion of complex III in mouse microglia causes impaired remyelination response, and, in an AD model, decreased amyloid ß plaque coverage. However, loss of CIII in microglia does not cause any clear defect during normal aging[45], which together with our results suggest that microglia function depends on OXPHOS only under certain stimuli, such as developmental activation or pathological conditions.

Methods

Mouse models. Mice were housed under controlled temperature (22 ºC) and humidity conditions in a 12 h light/dark cycle with *ad libitum* access to food and water. Housing was performed according to the animal care guidelines of the European Community Council (86/60/EEC). The competent Spanish entity approved all procedures with numbers 27/03/2020/044 and 17/10/2023/089 ("Consejería de agricultura, pesca y desarrollo rural. Dirección general de la producción agrícola y ganadera"). Experimental groups were homogeneously distributed by sex and assigned to each experiment without previous observation of the mice by the experimenter. Mice were euthanized by administration of a lethal dose of anesthesia (sodium thiopental, thiobarbital; Braun, #635573.2). Mouse lines: *Ndufs2*$^{Flox/Flox}$[8]. *B6J.B6N(Cg)-Cx3cr1*$^{tm1.1(cre)Jung}$*/J*[46] (Jackson, #025524). Control and *Cx3cr1*$^{Cre/+}$*; Ndufs2*$^{Flox/Flox}$ (MGcCI) were obtained by crossing the previous genotypes. As Cx3cr1-Cre/+ mice did not develop freezing behaviour nor premature death, we used *Cx3cr1*$^{Cre/+}$; *Ndufs2*$^{+/+}$ as control group at least specified in the figure legends. B6.129P2-Lyz2tm1(cre)Ifo/J (Jackson, #004781) were crossed with *Ndufs2*$^{Flox/Flox}$ to generate the LYcCI control and experimental mice.

For experiments of microglia depletion, AIN-76A rodent diet (Research Diets, #D10001; control) and the same with CSF1R antagonist PLX3397 (MedChemExpress, #D13050910; 600 ppm in chow) were used *ad libitum* from the corresponding stage (weaning or 45 days old) until the animal sacrifice.

Primary mouse microglial cell cultures. Primary microglia cultures were prepared as previously described[47] from brains of 2-4 days old wildtype (pooled mice for metabolomics) or control or *Cx3cr1-Cre; Ndufs2*$^{Flox/Flox}$ mice (individual mouse cultures). Briefly, meninges were removed from the brains and the brain tissue was triturated and trypsinized in 1x Trypsin-EDTA (Biowest, #L0940). Dissociated cells were cultured in DMEM-Ham's F12 (Biowest, #L0090) supplemented with 10% FBS (Gibco, #10270106), 2 mM L-glutamine (Gibco, #A2916801), 1% penicillin/streptomycin (Gibco, #15140122), 10 µg/mL gentamicine (Lonza, #17518Z), and 0.1 mM non-essential amino-acids (Gibco, #11140050) in poly-D-lysine (Sigma, #P6407) coated plates. The cultures were

maintained at 37ºC in a humidified incubator with 5% CO_2 for 21 days. The medium was changed every 2-3 days. Isolation of microglial cells was performed by mild trypsinization with 0.25% Trypsin-EDTA (Gibco, #25200056) diluted 1:3 in serum-free medium for 30-45 min. The detached cells were discarded. Treatments were performed during 24 h starting one day after microglia isolation: 5 µM rotenone (Sigma, #R8875), 3 nM IL-33 (RnD Systems, #3626-ML), TAU (supernatants of the SH-SY5Y cell line –CRL-2266– overexpressing *Mapt*[48]; three control cultures were non-treated and three treated with non-genetically modified SH-SY5Y, no differences were observed between control groups –see source data file–), or combinations of treatments.

Targeted metabolomics. Plates containing microglial cell cultures were placed over dry ice to stop metabolism, and then cells were washed twice with phosphate-buffered saline (PBS; PanReac AppliChem, #A0965). Metabolites extraction was performed by incubation with extraction buffer (50% methanol (Sigma, #34860), 30% acetonitrile (Sigma, #34851), and 20% water). First, 15 min on dry ice; second, 15 min at 4ºC and 1,400 rpm agitation; third, 1 h incubation at -20ºC. Then, a 10 min centrifugation at 21,000 g was performed to discard cellular debris. Samples were analyzed by chromatography and mass spectrometry. Chromatographic separation was performed with EXION HPLC (SCIEX). The injection volume was 5 µL. Mass spectrometry was performed with QTRAP 6500 System (SCIEX) for targeted profiling using multiple reaction monitoring approach. Electrospray ionization parameters were optimized for 0.3 mL/min flow rate. Analyzer parameters were optimized for each compound, and a calibration mixture with known dilutions of all the compounds analyzed was used to calculate the metabolite concentration.

Bioenergetic analysis of primary microglial cell cultures. The Seahorse Extracellular Flux (XFp) Analyzer (Agilent Seahorse) was used to carry out bioenergetic analysis of cells. Primary microglial cells (4 x 10^4 cells/well) were seeded (80 µL/well) in XFp cell culture miniplates (Agilent, #103025-100), and incubated at 37°C in a humidified incubator with 5% CO_2 for 24 h in their cell growth medium. The sensor cartridge was hydrated by adding 400 µL of Seahorse XF Calibrant Solution (Agilent, #100840-000) to each well, and left

overnight in a CO_2-free incubator at 37ºC. Following incubation, cells were washed twice with the assay medium: Agilent XF Base Medium (Agilent, #103334-100) supplemented with 17.5 mM glucose (Sigma, #G7021), 0.5 mM pyruvate (Sigma #P2256), 4.5 mM L-glutamine (Gibco, #A2916801), and 5 mM HEPES (Sigma, #H4034). This media was added to reach a final volume of 180 µL/well, and the plate incubated in a CO_2-free incubator at 37°C for 1 h. For the mitochondrial stress test, oligomycin (20 µM) (Agilent, #103010), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; 20 µM) (Agilent, #103010), rotenone (10 µM) (Sigma, #R8875), and antimycin A (10 µM) (Sigma, #A8674) were loaded into the appropriate ports for sequential delivery. For the glycolytic rate assay test, rotenone (10 µM) (Sigma, #R8875), antimycin A (10 µM) (Sigma, #A8674), and 2-deoxy-D-glucose (2-DG; 500 mM) (Agilent, #103010) were similarly loaded into the appropriate ports. Following calibration, oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured every 6 min for 90 min, and the compounds were injected sequentially at intervals of 18 min. OCR and ECAR were automatically calculated using Seahorse XFp software and 3 biological replicates were assessed for each condition. The resulting data was visualized by using Wave Desktop software.

qRT-PCR. TaqMan probes used were (Applied biosystem) *Gapdh* (#Mm99999915_g1), *Hmbs* (#Mm01143545_m), *Gfap* (#Mm01253033_m1), *Sst* (#Mm00436671_m1), *Pvalb* (#Mm00443100_m1), and *Ndufs2* (#Mm00467601_g1). RNA was extracted from primary microglial cell cultures using TRIzol reagent (Invitrogen, #15596026) following the manufacturer's instructions. For mouse brain samples, a previous tissue homogenization step was performed in a tissue homogenizer (Bullet Blender, Next Advance) in the presence of TRIzol. RNA samples (0.5 µg) were treated with DNase and used to generate cDNA using iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad, #1725035) (final volume: 20 µL) following the manufacturer's instructions. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed in a ViiA 7 Real-Time PCR System (Applied-Biosystems) (for primary cultures and FACS-isolated cells) or 7900-HT Real-Time PCR System (Applied-Biosystems) *(for mouse brain samples)* using iTaq Universal Probes Supermix

(Bio-Rad, #1725130). *Hmbs* (for primary cultures and FACS-isolated cells) *or Gapdh (for mouse brain samples)* RNA levels were used to normalize RNA input.

Western blot. Total proteins were extracted from both primary cultures and frozen adult mouse brain tissue after sequential RNA and DNA extraction using TRIzol reagent (Invitrogen, #15596026) following the manufacturer's instructions. Protein pellets were solubilized using 4% (w/v) SDS (Applichem-Panreac, A2263,1000), 8 M urea (Applichem-Panreac, 131754.1211), 40 mM Tris (Applichem-Panreac, A1379,1000)-HCl Applichem-Panreac, 141020.1611), pH 7.4 under rotation overnight at room temperature. All the samples were quantified using the BCA protein assay kit (Thermo Scientific, #23225) following manufacturer's guidelines. Western blot was performed using standard procedures (SDS-page). Antibodies used: anti-NDUFS2 (Abcam, #ab192022; 1:1000**),** anti-VGAT (Synaptic Systems, #131003;1:5,000), anti-VGLUT (Synaptic Systems, #135302; 1:2,000), anti-PSD95 (Cell Signaling, #2507S; 1:5,000), anti-GFAP (Sigma, #G3893; 1:1,000), anti-GAPDH (Cell Signaling, #2118S;1:10,000) and anti-ß-ACTIN (Sigma, #A5316; 1:10,000). Signal detection was performed using anti-rabbit (Cell Signaling, #7074S; 1:10,000) or anti-mouse (Cell Signaling, #7076; 1:10,000) antibodies, and Western Pierce™ ECL 2 Substrate kit (Themo Scientific, #PI80197), and quantified with Image Lab software.

Flow cytometry. We followed the previously described protocol for acute isolation of cortical astrocytes and microglia[2,49]. In brief, mice were anesthetized and transcardially perfused with HBSS (-$CaCl_2$/-$MgCl_2$; Merck, #H4891) and cortex was dissected and subsequently subjected to dissociation using a Tissue Chopper (Vibratome, 800 series). A chemical digestion was performed using a combination of papain (Worthington, #LS003126; 8 U/mL) and DNAse I (Sigma, #D4263-5VL; 80 Kunitz units/mL) followed by a Percoll gradient (Cytiva, #17089102) at 90 % in PBS (v/v) for microglia and astrocytes enrichment. Cells were stained with primary conjugated monoclonal antibodies CD11b-BV711 (BD Bioscience, #563045; 1:200), CD45-PE (eBioscience, #12-0451-82; 1:200) and ACSA-2-APC (Miltenyi, #130-117-386; 1:50) at 4°C for 30 min. Staining with isotypes control-BV711 (BD Bioscience, #563045; 1:200), control-PE

(eBioscience, #12-4031-82; 1:200), and control-APC (Miltenyi, 130-113-831; 1:50) was used as negative control. Cells were washed and sorted using FACS Aria Fusion (Beckton Dickinson) or MoFlo Astrios (Beckman Coulter) flow cytometers, and data were acquired and analyzed with FACS Diva software (Beckton Dickinson) or SUMMIT software (Beckman Coulter). Gating strategy and data analysis were made according to guidelines[50] (Extended Data Fig. 2a–e). Debris and dead cells were discarded by forward and side scatter patterns. FSC-A and FSC-H events distribution was used to gate single cells. Microglial cells were identified as reactive events for both CD11b and CD45 markers, and astrocytes as reactive events for ACSA-2 marker. Subpopulation gating was performed on contour density plot scaled at 15% probability. Percentages are relative to total single cells.

Global transcription profiling. Total RNA was extracted from primary mouse microglial cell culture, FACS-isolated microglia or astrocytes using TRIzol reagent, following the manufacturer's instructions. The quality of RNA was analyzed with Agilent 2100 Bioanalyzer (Agilent). RNA was amplified, and the cDNA generated, hybridized and stained using the Clariom S Pico Assay, mouse (Applied Biosystems, #902932). Array scanning was performed using the Expression Console Software and the GeneChip Scanner 3,000 (Affymetrix). Raw data was exported to R environment with LIMMA/Bioconductor packages (RStudio), which were used to assess data quality, normalize the data by the Robust Multi-Array (RMA) method, and analyze differential expression. Microglial transcription profile (RNAseq) of phagocytic microglia model was obtained from the Supplementary Data of recently published work[6], and analyzed with R (https://combine-australia.github.io/RNAseq-R/). Microglial transcription profiles of *APP*$_{751}$*SL/+* and *MAPT*p.P301S/+ mouse models were obtained from previous work of the group[2]. To identify underlying biological processes in microglia from MGcCI mice, we compared Control, APP, Tau and phagocytic microglia mouse models using the Gene Set Enrichment Analysis (GSEA)[51,52]. We analyzed the enrichment of gene sets from the Biological Processes Dataset C5-v5.2, the KEGG Dataset, the Hallmark gene set, and the previously used[3] custom gene sets HIF1/hypoxia-induced microglial module (HMM), disease-associated microglia (DAM), and microglial homeostatic signature (HOM).

Immunostaining. Mice were anesthetized with an overdose of thiobarbital and perfused with an intracardiac injection of PBS. The brains were dissected and immediately fixed overnight at 4°C with fixing solution (4% paraformaldehyde (PFA; Sigma, #P6148)–0.05% glutaraldehyde (Sigma, #G6257) in PBS). The brains were cryoprotected during 48 h with a solution of 30% sucrose (PanReac AppliChem, #A2211) in PBS and embedded in OCT (Tissue-Tek, #4583). Blocks were sliced in 40 µm thick coronal sections using a cryostat (CM 1,950, Leica). Tissues showing evident technical alterations (i.e., not properly fixed, stained or cut) were excluded. Immunostaining was performed on free-floating sections[2]. Brain sections were first incubated in fixing solution for 10 min, in 50 mM NH_4Cl (Sigma, #A9434) in PBS for 30 min, in 50 mM glycine (GE Healthcare, #17-1323-01) in Tris pH 8 (PanReac AppliChem, #A2264) for 5 min at 80ºC with gentle shaking, in $CaCl_2$-containing buffer with 0.1 mM $CaCl_2$ (Sigma, #7902); 0.1 mM $MgCl_2$ (Sigma, #M0250); 0.1 mM $MnCl_2$ (Sigma, #M3634) diluted in 0.1 M PBS pH 6.8, heated for 90 s in a microwave (600 W), and finally, in 0.3% Triton X-100 (Sigma, #X100) in PBS for 10 min. Then, sections were incubated for 72 h at 4ºC in blocking solution ($CaCl_2$-containing buffer, 0.05% Triton X-100 and 2% Normal Goat Serum (NGS; Gibco, #PCN5000) in 0.1 M PBS, using different primary antibodies: anti-IBA1 (Wako, #019-19741; 1:500 or Synaptic Systems, #234006; 1:500), anti-GFAP (Sigma, #G3893; 1:2,000), anti-Ki67 (**BD Biosciences,** #550609; 1:200) and anti-cleaved CASPASE-3 (Cell Signaling, #9661; 1:400). Subsequently, the appropriate secondary antibodies were incubated overnight: anti-rabbit IgG-Cy3 (Jackson, #111-165-003; 1:300), anti-rabbit IgG Alexa 647 (Jackson, #111-605-003; 1:400), anti-chicken IgY-Rhodamine Red (Jackson, #703-295-155; 1:100), and anti-mouse IgG-Alexa 488 (Jackson, #111-545-144; 1:400). Sections were finally stained with DAPI (Sigma, #D9542; 1:1,000). For parvalbumin immunostaining with anti-PV (Swant, #235, 1:500), the protocol was reduced to 30 min incubation in 0,3% Triton X-100 and 2% NGS and overnight incubation with primary antibodies embedded in the same solution. Subsequently, the appropriate secondary antibodies were incubated for 2 h at room temperature.

Electron microscopy. Mouse brains were processed according to standard protocol for transmission electron microscopy. 250 µm-thick vibratome sections were postfixed in osmium tetroxide (Electron Microscopy Science, #19150, 1%), and then stained with uranyl acetate (Electron Microscopy Science, #22400-4, 1%), dehydrated in acetone and embedded in Araldite (Electron Microscopy Science, #10900). Selected areas were cut in ultrathin sections and examined with an electron microscope (JEOL JEM 1400). Quantification of mitochondrial load (area occupied by mitochondria/microglial cytoplasmic area) and density (number of mitochondria/microglial cytoplasmic area) was performed using Fiji.

Imaging and image quantification. Imaging acquisition of immunostained samples was performed with Leica Thunder fluorescence microscope and confocal microscopes Nikon A1R^{+} and Leica Stellaris 8 Scan Head. The particular microscope and objective used is specified for each experiment. Unless otherwise specified, image analysis was carried out using FIJI (National Institutes of Health, USA). For the study of IBA1 and GFAP distribution, composite images of the whole brain were collected with Leica Stellaris 8 (objective 10x). Density was calculated as the number of nuclei surrounded by GFAP staining per mm^2 in a single plane of 0.55 µm of thickness (10–20 images per mice). Load was measured as the percentage occupied by GFAP immunostaining of a single plane of 0.55 µm of thickness (10–20 images per mice). For morphological analysis of microglia, brain cortex and striatum images collected with Leica Stellaris 8 (40x) were imported to IMARIS software (x64 V9.6.0), where microglial morphologic characteristics were measured using surface and filament functions respectively[18].

Phagocytic activity of microglia. Brain slices immunostained for IBA1, cCASPASE-3 and DAPI were analyzed to assess the ability of microglia for phagocytizing dying cells. For 1-month-old mice, hippocampal images were collected with Leica Stellaris 8 (objective 20x). For 3-month-old mice, composite images of the whole brain were collected with Leica Thunder (objective 10x). The resulting images were used to calculate different parameters: cell death: reflecting the number of cCASPASE-3 immunoreactive cells; cell death density: relativized to the analyzed area; dying cells engulfed by microglia: reflecting the

number of cCASPASE-3 immunoreactive cells surrounded by IBA1 immunoreactive cells. The phagocytic index was defined as the percentage of dying cells (cCASPASE-3 immunoreactive) that were surrounded by microglial projections (cCASPASE-3 immunoreactive and surrounded by IBA1 microglial marker). Inclusion criteria for dying cells included concomitance of cCASPASE-3 and DAPI signals, to validate pyknotic nuclei. This protocol was repeated in 3-5 brain sections per mouse, and the resulting average was calculated for each animal. A single dose of 10 mg/mL of kainic acid (Sigma, #K0250) in saline was administered intraperitoneally. Mice were processed for histology 24 h after the injection and phagocytosis was studied as described before.

Microglial turnover analysis. Brain cortex samples immunostained for IBA1, Ki67 (BD Biosciences, #550609) and DAPI were used to collect images with Nikon A1R^{+} (objective 60x). Those cells showing concomitance of the three markers were considered positive dividing microglia. Specifically, those nuclei that showed colocalization of Ki67 and DAPI signals, and that were surrounded by IBA1 signal.

Behavioral tests. Open field test consisted of a triple and separated squares arena (60 cm^2), enclosed by continuous 30-cm-high walls. Mice were tested during their dark phase, and placed in the middle of the square, freely wandering for 15 minutes. Mouse behavior was recorded with video cameras disposed above the arenas. Test sessions were scored with Biobserve Viewer3 software (v.17). All the arenas were carefully cleaned with ethanol 96º and rinsed with water after every test. The software calculated the following parameters: i) total number of ambulation events (a spontaneous short-term acceleration comparing three video frames, threshold 140 cm/s), ii) total number of freezing events (when the mouse velocity limit –0.5 cm/s– is not reached for a minimal duration threshold –2 s–), iii) track length (cm), and iv) average velocity (cm/s). The duration of freezing was estimated using a fully automatic startle and fear conditioning system (Panlab). Mice were introduced in a chamber equipped with a high sensitivity weight transducer system and the immobility was measured using the PACWINCSFR software (Panlab), with a lower bound threshold of 18% and a minimal duration of 1 s.

Statistical analysis. All individual measurements constitute biological replicates. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications[2,53]. Samples with an $n < 9$ were analyzed using parametric tests. Samples with an $n \geq 9$ were evaluated for normal distribution using D'Agostino and Pearson's *n* omnibus normality test and variances were compared using the F test. Comparisons between two groups were performed with two-tail unpaired Student's *t*-test (parametric data) or Mann-Whitney test (non-parametric data). Whereas comparisons between more than two groups were done with ANOVA with Tukey's test (parametric data) or Kruskal-Wallis test (non-parametric data). Data are expressed as mean ± standard error of the mean (s.e.m.); $p < 0.05$ was considered statistically significant. Data with significant differences but low effect size were not shown in the Extended Data Figure 1, but the raw data are included in Supplementary Table 1. Statistical analyses and graphs were generated in GraphPad Prism version 9.0 (GraphPad). When quantification included multiple measurements of the response variables per animal, we accounted for pseudo-replication by applying a linear mixed effects model (LMM), in which we included animal as a random effect term to control for heterogeneity between individual mouse samples. Two-sided **t**ype III ANOVA with Satterthwaite approximation was run to assess for significant differences between effects in the Tukey transformed LMM model. LMM analysis was performed with RStudio.

References

1. Beccari, S. *et al.* Microglial phagocytosis dysfunction in stroke is driven by energy depletion and induction of autophagy. *Autophagy* **00**, 1–30 (2023).
2. March-Diaz, R. *et al.* Hypoxia compromises the mitochondrial metabolism of Alzheimer's disease microglia via HIF1. *Nat. aging* **1**, 385–399 (2021).
3. Oosterhof, N. *et al.* Homozygous Mutations in CSF1R Cause a Pediatric-Onset Leukoencephalopathy and Can Result in Congenital Absence of Microglia. *Am. J. Hum. Genet.* **104**, 936–947 (2019).
4. Guo, L. *et al.* Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with Brain Malformation. *Am. J. Hum. Genet.* **104**, 925–935 (2019).
5. Hu, Y. *et al.* Dual roles of hexokinase 2 in shaping microglial function by gating glycolytic flux and mitochondrial activity. *Nat. Metab.* **4**, 1756–1774 (2022).
6. He, D. *et al.* Disruption of the IL-33-ST2-AKT signaling axis impairs neurodevelopment by inhibiting microglial metabolic adaptation and phagocytic function. *Immunity* **55**, 159-173.e9 (2022).
7. McFarland, R., Taylor, R. W. & Turnbull, D. M. A neurological perspective on mitochondrial disease. *Lancet Neurol.* **9**, 829–840 (2010).
8. Fernández-Agüera, M. C. *et al.* Oxygen Sensing by Arterial Chemoreceptors Depends on Mitochondrial Complex I Signaling. *Cell Metab.* **22**, 825–837 (2015).
9. González-Rodríguez, P. *et al.* Disruption of mitochondrial complex I induces progressive parkinsonism. *Nature* **2**, 133–7 (2021).
10. Altea-Manzano, P. *et al.* Reversal of mitochondrial malate dehydrogenase 2 enables anaplerosis via redox rescue in respiration-deficient cells. *Mol. Cell* **82**, 4537-4547.e7 (2022).
11. Kashani-Poor, N., Zwicker, K., Kerscher, S. & Brandt, U. A Central Functional Role for the 49-kDa Subunit within the Catalytic Core of Mitochondrial Complex I. *J. Biol. Chem.* **276**, 24082–24087 (2001).
12. Baradaran, R., Berrisford, J. M., Minhas, G. S. & Sazanov, L. A. Crystal structure of the entire respiratory complex I. *Nature* **494**, 443–448 (2013).
13. Haimon, Z. *et al.* Re-evaluating microglia expression profiles using RiboTag and cell isolation strategies. *Nat. Immunol.* **19**, 636–644 (2018).
14. Fornasiero, E. F. *et al.* Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. *Nat. Commun.* **9**, (2018).
15. Yu, W. *et al.* One-Carbon Metabolism Supports S-Adenosylmethionine and Histone Methylation to Drive Inflammatory Macrophages. *Mol. Cell* **75**, 1147-1160.e5 (2019).
16. Keren-Shaul, H. *et al.* A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* **169**, 1–15 (2017).
17. Ulland, T. K. *et al.* TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. *Cell* **170**, 649-663.e13 (2017).
18. Villadiego, J. *et al.* Full protection from SARS-CoV-2 brain infection and damage in susceptible transgenic mice conferred by MVA-CoV2-S vaccine candidate. *Nat. Neurosci.* **26**, 226–238 (2023).
19. Paolicelli, R. C. *et al.* Microglia states and nomenclature: A field at its

crossroads. *Neuron* **110**, 3458–3483 (2022).
20. Prinz, M., Jung, S. & Priller, J. Microglia Biology: One Century of Evolving Concepts. *Cell* **179**, 292–311 (2019).
21. Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483–495 (2010).
22. Lee, J., Lim, E., Kim, Y., Li, E. & Park, S. Ghrelin attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. *J. Endocrinol.* **205**, 263–270 (2010).
23. Askew, K. *et al.* Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. *Cell Rep.* **18**, 391–405 (2017).
24. Liddelow, S. A. *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481–487 (2017).
25. Molnar, M. J. & Kovacs, G. G. Mitochondrial diseases. in *Handbook of Clinical Neurology* **145**, 147–155 (Elsevier B.V., 2018).
26. Orre, M. *et al.* Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. *Neurobiol. Aging* **35**, 2746–60 (2014).
27. Goldmann, T. *et al.* A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat. Neurosci.* **16**, 1618–1626 (2013).
28. Wculek, S. K. *et al.* Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis. *Immunity* 1–15 (2023). doi:10.1016/j.immuni.2023.01.011
29. Schafer, D. P. *et al.* Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron* **74**, 691–705 (2012).
30. Arnold, T. D. *et al.* Impaired αVβ8 and TGFβ signaling lead to microglial dysmaturation and neuromotor dysfunction. *J. Exp. Med.* **216**, 900–915 (2019).
31. Ramos, B. *et al.* Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. *Neurobiol. Aging* **27**, 1658–1672 (2006).
32. Xiang, X. *et al.* Microglial activation states drive glucose uptake and FDG-PET alterations in neurodegenerative diseases. *Sci. Transl. Med.* **13**, eabe5640 (2021).
33. Li, Y. *et al.* Hexokinase 2-dependent hyperglycolysis driving microglial activation contributes to ischemic brain injury. *J. Neurochem.* **144**, 186–200 (2018).
34. Leng, L. *et al.* Microglial hexokinase 2 deficiency increases ATP generation through lipid metabolism leading to β-amyloid clearance. *Nat. Metab.* (2022). doi:10.1038/s42255-022-00643-4
35. Bernier, L. P. *et al.* Microglial metabolic flexibility supports immune surveillance of the brain parenchyma. *Nat. Commun.* **11**, (2020).
36. Baik, S. H. *et al.* A Breakdown in Metabolic Reprogramming Causes Microglia Dysfunction in Alzheimer's Disease. *Cell Metab.* **30**, 493-507.e6 (2019).
37. Fairley, L. H. *et al.* Mitochondrial control of microglial phagocytosis by the translocator protein and hexokinase 2 in Alzheimer's disease. *Proc. Natl. Acad. Sci.* **120**, 2017 (2023).
38. Peruzzotti-Jametti, L. *et al.* Mitochondrial complex I activity in microglia sustains neuroinflammation. *Nature* **628**, 195–203 (2024).

39. Pan, X. *et al.* A genetically encoded tool to increase cellular NADH/NAD+ ratio in living cells. *Nat. Chem. Biol.* 1–31 (2023). doi:10.1038/s41589-023-01460-w
40. Chandel, N. S. Mitochondria. *Cold Spring Harb. Perspect. Biol.* **13**, 1–23 (2021).
41. Seo, B. B. *et al.* Molecular remedy of complex I defects: Rotenone-insensitive internal NADH-quinone oxidoreductase of Saccharomyces cerevisiae mitochondria restores the NADH oxidase activity of complex I-deficient mammalian cells. *Proc. Natl. Acad. Sci.* **95**, 9167–9171 (1998).
42. Goodman, R. P. *et al.* Hepatic NADH reductive stress underlies common variation in metabolic traits. *Nature* **583**, 122–126 (2020).
43. Rojo, R. *et al.* Deletion of a Csf1r enhancer selectively impacts CSF1R expression and development of tissue macrophage populations. *Nat. Commun.* **10**, 1–17 (2019).
44. Valdearcos, M., Myers, M. G. & Koliwad, S. K. Hypothalamic microglia as potential regulators of metabolic physiology. *Nat. Metab.* **1**, 314–320 (2019).
45. NATMETAB-L23018231A
46. Yona, S. *et al.* Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. *Immunity* **38**, 79–91 (2013).
47. Saura, J., Tusell, J. M. & Serratosa, J. High-yield isolation of murine microglia by mild trypsinization. *Glia* **44**, 183–9 (2003).
48. Mailliot, C. *et al.* Pathological tau phenotypes. The weight of mutations, polymorphisms, and differential neuronal vulnerabilities. *Ann. N. Y. Acad. Sci.* **920**, 107–14 (2000).
49. Orre, M. *et al.* Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. *NBA* **35**, 1–14 (2014).
50. Herzenberg, L. a, Tung, J., Moore, W. a, Herzenberg, L. a & Parks, D. R. Interpreting flow cytometry data: a guide for the perplexed. *Nat. Immunol.* **7**, 681–5 (2006).
51. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15545–50 (2005).
52. Mootha, V. K. *et al.* PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267–273 (2003).
53. Alvarez-Vergara, M. I. *et al.* Non-productive angiogenesis disassembles Aß plaque-associated blood vessels. *Nat. Commun.* **12**, 3098 (2021).

Fig. 1. | CI deficiency triggers a metabolic rewiring in microglia. a, MGcCI mouse model was generated by crossing *Nfudfs2*^{Flox} (top) with *Cx3cr1*$^{Cre/+}$ (center) to produce the deletion of the *Ndufs2* gene in microglia (bottom, excised allele). **b**, *Ndufs2* mRNA expression in FACS-isolated microglia (MG) from 1- and 3-month-old (mo) control -C- and MGcCI mice was analyzed by qRT-PCR. *n* = 5 1mo and *n* = 6 3mo mice. **c**, *Ndufs2* mRNA and NDUFS2 protein expression in primary microglial cell cultures were analyzed respectively by qRT-PCR and western blot using GAPDH levels to normalize load. *n* = 4 mice. **d**, NADH and Aspartate levels in primary microglial cell cultures. *n* = 14 C and *n* = 6 MGcCI mice for NADH, and *n* = 7 C and *n* = 3 MGcCI mice for Aspartate. **e**, Oxygen consumption rate (OCR), basal respiration, and maximal respiration of primary microglial cultures. Dashed lines indicate injection times. *n* = 3 mice; Oli: oligomycin; FCCP: carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; Rot: rotenone; AntA: antimycin A. **f**, Extracellular acidification rate (ECAR), basal glycolysis, and maximal glycolysis of primary microglial cultures. Dashed lines indicate injection times. *n* = 3 mice; 2-DG: 2-deoxyglucose. **g**–**i**, GSEA of FACS-isolated microglia showing 1mo (**g**,**i**) and 3mo (**g**–**i**) MGcCI *versus* C mice. Left, enplot graphs and right, heat maps show the top-ranking genes for different gene sets. Red symbolizes upregulation and blue represents downregulation. FC: fold change; PPP: pentose phosphate pathway; 1-CP: one carbon pathway; DAM: disease-associated microglia. All data are presented as means ± s.e.m. *n* values represent the number of biologically independent experiments. *p*-values from two-sided Student's *t*-test.

Fig. 2 | Cl-deficient microglia develop progressive dystrophy and dysfunction. **a**,**b**, Morphologic analysis of control -C- and MGcCl cortical microglia from 1-month-old (1mo) (**a**) and 3mo mice (**b**). Left images show the microglia (IBA1); left-middle images show the surface of the reconstructed microglia; middle-right and right images show, respectively, the surface and the filament reconstruction of a microglia cell. Graphs show the morphologic parameters analyzed. Scale bars, 30 µm and 10 µm in low and high magnification images, respectively. (**a**) *n* = 3 mice and (**b**): *n* = 4 mice; *p*-values from two-sided type III ANOVA with Satterthwaite approximation on linear mixed effects models (LMM). **c**,**d**, Quantification of the number of dying cells (cleaved –c–CASPASE-3^+) and the percentage of dying cells phagocyted by microglia (Phagocytic index: cCASPASE-3^+-IBA1^+ cells/cCASPASE-3^+ cells) in the dentate gyrus (1mo mice; **c**) or whole brain (3mo mice; **d**). Microglia (IBA1; red), dying cells (cCASPASE-3; green), and nuclei (DAPI; blue) are shown. Yellow arrows indicate cCASPASE3^+ dying cells and yellow arrowheads the nuclei of microglia projecting towards the dying cells. Scale bar, 10 µm. (**c**): *n* = 4 mice; (**d**-top): *n* = 3 C and *n* = 4 MGcCl mice; (**d**-bottom): *n* = 6 C (126 dying cells analyzed) and *n* = 7 MGcCl (229 dying cells analyzed) mice. **e**, Quantification of the phagocytic index in the dentate gyrus of 3mo mice treated with kainate. *n* = 4 C (50 dying cells analyzed) and MGcCl (64 dying cells analyzed) mice. (**c**–**e**) *p*-values from two-sided Student *t*-test. **f**, Representative image of cCASPASE-3^+/IBA1^+ microglia. Arrowheads indicate the nuclei of microglial cells. *n* = 6 C and *n* = 7 MGcCl mice. All data are presented as means ± s.e.m. *n* values represent the number of biologically independent experiments.

Fig. 3 | MGcCl mice present widespread gliosis. a, Brain coronal sections of 3-month-old control –C– and MGcCl from mice immunostained for microglia (IBA1), astrocytes (GFAP), and nuclei (DAPI). Dashed lines rectangles corresponding to cortex (Cx) and hippocampus (Hp) are shown in the far left (C) and right (MGcCl) panels. Scale bar, 1 mm. **b**, Quantification of the cortical density of $GFAP^+$ astrocytes and the percentage of cortex occupied by GFAP signal (load). *n* = 4 C and *n* = 6 MGcCl mice. *p*-values from two-sided Student *t*-test. **c**, GSEA of FACS-isolated astrocytes showing MGcCl *versus* C 3mo mice. Left, enplot graphs and right, heat maps show the top-ranking genes for different gene sets. Red symbolizes upregulation and blue represents downregulation. **d**, Early (from postnatal day –P– 24) and late (from P45) PLX3397 treatment reduce astrocyte reactivity in the cortex of MGcCl mice. Left panels, Cortical sections stained for microglia (IBA1, red); astrocyte (GFAP, green), and nuclei (DAPI, blue). Scale bar, 50 µm. Top graphs, quantification of the microglia density and load; bottom graphs, quantification of the astrocyte density and load. *n* = 4 non-treated MGcCl (–); *n* = 6 MGcCl (PLX P24); *n* = 4 MGcCl (PLX P45) mice. *p*-values from ANOVA with post-hoc Tukey's test. All data are presented as means ± s.e.m. *n* values represent the number of biologically independent experiments.

Fig. 4 | MGcCI mice develop neuronal changes, behavioral dysfunction, and premature death. a, Pre (VGAT and VGLUT) and postsynaptic (PSD95) markers were analyzed by western blot in the cortex, hippocampus, and striatum of control –C– and MGcCI 3-month-old (mo) mice. GAPDH was used to normalize load. *n* = 3, C (cortex) and *n* = 4 in other samples. **b**, Top panels, cortical sections of 3-month-old (mo) mice were stained with the parvalbumin marker (PV). Scale bar, 100 µm. Bottom graphs, quantification of the cortical density of parvalbumin cells in 1mo and 3mo mice. *n* = 4. (**a**,**b**) *p*-values from two-sided Student *t*-test. **c**, 1 mo and 3mo C and MGcCI mice were recorded in a chamber equipped with a high sensitivity weight detection system for 3 min, Orange, no freezing, and green, freezing. 1mo: *n* = 9 C and *n* = 6 MGcCI mice; 3mo *n* = 20 C (*Cx3cr1*$^{Cre/+}$ *n* = 6 and *Ndufs2*$^{Flox/Flox}$ *n* = 14) and *n* = 11 MGcCI mice. *p*-values from 1mo, two-sided Student *t*-test; 3mo, two-sided Mann-Whitney test. **d**, Survival curve of C and MGcCI mice. *n* = 20 C (*Cx3cr1*$^{Cre/+}$ *n* = 9 and *Ndufs2*$^{Flox/Flox}$ *n* = 11) and *n* = 20 MGcCI. *p*-values from long rank Mantel-Cox test. **e**, Early (starting at postnatal day –P– 24) but not late (starting at P45) PLX3397 treatment extend life expectancy of MGcCI mice. Endpoint: 20% weight loss. *n* = 4 non-treated MGcCI (–); *n* = 6 MGcCI (PLX P24); *n* = 4 MGcCI (PLX P45) mice. *p*-values from two-sided ANOVA with post-hoc Tukey's test. All data are presented as means ± s.e.m. *n* values represent the number of biologically independent experiments.

ACKNOWLEDGMENTS

We thank David Carnero and Matilde Bustos for helping with the optimization of the metabolomic methods used here and Austeja Ciulkinyte, from Blanca Diaz-Castro's laboratory (UK DRI, University of Edinburgh, Edinburgh), for providing us with an LMM script. B.M.-R. was the recipient of a "Junta de Andalucía" predoctoral fellowship (2021), N.C.-C, M.I.A.-V., and E.M.M. were the recipient of an FPU/FPI fellowship from Spanish Ministry of Education (FPU20/03320, FPU15/02898, and PRE2019-087729), J.J.P.-M. and A.E.R.-N. were the recipients of JdlC-I and JdlC-F fellowships from MCIN/AEI/ 10.13039/501100011033 (IJC2019-038819-I and FJCI-2015-23708). Work was supported by grants to A.P./J.V./A.G./D.M. by MCIN/AEI/ 10.13039/501100011033, ISCIII (FORT23/00008 –A.P.–; PI21/00915 –J.V.–; and PI21/00915 –A.G.–), and FEDER (RTI2018-096629-B-100, PID2021-126894OB-I00 "y por FEDER Una manera de hacer Europa", SAF2017-90794-REDT, and PIE13/0004), by the regional Government of Andalusia ("Proyectos de Excelencia" P12-CTS-2138, P20_01312; BIOT22_00018_1, and ProyExcel_00845) co-funded by CEC, REC_EU, and FEDER funds, and by the "Ayuda de Biomedicina 2018", Fundación Domingo Martínez. We also thanks K. Levitsky and John Pearson (microscopy), M.J. Castro and Carl Henderson (flow cytometry), F.J. Moron and R. March-Diaz (genomics), and R. Duran (histology) for advice and technical assistance in experiments at IBiS core facilities; and C.A. Parejo-Perez at "Instituto de Bioquímica Vegetal y Fotosíntesis" (IBVF, Seville) metabolomic facility.

Extended and Supplementary Data files

Extended Data Figs. 1 to 7

Supplementary Data Tables 1 to 4

Supplementary Data Videos 1 and 2

Source Data Files

Data availability

Original data is provided as Source Data files in the Supplementary Data, indicating the correspondence with each main and extended figure. Transcriptomics data are available from the Gene Expression Omnibus Dataset GSE254585 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE254585.

Author contributions

A.P., J.J.P.M., B.M.R. and N.C.C. conceived of and designed the research. B.M.R., N.C.C., J.J.P.M., M.I.A.V., L.T.E., C.R.M., E.M.V., N.M.C., M.V., J.L.N.G., P.G.J.C, D.M., A.E.R.N. and A.P. performed the experiments. B.M.R., N.C.C., J.J.P.M., L.T.E., C.R.M., E.M.V., N.M.C., M.V., J.L.N.G., P.G.J.C, J.V., A.G., A.E.R.N. and A.P. analyzed the data. J.L.B. and J.V. contributed mouse models/samples. A.P. and J.J.P.M. wrote the manuscript.

Competing interests

The authors declare no competing interests.