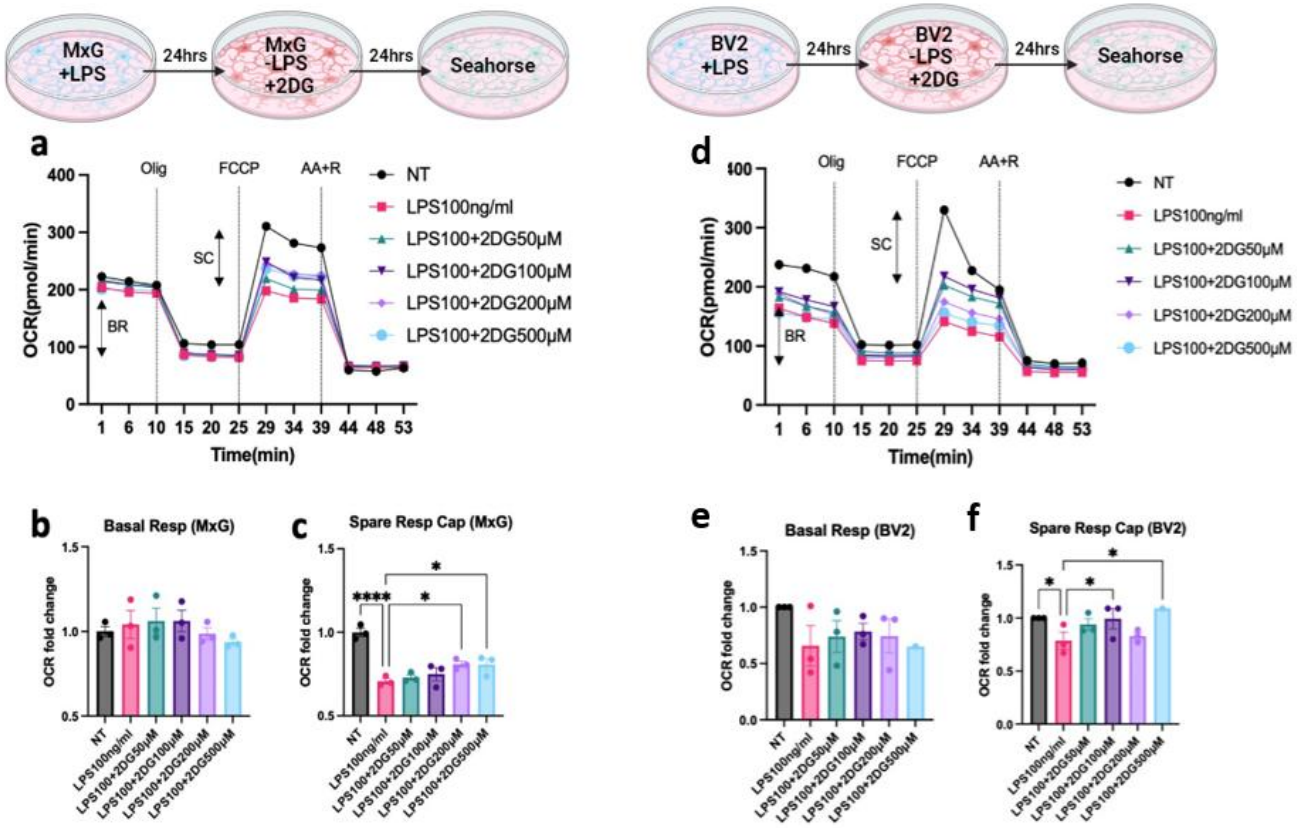

Figure S1: **Changes in OXPHOS induced by LPS (100 ng/ml) are mitigated by 2DG in MxG and BV2 cells**. Treatment with 2DG for 24 hours mitigated the metabolic shift induced by 100 ng/ml LPS by enhancing spare respiratory capacity in MxG (a-c) and BV2 (d-f) cells, measured via Seahorse extracellular flux assay. LPS = lipopolysaccharide; 2DG: 2-Deoxy-D-glucose; OCR = oxygen consumption rate; FCCP = carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; BR: basal respiration; SC: spare capacity; Olig: oligomycin; AA: antimycin a; R: rotenone; NT: no treatment. Data are derived from 3 independent experiments, each including 3 to 5 biological replicates. Bar graphs are presented as fold changes relative to the NT group and are expressed as mean ± SEM. *p ≤ 0.05, **p ≤ 0.01, ****p ≤ 0.001, One-way ANOVA with Tukey multiple comparison testing.

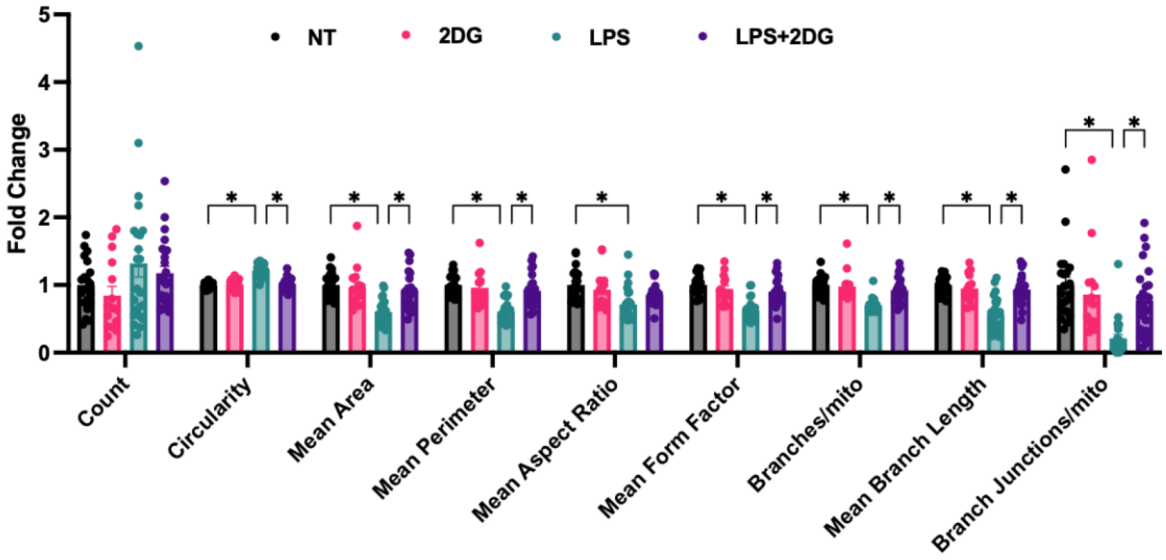

Figure S2: **MitoTracker-staining.** Images were processed using the open-source ImageJ/Fiji software with the Mitochondria Analyzer plugin, enabling a two-dimensional (2D) analysis of mitochondrial morphology and network connectivity. This graph shows all the data points of microglia mitochondrial staining using MitoTracker to quantify the mitochondrial morphology and network connectivity in LPS (200 ng/ml, 24 hours), followed by 2DG (200 µM, for 24 hours) – normalized to NT group. LPS = lipopolysaccharide; 2DG: 2-Deoxy-D-glucose; NT: no treatment. *p ≤ 0.05, One-way ANOVA, post hoc Tukey.

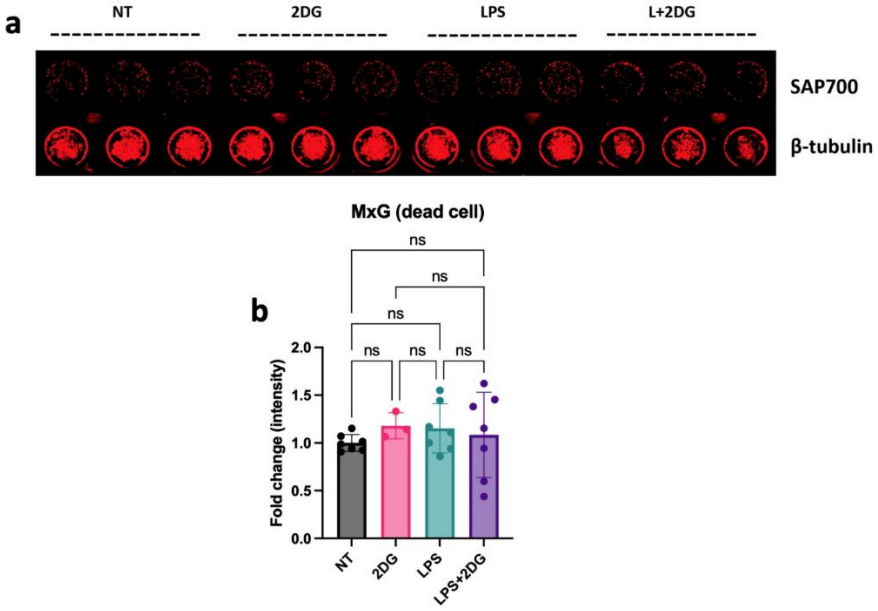

Figure S3: **Treatment of MxG with LPS (200ng/ml), 2DG (200μM), and LPS+2DG for 24 hours had no effects on cell viability**. Sapphire-700 was used to stain the dead cells (a: InCell Western assay) and the intensity of Sapphire-700 representing the dead cells was measured and then normalized to no treatment group (b). Data are derived from a minimum of 3 independent experiments per group, each including 3 biological replicates. MxG: mixed glial cells; SAP700: Sapphire-700; ns: not significant. Bar graphs are presented as fold change relative to the NT group and are expressed as mean ± SEM. One-way ANOVA with Tukey multiple comparison testing.

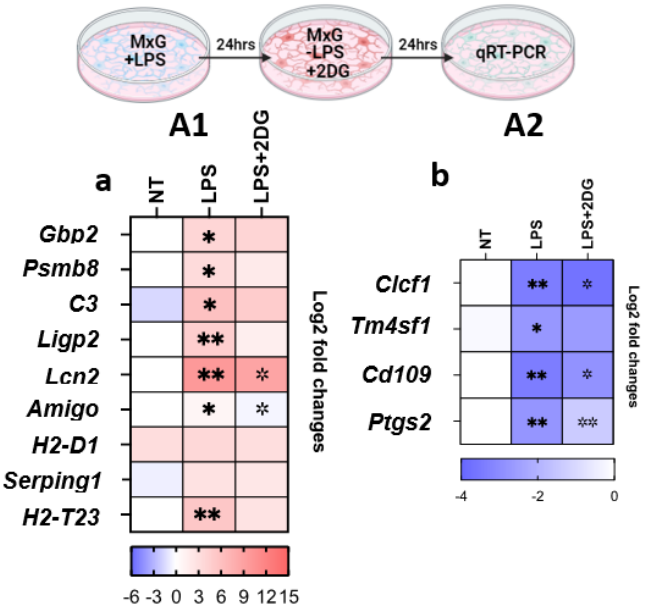

Figure S4: **2DG modulates reactive astrocyte gene expression**. LPS induced reactive astrocytes to upregulate A1 (pro-inflammatory) markers (a: qRT-PCR) and downregulate A2 (alternative activation) markers (b: qRT-PCR). However, 2DG partially reverted this effect. MxG: mixed glial cells. qRT-PCR data derived from 3 independent experiments, each including 3 biological replicates. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 (for qRT-PCR, solid asterisks refer to LPS vs NT; hollow asterisks refer to LPS+2DG vs. LPS), One-way ANOVA with Tukey multiple comparison testing.

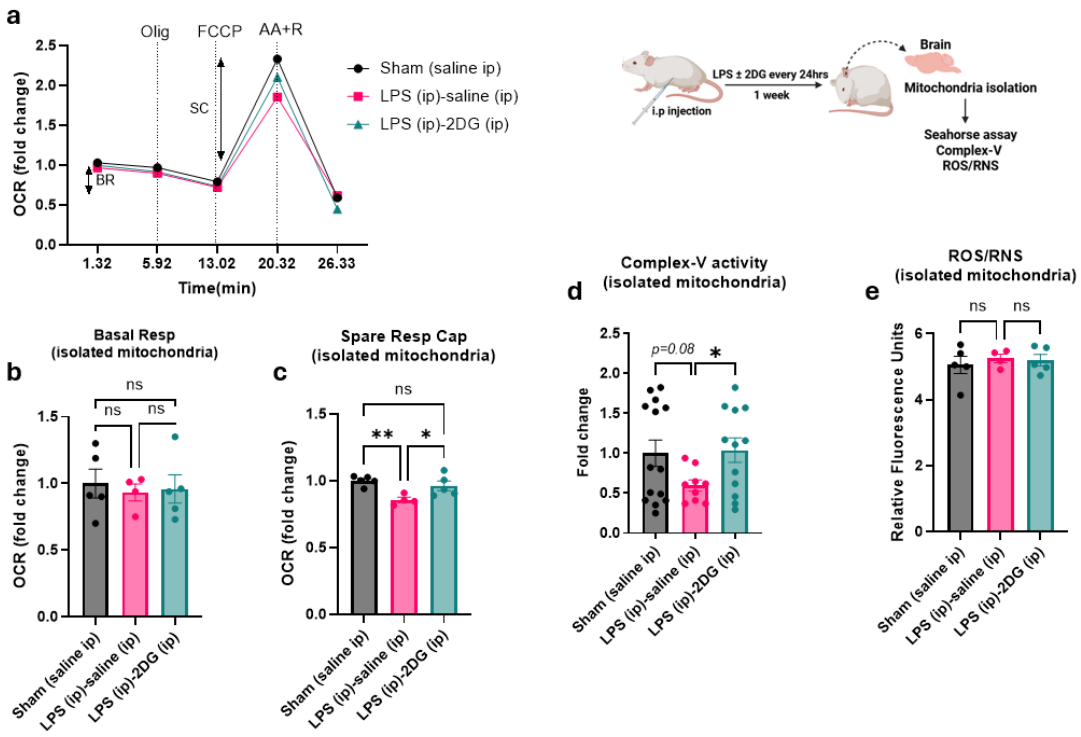

Figure S5: **2DG modulated mitochondrial respiratory function and complex-V activity in isolated brain mitochondria following systemic LPS challenge.** Seahorse XF assay tracing showing oxygen consumption rate in isolated brain mitochondria from mice injected intraperitoneally with saline (Sham), LPS + saline (vehicle), or LPS + 2DG once daily for 7 days. Arrows indicate sequential additions: oligomycin (Olig; ATP synthase inhibitor), FCCP (uncoupler), and antimycin A + rotenone (AA+R; complex III/I inhibitors) (a). Basal respiration (b) and spare respiratory capacity (c) (fold change relative to sham) in isolated mitochondria. Complex-V (ATP synthase) activity assay showing partial LPS-induced suppression, which was restored by 2DG treatment (d). ROS/RNS activity, expressed as Relative Fluorescence Units, measured in isolated mitochondria (e). Data are presented as mean ± SEM; Data derived from 4-5 mice per group, as indicated by individual points. Complex V activity (d) represents measurements obtained at minutes 3, 4, and 6; all data points from these time intervals are displayed in the figure. LPS = lipopolysaccharide; 2DG: 2-Deoxy-D-glucose; OCR = oxygen consumption rate; FCCP = carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; Olig: oligomycin; AA: antimycin a; R: rotenone; BR, basal respiration; SC, spare respiratory capacity. Statistical analysis by one-way ANOVA with post-hoc multiple comparison testing: *p < 0.05, **p < 0.01, ns = not significant.

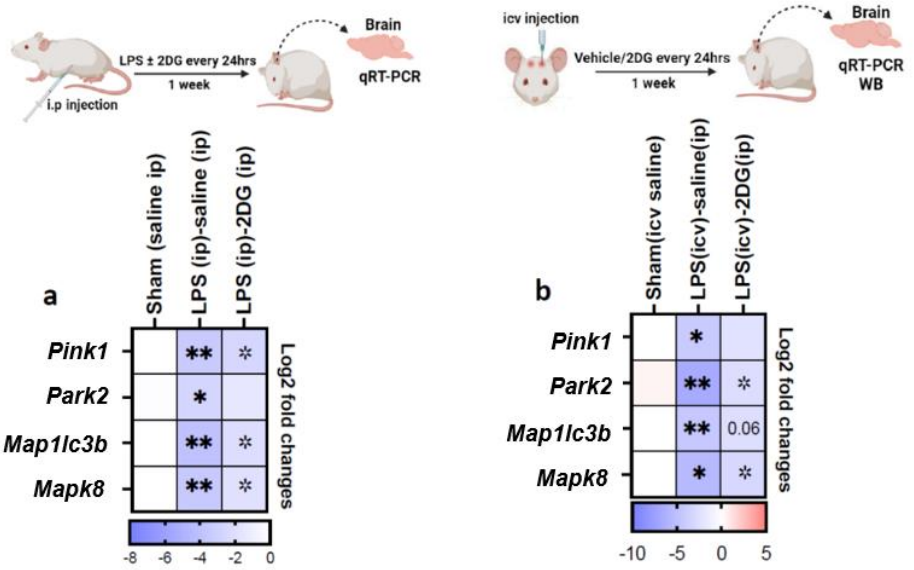

Figure S6: **2DG modulates autophagy-related gene expression**. 2DG mitigated the effects of LPS on autophagy-related genes in the mouse brain following intraperitoneal (a) and intracerebroventricular (b) injection of LPS *in vivo*. qRT-PCR data are derived from 3 animals, each including minimum 3 technical replicates. Graphs expressed as mean ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 (for qRT-PCR, solid asterisks refer to LPS vs sham; hollow asterisks refer to LPS+2DG vs. LPS), One-way ANOVA with Tukey multiple comparison testing.

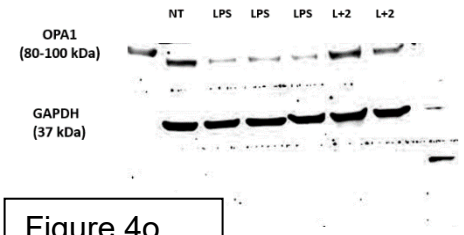

Figure 4o.

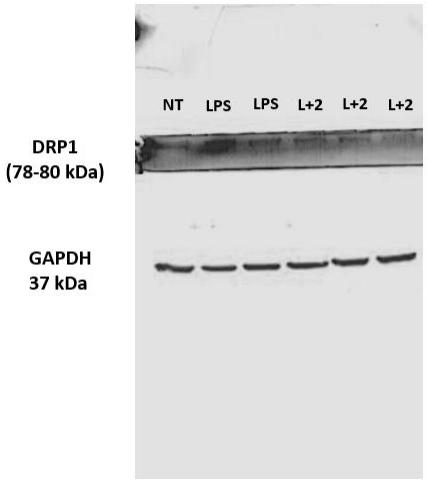

Figure 4p.

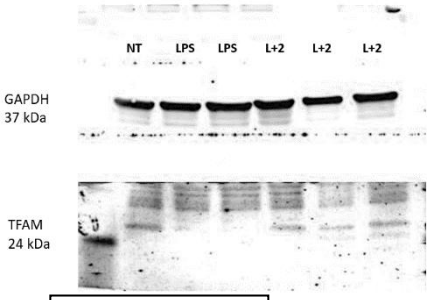

Figure 4q.

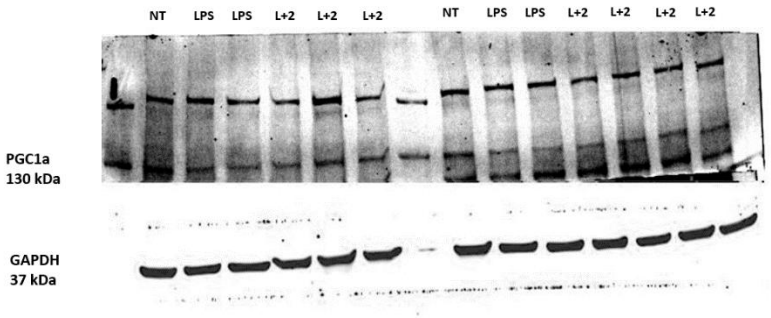

Figure 4r.

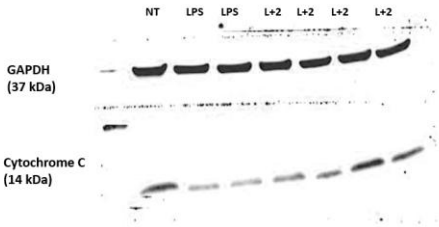

Figure 5j.

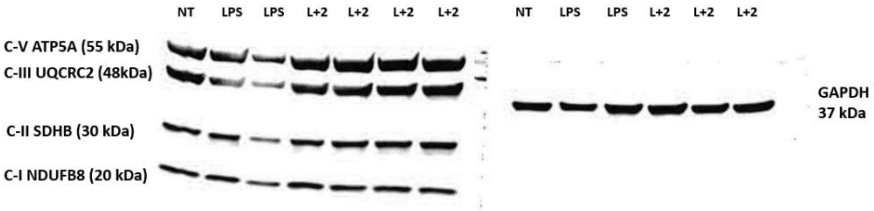

Figure 5k-n.

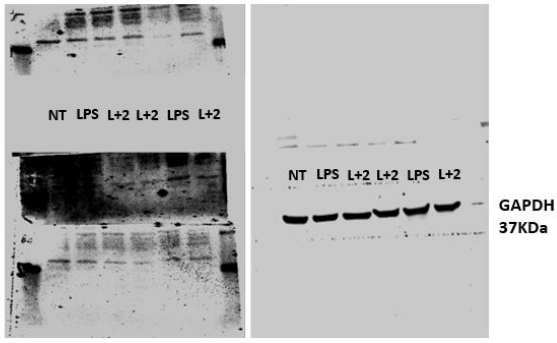

Figure 7c.

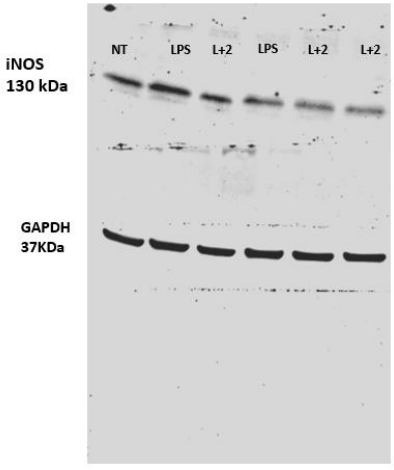

Figure 7d.

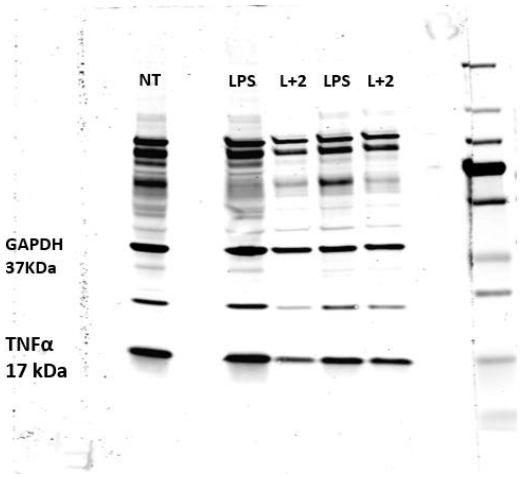

Figure 7e.

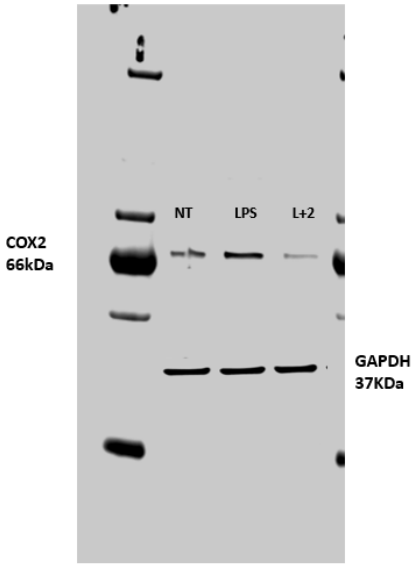

Figure 7f.

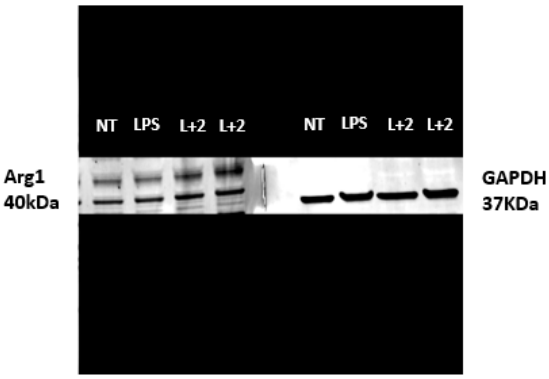

Figure 7g.

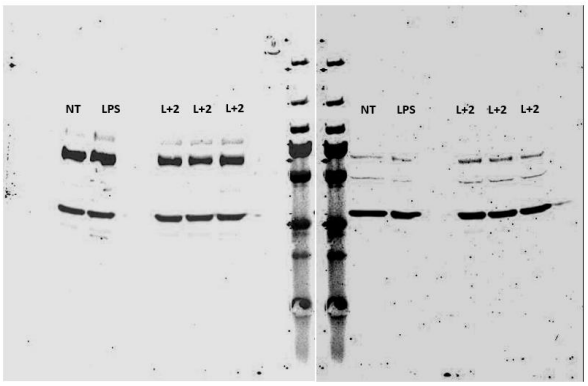

Figure 8g.

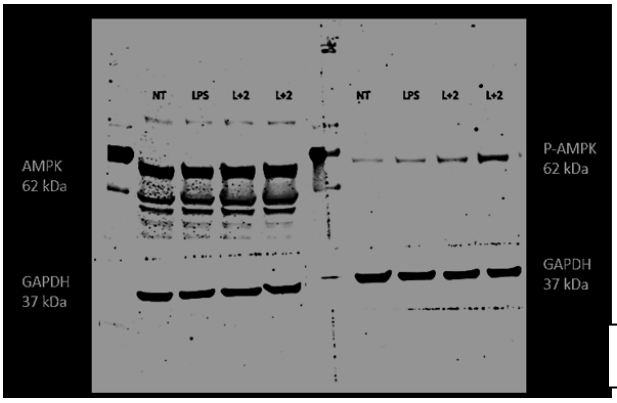

Figure 8h.

Figure S7: **Full scans of key Western blot data**. In most experiments, membranes were cut before probing with the antibody. In cases in which separate blots were exposed to the same piece of film, the full scan incorporating all blots is shown. In some cases, lanes were spliced together in the main figures in order to show different conditions next to one another while conserving space; however, any adjustments were applied to the entire blot equally before splicing.

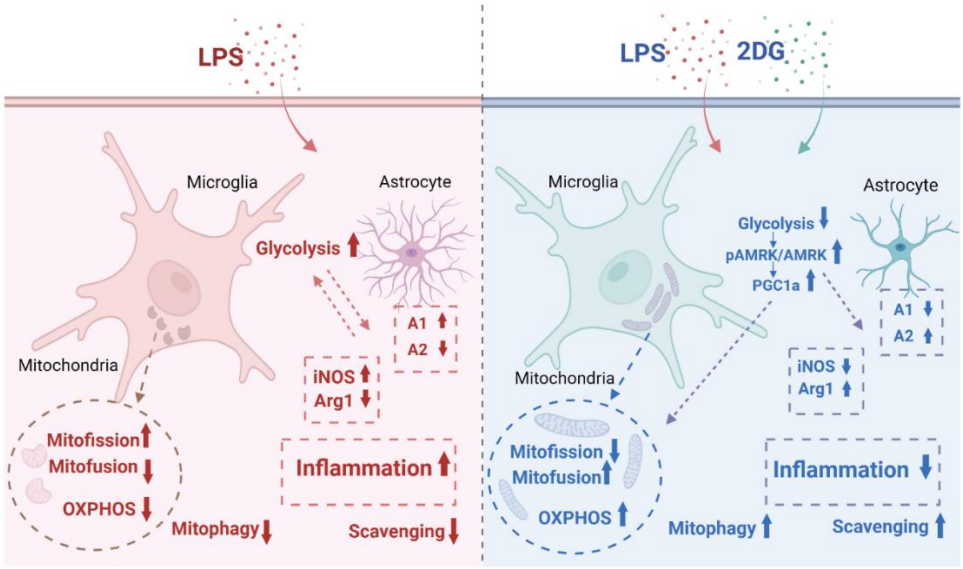

Figure S8 (summary figure): **Schematic representation illustrating the impact of 2DG on neuroinflammation in microglia and astrocytes**. LPS stimulation triggers a metabolic shift in microglia and astrocytes, resulting in the release of pro-inflammatory cytokines that drive neuroinflammation. In contrast, low-dose 2DG reduces LPS-induced neuroinflammation by promoting a compensatory increase in oxidative phosphorylation. LPS: Lipopolysaccharide; 2DG: 2-Deoxy-D-glucose; OXPHOS: oxidative phosphorylation; A1: neurotoxic astrocytes; A2: alternatively activated astrocytes.

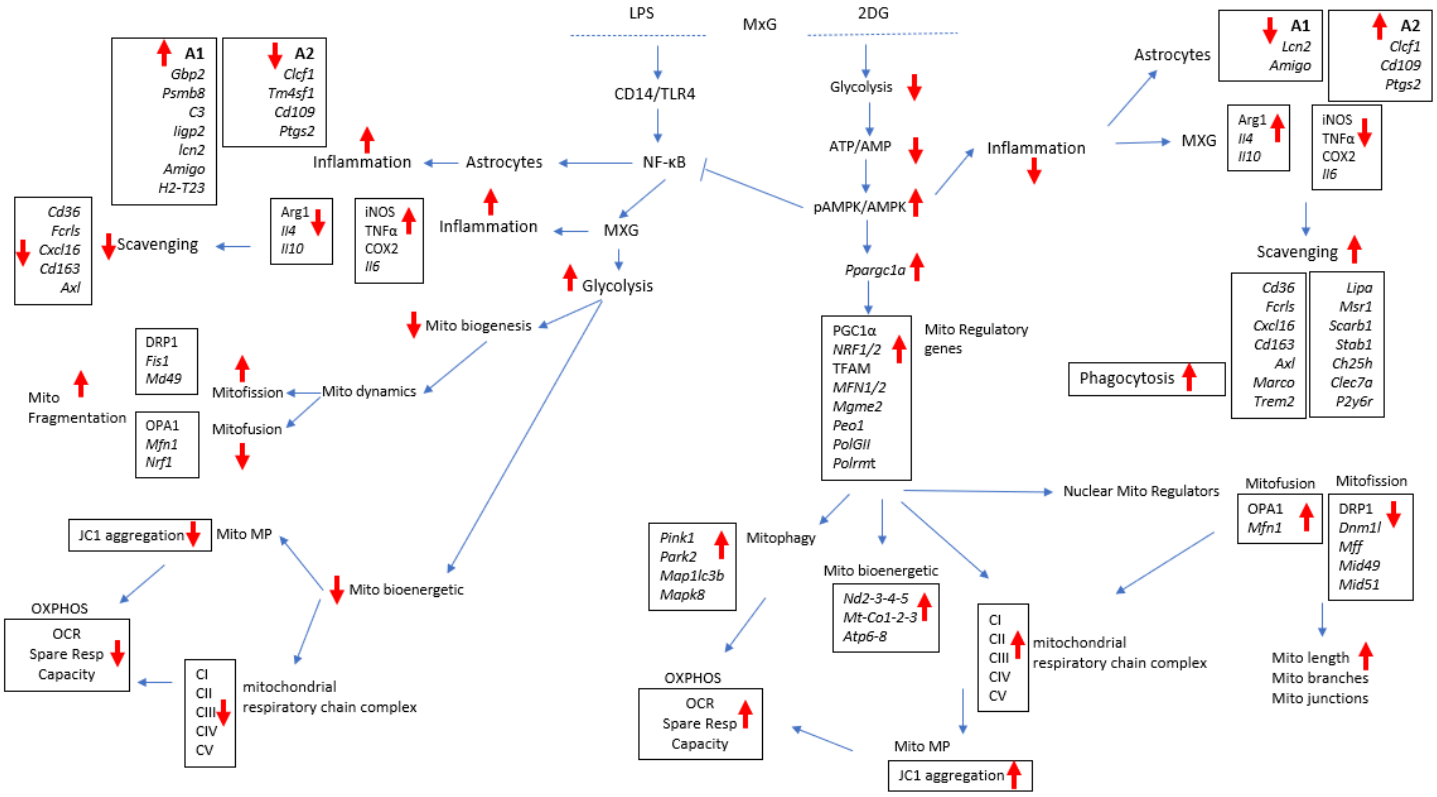

Figure S9: **The overall interplay between LPS and 2DG for mitigating neuroinflammatory responses**. MxG: mixed glial cells; LPS: Lipopolysaccharide; 2DG: 2-Deoxy-D-glucose; OXPHOS: oxidative phosphorylation; Mito: mitochondrial; MP: membrane potential; OCR: oxygen consumption rate.

Target	Forward	Reverse
Tfam	TCCACAGAACAGCTACCCAA	CCACAGGGCTGCAATTTTCC
Mgme2	TGTTAGGTTCTCCTGGGGTG	TTTGGAGAGGTGGAAAGAGC
ppargc1a	GCAGTCGCAACATGCTCAAG	GGGAACCCTTGGGGTCATTT
Peo1	GCCCAGTCACCAGTTTCCTA	ACTCTGGTCATTCACCCTCG
Polg2	CCGTTTTCCAGCGTAGTCTC	TTCTGTGTGGCCTGGCTATT
Polrmt	CTCATCTCAGGTGTGCCCTC	TCTGCAGCTCAAGAAGGAGC
Dnm1l	CGTGGACTAGCTGCAGAATG	TGCCTCAGATCGTCGTAGTG
Fis1	AGGAGCTGGAACGCCTGATTGATA	AGGATTTGGACTTGGAGACAGCCA
Mff	GCAGTTGGCAGGCTAAAAAG	TCAGGTAGCATATGGGGAGG
Mfn1	GCTTCCGACGGACTTACAAC	TGAATAACCGTTGGGATGCT
Mfn2	ATTGATCACGGTGCTCTTCC	GTCCTGGACGTCAAAGGGTA
Opa1	TCCTGGTGAAGAGCTTCAATG	TTTGCAGAAGACGGTGAGAA
Drp1	GGGCACTTAAATTGGGCTCC	TGTATTCTGTTGGCGTGGAAC
Nrf1	AGAAACGGAAACGGCCTCAT	CATCCAACGTGGCTCTGAGT
Nrf2	ATGGAGCAAGTTTGGCAGGA	GCTGGGAACAGCGGTAGTAT
Mid51	ATGTACTTGAGTGGCAGCCT	TCACGACGAACCAGGAAGAA
Mid49	TGTCAGCCTCTGTCAACTGG	AGTGAACTCCAGGCACTCGT
Cycs (Cyt-C)	CCAAATCTCCACGGTCTGTT	GTCTGCCCTTTCTCCCTTCT
Ndufb8	TGTTGCCGGGGTCATATCCTA	AGCATCGGGTAGTCGCCATA
Nd4-Nd5	CACTCAGACCCAAACATCAATCG	TCGTCCGTACCATCATCCAATTA
Cox3-Nd3-Nd4	ATGCGGATTCGACCCTACAAG	TGCTCATGGTAGTGGAAGTAGAA
Nd2-Cox1	AGCCTGAGCGGGAATAGTG	ATGGGCAGTTACGATAACATTGT
Cox1-2, Atp6-8	AATTGCTCTCCCCTCTCTACG	GGTTTTAGGTCGTTTGTTGGGAT
Mt-Co2	CTACAAGACGCCACAT	GAGAGGGGAGAGCAAT
Mt-Co3	GCAGGATTCTTCTGAGCGTTCT	GTCAGCAGCCTCCTAGATCATGT
Aif1 (Iba1)	CAGACTGCCAGCCTAAGACA	AGGAATTGCTTGTTGATCCC
Nos2	ATGGACCAGTATAAGGCAAGC	GCTCTGGATGAGCCTATATTG
Tnf	CCAGACCCTCACACTCAGATC	CACTTGGTGGTTTGCTACGAC
Il6	AGTCCGGAGAGGAGACTTCA	TTGCCATTGCACAACTCTTT

Il4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Il10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
C3	AGCTTCAGGGTCCCAGCTAC	GCTGGAATCTTGATGGAGACGC
Gbp2	GGGGTCACTGTCTGACCACT	GGGAAACCTGGGATGAGATT
Iigp1	GGGGCAATAGCTCATTGGTA	ACCTCGAAGACATCCCCTTT
Lcn2	CCAGTTCGCCATGGTATTTT	CACACTCACCACCCATTCAG
Psmb8	CAGTCCTGAAGAGGCCTACG	CACTTTCACCCAACCGTCTT
Amigo2	GAGGCGACCATAATGTCGTT	GCATCCAACAGTCCGATTCT
Clcf1	CTTCAATCCTCCTCGACTGG	TACGTCGGAGTTCAGCTGTG
H2-D1	TCCGAGATTGTAAAGCGTGAAGA	ACAGGGCAGTGCAGGGATAG
Serping1	ACAGCCCCCTCTGAATTCTT	GGATGCTCTCCAAGTTGCTC
Tm4sf1	GCCCAAGCATATTGTGGAGT	AGGGTAGGATGTGGCACAAG
Cd109	CACAGTCGGGAGCCCTAAAG	GCAGCGATTTCGATGTCCAC
Ptgs2	GCTGTACAAGCAGTGGCAAA	CCCCAAAGATAGCATCTGGA
H2-T23	GGACCGCGAATGACATAGC	GCACCTCAGGGTGACTTCAT
Axl	ATGGCCGACATTGCCAGTG	CGGTAGTAATCCCCGTTGTAGA
Cd36	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
Cd68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
Cd163	ATGGGTGGACACAGAATGGTT	CAGGAGCGTTAGTGACAGCAG
Ch25h	TGCTACAACGGTTCGGAGC	AGAAGCCCACGTAAGTGATGAT
Clec7a	GACTTCAGCACTCAAGACATCC	TTGTGTCGCCAAAATGCTAGG
Csfr1	TGTCATCGAGCCTAGTGGC	CGGGAGATTCAGGGTCCAAG
Cxcr1	GAGTATGACGATTCTGCTGAGG	CAGACCGAACGTGAAGACGAG
Cxcl16	CCTTGTCTCTTGCGTTCTTCC	TCCAAAGTACCCTGCGGTATC
Fcrls	CTTCTGGTCTTCGCTCCTGTC	ATGGTGTAGCTTGAAGCACTG
Lamp1	CAGCACTCTTTGAGGTGAAAAAC	ACGATCTGAGAACCATTCGCA
Lamp2	TGTATTTGGCTAATGGCTCAGC	TATGGGCACAAGGAAGTTGTC
Lipa	AGCGACGACTTGGTGTTCC	GCTGAGCAAGACTCCACCG
Lox1	GTTCCACACATCCGTTACACT	CCGAGTAAGCAACTGAACATGG

Lpi	CCAACTCTTTTGTGCCAGAGA	GGCTACATTGGTGTTGAGCTTTT
Lrp1	GCTGGGGTGTACGGAAATGG	GTGCTCGAATTTGTTCTGGACT
Marco	ACAGAGCCGATTTTGACCAAG	CAGCAGTGCAGTACCTGCC
Msr1	GCACAATCTGTGATGATCGCT	CCCAGCATCTTCTGAATGTGAA
P2y6r	TGAAAACAACGAGGAACACCAA	CAGCCTTTCCTATGCTCGGA
Trem2	CTGGAACCGTCACCATCACTC	CGAAACTCGATGACTCCTCGG
Scrab1	AAACAGGGAAGATCGAGCCAG	GGTCTGACCAAGCTATCAGGTT
Stab1	GGCAGACGGTACGGTCTAAAC	AGCGGCAGTCCAGAAGTATCT
Sra1	CACGCAATCCCACCTACAAAG	CGGGTCGTGATTCTCCCAG
Mapk8 (Jnk1)	AGCAGAAGCAAACGTGACAAC	GCTGCACACACTATTCCTTGAG
Map1lc3b	AATCACTGGGATCTTGGTGG	AGTCAGATCGTCTGGCTCG
Park2	ATCGACCTCCACTGGGAAG	GCGTAGGTCCTTCTCGACC
Pink1	GGATGTCGTCCTGAAGGGAG	GCTTCGCTGGAGGAACCTG
Ppia	TGTGCCAGGGTGGTGACTTT	CGTTTGTGTTTGGTCCAGCAT
B2m	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC

Table S1: List of the primers used for RT-qPCR.

Antibody name	Host	Concentration used	Company	Product number
Primary antibodies (WB) (InCell WB)				
TFAM	Rabbit	1:1000	Cell Signaling Technology	#7495
OPA1	Rabbit	1:1000	Cell Signaling Technology	#67589
DRP1	Rabbit	1:1000	Cell Signaling Technology	#5391
PGC1α	Rabbit	1:1000	Cell Signaling Technology	#2178
Cytochrome-C	Rabbit	1:1000	Cell Signaling Technology	#4272
OxPhos Rodent WB Antibody Cocktail	Mouse	1:500	Thermo Fisher Scientific	#45-8099
AIF-1/Iba1	Goat	1:1000	Novus Biologicals	#NB100-1028
iNOS	Rabbit	1:1000	Cell Signaling Technology	#2982
TNFα	Rabbit	1:1000	Cell Signaling Technology	#3707
Arginase-1	Rabbit	1:1000	Novus Biologicals	#NBP1-32731
Anti-COX2 / Cyclooxygenase 2	Rabbit	1:1000	Novus Biologicals	#NB100-689
CD206	Rabbit	1:1000	Novus Biologicals	#NBP1-90020
Galectin-3	Rabbit	1:1000	Cell Signaling Technology	#87985
AMPKα	Rabbit	1:1000	Cell Signaling Technology	#2532
p-AMPKα	Rabbit	1:1000	Cell Signaling Technology	#2535
GAPDH	Mouse	1:5000	Cell Signaling Technology	#97166
β-tubulin	Mouse	1:2000	Cell Signaling Technology	#2146
Secondary antibodies (WB) (InCell WB)				
IRDye 800CW	Mouse	1:15000	Li-Cor	926-32210
IRDye 800CW	Rabbit	1:15000	Li-Cor	926-32211
IRDye 680RD	Mouse	1:15000	Li-Cor	926-68070
IRDye 680RD	Goat	1:15000	Li-Cor	926-68074
Primary antibodies (ICC)				
AIF-1/Iba1	Goat	1:100	Novus Biologicals	#NB100-1028
iNOS	Rabbit	1:50	Cell Signaling Technology	#2982
Arg1	Rabbit	1:200	Novus Biologicals	#NBP1-32731
Secondary antibodies (ICC)				

anti-goat Alexa fluor 488	Donkey	1:2000	Invitrogen	A32814
anti-rabbit Alexa fluor 555	Donkey	1:2000	Invitrogen	A32794

Table S2: The antibodies used for IHC, WB and InCell Western assay.