

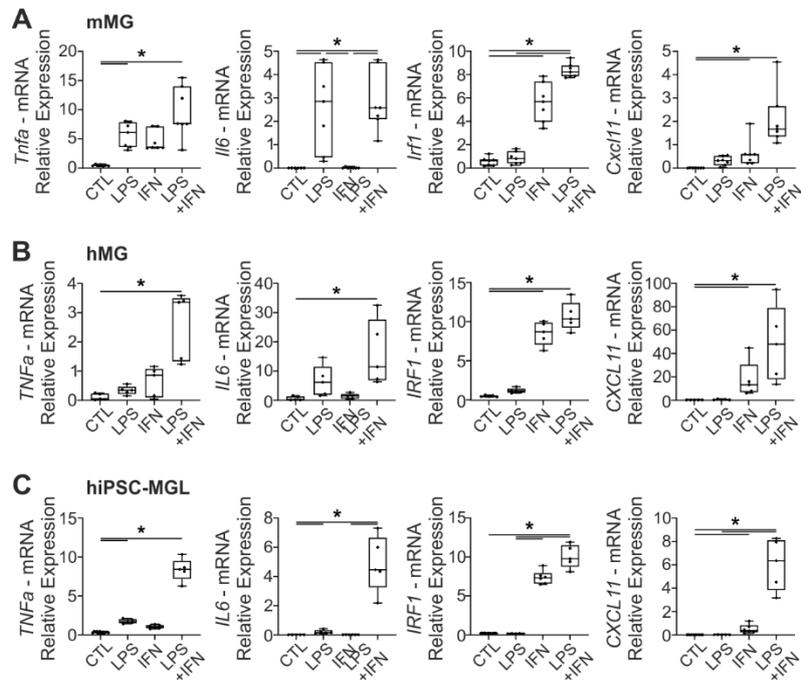
1 Supplemental information

2 Material and Methods

3 Primary mouse microglia from adult mice (14–16 weeks old) were isolated from whole brains
4 without the cerebellum, as previously described, with slight modifications [S1,S2]. In brief,
5 blood vessels and meninges were carefully removed from the resected brain tissue. The tissue
6 was then diced and dissociated using the Neural Tissue Dissociation Kit (P) (#130–092-628,
7 Miltenyi Biotec, Bergisch Gladbach, Germany). Microglia obtained from one mouse brain
8 were cultured as adherent cells in non-coated T25 flasks containing microglia culture medium
9 composed of DMEM/F12 supplemented with with 10% fetal calf serum and 1X penicillin–
10 streptomycin solution. One day after the isolation procedure, the medium was supplemented
11 with macrophage colony-stimulating factor (M-CSF, 100 ng/mL, PeproTech #315-02) and
12 granulocyte-macrophage colony-stimulating factor (GM-CSF, 100 ng/mL, PeproTech #315-
13 03). Microglia were cultured for 7 days, and the medium was changed every 3 days. After 1
14 week of incubation at 37°C and 5% CO₂, the cells were transferred to PLL-coated glass
15 coverslips for patch-clamp experiments. To detach the cells, the culture medium was removed,
16 and the flasks were washed with phosphate-buffered saline (PBS, without Mg²⁺ and Ca²⁺). The
17 cells were then incubated with 0.5 mM EDTA solution (diluted from 0.5 M solution,
18 Invitrogen, #15575020) at 37°C and 5% CO₂ for 10 minutes. To ensure complete detachment,
19 cells were gently scraped using a rubber cell scraper and seeded in microglia culture medium
20 without M-CSF and GM-CSF.

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24 **Fig. S1: mMG, hMG, and hiPSC-MGL upregulate cytokines upon proinflammatory**
 25 **stimulation**

26 **B.** mRNA of *TNFα*, *IL6*, *IRF1* and *CXCL11* were quantified by qPCR and are presented relative
 27 to the mRNA expression of housekeeping gene *TPP2*. Cells were collected after stimulation
 28 with the indicated stimuli.

29 Statistical analysis with the Kruskal-Wallis test followed by Dunn's test for multiple
 30 comparisons, $*P < 0.05$. Data represented box plots and median. Each dot represents one
 31 sample.

32 For n=samples: mMG (CTL n=7, LPS n=7, IFN n=7, LPS+IFN n=7), iPSC-MGL (CTL n=12,
 33 LPS n=5, IFN n=6, LPS+IFN n=5), hMG (CTL n=5, LPS n=5, IFN n=5, LPS+IFN n=5)

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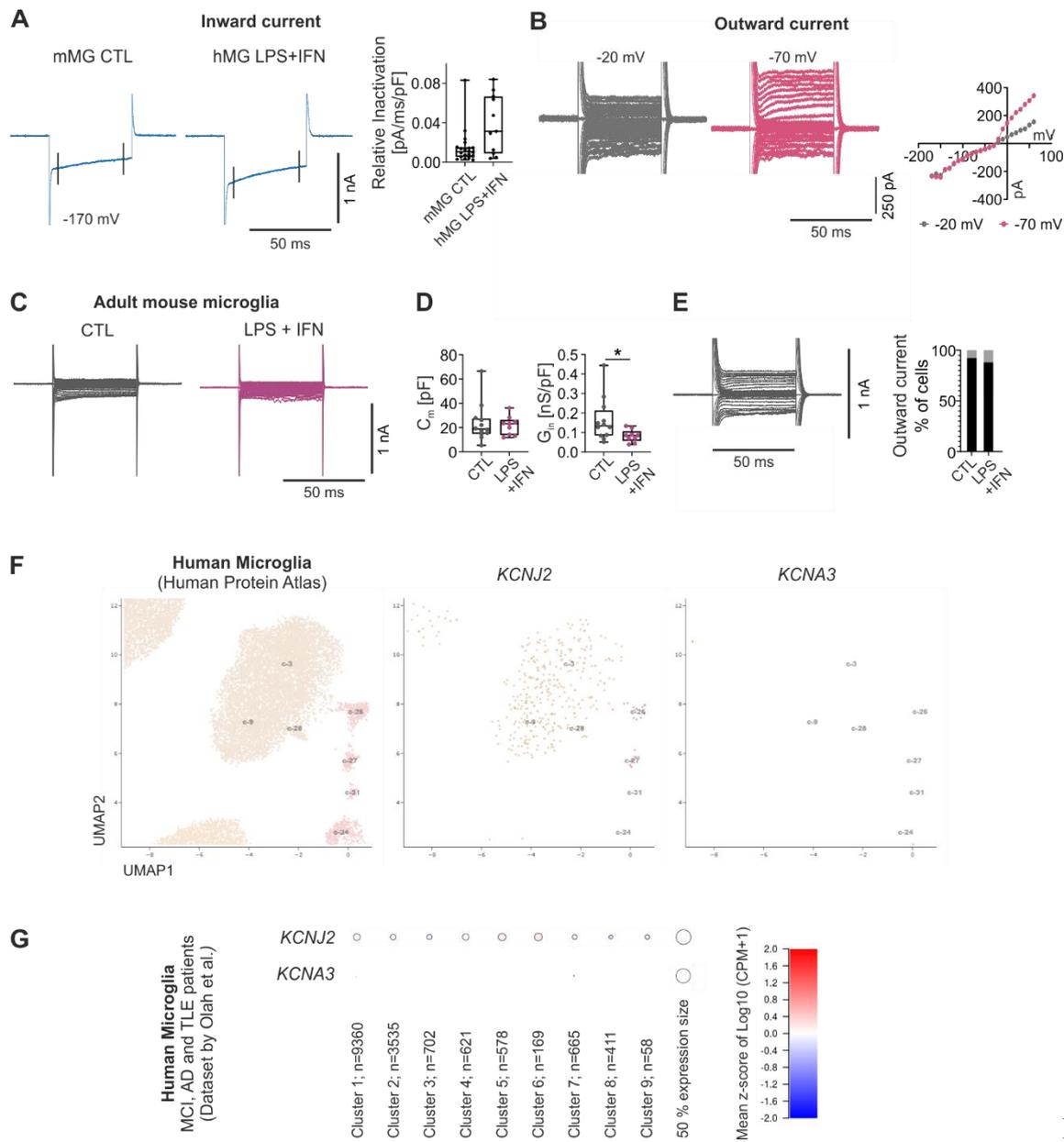


Fig.

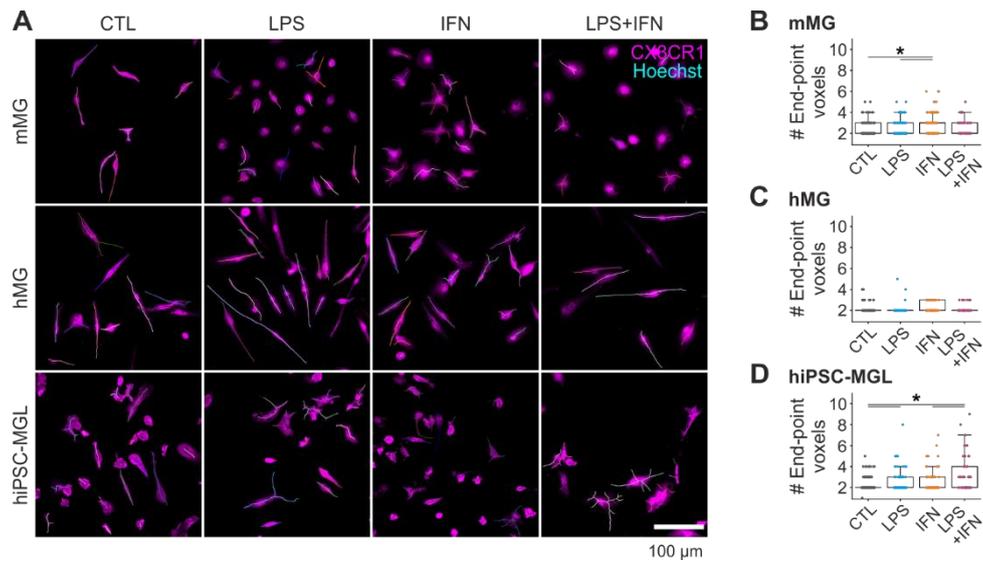
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37 **S2: K_v and $K_{ir2.1}$ characteristics, currents in adult mouse microglia and expression in**
 38 **human tissue**

39 **(A)** Time-dependent inactivation of inward currents at low voltages. Exemplary traces at -170
 40 mV and a holding potential of -20 mV are shown for mMG under control condition and hMG
 41 after stimulation with LPS+IFN- γ . The inactivation is calculated from the current at the
 42 indicated time points (black bars) and the decrease over time. The values are normalized to the
 43 capacity of each cell. For n=recodings: mMG CTL n=20, hMG LPS+IFN n=11 **(B)** Exemplary
 44 recording of mMG after stimulation with LPS+IFN- γ displaying voltage-dependent delayed

45 outward rectifying current and corresponding IV-curves. At a holding potential of -20 mV
46 (grey) the channel is inactive, whereas at a holding potential of -70 mV (pink) it displays the
47 characteristic delayed inward current. **(C)** Single-cell patch-clamp recordings of primary
48 mouse microglia isolated from adult mice were performed following stimulation with LPS and
49 IFN- γ . Representative traces of recordings at a holding potential of -20 mV are shown. **(D)** The
50 basic membrane characteristics of reversal potential (V_{rev}), membrane resistance (R_m),
51 capacitance (C_m) and specific inward conductance (G_{in}) were derived from the IV-curves.
52 Statistical analysis with the Mann-Whitney test, $*P < 0.05$. Data represented as box plots with
53 median, each dot represents one cell. **(E)** Percentage of cells exhibiting voltage-dependent,
54 delayed outward current at a holding potential of -70 mV and activation at stimulation of -20
55 mV with exemplary trace. For n=recordings: CTL n=12, LPS+IFN n=9. **(F)** UMAP from single
56 cell analysis in the human protein atlas (www.proteinatlas.org). Microglia are represented in
57 Cluster c-3, c-9, and c-28. *KCNJ2* had the following expression in the three microglia clusters:
58 c-3 189 reads/4332 cells, Expression 11.9 nCPM; c-9 155 reads/2534 cells, Expression 27.9
59 nCPM, c-28 9 reads/133 cells, Expression 12.7 nCPM. *KCNA3* had no reads in any of the three
60 microglia clusters. **(G)** RNA expression derived from the dataset of Olah et al. [1]. Microglia
61 were isolated from postmortem tissue from patients suffering from Alzheimer's disease (AD),
62 mild cognitive impairment (MCI) or from fresh surgery tissue of patients suffering from
63 temporal lobe epilepsy (TLE). Based on their expression profiles, the microglia were then
64 grouped into 9 different clusters. The data show induced expression of *KCNJ2* in clusters 5 and
65 6. Cluster 5 is marked by increased interleukin 10 signaling and both clusters 5 and 6 show
66 increased expression of CD83, overall relating clusters 5 and 6 to an anti-inflammatory
67 response. There is no relevant expression of *KCNA3* in any of the clusters.

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70 **Fig. S3: Analysis of bifurcation of microglial cell cultures**

71 **(A)** Maximum intensity projections of confocal laser scanning microscopy images for cultured
 72 cells from the studied cell lines under four treatment conditions, labeled with CX3CR1-AF488
 73 (magenta) and Hoechst (cyan). The colored lines represent the skeleton results of the
 74 morphological analysis. Scale bar: 100 μ m. **(B-E)** Statistical analysis of number of End-point
 75 Voxels, quantifying the ramification of cultured cells under the treatment conditions. Statistical
 76 analysis with the Kruskal-Wallis test followed by Dunn's test for multiple comparisons, $*P <$
 77 0.05. Data represented as box plot with median. Each dot represents one cell.

78 For n=cells: mMG (CTL n=135, LPS n=123, IFN n=107, LPS+IFN n=63), iPSC-MGL (CTL
 79 n=154, LPS n=63, IFN n=88, LPS+IFN n=57), hMG (CTL n=60, LPS n=38, IFN n=61,
 80 LPS+IFN n=39).

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83 **Suppl. Table 1: Characteristics of human donors**

ID#	Sex	Age	Pathology
Pat #1	male	55	TLE
Pat #2	male	59	TLE
Pat #5	male	40	TLE
Pat #6	male	39	TLE
Pat #7	male	29	TLE
Pat #9	female	37	TLE
Pat #14	male	58	TLE
hiPSC	Female	-	

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86 Reference:

- 87 1. Olah, M., et al., *Single cell RNA sequencing of human microglia uncovers a subset*
88 *associated with Alzheimer's disease*. Nature Communications, 2020. **11**(1).

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