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Supplemental information

Purinergic exposure induces epigenomic and transcriptomic-mediated preconditioning resembling epilepsy-associated microglial states

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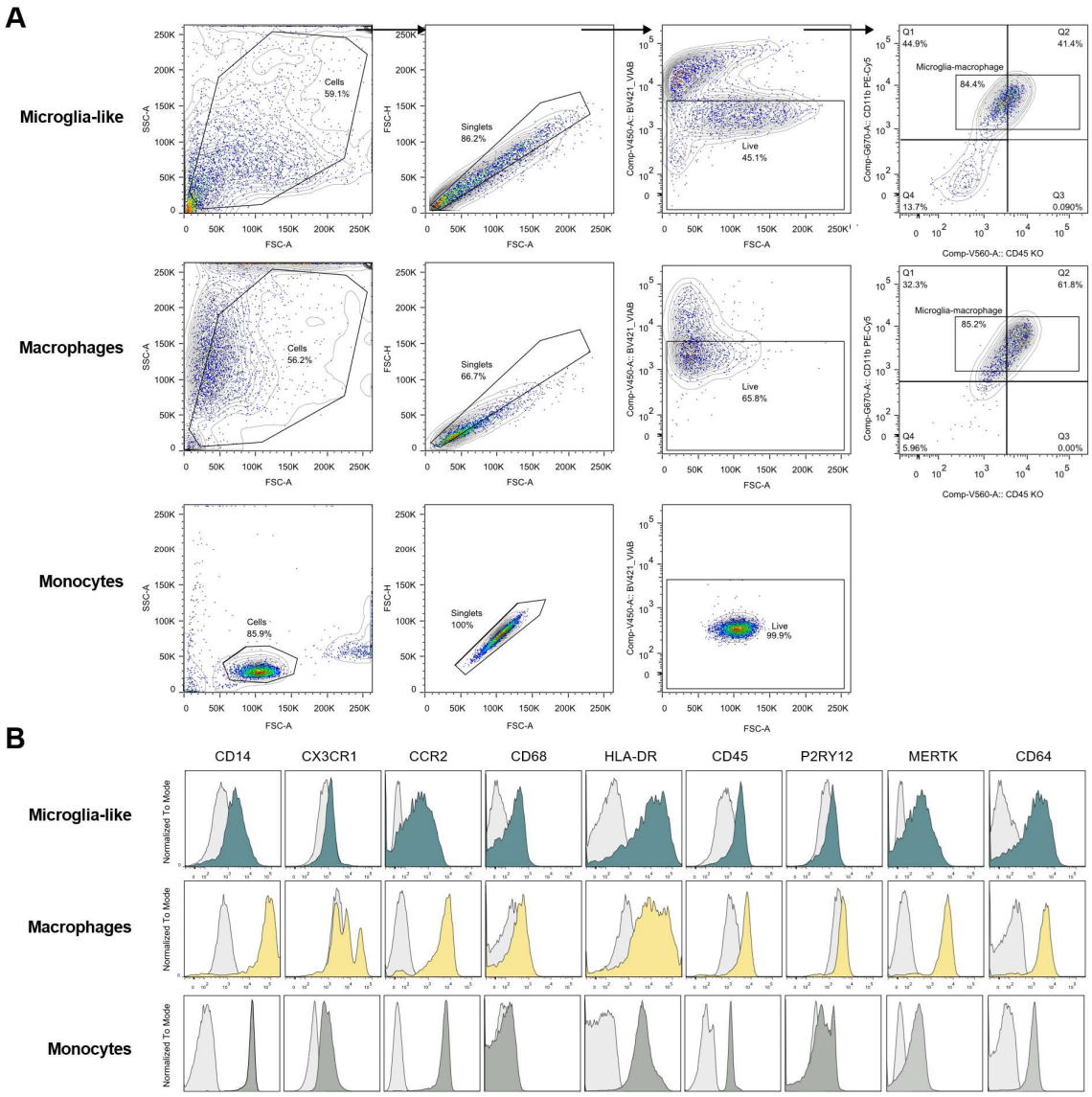

Figure S1. Characterization of surface markers, related to Figure 1. (A) Representation of the flow cytometry gating strategy used to single out the microglia/macrophage populations in the in vitro models of monocyte-derived microglia-like and monocyte-derived macrophages. Microglia and macrophages are CD11b+, and present low or high CD45 surface levels, respectively. For monocytes, we considered all live cells. **(B)** Histogram representation of the protein levels of CD14, CX3CR1, CCR2, CD68, HLA-DR, CD45, P2RY12, MERTK and CD64 obtained for freshly isolated monocytes, microglia-like and macrophages. The unstained controls are represented in light grey.

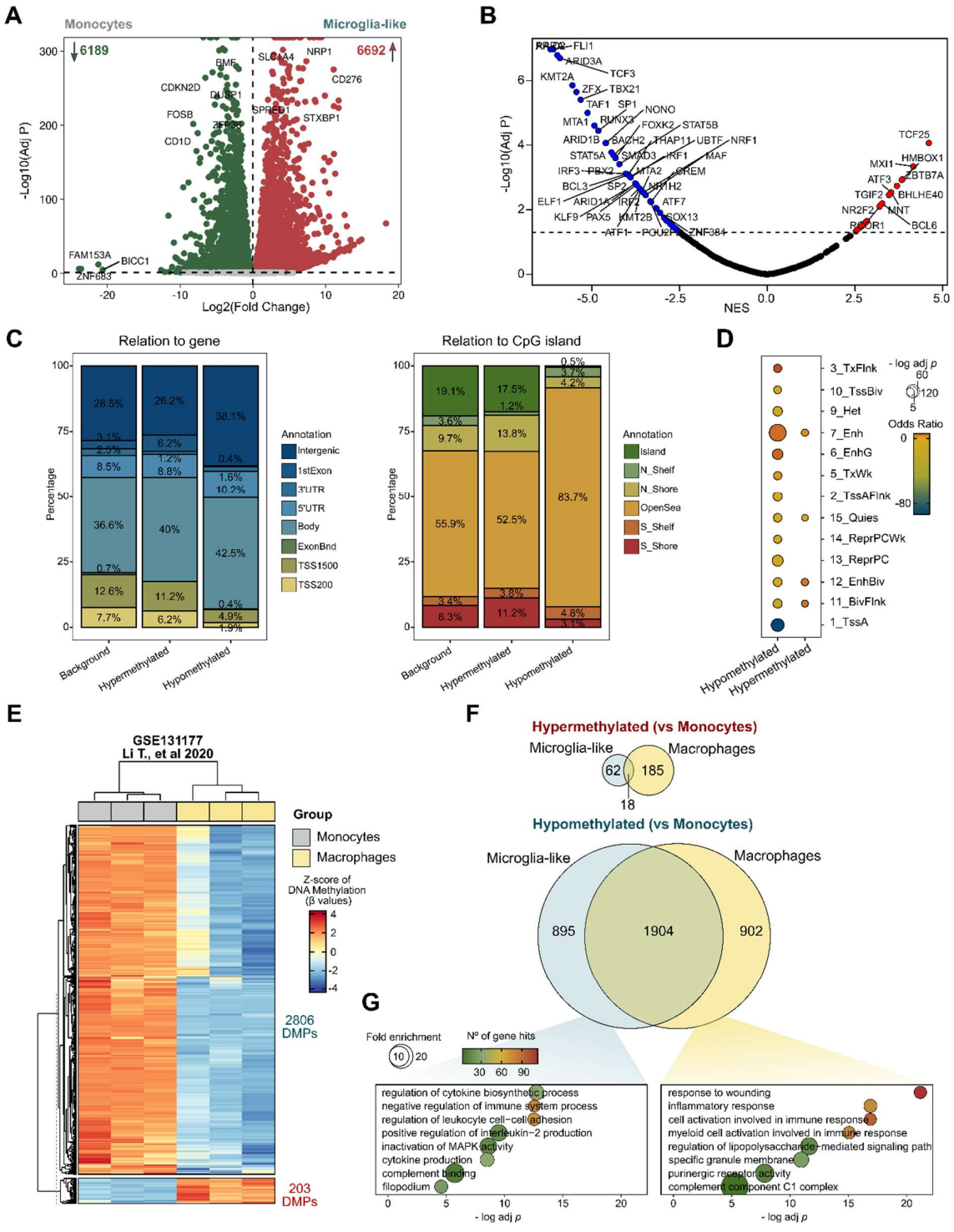
A
Monocytes
Microglia-like
6189
6692
-Log10(Adj P)
Log2(Fold Change)
BMF
SLC4A4
NRP1
CD276
CDKN2D
DUSP1
SPRED1
STXBP1
FOSB
ZFP36
CD1D
FAM153A
BICC1
ZNF683
B
-Log10(Adj P)
NES
FLI1
ARID3A
TCF3
KMT2A
ZFX
TBX21
TAF1
SP1
NONO
MTA1
RUNX3
FOXK2
STAT5B
ARID1B
SAC2
THAP11
UBTF
NRF1
STAT5A
SMAD3
IRF1
MAF
IRF3
PBX2
MTA2
CREM
BCL3
SP2
NFIH2
ELF1
ARID1A
IRF2
ATF7
KLF9
PAX5
KMT2B
SOX13
ATF1
POU2F2
ZNF384
TCF25
HMBOX1
MXI1
ZBTB7A
ATF3
TGIF2
BHLHE40
NR2F2
MNT
RORA
BCL6
C
Relation to gene
Percentage
Annotation
Intergenic
1stExon
3'UTR
5'UTR
Body
ExonBnd
TSS1500
TSS200
28.5%
3.1%
2.5%
8.5%
36.6%
0.7%
12.6%
7.7%
26.2%
6.2%
1.2%
8.8%
40%
11.2%
6.2%
38.1%
0.4%
1.6%
10.2%
42.5%
0.4%
4.9%
1.9%
Background
Hypermethylated
Hypomethylated
Relation to CpG island
Annotation
Island
N_Shelf
N_Shore
OpenSea
S_Shelf
S_Shore
19.1%
3.6%
9.7%
55.9%
3.4%
8.3%
17.5%
1.2%
13.8%
52.5%
3.8%
11.2%
0.5%
3.7%
4.2%
83.7%
4.8%
3.1%
D
- log adj p
Odds Ratio
3_TxFlnk
10_TssBiv
9_Het
7_Enh
6_EnhG
5_TxWk
2_TssAFlnk
15_Quies
14_ReprPCWk
13_ReprPC
12_EnhBiv
11_BivFlnk
1_TssA
E
GSE131177
Li T., et al 2020
Group
Monocytes
Macrophages
Z-score of DNA Methylation (β values)
2806 DMPs
203 DMPs
F
Hypermethylated (vs Monocytes)
Microglia-like
Macrophages
62
185
18
Hypomethylated (vs Monocytes)
Microglia-like
Macrophages
895
1904
902
G
Fold enrichment
Nº of gene hits
- log adj p
regulation of cytokine biosynthetic process
negative regulation of immune system process
regulation of leukocyte cell–cell adhesion
positive regulation of interleukin-2 production
inactivation of MAPK activity
cytokine production
complement binding
filopodium
response to wounding
inflammatory response
cell activation involved in immune response
myeloid cell activation involved in immune response
regulation of lipopolysaccharide-mediated signaling path
specific granule membrane
purinergic receptor activity
complement component C1 complex

Figure S2. Transcriptomic and epigenomic characterization of microglia-like cells, related to Figures 1 and 2. (A) Volcano plot depicting the differential expression between microglia-like cells and monocytes. Differentially expressed genes (DEGs) are considered for FDR < 0.05. Upregulated DEGs (log2(Fold Change) > 0) are highlighted in red, and downregulated DEGs (log2(Fold Change) < 0) in green. **(B)** Transcription factor (TFs) enrichment of all significant TFs (adjusted p value (FDR) < 0.05) in the differential expression comparison between microglia-like cells and monocytes. Significance is represented by the NES (Normalized Enrichment Score) and the negative of log of the adjusted p value (FDR). **(C)** Proportion distribution of the lists of hypermethylated and hypomethylated differentially methylated positions (DMPs) in microglia-like vs monocytes in relation to gene (left panel) and in relation to CpG islands (right panel), according to annotations from the Infinium MethylationEPIC array. The proportion of each category was calculated for background, hypermethylated and hypomethylated DMPs. N, north; S, south; 1stExon, first exon; UTR, untranslated region; ExonBnd, exon boundary; TSS, transcription start site. **(D)** Enrichment of hypermethylated and hypomethylated DMPs in microglia-like vs monocytes in ChromHMM categories of monocytes (Roadmap Epigenomics Project). Fisher's exact tests were calculated using all the positions annotated in the EPIC array as background. **(E)** Heatmap representation of the DNA methylation levels of the DMPs obtained for the monocyte-derived macrophages vs monocytes comparison. The data was collected from GSE131177. DNA methylation is represented as the z-score of the beta values. The significance cutoff was of adjusted p value (FDR) < 0.05 and difference in mean beta values > 0.2. **(F)** Overlap of the lists of DMPs obtained in macrophage vs monocyte comparison (GSE131177) and microglia-like vs monocytes (this study). **(G)** Selected list of significantly enriched gene ontology (GO) terms for the exclusive hypomethylated DMPs in microglia-like (n=895) and in macrophages (n=902) in comparison to monocytes. The significance of enrichment is represented by the negative of the log of the adjusted p value, the enrichment fold change and the number of gene hits.

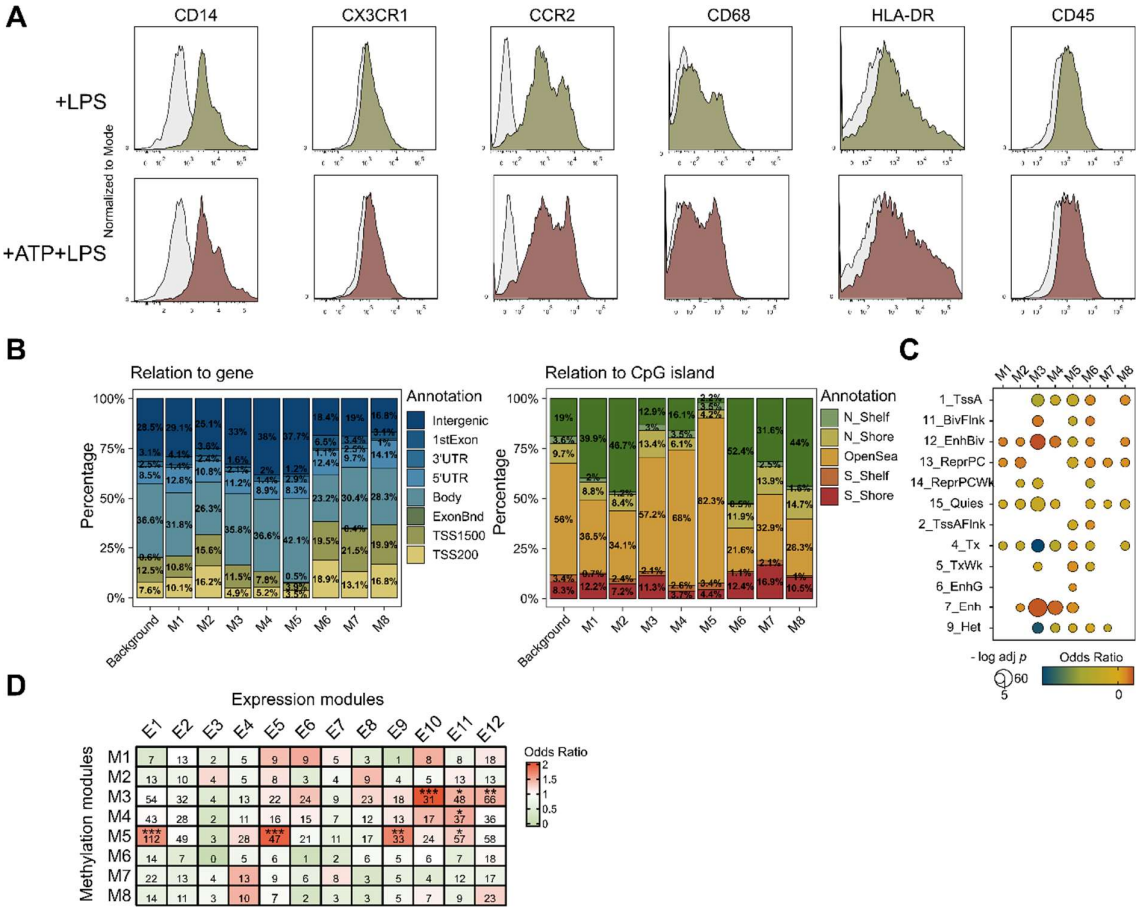

Figure S3. Effects of ATP preconditioning on microglia-like cell activation, related to Figures 3 and 4. (A) Histogram representation of the protein levels of CD14, CX3CR1, CCR2, CD68, HLA-DR and CD45 obtained for +LPS and +ATP+LPS microglia-like cells. The unstained controls are represented in light grey. **(B)** Proportion distribution of the eight modules of DMPs obtained in the pairwise comparison of all studied microglia-like conditions in relation to gene (left panel) and in relation to CpG islands (right panel), according to annotations from the Infinium MethylationEPIC array. The proportion of each category was calculated for background and M1-M8 DMPs. N, north; S, south; 1stExon, first exon; UTR, untranslated region; ExonBnd, exon boundary; TSS, transcription start site. **(C)** Enrichment of the modules of DMPs in ChromHMM categories of monocytes (Roadmap Epigenomics Project). Fisher's exact tests were calculated using all the positions annotated in the EPIC array as background. **(D)** Heatmap representation of the odds ratio of the overlap between the lists of the twelve modules of DEGs (E1-E12) and the lists of genes associated with each module of DMPs (M1-M8), obtained using GeneOverlap. Higher enrichment is represented by a higher odds ratio value (red). The significance of the overlap enrichment is based on p value and is represented by the asterisks (*p<0.05, **p<0.01, ***p<0.001). The number labels represent the number of genes shared between each pair of DEG and DMP modules.