Expanded View Figures

Figure EV1. Microglial isolation and characterization.

A FACS gating strategy representative of five independent experiments adopted to sort CD11b^{+}CD45^{int} microglial. Cells were distinguished from debris using forward (FSC-A) and side (SSC-A) scatters, followed by cell doublet and aggregate elimination (SSC-H/SSC-A). Dead cells were gated out by their strong positivity for the dead cell discrimination marker Hoechst. Single viable microglial cells were gated as CD11b^{+}CD45^{int}.

B Analysis of microglial purity by qPCR. Gene expression levels of microglial-specific genes (*Itgb5*, *Sall1*, *Hexb*, *Tgfb*, *Aif1*, *Cx3cr1*, *Mertk*, *Ctss*, *Tyrobp*, *Trem2*, *Itgam*, *Itgax*) in purified microglia compared to whole brain. Bars represent mean (n = 4; one female and one male per sample) of relative expression (*Gapdh* as housekeeping gene) ± SEM (*$P < 0.05$; **$P < 0.01$ by two-tailed Student's t-test).

C Quantification of CD11c, Ly6C and CCR2 expression in CD11b^{+}CD45^{int} microglia and CD11b^{+}CD45^{high} resident macrophages representative of five independent experiments. Values denote the percentage of the mean ± SEM of five independent experiments.

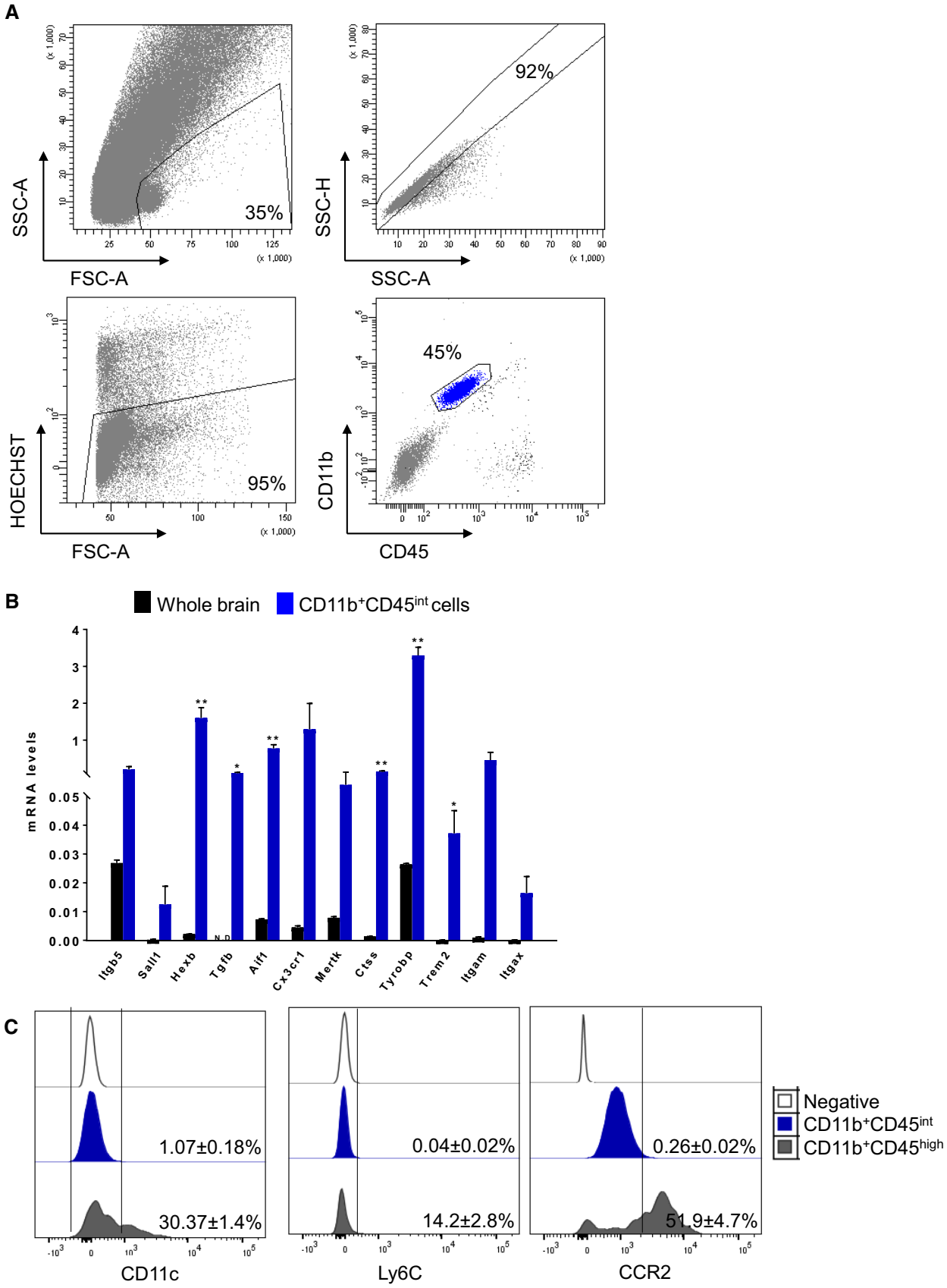

A
SSC-A
FSC-A
35%
SSC-H
SSC-A
92%
HOECHST
FSC-A
95%
CD11b
CD45
45%
B
Whole brain
CD11b+CD45int cells
mRNA levels
Itgb5
Sall1
Hexb
Tgfb
Aif1
Cx3cr1
Mertk
Ctss
Tyrobp
Trem2
Itgam
Itgax
C
1.07±0.18%
30.37±1.4%
0.04±0.02%
14.2±2.8%
0.26±0.02%
51.9±4.7%
CD11c
Ly6C
CCR2
Negative
CD11b+CD45int
CD11b+CD45high

Figure EV1.

Figure EV2. *Ex vivo* and *in vitro* microglial characterization.

A, B Three- to four-month-old mice were treated with an acute dose of LPS (4 μg/g body) or vehicle (saline). Microglial (pool of two mice per group; one female and one male) were isolated 24 h later. (A) Gene expression levels of microglial homeostatic genes (*Itgb5, Sall1, Hexb, Tgfb, Mertk, Ctss, Itgam, Cx3cr1*) were analysed by qPCR. Bars represent mean of relative expression (% of saline; *Gapdh* as housekeeping gene) ± SEM (***P* < 0.01 by two-tailed Student's *t*-test; *n* = 4). (B) Representative multicolour flow cytometry analysis of five independent experiments showing CD45, CD11b, CD86 and CD11c expression levels in $CD11b^{+}CD45^{int}$ microglia of saline or LPS-injected mouse brains.

C Representative results of two independent experiments showing the purity of MACS-isolated bone marrow monocytes based on the expression levels of the monocytic marker Ly6C.

D Comparison of the homeostatic signature (*Tmem119, Siglech, Gpr34, P2ry12*) between primary and acutely isolated microglia. Primary adult microglia were cultivated in the presence of TGF-β (50 μg/ml) and M-CSF (10 ng/ml), while neonatal cells were treated for 24 h with TGF-β or left untreated. Gene expression levels were analysed by qPCR and normalized using *Gapdh* as housekeeping gene. Bars represent mean ± SEM (***P* < 0.01 by two-tailed Student's *t*-test; *n* = 3).

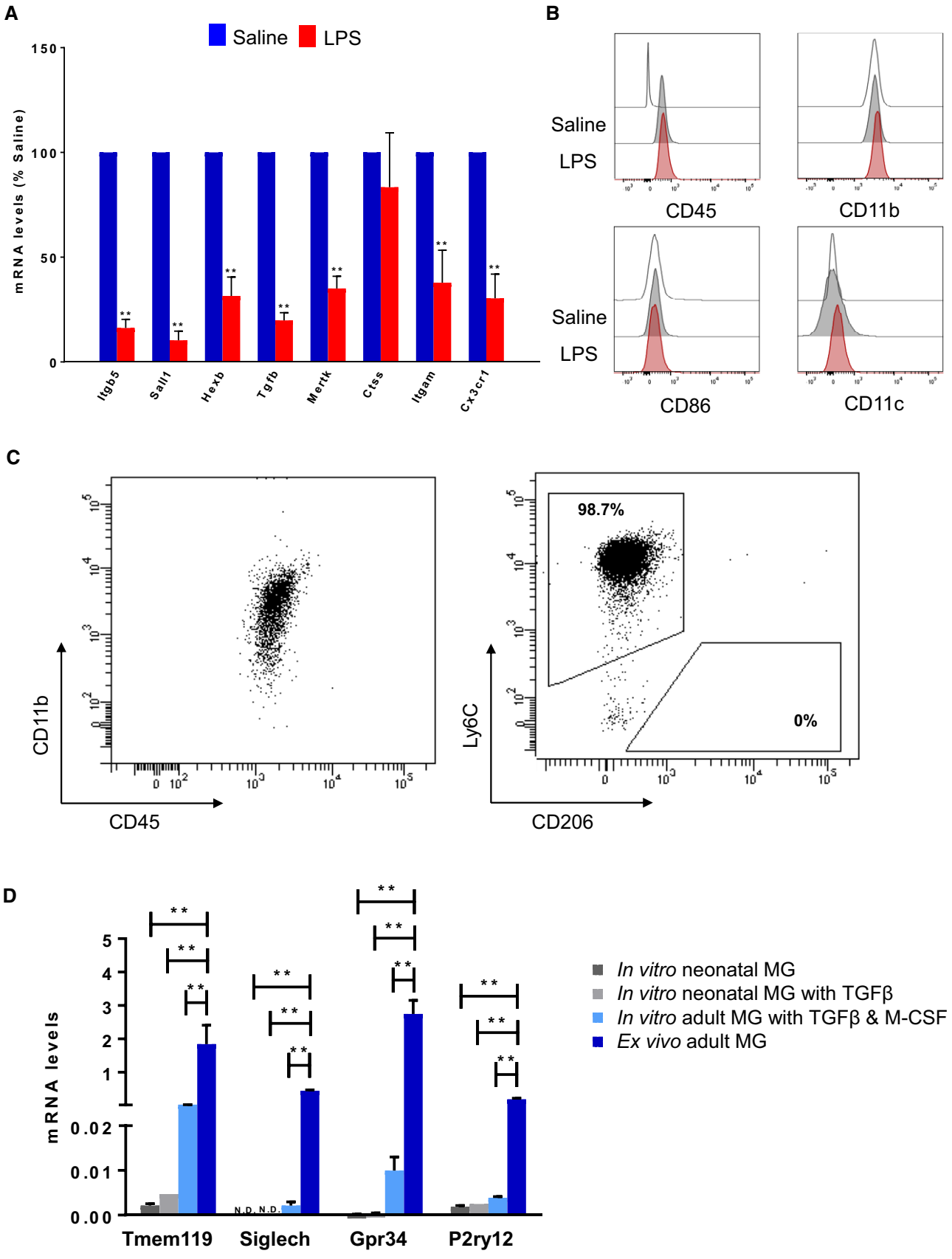

A
Saline
LPS
mRNA levels (% Saline)
150
100
50
0
Itgb5
Sall1
Hexb
Tgfb
Mertk
Ctss
Itgam
Cx3cr1
B
Saline
LPS
CD45
CD11b
CD86
CD11c
C
CD11b
CD45
98.7%
0%
Ly6C
CD206
D
mRNA levels
5
4
3
2
1
0.02
0.01
0.00
N.D. N.D.
Tmem119
Siglech
Gpr34
P2ry12
In vitro neonatal MG
In vitro neonatal MG with TGFβ
In vitro adult MG with TGFβ & M-CSF
Ex vivo adult MG

Figure EV2.

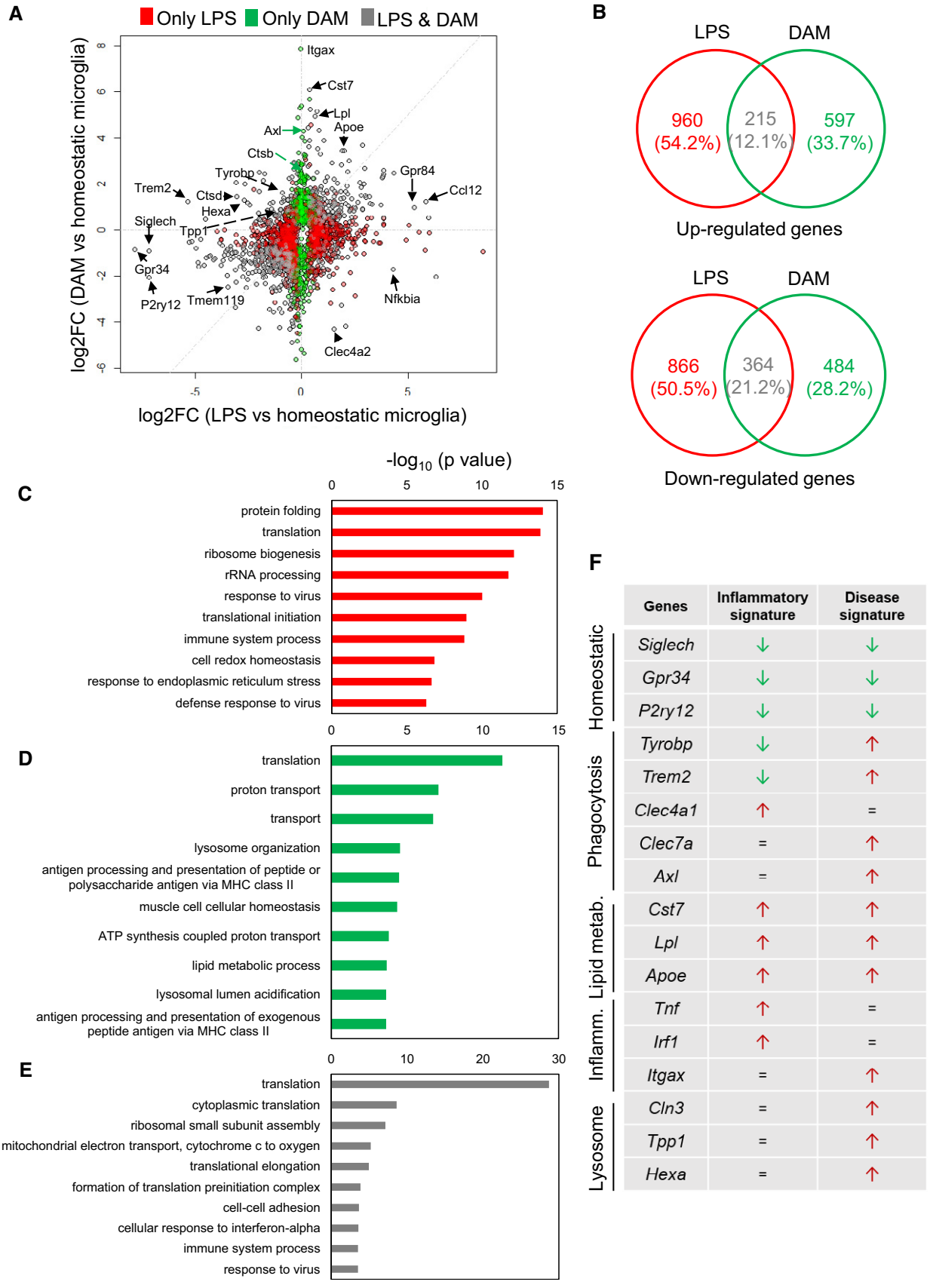
Figure EV3. Inflammatory- versus disease-associated signatures.

A Scatterplot comparing the fold change of genes (log2 scale) between microglia isolated from LPS-injected mice (*x*-axis) versus DAM (*y*-axis) compared to homeostatic microglia (FDR < 0.05).

B Venn diagrams showing the number of genes upregulated (left) or downregulated (right) uniquely under LPS treatment, exclusively by DAM and shared between LPS and DAM compared to their corresponding controls (FDR < 0.05).

C–E Top 10 biological processes identified by Database for Annotation, Visualization and Integrated Discovery (DAVID) resulting from (C) 960 uniquely upregulated genes under LPS treatment, (D) 597 increased genes specific for DAM and (E) 215 shared genes versus control conditions.

F Inflammatory- and disease-associated signatures identified by distinct genes corresponding to specific microglial functions (homeostasis, phagocytosis, lipid metabolism, inflammation and lysosomal activity).



F

	Genes	Inflammatory signature	Disease signature
Homeostatic	*Siglech*	↓	↓
	Gpr34	↓	↓
	P2ry12	↓	↓
Phagocytosis	*Tyrobp*	↓	↑
	Trem2	↓	↑
	Clec4a1	↑	=
	Clec7a	=	↑
	Axl	=	↑
Lipid metab.	*Cst7*	↑	↑
	Lpl	↑	↑
	Apoe	↑	↑
Inflamm.	*Tnf*	↑	=
	Irf1	↑	=
	Itgax	=	↑
Lysosome	*Cln3*	=	↑
	Tpp1	=	↑
	Hexa	=	↑

Figure EV3.

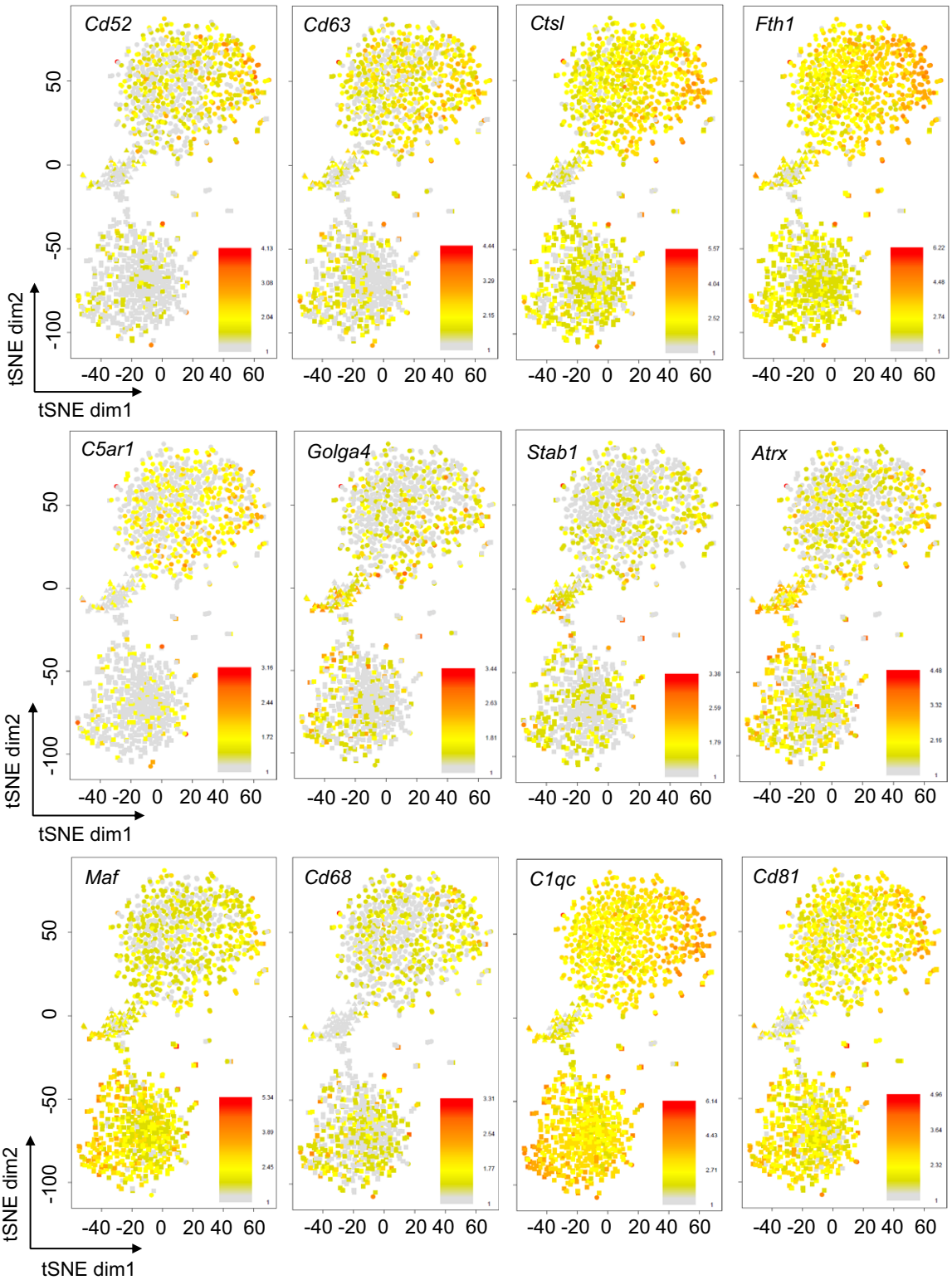


Figure EV4. Representation of mainly top deregulated genes in "main LPS" and "subset LPS".
Expression of specific genes uniquely upregulated in "main LPS" (*Cd52*, *Cd63*, *Ctsl*, *Fth1*, *C5ar1*) or "subset LPS" (*Golga4*, *Stab 1*, *Atrx*) and downregulated in "main LPS" (*Maf*) or "subset LPS" (*Cd68*, *C1qc*, *Cd81*) overlaid on the 2D-tSNE space. Bars represent log2 (Count + 1).

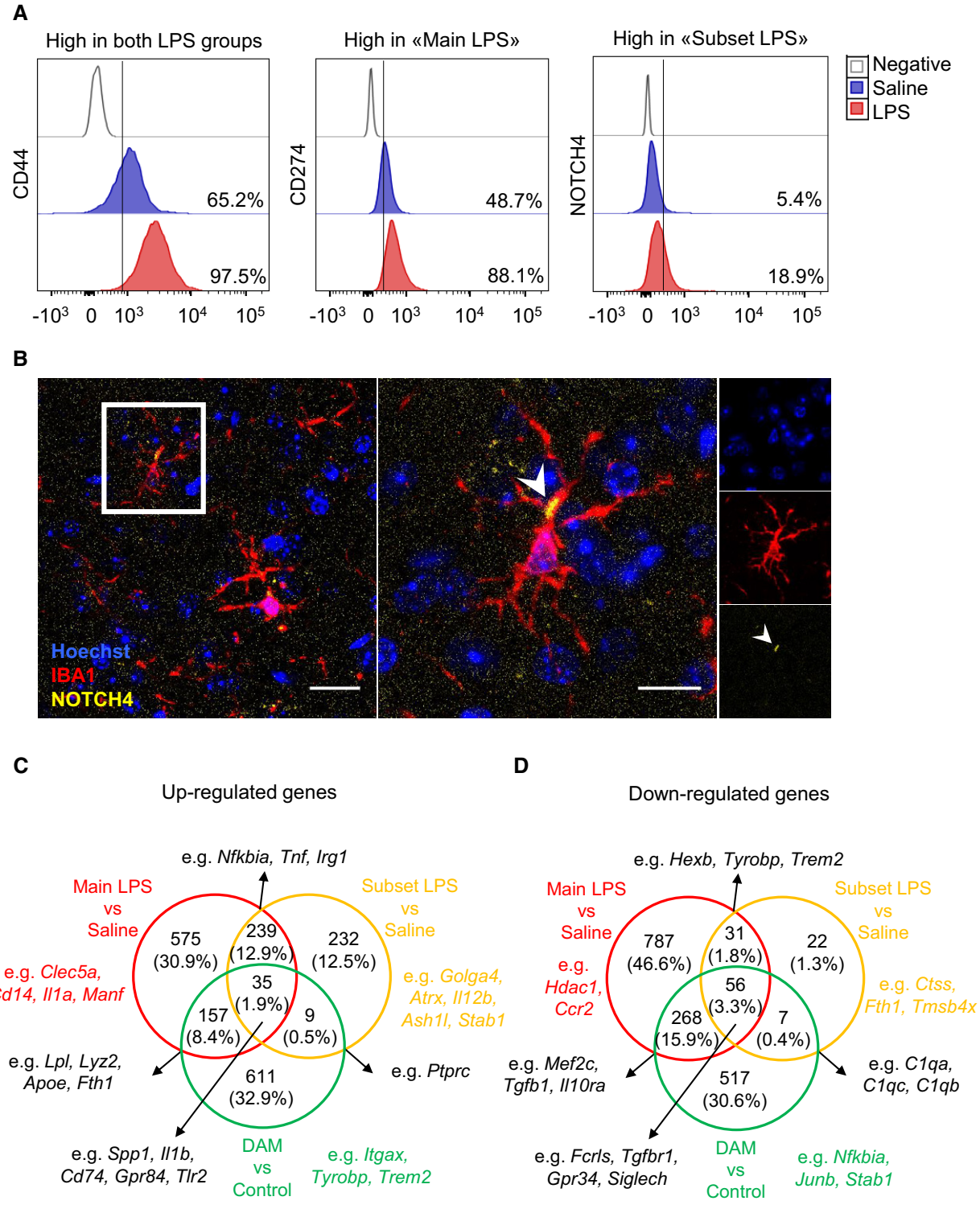


Figure EV5. FACS and immunohistochemistry analyses of "main LPS" and "subset LPS" upregulated genes and further comparison with DAM.

A Quantification of CD44, CD274 and NOTCH4 expression in $CD11b^{+}CD45^{int}$ microglia under homeostatic (in blue) and inflammatory (in red) conditions. Values denote the percentage of cells obtained from one representative experiment.

B Immunohistochemistry for "subset LPS" marker (NOTCH4) in brain sections of tissue isolated from LPS-injected mice after 24 h (IBA1 in red and NOTCH4 in yellow). Cell nuclei were counterstained with Hoechst (in blue). Scale bars: left panel 10 μm, right panel 20 μm. Arrows show NOTCH4 expression in IBA1+ cells.

C, D Venn diagrams showing unique and commonly (C) upregulated and (D) downregulated genes among "main LPS" cluster (red), "subset LPS" (yellow) and DAM (green) (FDR < 0.05).