Expanded View Figures

Figure EV1. SFRP1 enhances glial cell activation.

A On the left, diagram of the observed GFP distribution (green dots) after lentiviral (LV) particles' delivery into the lateral ventricle (*Lu*). The dashed boxes represent areas shown in (B) and (D), 1 points to the wall of the lateral ventricle, and 2 to the Rostral Migratory Stream. Images on the right show examples of immunostaining against GFP protein (GFP signal was no longer visible, unless detected with immunohistochemistry) in the Lv of brains transduced with LV-*IRES-Gfp* or LV-*Sfrp1-IRES-Gfp* 5 months (m) post-infusion. Scale bar: 100 μm.

B Double immunostaining against GFP (green) and GFAP (magenta) of brains transduced with LV-*Sfrp1-IRES-Gfp*, indicating that transduced cells include astrocytes. Arrowheads indicate GFP^+ astrocytes. Scale bar: 50 μm.

C The graphs show the number of Sox9 immunopositive cells, S100β and Cleac7a immune intensity level and CD45 immunopositive area in LV-*Sfrp1-IRES-Gfp*-infected brains normalized against the mean value obtained in LV-*IRES-Gfp*-infected control brains. Dots represent the mean value obtained by the analysis of 6 sections for each biological replica (n = 4–6, mice per group). Error bars represent standard error. Statistical significance: $*P < 0.05$; $**P < 0.01$ by two-sided Student's t-test.

D Coronal sections from LV-*IRES-Gfp*- or LV-*Sfrp1-IRES-Gfp*-infected brains 1 or 5 months (m) post-infusion (PI). Sections were immunostained for CD45. Arrowheads indicate $CD45^{hi}$-positive macrophages or lymphocytes infiltrated in the parenchyma after prolonged LV-*Sfrp1-IRES-Gfp* infection. High-power images 1 and 2 were taken from the regions indicated with grey dotted lines in A. Scale bar: 50 μm.

E Coronal sections from wt and $Sfrp1^{-/-}$ mouse brains three days after infusion of saline or LPS, immunostained for GFAP. The white arrows indicate the injection site; the area boxed with white lines is represented at high power in Fig 2. Scale bar: 100 μm.

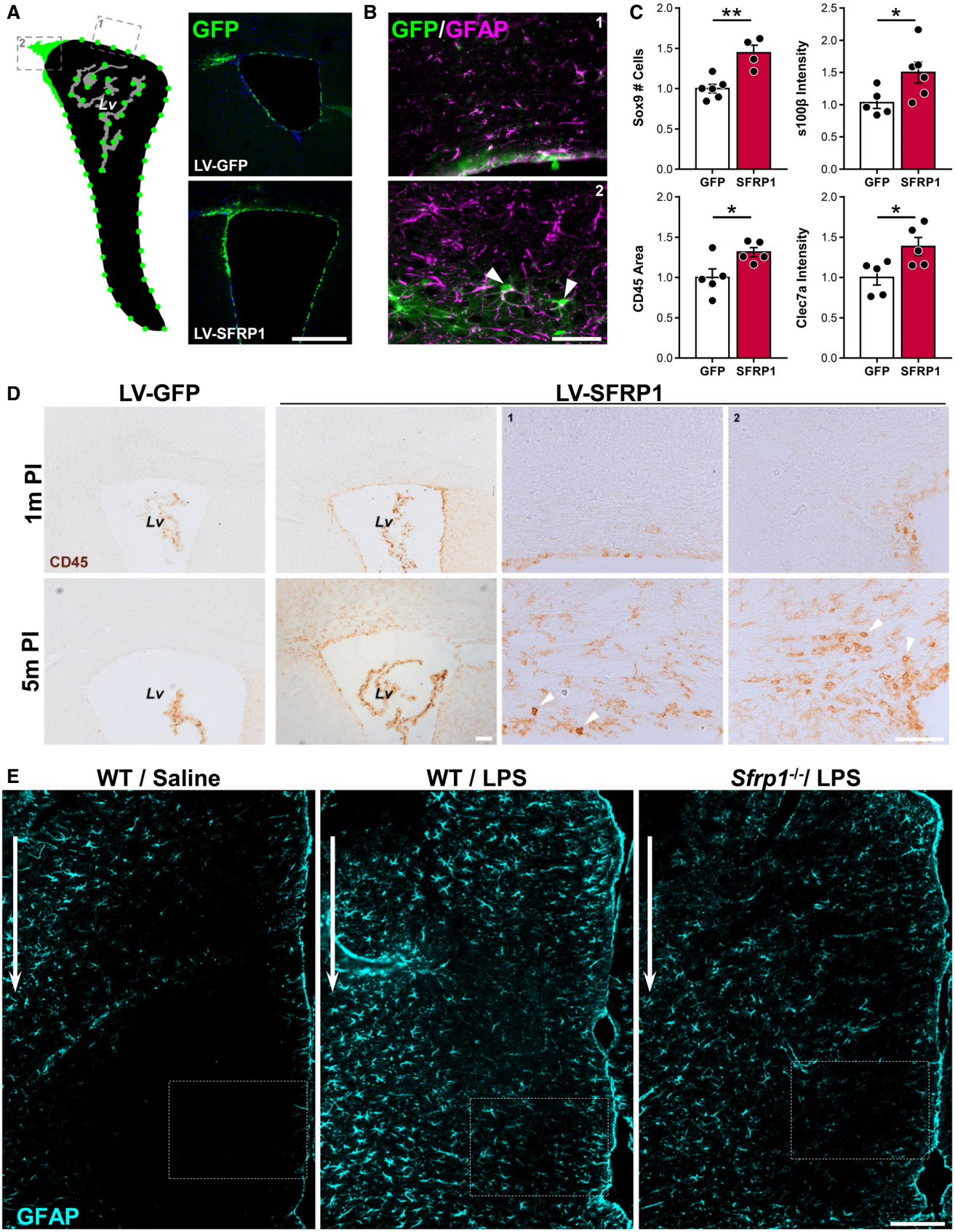
A
GFP
LV-GFP
LV-SFRP1
Lv
B
GFP/GFAP
C
Sox9 # Cells
s100β Intensity
CD45 Area
Clec7a Intensity
GFP
SFRP1
D
LV-GFP
LV-SFRP1
1m PI
5m PI
CD45
E
WT / Saline
WT / LPS
Sfrp1-/-/ LPS
GFAP

Figure EV1.

Figure EV2. SFRP1 enhances microglial cell activation and phagocytosis.

A The graph shows the % of $CD11b^+$ populations present in the cortex of $CX_3CR1^{GFP/+}$ or $CX_3CR1^{GFP/+}$;$Sfrp1^{-/-}$ mice 3 days after saline or LPS intraventricular infusion (n = 5 per genotype and condition). Gating was set for isolating the following myeloid cell populations: $CD11b^+$; $CD45^{lo}$; GFP^+ surveying microglia, $CD11b^+$; $CD45^+$; GFP^+ activated microglia and $CD11b^+$; $CD45^+$; GFP^- infiltrated monocytes. Error bars represent standard error.

B The graph shows the pHrodo mean fluorescent signal present in GFP/$CD45^{hi}$ and GFP/$CD45^{low}$ cell populations isolated from cortex of $CX_3CR1^{GFP/+}$ or $CX_3CR1^{GFP/+}$; $Sfrp1^{-/-}$ mice 3 days after pHrodo intraventricular infusion (n = 4/5 per genotype respectively). Error bars represent standard error. Statistical significance: $^{**}P < 0.01$ by two-way ANOVA followed by Bonferroni's multiple comparisons test.

C Representative cytometric plots of brain cells suspension showing gating of individualized live cells $CD11b^+$; $CX3CR1^+$ recognized as microglial cells for pHrodo-labelled *E. coli* BioParticles quantitation. Comparison of microglial cells exposed (GFP^+; $CD45^{lo}$ and GFP^+; $CD45^+$ or not exposed (Ctrl-) to pHrodo-labelled *E. coli* shows specific pHrodo recognition.

D Representative confocal image of FACS-sorted GFP^+ microglia. Note specific $pHrodo^+$ lysosomal inclusion of *E.coli* particles in phagocyting vs non-phagocyting microglia. Scale bar: 5μm.

E The graphs show the quantification of the normalized total fluorescence from phagocytized pHrodo-labelled *E. coli* BioParticles and percentage of $CD11b^+$ phagocyting cells in the presence of wt or $Sfrp1^{-/-}$ astrocytes (n = 4 cultures per condition). Error bars represent standard error. Statistical significance: Student's t-test.

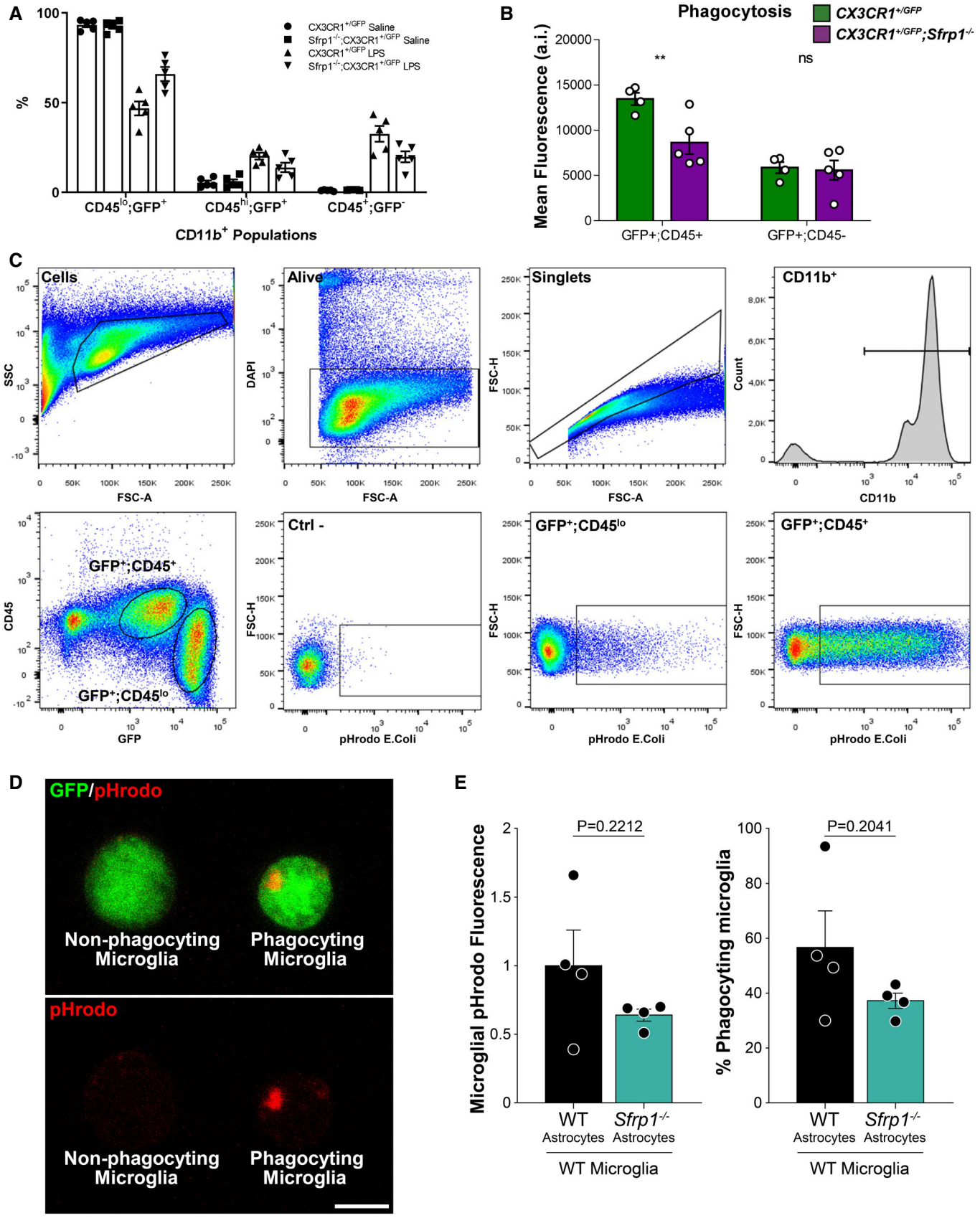
A
100
50
0
%
CX3CR1+/GFP Saline
Sfrp1-/-;CX3CR1+/GFP Saline
CX3CR1+/GFP LPS
Sfrp1-/-;CX3CR1+/GFP LPS
CD45lo;GFP+
CD45hi;GFP+
CD45+;GFP-
CD11b+ Populations
B
Phagocytosis
CX3CR1+/GFP
CX3CR1+/GFP;Sfrp1-/-
Mean Fluorescence (a.i.)
20000
15000
10000
5000
0
**
ns
GFP+;CD45+
GFP+;CD45-
C
Cells
SSC
FSC-A
Alive
DAPI
FSC-A
Singlets
FSC-H
FSC-A
CD11b+
Count
CD11b
GFP+;CD45+
GFP+;CD45lo
CD45
GFP
Ctrl -
FSC-H
pHrodo E.Coli
GFP+;CD45lo
FSC-H
pHrodo E.Coli
GFP+;CD45+
FSC-H
pHrodo E.Coli
D
GFP/pHrodo
Non-phagocyting Microglia
Phagocyting Microglia
pHrodo
Non-phagocyting Microglia
Phagocyting Microglia
E
Microglial pHrodo Fluorescence
P=0.2212
WT Astrocytes
Sfrp1-/- Astrocytes
WT Microglia
% Phagocyting microglia
P=0.2041
WT Astrocytes
Sfrp1-/- Astrocytes
WT Microglia

Figure EV2.

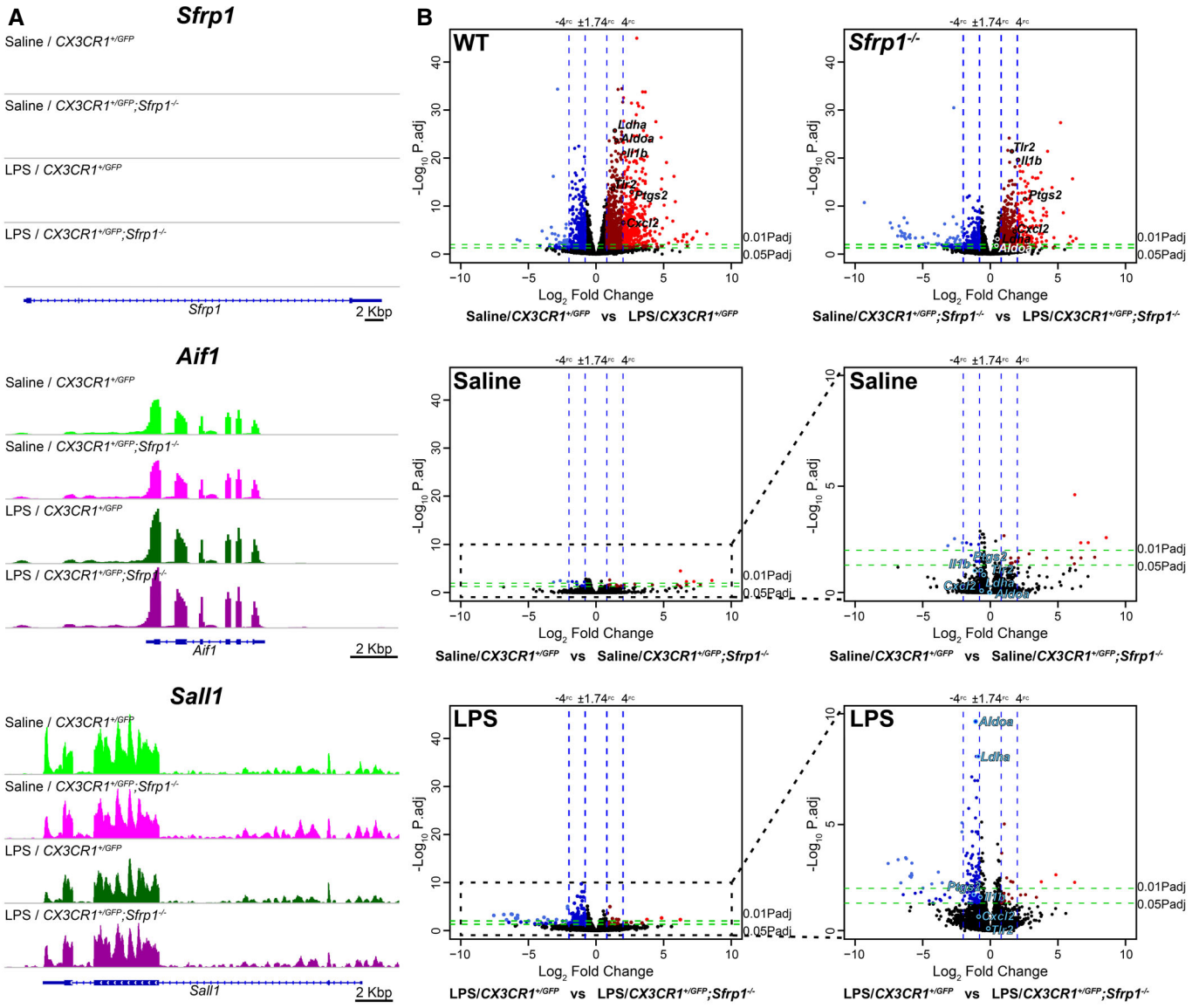

Figure EV3. Non-cell-autonomous effect of *Sfrp1* on microglial response to LPS.

A Integrative Genomics Viewer transcription profile of *Sfrp1* and the microglial specific genes *Aif1* and *Sall1* in microglial cells isolated from saline and LPS treatment of $CX_3CR1^{GFP/+}$ and $CX_3CR1^{GFP/+}$;$Sfrp1^{-/-}$ mice. Scale bar: 2 Kbp.

B Volcano plots of differential gene expression from $CX_3CR1^{GFP/+}$ or $CX_3CR1^{GFP/+}$;$Sfrp1^{-/-}$ microglial cell in response to saline or LPS. Data are represented as log2 fold change vs -Log10 adjusted *P*-value by the Wald test corrected for multiple comparisons by the Benjamini and Hochberg method. Note that expression variations associated with genotype in saline-treated animals are minimal. The effect of the genotype is evident only after LPS treatment. Genes with an expression change higher than 75% and an adjusted *P*-value < 0.05 are highlighted in dark red (+FC) and dark blue (−FC). Genes with an expression change higher than 400% and an adjusted *P*-value < 0.01 are highlighted in light red (+FC) and light blue (−FC).

Figure EV4. SFRP1 microglial inflammatory response depends on HIF factors.

A Representation of the four upregulated k-means unsupervised clusters of upregulated genes in response to LPS in $CX_3CR1^{GFP/+}$ or $CX_3CR1^{GFP/+}$; $Sfrp1^{-/-}$ microglial cells. Eight different clusters were generated by Z-score covariation. The clusters are indicated in the heatmap of Fig 5F. Dotted lines indicate the mean variation of the genes included in each cluster.

B Gene ontology enrichment analysis of different clusters and GO biological process annotated is represented by fold enrichment and colour-coded by adjusted *P*-value of enrichment by the hypergeometric test corrected for multiple comparisons by the Benjamini and Hochberg method, and number of genes of each cluster in that term.

C Regulatory element analysis of different clusters. Specifically enriched transcription factors are represented by fold enrichment relative to the overall transcripts present in the microglia transcriptome. Coloured by log odds detection threshold by the hypergeometric test corrected for multiple comparisons by the Benjamini and Hochberg method, and the percentage of total targets for each transcription factor present in the cluster.

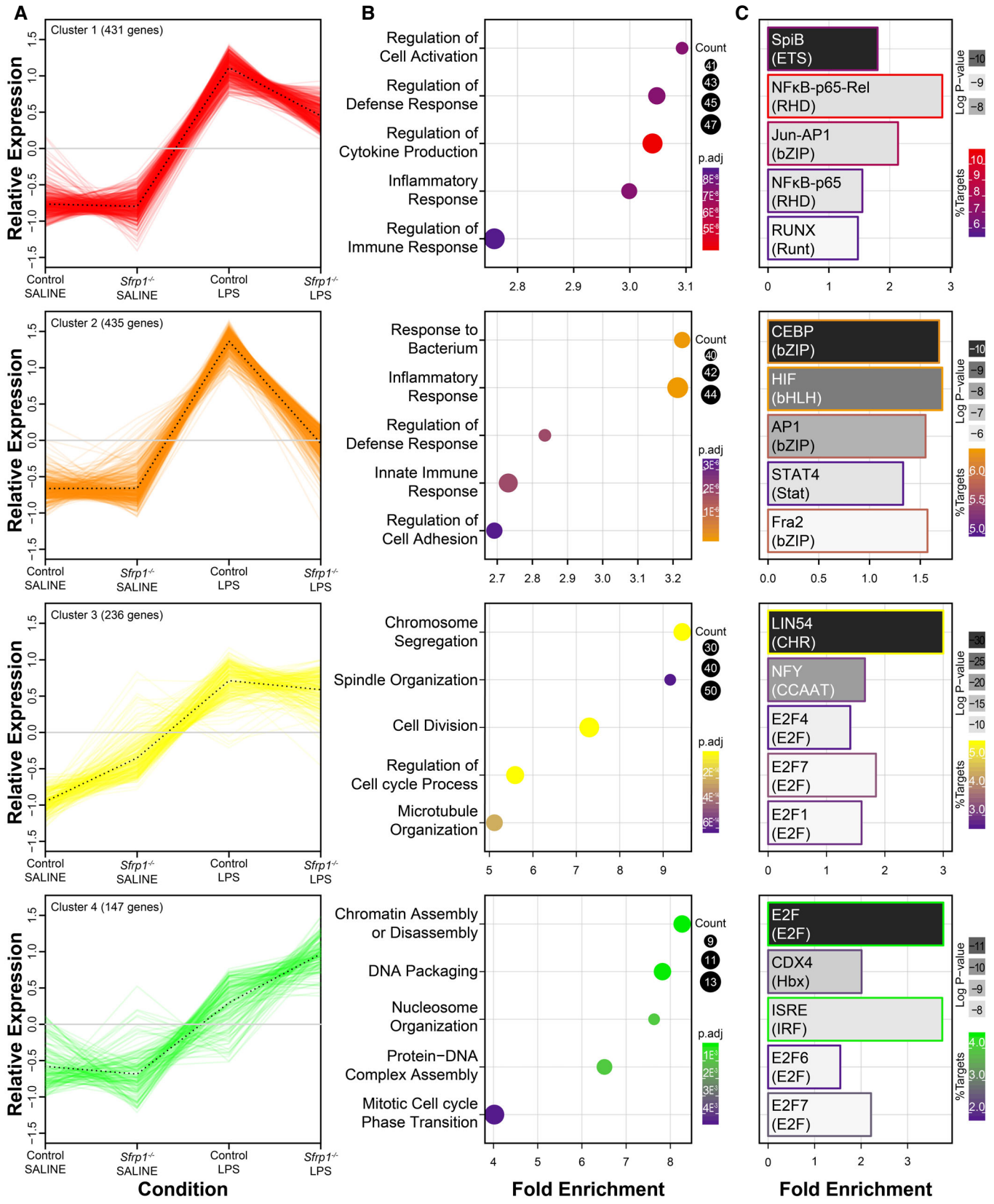

Figure EV4.