

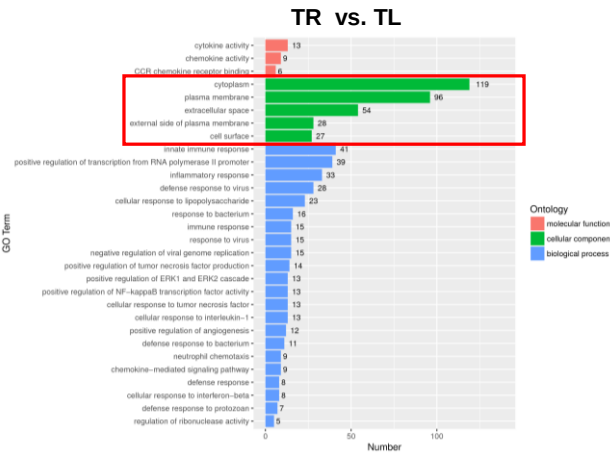
Supplementary materials

Hypoxia Inducible Factor-1 α regulates microglial innate immune memory and the pathology of Parkinson's disease

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Supplementary figures and legends

A



B

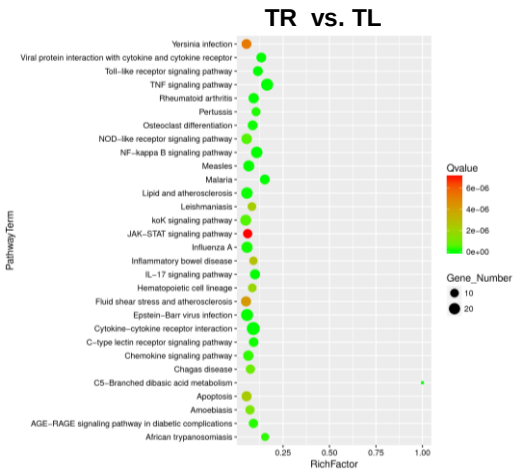


Fig. S1. RNA-seq analysis of the striatum between mice treated with 2xLPS or 4xLPS. TR, Training; TL, tolerance.

A. Gene ontology (GO) enrichment analysis of differential expressed genes. **B.** KEGG enrichment analysis of the enriched pathways.

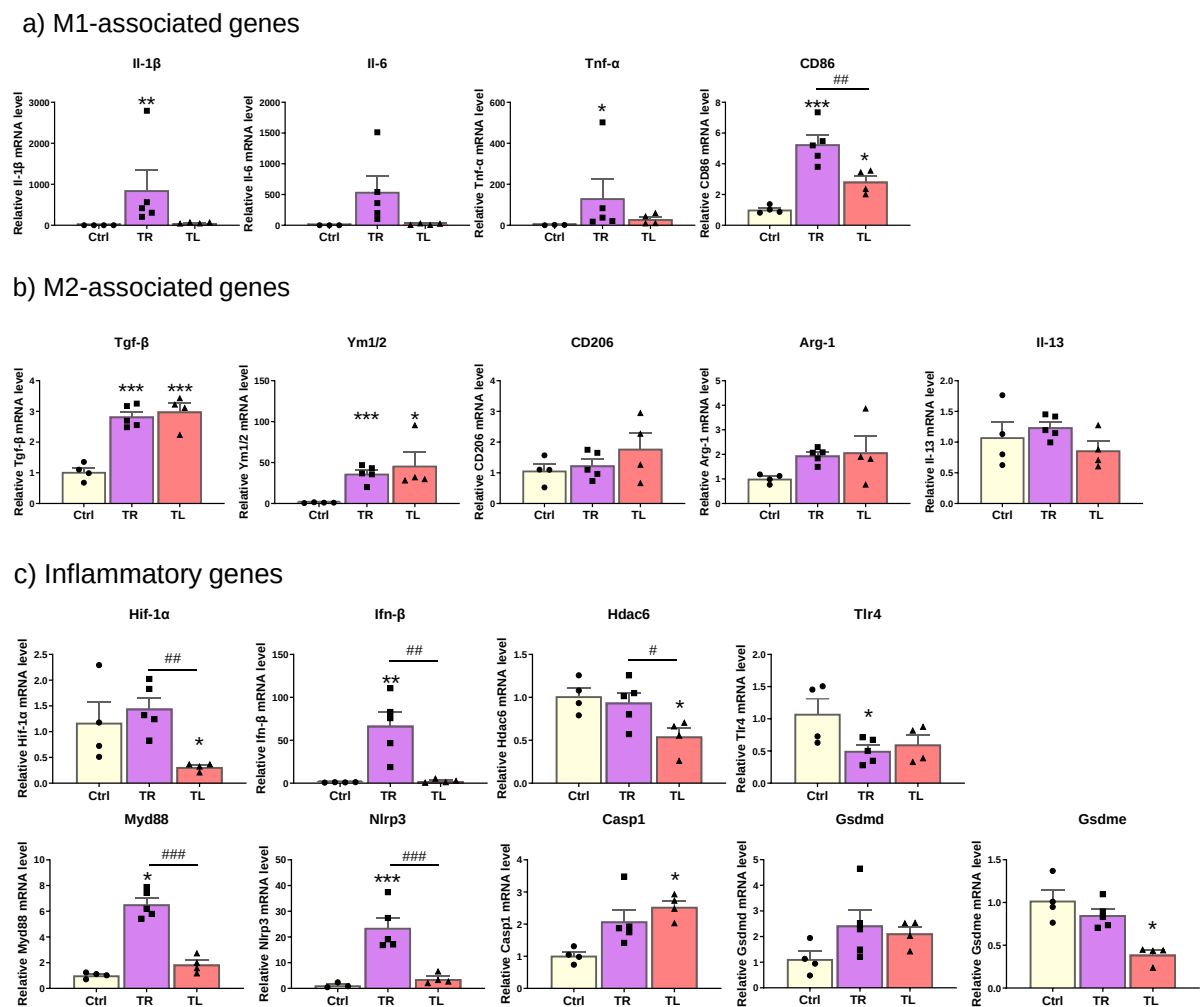


Fig. S2. Statistical graph of transcription of M1/M2-associated genes and inflammatory genes in the striatum of mice 3 hours after saline or LPS treatment. n = 4-5/group. Differences were analyzed by one-way ANOVA followed by LSD multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ vs control groups. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.**

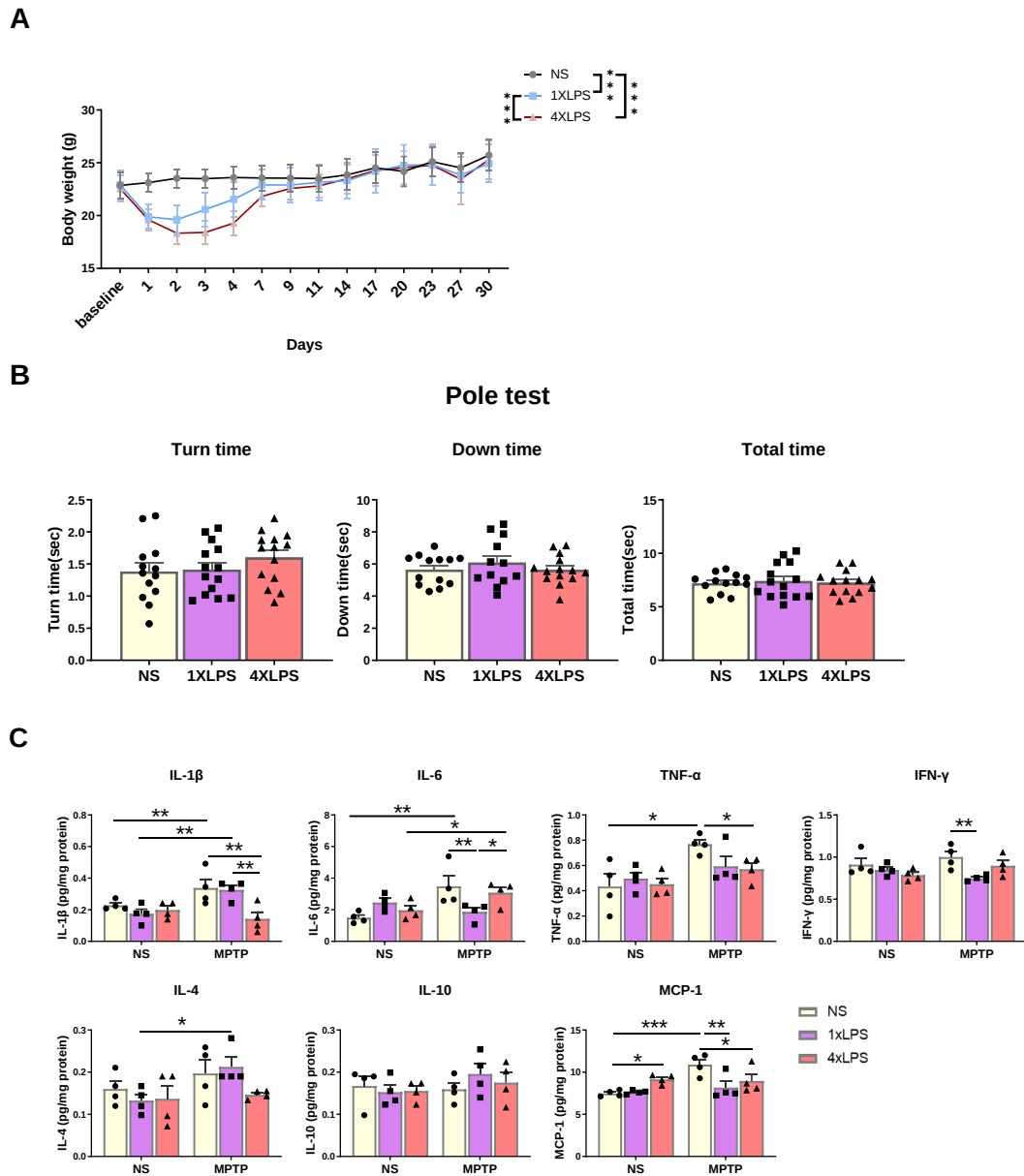


Fig. S3. Analysis of body weight changes, Pole test results, and cytokine protein levels in Mice following LPS Injections (A, B) and MPTP Treatment (C).

A. The changes of body weight during and after LPS injections. $n = 11-13$ /group. Differences were analyzed by two-way ANOVA followed by LSD multiple comparison tests. *** $p < 0.001$.

B. The Pole test results in mice 4 weeks after low-dose(s) of LPS administration.

Time to turn around, climb down and total time were showed. $n = 13\text{--}14$ /group.

C. Statistical graph of protein levels based on Luminex assay of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α and IFN- γ), anti-inflammatory cytokines (IL-4 and IL-10) and chemokine (MCP-1) in mouse striatum 3 days after saline or MPTP treatment. $n = 4$ /group. Differences were analyzed by two-way ANOVA followed by LSD multiple comparison tests. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

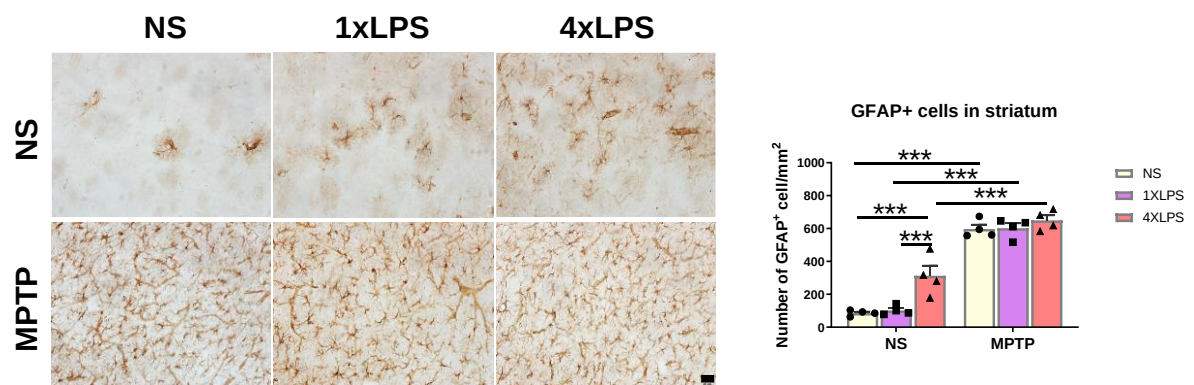


Fig. S4. Assessments of GFAP⁺ cells in the striatum of mice by Immunohistochemistry staining and quantification of GFAP⁺ astrocytes.

Scale bar, 20 μm . $n = 4$ /group. Differences were analyzed by two-way ANOVA followed by LSD multiple comparison tests. $***p < 0.001$.

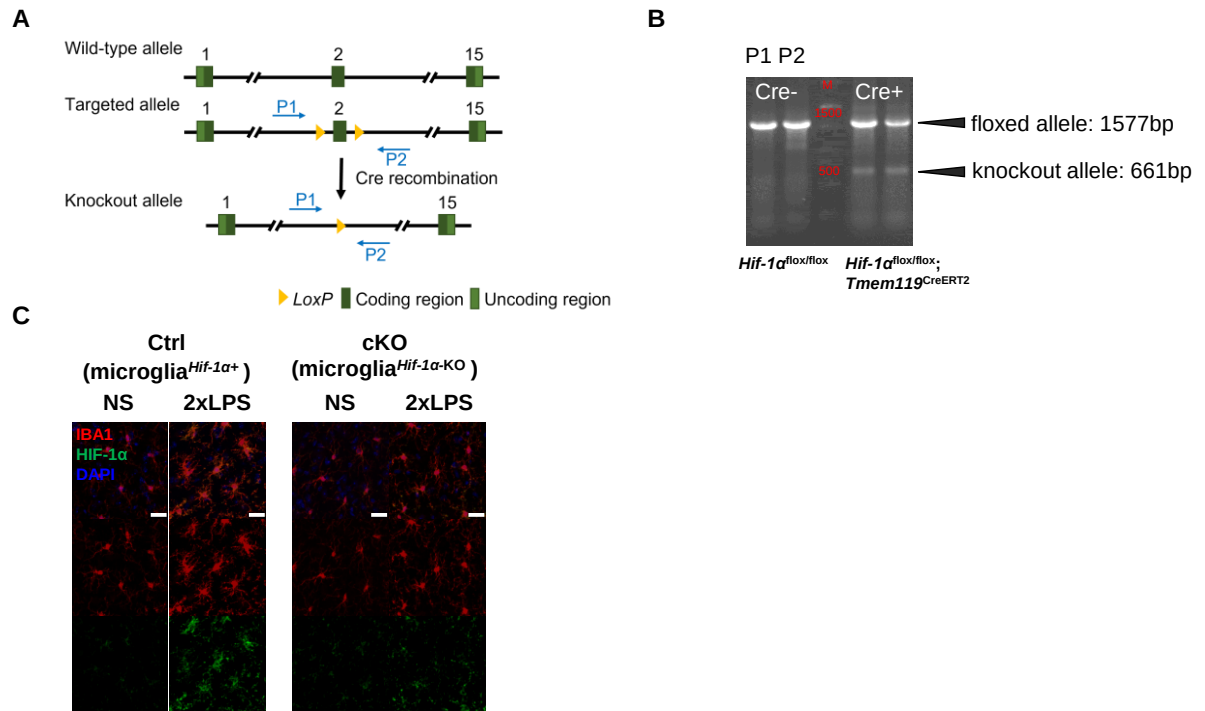


Fig. S5. The efficiency of *Hif-1α* gene deletion in microglia of the *Hif-1α*^{flox/flox}; *Tmem119*^{CreERT2} mice.

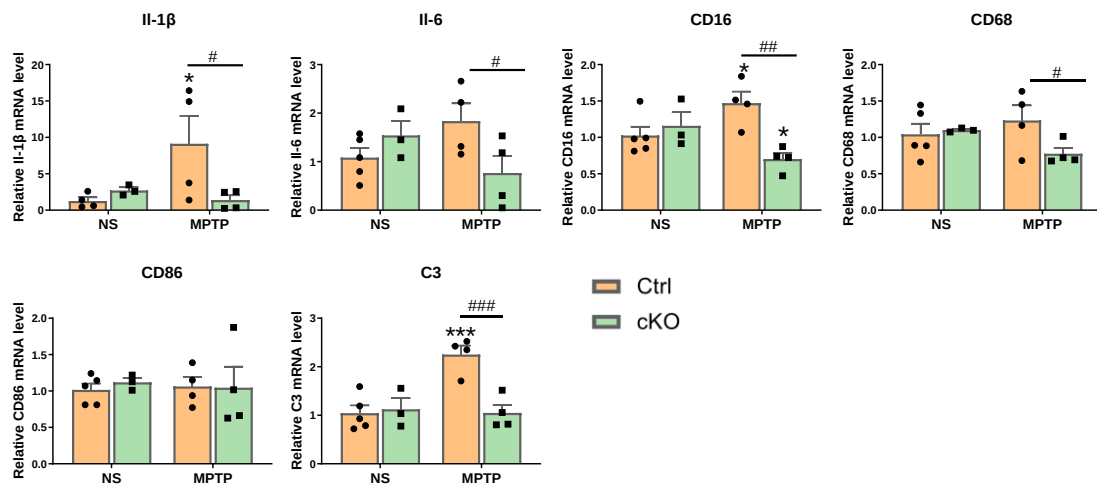
A. Targeting strategy to generate *Hif-1α*^{flox/flox} mice with *LoxP*-flanked exon 2 of mouse *Hif-1α* gene. In the presence of Cre, the *LoxP* sites were recombined and the floxed region of *Hif-1α* was deleted. P1 and P2, genotyping primers.

B. *Hif-1α*^{flox/flox} mice with or without Cre transgene (Cre⁺ or Cre⁻) 2 weeks after the induction of tamoxifen were genotyped by PCR. The PCR product from the floxed allele had a PCR product of 1577 bp, whereas the knockout allele had a PCR product of 661 bp. The DNA was extracted from mouse brain.

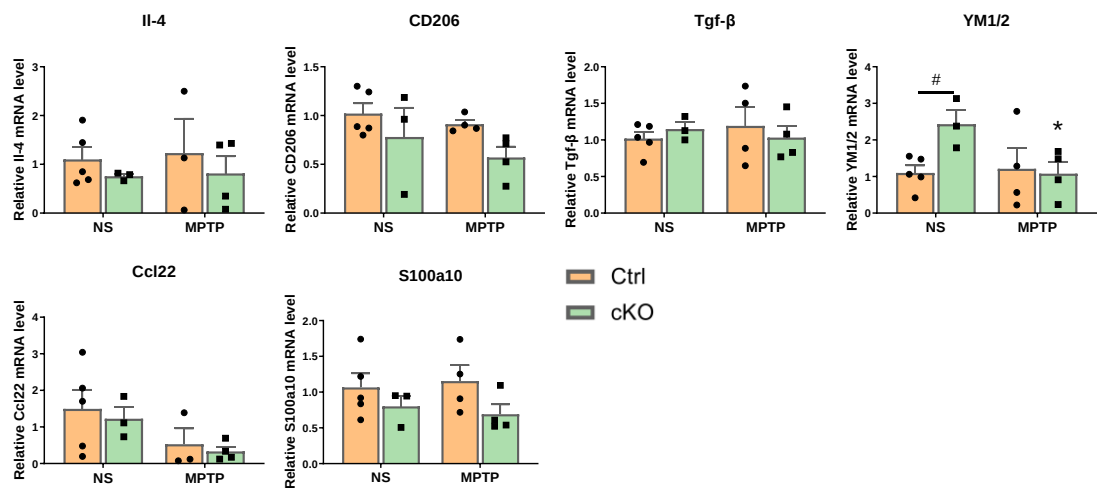
C. The expression of HIF-1α in microglia was assessed by IBA1/HIF-1α double-immunostaining in the brain slices of *Hif-1α*^{flox/flox} and *Hif1α*^{flox/flox}; *Tmem119*^{CreERT2} mice treated with NS or 2xLPS for 3 hours. Representative images of IBA1 (red) and HIF-1α (green) immunostaining in the striatum are

shown. Scale bar, 25 μ m.

a) pro-inflammatory markers



b) anti-inflammatory markers



c) immune response genes

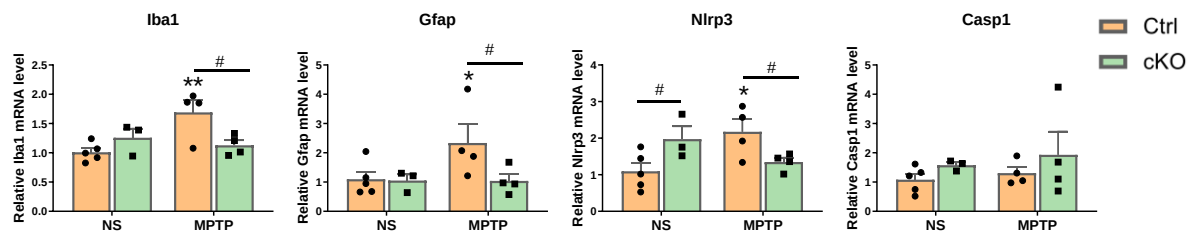


Fig. S6. Statistical graph of transcription of pro-inflammatory markers (*IL-1 β* , *IL-6*, *CD16*, *CD68*, *CD86* and *C3*) anti-inflammatory markers (*IL-4*, *CD206*, *Tgf- β* , *Ym1/2*, *Ccl22*, *S100a10*) and immune response genes (*Iba1*, *Gfap*, *Nlrp3*, *Casp1*) in the striatum of mice 7 days after NS or MPTP

administration. n = 3-5/group. Differences were analyzed by two-way ANOVA followed by LSD multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs normal saline (NS) control. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs Cre-negative control.

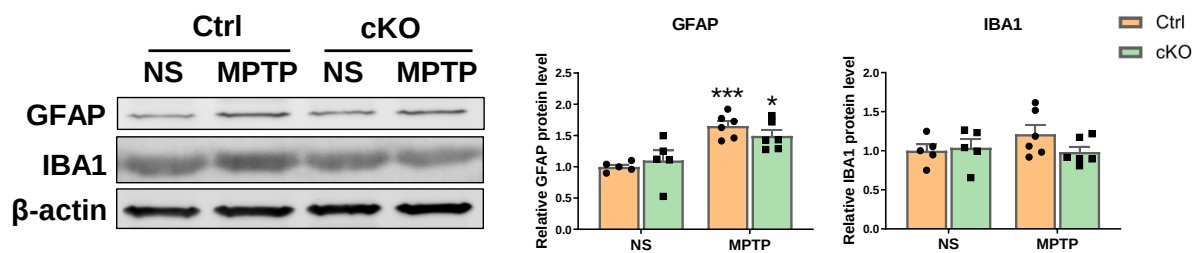


Fig. S7. Protein levels of GFAP and IBA1 in mouse striatum detected by Western Blot. n = 5–6 /group. Differences were analyzed by two-way ANOVA followed by LSD multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs normal saline (NS) control.

Supplementary table

Table S1. Primers used in the quantitative PCR.

Primers for qPCR	Sequence (5'→3')
mArg-1-F	AGCCAATGAAGAGCTGGCTGGT
mArg-1-R	AACTGCCAGACTGTGGTCTCCA
mC3-F	CAAAGGACCTGGAAGTCTGG
mC3-R	GGTCAGGCAGTCTTCTTCGG
mCasp1-F	AGGACATCCTTCATCCTCAGAAAC
mCasp1-R	GCAAGACGTGTACGAGTGGT
mCcl22-F	CTGATGCAGGTCCCTATGGT

mCcl22-R	GCAGGATTTTGAGGTCCAGA
mCD16-F	TTTGGACACCCAGATGTTTCAG
mCD16-R	GTCTTCCTTGAGCACCTGGATC
mCD206-F	ATGGATTGCCCTGAACAGCA
mCD206-R	CTCGTCAGCACCCCAGTTAG
mCD68-F	ACTTCGGGCCATGTTTCTCT
mCD68-R	GGGGCTGGTAGGTTGATTGT
mCD86-F	AGAAGCCGAATCAGCCTAGC
mCD86-R	CACTCTGCATTTGGTTTTGCTG
mGfap-F	GAGAACAACCTGGCTGCGTA
mGfap-R	CGGAGTTCTCGAACTTCCTCC
mGsdmd-F	GCTCAGTCTCCTGTCAGATGG
mGsdmd-R	GCTGGGCTGGTCCTGTAAAA
mGsdme-F	TTCCATCCCTTTGCGGTGTT
mGsdme-R	TACAGCCTGTGAGGACCAGA
mHdac6-R	CACCGCATTTCAGAGGGTTCT
mHdac6-R	CCTTAAGGTGGGGCCAGAAG
mHif-1α-F	CAAGCAGCAGGAATTGGAACAT
mHif-1α-R	GCTCCGTTCCATTCTGTTCAC
mlba1-F	GGCTGGAGGGGATCAACAAG
mlba1-R	CAAACCTCCATGTACTTCACCTTGA
mlfn-β-F	GTCCGAGCAGAGATCTTCAGG
mlfn-β-R	ACTACCAGTCCCAGAGTCCG
mll-10-F	AGCCGGGAAGACAATAACTG
mll-10-R	GGAGTCGGTTAGCAGTATGTTG
mll-13-F	TGAGGAGCTGAGCAACATCAC
mll-13-R	CCAGGGCTACACAGAACCCG
mll-1β-F	CTCCATGAGCTTTGTACAAGG
mll-1β-R	TGCTGATGTACCAGTTGGGG

mIl-4-F	TGCCTGGATTCATCGATAAGCTG
mIl-4-R	TTGCATGATGCTCTTTAGGCTT
mIl-6-F	CATAGCTACCTGGAGTACATGA
mIl-6-R	CATTCATATTGTCAGTTCTTCG
mMyd88-F	CCGCCTATCGCTGTTCTTGA
mMyd88-R	CTGCCAGGCATCCAACAAAC
mNlrp3-F	TGGGCAACAATGATCTTGGC
mNlrp3-R	GTACATTTCACCCAACTGTAGGC
ms100a10-F	GGTCTTCGGCACTAGCCTC
ms100a10-R	AGTCTGTGCGAAACCTGGGC
mTgf-β-F	TGCGCTTGCAGAGATTAAAA
mTgf-β-R	CGTCAAAAGACAGCCACTCA
mTlr4-F	CTTGAATCCCTGCATAGAGGTAG
mTlr4-R	TGTCATCAGGGACTTTGCTGAG
mTnf-α-F	GACCCTCACACTCAGATCATCTTCT-
mTnf-α-R	CCTCCACTTGGTGGTTTGCT
mYm1/2-F	CAGGGTAATGAGTGGGTTGG
mYm1/2-R	CACGGCACCTCCTAAATTGT
mβ-actin-F	CAGGATGCAGAAGGAGATTAC
mβ-actin-R	AACGCAGCTCAGTAACAGTC

Note: M stands for mouse