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Supplementary Materials for

Microglial Ffar4 deficiency promotes cognitive impairment in the context of metabolic syndrome

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Supplementary Figures

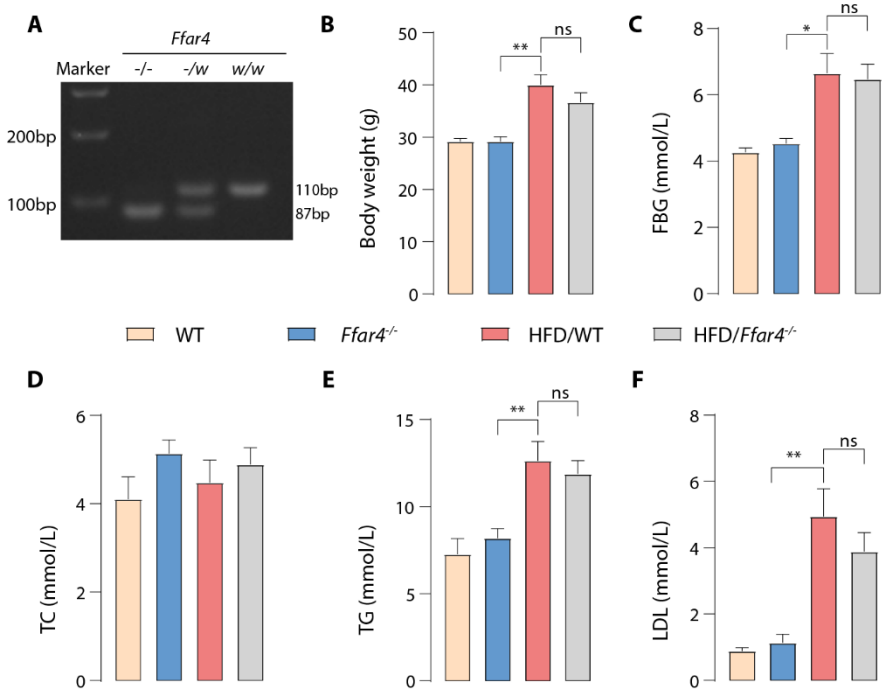

Fig. S1. Effects of *Ffar4* knockout on peripheral metabolic parameters in HFD-fed mice. (**A**) Genotyping primers amplify a 110 bp band in wild-type and an 87 bp band in knockout mice by PCR. (**B**) Body weight. (**C**) Fasting blood glucose. (**D**) Serum TC, (**E**) Serum TG, (**F**) Serum LDL. Data are presented as the mean ± SEM (n = 7-8 per group). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Detailed statistical analyses are presented in Method section.

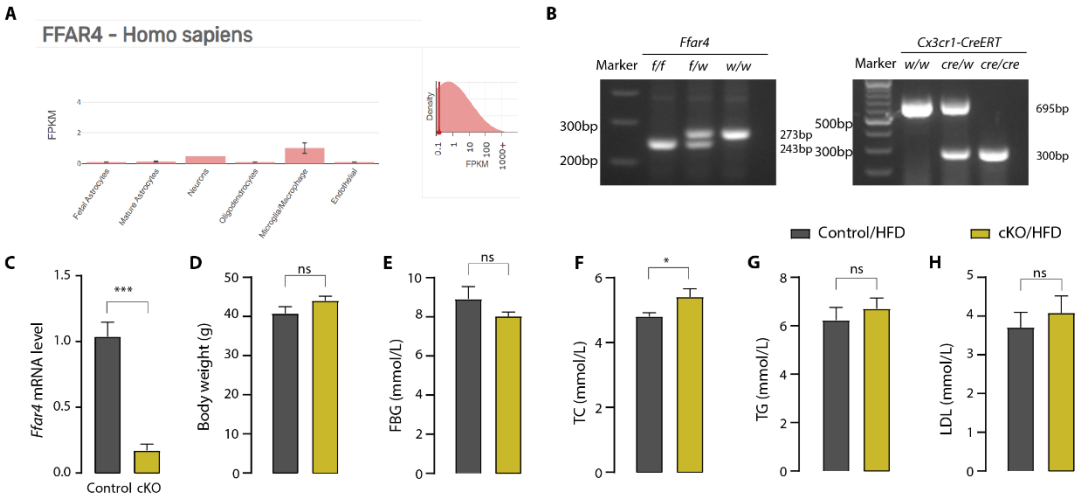

Fig. S2. Effects of microglial *Ffar4* knockout on peripheral metabolic parameters in HFD-fed mice. (**A**) *Ffar4* expression in the human brain (https://brainrnaseq.org/). (**B**) Genotyping primer amplification results: a 243 bp band in WT and a 273 bp band in *fl/fl* mice; a 300 bp band in WT and a 695 bp band in *cre/cre* mice. (**C**) Transcriptional levels of *Ffar4* in isolated microglial cells from cKO and littermate control mice. (**D**) Body weight. (**E**) Fasting blood glucose (FBG). (**F**) Serum TC, (**G**) serum TG, and (**H**) serum LDL. Data are presented as the mean ± SEM (n = 6-8 per group). **P* < 0.05, ****P* < 0.001. Detailed statistical analyses are presented in Method section.

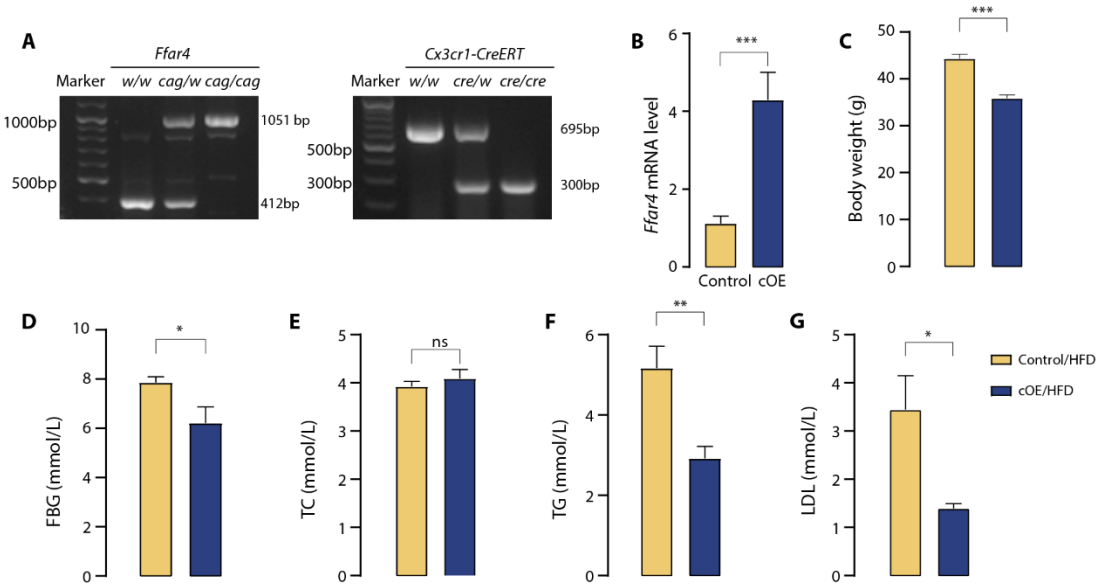

Fig. S3. Effects of microglial *Ffar4* overexpression on peripheral metabolic parameters in HFD-fed mice. (**A**) Genotyping primer amplification results: a 412 bp band in wild-type and a 1051 bp band in *cag/cag* mice; a 300 bp band in WT and a 695 bp band in *cre/cre* mice. (**B**) Transcriptional levels of *Ffar4* in isolated microglial cells from cOE and littermate control mice. (**C**) Body weight. (**D**) FBG. (**E**) Serum TC, (**F**) serum TG, and (**G**) serum LDL. Data are presented as the mean ± SEM (n = 6-8 per group). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Detailed statistical analyses are presented in Method section.

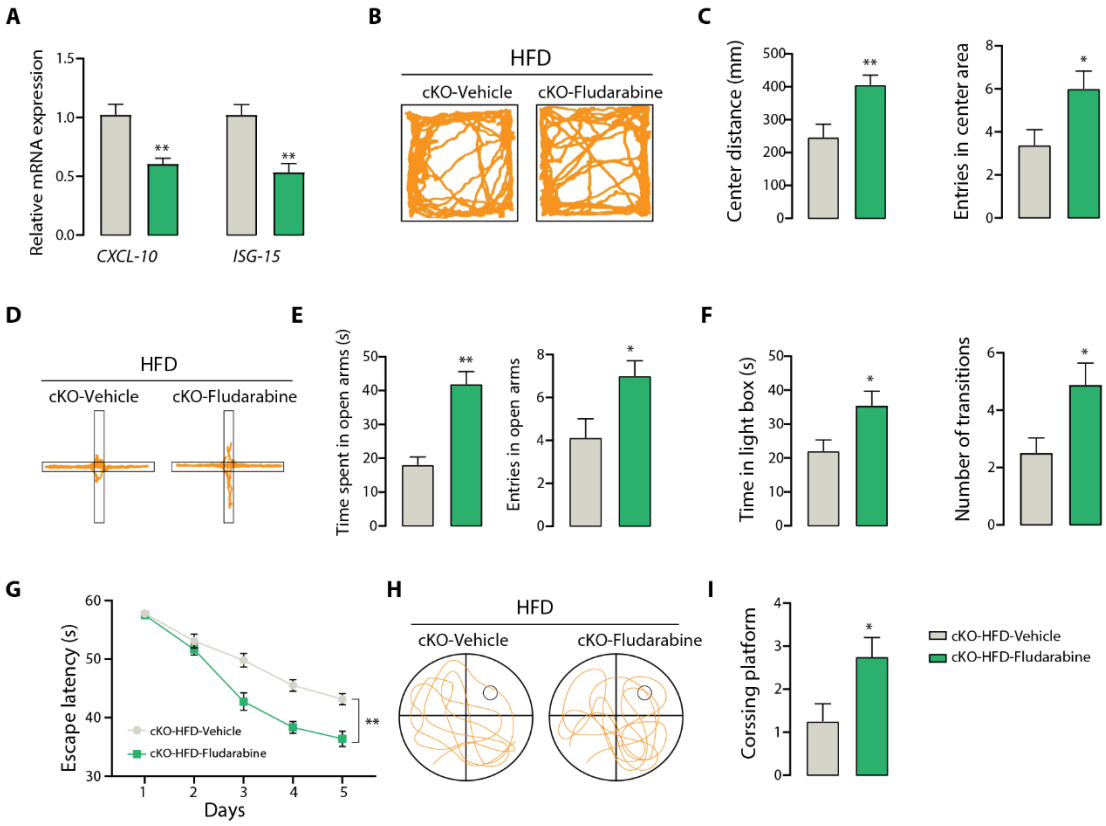

Fig. S4. Blocking type I interferon signaling improved *Ffar4* deletion induced cognitive impairment and anxiety in HFD-fed mice. (**A**) qPCR assay to test the expression levels of ISG-related gene (*CXCL-10*, *ISG-15*) expression in isolated microglial cells from cKO-HFD-Fludarabine and cKO-HFD-Vehicle mice (n = 6 per group). (**B**) Representative trajectory of mice in the OFT. (**C**) The center distance and entries into the center area traveled in the open field for cKO-HFD-Fludarabine and cKO-HFD-Vehicle mice (n=8 per group). (**D**) Representative trajectory of mice in the EPM. (**E**) The time spent in the open zones of the mazes and entries into the open area. (**F**) The time in light box and number of transitions in the LDB. MWM analysis shows escape latency (**G**) to target in the invisible platform trials. Representative trajectory of mice in the probe trials (**H**) and target cross number (**I**) in the MWM test. Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01. Detailed statistical analyses are presented in Method section.

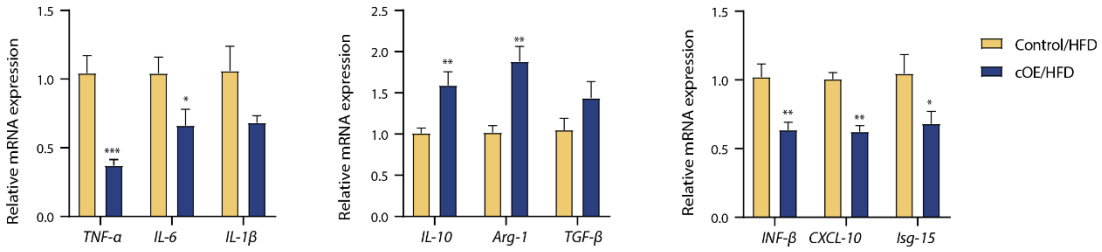

Fig. S5. Effects of microglial *Ffar4* overexpression on inflammatory response in HFD-fed mice. qPCR assay to test the expression levels of pro-, anti-inflammatory cytokines, and ISG-related gene expression in isolated microglial cells from cOE/HFD and Control/HFD mic (n = 6 per group). Data are presented as the mean ± SEM (n = 6 per group). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Detailed statistical analyses are presented in Method section.

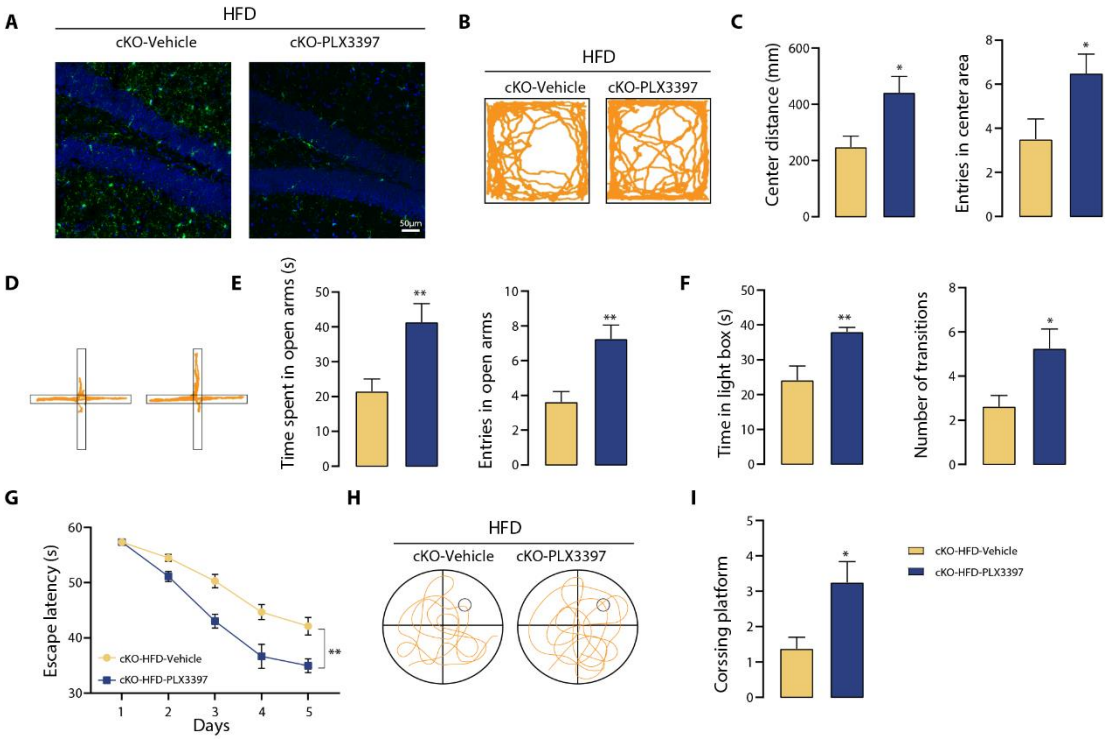

Fig. S6. PLX3397 treatment rescued *Ffar4* deletion induced cognitive impairment and anxiety in HFD-fed mice. (**A**) Representative images of Iba-1 immunostaining (green) in the hippocampus of mice. Scale bar = 50 μm. (**B**) Representative trajectory of mice in the OFT. (**C**) The center distance and entries into the center area traveled in the open field for cKO-HFD-PLX3397 and cKO-HFD-Vehicle mice (n=8 per group). (**D**) Representative trajectory of mice in the EPM. (**E**) The time spent in the open zones of the mazes and entries into the open area. (**F**) The time in light box and number of transitions in the LDB. MWM analysis shows escape latency (**G**) to target in the invisible platform trials. Representative trajectory of mice in the probe trials (**H**) and target cross number (**I**) in the MWM test. Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01. Detailed statistical analyses are presented in Method section.

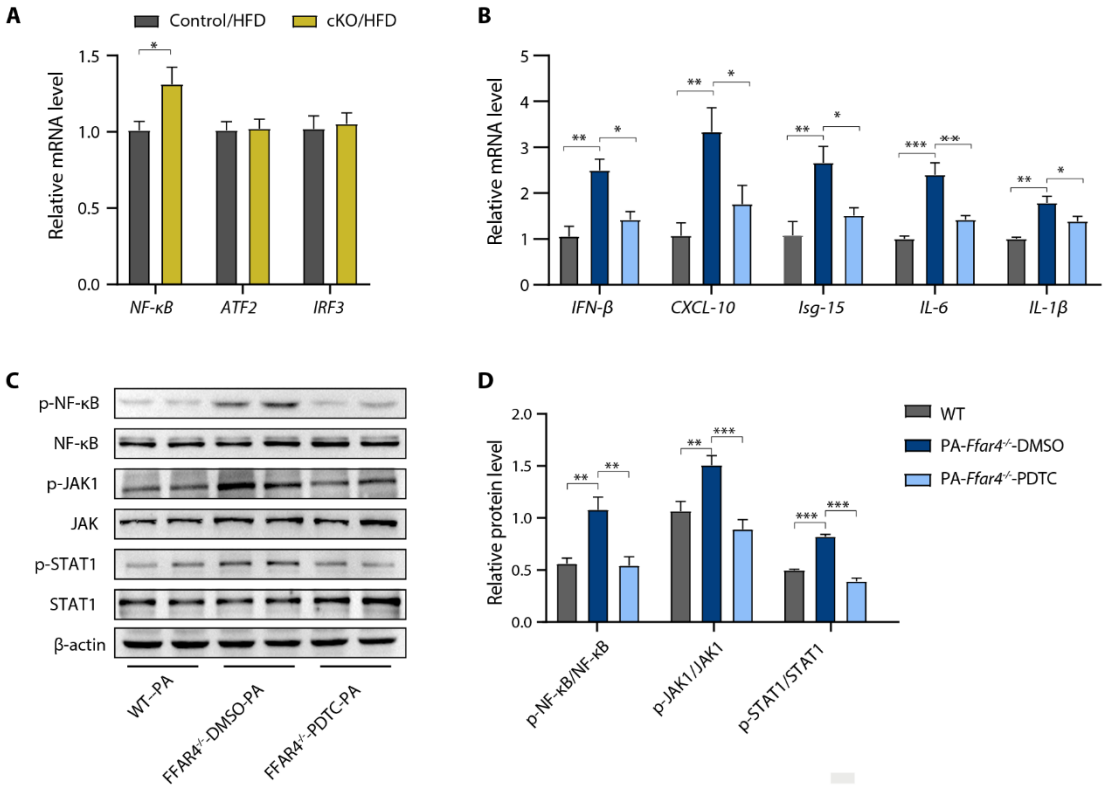

Fig. S7. Effects of *Ffar4* knockout on NF-κB activation. (**A**) qPCR assay to test the expression levels of *IRF3*, *ATF2*, and *NF-κB* in isolated microglial cells from control/HFD and cKO/HFD mice (n = 6 per group). (**B**) qPCR assay to test the expression levels of *IL-1β*, *IL-6*, *CXCL-10*, *IFN-β*, and *Isg-15* with or without PDTC (10 μM) for 4 h and then palmitate (200 μM) incubation for 24 h in primary microglial cell cultures of WT and *Ffar4* knockout mice (n = 3). (**C**) Immunoblotting and (**D**) statistical analysis of NF-κB, p-NF-κB, JAK1, p-JAK1, STAT1, and p-STAT1 levels with or without PDTC (10 μM) for 4 h and then palmitate (200 μM) incubation for 24 h in primary microglial cell cultures of WT and *Ffar4* knockout mice (n = 3). Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Detailed statistical analyses are presented in Method section.

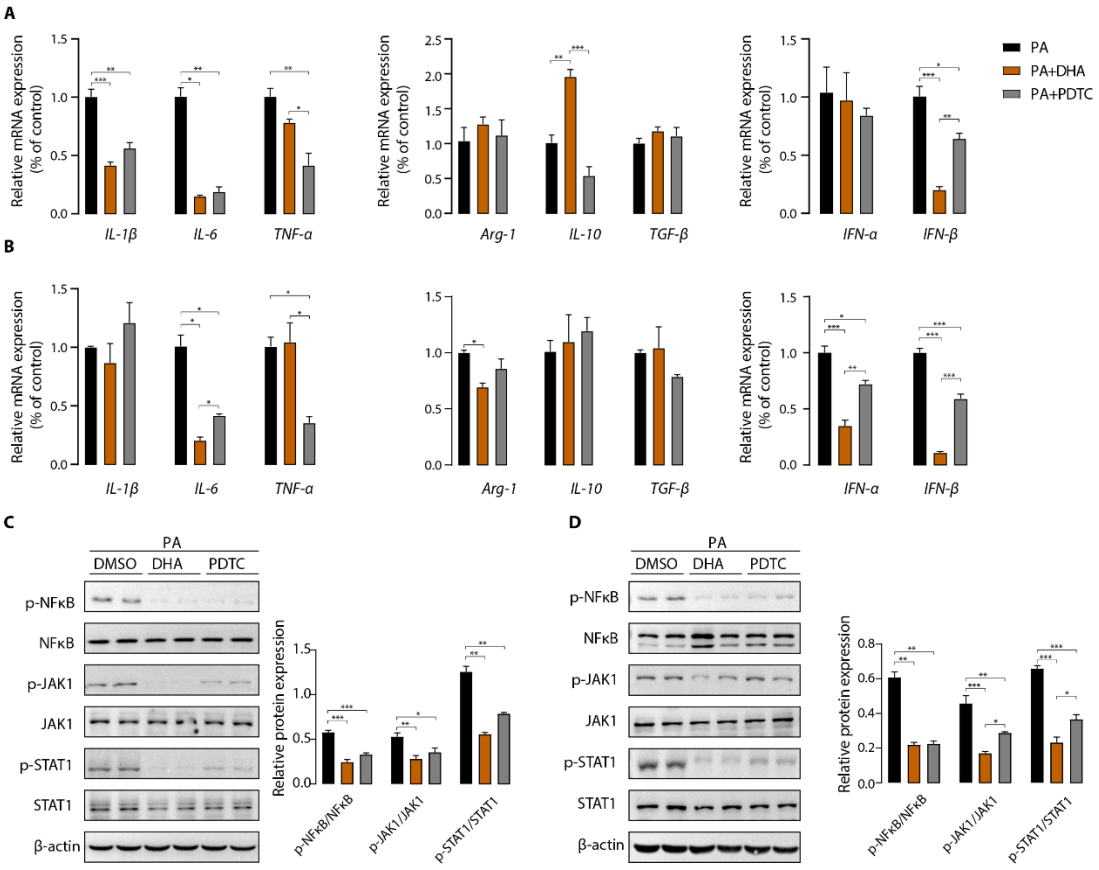

Fig. S8. Combination therapy with NF-κB and Ffar4 reduces the microglial inflammatory response. (**A**) Primary microglial and (**B**) BV2 cells were pretreated with PDTC (10 μM), TUG891(10 μM), or DHA (30 μM) and then incubated with palmitic acid (200 μM) for 12 h. qPCR assay to test the expression levels of pro- and anti-inflammatory cytokines, *IFN-α* and *IFN-β* (n = 3 per group). (**C**) Immunoblotting analysis of NF-κB, p-NF-κB, JAK1, p-JAK1, STAT1, and p-STAT1 expression in (**C**) primary microglia and (**D**) BV2 cells. Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Detailed statistical analyses are presented in Method section.

Table S1: The information of Primers.

Primers	Sequence
Mouse *IL-1β*	Forward: CTGAACTCAACTGTGAAATGC
	Reverse: TGATGTGCTGCTGCGAGA
Mouse *TGFβ*	Forward: TAGCAACAATTCCTGGCGTTAC
	Reverse: TGTATTCCGTCTCCTTGGTTCA
Mouse *Arg-1*	Forward: AAGCCAAGGTTAAAGCCACT
	Reverse: CGATTCACCTGAGCTTTGAT
Human *Ffar4*	Forward: ACACGCTTGAAGGGAGAGTG
	Reverse: CTCTGGCCTCAACTGACAGG
Mouse *Ffar4*	Forward: TGGCCATCCCTTTTCTTCTGG
	Reverse: AAATGGCTCCCTTCTCTGGAA
Mouse *CXCL10*	Forward: TCAGGCTCGTCAGTTCTAAGT
	Reverse: GATGGTGGTTAAGTTCGTCCTT
Mouse *Isg15*	Forward: ACAGCAACATCTATGAGGTCTT
	Reverse: CTGGTCTTCGTGGACTTGT
Mouse *IFN-α*	Forward: CAACAGATCCAGAAGGCTCAAG
	Reverse: GCATTCCAAGTAGCAGACAAGT
Mouse *IFN-β*	Forward: TCCTCAACTGCTCTCCACTT
	Reverse: ACCACCATCCAGGCGTAG
Mouse *IL-10*	Forward: CCCTTTGCTATGGTGTCCTT
	Reverse: TGGTTTCTCTTCCCAAGACC
Mouse *β-actin*	Forward: TGTTACCAACTGGGACGACA
	Reverse: CTGGGTCATCTTTTCACGGT
Mouse *IL-6*	Forward: CTCTGCAAGAGACTTCCATCCAGT
	Reverse: GAAGTAGGGAAGGCCGTGG
Mouse *TNF-α*	Forward: AGGGTCTGGGCCATAGAACT
	Reverse: CCACCACGCTCTTCTGTCTAC
Mouse *ATF2*	Forward: CCGTTGCTATTCCTGCATCAA
	Reverse: TTGCTTCTGACTGGACTGGTT
Mouse *IRF3*	Forward: GAGAGCCGAACGAGGTTCAG
	Reverse: CTTCCAGGTTGACACGTCCG
Mouse *NF-κB*	Forward: AGGCTTCTGGGCCTTATGTG
	Reverse: TGCTTCTCTCGCCAGGAATAC
Mouse *IFN-β* promoter	Forward: ATTCCTCTGAGGCAGAAAGGACCA
	Reverse: GCAAGATGAGGCAAAGGCTGTCAA