Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/7/4/eabc5539/DC1

Supplementary Materials for

Annexin-A1 SUMOylation regulates microglial polarization after cerebral ischemia by modulating IKKα stability via selective autophagy

Xing Li, Qian Xia, Meng Mao, Huijuan Zhou, Lu Zheng, Yi Wang, Zhen Zeng, Lulu Yan, Yin Zhao, Jing Shi*

*Corresponding author. Email: sj@mails.tjmu.edu.cn

Published 20 January 2021, *Sci. Adv.* **7**, eabc5539 (2021)
DOI: 10.1126/sciadv.abc5539

This PDF file includes:

Figs. S1 to S10
Tables S1 to S3

Supplementary Figures

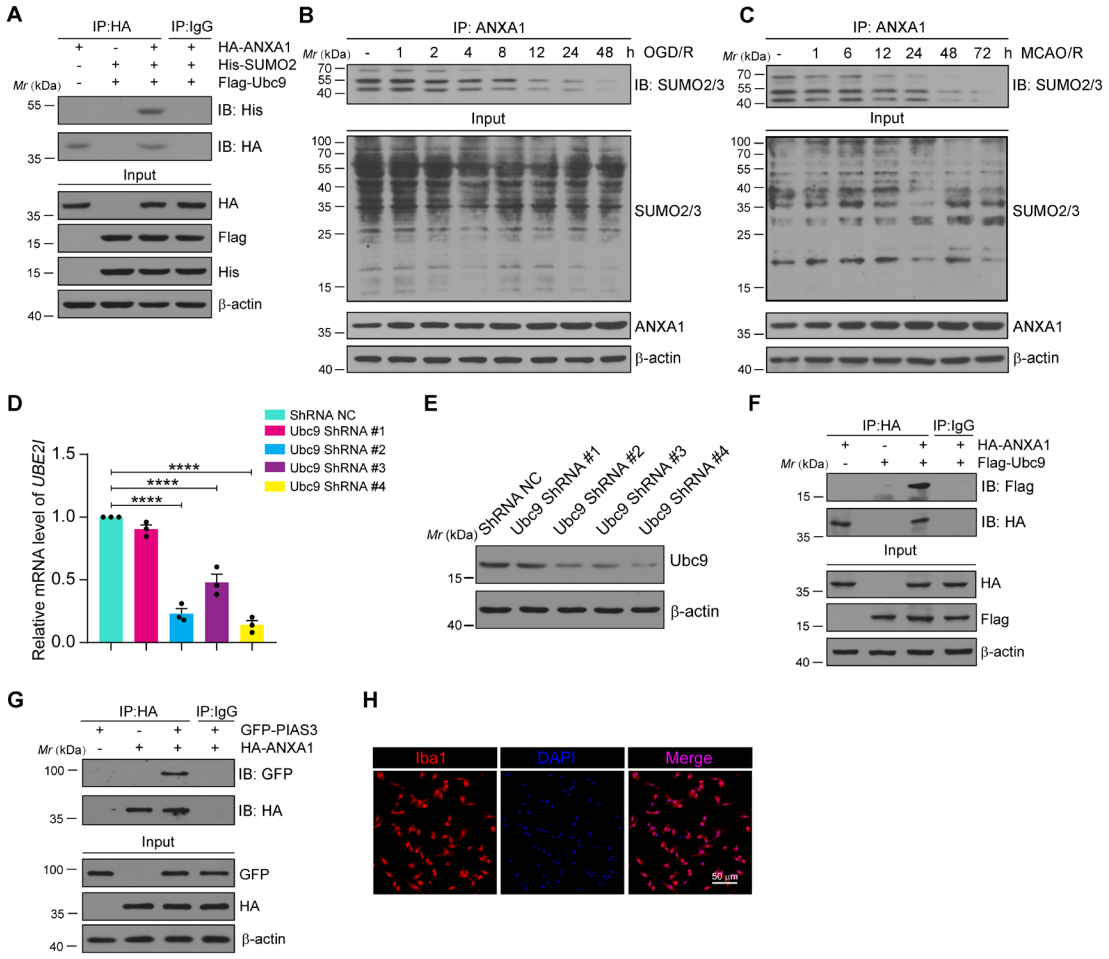

Fig. S1. ANXA1 is modified by SUMOylation. (A) SUMO2 is conjugated covalently to ANXA1. HEK293T cells were transduced with the indicated plasmids. Cell lysates were subjected to IP with anti-HA antibody, followed by immunoblotting with anti-His antibody. (**B**) The time course of SUMOylation of ANXA1 induced by 1 h of OGD treatment in primary microglial cells was assessed. Total lysates of primary microglial cells were obtained at various time points following OGD challenge. (**C**) The time course of SUMOylation of ANXA1 in microglia/macrophages isolated from sham-operated and 1 h MCAO-operated mice with varying durations of reperfusion as indicated. (**D** and **E**) The knockdown efficiency of four different Ubc9 ShRNA plasmids on the transcription (**D**) and expression (**E**) of Ubc9 in HEK293T cell lines. (**F**) Interaction between ANXA1 and Ubc9 in HEK293T cells was confirmed by the IP method. (**G**) Interaction between ANXA1 and PIAS3 in HEK293T cells was confirmed by the IP method. (**H**) Immunofluorescence analysis shows the purity of primary cultured microglial cells. Cells were fixed and stained for microglia specific marker Iba1 (red). Scale bar, 50 μm. Data in panel **D** are presented as the mean ± S.E.M. and analysed by one-way ANOVA followed by Dunnett's post hoc test. ****$P < 0.0001$, $n = 3$ per group.

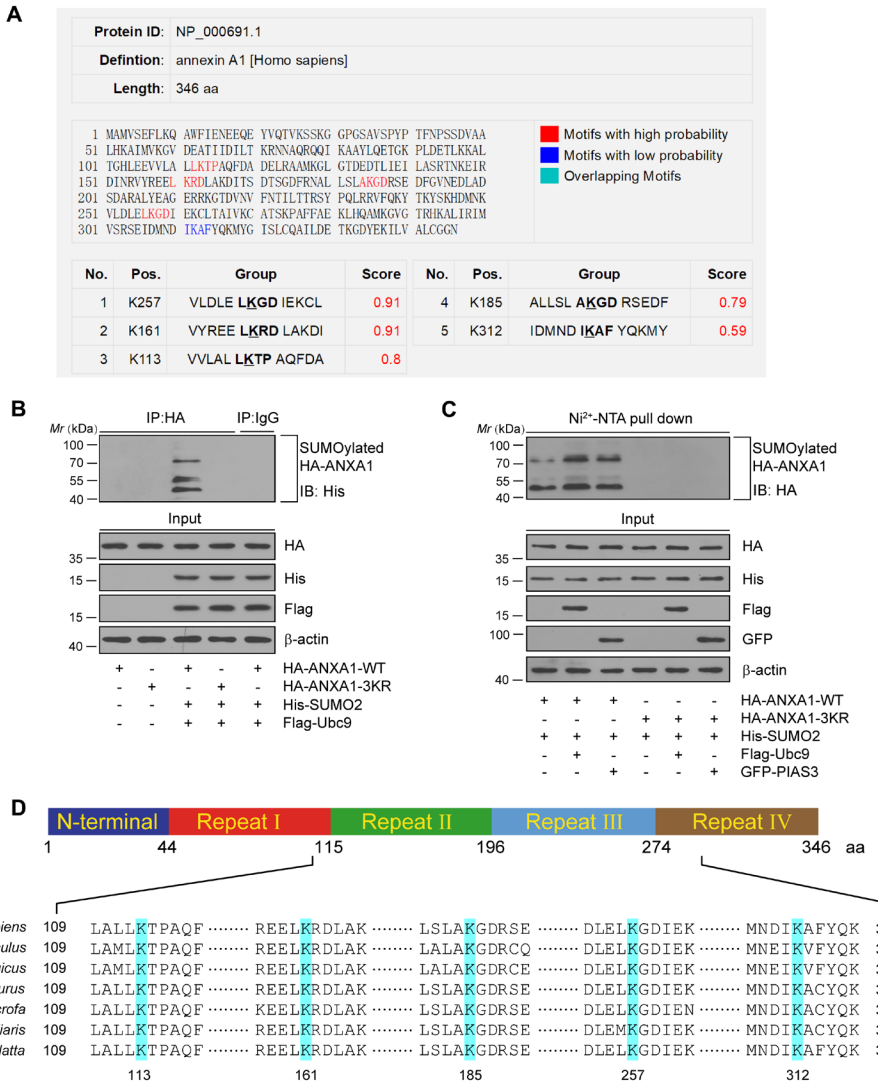

Fig. S2. K113, K161 and K257 are the major SUMOylation sites in ANXA1. (**A**) SUMOylation sites of human ANXA1 protein were predicted by the Abgent program SUMOplot™ (http://www.abgent.com/sumoplot). (**B**) ANXA1 triple-mutant greatly reduced SUMOylation of ANXA1. HEK293T cells were co-transfected with His-SUMO2 and HA-tagged wild-type ANXA1 (HA-ANXA1-WT) or ANXA1 triple-mutant (HA-ANXA1-3KR). After 24 h, cells lysates were used for IP experiment with anti-HA antibody or normal IgG. The precipitates and whole cell lysates were detected by immunoblotting assay. (**C**) Ubc9 and PIAS3 could not enhance SUMOylation level of ANXA1 triple-mutant (3KR). HEK293T cells were transduced with indicated plasmids and then lysed for Ni^{2+}-NTA affinity pull-down assay. Immunoblots were conducted to examine the levels of ANXA1 SUMO modification. (**D**) Schematic of human ANXA1 protein and amino acid sequence alignment of ANXA1 sequences from different species as indicated. The conserved SUMOylation motif lysines are shown in turquoise. Data represent of three independent experiments.

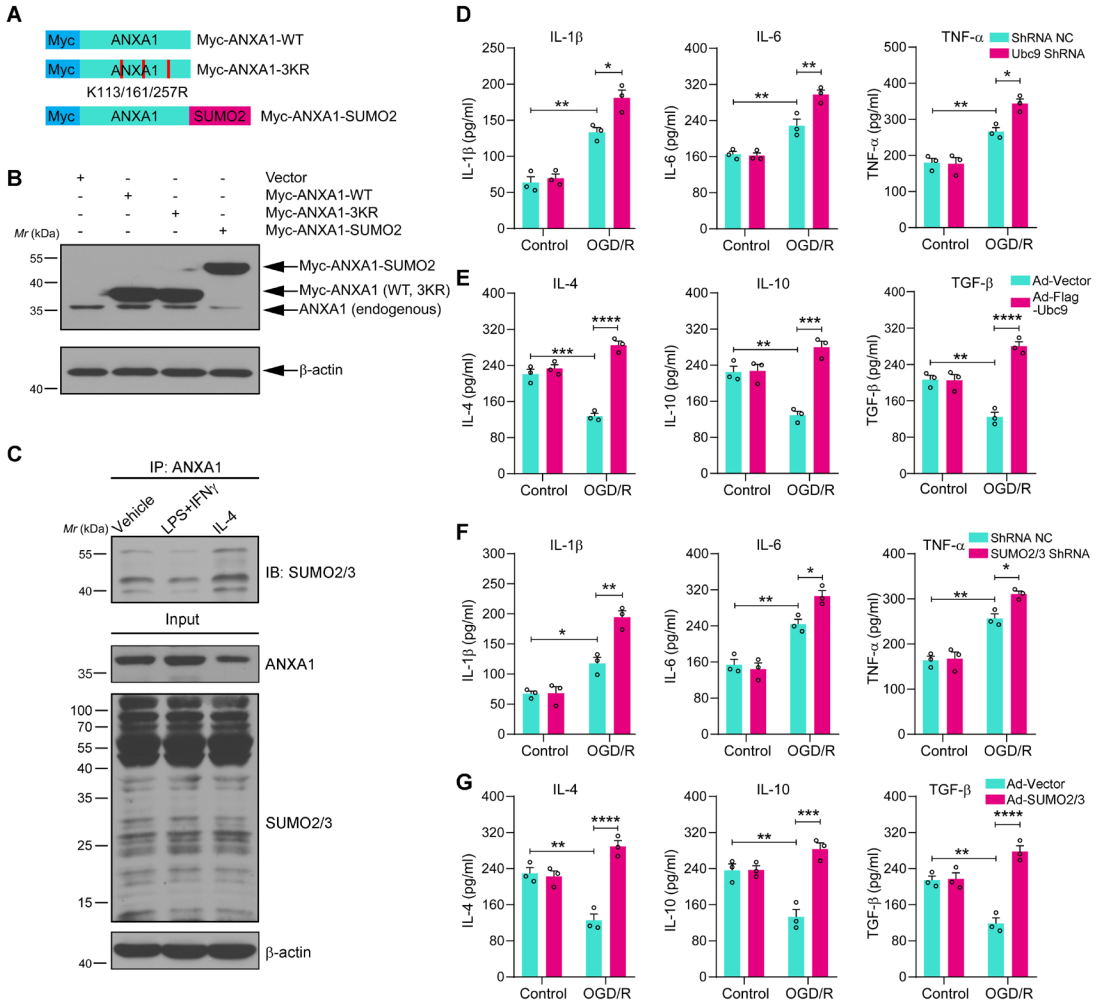

Fig. S3. SUMOylation of endogenous ANXA1 promoted the anti-inflammatory phenotype polarization of microglia. (**A**) Schematic diagram of Myc-ANXA1-WT, Myc-ANXA1-3KR, and Myc-ANXA1-SUMO2 fusion constructs. The location of the KR mutation is shown in red. (**B**) Immunoblot analysis of ANXA1 and Myc-ANXA1-WT, Myc-ANXA1-3KR, and Myc-ANXA1-SUMO2 using an anti-ANXA1 antibody in whole cell lysates prepared from HEK293T cells overexpressing the indicated Myc-tagged proteins. β-actin was used as a loading control. Data are representative of three independent experiments. (**C**) IL-4 treatment enhanced the SUMOylation of endogenous ANXA1, whereas LPS plus IFN-γ treatment decreased the SUMOylation of endogenous ANXA1 in primary cultured microglia. (**D** and **E**) Interference of Ubc9 expression exacerbated OGD/R-induced mRNA expression of pro-inflammatory marker genes (**D**), whereas Ubc9 overexpression promoted the mRNA expression of anti-inflammatory marker genes (**E**) in primary cultured microglia. (**F** and **G**) Interference of the expression of SUMO2/3 exacerbated OGD/R-induced mRNA expression of pro-inflammatory marker genes (**F**), whereas the overexpression of SUMO2/3 promoted the mRNA expression of anti-inflammatory marker genes (**G**) in primary cultured microglia. Data are presented as the mean ± S.E.M. and analysed by two-way ANOVA followed by Tukey's post hoc test. n.s. for $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

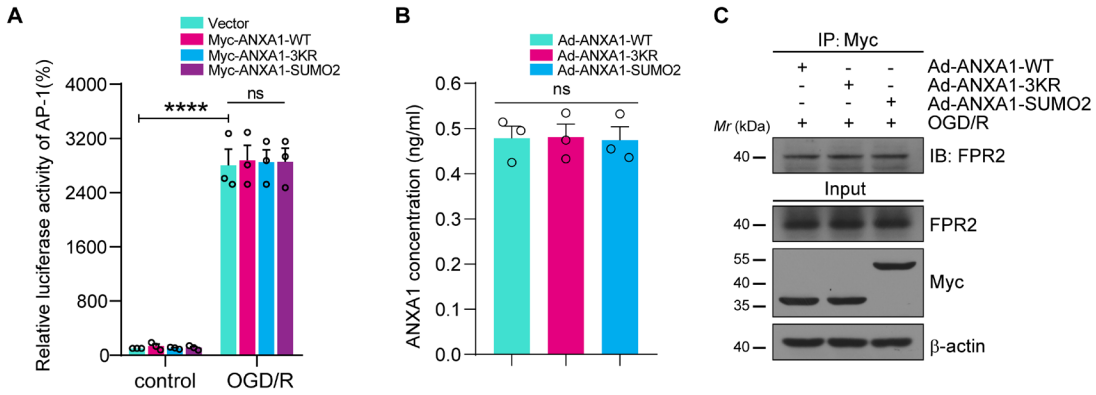

Fig. S4. SUMOylation modification of ANXA1 had no effect on its secretion and FPR2 receptor binding. (**A**) Dual luciferase reporter assay results showing the transcriptional activity of AP-1. HEK293T cells were transduced with pAP-1-luc reporter and pRL-TK-luc plasmids, together with empty vector or wild-type ANXA1, triple-mutant ANXA1 and the constitutive SUMOylation mimic plasmids and then subjected to OGD/R treatment. After 24 h, the AP-1 luciferase activity was analysed. (**B**) The secretion of ANXA1 in microglial cell supernatants upon the indicated stimulation and treatment were detected by ELISA. Quantitative analysis was performed. (**C**) Representative blots of co-IP experiments showing the interaction of ANXA1 with FPR2 in primary cultured microglia. Data are presented as the mean ± S.E.M. and analysed by two-way ANOVA (**A**) or one-way ANOVA (**B**) followed by Tukey's post hoc test. n.s. for $P > 0.05$, ****$P < 0.0001$.

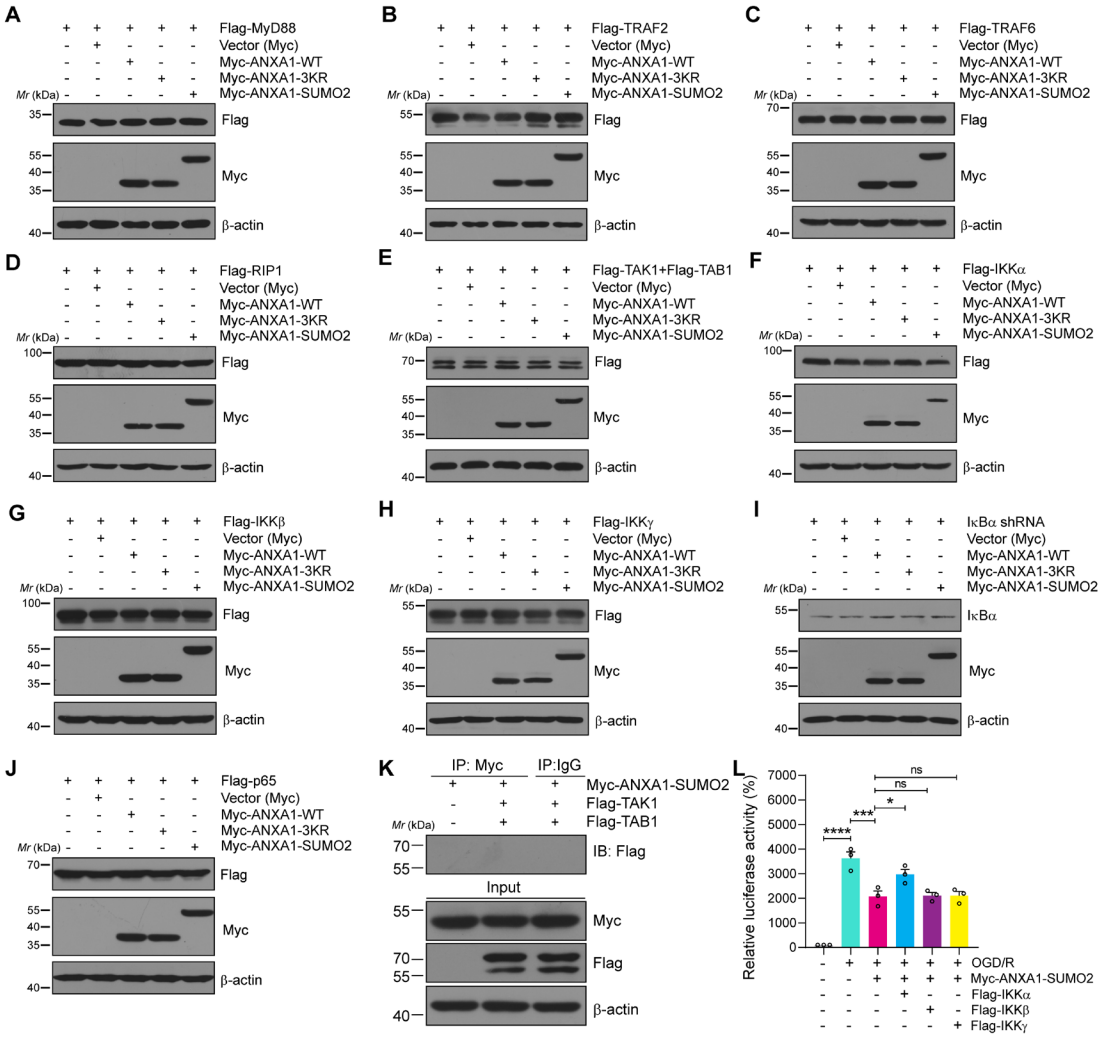

Fig. S5. **The cell lysates of Fig. 3F were detected by immunoblots analysis.** (**A** to **J**) HEK293T cells were transfected with NF-κB-luc, pRL-TK, MyD88, TRAF2, TRAF6, RIP1, TAK1+TAB1, IKKα, IKKβ, IKKγ, IκBα shRNA or p65, along with wild-type or SUMOylation mutants ANXA1. The protein expression levels were detected by immunoblots analysis using the indicated antibodies. (**K**) Representative blots of co-IP experiments show no interaction of ANXA1 with TAK1 or TAB1. Myc-ANXA1-SUMO2 expression plasmid together with empty vector or TAK1 and TAB1 were transduced into HEK293T cells. After 24 h, whole cell lysates were collected and used for the co-IP and immunoblotting experiments. (**L**) Dual luciferase reporter assay results show the transcriptional activity of NF-κB p65. HEK293T cells were transduced with pNF-κB-luc reporter and pRL-TK-luc plasmids, together with empty vector or ANXA1-SUMO2 plasmids and then subjected to OGD/R treatment. After 12 h, cells were transfected with IKKα, IKKβ, IKKγ, respectively. Then 12 h later, the NF-κB luciferase activity was analysed. Data are expressed as mean ± S.E.M. and analysed by one-way ANOVA followed by Tukey's post hoc test. n.s. for $P > 0.05$, *$P < 0.05$, ***$P < 0.001$ and ****$P < 0.0001$, $n = 3$ per group.

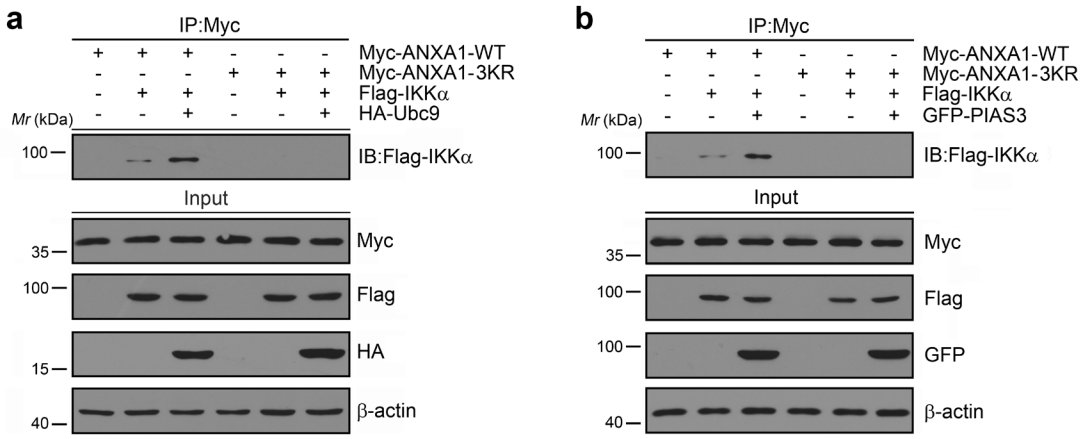

Fig. S6. Ubc9 and PIAS3 enhances the binding of ANXA1 with IKKα. (**A** and **B**) Ubc9 and PIAS3 enhances the binding of exogenous ANXA1 with IKKα. HEK293T cells were transfected with plasmids for Flag-IKKα and wild-type or triple-mutant of ANXA1 along with HA-Ubc9 or GFP-PIAS3. A co-IP assay was used to determine the interaction between Myc-ANXA1 and Flag-IKKα. Data are representative of three independent experiments.

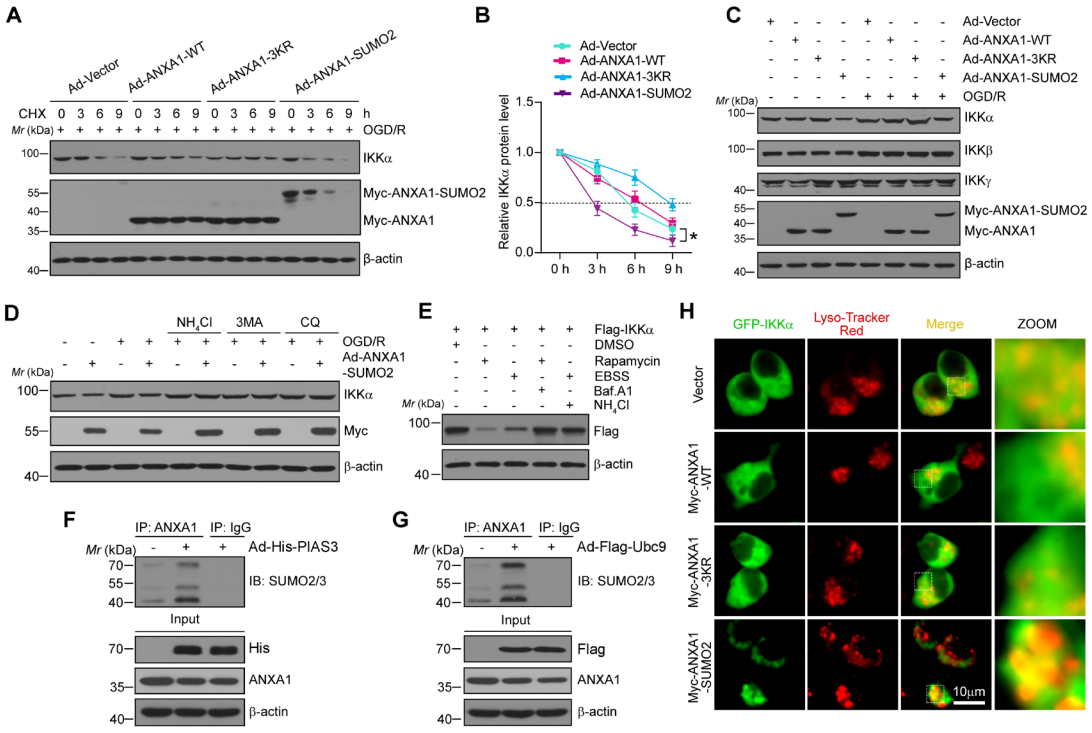

Fig. S7. SUMOylated ANXA1 mediates the autophagy-dependent degradation of IKKα. (**A** and **B**) SUMO conjugation of ANXA1 reduces the half-life of endogenous IKKα in primary microglial cells. Protein level of IKKα was analysed by immunoblotting. Representative blots are shown in (**A**), and the quantification of relative protein levels is shown in (**B**). (**C**) Representative immunoblots indicate the effects of SUMOylated ANXA1 on the protein levels of the endogenous IKKα, IKKβ or IKKγ in microglia. (**D**) Immunoblot analysis show endogenous IKKα of microglia cells infected with adenoviral vectors carrying Myc-tagged wild-type ANXA1 and then treated with DMSO, MG132, NH_4Cl, 3MA and CQ for 6 h. (**E**) Representative immunoblots show the protein level of Flag-IKKα. HEK293T cells were transduced with plasmid for Flag-IKKα and treated with Rapamycin (250 nM), Bafilomycin A1 (800 nM), NH_4Cl (20 mM), or incubated with EBSS. (**F** and **G**) PIAS3 and Ubc9 enhanced the SUMOylation level and decreased the protein level of endogenous ANXA1. Primary microglial cells were transduced with adenoviral vectors carrying His-PIAS3 and Flag-Ubc9, and a co-IP assay was used to determine the SUMOylation level and protein level of endogenous ANXA1. (**H**) HEK293T cells were transiently transfected with GFP-IKKα and Myc-tagged wild-type or SUMOylation mutations of ANXA1. The colocalization of GFP-IKKα and LysoTracker Red was analysed by fluorescence microscopy. Enlarged images show the colocalization of the two signals. Scale bar, 10 μm. Data in panel **B** are presented as the mean ± S.E.M. and analysed by two-way RM ANOVA followed by Tukey's post hoc test. *$P < 0.05$, $n = 3$ per group.

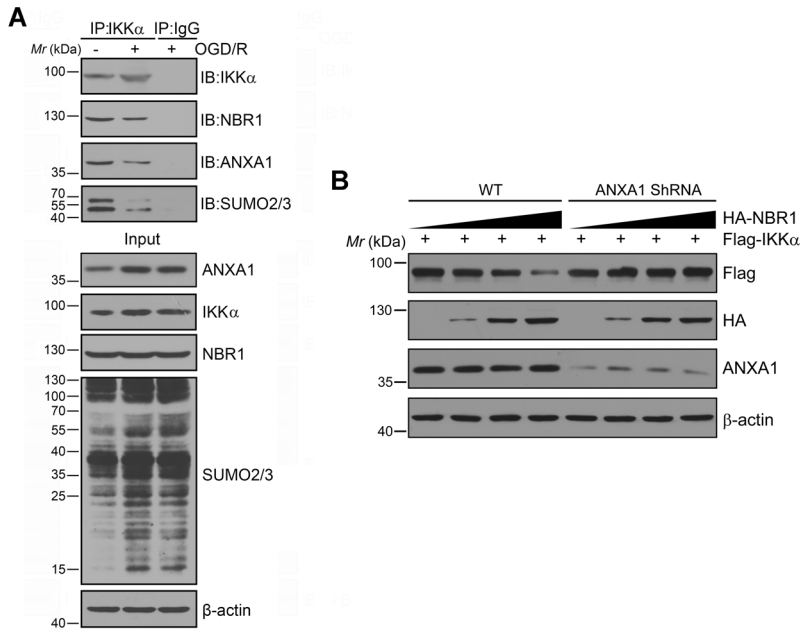

Fig. S8. ANXA1 forms a complex with SUMO2, IKKα and NBR1 and mediates the degradation of IKKα by NBR1. (**A**) OGD/R decreased the interaction of IKKα with NBR1, ANXA1, and SUMO2. Primary microglial cells were stimulated with OGD/R or left unstimulated. Then, the cells were lysed, and cell lysates was subjected to co-IP analysis by anti-IKKα followed by immunoblotting. (**B**) ANXA1 mediates the degradation of IKKα by NBR1. Wild-type or ANXA1-knockdown HEK293T cells were transfected with increasing amounts of HA-NBR1 plasmids (0, 1, 2, 4 μg), along with the Flag-IKKα plasmids. Cells were harvested at 24 h after transfection and analysed by immunoblotting with the indicated antibodies. Data are representative of three independent experiments.

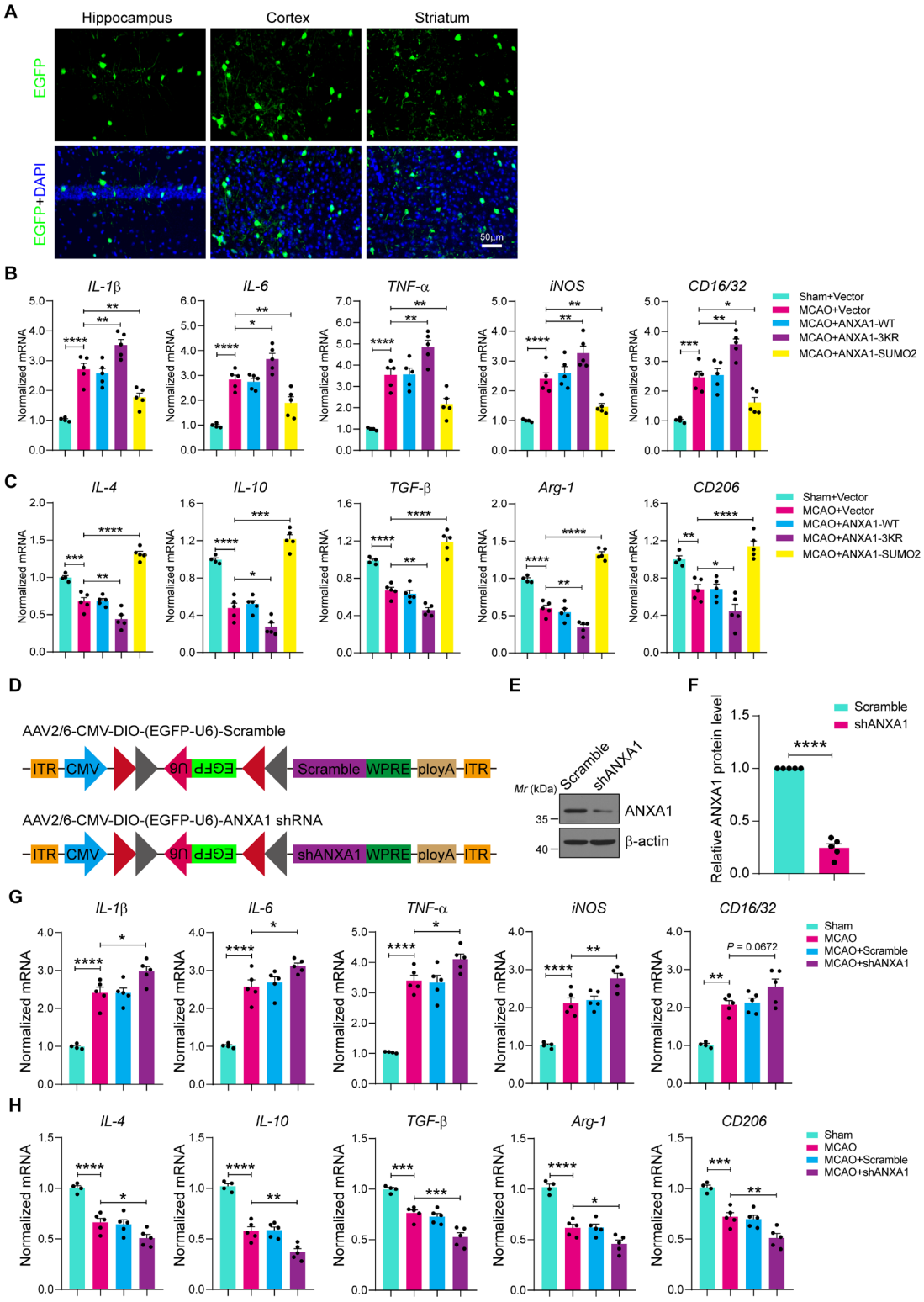

Fig. S9. AAV-mediated introduction or inhibition of SUMOylated ANXA1 altered the phenotypic polarization of microglia/macrophages in ischemic stroke mice. (**A**) Representative images of GFP signals in the hippocampus CA1 region, cerebral cortex, and striatum of Cx3cr1-Cre mice injected with the AAV vectors at day 49 after the injection. Scale bar, 50 μm. (**B** and **C**) SUMOylated ANXA1 but not the wild-type or the triple-mutant of ANXA1 reversed MCAO-induced mRNA expression of pro-inflammatory marker genes (**B**) and promoted the mRNA

expression of anti-inflammatory marker genes (**C**) in microglia/macrophages isolated from ischemic stroke mice. (**D**) Schematic of Cx3cr1-Cre-dependent AAV vectors for microglia/macrophages ANXA1 silencing. (**E** and **F**) Representative immunoblotting of ANXA1 and quantification of ANXA1 expression in the isolated microglia/macrophages from Cx3cr1-Cre mice injected with AAV at 4 wk. (**G** and **H**) AAV mediated microglia/macrophages ANXA1 silencing exacerbated MCAO-induced mRNA expression of pro-inflammatory marker genes (**G**) and attenuated the mRNA expression of anti-inflammatory marker genes (**H**) in microglia/macrophage cells isolated from ischemic stroke mice. The mRNA levels of pro-inflammatory mediators and anti-inflammatory mediators were detected by qPCR. Data are presented as the mean ± S.E.M. and analysed by two-tailed unpaired t test (**F**) or one-way ANOVA followed by Dunnett's post hoc test (**B**, **C**, **G**, **H**). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

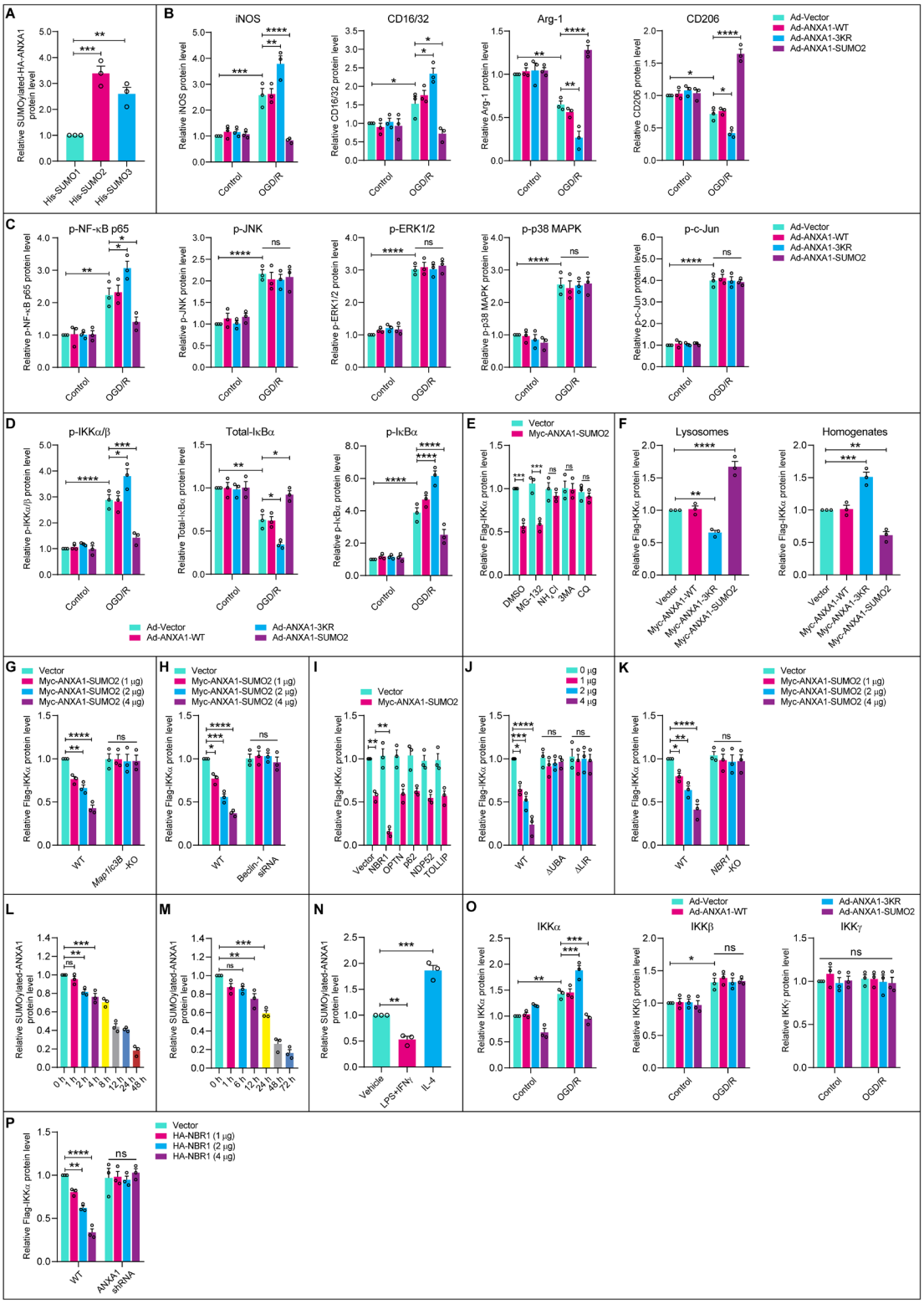

Fig. S10. Quantification of the immunoblot results (Figs. 1, 2, 3, 5, 6 and Figs. S1, S2, S7, S8). (**A**) The quantification analysis of the immunoblots of **Fig. 1A**. Normalized SUMOylated-HA-ANXA1 to corresponding loading control is summarized for three independent experiments. (**B**) The quantification analysis of immunoblots of **Fig. 2C**. Normalized iNOS, CD16/32, Arg-1 and CD206 to corresponding loading control are summarized for three independent experiments. (**C** and **D**) The quantification analysis of immunoblots of **Fig. 3, A** and **B**. Normalized phospho-NF-κB p65,

-JNK, -ERK1/2, -p38 MAPK, -c-Jun, -IKKα/β, -IκBα and total IκBα to corresponding loading control are summarized for three independent experiments. (**E** to **H**) The quantification analysis of immunoblots of **Fig. 5, F, I, L** and **M**. Normalized Flag-IKKα to corresponding loading control are summarized for three independent experiments. (**I** to **K**) The quantification analysis of immunoblots of **Fig. 6, B, K** and **L**. Normalized Flag-IKKα to corresponding loading control are summarized for three independent experiments. (**L** and **M**) The quantification analysis of immunoblots of **Fig. S1, B** and **C**. Normalized SUMOylated-ANXA1 to corresponding loading control are summarized for three independent experiments. (**N**) The quantification analysis of immunoblots of **Fig. S3C**. Normalized SUMOylated-ANXA1 to corresponding loading control is summarized for three independent experiments. (**O**) The quantification analysis of immunoblots of **Fig. S7C**. Normalized IKKα, IKKβ, IKKγ to corresponding loading control is summarized for three independent experiments. (**P**) The quantification analysis of immunoblots of **Fig. S8B**. Normalized Flag-IKKα to corresponding loading control were summarized for three independent experiments. Statistical difference in panel **A**, **F**, **L**, **M**, **N** were determined using one-way ANOVA followed by Dunnett's post hoc test, and all others were analysed by two-way ANOVA followed by Tukey's post hoc test. Data are presented as mean ± S.E.M. n.s. for $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

Supplementary Table 1. Antibodies employed in this study.

Antibody	Species	Type	IB	IF	Source	Identifier
HA	Mouse	Mono-	1:1000		Santa Cruz	sc-7392
Flag	Mouse	Mono-	1:2000		Santa Cruz	sc-166355
His	Rabbit	Poly-	1:1000		Sigma-Aldrich	SAB1306085
Ubc9	Mouse	Mono-	1:1000		Santa Cruz	sc-271057
GFP	Mouse	Mono-	1:2000		Santa Cruz	sc-9996
ANXA1	Mouse	Mono-	1:1000	1:200	Santa Cruz	sc-12740
SUMO-2/3	Rabbit	Mono-	1:1000	1:200	Cell Signaling	#4971
β-actin	Mouse	Mono-	1:1000		Santa Cruz	sc-47778
iNOS	Rabbit	Poly-	1:500	1:200	Proteintech	18985-1-AP
CD16/32	Mouse	Poly-	1:500		R&D systems	AF1460
Arg-1	Rabbit	Mono-	1:1000	1:200	Cell Signaling	#93668
CD206	Mouse	Poly-	1:500		R&D systems	AF2535
NF-κB p65	Rabbit	Mono-	1:1000	1:200	Cell Signaling	#8242
Phospho-NF-κB p65	Rabbit	Mono-	1:1000		Cell Signaling	#3033
JNK	Rabbit	Poly-	1:2000		R&D systems	AF1387
Phospho-JNK	Rabbit	Mono-	1:1000		R&D systems	MAB1205
ERK1/2	Mouse	Mono-	1:1000		R&D systems	MAB1576
Phospho-ERK1/2	Rabbit	Poly-	1:1000		R&D systems	AF1018
p38 MAPK	Rabbit	Mono-	1:1000		Cell Signaling	#8690
Phospho-p38 MAPK	Rabbit	Poly-	1:1000		R&D systems	AF869
c-Jun	Rabbit	Mono-	1:1000		Cell Signaling	#9165
Phospho-c-Jun	Rabbit	Mono-	1:1000		Cell Signaling	#3270
Phospho-IKKα/β	Rabbit	Mono-	1:1000		Cell Signaling	#2697
IκBα	Mouse	Mono-	1:1000		Cell Signaling	#4814
Phospho-IκBα	Rabbit	Mono-	1:1000		Cell Signaling	#2859
α-tubulin	Mouse	Mono-	1:2000		Santa Cruz	sc-8035
Histone H3	Rabbit	Mono-	1:2000		Cell Signaling	#4499
Myc	Mouse	Mono-	1:1000		Santa Cruz	sc-40
IKKα	Mouse	Mono-	1:1000		Cell Signaling	#11930
IKKβ	Rabbit	Mono-	1:1000		Cell Signaling	#8943
IKKγ	Mouse	Mono-	1:1000		Santa Cruz	sc-8032
LAMP2A	Rabbit	Mono-	1:1000		Abcam	ab125068
LC3B	Rabbit	Poly-	1:1000		Abcam	ab48394
Beclin1	Rabbit	Poly-	1:1000		Abcam	ab62557
NBR1	Rabbit	Mono-	1:1000		Cell Signaling	#9891
Iba1	Rabbit	Poly-		1:500	Wako	#019-19741

Abbreviations: IB, Immunoblotting; IF, Immunofluorescence.

Supplementary Table 2. Primers used in this study.

Primer name	Primer sequences (5'- 3')	
	Forward	Reverse
Quantitative RT-PCR primers		
IL-1β	GAAAGACGGCACACCCAC	TGTGACCCTGAGCGACCT
IL-6	TCTCTGGGAAATCGTGGAA	GATGGTCTTGGTCCTTAGCC
TNF-α	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
iNOS	GCTTGTCTCTGGGTCCTCTG	CTCACTGGGACAGCACAGAA
CD16	GGCTGTGGTGAAACTGGAC	GGTTGGCTTTTGGGATAGA
Arg-1	CAAGACAGGGCTCCTTTCAG	TGGCTTATGGTTACCCTCCC
IL-4	CCCCCAGCTAGTTGTCATCC	AGGACGTTTGGCACATCCAT
IL-10	CTGCCTGCTCTTACTGACTG	AAATCACTCTTCACCTGCTC
TGF-β	TGCGCTTGCAGAGATTAAAA	CGTCAAAAGACAGCCACTCA
CD206	TCAGCTATTGGACGCGAGGCA	TCCGGGTTGCAAGTTGCCGT
UBE2I	GAAAGGGACTCCGTGGGAAG	GCTTGAGCTGGGTCTTGGAT
CHUK	GACTTGATGGAATCTCTGGA	GATGCCATATTTCTTTCTGC
GAPDH	AGGAGCGAGACCCCACTAACA	AGGGGGGCTAAGCAGTTGGT
Genotyping primers		
Cx3cr1 Cre	CAACGAGTGATGAGGTTCGCAAG	ACACCAGAGACGGAAATCCATCG

Supplementary Table 3. The statistical analysis results for all figures.

Figure Number	*n*	Primary statistic	Post-hoc test	*P* value	Degrees of Freedom & *F* Value
2A	*n* = 4 per group	two-way ANOVA	Tukey's post hoc test	*IL-1β*, $P < 0.0001$	$F_{3,24} = 30.91$
				IL-6, $P < 0.0001$	$F_{3,24} = 14.84$
				TNF-α, $P < 0.0001$	$F_{3,24} = 21.18$
				iNOS, $P < 0.0001$	$F_{3,24} = 28.56$
				CD16/32, $P < 0.0001$	$F_{3,24} = 29.92$
2B	*n* = 4 per group	two-way ANOVA	Tukey's post hoc test	*Arg-1*, $P < 0.0001$	$F_{3,24} = 16.60$
				TGF-β, $P < 0.0001$	$F_{3,24} = 20.61$
				IL-4, $P < 0.0001$	$F_{3,24} = 20.91$
				IL-10, $P < 0.0001$	$F_{3,24} = 31.83$
				CD206, $P < 0.0001$	$F_{3,24} = 30.16$
2D	*n* = 4 per group	two-way ANOVA	Tukey's post hoc test	IL-1β, $P < 0.0001$	$F_{3,24} = 34.60$
				IL-6, $P < 0.0001$	$F_{3,24} = 36.65$
				TNF-α, $P < 0.0001$	$F_{3,24} = 29.56$
2E	*n* = 4 per group	two-way ANOVA	Tukey's post hoc test	IL-4, $P < 0.0001$	$F_{3,24} = 23.40$
				IL-10, $P < 0.0001$	$F_{3,24} = 21.90$
				TGF-β, $P < 0.0001$	$F_{3,24} = 34.72$
2G	*n* = 6 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,40} = 116.5$
2H	*n* = 6 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,40} = 61.02$
3C	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 29.61$
3D	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 27.94$
3F	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	$P = 0.0007$	$F_{3,16} = 9.734$
3G	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 25.90$
3I	*n* = 50 cells per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{7,784} = 2032$
4A	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	MyD88, $P < 0.0001$	$F_{5,12} = 39.94$
				TRAF2, $P < 0.0001$	$F_{5,12} = 42.15$
				TRAF6, $P < 0.0001$	$F_{5,12} = 31.86$
				RIP1, $P < 0.0001$	$F_{5,12} = 31.39$
				TAK1+TAB1, $P < 0.0001$	$F_{5,12} = 49.68$
				IKKα, $P < 0.0001$	$F_{5,12} = 40.42$
				IKKβ, $P < 0.0001$	$F_{5,12} = 72.41$
				IKKγ, $P < 0.0001$	$F_{5,12} = 27.17$

				IκBα shRNA, $P < 0.0001$	$F_{5,12} = 27.70$
				p65, $P < 0.0001$	$F_{5,12} = 44.22$
4G	n = 50 cells per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{3,196} = 590.4$
5B	n = 3 per group	two-way repeated measures (RM) ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{9,24} = 8.926$
5C	n = 3 per group	two-way ANOVA	Tukey's post hoc test	$P = 0.9756$	$F_{9,32} = 0.2799$
5E	n = 3 per group	two-way repeated measures (RM) ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{9,24} = 7.278$
5K	n = 50 cells per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{3,196} = 967.3$
6H	n = 50 cells per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{3,196} = 780.6$
7E	n =9 mice per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{3,32} = 40.47$
7F	n =10 or 12 mice per group	Kruskal–Wallis non-parametric test	Dunnett's post hoc test	$P < 0.0001$	
7G	n =10 or 12 mice per group	two-way RM ANOVA	Tukey's post hoc test	*$P = 0.0443$*	$F_{20,265} = 1.637$
7H	n =10 or 12 mice per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{4,53} = 19.62$
7I	n =10 or 12 mice per	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{4,53} = 18.52$

	group				
7J	n =10 or 12 mice per group	Kruskal–Wallis non-parametric test	Dunnett's post hoc test	$P < 0.0001$	
7L	n =10 or 12 mice per group	two-way ANOVA	Tukey's post hoc test	$P = 0.8770$	$F_{4,106} = 0.3006$
7M	n =10 or 12 mice per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{4,53} = 19.05$
7N	n =10 or 12 mice per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{4,53} = 42.91$
S1D	n = 3 per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{4,10} = 92.89$
S3D	n = 3 per group	two-way ANOVA	Tukey's post hoc test	IL-1β, $P = 0.0322$	$F_{1,8} = 6.698$
				IL-6, $P = 0.0064$	$F_{1,8} = 13.39$
				TNF-α, $P = 0.0166$	$F_{1,8} = 9.103$
S3E	n = 3 per group	two-way ANOVA	Tukey's post hoc test	IL-4, $P < 0.0001$	$F_{1,8} = 68.76$
				IL-10, $P = 0.0003$	$F_{1,8} = 35.22$
				TGF-β, $P < 0.0001$	$F_{1,8} = 55.58$
S3F	n = 3 per group	two-way ANOVA	Tukey's post hoc test	IL-1β, $P = 0.0034$	$F_{1,8} = 16.84$
				IL-6, $P = 0.0168$	$F_{1,8} = 9.059$
				TNF-α, $P = 0.0414$	$F_{1,8} = 5.891$
S3G	n = 3 per group	two-way ANOVA	Tukey's post hoc test	IL-4, $P = 0.0002$	$F_{1,8} = 43.48$
				IL-10, $P = 0.0006$	$F_{1,8} = 30.30$
				TGF-β, $P = 0.0002$	$F_{1,8} = 42.19$
S4A	n = 3 per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 29.61$
S4B	n = 3 per group	one-way ANOVA	Tukey's post hoc test	$P = 0.9885$	$F_{2,6} = 0.01161$
S5L	n = 3 per group	one-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{5,12} = 41.36$
S7B	n = 3 per group	two-way RM ANOVA	Tukey's post hoc test	$P = 0.0051$	$F_{9,24} = 3.681$
S9B	n =4 or 5 mice	one-way ANOVA	Dunnett's post hoc test	*IL-1β*, $P < 0.0001$	$F_{4,19} = 31.69$
				IL-6, $P < 0.0001$	$F_{4,19} = 26.80$

	per group			*TNF-α, P* < 0.0001	$F_{4,19}$ = 27.26
				iNOS, P < 0.0001	$F_{4,19}$ = 23.50
				CD16/32, P < 0.0001	$F_{4,19}$ = 27.47
S9C	*n* =4 or 5 mice per group	one-way ANOVA	Dunnett's post hoc test	*IL-4, P* < 0.0001	$F_{4,19}$ = 66.11
				IL-10, P < 0.0001	$F_{4,19}$ = 86.88
				TGF-β, P < 0.0001	$F_{4,19}$ = 64.09
				Arg-1, P < 0.0001	$F_{4,19}$ = 105.8
				CD206, P < 0.0001	$F_{4,19}$ = 27.74
S9F	*n* = 5 mice per group	two-tailed unpaired t test		*P* < 0.0001	
S9G	*n* =4 or 5 mice per group	one-way ANOVA	Dunnett's post hoc test	*IL-1β, P* < 0.0001	$F_{3,15}$ = 38.58
				IL-6, P < 0.0001	$F_{3,15}$ = 44.40
				TNF-α, P < 0.0001	$F_{3,15}$ = 47.69
				iNOS, P < 0.0001	$F_{3,15}$ = 35.21
				CD16/32, P < 0.0001	$F_{3,15}$ = 19.88
S9H	*n* =4 or 5 mice per group	one-way ANOVA	Dunnett's post hoc test	*IL-4, P* < 0.0001	$F_{3,15}$ = 26.83
				IL-10, P < 0.0001	$F_{3,15}$ = 55.83
				TGF-β, P < 0.0001	$F_{3,15}$ = 32.75
				Arg-1, P < 0.0001	$F_{3,15}$ = 41.70
				CD206, P < 0.0001	$F_{3,15}$ = 27.12
S10A	*n* = 3 per group	one-way ANOVA	Dunnett's post hoc test	*P* = 0.0008	$F_{2,6}$ = 29.91
S10B	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	iNOS, *P* < 0.0001	$F_{3,16}$ = 21.86
				CD16/32, *P* = 0.0005	$F_{3,16}$ = 10.19
				Arg-1, *P* < 0.0001	$F_{3,16}$ = 33.34
				CD206, *P* < 0.0001	$F_{3,16}$ = 58.55
S10C	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	p-p65, *P* = 0.0014	$F_{3,16}$ = 8.358
				p-JNK, *P* = 0.6773	$F_{3,16}$ = 0.5157
				p-ERK1/2, *P* = 0.7737	$F_{3,16}$ = 0.3728
				p-p38, *P* = 0.7009	$F_{3,16}$ = 0.4798
				p-c-Jun, *P* = 0.8994	$F_{3,16}$ = 0.1933
S10D	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	p-IKKα/β, *P* < 0.0001	$F_{3,16}$ = 15.42
				p-IκBα, *P* < 0.0001	$F_{3,16}$ = 21.51
				total IκBα, *P* = 0.0005	$F_{3,16}$ = 10.34
S10E	*n* = 3 per group	two-way ANOVA	Tukey's post hoc test	*P* = 0.0004	$F_{4,20}$ = 8.517
S10F	*n* = 3 per group	one-way ANOVA	Dunnett's post hoc test	Lysosomes, *P* < 0.0001	$F_{3,8}$ = 67.11

				Homogenates, $P < 0.0001$	$F_{3,8} = 44.97$
S10G	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P = 0.0010$	$F_{3,16} = 9.023$
S10H	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 18.08$
S10I	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P = 0.0027$	$F_{5,24} = 5.023$
S10J	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P = 0.0002$	$F_{6,24} = 7.351$
S10K	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P = 0.0027$	$F_{3,16} = 7.288$
S10L	$n = 3$ per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{7,16} = 110.0$
S10M	$n = 3$ per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{6,24} = 110.0$
S10N	$n = 3$ per group	one-way ANOVA	Dunnett's post hoc test	$P < 0.0001$	$F_{2,6} = 102.7$
S10O	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	IKKα, $P = 0.0219$	$F_{3,16} = 4.245$
				IKKβ, $P = 0.9052$	$F_{3,16} = 0.1848$
				IKKγ, $P = 0.8804$	$F_{3,16} = 0.2210$
S10P	$n = 3$ per group	two-way ANOVA	Tukey's post hoc test	$P < 0.0001$	$F_{3,16} = 16.27$