Transcription Factor ELF-1 Drives Inflammatory Macrophage to Exacerbate AKI by GBP4/NLRs/NF-κB Axis

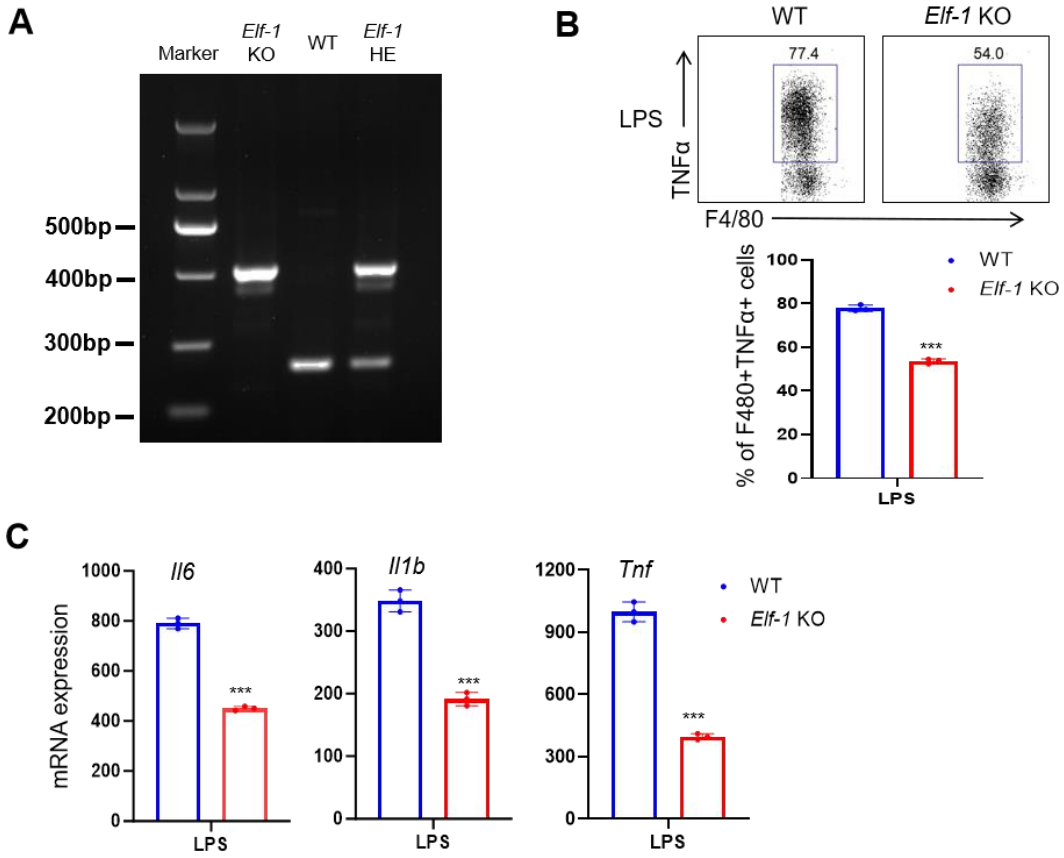
Yangxiao Hou[1,4,*], Chenghao Feng[1,4], Zhaoqi Zhang[2,4], Yang Qiu[1,4], Xu Zhang[1], Yuanbo Qi[1], Zhigang Wang[1], Haowei Zhu[1], Xinyue Hou[1], Peng Wang[3,*], Wenjun Shang[1,*]

[1]Department of Kidney Transplantation, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450001, China; [2]Department of Rheumatology and Immunology, Peking University People's Hospital & Beijing Key Laboratory for Rheumatism Mechanism and Immune Diagnosis (BZ0135), Beijing, 100044, China;

[3]Department of Microbiology and Immunology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, 450001 China;

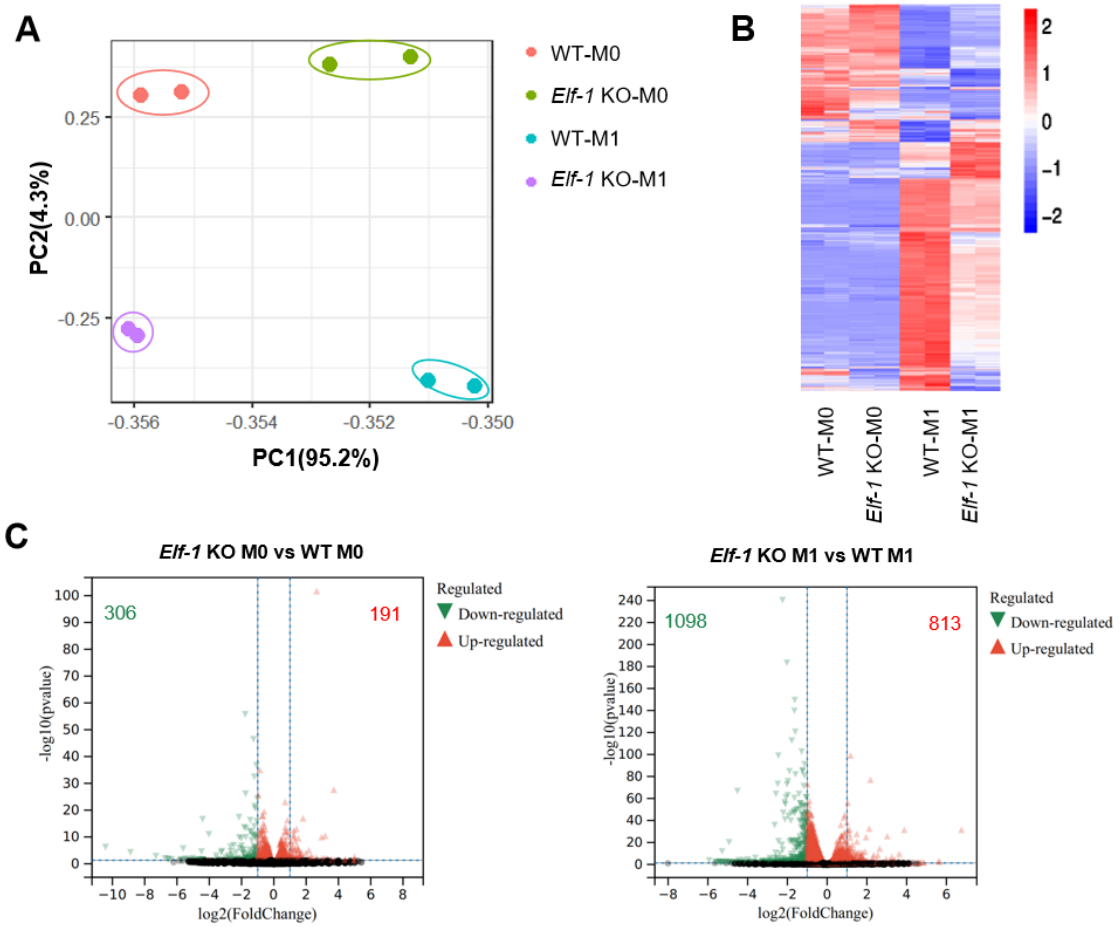
[4] These authors contributed equally

[*]Corresponding Authors



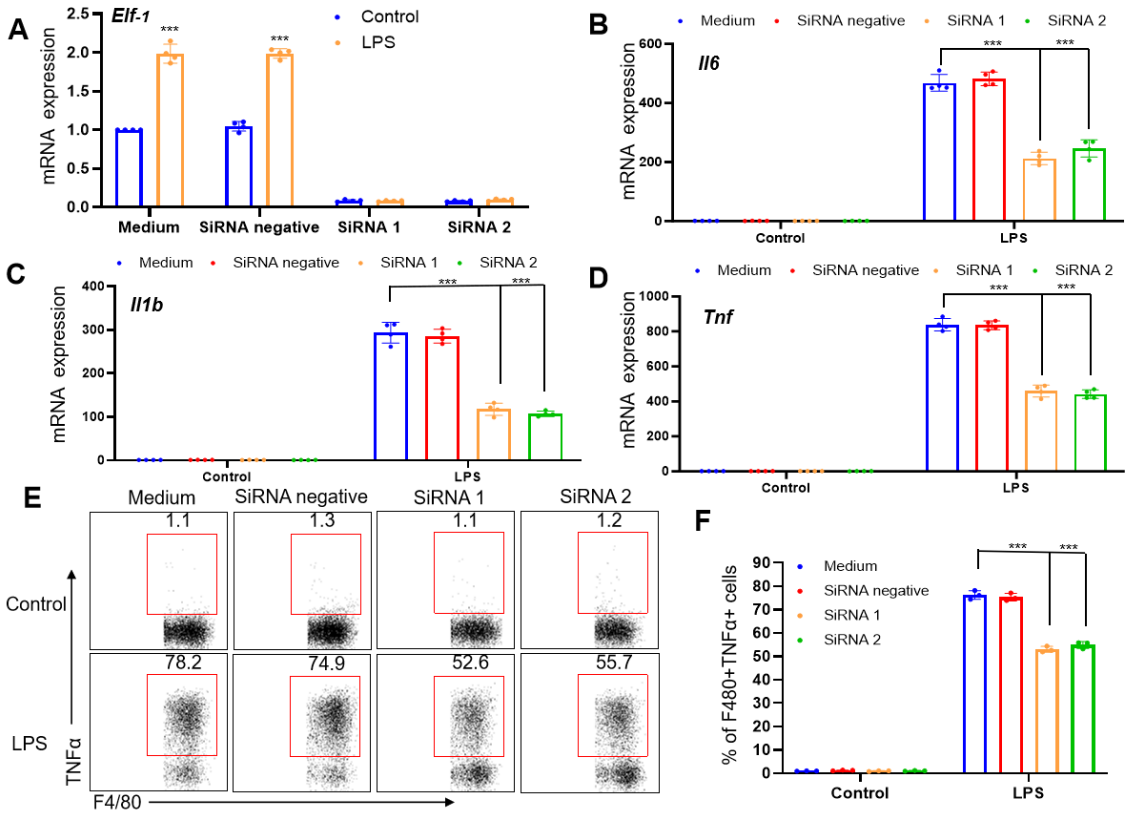
Supplementary Figure 1. The deletion of ELF-1 reduces the M1 polarization of TG-induced PEMs.

Peritoneal macrophages (PEMs) were obtained from WT and *Elf-1* KO mice after 5 days of 3% TG treatment. (A) *Elf-1* gene DNA level detection. WT indicates wild type mice, *Elf-1* KO indicates *Elf-1* null mice, and *Elf-1* HE indicates *Elf-1* heterozygous mice. (B) TNFα expression in $F4/80^{+}CD11b^{+}$ cells in WT and *Elf-1* KO PEMs with LPS stimulation for 6h was measured by flow cytometry (n=3). (C) The gene expressions of *Tnf*, *Il6* and *Il1b* in WT and *Elf-1* KO PEMs with LPS stimulation for 6h were detected by qPCR (n=3). Data are representative of three independent experiments. Data are presented as mean ± S.D. ***$p < 0.001$, one-way ANOVA.



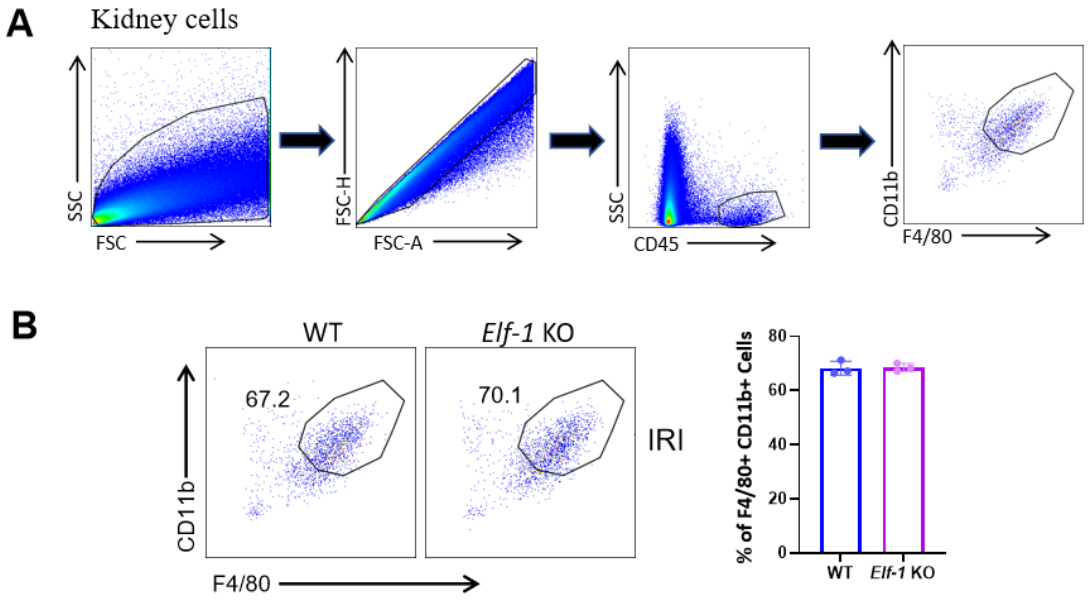
Supplementary Figure 2. Analysis process of RNA-seq data.

(A) PCA plots of M0 or M1 in WT and *Elf-1* KO mice. (B) Heatmap showing the differential expression of all genes in M0 or M1 of WT and *Elf-1* KO mice. Red and blue represent the up-regulated and down-regulated genes, respectively. (C) volcano plots showing the differential expression of all genes in M0(left) or M1(right) of WT and *Elf-1* KO mice. Red and green represent the up-regulated and down-regulated genes, respectively.



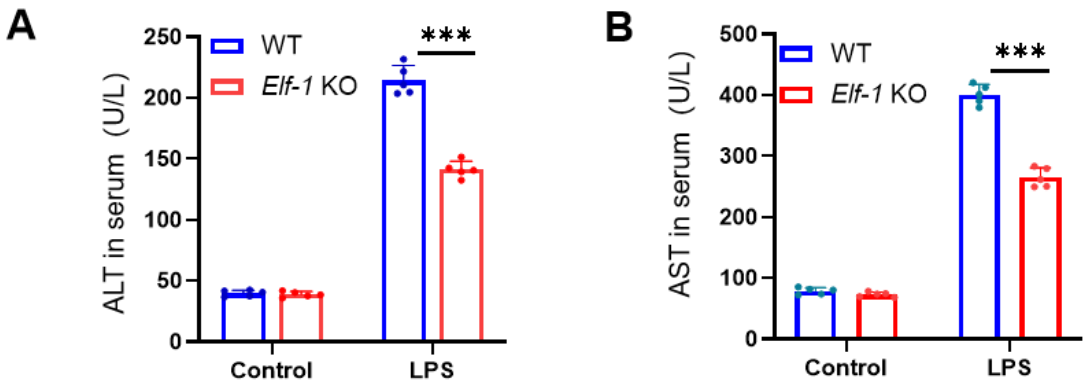
Supplementary Figure 3. Knockdown of ELF-1 expression by siRNA in macrophages decreases LPS-induced pro-inflammatory response.

BMDMs were obtained from M-CSF-induced bone marrow cells for 7 days. (A) qPCR analysis showing the *Elf-1* gene expressed in WT and *Elf-1* KO macrophages under different SiRNA treatments (n=4). (B-D) Expression of M1 marker genes *Il6* (B), *Il1b* (C) and *Tnf* (D) in WT and *Elf-1* KO macrophages under different SiRNA treatments was detected by qPCR (n=4). (E-F) The expression of TNFα in $F4/80^{+}CD11b^{+}$ BMDMs of WT and *Elf-1* KO mice under different SiRNA treatments was detected by flow cytometry analysis (E). "F" represents the statistical histogram (n=3). Data are representative of three independent experiments. Data are presented as mean ± S.D. ***$p < 0.001$, one-way ANOVA.



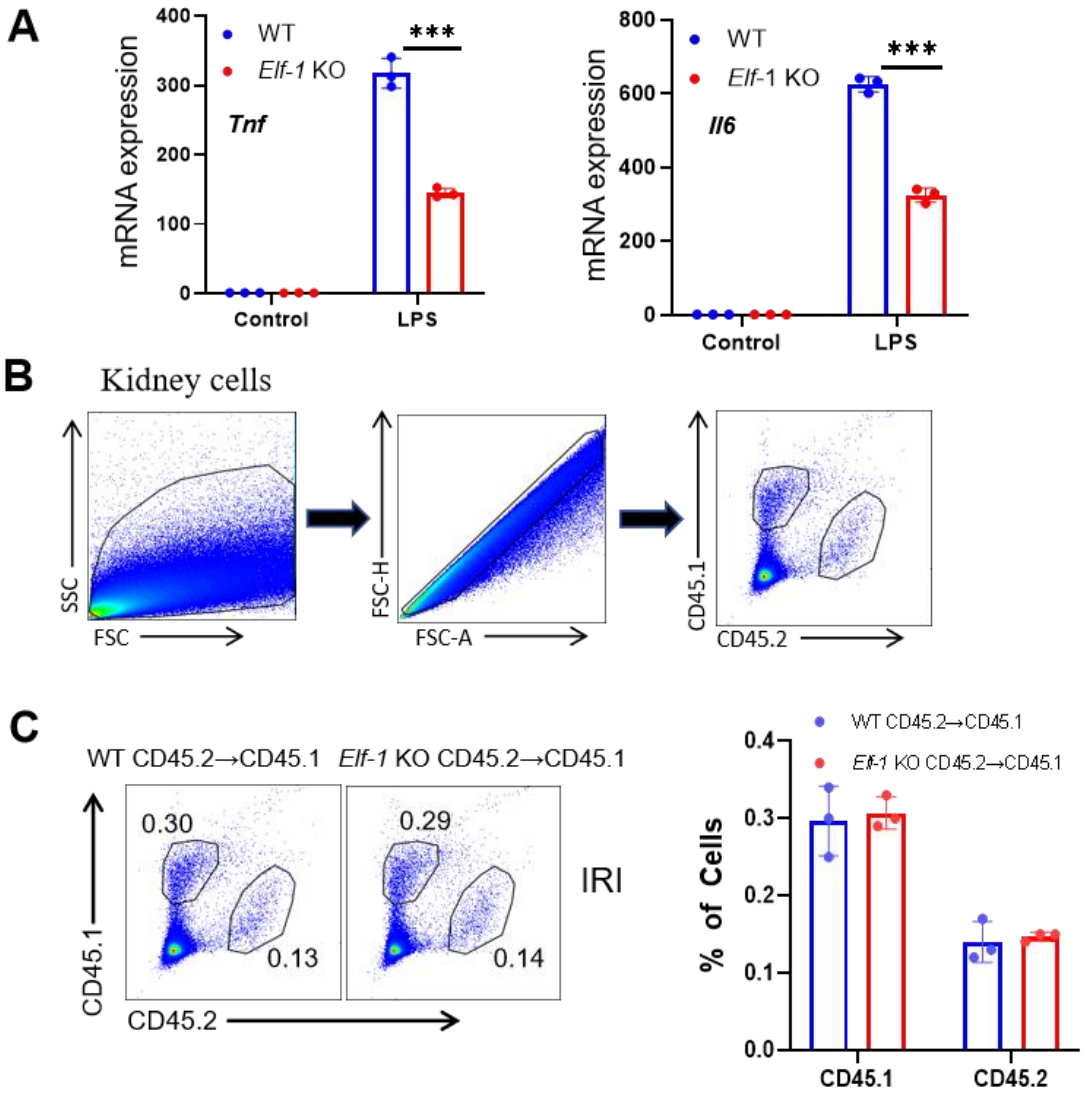
Supplementary Figure 4. ELF-1 deficiency alleviates AKI in an Ischemia-reperfusion mouse model.

(A) Flow cytometry loop gate strategy for kidney cells in WT and *Elf-1* KO mice. (B) The proportion of $F4/80^{+}CD11b^{+}$ cells were examined in the $CD45^{+}$ kidney cells of WT and *Elf-1* KO mice by flow cytometry (n=3). Data are representative of three independent experiments. Data are presented as mean ± S.D. One-way ANOVA.



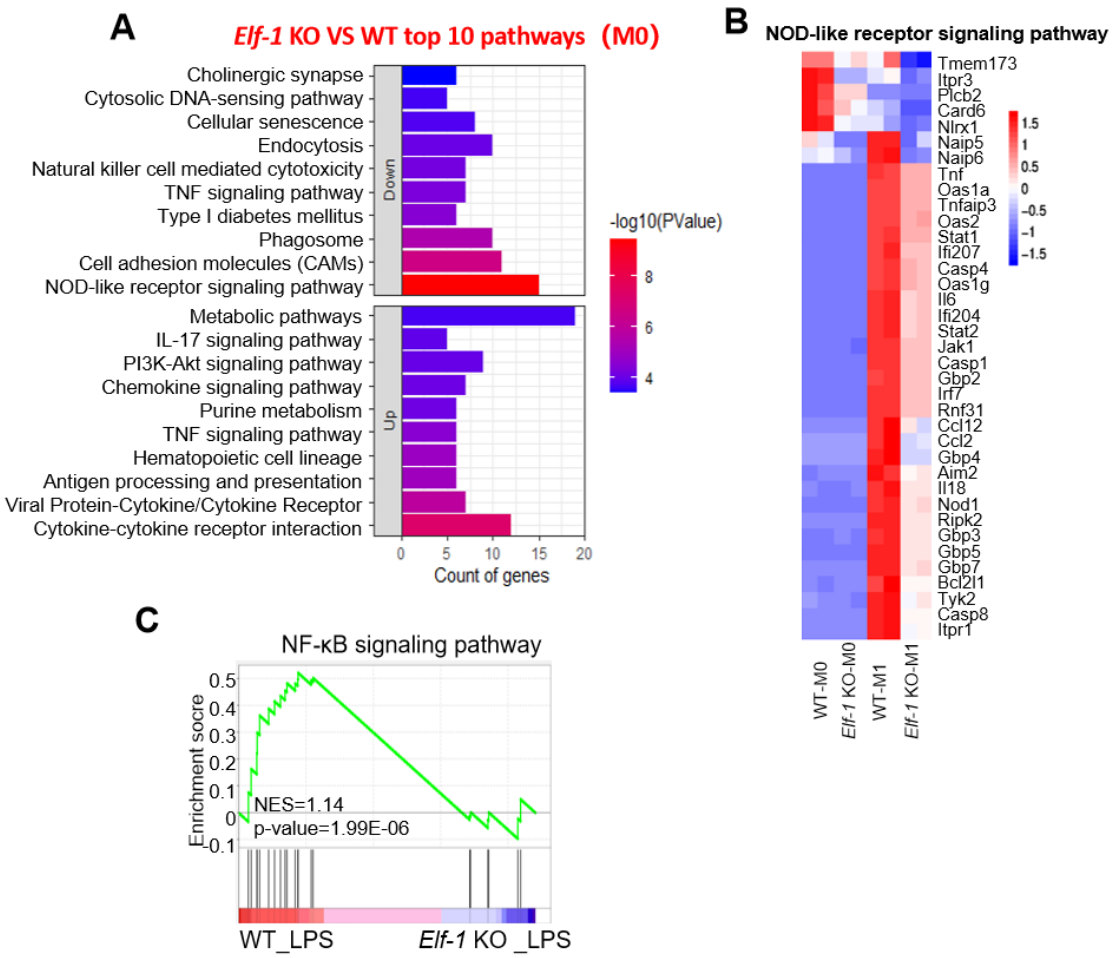
Supplementary Figure 5. ELF-1 deficiency alleviates acute liver injury in a sepsis mouse model.

(A-B) The concentrations of ALT (A) and AST (B) in the serum of WT and *Elf-1* KO mice 6 hours after LPS injection. Data are representative of three independent experiments. Data are presented as mean ± S.D. ***p < 0.001, one-way ANOVA.



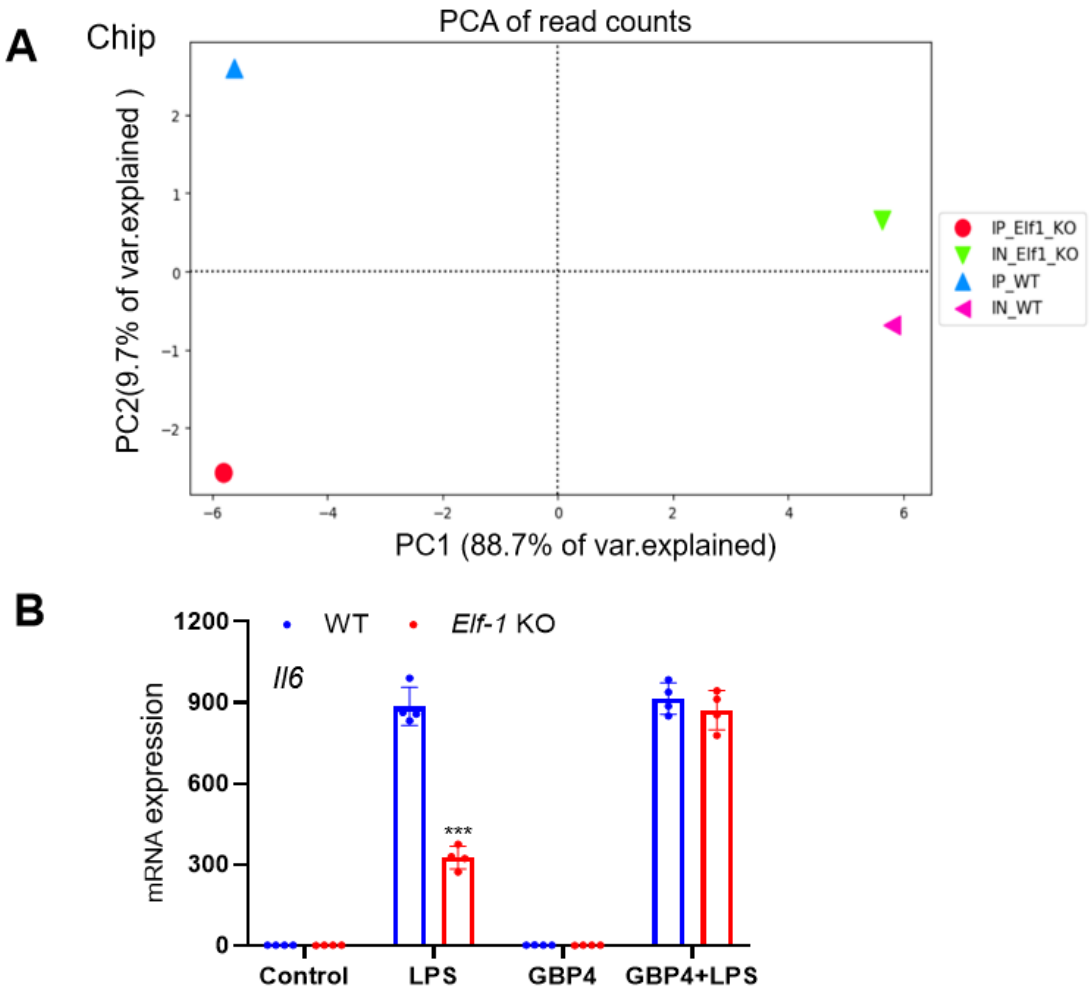
Supplementary Figure 6. ELF-1 deficiency alleviates IRI-AKI by regulating M1 macrophages.

(A) The gene expressions of *Tnf* and *Il6* in WT and *Elf-1* KO BMDMs with or without LPS stimulation at 6h were detected by qPCR. (B) Flow cytometry loop gate strategy for kidney cells in WT CD45.2→CD45.1 and *Elf-1* KO CD45.2→CD45.1 mice. (C) Flow cytometry revealed the expression of CD45.1 and CD45.2 antibodies in kidney cells of WT CD45.2→CD45.1 and *Elf-1* KO CD45.2→CD45.1 mice (n=3). Data are representative of three independent experiments. Data are presented as mean ± S.D. One-way ANOVA.



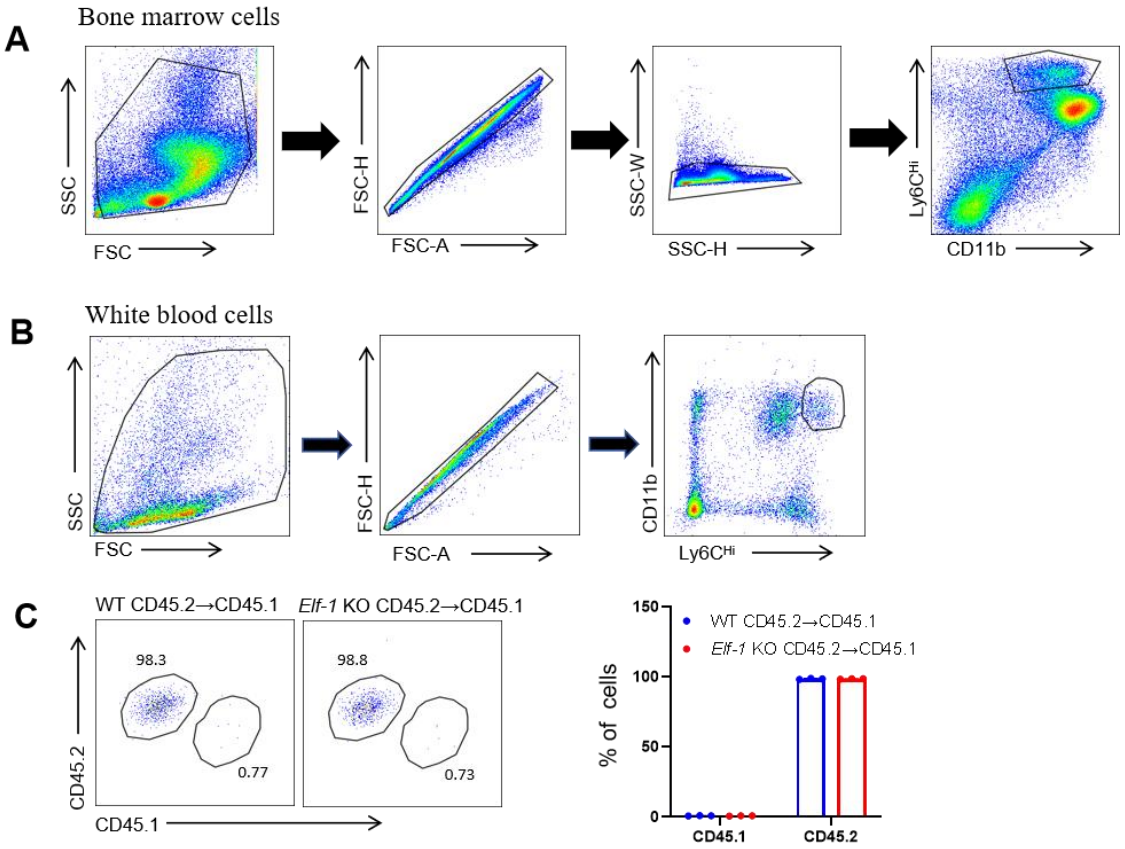
Supplementary Figure 7. ELF-1 regulates the activation of NOD-like receptors and the NF-κB signaling pathway.

(A) Top 10 regulated KEGG pathways in *Elf-1* KO vs WT (M0) up or down regulated enrichment pathways. (B) The heatmap of DEGs in NOD-like receptor signaling pathway. (C) GSEA enrichment of NF-κB signaling pathway.



Supplementary Figure 8. ChIPseq PCA plot and the role of supplementing GBP4 in vitro.

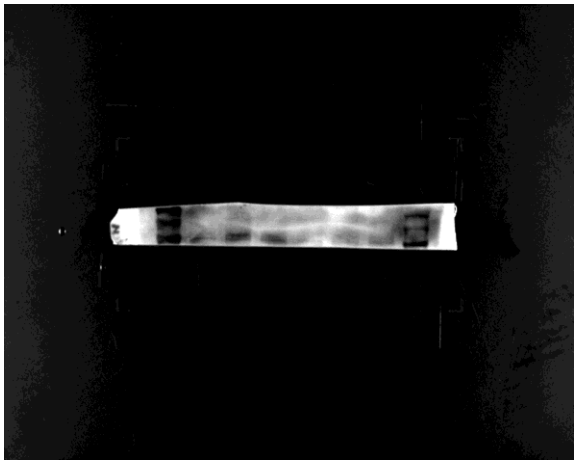
(A) ChIPseq PCA plot in WT and *Elf-1* KO mice. (B) qPCR analysis of *Il6* expression that was pretreated by recombinant GBP4 protein (10ng/mL) for 20h and thus stimulated with LPS for 6h in WT and *Elf-1* KO BMDMs (n=4). Data are representative of three independent experiments. Data are presented as mean ± S.D. ***p < 0.001, one-way ANOVA.



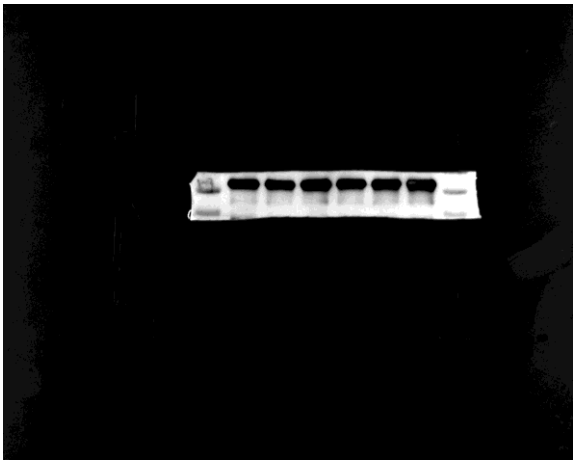
Supplementary Figure 9. The loop gate strategy in flow cytometry analysis and the detection of chimeric efficiency.

(A) Flow cytometry loop gate strategy for sorting WT bone marrow monocytes. (B) Flow cytometry loop gate strategy for white blood cells in WT CD45.2→CD45.1 and *Elf-1* KO CD45.2→CD45.1 mice. (C) Flow cytometry revealed the expression of CD45.1 and CD45.2 antibodies in white blood cells of WT CD45.2→CD45.1 and *Elf-1* KO CD45.2→CD45.1 mice (n=3). Data are representative of three independent experiments. Data are presented as mean ± S.D. One-way ANOVA.

NLRP3

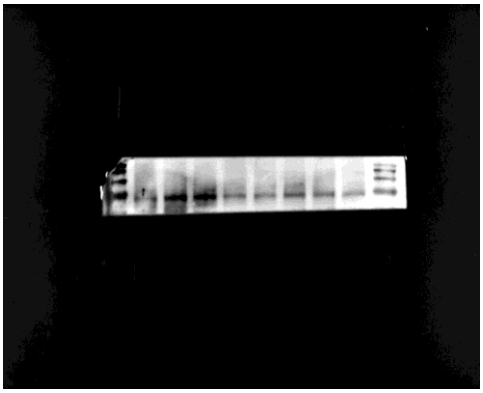


β-actin

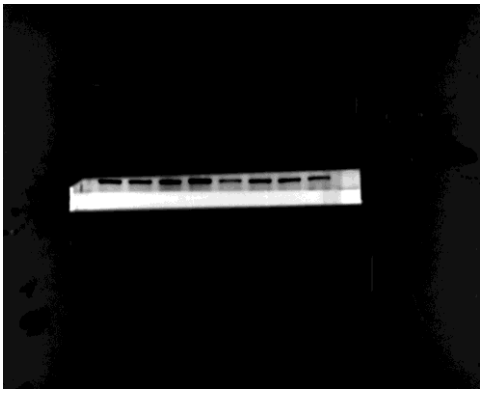


Supplementary Figure 10. Uncropped blots of Figure 6C

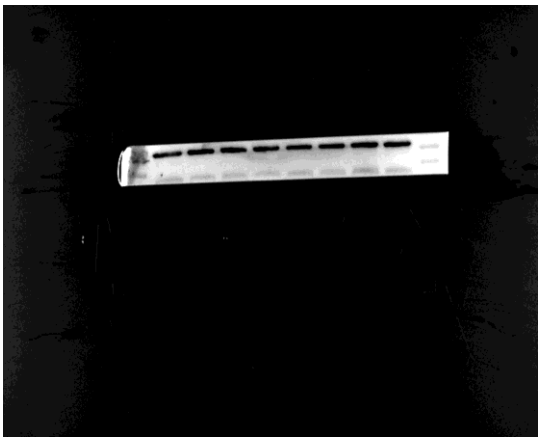
p-P65



P65



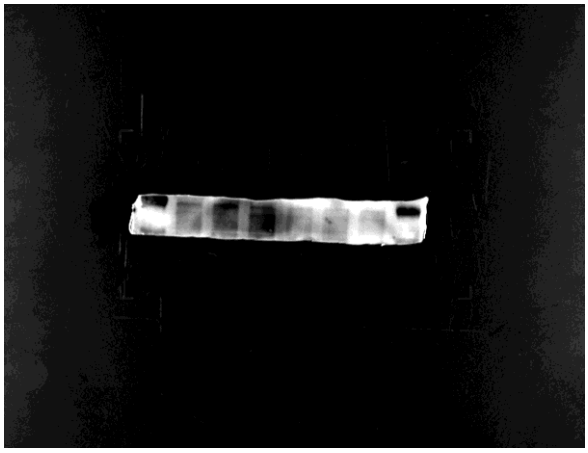
β-actin

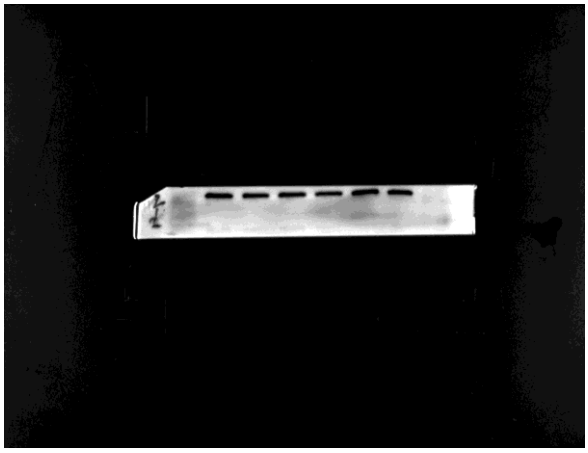


Supplementary Figure 11. Uncropped blots of Figure 6H

GBP4

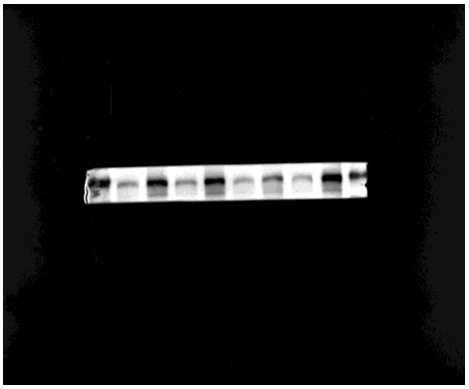
β-actin



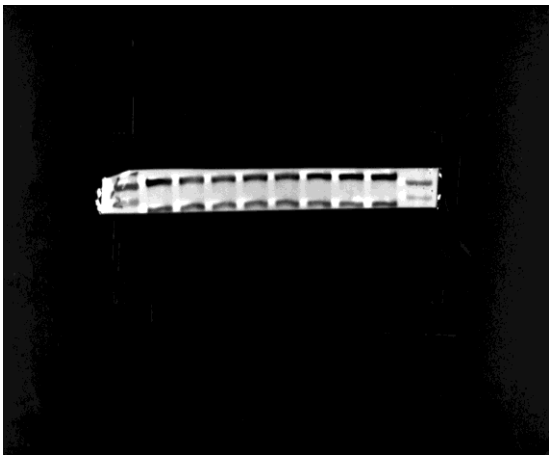


Supplementary Figure 12. Uncropped blots of Figure 7E

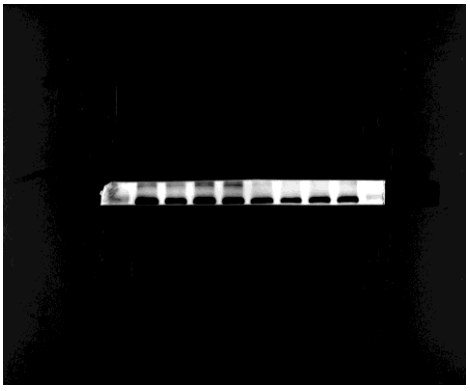
p-P65



β-actin



P65



Supplementary Figure 13. Uncropped blots of Figure 7H

Supplementary Table 1. Clinical baseline data of patients in the kidney transplantation group and the non-transplantation group

Patient characteristics	Non-kidney transplantation group	Kidney transplantation group	*P*
Cases (n)	10	10	
Age (y)	36.4±11.5	41.9±16.1	0.393
BMI (kg/m^2)	21.2±3.28	20.5±4.51	1
Gender (n)			0.325
Male	5	3	
Female	5	7	
Dialysis time (M)	28.5±20.27	37.4±24.5	0.529
Dialysis mode (n)			0.314
Hemodialysis	6	8	
Peritoneal dialysis	4	2	
Preoperative UN (mmol/L)	20.1±6.72	20.1±7.43	0.971
Preoperative Scr (μmol/L)	963±255	832±334	0.436
Comorbidity (n)			
Hypertension	9	8	0.500
Anemia	5	4	0.500
hepatitis B	1	0	0.500
Diabetes mellitus	0	1	0.500
Primary disease (n)			
IgA Nephropathy	2	2	0.709
Polycystic kidney	1	0	0.500
Focal glomerulonephritis	1	1	0.763
Presumed glomerulonephritis	6	6	0.675
Membranous nephropathy	0	1	0.500

Supplementary Table 2. Primers used for genotyping and qPCR

Gene	Primer sequence (5′–3′)	Use
M-*Elf-1*- forward	GCTCATTGGATGTTGCTGAAG	qPCR
M-*Elf-1*- reverse	GAGGGCTGGGAGAATCTATATTG	qPCR
M-*Tnf*- forward	CTTCTGTCTACTGAACTTCGGG	qPCR
M-*Tnf*- reverse	CAGGCTTGTCACTCGAATTTTG	qPCR
M-*Il6*- forward	CAAAGCCAGAGTCCTTCAGAG	qPCR
M-*Il6*- reverse	GTCCTTAGCCACTCCTTCTG	qPCR
M-*Hprt*- forward	AGTACAGCCCCAAAATGGTTAAG	qPCR
M-*Hprt*- reverse	CTTAGGCTTTGTATTTGGCTTTTC	qPCR
M-*Il1b*- forward	ACGGACCCCAAAAGATGAAG	qPCR
M-*Il1b*- reverse	TTCTCCACAGCCACAATGAG	qPCR
M-*Ccl4*- forward	TGACCAAAAGAGGCAGACAG	qPCR
M-*Ccl4*- reverse	GTGAGAAGCATCAGGGCTG	qPCR
M-*Ptgs2*- forward	CTCACGAAGGAACTCAGCAC	qPCR
M-*Ptgs2*- reverse	GGATTGGAACAGCAAGGATTTG	qPCR
M-*Gadd45b*- forward	CCTGGTCACGAACTGTCATAC	qPCR
M-*Gadd45b*- reverse	GTTGCTTTAGATGTTTGGAGTGG	qPCR
M-*Ddx58*- forward	GATGAAGGAGACAGAGAAGCTAG	qPCR
M- *Ddx58*- reverse	TCTGCCATCTGAAACACTGAG	qPCR
H-*ELF-1*- forward	CCTCAATATGGATTCCCCTGG	qPCR
H-*ELF-1*- reverse	CTCTTAGGCTGTTCTGGTGATG	qPCR
H-*TNFA*- forward	ACTTTGGAGTGATCGGCC	qPCR
H-*TNFA*- reverse	GCTTGAGGGTTTGCTACAAC	qPCR
H-*IL6*- forward	CCACTCACCTCTTCAGAACG	qPCR
H-*IL6*- reverse	CATCTTTGGAAGGTTCAGGTTG	qPCR
H-*GAPDH*- forward	GGAGCGAGATCCCTCCAAAAT	qPCR
H-*GAPDH*- reverse	GGCTGTTGTCATACTTCTCATGG	qPCR
H-*IL1B*- forward	ATGCACCTGTACGATCACTG	qPCR
H-*IL1B*- reverse	ACAAAGGACATGGAGAACACC	qPCR
Elf-1- forward WT	CGGGACTAAATAGGGTTG	Genotyping
Elf-1- forward WT	GAGATGGAAGGGACGAGA	Genotyping
Elf-1- forward KO	ATCAGGCTTCCATGCCAAGT	Genotyping
Elf-1- reverse KO	TTCTCCCTTGTCAAAGGCCC	Genotyping

Supplementary Table 3. Detailed description of the antibodies used.

Antibody name	Company	Concentration	Product number
Anti-β-actin	Sigma	WB 1:40000	A5441
Anti-P65	Cell Signaling Technology	WB 1:1000	6956
Anti-p-P65	Cell Signaling Technology	WB 1:1000	3033
Anti-NLRP3	Cell Signaling Technology	WB 1:1000	15101
Anti-GBP4	Abcam	WB 1:500	AB232689
PE/Cy5 anti-mouse CD11b Antibody	ThermoFisher	FLOW 1:3200	15-0112-83
PE anti-mouse F4/80 Antibody	BioLegend	FLOW 1:250	123110
FITC anti-mouse TNF-α Antibody	ThermoFisher	FLOW 1:400	12-7321-82
FITC anti-mouse CD86 Antibody	eBioscience	FLOW 1:400	105006
FITC anti-mouse CD206 Antibody	BioLegend	FLOW 1:200	141703
APC anti-mouse CD45 Antibody	BioLegend	FLOW 1:200	103112
PE/Cyanine7 anti-mouse CD45.1 Antibody	BioLegend	FLOW 1:400	110730
PE/Cy5.5 anti-anti-mouse CD45.2 Antibody	BioLegend	FLOW 1:400	109827
FITC anti-mouse Ly-6C Antibody	BioLegend	FLOW:1:400	128005

Supplementary Table 4. The sequences of siRNA used to knock down Elf-1

Name	sequence (5′–3′)
SiElf-1-1 sense	CCGACGACUACACCAAAUA
SiElf-1-1 antisense	UAUUUGGUGUAGUCGUCGG
SiElf-1-2 sense	UAUUUGGUGUAGUCGUCGG
SiElf-1-2 antisense	UAGUCCUAAUACUUGGAAC