
Supplemental Material

Macrophage ILF3 promotes abdominal aortic aneurysm by inducing inflammatory imbalance in male mice

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Fig. S1-S12

Supplemental Table 1-4

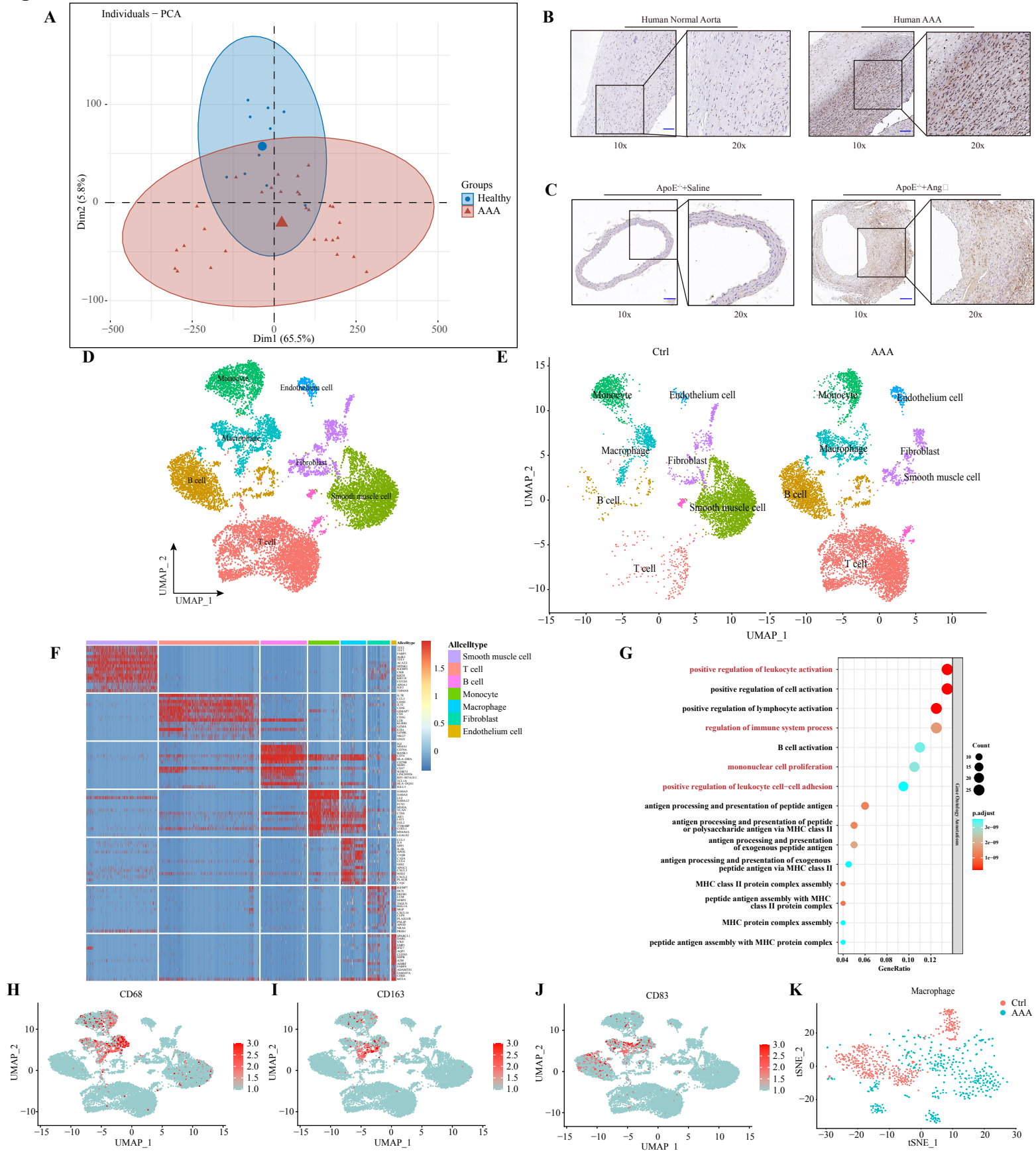
Fig. S1

Fig. S1. Relation of ILF3 expression to the progression of abdominal aortic aneurysm (AAA).

A, Principal component analysis (PCA) for human and mouse samples.

B-C, Representative immunostaining for ILF3 on aortic cross sections of controls without AAA and patients (B) or mice (C) with AAA. Colorimetric images showing ILF3 in brown (n=6 per group, scar bar =100 μ m).

D, UMAP plots showing the seven cell types colored according to cell cluster from samples of human AAA and controls.

E, UMAP plots showing the seven cell types colored according to cell cluster in human AAA and non-aneurysm samples.

F, Dot plots showing expression of selected marker genes for each cluster.

G, Representative Gene Ontology (GO) terms for DEGs enriched in ILF3⁺ cell clusters based on functional enrichment analysis.

H-J, UMAP feature plot of macrophage cell types displaying CD68(H), CD163(I) and CD83(J) expression.

K, tSNE projections of macrophage clusters colored according to human AAA and non-aneurysm samples.

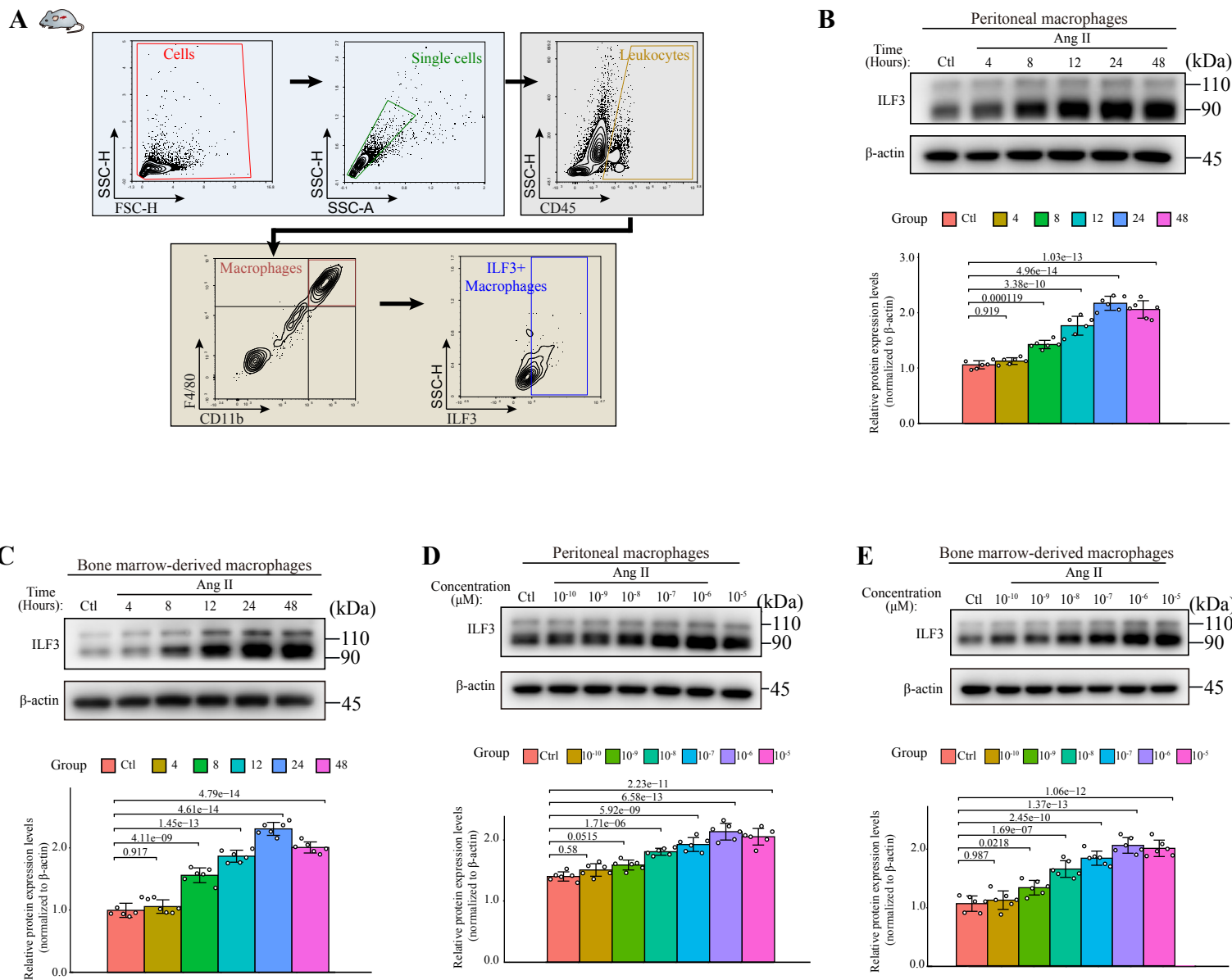
Fig. S2

Fig. S2. Relation of ILF3 expression to the progression of AAA.

A, Gating strategy for ILF3⁺ macrophages derived from the suprarenal aortic regions of AAA model mice, post their exposure to Ang II infusion at specified time points.

B-C, Representative images of western blot and analysis for the level of ILF3 in peritoneal macrophages (B) and bone marrow-derived macrophages (C) treated with Ang II at different time points (1μM; n=6 per group).

D-E, Representative images of western blot and analysis for the level of ILF3 in peritoneal macrophages (D) and bone marrow-derived macrophages (E) treated with Ang II at different concentrations (24 hours; n=6 per group).

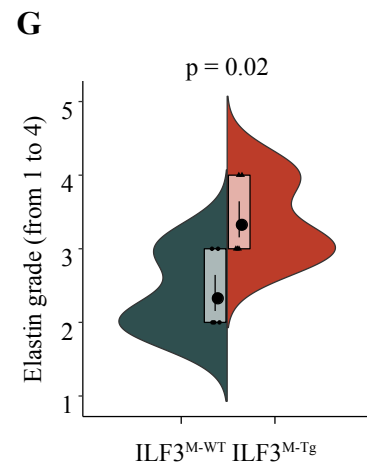
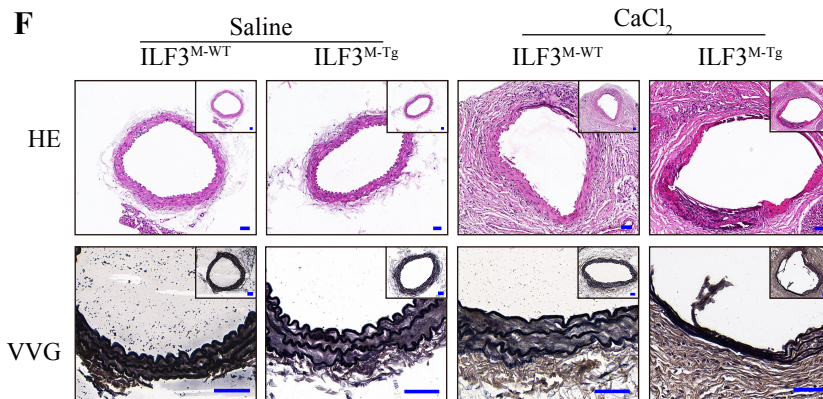
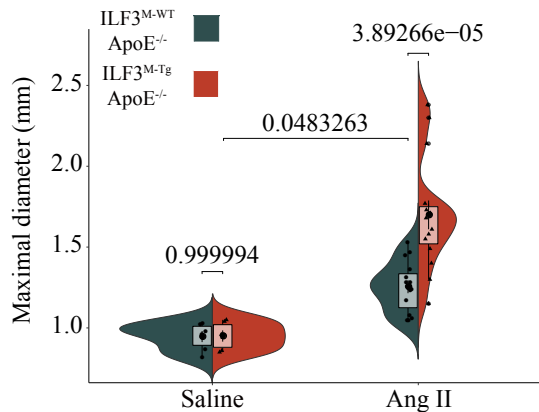
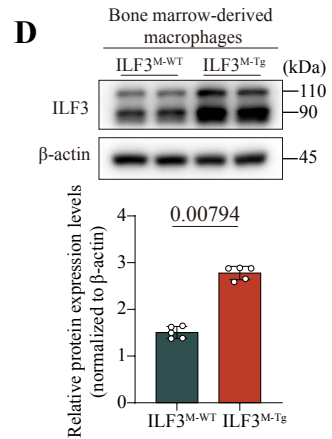
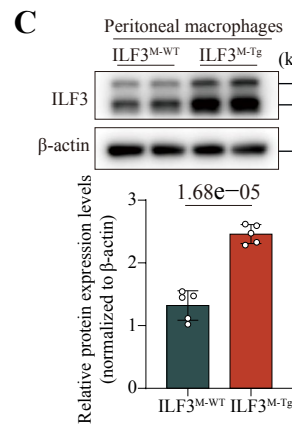
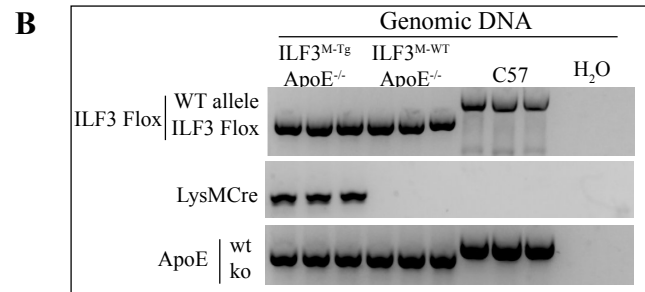
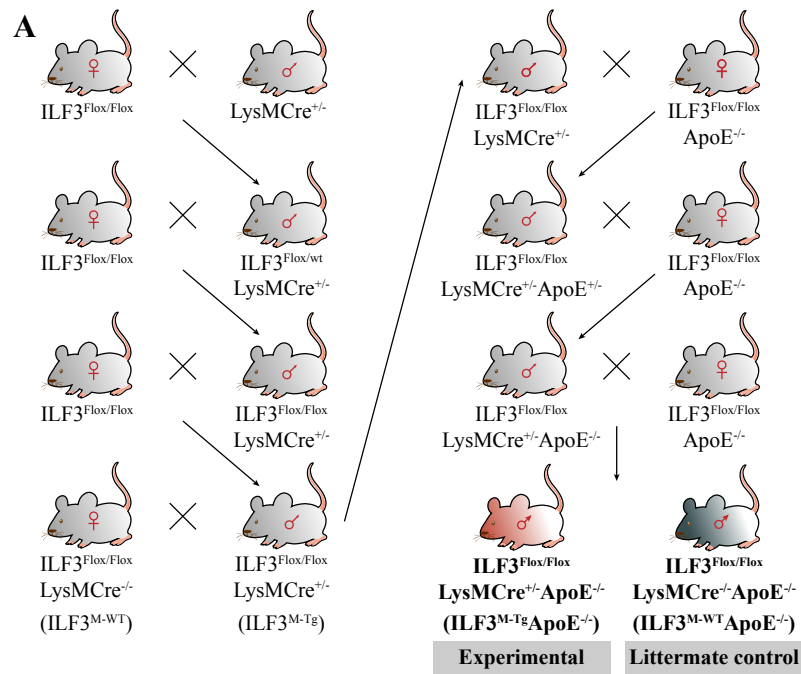
Fig. S3

Fig. S3. Macrophage ILF3 overexpression promoted AAA formation.

A, Schematic illustration of generation of macrophage-specific ILF3 transgenic mice on an ApoE-deficient background.

B, Genomic PCR confirms the phenotype of ILF3^{M-Tg} ApoE^{-/-} mice.

C-D, Western blot analysis of ILF3 levels in peritoneal macrophages (C) and bone marrow-derived macrophages (D) from ILF3^{M-Tg} and ILF3^{M-WT} mice (n=5).

E, Quantification of maximal external diameters of suprarenal abdominal aortas (sham group, n=6; Ang II group, n=15).

F-G, Representative images of HE and VVG staining (F), and the elastin degradation score (G) of abdominal aortas from Saline-treated and CaCl₂-treated mice (sham group, n=3; CaCl₂ group, n=5; Scale bar= 100 μm).

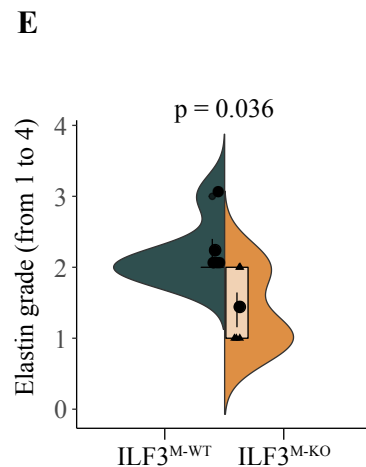
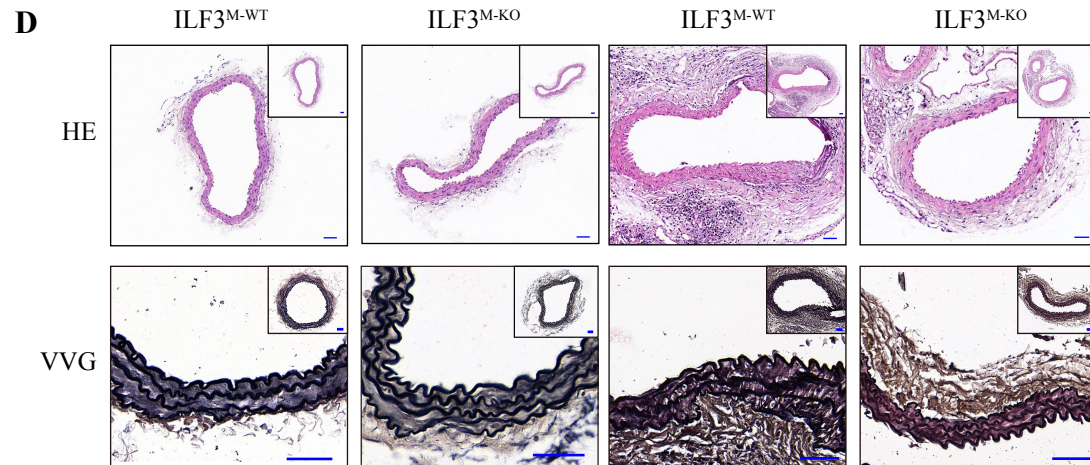
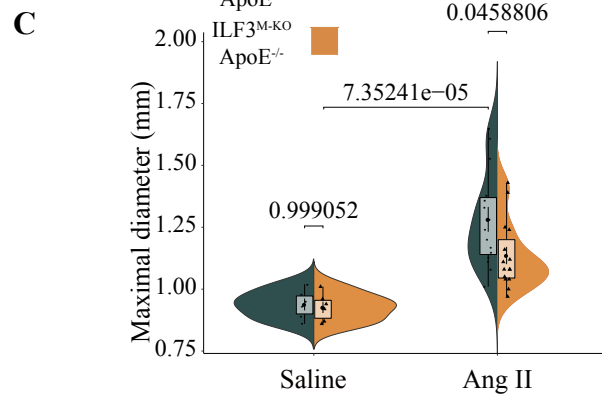
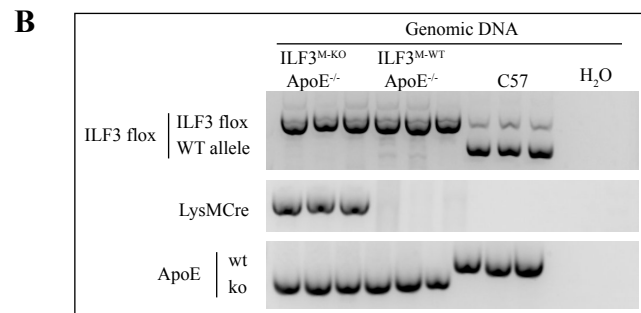
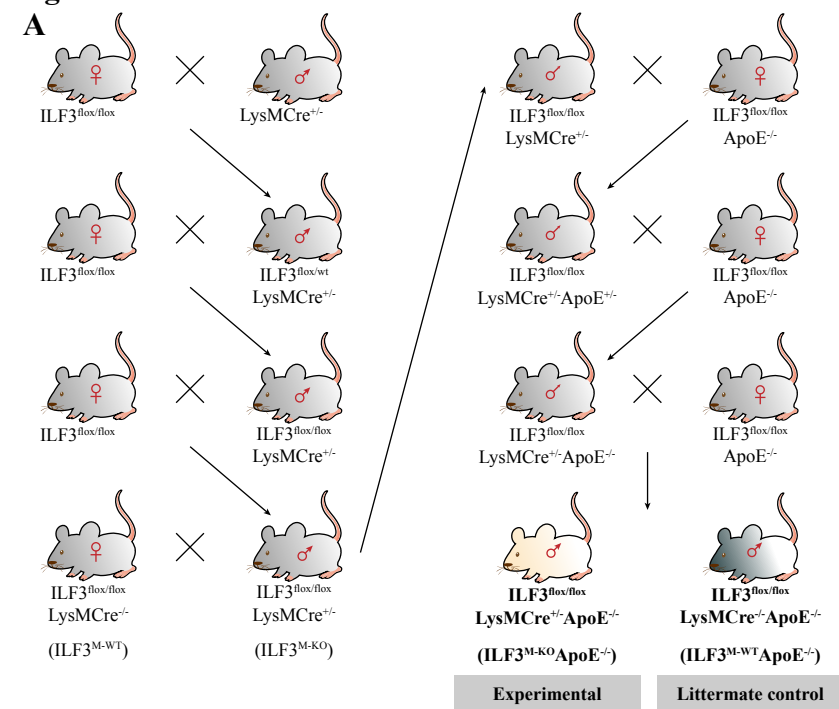
Fig. S4

Fig. S4. Macrophage ILF3 deficiency attenuates CaCl₂-induced AAA formation.

A, Schematic illustration of the generation of macrophage-specific ILF3 deficient mice on an ApoE-deficient background.

B, Genomic PCR confirms the phenotype of ILF3^{M-KO} ApoE^{-/-} mice.

C, Quantification of maximal external diameters of suprarenal abdominal aortas. (sham group, n=6; Ang II group, n=15).

D-E, Representative images of HE and VVG staining (D), and the elastin degradation score (E) of abdominal aortas from Saline-treated and CaCl₂-treated mice (sham group, n=3; CaCl₂ group, n=5; Scale bar= 100 μm).

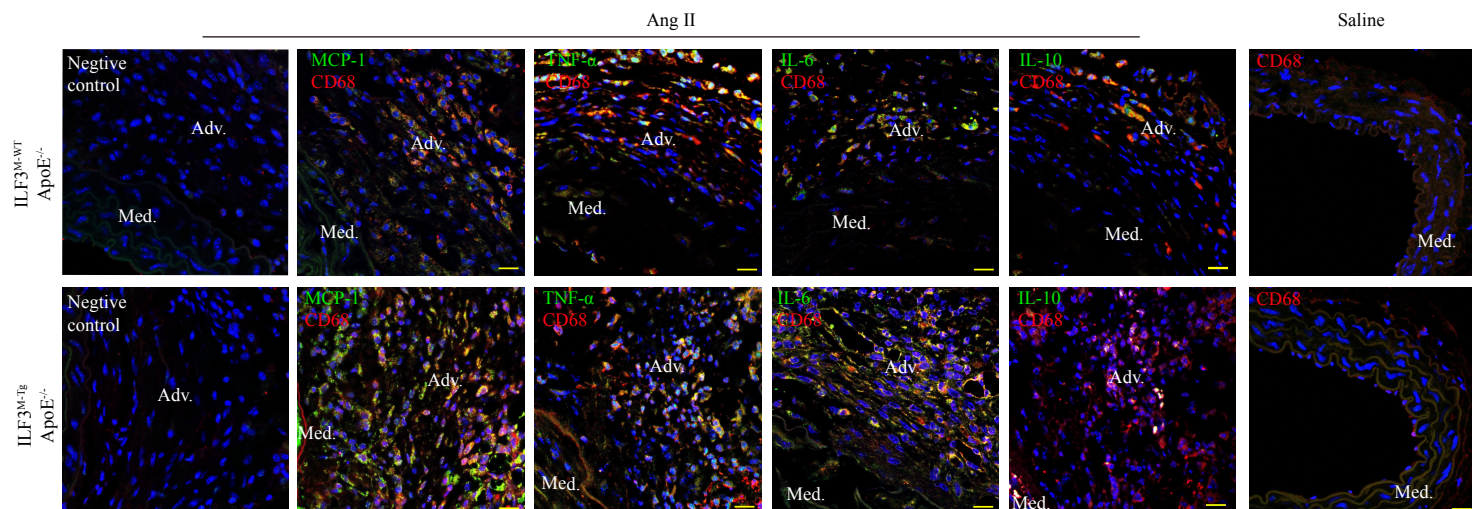
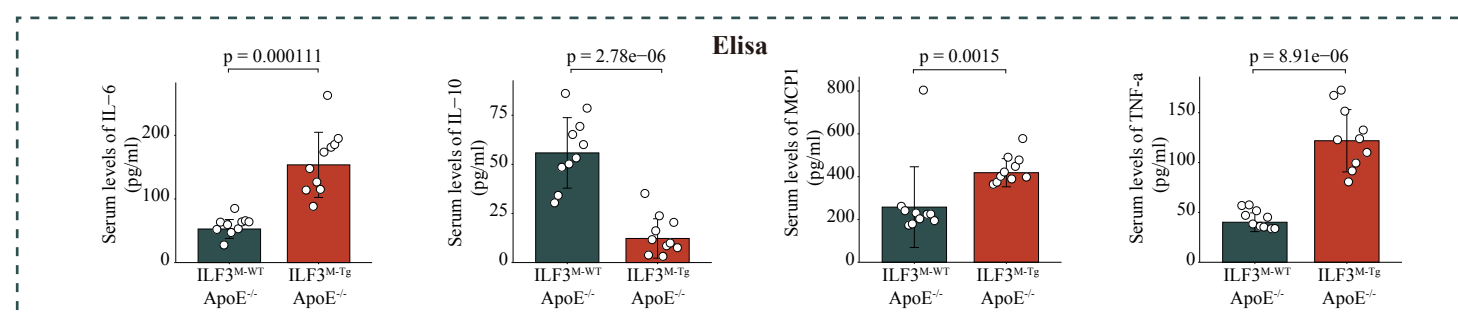
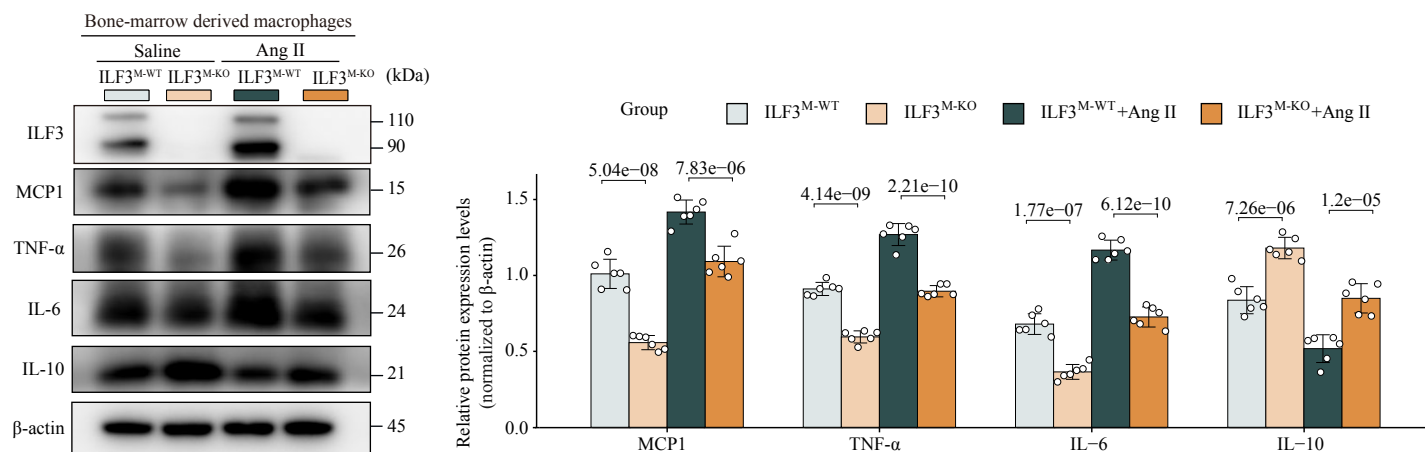
Fig. S5**A****B****C**

Fig. S5. Macrophage ILF3 mediates the macrophage inflammatory response in AAA lesions.

A, Immuno-staining of macrophage-derived MCP1, IL-6, IL-10 and TNF- α levels in AAA lesions of ILF3^{M-WT} ApoE^{-/-} and ILF3^{M-Tg} ApoE^{-/-} mice treated with Ang II for four weeks. (n=5 per group; Scale bar=25 μ m).

B, Plasma IL-6, TNF- α , MCP1, IL10 levels in ILF3^{M-WT} ApoE^{-/-} and ILF3^{M-Tg} ApoE^{-/-} mice treated with Ang II for four weeks. (n=10 per group).

C, Western blot analysis and quantification of ILF3, MCP1, TNF- α , IL-6, and IL-10 expression in bone marrow-derived macrophages from the ILF3 deficiency and control groups with or without Ang II treatment (1 μ M; n=6 per group).

Fig. S6

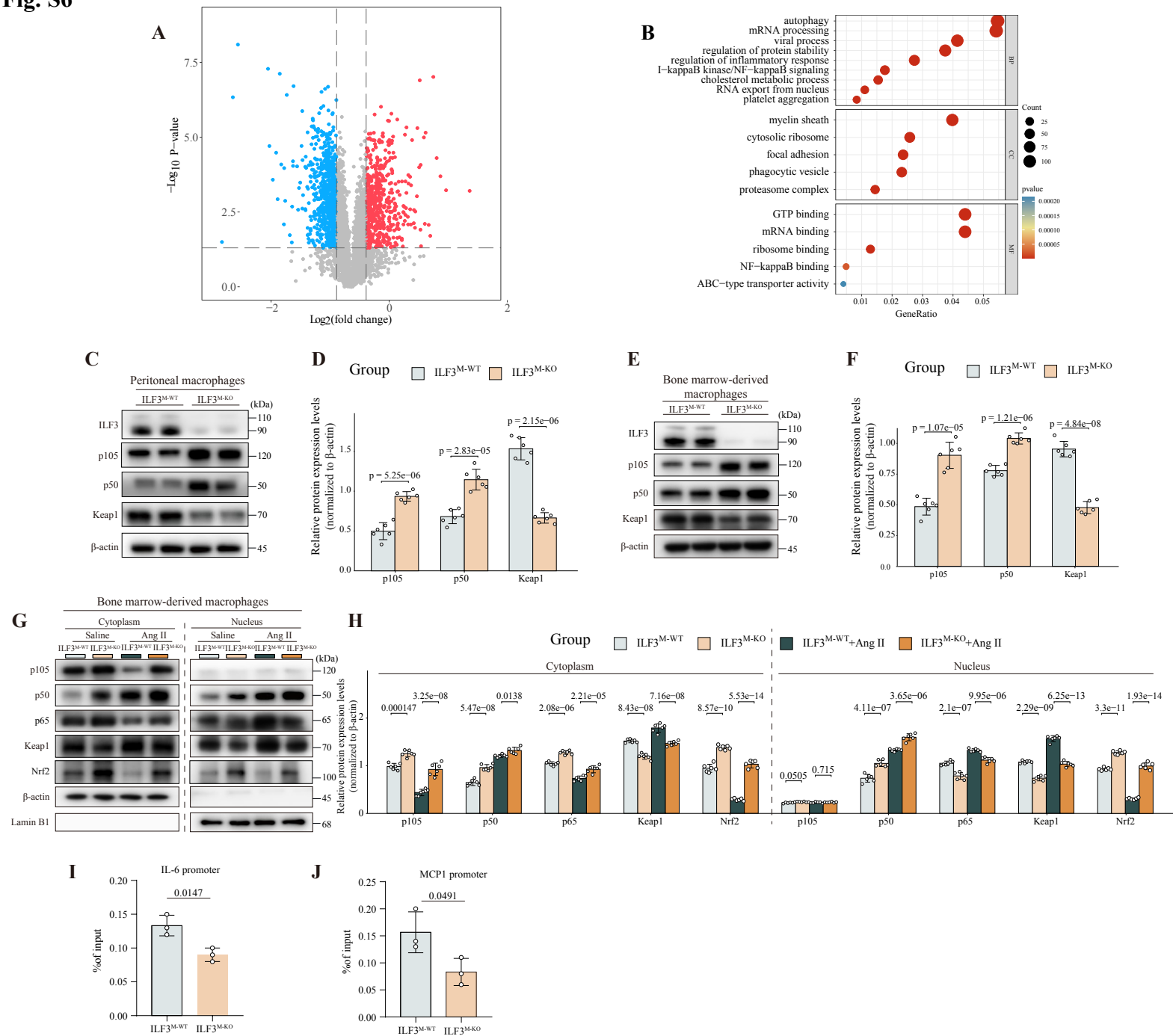


Fig. S6. The influence of ILF3 on the Keap1-Nrf2 and NF- κ B pathways.

A, Volcano plot displaying all proteins differentially expressed between the ILF3^{M-WT} and ILF3^{M-KO} groups. Proteins with significantly decreased levels in the ILF3^{M-KO} group ($P < 0.05$) are shown in blue, while the proteins with significantly increased levels are visualized in red.

B, Gene Ontology enrichment of differentially expressed genes (DEGs). The dot size represents the number of genes under a specific term. The color of the dots represents the P-value.

C-D, Western blot analysis and quantification of p105, p50, and Keap1 expression in peritoneal macrophages from ILF3^{M-WT} and ILF3^{M-KO} mice under basal conditions (n=6 per group).

E-F, Western blot analysis and quantification of p105, p50, and Keap1 expression in bone marrow-derived macrophages from ILF3^{M-WT} and ILF3^{M-KO} mice under basal conditions (n=6 per group).

G-H, Western blot analysis and quantification of cytoplasmic and nuclear p105, p50, p65, Keap1, and Nrf2 expression in bone marrow-derived macrophages from ILF3^{M-WT}, ILF3^{M-KO} treated with saline or Ang II (n=6 per group).

I-J, Chromatin immunoprecipitation (ChIP) assay was applied to confirm the Nrf2 binding at the ARE sequence of IL-6 and MCP1 promoters in macrophages (n=3 per group).

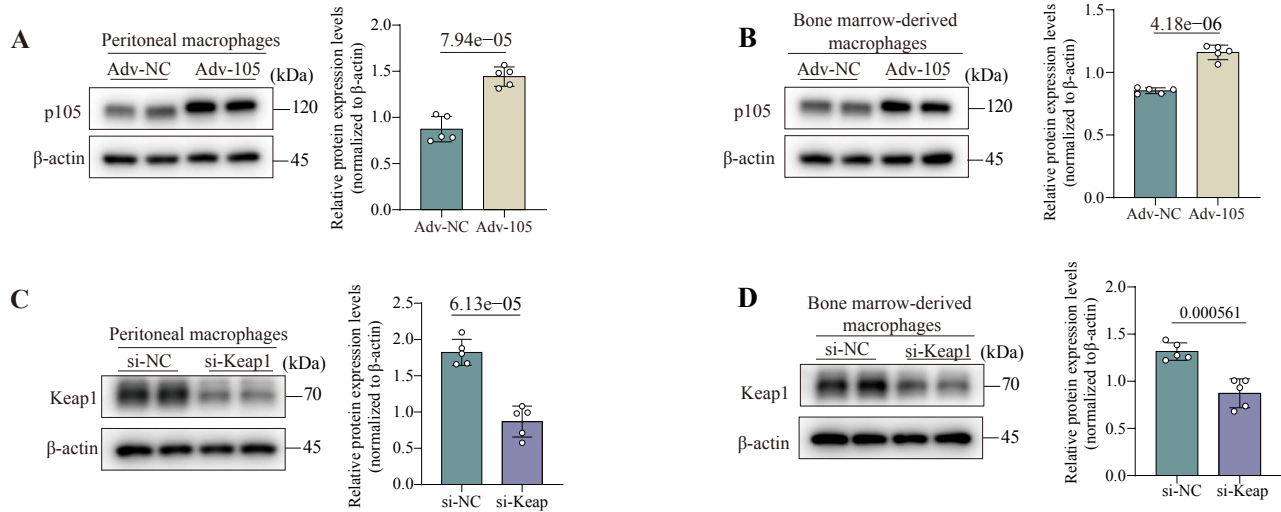
Fig. S7

Fig. S7. Assessment of Adv-105-mediated overexpression and siRNA-induced knockdown of Keap1 in macrophages.

A-B, Quantification of p105 protein levels in peritoneal macrophages (A) and bone marrow-derived macrophages (B) following treatment with either Adv-105 or a control vector (Adv-NC; n=5).

C-D, Evaluation of Keap1 protein expression in peritoneal macrophages (C) and bone marrow-derived macrophages (D) after siRNA knockdown (si-Keap1) compared with control siRNA (si-NC; n=5).

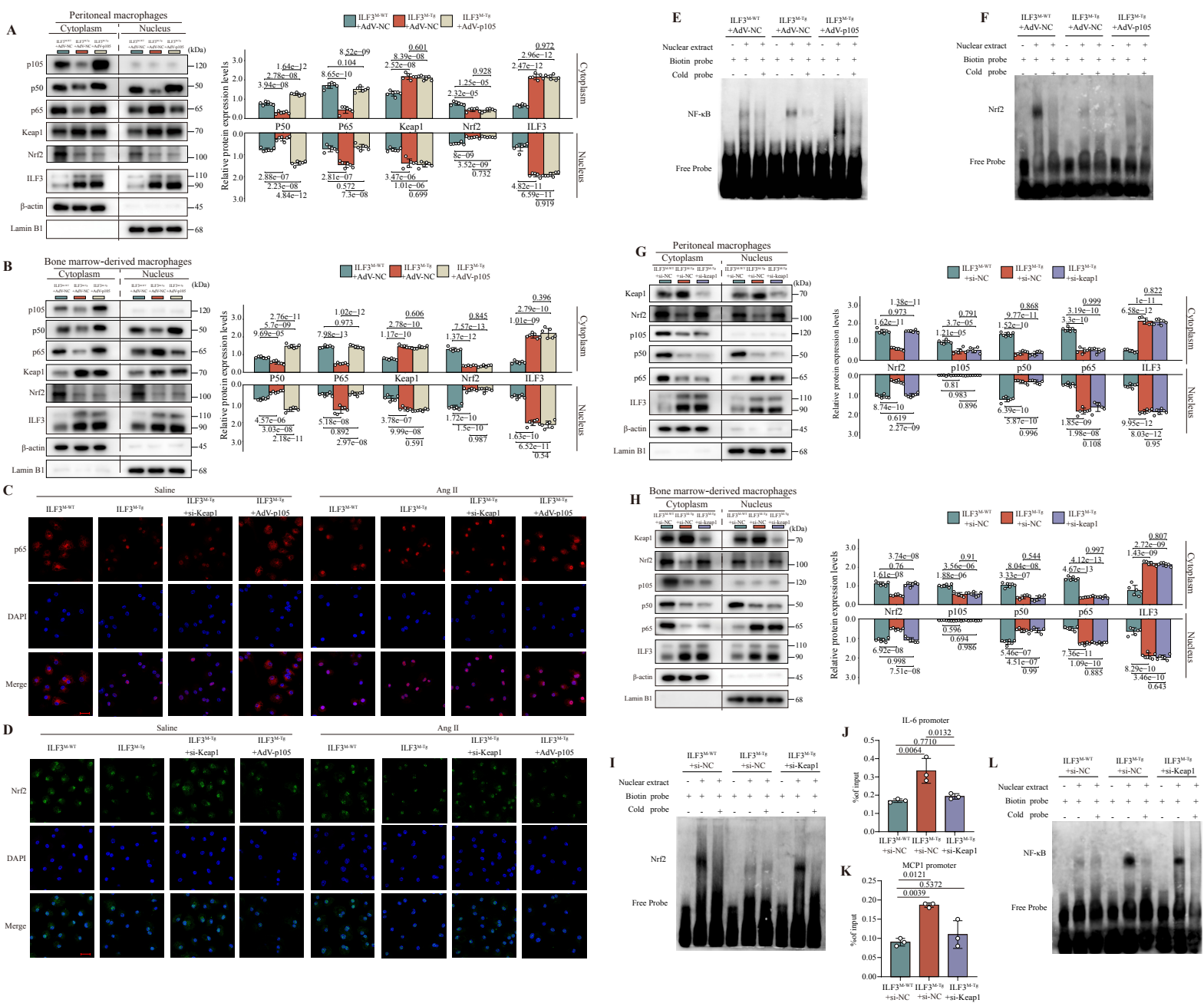


Fig. S8. Effect of p105 overexpression and Keap1 deficiency on ILF3-induced activation of the NF-κB and Keap1-Nrf2 pathways.

A, Western blot analysis and quantification of cytoplasmic and nuclear p105, p50, p65, Keap1, and Nrf2 expression in peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with adenovirus overexpressing p105(AdV-p105), which were all under stimulus with Ang II (n=6 per group).

B, Western blot analysis and quantification of cytoplasmic and nuclear p105, p50, p65, Keap1, and Nrf2 expression in bone marrow-derived macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with adenovirus overexpressing p105(AdV-p105), which were all under stimulus with Ang II (n=6 per group).

C, Immunofluorescence staining for p65 (red) in peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with AdV-p105 or Keap1 small interfering RNA (si-Keap1); nuclei were stained with DAPI (blue, Scale bar= 20 μm; n=6 per group).

D, Immunofluorescence staining for Nrf2 (green) in peritoneal macrophages from groups of ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} treated with AdV-p105 or si-Keap1; nuclei were stained with DAPI (blue, Scale bar= 20 μm; n=6 per group).

E, EMSA analysis showing the DNA binding activity of NF-κB in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with AdV-p105 under stimulus with Ang II (1μM, 24h; n=6 per group).

F, EMSA analysis showing the DNA binding activity of Nrf2 in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with AdV-p105 under stimulus with Ang II (1μM, 24h; n=6 per group).

G, Western blot assessment quantifying cytoplasmic and nuclear Keap1, Nrf2, p105, p50, and p65 expression in peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with si-Keap1 under stimulus with Ang II. (n=6 per group).

H, Western blot assessment quantifying cytoplasmic and nuclear Keap1, Nrf2, p105, p50, and p65 expression in bone marrow-derived macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with si-Keap1 under stimulus with Ang II. (n=6 per group).

I, EMSA analysis showing the DNA binding activity of Nrf2 in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with si-Keap1 under stimulus with Ang II (1μM, 24h; n=6 per group).

J-K, ChIP assay was applied to confirm the Nrf2 binding at the ARE sequence of IL-6 and MCP1 promoters in macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with si-Keap1 (n=3 per group).

L, EMSA analysis showing the DNA binding activity of NF-κB in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with si-Keap1 under stimulus with Ang II (1μM, 24h; n=6 per group).

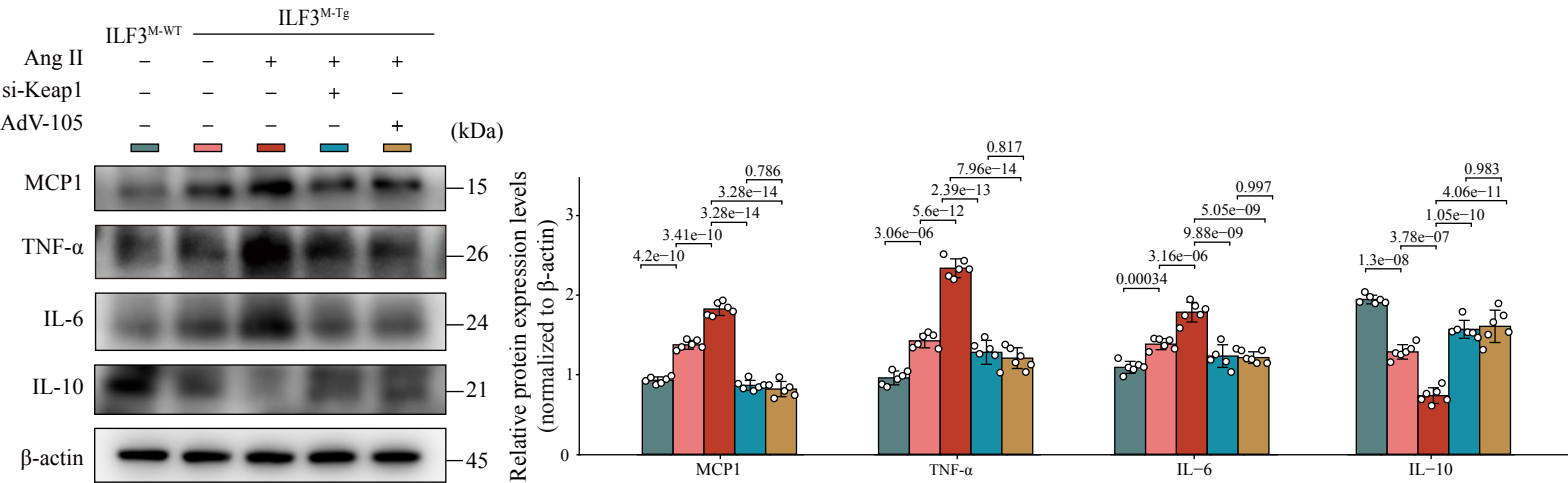
Fig. S9**Bone marrow-derived macrophages**

Fig. S9. Effect of p105 overexpression and Keap1 deficiency on the ILF3-induced inflammatory response.

Western blot analysis and quantification of MCP1, TNF- α , IL-6, and IL-10 expression in bone marrow-derived macrophages from ILF3^{M-WT} and ILF3^{M-Tg} mice treated with si-Keap1 and AdV-p105 under stimulus without or with Ang II (1 μ M; n=6 per group).

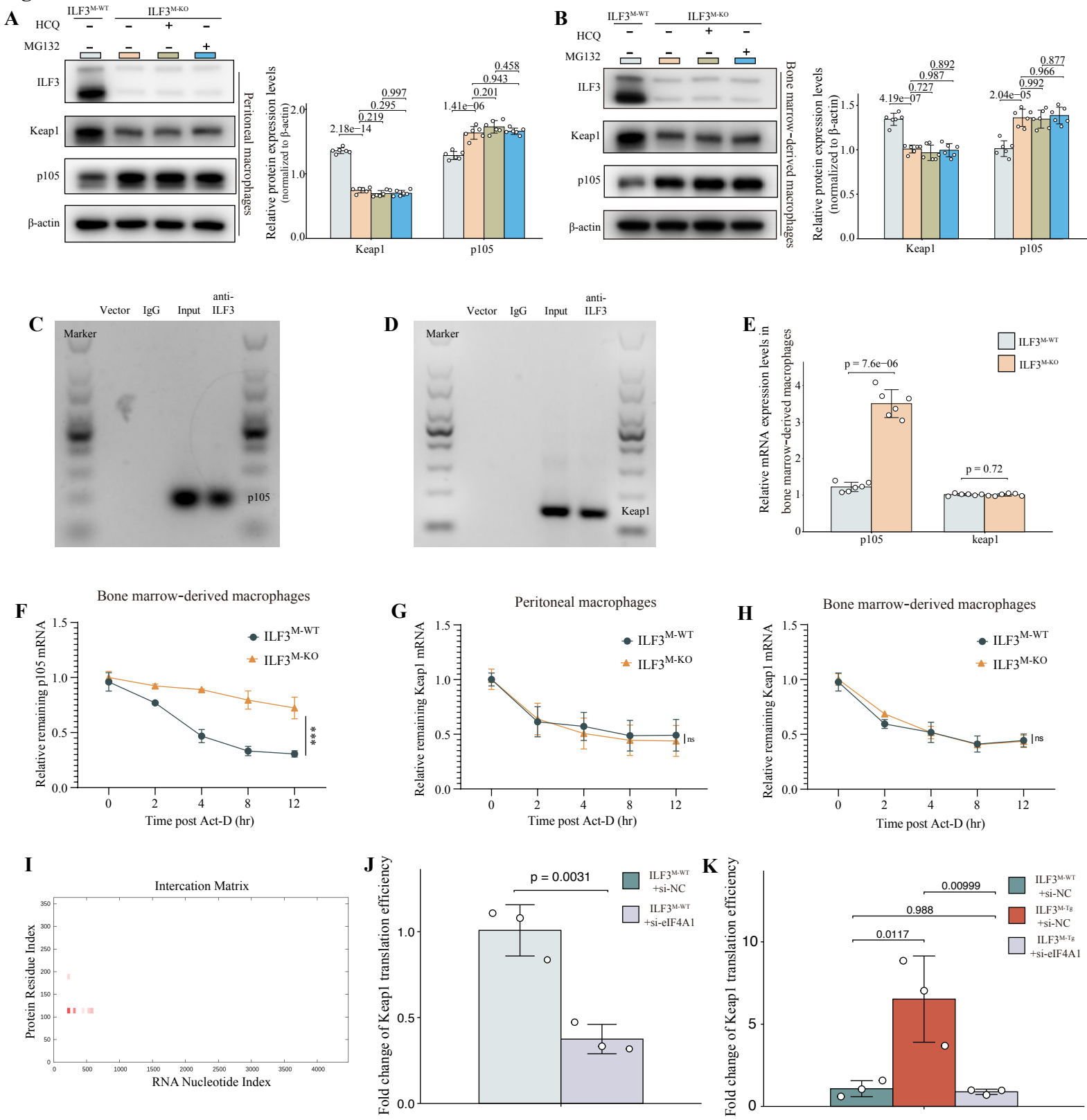
Fig. S10

Fig. S10. Effect of ILF3 on Keap1 and Nrf2 protein and RNA expression.

A and B, Immunoblot and quantification of protein levels of Keap1 and p105 after inhibition of proteasomal and lysosomal degradation in peritoneal macrophages and bone marrow-derived macrophages from ILF3^{M-WT} and ILF3^{M-KO} mice (n=6 per group).

C and D, RIP assay for enrichment of p105(C) and Keap1(D) mRNA with ILF3 in wild-type peritoneal macrophages; IgG served as an internal control (n=6 per group).

E, Relative mRNA levels of Keap1 and p105 in bone marrow-derived macrophages from ILF3^{M-WT} and ILF3^{M-KO} mice (n=6 per group).

F, Relative p105 RNA levels at different time points after actinomycin-D treatment in bone marrow-derived macrophages from ILF3^{M-WT} and ILF3^{M-KO} mice (n=6 per group).

G and H, Relative Keap1 RNA levels at different time points after actinomycin-D treatment in peritoneal macrophages (G) and bone marrow-derived macrophages (H) from ILF3^{M-WT} and ILF3^{M-KO} mice (n=6 per group).

I, The interaction matrix showing catRAPID prediction of interaction between eIF4A1 protein and the Keap1 mRNA. The x- and the y-axes represent the indexes of the RNA and protein sequences, respectively.

J, Box plot illustrating the translation efficiency of Keap1 mRNAs in peritoneal macrophages from ILF3^{M-WT} mice treated with si-NC or si-eIF4A1 (n=3 per group).

K, Box plot showing the translation efficiency of Keap1 mRNAs in peritoneal macrophages of different groups (n=3 per group).

Fig. S11

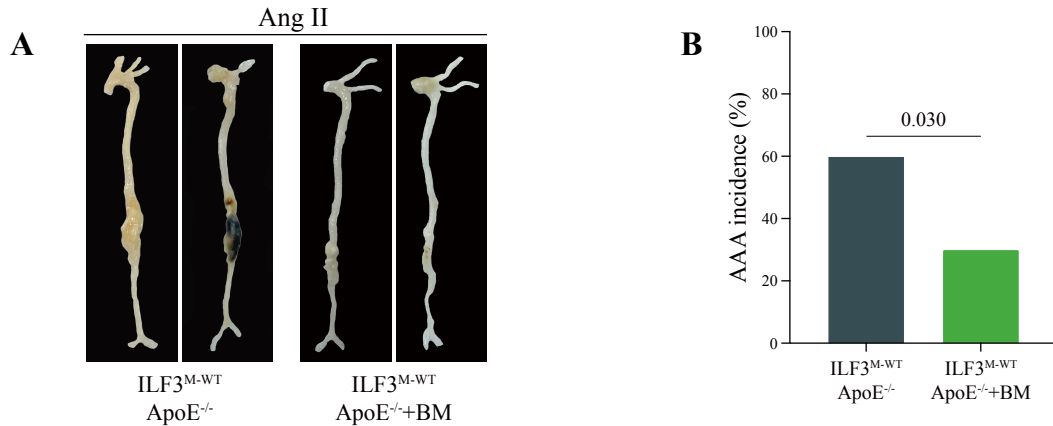


Fig. S11. Bardoxolone methyl (BM) treatment attenuates AAA formation.

A, The representative images showing the morphology of the whole aorta from all groups showing the macroscopic characteristics of aneurysms.

B, The AAA incidence across different groups (n=15).

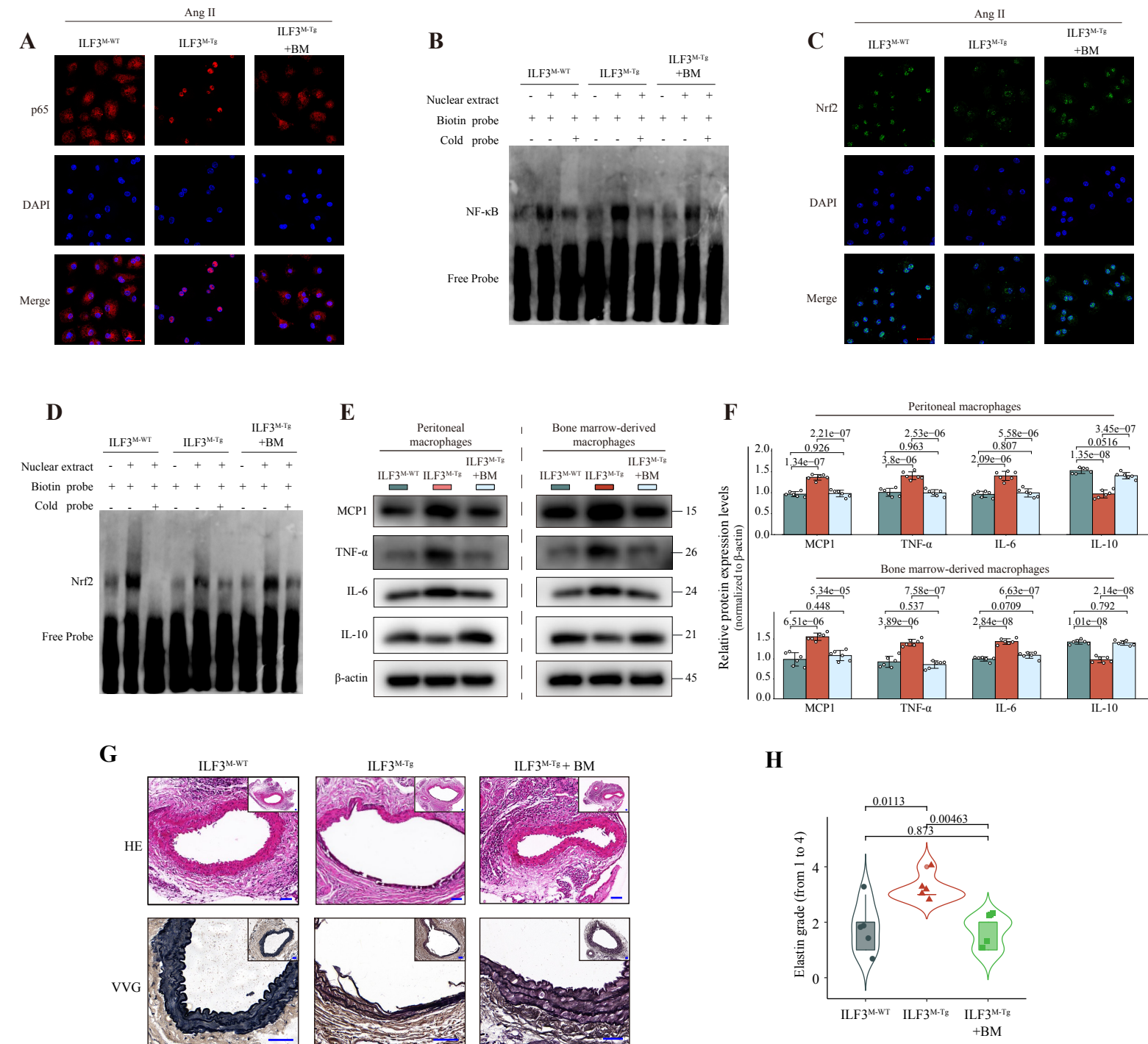
Fig. S12

Fig. S12. BM treatment mitigates the exacerbation of AAA progression induced by ILF3 overexpression.

A, Immunofluorescence staining for p65 (red) in peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with BM; nuclei were stained with DAPI (blue, Scale bar= 20 μ m; n=6 per group).

B, EMSA analysis showing the DNA binding activity of NF- κ B in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with BM under stimulus with Ang II (1 μ M, 24h; n=6 per group).

C, Immunofluorescence staining for Nrf2 (green) in peritoneal macrophages from groups of ILF3^{M-WT}, ILF3^{M-Tg} and ILF3^{M-Tg} treated with BM; nuclei were stained with DAPI (blue, Scale bar= 20 μ m; n=6 per group).

D, EMSA analysis showing the DNA binding activity of Nrf2 in nuclear extracts of peritoneal macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with BM under stimulus with Ang II (1 μ M, 24h; n=6 per group).

E-F, Western blot analysis and quantification of MCP1, TNF- α , IL-6, and IL-10 expression in peritoneal macrophages and bone marrow-derived macrophages from ILF3^{M-WT}, ILF3^{M-Tg}, and ILF3^{M-Tg} mice treated with BM under stimulus with Ang II (1 μ M, 24h; n=6 per group).

G and H, Representative images of HE and VVG staining (G) and elastin degradation scoring (H) of abdominal aortas from CaCl₂-treated mice (n=5 per group; Scale bar= 100 μ m).

Supplemental Table 1. Characteristics of the patients enrolled in the human tissue study.

	Control (n=10)	AAA (n=10)	Summary
Age (yrs)	54.00 ± 5.98	53.60 ± 7.57	ns
Male gender (n,%)	10 (100)	10 (100)	ns
Smoking (n,%)	6 (60)	6 (60)	ns
Body mass index (kg/m ²)	24.02 ± 1.78	24.75 ± 2.65	ns
Hypertension (n,%)	6 (60)	8 (80)	ns
Diabetes Mellitus (n,%)	0 (0)	0 (0)	ns
Total cholesterol (mmol/L)	3.59 ± 0.98	4.00 ± 0.35	ns
HDL (mmol/L)	1.19 ± 0.34	1.15 ± 0.18	ns
Triglyceride (mmol/L)	1.24 ± 0.42	1.40 ± 0.64	ns
CCB (n,%)	4 (40)	7 (70)	ns
ACEI/ARB (n,%)	4 (40)	5 (50)	ns
Beta-blockers (n,%)	6 (60)	7 (70)	ns
Anti-diabetic medications (n,%)	0 (0)	0 (0)	ns

Data are shown as mean ± SD or frequencies (%). Ten study patients (10 AAA patients and 10 matched control subjects) tissue samples were obtained. The patients who underwent surgical repair with abdominal aortic diameters of ≥ 55 mm measured by computed tomographic angiography in accordance with the Society for Vascular Surgery Practice Guidelines. Continuous variables were compared by Student's t-test for normally distributed variables or the Mann–Whitney U test for variables that were not normally distributed. Categorical variables were compared with the Chi-squared test or Fisher's exact test. Abbreviations: HDL, high-density lipoprotein; CCB, calcium channel blocker; ACEi, angiotensin-converting enzyme inhibitor; ARb, angiotensin receptor blocker.

Supplemental Table 2. Body weight and blood pressure in ILF3^{M-WT}ApoE^{-/-} mice and ILF3^{M-Tg}ApoE^{-/-} mice pre and post Ang-II infusion.

Group	Pre Ang-II infusion		Post Ang-II infusion	
	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-}	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-}
BW(g)	25.75±1.59	26.16±1.46	26.97±1.31	27.31±1.30
SBP (mmHg)	99.17±7.72	95.58±8.69	141.50±15.35***	149.83±19.60###
DBP (mmHg)	70.08±2.27	71.58±6.69	96.08±10.16***	100.58±14.20###
MAP (mmHg)	79.78±2.65	79.58±5.33	111.22±9.38***	117.00±15.39###

Data are presented as mean ± SD, n = 12 in each group. Data were analyzed by one-way ANOVA followed by Tukey *post-hoc* test for multiple comparisons. $P < 0.05$ was statistically significant. *** $P < 0.001$ vs. ILF3^{M-WT} ApoE^{-/-} mice pre Ang-II infusion. ### $P < 0.001$ vs. ILF3^{M-Tg}ApoE^{-/-} mice pre Ang-II infusion. Abbreviations: BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Supplemental Table 3. Body weight and blood pressure in ILF3^{M-WT}ApoE^{-/-} mice and ILF3^{M-KO}ApoE^{-/-} mice pre and post Ang-II infusion.

Group	Pre Ang-II infusion		Post Ang-II infusion	
	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-KO} ApoE ^{-/-}	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-KO} ApoE ^{-/-}
BW(g)	25.05±3.17	26.69±2.75	25.68±3.45	27.30±2.68
SBP (mmHg)	102.17±10.43	98.50±15.50	147.00±11.59***	143.92±12.24###
DBP (mmHg)	69.75±3.41	65.80±8.46	104.00±12.48***	99.25±9.26###
MAP(mmHg)	80.56±3.76	76.72±9.62	118.33±10.88***	114.14±9.11###

Data are presented as mean ± SD, n = 12 in each group. Data were analyzed by one-way ANOVA followed by Tukey *post-hoc* test for multiple comparisons. $P < 0.05$ was statistically significant. *** $P < 0.001$ vs. ILF3^{M-WT} ApoE^{-/-} mice pre Ang-II infusion. ### $P < 0.001$ vs ILF3^{M-KO}ApoE^{-/-} mice pre Ang-II infusion. Abbreviations: BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Supplemental Table 4. Body weight and blood pressure in ILF3^{M-WT}ApoE^{-/-} mice、 ILF3^{M-Tg}ApoE^{-/-} mice and BM-treated ILF3^{M-Tg}ApoE^{-/-} mice pre and post Ang-II infusion.

Group	Pre Ang-II infusion			Post Ang-II infusion		
	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-} +BM	ILF3 ^{M-WT} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-}	ILF3 ^{M-Tg} ApoE ^{-/-} +BM
BW(g)	25.17±1.95	24.65±2.28	25.08±2.08	26.36±2.10	25.83±2.35	26.49±2.21
SBP (mmHg)	103.70±7.89	105.80±9.68	99.10±13.47	156.50±12.93 ^{**}	147.90±9.88 [#]	145.50±11.70 ^{\$\$\$}
DBP (mmHg)	75.30±6.41	73.60±6.43	69.40±6.98	94.40±19.84 ^{**}	99.50±6.28 ^{###}	98.00±13.14 ^{\$\$\$}
MAP(mmHg)	84.77±6.29	84.33±5.08	79.30±7.33	115.10± 12.94 ^{***}	115.63± 4.40 ^{###}	113.83±8.65 ^{\$\$\$}

Data are presented as mean ± SD, n = 10 in each group. Data were analyzed by one-way ANOVA followed by Tukey *post-hoc* test for multiple comparisons. $P < 0.05$, was statistically significant. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. ILF3^{M-WT} ApoE^{-/-} mice pre Ang-II infusion. $^{###}P < 0.001$ vs. ILF3^{M-Tg}ApoE^{-/-} mice pre Ang-II infusion. $^{$$$}P < 0.001$ vs. BM-treated ILF3^{M-Tg}ApoE^{-/-} mice pre Ang-II infusion. Abbreviations: BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.