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Supplementary data

Fig. S1

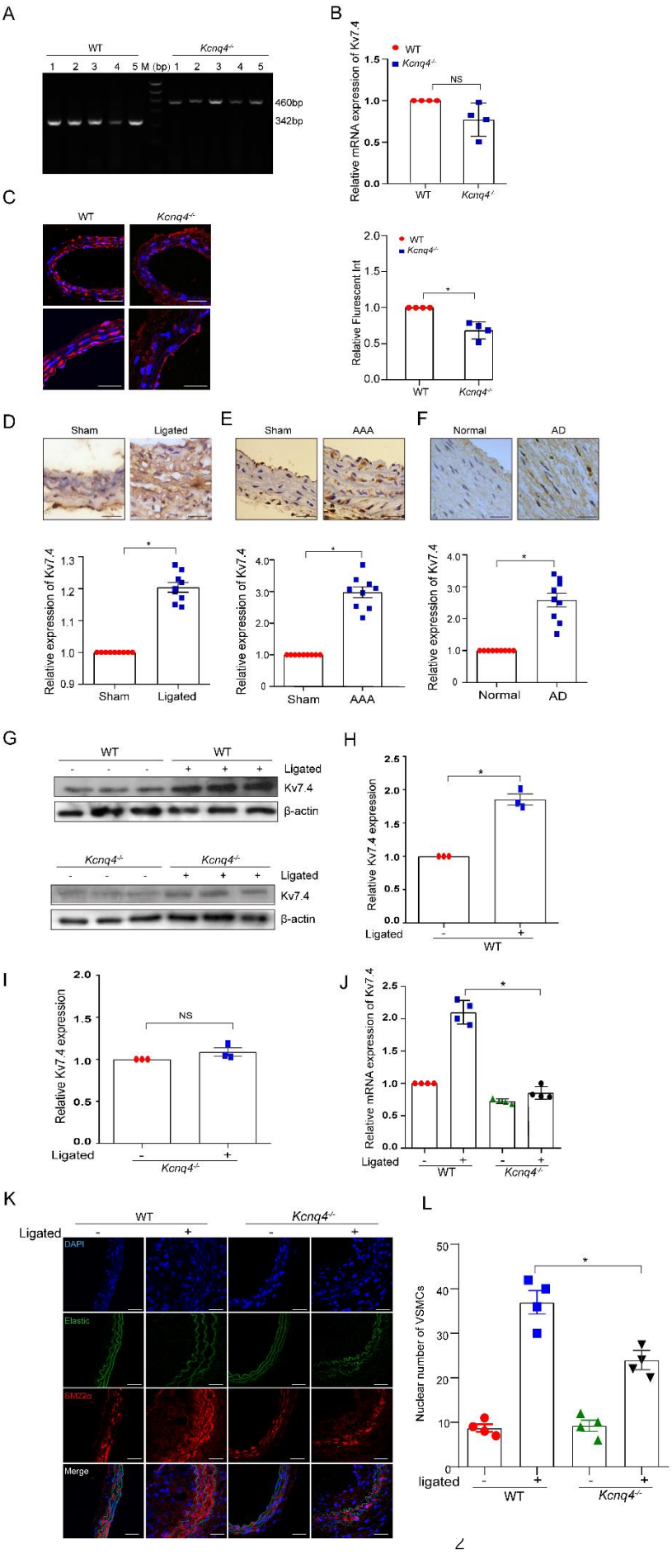
A
WT
Kcnq4-/-
1 2 3 4 5 M (bp) 1 2 3 4 5
460bp
342bp
B
Relative mRNA expression of Kv7.4
WT
Kcnq4-/-
NS
C
Relative Fluorescent Int
D
Sham
Ligated
Relative expression of Kv7.4
E
Sham
AAA
F
Normal
AD
G
Ligated
Kv7.4
β-actin
H
Relative Kv7.4 expression
I
NS
J
Relative mRNA expression of Kv7.4
K
DAPI
Elastic
SM22α
Merge
L
Nuclear number of VSMCs

Fig. S1 Kv7.4 channel expression is induced in mouse model of neointimal formation and aortic aneurysms and in dissected human aortic aneurysms. (A) The genotyping results from WT (342bp) and *Kcnq4*$^{-/-}$(460bp) mice. **(B)** qRT-PCR for the expression of Kv7.4 mRNA in WT and *Kcnq4*$^{-/-}$ mice. Data are summarized as mean ± SEM, n=4 per group, *P<0.05. **(C)** Representative immunofluorescence staining of Kv7.4 in the carotid arteries from the WT or *Kcnq4*$^{-/-}$ mice. Bar=50 μm (upper), Bar=25 μm (lower). The red staining is Kv7.4. Fluorescence intensity from images was summarized as mean ± SEM. n=4 per group, *P<0.05. **(D)** Example immunohistochemical staining of Kv7.4 in the aortas from WT mice with or without carotid artery ligation (imaged at day 14 after ligation). Bar=20 μm. Data are presented as the mean ± SEM. n=9 per group, *P<0.05. (**E)** Example immunohistochemistry staining of Kv7.4 in the aortas from AngII infused WT mice with and without AAA. Bar=20 μm. Data are presented as the mean ± SEM. n=9 per group, *P<0.05. Mice were infused with saline or AngII (1000 ng/kg/min) via subcutaneouse osmotic minipumps for 4 weeks. (**F)** Example immunohistochemistry staining of Kv7.4 in the normal and dissection of normal human aortas from the aortic aneurisms (AD). Bar=20 μm. Data are summarized as mean ± SEM. n=9 per group, *P<0.05. **(G-I)** Western blot analysis of the expression of Kv7.4 in the aortas from WT and *Kcnq4*$^{-/-}$ mice with or without carotid artery ligation. Data are summarized as mean ± SEM, n=3 per group. N.S., not significantly different; *P<0.05. **(J)** qRT-PCR for Kv7.4 expression in aortas from WT and *Kcnq4*$^{-/-}$ mice with or without carotid artery ligation. Data are summarized as mean ± SEM. n=4 per group, **P*<0.05. **(K, L)** Representative immunofluorescence staining with SM22α^{+} VSMCs (red) in the neointima from the WT or *Kcnq4*$^{-/-}$ mice after carotid artery ligation, bars=25 μm. The green fluorescence is the autofluorescence of the elastic plate; blue staining is DAPI. Cell counting by nuclear DAPI was summarized as mean ± SEM. n=4 per group, *P<0.05.

Fig. S2

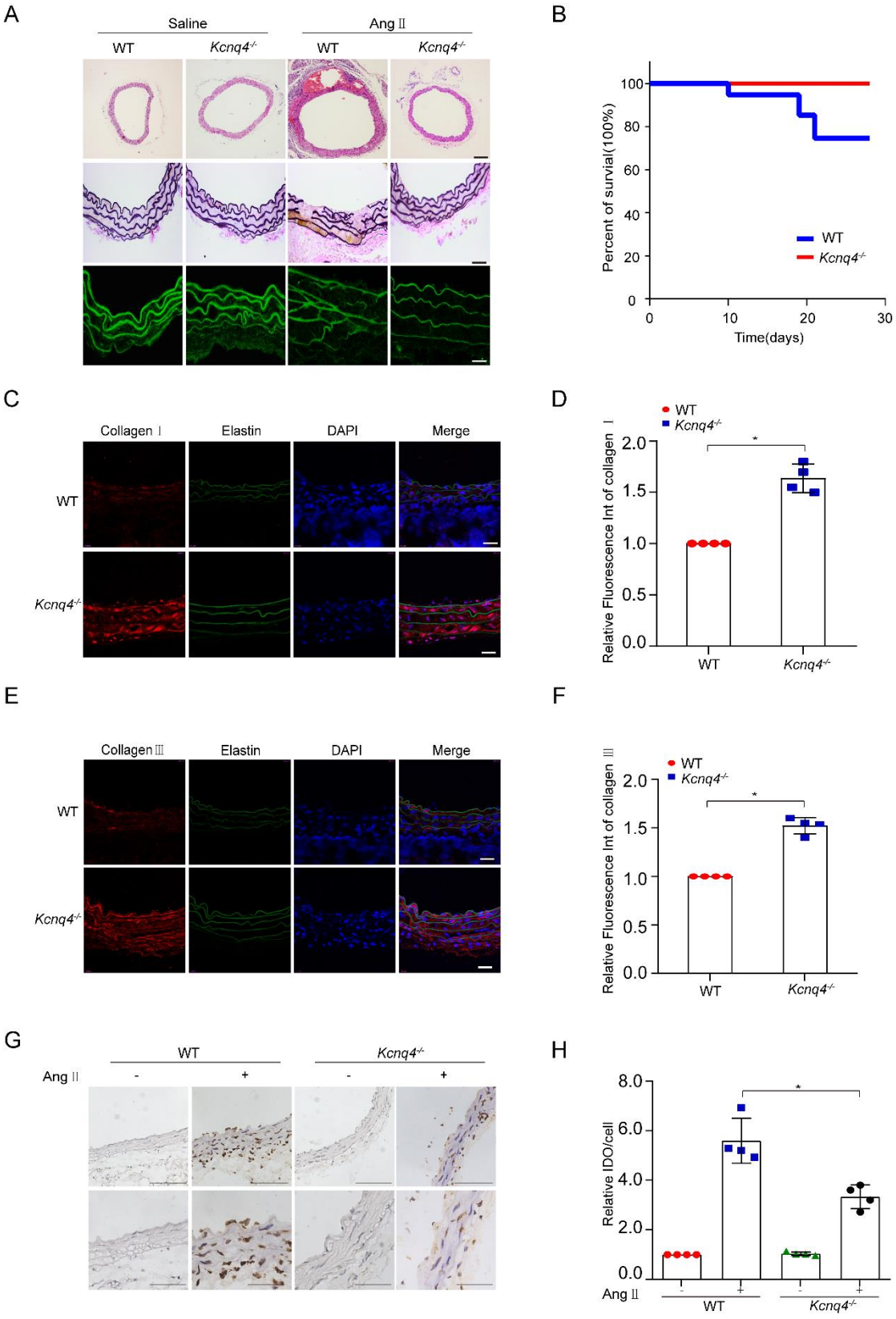

Fig. S2 Vascular lesions are alleviated in AngII-infused AAA models of *Kcnq4*$^{-/-}$

mice. (A) Representative staining of HE, Elastin Van Gieson (EVG) and immunofluorescence staining with elastin and elastin degradation score in the aortas from saline- and Ang II-infused mice. Bar=200 μm (upper), Bar=20 μm (middle), Bar=25 μm (lower). **(B)** Kaplan-Meier survival curve of WT and *Kcnq4*$^{-/-}$ mice infused with saline or Ang II for 4 weeks. *P<0.05. **(C-F)** Immunofluorescence staining for type I and type III collagen in abdominal aortas from saline- or Ang II-infused mice. Bar=25 μm. Statistical analyses of relative fluorescence intensity of collagen I and III are shown in panels **D** and **F**, respectively. n=4 per group, *P<0.05. **(G, H)** TUNEL staining for VSMC apoptosis in the abdominal aortas of WT and *Kcnq4*$^{-/-}$ mice. Bar=100 μm (upper), Bar=50 μm (lower). Data are summarized as mean ± SEM. n=4 per group, N.S., not significantly different.

Fig. S3

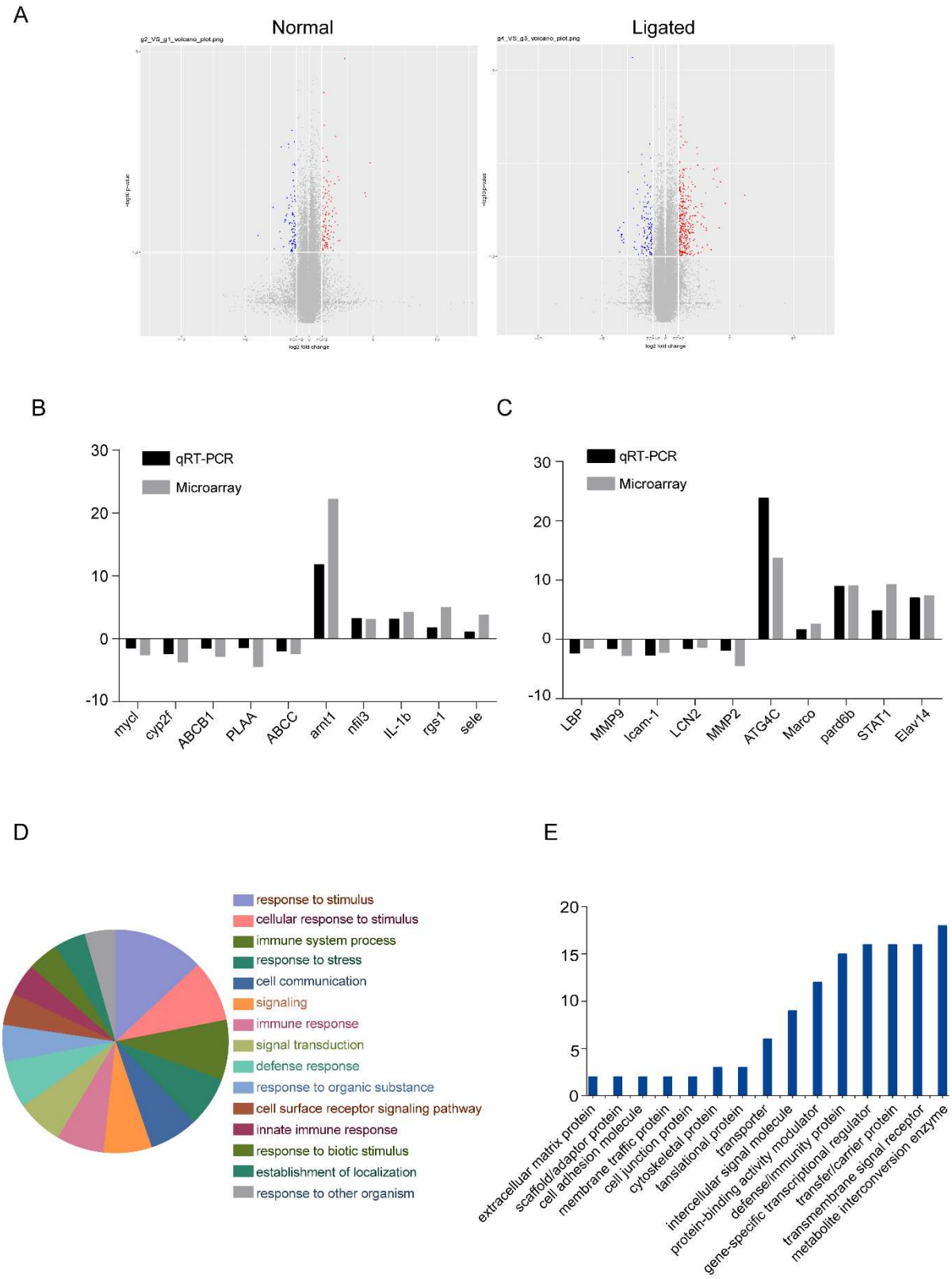

Fig. S3 Kv7.4 knockdown changes the gene expression response in neointimal hyperplasia. (A) Volcano plots illustrating the number and distribution of

differentially expressed mRNAs of WT and *Kcnq4*$^{-/-}$ mice in control groups and carotid artery ligated groups. **(B)** Comparison the fold change (log2; *Kcnq4*$^{-/-}$ *vs.* WT) values of 10 selected transcripts using microarray and qRT-PCR in the control group. **(C)** Comparison the fold change (log2; *Kcnq4*$^{-/-}$ *vs.* WT) values of 10 selected transcripts using microarray and qRT-PCR in the artery ligation group. **(D)** GO enrichment analysis of mRNAs with differential expression in the ligated groups. The differentially expressed genes enriched in biological process (BP). **(E)** The protein classification of differentially expressed genes in ligated groups of WT and *Kcnq4*$^{-/-}$ mice were shown by PANTHER analysis (n=3 per group).

Fig. S4

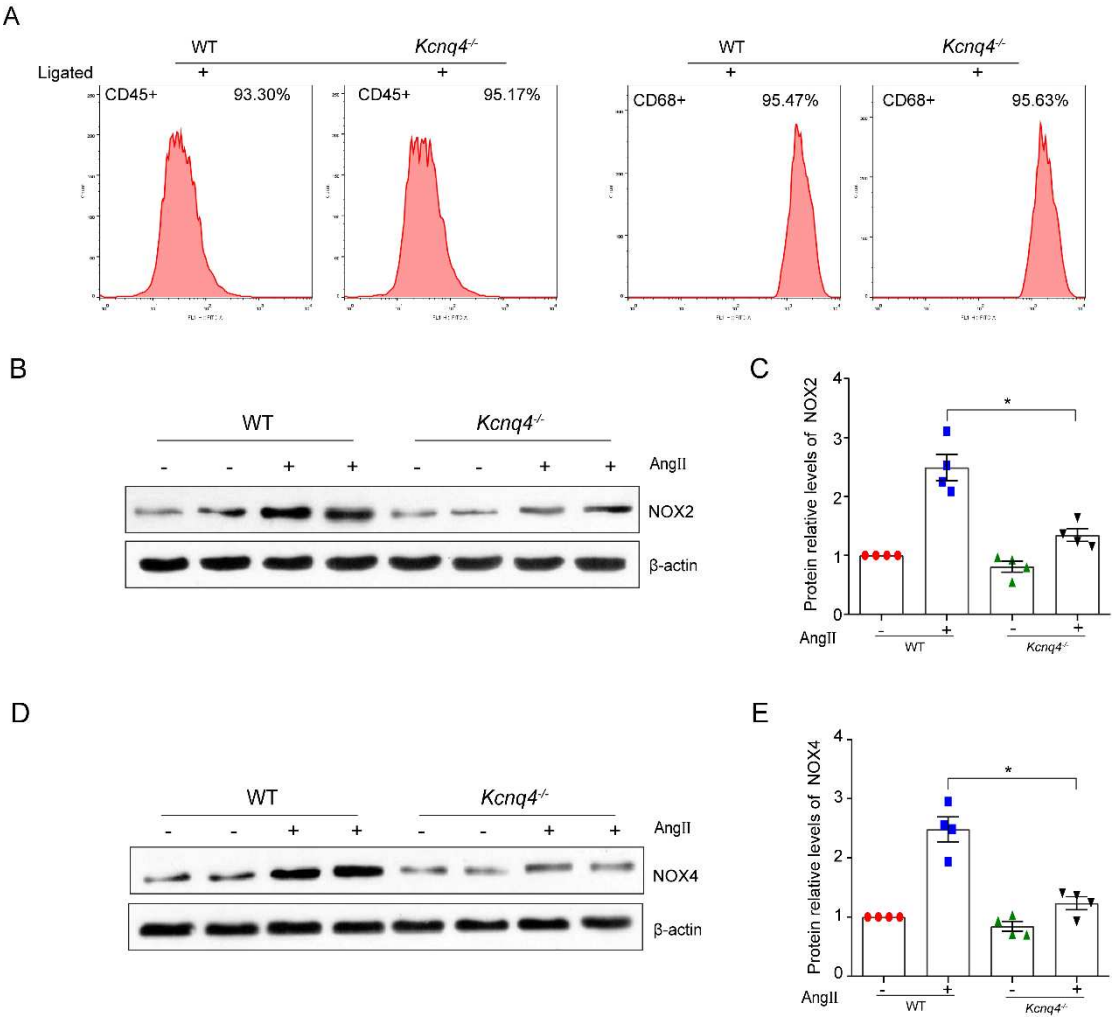

Fig. S4 Effect of Kv7.4 deletion on infiltration of immune cells and oxidative stress

(A) The ligated carotid arteries of WT and *Kcnq4*$^{-/-}$ mice were measured by flow cytometry. Data are summarized as mean ± SEM. n=3 per group, P=0.381206 (CD45+) and P=0.497418 (CD68+). **(B-E)** Western blot analysis for the expression of NOX2 (**B**, **C**) and NOX4 (**D**, **E**) in the aortas from saline- and Ang II-infused WT and *Kcnq4*$^{-/-}$ mice. Data are summarized as mean ± SEM, n=4 per group, *P<0.05.

Fig. S5

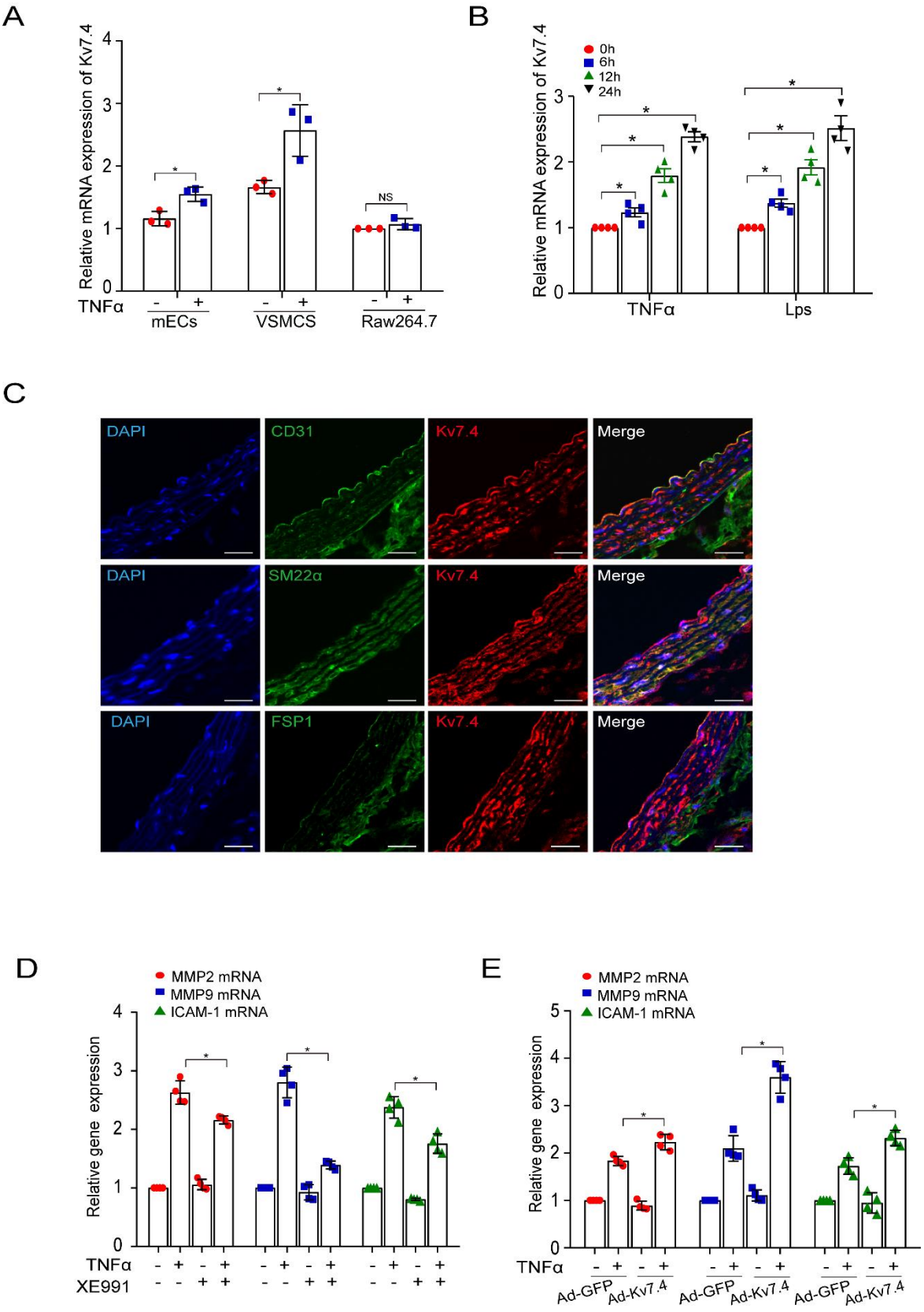
A
Relative mRNA expression of Kv7.4
TNFα
mECs
VSMCS
Raw264.7
NS
B
0h
6h
12h
24h
Relative mRNA expression of Kv7.4
TNFα
Lps
C
DAPI
CD31
Kv7.4
Merge
DAPI
SM22α
Kv7.4
Merge
DAPI
FSP1
Kv7.4
Merge
D
MMP2 mRNA
MMP9 mRNA
ICAM-1 mRNA
Relative gene expression
TNFα
XE991
E
MMP2 mRNA
MMP9 mRNA
ICAM-1 mRNA
Relative gene expression
TNFα
Ad-GFP
Ad-Kv7.4
Ad-GFP
Ad-Kv7.4
Ad-GFP
Ad-Kv7.4

Fig. S5 Kv7.4 enhances inflammatory response in VSMCs. **(A)** Relative mRNA levels of *Kcnq4* in mECs, VSMCs and Raw264.7 cells were analyzed by qRT-PCR before and after TNF-α (20 ng/ml) treatment 24 hours. Data are summarized as mean ± SEM. n=3 per group, *P<0.05. **(B)** Relative mRNA levels of *Kcnq4* in VSMCs treated with TNF-α (20 ng/ml) or LPS (1 μg/ml) for 6, 12 and 24 hours. Data are summarized as mean ± SEM. n=4 per group, *P<0.05. **(C)** Representative images of Kv7.4 and the cell type-specific marker co-localization in the tissue section of mouse abdominal aortic aneurysm using immunofluorescence staining, bars=25 μm. (**D)** VSMCs were pretreated with Kv7.4 blocker XE991 (3 μM) followed by stimulation with TNF-α for 24 hours. The expression of MMP2, MMP9 and ICAM-1 was analyzed by qRT-PCR. Data are summarized as mean ± SEM. n=4 per group, *P<0.05. (**E)** qRT-PCR was conducted to detect MMP2, MMP9 and ICAM-1 expression in Ad-GFP or Ad-Kv7.4 treated (36 hours) VSMCs from *Kcnq4*$^{-/-}$ mice, stimulation with TNF-α for 24 hours. Data are summarized as mean ± SEM. n=4 per group, *P<0.05.

Fig. S6

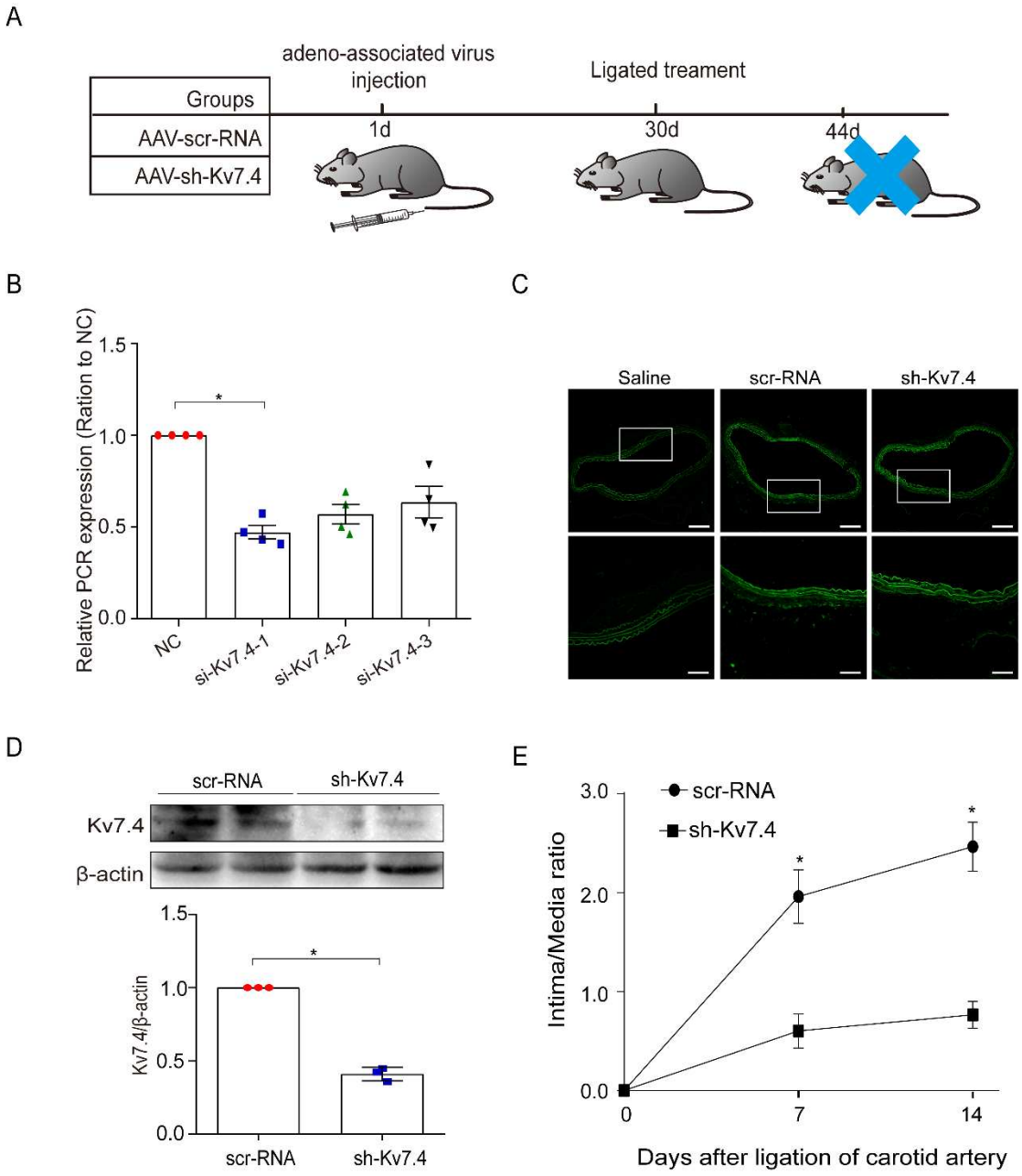

Fig. S6 Knockdown of Kv7.4 channel in VSMCs using viral delivery of *Kcnq4*-targeting shRNA under control of VSMC-specific promotor. (A) Flow diagram of adeno-associated virus (AAV)-mediated Kv7.4 knockdown in VSMC *in vivo*. AAV-shKv7.4 and AAV-scrRNA were injected into the mice via the tail vein. Thirty days after the initial virus transfection, mice had their carotid arteries ligated for up to 14 days. **(B)** qRT-PCR analysis of Kv7.4 mRNA in cultured VSMCs after the transfection of 3 different types of small-interfering RNA (siRNA) sequences. Data are summarized as mean ± SEM. n=4 per group, *P<0.05. **(C)** Representative

immunofluorescence staining of virus-borne green fluorescent protein (GFP) in the WT male mice aortas in different virus-mediated groups and the saline group. Bar=100 μm (upper), 50 μm (lower). **(D)** Western blot analysis of the expression levels of Kv7.4 after Kv7.4 knockdown in the aortas. Data are summarized as mean ± SEM. n=3 per group, *P<0.05. **(E)** The ratio of I/M was measured in the injured carotid arteries from mice injected with AAV-shKv7.4 and AAV-scrRNA at 7 and 14 days. Data are summarized as mean ± SEM. n=7 per group, *P<0.05.

Fig. S7

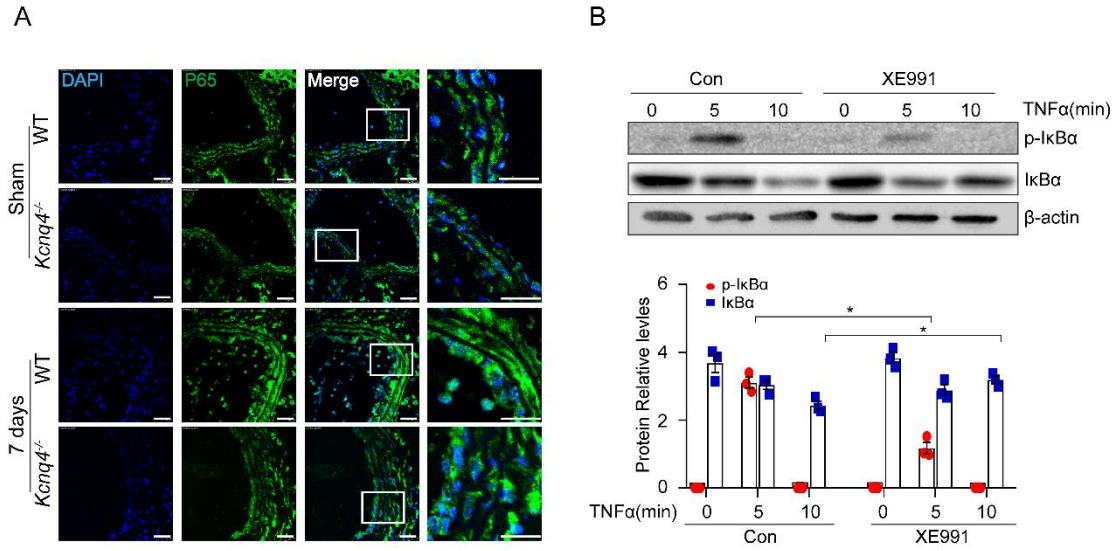

Fig. S7 Kv7.4 promotes TNFα-induced NF-κB nuclear translocation in injured arteries of neointimal hyperplasia. (A) Examples of immunofluorescence staining for NF-κB (P65) of ligated aortic cross sections from WT and *Kcnq4*$^{-/-}$ mice. Scale bars=25 μm. (**B)** Western blot for p-IκBα and IκBα in VSMCs preincubated with XE991 (3 uM) before stimulation with TNFα for 10 minutes. Data are presented as the mean ± SEM. n=3 per group, *P < 0.05.