Supplemental methods:

RNA extraction and Quantitative RT-PCR analysis — Cells were treated as indicated and then harvested for total RNA. Abdominal aorta tissues from saline or AngII treated mice were snap-frozen in liquid nitrogen. Total RNA was isolated from cultured cells or tissues with Trizol (Invitrogen Corp., Carlsbad, CA) as described by the manufacturer. Total RNA (1 μg) was reverse transcribed using Bio-Rad iScript Reverse Transcription Supermix kit. The resulting cDNA was diluted to 100 μl and used in subsequent real time PCR reactions. Gene expression was assessed by the Roche SYBR green or Taqman system. Gene expression was standardized to GAPDH, β-actin or 18S using the ΔΔCt method.

MMP2 and MMP9 ELISA assay — Blood was drawn from the vena cava into heparinized tubes. Plasma was obtained by centrifugation at 2500xg for 30 minutes at 4°C. Plasma MMP2 and MMP9 levels were assayed using Quantikine MMP2 and MMP9 ELISA kit from R&D Systems according to manufacturer's instructions.

Histology and Immunohistochemistry — All mice were euthanized and tissues were harvested, rinsed in PBS, fixed in 4% paraformaldehyde for 48 hours, embedded in paraffin, and serial 5 μm sections were cut. Morphology was evaluated by hematoxylin and eosin (H&E) staining. Aorta wall elastin integrity was assessed by Verhoeff-van Giesen staining using the Accustain Elastic Stain kit (Sigma). Elastin fragmentation was graded as follows according to Libby et al (1): grade 1, intact, well-organized elastic laminae; grade 2, elastic laminae with some interruptions and breaks; grade 3, severe

elastin fragmentation or loss; and grade 4, severe elastin degradation with visible ruptured sites. Representative elastin fragmentation scale is illustrated in Supplemental Figure 20. Immunostaining studies were performed according to standard procedure using the indicated antibodies. CCN3 expression in mouse vessels was determined using CCN3 antibody from Abcam (ab10888). Immunohistochemical analysis of formalin-fixed tissues was performed according to standard procedures. Immunohistochemical analysis of formalin-fixed frozen human control and AAA sections was performed using a rabbit polyclonal antibody against CCN3 (K19M, 1:200) (2). Other primary antibodies are listed in Supplemental Table II.

Smooth muscle cell isolation: Aortas were dissected from 8-10 weeks old male mice and used for smooth muscle cells isolation. SMCs were isolated from aortas using standard techniques as previously described (3). Cells were cultured and propagated in DMEM/F12 (Invitrogen) supplemented with 10% FBS (Atlantics) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. For each isolation, cells were pooled from 5 mice per genotype.

Supplemental references:

1. Tang, E.H., Shvartz, E., Shimizu, K., Rocha, V.Z., Zheng, C., Fukuda, D., Shi, G.P., Sukhova, G., and Libby, P. 2011. Deletion of EP4 on bone marrow-derived cells enhances inflammation and angiotensin II-induced abdominal aortic aneurysm formation. *Arterioscler Thromb Vasc Biol* 31:261-269.
2. Chevalier, G., Yeger, H., Martinerie, C., Laurent, M., Alami, J., Schofield, P.N., and Perbal, B. 1998. novH: differential expression in developing kidney and Wilm's tumors. *Am J Pathol* 152:1563-1575.
3. Kuang, S.Q., Kwartler, C.S., Byanova, K.L., Pham, J., Gong, L., Prakash, S.K., Huang, J., Kamm, K.E., Stull, J.T., Sweeney, H.L., et al. 2012. Rare, nonsynonymous variant in the smooth muscle-specific isoform of myosin heavy chain, MYH11, R247C, alters force generation in the aorta and phenotype of smooth muscle cells. *Circ Res* 110:1411-1422.

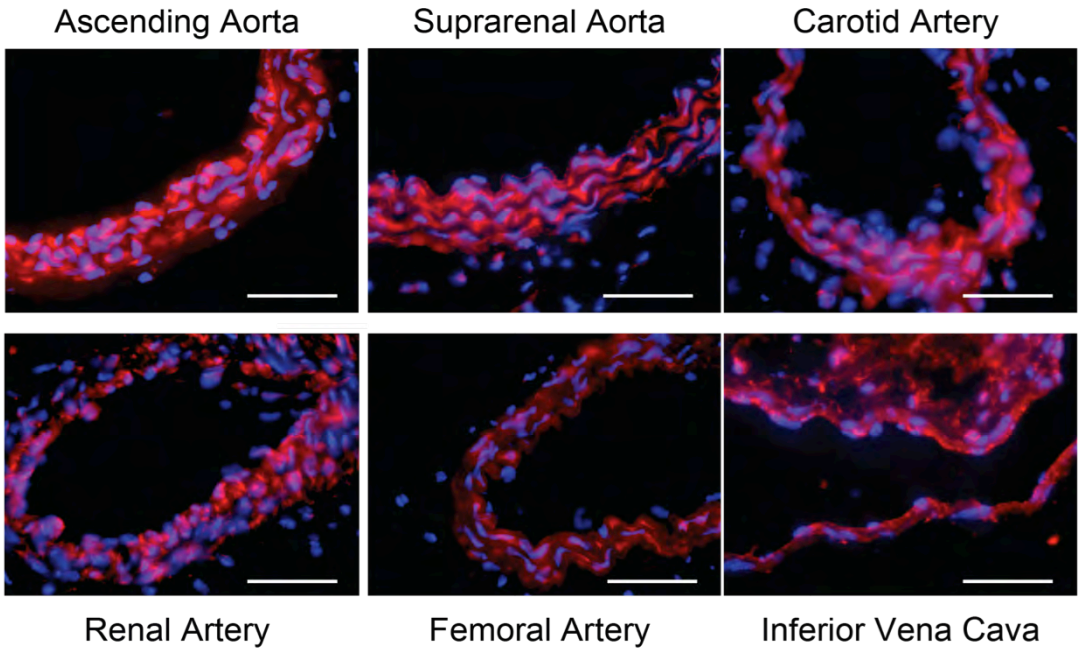
Supplemental Table I. Primary Antibodies Used in Western Blotting

Antibody Name Catalog number	Supplier	Species	IHC dilution
P-ERK1/2 4370S	Cell Signaling Technology	Rabbit IgG	1:2000
P-JNK, 4668P	Cell Signaling Technology	Rabbit IgG	1:1000
P-SMAD2, 3101S	Cell Signaling Technology	Rabbit IgG	1:1000
P-P65, 3033S	Cell Signaling Technology	Rabbit IgG	1:1000
P-P38, 4511P	Cell Signaling Technology	Rabbit IgG	1:1000
P-MEK1/2, 8211	Cell Signaling Technology	Rabbit IgG	1:1000
P-AKT1, 9018P	Cell Signaling Technology	Rabbit IgG	1:1000
ERK1/2, 4695S	Cell Signaling Technology	Rabbit IgG	1:3000
JNK, 9258P	Cell Signaling Technology	Rabbit IgG	1:2000
SMAD2, 3122	Cell Signaling Technology	Rabbit IgG	1:1000
P65, 4764S	Cell Signaling Technology	Rabbit IgG	1:1000
P38, 8690	Cell Signaling Technology	Rabbit IgG	1:1000
MEK1/2, 9122	Cell Signaling Technology	Rabbit IgG	1:1000
AKT1, 2938	Cell Signaling Technology	Rabbit IgG	1:1000
β-actin, sc-69879	Santa Cruz Biotechnology	Mouse monoclonal	1:5000
Anti mouse IgG-HRP, 7076	Cell Signaling Technology	Goat	1:3000
Anti Rabbit IgG-HRP, 7074	Cell Signaling Technology	Goat	1:3000

Supplemental Table II. Primary Antibodies Used in Immunohistochemistry

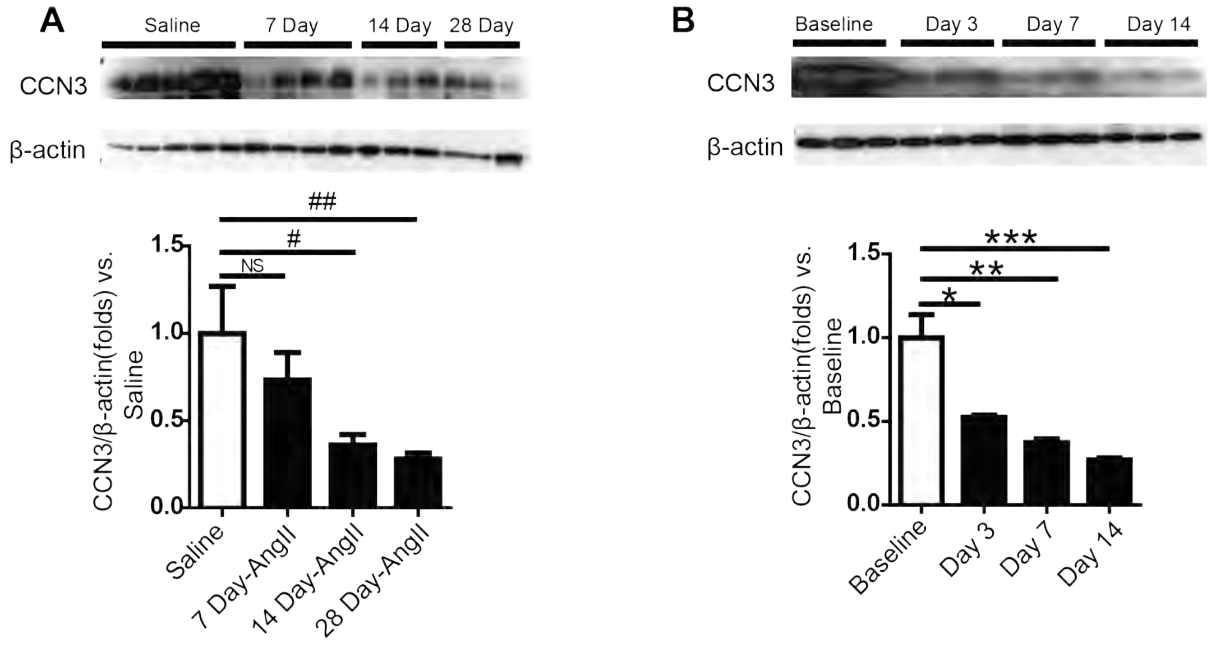
Antibody Name Catalog number	Supplier	Species	IHC dilution
CD3, A0452	Dako, Denmark	Polyclonal rabbit anti-human,mouse	1:200
MAC3, 550292	Fisher Scientific Inc	Monoclonal rat anti-mouse	1:200
MCP-1, sc-1784	Santa Cruz Biotechnology	Monoclonal goat anti-mouse	1:200
VCAM-1, sc-1504	Santa Cruz Biotechnology	Monoclonal goat anti-mouse	1:150
MMP2, AF1488	R&D System	Monoclonal goat anti-mouse	1:200
MMP9, AF909	R&D System	Monoclonal goat anti-mouse	1:200
P-ERK1/2 4370S	Cell Signaling Technology	Rabbit IgG	1:200
P-JNK, 4668P	Cell Signaling Technology	Rabbit IgG	1:200
P-SMAD2, 3101S	Cell Signaling Technology	Rabbit IgG	1:200
4-HNE, 24327	OxisResearch products	Monoclonal mouse	25ug/ml
Cy3-Sm-α-actin, C6198	Sigma-Aldrich	Monoclonal mouse anti human, mouse	1:500
CD68, MCA1957GA	AcD Serotec	Monoclonal Rat anti mouse	1:50

Supplemental Figure 1



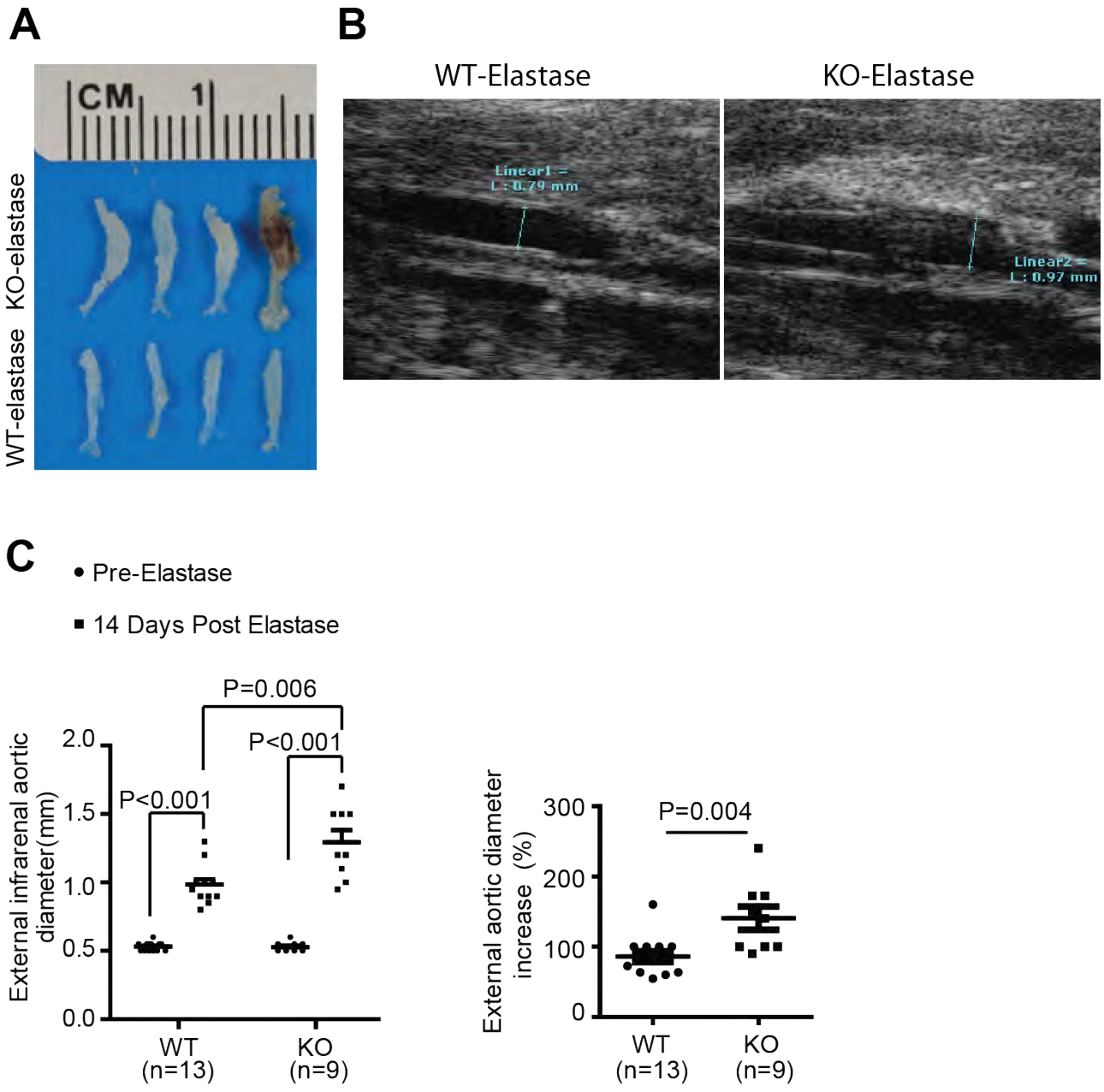
Supplemental Figure 1: Vascular expression of CCN3 in mouse. Red: CCN3 staining, Blue: DAPI. Scale bar=50μm.

Supplemental Figure 2



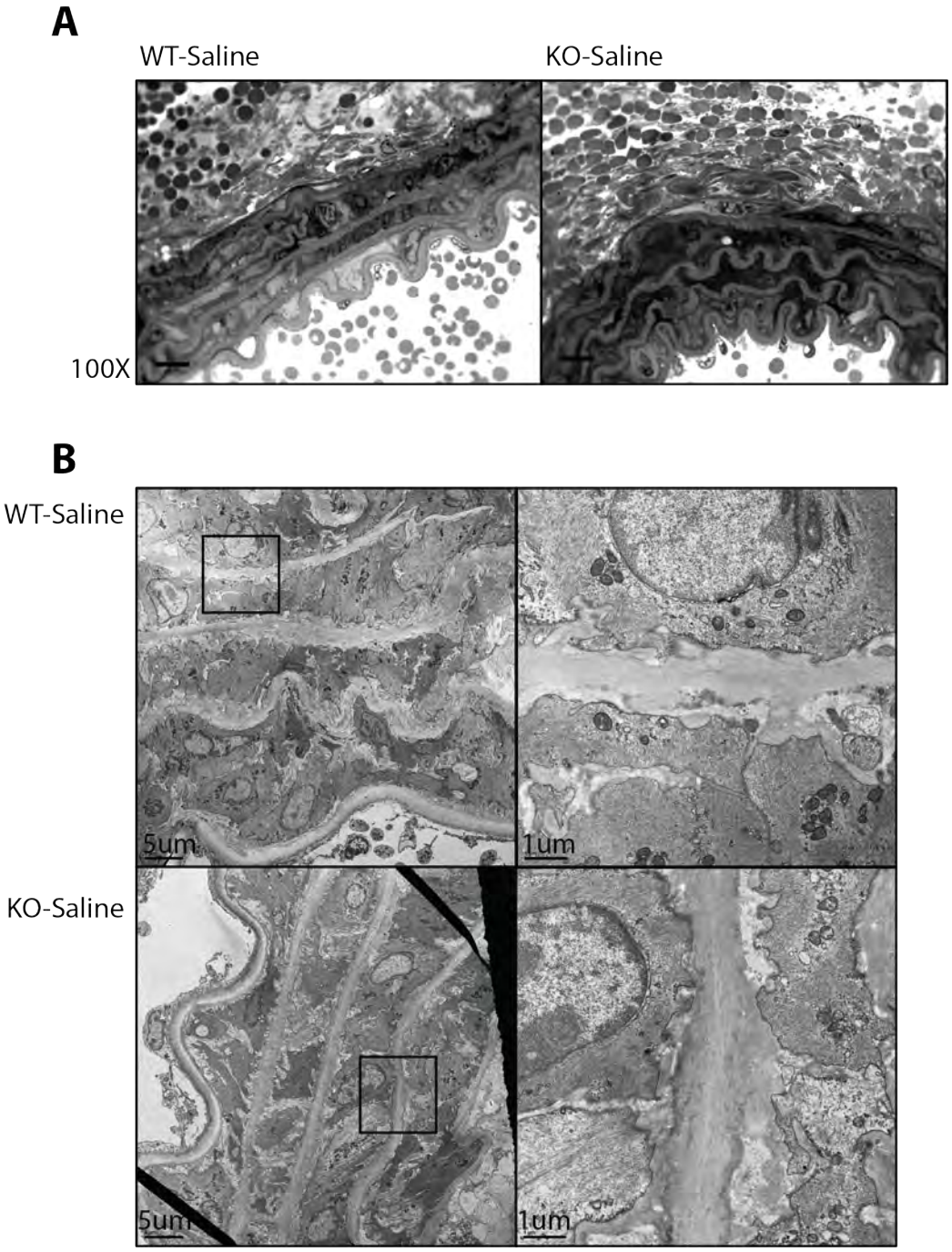
Supplemental Figure 2: Reduction of CCN3 expression in rodent abdominal aortic aneurysm (AAA). (A) Western blot of CCN3 protein in mouse suprarenal abdominal aortic tissues from *Apoe*-null mice infused with AngII for 7, 14 and 28 days; saline, n=5; AngII 7days, n=6; AngII 14 days, n=7; AngII 28 days, n=11. # saline versus 14 days AngII, P<0.01; ## Saline versus 28 days AngII, P<0.001. (B) Western blot of CCN3 protein in mouse infrarenal abdominal aortic tissues from C57Bl6 mice 3, 7 and 14 days post elastase perfusion; n=5 per time point. Saline, n=7; n=6 for elastase perfusion at 3, 7 and 14 days. * baseline versus 3 days of elastase perfusion, P<0.002; ** baseline versus 7 days of elastase perfusion, P<0.001; *** baseline versus 14 days of elastase perfusion, P<0.001. One way ANOVA followed by Bonferroni correction.

Supplemental Figure 3



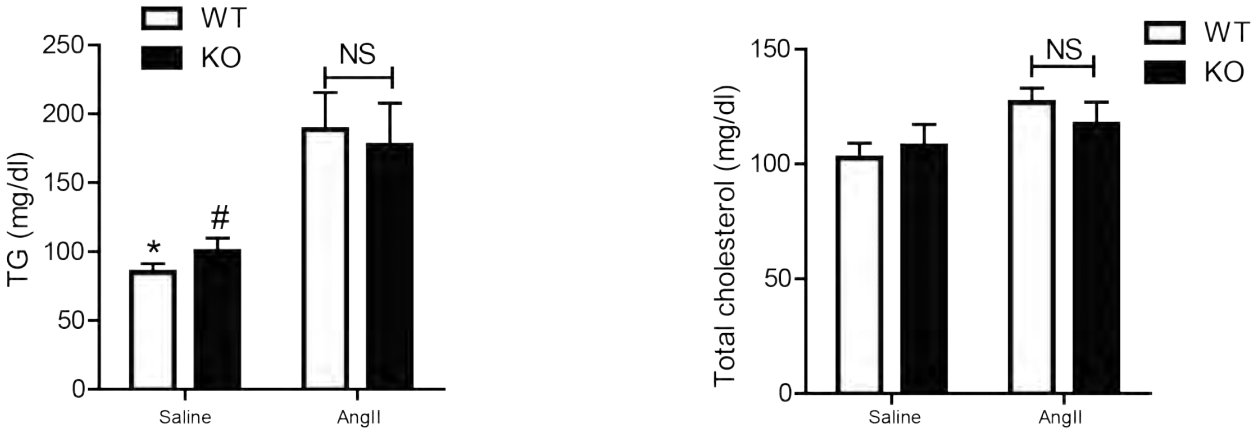
Supplemental Figure 3: Ablation of CCN3 leads to enhanced AAA formation in elastase perfusion model. (A) Representative pictures of whole aortae from WT and CCN3-deficient mice 2 weeks post elastase perfusion; (B) Ultrasound image of infrarenal aorta following 2 weeks after elastase perfusion; (C) Changes of external aortic diameter and lumen size in WT and CCN3-deficient mice. Left panel: external infrarenal aortic diameter; right panel: percentage of external aortic increase over baseline. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$. Two-way repeated-measures ANOVA test followed by Bonferroni post-hoc correction.

Supplemental Figure 4



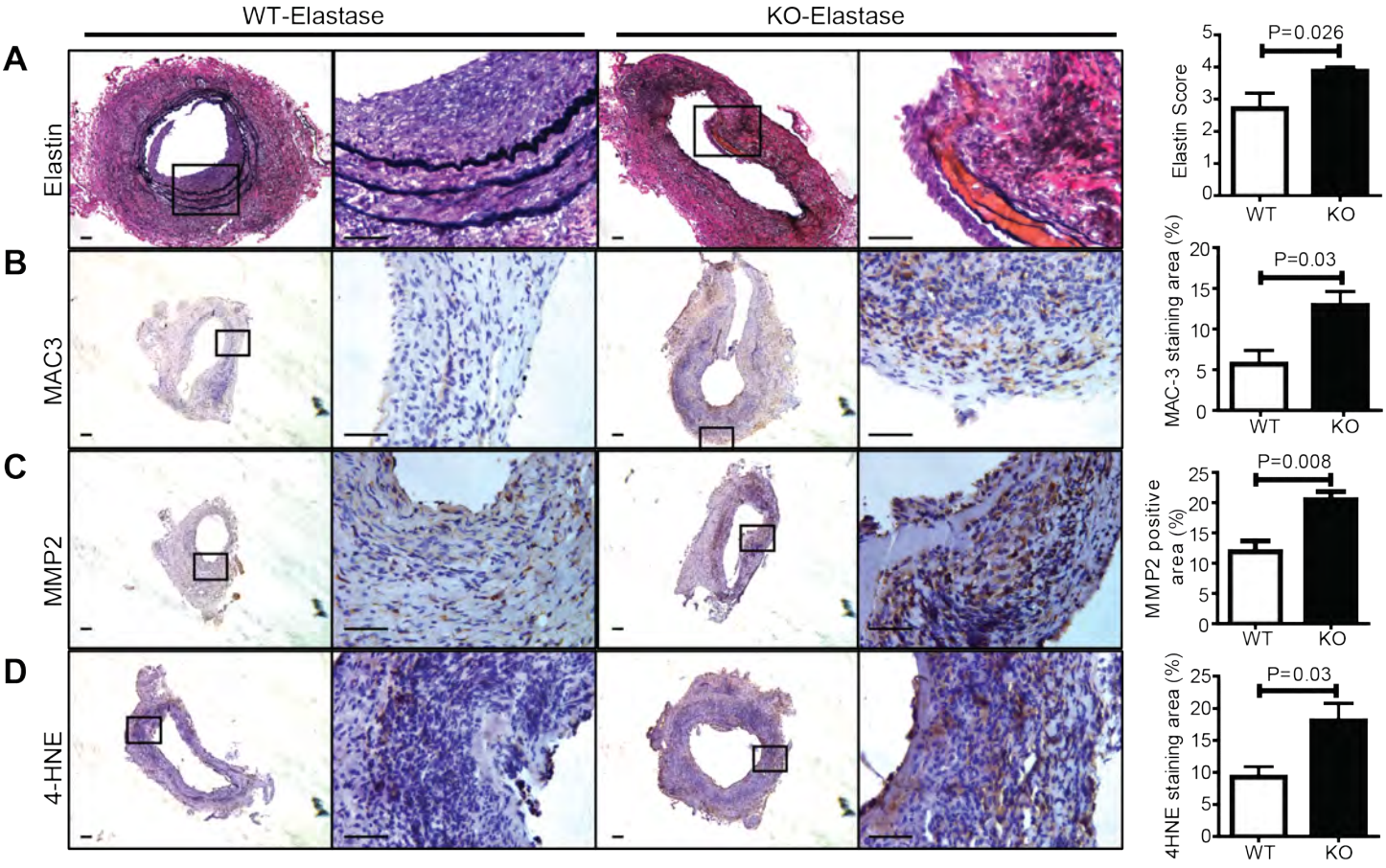
Supplemental Figure 4: Electron micrograph of abdominal aortic structure from wild type and CCN3-deficient mice at 100X magnification (A) and higher magnification, scale bar=5μm (left panel), scale bar=1μm (right panel) (B). n=3 per genotype. WT=*Ccn3*$^{+/+}$, KO=*Ccn3*$^{-/-}$.

Supplemental Figure 5



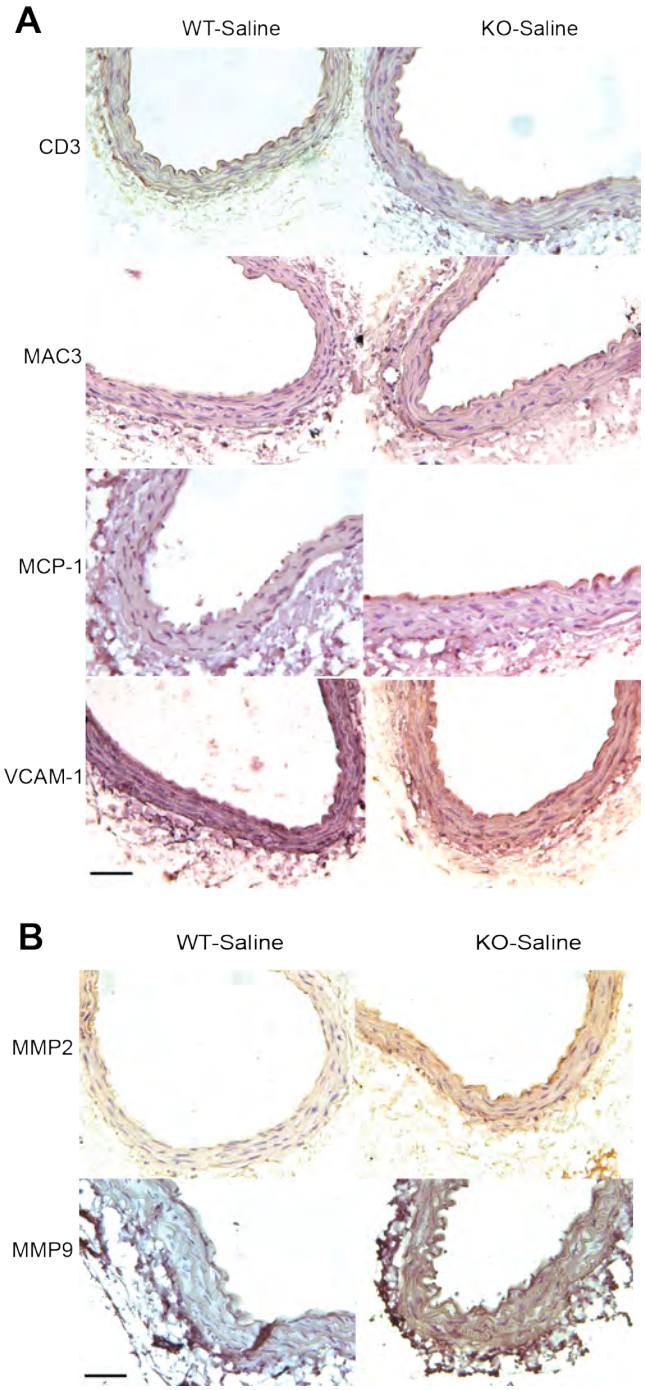
Supplemental Figure 5: Cholesterol and triglyceride levels from wild type and CCN3-deficient mice following 4 weeks of saline or AngII infusion. * WT saline vs WT AngII, P<0.01. # KO Saline vs KO AngII, P<0.01. WT, n=9; KO, n=10. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$. NS=Not significant. Two-way ANOVA followed by Bonferroni post-hoc correction was used.

Supplemental Figure 6



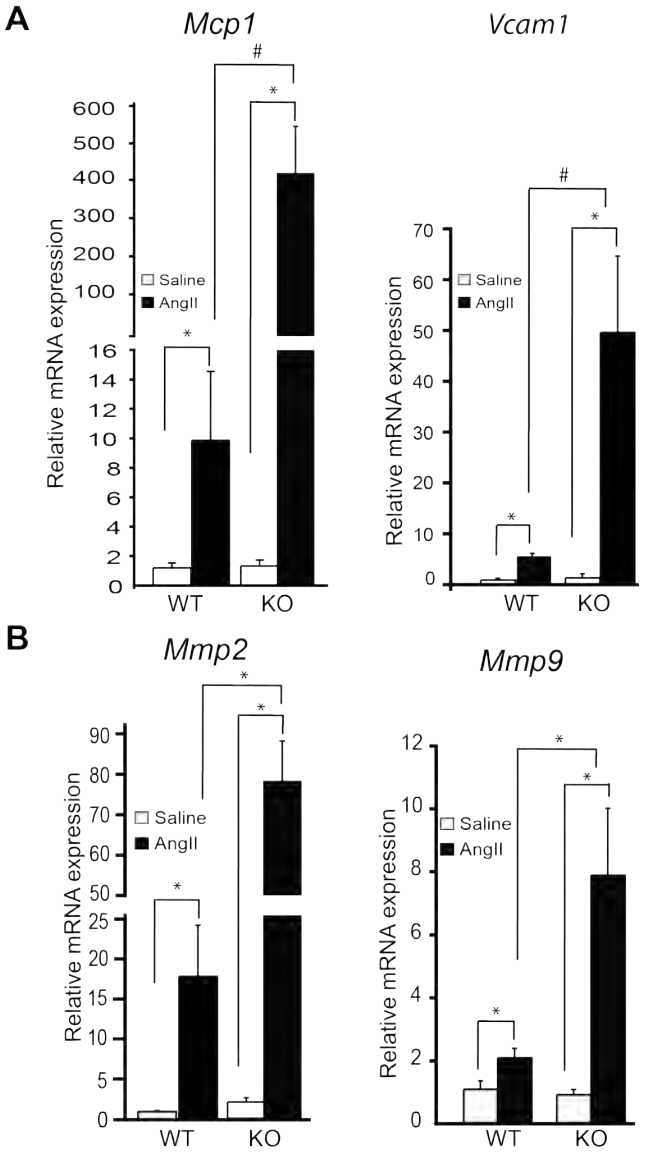
Supplemental Figure 6: CCN3 deficiency promotes elastin breakdown, macrophage infiltration, MMP2 expression and oxidative stress in CCN3-deficient aortae 2 weeks post elastase perfusion. (A) Elastin integrity grading in WT vs CCN3-deficient aortas. Data are presented as mean ± SEM. wild type: n=7; knockout: n=8. (B) Immunohistochemical staining for macrophages (MAC3) in WT vs CCN3-deficient aortae; wild type: n=5; knockout: n=6, quantification shown on the right panel. (C) Immunohistochemical staining for MMP2 (n=5 each group); quantification shown on the right panel. (D) ROS assessment by immunohistochemical detection of 4-HNE in abdominal aortae; wild type: n=5; knockout: n=6. Scale bar = 50 μm; WT= *Ccn3*$^{+/+}$, KO=*Ccn3*$^{-/-}$. Mann Whitney test was used.

Supplemental Figure 7



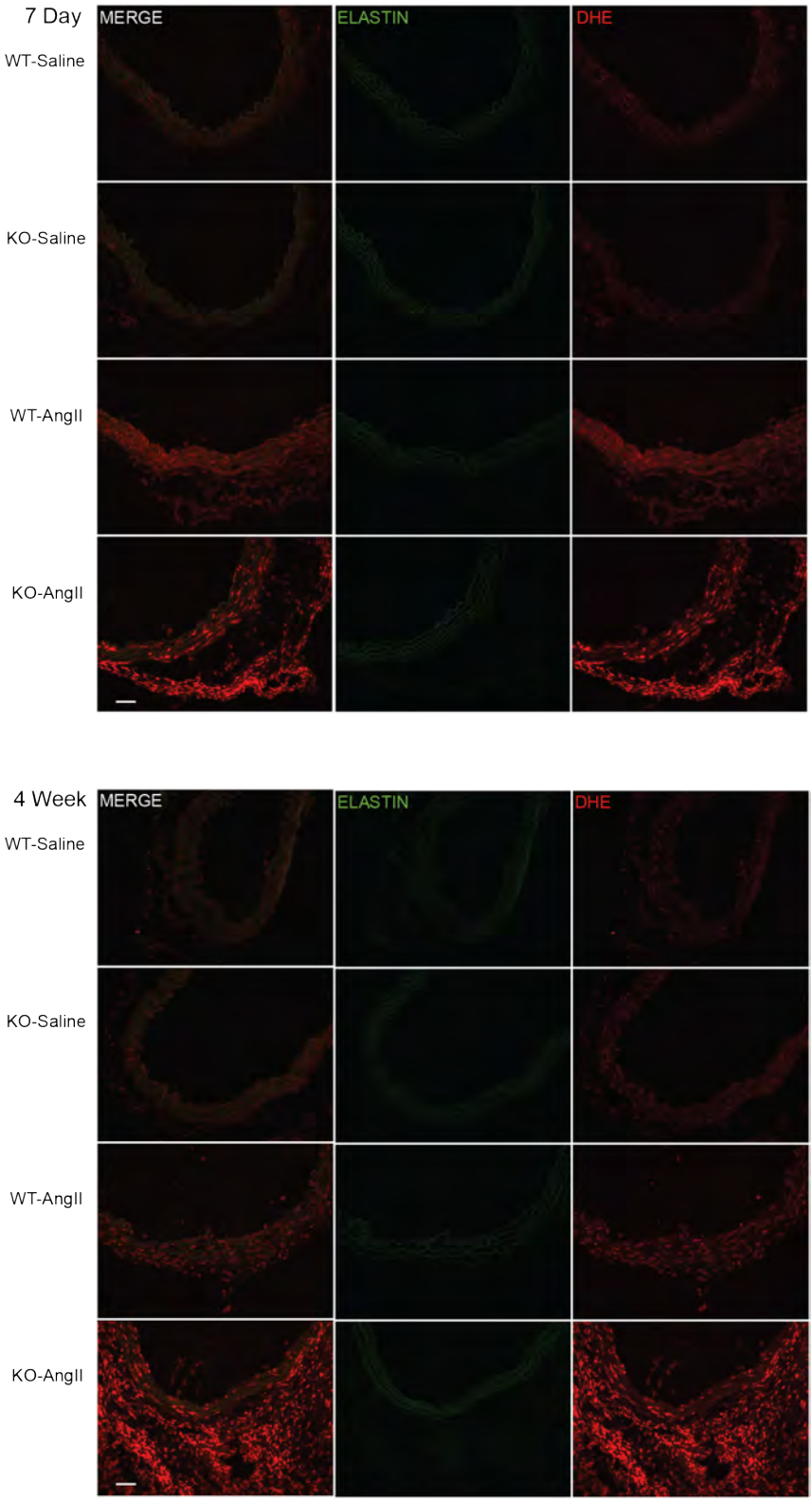
Supplemental Figure 7: CCN3 deficiency does not alter the pro-inflammatory response or MMP protein expression. (A) Representative images of immunohistochemical staining for macrophages (MAC3), T cells (CD3), MCP1 and VCAM1 in WT vs CCN3-deficient aortae following 28 days of saline infusion; (B) Immunohistochemical staining for MMP2 and MMP9. WT= *Ccn3*$^{+/+}$, KO=*Ccn3*$^{-/-}$. Scale bar = 50 μm.

Supplemental Figure 8



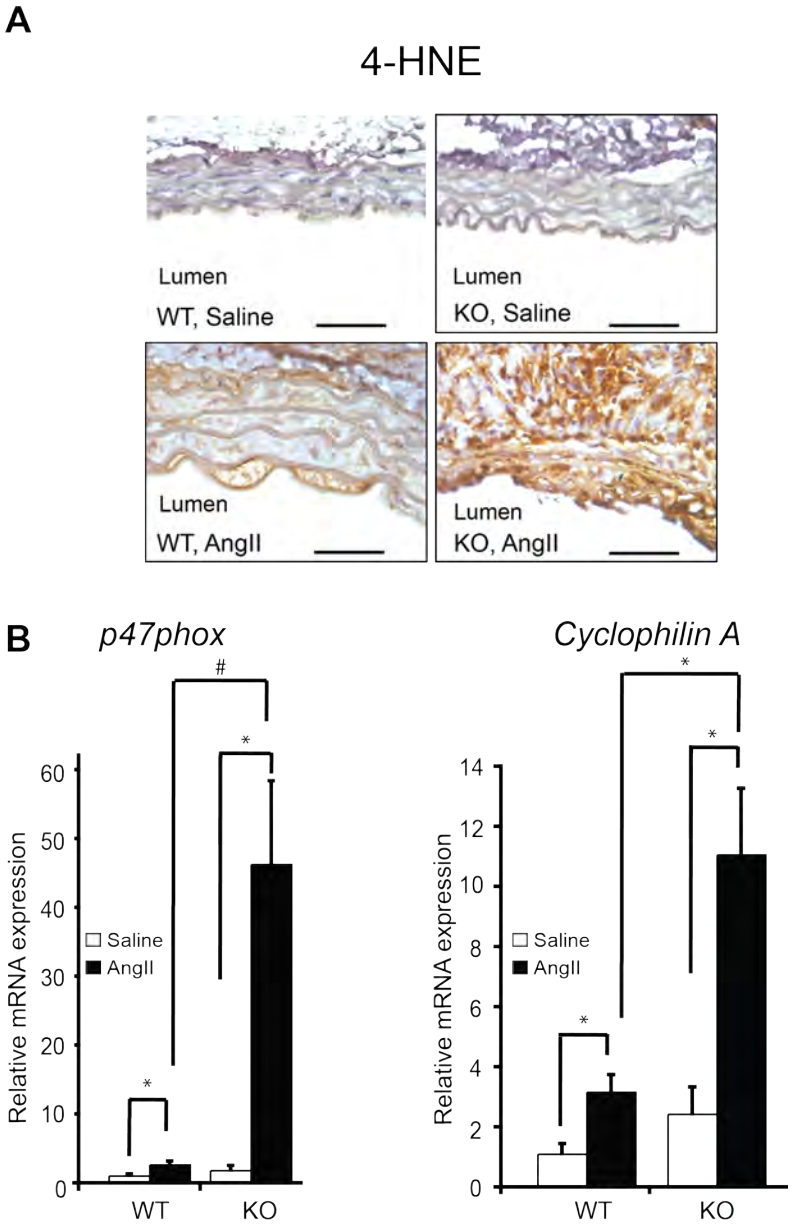
Supplemental Figure 8: CCN3 deficiency results in increased inflammation in abdominal aortas post 4-week AngII treatment. (A) Analysis of mRNA expression of *Vcam1* and *Mcp1* in abdominal aortas by qPCR, n= 6~8; (B) Analysis of mRNA expression of *Mmp2*, *Mmp3* and *Mmp9* by qPCR, n=5-7. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$. *P<0.05; # P<0.01. Two-way ANOVA followed by Bonferroni post-hoc correction was used.

Supplemental Figure 9



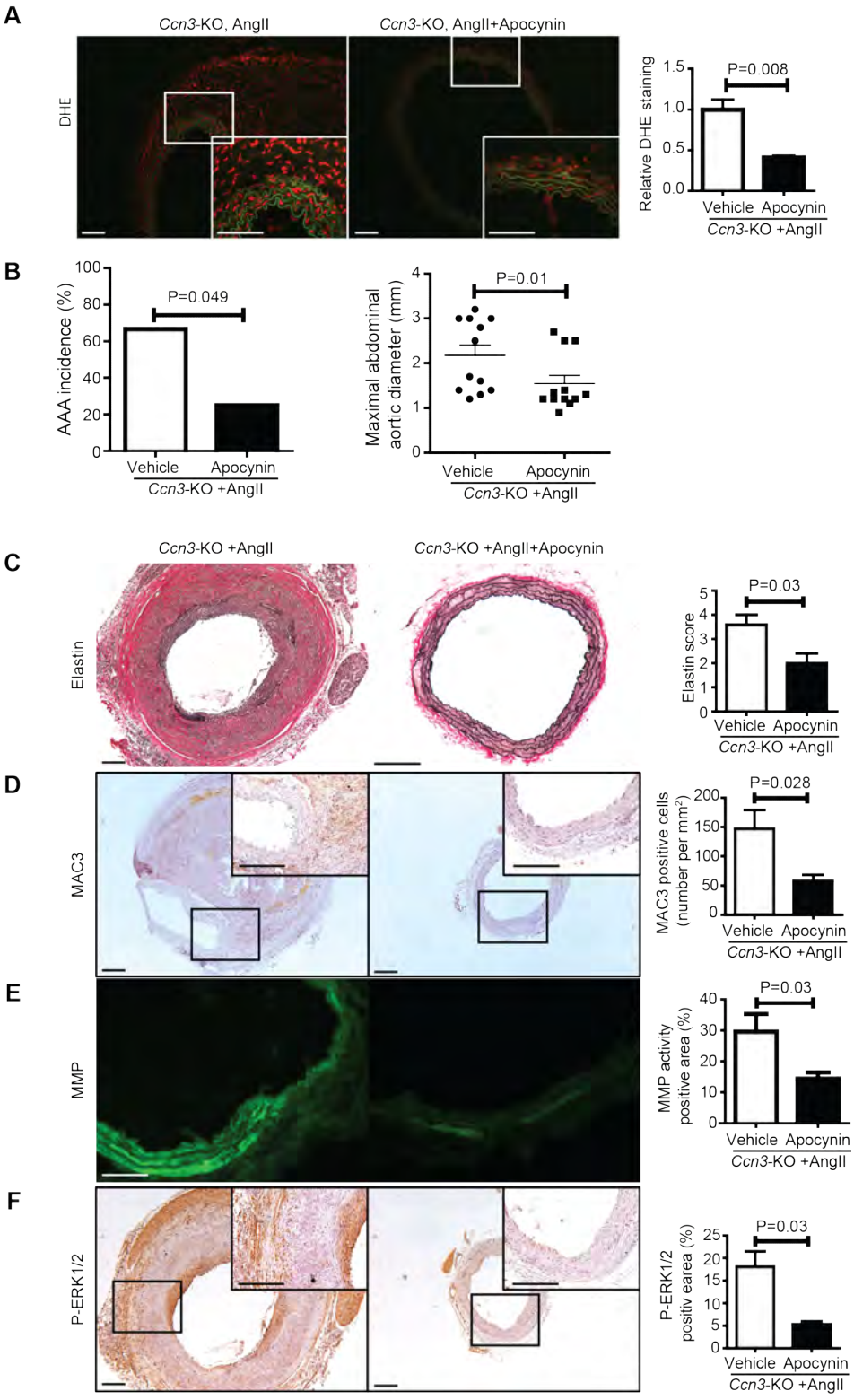
Supplemental Figure 9: CCN3 deficiency results in enhanced ROS production in abdominal aortas post-AngII treatment. ROS assessment by DHE staining in abdominal aortae following 7 days or 4 weeks of AngII infusion; For each treatment, Left: Merge image, middle: auto green fluorescence from elastin; right: DHE (red) image. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$. Scale bar = 50μm.

Supplemental Figure 10



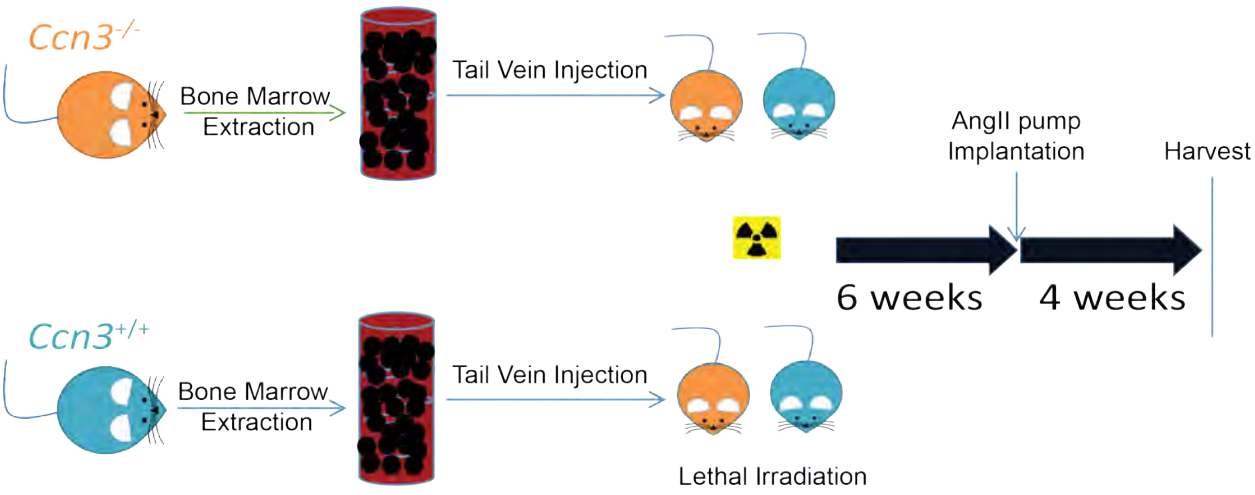
Supplemental Figure 10: CCN3 deficiency results in enhanced ROS production in abdominal aortae post 4-week AngII treatment. (A) ROS assessment by immunohistochemical detection of 4-HNE in abdominal aortae; scale bar = 50 μm; (B) Quantitative RT-PCR assessment of *p47* and cyclophilin A mRNA levels in WT and CCN3-deficient abdominal aortae, n= 6~8 per group, p<0.005; WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$. Two-way ANOVA followed by Bonferroni post-hoc correction was used.

Supplemental Figure 11



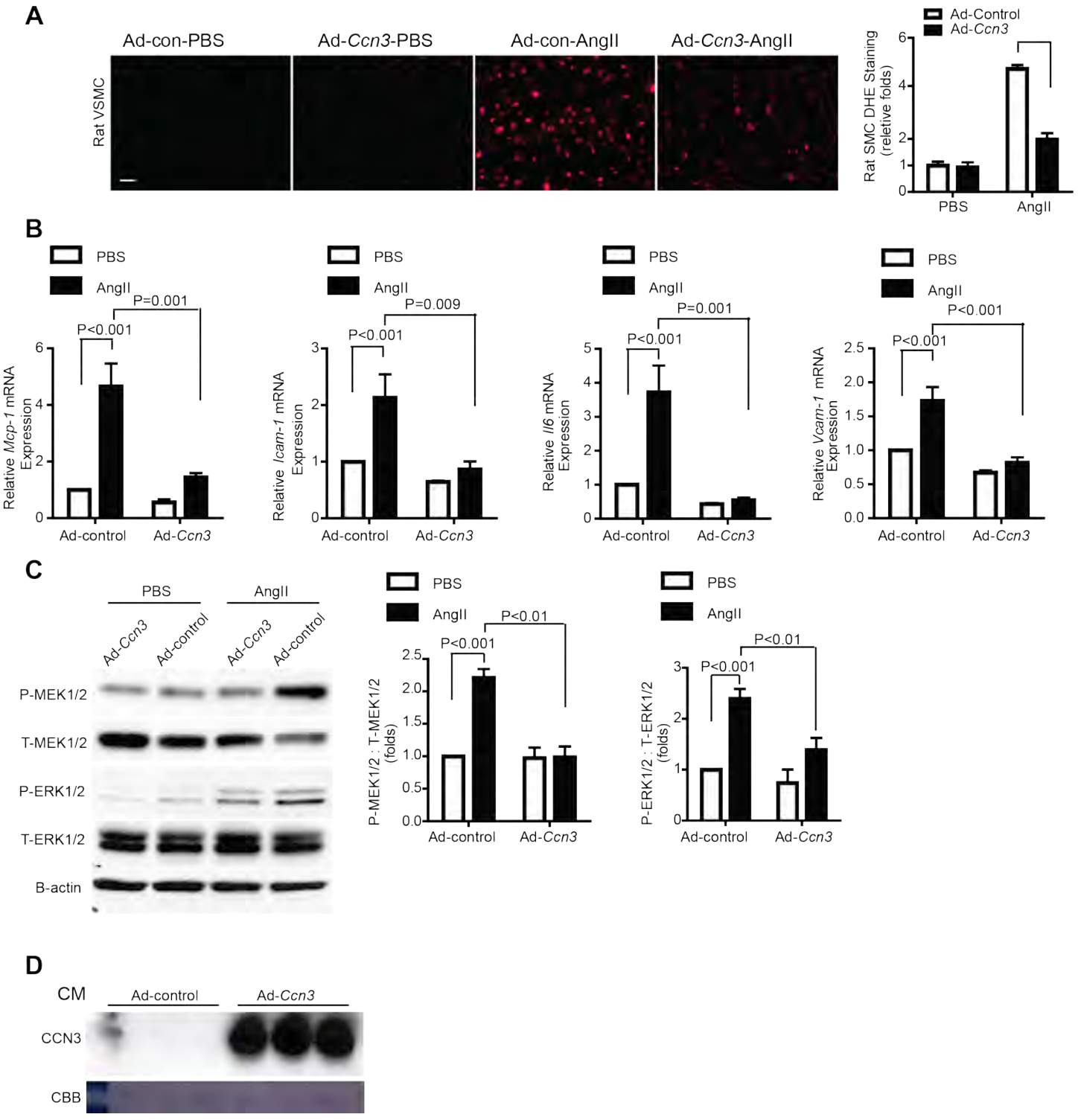
Supplemental Figure 11: ROS inhibition by apocynin ameliorates AngII-induced AAA formation in CCN3-deficient mice. (A) Confirmation of the inhibition of ROS production by DHE staining (n=5 per group). (B) Effects of ROS inhibition on AngII-induced AAA formation in CCN3-deficient mice. Left panel: AAA incidence; right panel: maximal external abdominal aortic diameter (n=12 per group). (C) Effects of ROS inhibition on elastin breakdown in CCN3-deficient aortae (n=5 per group). (D) Macrophage infiltration assessed by MAC3 staining (n=4 per group). (E) In situ MMP activity analysis in aorta. *Ccn3*-KO +AngII, n=5; *Ccn3*-KO + AngII + apocynin, n=6. (F) Immunohistochemical analysis of ERK1/2 activation in aortae of CCN3-deficient mice following vehicle and apocynin treatment in AngII-induced AAA model (n=4 per group). Scale bar = 100 μm. Mann Whitney test was used.

Supplemental Figure 12



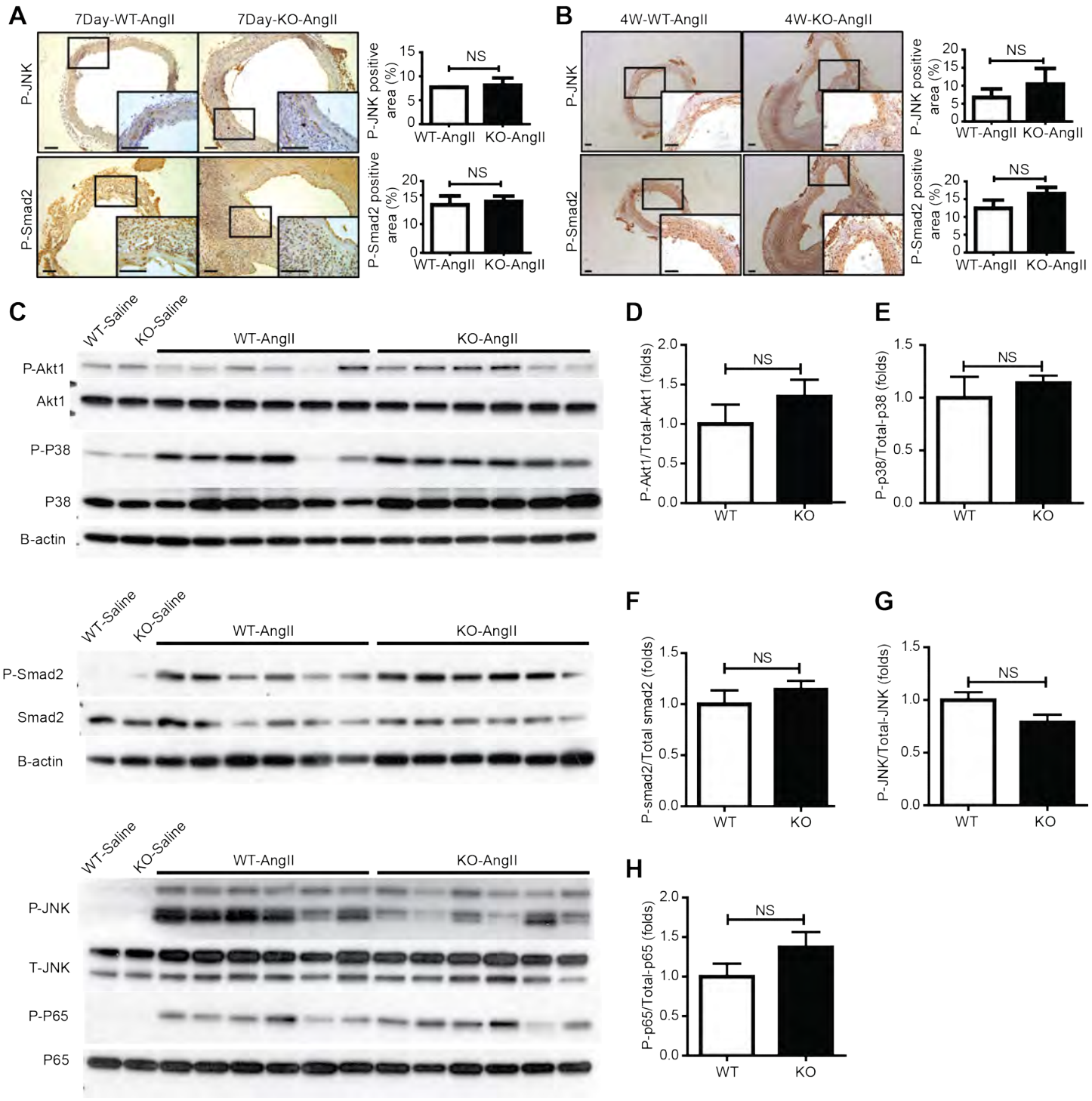
Supplemental Figure 12: Schema of bone marrow transplantation experiment.

Supplemental Figure 13



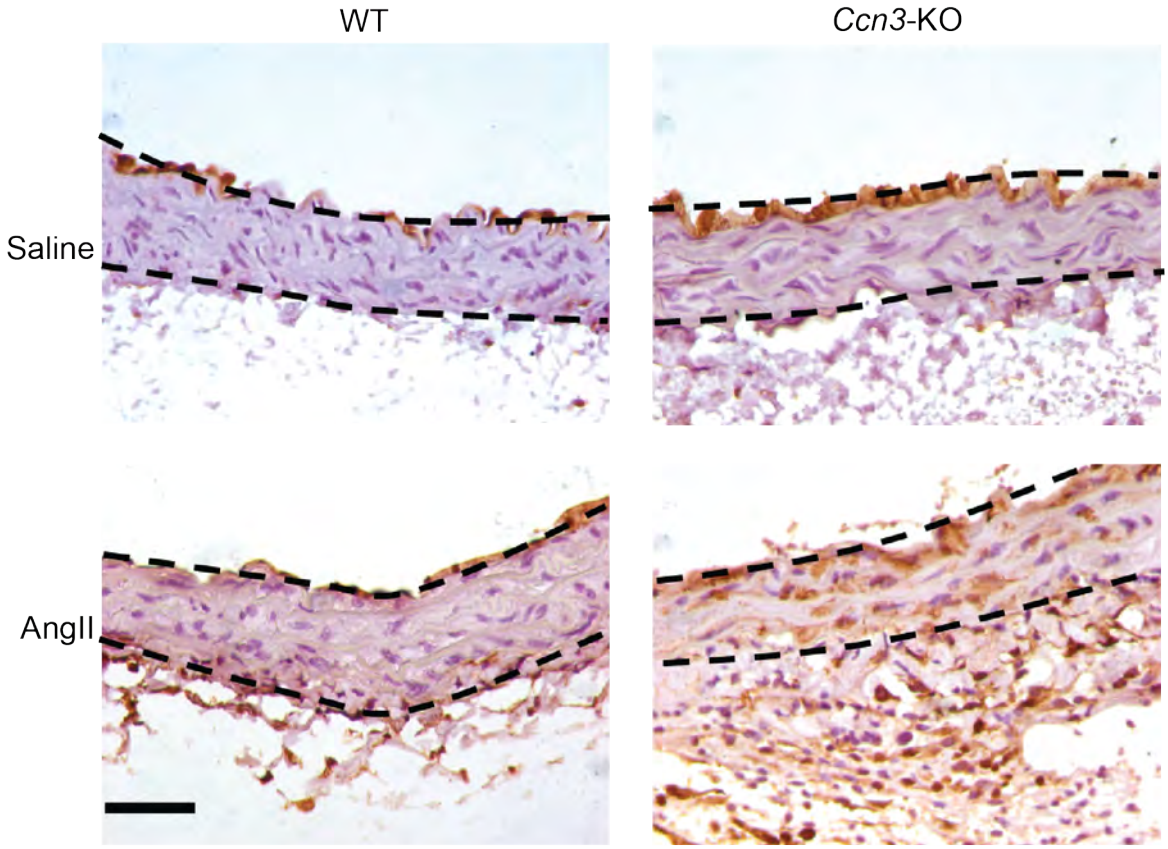
Supplemental Figure 13: CCN3 inhibits ROS production and inflammatory response following AngII treatment in rat aortic smooth muscle cells (RASMC). (A) Assessment of ROS generation by DHE staining (Scale bar = 50 μm.). RASMCs were infected with control or Ccn3 adenovirus, followed by treatment with AngII (1 μM) for 12 hours, n=4. (B) qRT-PCR assessment of *Mcp1*, *Icam1, Il6* and *Vcam1*, n=3 per group. (C) Western blot analysis of p-ERK1/2, total ERK1/2, p-MEK1/2, total MEK1/2 following 30-minute AngII treatment (n=3 per group). (D) Confirmation of CCN3 release into the medium, Coomassie Brilliant Blue staining (CBB) was performed to verify equal loading of conditional medium. CM: Conditional medium. Two-way ANOVA followed by Bonferroni correction was used.

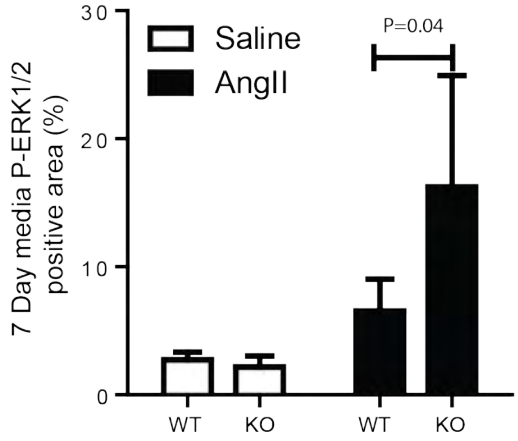
Supplemental Figure 14



Supplemental Figure 14: Assessment of several pathways implicated in AAA formation. Immunohistochemical analysis of TGFB (represented by p-Smad2) and p-JNK in aortae following 7 days (A) and 4 weeks (B) of AngII treatment; representative images shown on the left (scale bar = 100 μm), results are quantified in the right panels in WT vs CCN3-deficient aortae. n=3-5 per group; NS= not significant. (C) Protein lysates (same set of samples used in Figure 7C) were extracted from aortae following 1-week saline or AngII infusion and subjected to western blot analysis. TGFB (represented by p-Smad2), NFkB (represented by p-p65), JNK1/2, Akt and p38 pathways were assessed. Quantification is shown in (D) p-Akt1; (E) P-P38; (F) P-Smad2; (G) P-JNK; (H) P-p65. WT= *Ccn3*$^{+/+}$, KO=*Ccn3*$^{-/-}$. Mann Whitney test was used.

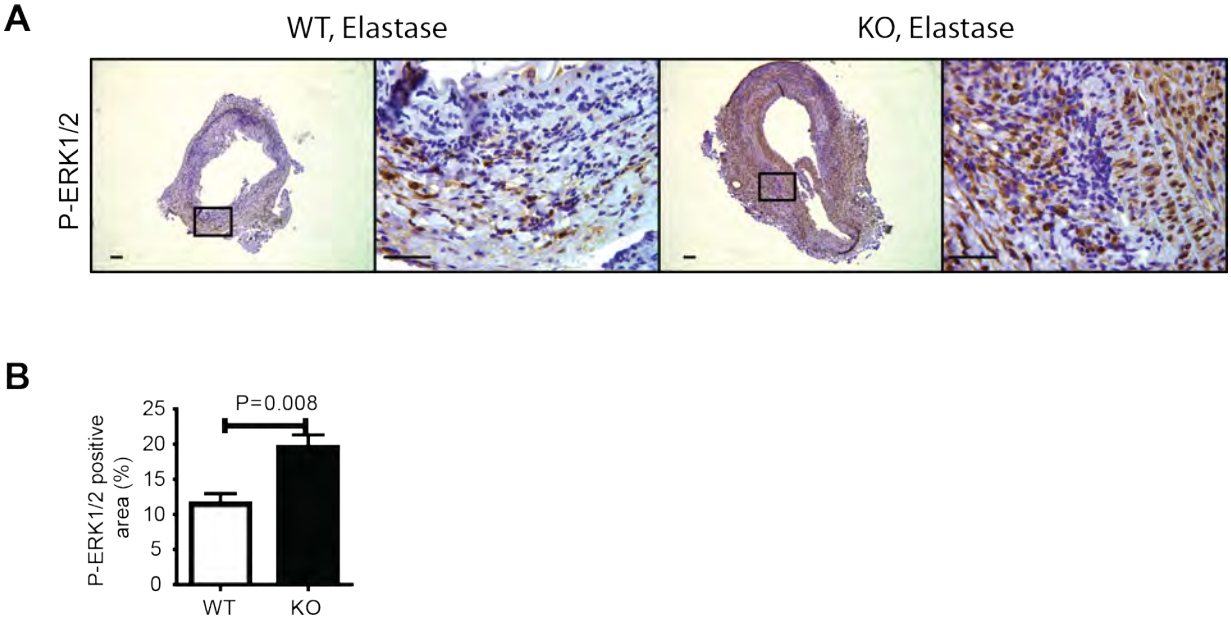
Supplemental Figure 15





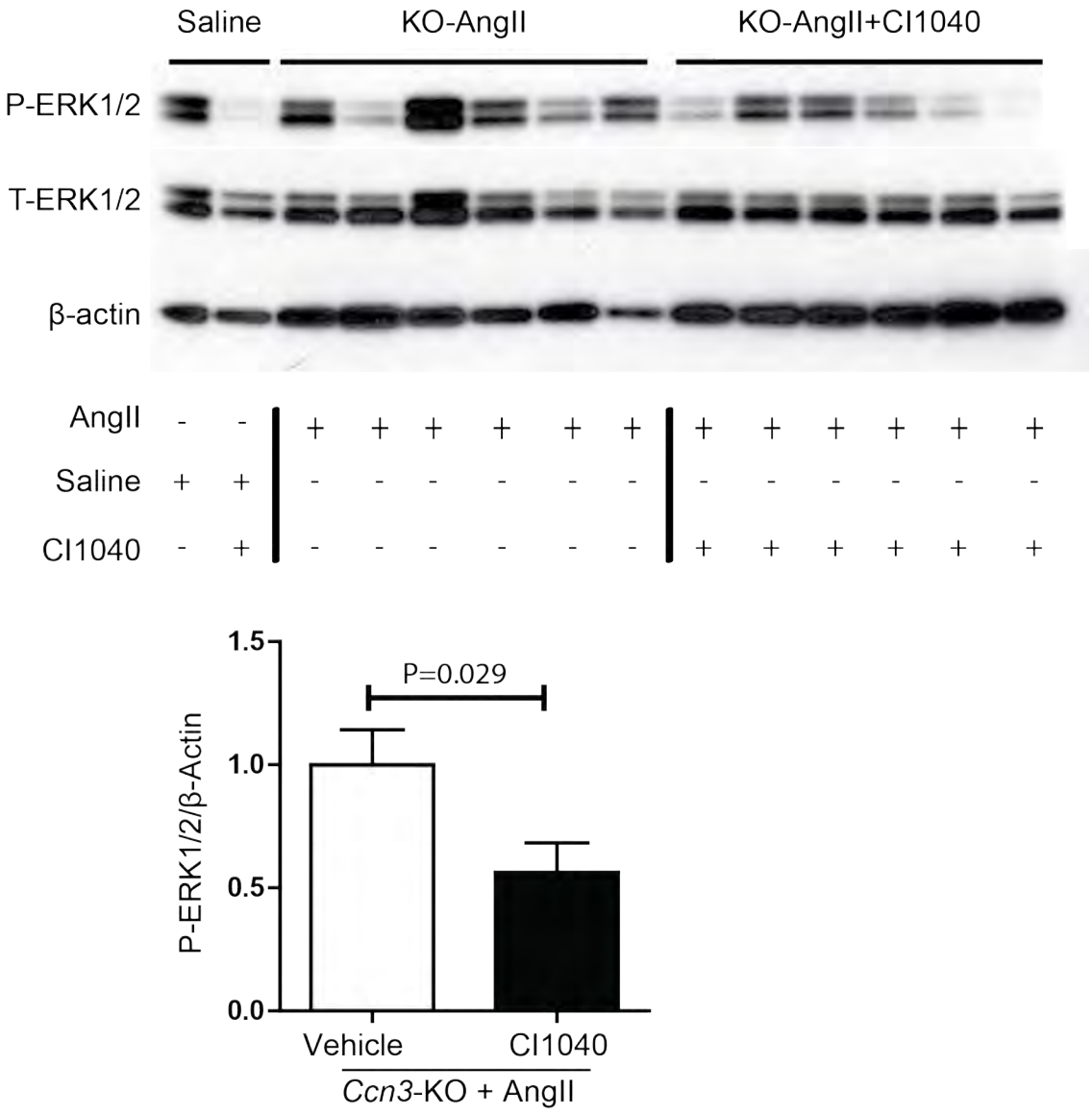
Supplemental Figure 15: Enhanced p-ERK1/2 in the media of CCN3-deficient aorta in response to AngII infusion. P-ERK1/2 in the media of aorta was quantified and standardized to the total area of aortic cross section. Scale bar = 50 μm. n=3-4 for saline group, n=5-6 for AngII group. Two-way ANOVA followed by Bonferroni correction was used. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$.

Supplemental Figure 16



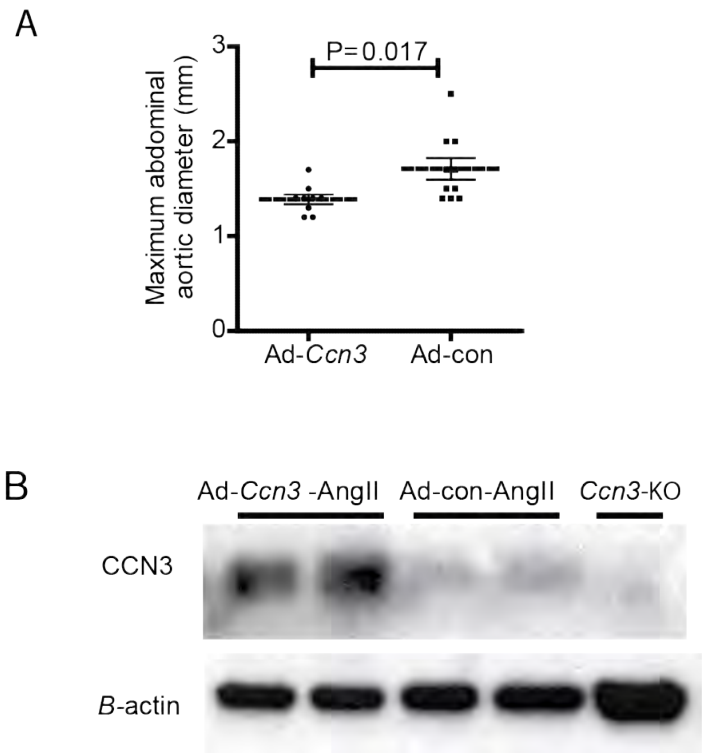
Supplemental Figure 16: Increased ERK1/2 activation in elastase perfused infrarenal aorta of CCN3-deficient mice**.** (A) Immunohistochemical analysis of ERK1/2 activation in elastase perfused aorta of WT and CCN3-deficient mice (scale bar = 50 μm); (B) Quantitation of p-ERK1/2 in infrarenal aorta. n=6 per group. Mann Whitney test was used. WT=*Ccn3*$^{+/+}$; KO=*Ccn3*$^{-/-}$.

Supplemental Figure 17



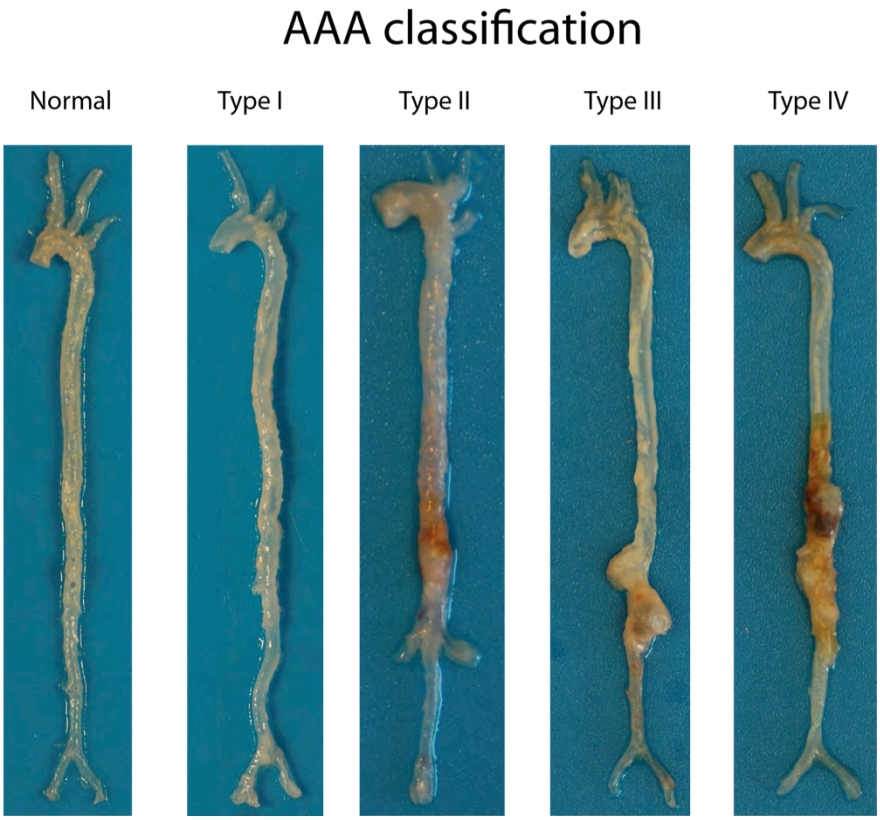
Supplemental Figure 17: Confirmation of inhibition of ERK1/2 activation by CI-1040. Western blot was performed to assess the efficacy of CI-1040 treatment on ERK1/2 activation. KO=*Ccn3*$^{-/-}$, n=6. Mann Whitney test was used.

Supplemental Figure 18



Supplemental Figure 18: Inhibition of aortic dilation in *Apoe*-null mice by CCN3 overexpression in response to 2-week AngII treatment. (A) Measurement of suprarenal abdominal aortic diameter, n=9 per group. (B) Confirmation of adenovirus-mediated CCN3 overexpression in the suprarenal abdominal aorta.

Supplemental Figure 19



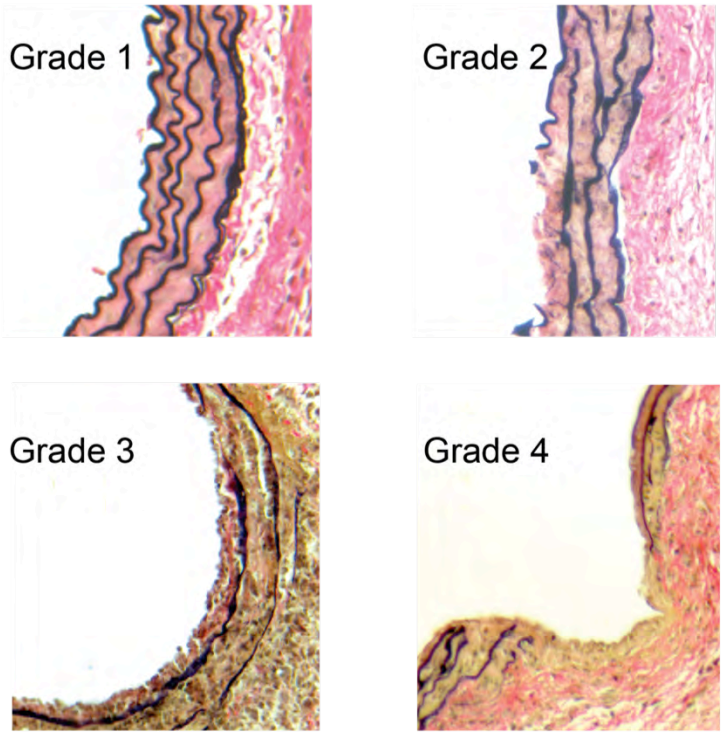
Supplemental Figure 19: AAA classification. **Type I**, dilated lumen without thrombus;

Type II, remodeled aneurysmal tissue with little thrombus;

Type III, a pronounced bulbous form of Type II with thrombus;

Type IV, multiple, often overlapping aneurysms containing thrombus.

Supplemental Figure 20



Supplemental Figure 20: Elastin grading criteria. Grade 1, intact, well-organized elastic laminae; Grade 2, elastic laminae with some interruptions and breaks; Grade 3, severe elastin fragmentation or loss; and Grade 4, severe elastin digestion with visible ruptured sites.