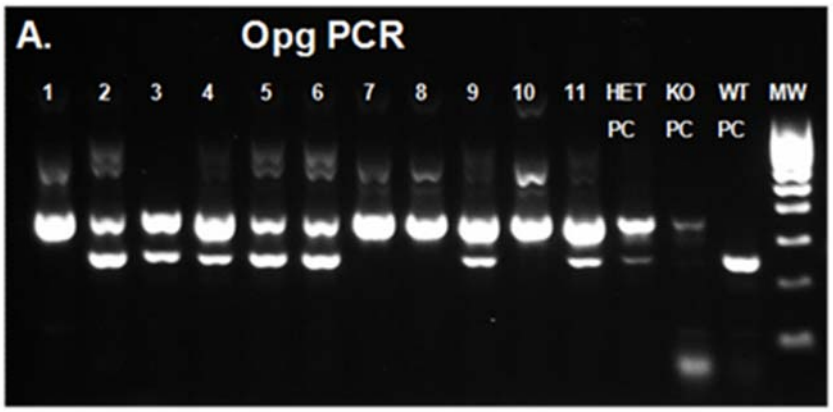
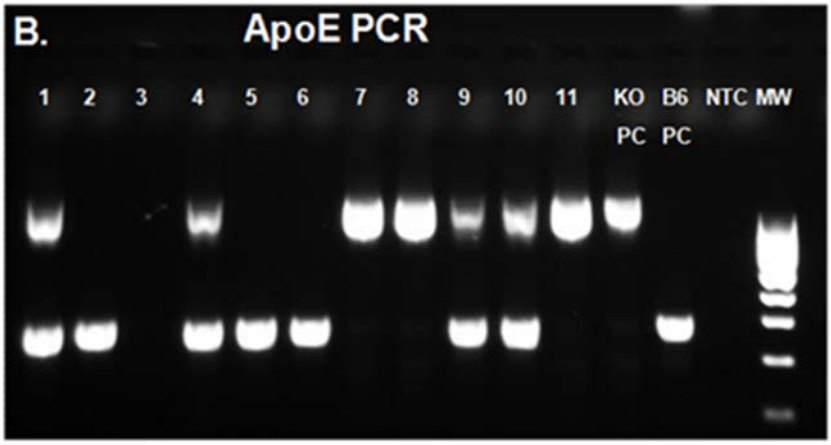
SUPPLEMENTAL MATERIAL

Supplemental Figures



	Opg(+/+)	Opg(+/-)	Opg(-/-)
WT allele	+ (250bp)	+ (250bp)	-
Opg null allele	-	+ (366bp)	+ (366bp)



	ApoE(+/+)	ApoE(+/-)	ApoE(-/-)
WT allele	+ (300bp)	+ (300bp)	-
ApoEnull allele	-	+ (1.2Kb)	+ (1.2Kb)

Note: 7 and 8 are ApoE.OPG double knockouts

Figure I: Homozygous null mutations for osteoprotegerin (*Opg*$^{-/-}$) generated in apolipoprotein E-deficient C57Bl/6 (*ApoE*$^{-/-}$) mice. Representative agarose gel electrophoretic profiles of PCR-amplified products of *Opg* (**A**) and *ApoE* (**B**) PCR. *PC, positive control; NTC, no template control; WT, wild-type; KO, knock-out; MW, molecular weight ladder (100bp).*

Figure II: Aortic region boundaries. *Aortic arch*: from heart to left subclavian artery; *thoracic aorta* (TA): from left subclavian artery to the aortic hiatus in the diaphragm; *suprarenal aorta* (SRA): from the aortic hiatus to the left renal artery; *infrarenal aorta* (IRA): from left renal artery to the aortic bifurcation at the left and right common iliac arteries.

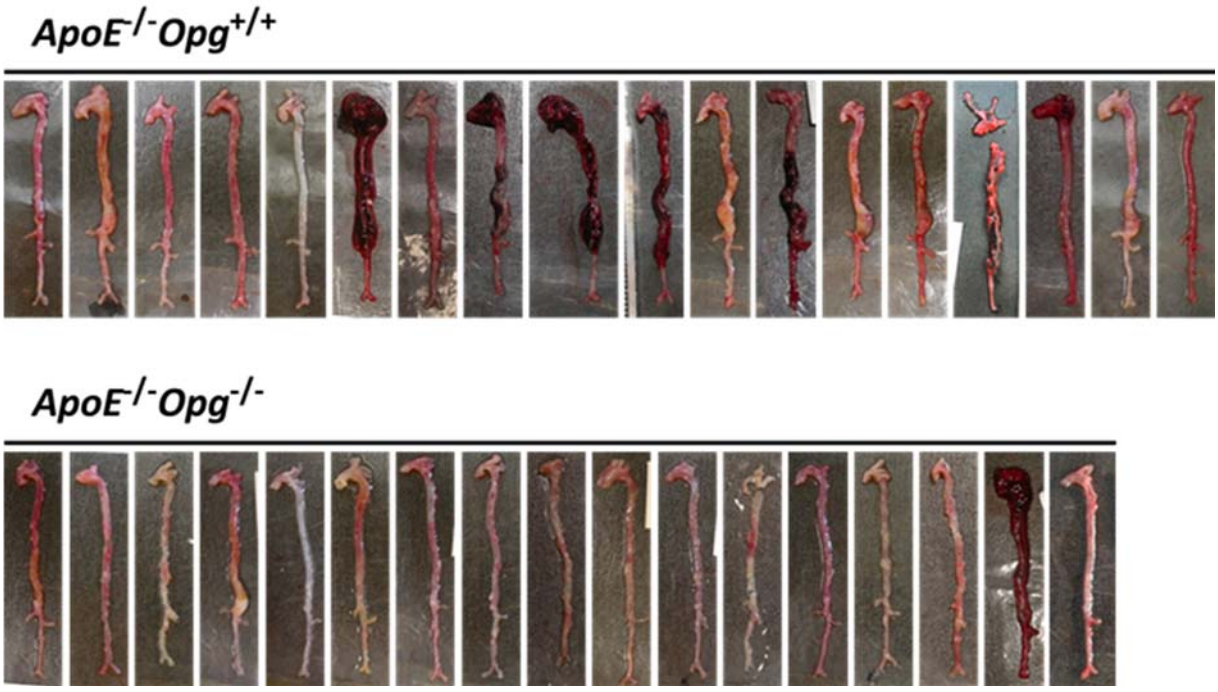


Figure III: Effect of *Opg* deficiency on AngII-induced aortic dilatation in *ApoE*$^{-/-}$ mice. Gross morphology of aortas harvested from *ApoE*$^{-/-}$*Opg*$^{+/+}$ (control) and *ApoE*$^{-/-}$*OPG*$^{-/-}$ mice subcutaneously infused with AngII (1.0 µg/kg/min) for 28 days.

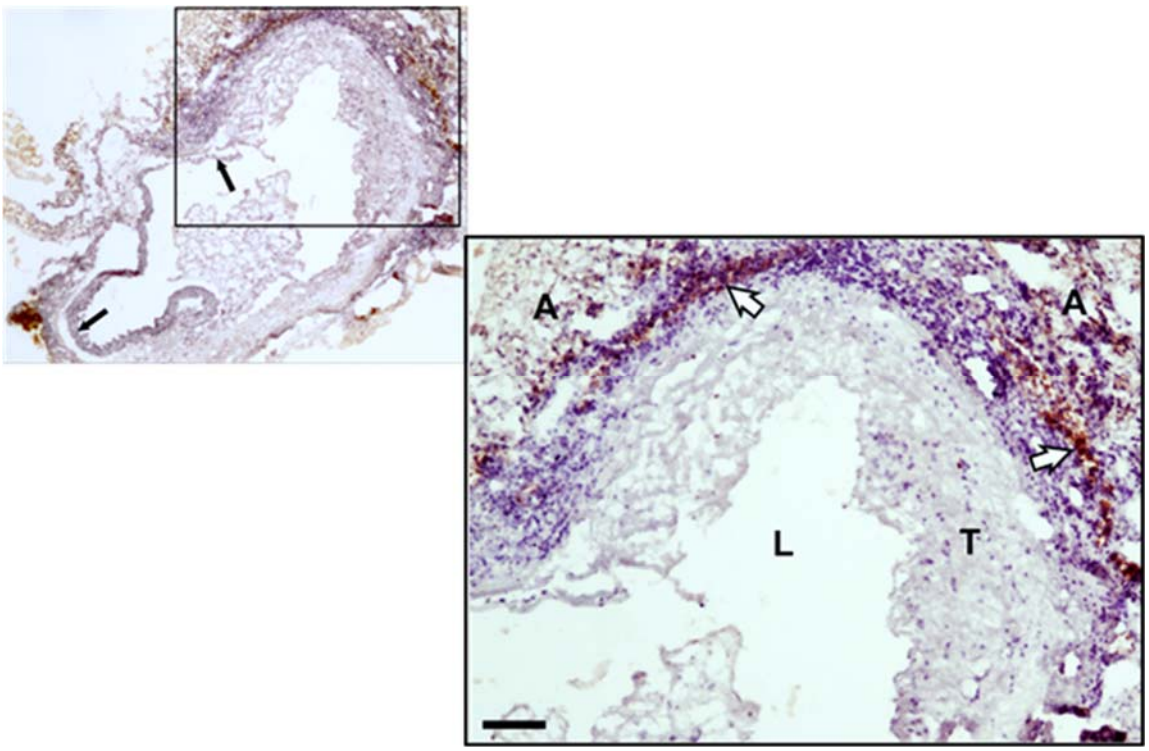


Figure IV: Immuno-detection of monocyte/macrophages within *ApoE*$^{-/-}$*Opg*$^{-/-}$ aorta. Immunohistochemical localisation of MOMA-2 (brown stain) identifying monocyte/macrophages (white arrows) within the adventitia of 5μm frozen-sectioned SRA from an *Opg*-deficient *ApoE*$^{-/-}$ mouse infused with AngII over 28 days. **A**, adventitia; **L**, lumen; **T**, intramural thrombus; **black arrows** indicating elastic lamellae (media); Scale bar = 0.1 mm.

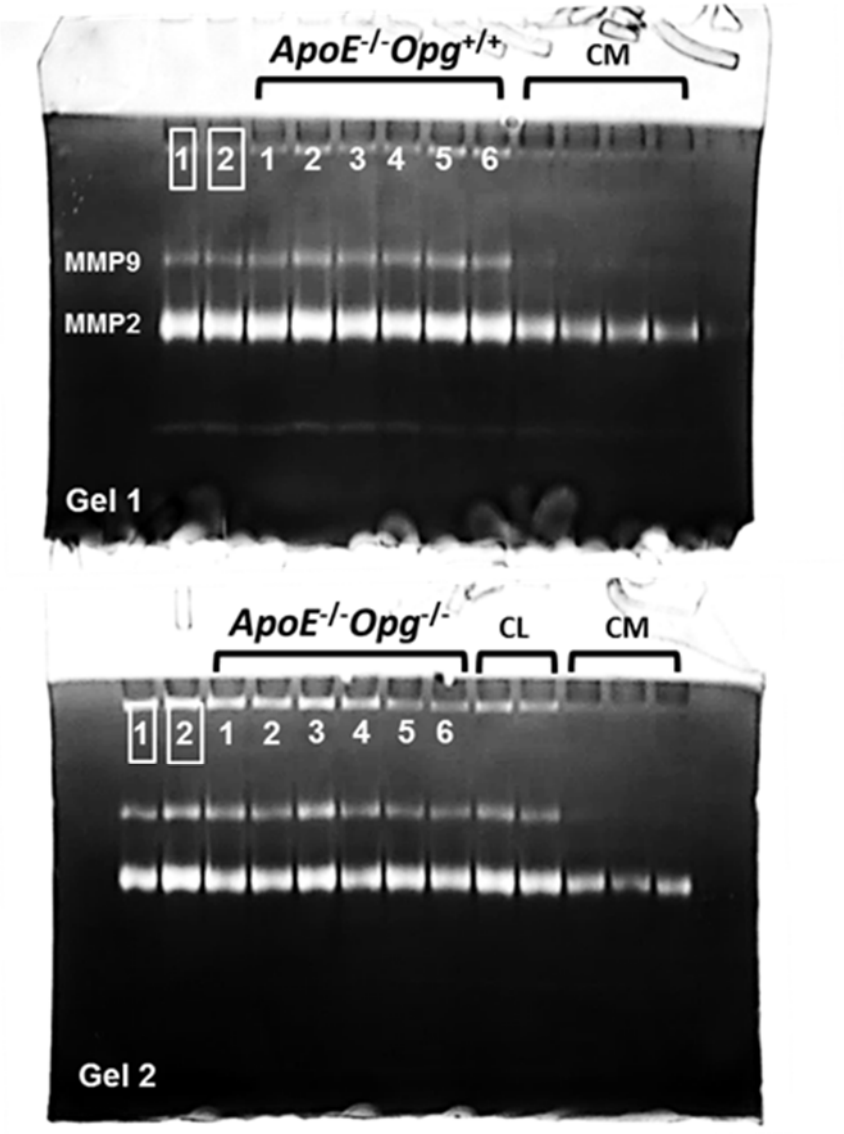


Figure V: Effect of *Opg* deficiency on aortic levels of MMP2 and MMP9 in AngII-infused *ApoE*$^{-/-}$ mice. Zymographic detection of MMP2 and MMP9 in aortic tissue from *ApoE*$^{-/-}$*OPG*$^{+/+}$ (n=6; Gel 1) and *ApoE*$^{-/-}$*Opg*$^{-/-}$ (n=6; Gel 2) mice following infusion of AngII for seven days. Boxed numerals 1 and 2 are duplicate samples from Gel 1 included on Gel 2 for normalisation of densitometry between gels; **CL**, cell lysate and conditioned media (**CM**) from cultured vascular smooth muscle cells as positive control (marker) for MMP2 and 9.

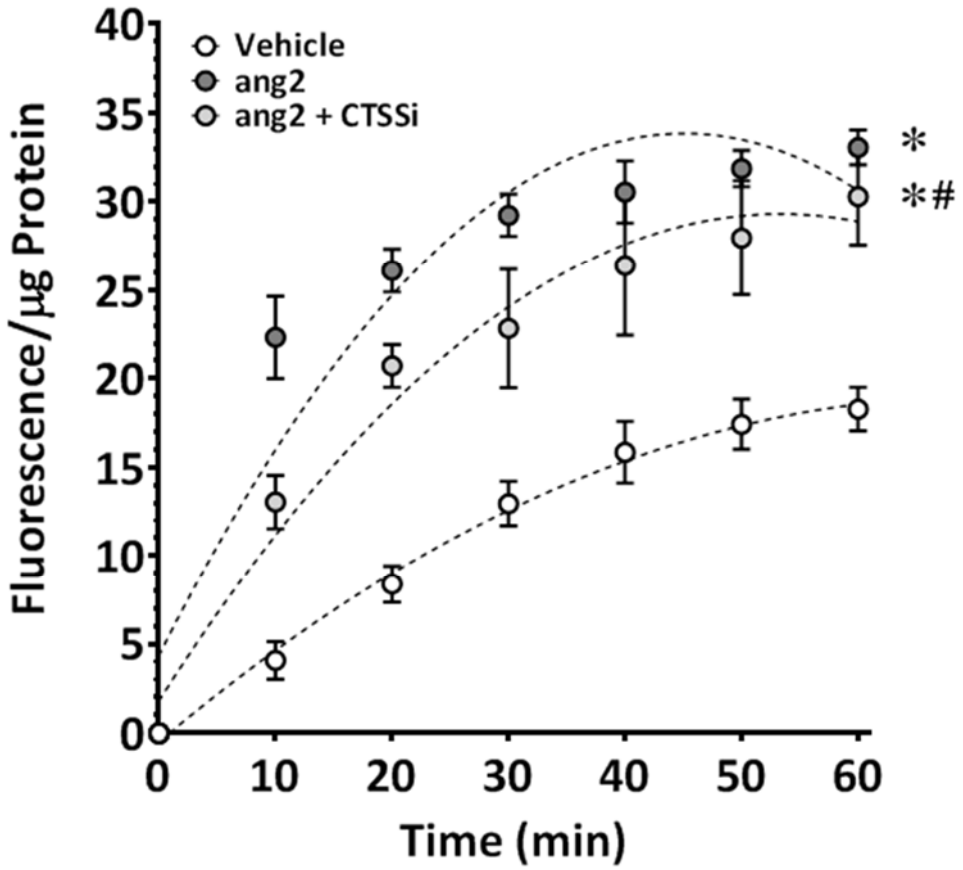


Figure VI: AngII-induced CTSS-derived elastase activity in AoSMC *in vitro*. Elastase activity in healthy human AoSMC cultured in the presence and absence of AngII (100 nM) over 36 hours measured using an elastin degradation assay. Addition of a specific CTSS inhibitor Z-FL-COCHO (CTSSi, 20 nM) to AngII-activated AoSMC confirmed CTSS-derived elastase activity induced by AngII. *AngII, angiotensin II; CTSS, cathepsin S; AoSMC, aortic smooth muscle cells; Data expressed at each time point (n=6 cultures) as mean and standard deviation CTSS activity (fluorescence/µg protein); $^{*}P<0.001$ and $^{\#}P=0.042$ calculated by mixed-effects linear regression for difference between vehicle and AngII and AngII+CTSSi, and between AngII and AngII+CTSSi, respectively.*

Supplementary Table

Table I: Regional aortic diameters in AngII-infused wild-type (*ApoE*$^{+/+}$*Opg*$^{+/+}$) and *Opg*-null (*ApoE*$^{+/+}$*Opg*$^{-/-}$) mice

	ApoE$^{+/+}$*Opg*$^{+/+}$	*ApoE*$^{+/+}$*Opg*$^{-/-}$	P
N	30	28	
Arch	1.48 (1.39-1.67)	1.41 (1.27-1.50)	0.019
TA	1.31 (1.22-1.48)	1.23 (1.12-1.33)	0.014
SRA	1.37 (1.26-1.69)	1.27 (1.17-1.48)	0.065
IRA	0.79 (0.67-0.84)	0.77 (0.68-0.88)	0.562

Opg, osteoprotegerin; n, number; TA, thoracic aorta; SRA, suprarenal aorta; IRA, infra-renal aorta; data presented as median (interquartile range) maximum diameter (mm); P, 2-sided P-value for comparison between groups by Mann-Whitney U test.