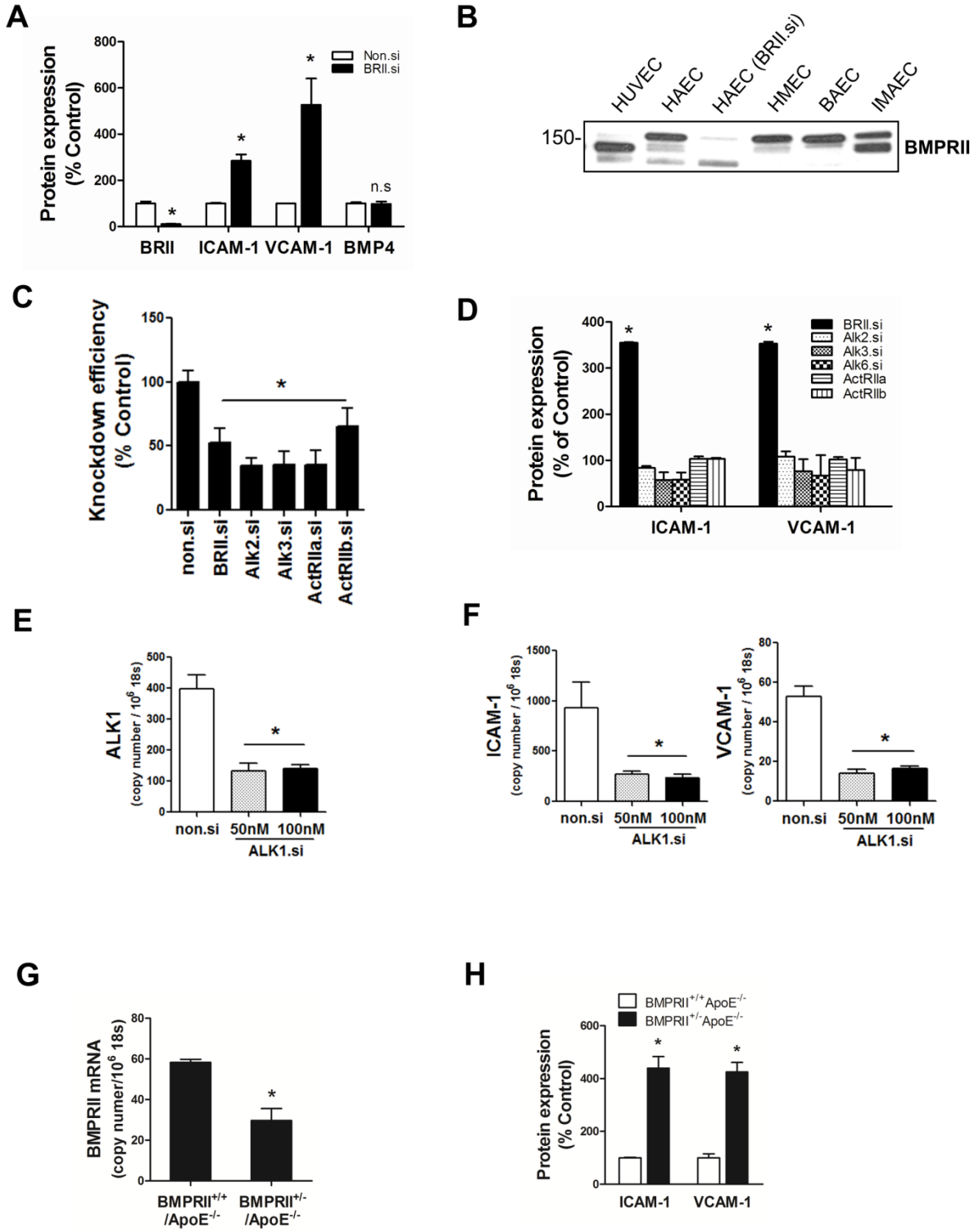
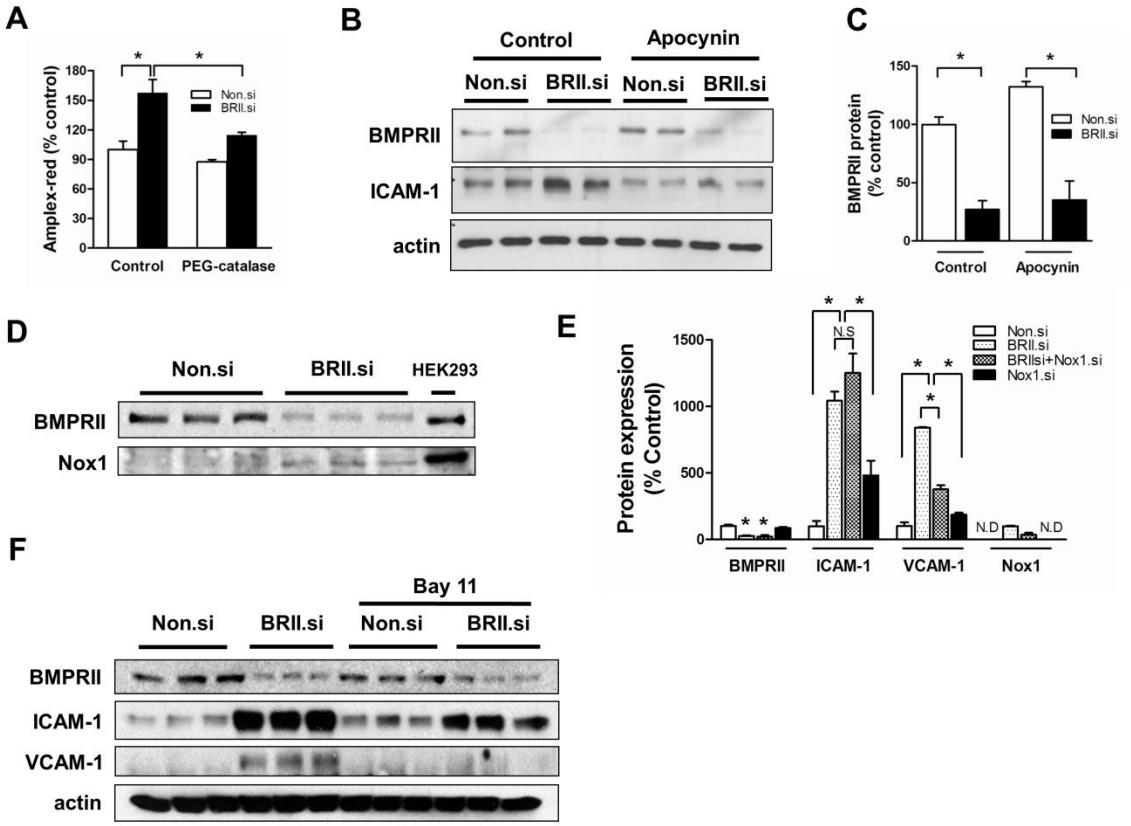
Supplemental Material: Figures I-VI and Table I-II



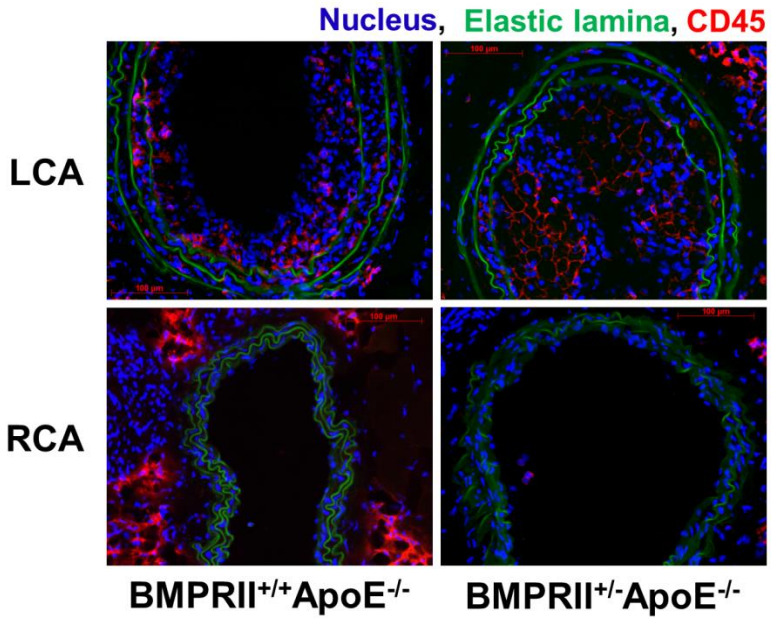
Supplemental Figure I.

(A) Quantification of BMPRII, ICAM-1, VCAM-1 and BMP4 protein expression by Western blot. Cell lysates obtained from HUVECs transfected with BMPRII siRNA (BRII.si) or non-silencing control siRNA (Non.si) were analyzed by Western blot using antibodies to BMPRII, ICAM-1, VCAM-1 and BMP4. The representative Western blot is shown in main Figure 1B. (B) Western blot showing BMPRII expression in various endothelial cell sources. BAEC- bovine arterial endothelial cells; HAEC- human aortic endothelial cells HMEC-human microvascular endothelial cells; iMAEC-immortalized mouse aortic endothelial cells. Some HAECs were treated with BRII.si to determine specificity of Western blot analysis. (C, D) HAECs were transfected with siRNAs specific for BMPRII, Alk2, Alk3, Alk6, ActRIIa, ActRIIb or control (Non.si). Transfection efficiency was quantified by qPCR (n=3, *p<0.05). (C). Note that Alk6 was nearly undetectable in ECs even without the Alk6 siRNA treatment. Western blot analyses using antibodies for BMPRII, VCAM-1, ICAM-1 and b-actin antibodies were carried out and graph in (D) shows quantification of Western blot results (n=3, *p<0.05). The representative Western blot is shown in main Figure 1D. (E) Knockdown efficiency of Alk1 siRNA in HUVECs is shown by qPCR (n=3, *p<0.05). (F) Effect of Alk1 siRNA treatment on ICAM1 and VCAM1 in HUVECs is shown by qPCR (n=3, *p<0.05). (G) Graph shows BMPRII mRNA expression from the endothelial-enriched RNA isolated from thoracic aortas of $BMPRII^{+/-}ApoE^{-/-}$ and $BMPRII^{+/+}ApoE^{-/-}$ mice, respectively, (n=4 each). (H) Quantification of immunofluorescence staining using ICAM-1 and VCAM-1 antibodies on the cross-sections of thoracic aortas obtained from $BMPRII^{+/-}ApoE^{-/-}$ and $BMPRII^{+/+}ApoE^{-/-}$ mice, respectively, (n=6).



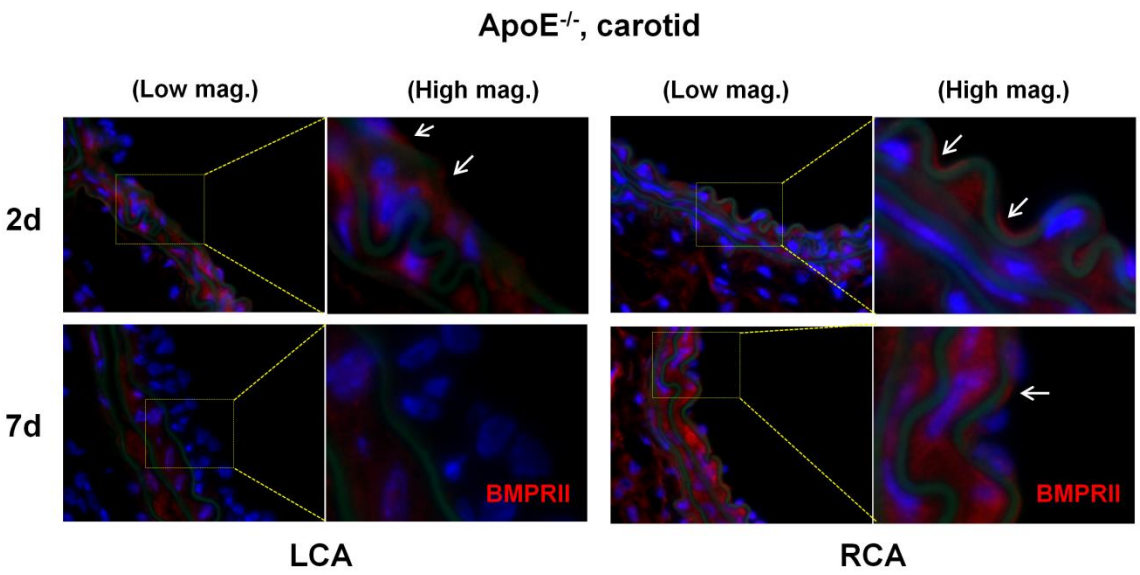
Supplemental Figure II

(A) Quantification of Amplex-Red assay. HUVECs were treated with 100 nM BMPRII siRNA (BRII.si) or control (Non.si) siRNA. PEG-catalase was used to scavenge ROS generation. (*, $p<0.05$, n=4) (B and C) BRII.si and non.si transfected ± apocynin-treated cells were lysed for immunoblot with antibodies to BMPRII, and ICAM-1, using β-actin as loading control. (C) Graph shows quantification of Western blot data shown in B; mean ± SEM (n=4, *$p< 0.05$). (D) Western blot shows expression of nox1 and BMPRII on cell lysates from HUVECs transfected with BRII.si or Non.si. (E) Graph shows quantification of Western blot analysis shown in main Fig. 2E. Data are expressed as mean ± SEM (n=4, *$p< 0.05$). (F) Western blot show the expression of BMPRII, ICAM1 and VCAM1 on HUVECs transfected with BRII siRNA or non.si and treated with or without NFκB inhibitor, BAY11-7082 (10μM, 24 h).



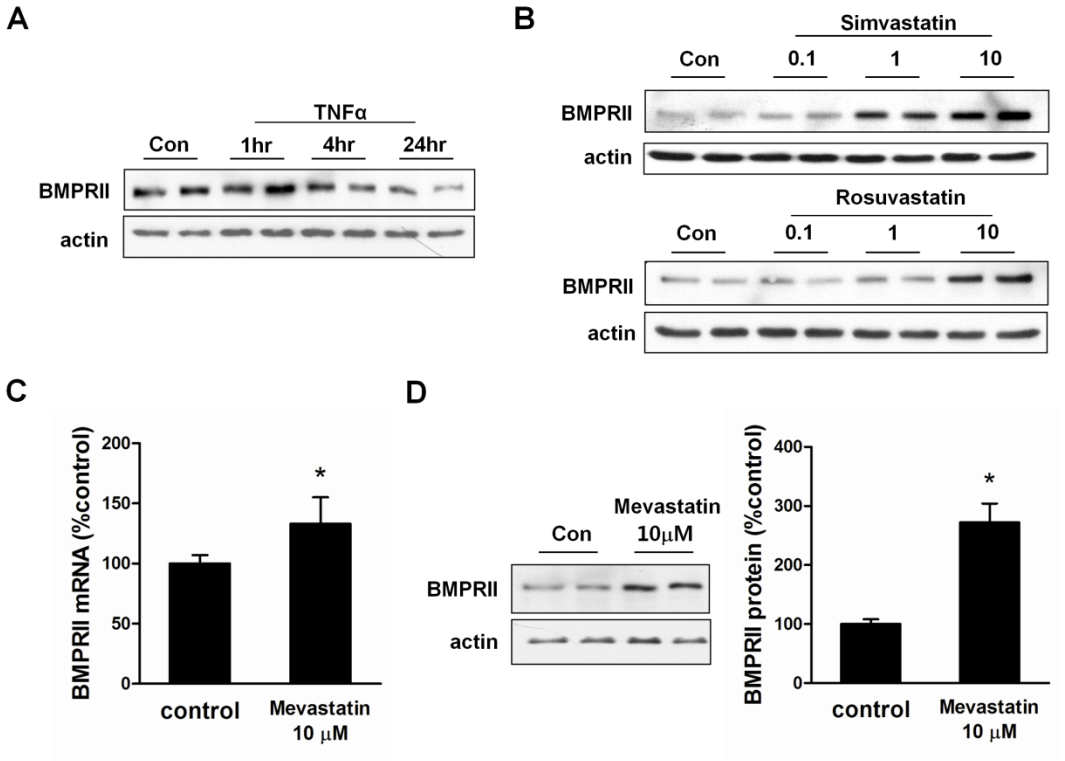
Supplementary Figure III. BMPRII deficiency exacerbates CD45+ leukocyte infiltration to atherosclerotic plaques in ApoE$^{-/-}$ mice.

BMPRII$^{+/-}$ApoE$^{-/-}$ and littermate control BMPRII$^{+/+}$ApoE$^{-/-}$ mice were partially ligated and fed a high-fat diet for 2 weeks. Frozen sections from the ligated LCA (*d-flow*) and contralateral RCA (*s-flow*) were stained with CD45 antibody as shown by representative (n=4) microscopy images. Blue= DAPI nuclear staining; Green= elastic laminas, Red= CD45 staining.



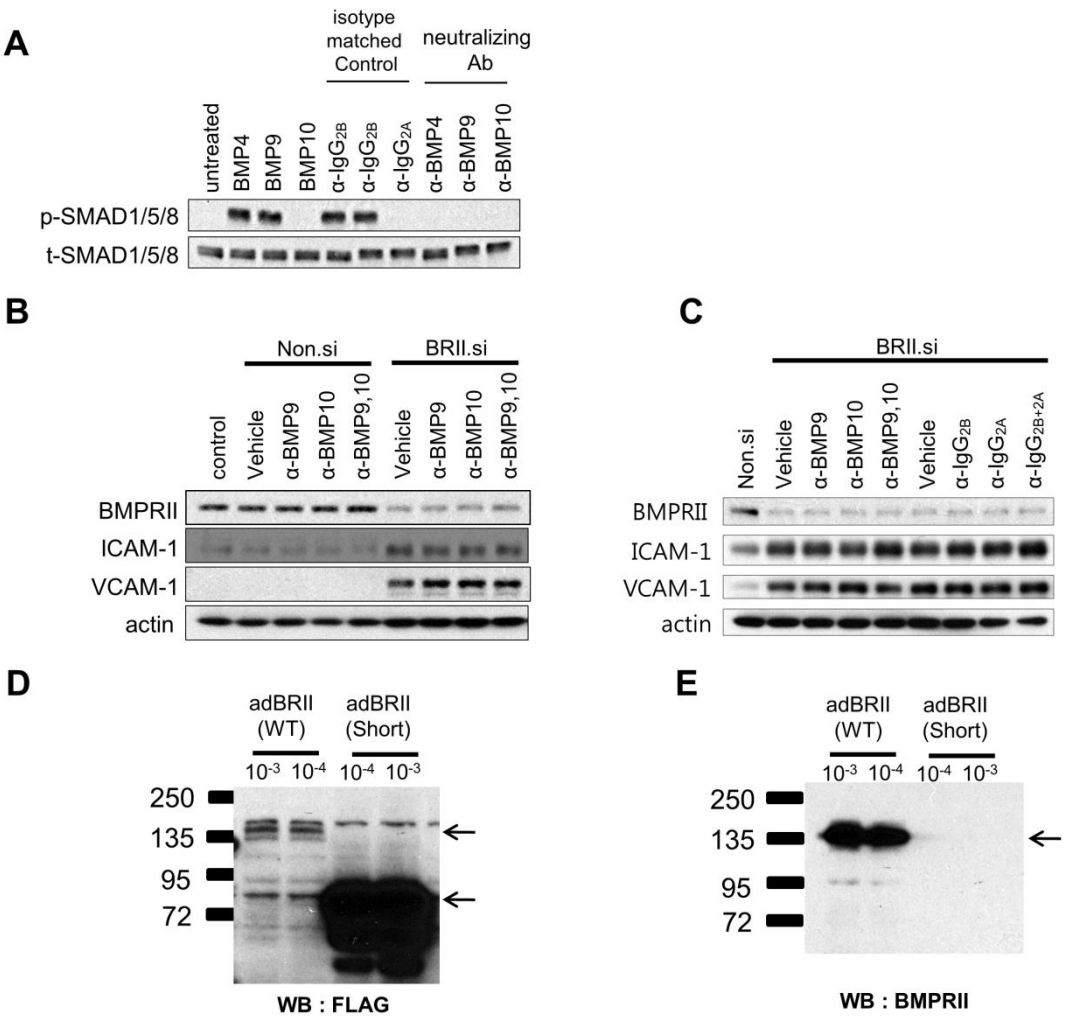
Supplemental Figure IV

ApoE$^{-/-}$ mice were partially ligated and fed a high-fat diet for 2 or 7 days. Frozen sections of ligated LCA and RCA exposed to *d-flow* were stained with BMPRII antibody. Representative confocal microscopy images of mouse carotids show loss of BMPRII expression. DAPI staining for nuclei (blue); BMPRII antibody (red) and auto-fluorescence signal for elastic lamina (green). Arrows point to BMPRII expression in the endothelial layer. Images shown on the right are magnified regions indicated by broken boxes (low-magnification).



Supplemental Figure V

(A) Representative immunoblots show the expression of BMPRII in HUVECs treated with 20ng/ml TNF-α for 1 h, 4 h, and 24 h. (B) Immunoblot shows expression of BMPRII in HUVECs treated with simvastatin and Rosuvastatin (μM). (C) qPCR and (D) immunoblot showing the expression of BMPRII in HUVECs treated with mevastatin (10μM). Graph shows quantification of the immunoblot data (*, $p<0.05$, n=6).



Supplemental Figure VI

(A) HUVECs were treated with BMP4 (50 ng/ml), BMP9 (10 ng/ml), BMP10 (50 ng/ml), BMP4 neutralizing antibody (1 μg/ml), BMP9 neutralizing antibody (10 μg/ml), BMP10 neutralizing antibody (10 μg/ml), or each IgG control. Cells were lysed and analyzed by Western blot with antibodies specific to phospho-SMAD1/5/8 and total SMAD1/5/8. (B, C) HUVECs were treated with BRII siRNA or Non.si. One day later, cells were incubated in complete medium containing neutralizing antibodies specific to BMP9 or BMP10 (10 μg/ml each) or IgG controls for 1 day and analyzed by Western blot using antibodies to BMPRII, ICAM1, VCAM1 or actin. Representative immunoblots (n=3) show that increased expression of ICAM1 and VCAM1 by BRII.si was not affected by either antibodies used. (D, E) HEK293 cells were infected with adenoviral BMPRII WT or BMPRII Short-form for 24 h, Western blots show expression of FLAG and BMPRII. Note that the BMPRII antibody used here was raised against the C-terminal domain and could not detect the short-form.

Supplemental Table I

Serum lipid profile of partially ligated BMPRII$^{+/+}$ApoE$^{-/-}$ and BMPRII$^{+/-}$ApoE$^{-/-}$ mice fed with a high-fat diet (two weeks).

Mouse	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
BMPRII$^{+/+}$ApoE$^{-/-}$ (n=12)	1384.56±229.62	66.56±47.54	13.53±5.20	786.67±130.24
BMPRII$^{+/-}$ApoE$^{-/-}$ (n=14)	1501.11±232.72	48.79±12.45	17.12±20.66	842.89±187.44

Supplemental Table II. List of q-PCR primers

Target Gene	Species		Sequence
BMPRII	Human	Forward	5'-TCTTTCAGCCACAAATGTCCT-3'
		Reverse	5'-TGCCATCTTGTGTTGACTCAC-3'
BMPRII	Mouse	Forward	5'-GAGCCCTCCCTTGACCTG-3'
		Reverse	5'-GTATCGACCCCGTCCAATC-3'
ICAM-1	Human	Forward	5'-CCTTCCTCACCGTGTACTGG-3'
		Reverse	5'-AGCGTAGGGTAAGGTTCTTGC-3'
ICAM-1	Mouse	Forward	5'-AGGTGGTTCTTCTGAGCGGC-3'
		Reverse	5'-AAACAGGAACTTTCCCGCCA-3'
VCAM-1	Human	Forward	5'-TGGACATAAGAAACTGGAAAAGG-3'
		Reverse	5'-CCACTCATCTCGATTTCTGGA-3'
VCAM-1	Mouse	Forward	5'-TCTTGGGAGCCTCAACGGTA-3'
		Reverse	5'-CAAGTGAGGGCCATGGAGTC-3'