Homeostatic, non-canonical role of macrophage elastase in vascular integrity

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Supplemental Materials

Supplemental Figures

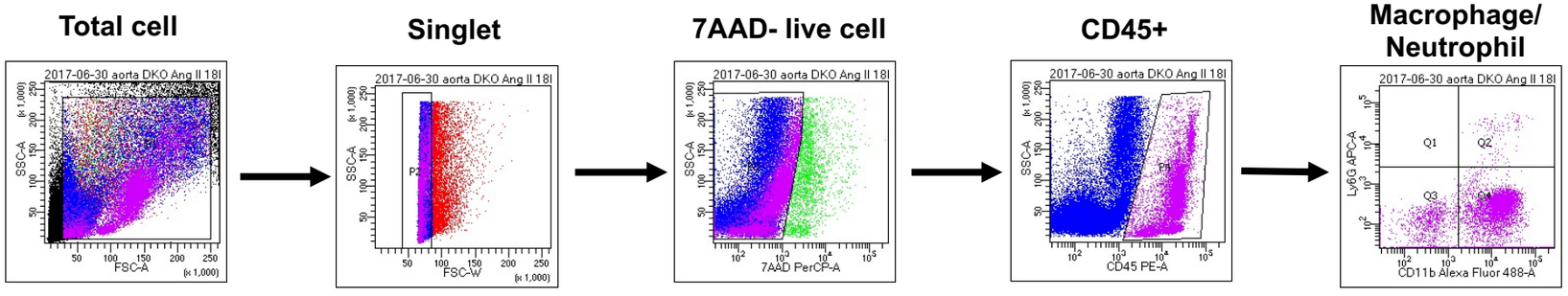

Figure S1. Gating Strategy for Flow Cytometry. Forward and side scatter density plots were used to exclude debris and single cells were identified based on FSC-W / SSC-A plots. Live cells were identified from 7AAD- density plots and CD45+ cells were gated on. Finally, the CD45+ population was further analyzed for the CD11b and Ly6g signal to identify macrophage and neutrophil populations.

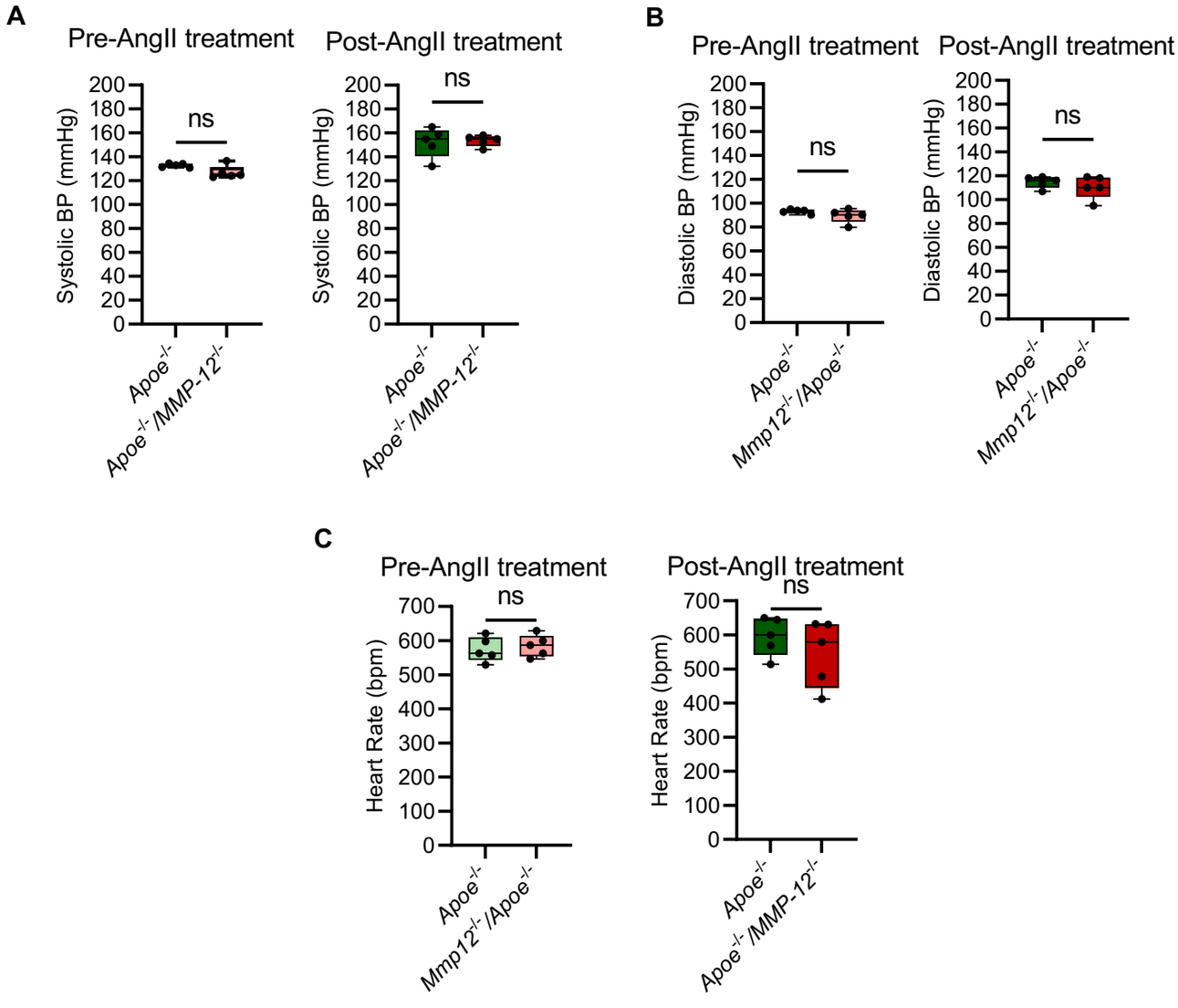

Figure S2. Effect of *Mmp12* deletion on blood pressure before and after AngII infusion in *Apoe*$^{-/-}$ mice. Average systolic (**A**) and diastolic (**B**) blood pressure (BP) and heart rate (**C**) over 24 hours prior to minipump implantation (pre-AngII), and the first 12 h of Ang-II infusion. n = 5 per group; *P < 0.05 by two-tailed Mann-Whitney U test.

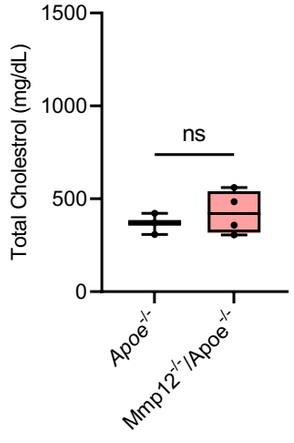

Figure S3. Total cholesterol level in *Apoe*$^{-/-}$ (n=3) and *Mmp12*$^{-/-}$*/Apoe*$^{-/-}$ (n=4) mice.
ns: not significant by two-tailed Mann-Whitney U test.

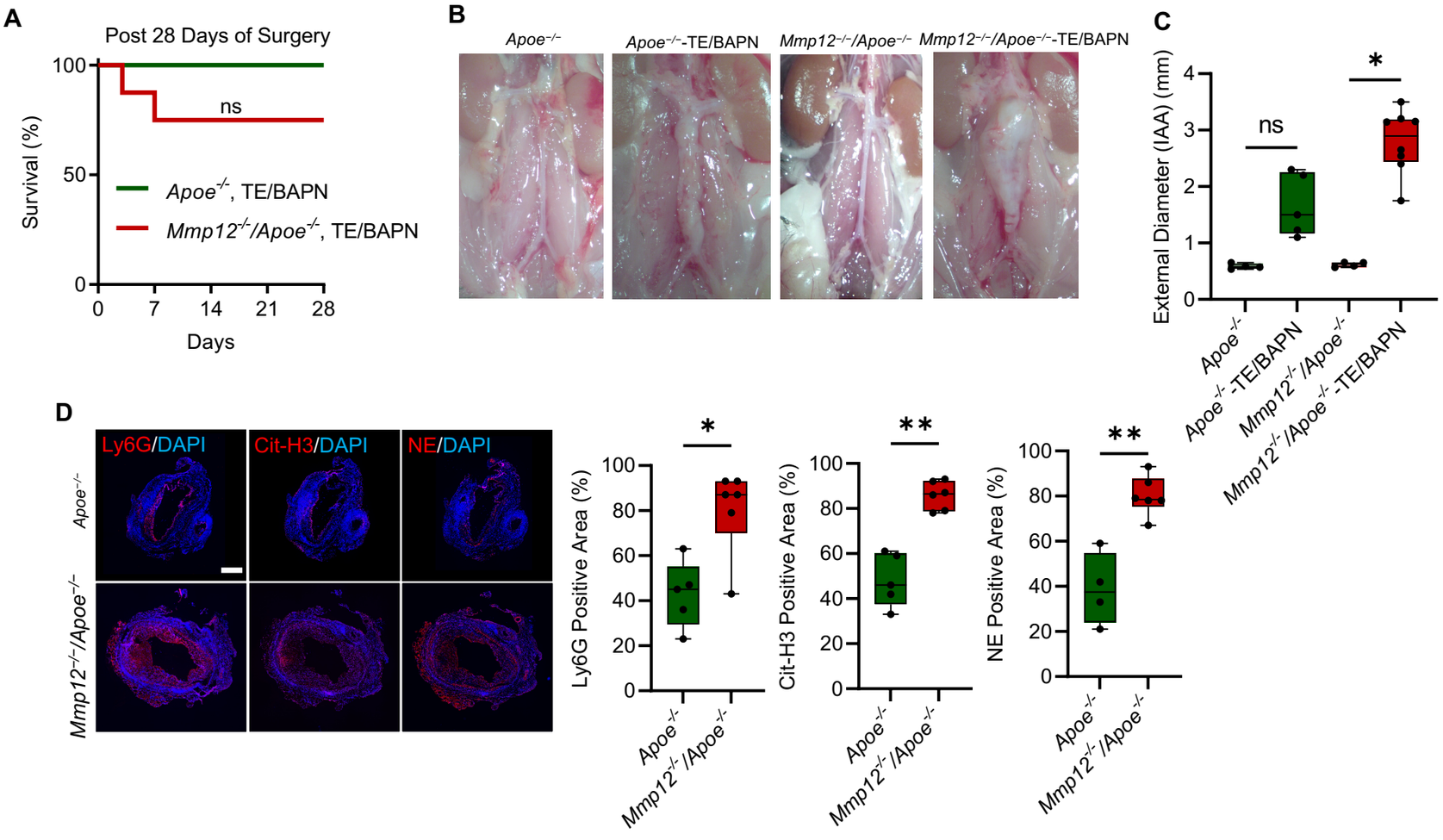

Figure S4. Effect of *Mmp12* deletion on BAPN/elastase-induced AAA development, progression, and survival in Apoe$^{-/-}$ mice. A. Kaplan-Meier survival curves of BAPN/elastase-induced AAA in *Apoe-/-* (n = 5) and *Mmp12*$^{-/-}$*/Apoe*$^{-/-}$ (n = 8) mice. The curves were compared using Log-rank (Mantel-Cox) test. ns: not significant. **B**, **C**. Representative images of infrarenal abdominal aorta (**B**) and quantification of its maximal external diameter (**C**) at baseline (no surgery) and 28 days post-surgery to induce AAA in *Apoe*$^{-/-}$ and *Mmp12*$^{-/-}$*/Apoe*$^{-/-}$ mice; n = 4 to 8. *P < 0.05 by Kruskal-Wallis test with Dunn's multiple comparisons test. **D**. Representative images of immunofluorescence staining and quantification of neutrophils (Ly6G, red), neutrophil extracellular traps (Cit-H3, red), and NE (red) in infrarenal abdominal aortae of surviving *Apoe*$^{-/-}$ and *Mmp12*$^{-/-}$*/Apoe*$^{-/-}$ mice at 28 days post-surgery. Nuclei are stained blue with DAPI. n = 5 per group. Scale bar: 500 µm. *P < 0.05, **P < 0.01 by two-tailed Mann-Whitney U test. BAPN: β-aminopropionitrile, Cit-H3: citrullinated histone 3, NE: neutrophil elastase.

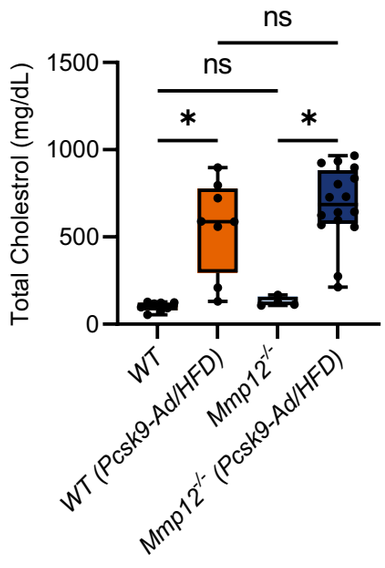

Figure S5. Effect of Mmp12 deletion on serum cholesterol levels. Total serum cholesterol level in wildtype (WT) and Mmp12-/- mice before, and 14 days after AAV8-PCSK9 (Pcsk9-Ad) injection and high fat diet (HFD). n = 4 to 16 per group; *P < 0.05 by Kruskal-Wallis test with Dunn's multiple comparisons test. ns: not significant.

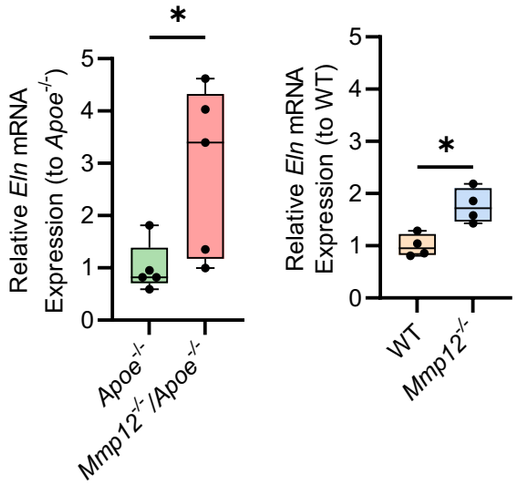

Figure S6. Effect of Mmp12 deletion on elastin gene expression. Elastin (*Eln*) gene expression levels in the aorta of *Apoe*$^{-/-}$ and *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$ (left), and wild-type (WT) and *Mmp12*$^{-/-}$ (right) mice. Eln expression was normalized to β-actin, n = 4 to 5 per group. *P < 0.05 by two-tailed Mann-Whitney U test.

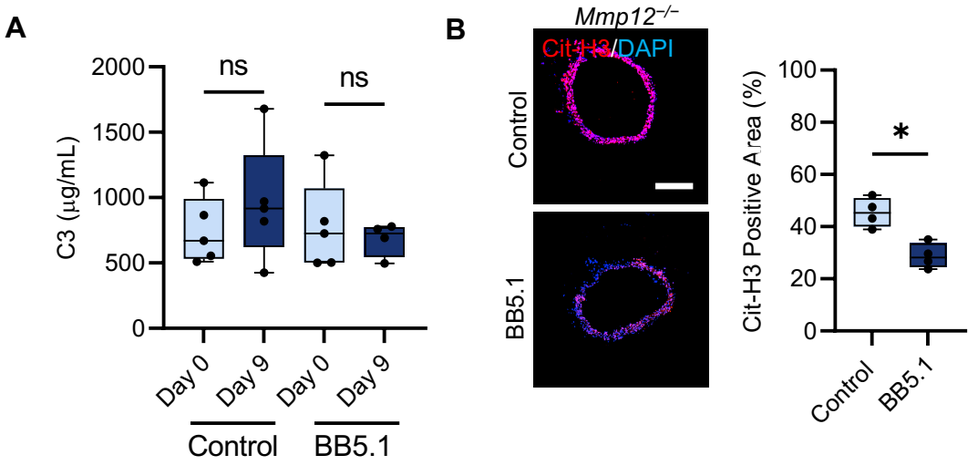

Figure S7. Effect of anti-C5 antibody (BB5.1) on complement system activation and aortic wall NETosis in *Mmp12*$^{-/-}$ mice. A. Plasma C3 levels in *Mmp12*$^{-/-}$ mice at baseline and 9 days posttreatment with anti-C5 (BB5.1) or MOPC (control) antibodies. n = 5 per group; ns: not significant by Kruskal-Wallis test with Dunn's multiple comparisons post hoc test. **B**. Representative images of immunofluorescence staining and quantification of suprarenal abdominal aorta Cit-H3 (red) in *Mmp12*$^{-/-}$ at 9 days post-treatment with BB5.1 or MOPC antibodies. n = 4 per group ; Scale bar: 500 µm. *P < 0.05 by two-tailed Mann-Whitney U test.

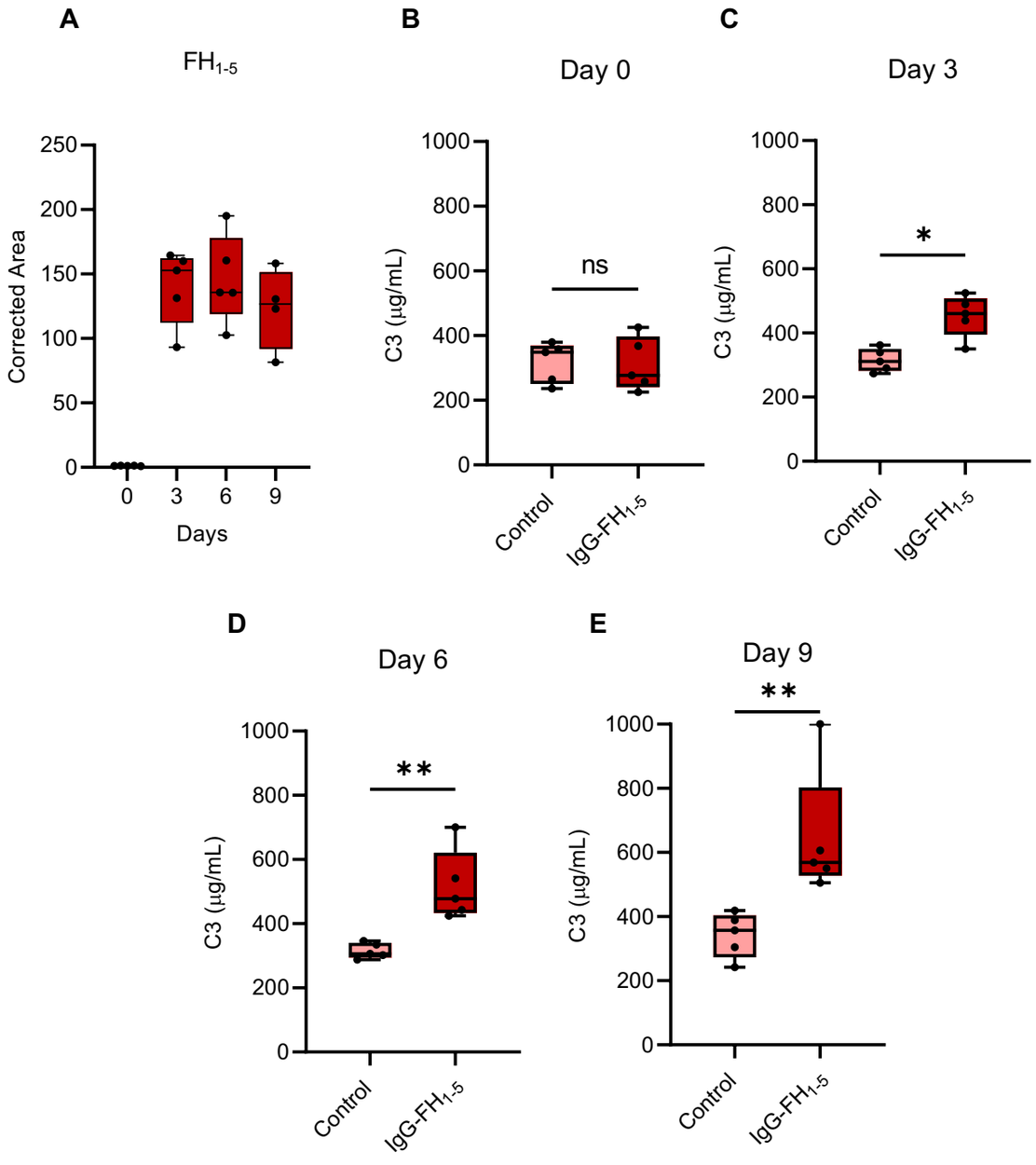

Figure S8. Effect of IgG-FH1-5 on complement system activation in *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$ mice. A. Western blotting-based quantification of plasma FH1-5 levels at 0, 3, 6, and 9 days post-injection with IgGFH1-5. **B-E**. Plasma C3 levels in *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$ mice at 0, 3, 6, and 9 days post treatment of IgGFH1-5 or MOPC antibody (control). n = 5 per group. *P < 0.05, **P < 0.01 by two-tailed Mann-Whitney U test.

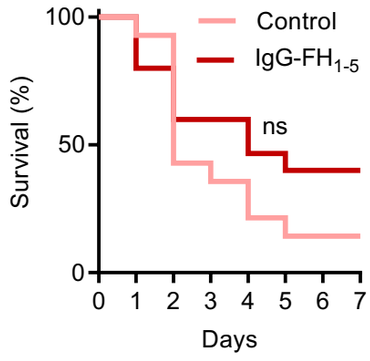

Figure S9. Effect of IgG-FH1-5 on survival post AngII infusion in *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$ mice. Kaplan-Meier survival curves of AngII-induced AAA in *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$ mice post treatment with IgG-FH1-5 (n = 15) or MOPC antibody (control) (n = 14). The curves were compared using Log-rank (Mantel Cox) test. ns: not significant.

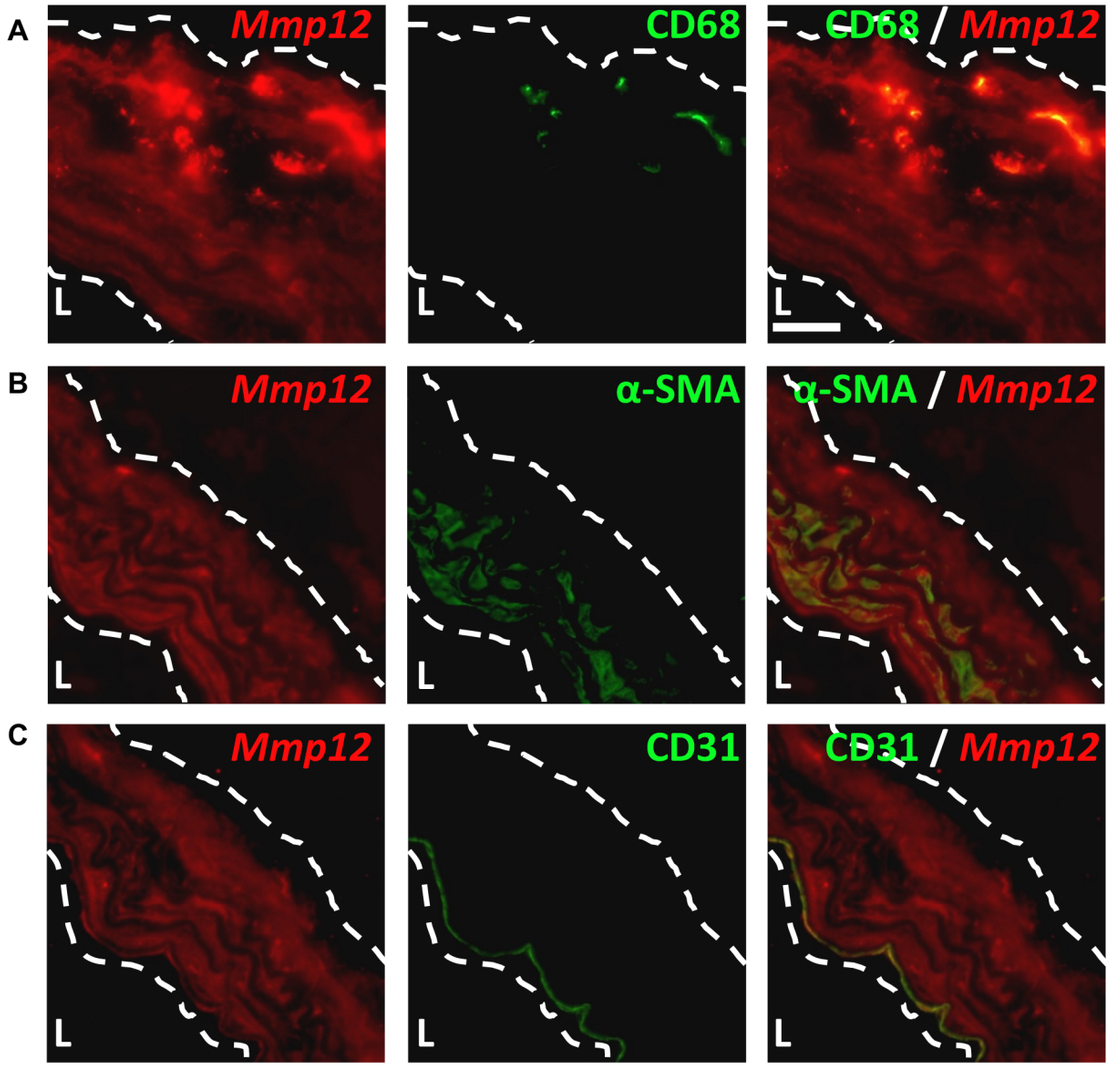

Figure S10. *Mmp12* mRNA expression detected by fluorescence in situ hybridization in suprarenal abdominal aortae of *Mmp12*$^{flox/flox}$/*Apoe*$^{-/-}$ mice. Representative immunofluorescence images of *Mmp12* mRNA (in red) co-stained with CD68 (**A**), α-smooth muscle actin (SMA, **B**), or CD31 (**C**) in green. White dashed lines indicate the boundaries of the aorta on the luminal and adventitial sides. L: lumen. Scale bar = 50 μm.

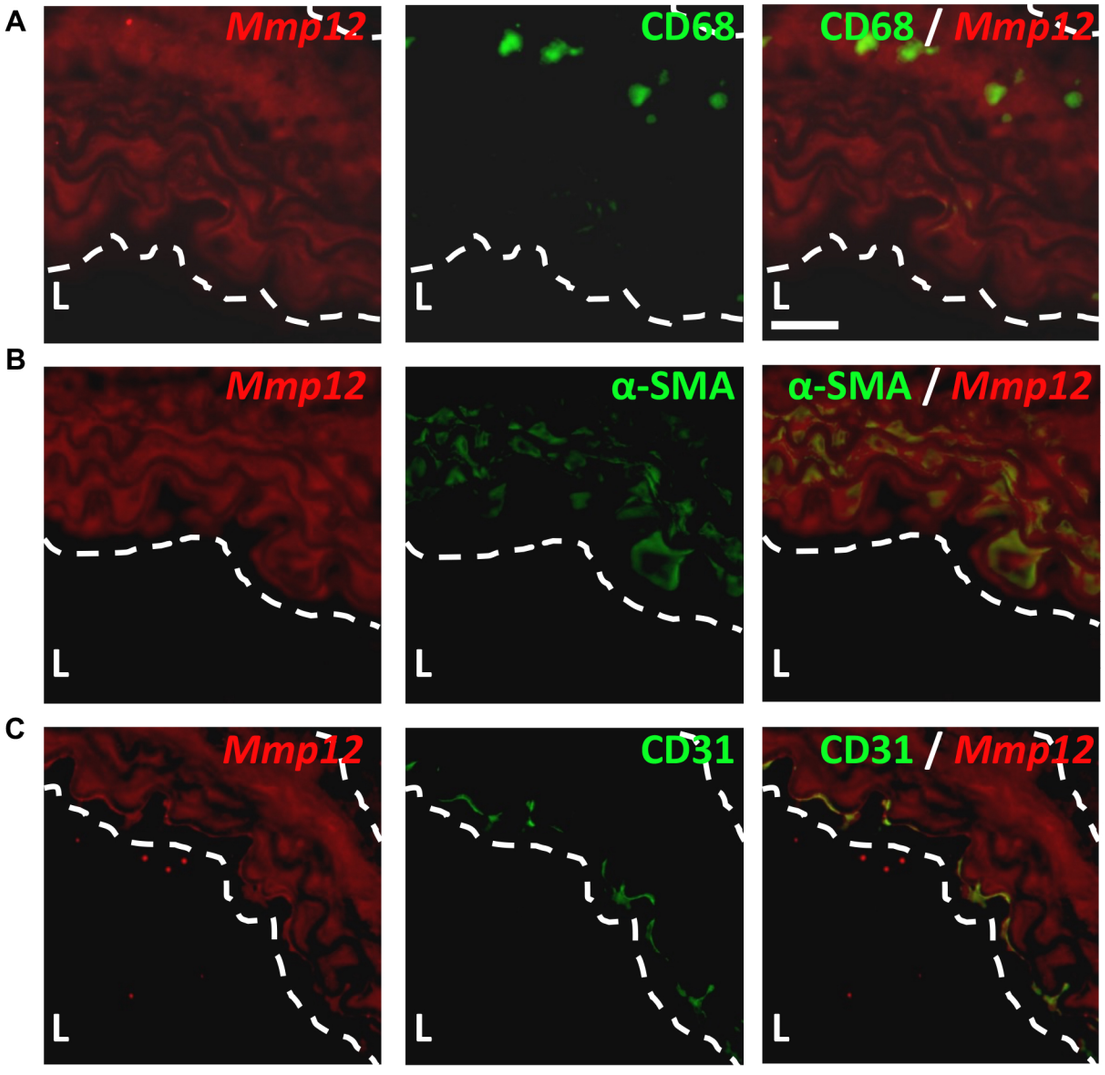

Figure S11. Cellular selectivity of *Mmp12* mRNA expression detected by fluorescence in situ hybridization in suprarenal abdominal aortae of tamoxifen-treated *Mmp12* $^{flox/flox}$*/Apoe*$^{-/-}$*/Csf1r-iCre* mice. Representative immunofluorescence images of *Mmp12* mRNA (in red) co-stained with CD68 (**A**), α-smooth muscle actin (SMA, **B**), or CD31 (**C**) in green. White dashed lines indicate the boundaries of the aorta on the luminal and adventitial sides. L: lumen. Scale bar = 50 μm.

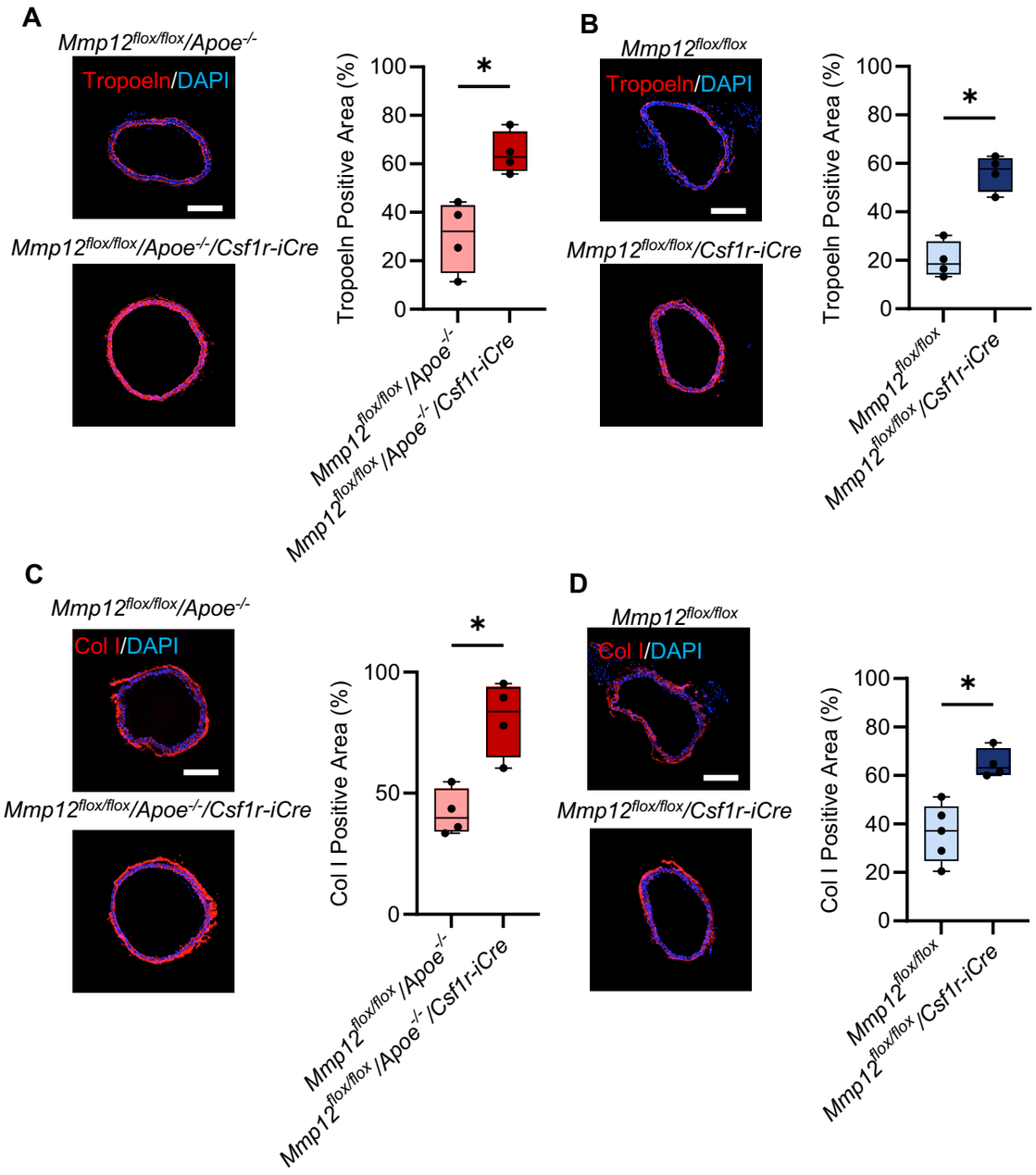

Figure S12. Effect of macrophage *Mmp12* deficiency on extracellular matrix composition of aortic wall. Representative images of immunofluorescence staining and quantification of tropoelastin (red, **A** and **B**) and collagen type I (red, **C** and **D**) in suprarenal abdominal aorta of *Mmp12*$^{flox/flox}$*/Apoe*$^{-/-}$ and *Mmp12*$^{flox/flox}$*/Apoe*$^{-/-}$ */Csf1r-iCre* (**A** and **C**) *Mmp12*$^{flox/flox}$ and *Mmp12*$^{flox/flox}$*/Csf1r-iCre* (**B** and **D**) mice following tamoxifen administration. Nuclei are stained blue with DAPI. n = 4 per group; Scale bar: 500 μm. *P < 0.05 by two-tailed Mann-Whitney U test.

Supplemental Statistical Analysis Data

Figure 1

A

Log-rank (Mantel-Cox) test	
Chi square	46.01
df	1
P value	<0.0001
P value summary	****
Are the survival curves sig different?	Yes

B

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
Apoe$^{-/-}$ vs. Apoe$^{-/-}$- Ang II	-9.607	No	ns	0.2704
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	-0.4167	No	ns	>0.9999
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-13.19	Yes	*	0.0246
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	9.19	No	ns	0.1847
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-3.587	No	ns	>0.9999
Mmp12$^{-/-}$/Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-12.78	Yes	**	0.0092

C

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
Apoe$^{-/-}$ vs. Apoe$^{-/-}$- Ang II	-8.821	No	ns	0.3945
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	-0.5833	No	ns	>0.9999
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-9.361	No	ns	0.2501
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	8.238	No	ns	0.3172
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-0.5397	No	ns	>0.9999
Mmp12$^{-/-}$/Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-8.778	No	ns	0.1767

D

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
Apoe$^{-/-}$ vs. Apoe$^{-/-}$- Ang II	-8.875	No	ns	0.384
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	-1.208	No	ns	>0.9999
Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-15.04	Yes	**	0.0064
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$	7.667	No	ns	0.4287
Apoe$^{-/-}$- Ang II vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-6.167	No	ns	0.6567
Mmp12$^{-/-}$/Apoe$^{-/-}$ vs. Mmp12$^{-/-}$/Apoe$^{-/-}$- Ang II	-13.83	Yes	**	0.0036

E

Ly6G		Cit-H3		NE	
Mann Whitney test		Mann Whitney test		Mann Whitney test	
P value	0.0286	P value	0.0286	P value	0.0286
Exact or approximate P value?	Exact	Exact or approximate P value?	Exact	Exact or approximate P value?	Exact
P value summary	*	P value summary	*	P value summary	*
Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed	One- or two-tailed P value?	Two-tailed	One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26	Sum of ranks in column A,B	10 , 26	Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0	Mann-Whitney U	0	Mann-Whitney U	0

F

Ly6G		Cit-H3		NE	
Mann Whitney test		Mann Whitney test		Mann Whitney test	
P value	0.0286	P value	0.0286	P value	0.0286
Exact or approximate P value?	Exact	Exact or approximate P value?	Exact	Exact or approximate P value?	Exact
P value summary	*	P value summary	*	P value summary	*
Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed	One- or two-tailed P value?	Two-tailed	One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26	Sum of ranks in column A,B	10 , 26	Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0	Mann-Whitney U	0	Mann-Whitney U	0

Figure 2

A

Log-rank (Mantel-Cox) test	
Chi square	4.176
df	1
P value	0.041
P value summary	*
Are the survival curves sig different?	Yes

B

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
Mmp12$^{-/-}$-Ang II vs. WT	14.88	Yes	**	0.0023
Mmp12$^{-/-}$-Ang II vs. WT-Ang II	12.06	Yes	**	0.0022
Mmp12$^{-/-}$-Ang II vs. Mmp12$^{-/-}$	11	Yes	*	0.0383

C

Ly6G	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Cit-H3	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

D

Ly6G	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Cit-H3	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Figure 3

B

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

F

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

C

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

G

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

D

Mann Whitney test	
P value	0.0476
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	18 , 37
Mann-Whitney U	3

E

Mann Whitney test	
P value	0.0317
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	17 , 38
Mann-Whitney U	2

H

DTA			SAA	
Mann Whitney test			Mann Whitney test	
P value	0.0159		P value	0.0303
Exact or approximate P value?	Exact		Exact or approximate P value?	Exact
P value summary	*		P value summary	*
Significantly different (P < 0.05)?	Yes		Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed		One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	39 , 16		Sum of ranks in column A,B	42 , 24
Mann-Whitney U	1		Mann-Whitney U	3

Figure 4

A

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 40
Mann-Whitney U	0

B

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 40
Mann-Whitney U	0

C

C3	
Mann Whitney test	
P value	0.0002
Exact or approximate P value?	Exact
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	81 , 55

C5a	
Mann Whitney test	
P value	0.04
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	23 , 97

D

C3	
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	129 , 171
Mann-Whitney U	0

C5a	
Mann Whitney test	
P value	0.014
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	32 , 59
Mann-Whitney U	4

E

Control	
Mann Whitney test	
P value	0.5476
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	31 , 24
Mann-Whitney U	9

IgG-FH1-5	
Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 40
Mann-Whitney U	0

F

Control	
Mann Whitney test	
P value	0.5476
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	24 , 31
Mann-Whitney U	9

IgG-FH1-5	
Mann Whitney test	
P value	0.0159
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	16 , 39
Mann-Whitney U	1

G

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	40 , 15
Mann-Whitney U	0

H

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	40 , 15
Mann-Whitney U	0

I

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	40 , 15
Mann-Whitney U	0

J

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	26 , 10
Mann-Whitney U	0

Figure 5

A

Log-rank (Mantel-Cox) test	
Chi square	5.194
df	1
P value	0.0227
P value summary	*
Are the survival curves sig different?	Yes

B

Mann Whitney test	
P value	0.0162
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	40 , 38
Mann-Whitney U	2

C

Mann Whitney test	
P value	0.0061
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	38 , 28
Mann-Whitney U	0

D

Ly6G	
Mann Whitney test	
P value	0.0242
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	36 , 30
Mann-Whitney U	2

Cit-H3	
Mann Whitney test	
P value	0.0061
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	38 , 28
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0061
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	38 , 28
Mann-Whitney U	0

Figure 6

A

Mann Whitney test	
P value	0.0159
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	35 , 10
Mann-Whitney U	0

B

Mann Whitney test	
P value	0.0159
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	35 , 10
Mann-Whitney U	0

C

Ly6G	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Cit-H3	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

C3d	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

D

Ly6G	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Cit-H3	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

C3d	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

E

Log-rank (Mantel-Cox) test	
Chi square	4.29
df	1
P value	0.0383
P value summary	*
Are the survival curves sig different?	Yes

F

Mann Whitney test	
P value	0.0016
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	47 , 73
Mann-Whitney U	2

Figure S2

A

Pre-AngII treatment	
Mann Whitney test	
P value	0.1508
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	35 , 20
Mann-Whitney U	5

Post-AngII treatment	
Mann Whitney test	
P value	0.8889
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	28.50 , 26.50
Mann-Whitney U	11.5

B

Pre-AngII treatment	
Mann Whitney test	
P value	0.2222
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	34 , 21
Mann-Whitney U	6

Post-AngII treatment	
Mann Whitney test	
P value	0.6508
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	30 , 25
Mann-Whitney U	10

C

Pre-AngII treatment	
Mann Whitney test	
P value	0.5952
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	24.50 , 30.50
Mann-Whitney U	9.5

Post-AngII treatment	
Mann Whitney test	
P value	0.4206
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	32 , 23
Mann-Whitney U	8

Figure S3

Mann Whitney test	
P value	0.8571
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	11 , 17
Mann-Whitney U	5

Figure S4

A

Log-rank (Mantel-Cox) test	
Chi square	1.338
df	1
P value	0.2473
P value summary	ns
Are the survival curves sig different?	No

C

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
$Apoe^{-/-}$ vs. $Apoe^{-/-}$-TE/BAPN	-7.9	No	ns	0.3462
$Apoe^{-/-}$ vs. $Mmp12^{-/-}/Apoe^{-/-}$	-2	No	ns	>0.9999
$Apoe^{-/-}$ vs. $Mmp12^{-/-}/Apoe^{-/-}$-TE/BAPN	-13.75	Yes	**	0.0018
$Apoe^{-/-}$-TE/BAPN vs. $Mmp12^{-/-}/Apoe^{-/-}$	5.9	No	ns	0.9381
$Apoe^{-/-}$-TE/BAPN vs. $Mmp12^{-/-}/Apoe^{-/-}$-TE/BAPN	-5.85	No	ns	0.589
$Mmp12^{-/-}/Apoe^{-/-}$ vs. $Mmp12^{-/-}/Apoe^{-/-}$-TE/BAPN	-11.75	Yes	*	0.0119

D

Ly6G	
Mann Whitney test	
P value	0.026
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	18 , 48
Mann-Whitney U	3

Cit-H3	
Mann Whitney test	
P value	0.0043
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 51
Mann-Whitney U	0

NE	
Mann Whitney test	
P value	0.0048
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 45
Mann-Whitney U	0

Figure S5

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
WT vs. WT (Pcsk9-Ad/HFD)	-15.88	Yes	*	0.0155
WT vs. Mmp12$^{-/-}$	-2.875	No	ns	>0.9999
WT vs. Mmp12$^{-/-}$ (Pcsk9-Ad/HFD)	-20.31	Yes	****	<0.0001
WT (Pcsk9-Ad/HFD) vs. Mmp12$^{-/-}$	13	No	ns	0.2635
WT (Pcsk9-Ad/HFD) vs. Mmp12$^{-/-}$ (Pcsk9-Ad/HFD)	-4.438	No	ns	>0.9999
Mmp12$^{-/-}$ vs. Mmp12$^{-/-}$ (Pcsk9-Ad/HFD)	-17.44	Yes	*	0.0184

Figure S6

Apoe$^{-/-}$	
Mann Whitney test	
P value	0.0317
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	17 , 38
Mann-Whitney U	2

WT	
Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

Figure S7

A

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
Day 0 vs. Day 9	-3	No	ns	>0.9999
Day 0 vs. Day 0	0.6	No	ns	>0.9999
Day 0 vs. Day 9	2.05	No	ns	>0.9999
Day 9 vs. Day 0	3.6	No	ns	>0.9999
Day 9 vs. Day 9	5.05	No	ns	>0.9999
Day 0 vs. Day 9	1.45	No	ns	>0.9999

B

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	26 , 10
Mann-Whitney U	0

Figure S8

B

Mann Whitney test	
P value	>0.9999
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	28 , 27
Mann-Whitney U	12

C

Mann Whitney test	
P value	0.0159
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	16 , 39
Mann-Whitney U	1

D

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 40
Mann-Whitney U	0

E

Mann Whitney test	
P value	0.0079
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 40
Mann-Whitney U	0

Figure S9

Log-rank (Mantel-Cox) test	
Chi square	1.579
df	1
P value	0.2089
P value summary	ns
Are the survival curves sig different?	No

Figure S12

A

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

B

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

C

Mann Whitney test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10 , 26
Mann-Whitney U	0

D

Mann Whitney test	
P value	0.0159
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 30
Mann-Whitney U	0

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jackson Laboratory	B6.129P2-*Apoe*^{tm1Unc}/J mice	M/F	https://www.jax.org/strain/002052
Mouse	Jackson Laboratory	B6.129X-Mmp12^{tm1Sds}/J	M/F	https://www.jax.org/strain/004855
Mouse	Infrafrontier GmbH	C57BL/6N *Mmp12*$^{tm1a(EUCOMM)Hmgu/H}$	M/F	https://www.infrafrontier.eu/emma/strain-search/straindetails/?q=5321
Mouse	Jackson Laboratory	B6.Cg-Tg(Pgk1-flpo)10Sykr/J	M/F	https://www.jax.org/strain/011065
Mouse	Jackson Laboratory	Tg(Csf1r-Mer-iCre-Mer)1Jwp	M/F	https://www.jax.org/strain/019098
Mouse	Jackson Laboratory	B6.129S4-*Gt(ROSA)26Sor*$^{tm2(FLP*)Sor}$/J	M/F	https://www.jax.org/strain/012930

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	*Mmp12$^{flox/flox}$/Apoe$^{-/-}$/Csf1r-iCre*	In-house	C57BL/6, 129 S4		N/A
Parent - Female	*Mmp12$^{flox/flox}$/Apoe$^{-/-}$/Csf1r-iCre*	In-house	C57BL/6, 129 S4		N/A
Parent - Male	*Mmp12$^{flox/flox}$/Csf1r-iCre*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Female	*Mmp12$^{flox/flox}$/Csf1r-iCre*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Male	*Mmp12$^{flox/flox}$/Apoe$^{-/-}$*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Female	*Mmp12$^{flox/flox}$/Apoe$^{-/-}$*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Male	*Mmp12$^{flox/flox}$*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Female	*Mmp12$^{flox/flox}$*	In-house	C57BL/6, 129 S4/SvJaeSor, FVB/N		N/A
Parent - Male	*Mmp12$^{-/-}$/Apoe$^{-/-}$*	In-house	C57BL/6		N/A

Parent - Female	*Mmp12*$^{-/-}$/*Apoe*$^{-/-}$	In-house	C57BL/6		N/A

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Anti-Ly6G clone 1A8	Bio X Cell	BP0075-1	1:100		https://bioxcell.com/invivoplus-anti-mouse-ly6g-bp0075-1
Anti-Histone H3 (citrulline R2 + R8 + R17):	Abcam	ab5103	1:100		https://www.abcam.com/histone-h3-citrulline-r2--r8--r17-antibody-ab5103.html
Anti-Neutrophil elastase	Abcam	ab68672	1:100		https://www.abcam.com/neutrophil-elastase-antibody-ab68672.html
Anti-Complement C3	MP Biomedicals	55463	1:100		https://www.mpbio.com/0855463-goat-igg-fraction-to-mouse-complement-c3
Anti-Tropoelastin	Abcam	ab21600	1:100		https://www.abcam.com/tropoelastin-antibody-ab21600.html
Anti-C3d	R&D Systems	BAF2655	1:200		https://www.rndsystems.com/products/mouse-complement-component-c3d-biotinylated-antibody_baf2655
Anti-CD68	Abcam	ab53444	1:100		https://www.abcam.com/cd68-antibody-fa-11-ab53444.html
Anti-CD31	Millipore Sigma	MAB1398Z	1:100		https://www.emdmillipore.com/US/en/product/Anti-PECAM-1-Antibody-clone-2H8-Azide-Free,MM_NF-MAB1398Z
Anti-α-Smooth muscle actin	Millipore Sigma	A2547	1:100		https://www.sigmaaldrich.com/US/en/product/sigma/a2547
Anti-Rat Alexa Fluor 555	Thermo Fisher Scientific	A-48270	1:200		https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A48270
Anti-Rat Alexa Fluor 594	Thermo Fisher Scientific	A-11007	1:200		https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007
Anti-Rabbit	Thermo Fisher Scientific	A-11012	1:200		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11012

Alexa Fluor 594					
Mmp12	HuluFish	Q2022016	1:100 (Probe to HuluHyb solution)		https://www.pixelbiotech.com/product-page/copy-of-hulufish-plus-kit-nano-ideal-for-ffpe-sample
Collagen Hybridizing Peptide	3Helix	R-CHP	20 µM		https://www.3helix.com/product/red300/
Anti-Complement C3	Novus	NB200-540	100 µg/mL		https://www.novusbio.com/products/complement-c3-antibody-11h9_nb200-540
Anti-C5a	R&D Systems	MAB21501	100 µg/mL		https://www.rndsystems.com/products/mouse-complement-component-c5a-antibody-295108_mab21501
Anti-C5a	R&D Systems	AF2150	5 µg/mL		https://www.rndsystems.com/products/mouse-complement-component-c5a-antibody_af2150
Anti-CD45	BioLegend	103105	2 µg/mL		https://www.biolegend.com/en-us/products/pe-anti-mouse-cd45-antibody-100
Anti-Ly6G	BD Biosciences	560599	2 µg/mL		https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-ly-6g.560599
Anti-CD11b	BD Biosciences	557672	2 µg/mL		https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-488-rat-anti-cd11b.557672
MOPC	Alexion Pharmaceuticals	N/A	40 mg/kg		Provided by Alexion Pharmaceuticals, New Haven, CT.
BB5.1	Alexion Pharmaceuticals	N/A	40 mg/kg		Provided by Alexion Pharmaceuticals, New Haven, CT.

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Mmp12$^{-/-}$ bone marrow-derived macrophages	Csf1r-Mer-iCre-Mer:*Mmp12*$^{flox/flox}$	unknown	N/A

Data & Code Availability

Description	Source / Repository	Persistent ID / URL

FlowJo	BD Biosciences	https://www.bdbiosciences.com/en-ca/products/software/flowjo-v10-software
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij/download.html
GraphPad Prism	GraphPad	https://www.graphpad.com

Other

Description	Source / Repository	Persistent ID / URL
Recombinant mouse C5a	R&D, 2150-C5-025	https://www.rndsystems.com/products/recombinant-mouse-complement-component-c5a-protein_2150-c5
C5-deficient NOD-SCID mouse plasma	BIOIVT	MSE61PLKZYNN
IgG-FH_{1-5}	Alexion Pharmaceuticals	Working concentration: 56 mg/kg Provided by Alexion Pharmaceuticals, New Haven, CT.
β-actin	Thermo Fisher Scientific	Mn00607939_s1
Elastin	Thermo Fisher Scientific	Mm00514670_m1
Eln	Thermo Fisher Scientific	Mm00500554_m1

ARRIVE GUIDELINES

The ARRIVE guidelines (https://arriveguidelines.org/) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*Apoe*$^{-/-}$	M	12w	66	42	No	Ang II treatment studies
Group 2 *Mmp12*$^{-/-}$/*Apoe*$^{-/-}$	M	12w	36	5	No	Ang II treatment studies
Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*WT*	M	12w	17	13	No	PCSK9/HFD/Ang II treatment studies
Group 2 *Mmp12*$^{-/-}$	M	12w	20	9	No	PCSK9/HFD/Ang II treatment studies
Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*MOPC-Mmp12*$^{-/-}$	M	12w	10	4	No	PCSK9/HFD/MOPC/Ang II treatment studies
Group 2 *FH-Mmp12*$^{-/-}$	M	12w	10	8	No	PCSK9/HFD/IgG-FH_{1-5}/Ang II treatment studies
Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*Mmp12*$^{flox/flox}$/*Apoe*$^{-/-}$	M	12w	9	9	No	Ang II treatment studies
Group 2 *Mmp12*$^{flox/flox}$/*Apoe*$^{-/-}$/*Csf1r-iCre*	M	12w	10	6	No	Ang II treatment studies
Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*TE/BAPN-Apoe*$^{-/-}$	M	12w	5	5	No	TE/BAPN treatment studies
Group 2 *TE/BAPN-Mmp12*$^{-/-}$/*Apoe*$^{-/-}$	M	12w	8	6	No	TE/BAPN treatment studies

Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Group 1 Control-*MOPC-Mmp12*$^{-/-}$*/Apoe*$^{-/-}$	M	12w	14	2	No	MOPC/Ang II treatment studies
Group 2 *FH-Mmp12*$^{-/-}$*/Apoe*$^{-/-}$	M	12w	15	6	No	IgG-FH_{1-5}/Ang II treatment studies

Sample Size: Please explain how the sample size was decided Please provide details of any a *prior* sample size calculation, if done.
Samples sizes were determined based on literature and previous experience/studies.

Inclusion Criteria
All animals within a group were included for analysis

Exclusion Criteria
Animals were not excluded.

Randomization
Randomization was not performed when assigning animals to groups.

Blinding
Blinding of investigators were not performed for data acquisition and analysis.