

Online Table 1. Source and catalog number of reagents.		
Reagent	Vendor	Catalog Number
Fast Green FCF	Sigma	F7252-5G
Direct Red 80	Sigma	365548
Alcian Blue solution (pH2.5)	Sigma	B8438
Nuclear fast red solution	Sigma	N8002
3-aminopropionitrile fumarate salt (BAPN)	Sigma	A3134
porcine pancreas elastase	Sigma	E1250
Van Gieson Elastic stain kit (Richard-Allan Scientific)	Thermo Scientific	87017
Rabbit F4/80 monoclonal antibody	Cell Signaling Technology	#30325
Mouse anti- α -smooth muscle actin monoclonal antibody	DAKO	M0851
Rabbit anti-NF- κ B p65 monoclonal antibody	Cell Signaling Technology	#8242
Rat anti-Mac2 monoclonal antibody	Cedarlane	CL8942AP
Biotinylated goat anti-mouse IgG antibody	Vector Laboratories	BA-9200
Biotinylated goat anti-rabbit IgG antibody	Vector Laboratories	BA-1000
Goat anti-rabbit IgG Secondary Antibody, Alexa Fluor Plus 594	Invitrogen	# A32740
Avidin-biotinylated enzyme complex	Vector Laboratories	PK-6100
DAB Peroxidase Substrate Kit	Vector Laboratories	SK-4100
Serum-free blocking solution	Dako	X0909
Antibody diluent	Dako	S080983-2
ProLong TM Gold Antifade Mountant	ThermoFisher Scientific	P10144
RPMI-1640 Medium	Sigma	R8758
Gibco TM Fetal Bovine Serum	ThermoFisher Scientific	10437028
Gibco TM Penicillin-Streptomycin-Glutamine	ThermoFisher Scientific	10378016
Recombinant murine TNF- α	PeproTech	315-01A
RNeasy Mini Kit	QIAGEN	74104
iScript TM cDNA Synthesis Kit	BIO-RAD	1708891
iQ TM SYBR [®] Green Supermix	BIO-RAD	1708882
Mouse IgG2a kappa Isotype Control	Invitrogen	# 14-4724-82
Rabbit IgG Isotype Control	Invitrogen	#02-6102
Rat IgG2a isotype control	Cedarlane	#CLCR2A00
CyQUANT LDH Cytotoxicity Assay kit	Invitrogen	C20300

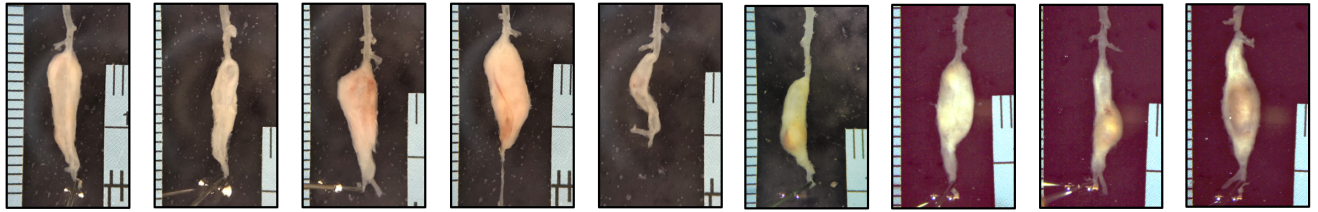
Online Table 2. Statistics

Figures	Shapiro-Wilk normality test	Brown-Forsythe test	Test used (Graphpad prism 8)	
Figure 1	Figure 1C Prevention-Maximal aortic width	Accept normality	Reject constancy of variance	Parametric: Welch ANOVA with Dunnett's T3 post-hoc test
	Figure 1D Prevention-Percentage increase of maxial aortic width	Accept normality	Accept constancy of variance	Parametric: unpaired Student's t-test
	Figure 1E Prevention-Total aneurysmal area	Accept normality	Reject constancy of variance	Parametric: Welch ANOVA with Dunnett's T3 post-hoc test
Figure 2	Figure 2C Echo diastole internal diameter	Accept normality	Accept constancy of variance	Parametric: paired Student's t-test
	Figure 2E Intervention-Maximal aortic width	Accept normality	Accept constancy of variance	Parametric: unpaired Student's t-test
	Figure 2F Intervention-Total aneurysmal area	Accept normality	Accept constancy of variance	Parametric: unpaired Student's t-test
Figure 3	Figure 3B Elastin content in media area	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
	Figure 3D Media α -SMA intensity	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
Figure 4	Figure 4C Proteoglycan content in media area	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
Figure 5	Figure 5B F4/80 staining area	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
Figure 6	Figure 6B p65 nuclear translocation in macrophages	Accept normality	Reject constancy of variance	Parametric: Welch ANOVA with Games-Howell post-hoc test
	Figure 6C TNF- α mRNA level in macrophages	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
	Figure 6D IL-1 β mRNA level in macrophages	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
	Figure 6F p65 staining area	Accept normality	Accept constancy of variance	Parametric: one-way ANOVA with Holm-Sidak's post-hoc test
Figure S11	Figure S11 TNF- α mRNA level of infrarenal aortas	Accept normality	Reject constancy of variance	Parametric: Welch's t-test

Supplementary Figure S1

A

Saline/Elastase



Mice # 1

2

3

4

5

6

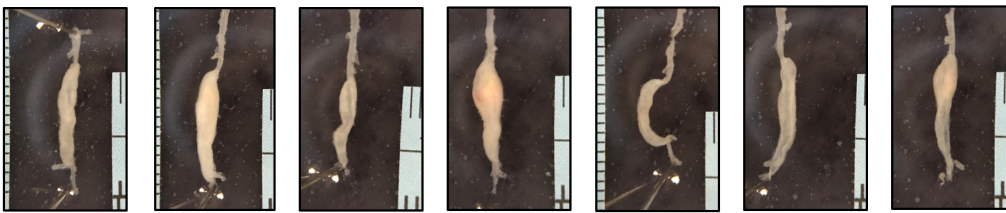
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8

9

B

Vinpocetine/Elastase



Mice # 1

2

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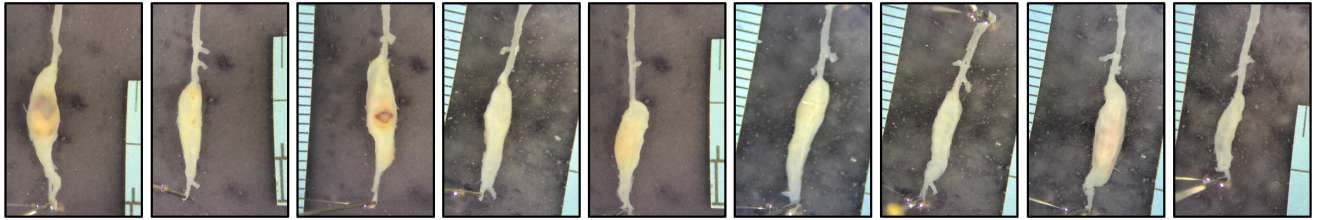
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Supplementary Figure S1. Microscopic images of abdominal aortas included for quantification for vinpocetine pretreatment model. A, Saline/elastase group. One animal died due to rupture at 10 days before harvest and was not shown here. B, Vinpocetine/elastase group. Mice #1-5 in the elastase/Saline group (A) and Mice #1-7 in the elastase/vinpocetine group (B) were included for histology and immunostaining analysis.

Supplementary Figure S2

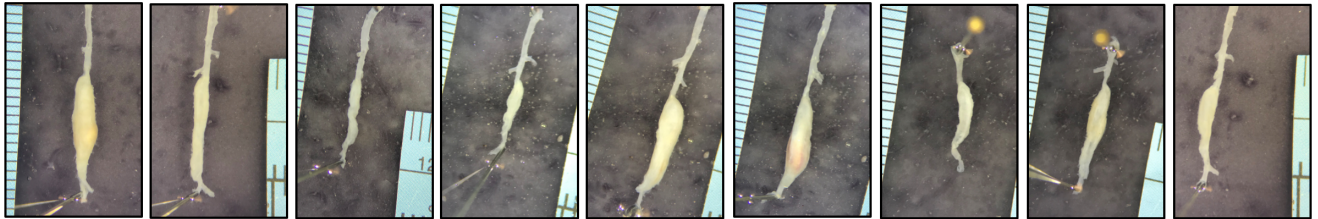
A

Saline/Elastase



B

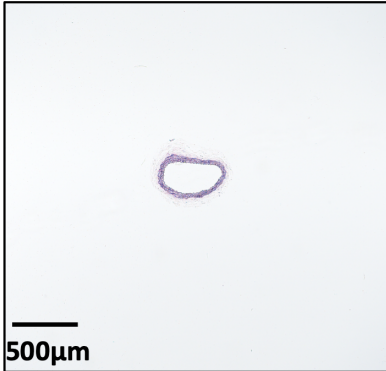
Vinpocetine/Elastase



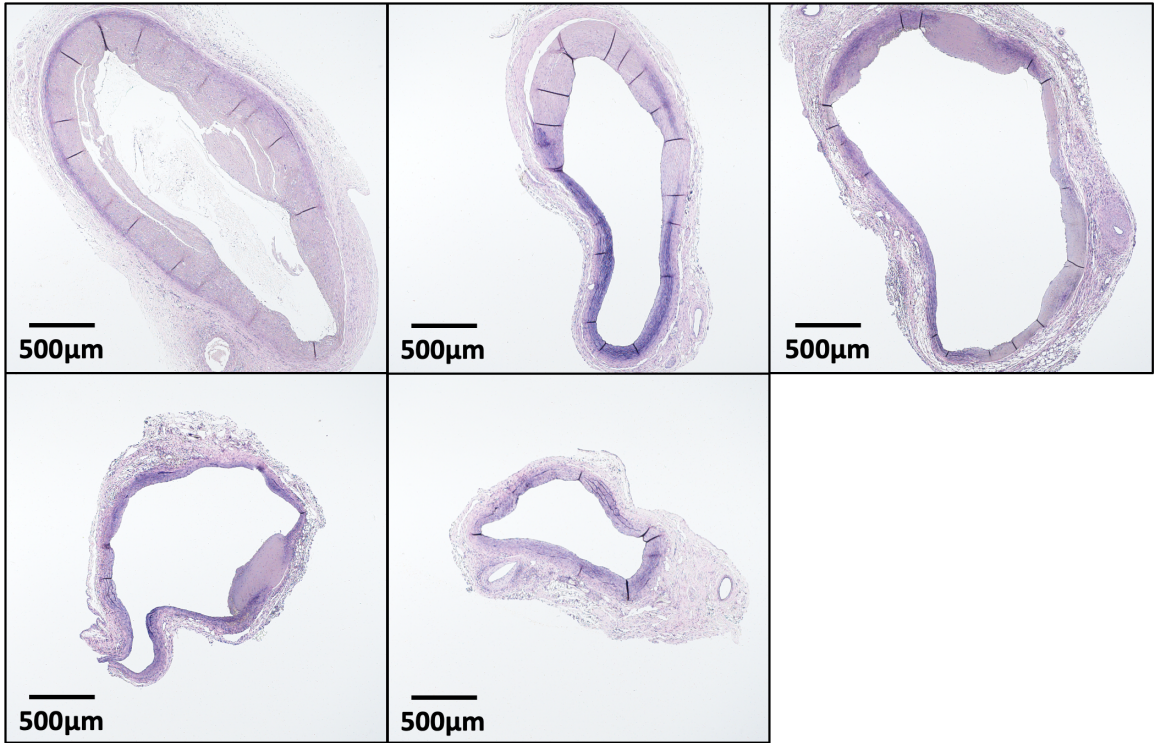
Supplementary Figure S2. Microscopic images of abdominal aortas included in quantification for the experimental post-intervention model. A, Saline/elastase group. B, Vinpocetine/elastase group.

Supplementary Figure S3

A Saline/Sham



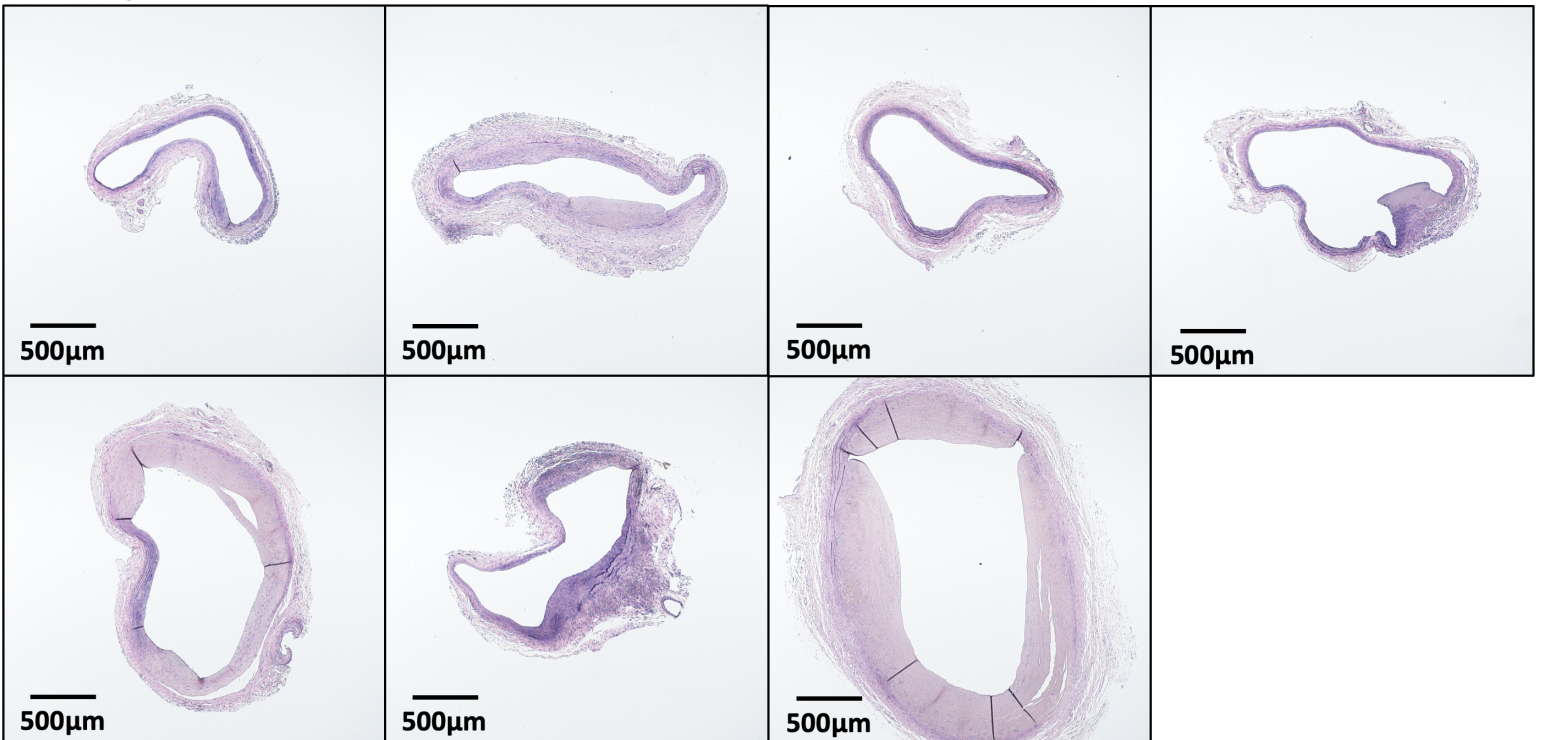
C Saline/Elastase



B Vinpocetine/Sham



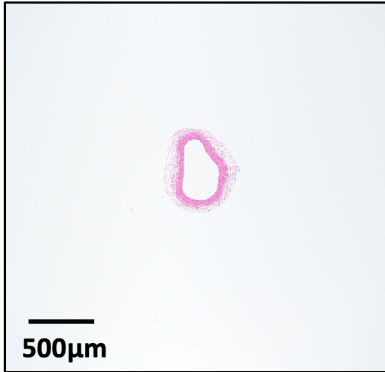
D Vinpocetine/Elastase



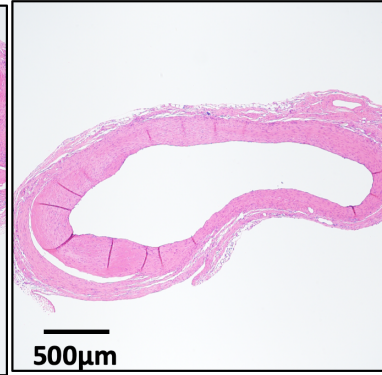
Supplementary Figure S3. Van Gieson staining of abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. The section from the level of the largest diameter of AAA for each animal was selected for staining.

Supplementary Figure S4

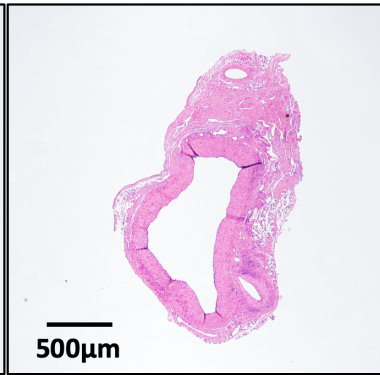
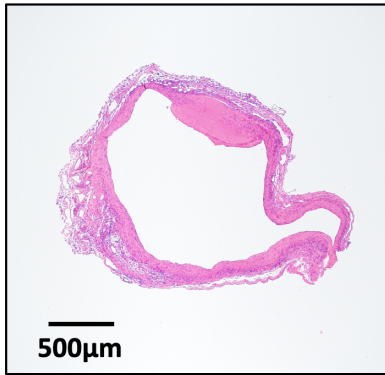
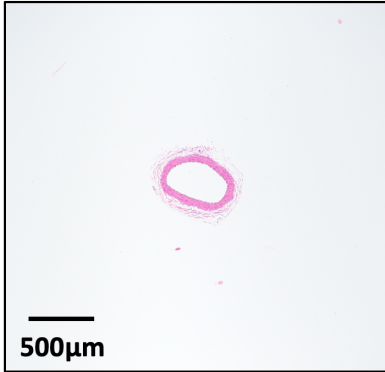
A Saline/Sham



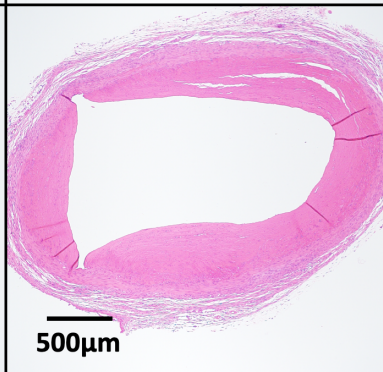
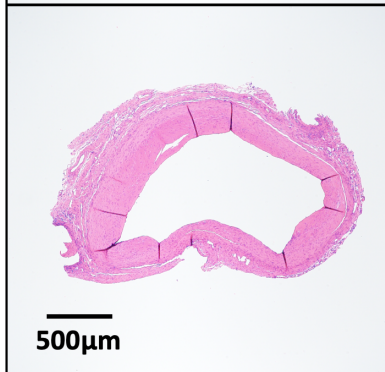
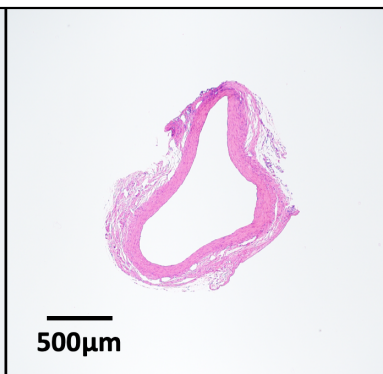
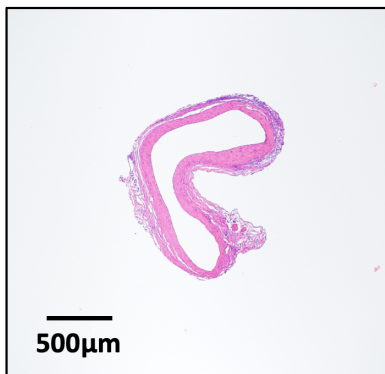
C Saline/Elastase



B Vinpocetine/Sham



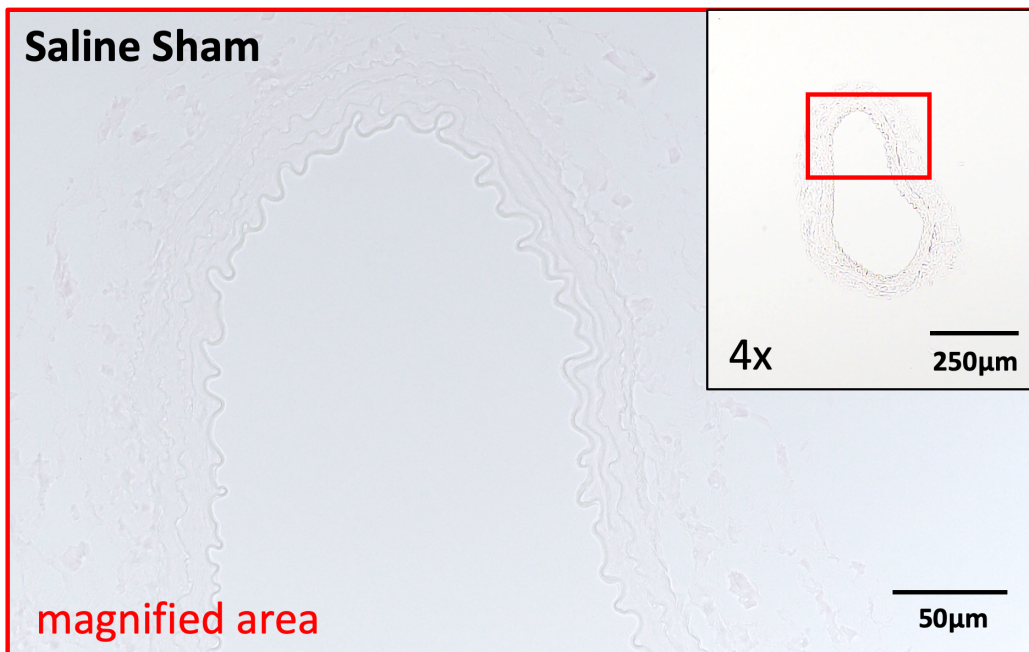
D Vinpocetine/Elastase



Supplementary Figure S4. Hematoxylin and eosin staining of abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. The section from the level of the largest diameter of AAA for each animal was selected for staining.

Supplementary Figure S5

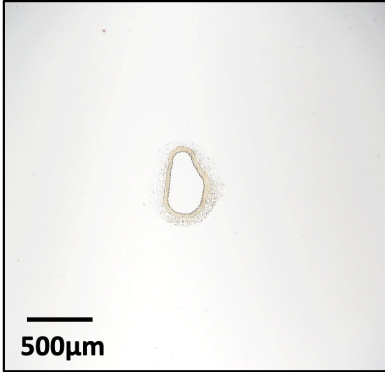
Negative control of α -SMA immunohistochemistry



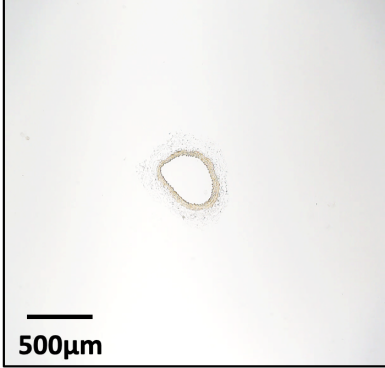
Supplementary Figure S5. Negative control of α -SMA immunohistochemistry staining in adjacent section of saline sham used in Fig. 3C (upper left panel). The inset is the 4x image of the whole section. Image outlined red is the magnification of the area highlighted in red box in inset. Negative controls were imaged using same acquisition parameters as Fig. 3C.

Supplementary Figure S6

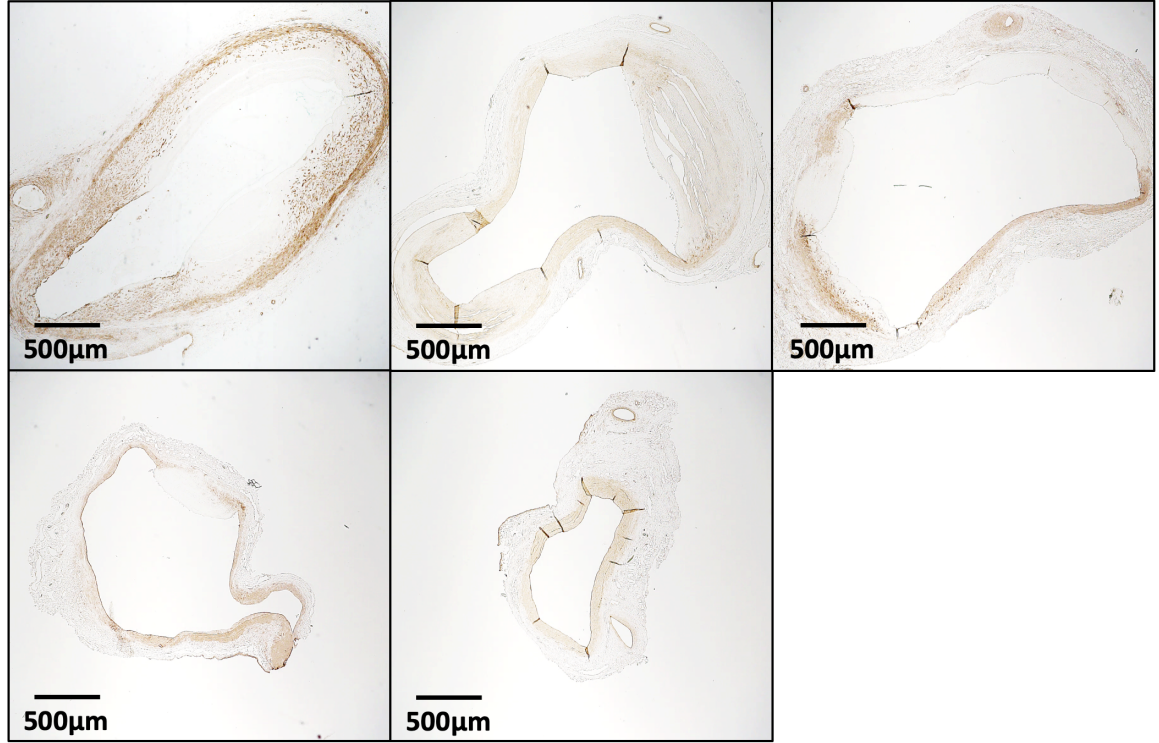
A Saline/Sham



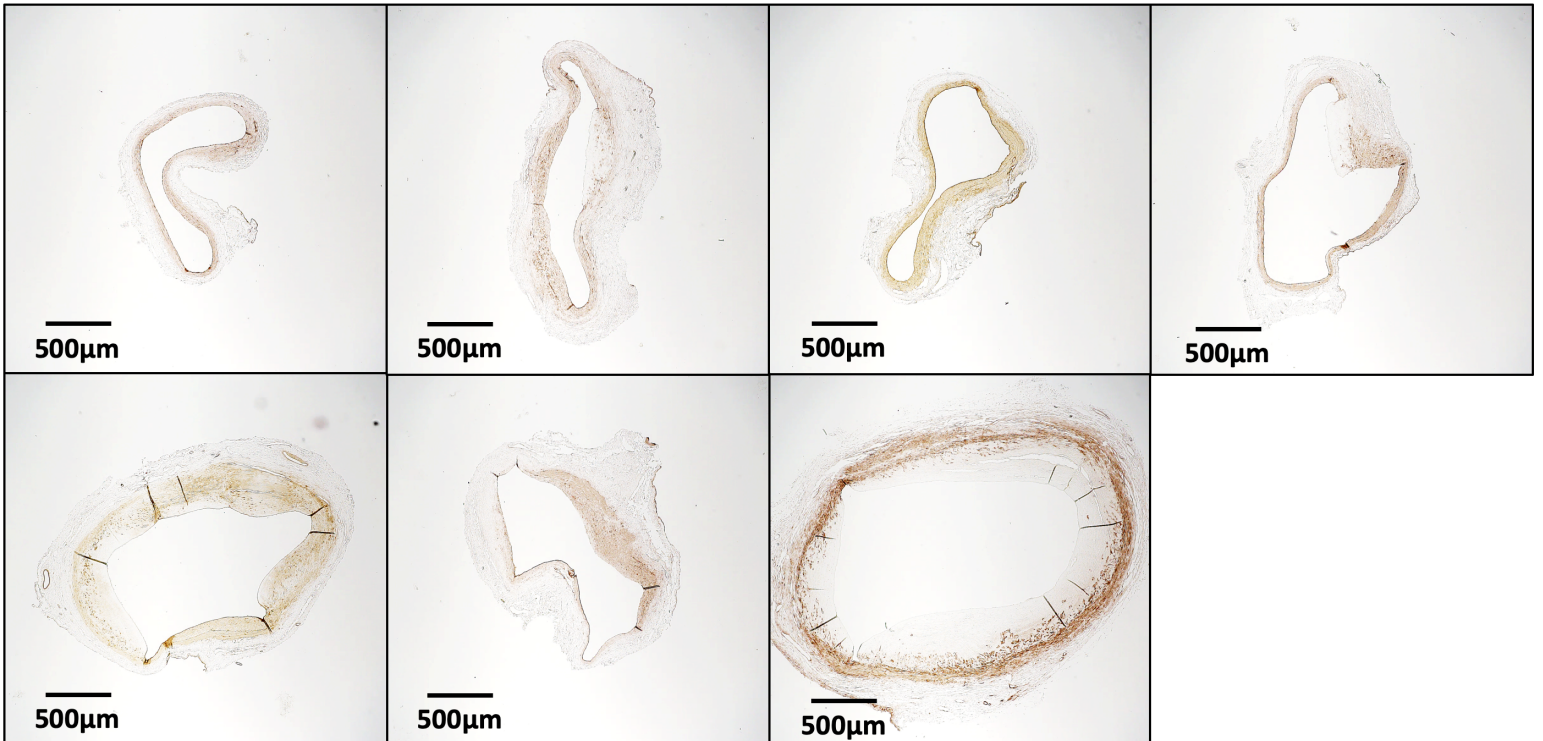
B Vinpocetine/Sham



C Saline/Elastase



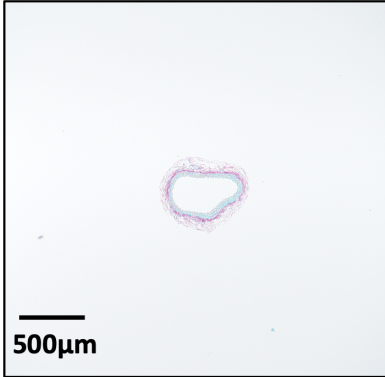
D Vinpocetine/Elastase



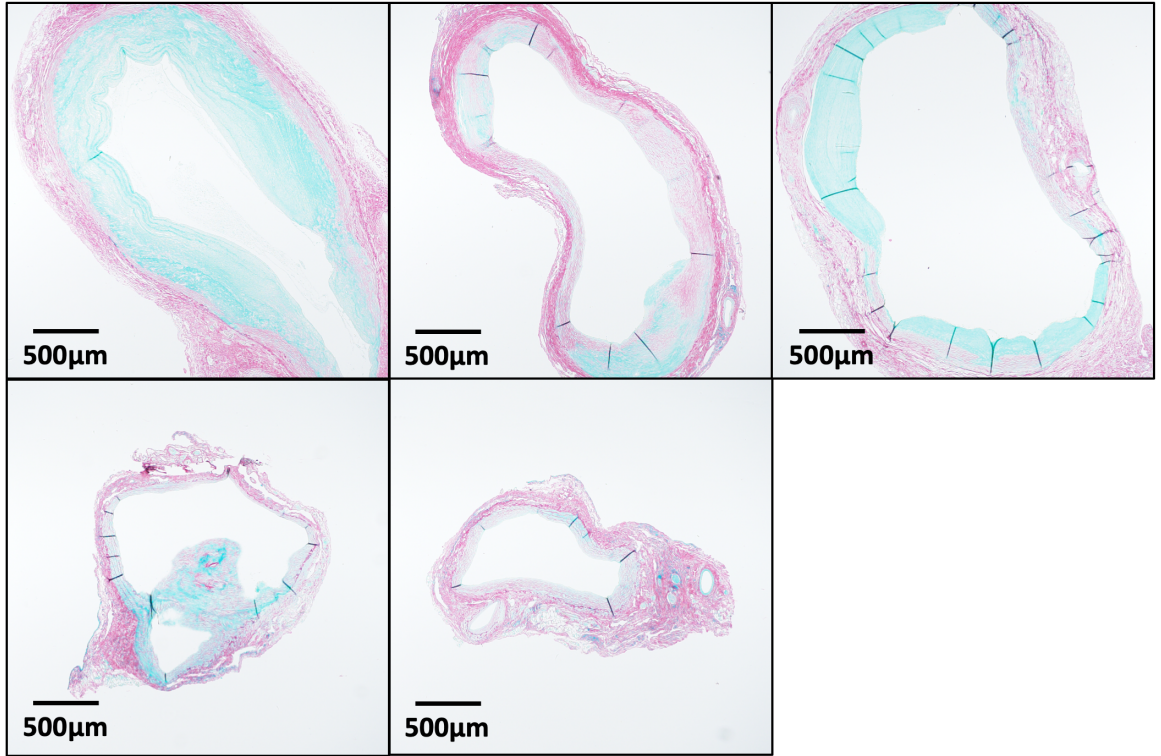
Supplementary Figure S6. Immunostaining of α -SMA in abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. Three sections at 300 μ m intervals from aneurysmal center segment (segment of largest diameter) of each animal were included in quantification. Representative images from one level are shown here due to space limitation.

Supplementary Figure S7

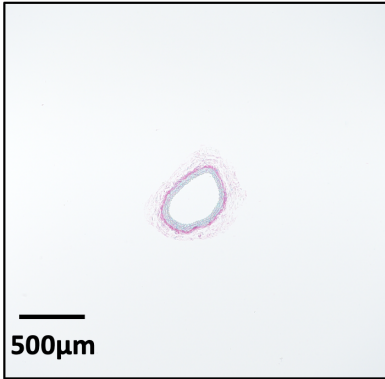
A Saline/Sham



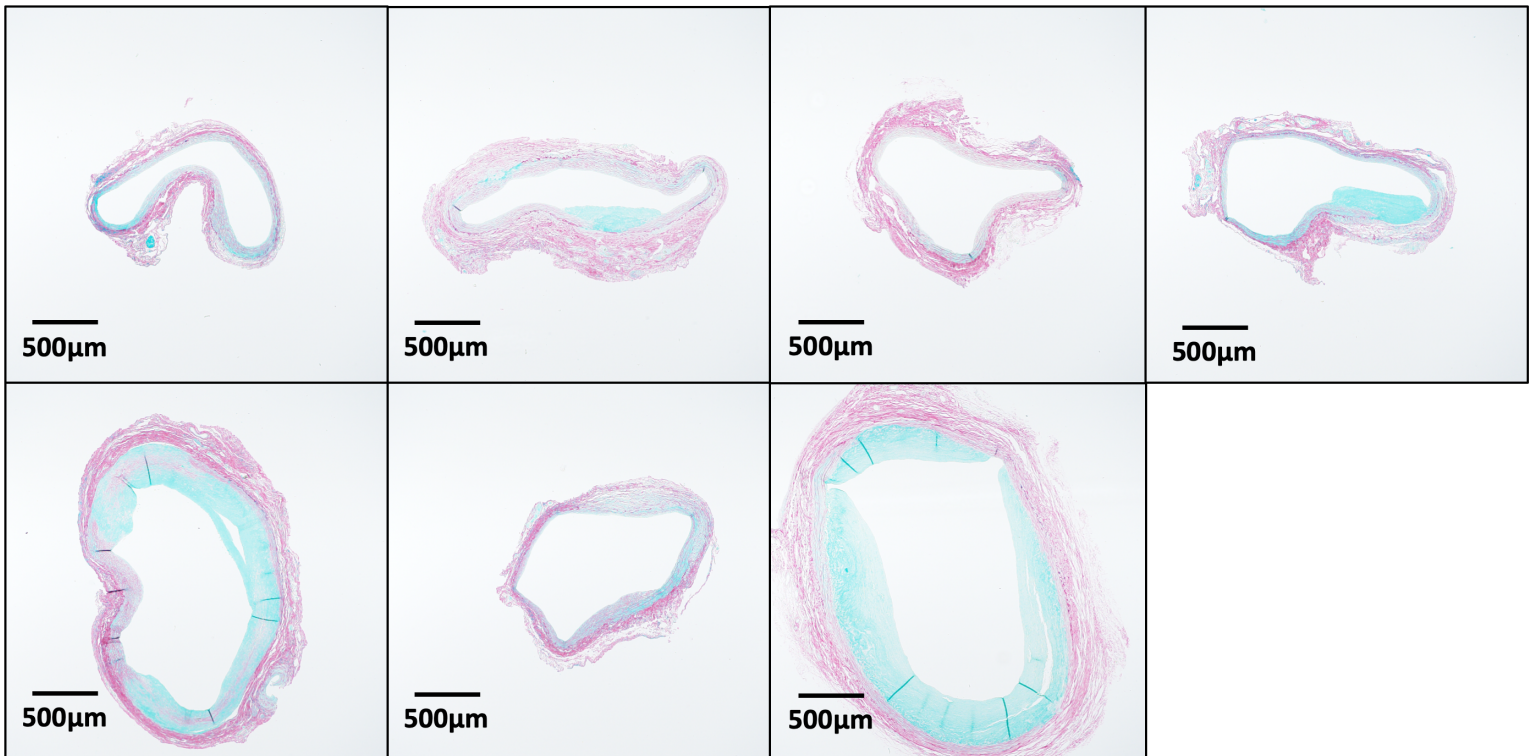
C Saline/Elastase



B Vinpocetine/Sham



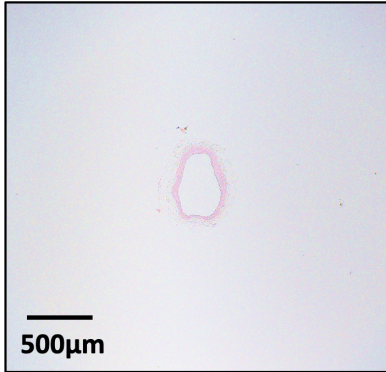
D Vinpocetine/Elastase



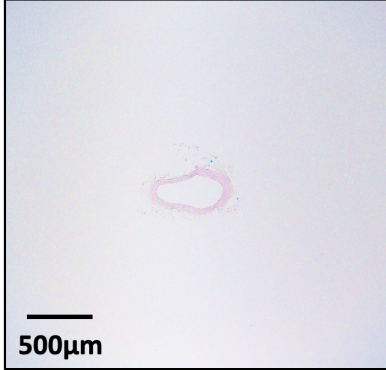
Supplementary Figure S7. Sirius red/Fast green staining of abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. The section from the level of the largest diameter of AAA for each animal was selected for staining.

Supplementary Figure S8

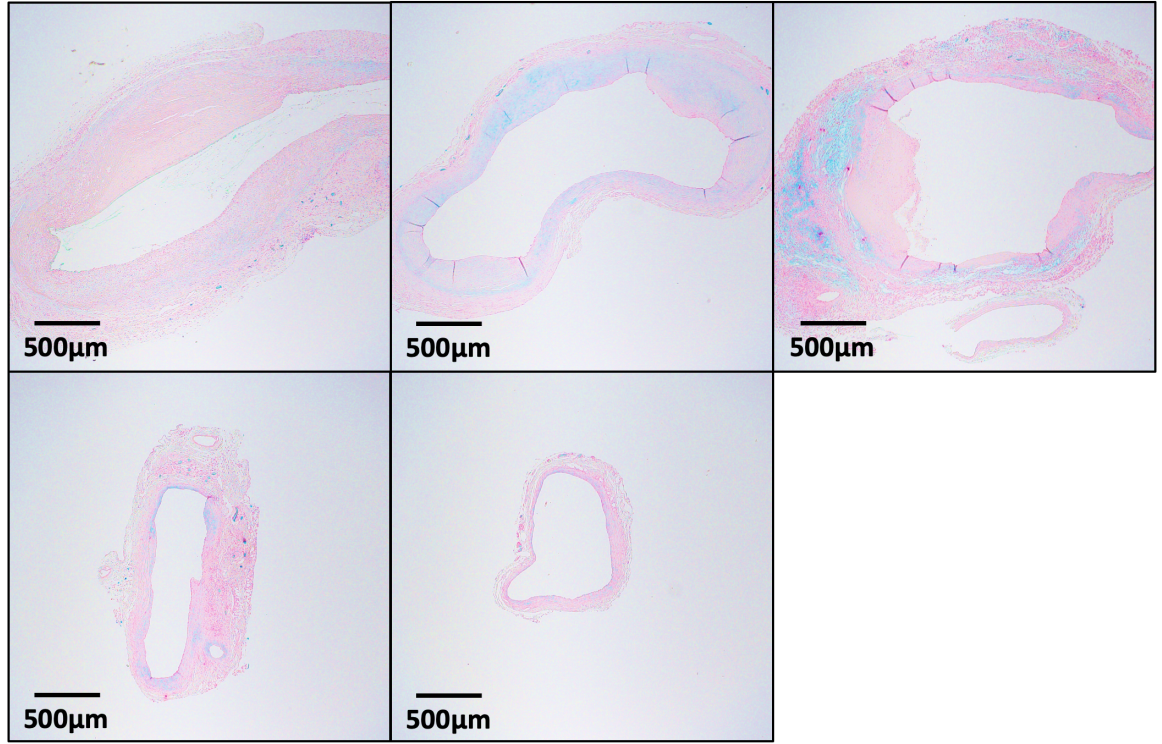
A Saline/Sham



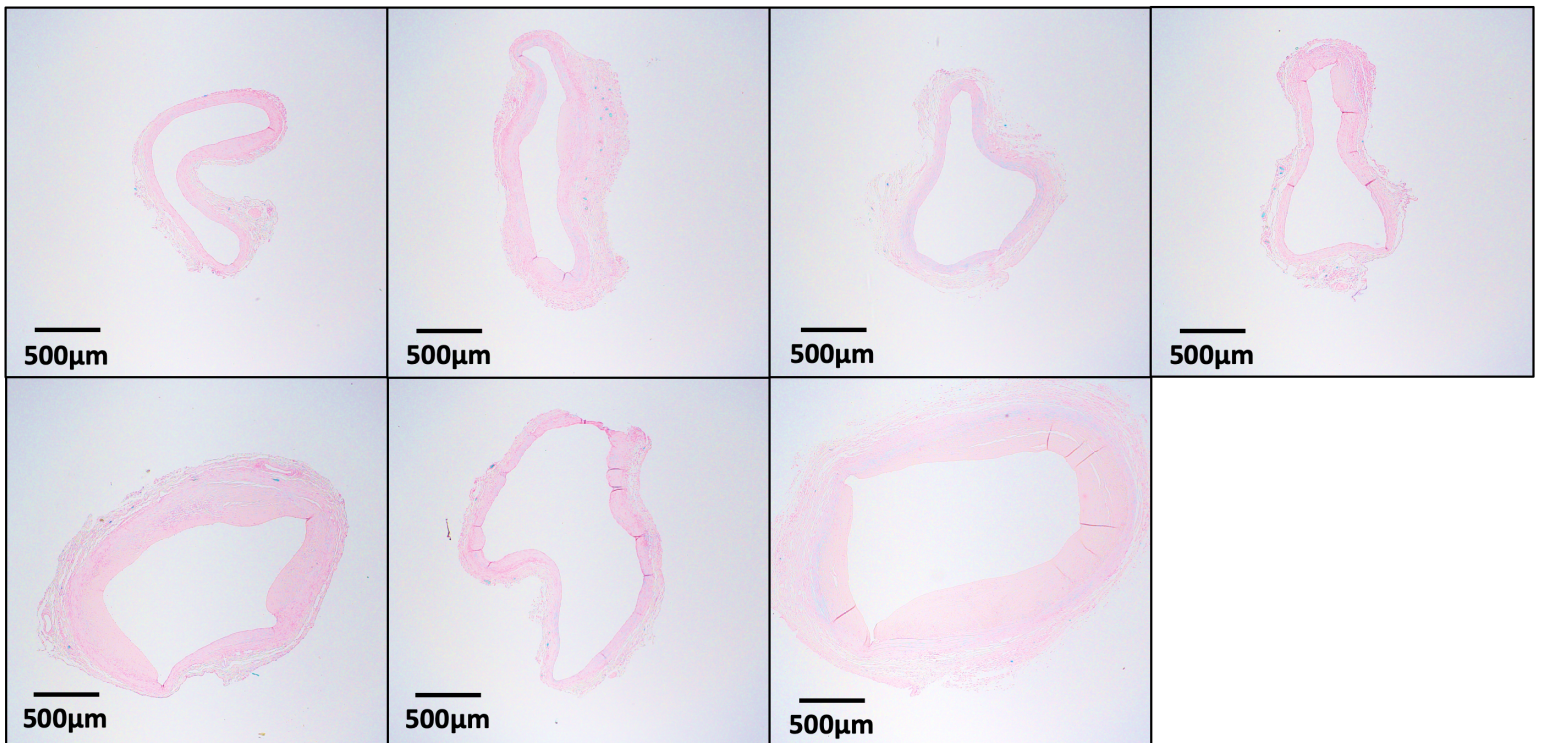
B Vinpocetine/Sham



C Saline/Elastase



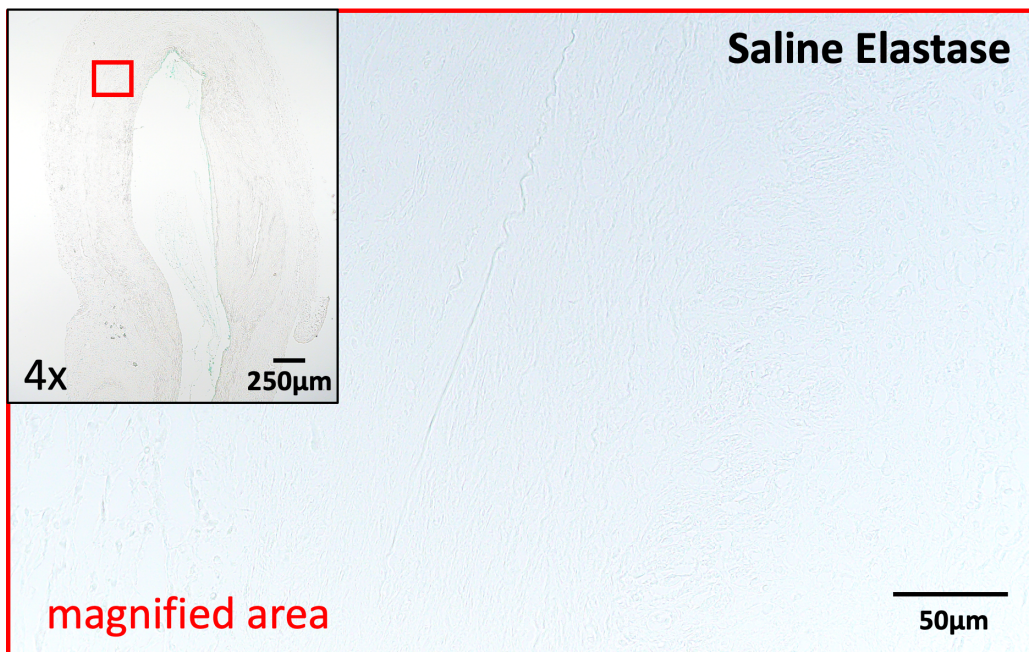
D Vinpocetine/Elastase



Supplementary Figure S8. Alcian blue staining of abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. Three sections at 300 μm intervals from aneurysmal center segment (segment of largest diameter) of each animal were included in quantification. Representative images from one level are shown here due to space limitation.

Supplementary Figure S9

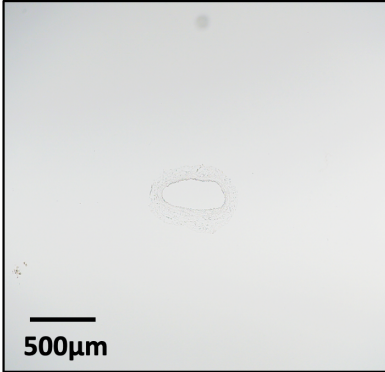
Negative control of F4/80 immunohistochemistry



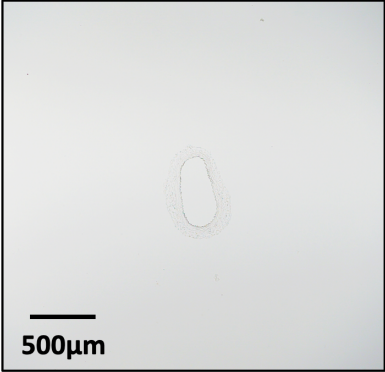
Supplementary Figure S9. Negative control of F4/80 immunohistochemistry staining in adjacent section of saline elastase induced-AAA used in Fig. 5A (lower left panel). The inset is the 4x image of the whole section. Image outlined red is the magnification of the area highlighted in red box in inset. The magnification shows the same area as Fig. 5A. Negative controls were imaged using same acquisition parameters as Fig. 5A.

Supplementary Figure S10

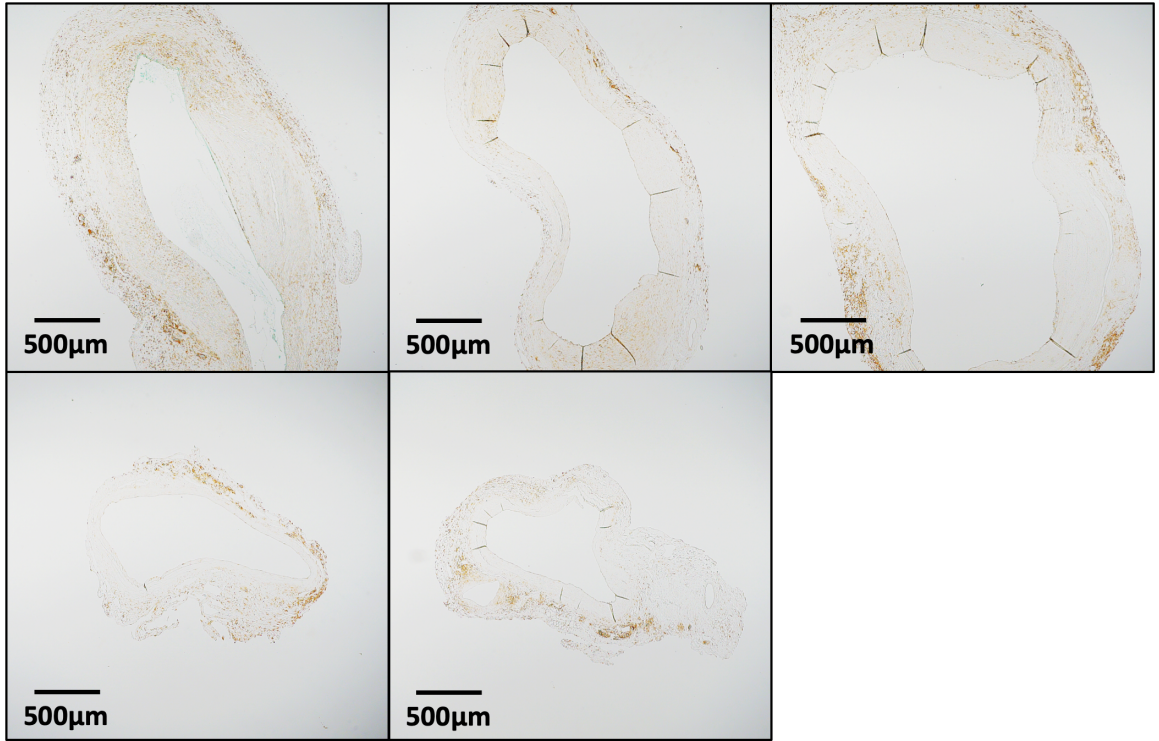
A Saline/Sham



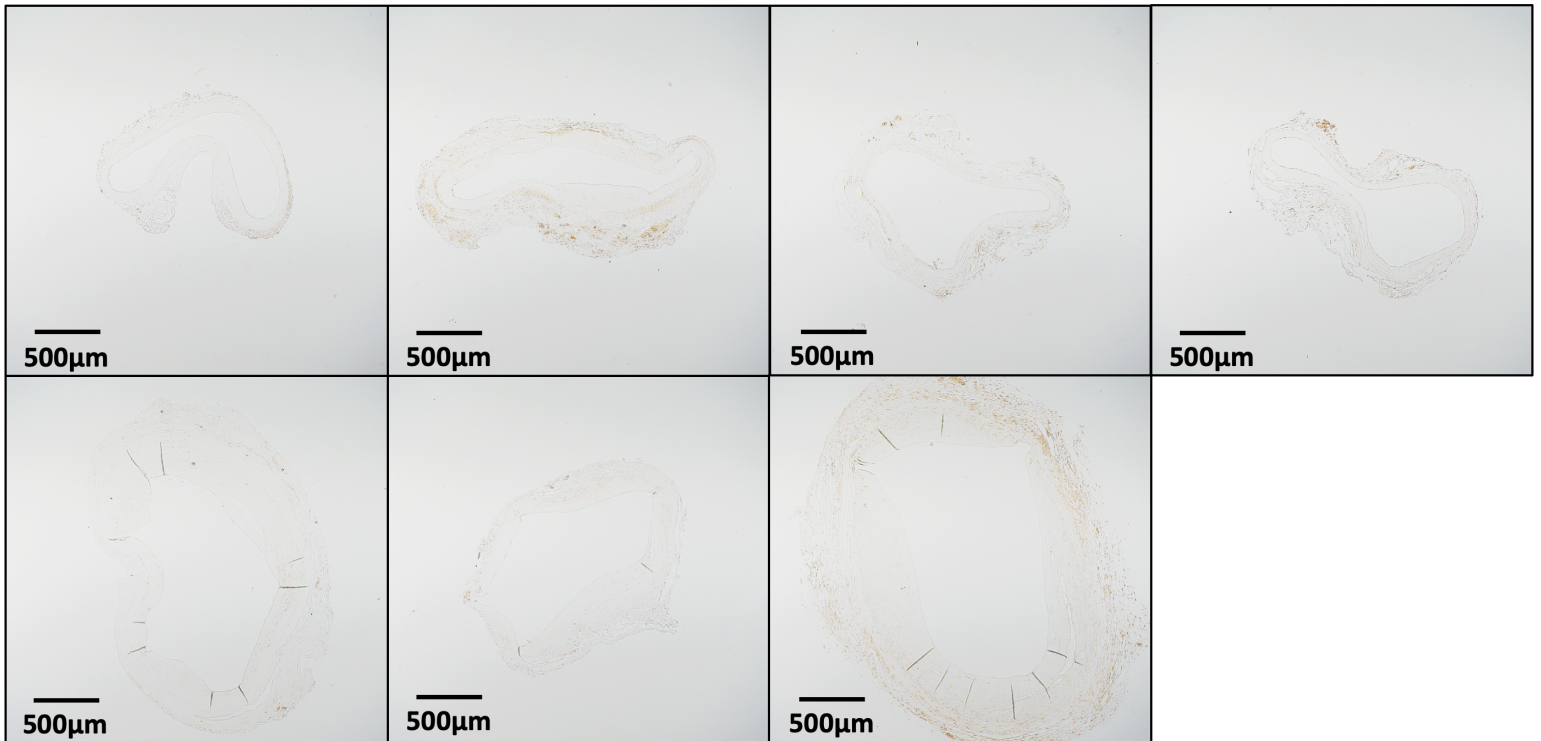
B Vinpocetine/Sham



C Saline/Elastase

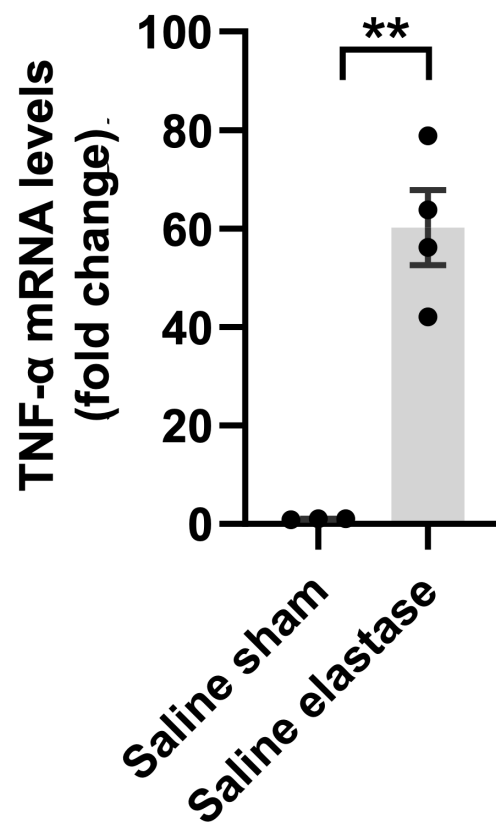


D Vinpocetine/Elastase



Supplementary Figure S10. Immunostaining of F4/80 in abdominal aorta cross sections. A, Saline/sham group. B, Vinpocetine/sham group. C, Saline/elastase group. D, Vinpocetine/elastase group. Three sections at 300 μm intervals from aneurysmal center segment (segment of largest diameter) of each animal were included in quantification. Representative images from one level are shown here due to space limitation.

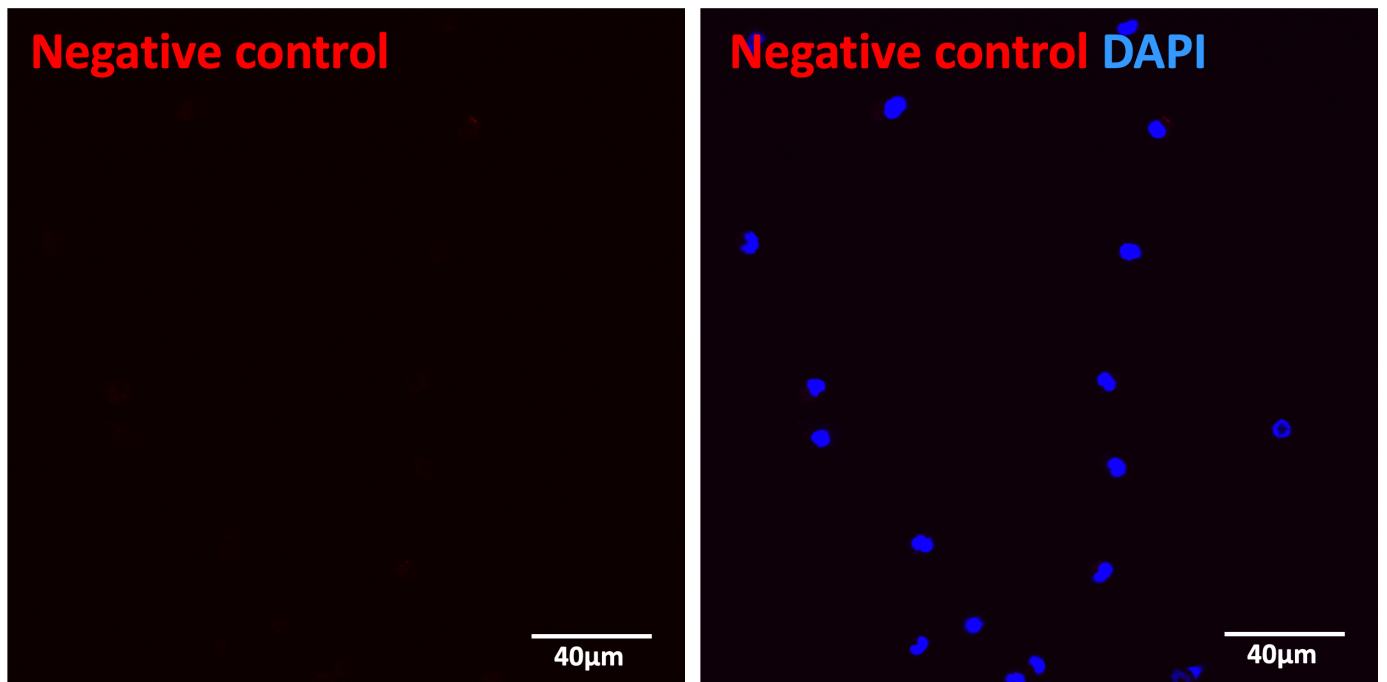
Supplementary Figure S11



Supplementary Figure S11. TNF α mRNA level in infrarenal aortas of mice. 13-week-old wild type C57BL/6 male mice were treated with 0.2% BAPN in drinking water that started from 2 days before surgery. Mice were given either sham (n=3) or perivascular elastase surgery (n=4), and infrarenal aortas were collected on the 7th day post-surgery. TNF α mRNA levels in the samples were assessed by real time PCR and normalized to saline sham, shown as fold change. Statistics were performed with Parametric Welch's t-test. Data are mean \pm SEM. **P < 0.01.

Supplementary Figure S12

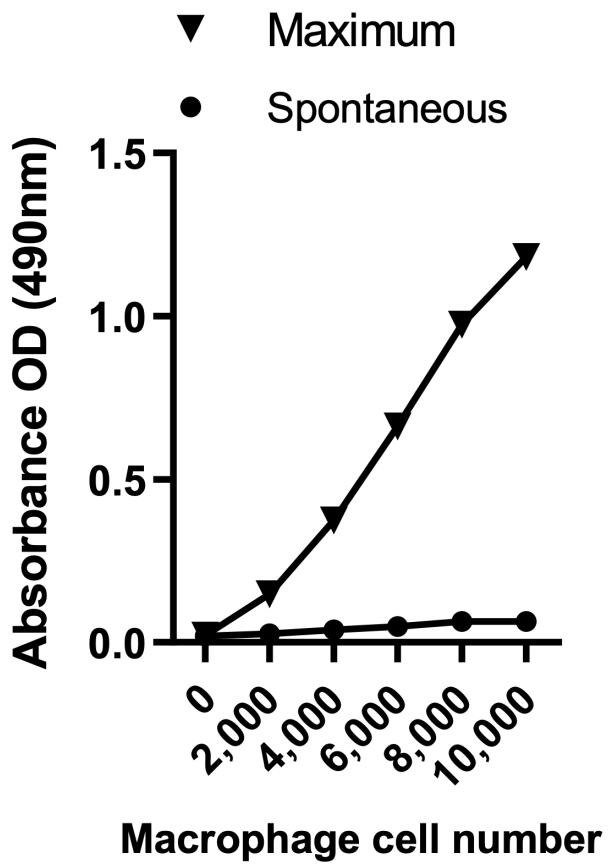
Negative control of p65 immunocytochemistry



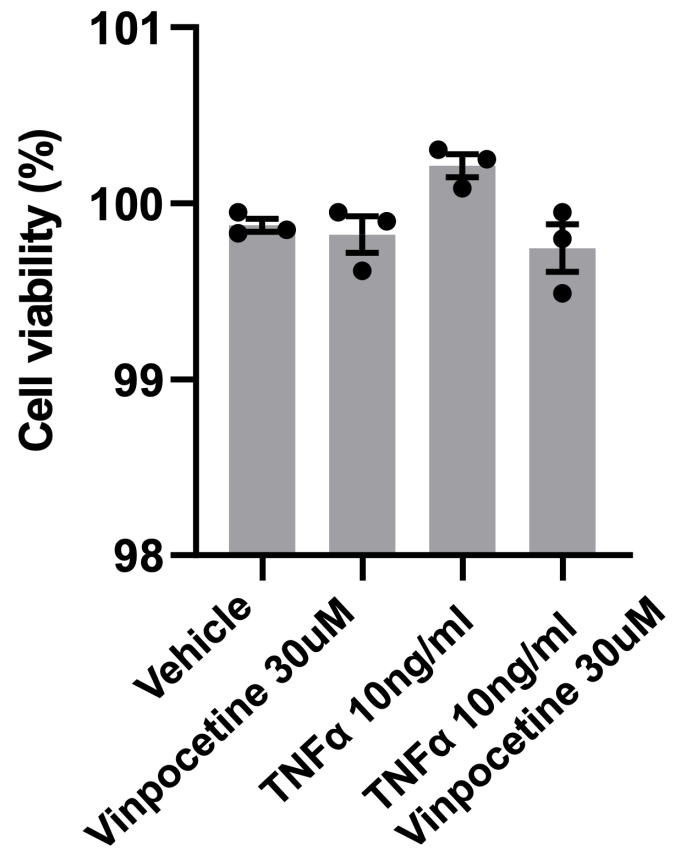
Supplementary Figure S12. Negative control of p65 immunocytochemistry staining in macrophages. The left panel is negative control without DAPI, the right panel shows negative control image merged with DAPI. Negative controls were imaged using same acquisition parameters as Fig. 6A.

Supplementary Figure S13

A



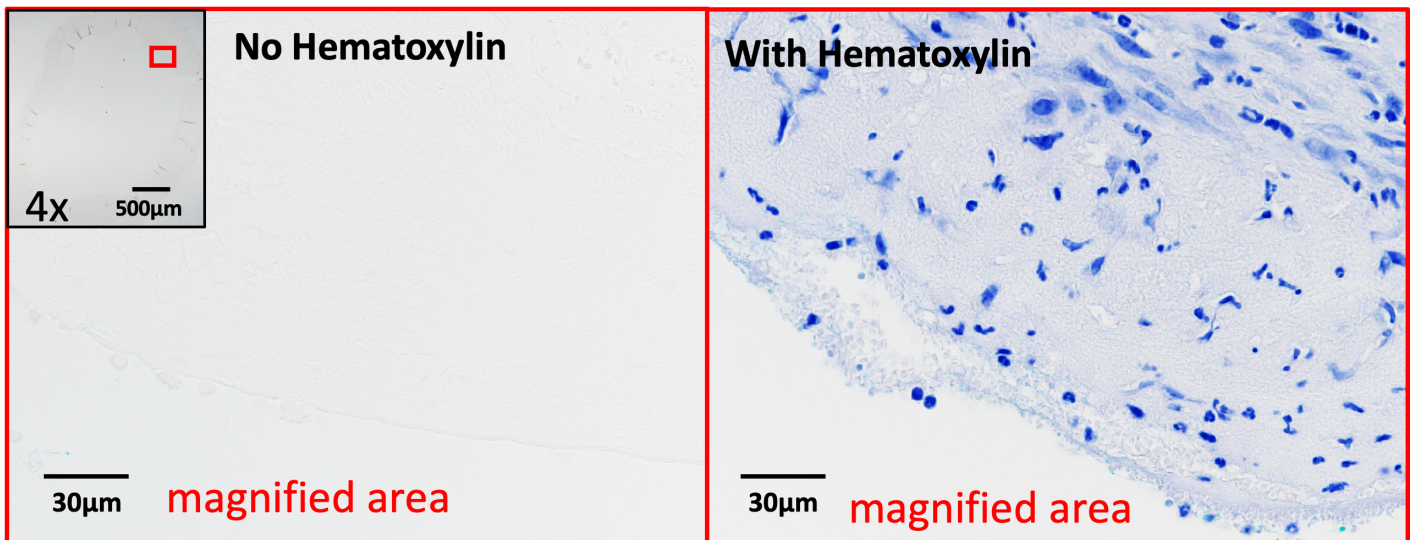
B



Supplementary Figure S13. Macrophage cytotoxicity evaluated by lactate dehydrogenase (LDH)-releasing assay. A, Absorbance versus macrophage cell number to determine optimum cell number to use for LDH cytotoxicity assay according to manufacturer's instructions. Primary mouse resident peritoneal macrophages were isolated and seeded in a serial dilution in two sets. One set of 0-10,000 macrophages series was used to determine the Spontaneous LDH Release. Another set 0-10,000 cells series was used to determine the Maximum LDH release. The Maximum LDH Release absorbance and Spontaneous LDH Release absorbance were plotted versus macrophage cell number. The greatest difference of absorbance between the Spontaneous and Maximum determined the optimal cell number to use in cytotoxicity experiments in panel B. B, Effects of vinpocetine and/or TNF α on macrophage viability. 10,000 macrophages in triplicate wells in a 96-well tissue culture plate were used for experimental groups (Vehicle control, Vinpocetine, TNF α , TNF α and Vinpocetine), Spontaneous LDH Activity group and Maximum LDH Activity group. Cells of experimental groups were starved in serum free RPMI1640 medium for 4 hours, followed by pretreatment with 30uM vinpocetine for 60min before treatment with or without murine TNF- α (10ng/ml) for 6 hours in the continued presence or absence of vinpocetine (30uM). Data were from 3 experiments.

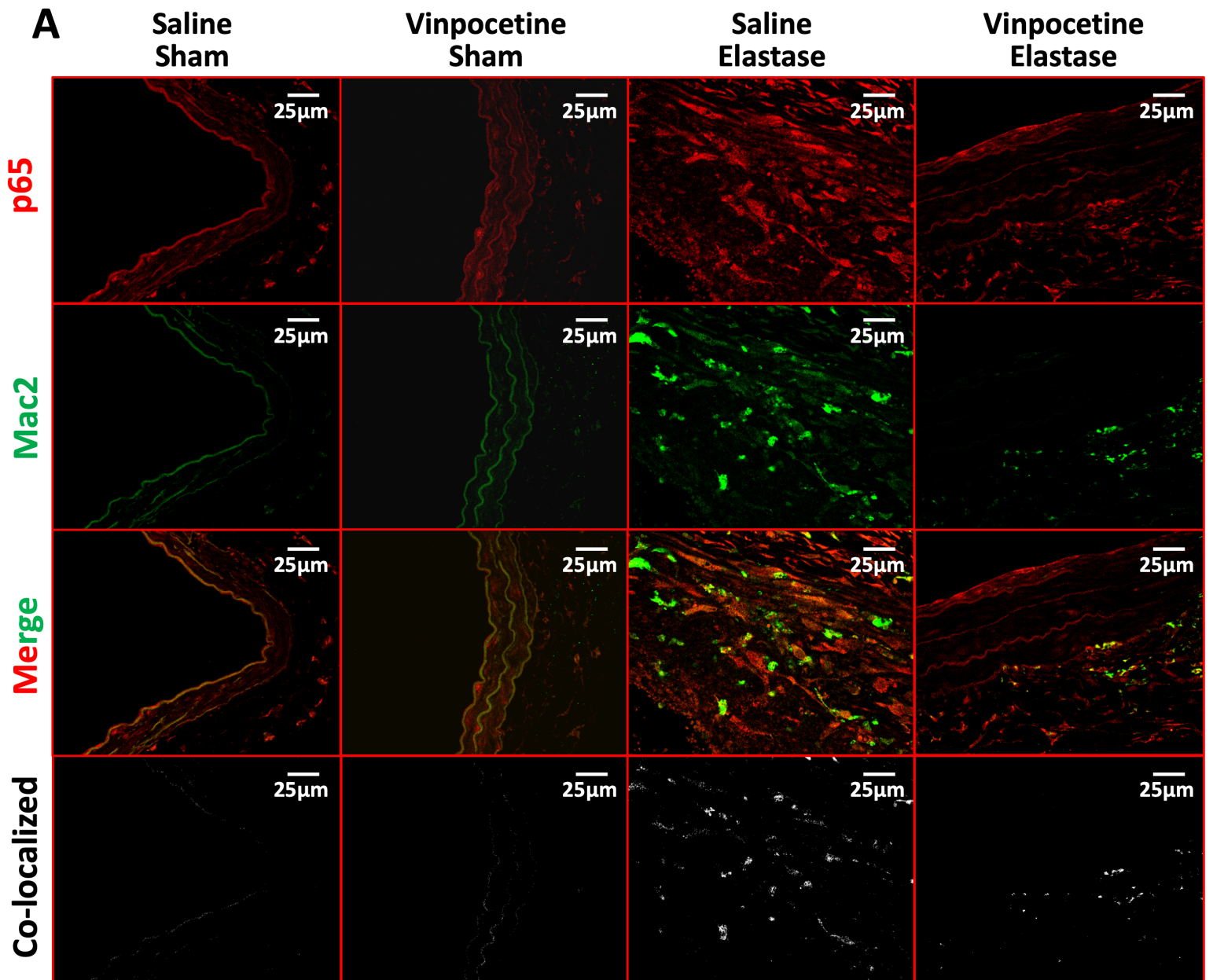
Supplementary Figure S14

Negative control of p65 immunohistochemistry

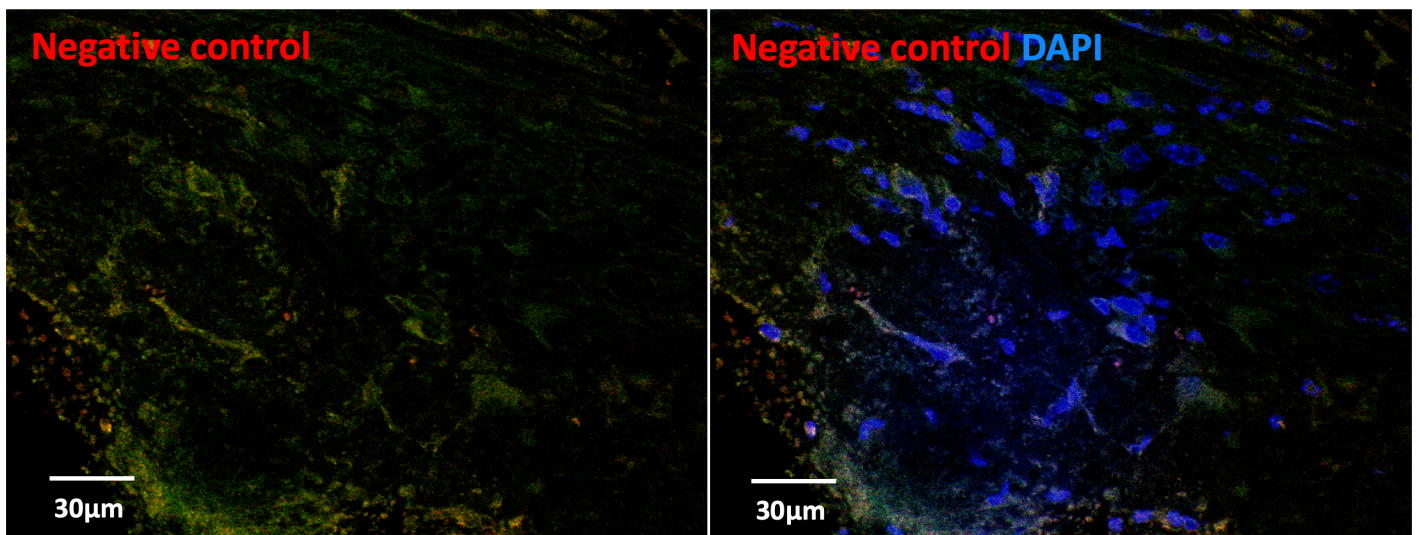


Supplementary Figure S14. Negative control of p65 immunostaining in tissue sections. A, negative control of p65 immunohistochemistry staining in adjacent sections of saline elastase induced-AAA used in Fig. 6E (lower left panel). The inset is the 4x image of the whole section without hematoxylin counterstain. Image outlined red is the magnification of the area highlighted in red box in inset. The magnification shows the same area as Fig. 6E. The left panel is negative control without hematoxylin counterstain, the right panel is negative control with hematoxylin counterstain.

Supplementary Figure S15



B Negative control of p65 and Mac2 immunofluorescence



Supplementary Figure S15. Double immunofluorescence staining of p65 and Mac2 on abdominal aortic cross sections from saline/sham, vinpocetine/sham, saline/elastase, vinpocetine/elastase samples. A, Immunostaining of sections from the same level used for p65 immunohistochemistry (Fig. 6E) are shown (same area). The first three row show p65, Mac2 and merged images. The fourth row shows colocalized points in white by colocalization analysis. B, Negative control of p65 and Mac2 immunostaining in saline elastase section (same area as panel A). The left panel is negative control without DAPI, the right panel shows negative control image merged with DAPI. Negative controls were imaged using same acquisition parameters as panel A.