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Original Article

Vasohibin-2 Aggravates Development of Ascending Aortic Aneurysms But Not Abdominal Aortic Aneurysms Nor Atherosclerosis in ApoE Deficient Mice

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Short Title:

VASH2 effect on AngII-induced vascular pathology

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Abstract

Background: Vasohibin-2 (VASH2) has been isolated as a homologue of vasohibin-1 (VASH1) that promote angiogenesis counteracting with VASH1. Chronic angiotensin II (AngII) infusion promotes both ascending and abdominal aortic aneurysms (AAs) in mice. The present study aimed to investigate whether exogenous VASH2 influenced AngII-induced vascular pathology in apolipoprotein E deficient (*ApoE*$^{-/-}$) mice.

Methods: Male, *ApoE*$^{-/-}$ mice (9 to 14 weeks old) were injected with Ad LacZ or Ad VASH2. After a week, saline or AngII (1,000 ng/kg/min) was infused into the mice subcutaneously via mini-osmotic pumps for 3 weeks. Consequently, all these mice were divided into 4 groups: saline + LacZ (n=5), saline + VASH2 (n=5), AngII + LacZ (n=18), and AngII + VASH2 (n=17).

Results: Exogenous VASH2 had no significant effect on *ex vivo* maximal diameters of abdominal aortas (AngII + LacZ; 1.67±0.17 mm, AngII + VASH2; 1.52±0.16 mm, n.s.) or elastin fragmentation and accumulation of inflammatory cells. Conversely, exogenous VASH2 significantly increased intima areas of aortic arches (AngII + LacZ; 16.6±0.27 mm^2, AngII + VASH2; 18.6±0.64 mm^2, p=0.006). VASH2 effect of AngII-induced ascending AAs was associated with

increased cleaved caspase-3 abundance. AngII-induced atherosclerosis was not altered by VASH2.

Conclusion: The present study demonstrated that augmented VASH2 expression had no effect of AngII-induced abdominal AAs or atherosclerosis, while increasing dilation in the ascending aorta.

Abbreviations

AA; aortic aneurysm

AngII; angiotensin II

apoE; apolipoprotein E

VEGF; vascular endothelial growth factor

VASH1; Vasohibin-1

VASH2; Vasohibin-2

NOX; Nicotinamide adenine dinucleotide phosphate oxidase

ROS; reactive oxygen species

DT-α-tubulin; detyrosinated-alpha-tubulin

VSMC; vascular smooth muscle cell

Introduction

Abdominal aortic aneurysms (abdominal AA) in humans are predominantly located in the aortas, distal to the renal arteries and are usually defined by an external diameter that is greater than 30 mm. Surgical repair is performed when the abdominal AA expands ≥ 55 mm because the risk of rupture increases markedly.[1,2] Survival rate from ruptured abdominal AA is only 10% to 25%.[3] The disease mainly affects elderly people over aged 65 years with a history of smoking, family history of AA, chronic kidney disease and male gender.[4] However, the mechanisms of abdominal AA onset and progression remain unknown. Clinical practice lacks effective treatment other than surgical approaches to repair enlarged aneurysm. Therefore, greater insight into the mechanisms of the disease are needed to develop new strategy to treat with abdominal AA.

Abdominal AA is a chronic inflammatory disease whose vascular wall is expanded by destruction of the aortic wall structure. It is often accompanied with infiltration of monocytes and macrophages, differentiation and proliferation of smooth muscle, elevation of matrix metalloproteinases activity, and

degradation of extracellular matrix including elastic fibers and collagenous fibers. [5]

Unlike abdominal AA, the involvement of inflammation in the development of ascending AA is unclear.[6] Recent studies reported that Marfan model mice with mutated fibrillin 1 disrupted genes of TGF-β receptor, thereby ascending AA dilatation increased. Furthermore, the influence of smooth muscle contractile function might contribute to dilatation of ascending AA. Aortic tissues of Marfan syndrome patients had increased levels of oxidative stress and increased expression of nicotinamide adenine dinucleotide phosphate oxidase (NOX) subunits.[6]

Angiotensin II (AngII) is implicated in oxidative stress and vascular inflammation, and promotes formation of atherosclerosis and induces formation of abdominal AAs, especially in hyperlipidemic mice.[7] Currently, the most commonly used model mouse for atherosclerosis and abdominal AA is apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice.[8] Tedesco et al. reported that vascular endothelial growth factor (VEGF) receptor expressions in areas of neovessel formation increased and there was an abundance of adventitial neovessel formation in intact quadrants within aneurysmal segments. The association

between neovessel formation and AAA progression was confirmed by treatment with an oral angiogenesis agent.[9] It has also been reported that VEGF-A production increases in medial smooth muscle cells of human aorta in early stages of atherosclerotic lesions and abdominal AA compared to normal aorta.[10,11] VEGF promotes angiogenesis of fragile vasa vasorum in the media and adventitia, and it has been proposed that abdominal AA rupture occurs due to collapse of vasa vasorum.[12]

Vasohibin-1 (VASH1) was isolated as a negative feedback regulator of angiogenesis produced by VEGF-inducible endothelial cells. Subsequently vasohibin-2 (VASH2) was discovered and shown to be a homologue of VASH1.[13] VASH2 is expressed in mononuclear cells that promotes sprout growth and angiogenesis.[13] VASH2 has been linked to tumor growth through stimulation of angiogenesis.[14-16] Cancer treatment has been proposed with neutralizing monoclonal antibodies that target human VASH2.[17] Furthermore, a recent investigation demonstrated that VASH2 exerted its activity as an enzyme that detyrosinates the c-terminus of α-tubulin which forms microtubules.[18] Additionally, other studies have reported that physiologic stretch rapidly activates reduced-form NOX to produce reactive oxygen species

(ROS) in a process dependent on microtubules.[19] To date, few reports have been reported regarding abdominal AA. We reported the possible relationship between VASH2 and ascending AA in association with increment of apoptosis in male C57BL/6J mice.[20]

On the basis of these previous findings, we hypothesized that exogenous VASH2 promotes angiogenesis and increases death from abdominal AA rupture in AngII-infused *ApoE*$^{-/-}$ mice. The present study aimed to investigate the severity of abdominal AA and histological changes using mouse model. The results demonstrated that that exogenous VASH2 influenced development of AngII-induced ascending AA, but not abdominal AA in *ApoE*$^{-/-}$ mice.

Materials and Methods

Study mice

All animal experiments were performed to conform the NIH guidelines (Guide for the care and use of laboratory animals). *ApoE*$^{-/-}$ mice were purchased from The Jackson Laboratory (Bar Harbor, Cat. No. 2052) and maintaining in the Department of Animal Resources, Advanced Science Research Center of Okayama University. All mice were maintained in a barrier facility and fed a normal rodent laboratory diet. Male, 9-14 week-old, *ApoE*$^{-/-}$ mice were injected with adenoviral vectors encoding for either hVASH2 or LacZ. The mice were divided into 4 groups: saline + LacZ (n=5), saline + VASH2 (n=5), AngII + LacZ (n=18) and AngII + VASH2 (n=17). The experimental protocols were approved by the Ethics Review Committees for Animal Experimentation of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical sciences (approved numbers: OKU-2016216, OKU-2019481).

Adenovirus injection

The mice were injected with adenoviral vectors (Ad LacZ or Ad hVASH2; 7.5 x 10^9 vp/100μL) intravenously (via tail vein) as described previously.[21]

Injections started one week prior to AngII infusion, and were repeated at two-week interval as described previously.[21]

AngII infusion

The mice were anesthetized using pentobarbital sodium (25 μg/g, intraperitoneally). Saline or AngII (1,000 ng/kg/min; Bachem, Cat. # H-1705) was infused into mice subcutaneously via ALZET mini-osmotic pumps (Model 2004, DURECT Corp, Lot No. 10274-12) for 21 days as described previously.[8]

Blood pressure measurements

Systolic blood pressure was measured in conscious mice using a tail cuff system (#BP-98A, Softron). The mean value of the 5-10 readings was recorded.[22,23]

Plasma measurements

Blood was obtained by cardiac puncture under anesthesia. Plasma total cholesterol concentrations were measured in individual samples using commercially available enzymatic-based kits (Waco Chemicals, Cat. #

439-17501).[24]

Quantification of ascending aortic aneurysm and abdominal aortic aneurysm

After 21 days of saline or AngII infusion, mouse aortas were harvested. Aortas were perfused with saline via a left ventricular puncture and fixed in 10% formalin overnight. Intima area of thoracic aortas were measured by an *en face* method.[25] Ex vivo width of ascending aorta was measured toward the vertical direction of the tangential line of the ascending aortic greater curvature. Abdominal aortic width was measured by *ex vivo* outer diameter of abdominal aorta.[22]

AngII-induced abdominal AAs were classified in four types, as described previously.[26] In brief, type I represents a small single dilation (1.5–2.0 times of a normal diameter). Type II denotes a large single dilation (> 2 times of a normal diameter). Type III is multiple dilations and Type IV is aortic rupture.

Analysis of atherosclerosis

Areas of atherosclerotic lesions covering the aortic arch were measured

after 21 days of saline or AngII infusion as described previously.[27] Atherosclerosis lesions were represented as a total volume and a percentage per area.

Histology and immunostaining

Histological staining and immunostaining were performed on formalin-fixed frozen sections, with appropriate negative controls. Elastica Van Gieson staining were performed. Macrophages were detected in tissue sections of ascending and abdominal aortas using a rat anti-mouse CD68 (1:500, Serotec Raleigh, Cat. # MCA1957). Endothelial cells were detected using a rabbit anti-mouse CD31 (1:500, Abcam, Cat. # GR244952-5).

Western blotting analyses

Liver and aortic tissue lysates were extracted in RIPA lysis buffer, and concentrations of extracted proteins were measured using a DC Protein Assay Kit (Bio Rad Inc., Hercules, CA). Protein extracts (10-20 μg) were resolved by SDS-PAGE (10% wt/vol) and transferred electrophoretically to PVDF membranes.[28] After blocking with non-dry fat milk (5 % wt/vol), membranes

were probed with antibodies against VASH2 (clone 1760; provided by Tohoku University, Sendai, Japan),[29] α-tubulin (abcam, ab195889), detyrosinated (DT)-α-tubulin (Sigma-Aldrich, Cat. # AB3201), cleaved caspase-3 (Cell Signaling, Lot #9661), NOX-1 (abcam, ab121009) and β-actin (Sigma-Aldrich, Cat. # A5441).

Statistics

All data were presented as mean ± SEM. All plot and bar graphs were created with SigmaPlot (version 14.0, Systat Software Inc.). All statistical analyses were performed using SigmaStat v3.5, incorporated into SigmaPlot. Statistical significance between the 4 groups was assessed by two-way analysis of variance with Holm-Sidak post hoc. The level of significance was set to $p < 0.05$.

Results

Detection of VASH2 protein expression in liver tissue

To confirm successful delivery of the *LacZ* and *VASH2* gene, LacZ and VASH2 protein abundance was evaluated using mice liver tissues. Western blotting analyses using antibodies against VASH2 displayed the presence of VASH2 protein bands only in mice infected with VASH2 groups (Supplement Figure 1A). Similar to VASH2, the presence of LacZ protein bands only in mice infected with LacZ groups (Supplement Figure 1B).

Characteristics of mice

After 21 days of saline or AngII infusion, systolic blood pressure and heart weight were significantly higher and body weight were lower in AngII infused groups (Table 1). No significant differences were observed in plasma total cholesterol concentrations (Table 1). The differences between LacZ and VASH2 groups were not significant.

Exogenous VASH2 had no effect on AngII-induced abdominal AA formation

Abdominal aortic width was measured by *ex vivo* outer diameter. A representative image of an abdominal AA is shown in Figure 1A. The width was significantly increased in AngII-infused groups compared to saline-infused groups (p<0.001, Figure 1B). However, there was no difference in the width between the AngII + LacZ group and the AngII + VASH2 group (1.67 ± 0.14 mm vs 1.52 ± 0.14 mm, p=0.453, Figure 1B).

Furthermore, there was no significant difference in the classification of abdominal AAs between the AngII + LacZ and the AngII + VASH2 group (Figure 1C).

The medial thickness was increased and elastin fragmentation by Elastic-Van Gieson staining in AngII infused groups (Figure 1D-G). Again, no difference in prominent macrophage accumulation demonstrated by CD68 nor neovascularization in tunica media by CD31 was observed between LacZ and VASH2 groups (Figure 1H-O).

Exogenous VASH2 had no influence on AngII-induced atherosclerosis

Areas of atherosclerosis were significantly increased in AngII-infused groups compared with saline-infused groups (p<0.001, Figure 2). No

significant differences in atherosclerosis area in aortic arch were found between LacZ and VASH2 groups.

Exogenous VASH2 exacerbated development of AngII-induced ascending AA.

A representative image of an AngII-induced ascending AA is shown in Figure 3A. Areas of ascending aortas significantly increased in AngII-infused groups compared to saline-infused groups ($p<0.001$, Figure 3B). Furthermore, the areas of the AngII + VASH2 group was significantly larger than those of the AngII + LacZ group (18.6 ± 2.0 mm^2 vs 16.6 ± 0.8 mm^2, $p=0.013$, Figure 3B). Similar to the areas of *en face*, ex vivo width of ascending AA significantly expanded (Figure 3C).

The medial thickness was increased by Elastic-Van Gieson staining in AngII infused groups (Figure 3D-G). However, no differences in macrophage accumulation evaluated by CD68 staining were observed between LacZ and VASH2 groups, and little neovascularization in tunica media evaluated by CD31 staining was detected in both LacZ and VASH2 groups, as well as abdominal AA (Figure 3H-O).

Exogenous VASH2 led to apoptosis in ascending aorta but not in abdominal aorta

To investigate the difference between LacZ and VASH2 groups in aortic dilation, the protein expression of cleaved caspase-3 in the ascending and abdominal aorta were investigated (Figure 4). No difference in the cleaved caspase-3 expression in the abdominal aorta was observed between LacZ and VASH2 groups. On the other hand, in the ascending aorta, the cleaved caspase-3 expression increased in VASH2 groups, which was enhanced by AngII infusion. These results suggest that VASH2 affects the apoptosis in AngII-induced ascending AA in association with apoptosis.

Exogenous VASH2 did not influence protein expression of NOX-1, α-tubulin and DT-α-tubulin

Abundance of NOX-1 in extracts from ascending aortas were not significantly different in VASH2 groups compared with LacZ groups (Figure 4). In contrast, α-tubulin were increased in the VASH2 group compared to the LacZ group in the ascending aorta (Figure 4). In the abdominal aorta, there was no

difference between the two groups. In contrast, DT-α-tubulin, there was no difference between the LacZ and VASH2 groups in either the abdominal or the ascending aorta.

Discussion

Based on previous studies, we hypothesized that exogenous VASH2 would exacerbate AngII-induced abdominal AA in *ApoE*$^{-/-}$ mice. Contrary to our hypothesis, exogenous VASH2 failed to affect AngII-induced abdominal AA and atherosclerosis. However, similar to our previous study using C57BL/6J mice, exogenous VASH2 exacerbated AngII-induced ascending AA in *ApoE*$^{-/-}$ mice, in association with VASH2-enhanced vascular apoptosis.

Previous studies have reported that exogenous VASH2 contributed to tumor growth, microvessel density and hemoglobin concentration in several carcinoma. Further analyses showed that the increase of angiogenesis may be via the transcription of fibroblast growth factor-2 and VEGF.[30] In tumor tissues, cancer cells themselves produce VASH2 by autocrine and reach local areas easily.[31] Given these reports, it is likely that exogenous VASH2 would produce fragile vasa vasorum in the adventitia in abdominal aorta, consequently, exacerbate abdominal AA. However, in our study, exogenous VASH2 did not significantly affect abdominal AA dimensions. In addition, exogenous VASH2 was not associated with angiogenesis. This result consists with our previously study using C57BL/6J.[20] These observations imply that exogenous VASH2 in

abdominal aorta might exert different effects from that in the tumor tissue regarding angiogenesis. Indeed, Sonoda et al. reported that transcripts of splicing variant 290aa of hVASH2 had anti-angiogenic activity.[32] In this report, angiogenesis was associated with high expression of hVASH2 in mononuclear cells. Meanwhile, anti-angiogenic activity of a specific splicing variant of hVASH2 was confirmed in vascular endothelial cells.[33] Thus, there is clear evidence that splicing variants have diverse functions.

Regarding atherosclerosis, several reports have indicated that VASH1 influences atherosclerosis.[34] VASH1 was downregulated via miR-22 during replicative senescence of endothelial cells. The endothelial cell senescence might be response for aging-associated vascular diseases including atherosclerosis.[34] Meanwhile, no evidence has been reported in the association between VASH2 and atherosclerosis. We evaluated how both VASH2 and AngII affect atherosclerosis in hyperlipidemic model mice. However, VASH2 had little influence on atherosclerosis. In case we fed high fat diet, different conclusions might have been reached. Chronic AngII infusion into normolipidemic mice promotes dilatation of the ascending aorta, as well as hyperlipidemic mice.[25] The current study demonstrated a similar effect, with

AngII infusion into *ApoE*$^{-/-}$ mice enlarging the ascending aorta. Furthermore, the overexpression of VASH2 with AngII infusion into *ApoE*$^{-/-}$ mice exacerbated dilatation of ascending aorta, in agreement with our previous study.[20] Thus, exogenous VASH2 in mice may have a significant role on the enlargement of ascending AA by enhancing the vascular apoptosis. In human tissue, it has been reported that thoracic AA group did not differ from the control group in the protein levels of VEGF-A[35], suggesting that angiogenesis might not be involved in the development of thoracic AA. Indeed, our study failed to demonstrate angiogenesis in ascending aortic tissue.

Apoptosis in vascular smooth muscle cells (VSMCs) occurs in many arterial diseases, including atherosclerosis and aortic aneurysm formation.[36,37] Recently, it was reported that lncRNA HIF 1α-antisense RNA 1, which was associated with apoptosis in VSMCs, was overexpressed in the site of thoracic and abdominal AA in mice.[38] These observations suggest that apoptosis might be involved in AA formation. Interestingly, the current study revealed that exogenous VASH2 by itself induced the apoptosis in aortas in *ApoE*$^{-/-}$ mice, unlike C57BL6 mice.[20] Nevertheless, this increment appears to exert little effect on the vascular phenotype. Only exogenous VASH2 under AngII infusion

could be associated with ascending aortic apoptosis and dilatation in *ApoE*$^{-/-}$ mice, similar to C57BL6 mice.[20]

We further examined the mechanism of VASH2 which was involved in the dilatation of the ascending AA. As described above, VASH2 operates as an enzyme, in which it detyrosinates the c-terminus of α-tubulin which forms microtubules.[18] Recent investigations have demonstrated that DT-microtubules in human cardiomyocytes are frequently observed in patients with heart failure.[39] A dense, heavily DT-microtubule network is associated with increased myocyte stiffness and impaired contractility.[39] By reducing detyrosination, cytoskeletal stiffness decreased and the speed of muscle contraction and relaxation increased.[19] Benjamin et al. proposed that physiologic stretch rapidly activates reduced-form NOX-2 to produce ROS in heart cells.[40] Altogether, we hypothesized that increased DT-α-tubulin would stimulate ROS production in ascending Aorta. In the present study, α-tubulin in aortas was increased in the VASH2 groups. However, neither DT-α-tubulin nor NOX-1 was influenced by VASH2. Taken together, regarding ascending aorta, DT-microtubule network was unlikely associated with aortic dilatation.

In conclusion, the current study demonstrated that exogenous VASH2

failed to influence AngII-induced abdominal AA in *ApoE*$^{-/-}$ mice. In contrast, exogenous VASH2 significantly exacerbated AngII-induced ascending AA, in part, associated with apoptosis of VSMCs. VASH2 may exert differential effects on AngII-induced vascular pathology.

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Disclosure

None of the authors declare any potential conflict of interests regarding this study.

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Figure legend

Figure 1. Effect of VASH2 on AngII–induced abdominal aortic aneurysm formation.

A. Representative images of abdominal aortas. **B.** Quantification of mouse abdominal aortic aneurysm. AngII infusion significantly increased ex vivo maximal diameters of abdominal aortas. Square box represents mean and bar represents SEM. **C.** Severity of abdominal aortic aneurysm. Statistical analysis was performed by two-way ANOVA, followed by post hoc test of Holm-Sidak method. **D-G.** Elastica van Gieson (EVG) staining. **H-K.** CD68. **L-O.** CD31 (scale bars: 100 μm)

Figure 2. Effect of VASH2 on AngII–induced atherosclerosis.

A. Atherosclerosis area in aortic **B.** Percent atherosclerosis area in aortic arch. AngII infusion significantly increased atherosclerosis area. Square box represents mean and bar represents SEM.

Figure 3. Effect of VASH2 on AngII–induced ascending aortic aneurysm

formation.

A. Representative images of *en face* ascending aortas. **B.** Intima area of ascending aortas. AngII infusion significantly enhanced ascending aortic aneurysms. Overexpression of VASH2 exacerbated ascending aortic aneurysms. **C.** Ex vivo aortic width of ascending aorta. Square box represents mean and bar represents SEM. Statistical analysis was performed by two-way ANOVA. **D-F.** Elastica van Gieson (EVG) staining. **H-K.** CD68. **L-O.** CD31 (scale bars: 100 μm)

Figure 4. Relationship between apoptosis and individual protein expression.

Overexpression of VASH2 increased the expression of cleaved caspase-3 and α-tubulin. Meanwhile, failed to increase the expression of NOX-1 and DT-α-tubulin. *p<0.05 vs Saline + LacZ group, ** p<0.05 vs AngII + LacZ group. Square box represents mean and bar represents SEM. Statistical analysis was performed by two-way ANOVA.

NOX-1; nicotinamide adenine dinucleotide phosphate oxidase-1, DT-α-tubulin; Detyrosinated-alpha-tubulin, n.s.; not significant

Table 1. Characteristics of study groups.

	Saline + LacZ	Saline + VASH2	AngII + LacZ	AngII + VASH2
N	5	5	18	17
SBP (mmHg)	103 ± 1	98 ± 1	139 ± 3[*]	131 ± 6[†]
BW (g)	29.4 ± 0.9	30.4 ± 1.2	27.1 ± 0.5[‡]	27.7 ± 0.5[§]
T-Cho (mg/dL)	706 ± 25	731 ± 35	628 ± 45	613 ± 46
HW/BW (mg/g)	4.6 ± 0.1	4.6 ± 0.1	6.0 ± 0.2[‖]	5.7 ± 0.1[¶]

SBP; systolic blood pressure, BW; body weight, T-Cho; total cholesterol concentration, HW; heart weight. [*]*p<0.001* vs Saline + LacZ group, [†]*p<0.001* vs Saline + VASH2 group, [‡]*p<0.05* vs Saline + LacZ group, [§]*p<0.05* vs Saline + VASH2 group, [‖]*p<0.001* vs Saline + LacZ group, [¶] *p<0.05* vs Saline + VASH2 group. Statistical analyses were performed by two-way ANOVA. Differences in means were analyzed using a Holm-Sidak post hoc test.

Figure 1

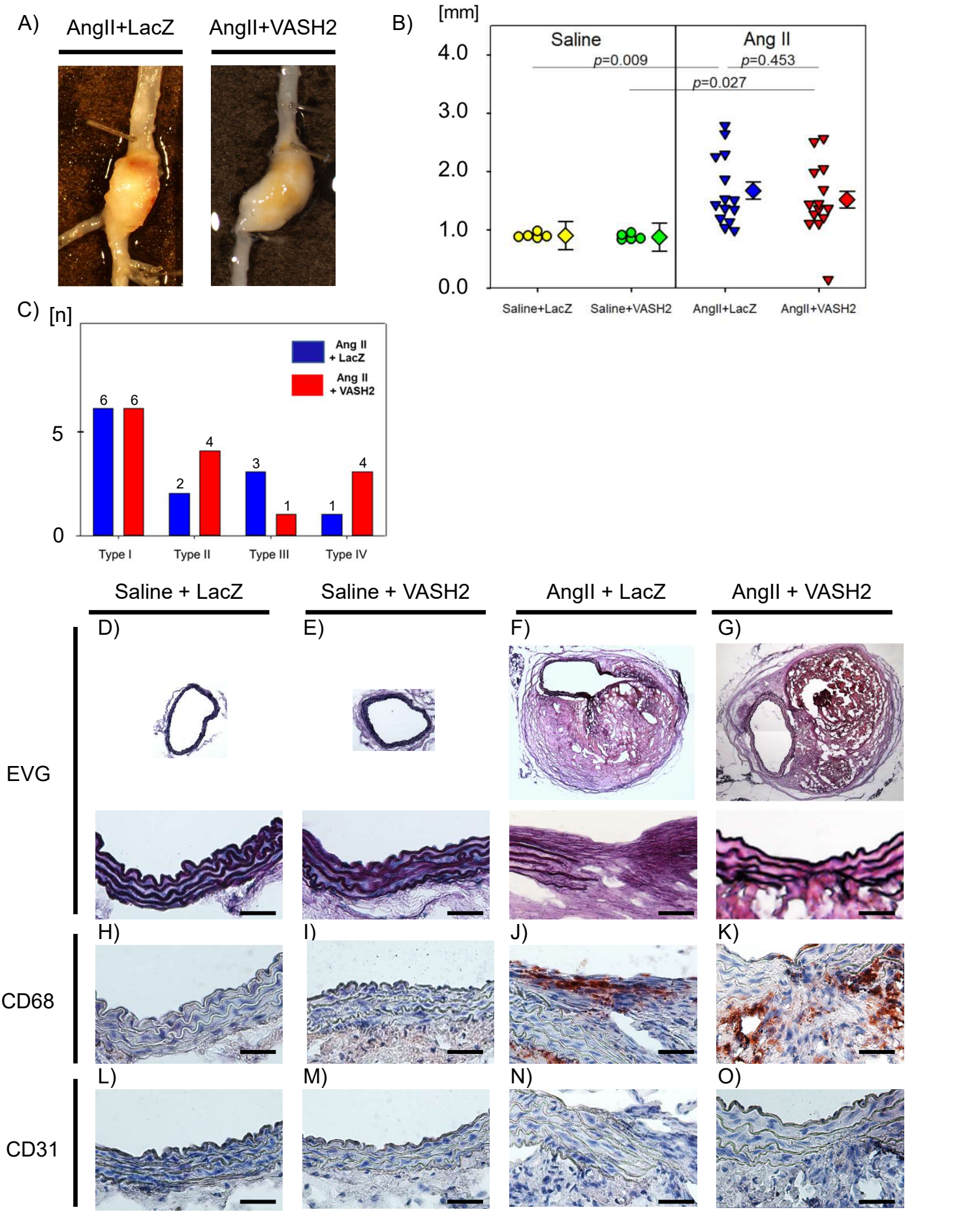
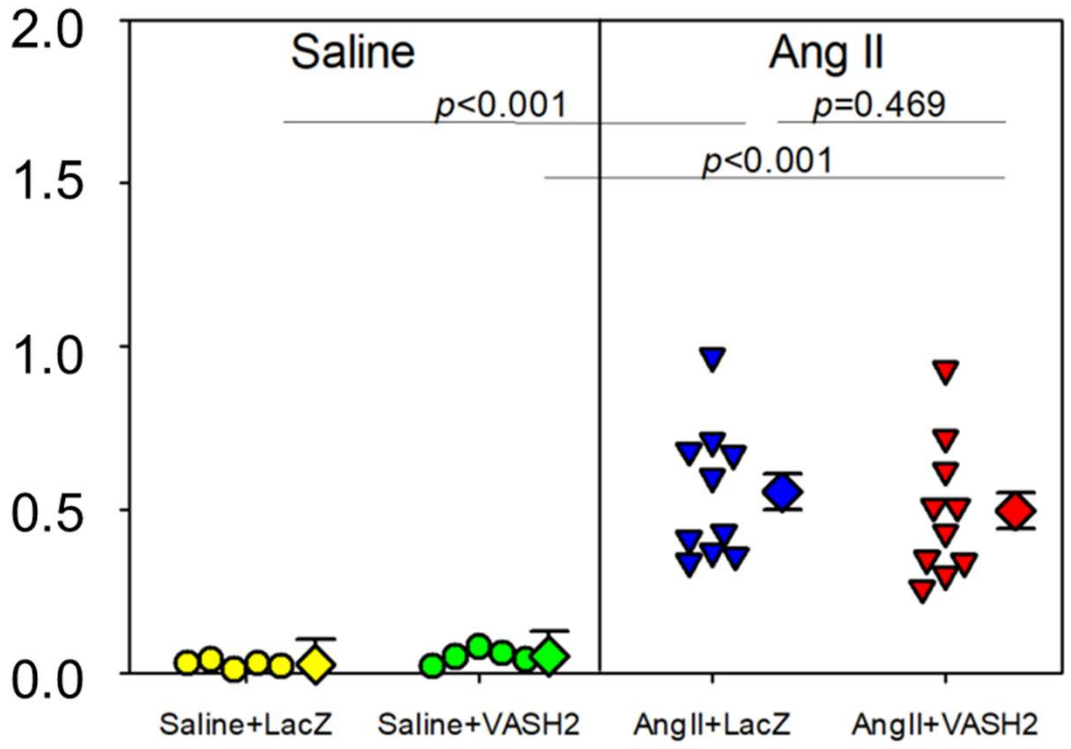

A)
AngII+LacZ
AngII+VASH2
B)
[mm]
Saline
Ang II
p=0.009
p=0.453
p=0.027
4.0
3.0
2.0
1.0
0.0
Saline+LacZ
Saline+VASH2
AngII+LacZ
AngII+VASH2
C)
[n]
Ang II + LacZ
Ang II + VASH2
5
0
6
6
2
4
3
1
1
4
Type I
Type II
Type III
Type IV
Saline + LacZ
Saline + VASH2
AngII + LacZ
AngII + VASH2
D)
E)
F)
G)
EVG
H)
I)
J)
K)
CD68
L)
M)
N)
O)
CD31

Figure 2

A) [mm²]



B) [%]

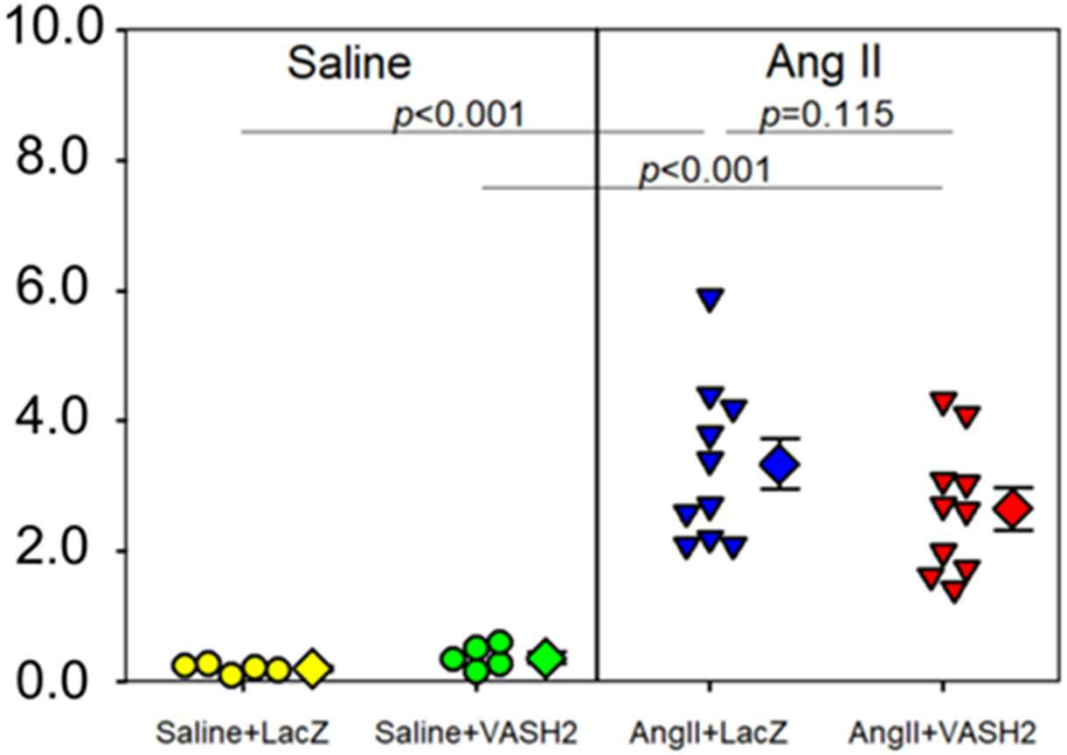


Figure 3

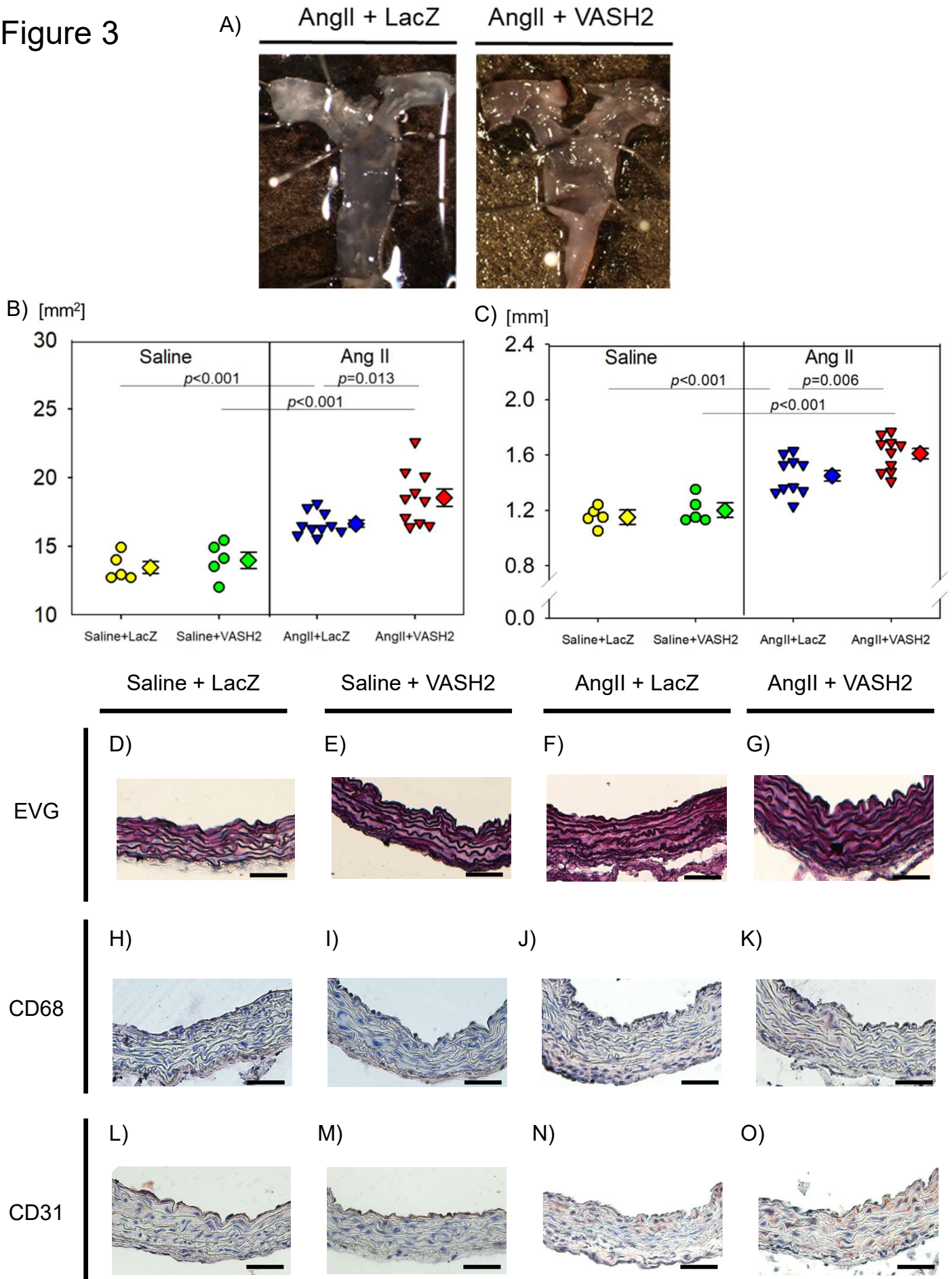
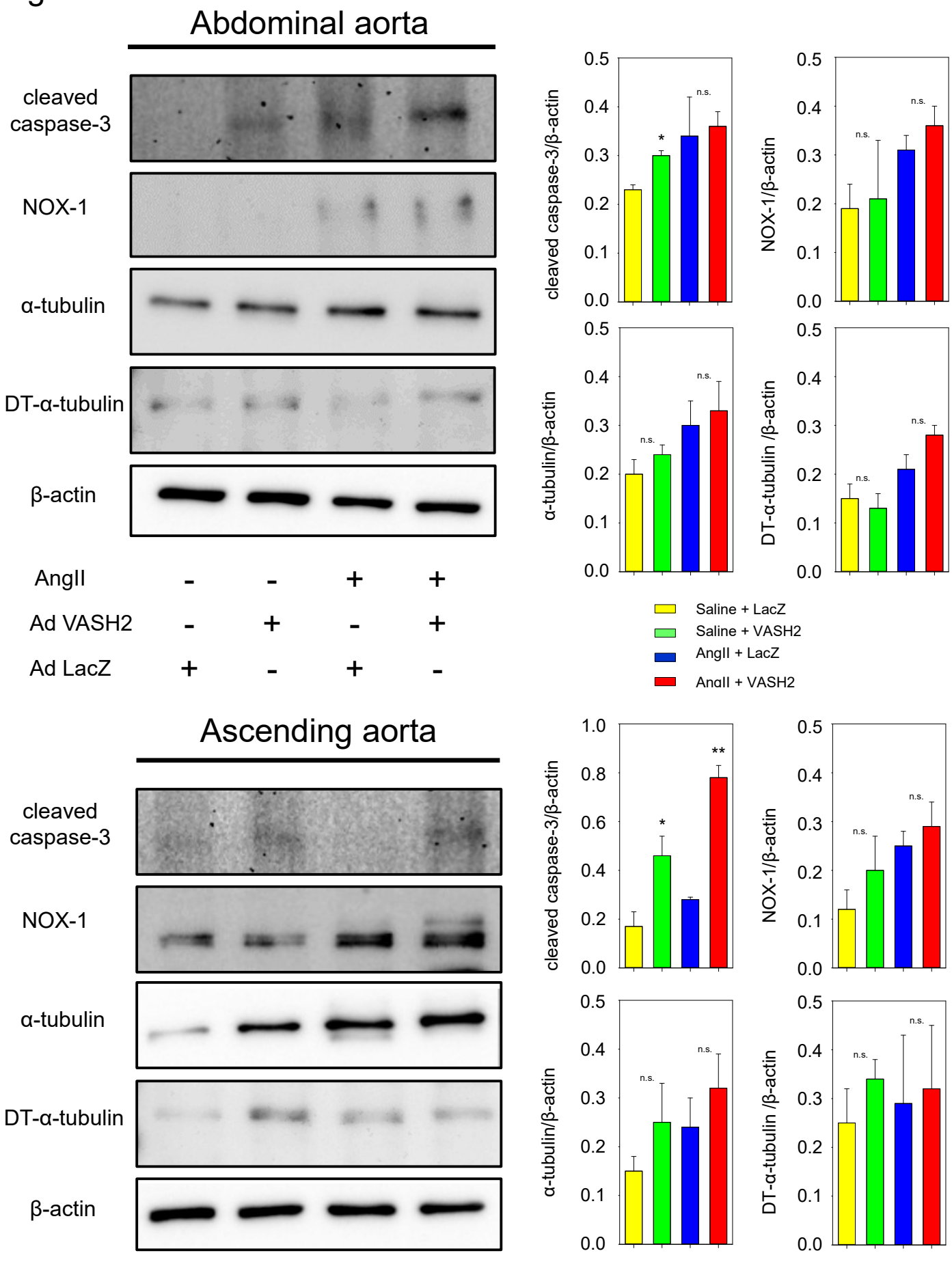
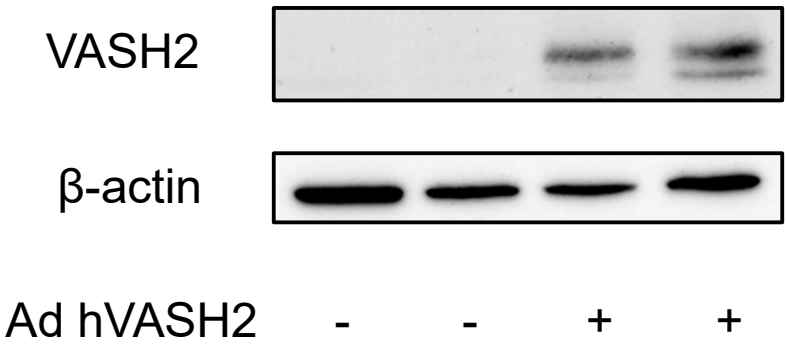

A)
AngII + LacZ
AngII + VASH2
B) [mm²]
Saline
Ang II
p<0.001
p=0.013
p<0.001
30
25
20
15
10
Saline+LacZ
Saline+VASH2
AngII+LacZ
AngII+VASH2
C) [mm]
Saline
Ang II
p<0.001
p=0.006
p<0.001
2.4
2.0
1.6
1.2
0.8
0.0
Saline+LacZ
Saline+VASH2
AngII+LacZ
AngII+VASH2
Saline + LacZ
Saline + VASH2
AngII + LacZ
AngII + VASH2
EVG
CD68
CD31
D)
E)
F)
G)
H)
I)
J)
K)
L)
M)
N)
O)

Figure 4


Abdominal aorta
cleaved caspase-3
NOX-1
α-tubulin
DT-α-tubulin
β-actin
AngII - - + +
Ad VASH2 - + - +
Ad LacZ + - + -
cleaved caspase-3/β-actin
NOX-1/β-actin
α-tubulin/β-actin
DT-α-tubulin /β-actin
n.s.
*
Saline + LacZ
Saline + VASH2
AngII + LacZ
AngII + VASH2
Ascending aorta
cleaved caspase-3
NOX-1
α-tubulin
DT-α-tubulin
β-actin
cleaved caspase-3/β-actin
NOX-1/β-actin
α-tubulin/β-actin
DT-α-tubulin /β-actin
n.s.
*
**

Supplement Figure 1 Confirmation of successful delivery of the VASH2 and LacZ gene in liver tissues. (A) Western blotting identified the presence of VASH2 protein bands only in mice infected with VASH2 groups. (B) Western blotting identified the presence of LacZ protein bands only in mice infected with LacZ groups.

A)



B)