## Supplemental Table I. Primers for real-time RT-PCR.

| Gene | F/R | Sequence | Product size (bp) | Annealing Temp. (°C) | Gene bank No. |
|---|---|---|---|---|---|
| Rabbit IL-1β | F | GGAGAGCTCTTTCCCACCAG | 138 | 58 | NM_001082201.1 |
| | R | TGGGTACCAAGGTTCTTTGAA | | | |
| Rabbit TNF-α | F | ATGGTCACCCTCAGATCAGC | 150 | 58 | NM_001082263.1 |
| | R | CTGGTTGTCCGTGAGCTTC | | | |
| Rabbit IL-6 | F | AGACGACCACGATCCACTTC | 145 | 58 | NM_001082064.2 |
| | R | CAGGATGGTGTGTTCTGACC | | | |
| Rabbit PAI-1 | F | ATTGTTCCGAACCACGGTCA | 104 | 60 | XM_002722823.1 |
| | R | CGCTGATCATGCCTTTCGTG | | | |
| Rabbit MMP-9 | F | CACGTACTTTGGAAACGCCG | 232 | 60 | NM_001082203.1 |
| | R | GCCCTGGAAGATGAACGGAA | | | |
| Rabbit Collagen Type I | F | CGGTGGTTACGACTTTGGTT | 137 | 58 | NM_001195668.1 |
| | R | TGAGGAGGGTCTCAATCTGG | | | |
| Rabbit Collagen Type III | F | TCAACCAGTACAAGTGACCAAC | 113 | 58 | XM_002712333.2 |
| | R | ATGTGTTTGGTGGAACAGCA | | | |
| Rabbit MCP-1 | F | AGCACCAAGTGTCCCAAAGA | 163 | 60 | NM_001082294.1 |
| | R | TGTGTTCTTGGGTTGTGGAA | | | |

**Supplemental Table II. List of antibodies used in the current study**

| Abs | Manufactures | Cat. No. | Dilution and concentrations |
|---|---|---|---|
| **Macrophage (RAM11)** (36.2 μg/ml) | Dako Co., Carpinteria, CA | M0633 | 1:400 (90.5 ng/ml) |
| **Muscle actin (HHF35)** (36.8 μg/ml) | Dako Co., Carpinteria, CA | M0635 | 1:300 (122.6 ng/ml) |
| **MMP-1** (500 μg/ml) | Daiichi Fine Chemical, Toyama, Japan | F-67 | 1:400 (1.25 μg/ml) |
| **MMP-2** (500 μg/ml) | Daiichi Fine Chemical, Toyama, Japan | F-68 | 1:200 (2.5 μg/ml) |
| **MMP-9** (500 μg/ml) | Daiichi Fine Chemical, Toyama, Japan | F-69 | 1:25 (20 μg/ml) |
| **MMP-12** (500 μg/ml) | R&D Systems Inc., Minneapolis, MN | mAb919 | 1:20 (25 μg /ml) |
| **Mouse IgG** (1.4 mg/ml) | Sigma-Aldrich Co. St. Louis, MO | i8765 | 1:20 (70 μg /ml) |

**Supplemental Table III. The plasma levels of TC, TG and HDL-C.**

| Experiment. 1 | | Vehicle (n=8) | Low (n=10) | High (n=9) |
|---|---|---|---|---|
| 0W | TC (mg/dL) | 940±130 | 839±111 | 1154±80 |
| | TG (mg/dL) | 228±47 | 262±88 | 310±81 |
| | HDL-C (mg/dL) | 7.1±0.48 | 8±1.12 | 8±0.33 |
| | | **Vehicle (n=8)** | **Low (n=5)** | **High (n=1)** |
| 4W | TC (mg/dL) | 825±131 | 868±274 | 1400 |
| | TG (mg/dL) | 265±64 | 379±166 | 308 |
| | HDL-C (mg/dL) | 7.8±0.64 | 8±1.1 | 7.8 |

| Experiment. 2 | | Vehicle (n=6) | Low (n=7) | High (n=7) |
|---|---|---|---|---|
| 0W | TC (mg/dL) | 1249±183 | 1172±74 | 1416±162 |
| | TG (mg/dL) | 298±81 | 183±48 | 362±128 |
| | HDL-C (mg/dL) | 8.47±0.74 | 7.7±1.20 | 8.87±0.59 |
| | | **Vehicle (n=6)** | **Low (n=6)** | **High (n=7)** |
| 4W | TC (mg/dL) | 975±187 | 999±89 | 1057±202 |
| | TG (mg/dL) | 185±47 | 243±58 | 257±159 |
| | HDL-C (mg/dL) | 6.25±0.79 | 7.25±1.60 | 6.85±1.01 |
| | | **Vehicle (n=6)** | **Low (n=5)** | **High (n=2)** |
| 8W | TC (mg/dL) | 1039±53 | 1005±118 | 1035±134 |
| | TG (mg/dL) | 198±30 | 227±60 | 196±16 |
| | HDL-C (mg/dL) | 7.78±0.75 | 8.1±2.42 | 7.9±0.07 |

Values are mean ± SD. TC, Total cholesterol; TG, triglycerides; HDL-C, High-density lipoprotein cholesterol. We compared plasma lipids at different time points (0, 4, and 8 weeks) using one way ANOVA with post-hoc Tukey test. In the experiment 1, comparison was only made between the low group and vehicle group using Student's t-test because the number of high group was 1 (not enough for comparison). No statistical significance was found between Ang II groups and the vehicle group.

**Supplemental Table IV. The number of coronary erosions, rupture and thrombosis in each block of experiment 2.**

**Erosions**

| | Block I | Block II | Block III | Block IV | Block V |
|---|---|---|---|---|---|
| **Vehicle** | 0.333 ± 0.5 | 0.33 ± 0.52 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=6)** | (2/6) | (2/6) | (0/6) | (0/6) | (0/6) |
| **Ang II-L** | 1.142 ± 0.69 | 1.0 ± 0.58 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (6/7) | (6/7) | (0/7) | (0/7) | (0/7) |
| **Ang II-H** | 0.71 ± 0.76 | 0.57 ± 0.53 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (4/7) | (4/7) | (0/7) | (0/7) | (0/7) |

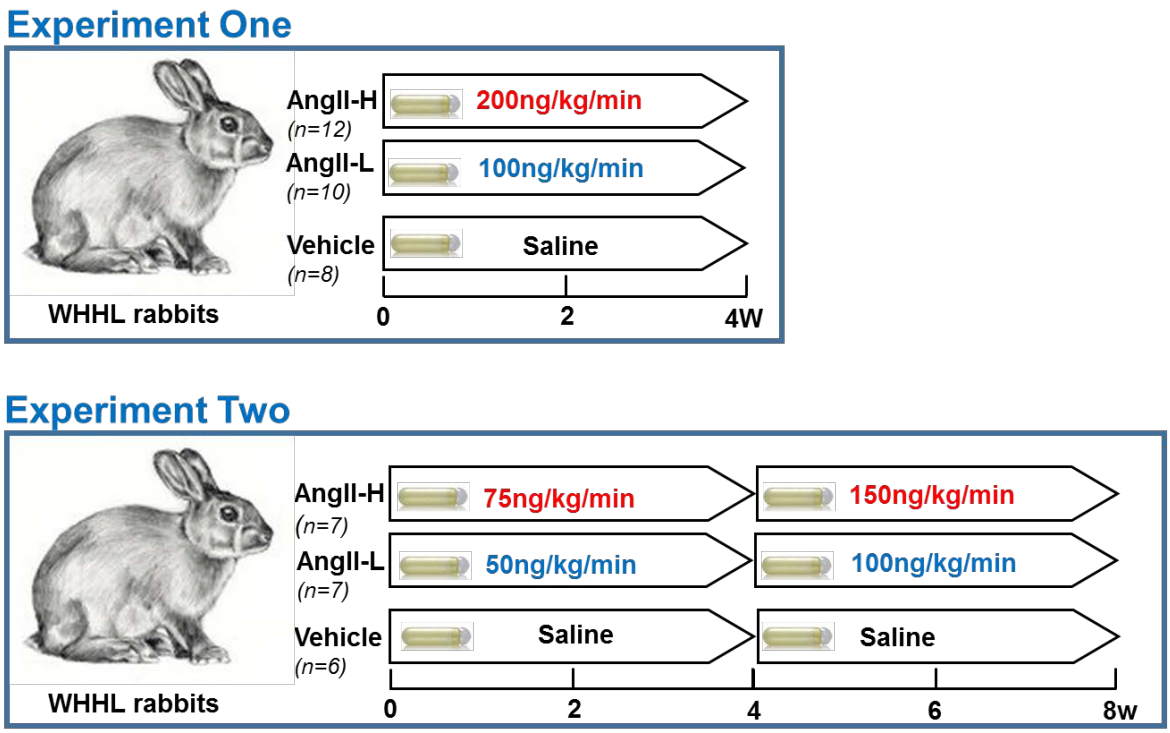
**Ruptures**

| | Block I | Block II | Block III | Block IV | Block V |
|---|---|---|---|---|---|
| **Vehicle** | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=6)** | (0/6) | (0/6) | (0/6) | (0/6) | (0/6) |
| **Ang II-L** | 0.428 ± 0.79 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (2/7) | (0/7) | (0/7) | (0/7) | (0/7) |
| **Ang II-H** | 0.71 ± 0.49* | 0.14 ± 0.38 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (5/7) | (1/7) | (0/7) | (0/7) | (0/7) |

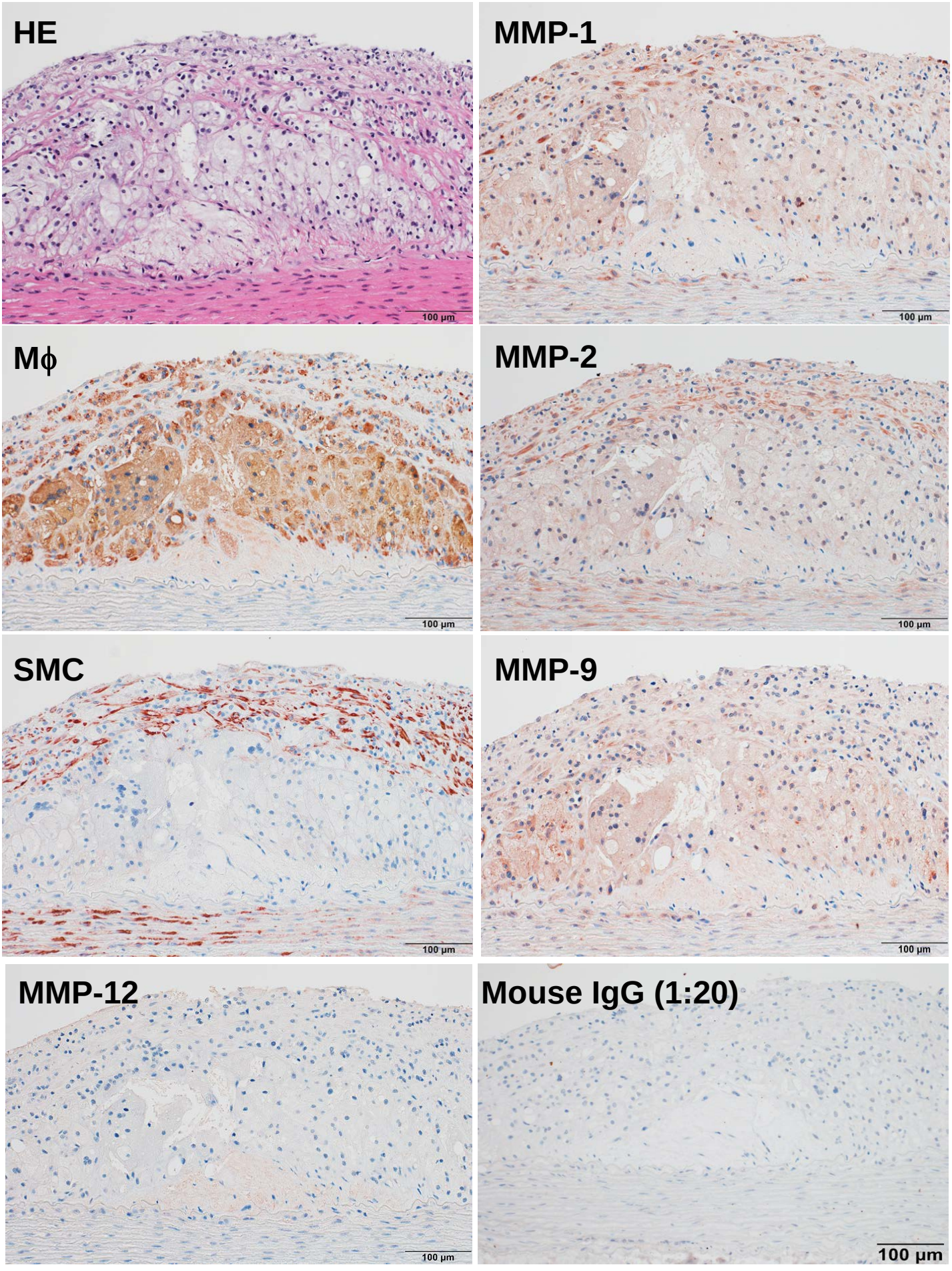
**Erosions/rupture-associated thrombosis**

| | Block I | Block II | Block III | Block IV | Block V |
|---|---|---|---|---|---|
| **Vehicle** | 0 ± 0 | 0.17 ± 0.41 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=6)** | (0/6) | (1/6) | (0/6) | (0/6) | (0/6) |
| **Ang II-L** | 1.0 ± 1.0 | 1.0 ± 0.58* | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (4/7) | (6/7) | (0/7) | (0/7) | (0/7) |
| **Ang II-H** | 1.14 ± 0.69** | 0.71 ± 0.49 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **(n=7)** | (6/7) | (5/7) | (0/7) | (0/7) | (0/7) |

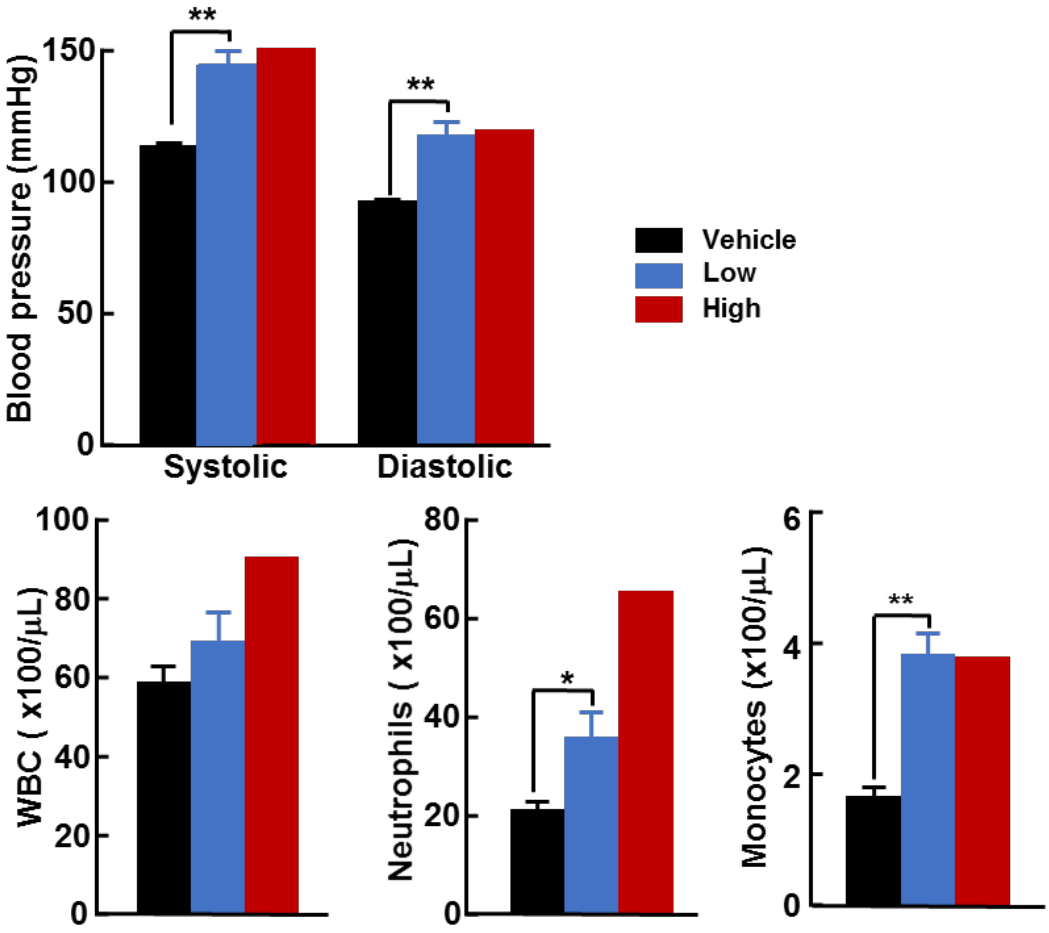
The number of each type of lesions was calculated from all sections of each block. The largest number on the section was used to represent each block. The data are expressed as the mean ± SD. *P<0.05, **P<0.01 vs vehicle group using the Mann-Whitney U test. Parentheses indicate the ratio of animals with the lesions to total animal number. Thrombosis includes both partial and occluded thrombi.



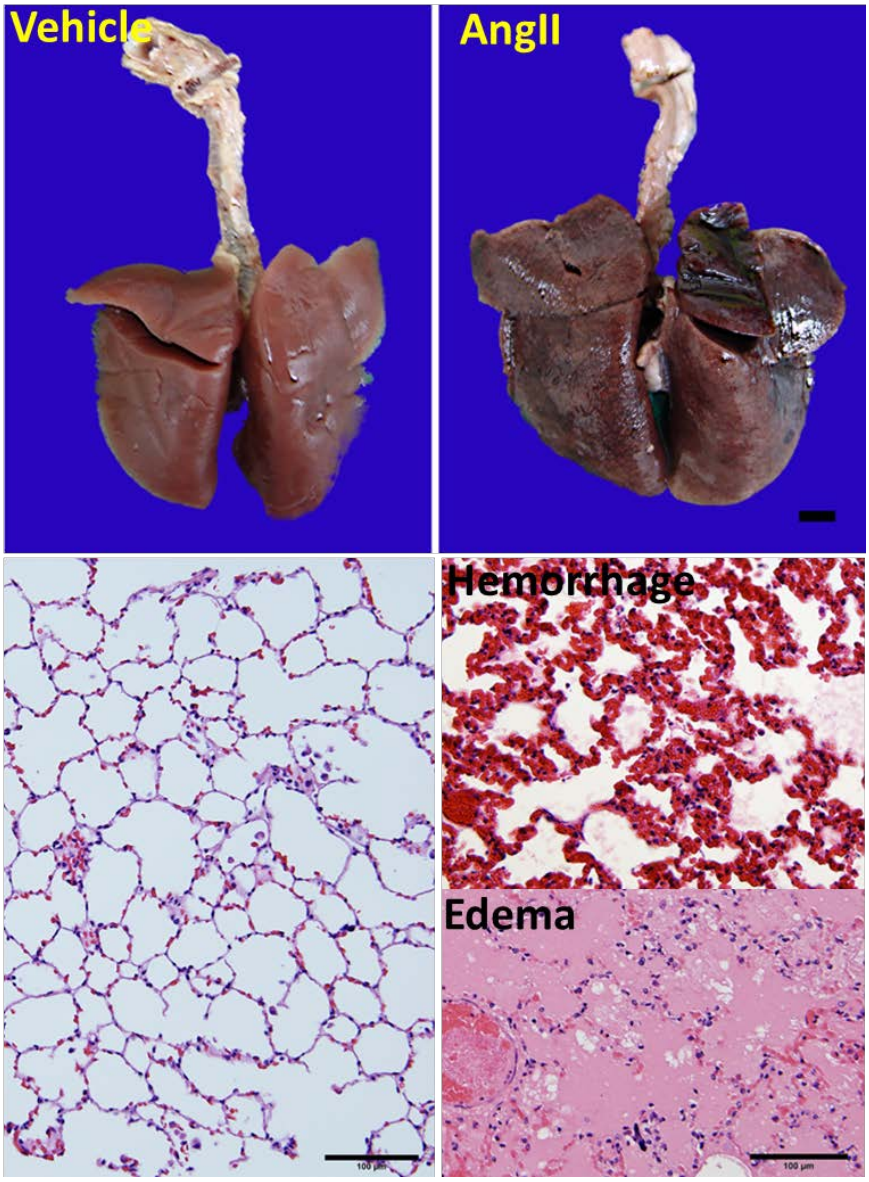

**Supplemental figure I. Schematic diagram showing the experimental design.** Two experiments were designed to illustrate the effects of Ang II infusion administered differently: acute increase of Ang II in circulation by a single high-dose infusion (upper panel) and escalation increase of Ang II in circulation by two different doses (from low- to high-dose infusion) (lower panel).


HE
MMP-1
Mϕ
MMP-2
SMC
MMP-9
MMP-12
Mouse IgG (1:20)
100 μm

**Supplemental figure II. Immunohistochemical staining specificity of each antibody used for the current study.**
Serial sections of WHHL rabbit aorta were immunohistochemically stained with antibodies against rabbit macrophage (Mϕ), smooth muscle cells (SMC), MMP-1, -2, -9, -12 **(See S-Table II)** along with non-specific mouse IgG as a negative control staining (bottom right).



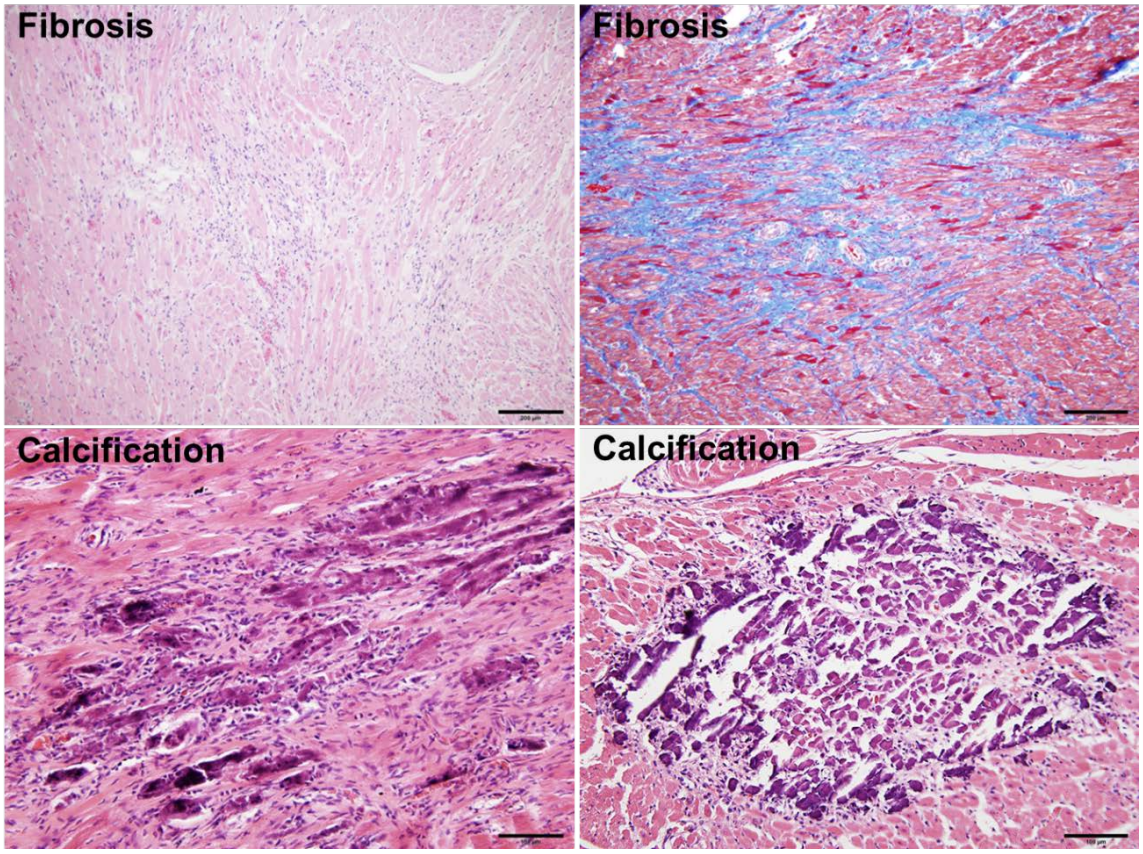

**Supplemental figure III. Blood pressure and blood leukocytes at 4 weeks.**
Blood pressure (upper) and blood leukocyte count (lower panel) were measured at 4 weeks after Ang II infusion. Data are expressed as mean ± SEM. * *P*<0.05 or ** *P*<0.01 versus the vehicle. n=7 for the vehicle, n=5 for low-Ang II and n=1 for high-Ang II groups.



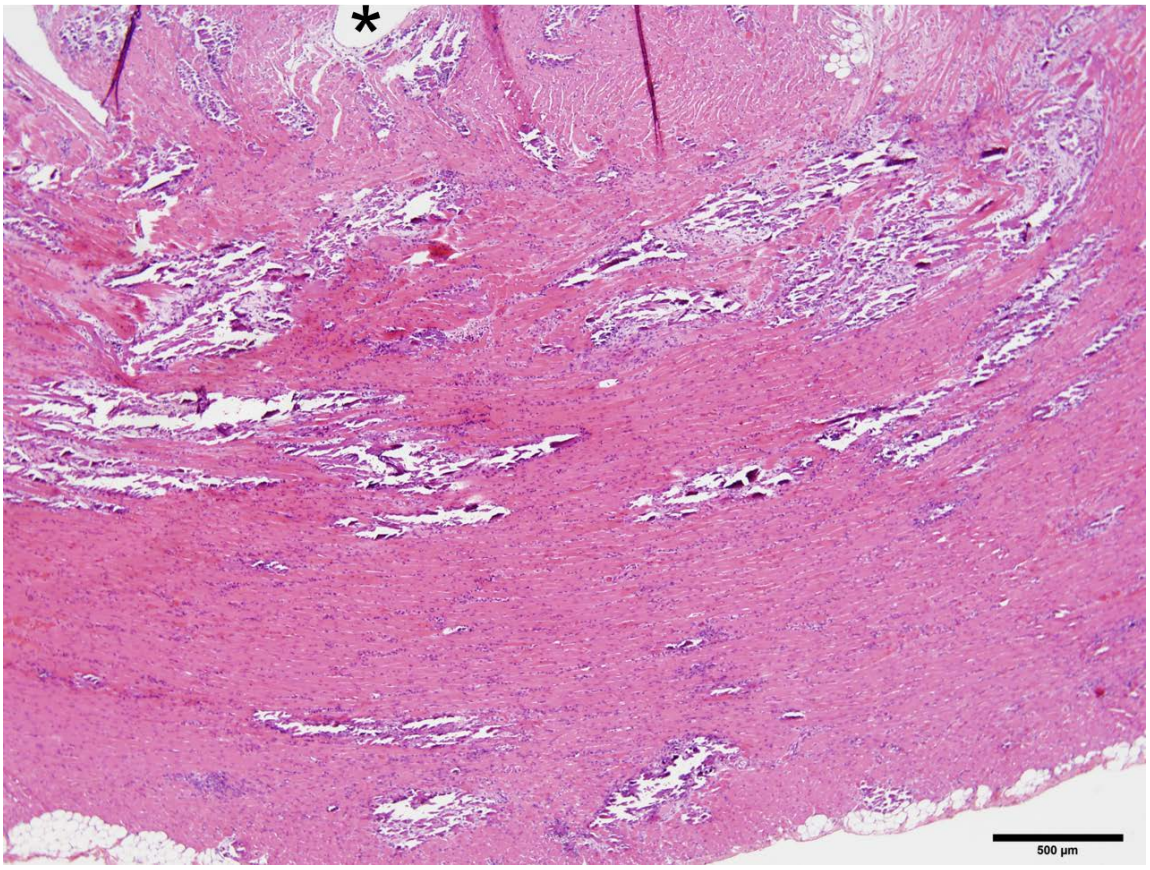

**Supplemental figure IV. Pulmonary pathological changes in Ang II-infused rabbits.**

Representative photographs of rabbit lungs of the vehicle group (left) and Ang II group (right) are shown. The lungs of a rabbit that died at 10 days after Ang II infusion showed pulmonary congestion (upper right). Microscopically, alveolar space was filled with exudate fluid and blood cells (lower right) compared with normal alveoli (lower left). Paraffin sections were stained with HE staining.

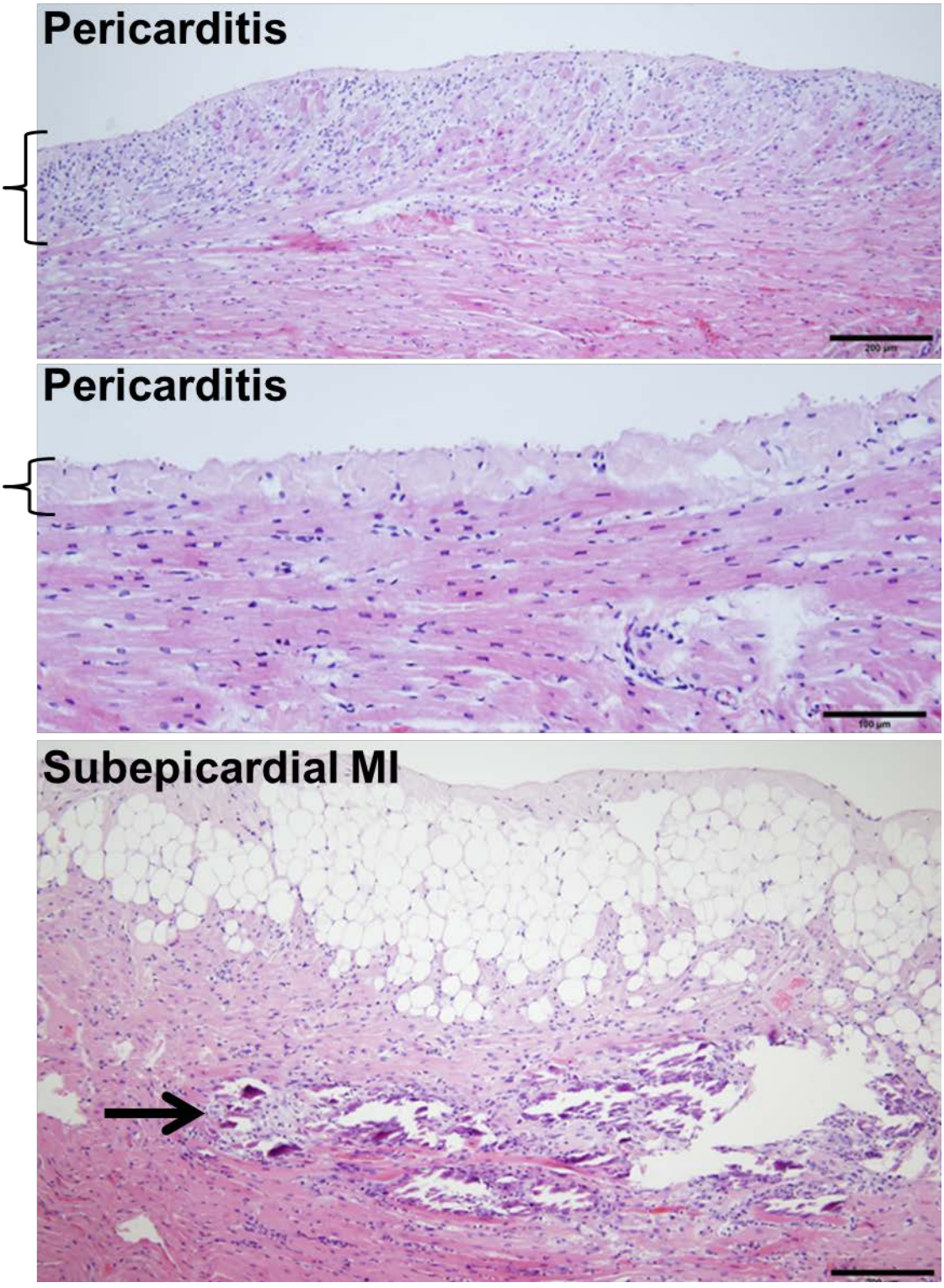
**A.**




**B.**



C.

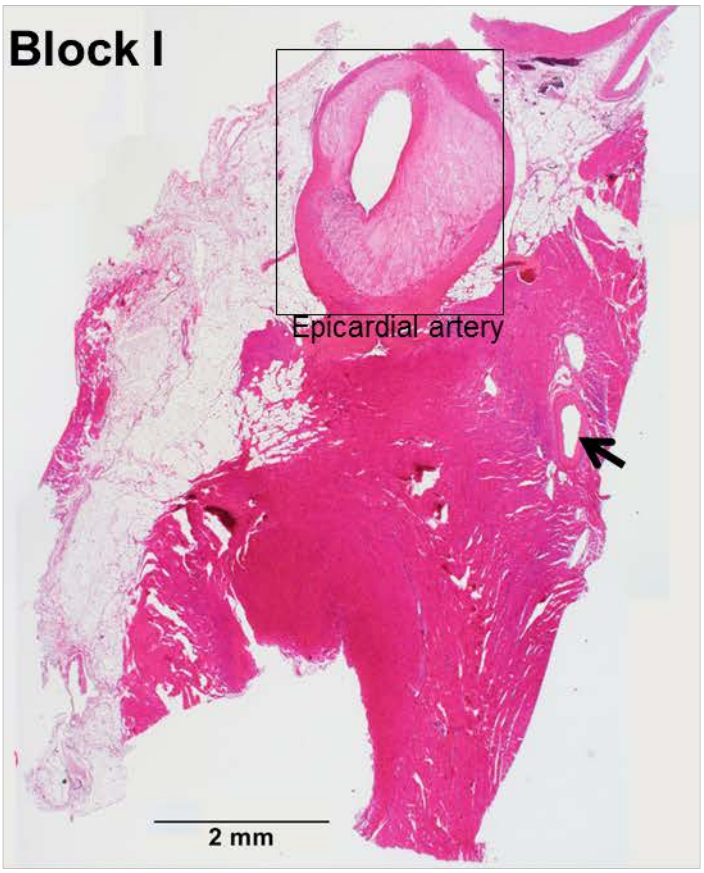


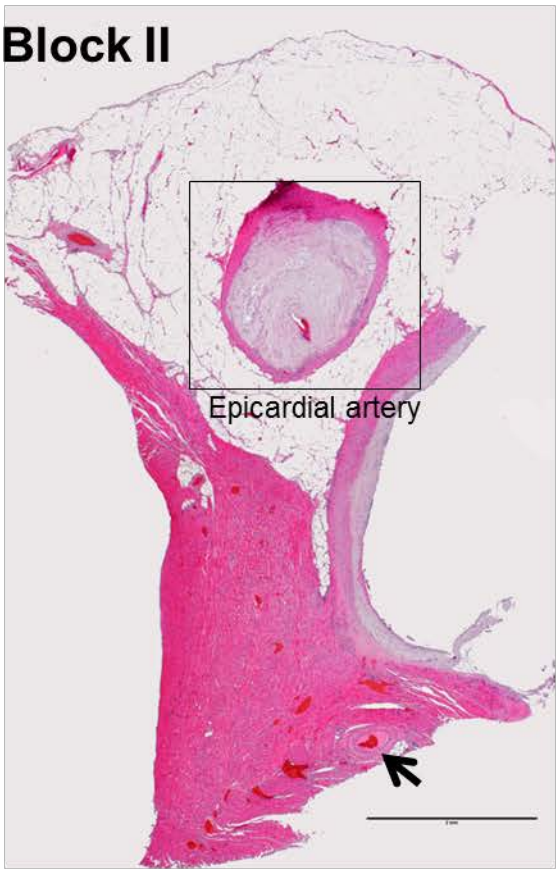
**Supplemental figure V. Micrographs of myocardial infarction in Ang II-infused WHHL rabbits.**

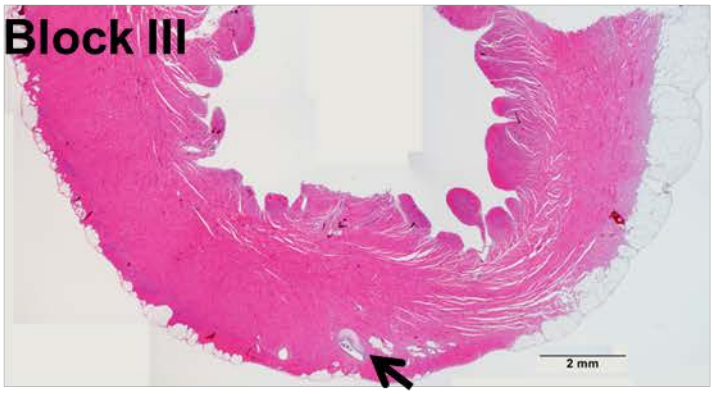
**A.** Necrotic cardiac myocytes were partly replaced by fibrosis (upper panels, HE and Masson's trichrome staining) and calcification (lower panels, HE staining), suggesting that healing process after myocardial infarction. Fibrosis (stained as blue) is more distinctive when the specimens are stained with Masson's trichrome staining.

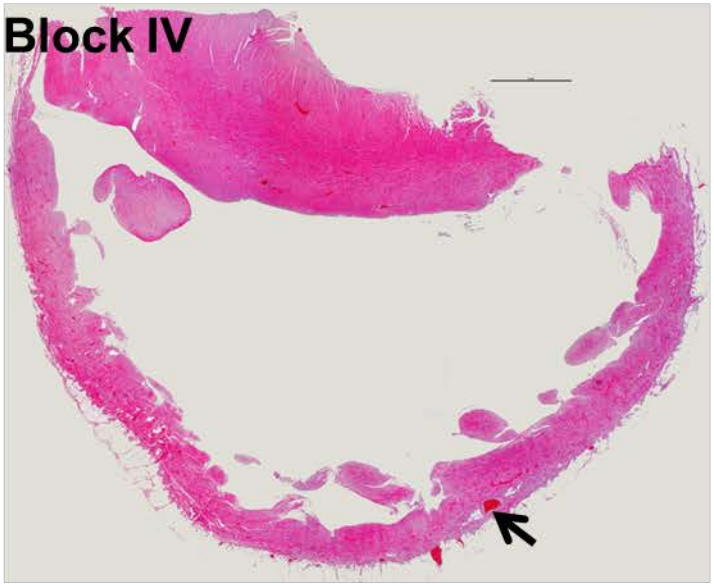
**B.** Representative micrograph of transmural myocardial infarction (HE staining). The whole layer of the left ventricle wall of a dead WHHL rabbit of the Ang II group showed diffuse myocardial infarction and many necrotic foci (an asterisk indicates the ventricle cavity).

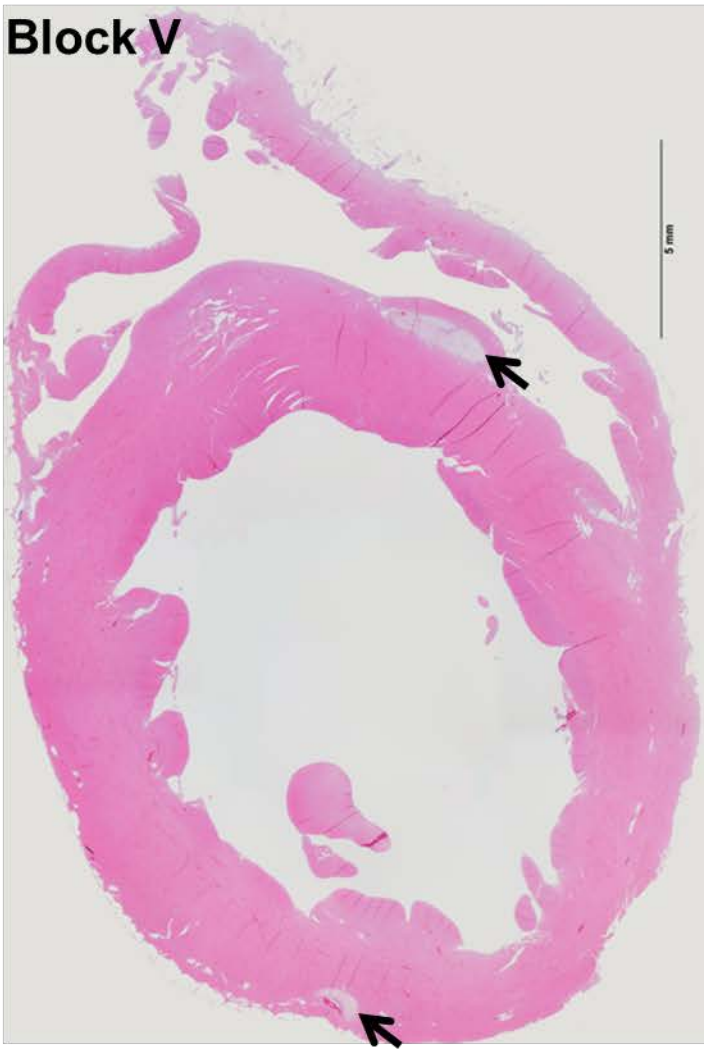
**C.** Pathological features of pericarditis and subepicardial infarction. In some areas, pericardium was either infiltrated by chronic inflammatory cells (top, arrow head) or the presence of fibrinous exudative stained pink (middle, arrow head). Beneath the subepicardial area, there are foci of necrosis (indicated by an arrow), indicating the presence of subepicardial infarction (bottom).


Block I
Epicardial artery
2 mm


Block II
Epicardial artery


Block III
2 mm


Block IV


Block V
5 mm

**Supplemental figure VI. Representative micrographs of 5 blocks of the WHHL rabbit heart dissected for the analysis (HE staining).**

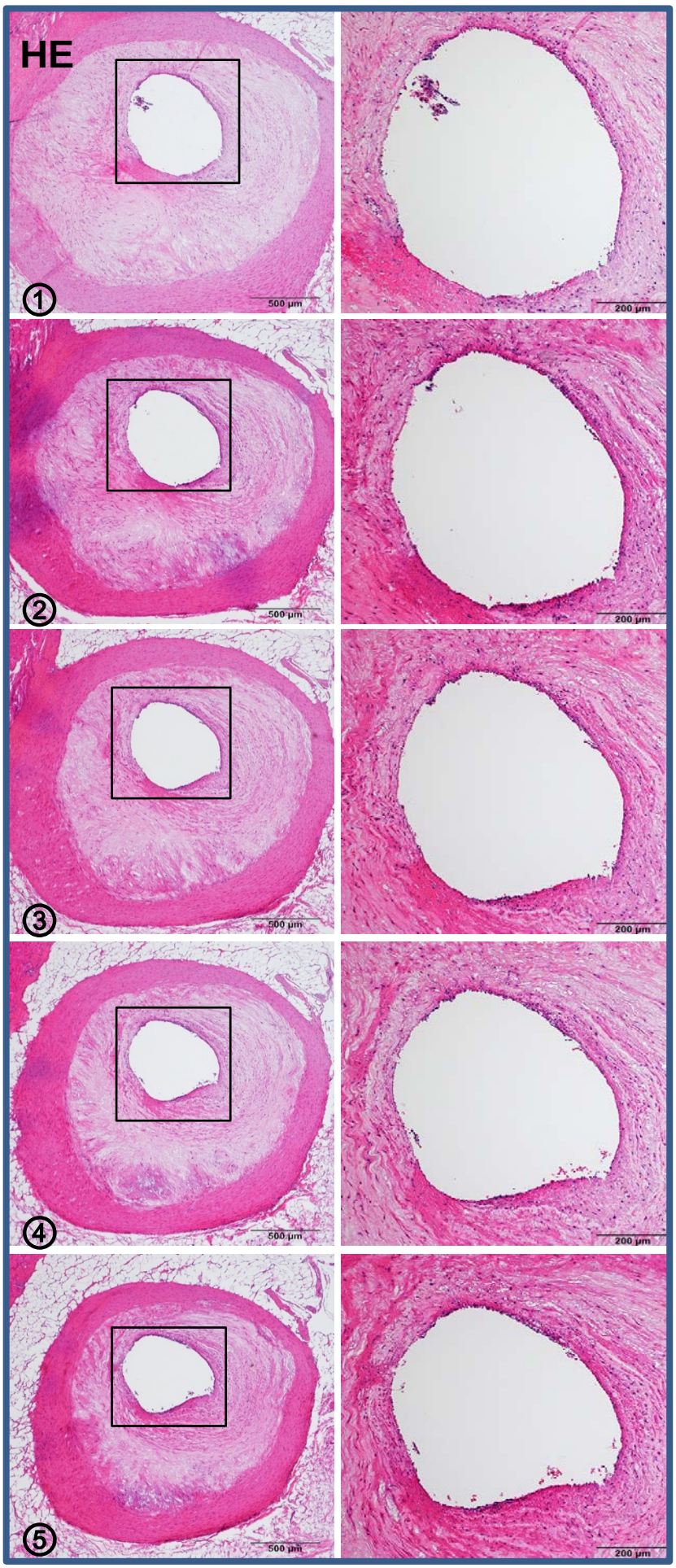
Block I usually contains one large left coronary main trunk (highlighted by a square) located in the epicardium and several small arteries and arterioles (indicated by arrowheads) in the heart muscle (interventricular septum). The diameter of the epicardial arteries is arranged from 1.0 to 3.0 mm (n=10). Small arteries and arterioles are ~0.8 mm in diameter (arterioles less than 300 μm are not calculated in the current study because they do not have any lesions). In general, 1-2 arteries have atherosclerosis in epicardial arteries or small arteries and arterioles in this block.

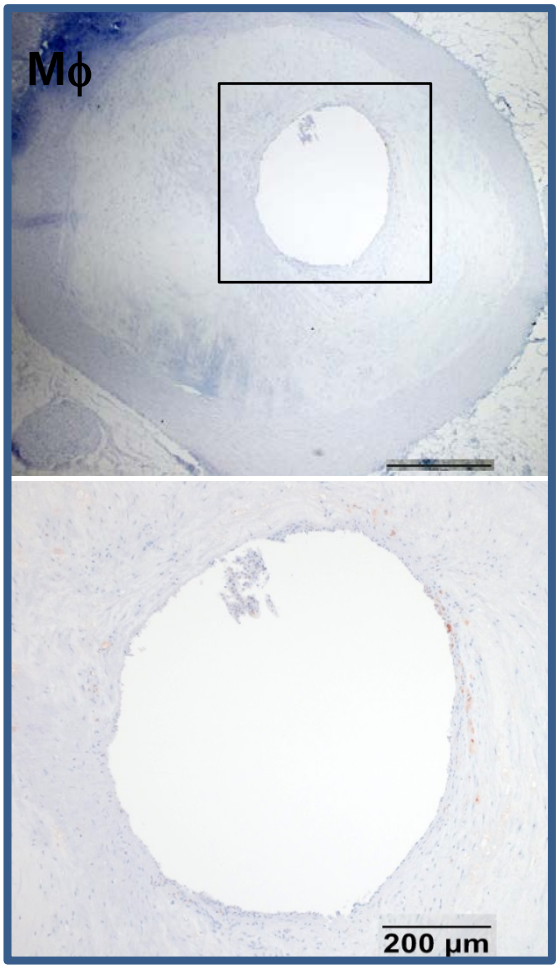
Similar to the block I, block II also contains one large right coronary main trunk located in the epicardium with several small arteries and arterioles (indicated by arrowheads) in the heart muscle (interventricular septum). The diameter of the epicardial arteries is up to 2.0 mm. In general, 1-2 arteries have atherosclerosis in epicardial arteries or small arteries and arterioles in this block.
.
Block III is a part of the left ventricle. The number of small arteries and arterioles are varying from 4 to 9 but the number of the arteries with lesions is 0~2.

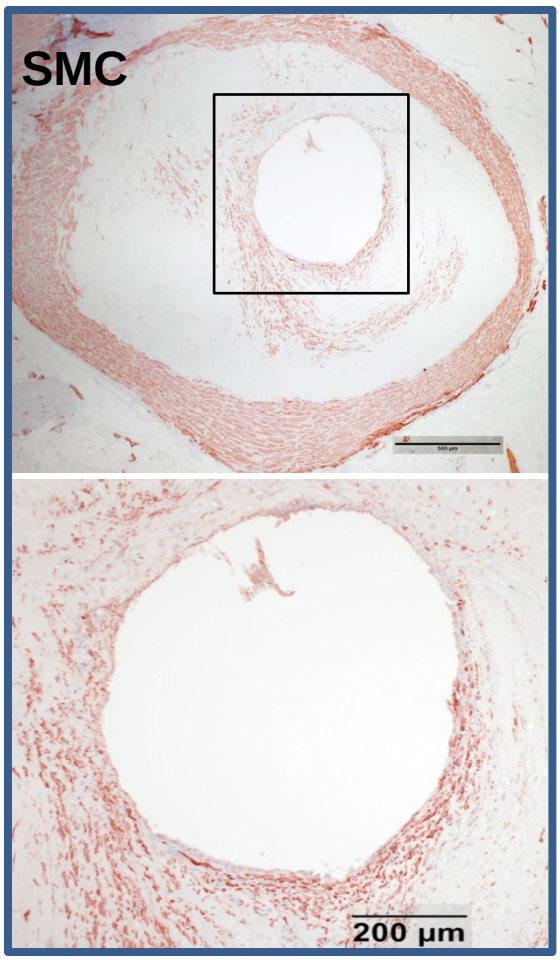
Block IV contains the right ventricle and part of the interventricular septum. The number of small arteries and arterioles are varying from 4 to 9 but the number of the arteries with lesions is 0~2.

Block V shows both left and right ventricle. The number of small arteries and arterioles are varying from 6 to 18 but the number of the arteries with lesions is 0~3. Arteriolosclerosis can be seen in both the septum and left ventricle but rarely seen in the right ventricle.
Arrowheads indicate small arteries.

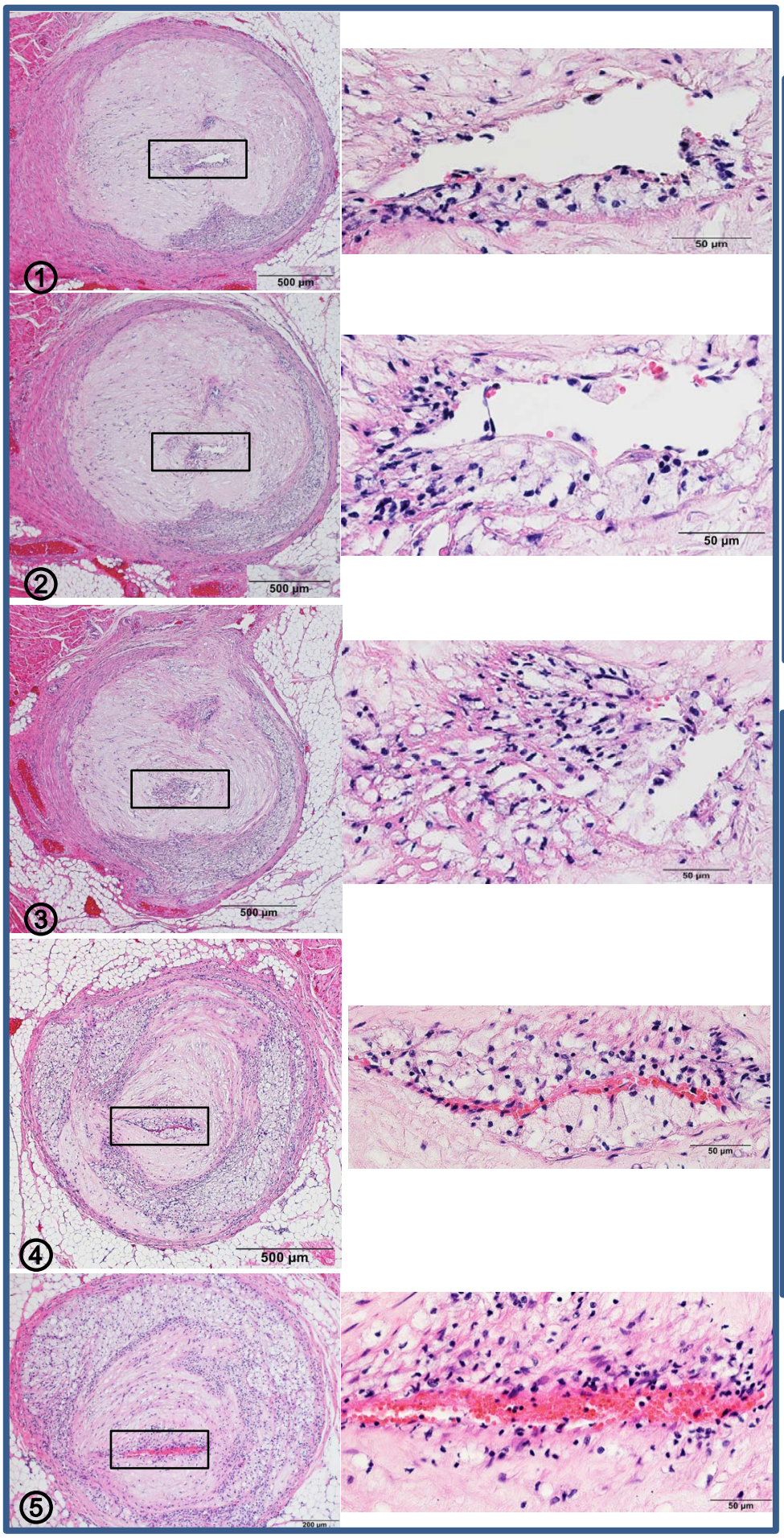
A.

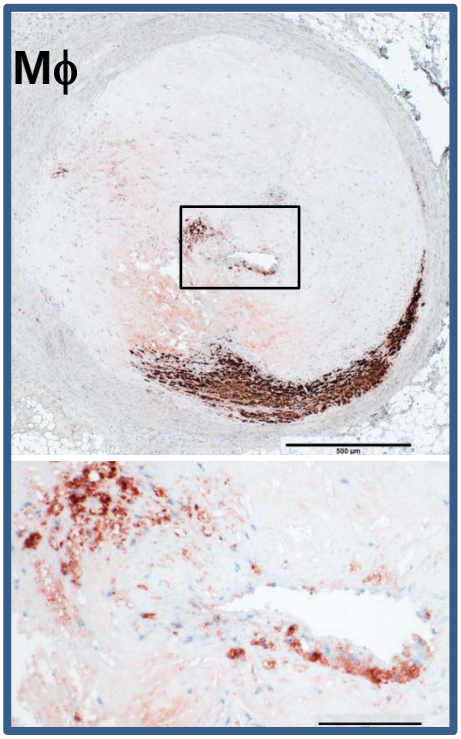


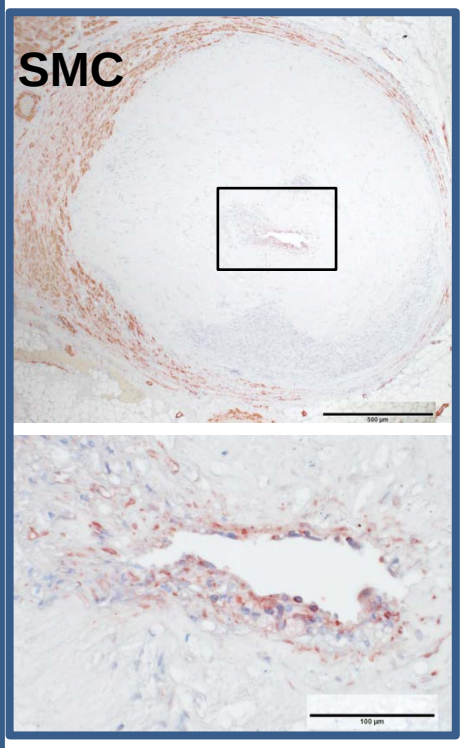



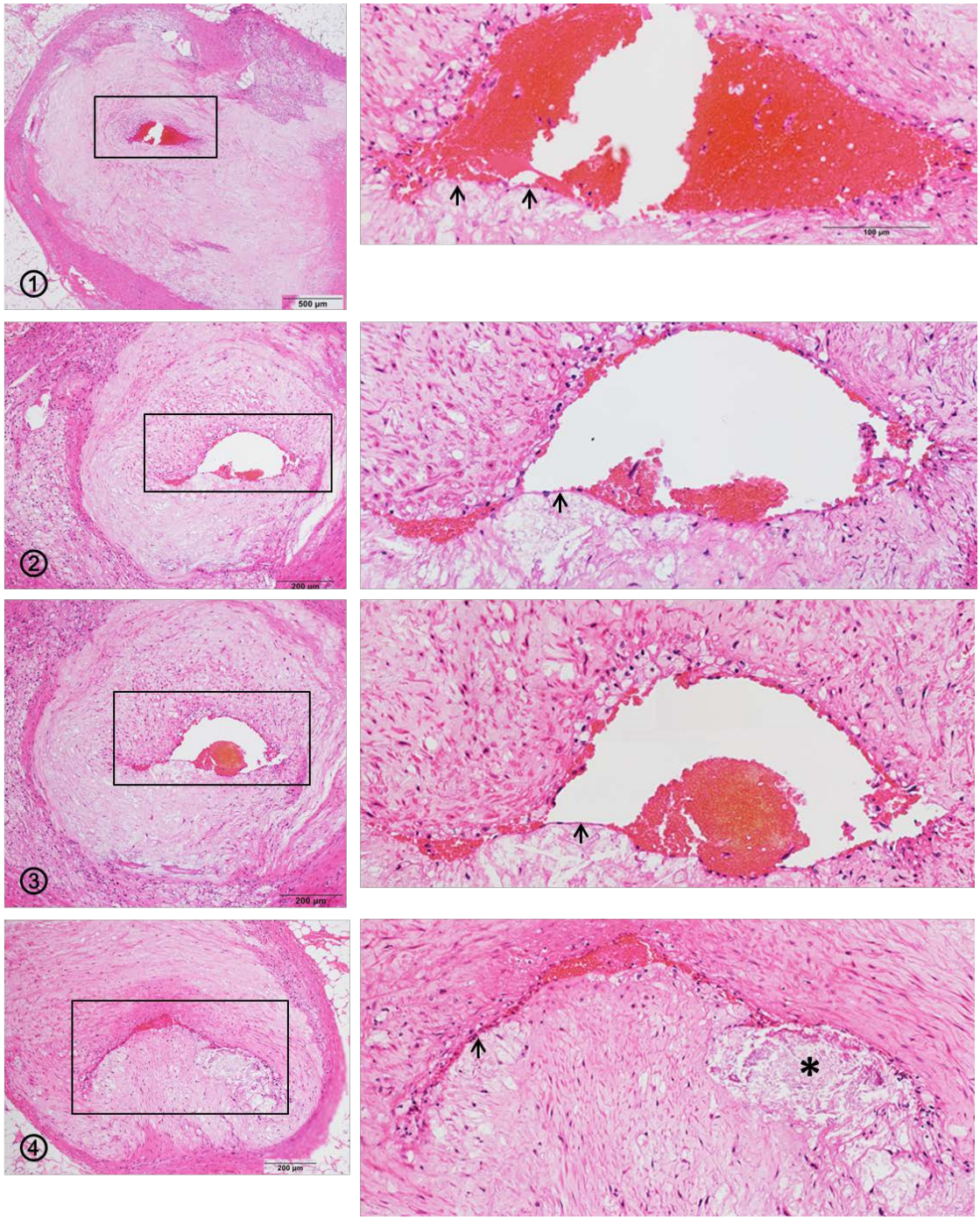
B.


①
500 μm
50 μm
②
500 μm
50 μm
③
500 μm
50 μm
④
500 μm
50 μm
⑤
200 μm
50 μm


Mϕ


SMC

C.

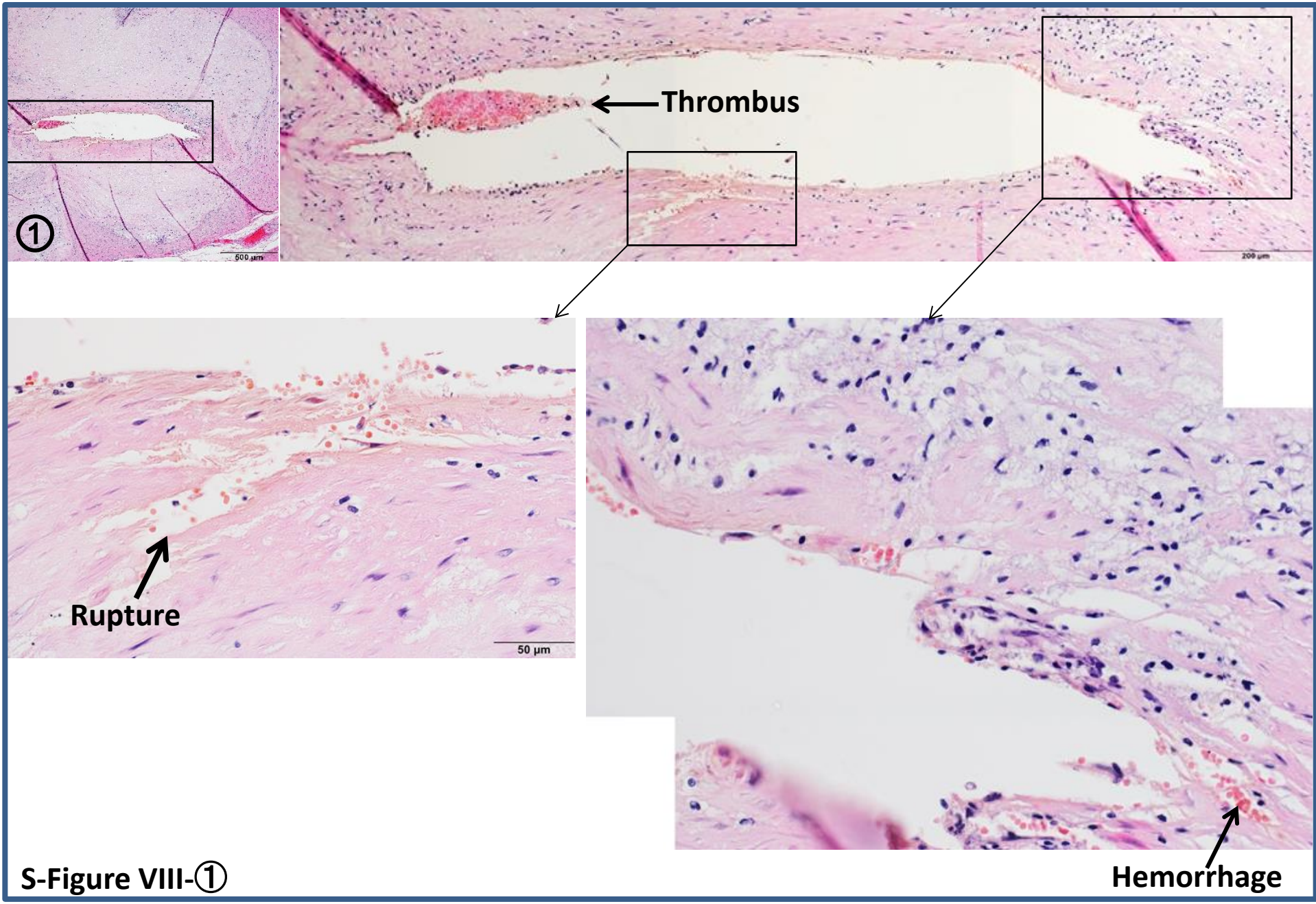


**Supplemental figure VII. Representative micrographs of coronary atherosclerotic lesions showing either various histological features in WHHL rabbits.**

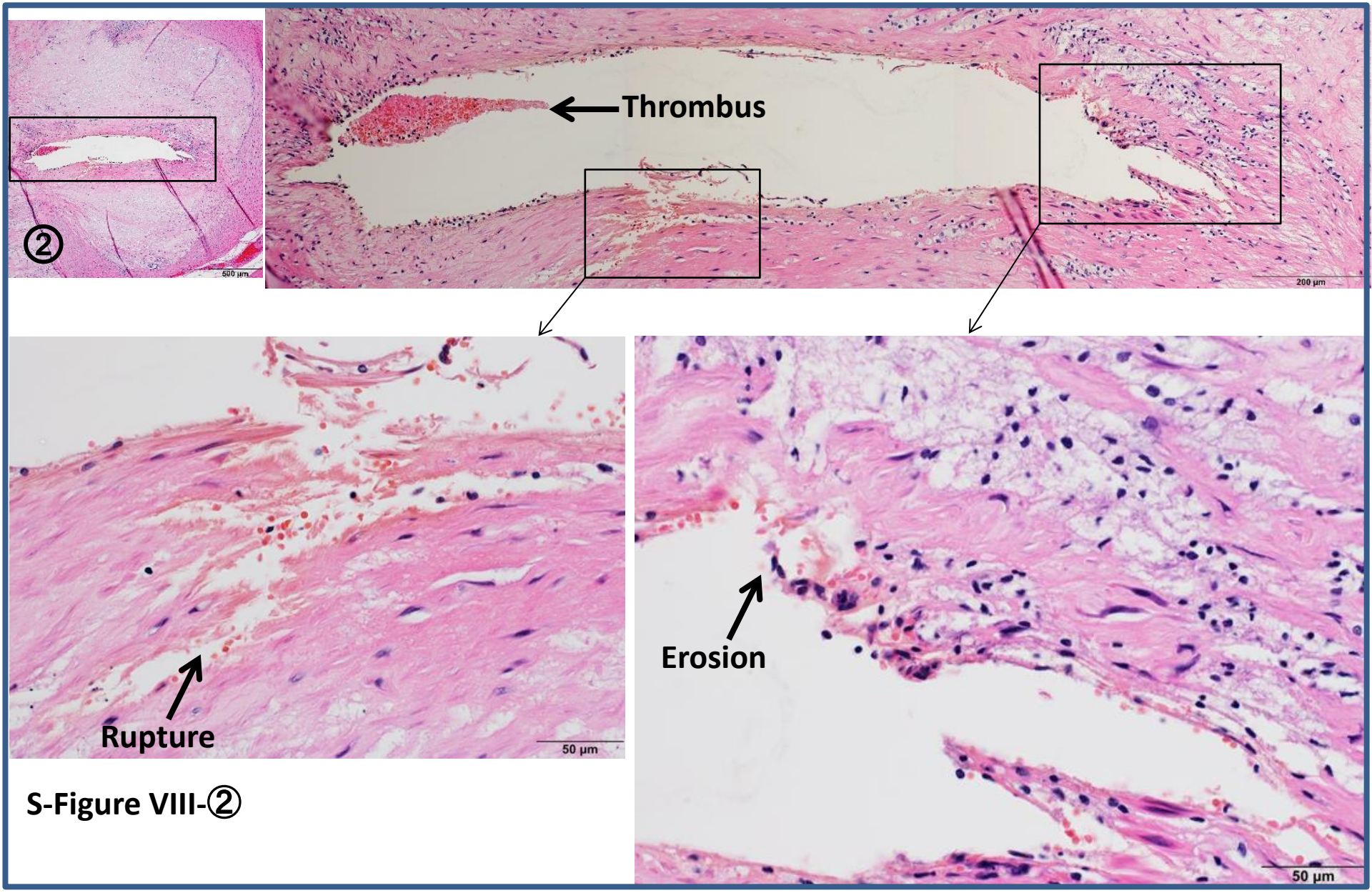
**A.** Step sections (the distance from coronary artery orifice to each section: ①orifice, ②50μm, ③100μm, ④150μm, ⑤200μm) of “stable” lesions show that the lesions contain few macrophage-derived foam cells and almost totally consist of fibrotic tissue with smooth muscle cells sparsely. Immunohistochemical staining using Abs against either macrophages (Mϕ) or smooth muscle cells (SMC) was performed using the serial sections of No. ① and is shown on the right.

**B.** Step sections (the distance from coronary artery orifice to each section: ①50μm, ②100μm, ③150μm, ④300μm, ⑤400μm) of “unstable” lesions reveal that the lesions show not only severe stenosis but also accumulation of foam cells in both lumen and peripheral area (beneath the adventitia). The surface foam cells seems to be exposed to the lumen (①~③), which is filled with a blood clot (④~⑤). Immunohistochemical staining using Abs against either macrophages (Mϕ) or smooth muscle cells (SMC) was performed using the serial sections of No. ② and shown on the right.

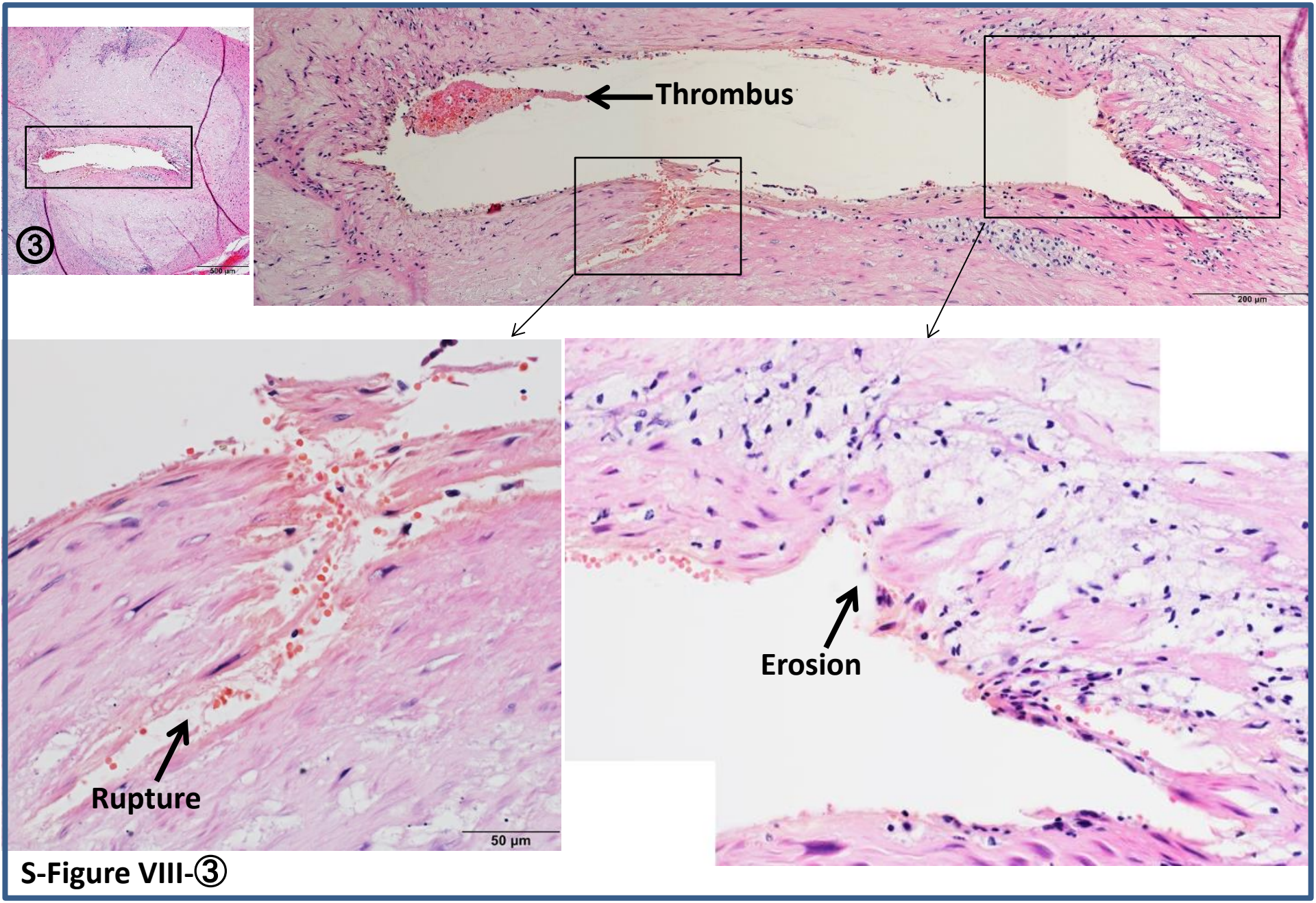
**C.** Step sections (the distance from coronary artery orifice to each section: ①orifice, ②350μm, ③400μm, ④900μm) of another “unstable” lesions contain a thin cap (arrowheads, ①~④) and calcification (asterisk, ④). A blood clot is either attached to the surface of the lesions or filled with the lumen.



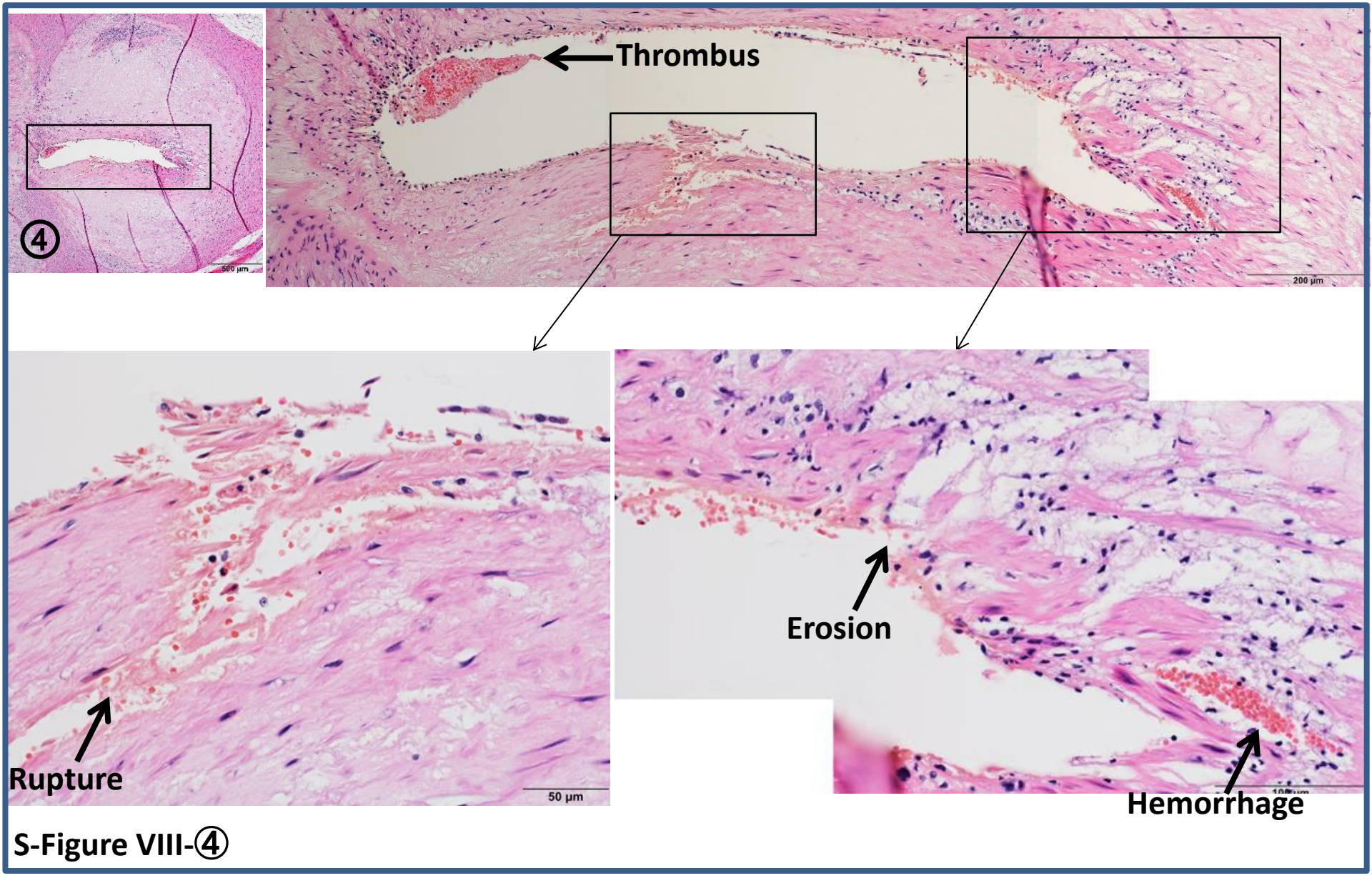

S-Figure VIII-①



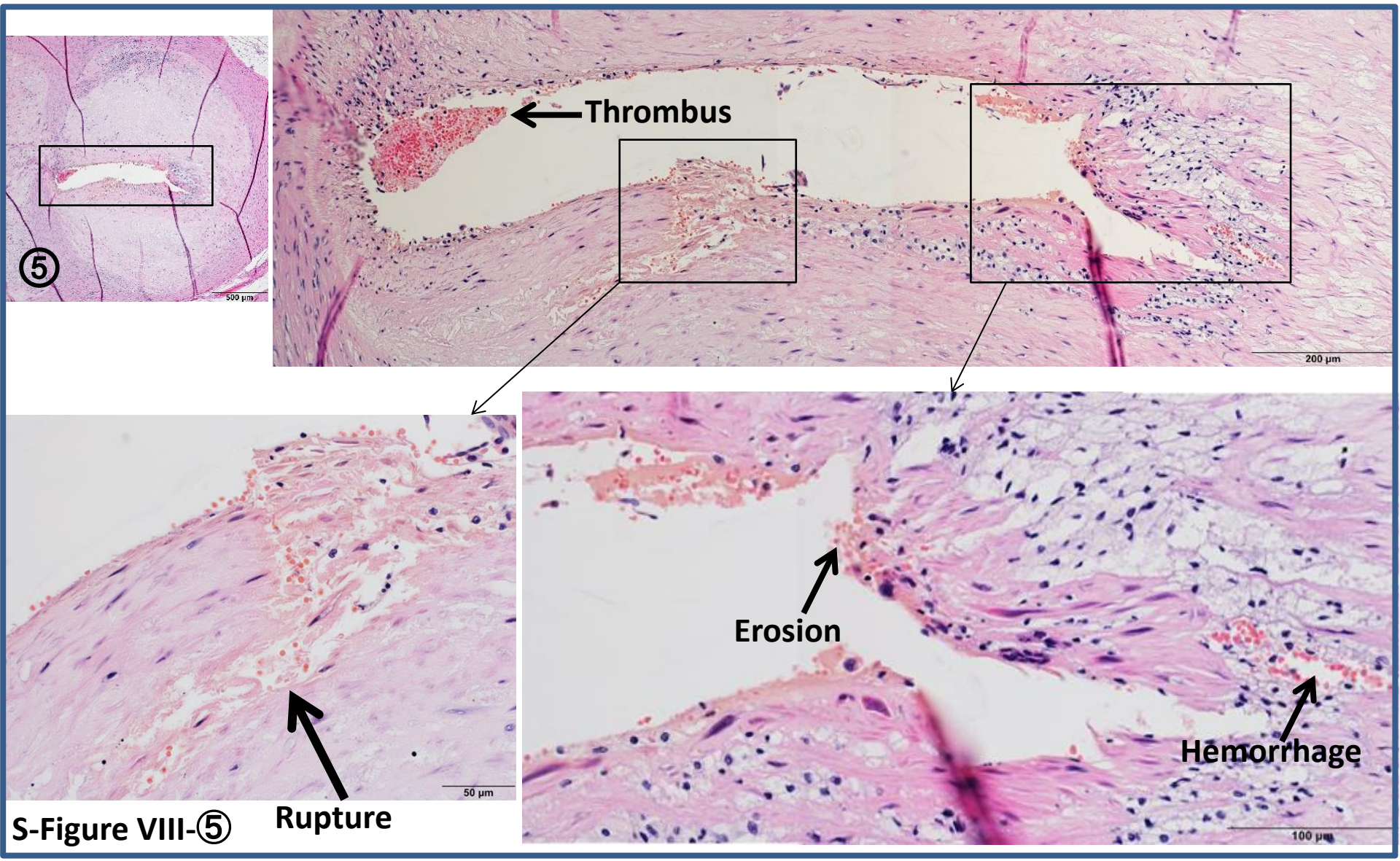

S-Figure VIII-②



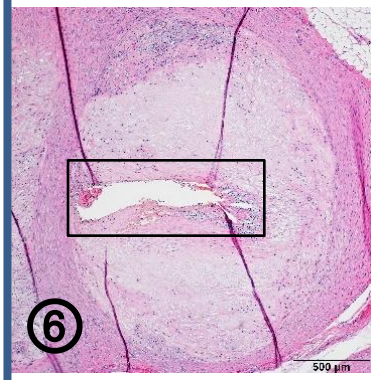

S-Figure VIII-③

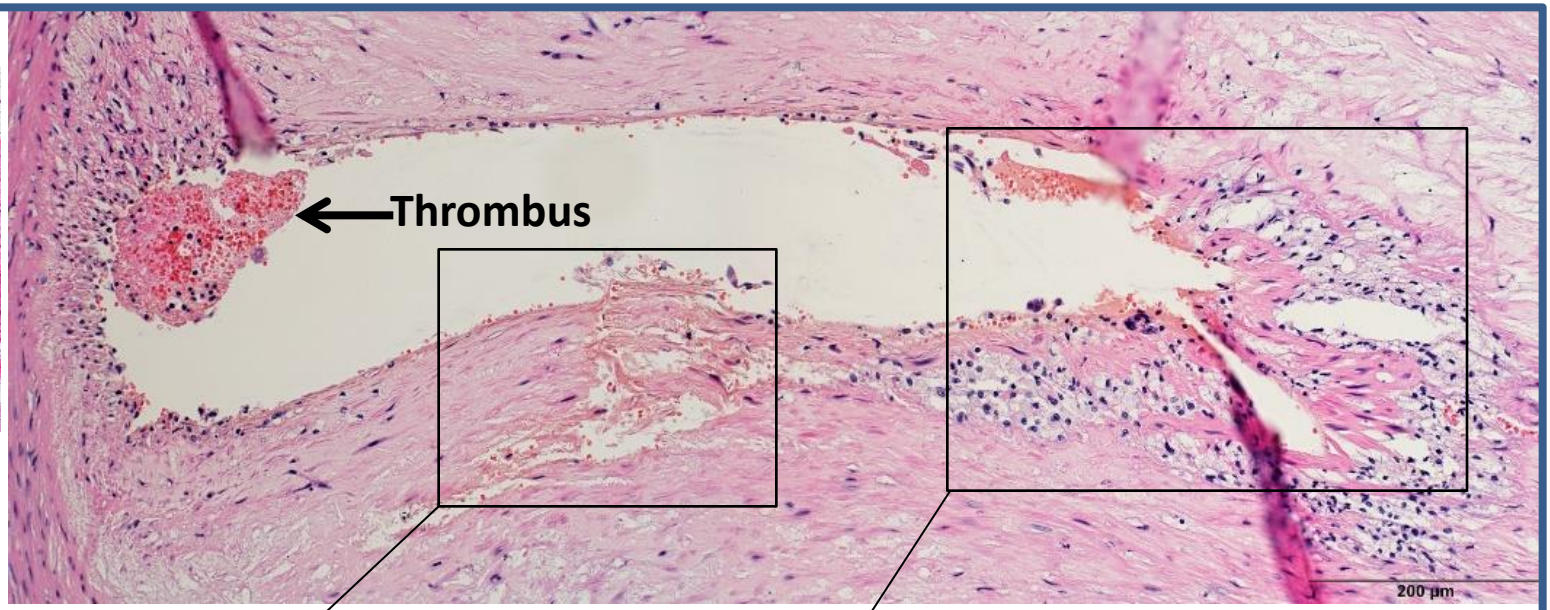


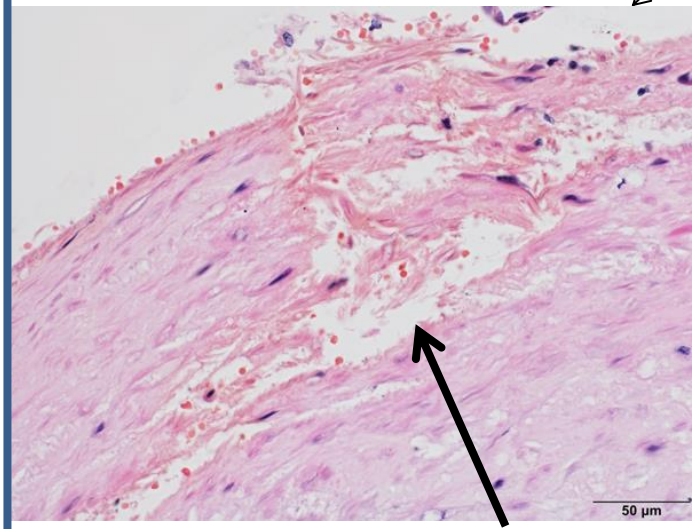

S-Figure VIII-④

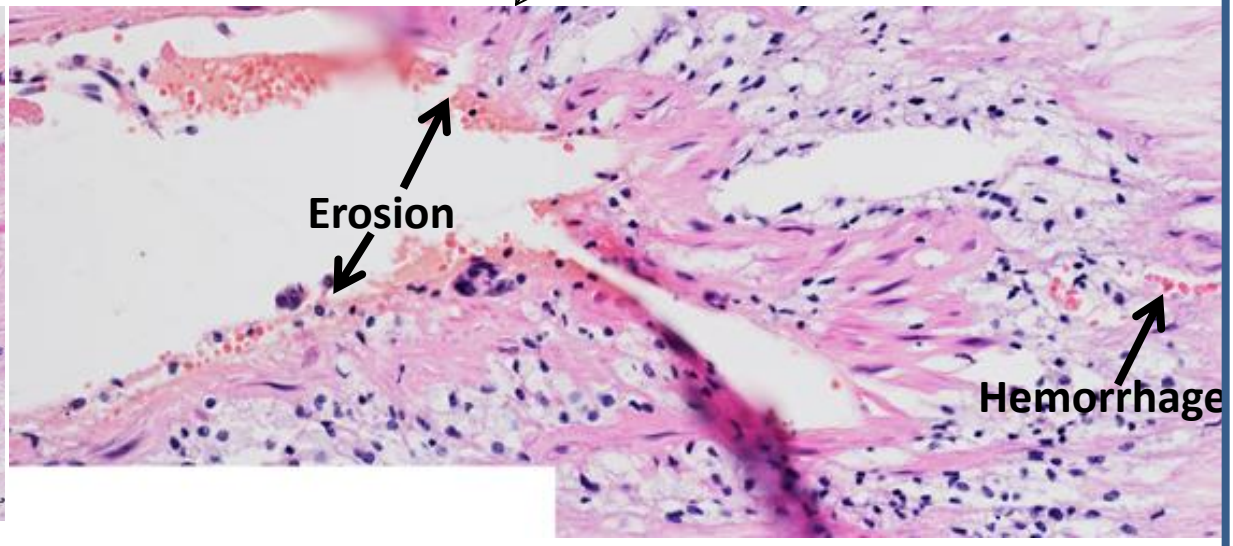



S-Figure VIII-⑤



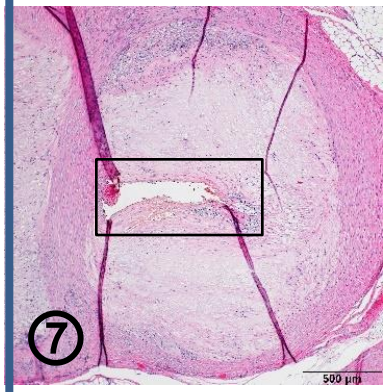



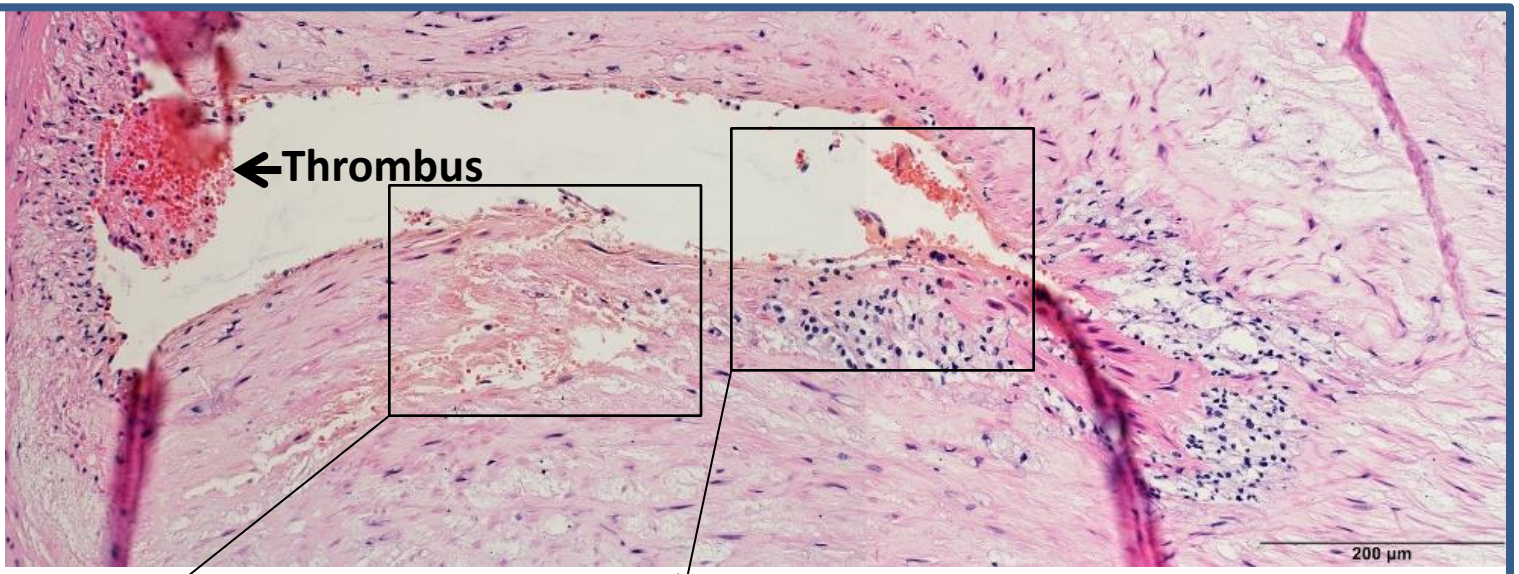



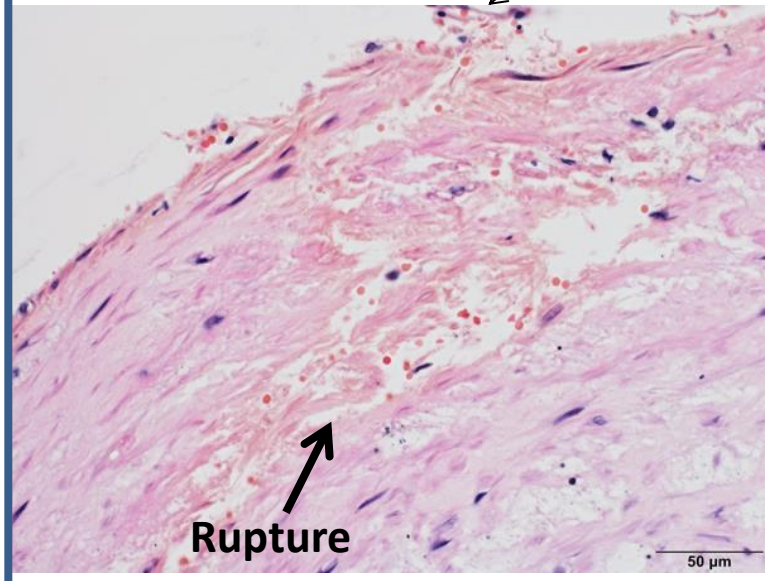



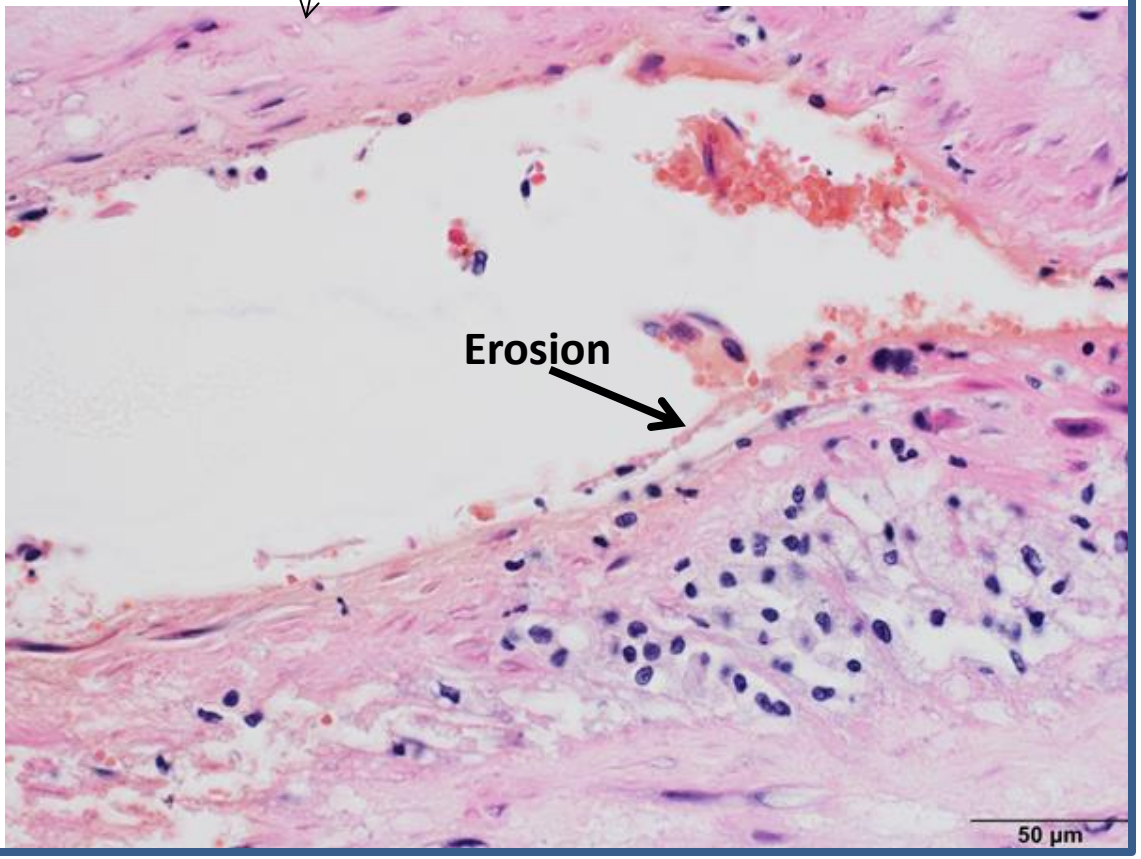

Rupture

S-Figure VIII-⑥

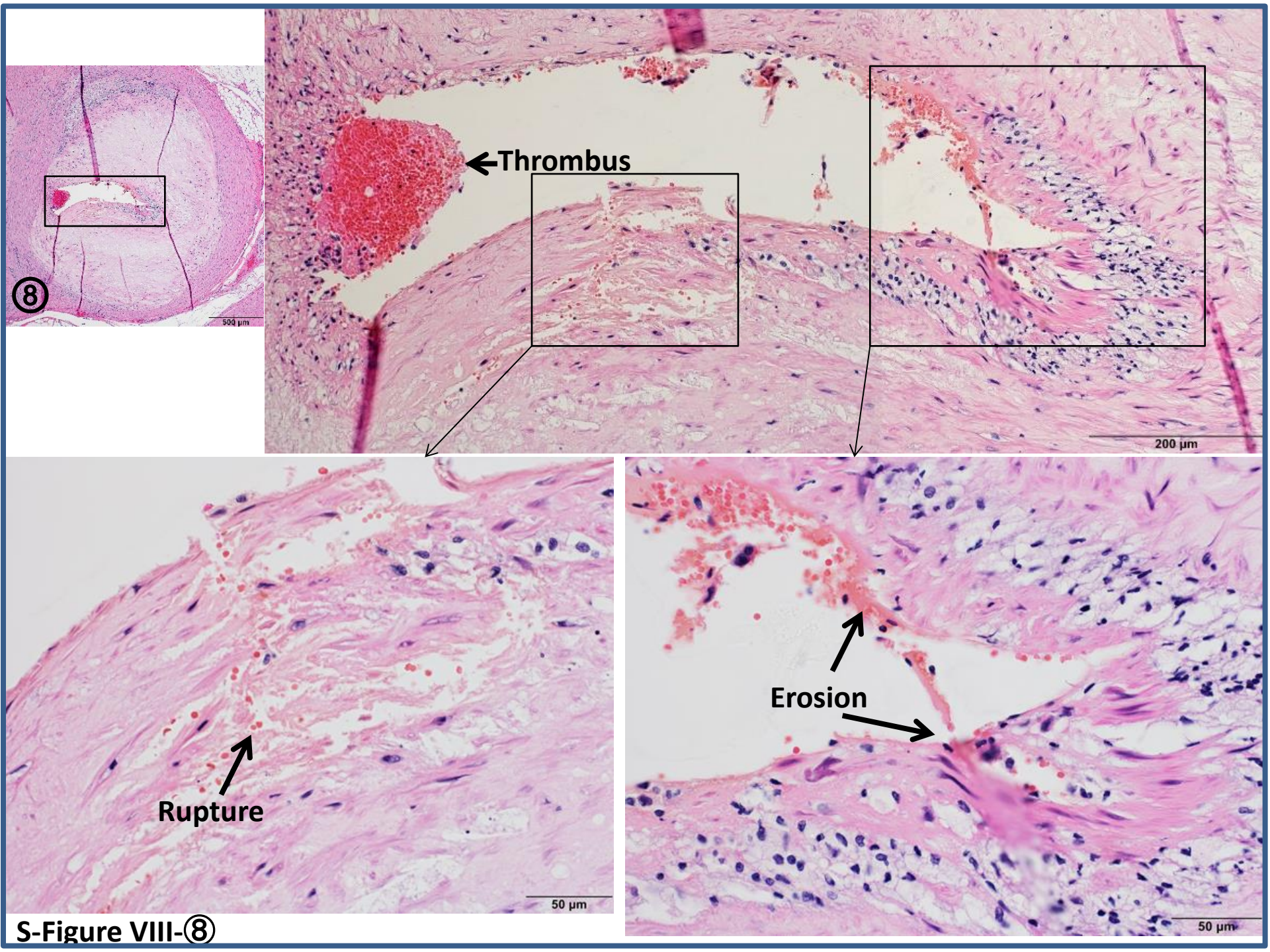


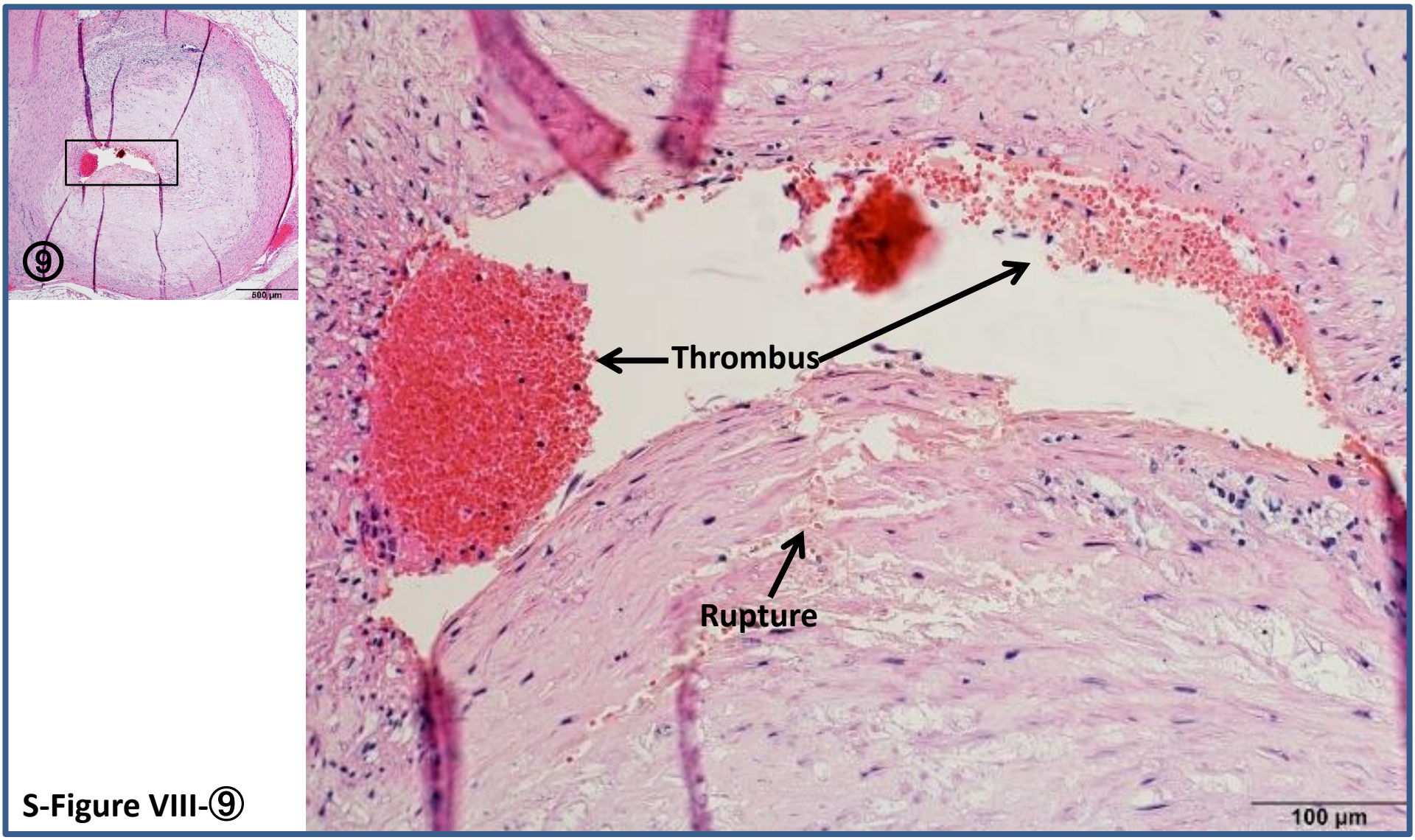



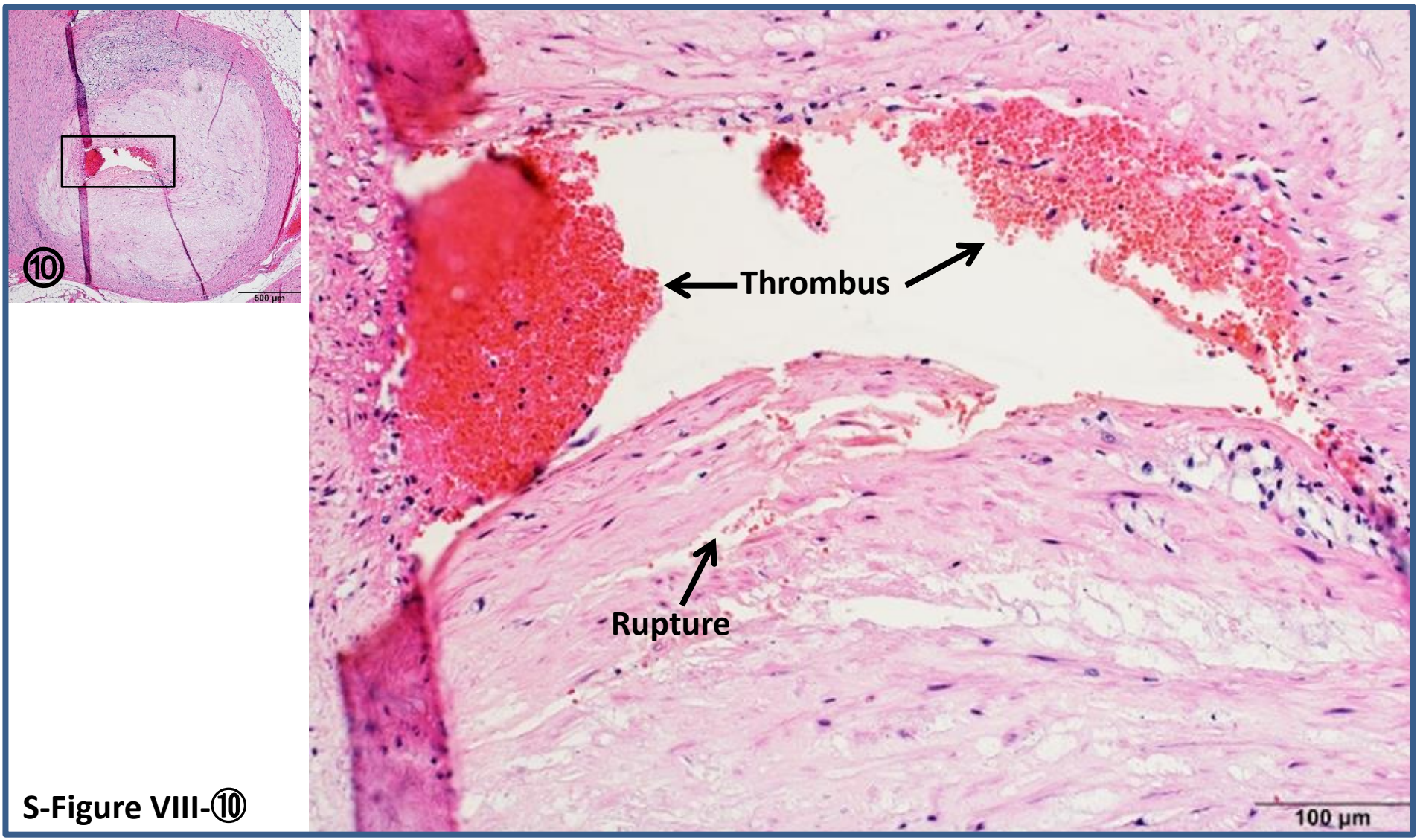

**S-Figure VIII-⑦**




S-Figure VIII-⑧




S-Figure VIII-⑨

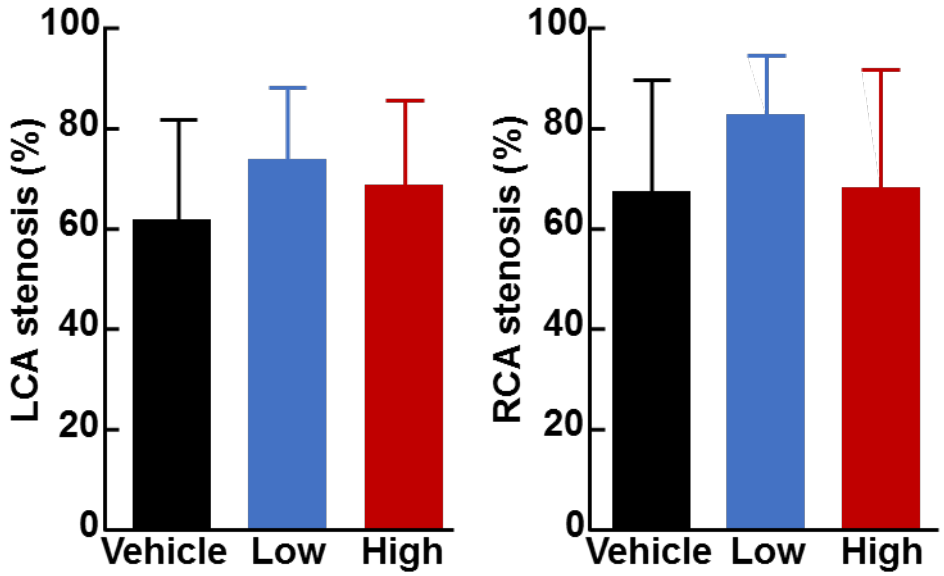



S-Figure VIII-⑩

**Supplemental figure VIII. Representative micrographs showing the continuity of the coronary plaque rupture/erosion and thrombosis.**

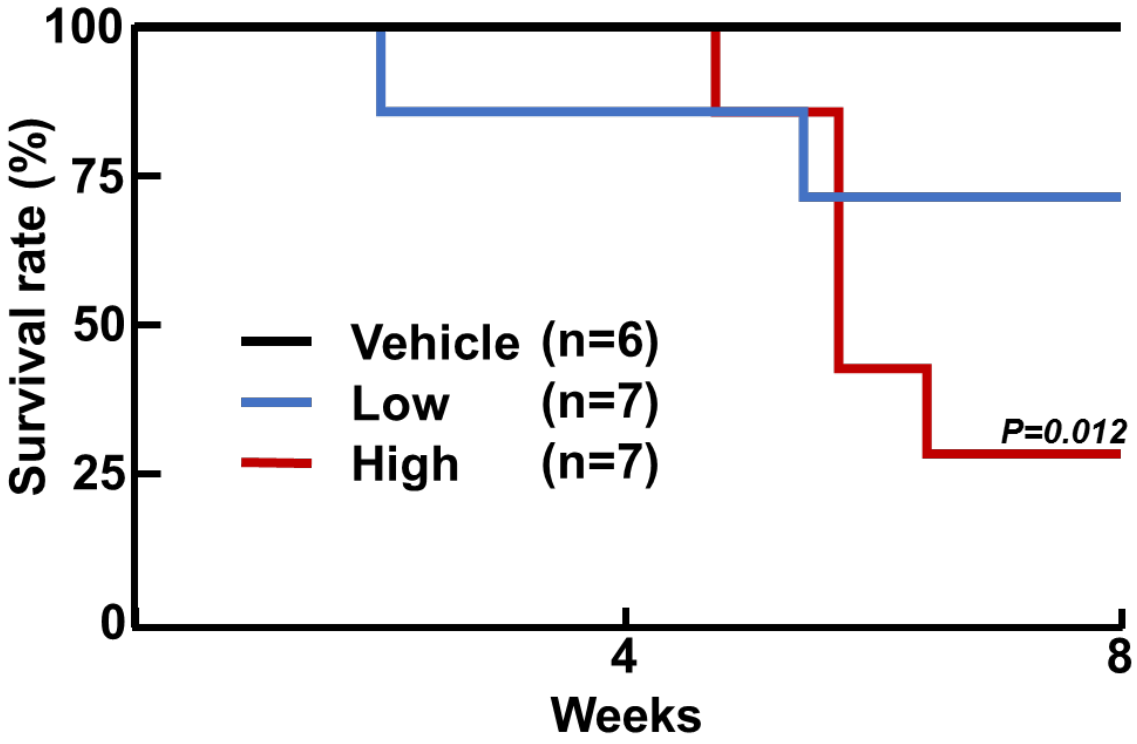
Step sections (numbered from ① to ⑩) of the left coronary artery from a dead WHHL rabbits infused with Ang II were cut at 50 μm interval and stained with HE staining.

The plaque shows a distinctive disruption (indicated by arrows, from ① to ⑩ on the left, bottom panel) in which many red blood cells are contained. Accumulation of foam cells associated with the surface erosion is present (indicated by arrows, ②-⑧). Discernible hemorrhage within the lesions is seen in sections ①, ④-⑥. Thrombi are present in sections ①-⑩.

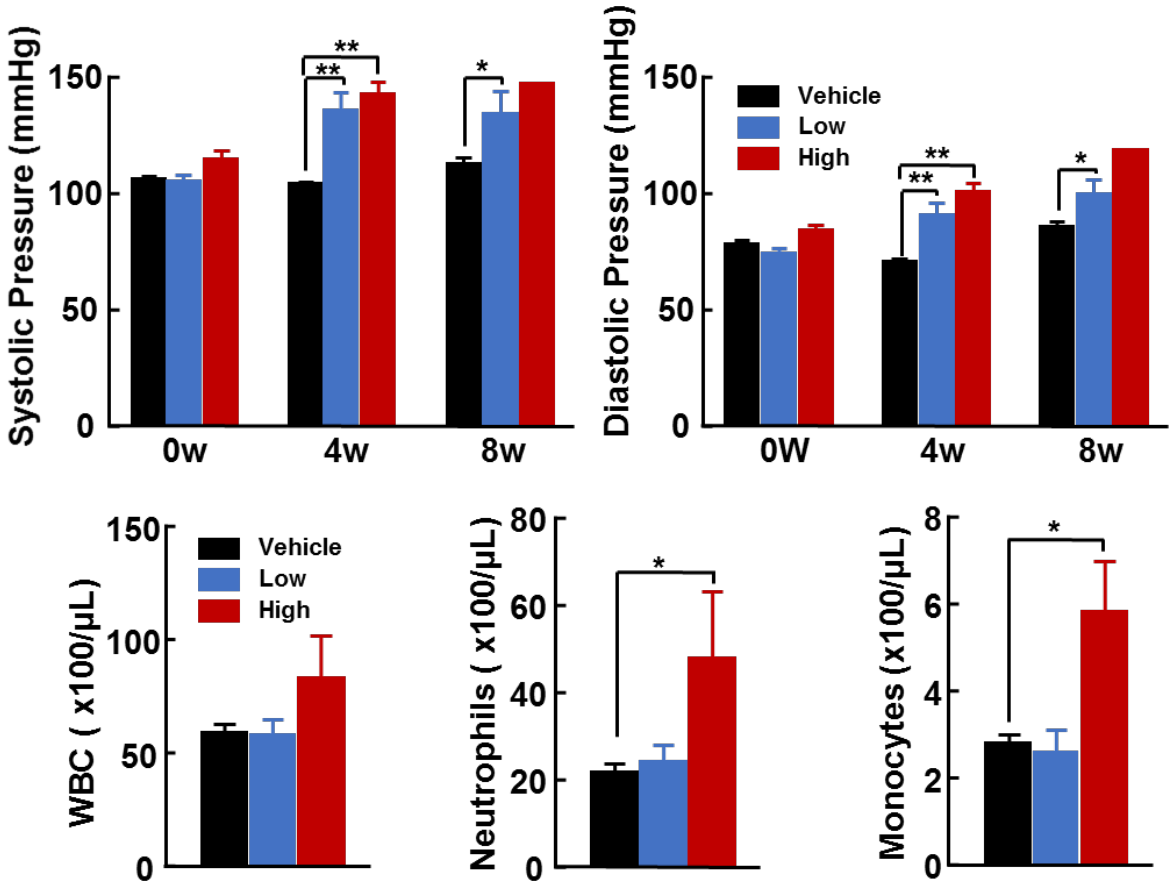



**Supplemental figure IX. Comparison of coronary stenosis of Ang II groups with the vehicle group.**

Data are expressed as mean ± SEM. n=8 for the vehicle, n=10 for low-Ang II and n=9 for high-Ang II groups. No statistical significance was found between Ang II groups and the vehicle group.

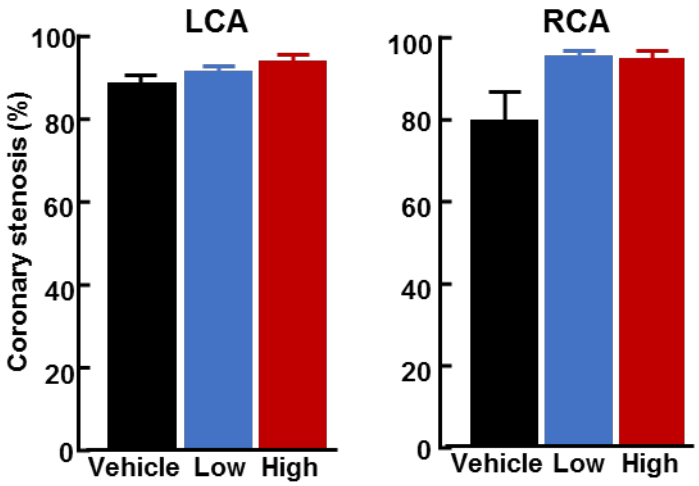



**Supplemental figure X. Kaplan-Meier analysis of cumulative rates of survival in WHHL rabbits in experiment two.**



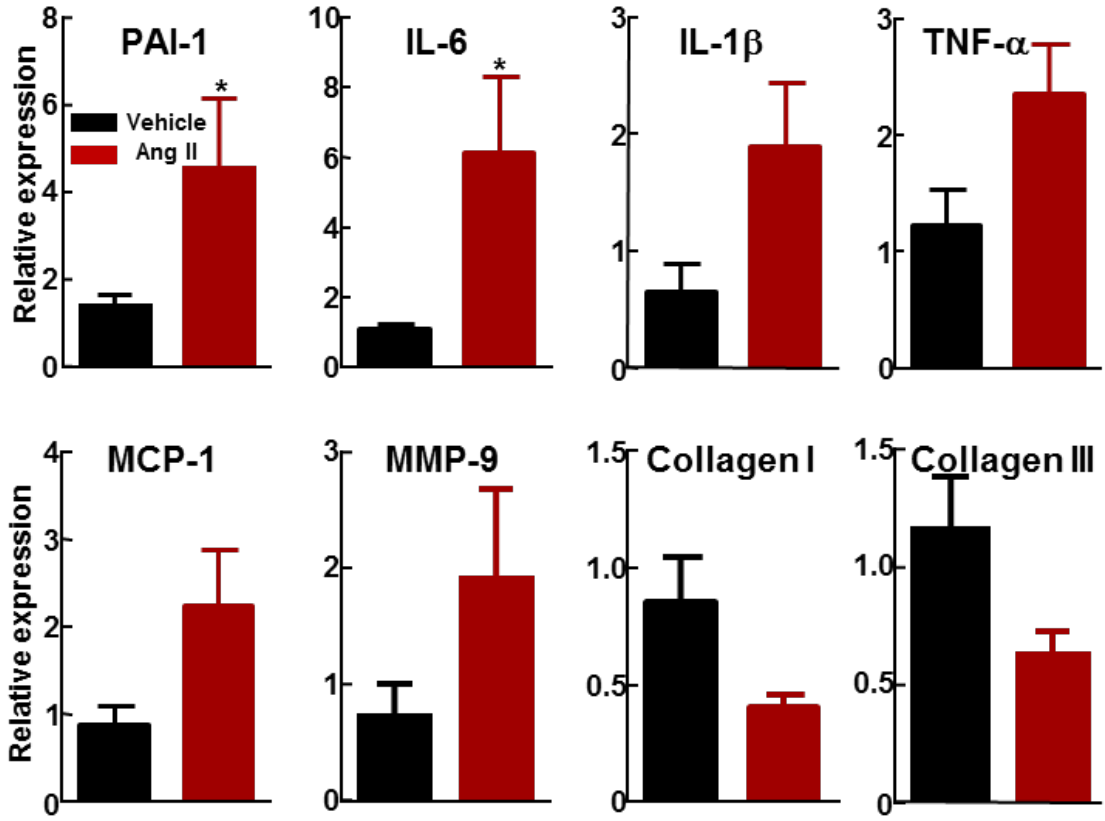

**Supplemental figure XI. Blood pressure and blood leukocytes in experiment two.**

Blood pressure (upper) at 0, 4, 8 wks and blood leukocyte count (lower panel) were measured at 6 wks. We compared the blood pressure at different time points (0, 4, and 8 weeks) using one way ANOVA with post-hoc Tukey test (0 and 4 weeks) or Student's t-test (8 weeks, low group vs vehicle). Data are expressed as mean ± SEM. *$P$<0.05 or **$P$<0.01 versus the vehicle. n=6 for the vehicle, n=5-7 for low-Ang II and n=1-7 for high-Ang II groups.




**Supplemental figure XII. Quantitative analysis of the coronary stenosis of experiment 2.**

Coronary stenosis of both left (LCA) and right coronary arteries (RCA) was compared between Ang II and vehicle groups. N=6 for vehicle group, n=7 for low- and high-Ang II groups.




**Supplemental figure XIII. RT-PCR analysis of mRNA expression of aortic lesions of the aortic arch.**

Total RNA was extracted as described in the Materials and Methods and quantitative real-time RT-PCR was used to quantify each gene expression. N=5 for each group. *P<0.05 vs. vehicle group.