Supplemental Material

Figure legends

Figure 1. Systolic blood pressure (SBP) after two days of infusion of Ang II (200 ng/kg/min) via an osmotic minipump in young F344XBN rats (Y-200) increased significantly compared to young untreated rats (Y). At the endpoint of the experiment (28 days) the SBP of young rats infused with Ang II (200 ng/kg/min) (Y-200) did not differ from that of old rats (O). In young rats infused with Ang II 50 ng/kg/min (Y-50) SBP did not differ from control young rats. *$p<0.05$ vs. young untreated rats.

Figure 2. Vessel diameter (left panel), the ratio of wall-lumen, (W/L, middle panel), and the vascular mass, (VM, right panel) of young rats treated with chronic Ang II infusion. *$p<0.01$ versus untreated young rats. †$p<0.05$ vs. untreated old rats.

Figure 3. A) Representative photomicrographs of paraffin sections of carotid arteries immunostained with antibodies against collagen types I and III and TGF-β_1, respectively. The brown color indicates the areas positive for collagen type I, type III, or TGF-β_1 staining. L: lumen; M: media. Magnification: 400X. B) Average collagen types I and III and TGF-β_1 staining within the carotid wall, respectively. *$p<0.01$, vs. young untreated group; †$p<0.05$ vs. untreated old rats.

Figure 4. Double immunostaining fluorescence of paraffin sections (400X magnification) from an old rat carotid artery with α-SMA or CD31 monoclonal antibodies, detected by FITC (green color) (left panels), or a polyclonal MMP2 antibody, detected by TRIC (red color) (middle

panels). Merged images are presented in the right panels; yellow color indicates co-localization of the two proteins. Interestingly, staining of both cell types in the adventitia is consistent with the presence of vasa vasorum. L: lumen; M: media.

Figure 5. Average MMP2/TIMP2 or MT1MMP/TIMP2 staining within the intima (A) or media (B). *p<0.01, vs. young untreated group; † p<0.05 vs. untreated old rats.

Figure 6. Average MMP2/TIMP2 or MT1MMP/TIMP2 mRNA staining within the intima (A) or media (B). Y: untreated young rats; Y-200: young rats infused with Ang II 200 ng/kg/min. *p<0.01, vs. young untreated group.

Figure 7. Average PDGF-BB staining within the intima (A) or media (B). Y: untreated young rats (n=4); Y-200 (n=4): young rats infused with Ang II 200 ng/kg/min (n=4); O: untreated old rats (n=4). *p<0.01, vs. young untreated group; † p<0.05, vs. untreated old rats.

Figure 8. A) Representative photomicrographs of carotid sections stained with an anti-ET-1 antibody (brown color). B) Average ET-1 staining fractions (right panel). C) Representative immunoblotting of carotid arteries with anti-ET-1 antibody. D) Representative zymogram of VSMC with or without ET-1 treatment. *p<0.01, vs. young untreated group; † p<0.05 vs. untreated old rats.

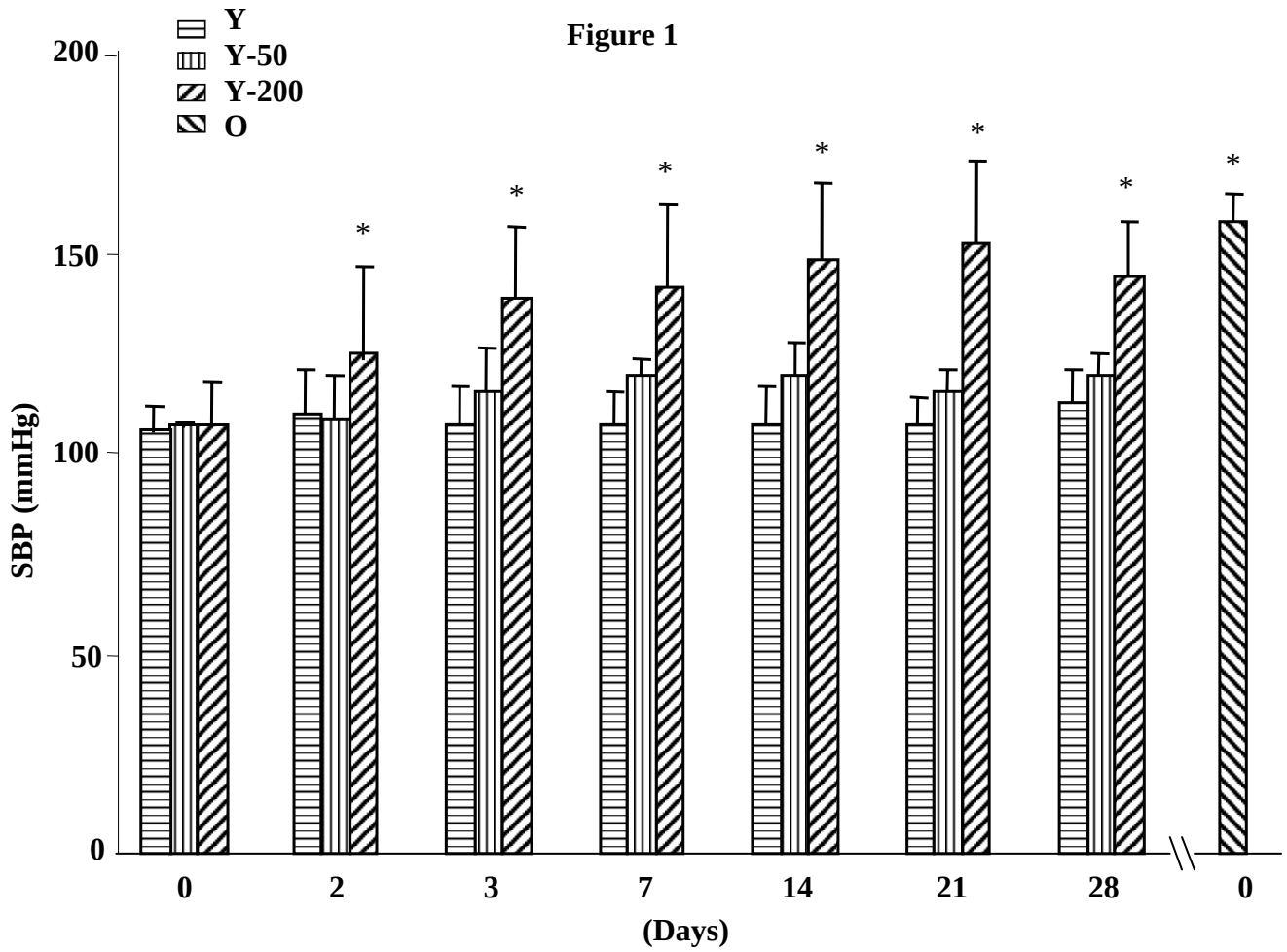


Figure 1

Figure 2

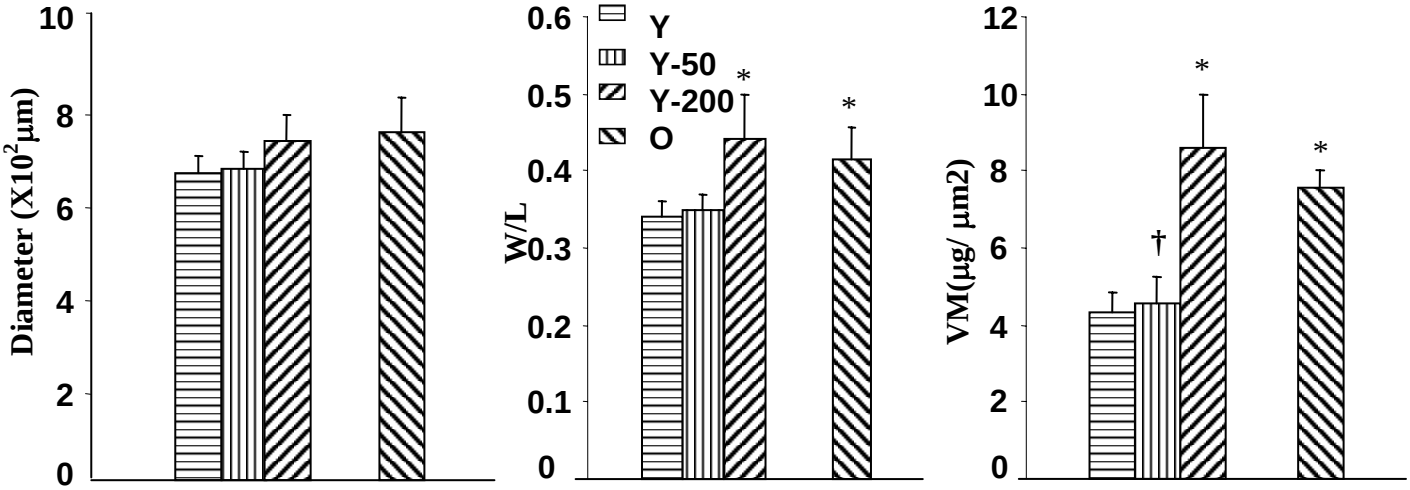

Diameter (X10²μm)
10
8
6
4
2
0
W/L
0.6
0.5
0.4
0.3
0.2
0.1
0
Y
Y-50
Y-200
O
*
*
VM(μg/ μm2)
12
10
8
6
4
2
0
†
*
*

Figure 3A

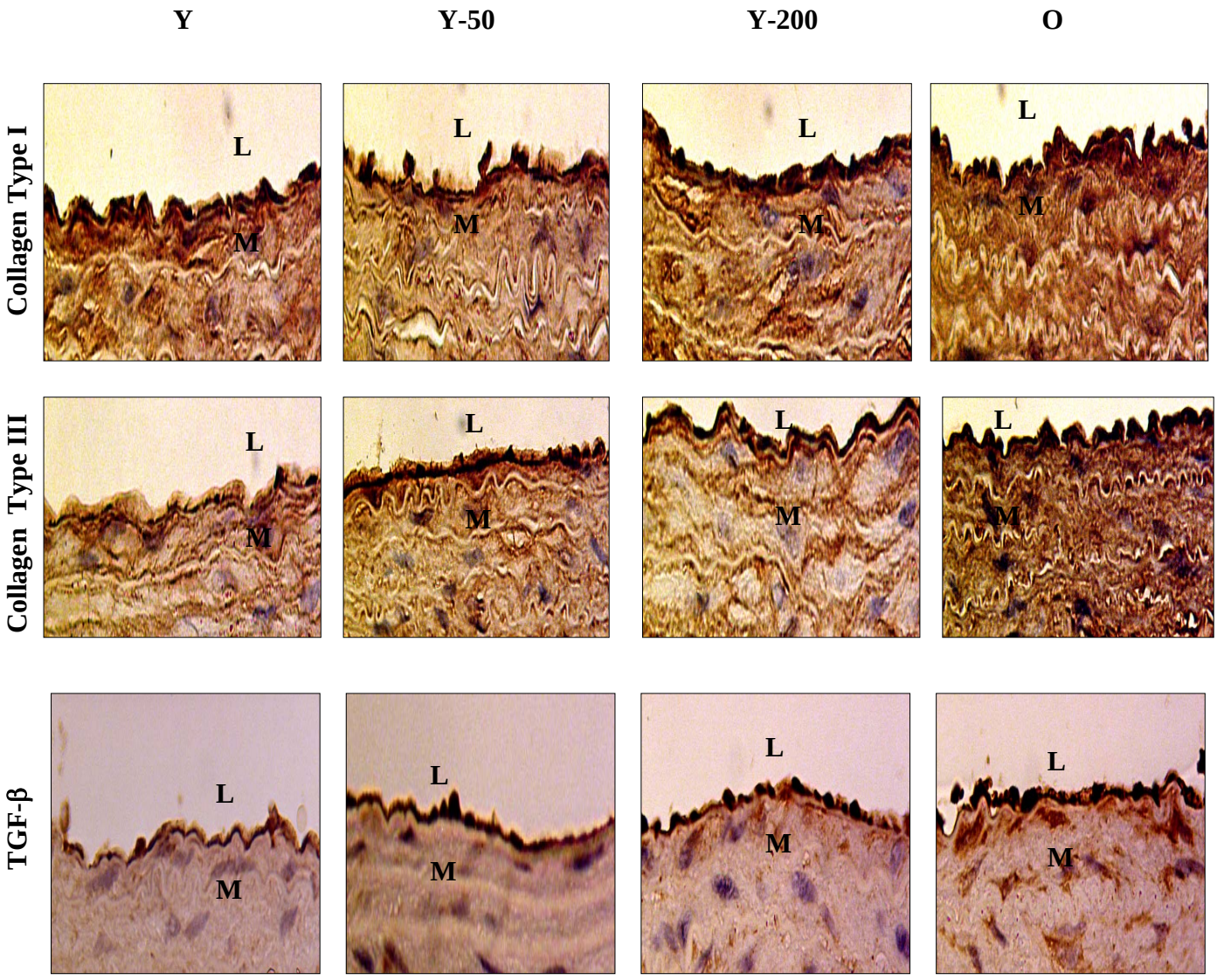


Figure 3B

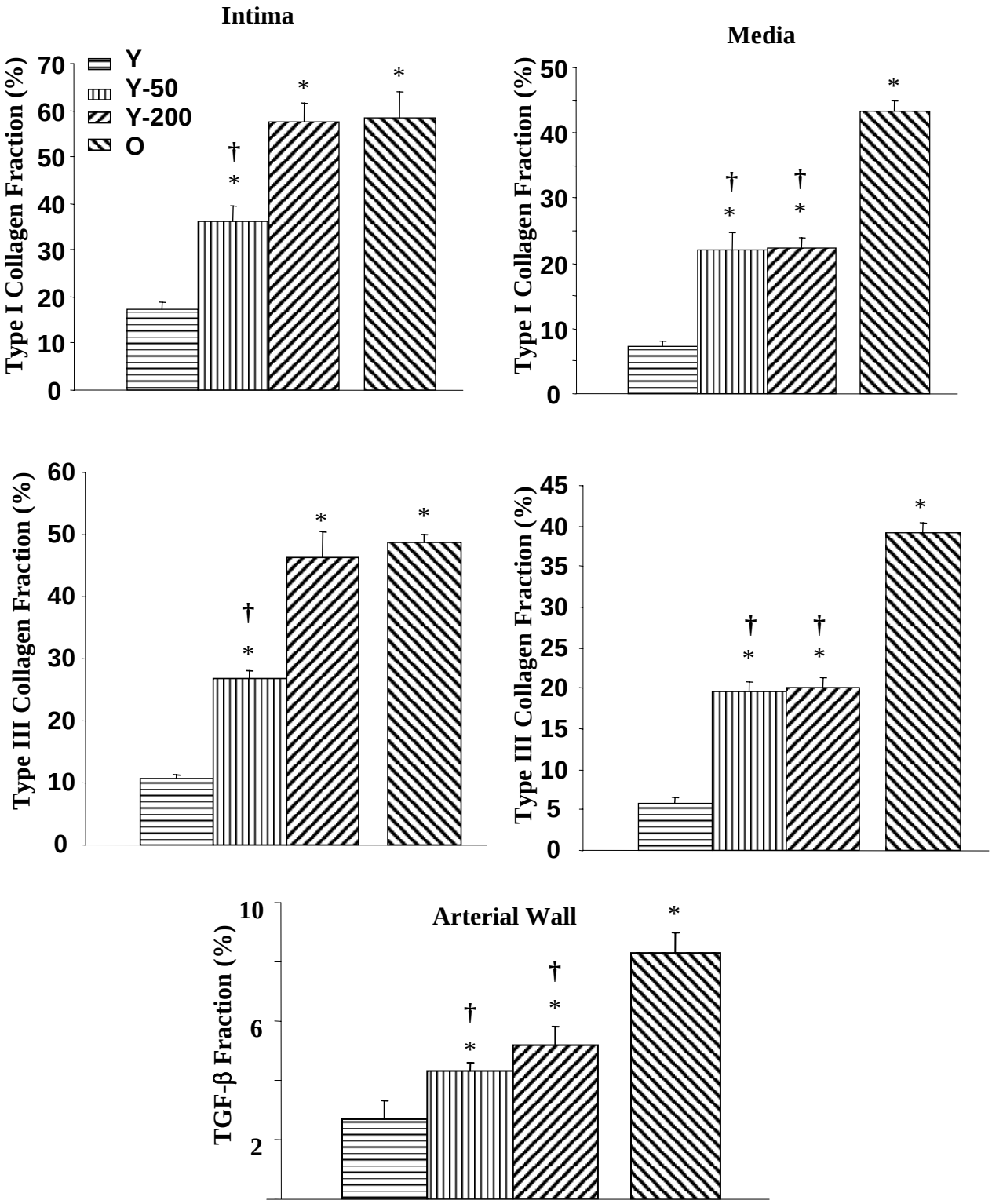
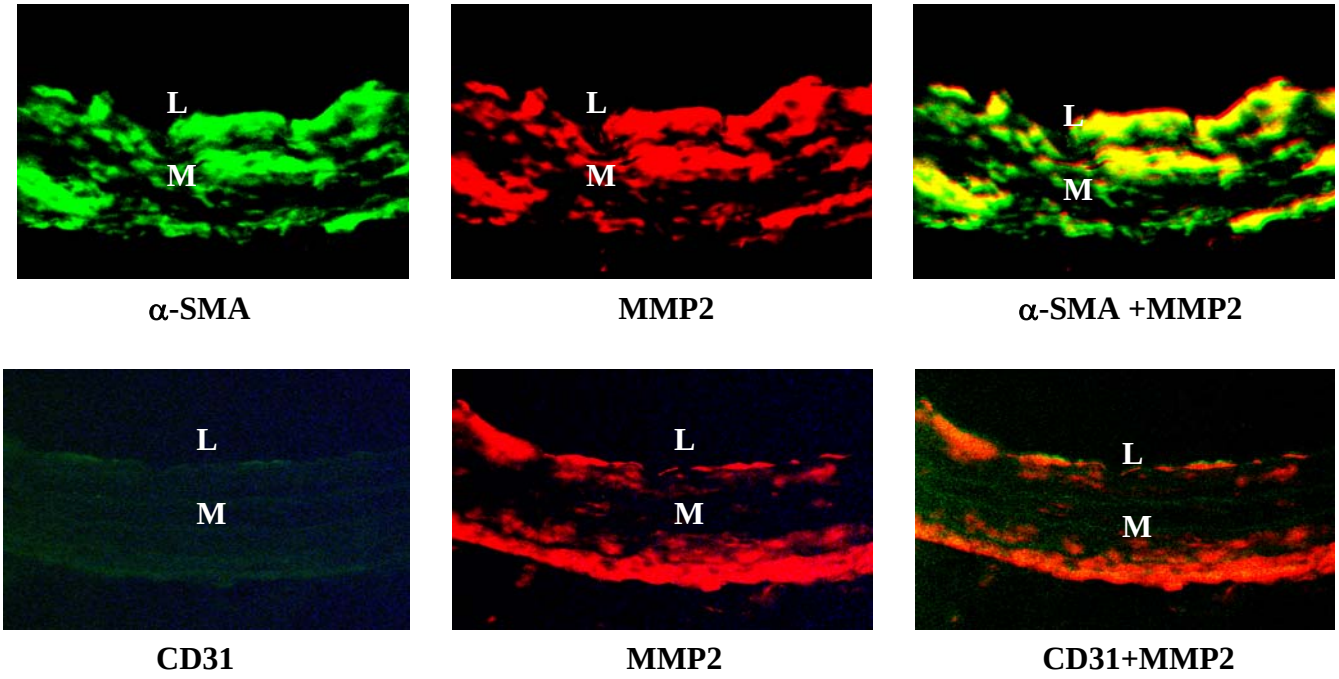

Intima
Media
Y
Y-50
Y-200
O
Type I Collagen Fraction (%)
Type III Collagen Fraction (%)
TGF-β Fraction (%)
Arterial Wall
0
10
20
30
40
50
60
70
2
6
5
15
25
35
45
*
†

Figure 4



α-SMA **MMP2** **α-SMA +MMP2**

CD31 **MMP2** **CD31+MMP2**

Figure 5

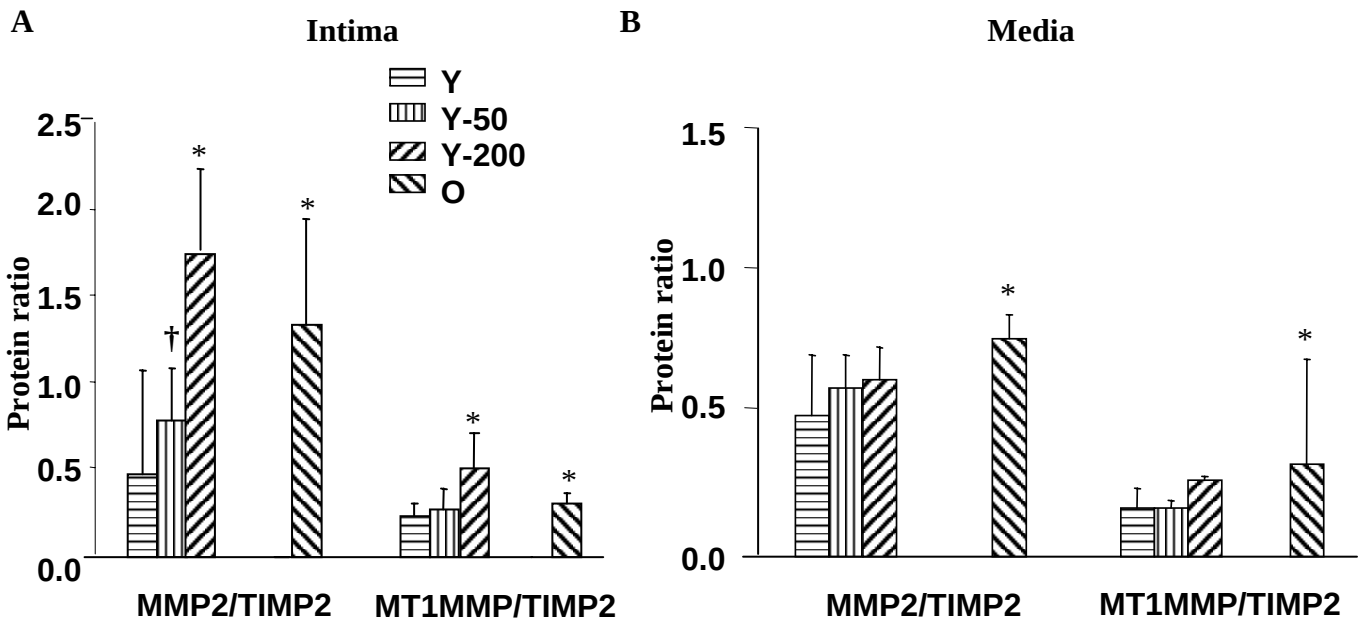

A
Intima
Y
Y-50
Y-200
O
Protein ratio
2.5
2.0
1.5
1.0
0.5
0.0
*
†
MMP2/TIMP2
MT1MMP/TIMP2
B
Media
1.5
1.0
0.5
0.0
MMP2/TIMP2
MT1MMP/TIMP2

Figure 6

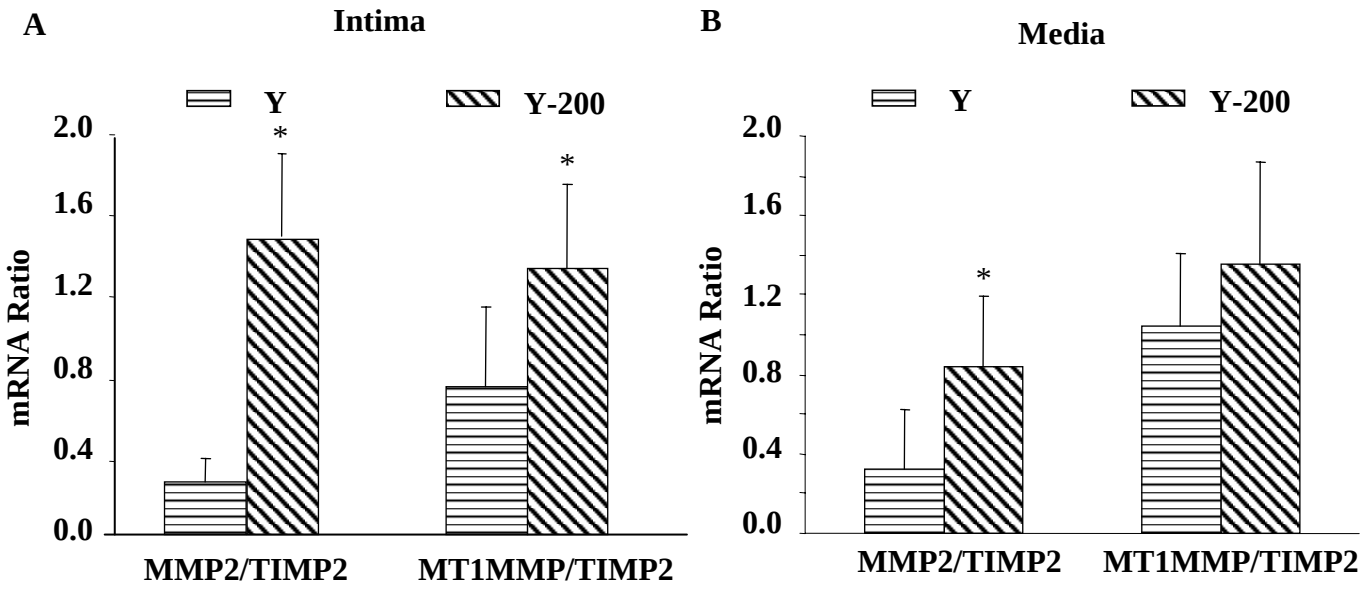

A
Intima
Y
Y-200
2.0
1.6
1.2
0.8
0.4
0.0
mRNA Ratio
*
*
MMP2/TIMP2
MT1MMP/TIMP2
B
Media
Y
Y-200
2.0
1.6
1.2
0.8
0.4
0.0
mRNA Ratio
*
MMP2/TIMP2
MT1MMP/TIMP2

Figure 7

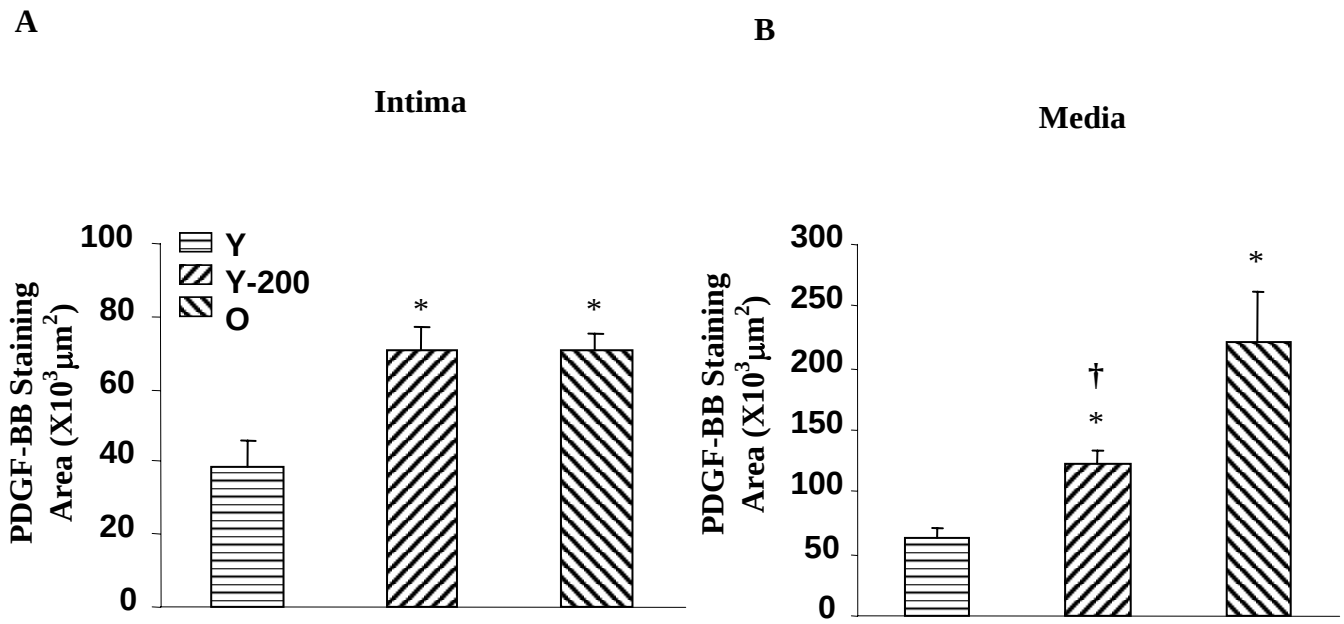
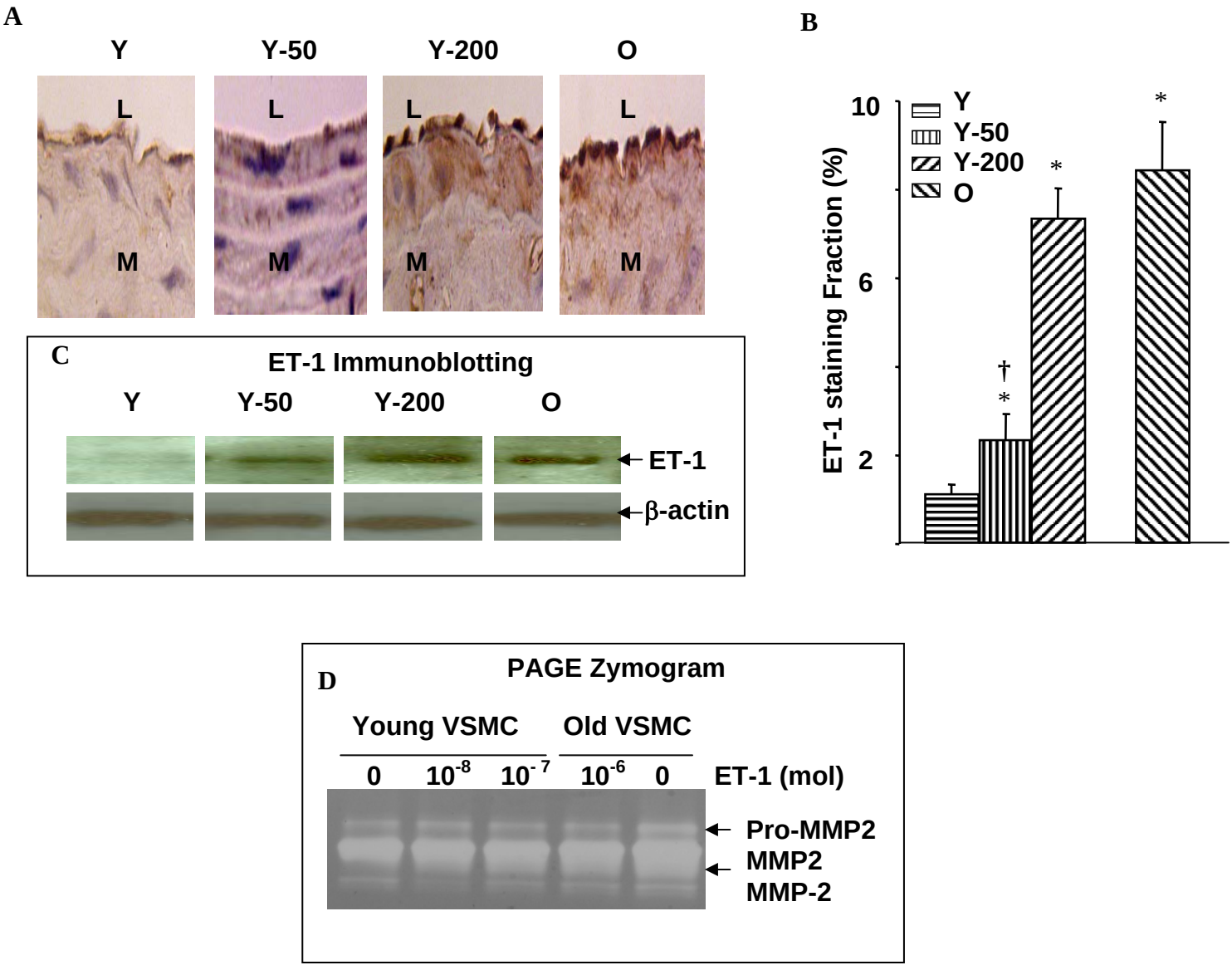

A
Intima
PDGF-BB Staining
Area (X10³μm²)
100
80
60
40
20
0
Y
Y-200
O
*
*
B
Media
PDGF-BB Staining
Area (X10³μm²)
300
250
200
150
100
50
0
†
*
*

Figure 8


A
Y
Y-50
Y-200
O
L
M
B
ET-1 staining Fraction (%)
10
6
2
Y
Y-50
Y-200
O
*
†
C
ET-1 Immunoblotting
ET-1
β-actin
D
PAGE Zymogram
Young VSMC
Old VSMC
0
10-8
10-7
10-6
ET-1 (mol)
Pro-MMP2
MMP2
MMP-2