# Supplement Figure legends

**Supplement Figure 1.** Strategy for construction of $LacZ^{flox}$-CyPA mice and VSMC-specific overexpression of FLAG-CyPA. $LacZ^{flox}$-CyPA construct using the pZ/EG vector.

**Supplement Figure 2.** Evaluation of intimal, medial, and adventitial area. (**A**) Graphic representation of ligated carotids and preparation of sections at equal interval. Actual average values obtained from measurements of 5 different levels of section (section 1–5) in each animal were used for the evaluation of intimal, medial, and adventitial areas. (**B**) Masson-Trichrome staining at high magnification show the internal elastic lamina (IEL) and external elastic lamina (EEL) in sham and ligated carotid of $CyPA^{+/+}$ mice. Intima and media areas were determined by the areas surrounded by the luminal surface, IEL, and EEL. The intimal area was calculated by subtraction of the luminal area from the area defined by the IEL. The medial area was calculated by subtraction of the area defined by the IEL from the area defined by the EEL. In control and sham arteries, intimal thickening was not observed. The I/M ratio was calculated by dividing the value for intima and media for each animal and then averaging the values for each group.

**Supplement Figure 3.** Representative immunostaining of Ki67 and α-smooth muscle actin (αSMA) and counterstaining with hematoxylin in ligated carotids from wild-type (WT) mice. Immunostaining for Ki67 (**A**), αSMA (**B**), and CyPA (**D**) alone, and double-immunostaining for both Ki67 and αSMA (**C**) on serial sections of ligated carotids from WT mice. Scale bars, 50 μm.

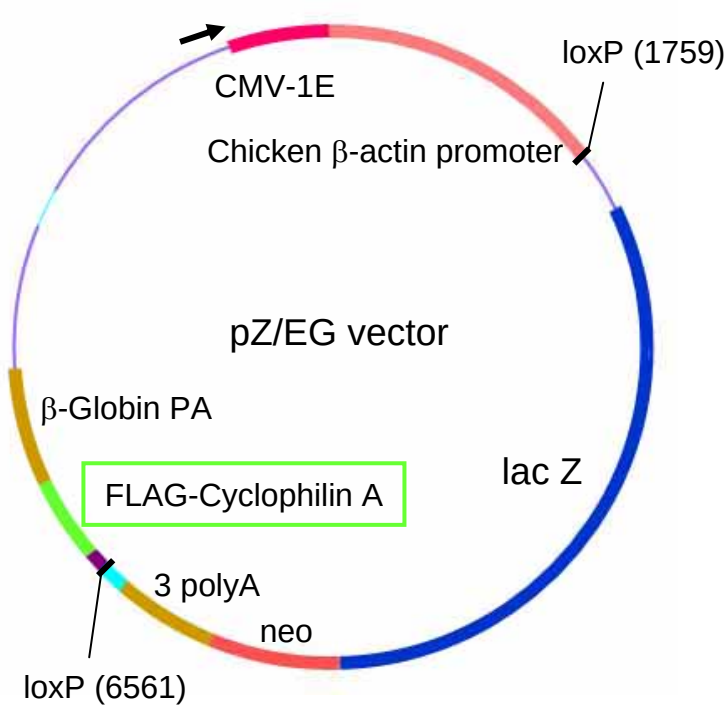
**Supplement Figure 4.** Representative immunostaining of phospho-ERK1/2 (pERK1/2) and counterstaining with hematoxylin in sham carotids and ligated carotids from wild-type (WT) and $CyPA^{-/-}$. (**A**–**E**) Strong expression of pERK1/2 in ligated WT carotids compared with $CyPA^{-/-}$ carotids. Scale bars, 50 μm. (**F**) Western blot comparison of pERK1/2 in carotid homogenates of WT (n = 8, black bars) and $CyPA^{-/-}$ mice (n = 6, white bars) 7 days after ligation. Equal protein loading was confirmed with tubulin antibody.

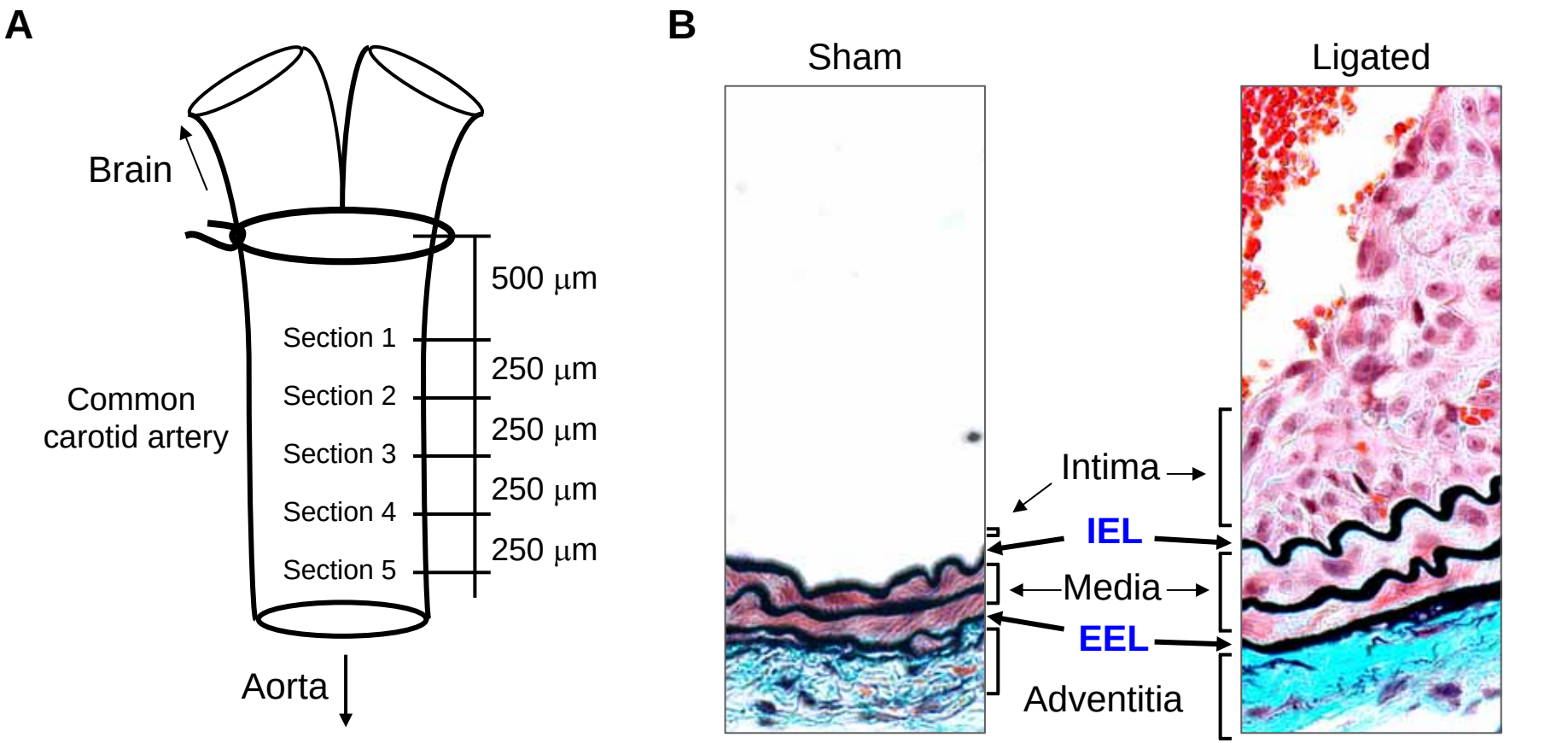
**Supplement Figure 5.** Scratch wound migration assay. (**A**) Representative pictures (hematoxylin staining) of scratch wound migration assay. WT-MASM were seeded in 35 mm dishes in DMEM supplemented with 10% FBS, starved for 24 hours, scratch wound made with a

pipette tip, and stimulated with Tg-CM, Cont-CM, WT-CM or KO-CM for 24 hours. (**B**) Quantification of number of migrating cells in scratch wound migration assay. Data are mean ± SD. $^{\star}P<0.01$. $n = 8$ in each group.

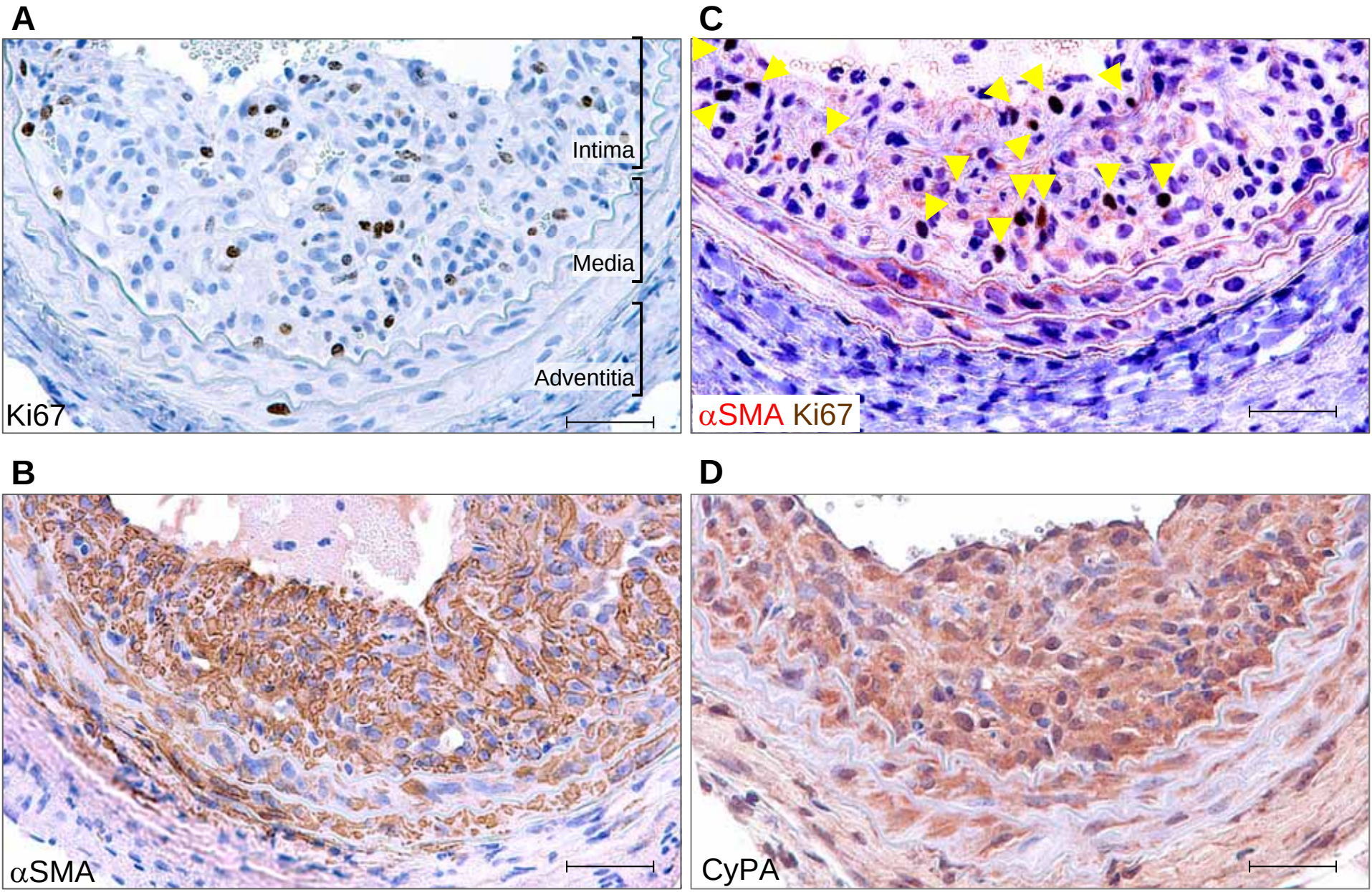
**Supplement Figure 6.** CyPA plays a crucial role in vascular remodeling. Decreased blood flow increases reactive oxygen species (ROS) generation which induces secretion of CyPA. Secreted CyPA promotes VSMC proliferation and migration, VCAM-1 expression and inflammatory cell migration, resulting in vascular remodeling.

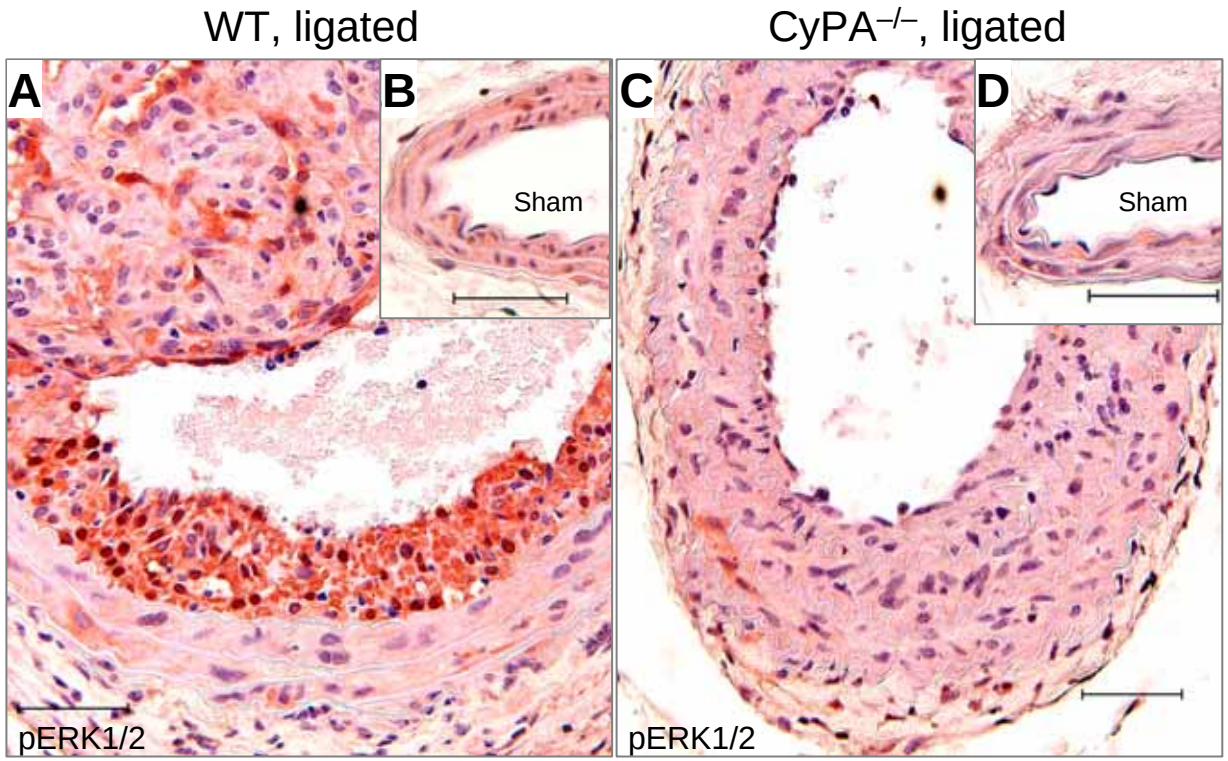
LacZ$^{flox}$-CyPA construct

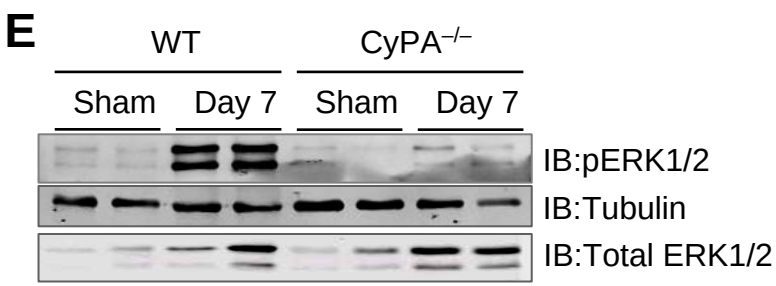


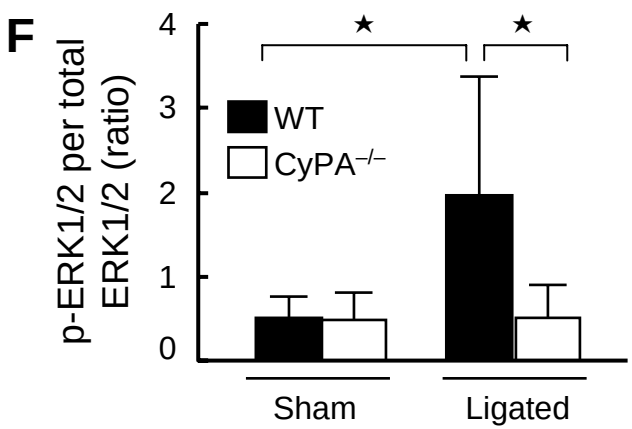

A
Brain
Common carotid artery
Section 1
Section 2
Section 3
Section 4
Section 5
500 μm
250 μm
250 μm
250 μm
250 μm
Aorta
B
Sham
Ligated
Intima
IEL
Media
EEL
Adventitia

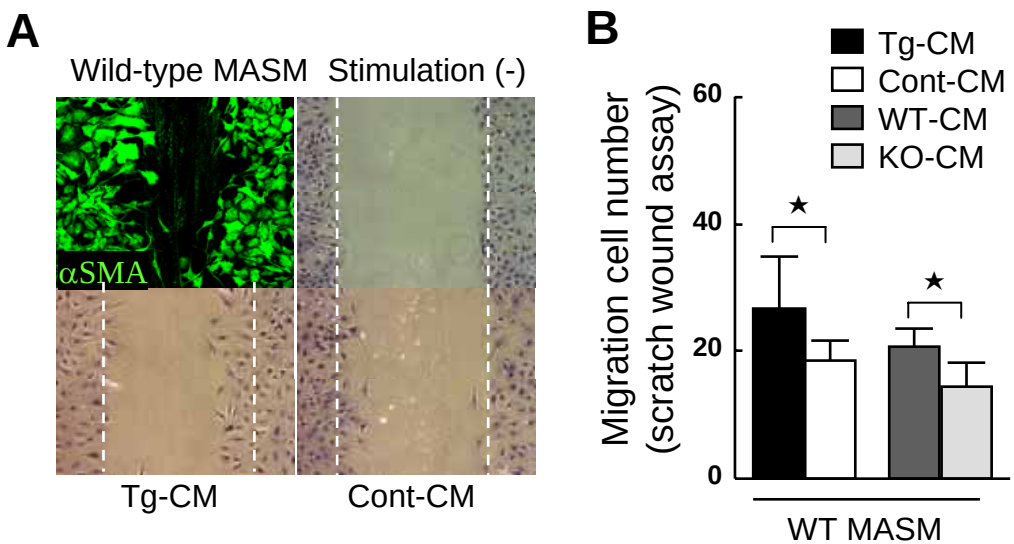
WT, ligated (Serial sections)

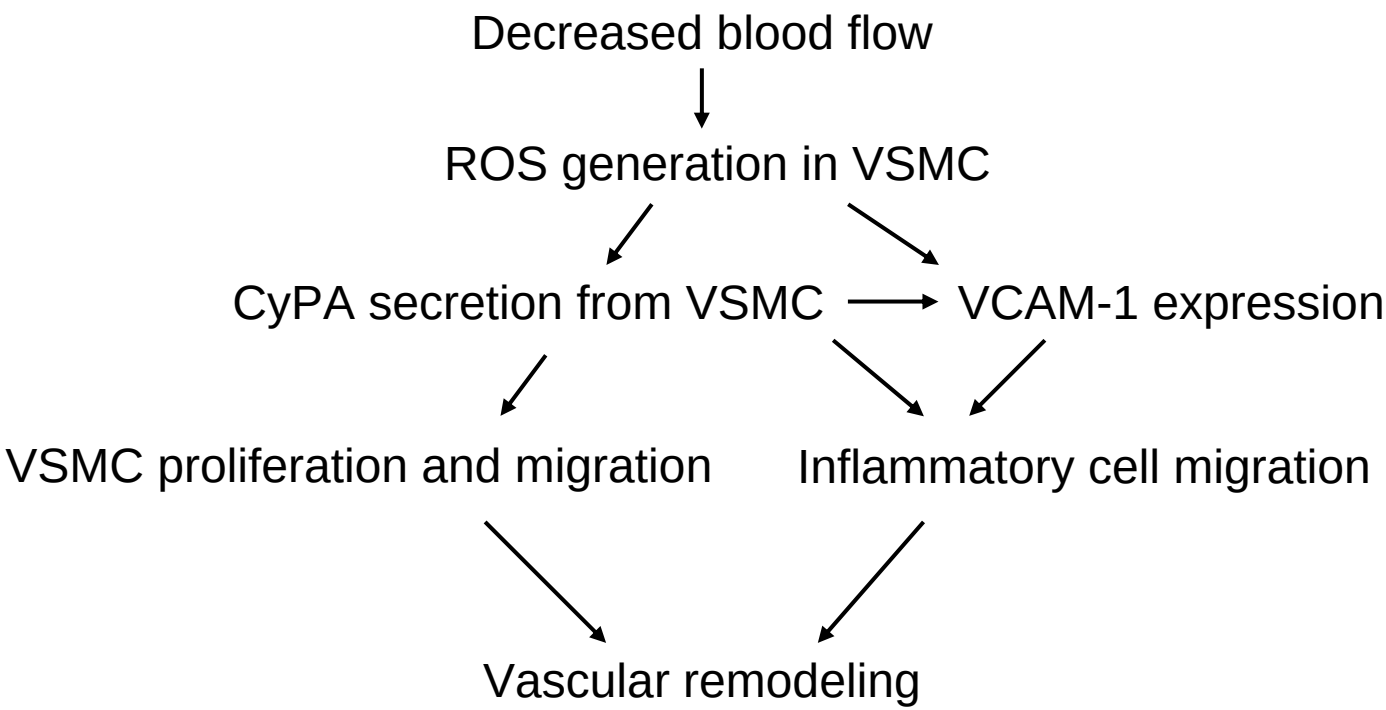



WT, ligated
CyPA−/−, ligated
A
B
Sham
C
D
Sham
pERK1/2
pERK1/2


E
WT
CyPA−/−
Sham
Day 7
Sham
Day 7
IB:pERK1/2
IB:Tubulin
IB:Total ERK1/2


F
p-ERK1/2 per total ERK1/2 (ratio)
4
3
2
1
0
★
★
WT
CyPA−/−
Sham
Ligated


A
Wild-type MASM
Stimulation (-)
αSMA
Tg-CM
Cont-CM
B
Tg-CM
Cont-CM
WT-CM
KO-CM
Migration cell number
(scratch wound assay)
60
40
20
0
★
★
WT MASM


Decreased blood flow
ROS generation in VSMC
CyPA secretion from VSMC
VCAM-1 expression
VSMC proliferation and migration
Inflammatory cell migration
Vascular remodeling