SUPPLEMENTAL MATERIAL

LMO7 is a negative feedback regulator of TGF-β signaling

Supplemental Methods

Histological staining. Verhoeff's van Gieson (EVG), Masson's Trichrome and Picro-Sirius Red stainings were performed at the Yale Pathology Tissue Service using standard protocols. The quantification of collagen in Picro-Sirius Red staining by polarized light microscopy was performed using ImageJ software. A background threshold was applied to intimal and medial area of the images to identify the collagen positive area. Integrated density was defined as the intensity of this signal normalized to combined area of the media and intima to represent the average collagen expression per area.

Immunohistochemistry and immunofluorescence. For TGF-β Immunohistochemistry, cryosections were air-dried completely and fixed/permeabilized in cold acetone for 10 min. OCT was removed by washing in distilled H_2O. Staining was performed using a Mouse-on-Mouse (M.O.M.™, Vector Laboratories) kit and developed using DAB kit (Vector Laboratories) per the manufacturer's protocol, followed by hematoxylin counterstain. The sections were mounted in DPX mounting media (Electron Microscopy Sciences) and imaged with Nikon Eclipse 80i microscope running NIS Elements software (Nikon). The antibody against TGF-β (MAB1835, clone #1D11, R&D Systems) was used at a dilution of 1: 25, and antibody for CTGF (ab6992, Abcam) was used at a dilution of 1: 200. IHC of Ki67 was performed at the Yale Pathology Tissue Service using standard protocols. For Immunofluorescence, cryosections were air-dried completely and washed with distilled H_2O to remove OCT. The sections were then permeabilized/blocked in blocking buffer (4% goat normal serum, 1% BSA and 0.5% Triton X-100 in PBS) at room temperature (R.T.) for 1 h, and incubated with primary antibodies at 4°C for 18 h and then secondary antibodies at R.T. for 1 h. Antibodies were diluted in blocking buffer. Finally, the sections were stained with 2ug/ml DAPI (4',6-diamidino-2-phenylindole), to identify nuclei, at room temperature for 5 min and mounted with Vectashield Mounting Media (Vector Laboratories). The following primary antibodies were used: LMO7 (sc-98422x, 1: 100, Santa Cruz), p-SMAD3 (S423/425) (ab52903, 1: 100, Abcam), Integrin β3 (ab75872, 1: 100, Abcam), Integrin αV (MCA2461, 1: 100, Bio-Rad), αSMA (Cy3-conjugated, C6198, 1: 300, Sigma), Ki67 (MA5-14520, 1: 200, Thermo). Secondary antibodies Goat anti-Rabbit IgG, Alexa Fluor 488 (A-11008, Molecular Probes) and Goat anti-Rat IgG, Alexa Fluor 647 (A-21247, Molecular probes) were used at a 1:300 dilution. Fluorescent micrographs were obtained with a Nikon Eclipse Ti Confocal Microscope using Volocity software (Perkin Elmer).

Primary tissue or cell isolation and cell culture. PBS perfused mouse descending aortas were digested (175U/ml Collagenase II and 1.25U/ml elastase in HBSS) for 10 min, and the adventitia was peeled off under a dissecting microscope. To obtain tissue lysates the medial layers were homogenized in cold RIPA buffer supplemented with protease inhibitor cocktail and PhosSTOP (Roche) for protein, or in RLT buffer (Qiagen) with 1% β-mercaptoethanol for RNA. Lysates were homogenized in a Tissuelyzer II (Qiagen). To culture primary SMCs, vessels were minced with a sterile blade and digested in digestion mix (400U/ml collagenase II, 2.5U/ml

elastase and 0.2mg/ml soybean trypsin inhibitor in HBSS) by shaking at 700 rpm at 37°C for 1 h. The cell suspension was centrifuged at 500rpm for 5 min, washed once with DMEM supplemented with 20% FBS, 100U/ml penicillin and 100ug/ml streptomycin (Thermo) and plated on culture dishes in the same media. Cells were subcultured 1: 4 once confluent. Primary human coronary artery SMCs (Lonza) were propagated in M199 media supplemented with 10% FBS, 100U/ml penicillin, 100ug/ml streptomycin, 2.7 ng/ml EGF (Promega), and 2 ng/ml FGF (Promega) (complete M199). Cells at passage 4-7 were used for experiments. HEK293A cells were maintained in DMEM media with 10% FBS and penicillin/streptomycin. Drugs and cytokines used: TGF-β1 (BioLegend), SB431542 (Sigma), Cilengitide (ApexBio), T5224 (ApexBio), Cycloheximide (Tocris), MG132 (Sigma).

Transient transfection of siRNA and plasmids. Transient transfection of hCASMCs with small interfering RNA (siRNA) was performed using Lipofectamine® RNAiMAX (Life Technologies). Cells were plated the day before transfection to reach a confluency of 70% at transfection. Transfection reagent and siRNA were mixed in OPTI-MEM (Gibco) and applied to the cells. M199 media with 20% FBS was added to the cells after 6 hours. After 24 hours, the media was changed to complete M199. Nonsilencing siRNA (siControl) and siRNA targeting LMO7 were purchased from Ambion. Human *FOS* and *JUN* plasmids were purchased from Addgene (deposited by Drs. William Hahn and Alexander Dent, respectively). A partial mouse *Lmo7* cDNA (lacking the N-terminus) was purchased from Transomic Technologies. The full engthcDNA was assembled by ligating a synthetic N-terminal DNA fragment (synthesized by Genscript). The mutant *Lmo7* cDNA lacking C-terminal sequence encoding the LIM domain was generated by PCR-based mutagenesis. Plasmid DNA was prepared using Endotoxin-free Maxiprep Kit (Qiagen) and hCASMCs were transfected with 4-8 ug of plasmid DNA using the Nucleofector™ system (Lonza). HEK293A cells were transfected with plasmid DNA using Lipofectamine 2000 (Invitrogen).

Cell lysis and western blotting. Cells were briefly washed with PBS and scraped in cold 1X RIPA buffer supplemented with Protease inhibitor cocktail and PhosSTOP (Roche) on ice. Cell lysates were centrifuged at 20,000 X g for 3 min at 4°C. Supernatants were mixed with loading buffer, and denatured at 95°C for 5 min. Equal amounts of protein from each sample was separated on SDS-PAGE gels, transferred onto PVDF membranes and immunoblotted with primary antibodies at 4°C for 18 h and secondary antibodies (Thermo) at R.T. for 1 h. Blots were developed using SuperSignal® Chemiluminescence Substrate (Thermo). Digital images were taken with a Gel Doc™ XR+ System using ImageLab software (BioRad). Primary antibodies used: LMO7 for human samples (H00004008, 1: 1000, Abnova), LMO7 for mouse samples (sc-98422x, 1: 2000, Santa Cruz), SMAD3 (9523, 1: 1000, Cell Signaling), p-SMAD3 (S423/425) (ab52903, 1: 1000, Abcam), Integrin β3 (ab75872, 1: 1000, Abcam), TGFβ-RI (sc-398, 1: 1000, Santa Cruz), c-JUN (9165, 1: 1000, Cell Signaling), c-FOS (2250, 1: 1000, Cell Signaling), Ubiquitin (13724, 1: 1000, Cayman), β-Tubulin (sc-9104, 1: 500, Santa Cruz), GAPDH (2118, 1: 2000, Cell Signaling). Secondary antibodies Rabbit anti-Mouse IgG, HRP (31450, Invitrogen) and Goat anti-Rabbit IgG, HRP (31460, Invitrogen) were used in a 1: 3000 dilution. Densitometry of bands was quantitated using ImageJ.

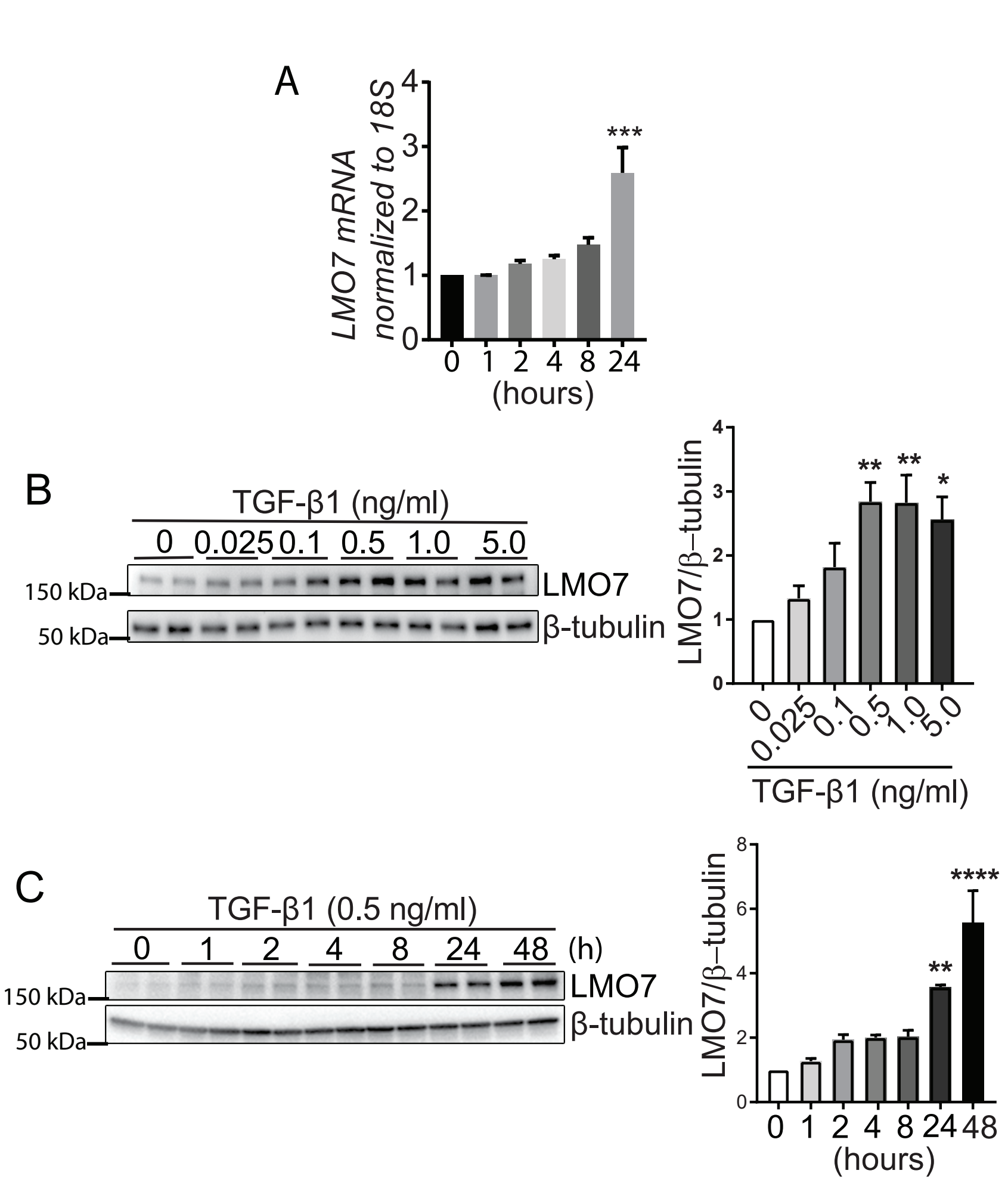
Immunoprecipitation. Cells were washed and scraped as above except using 1X Cell Lysis Buffer (Cell Signaling). After centrifugation, equal amounts of cell lysates from each sample were mixed with the appropriate amount of primary antibody with gentle rotation at 4°C for 18 h. Magnetic beads (Bio-Rad) were added and rotated for another 2 hrs to pull down antibody-protein complex. Beads were washed with 1x Cell Lysis Buffer twice and heated in the presence of loading buffer at 95°C for 5 min. Primary antibodies used for IP: LMO7 (sc-49827, 1: 500, Santa Cruz), c-JUN (9165, 1: 100, Cell Signaling), c-FOS (2250, 1: 100, Cell Signaling), normal Rabbit IgG (2729, Cell Signaling), normal Goat IgG (sc-2028, Santa Cruz). Western analysis was as above.

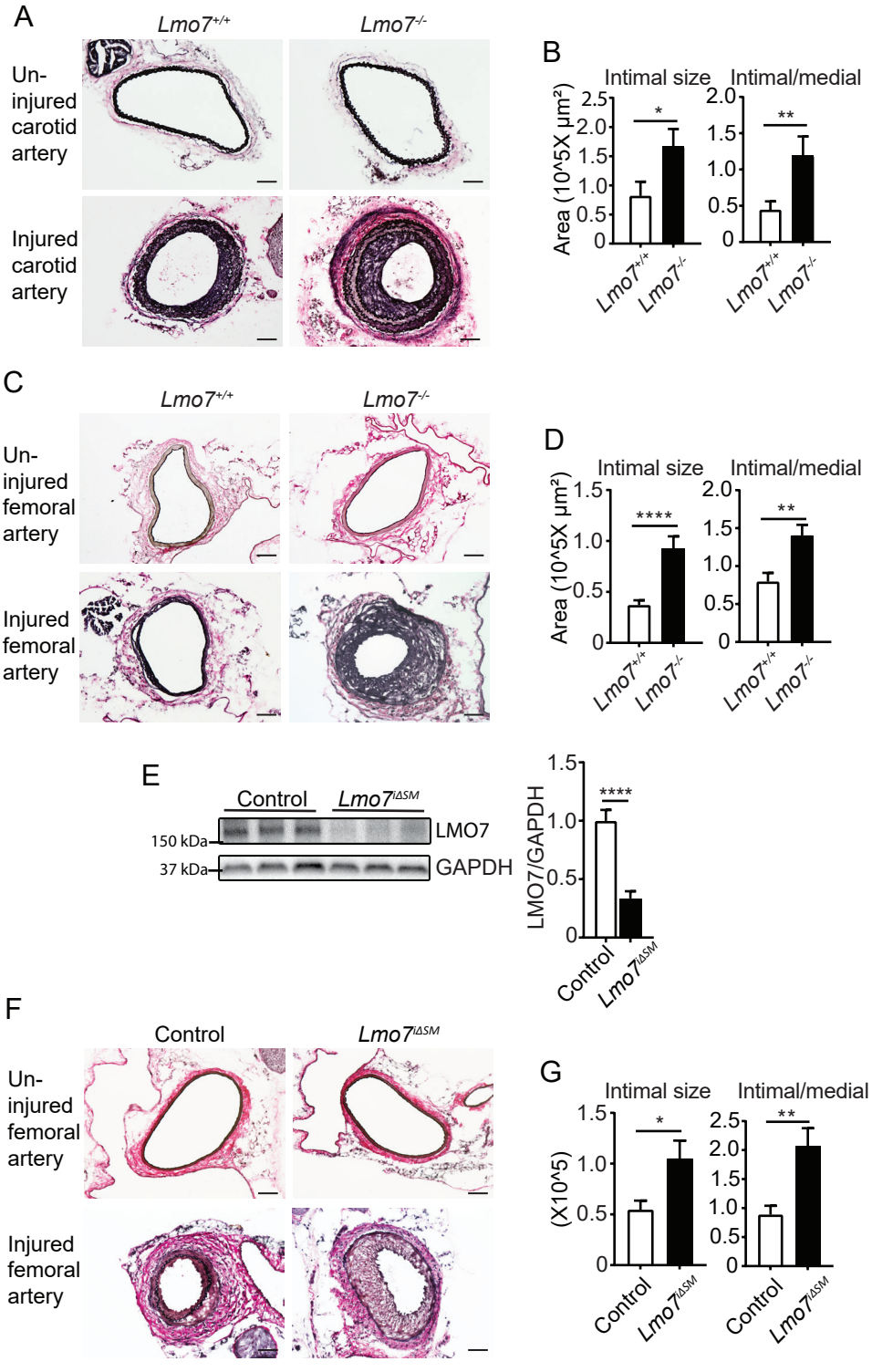
qRT-PCR. Cells were briefly washed with PBS and scraped in RLT buffer with 1% β-mercaptoethanol and RNA was extracted and purified using RNAeasy kit (Qiagen). cDNA was reverse transcribed using iScript Supermix (Bio-Rad) with equal amounts of RNA from each sample. cDNA was then used for quantitative real-time PCR analysis (Bio-Rad CFX96 Real-Time System) using Ssofast Evagreen Supermix (Bio-Rad). qPCR was analyzed using the delta delta CT method. Mouse qPCR primer sequences were obtained from Origene except *Lmo7*, *18S* and *Hprt1*. In-house designed qPCR primers for mouse *Lmo7* are (5' to 3'): forward - CCCGGGAACAAACCTGTCTT, reverse - TGGGCTACCTTGTTCGACTG. *Hprt1* qPCR primers are forward - TGGATACAGGCCAGACTTTGTT, reverse - CAGATTCAACTTGCGCTCATC. *18S* qPCR primers are forward - CGCCGCTAGAGGTGAAATTC, reverse - TTGGCAAATGCTTTCGCTC[1]. All samples were run in duplicate, and signals were normalized to the indicated housekeeping genes.

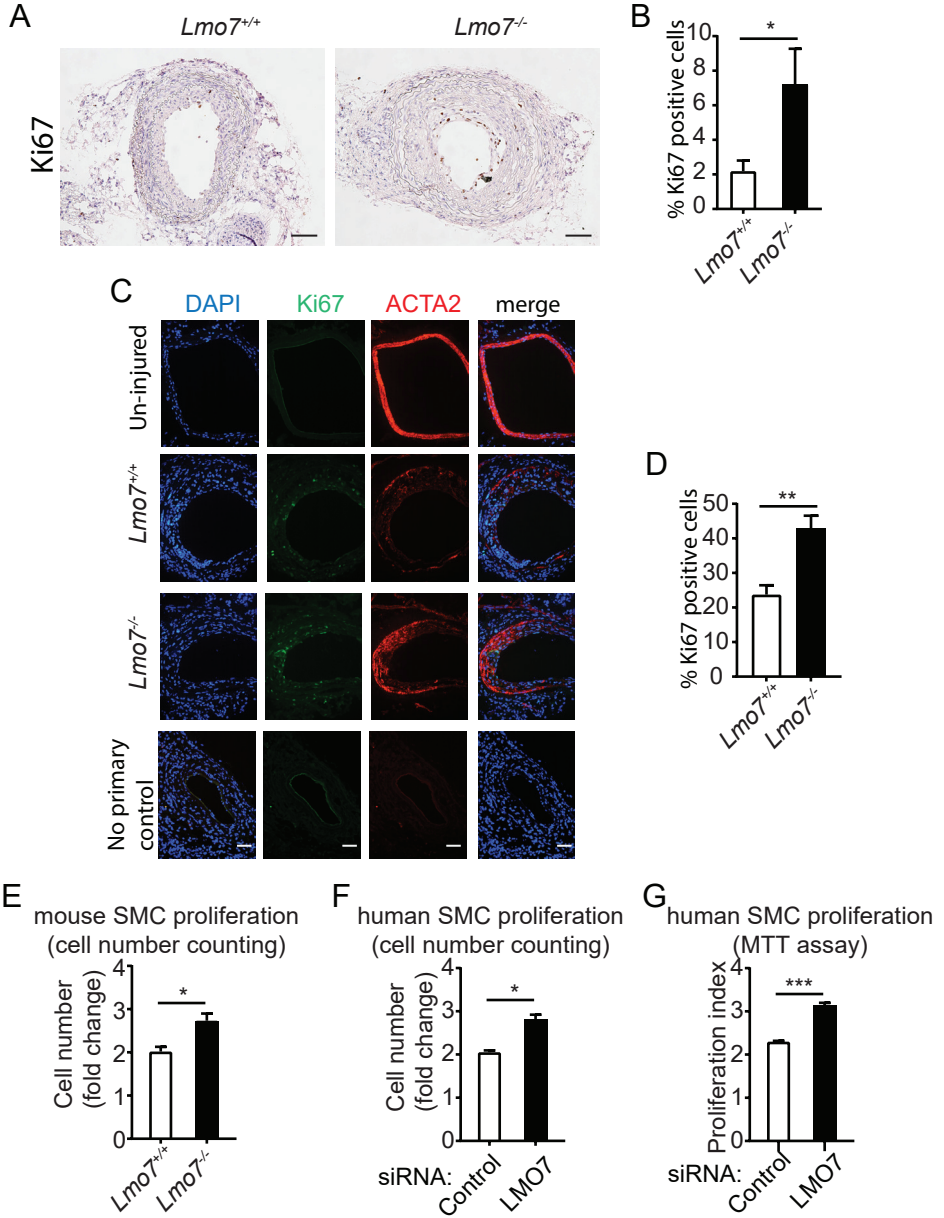
Chromatin immunoprecipitation-qRT-PCR. hCASMCs were fixed in plates with 1% formaldehyde and cross-linked protein-DNA complexes were prepared with SimpleChIP® Enzymatic Chromatin IP Kit (Cell Signaling). Equal volumes of protein-DNA complex from each sample was mixed with primary antibodies (c-JUN, 9165, 1: 100, Cell Signaling; c-FOS, 2250, 1: 100, Cell Signaling) with gentle rotation at 4°C for 18 h. Magnetic beads were added and rotated for another 2 hrs. Immunoprecipitates were eluted from the beads and treated with protease K to digest protein. DNA was then purified as per the manufacturer's instructions and analyzed in qRT-PCR assays using primers flanking the AP-1 binding sites on human *TGFB1* or *ITGB3* promoters. qPCR primers for human *TGFB1* promoter AP-1 site are (5' to 3'): forward - TTGTTTCCCAGCCTGACTCT, reverse - AAAGCGGGTGATCCAGATG[2]. In-house designed qPCR primers for human *ITGB3* promoter AP-1 site are forward - TCCCACCGCTCCCTCTC, reverse - TTCCTCCCCTCCGGCTTC. All samples were run in triplicate, and signals were normalized to preserved input control DNA (2% of that used for IP).

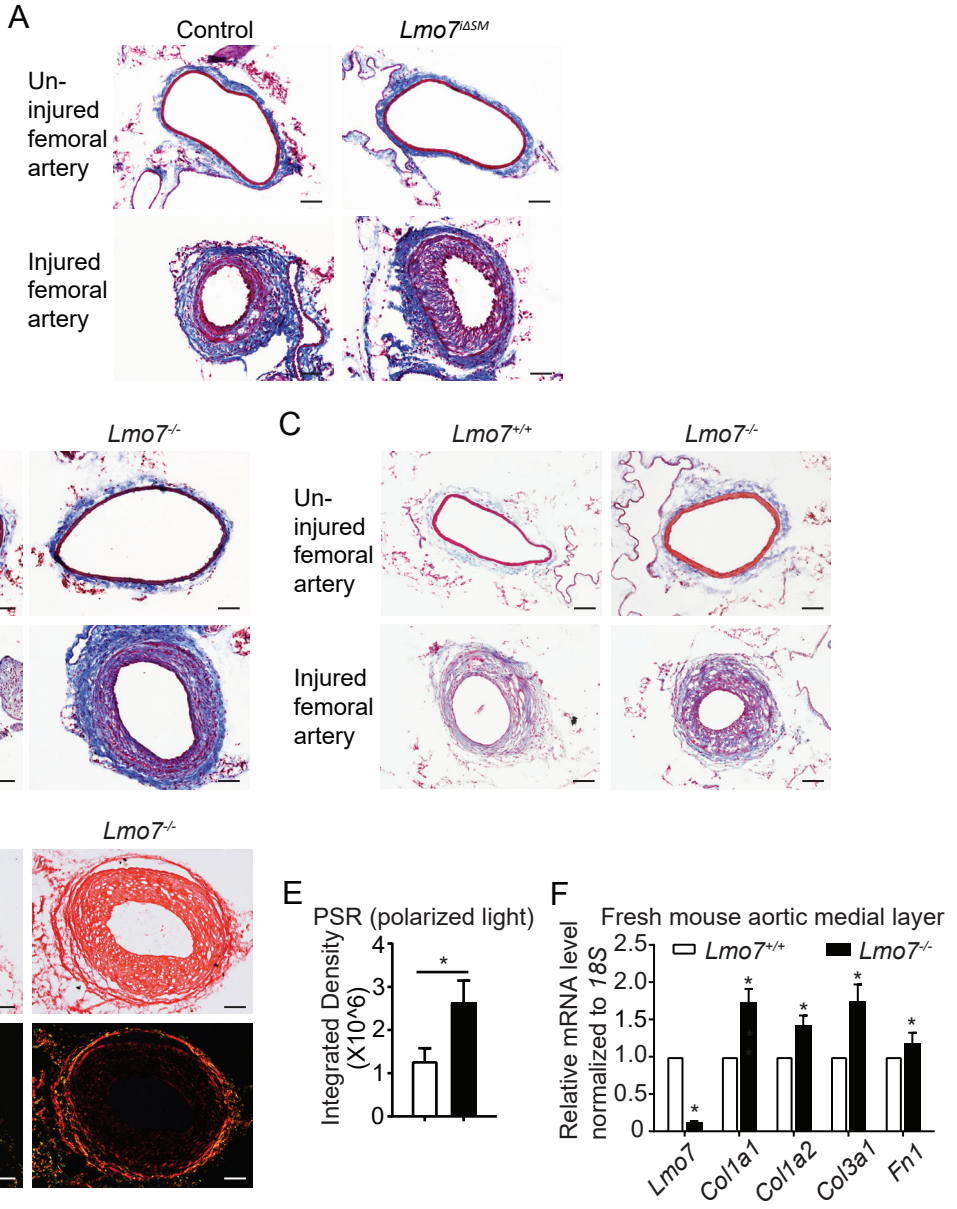
ELISA. Conditioned media with 0.5% FBS from cultured mouse SMCs was harvested and cleared by brief centrifugation. The secreted cytokines were concentrated with Amicon centrifugal filter units with a 3kDa cut-off (Millipore). 1/5 volume of 1N HCl was added to the concentrated media for 20 min to activate latent TGF-β and neutralized by and equal volume of 1N NaOH. Total TGF-β was then quantified using Mouse TGF-β DuoSet ELISA kit (R&D System). All samples were run in duplicate. Blank media with 0.5% FBS showed negligible signal.

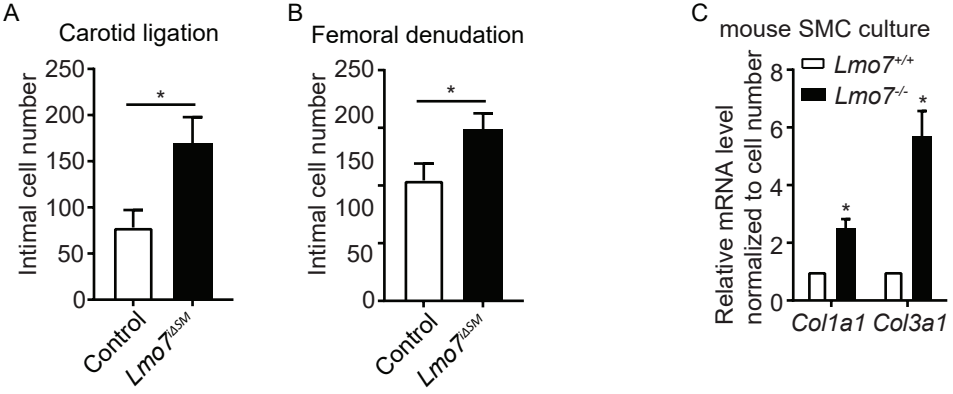
Proliferation – cell number counting and MTT assays. For cell number counting assays, 60,000 cells per well were plated in 6-well plates and cultured in full media for 72 hrs before trypsinization for counting using a Cellometer (Nexcelom). All samples were run in triplicate. For MTT assays, 2,000 cells were plated per well in 96-well plates, cultured in full media for 72 hrs, and analyzed using CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega). Eight biological replicates were performed. The same number of cells were seeded in another plate in parallel and assayed after ~8 hrs as an input control.

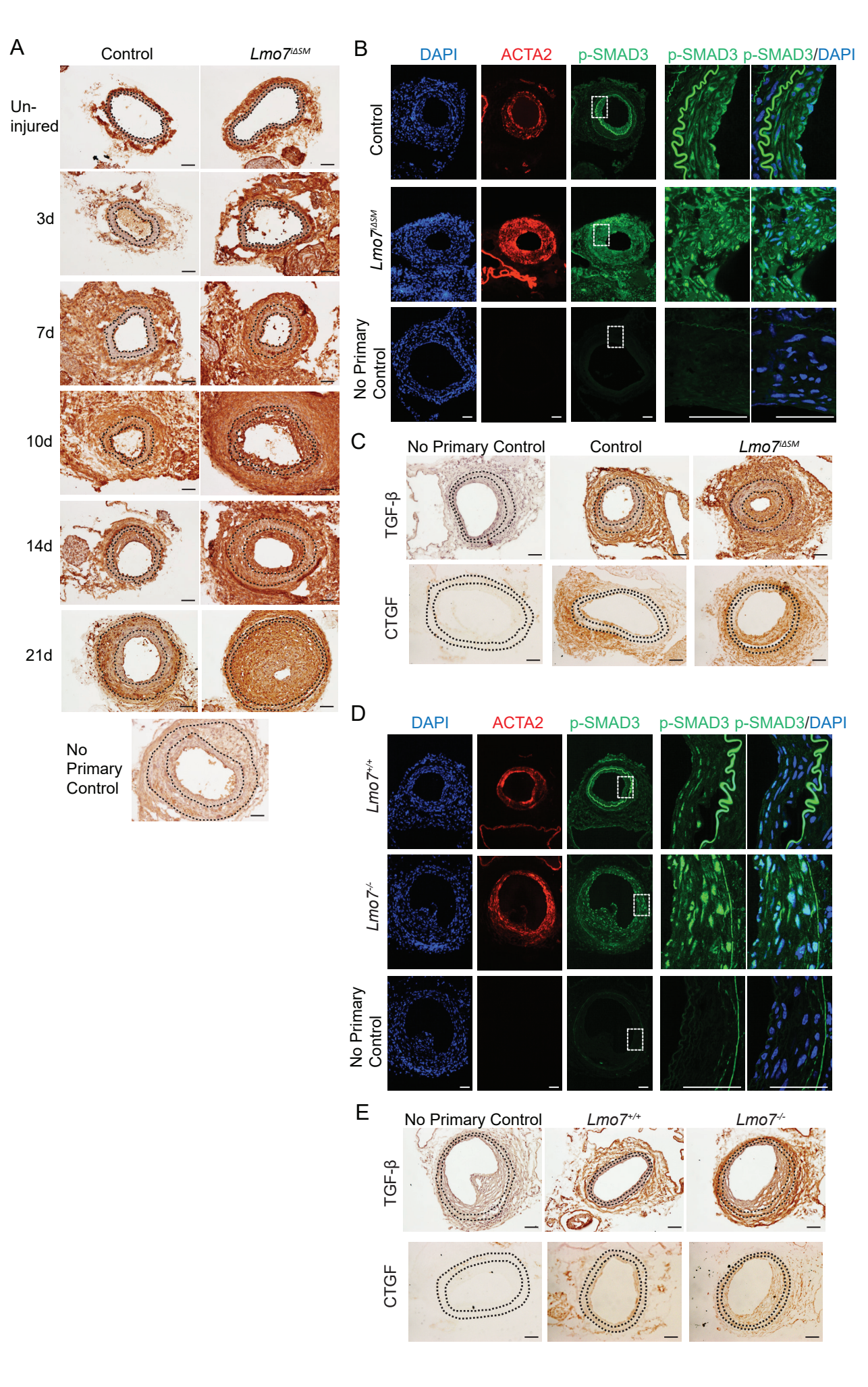

A
LMO7 mRNA normalized to 18S
0
1
2
3
4
***
0 1 2 4 8 24
(hours)
B
TGF-β1 (ng/ml)
0 0.025 0.1 0.5 1.0 5.0
150 kDa
LMO7
50 kDa
β-tubulin
LMO7/β–tubulin
0
1
2
3
4
**
**
*
0 0.025 0.1 0.5 1.0 5.0
TGF-β1 (ng/ml)
C
TGF-β1 (0.5 ng/ml)
0 1 2 4 8 24 48 (h)
150 kDa
LMO7
50 kDa
β-tubulin
LMO7/β–tubulin
0
2
4
6
8
**
****
0 1 2 4 8 24 48
(hours)

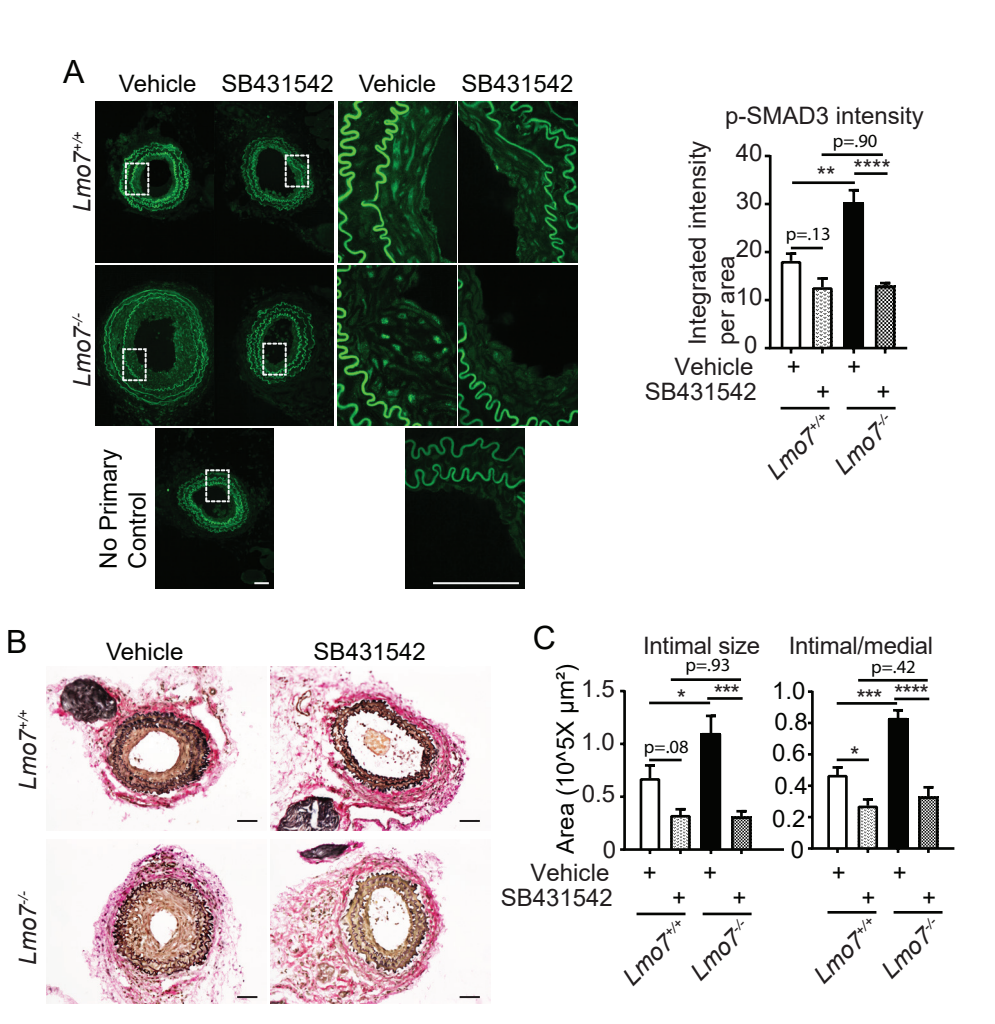

A
Lmo7+/+
Lmo7-/-
Un-injured carotid artery
Injured carotid artery
B
Intimal size
Intimal/medial
Area (10^5X µm²)
C
Un-injured femoral artery
Injured femoral artery
D
E
Control
Lmo7iΔSM
150 kDa
37 kDa
LMO7
GAPDH
LMO7/GAPDH
F
Control
Lmo7iΔSM
Un-injured femoral artery
Injured femoral artery
G
(X10^5)

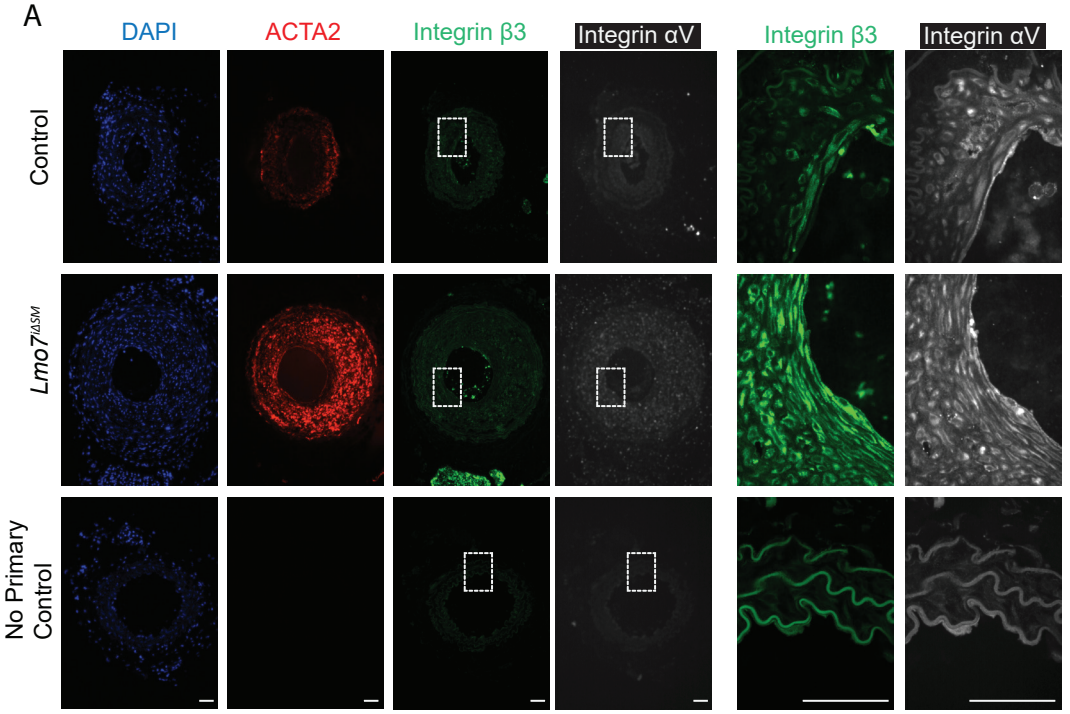

A
Lmo7+/+
Lmo7-/-
Ki67
B
% Ki67 positive cells
10
8
6
4
2
0
*
Lmo7+/+
Lmo7-/-
C
DAPI
Ki67
ACTA2
merge
Un-injured
Lmo7+/+
Lmo7-/-
No primary control
D
% Ki67 positive cells
50
40
30
20
10
0
**
Lmo7+/+
Lmo7-/-
E
mouse SMC proliferation
(cell number counting)
Cell number
(fold change)
4
3
2
1
0
*
Lmo7+/+
Lmo7-/-
F
human SMC proliferation
(cell number counting)
Cell number
(fold change)
4
3
2
1
0
*
siRNA:
Control
LMO7
G
human SMC proliferation
(MTT assay)
Proliferation index
4
3
2
1
0
***
siRNA:
Control
LMO7

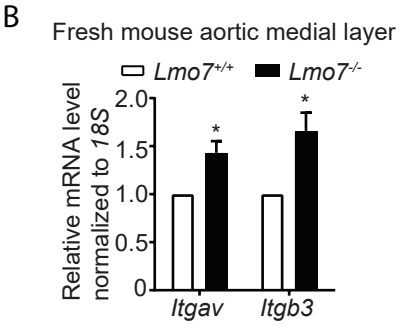

A
Control
Lmo7iΔSM
Un-injured femoral artery
Injured femoral artery
B
Lmo7+/+
Lmo7-/-
Un-injured carotid artery
Injured carotid artery
C
Lmo7+/+
Lmo7-/-
Un-injured femoral artery
Injured femoral artery
D
Lmo7+/+
Lmo7-/-
Phase contrast
Polarized light
E
PSR (polarized light)
Integrated Density (X10^6)
0
1
2
3
4
*
F
Fresh mouse aortic medial layer
Relative mRNA level normalized to 18S
Lmo7+/+
Lmo7-/-
0
0.5
1.0
1.5
2.0
2.5
*
Lmo7
Col1a1
Col1a2
Col3a1
Fn1

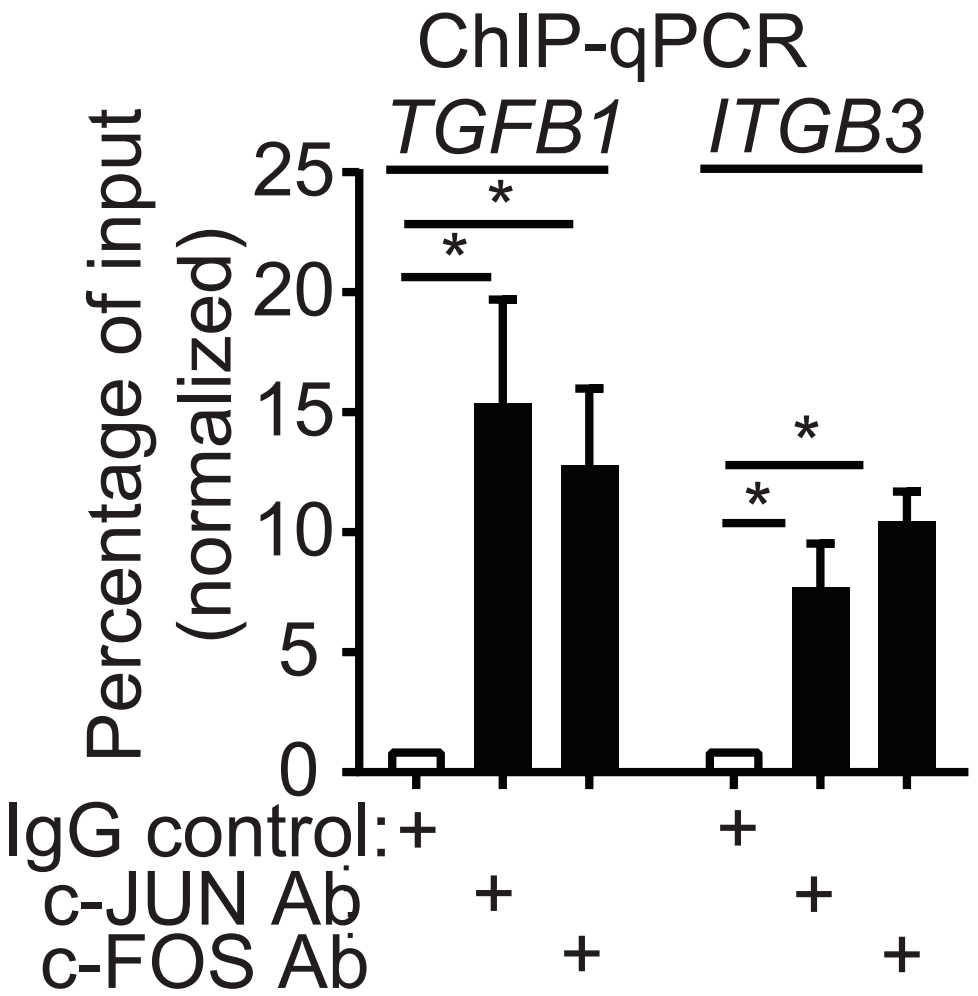

A
Carotid ligation
Intimal cell number
250
200
150
100
50
0
*
Control
Lmo7ΔSM
B
Femoral denudation
Intimal cell number
250
200
150
100
50
0
*
Control
Lmo7ΔSM
C
mouse SMC culture
Lmo7+/+
Lmo7-/-
Relative mRNA level normalized to cell number
8
6
4
2
0
*
*
Col1a1
Col3a1

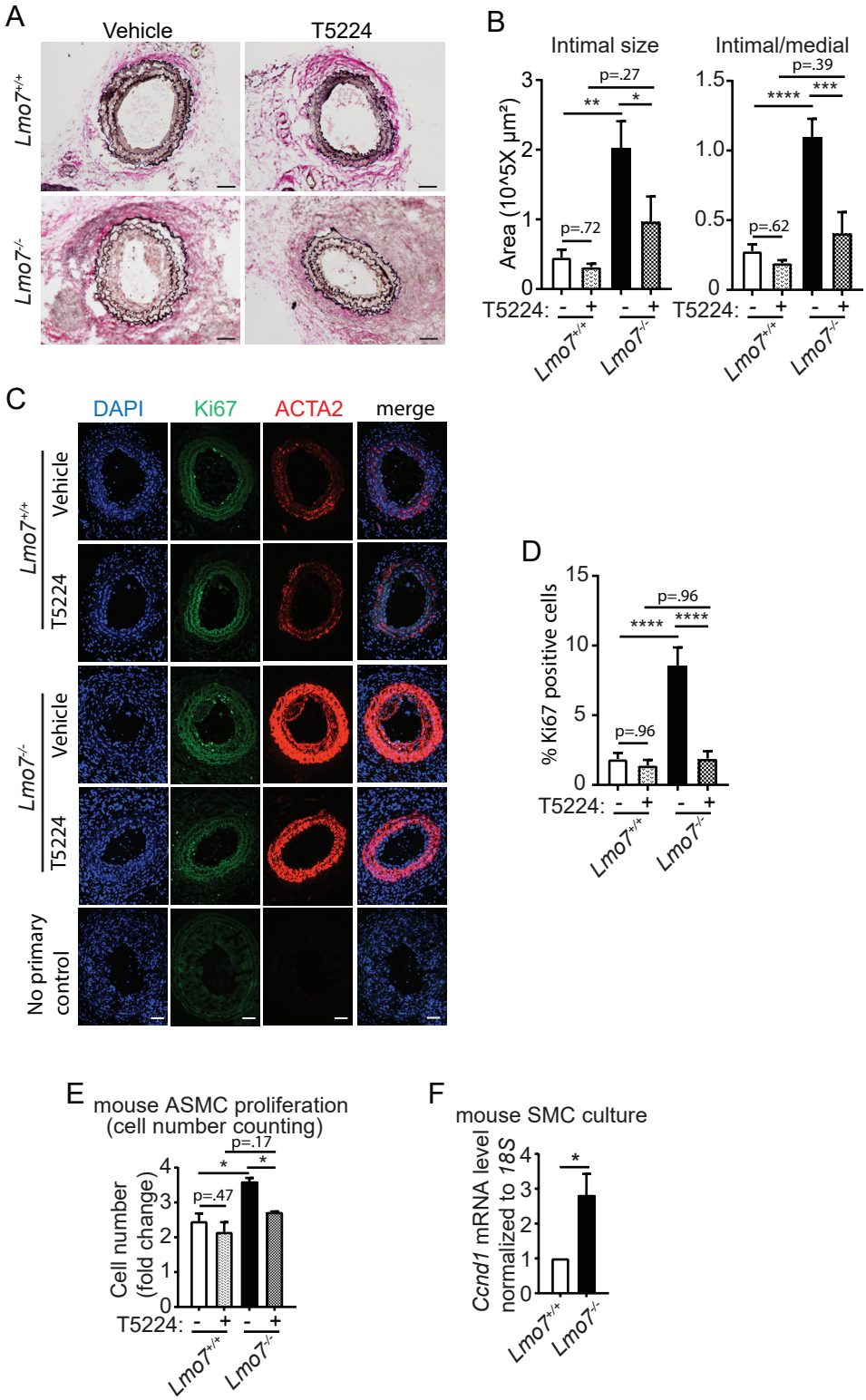

A
Control
Lmo7ΔSM
Un-injured
3d
7d
10d
14d
21d
No Primary Control
B
DAPI
ACTA2
p-SMAD3
p-SMAD3 p-SMAD3/DAPI
Control
Lmo7ΔSM
No Primary Control
C
No Primary Control
Control
Lmo7ΔSM
TGF-β
CTGF
D
DAPI
ACTA2
p-SMAD3
p-SMAD3 p-SMAD3/DAPI
Lmo7+/+
Lmo7-/-
No Primary Control
E
No Primary Control
Lmo7+/+
Lmo7-/-
TGF-β
CTGF


A
Vehicle
SB431542
Vehicle
SB431542
Lmo7+/+
Lmo7-/-
No Primary Control
p-SMAD3 intensity
Integrated intensity per area
40
30
20
10
0
p=.90
**
****
p=.13
Vehicle
SB431542
+
+
+
+
Lmo7+/+
Lmo7-/-
B
Vehicle
SB431542
Lmo7+/+
Lmo7-/-
C
Intimal size
Intimal/medial
Area (10^5X µm²)
1.5
1.0
0.5
0
p=.93
*
***
p=.08
p=.42
***
****
*
0.8
0.6
0.4
0.2
Vehicle
SB431542
+
+
+
+
Lmo7+/+
Lmo7-/-


A
DAPI
ACTA2
Integrin β3
Integrin αV
Integrin β3
Integrin αV
Control
Lmo7ΔSM
No Primary Control


B
Fresh mouse aortic medial layer
Lmo7+/+
Lmo7-/-
Relative mRNA level normalized to 18S
2.0
1.5
1.0
0.5
0
*
*
Itgav
Itgb3


ChIP-qPCR
TGFB1
ITGB3
Percentage of input
(normalized)
25
20
15
10
5
0
*
*
*
*
IgG control:
c-JUN Ab
c-FOS Ab
+
+
+
+
+
+


A
Vehicle
T5224
Lmo7+/+
Lmo7-/-
B
Intimal size
Intimal/medial
Area (10^5X µm²)
p=.27
**
*
p=.72
p=.39
****
***
p=.62
T5224: - + - +
Lmo7+/+
Lmo7-/-
C
DAPI
Ki67
ACTA2
merge
Lmo7+/+
Vehicle
T5224
Lmo7-/-
No primary control
D
% Ki67 positive cells
p=.96
****
p=.96
T5224: - + - +
Lmo7+/+
Lmo7-/-
E
mouse ASMC proliferation
(cell number counting)
Cell number (fold change)
p=.17
*
p=.47
T5224: - + - +
Lmo7+/+
Lmo7-/-
F
mouse SMC culture
Ccnd1 mRNA level normalized to 18S
*
Lmo7+/+
Lmo7-/-

Supplemental Figure Legends

Supplemental Figure 1 Time-dependent induction of LMO7 with TGF-β1 treatment. (A) Human CASMCs were treated with 0.5 ng/ml TGF-β1 for as the hours indicated and harvested for qPCR analysis of *LMO7* mRNA. (B, C) Human CASMCs were treated with (B) TGF-β1 at various doses for 24 hrs or (C) with 0.5 ng/ml TGF-β1 for the hours indicated, and harvested for western analysis. Densitometric quantification of LMO7 is shown at right (n=3 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

Supplemental Figure 2 Loss of LMO7 globally or in SMC exacerbates intimal hyperplasia in vascular injury models. (A, C) EVG staining of tissue sections from *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice (A) 28 days after carotid artery ligation or (C) 21 days after femoral artery injury. (B, D) Quantification of intimal size and intimal/medial ratio of (B) injured carotid arteries as in (A) (n=11 for *Lmo7*$^{+/+}$and n=10 for *Lmo7*$^{-/-}$) or (D) femoral arteries as in (C) (n=10 for *Lmo7*$^{+/+}$and n=15 for *Lmo7*$^{-/-}$). (E) Western blot analysis of aortic tissue with adventitia removed from *Lmo7*$^{i\Delta SM}$ mice following tamoxifen treatment and carotid ligation surgery. Quantification of LMO7 is shown on right (n=10). (F) EVG staining of tissue sections from control or *Lmo7*$^{i\Delta SM}$ mice 21 days after femoral artery injury. (G) Quantification of intimal size and intimal/medial ratio of injured femoral arteries (n=11 for *Lmo7*$^{+/+}$and n=9 for *Lmo7*$^{fl/fl}$). Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ****P < 0.0001. Scale bar = 50 μm.

Supplemental Figure 3 Loss of LMO7 enhances cell proliferation *in vivo* and *in vitro*. (A) Ki67 immunohistochemistry of cross-sections of injured arteries from *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice 28 days after carotid artery ligation. Scale bar = 50 μm. (B) Quantification of Ki67-positive cells normalized to total cell number in medial and intimal layers as in (A) (n=5 for *Lmo7*$^{+/+}$and n=7 for *Lmo7*$^{-/-}$). (C) Immunofluorescent images (DAPI, Ki67, ACTA2) of cross-sections of injured arteries from *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice 10 days after femoral artery injury. ("No primary control" samples are treated with secondary antibody only.) Scale bar = 50 μm. (D) Quantification of Ki67-positive cells normalized to total cell number (DAPI) in medial and intimal layers as in (C) (n=7 for *Lmo7*$^{+/+}$and *Lmo7*$^{-/-}$). (E) Proliferation (cell number counting) of mouse *Lmo7*$^{+/+}$ and *Lmo7*$^{-/-}$ SMCs (n=5 independent experiments). (F, G) Proliferation of control or LMO7 knockdown human CASMC assessed by cell number counting (F, n=3 independent experiments) or MTT assay (G, n=8 biological replicates). Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bar = 50 μm.

Supplemental Figure 4 Loss of LMO7 globally or in SMC enhances ECM deposition in vascular injury models. (A) Trichrome staining of cross-sections of contralateral uninjured and injured arteries of control or *Lmo7*$^{i\Delta SM}$ mice after femoral artery injury. (B, C) Trichrome staining of cross-sections of contralateral uninjured and injured arteries of *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice after (B) carotid ligation or (C) femoral artery injury. (D) Picro-Sirius Red staining of cross-sections of injured arteries of *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice after carotid ligation by phase contrast (upper panel) or polarized light (lower panel) microscopy. (E) Quantification of relative integrated intensity of signal in medial and intimal layers shown in polarized light images in (D) (n=6 for *Lmo7*$^{+/+}$and n=7 for *Lmo7*$^{-/-}$). (F) ECM gene expression was assessed in medial layers of freshly isolated mouse aortas (no injury) by qPCR (n=4 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05. Scale bar = 50 μm.

Supplemental Figure 5 Lmo7 effect on intimal cell number and ECM gene mRNA per cell. (A, B) Intimal cell numbers were counted per section from control or *Lmo7*$^{i\Delta SM}$ mice with (A) carotid ligation or (B) femoral denudation (n=4). (C) Mouse aortic SMCs were cultured and the

cell numbers were counted prior to lysis. The cells were then processed for qPCR analysis for *Col1a1* and *Col3a1* mRNA expression, which was normalized to cell number. Data represent ECM gene mRNA expression per cell in control vs *Lmo7*$^{-/-}$ SMC. (n=4 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05.

Supplemental Figure 6 Loss of LMO7 induces elevated TGF-β-SMAD3 signaling in injured femoral arteries. (A) TGF-β Immunohistochemistry of arterial sections of control and *Lmo7*$^{i\Delta SM}$ mice after carotid artery ligation for indicated durations post-injury. (B, C) Immunostaining of (B) p-SMAD3 (green) or ACTA2 (red) or (C) TGF-β in injured femoral artery sections from control and *Lmo7*$^{i\Delta SM}$ mice. (D, E) Immunostaining of (D) p-SMAD3 (green) or ACTA2 (red) or (E) TGF-β on injured femoral artery sections from *Lmo7*$^{+/+}$ and *Lmo7*$^{-/-}$ mice. ("No primary control" samples are treated with secondary antibody only.) Scale bar=50 μm.

Supplemental Figure 7 Administration of the TGFβ-R1/ALK5 inhibitor SB431542 attenuates TGF-β signaling and neointimal formation. (A, B) (A) p-SMAD3 Immunostaining or (B) EVG staining of cross-sections of injured arteries from *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice subjected to carotid ligation and daily IP injection with SB4341542 or Vehicle days 7-28 days post-injection. Scale bar=50 μm. p-SMAD3 staining intensity per cell area was measured and shown at right (n=4). (C) Quantification of intimal size and intimal/medial ratio of injured carotid arteries (n=6 for each group). Two-way ANOVA revealed a significant effect of LMO7 depletion and SB431542 treatment on p-SMAD3 staining intensity and intimal expansion. As determined by Two-way ANOVA, the magnitude of reduction by SB431542 treatment in *Lmo7*$^{-/-}$ mice was greater for p-SMAD3 (p=0.0048) and intimal/medial ratio (p=0.0062), indicating LMO7 regulates TGF-β signaling and intimal hyperplastic response via TGF-β receptor activity. Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Scale bar = 50 μm.

Supplemental Figure 8 Deficiency of LMO7 in smooth muscle induces expression of Integrin αV and β3 subunits. (A) Immunostaining of ACTA2 (red), β3 Integrin (green) and αV Integrin (white) in injured carotid artery sections from control and *Lmo7*$^{i\Delta SM}$ mice. Scale bar=50 μm. (B) qPCR analysis of *Itgav* and *Itgb3* genes from medial layers of freshly isolated mouse aortas (n=4 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05. Scale bar = 50 μm.

Supplemental Figure 9 Endogenous c-JUN and c-FOS bind to AP-1 sites in the *TGFB1* and *ITGB3* promoters. Chromatin immunoprecipitation (ChIP) assay in human CASMCs. DNA was immunoprecipitated with antibody to c-JUN or c-FOS (or IgG negative control) followed by qPCR analysis using primers flanking the AP-1 binding sites in *TGFB1* or *ITGB3* promoters (n=4 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05.

Supplemental Figure 10 The AP-1 inhibitor T5224 treatment inhibits intimal expansion and the hyper-proliferation of *Lmo7*$^{-/-}$ mouse SMCs. (A, C) (A) EVG or (C) Ki67 (green) and ACTA2 (red) staining of cross-sections of injured arteries from *Lmo7*$^{+/+}$ or *Lmo7*$^{-/-}$ mice subjected to carotid ligation and injection with T5224 or Vehicle daily post injury (as in Fig 4). Scale bar=50 μm. (B) Quantification of intimal size and intimal/medial ratio of injured carotid arteries (n=6 for each group). (D) Quantification of Ki67-positive cells normalized to total cell number (DAPI) in medial and intimal layers as in (C) (n=6 for each group). (E) Proliferation (cell number counting) of mouse *Lmo7*$^{+/+}$ and *Lmo7*$^{-/-}$ SMCs treated with 40 μM T5224 (n=3 independent experiments). Two-way ANOVA revealed a significant effect of LMO7 depletion and T5224 treatment on intimal expansion, percentage of Ki67+ cells and *in vitro* cell proliferation. As determined by Two-way ANOVA, the magnitude of reduction by T5524

treatment was greater in *Lmo7*$^{-/-}$ mice (intimal/medial ratio, p=0.0080, Ki67 staining p=0.0005), indicating LMO7 regulates intimal hyperplastic response and SMC proliferation via AP-1 activity. (F) Mouse SMCs were harvested for qPCR analysis of *Ccnd1* gene (n=3 independent experiments). Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Scale bar = 50 μm.

Supplemental References

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