SUPPLEMENTAL FIGURES

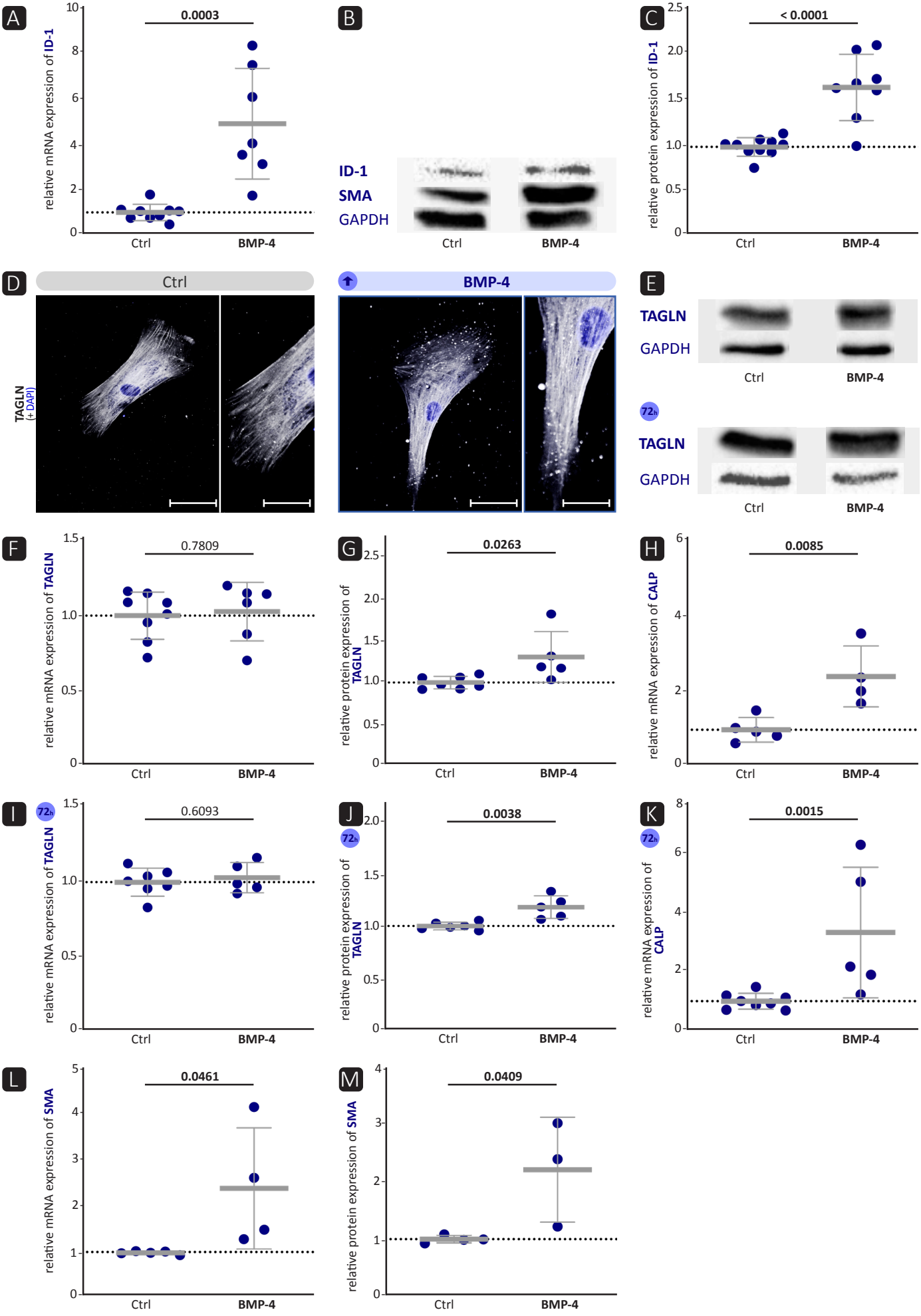
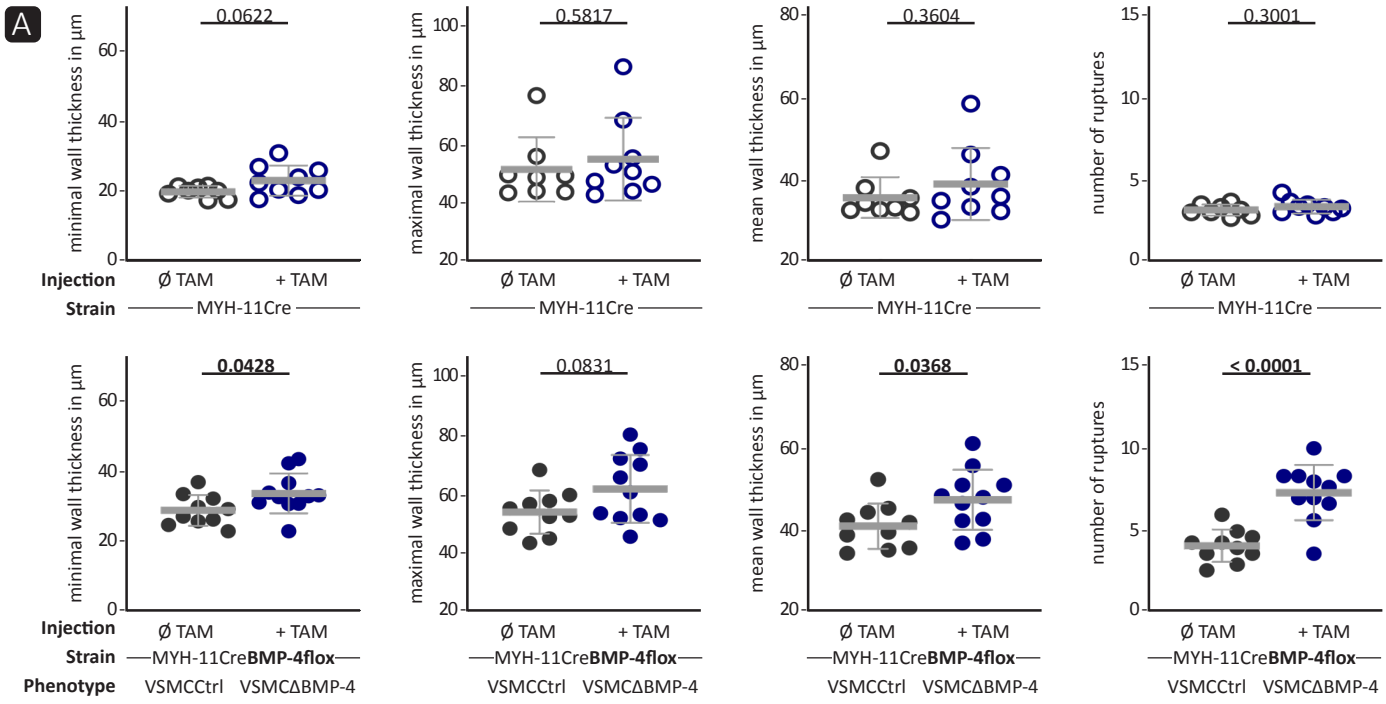
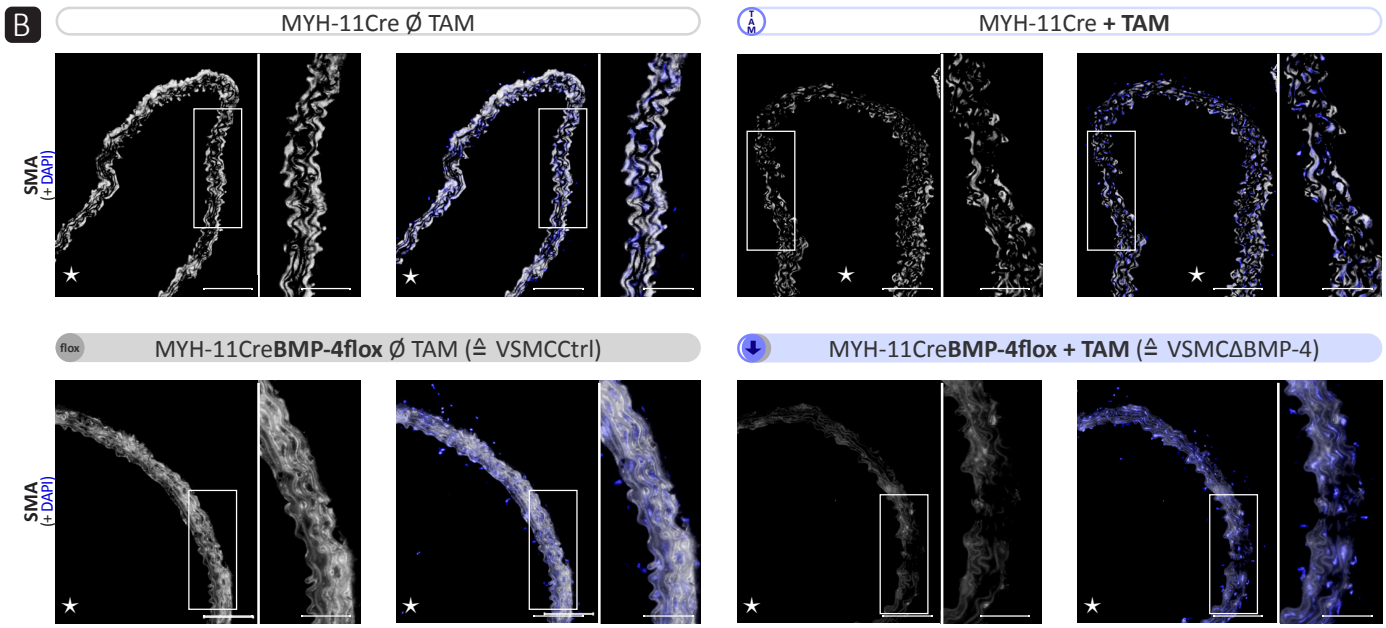

A
relative mRNA expression of ID-1
0.0003
Ctrl
BMP-4
B
ID-1
SMA
GAPDH
Ctrl
BMP-4
C
relative protein expression of ID-1
< 0.0001
Ctrl
BMP-4
D
Ctrl
BMP-4
TAGLN (+ DAPI)
E
TAGLN
GAPDH
Ctrl
BMP-4
72h
TAGLN
GAPDH
Ctrl
BMP-4
F
relative mRNA expression of TAGLN
0.7809
Ctrl
BMP-4
G
relative protein expression of TAGLN
0.0263
Ctrl
BMP-4
H
relative mRNA expression of CALP
0.0085
Ctrl
BMP-4
I
72h
relative mRNA expression of TAGLN
0.6093
Ctrl
BMP-4
J
72h
relative protein expression of TAGLN
0.0038
Ctrl
BMP-4
K
72h
relative mRNA expression of CALP
0.0015
Ctrl
BMP-4
L
relative mRNA expression of SMA
0.0461
Ctrl
BMP-4
M
relative protein expression of SMA
0.0409
Ctrl
BMP-4

Figure S1: Supplementary data to figure 1. Human VSMC were either incubated in 0.4% FBS/EBM (Ctrl) or stimulated with BMP-4 (40 ng/mL). Experiments were performed after 48 hours of treatment unless otherwise indicated. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. (**A+F+H+I+K+L**) qRT-PCR of (A) ID-1 (n≥7), (F) TAGLN (n≥6), (H) CALP (n≥4), (I) TAGLN (n≥5), (K) CALP (n≥5) and (L) SMA (n≥4). (**B+C+E+G+J+M**) Western blot analysis of (C) ID-1 (n≥8), (G) TAGLN (n≥5), (J) TAGLN (n≥5) and (M) SMA (n≥3) with GAPDH as loading control. Representative blots in B and E. (**D**) Representative photomicrographs of immunofluorescence staining for TAGLN after 72 hours. Nuclei were stained with DAPI (blue). Left image: scale bar 50 μm; right image: zoom in to 200%, scale bar 25 μm.


A
minimal wall thickness in µm
maximal wall thickness in µm
mean wall thickness in µm
number of ruptures
0.0622
0.5817
0.3604
0.3001
0.0428
0.0831
0.0368
< 0.0001
Injection
Ø TAM
+ TAM
Strain
MYH-11Cre
MYH-11CreBMP-4flox
Phenotype
VSMCCtrl
VSMCΔBMP-4


B
MYH-11Cre Ø TAM
MYH-11Cre + TAM
MYH-11CreBMP-4flox Ø TAM (≙ VSMCCtrl)
MYH-11CreBMP-4flox + TAM (≙ VSMCΔBMP-4)
SMA
(+ DAPI)

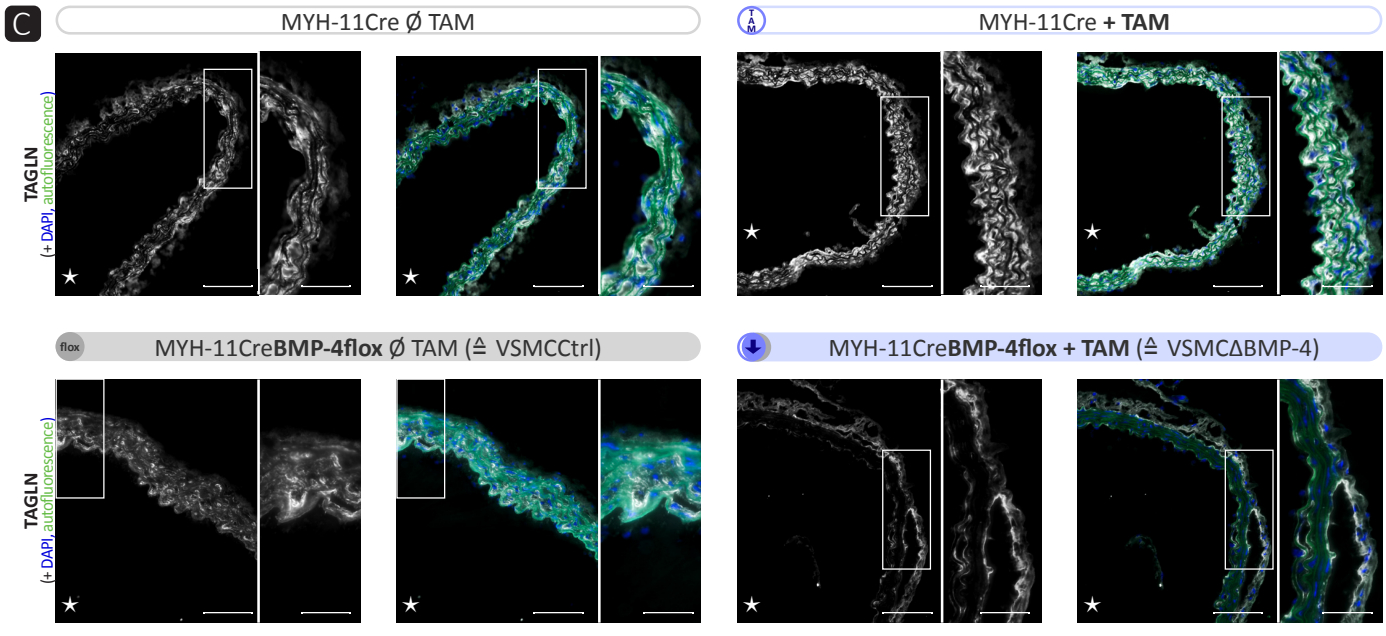
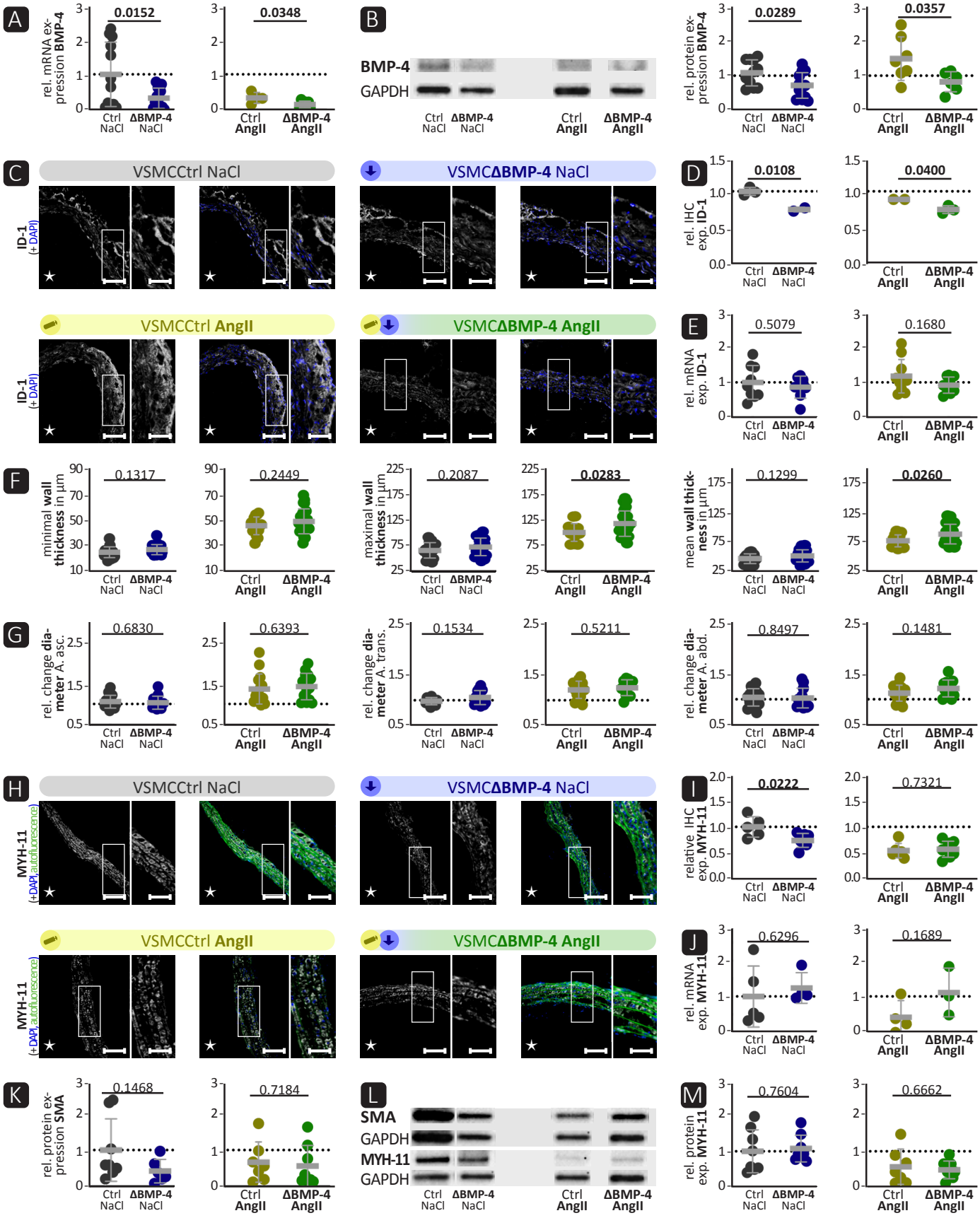

C
MYH-11Cre Ø TAM
MYH-11Cre + TAM
MYH-11CreBMP-4flox Ø TAM (≙ VSMCCtrl)
MYH-11CreBMP-4flox + TAM (≙ VSMCΔBMP-4)
TAGLN
(+ DAPI, autofluorescence)

Figure S2: Analysis of the impact of Cre-activity in VSMC in the MYH-11Cre^{ERT} model. Six to eight weeks old male MYH-11Cre or MYH-11CreBMP-4flox mice, were injected intraperitoneally with tamoxifen (+ TAM) over 5 days. At 16-18 weeks after the last injection, the aorta was isolated. Mice of the same genotype without tamoxifen injection (∅ TAM) served as controls. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. (**A**) Quantification of the Elastica van Gieson staining of thoracic aortic tissue sections. Analysis of minimal, maximal and mean wall thickness and number of ruptures (n≥7). (**B+C**) Representative photomicrographs of cross-sections of thoracic aortic tissue with immunostaining against (B) SMA, (C) TAGLN. Left image: scale bar 100 μm; right image: zoomed to 200% of the highlighted area, scale bar 50 μm. Vascular lumen is marked with an asterisk. DAPI stain visualizes nuclei (blue). Elastic fibres in green autofluorescence.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0.0348
Ctrl NaCl
ΔBMP-4 NaCl
Ctrl AngII
ΔBMP-4 AngII
B
BMP-4
GAPDH
rel. protein expression BMP-4
0.0289
0.0357
C
VSMCCtrl NaCl
VSMCΔBMP-4 NaCl
VSMCCtrl AngII
VSMCΔBMP-4 AngII
ID-1 (+ DAPI)
D
rel. IHC exp. ID-1
0.0108
0.0400
E
rel. mRNA exp. ID-1
0.5079
0.1680
F
minimal wall thickness in µm
0.1317
0.2449
maximal wall thickness in µm
0.2087
0.0283
mean wall thickness in µm
0.1299
0.0260
G
rel. change diameter A. asc.
0.6830
0.6393
rel. change diameter A. trans.
0.1534
0.5211
rel. change diameter A. abd.
0.8497
0.1481
H
MYH-11 (+DAPI, autofluorescence)
I
relative IHC exp. MYH-11
0.0222
0.7321
J
rel. mRNA exp. MYH-11
0.6296
0.1689
K
rel. protein expression SMA
0.1468
0.7184
L
SMA
GAPDH
MYH-11
GAPDH
M
rel. protein exp. MYH-11
0.7604
0.6662

Figure S3: Supplementary data to figure 2 A subcutaneous pump with AngII (flow rate = 1 μg/kg/min) or 0.9% NaCl was implanted in phenotypic wild-type littermates VSMCCtrl (Ctrl) or VSMCΔBMP-4 mice (ΔBMP-4) 28 days before the thoracic aorta was isolated. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. Rel.=relative; exp.=expression. (**A+E+J**) qRT-PCR of (A) BMP-4 (n≥4), (E) ID-1 (n≥4), (J) MYH-11 (n≥4). (**B+K+L+M**) Western blots analyses of (B) BMP-4 (n≥4), (K) SMA (n≥5), (M) MYH-11 (n≥5) with GAPDH as loading control. Representative blots in B and L. (**C+H**) Representative photomicrographs of immunostaining against (C) ID-1, (H) MYH-11. Vascular lumen is marked with an asterisk. Left image: scale bar 100 μm; right image: zoomed to 200% of the highlighted area, scale bar 50 μm. DAPI stain visualized nuclei (blue) in immunohistological stainings. Autofluorescence of elastic fibers is in green when indicated in the figure. (**D+I**) Quantification of mean density of IHC of (D) ID-1 (n≥3), (I) MYH-11 (n≥3). (**F**) Change over 28 days in minimal, maximal and mean wall thickness (n≥10). (**G**) Relative change over 28 days in aortic diameter of the aorta ascendens (A. asc.), aorta transversalis (A. trans.) and aorta abdominalis (A. abd.) (n≥17).

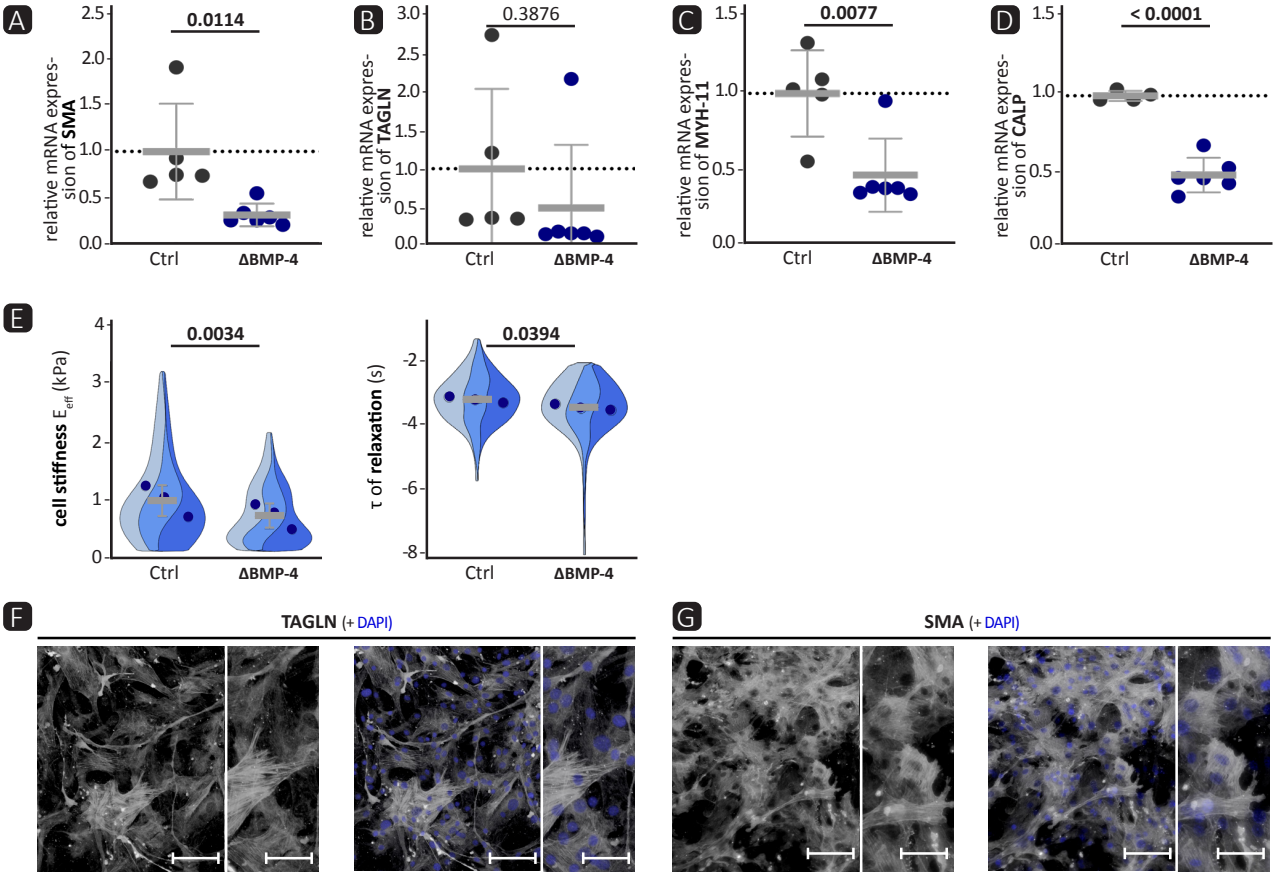

A
0.0114
relative mRNA expression of SMA
Ctrl
ΔBMP-4
B
0.3876
relative mRNA expression of TAGLN
C
0.0077
relative mRNA expression of MYH-11
D
< 0.0001
relative mRNA expression of CALP
E
0.0034
cell stiffness E_eff (kPa)
0.0394
τ of relaxation (s)
F
TAGLN (+ DAPI)
G
SMA (+ DAPI)

Figure S4: Isolated aortic mouse VSMC with BMP-4 deficiency show a lower expression of contractile markers. Four to six weeks old male VSMCCtrl (Ctrl) or VSMCΔBMP-4 mice (ΔBMP-4) were used. At 10 days after the last injection, the aorta was isolated and VSMC were extracted by enzymatic digestion. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. (**A+B+C+D**) qRT-PCR of (A) SMA (n≥5), (B) TAGLN (n≥5), (C) MYH-11 (n≥5) and (D) CALP (n≥4) of isolated murine VSMC. (**E**) Nanoindentation: Effective Young's modulus E_{eff} and time constant τ of relaxation of isolated VSMCΔBMP-4 vs Ctrl (3 experiments, each with n≥13). (**F+G**) Representative photomicrographs of immunostaining against (F) SMA and (G) TAGLN to verify culture purity of isolated murine VSMC. Left image: scale bar 100 μm; right image: zoom in to 200%, scale bar 50 μm. Nuclei were stained with DAPI (blue).

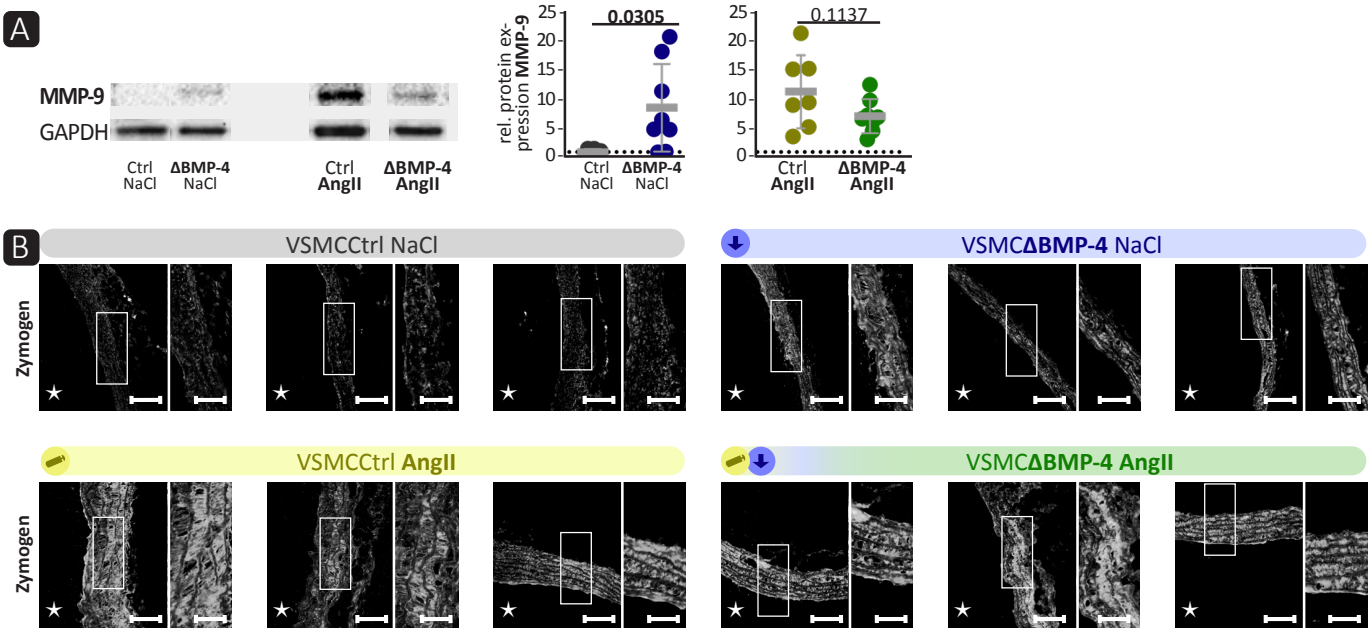

A
MMP-9
GAPDH
Ctrl NaCl
ΔBMP-4 NaCl
Ctrl AngII
ΔBMP-4 AngII
rel. protein expression MMP-9
0.0305
0.1137
B
VSMCCtrl NaCl
VSMCΔBMP-4 NaCl
VSMCCtrl AngII
VSMCΔBMP-4 AngII
Zymogen
Zymogen

Figure S5: Supplementary data to figure 3 A subcutaneous pump with AngII (flow rate = 1 μg/kg/min) or 0.9% NaCl was implanted in phenotypic wild-type littermates VSMCCtrl (Ctrl) or VSMCΔBMP-4 mice (ΔBMP-4) 28 days before the thoracic aorta was isolated. (**A**) Western blot analysis of MMP-9 (n≥7) with GAPDH as loading control. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. (**B**) Representative photomicrographs of zymogen assay from three different mice. Vascular lumen is marked with an asterisk. Left image: scale bar 100 μm; right image: zoomed to 200% of the highlighted area, scale bar 50 μm. DAPI stain visualized nuclei (blue) in immunohistological stainings.

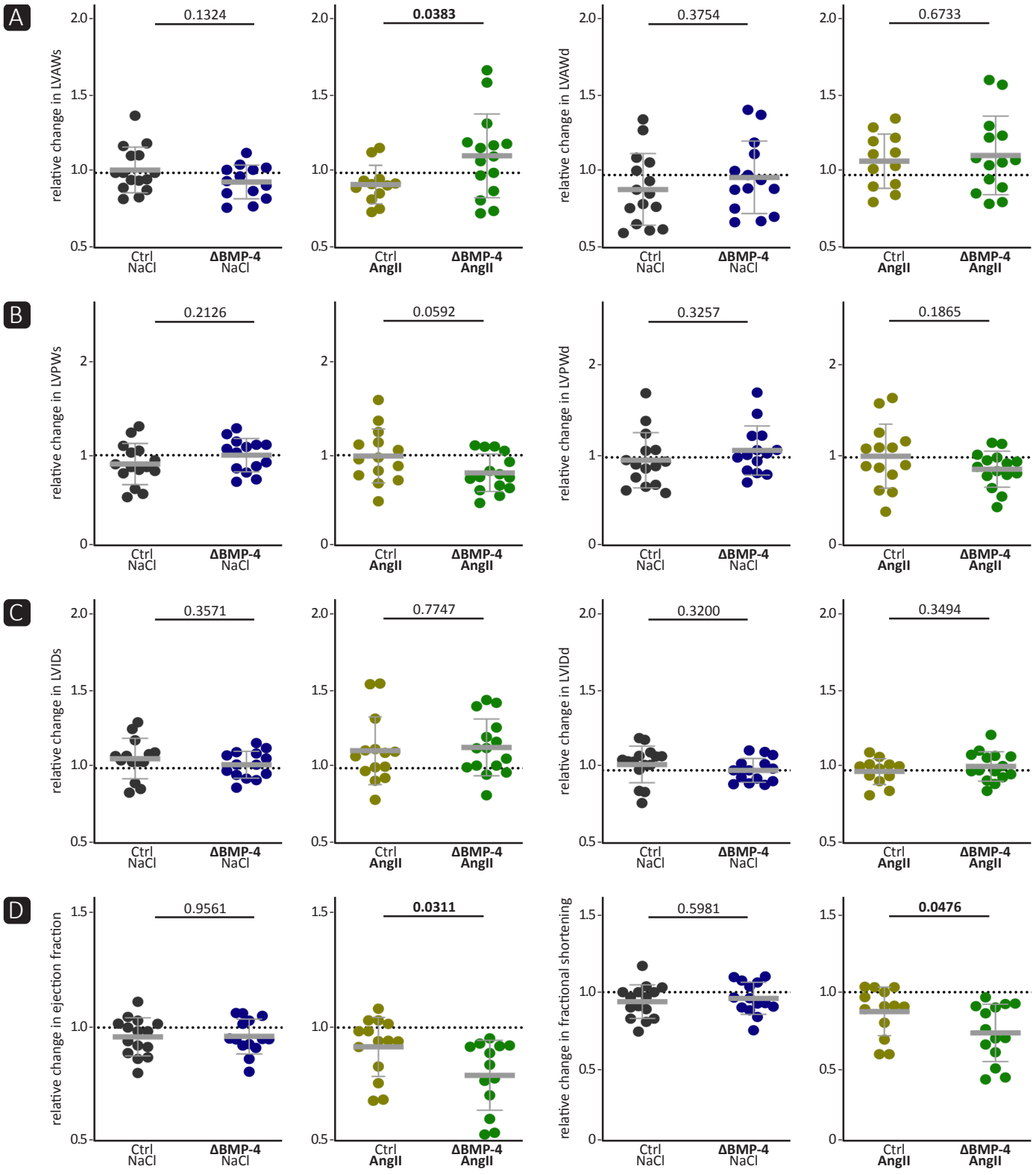

A
relative change in LVAWs
0.1324
0.0383
relative change in LVAWd
0.3754
0.6733
B
relative change in LVPWs
0.2126
0.0592
relative change in LVPWd
0.3257
0.1865
C
relative change in LVIDs
0.3571
0.7747
relative change in LVIDd
0.3200
0.3494
D
relative change in ejection fraction
0.9561
0.0311
relative change in fractional shortening
0.5981
0.0476
Ctrl NaCl
ΔBMP-4 NaCl
Ctrl AngII
ΔBMP-4 AngII

Figure S6: Echocardiographic studies analysing the effect of BMP-4 deficiency on cardiac function with and without Angiotensin II influence. A subcutaneous pump with AngII (flow rate = 1 μg/kg/min) was implanted in phenotypic wild-type littermates VSMCCtrl (Ctrl) or VSMCΔBMP-4 mice (ΔBMP-4) and echocardiography was performed. After 28 days a second echo was performed, and the heart was isolated. Data are presented as mean±SD and comparisons were calculated by an unpaired Student's t-test; p-values as indicated. (**A**) Relative change over 28 days of left ventricular anterior wall thickness end systole (LVAWs) and end diastole (LVAWd) (n≥15). (**B**) Relative change over 28 days of left ventricular posterior wall thickness end systole (LVPWs) and end diastole (LVPWd) (n≥15). (**C**) Relative change over 28 days of left ventricular internal diameter end systole (LVIDs) and end diastole (LVIDd) (n≥15). (**D**) The ejection fraction (EF) and fractional shortening (FS) were calculated from LVIDs and LVIDd: EF (%) = $((LVIDd^3 – LVIDs^3) / LVIDd^3) * 100$; FS (%) = ((LVIDd – LVIDs) / LVIDd) * 100. Relative change over 28 days is shown (n≥16).

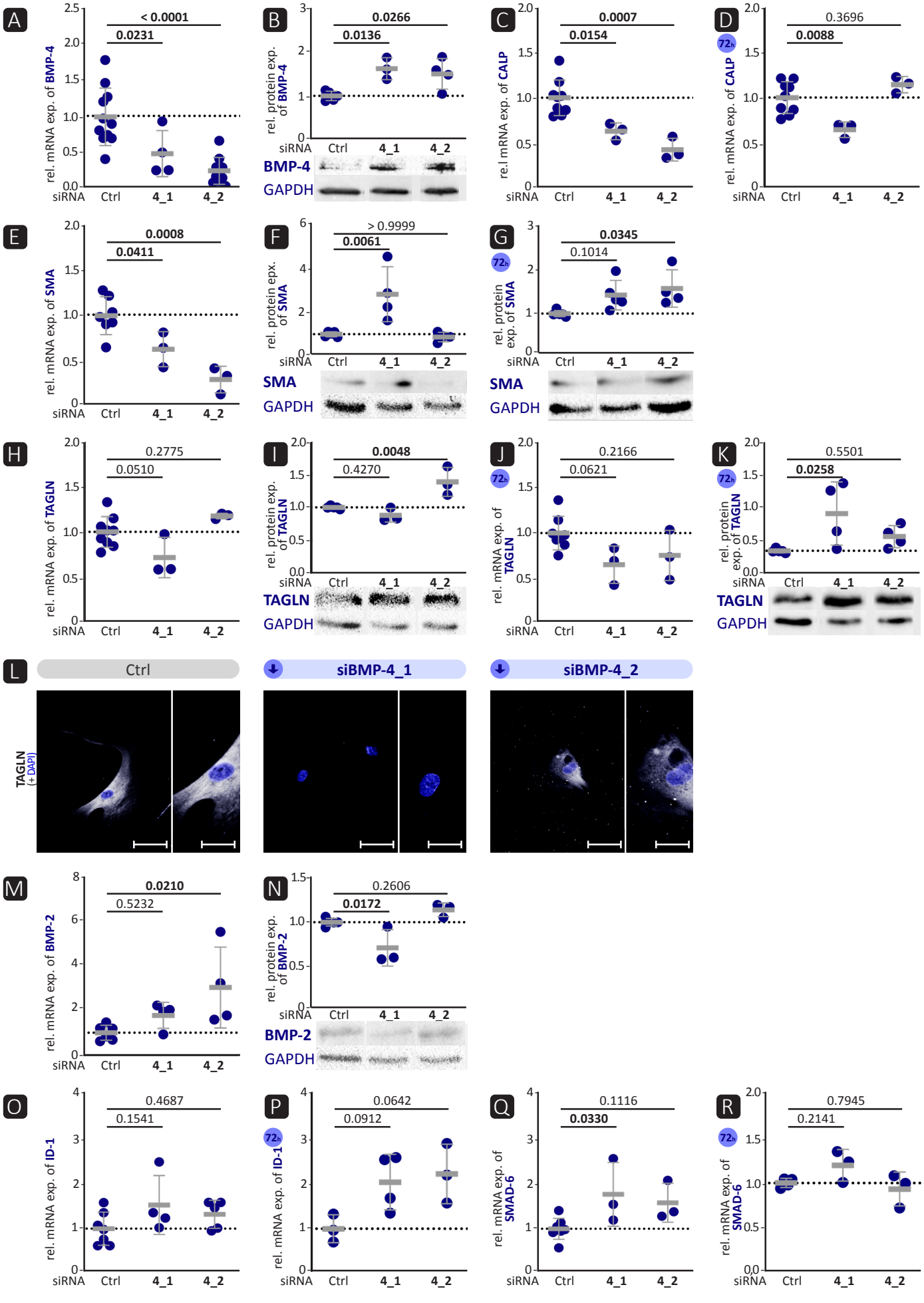

A
rel. mRNA exp. of BMP-4
< 0.0001
0.0231
siRNA Ctrl 4_1 4_2
B
rel. protein exp. of BMP-4
0.0266
0.0136
siRNA Ctrl 4_1 4_2
BMP-4
GAPDH
C
rel. mRNA exp. of CALP
0.0007
0.0154
siRNA Ctrl 4_1 4_2
D
72h
rel. mRNA exp. of CALP
0.3696
0.0088
siRNA Ctrl 4_1 4_2
E
rel. mRNA exp. of SMA
0.0008
0.0411
siRNA Ctrl 4_1 4_2
F
rel. protein exp. of SMA
> 0.9999
0.0061
siRNA Ctrl 4_1 4_2
SMA
GAPDH
G
72h
rel. protein exp. of SMA
0.0345
0.1014
siRNA Ctrl 4_1 4_2
SMA
GAPDH
H
rel. mRNA exp. of TAGLN
0.2775
0.0510
siRNA Ctrl 4_1 4_2
I
rel. protein exp. of TAGLN
0.0048
0.4270
siRNA Ctrl 4_1 4_2
TAGLN
GAPDH
J
72h
rel. mRNA exp. of TAGLN
0.2166
0.0621
siRNA Ctrl 4_1 4_2
K
72h
rel. protein exp. of TAGLN
0.5501
0.0258
siRNA Ctrl 4_1 4_2
TAGLN
GAPDH
L
Ctrl
siBMP-4_1
siBMP-4_2
TAGLN (+ DAPI)
M
rel. mRNA exp. of BMP-2
0.0210
0.5232
siRNA Ctrl 4_1 4_2
N
rel. protein exp. of BMP-2
0.2606
0.0172
siRNA Ctrl 4_1 4_2
BMP-2
GAPDH
O
rel. mRNA exp. of ID-1
0.4687
0.1541
siRNA Ctrl 4_1 4_2
P
72h
rel. mRNA exp. of ID-1
0.0642
0.0912
siRNA Ctrl 4_1 4_2
Q
rel. mRNA exp. of SMAD-6
0.1116
0.0330
siRNA Ctrl 4_1 4_2
R
72h
rel. mRNA exp. of SMAD-6
0.7945
0.2141
siRNA Ctrl 4_1 4_2

Figure S7: Supplementary data to figure 4. BMP-4 deficiency was induced in VSMC with either of two specific siRNA targeted against BMP-4 (4_1, 4_2). Scrambled siRNA was used as a control (Ctrl). Experiments were performed 48 hours post-siRNA transfection unless otherwise indicated. Data are presented as mean±SD and comparisons were calculated by an ordinary one-way ANOVA-test with Bonferroni correction for multiple testing; p-values as indicated. Rel.=relative; exp.=expression. (**A+C+D+E+H+J+M+O+P+Q+R**) qRT-PCR of (A) BMP-4 (n≥4), (C+D) CALP (n≥3), (E) SMA (n≥3), (H+J) TAGLN (n≥3), (M) BMP-2 (n≥4), (O+P) ID-1 (n≥4), (Q+R) SMAD-6 (n≥3). (**B+F+G+I+K+N**) Western blot analysis of (B) BMP-4 (n≥3), (F+G) SMA (n≥4), (I+K) TAGLN (n≥3), (N) BMP-2 (n≥3) with GAPDH as loading control. (**L**) Representative photomicrographs of immunostaining against TAGLN 72 hours *post* transfection. Left image: scale bar 50 μm; right image: zoom in to 200%, scale bar 25 μm.

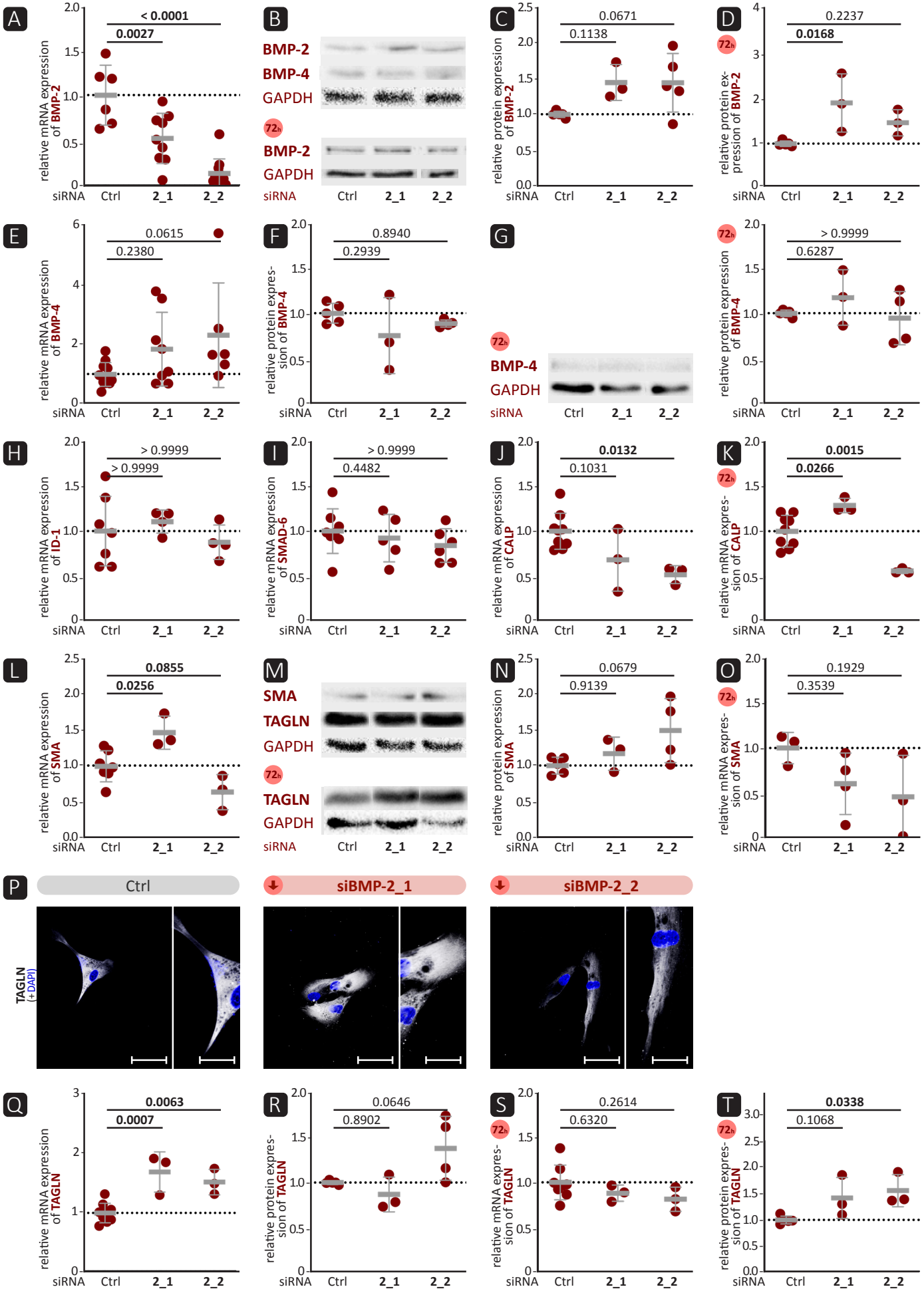

A
relative mRNA expression of BMP-2
< 0.0001
0.0027
siRNA Ctrl 2_1 2_2
B
BMP-2
BMP-4
GAPDH
72h
BMP-2
GAPDH
siRNA Ctrl 2_1 2_2
C
relative protein expression of BMP-2
0.0671
0.1138
siRNA Ctrl 2_1 2_2
D
72h
relative protein expression of BMP-2
0.2237
0.0168
siRNA Ctrl 2_1 2_2
E
relative mRNA expression of BMP-4
0.0615
0.2380
siRNA Ctrl 2_1 2_2
F
relative protein expression of BMP-4
0.8940
0.2939
siRNA Ctrl 2_1 2_2
G
72h
BMP-4
GAPDH
siRNA Ctrl 2_1 2_2
72h
relative protein expression of BMP-4
> 0.9999
0.6287
siRNA Ctrl 2_1 2_2
H
relative mRNA expression of ID-1
> 0.9999
> 0.9999
siRNA Ctrl 2_1 2_2
I
relative mRNA expression of SMAD-6
> 0.9999
0.4482
siRNA Ctrl 2_1 2_2
J
relative mRNA expression of CALP
0.0132
0.1031
siRNA Ctrl 2_1 2_2
K
72h
relative mRNA expression of CALP
0.0015
0.0266
siRNA Ctrl 2_1 2_2
L
relative mRNA expression of SMA
0.0855
0.0256
siRNA Ctrl 2_1 2_2
M
SMA
TAGLN
GAPDH
72h
TAGLN
GAPDH
siRNA Ctrl 2_1 2_2
N
relative protein expression of SMA
0.0679
0.9139
siRNA Ctrl 2_1 2_2
O
72h
relative mRNA expression of SMA
0.1929
0.3539
siRNA Ctrl 2_1 2_2
P
Ctrl
siBMP-2_1
siBMP-2_2
TAGLN (+DAPI)
Q
relative mRNA expression of TAGLN
0.0063
0.0007
siRNA Ctrl 2_1 2_2
R
relative protein expression of TAGLN
0.0646
0.8902
siRNA Ctrl 2_1 2_2
S
72h
relative mRNA expression of TAGLN
0.2614
0.6320
siRNA Ctrl 2_1 2_2
T
72h
relative protein expression of TAGLN
0.0338
0.1068
siRNA Ctrl 2_1 2_2

Figure S8: Supplementary data to figure 5. BMP-2 deficiency was induced in human VSMC with either of two specific siRNA targeted BMP-2 (2_1, 2_2). Scrambled siRNA was used as control (Ctrl). Experiments were performed 48 hours *post* siRNA transfection, unless otherwise stated. Data are presented as mean±SD and comparisons were calculated by an ordinary one-way ANOVA-test with Bonferroni correction for multiple testing; p-values as indicated. (**A+E+H+I+J+K+L+O+Q+S**) qRT-PCR of (A) BMP-2 (n≥6), (E) BMP-4 (n≥6), (H) ID-1 (n≥4), (I) SMAD-6 (n≥5), (J+K) CALP (n≥3), (L+O) SMA (n≥3), (Q+S) TAGLN (n≥3). (**B+C+D+F+G+M+N+R+T**) Western blot analysis of (C+D) BMP-2 (n≥3), (F+G) BMP-4 (n≥3), (N) SMA (n≥3), (R+T) TAGLN (n≥3) with GAPDH as loading control. Representative blots in B, G, M. (**P**) Representative photomicrographs of immunostaining against TAGLN 72 hours *post* transfection. Left image: scale bar 50 μm; right image: zoom in to 200%, scale bar 25 μm.

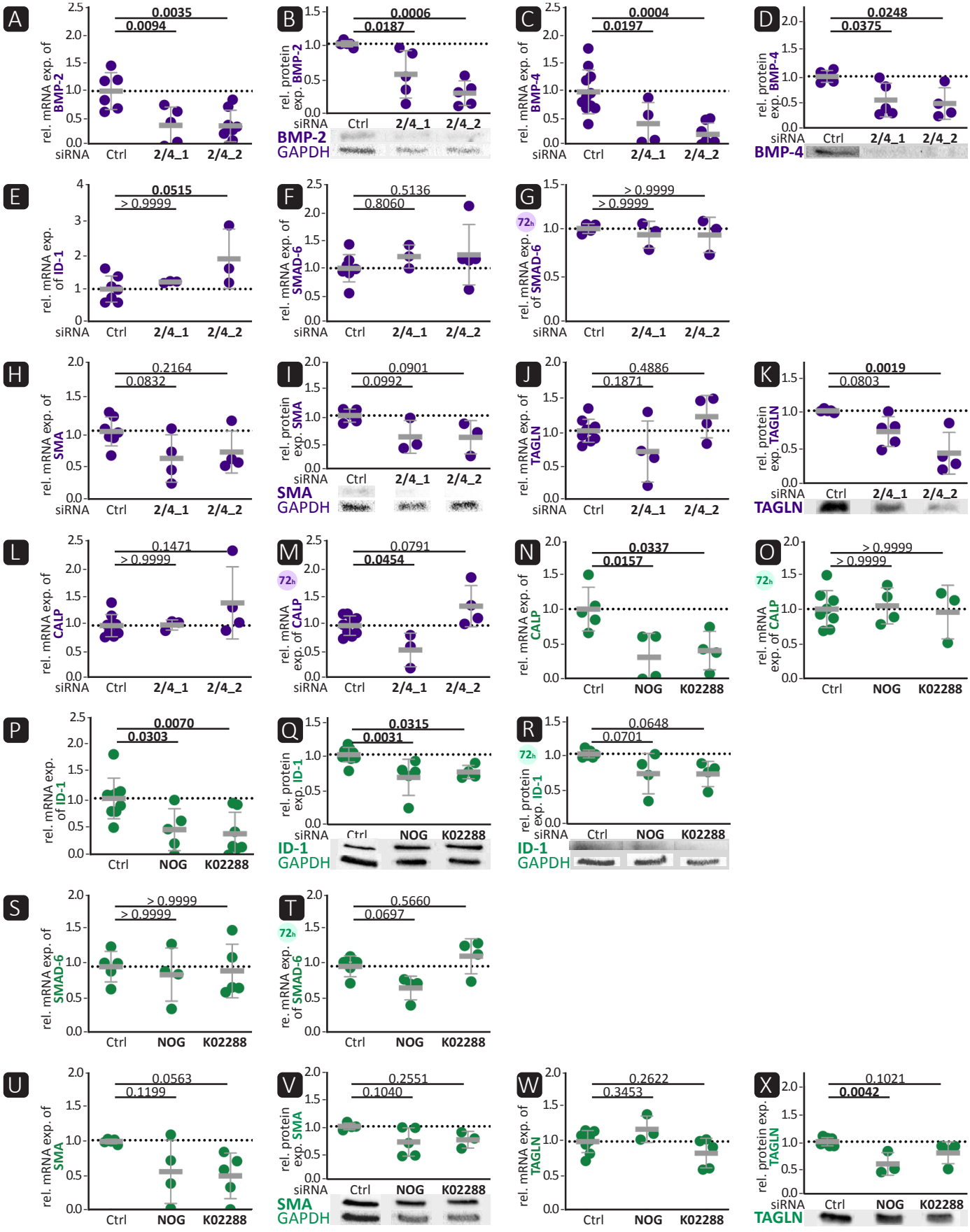

A
rel. mRNA exp. of BMP-2
0.0035
0.0094
siRNA Ctrl 2/4_1 2/4_2
B
rel. protein exp. BMP-2
0.0006
0.0187
BMP-2
GAPDH
C
rel. mRNA exp. of BMP-4
0.0004
0.0197
D
rel. protein exp. BMP-4
0.0248
0.0375
BMP-4
E
rel. mRNA exp. of ID-1
0.0515
> 0.9999
F
rel. mRNA exp. of SMAD-6
0.5136
0.8060
G
72h
rel. mRNA exp. of SMAD-6
> 0.9999
> 0.9999
H
rel. mRNA exp. of SMA
0.2164
0.0832
I
rel. protein exp. SMA
0.0901
0.0992
SMA
GAPDH
J
rel. mRNA exp. of TAGLN
0.4886
0.1871
K
rel. protein exp. TAGLN
0.0019
0.0803
TAGLN
L
rel. mRNA exp. of CALP
0.1471
> 0.9999
M
72h
rel. mRNA exp. of CALP
0.0791
0.0454
N
rel. mRNA exp. of CALP
0.0337
0.0157
Ctrl NOG K02288
O
72h
rel. mRNA exp. of CALP
> 0.9999
> 0.9999
P
rel. mRNA exp. of ID-1
0.0070
0.0303
Q
rel. protein exp. ID-1
0.0315
0.0031
ID-1
GAPDH
R
72h
rel. protein exp. ID-1
0.0648
0.0701
S
rel. mRNA exp. of SMAD-6
> 0.9999
> 0.9999
T
72h
re. mRNA exp. of SMAD-6
0.5660
0.0697
U
rel. mRNA exp. of SMA
0.0563
0.1199
V
rel. protein exp. SMA
0.2551
0.1040
SMA
GAPDH
W
rel. mRNA exp. of TAGLN
0.2622
0.3453
X
rel. protein exp. TAGLN
0.1021
0.0042
TAGLN

Figure S9: Supplementary data to figure 6 and 7. VSMC were either transfected with a combination of BMP-2 and BMP-4 targeting siRNA (2/4_1, 2/4_2) compared to scrambled siRNA (Ctrl) as control or incubated with BMP inhibitors noggin (NOG; 100ng/mL) or K02288 (1 μM) in 0.4% FBS/EBM (Ctrl). Data are presented as mean±SD and comparisons were calculated by an ordinary one-way ANOVA-test with Bonferroni correction for multiple testing; p-values as indicated. Rel.=relative; exp.=expression. (**A+C+E+F+G+H+J+L+M+N+N+O+P+S+T+U+W**) qRT-PCR of (A) BMP-2 (n≥5), (C) BMP-4 (n≥4), (E+P) ID-1 (n≥3), (F+G+S+T) SMAD-6 (n≥3), (H+U) SMA (n≥4), (J+W) TAGLN (n≥3), (L+M+N+O) CALP (n≥3). (**B+D+I+K+Q+R+V+X**) Western blot analysis of (B) BMP-2 (n≥5), (D) BMP-4 (n≥4, representative blots of GAPDH shown in B), (I+V) SMA (n≥3), (K+X) TAGLN (n≥3; representative blots of GAPDH shown in I, V), (Q+R) ID-1 (n≥4) with GAPDH as loading control.

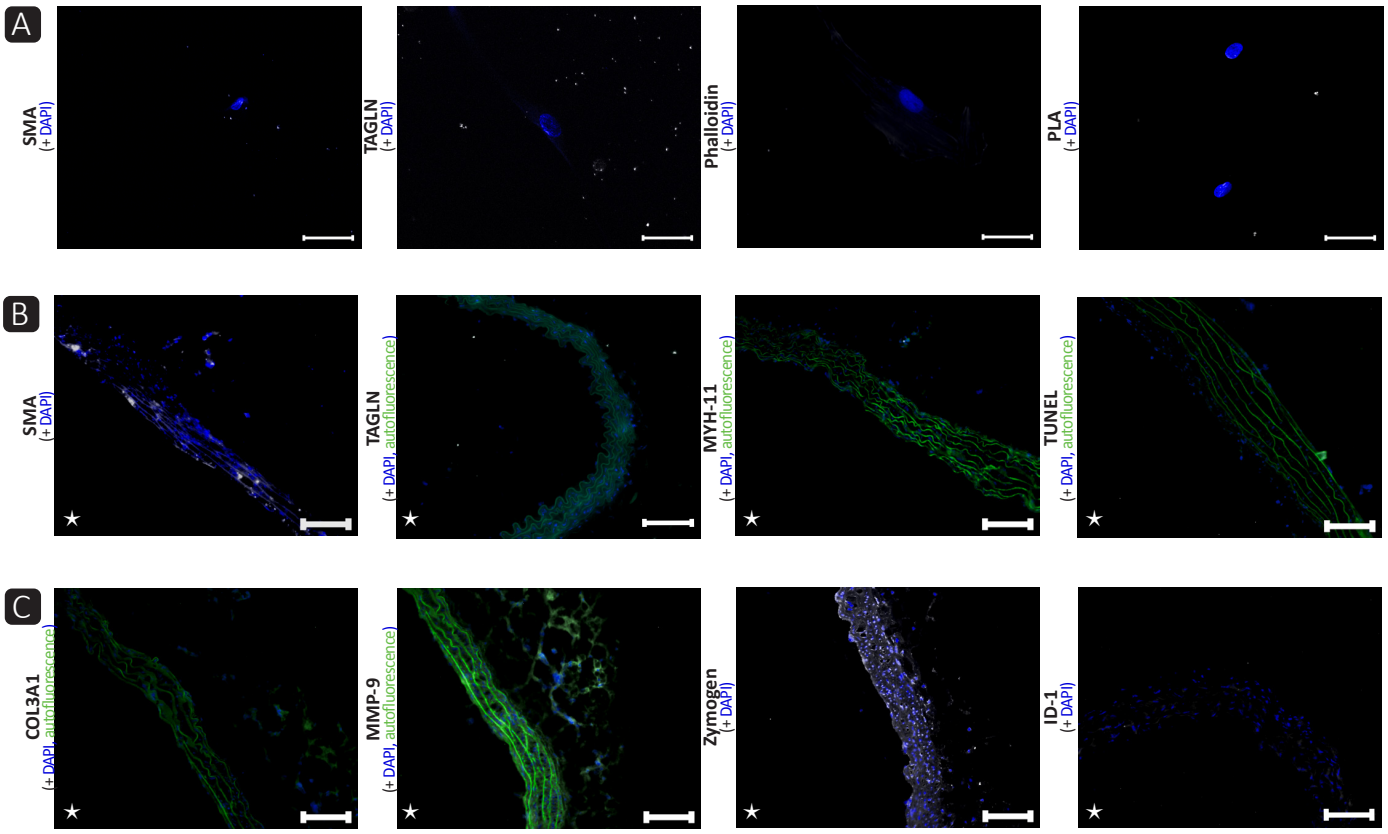
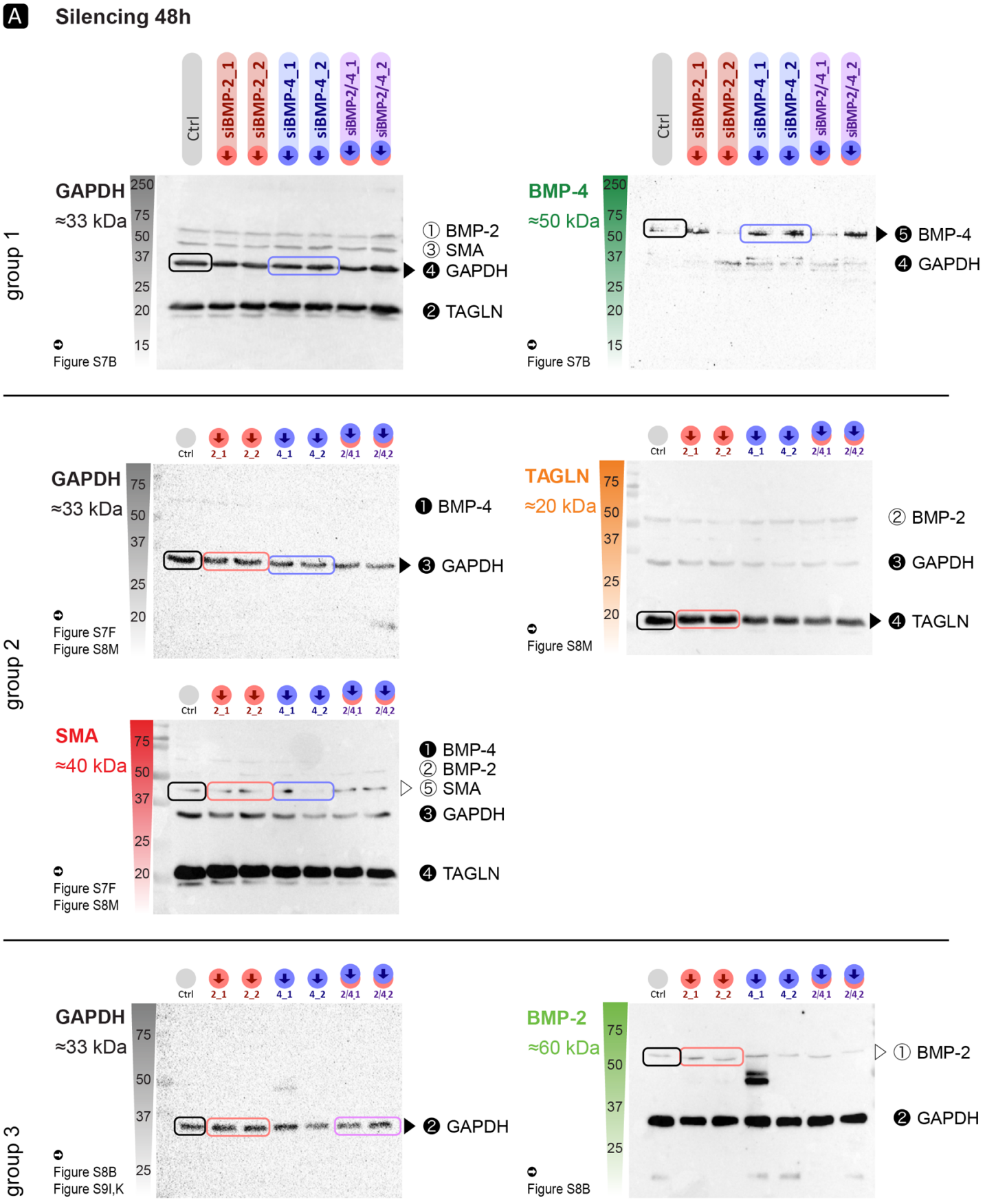

A
SMA
(+ DAPI)
TAGLN
(+ DAPI)
Phalloidin
(+ DAPI)
PLA
(+ DAPI)
B
SMA
(+ DAPI)
TAGLN
(+ DAPI, autofluorescence)
MYH-11
(+ DAPI, autofluorescence)
TUNEL
(+ DAPI, autofluorescence)
C
COL3A1
(+ DAPI, autofluorescence)
MMP-9
(+ DAPI, autofluorescence)
Zymogen
(+ DAPI)
ID-1
(+ DAPI)

Figure S10: Negative controls of immunocytological and immunohistological stainings. To investigate the background staining, a non-specific antibody at the same concentration was used instead of the target-specific antibody. Otherwise, the same protocol was used for the stainings and for image acquisition. The basal control condition was stained in each case. Nuclei were stained with DAPI (blue). Autofluorescence of elastic fibers in green when indicated in the figure. Vascular lumen is marked with an asterisk.(**A**) Representative photomicrographs of negative controls for immunocytological staining against alpha smooth muscle actin (SMA), transgelin (TAGLN), phalloidin and negative controls of *in-situ* proximity ligation assay (PLA) for the detection of BMPR1a phosphorylation. Scale bars, 50 μm. (**B**) Representative photomicrographs of negative controls of immunohistological staining of thoracic aortic tissue against SMA, TAGLN and myosin heavy chain 11 (MYH-11) and TUNEL assay. Scale bar 100 μm. (**C**) Representative photomicrographs of negative controls of immunohistological staining of thoracic aortic tissue against collagen3A1 (COL3A1), matrix metalloproteinase 9 (MMP-9), background activity of Zymogen assay and ID-1. Scale bar 100 μm.


A
Silencing 48h
group 1
Ctrl
siBMP-2_1
siBMP-2_2
siBMP-4_1
siBMP-4_2
siBMP-2/-4_1
siBMP-2/-4_2
GAPDH
≈33 kDa
250
75
50
37
25
20
15
① BMP-2
③ SMA
❹ GAPDH
❷ TAGLN
Figure S7B
BMP-4
≈50 kDa
❺ BMP-4
❹ GAPDH
Figure S7B
group 2
Ctrl
2_1
2_2
4_1
4_2
2/4_1
2/4_2
GAPDH
≈33 kDa
❶ BMP-4
❸ GAPDH
Figure S7F
Figure S8M
TAGLN
≈20 kDa
② BMP-2
❸ GAPDH
❹ TAGLN
Figure S8M
SMA
≈40 kDa
❶ BMP-4
② BMP-2
⑤ SMA
❸ GAPDH
❹ TAGLN
Figure S7F
Figure S8M
group 3
GAPDH
≈33 kDa
❷ GAPDH
Figure S8B
Figure S9I,K
BMP-2
≈60 kDa
① BMP-2
❷ GAPDH
Figure S8B

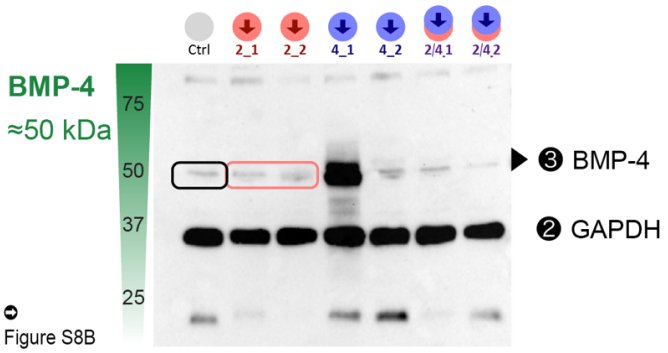


Figure S8B

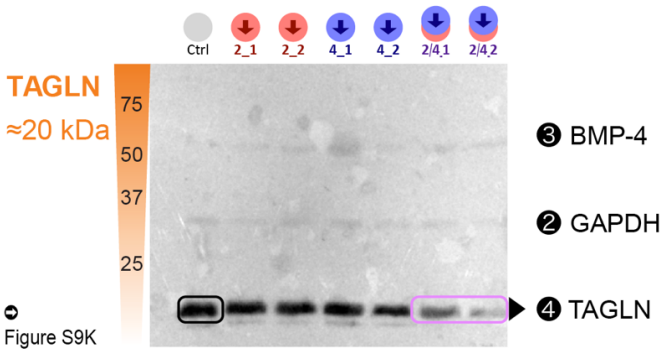


Figure S9K

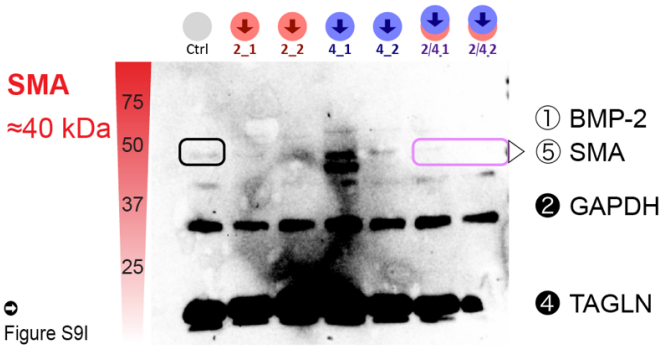


Figure S9I

group 4

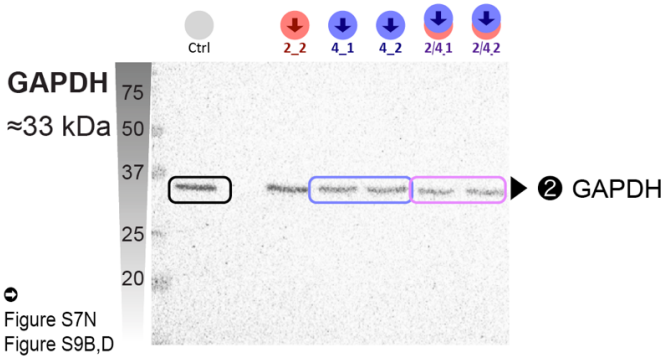


Figure S7N
Figure S9B,D

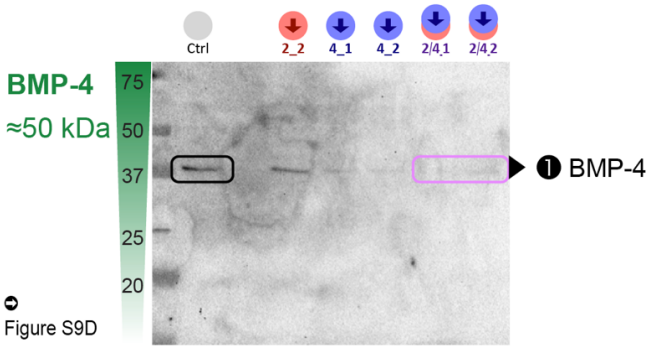


Figure S9D

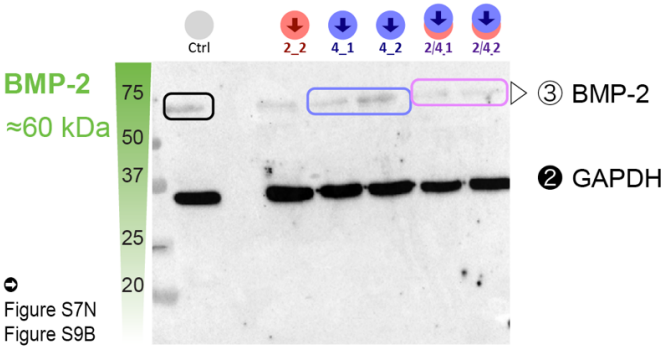


Figure S7N
Figure S9B

group 5

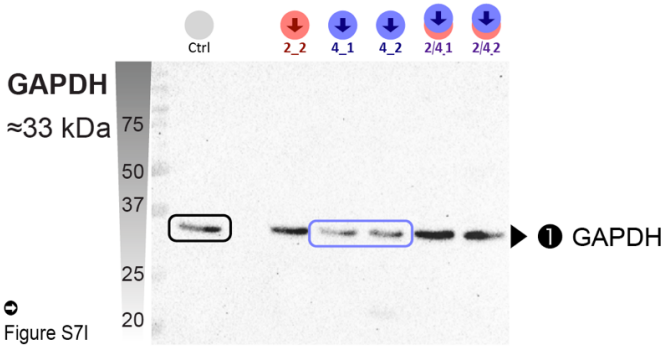


Figure S7I

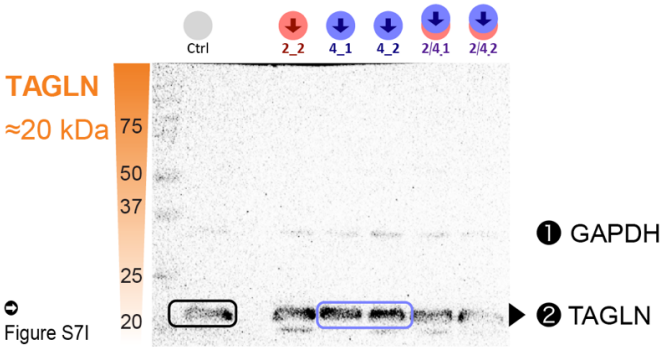


Figure S7I

B **Silencing 72h**

group 6

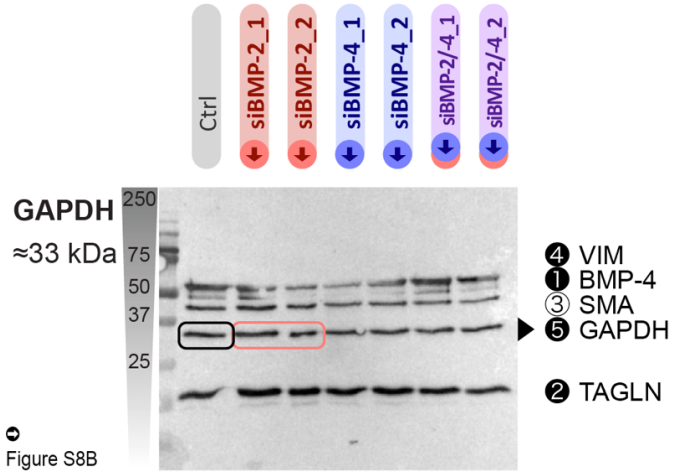


Figure S8B

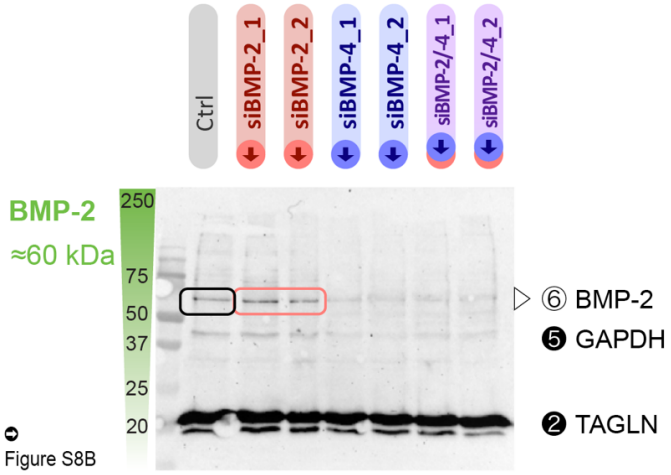


Figure S8B

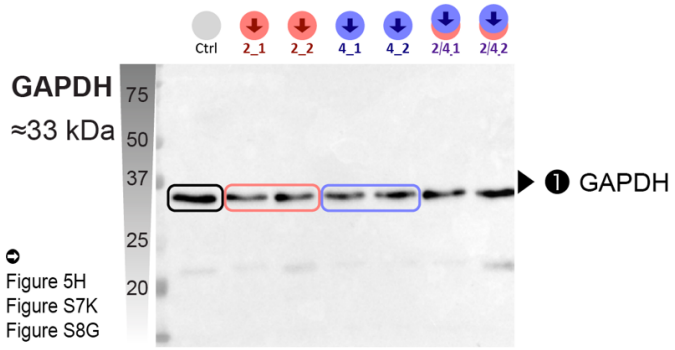


Figure 5H
Figure S7K
Figure S8G

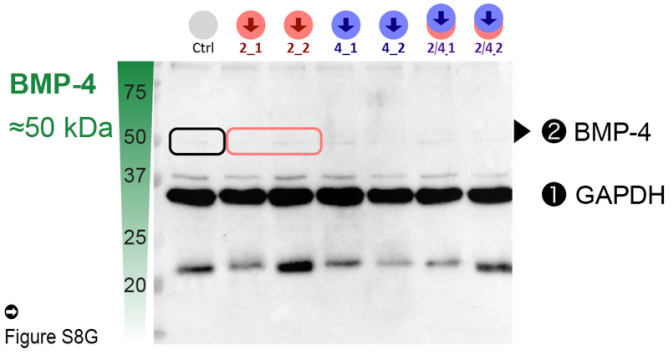


Figure S8G

group 7

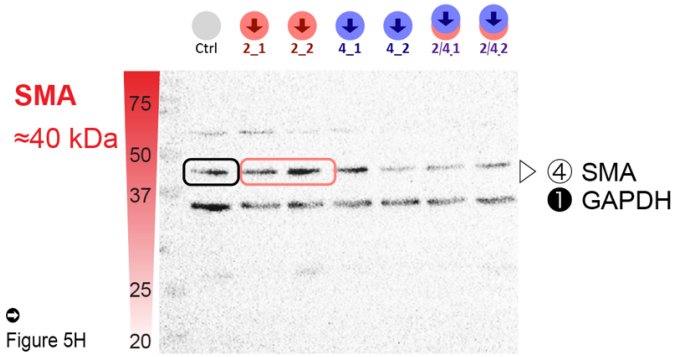


Figure 5H

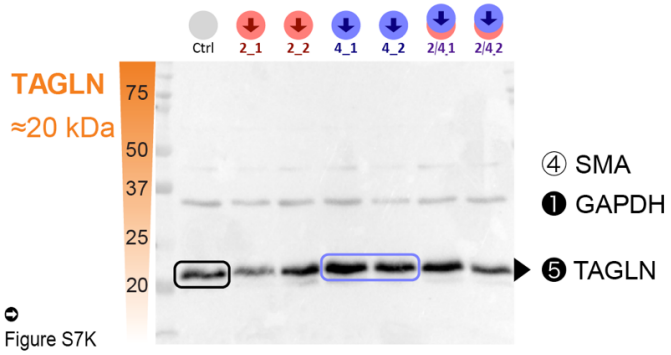


Figure S7K

group 8

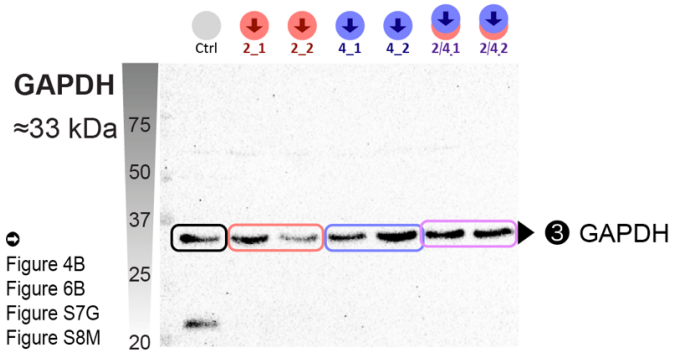


Figure 4B
Figure 6B
Figure S7G
Figure S8M

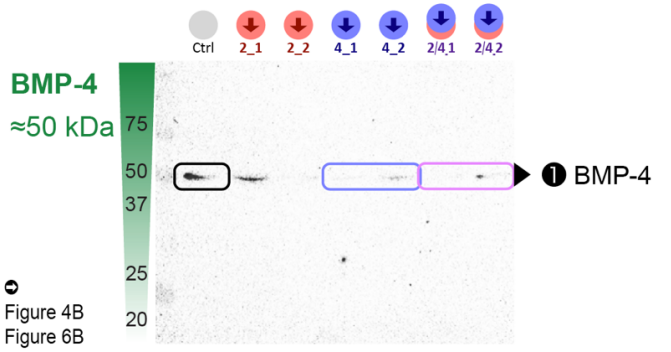
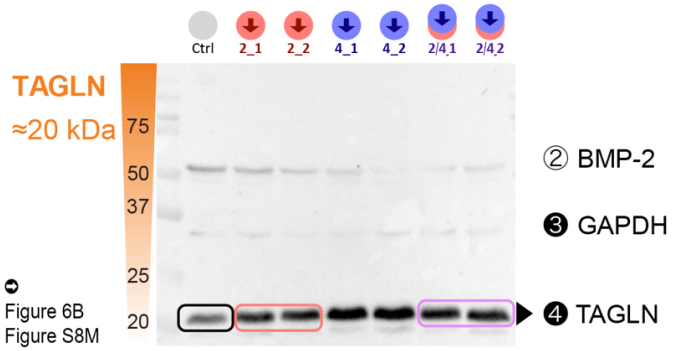
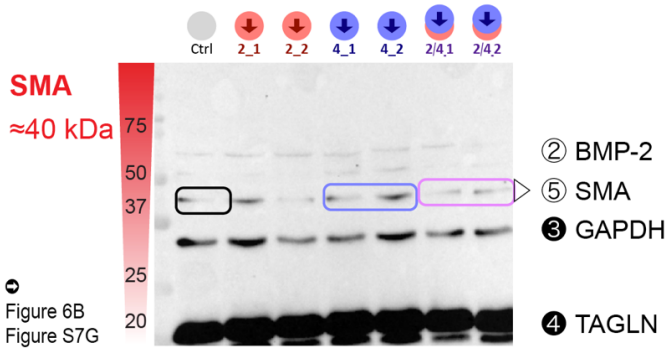
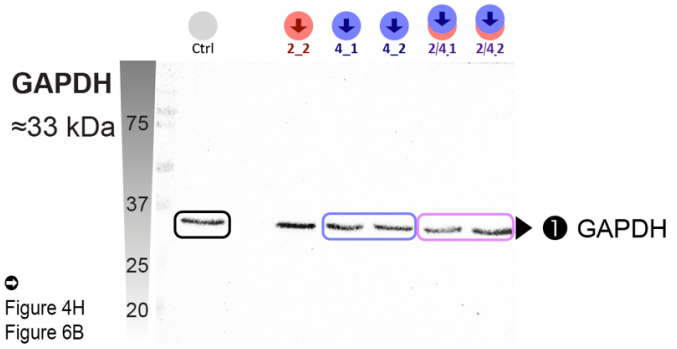


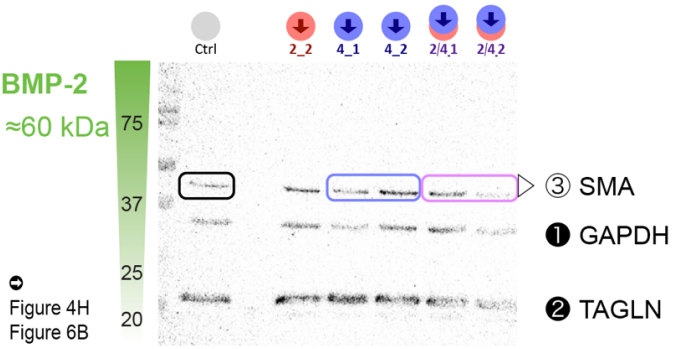
Figure 4B
Figure 6B





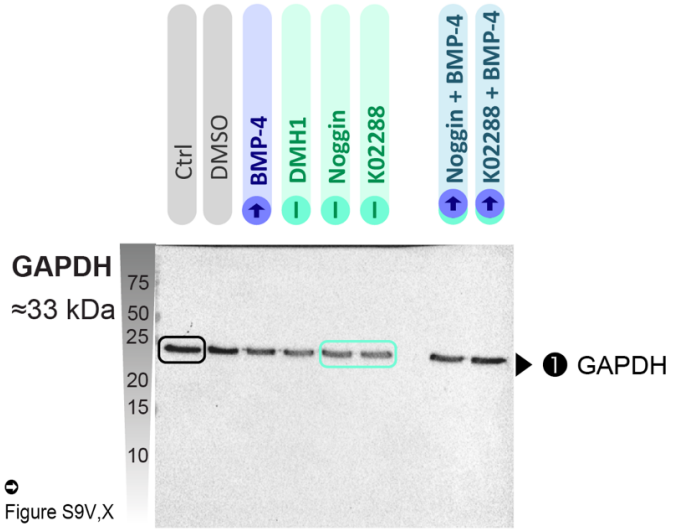
group 9

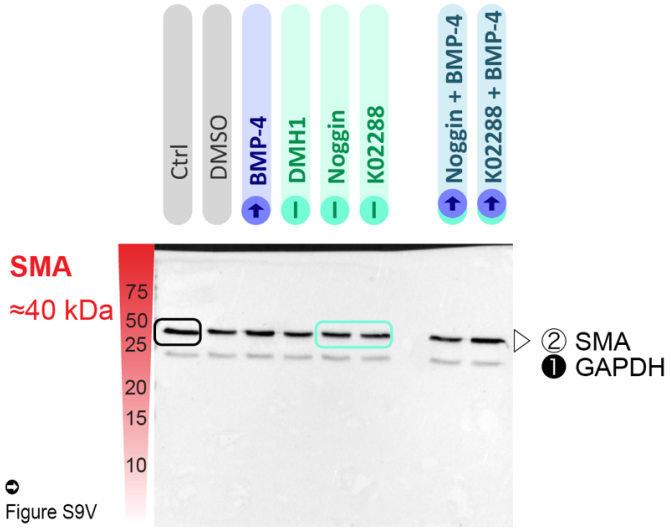


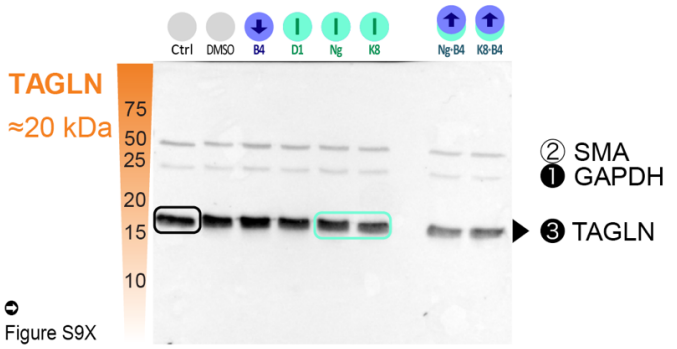


C Stimulation / Inhibition 48h

group 10







group 11

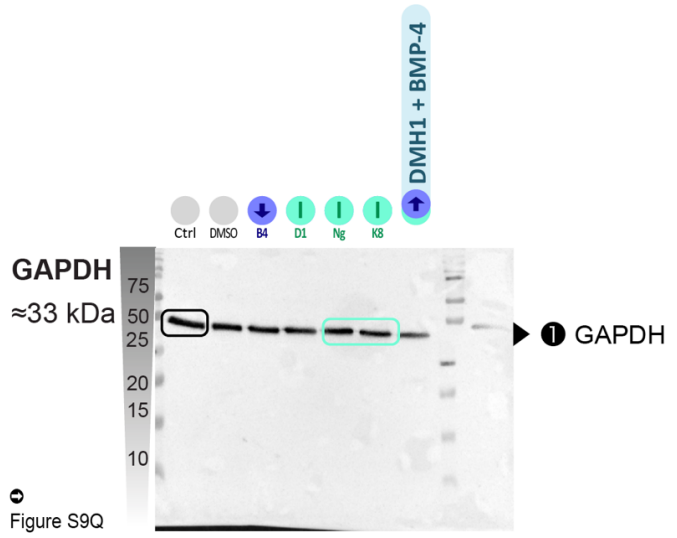


Figure S9Q

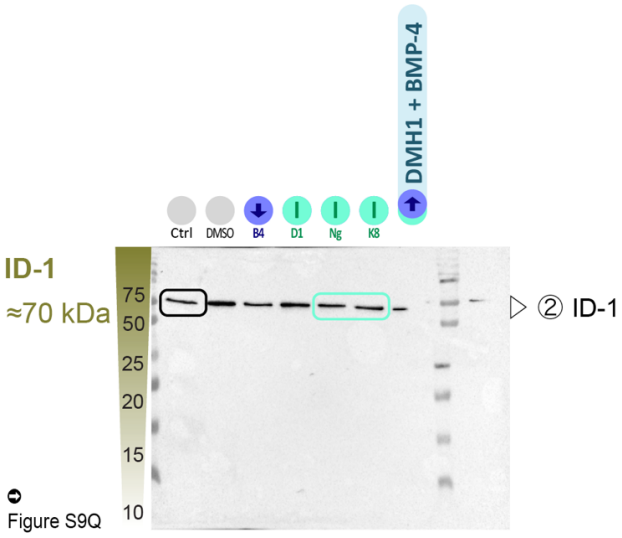


Figure S9Q

group 12

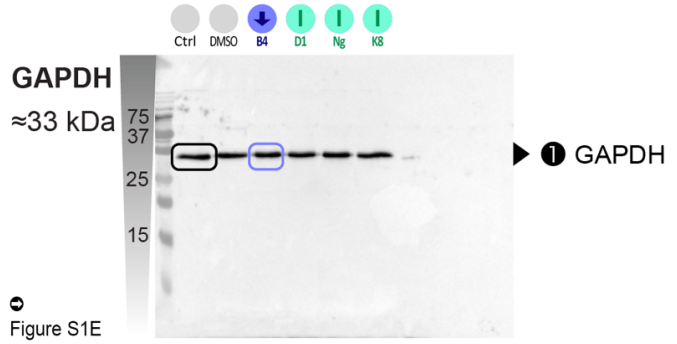


Figure S1E

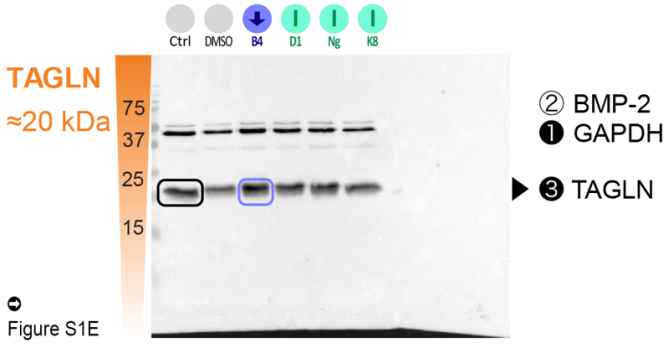


Figure S1E

group 13

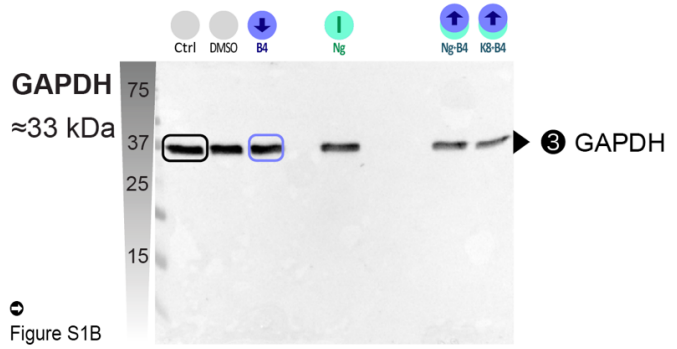


Figure S1B

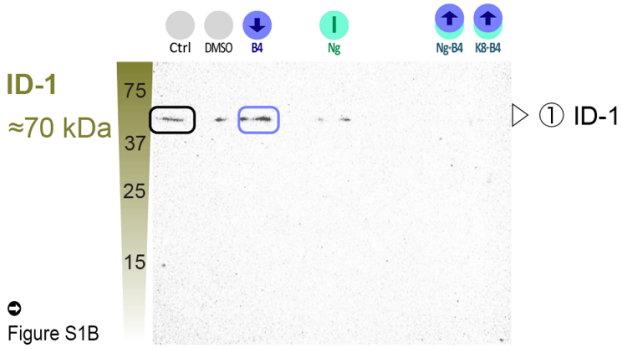


Figure S1B

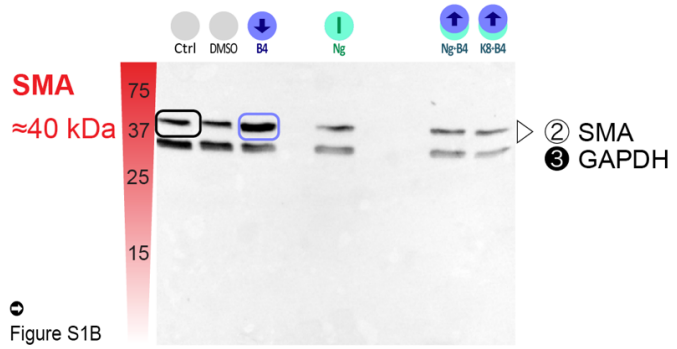


Figure S1B

D **Stimulation / Inhibition 72h**

group 14

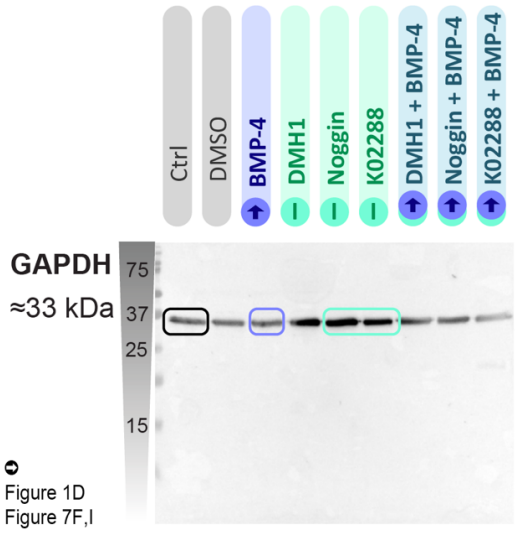


Figure 1D
Figure 7F,I

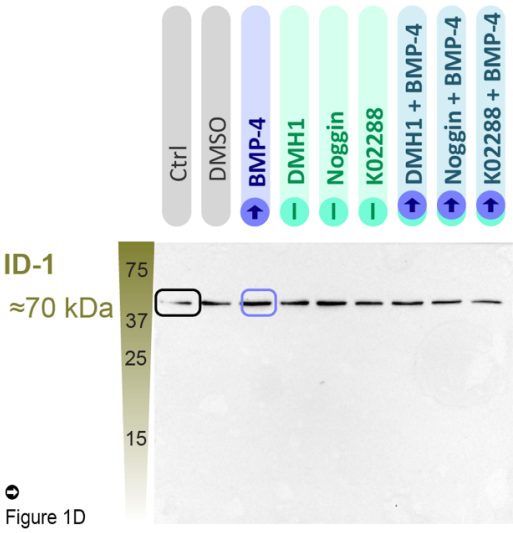


Figure 1D

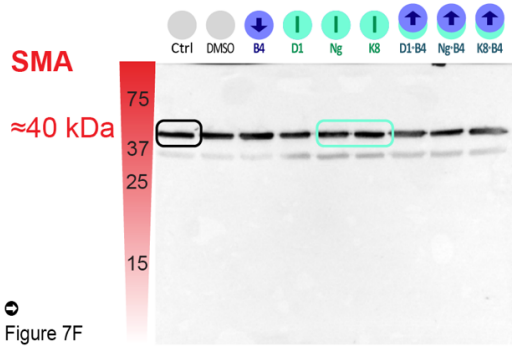


Figure 7F

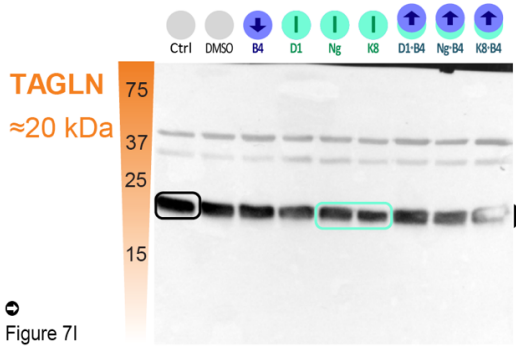


Figure 7I

group 15

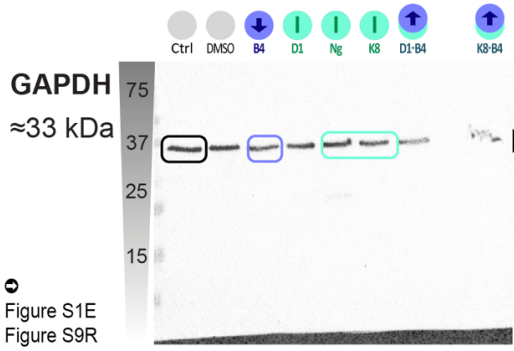


Figure S1E
Figure S9R

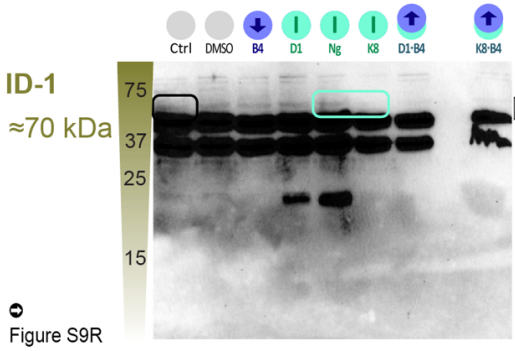


Figure S9R

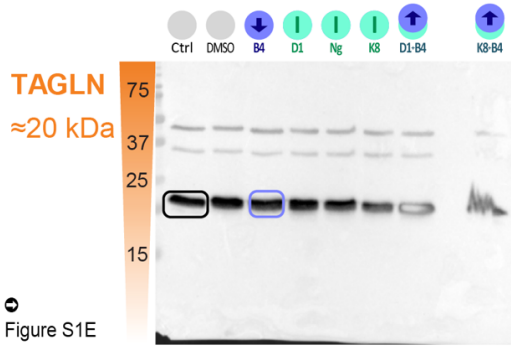


Figure S1E

group 16

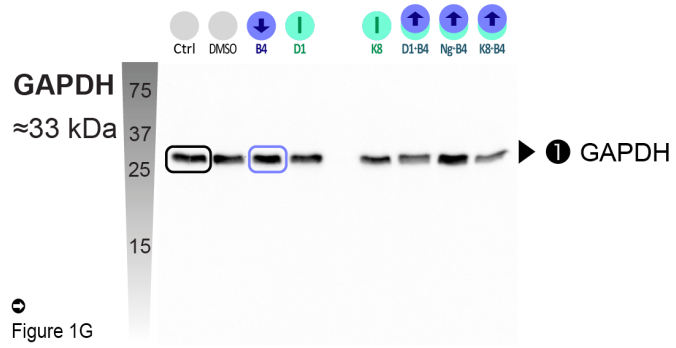


Figure 1G

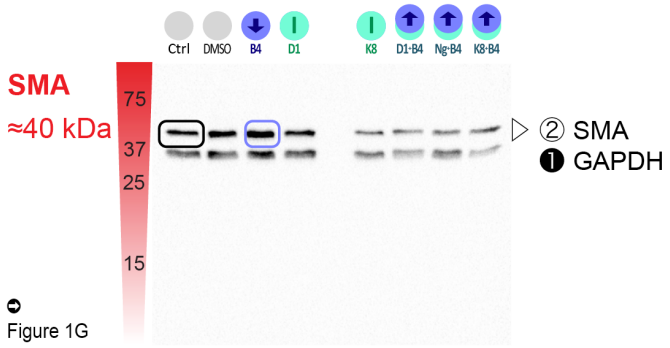


Figure 1G

E Murine proteins

group 17

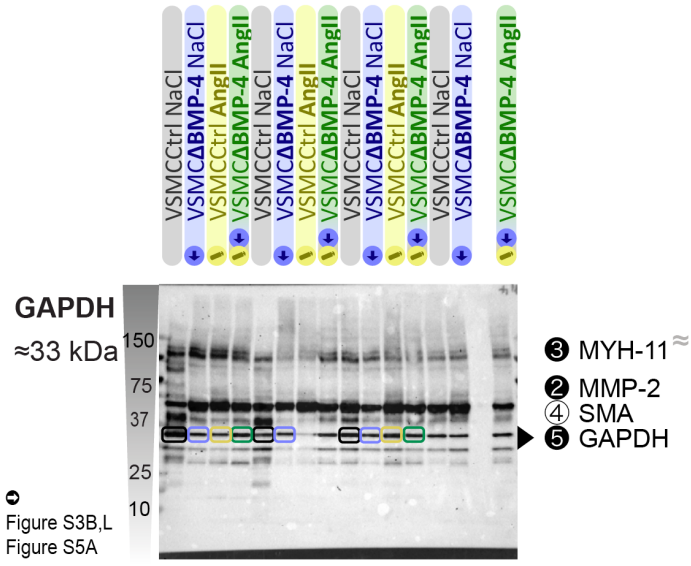


Figure S3B,L
Figure S5A

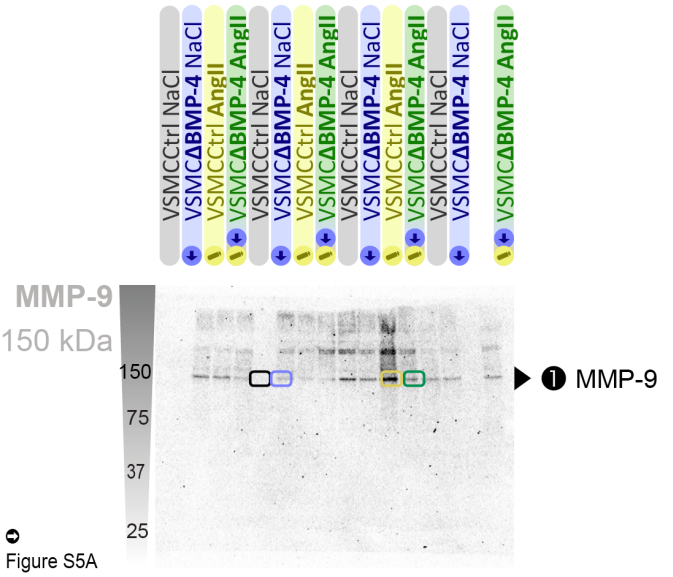


Figure S5A

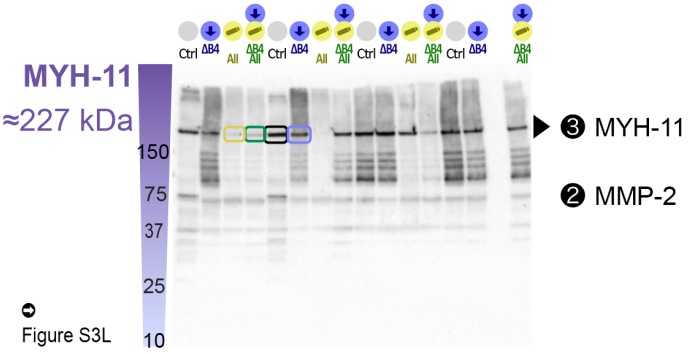


Figure S3L

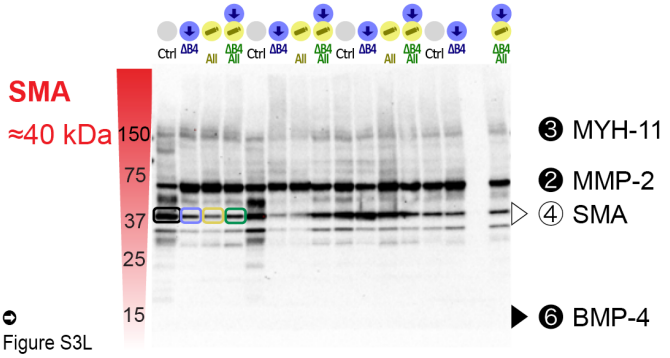


Figure S3L

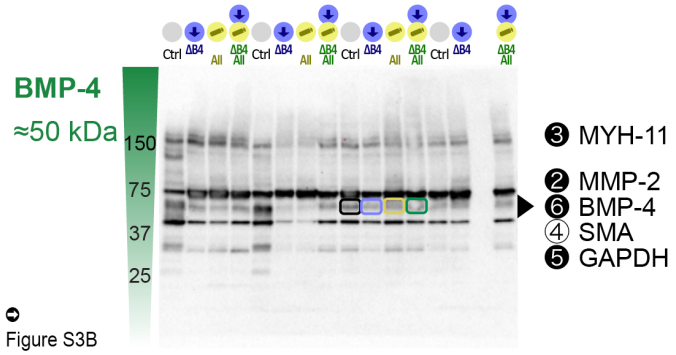


Figure S3B

Figure S11 Full unedited western blots. The same loading scheme was used for all gels of a subpanel (siBMP-2_1 = 2_1, siBMP-2_2 = 2_2, siBMP-4_1 = 4_1, siBMP-4_2 = 4_2, BMP-4 = B4, DMH1 = D1, Noggin = Ng, K02288 = K8, VSCMCtrl NaCl = Ctrl VSMCΔBMP-4 NaCl = ΔB4, VSMCCtrl AngII = AII, VSMCΔBMP-4 AngII = B4+AII). The antibodies that led to the detection of the corresponding bands and their order are indicated on the right of each image. Numbers with black background are rabbit antibodies, numbers with white background are mouse antibodies. The membrane used for analysis after incubation with the antibody of interest is indicated by an arrow in front of the number and the relevant sections are marked. In addition, antibodies against vimentin (VIM) and MMP-2 are visible on some of the membranes, but these were not analysed any further. An approximate scale is always given to the left of the membrane for reference. The bands have been identified based on their size, the order of the antibodies used and the type of secondary antibody used.

MAJOR RESOURCES TABLE

Animals (*in-vivo* studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID/URL
Mus musculus	Charles River	C57/BL6N	male	www.criver.com/products-services/find-model/c57bl6-mouse?region=23

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Persistent ID / URL
B6.129BS-Bmp4^{tm4Blh}	Mus musculus	Bridgid Hogan's lab	C57/BL6N	www.cellbio.duke.edu/primary-faculty/brigid-lm-hogan
B6.Tg(MYH11-icre/ERT2)^{1Soff}	Mus musculus	The Jackson Laboratory	C57/BL6N	www.jax.org/strain/019079

Antibodies Immunhistology

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
Anti-Actin α-Smooth muscle monoclonal mouse FITC conjugated	Sigma-Aldrich, Schnelldorf, Germany	F3777	1:300	www.sigmaaldrich.com/AT/de/product/sigma/f3777
Anti-SMHC monoclonal rabbit	Abcam®, Cambridge, UK	ab124679	1:200	www.abcam.com/smooth-muscle-myosin-heavy-chain-11-antibody-epr5335-ab124679.htmL
Anti-Transgelin polyclonal rabbit	Abcam®, Cambridge, UK	ab14106	1:200	www.abcam.com/tagln-transgelin-antibody-ab14106.htmL
Anti-Collagen III polyclonal rabbit	Abcam®, Cambridge, UK	ab7778	1:200	www.abcam.com/collagen-iii-antibody-ab7778.htmL
Anti-MMP-9 polyclonal rabbit	Abcam®, Cambridge, UK	ab38898	1:200	www.abcam.com/mmp9-antibody-ab38898.htmL
Anti-ID-1 polyclonal rabbit	Abcam®, Cambridge, UK	ab203202	1:100	www.abcam.com/id1-antibody-ab203202.htmL
Negative control mouse IgG2A-FITC	Sigma-Aldrich, Schnelldorf, Germany	MABC004F	-	https://www.sigmaaldrich.com/AT/de/product/mm/mabc004f

Negative control rabbit IgG	Dako Cytomation, Hamburg, Germany	X0936	-	-
Alexa Fluor 555 donkey anti-rabbit IgG (H+L)	Thermo Fisher Scientific, Schwerte, Germany	A-31572	1:500	www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572

Antibodies Immuncytology

Target antigen	**Vendor or Source**	**Catalog #**	**Working concentration**	**Persistent ID / URL**
Anti-Actin α-Smooth muscle monoclonal mouse FITC conjugated	Sigma-Aldrich, Schnelldorf, Germany	F3777	1:300	www.sigmaaldrich.com/AT/de/product/sigma/f3777
Anti-Transgelin polyclonal rabbit	Abcam®, Cambridge, UK	ab14106	1:100	www.abcam.com/tagln-transgelin-antibody-ab14106.htmL
Negative control mouse IgG2A-FITC	Sigma-Aldrich, Schnelldorf, Germany	MABC004F	-	www.sigmaaldrich.com/AT/de/product/mm/mabc004f
Negative control rabbit IgG	Dako Cytomation, Hamburg, Germany	X0936	-	-
Alexa Fluor 555 donkey anti-rabbit IgG (H+L)	Thermo Fisher Scientific, Schwerte, Germany	A-31572	1:400	www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572

Antibodies western blot

Target antigen	**Vendor or Source**	**Catalog #**	**Working concentration**	**Persistent ID / URL**
Anti-Actin α-Smooth muscle monoclonal mouse	Sigma-Aldrich, Schnelldorf, Germany	A 2547	1:5000	www.sigmaaldrich.com/AT/de/product/sigma/f3777
Anti-SMHC monoclonal rabbit	Abcam®, Cambridge, UK	ab124679	1:10000	www.abcam.com/smooth-muscle-myosin-heavy-chain-11-antibody-epr5335-ab124679.htmL
Anti-Transgelin polyclonal rabbit	Abcam®, Cambridge, UK	ab14106	1:2000	www.abcam.com/tagln-transgelin-antibody-ab14106.htmL
Anti-MMP-9 polyclonal rabbit	Abcam®, Cambridge, UK	ab38898	1:1000	www.abcam.com/mmp9-antibody-ab38898.htmL
Anti-ID-1 polyclonal rabbit	Abcam®, Cambridge, UK	ab203202	1:1000	www.abcam.com/id1-antibody-ab203202.htmL

Anti-BMP-2 monoclonal mouse	R&D Systems, Wiesbaden, Germany	MAB3551	1:500	www.rndsystems.com/products/human-bmp-2-antibody-100221_mab3551
Anti-BMP-4 polyclonal rabbit	ThermoFisher Scientific, Schwerte, Germany	PA5-19683	1:1000	www.thermofisher.com/antibody/product/BMP-4-Antibody-Polyclonal/PA5-19683
Anti-GAPDH polyclonal rabbit	EnoGene Biotech, New York, USA	E1C604	1:5000	www.enogene.com/product/E1C604-1
Anti-mouse IgG/HRP conjugated	R&D Systems, Wiesbaden, Germany	HAF007	1:5000	www.rndsystems.com/products/mouse-igg-hrp-conjugated-antibody_haf007
Anti-rabbit IgG/HRP conjugated	ThermoFisher Scientific, Schwerte, Germany	31460	1:10000	www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/31460

Recombinant proteins

Target antigen	**Vendor or Source**	**Catalog #**	**Working concentration**	**Lot # (preferred but not required)**	**Persistent ID / URL**
Recombinant human BMP-4	R&D Systems, Wiesbaden, Germany	314-BP-010	50 ng/mL	BEM9514081	www.rndsystems.com/products/recombinant-human-bmp-4-protein_314-bp
Recombinant human Noggin	R&D Systems, Wiesbaden, Germany	6057-NG-025	100 ng/mL	TNT2820061	https://www.rndsystems.com/products/recombinant-human-noggin-protein_6057-ng
K02288	TOCRIS, Bristol, UK	4986	1µM	1	https://www.tocris.com/products/k-02288_4986

siRNA

siRNA	**Clone Name**	**Sequence**	**Source / Repository**
2_1	BMP2HSS101044	(RNA)-GGA AAC GCC UUA AGU CCA GCU GUA A (RNA)-UUA CAG CUG GAC UUA AGG CGU UUC C	Thermo Fisher Scientific, Schwerte, Germany
2_2	BMP2HSS101045	(RNA)-GGU CAA CUC UGU UAA CUC UAA GAU U (RNA)-AAU CUU AGA GUU AAC AGA GUU GAC C	
4_1	BMP4HSS101052	(RNA)-GCU UCC ACC GUA UAA ACA UUU AUG A (RNA)-UCA UAA AUG UUU AUA CGG UGG AAG C	
4_2	BMP4HSS101050	(RNA)-GCQ UGU CAG GAU UAG CCG AUC GUU A (RNA)-UAA CGA UCG GCU AAU CCU GAC AUG C	

Primer for qRT-PCR

Clone Name	**Sequence**	**Source / Repository**	**Persistent ID / URL**
ID-1 (human)	Fwd: 5′-CTACGACATGAACGGCTGCTACTC-3′ Rev: 5′- GTAGAGCAGGACGTTCACCT-3′		eurofinsgenomics.com

ID-1 (murine)	Fwd: 5′- CCTAGCTGTTCGCTGAAGGC-3′ Rev: 5′-CTTGCTCACTTTGCGGTTCT-3′	Eurofins MWG Operon, Ebersberg, Germany	
SMA (human)	Fwd: 5′-CCCAGACATCAGGGAGTAATG-3′ Rev: 5′-TCTATCGGATACTTCAGCGTC-3′		
SMA (murine)	Fwd: 5′- CCCAGACATCAGGGAGTAATGG-3′ Rev: 5′- TCTATCGGATACTTCAGCGTCA-3′		
TAGLN (human)	Fwd: 5′- ACCAAAAACGATGGAAACTACCG-3′ Rev: 5′- GTGAAGTCCCTCTTATGFTCCT-3′		
TAGLN (murine)	Fwd: 5′-AACAGCCTGTACCCTGATGG-3′ Rev: 5′-CGGTAGTGCCCATCATTCTT-3′		
MYH-11	Fwd: 5′- AAGCTGCGGCTAGAGGTCA-3′ Rev: 5′- CCCTCCCTTTGATGGCTGAG-3′		
CALP	Fwd: 5′-AGCTAAGAGAAGGGCGGAAC-3′ Rev: 5′-CATCTGCAGGCTGACATTGA-3′		
Col3A1	Fwd: 5′-GCCAAATATGTGTCTGTGACTCA-3′ Rev: 5′-GGGCGAGTAGGAGCAGTTG-3′		
MMP-9	Fwd: 5′-CCTGGAGACCTGAGAACCAATC-3′ Rev: 5′-CCACCCGAGTGTAACCATAGC-3′		
BMP-2	Fwd: 5′-AACACTGTGCGCAGCTTCC-3′ Rev: 5′-CTCCGGGTTGTTTTCCCAC-3′		
BMP-4	Fwd: 5′-CACGAAGAACATCTGGAGAAC-3′ Rev: 5′-CCCTTGAGGTAACGATCAGCT-3′		
SMAD-6	Fwd: 5′-CCCCCGGCTACTCCATCAAGGTGT-3′ Rev: 5′-GTCCGTGGGGGCTGTGTCTCTGG-3′		
hRPII	Fwd: 5′-GCACCACGTCCAATGACAT-3′ Rev: 5′-GTGCGGCTGCTTCCATAA-3′		
M36B4	Fwd: 5′-AAGCGCGTCCTGGCATTGTCT-3′ Rev: 5′-CCGCAGGGGCAGCAGTGGT-3′		

Cultured Cells

Name	**Vendor or Source**	**Sex (F, M, or unknown)**	**Persistent ID / URL**
Human Aortic Smooth Muscle Cells	PELO biotech	Unknown	Catalog No: PB-CH-280-2011

Software

Software	**Source / Repository**
GraphPad Prism 9	GraphPad Software, La Jolla, US-CA
Microsoft Word Version 16	Microsoft Corporation, Redmond, USA
Bio-Rad CFX Manager Version 3.1	Bio-Rad GmbH, Munich, Germany
Image Lab Version 4	Bio-Rad GmbH, Munich, Germany
ZEN 3.1 blue edition	Carl Zeiss GmbH, Jena, Germany
Pixelmator Pro Version 2	Pixelmator Team, Vilnius, Lithuania
Affinity Designer 2	Serif Nottingham, UK

Affinity Publisher 2	Serif Nottingham, UK
ImageJ2 Version 2.3.0/1.53q	Open Source
Matlab script Version 9.6.0.1214997	MatWorks Inc, Natick, USA

ARRIVE GUIDELINES

Study Design

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
MYH11Cre-BMP4flox TAM- NaCl	male	18-21 weeks	18	17	Yes	Pump implantation, Histology qRT-PCR, western Blot
MYH11Cre-BMP4flox TAM+ NaCl	male	18-21 weeks	19	19	Yes	Pump implantation, Histology qRT-PCR, western Blot
MYH11Cre-BMP4flox TAM- AngII	male	18-21 weeks	19	17	Yes	Pump implantation, Histology qRT-PCR, western Blot
MYH11Cre-BMP4flox TAM+AngII	male	18-21 weeks	20	20	Yes	Pump implantation, Histology qRT-PCR
MYH11Cre-BMP4flox TAM-	male	7-9 weeks	15	15	Yes	Nanoindentation, Histology, qRT-PCR,
MYH11Cre-BMP4flox TAM+	male	7-9 weeks	16	16	Yes	Nanoindentation, Histology, qRT-PCR
MYH11Cre TAM-	male	6-9 weeks	8	8	Yes	Histology
MYH11Cre TAM+	male	6-9 weeks	9	9	Yes	Histology

Sample Size:
Given the investigator's assumed standardised effect size of 1.06, a desired power of 80%, and a two-sided significance level of 5%, a sample size of at least 7-15 animals per method and group is required to detect a statistically significant difference using the unpaired t-test, depending on the method used. A statistical assessment was also performed.

Inclusion Criteria
Only healthy male animals with inconspicuous behaviour at the age of 6 or 24 weeks were included in the experiment.

Exclusion Criteria
In the presence of conditions that may affect the outcome of the test or in the presence of severe functional impairment or symptoms of disease, the test should be terminated. For this purpose, the animals were observed daily for the first 48 h postoperatively. Withdrawal criteria include a 20% decrease in body weight from baseline, convulsions, paralysis (trunk muscles, extremities), respiratory noises, tremors and ataxia. If there is any doubt about an animal's distress, the animal welfare officer will be consulted immediately. Outside normal working hours, the experiment will be stopped in case of doubt. The animals are killed by cervical dislocation.

Randomization
The allocation of animals to each experimental group was randomised on the basis of the randomly assigned animal numbers.

Blinding
Data were analysed without knowledge of genotype and treatment and assigned to groups only after analysis using the corresponding animal number.