Supplemental Table I. Plasma PDGF-BB concentrations were detected in $CatS^{+/+}$ and $CatS^{-/-}$ mice followed ligation injury in the indicated days

Day after ligation injury	Group	Number	PDGF-BB (pg/mL)
day 0	$CatS^{+/+}$	8	45.8±2.1
	$CatS^{-/-}$	8	49.9±5.6
day 4	$CatS^{+/+}$	9	128.0±7.6*
	$CatS^{-/-}$	9	61.6±7.4†
day 28	$CatS^{+/+}$	11	81.7±20.2
	$CatS^{-/-}$	9	60.7±12.1
day 14	Control	7	132.7±25.8
	Tubastatin A	6	85.7±16.2

All of the results are presented as mean ± SEM. *P <0.01 vs $CatS^{+/+}$ mice group in day 0; †P <0.01 vs $CatS^{+/+}$ mice group in day 4.

Supplemental Table II. Primer sequences used in the quantitative real-time PCR

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	GenBank no.
MCP-1	GCCCCACTCACCTGCTGCTACT	CCTGCTGCTGGTGATCCTCTTGT	NM_011333
TLR2	AAGAAGCTGGCATTCCGAGGC	CGTCTGACTCCGAGGGGTTGA	NM_011905
TLR4	AGTGGGTCAAGGAACAGAAGCA	CTTTACCAGCTCATTTCTCACC	NM_021297
CatS	GTGGCCACTAAAGGGCCTG	ACCGCTTTTGTAGAAGAAGAAGGAG	NM_021281
CatK	AGCAGGCTGGAGGACTAAGGT	TTTGTGCATCTCAGTGGAAGACT	NM_007802
Cystatin C	AACAAGGGCAGCAACGATG	CGAGCTGCTTACGAGCTCTCAC	NM_009976
HDAC1	TCTGAATACAGCAAGCAGATGCA	ACAGAACTCAAACAAGCCATCAAAC	NM_008228
HDAC2	AGAAGATTGTCCGGTGTTTGATG	CACAGCCCCAGCAACTGAA	NM_008229
HDAC3	TCAGCCCCACCAATATGCA	GAACTCGAAAAGTCCTGGAAACA	NM_010411
HDAC4	CTGGCATCCCTGTGTCATTTG	ACACAAGACCTGTGGTGAACCTT	NM_207225
HDAC5	GCAACAAGGAGAAGAGCAAAGAG	TCCTGGAGCCTCAGCTTTACC	NM_010412
HDAC6	GCTGAGGGAGCCTGGTTAAA	AGGACTGCCCCTTTCGATCA	NM_010413
HDAC7	CCCACCTGTCAGACCCAAGT	AGTCATAGACCAGCCCTGTAGCA	NM_019572
HDAC8	AGGTACAATCACAGCTGCCC	TCTTTGCATGATGCCACCCT	NM_027382
HDAC9	TGGCAGAATCCTCGGTCAGT	CCCAGCAGGGCCATTGT	NM_024124
HIF-1α	GCAGCAGGAATTGGAACATT	GCATGCTAAATCGGAGGGTA	NM_176958
GAPDH	ATGTGTCCGTCGTGGATCTGA	ATGCCTGCTTCACCACCTTCT	NM_008084

Supplemental Fig. I. Ligation injuries induced CatS expression in carotid arteries. **A:** The mRNA levels of CatS were increased greatly in ligated arteries over those of the uninjured control vessels of $CatS^{+/+}$ mice on day 1, day 2, day 4, day 14 and day 28 after ligation injury (n=4–8). **B**: The representative image and quantitative data of CatS protein expression on day 4 after ligation (n=5). **C**: PCR bands show the image of the genotyping used $CatS^{+/+}$ and $CatS^{-/-}$ mice tails. **D**: Western blots images show the levels of CatS protein in the $CatS^{+/+}$ and $CatS^{-/-}$ VSMCs.

Supplemental Fig. II. $CatS^{-/-}$ decreases inflammatory reaction in injured arteries. The quantitative PCR revealed that the lesions in $CatS^{-/-}$ mice that received a ligation injury had lower mRNA levels of TLR2 (**A**) as well as MCP-1 (**B**), whereas TLR4 (**C**) exhibited no significant difference from the $CatS^{+/+}$ mice. There was no significant difference in the CatK (**D**) or Cystatin C (**E**) mRNA expressions between the $CatS^{+/+}$ and $CatS^{-/-}$ mice. **F:** Representative immunostaining images and quantitative data show $TLR2^{+}$ cells in injured arterial tissues of $CatS^{+/+}$ and $CatS^{-/-}$ mice (n=5). Triangles indicate TLR2+ cells. **G:** Quantitative real-time PCR data revealed that the levels of HIF-1α mRNA were increased in injured arterial tissues of $CatS^{+/+}$ mice (n= 5). **H:** Quantitative PCR revealed that the levels of HIF-1α mRNA were lower in the lesions of $CatS^{-/-}$ mice compared to $CatS^{+/+}$ mice. Data are mean ± SEM; Student's unpaired t-test.

Supplemental Fig. III. HDAC6 mRNA expression on day 1 to day 4 following ligation in wild-type mice. **A:** HDAC1-9 mRNA expressions on day 4 after ligation injury. Left artery, no ligation side. Right artery, ligation side. n=7. **B:** HDAC6 mRNA expression on day 0, day 1, day 2 and day 4 following ligation in wild-type mice (n= 6-8). Data are mean ± SEM, One-way ANOVA and Bonferroni post-hoc test.

Supplemental Fig. IV. HDAC6 inhibition reduced HDAC6 phophorylation. Representative image (**A**) and quantitative data (**B**) of the western blot show that tubastatin A (10 μM) decreased the levels of pHDAC6 (n= 3). Data are mean ± SEM, ANOVA and Bonferroni post hoc tests.

Supplemental Fig. V. HDAC6 silencing decreased VSMC proliferation and migration. A, siHDAC6 inhibited PDGF-BB (10 ng/mL)-induced VSMC proliferation (n=5). **B,** The representative image and quantitative data show siHDAC6 mitigated PDGF-BB (50 ng/mL)-induced VSMC migration (n= 6). Data are mean ± SEM, Student's unpaired t-test.

Supplemental Fig. VI. Role of CatS in mitogen-induced VSMC proliferation and migration. CatS inhibitor (CatS-I, 10 μM, **A**) and CatS deficiency (CatS KO, **B**) inhibited the PDGF-BB (50 ng/mL)-induced VSMC proliferation (n=8). The representative image and quantitative data show that CatS inhibitor (CatS-I, 10 μM) impaired PDGF-BB (50 ng/mL, **C**)- or 2% FBS (**D**)-induced VSMC migration. Data are mean ± SEM, Student's unpaired t-test.

Supplemental Fig. VII. CatS$^{-/-}$ impaired VSMC migration ex vivo. A: Representative figures of VSMC migration from arterial explants from day 1 to day 7 in the two groups. The quantitative data show that the sprouting VSMC numbers (**B**) and cell sprouted area (**C**) were markedly decreased in the CatS$^{-/-}$ mice (n= 7). Data are mean ± SEM, 2-way repeated measures ANOVA ad Bonferroni post hoc tests.

Supplemental Fig. VIII. p38MAPK/Akt signaling pathway-mediated regulation of HDAC6 activity in VSMCs. Mouse aortic VSMCs were cultured in 10% FBS/DMEM medium and then subjected to serum-free medium for 12 h before the following treatment. Protein samples were isolated and used for a western blotting analysis as indicated. Representative immunoblots (**A**) and quantitative data (**B**) show the effect of PDGF-BB (20 ng/mL) on phosphorylation of HDAC6, p38MAPK, Akt, Erk1/2 in CatS$^{+/+}$ VSMCs (n=3). CatS $^{+/+}$ VSMCs were pretreated with or without p38MAPK inhibitor (SB203580, 20 μM, **C**) or Akt inhibitor (10 μM, **D, E**) for 30 min and then treated with or without PDGF-BB (20 ng/mL) for 10 min (n=4). Representative images and quantitative data show that SB203580 or Akt inhibitor decreased the levels of p-HDAC6 and/or p-eEF2 (n=4) Data are mean ± SEM, 1-way, or 2-way ANOVA and Bonferroni post hoc tests.

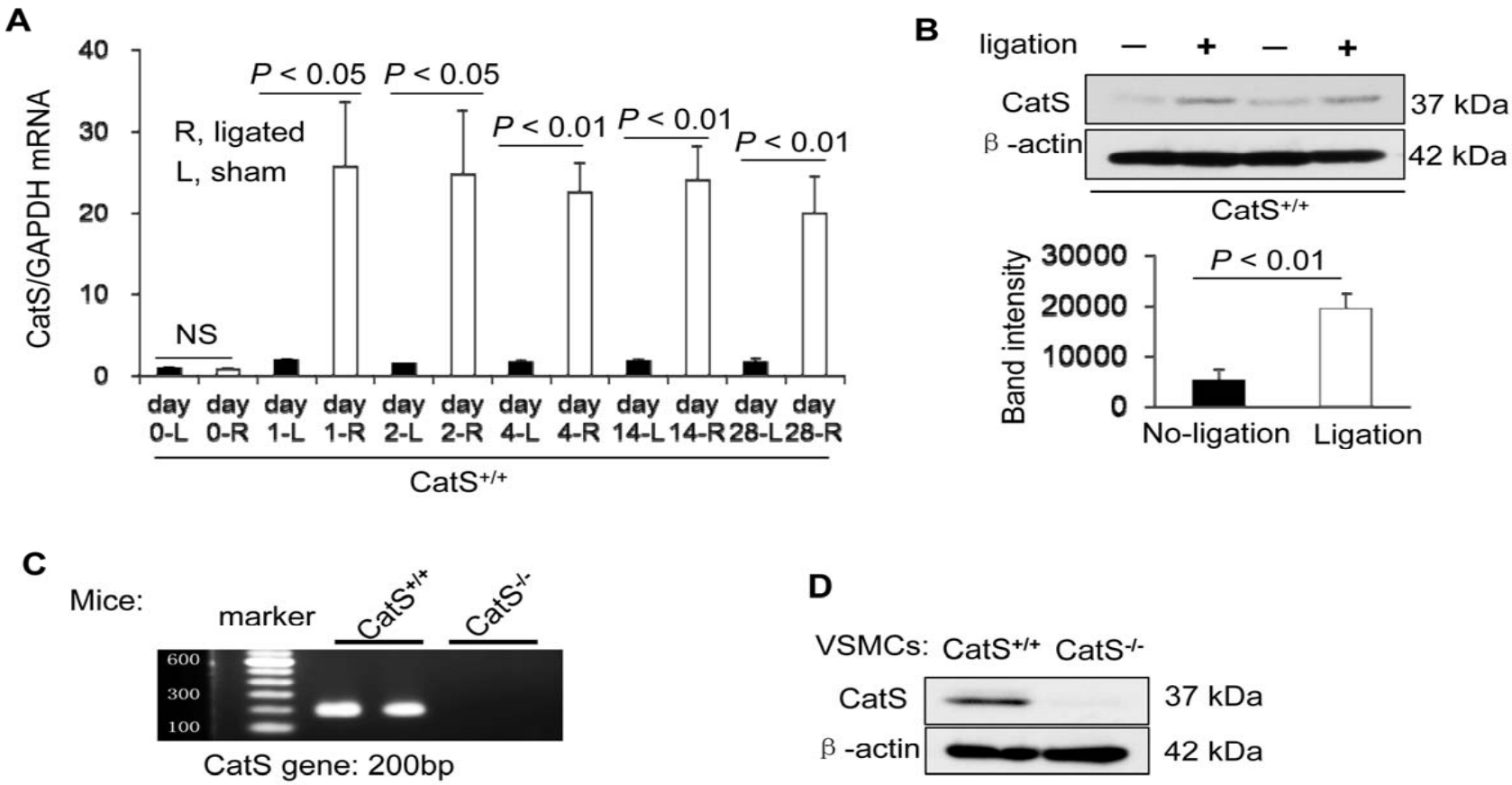
Supplemental Fig. IX. The effect of HDAC6 transfection on the expression of p-HDAC6. Mouse VSMCs were transfected with control vector or HDAC6 plasmid for 48 h and then subjected to serum-free medium for 12 h before the following treatment. Protein samples were isolated and used for a western blotting analysis as indicated. **A:** Representative immunoblots and quantitative data show that VSMCs transfected with HDAC6 plasmid increased HDAC6 expression. **B**: Representative immunoblots and quantitative data show the effect of HDAC6 transfection on the levels of p-HDAC6 protein (n=3). Data are mean±SEM; Student's paired t-test.

Supplemental. Fig. X. HDAC6 plasmid transfection increased the in vitro VSMC proliferation, migration, and cell-cycle progression. VSMCs were transfected with control (vector) or HDAC6 plasmid (HDAC6) for 48–72 h and then used in the following experiments: **A:** Cells were treated with PDGF-BB (20 ng/mL) for 48 h, and cell proliferation was then measured by an MTS assay (n=8). **B:** Cells were scratched with a 1-ml pipet tip and cultured for 24 h with PDGF-BB (20 ng/mL, n=7). Representative immunoblots (**B**) and quantitative data (**C**) show that HDAC6 plasmid transfection increased the in vitro VSMC migration. **D**,**E**: The transfected cells were re-grown in a 6-well plate for 24 h and starved for 12 h before stimulation with 10% FBS for 36 h for the cell-cycle assay (n= 4). Distribution of cells in S/G2/M expressed as a percentage of total cells. Data are mean ± SEM, Student's unpaired t-test.

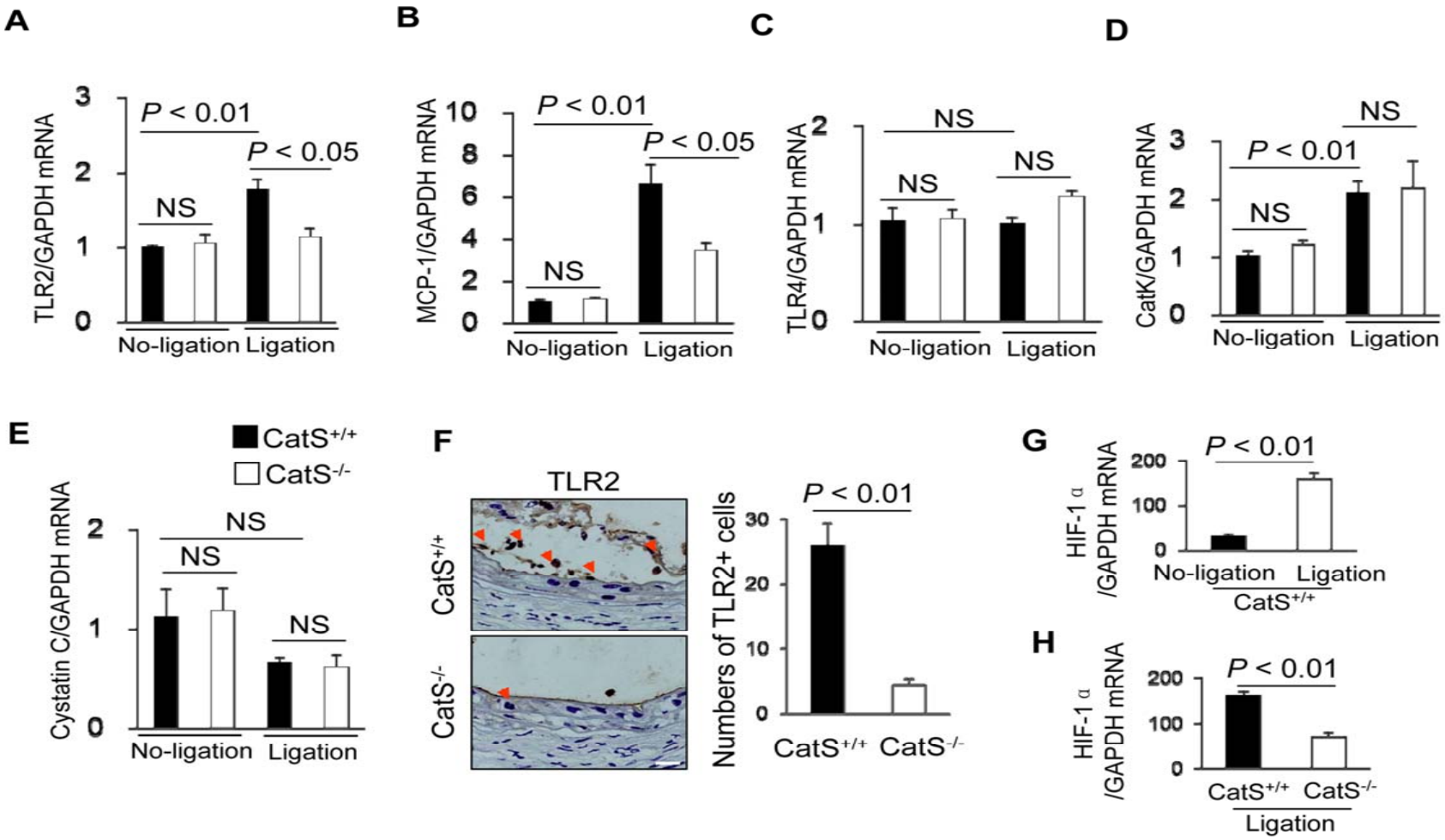
Supplemental Fig. XI. TLR2 silencing decreased not only the levels of p-HDAC6, p-p38MAPK and p-Akt and but also VSMC proliferation and migration. Representative immunoblots (**A**) and quantitative data (**B**) show TLR2 silencing inhibited the levels of p-HDAC6, p-p38MAPK and p-Akt induced by PDGF-BB (20 ng/mL) for 10 min (n= 3). **C**, siTLR2 reduced VSMC proliferation in response to PDGF-BB (10 ng/mL, n= 5). **D,** Rresentative image and quantitative data show siTLR2 mitigated VSMC migration in response to PDGF-BB (50 ng/mL, n=6). Data are mean ± SEM, Student's unpaired t-test.

Supplemental Fig. XII. The proposed mechanism of ligation-induced vascular repair in mice. TLR2, toll-like receptor 2; CatS, cathepsin S; PDGF-BB, platelet-derived growth factor BB; HDAC6, histone deacetylase 6; VSMC, vascular smooth muscle cell; HIF1α, hypoxia-inducible factor 1 alpha.

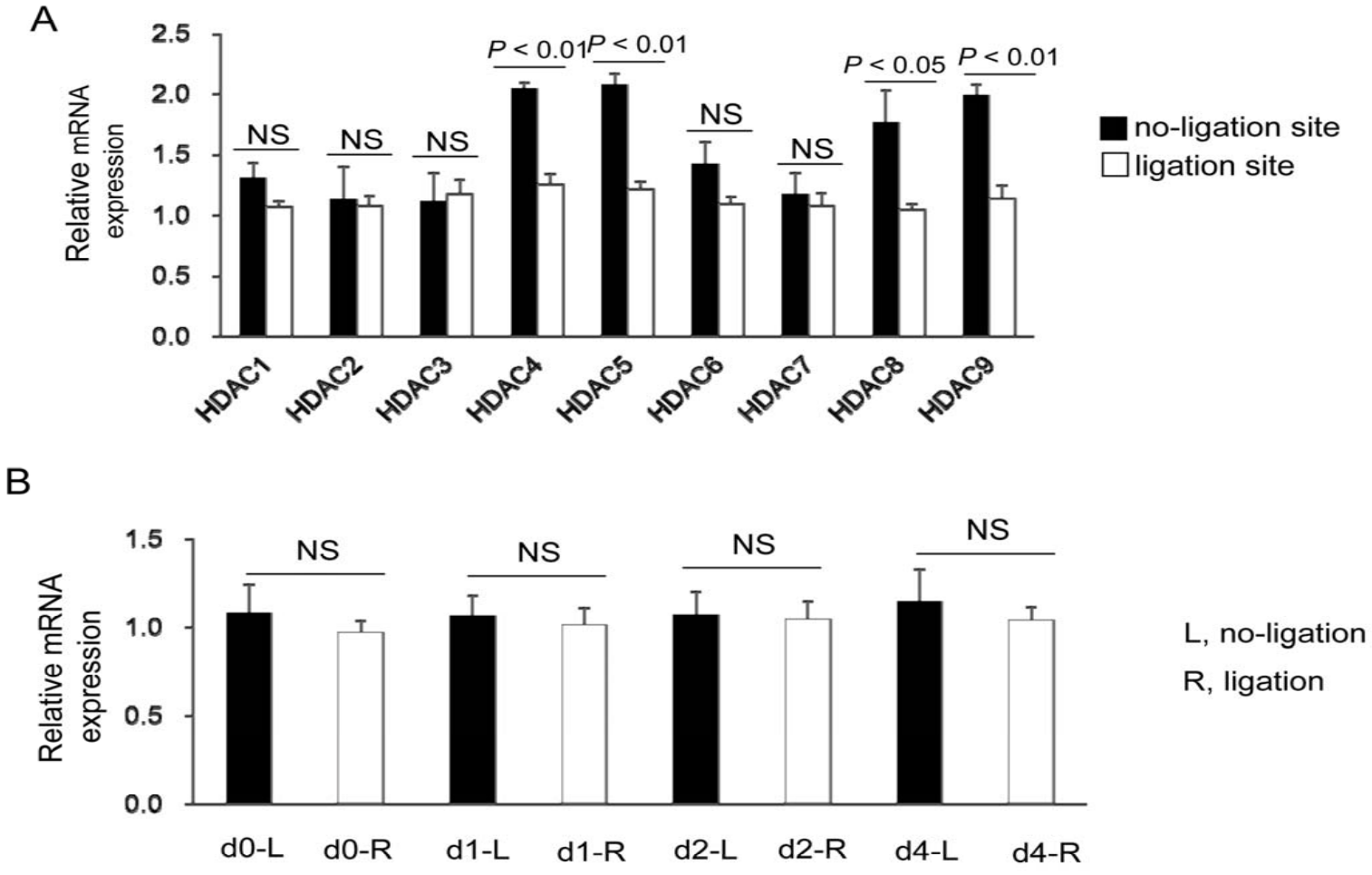
Sup. Fig. I


A
CatS/GAPDH mRNA
40
30
20
10
0
P < 0.05
P < 0.05
P < 0.01
P < 0.01
P < 0.01
R, ligated
L, sham
NS
day 0-L
day 0-R
day 1-L
day 1-R
day 2-L
day 2-R
day 4-L
day 4-R
day 14-L
day 14-R
day 28-L
day 28-R
CatS+/+
B
ligation — + — +
CatS
β -actin
37 kDa
42 kDa
CatS+/+
Band intensity
30000
20000
10000
0
P < 0.01
No-ligation
Ligation
C
Mice:
marker
CatS+/+
CatS-/-
600
300
100
CatS gene: 200bp
D
VSMCs: CatS+/+ CatS-/-
CatS
β -actin
37 kDa
42 kDa

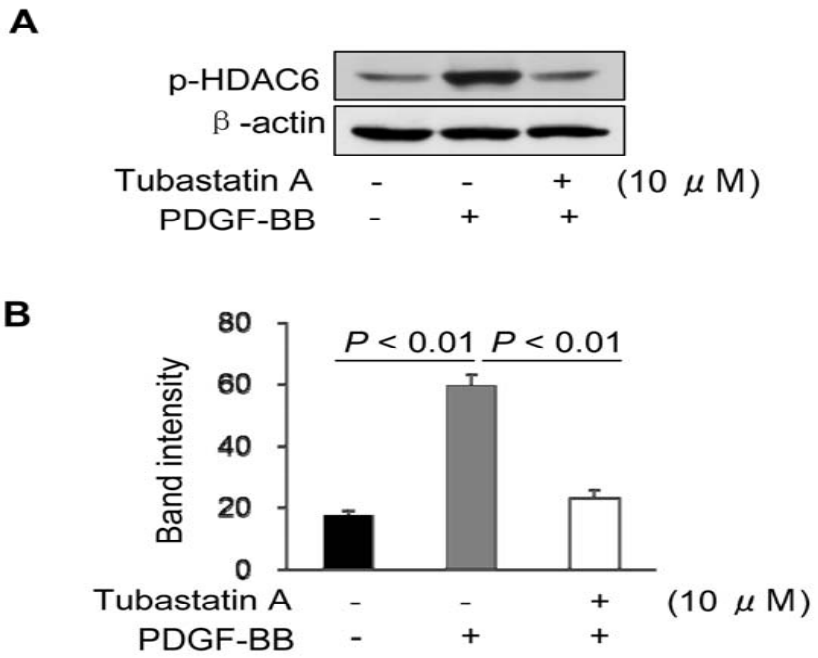
Sup. Fig. II


A
TLR2/GAPDH mRNA
P < 0.01
P < 0.05
NS
No-ligation Ligation
B
MCP-1/GAPDH mRNA
P < 0.01
P < 0.05
NS
No-ligation Ligation
C
TLR4/GAPDH mRNA
NS
NS
NS
No-ligation Ligation
D
CatK/GAPDH mRNA
P < 0.01
NS
NS
No-ligation Ligation
E
CatS+/+
CatS-/-
Cystatin C/GAPDH mRNA
NS
NS
NS
No-ligation Ligation
F
TLR2
CatS+/+
CatS-/-
Numbers of TLR2+ cells
P < 0.01
CatS+/+ CatS-/-
G
HIF-1α /GAPDH mRNA
P < 0.01
No-ligation Ligation
CatS+/+
H
HIF-1α /GAPDH mRNA
P < 0.01
CatS+/+ CatS-/-
Ligation

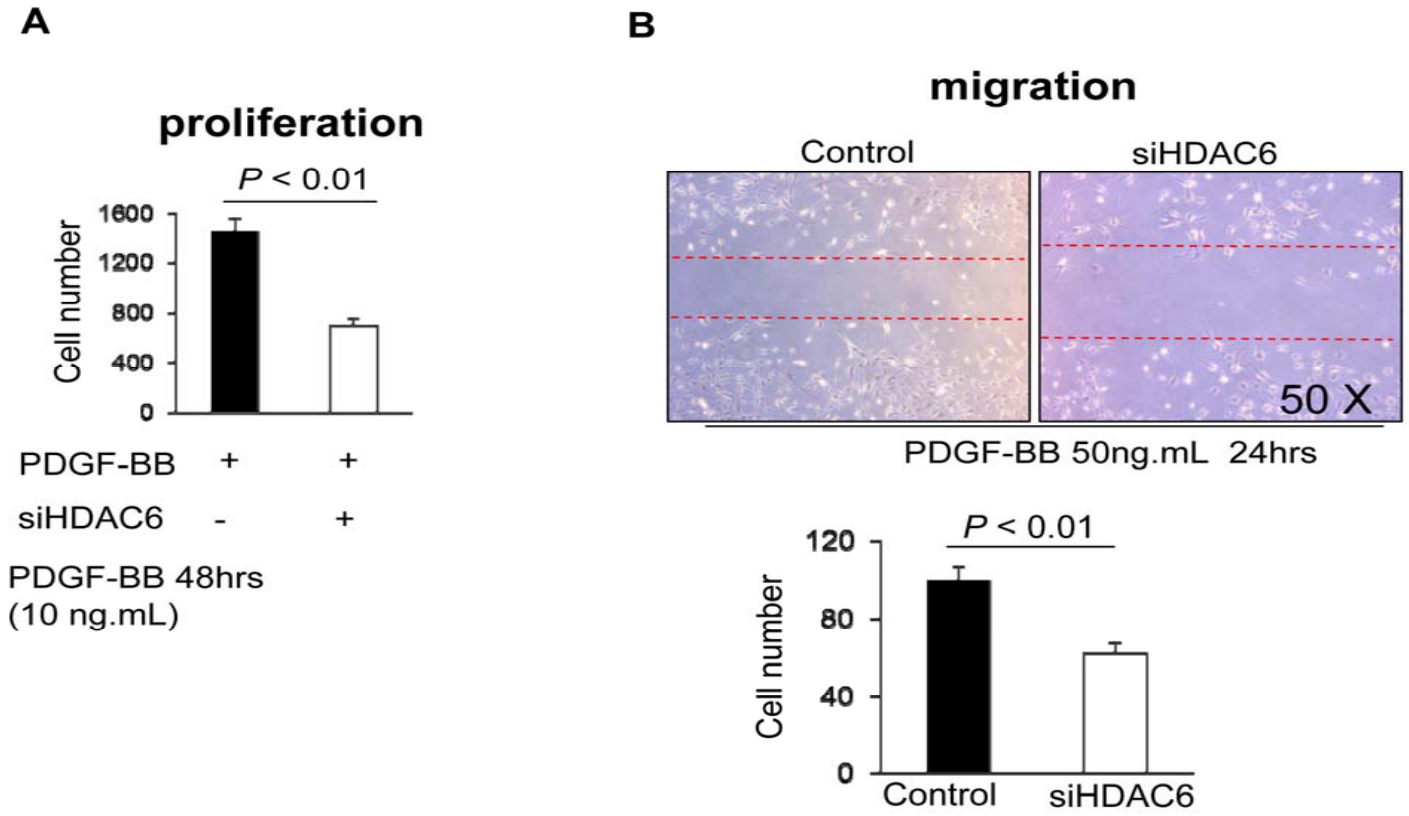
Sup. Fig. III


A
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
NS
NS
NS
P < 0.01
P < 0.01
NS
NS
P < 0.05
P < 0.01
HDAC1
HDAC2
HDAC3
HDAC4
HDAC5
HDAC6
HDAC7
HDAC8
HDAC9
no-ligation site
ligation site
B
Relative mRNA expression
1.5
1.0
0.5
0.0
NS
NS
NS
NS
d0-L
d0-R
d1-L
d1-R
d2-L
d2-R
d4-L
d4-R
L, no-ligation
R, ligation

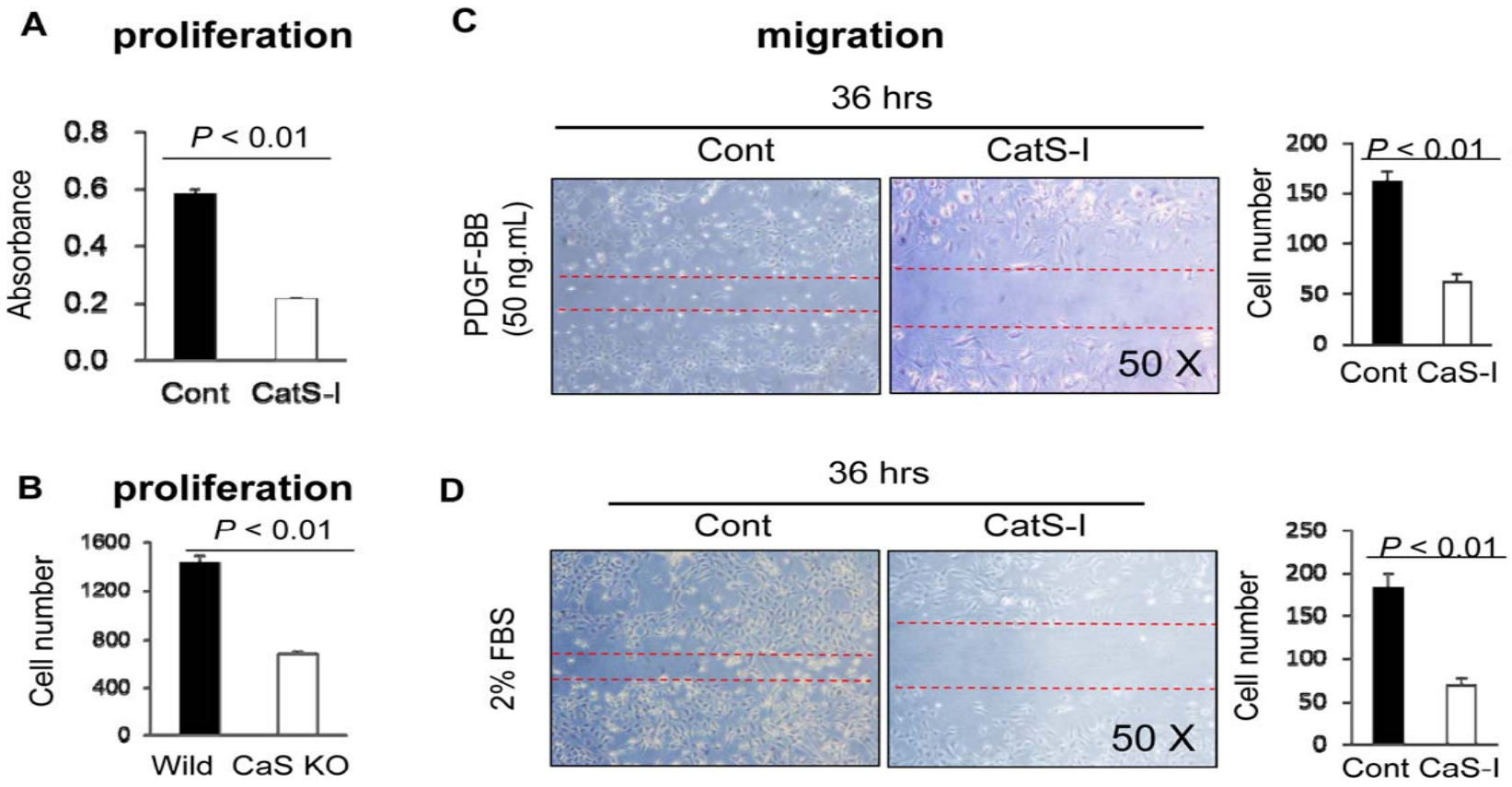
Sup. Fig. IV


A
p-HDAC6
β -actin
Tubastatin A - - + (10 μ M)
PDGF-BB - + +
B
Band intensity
80
60
40
20
0
P < 0.01
P < 0.01
Tubastatin A - - + (10 μ M)
PDGF-BB - + +

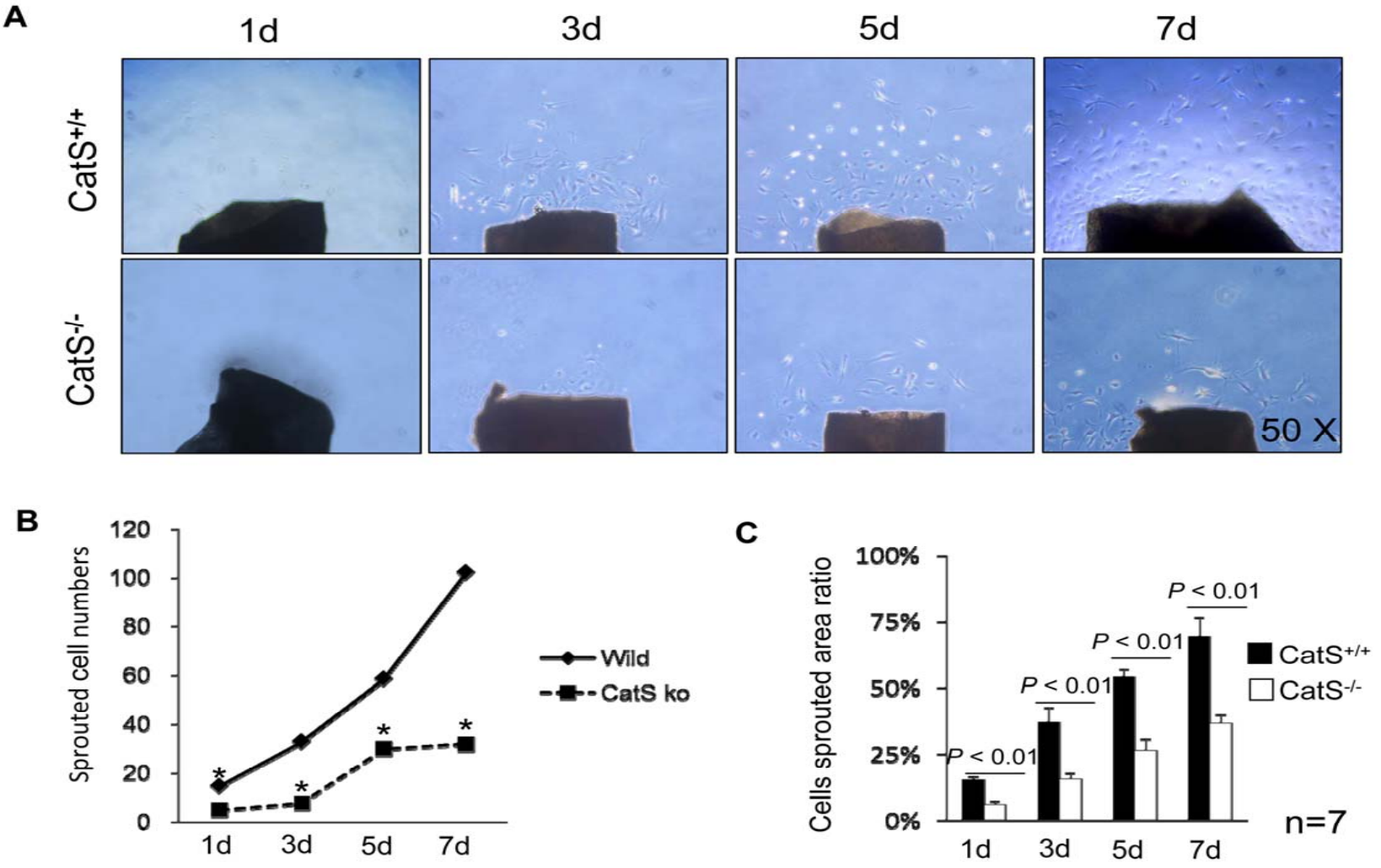
Sup. Fig. V


A
proliferation
P < 0.01
Cell number
1600
1200
800
400
0
PDGF-BB + +
siHDAC6 - +
PDGF-BB 48hrs
(10 ng.mL)
B
migration
Control
siHDAC6
50 X
PDGF-BB 50ng.mL 24hrs
P < 0.01
Cell number
120
80
40
0
Control
siHDAC6

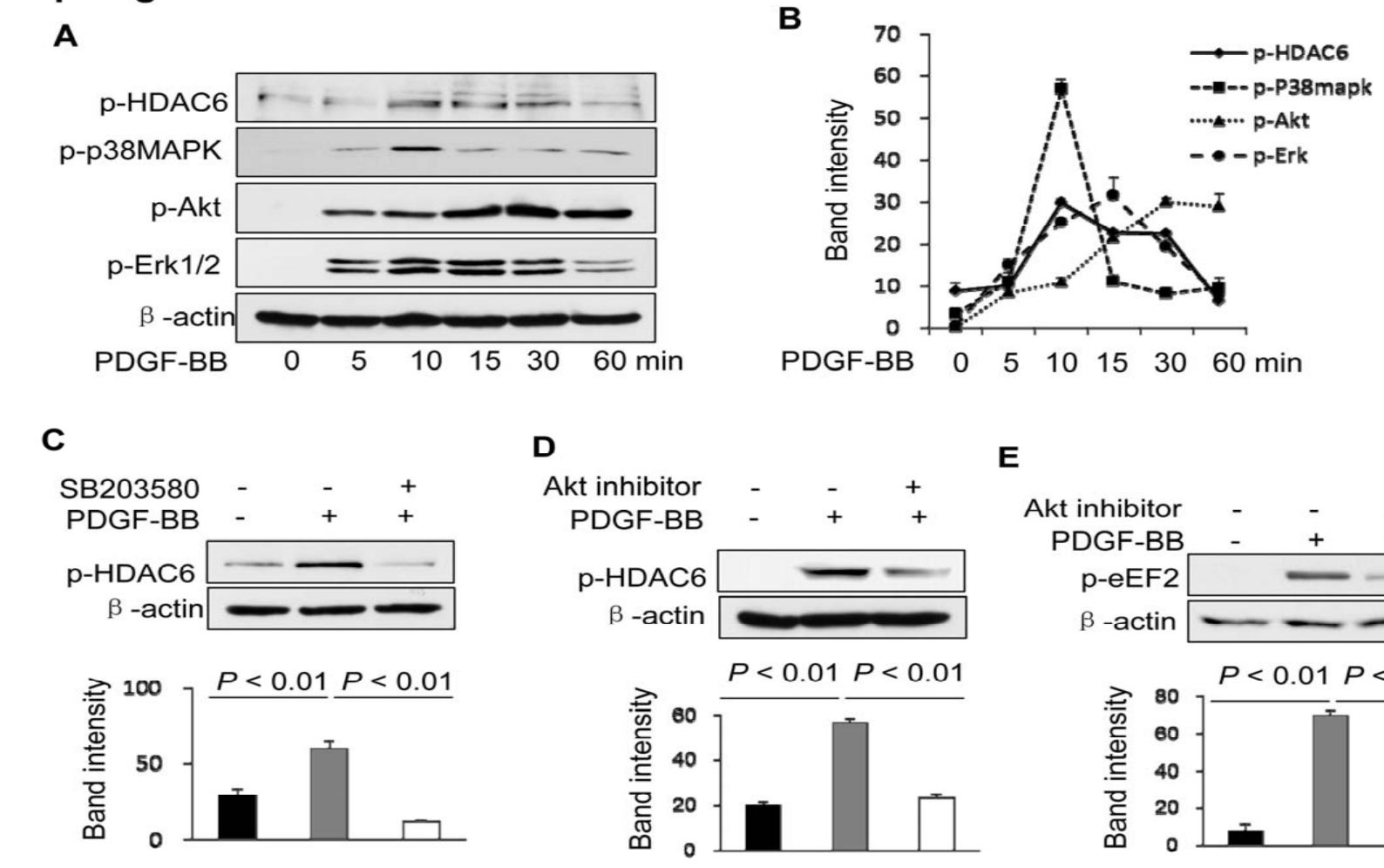
Sup. Fig. VI


A
proliferation
Absorbance
0.8
0.6
0.4
0.2
0.0
P < 0.01
Cont
CatS-I
B
proliferation
Cell number
1600
1200
800
400
0
P < 0.01
Wild
CaS KO
C
migration
36 hrs
Cont
CatS-I
PDGF-BB
(50 ng.mL)
50 X
Cell number
200
150
100
50
0
P < 0.01
Cont
CaS-I
D
36 hrs
Cont
CatS-I
2% FBS
50 X
Cell number
250
200
150
100
50
0
P < 0.01
Cont
CaS-I

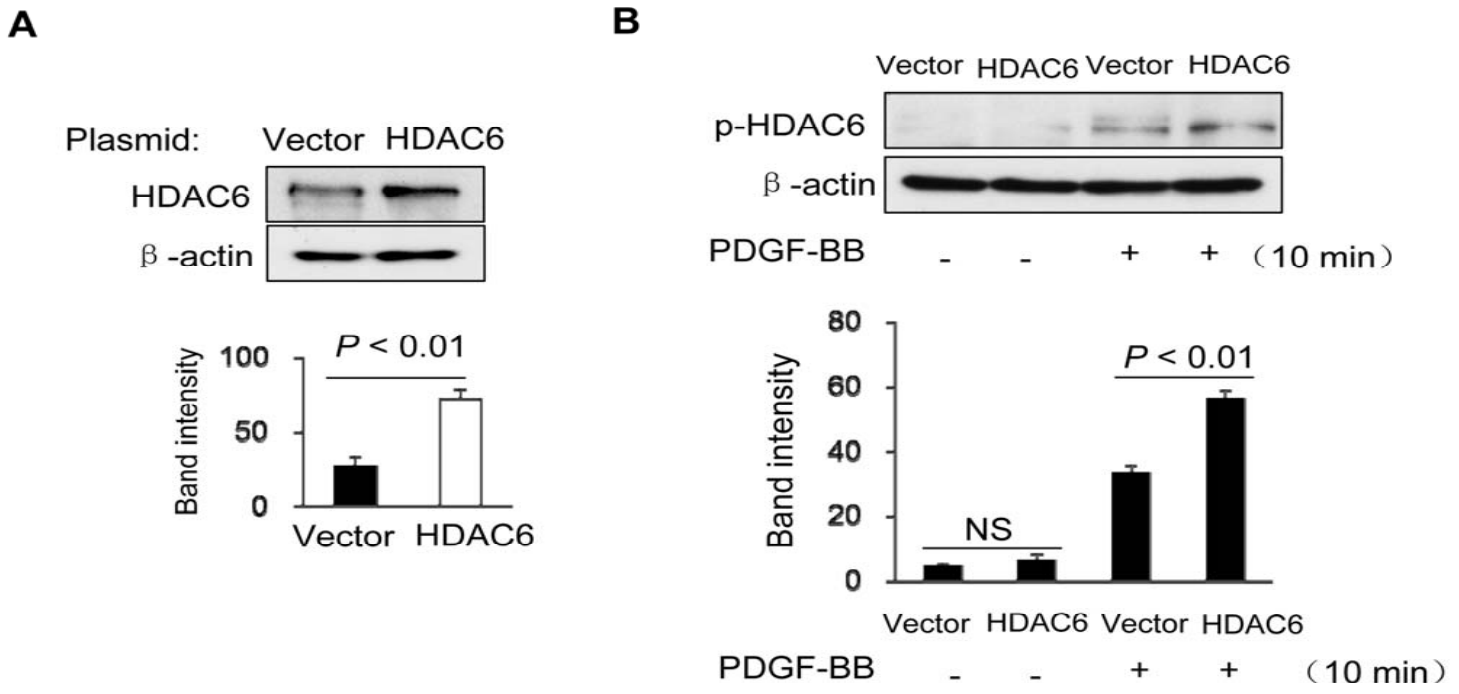
Sup. Fig. VII


A
1d
3d
5d
7d
CatS+/+
CatS-/-
50 X
B
Sprouted cell numbers
120
100
80
60
40
20
0
1d
3d
5d
7d
Wild
CatS ko
C
Cells sprouted area ratio
100%
75%
50%
25%
0%
P < 0.01
P < 0.01
P < 0.01
P < 0.01
CatS+/+
CatS-/-
n=7
1d
3d
5d
7d

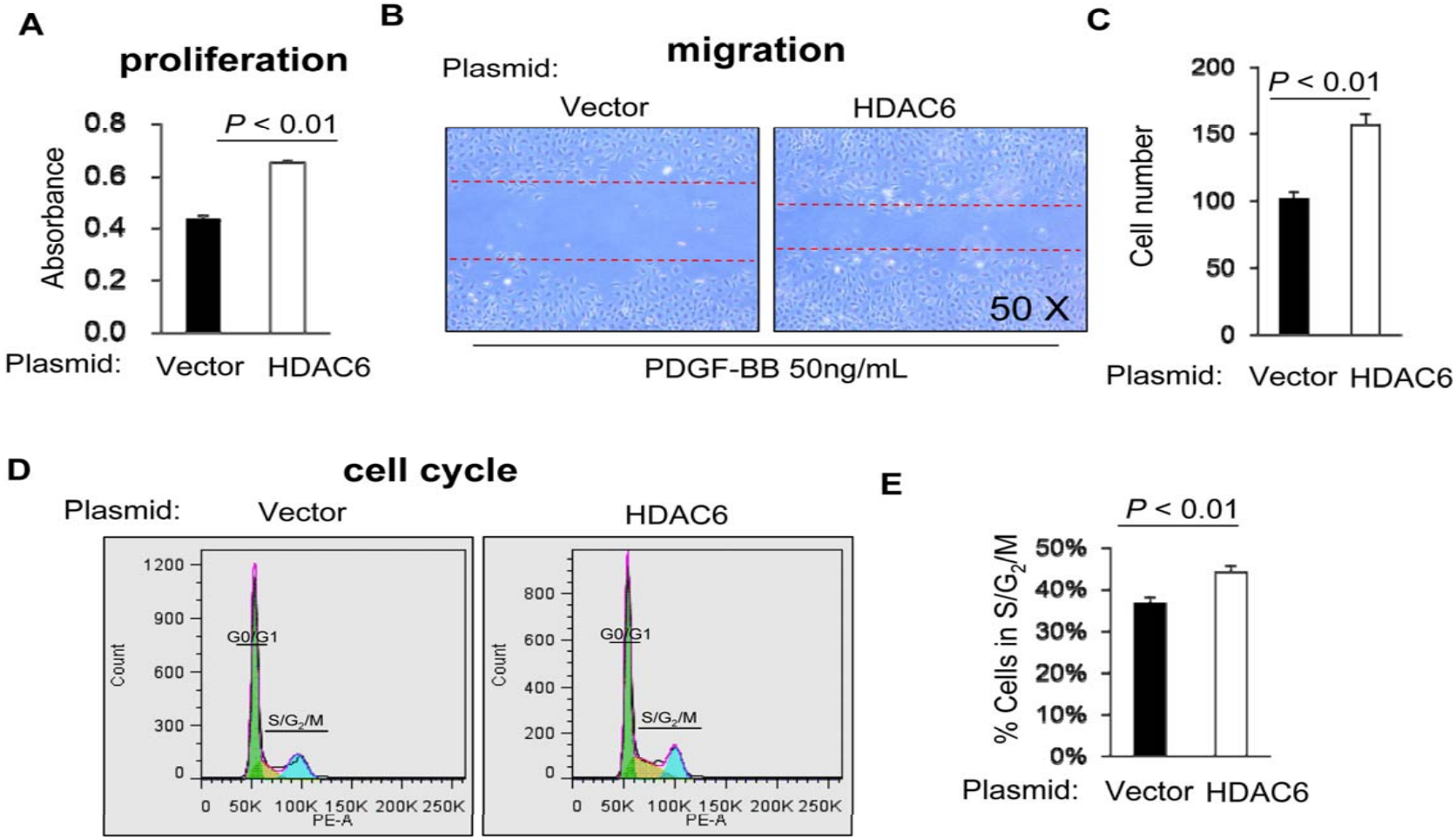
Sup. Fig. VIII


A
p-HDAC6
p-p38MAPK
p-Akt
p-Erk1/2
β -actin
PDGF-BB 0 5 10 15 30 60 min
B
Band intensity
p-HDAC6
p-P38mapk
p-Akt
p-Erk
PDGF-BB 0 5 10 15 30 60 min
C
SB203580 - - +
PDGF-BB - + +
p-HDAC6
β -actin
Band intensity
P < 0.01 P < 0.01
D
Akt inhibitor - - +
PDGF-BB - + +
p-HDAC6
β -actin
Band intensity
P < 0.01 P < 0.01
E
Akt inhibitor - - +
PDGF-BB - + +
p-eEF2
β -actin
Band intensity
P < 0.01 P < 0.01

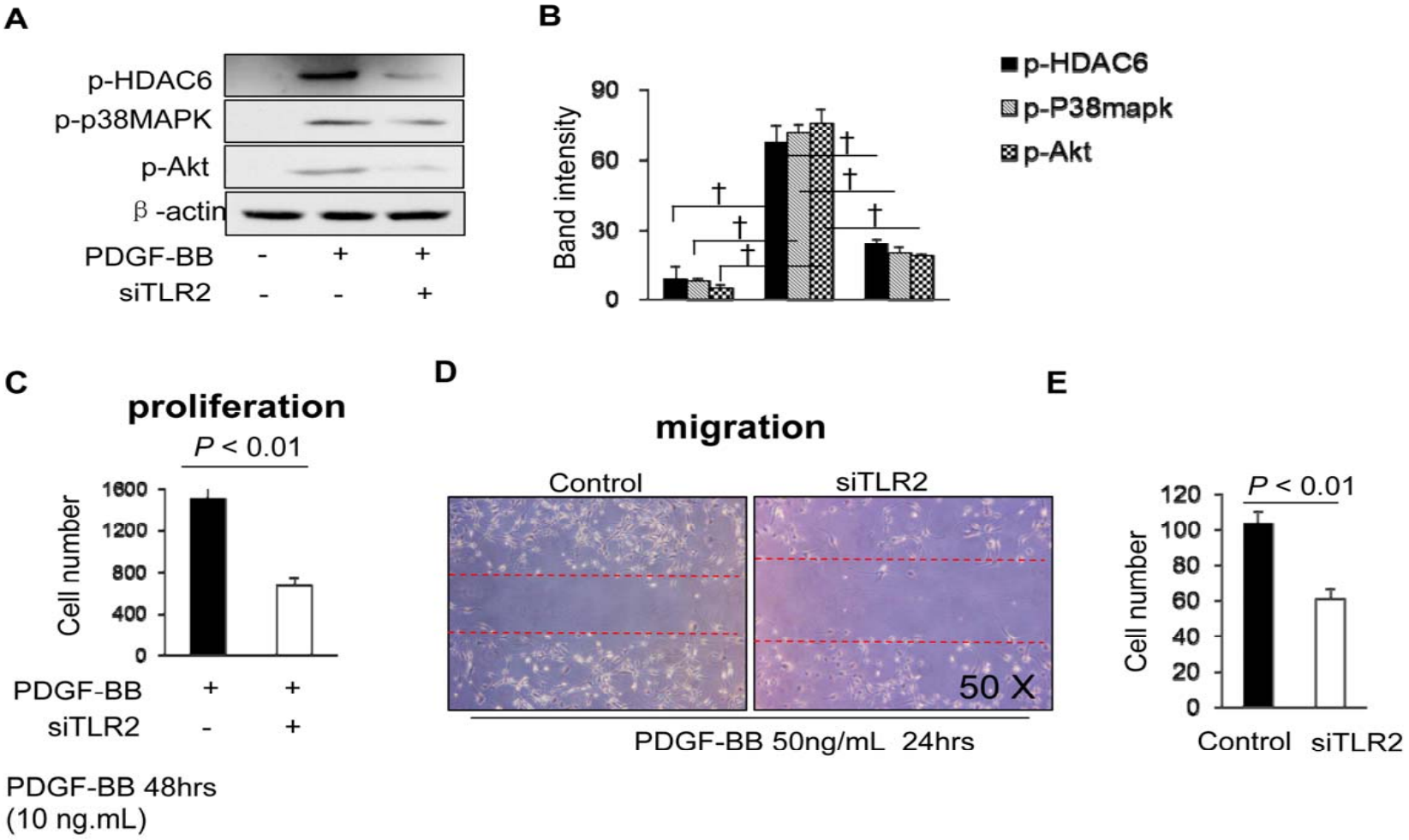
Sup. Fig. IX


A
Plasmid: Vector HDAC6
HDAC6
β -actin
Band intensity
100
50
0
P < 0.01
Vector HDAC6
B
Vector HDAC6 Vector HDAC6
p-HDAC6
β -actin
PDGF-BB - - + + (10 min)
Band intensity
80
60
40
20
0
P < 0.01
NS
Vector HDAC6 Vector HDAC6
PDGF-BB - - + + (10 min)

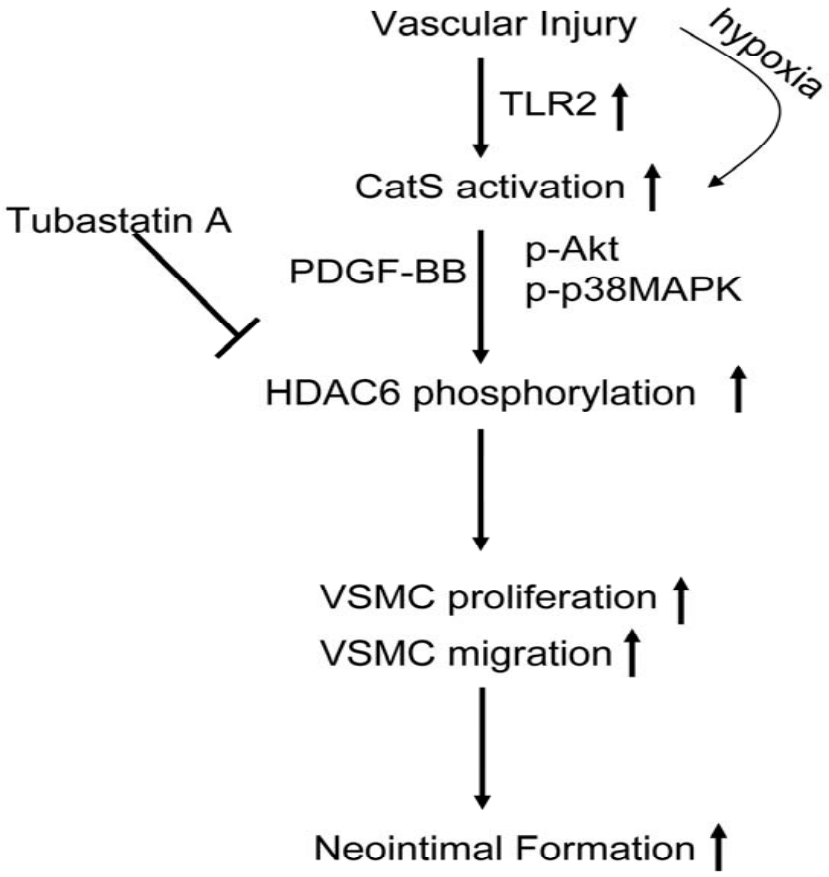
Sup. Fig. X


A
proliferation
Absorbance
0.8
0.6
0.4
0.2
0.0
P < 0.01
Plasmid:
Vector
HDAC6
B
migration
Plasmid:
Vector
HDAC6
50 X
PDGF-BB 50ng/mL
C
Cell number
200
150
100
50
0
P < 0.01
Plasmid:
Vector
HDAC6
D
cell cycle
Plasmid:
Vector
HDAC6
Count
G0/G1
S/G2/M
PE-A
E
P < 0.01
% Cells in S/G2/M
50%
40%
30%
20%
10%
0%
Plasmid:
Vector
HDAC6

Sup. Fig. XI


A
p-HDAC6
p-p38MAPK
p-Akt
β -actin
PDGF-BB - + +
siTLR2 - - +
B
Band intensity
90
60
30
0
p-HDAC6
p-P38mapk
p-Akt
C
proliferation
P < 0.01
Cell number
1600
1200
800
400
0
PDGF-BB + +
siTLR2 - +
PDGF-BB 48hrs
(10 ng.mL)
D
migration
Control
siTLR2
50 X
PDGF-BB 50ng/mL 24hrs
E
P < 0.01
Cell number
120
100
80
60
40
20
0
Control siTLR2

Sup. Fig. XII


Vascular Injury
hypoxia
TLR2
CatS activation
Tubastatin A
PDGF-BB
p-Akt
p-p38MAPK
HDAC6 phosphorylation
VSMC proliferation
VSMC migration
Neointimal Formation