Supplemental Appendix

Supplemental Table 1. siRNA sequences used in the study.

Target	**Species**	**sense（5'-3'）**	**antisense（5'-3'）**
OTUB1 #1	Homo Sapiens	UUAACUGUCUGGCCUAUGATT	UCAUAGGCCAGACAGUUAATT
OTUB1 #2	Homo Sapiens	CCAUGUGCAAGGAGAGCGATT	UCGCUCUCCUUGCACAUGGTT
OTUB2	Homo Sapiens	GCAGCCGAUAAACAUUGAUTT	AUCAAUGUUUAUCGGCUGCTT
OTUD1	Homo Sapiens	GCCAAAUCUAUGGCCAUAUTT	AUAUGGCCAUAGAUUUGGCTT
OTUD2	Homo Sapiens	GACGCCUCAUAGCACAAAUTT	AUUUGUGCUAUGAGGCGUCTT
OTUD3	Homo Sapiens	CUGGCAAUGAUGCAAUUGUTT	ACAAUUGCAUCAUUGCCAGTT
OTUD4	Homo Sapiens	GGGUAGGACAAGUGGAAAUTT	AUUUCCACUUGUCCUACCCTT
OTUD5	Homo Sapiens	GGAGGAGUCAUGGAUUGAATT	UUCAAUCCAUGACUCCUCCTT
OTUD6A	Homo Sapiens	GCCCACAGAAAGAGAGAAATT	UUUCUCUCUUUCUGUGGGCTT
OTUD6B	Homo Sapiens	GCUAGACAGUUAGAAAUUATT	UAAUUUCUAACUGUCUAGCTT
PDGFRβ	Homo Sapiens	GCCGUCAAGAUGCUUAAAUTT	AUUUAAGCAUCUUGACGGCTT
c-CBL	Homo Sapiens	CUGCCGAUGUGAAAUUAAATT	UUUAAUUUCACAUCGGCAGTT
Negative control	-	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

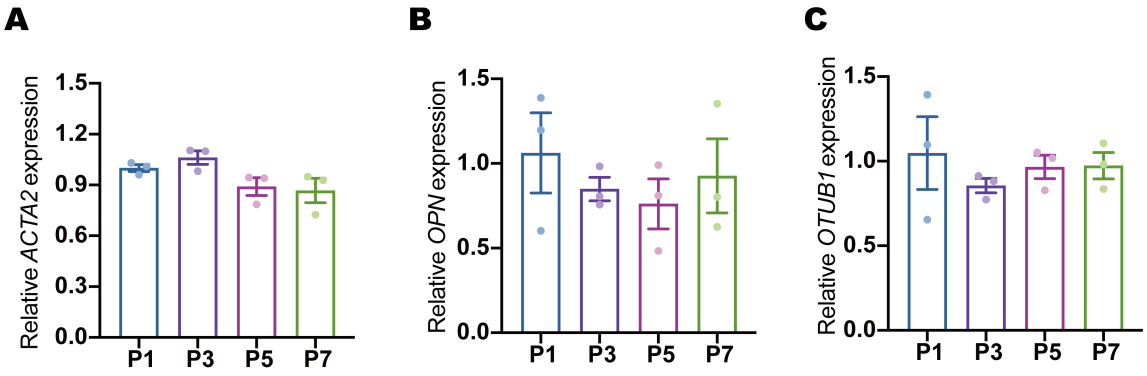
Supplemental Table 2. qPCR primers used in the study.

Target	Species	Foward	Reverse
OTUB1	Homo Sapiens	GCTGGATGACAGCAAGGAGTTG	CTTCTCCACCTGCTCAATCAGG
OTUB2	Homo Sapiens	GCTGGCTTTGAGGAGCACAAGT	CTGGTCGTTGAACACCTTCAGC
OTUD1	Homo Sapiens	CAGTTGGCTCAGTAACGGACAC	GATTTGGCAAGTTCTTCGTCGCG
OTUD2	Homo Sapiens	CCATTCTGGAAGACTTGCCCATC	ACCACGGTTCTGGTAAGCACAG
OTUD3	Homo Sapiens	AGCACTACGACAGTGTTCGGAG	GGTCGTCTTCAGAGTCCATTCC
OTUD4	Homo Sapiens	CTAACTCCTGCGGTGCCTTCTT	GCTGAATCAGGTCCAGTGGTCA
OTUD5	Homo Sapiens	CAGGCTACAACAGTGAGGACGA	GAAGCCCTTCTTGTCTCGTAGG
OTUD6A	Homo Sapiens	GTTCAGCGTGTCTGTGGAGATG	GCACGATGTTGTCGCAGTAGATC
OTUD6B	Homo Sapiens	CTGCTGAGAAGGCATCGCAAAG	GCCACATCTTCGGTGAGTTGCT
PDGFRβ	Homo Sapiens	TGCAGACATCGAGTCCTCCAAC	GCTTAGCACTGGAGACTCGTTG
SLC3A2	Homo Sapiens	CCAGAAGGATGATGTCGCTCAG	GAGTAAGGTCCAGAATGACACGG
SLC7A5	Homo Sapiens	GCCACAGAAAGCCTGAGCTTGA	ATGGTGAAGCCGATGCCACACT
ACTA2	Homo Sapiens	CTATGCCTCTGGACGCACAACT	CAGATCCAGACGCATGATGGCA
TAGLN	Homo Sapiens	TCCAGGTCTGGCTGAAGAATGG	CTGCTCCATCTGCTTGAAGACC
OPN	Homo Sapiens	CGAGGTGATAGTGTGGTTTATGG	GCACCATTCAACTCCTCGCTTTC
GAPDH	Homo Sapiens	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
Otub1	Mus musculus	GCTGGAACTCTCAGTCCTGTAC	CGGTAGAAGCAGTTGCCATCAG
Gapdh	Mus musculus	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

Supplemental Table 3.

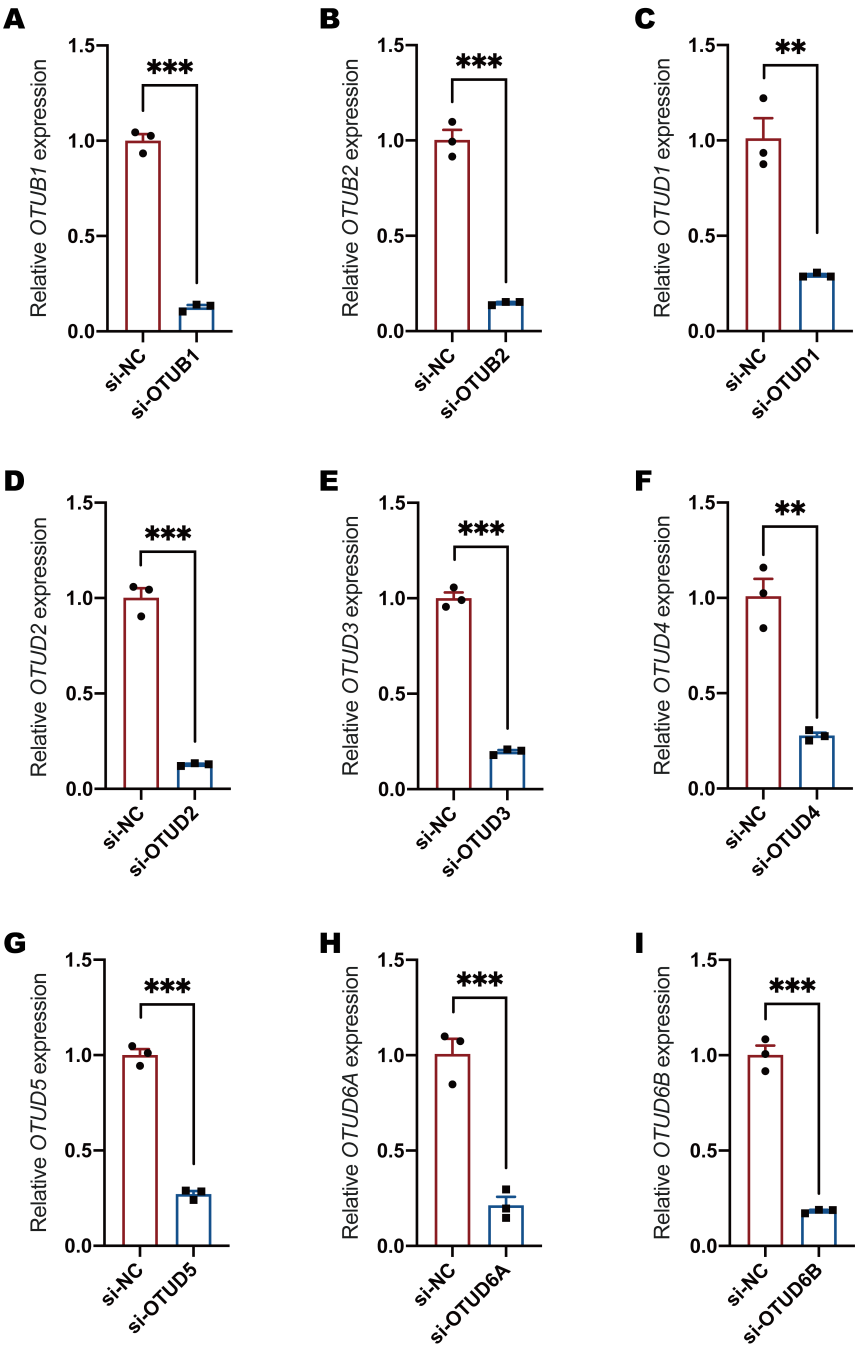
Ubibrowser website predicted binding site on PDGFRβ of OTUB1.

1	MRLPGAMPALALKGELLLLSLLLLLEPQISQGLVVTPPGPELVLNV
47	SSTFVLTCSGSAPVVWERMSQEPPQEMAKAQDGTFSSVLTLTNLTG
93	LDTGEYFCTHNDSRGLETDERKRLYIFVPDPTVGFLPNDAEELFIF
139	LTEITEITIPCRVTDPQLVVTLHEKKGDVALPVPYDHQRGFSGIFE
185	DRSYICKTTIGDREVDSDAYYVYRLQVSSINVSVNAVQTVVRQGEN
231	ITLMCIVIGNEVVNFEWTYPRKESGRLVEPVTDFLLDMPYHIRSIL
277	HIPSAELEDSGTYTCNVTESVNDHQDEKAINITVVESGYVRLLGEV
323	GTLQFAELHRSRTLQVVFEAYPPPTVLWFKDNRTLGDSSAGEIALS
369	TRNVSETRYVSELTLVRVKVAEAGHYTMRAFHEDAEVQLSFQLQIN
415	VPVRVLELSESHPDSGEQTVRCRGRGMPQPNIIWSACRDLKRCPRE
461	LPPTLLGNSSEEESQLETNVTYWEEEQEFEVVSTLRLQHVDRPLSV
507	RCTLRNAVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIIL
553	IMLWQKKPRYEIRWKVIESVSSDGHEYIYVDPMQLPYDSTWELPRD
599	QLVLGRTLGSGAFGQVVEATAHGLSHSQATMKVAVKMLKSTARSSE
645	KQALMSELKIMSHLGPHLNVVNLLGACTKGGPIYIITEYCRYGDLV
691	DYLHRNKHTFLQHHSD**K**RRPPSAELYSNALPVGLPLPSHVSLTGES
737	DGGYMDMSKDESVDYVPMLDMKGDVKYADIESSNYMAPYDNYVPSA
783	PERTCRATLINESPVLSYMDLVGFSYQVANGMEFLASKNCVHRDLA
829	ARNVLICEGKLVKICDFGLARDIMRDSNYISKGSTFLPLKWMAPES
875	IFNSLYTTLSDVWSFGILLWEIFTLGGTPYPELPMNEQFYNAIKRG
921	YRMAQPAHASDEIYEIMQKCWEEKFEIRPPFSQLVLLLERLLGEGY
967	KKKYQQVDEEFLRSDHPAILRSQARLPGFHGLRSPLDTSSVLYTAV
1013	QPNEGDNDYIIPLPDPKPEVADEGPLEGSPSLASSTLNEVNTSSTI
1059	SCDSPLEPQDEPEPEPQLELQVEPEPELEQLPDSGCPAPRAEAEDS
1105	FL



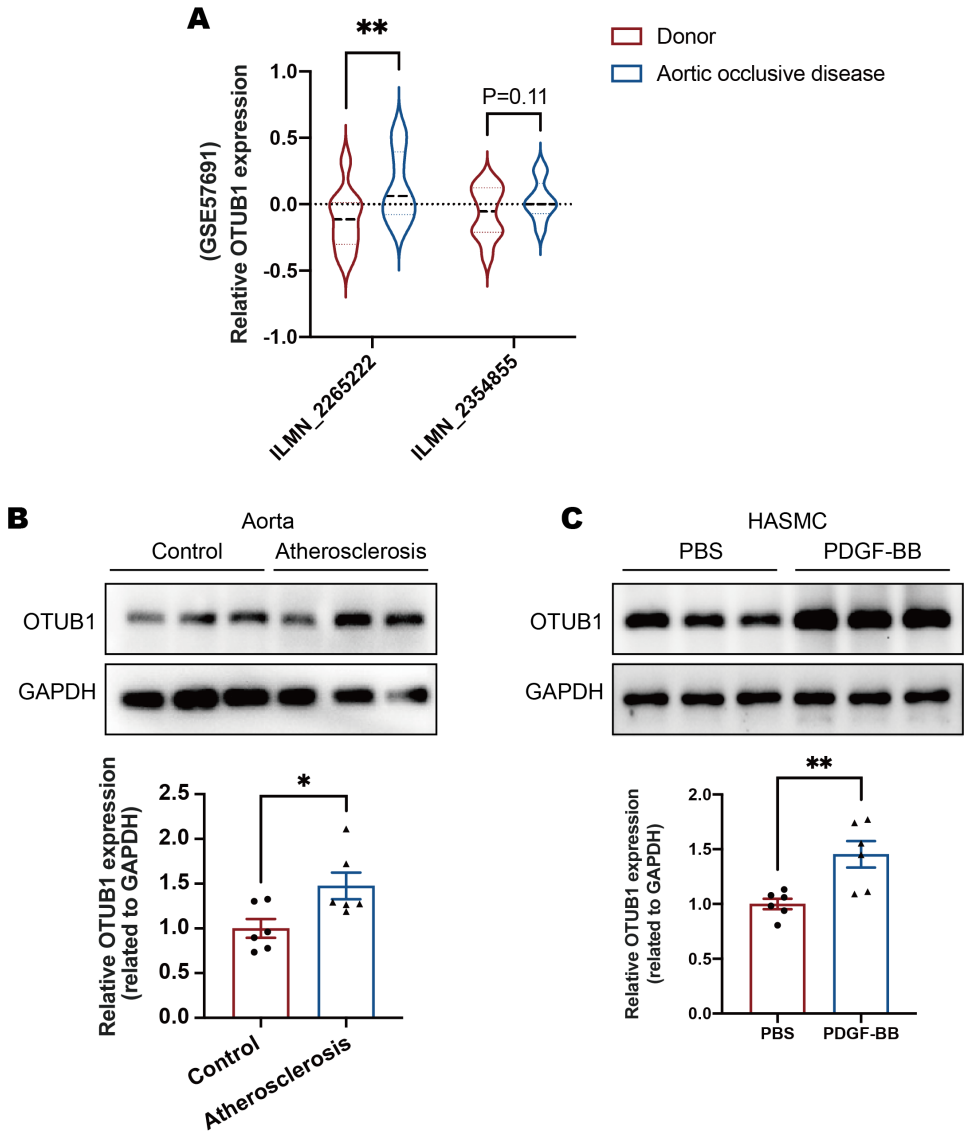
Supplemental Figure 1

qRT-PCR analysis for the relative mRNA expression of *ACTA2* **(A)**, *OPN* **(B)**, and *OTUB1* **(C)** on HASMCs from the 1st to 7th passages, showing the expression of phenotype markers (*ACTA2* and *OPN*) and *OTUB1* are relatively stable among these passages. Data were normalized to *GAPDH*, and shown as mean values ± SEM of three independent experiments.



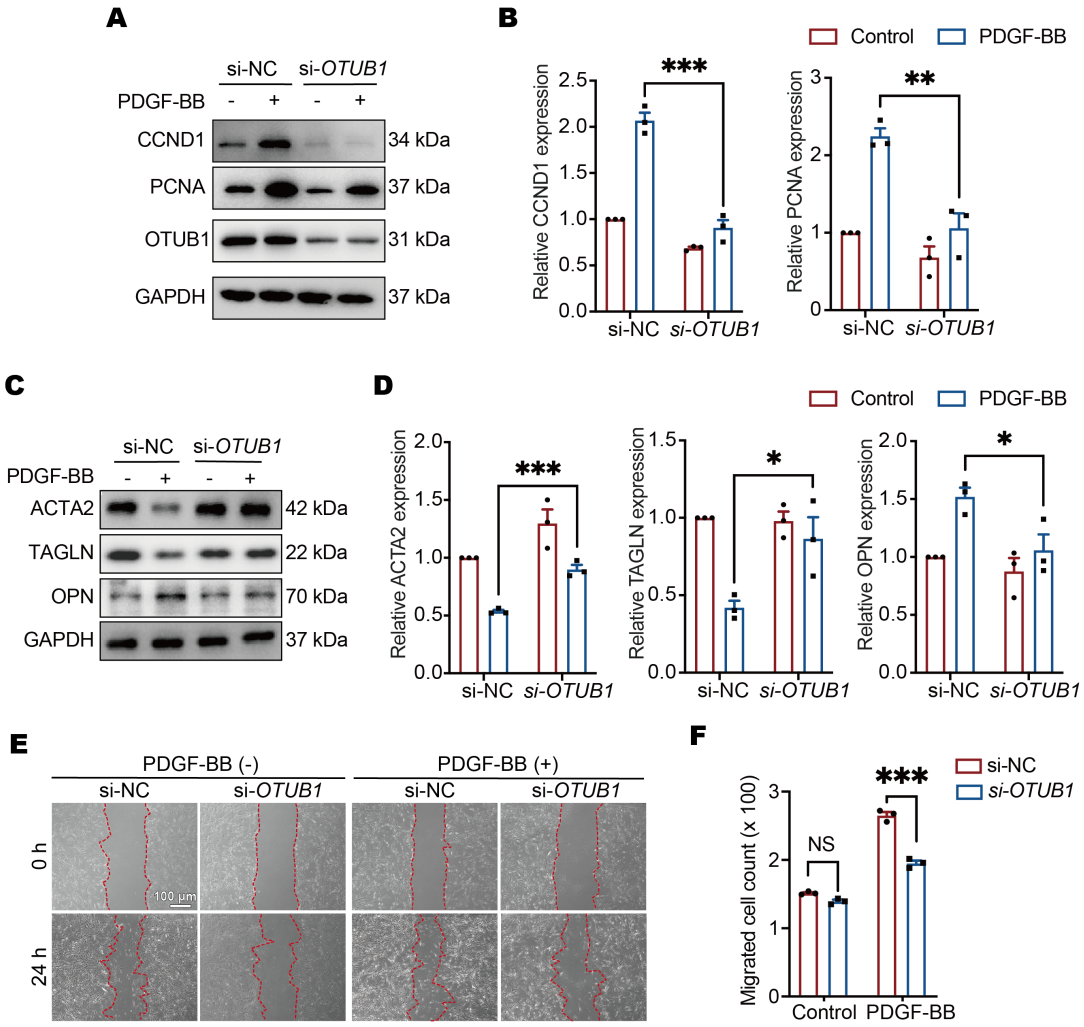
Supplemental Figure 2

Individual qRT-PCR results of the efficiency of siRNAs targeting OTU family deubiquitinases *OTUB1* **(A)**, *OTUB2* **(B)**, *OTUD1* **(C)**, *OTUD2* **(D)**, *OTUD3* **(E)**, *OTUD4* **(F)**, *OTUD5* **(G)**, *OTUD6A* **(H)**, and *OTUD6B* **(I)**. The data were normalized to *GAPDH*, and shown as mean values ± SEM of three independent experiments. For comparisons with si-NC group: ***P*<0.01, ****P*<0.001.



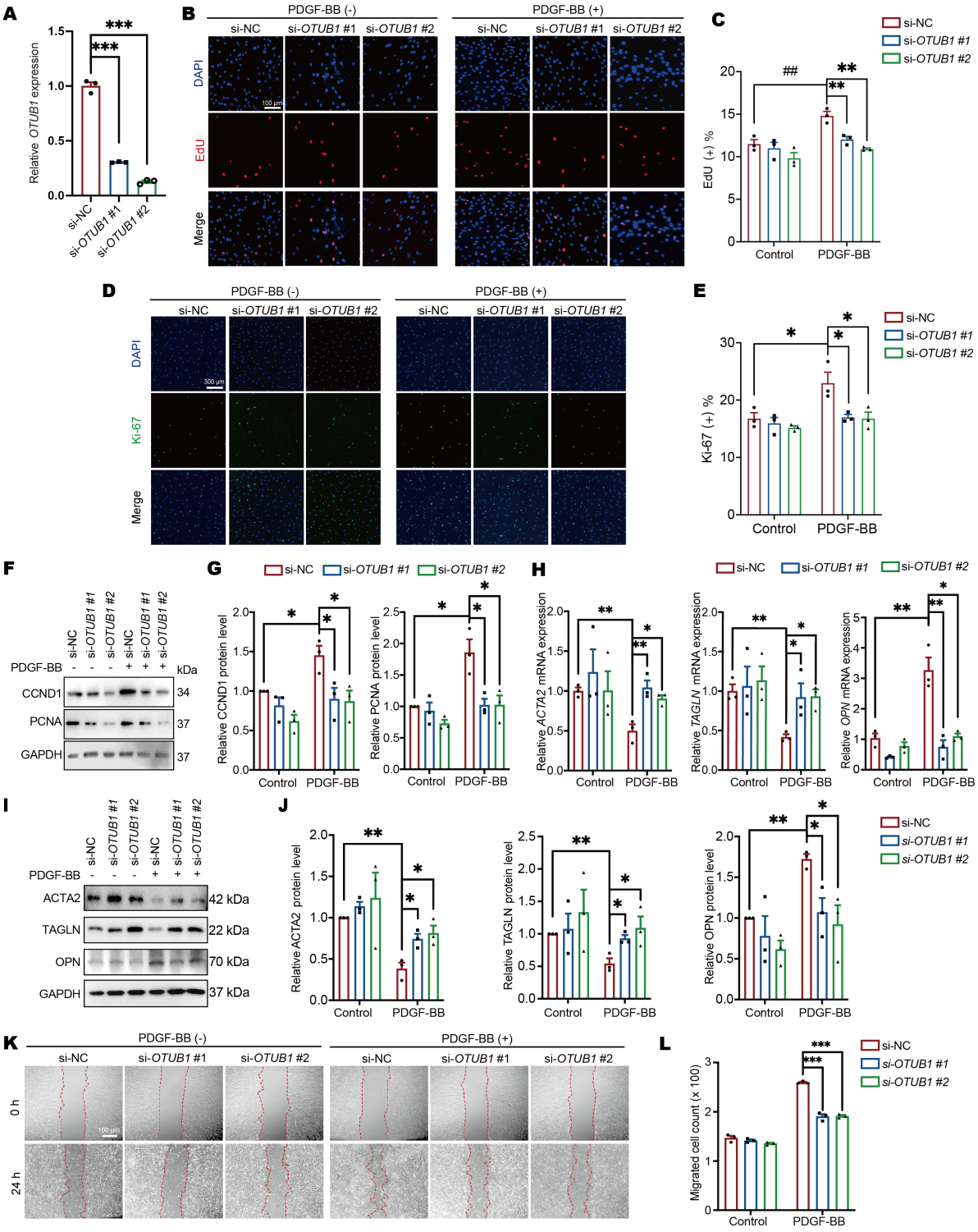
Supplemental Figure 3

A Normalized OTUB1 expression with 2 probes in GEO dataset GSE57691 showed that OTUB1 increases in aortic occlusive disease specimens compared to healthy donor arteries (n=9 per group). **B** Representative images and quantification of OTUB1 from Western blotting in control and atherosclerosis *Apoe*$^{-/-}$ mouse aortas (n=6 per group). GAPDH was used as an internal control, and data were compared to the control group. **C** Representative images and quantification of OTUB1 from Western blotting in PBS or PDGF-BB-treated HASMCs (n=6 per group). GAPDH was used as an internal control, and data were compared to the PBS group. *P<0.05, **P<0.01.



Supplemental Figure 4

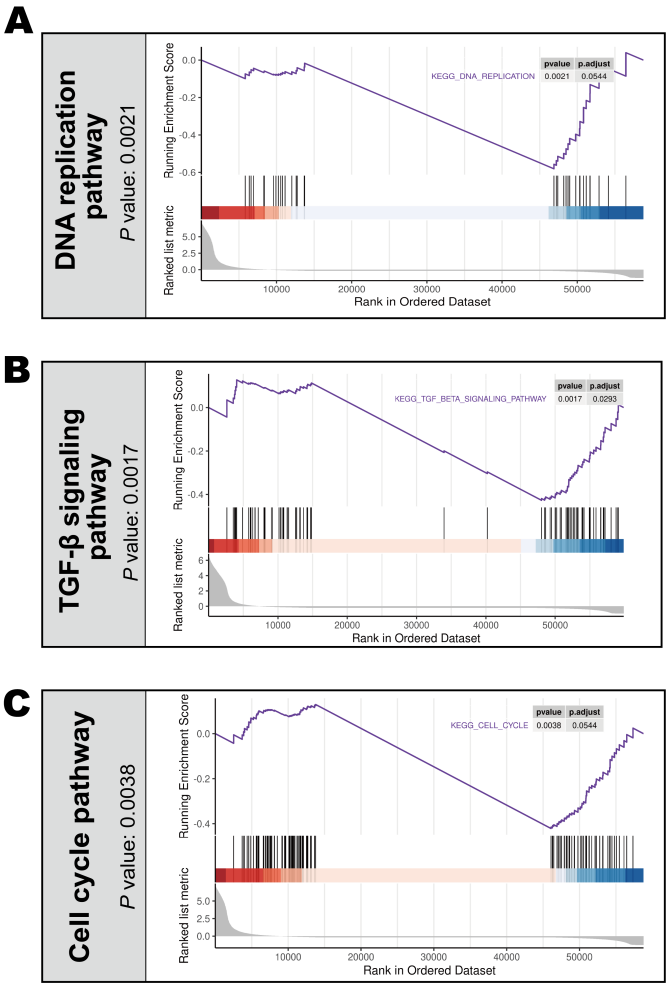
A-B Expression of markers related to proliferation (CCND1 and PCNA) in HASMCs were analyzed using western blotting. **(A)** shows representative Western blotting images, and **(B)** shows the quantification of proliferation markers' expression levels. GAPDH was used as an internal control, and data were compared to si-NC/PDGF-BB (-) group on the same image (n=3 independent experiments). **C-D** Expression of VSMC phenotype markers (ACTA2, TAGLN, and OPN) in HASMC were analyzed using Western blotting. **(C)** shows representative Western blotting images, and **(D)** shows the quantification of phenotype markers' expression levels. GAPDH was used as an internal control, and data were compared to si-NC/PDGF-BB (-) group on the same image (n=3 independent experiments). **E-F** Representative images **(E)** and quantification of migrated cells per microscopic field **(F)** from cell scratch assay. **P*<0.05, ***P*<0.01, ****P*<0.001.



Supplemental Figure 5

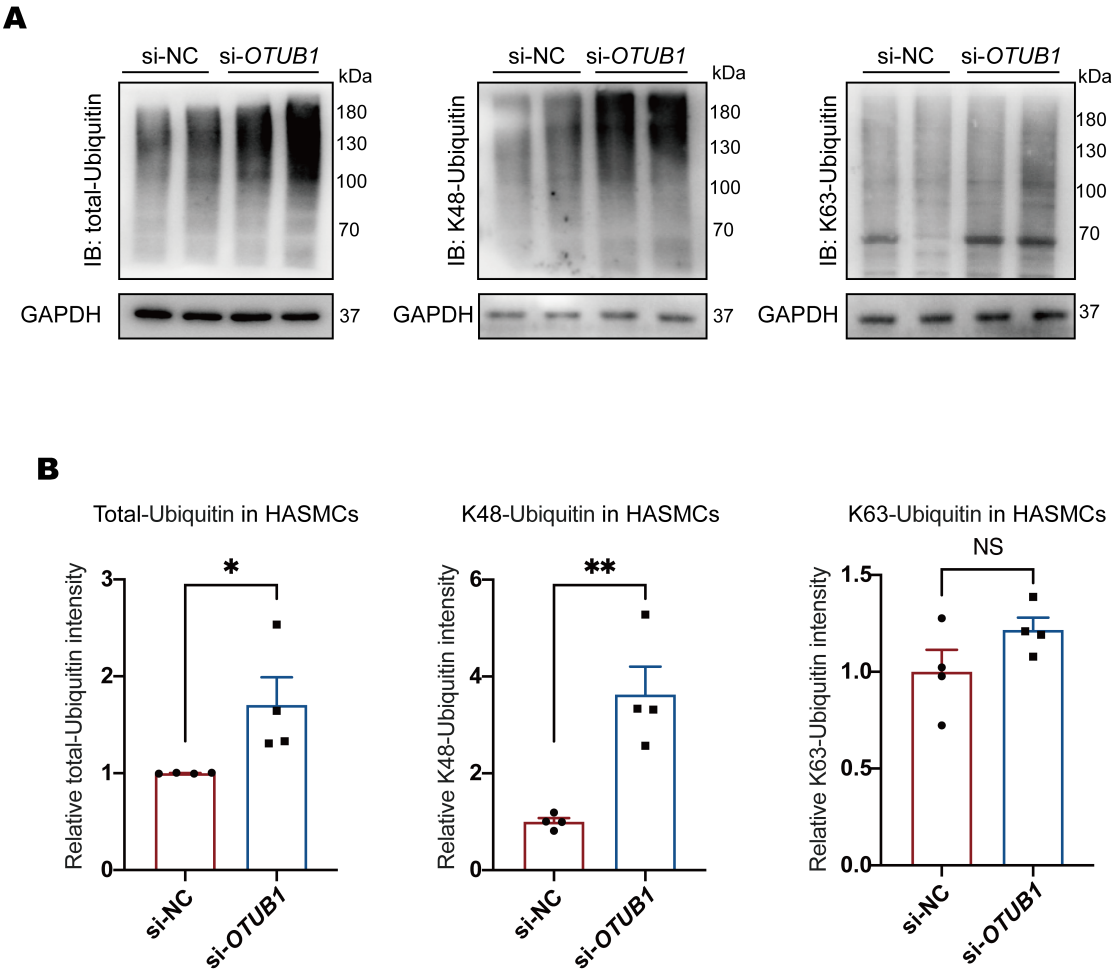
Results of experiments on HASMCs' phenotypes using two pairs of siRNAs targeting *OTUB1*. **A** qRT-PCR results of *OTUB1* mRNA expression in HASMCs transfected with 2 different si-*OTUB1*s or normal control siRNA (si-NC). si-*OTUB1* #1 is only used in experiments in this figure, while si-*OTUB1* #2 was used throughout the manuscript. Data were normalized to *GAPDH*, and shown as mean values ± SEM of three independent experiments. **B-C** Representative EdU staining images **(B)** and quantitative analyses of EdU-positive cells **(C)** in HASMCs transfected with control

siRNA (si-NC) and two pairs of si-*OTUB1*s (n=3 independent experiments). **D-E** Representative Ki-67 immunofluorescence staining images **(D)** and quantitative analyses of Ki-67-positive cells **(E)** in HASMCs (n=3 independent experiments). **F-G** Representative images **(F)** and quantification **(G)** of proliferation markers (CCND1, PCNA) in HASMCs were analyzed using western blotting, and GAPDH was used as an internal control (n=3 independent experiments). **H** qRT-PCR analysis on the relative mRNA expression of HASMC phenotype markers *ACTA2*, *TAGLN,* and *OPN* (n=3 independent experiments). The qRT-PCR data were normalized to *GAPDH* (n=3 independent experiments). **I-J** Representative images **(I)** and quantification **(J)** of phenotype markers (ACTA2, TAGLN, OPN) in HASMCs were analyzed using Western blotting, and GAPDH was used as an internal control (n=3 per group). **K-L** Representative images **(K)** and quantification of migrated cells per microscopic field **(L)** from cell scratch assay (n=3 independent experiments). All data are shown as mean values ± SEM of three independent experiments. NS: not significant, **P*<0.05, ***P*<0.01, ****P*<0.001.



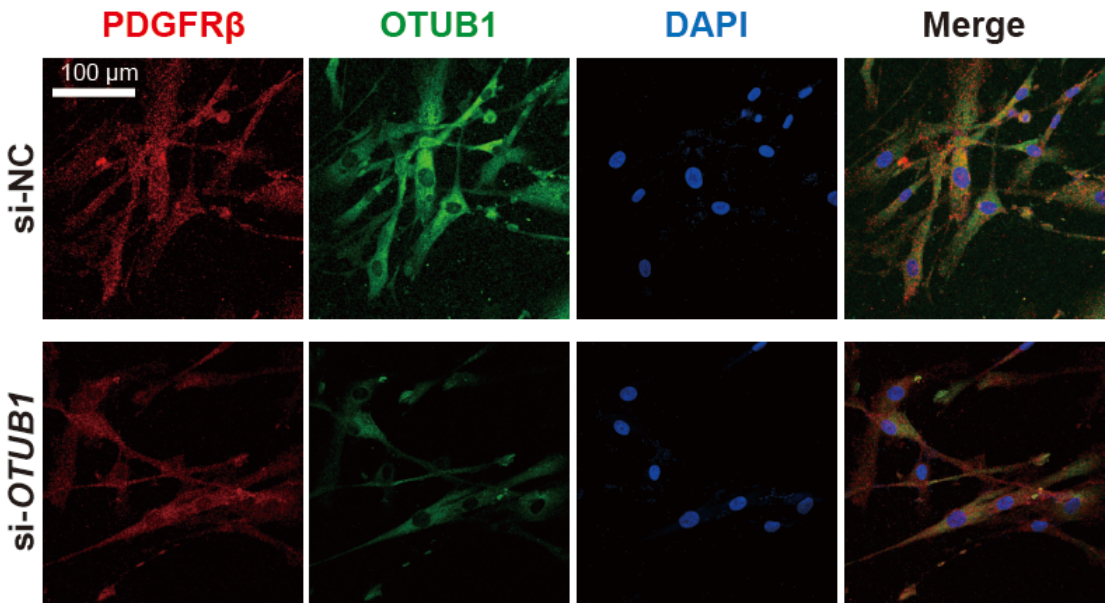
Supplemental Figure 6

GSEA analyses reveal that after *OTUB1* knockdown, genes related to DNA replication pathway **(A)**, TGF-β signaling pathway **(B),** and cell cycle pathway **(C)** were significantly negatively regulated.



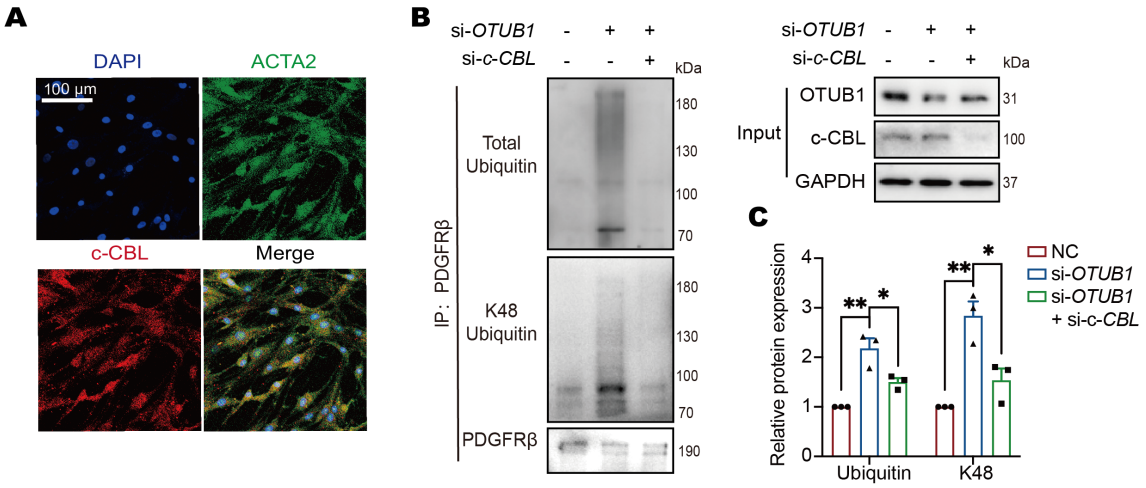
Supplemental Figure 7

A Western blotting assay of the total, K48-linked, and K63-linked ubiquitin in HASMCs transfected with control siRNA (si-NC) or si-*OTUB1*. GAPDH was used as an internal control. **B** Quantification of the total, K48-linked, and K63-linked ubiquitin protein expression levels in control or *OTUB*1-knock-down HASMCs (n=4 per group). Data were analyzed as relative to GAPDH, and shown as relative expression compared to the si-NC group. NC: normal control. NS: not significant, **P*<0.05, ***P*<0.01.



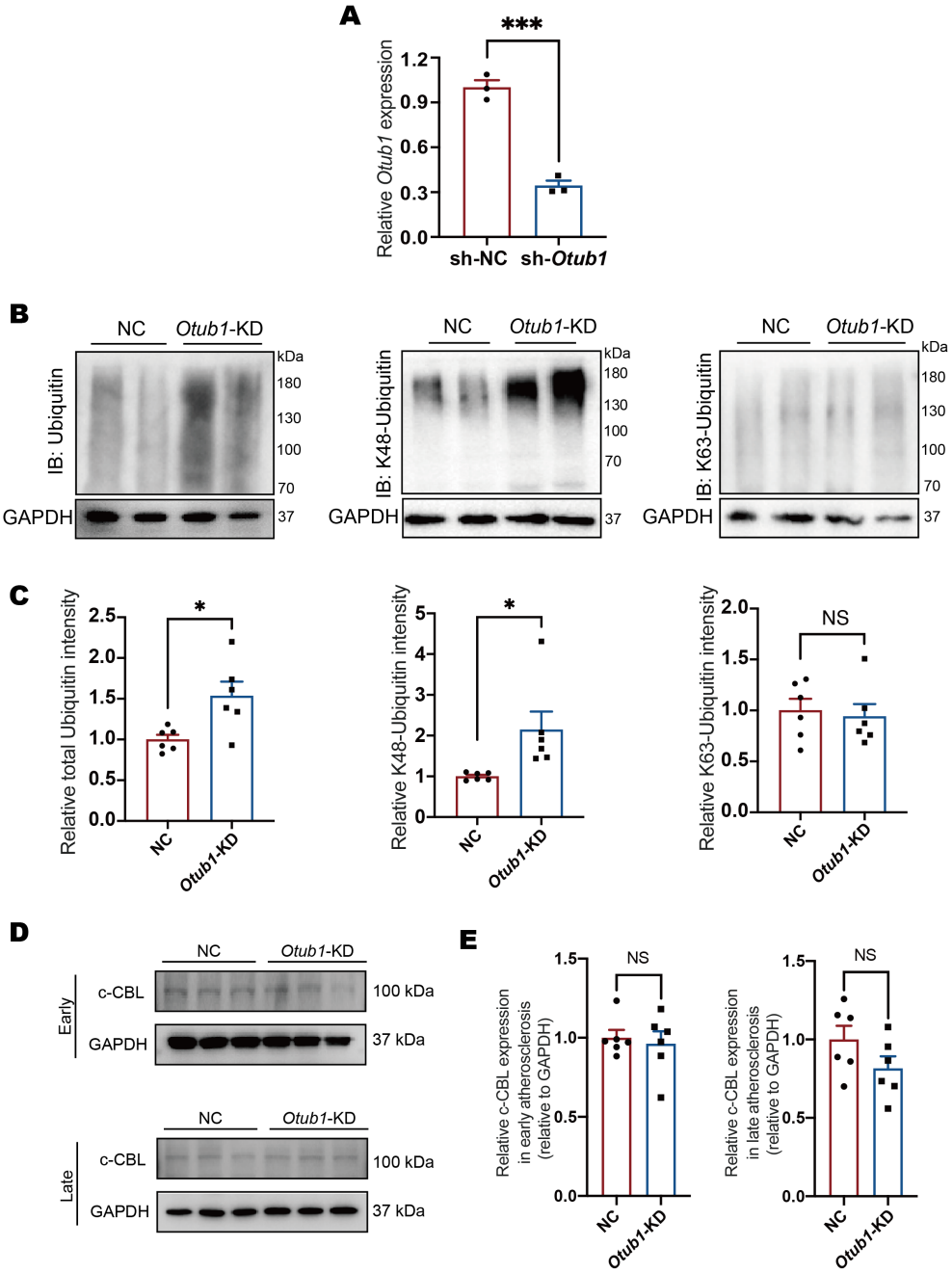
Supplemental Figure 8

Immunofluorescence staining showing the location of PDGFRβ (red) did not alter in HASMCs transfected with control siRNA or *OTUB1*-siRNA.



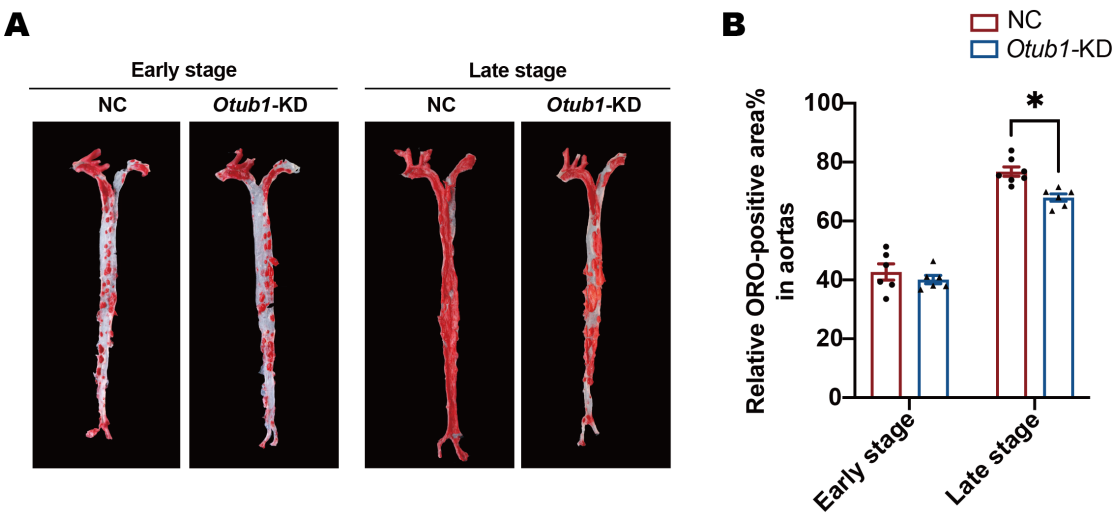
Supplemental Figure 9

A Immunofluorescence staining showing the location of c-CBL in HASMCs. ACTA2 was used as a positive control. **B** PDGFRβ-immunoprecipitation indicates that knocking-down *c-CBL* inhibits si-*OTUB1*-induced ubiquitylation. **C** Quantification of ubiquitin levels in PDGFRβ-immunoprecipitated samples in **(B)**. Data were normalized to immunoprecipitated PDGFRβ (n=3 independent experiments). **P*<0.05, ***P*<0.01.



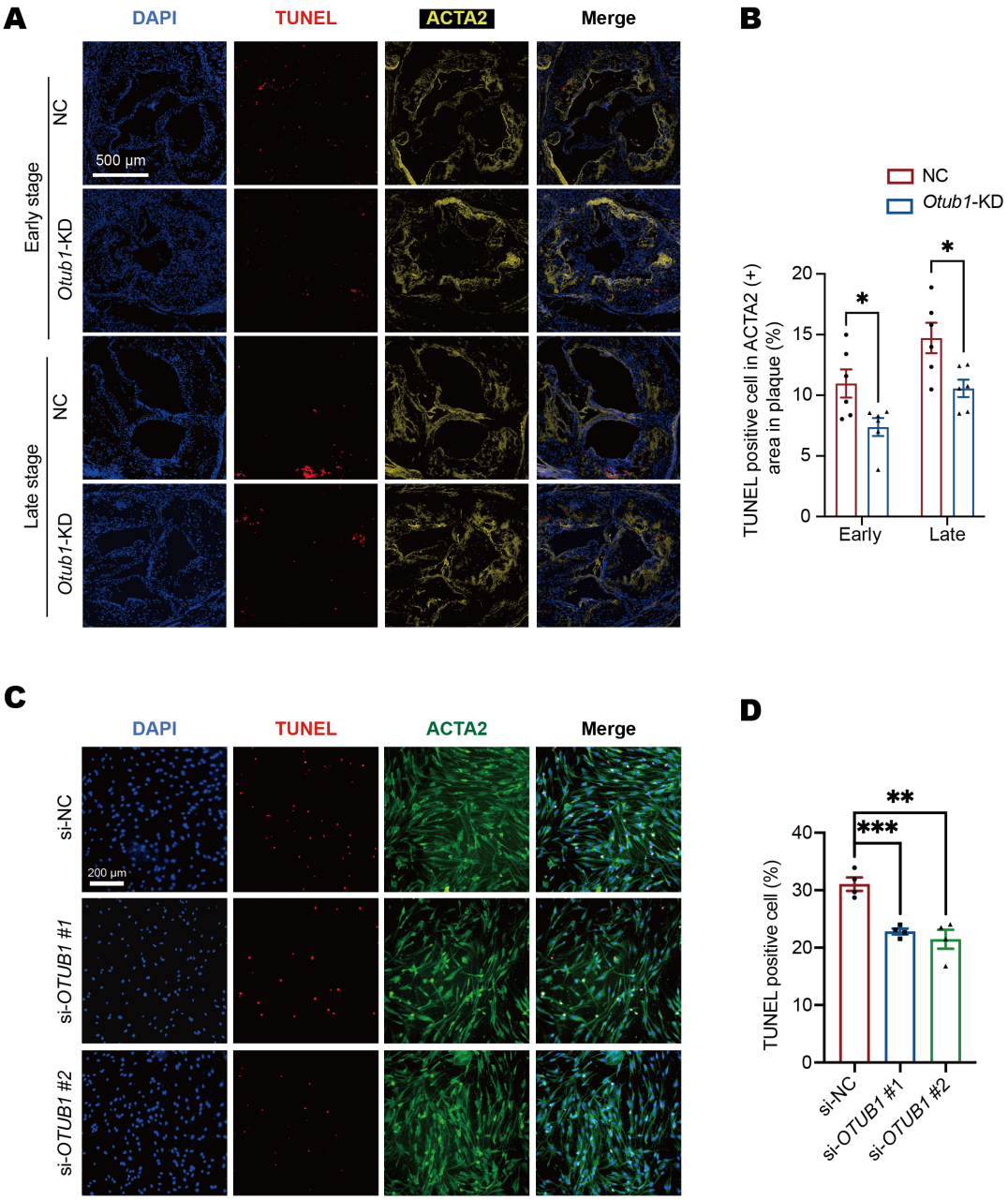
Supplemental Figure 10

A qRT-PCR in a mouse MOVAS cell line showed that sh*Otub1* could effectively knock down *Otub1* expression. Data were normalized to *Gapdh* (n=3). **B-C** Representative images **(B)** and quantification **(C)** of total, K48-linked, and K63-linked ubiquitin protein levels in control (sh-NC) or VSMC-specific *Otub1*-depleted (sh-*Otub1*) mice aortas (n=6 per group). GAPDH was used as an internal control, and data were compared to the NC group. **D-E** Representative images **(D)** and quantification **(E)** of c-CBL expression in control or VSMC-specific OTUB1-depleted mice aortas (n=6 per group). GAPDH was used as an internal control, and data were compared to the NC group. NC: normal control, KD: knock down, NS: not significant, **P*<0.05, ***P*<0.01, ****P*<0.001.



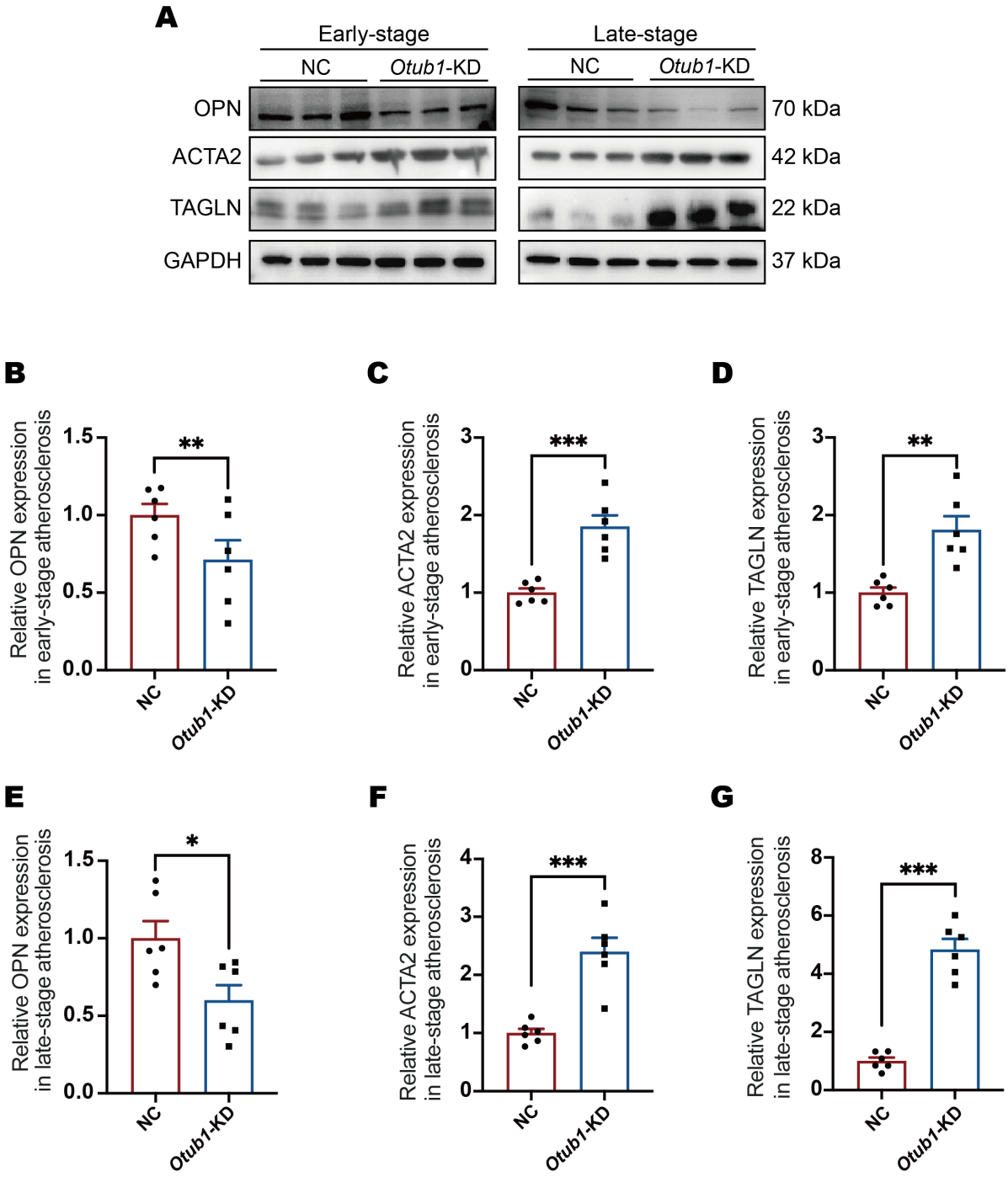
Supplemental Figure 11

Representative images **(A)** and quantification **(B)** of Oil-Red-O-positive areas in the gross observation of aortas in early- and late-stage atherosclerotic mice (n=6-7 per group). The Oil-Red-O-positive area was divided by the total surface area for the quantitative analysis. ORO: Oil-Red O, NC: normal control, KD: knock down, **P*<0.05**.**



Supplemental Figure 12

A-B Representative immunofluorescence staining **(A)** and quantification **(B)** show the percentage of TUNEL-positive cells in ACTA2-positive area within plaque is significantly decreased in *Otub1*-KD group (n=6 per group). **C-D** Representative immunofluorescence staining **(C)** and quantification **(D)** shows the percentage of TUNEL-positive cells is significantly decreased in PDGF-BB-treated HASMCs transfected with si-*OTUB1* (n=3). **P*<0.05, ***P*<0.01, ****P*<0.001.



Supplemental Figure 13

Representative images **(A)** and quantification **(B-G)** of phenotype markers' expression in control or VSMC-specific OTUB1-depleted mice aortas (n=6 per group). Figures **B-D** show the protein expression in early-stage atherosclerosis, and Figures **E-G** show the protein expression in late-stage atherosclerosis. GAPDH was used as an internal control, and data were compared to the NC group. NC: normal control, KD: knock down, **P*<0.05, ***P*<0.01, ****P*<0.001.