ONLINE SUPPLEMENT

Non-standard Abbreviations and Acronyms	
ACTA2	actin alpha 2, smooth muscle (aSMA)
AKT1	AKT serine/threonine kinase 1
AVF	arteriovenous fistula
CKD	chronic kidney disease
COL3A1	collagen type III alpha 1
CQ	chloroquine
Ctl	control
CVD	cardiovascular risk
Cyc	cyclopamine
EEA1	early endosome antigen 1
ESRD	end-stage kidney disease
FN1	Fibronectin 1
S100A4	S100 calcium binding protein A4（FSP1）
GFP	green fluorescent protein
GLI1	GLI family zinc finger 1
GMC	adventitial GLI1^{+} mesenchymal stem cells
KO	knockout
LacZ	beta-galactosidase
Ly6a	lymphocyte antigen 6 complex, locus A (Sca1)
MSCs	mesenchymal stem cells

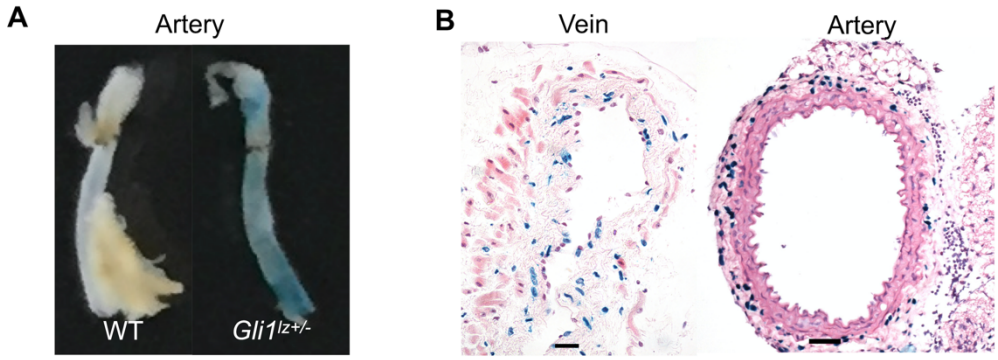
NH_4Cl	ammonium chloride
PDGFA	platelet derived growth factor subunit A
PDGFB	platelet derived growth factor subunit B
PDGFRA	platelet derived growth factor receptor alpha
PDGFRB	platelet derived growth factor receptor beta
PTCH1	patched 1
RT-PCR	reverse transcription polymerase chain reaction
QPCR	real-time polymerase chain reaction
SAG	smoothened agonist
SCA1	stem cells antigen 1
SHH	sonic hedgehog signaling molecule
SM22	smooth muscle protein 22 alpha
SMO	smoothened
TAGLN	transgelin (SM22)
TGFB1	transforming growth factor beta 1
TGFBR2	transforming growth factor beta receptor 2
VEGFA	vascular endothelial growth factor A
VIM	vimentin
VSMC	vascular smooth muscle cells
WT	wild type

Supplemental Table 1. Primer pairs used for qPCR

Gene	Sequence
Pdgfra	Fw 5'- TGGCATGATGGTCGATTCTA -3' Rv 5'- CGCTGAGGTGGTAGAAGGAG -3'
Pdgfrb	Fw 5'-CACCTTCTCCAGTGTGCTGA -3' Rv 5'-GGAGTCCATAGGGAGGAAGC -3'
Tgfbr2	Fw 5'- ACTGTCCACTTGCGACAACCAGA A -3' Rv 5'- AGAAGCGGCATCTTCCAGAGTGAA -3'
Shh	Fw 5'- CTGGCCAGATGTTTTCTGGT -3' Rv 5'- TAAAGGGGTCAGCTTTTTGG -3'
Smo	Fw 5'- GACCACTCCCATAAGGGCTA -3' Rv 5'- GAAGAGGTTGGCCTAGTGGA -3'
Ptch1	Fw 5'- ATGCTCCTTTCCTCCTGAAACC -3' Rv 5'- TGAACTGGGCAGCTATGAAGTC -3'
Gli1	Fw 5'- TCGACCTGCAAACCGTAATCC -3' Rv 5'- TCCTAAAGAAGGGCTCATGGTA -3'

Gli2	Fw 5'- GGCAGGGGTATCCCACAGA -3' Rv 5'- AGAAGGACCCATGTTGGAGTC- 3'

Supplemental figure legends

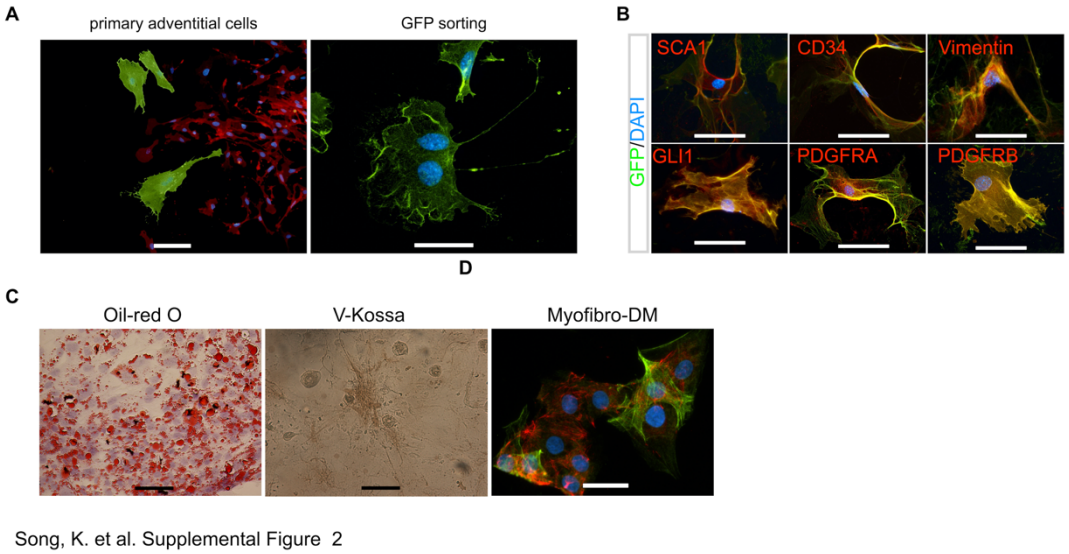


Song, K. et al. Supplemental Figure 1

Figure S1. GLI1 positive signals were restricted to the adventitial layers in *Gli1*$^{lz+/-}$ mice.

(**A**) Common carotid arteries from wide type and *Gli1*$^{lz+/-}$ mice were isolated. The arteries of *Gli1*$^{lz+/-}$ mice showed the blue color by X-gal staining.

(**B**) GLI1 positive signals were restricted to the adventitial layers of arteries and veins by X-gal staining. Scale bars are 100 μm.



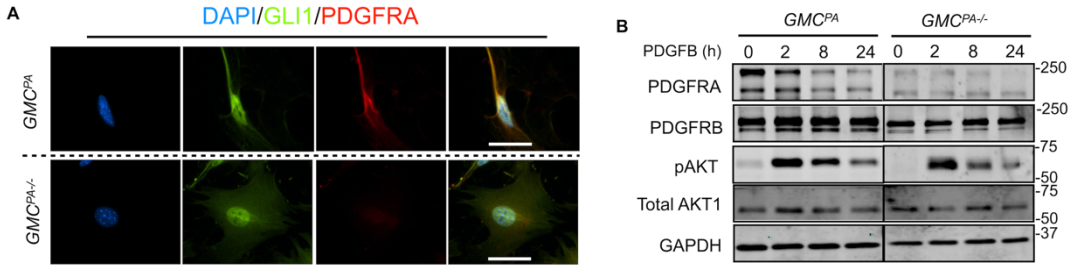
Song, K. et al. Supplemental Figure 2

Figure S2. GMCs isolated from the adventitia of aortas are MSC-like progenitors

(**A**) Primary adventitia cells were isolated from *mT/mG/GLI1*^{CreERt2} mice (left panel) and purified by sorting the GFP^{+} cells (right panel). Scale bars are 10 μm.

(**B**) Immunostaining revealed that GMCs were not only co-expressed with VIM and the stem cell/progenitor markers, SCA1 and CD34, but also expressed PDGFRA and PDGFRB. Scale bars are 10 μm.

(**C**) GMCs had the multipotential differentiation characteristics when cultured in differentiation medium. Scale bars are 50 μm for Oil-red O staining and Von Kossa staining, Scale bars are 10 μm for myofibroblasts differentiation.



Song, K. et al. Supplemental Figure 3

Figure S3. GMCs were treated with PDGFB.

(**A**)GMCs were isolated from control (*GMC*^{PA}) and *GMC*$^{PA-/-}$ mice. After tamoxifen treatment, the expressions of PDGFRA and GLI1 were determined by immunostaining. Scale bars are 10 μm.

(**B**)Representative WB images of indicated proteins in both control and *Pdgfra* KO GMCs after treatment with PDGFB (10 ng/ml) for different time. The lanes of gels were run on the same gel but were noncontiguous.

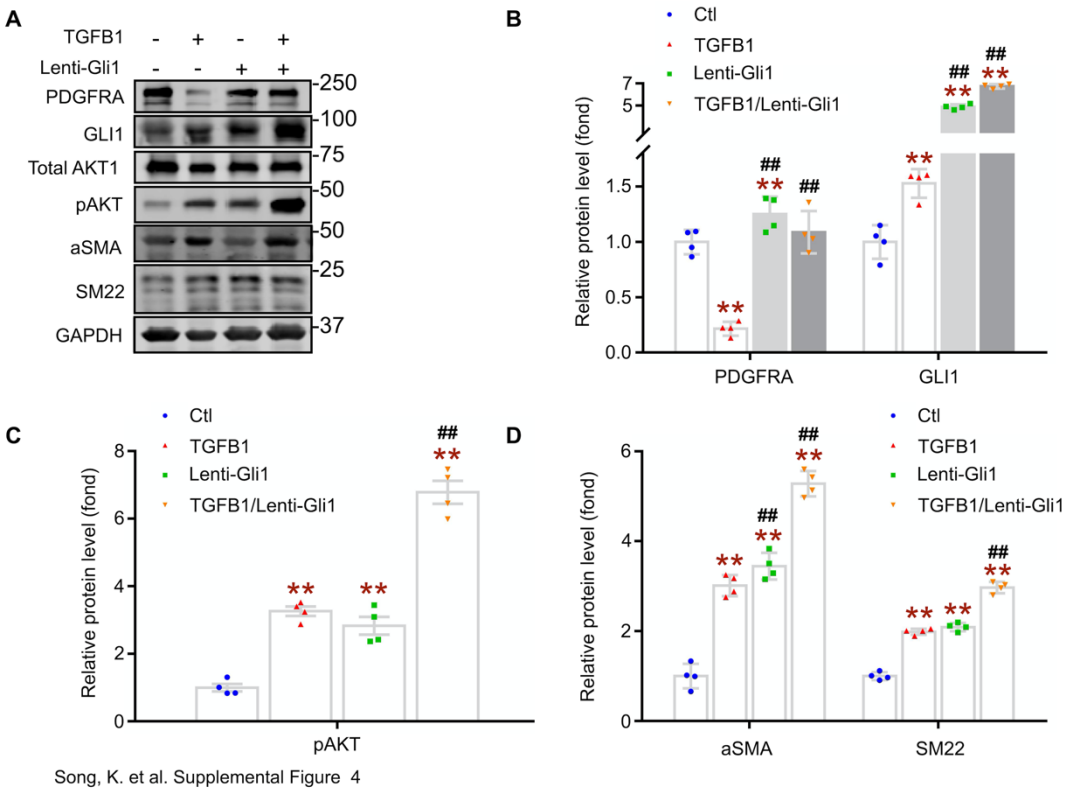


Figure S4: Lenti-Gli1 promotes PDGFRA upregulation to enhance TGFB1-induced myofibroblast transformation of GMCs.

(**A-D**) WB and summary densitometry graph showing the changes of Lenti-Gli1 on the TGFB1-induced PDGFRA degradation and activation of downstream signals in GMCs. Protein expression was normalized to GAPDH and expressed as fold change of control GMCs(**B-D**). Total replicates representing 4 independent experiments (*n* = 4).

Data are presented as mean ± SEM. One-way ANOVA was used for statistical analysis. $^{**}P < 0.01$ *versus* control; $^{\#\#}P < 0.01$ *versus* TGFB1-treated group.

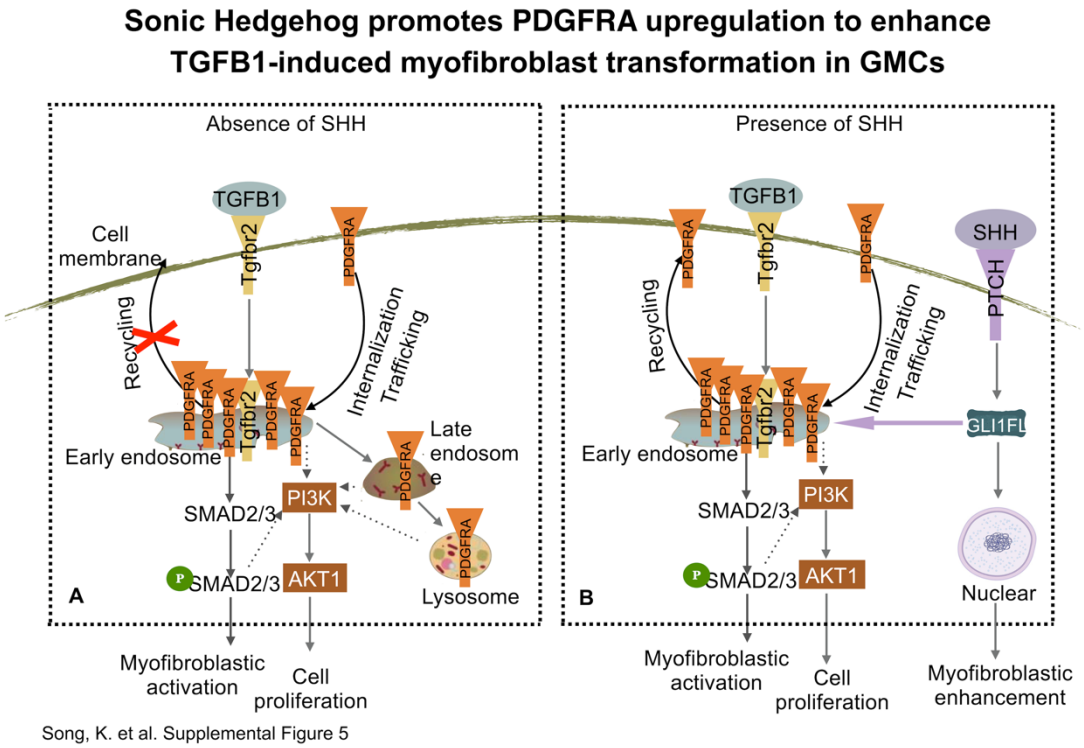


Figure S5. Sonic hedgehog promotes PDGFRA upregulation to enhance TGFB1-induced myofibroblast transformation in GMCs.

(**A**) In the absence of hedgehog ligands, TGFB1 treatment induces the degradation of PDGFRA through early endosome, leading to GMCs differentiation into myofibroblasts.

(**B**) In the presence of hedgehog ligands, the activated hedgehog signaling not only directly promotes GMCs differentiation, it also enhances PDGFRA accumulation in the early endosome, thus amplifying the TGFB1 induced GMCs activation and differentiation. These responses stimulate VSMC proliferation and extracellular matrix deposition, resulting in neointima formation in AVF in CKD.