

SUPPLEMENTAL MATERIAL

Table S1. Primers used for qRT-PCR.

Target	Forward	Reverse
Gene expression		
Smad3	GCCTGTGCTGGAACATCATC	TTGCCCTCATGTGTGCTCTT
NrP2	GCATGGCAAAAACCACAAGGTAT	TGGAGCGTGGAGCTTGTTCA
CD68	TCAGCTTTGGATTCATGCAG	AGGTGGACAGCTGGTGAAAG
ACTA2	TGACAATGGCTCTGGGCTCTGTAA	TTCGTCACCCACGTAGCTGTCTTT
GAPDH	CATGTTTCGTCATGGGTGTGAACCA	ATGGCATGGACTGTGGTCATGAGT
ChIP		
S1	TCTGAGATTAACAGTGTGA	CTTAACTGTTATTTACACT
S2	TAGTAAACGCAGCGAGTGT	GGTAGCGAGCCGGTCGGCA
S3	TCGGCCTGAAAAGGCAGTG	TGTCTGTGCGGCTGAGGAT
S4	GTGTTCTCTTCCCGGCTTGT	TCCTGGTGGCTTGTCTTCAC

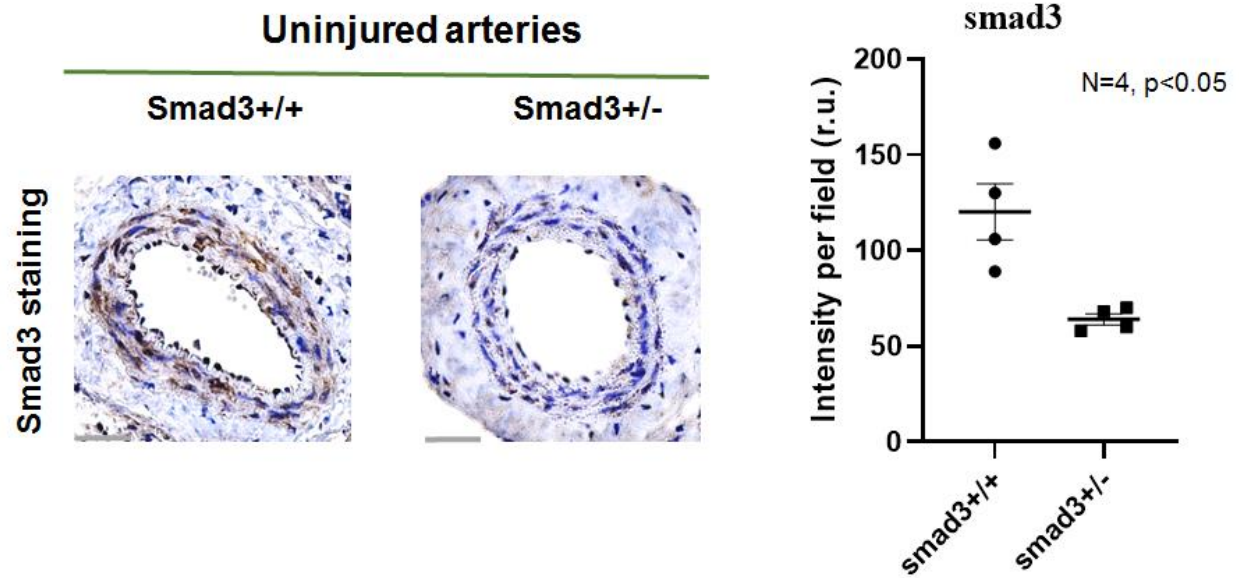
Table S2. Primers for NrP2 promoter and/or 5'UTR reporter clone.

Cloned region	Forward	Reverse
Promoter (-1.5k ~ 0 bp)	GACTCATACAGCTGGCAGAGC T CGTGTGATACTCAGCCTGTG	GACTCATACAGCTAGCTAAGC T TCTCCCCACTGCCTTTTCA
Promoter+5'UTR R (-1.5k ~ +791 bp)	GACTCATACAGCTGGCAGAGC T CGTGTGATACTCAGCCTGTGG	GACTCATACAGCTAGCTAAGC T TCTTTCCAGAGCTGGGTTTCGT
5' UTR (0 ~ +791 bp)	GACTCATACAGCTGGCAGACG T CCCTGAAAAGGCAGTGGGGAG	GACTCATACAGCTAGCTCCAT G GCTTTCCAGAGCTGGGTTTCGT

Table S3. Antibodies for Western blotting.

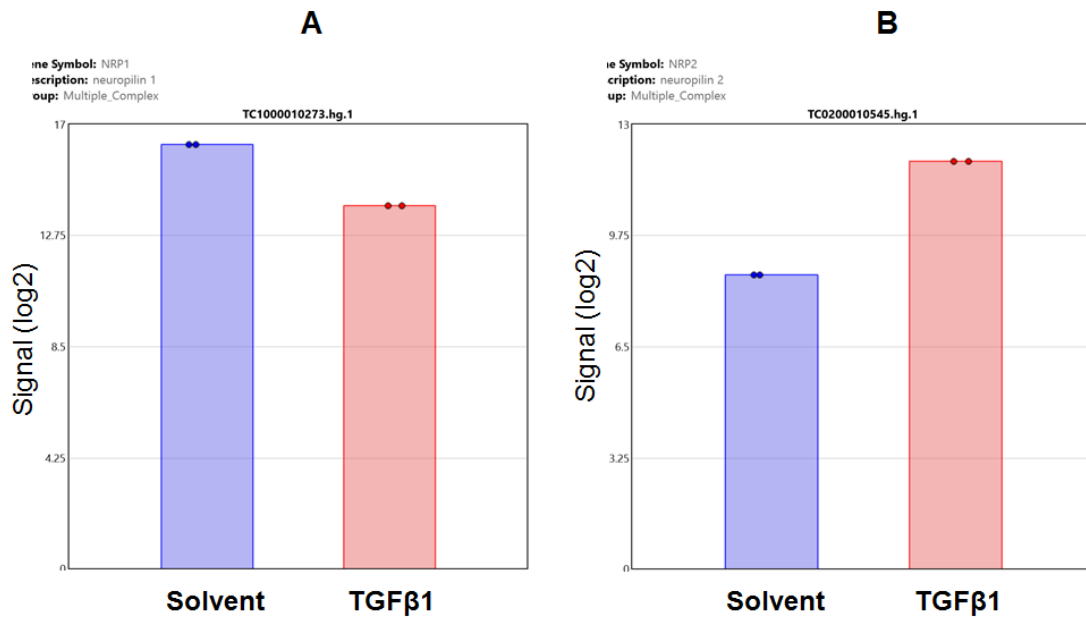
Antibody	Company	Catalog number	Dilution
Smad3	Abcam	ab40854	1:1000 (WB); 1:500 (IHC)
Phospho-smad3	Thermofisher Scientific	MA5-14936	1:100 (CHIP)
Phospho-smad3	Abcam	ab52903	1:2000 (WB)
Nrp2	Thermofisher scientific	PA5-47274	1:100 (IHC); 1:1000 (WB)
CD68	Santa Cruz	sc-20060	1:200 (WB)
aSMA	Abcam	ab5694	1:2000 (WB)
GAPDH	Cell Signaling Technologies	2118S	1:3000
β -ACTIN	Abcam	ab6276	1:3000

Figure S1. Reduced Smad3 protein levels in Smad3-haploinsufficient mice (*vs* WT).



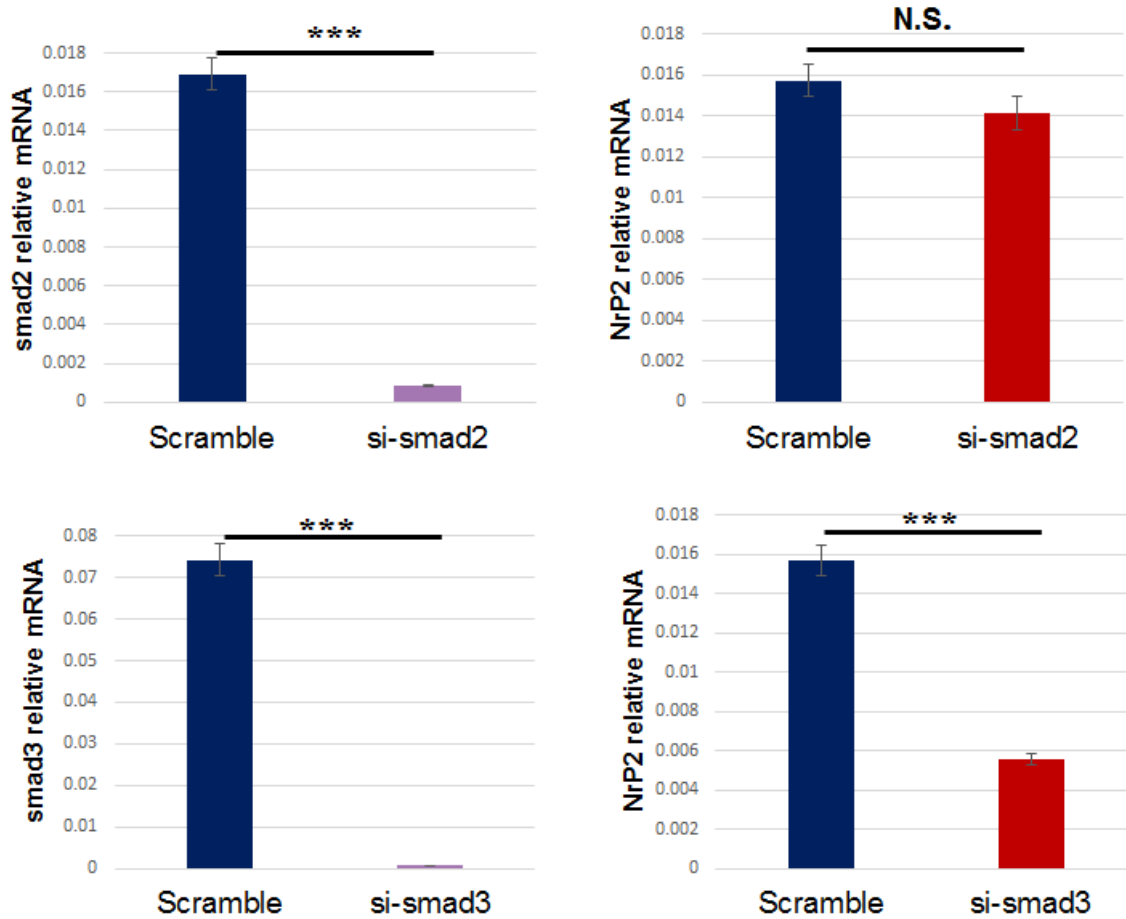
Uninjured mouse femoral artery sections were used for immunostaining of Smad3, which was performed as described in Figure 1. Scale bar: 50 μ m.

Figure S2. NRP2 expression is stimulated by TGF- β treatment of human AoSMCs.



In order to determine the different gene sets responding to TGF- β 1 stimulation, human AoSMCs were cultured, starved, and then treated with solvent control or TGF β 1 (10 ng/ml) for 20 hours. The cells were then used for Affymetrix gene array. Relative NRP1 and NRP2 gene expression levels are presented as log₂ values.

Figure S3. Smad3 but not Smad2 silencing down-regulates NRP2 expression.



To examine the effect of knockdown of smad2 or smad3 on expression of NRP2, AoSMCs were cultured, transfected with Smad2- or Smad3-specific siRNA. Cells were collected 48 hours post transfection for qPCR assay.