Supplementary Materials for

CCN2 deficiency in smooth muscle cells triggers cell reprogramming and aggravates aneurysm development

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Supplemental Figures

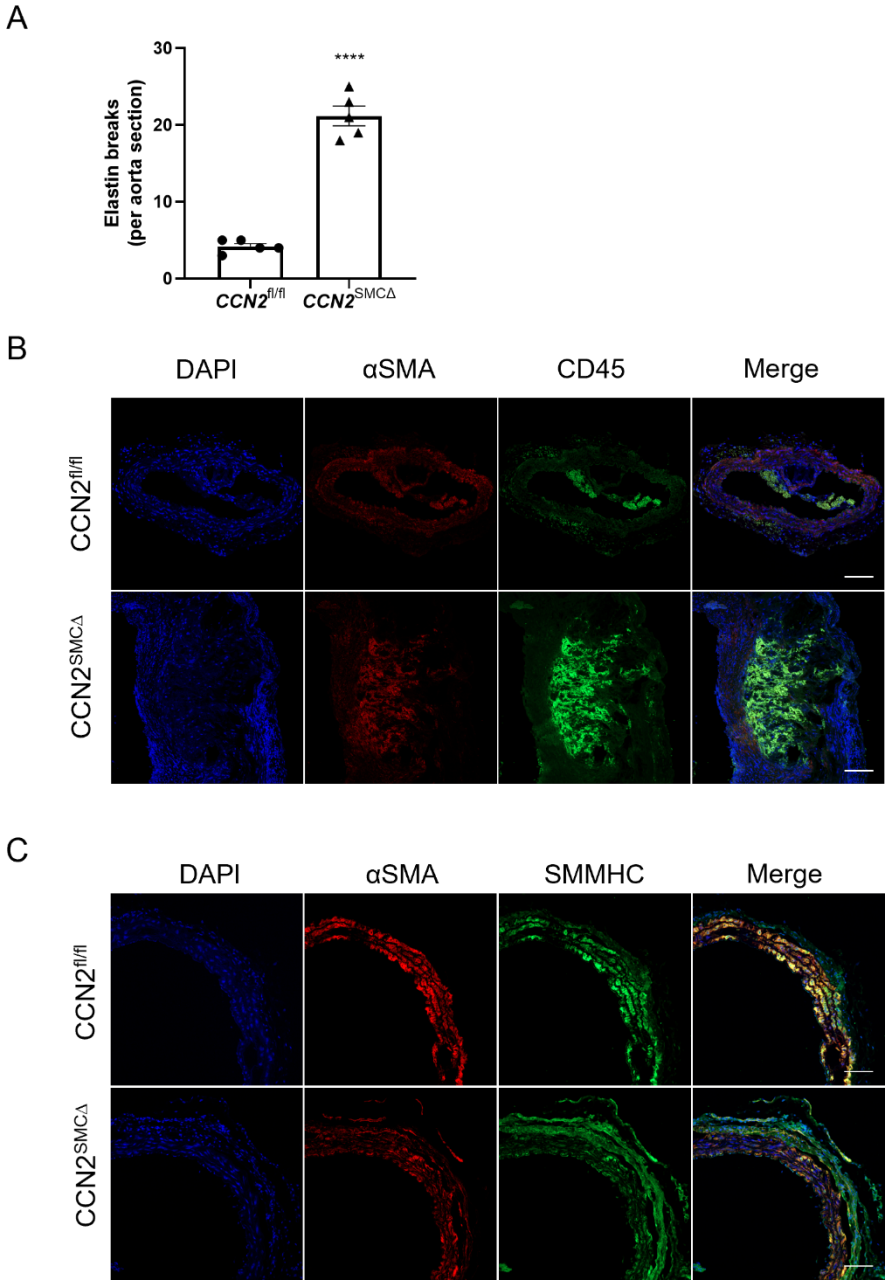

Figure S1. (A) Quantification of elastin breaks (counts per aorta section, 42-day Ang II infusion), n=5. Data were quantified and represented as mean ± SEM, ****, P<0.0001, 2-tailed Student's t test. **(B)** Immunofluorescence double staining of infrarenal aortic sections from $CCN2^{fl/fl}$ and $CCN2^{SMC\Delta}$ mice with specific antibodies against SM-α-actin (red) and CD45 (green). Nuclei were stained with DAPI (blue), n=3. **(C)** Immunofluorescence double staining of upper abdominal aortic sections (no dilations) from both groups with specific antibodies against SM-α-actin (red) and SMMHC (green). Nuclei were stained with DAPI (blue), n=3. Scale bars, 100 μm.

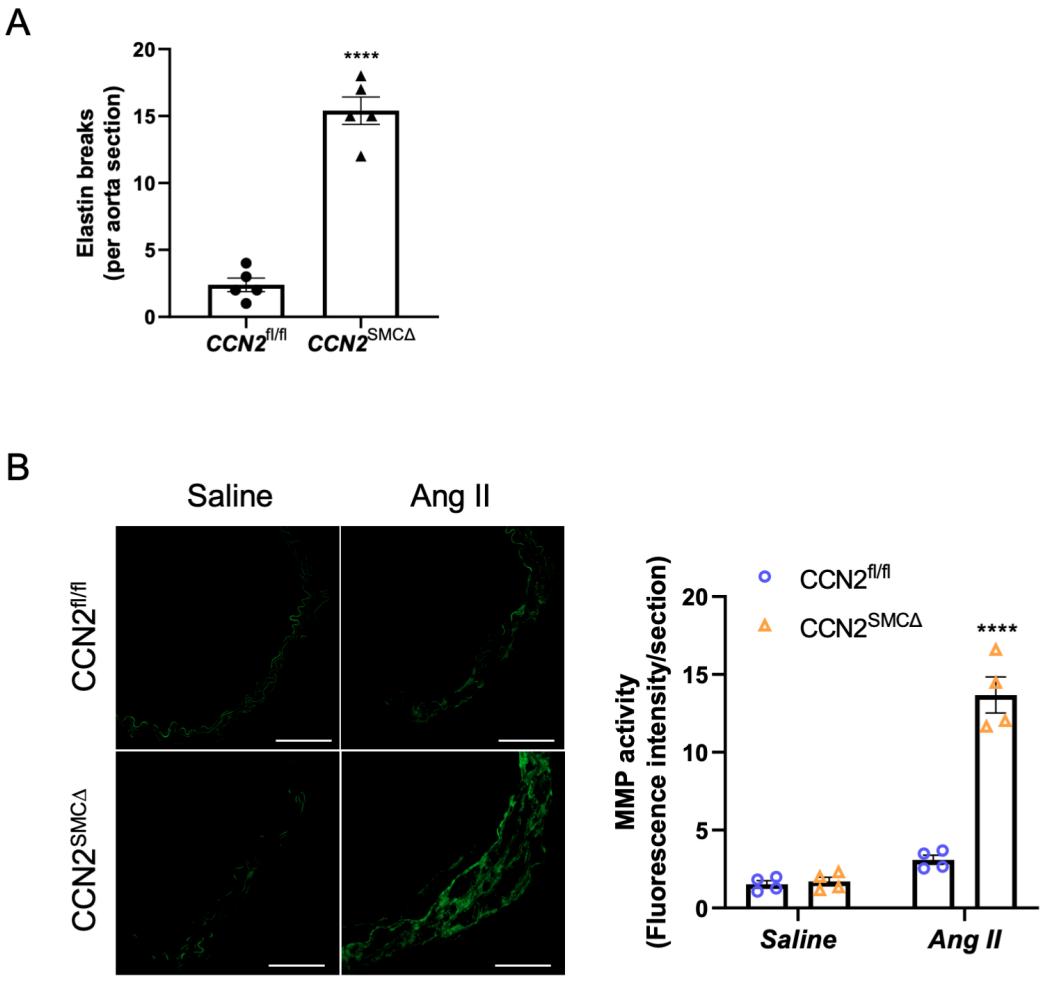

Figure S2. (A) Quantification of elastin breaks (counts per aorta section, 7-day Ang II infusion), n=5. **(B)** In situ zymography analysis of MMPs activity in infrarenal aortic sections from $CCN2^{fl/fl}$ and $CCN2^{SMC\Delta}$ mice infused with saline or Ang II (n=4). Total MMPs activity are indicated by positive green signals and quantified as fluorescence intensity per section. Scale bars, 100 μm. Data were represented as mean ± SEM, ****, P<0.0001, 2-way ANOVA.

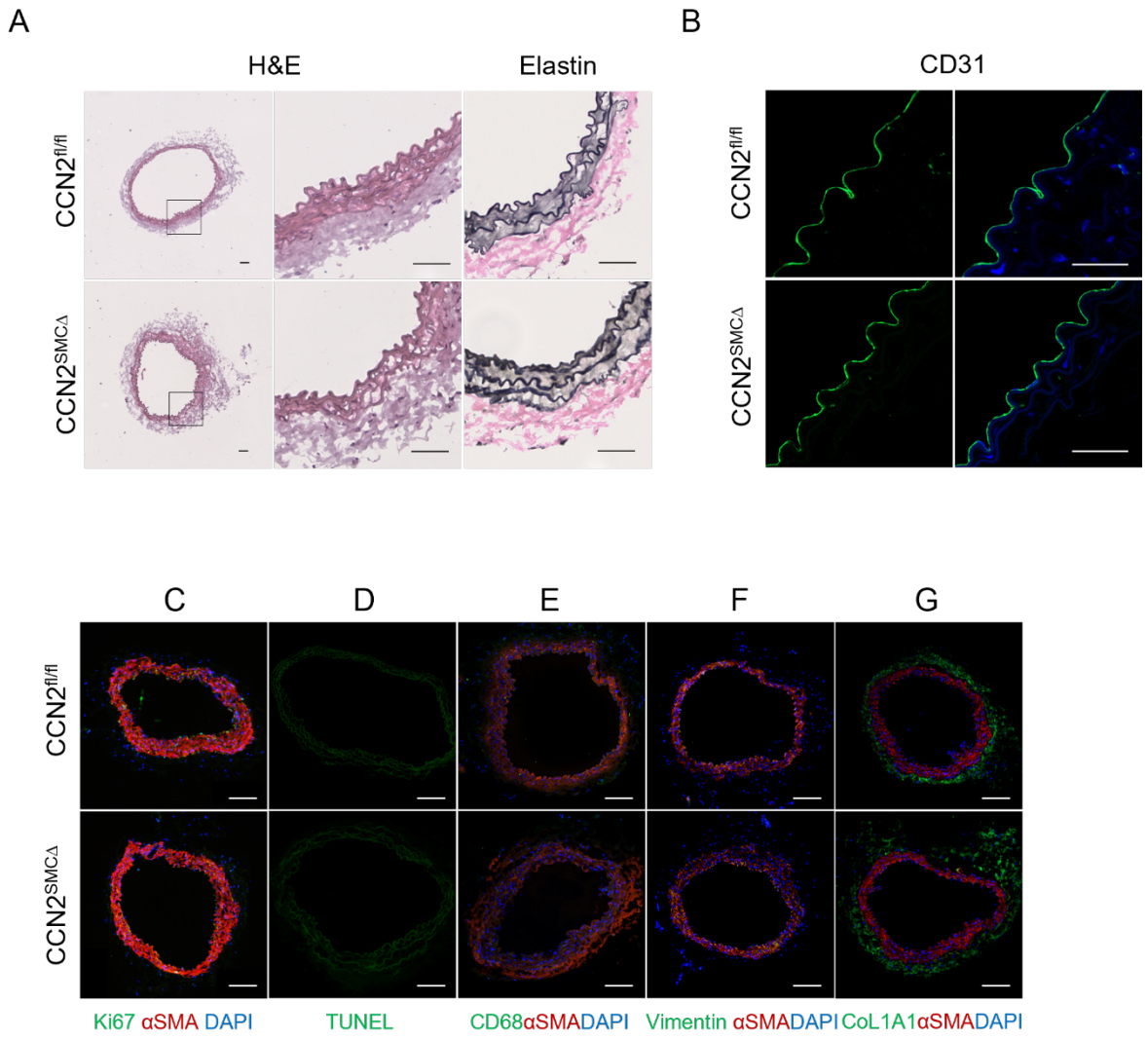

Figure S3. **(A)** Representative images of hematoxylin/eosin-stained (5x and 20x) and Elastin-stained (20x) abdominal aortic sections from saline- infused $CCN2^{fl/fl}$ and $CCN2^{SMC\Delta}$ mice (n=4). Scale bars, 100 µm. **(B)** Immunofluorescence staining of abdominal aortic sections from both groups with specific antibodies against CD31 (green). Nuclei were stained with DAPI (blue). Scale bars, 100 µm, n=4. Abdominal aortic sections (non-aneurysm) from saline- infused $CCN2^{fl/fl}$ and $CCN2^{SMC\Delta}$ mice stained with Ki67/αSMA **(C)**, TUNEL **(D)**, CD68/αSMA **(E)**, Vimentin/αSMA **(F)** and CoL1A1/αSMA **(G)**.

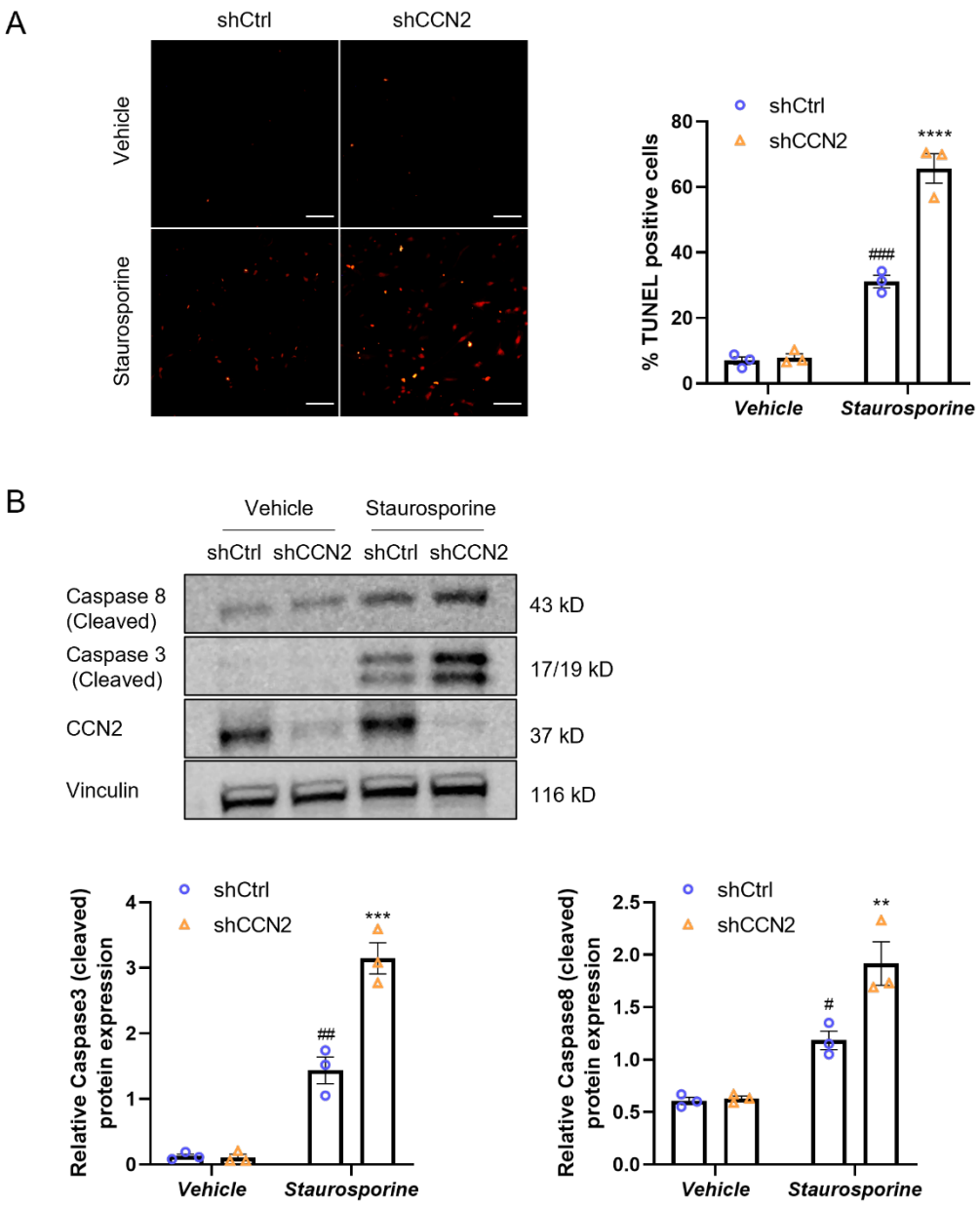

Figure S4. SMC-specific deficiency of CCN2 exacerbates cell apoptosis in vitro. (A) Representative images of TUNEL stained control and CCN2 deficient HASMCs treated with Vehicle or Staurosporine (n=3). Apoptotic cells were stained red. Scale bar, 100μm. **(B)** Western blot analysis of the active forms of caspase 8 and caspase 3 in control and CCN2 deficient HASMCs treated with Vehicle or Staurosporine (n=3). Target protein levels were normalized to vinculin. Data were quantified and represented as mean ± SEM. **, P<0.01, ***, P<0.001, ****, P<0.0001, effect of CCN2 deficiency within same treatment group; #, p<0.05, ##, p<0.01, ###, p< 0.001, effect of Staurosorine within same genotype group, 2-way ANOVA.

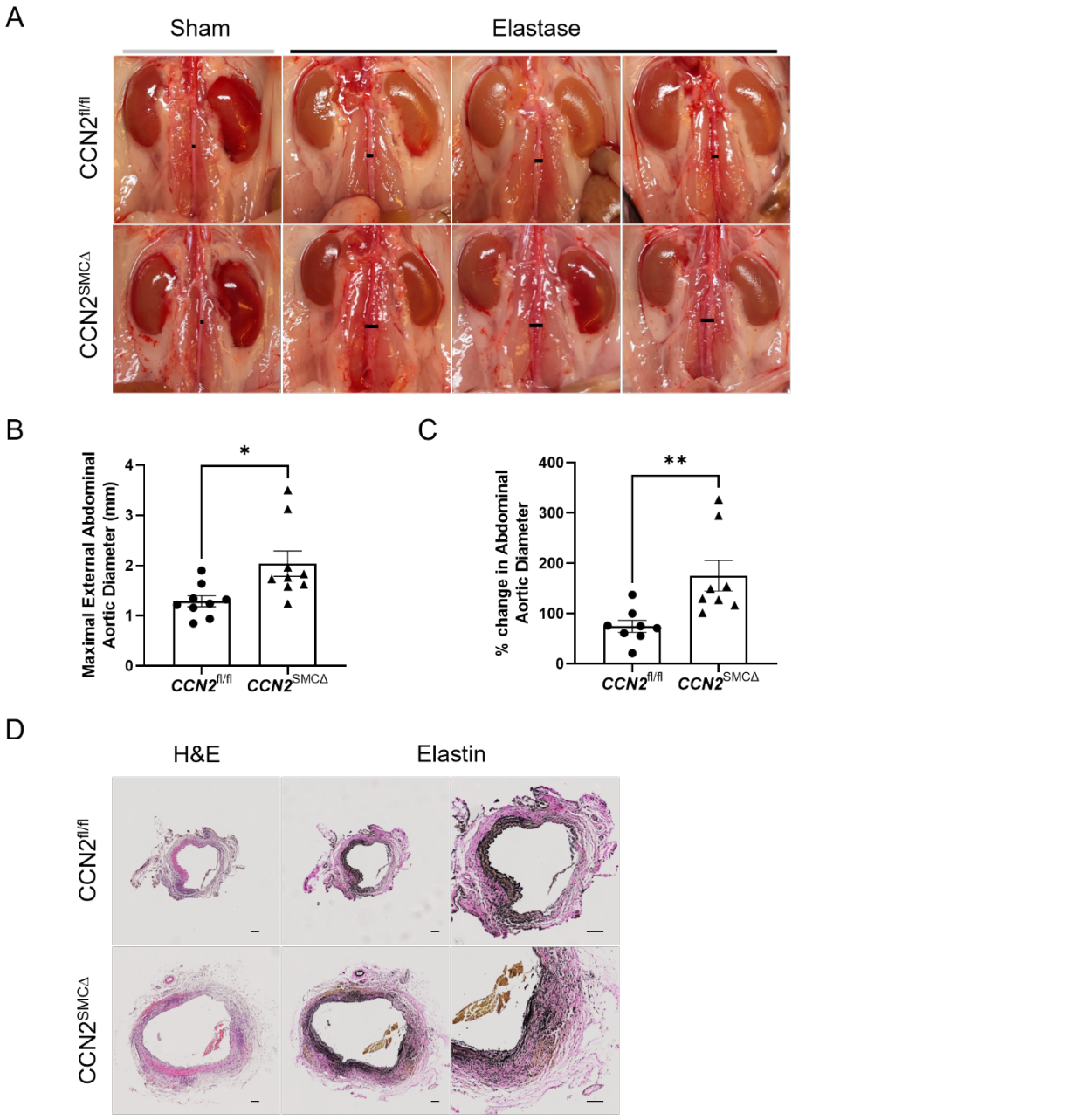

Figure S5. Exacerbation of elastase-BAPN induced infrarenal aortic aneurysm in CCN2$^{SMC\Delta}$ mice. (A) Representative gross images of abdominal aortas from sham-operated (n=3) and elastase-BAPN treated CCN2$^{fl/fl}$ and CCN2$^{SMC\Delta}$ mice (n=9). **(B)** Maximal external aortic diameters at study endpoint in mice from both groups. **(C)** % change in maximal external aortic diameters. Data were quantified and represented as mean ± SEM, *, P<0.05, **, P<0.01, 2-tailed Student's t test. **(D)** Representative images of H&E-stained (5x) and Elastin-stained (5x and 10x) abdominal aortic sections from both groups (n=5). Scale bars, 100 μm.

Supplemental Tables

Table 1. List of primers used

Gene Name (Mus)	**Primer sequence (Forward 5' to 3')**	**Primer sequence (Reverse 5' to 3')**
Ccn2	TGACCTGGAGGAAAACATTAAGA	AGCCCTGTATGTCTTCACACTG
Myh11	GGGTAGCCAAGAAGCTGGTGT	TTTTCCACCAACTCCACGACC
Acta2	CCCACCCAGAGTGGAGAA	ACATAGCTGGAGCAGCGTCT
Cnn1	GAAGGTCAATGAGTCAACTCAGAA	CCATACTTGGTAATGGCTTTGA
Tagln (Sm22)	CCTTCCAGTCCACAAACGAC	GTAGGATGGACCCTTGTTGG
Eln	TGGAGCAGGACTTGGAGGT	CCTCCAGCACCATACTTAGCA
Fn1	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTCAGCAAAGG
Lgals3	TCCTGGTTGAAGCTGACCAC	CGCTGGTGAGGGTTATGTCA
Tnf	AGTCCGGGCAGGTCTACTTT	GAGTTGGACCCTGAGCCATA
Gapdh	CATGGCCTTCCGTGTTCCTA	CCTGCTTCACCACCTTCTTGAT
Actb	CGCCACCAGTTCGCCATGGA	TACAGCCCGGGGAGCATCGT
Gene Name (Homo)	**Primer sequence (Forward 5' to 3')**	**Primer sequence (Reverse 5' to 3')**
TGFBR1	GTGACAGATGGGCTCTGCTT	AAGGGCCAGTAGTTGGAAGT
TGFBR2	GCACGTTCAGAAGTCGGATG	CTGCACCGTTGTTGTCAGTG
ACTA2	GCGTGTAGCACCTGAAGAG	GAATGGCGACGTACATGGCA
TAGLN (SM22)	CACTGGCAGTTATAGGTGTTGAC	CCCACCCAGATTCATCAGCG
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC

Table 2. List of antibodies used

Target antigen	Vendor/Source	Catalog #	Working dilution
CCN2	Cell signaling Technology	10095	1:500
SMMHC	Abcam	ab53219	1:500
αSMA	Cell signaling Technology	56856	1:1000
SM22	Cell signaling Technology	40471	1:5000
Calponin	Cell signaling Technology	17819	1:500
KLF4	R&D Systems	AF3158	1:500
p-SRF	Cell signaling Technology	4261	1:500
TGFβ1	Abcam	ab92468	1:500
TGFBR2	Cell signaling Technology	41896	1:500
p-TGFBR2	Abcam	ab183037	1:500
p-Smad3	Abcam	ab52903	1:500
Caspase 8 (cleaved)	Cell signaling Technology	9746	1:500
Caspase 3 (Cleaved)	Cell signaling Technology	9661	1:500
GAPDH	Santa Cruz	sc-365062	1:1000
β-actin	Cell signaling Technology	3700	1:10000
Vinculin	Sigma-Aldric	V9131	1:3000
SMMHC (IF)	Abcam	ab125884	1:500
αSMA-Alexa 594 (IF)	Abcam	ab202368	1:500
Ki67 (IF)	Abcam	ab15580	1:500
CD68 (IF)	Bio-Rad	MCA1957GA	1:500
Vimentin (IF)	Sigma-Aldric	AB1620	1:50
CD45	BD Biosciences	550539	1:100
CD31	BD Biosciences	553370	1:100
COL1A1	Cell signaling Technology	72026	1:100
Alexa Fluor 488 goat anti-rat IgG	Invitrogen	A11006	1:1000
Alexa Fluor 488 goat anti-rabbit IgG	Invitrogen	A11008	1:1000
Anti-mouse IgG-HRP	Cell signaling Technology	7076	1:5000
Anti-Rabbit IgG-HRP	Cell signaling Technology	7074	1:5000