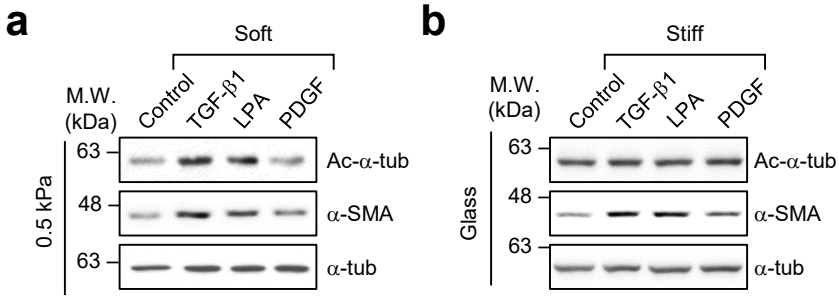
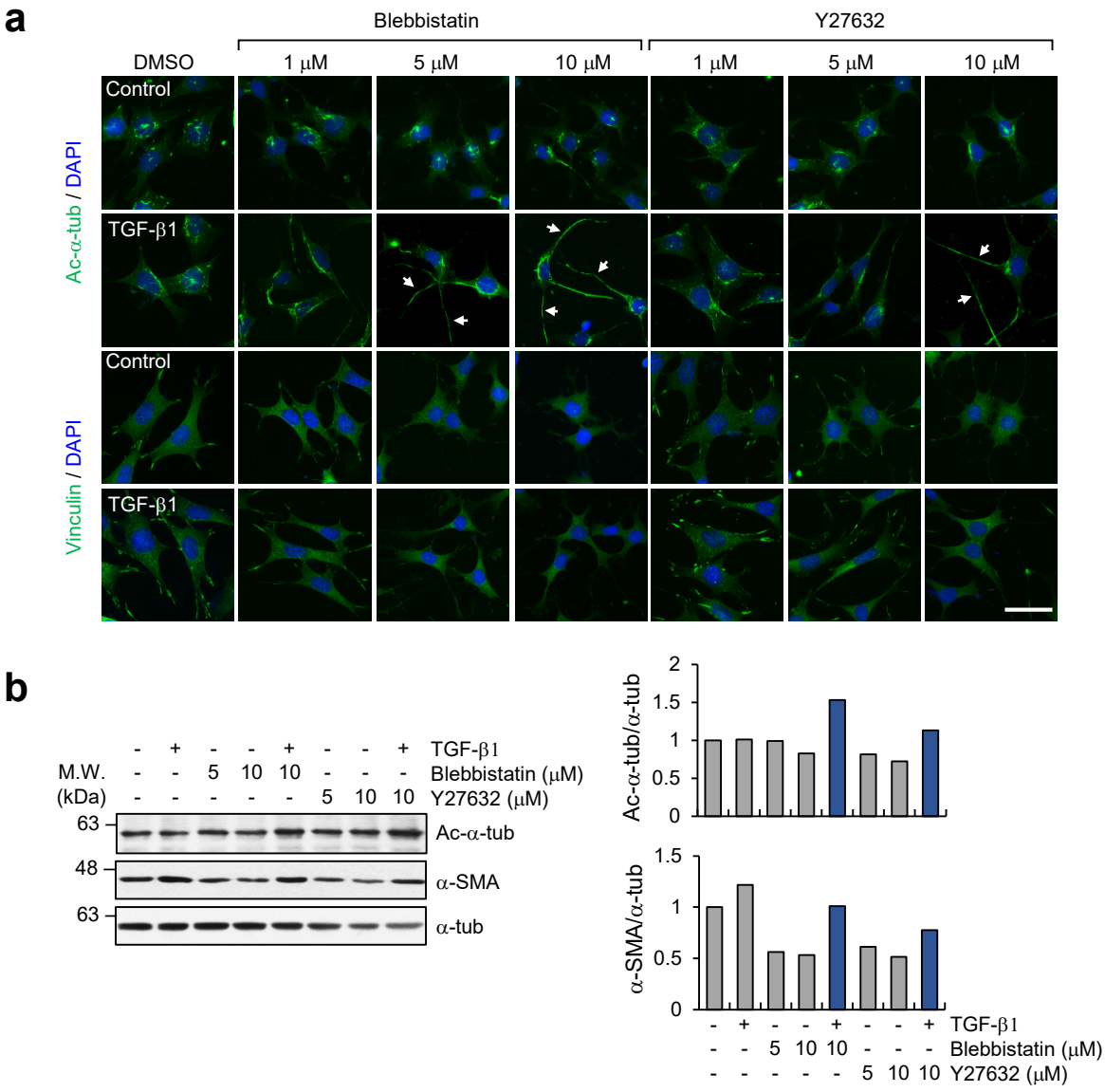
Supplementary Fig. 1



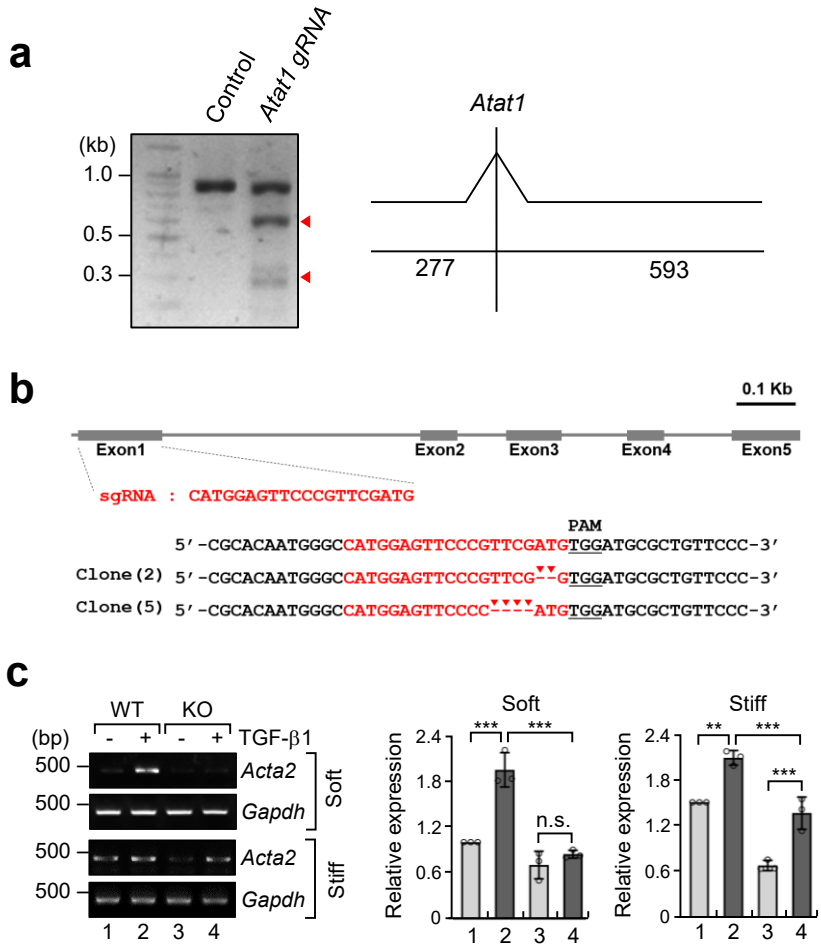
Supplementary Fig. 1 Increase in acetylated-α-tubulin in MEFs grown on soft matrices is correlated with α-SMA expression. a, b Cells were incubated on 0.5 kPa or glass and treated with TGF-β1 (2 ng ml^{-1}), LPA (10 μM), or PDGF (10 ng ml^{-1}) for 8 h. Then, western blotting was performed using antibodies specific for acetylated-α-tubulin and α-SMA.

Supplementary Fig. 2



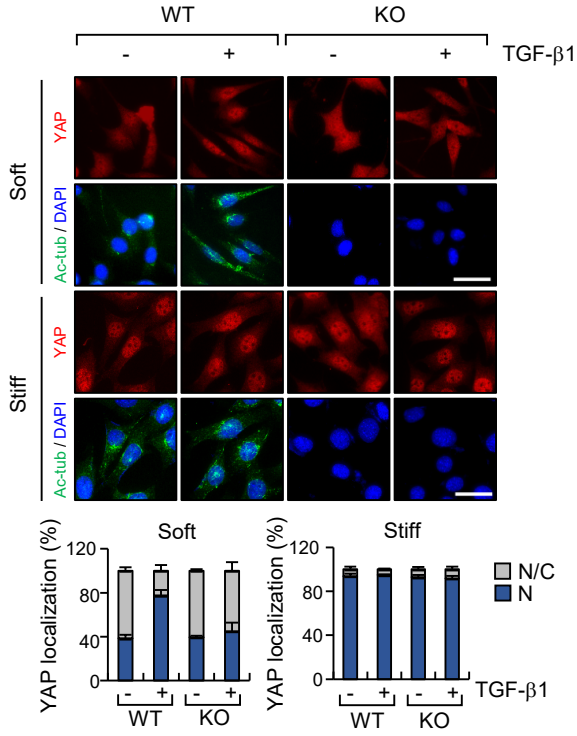
Supplementary Fig. 2 Acetylated-α-tubulin is increased in low-tension environment.
a Immunofluorescence imaging of MEFs, incubated on fibronectin-coated coverslips and treated with TGF-β1 combined with blebbistatin and Y27632 (1, 5, 10 μM), and immunolabeled with antibodies specific for acetylated-α-tubulin and vinculin. Arrow indicates long extended acetylated-α-tubulin. Scale bar, 50 μm. **b** Western blotting for acetylated-α-tubulin and α-SMA in cells incubated as described in (a). Graph shows relative expression of acetylated-α-tubulin and α-SMA normalized to that of α-tubulin.

Supplementary Fig. 3



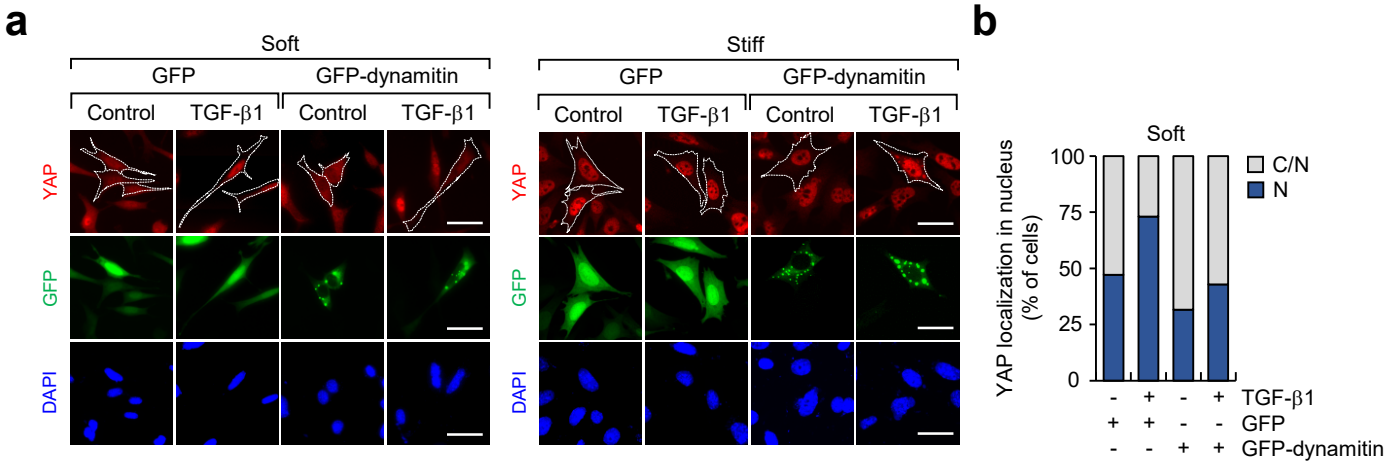
Supplementary Fig. 3 Generation of α-TAT1 KO MEFs, and comparison of α-SMA expression between WT and α-TAT KO. a Empty PX458 and guide RNA (gRNA) constructs used for insertion were transfected with B16F10 cells, and then selected with puromycin for 1 week. Genomic DNA obtained from puromycin resistant B16F10 cells was amplified (870 bp). PCR products were denatured, re-annealed, and incubated with T7 endonuclease (T7E1) to cleave mismatched DNA. PCR products were excised into two fragments (593 and 277 bp). **b** Validation of α-TAT1 KO MEFs by genomic DNA sequencing. **c** TGF-β1-induced *Acta2* (encoding α-SMA) expression was compared between WT and α-TAT1 KO MEFs in transcripts levels expressed under soft and stiff conditions. Soft: one-way ANOVA, $F_{3,8} = 44.21$, stiff: one-way ANOVA, $F_{3,8} = 69.01$. The graphs represent the mean of three independent experiments ± S.D. with p values $p < 0.05$ considered being significant. $^{**}p < 0.01$, $^{***}p < 0.005$.

Supplementary Fig. 4



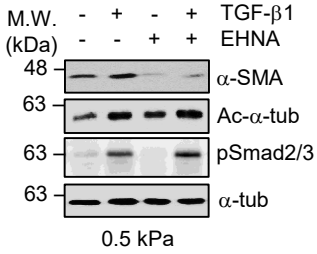
Supplementary Fig. 4 Microtubule acetylation increases YAP nuclear translocation in MEFs grown on a soft matrix. WT and α-TAT1 KO MEFs were seeded on fibronectin-coated soft and stiff matrices and stimulated with TGF-β1 for 8 h. Cells were fixed and labelled with antibodies specific for YAP and acetylated α-tubulin. Graphs show the quantification of YAP cellular localization in MEFs grown on soft and stiff matrices. N; nucleus, C; cytosol. Scale bar, 50 μm.

Supplementary Fig. 5



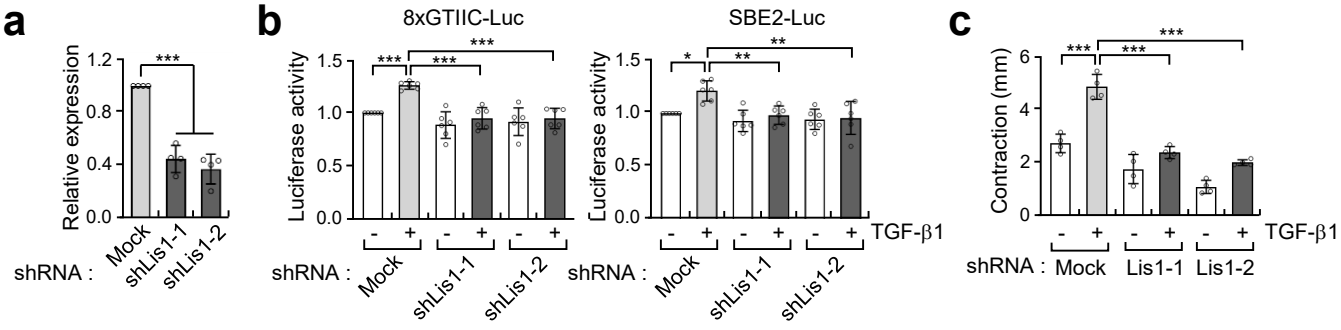
Supplementary Fig. 5 Overexpression of dynamitin suppresses TGF-β1-induced YAP nuclear translocation on soft matrices. a WT MEFs were transfected with GFP and GFP-dynamitin for 24 h and seeded on fibronectin-coated 0.5 kPa PAG and glass with or without TGF-β1 for 8 h. Cells were then fixed and immunocytochemistry was performed using an antibody specific for YAP. Dotted lines indicate GFP-expressing cells. Scale bar, 50 μm. **b** Quantification of YAP cellular localization in MEFs grown on soft matrices. N; nucleus, C; cytosol.

Supplementary Fig. 6



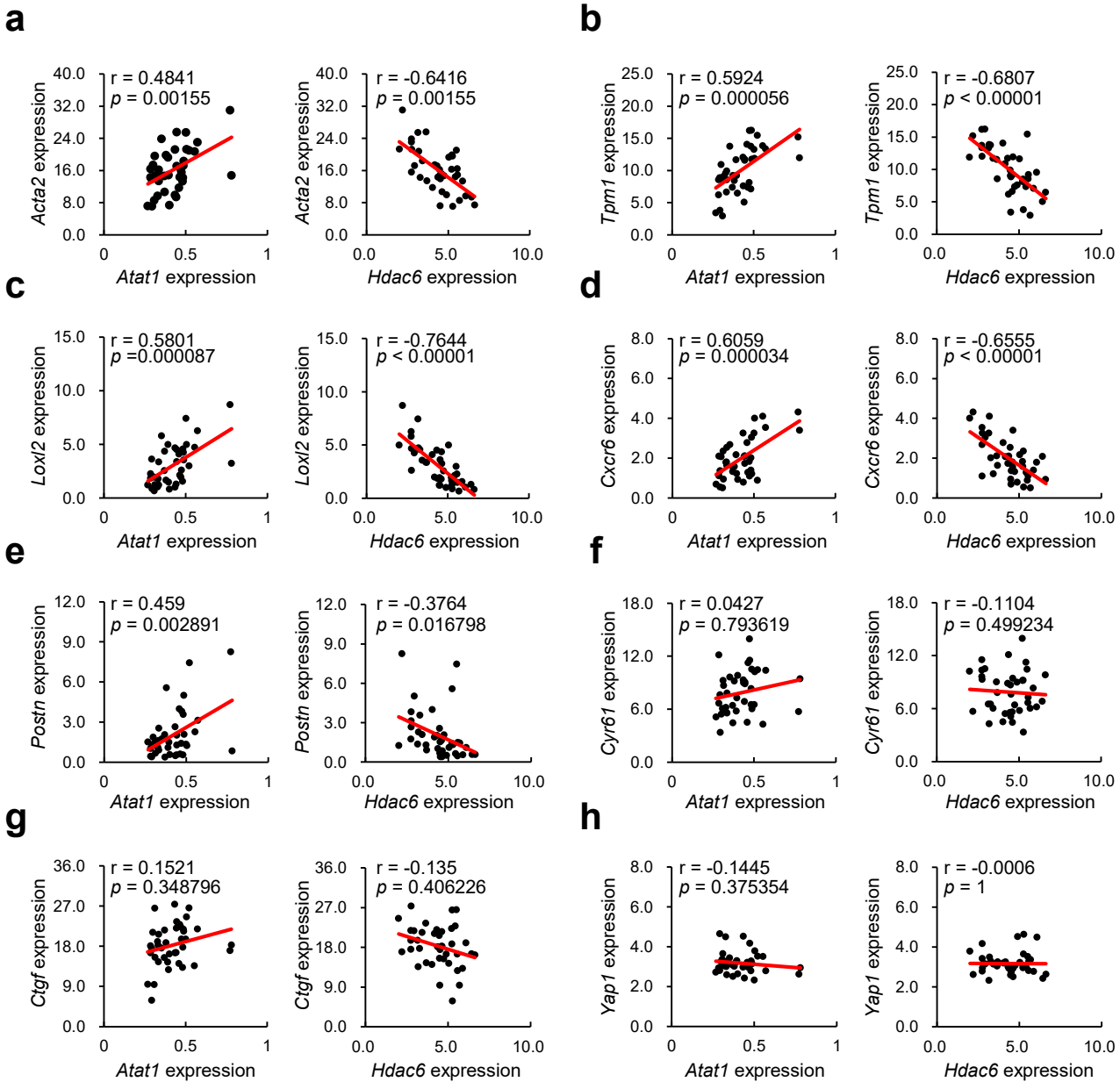
Supplementary Fig. 6 Inhibition of dynein activity does not affect TGF-β1-induced microtubule acetylation and total Smad phosphorylation in MEFs grown on soft matrices. MEFs were incubated on a soft matrix and treated with TGF-β1 and/or EHNA for 8 h. Then the cells were lysed and subjected to western blotting using antibodies specific for α-SMA, acetylated α-tubulin, and phospho-Smad2/3.

Supplementary Fig. 7



Supplementary Fig. 7 Knockdown of Lis1 to inhibit the dynein function suppresses transcriptional activities of YAP and Smad upon TGF-β1 on soft matrices. a Generation of Lis1 knockdown cell line using shRNA lentiviral infection. Knockdown efficiency of Lis1 was verified by RT-qPCR. n = 4 from two independent experiments are presented. One-way ANOVA, $F_{2,\ 9}$ = 61.26. **b** Transcriptional activity of YAP and Smad was compared by the luciferase assay in Lis1 KD cell lines upon TGF-β1 stimulation on soft matrix. n = 6 from three independent experiments are presented. 8xGTIIIC-Luc: one-way ANOVA, $F_{5,\ 30}$ = 12.40, SBE2-Luc: one-way ANOVA, $F_{5,\ 30}$ = 6.622. **c** Collagen matrices containing Mock and Lis1 KD cells (shLis1-1 and shLis1-2) were incubated for 12 h under floating condition with TGF-β1. The contractility of cells was measured using the reduction in the perimeter of the collagen gel. n = 4 from two independent experiments are presented. One-way ANOVA, $F_{5,\ 30}$ = 52.37. Data are represented as mean ± S.D. with p values $p < 0.05$ considered being significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

Supplementary Fig. 8



Supplementary Fig. 8 Correlation plot of genes expression regulated by α-TAT1 depending on *Atat1* and *Hdac6* in liver fibrosis tissue. a-h mRNA expression level of *Acta2, Tpm1, Loxl2, Cxcr6, Postn, Cyr61, Ctgf* and *Yap1* with respect to *Atat1* and *Hdac6* mRNA expression in the liver tissue (40 liver cirrhosis patients; GSE25097). Pearson correlation coefficient (r) and *p*-value (*p*) from r were calculated.

Supplementary Table 1

Gene	Direction	Sequence (5' → 3')
shYAP	Forward	CCGGGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTCTTTTTG
	Reverse	AATTCAAAAAGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTC
shSmad2	Forward	CCGGTGGTGTTCAATCGCATACTATCTCGAGATAGTATGCGATTGAACACCATTTTTG
	Reverse	AATTCAAAAATGGTGTTCAATCGCATACTATCTCGAGATAGTATGCGATTGAACACCA
shLis1-1	Forward	CCGGGATCACAATGTCTCTTCAGTACTCGAGTACTGAAGAGACATTGTGATCTTTTTTG
	Reverse	AATTCAAAAAAGATCACAATGTCTCTTCAGTACTCGAGTACTGAAGAGACATTGTGATC
shLis1-2	Forward	CCGGGCAGATTATCTTCGTTCAAATCTCGAGATTTGAACGAAGATAATCTGCTTTTTTG
	Reverse	AATTCAAAAAAGCAGATTATCTTCGTTCAAATCTCGAGATTTGAACGAAGATAATCTGC

Supplementary Table 2

Gene	Direction	Sequence (5' → 3')
Acta	Forward	TCTTCCAGCCATCTTTCA
	Reverse	CCTGGGTACATGGTGGTA
Tagln	Forward	CAGGTGGCTCAATTCTTG
	Reverse	TTTGGTCACAGCCAAACT
Tpm1	Forward	AGCTGGTTGAGGAGGAGT
	Reverse	TTGGGCTCGGCTTTCAAT
Cxcr6	Forward	GCATACTTTCGGGCTTGC
	Reverse	TGAGAGAGGCAGCCGATA
Loxl2	Forward	TGTGCCAACTTTGGAGAACA
	Reverse	GGCACTTCATAGTTGGGGTTA
Postn	Forward	ACCTGCAATGACGAAGATCC
	Reverse	GGATCACTTCTGTCACCGTT
Cyr61	Forward	TTCCAGCCCAACTGTAAA
	Reverse	AACCCACTCTTCACAGCA
Ctgf	Forward	TGCACCAGTGTGAAGACA
	Reverse	AGGCACAGGTCTTGATGA
Yap1	Forward	TGCGAGGTCATAGGTAAAGT
	Reverse	AATGGCCTCAAATGACTGAC
Smad2	Forward	GAGAGTTGAGACCCCAGT
	Reverse	TCCGAGTTTGATGGGTCT
Pafah1b1 (Lis1)	Forward	ACGTGGAGTTCTGTTCCATT
	Reverse	GTCTTCATGCATCGCTTGTT
Gapdh	Forward	TGGCAAAGTGGAGATTGT
	Reverse	CTTCCCGTTGATGACAAG