Supplementary Information

Deletion of ASPP1 in myofibroblasts alleviates myocardial fibrosis by reducing p53 degradation

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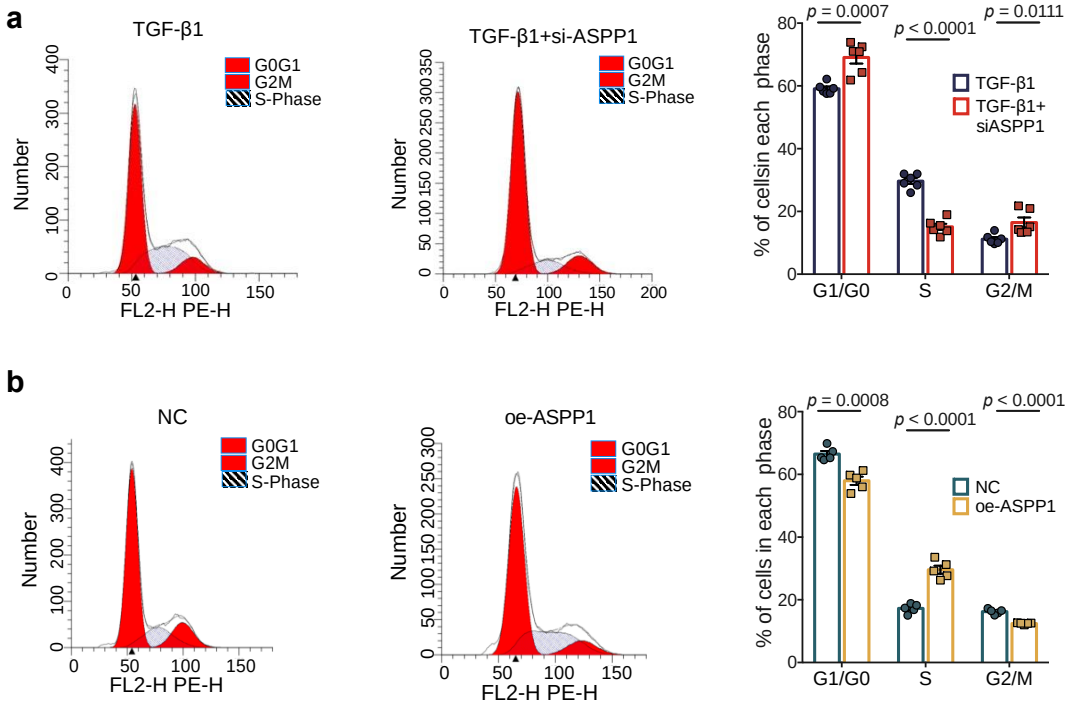
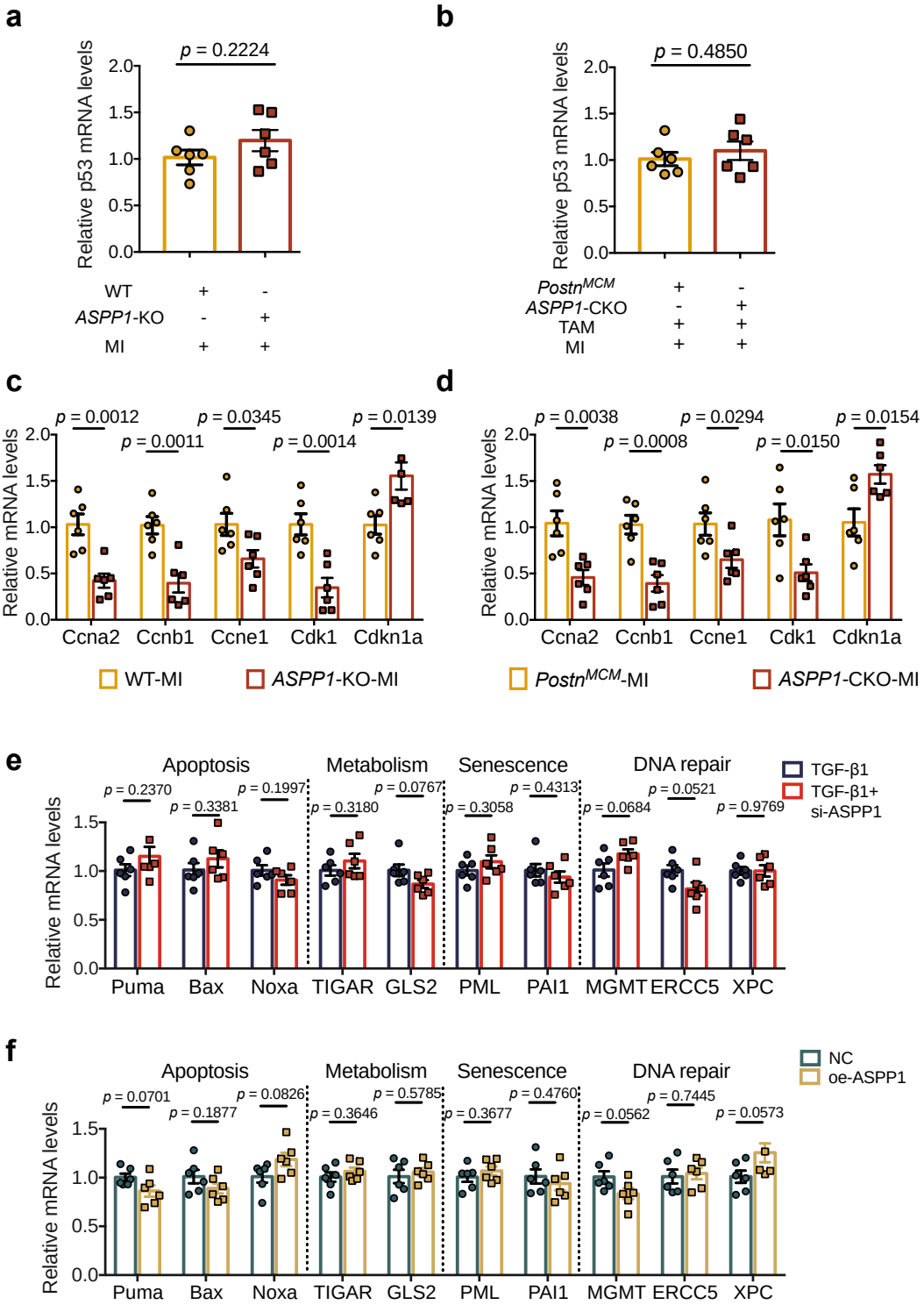
Supplementary Figure 1



Supplementary Figure 1. ASPP1 regulates cell proliferation. (a) Representative flowcytometry plots of fibroblasts in different stages of cell cycle by flow cytometry and quantification of fibroblasts in each phase of cell cycle. n=6 independent samples. **(b)** Effects of ASPP1 overexpression on the proliferation of primary mouse cardiac fibroblasts (PMCFs). n=5 independent samples. Data are represented as mean ± SEM. Statistics: Two-tailed Student's t test was used to calculate *P* values (**a** and **b**). Source data are provided as a Source Data file.

Supplementary Figure 2

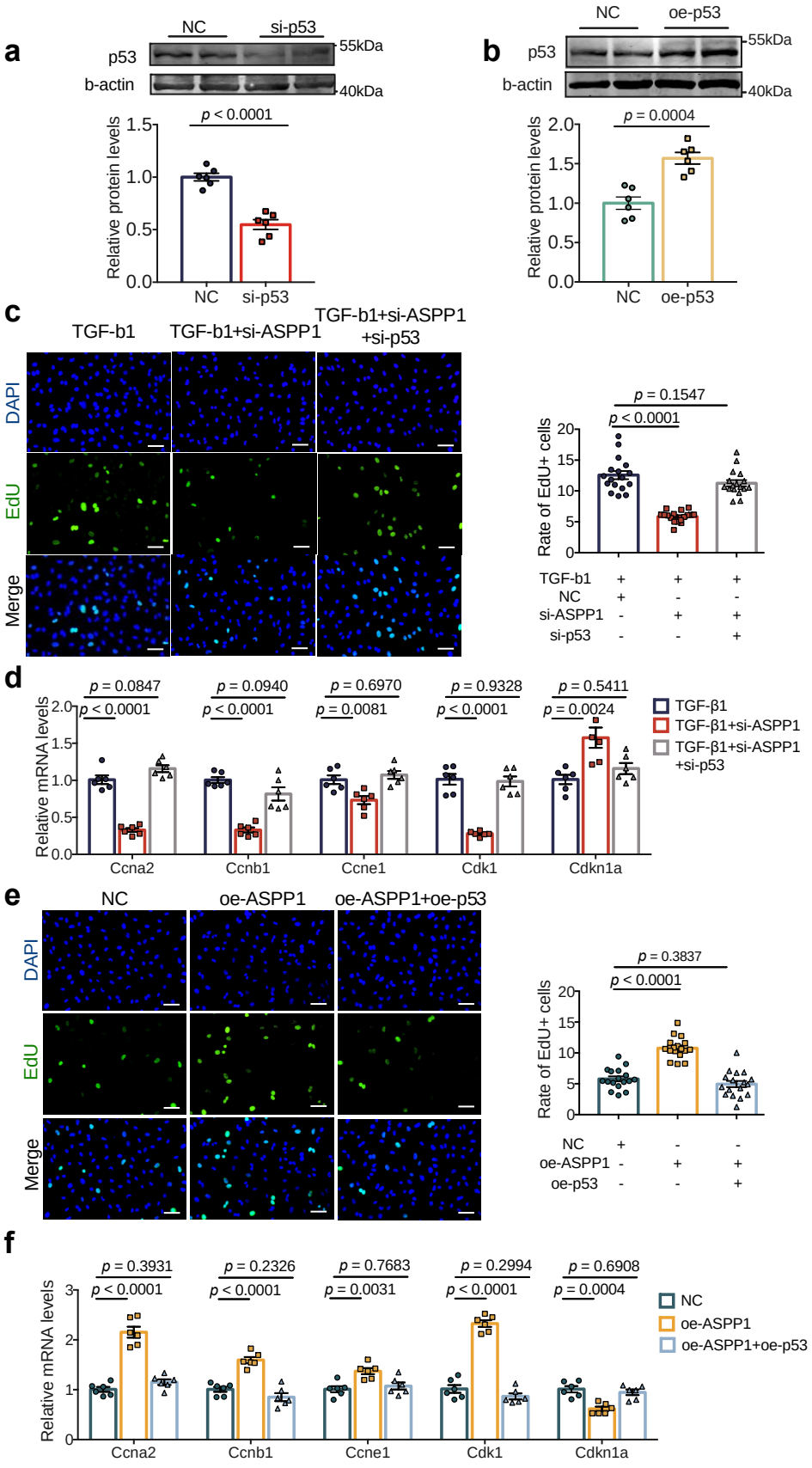


Supplementary Figure 2. ASPP1 modulates p53 signaling in cardiac fibroblasts.

(a) Effects of *ASPP1* global knockout mice on p53 mRNA levels in infarct area. n=6

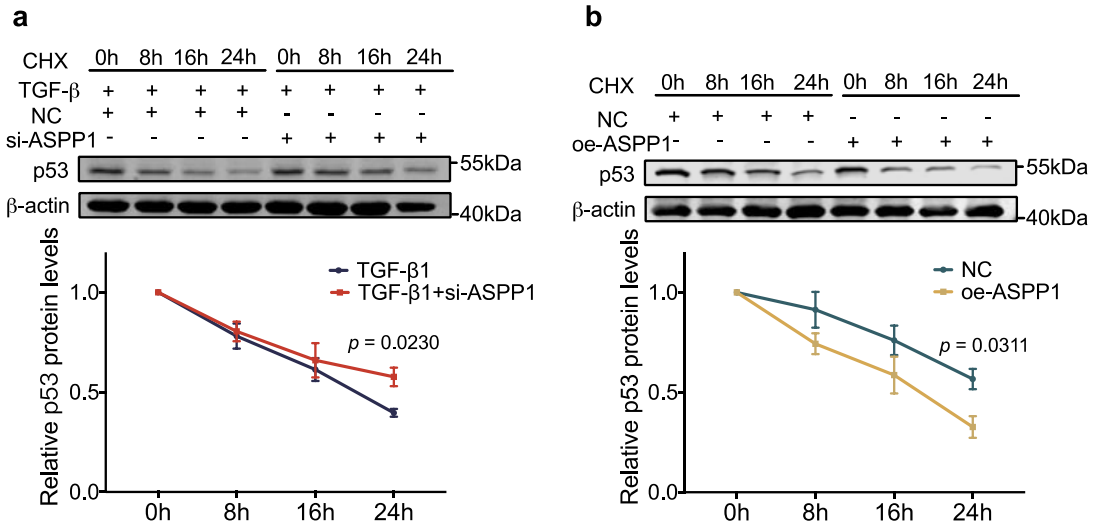
independent samples. **(b)** p53 mRNA levels in infarct area of myofibroblasts *ASPP1* knockout mice. n=6 independent samples. **(c)** qRT-PCR was used to evaluate genes encoding the cell cycle regulator in *ASPP1* global knockout mice. n=6 independent samples. **(d)** Relative mRNA levels of cell cycle regulator genes in myofibroblasts *ASPP1* deletion mice. n=6 independent samples. **(e)** qRT-PCR was used to evaluate the expression of p53 target genes in ASPP1-silenced cells treated with TGF-β1. n=6 independent samples. **(f)** Relative mRNA levels of p53 target genes in ASPP1-overexpressed PMCFs. n=6 independent samples. Data are represented as mean ± SEM. Statistics: Two tails Student's t test was used to calculate *P* values in **a**, **b**, **c**, **d**, **e** and **f**. Source data are provided as a Source Data file.

Supplementary Figure 3



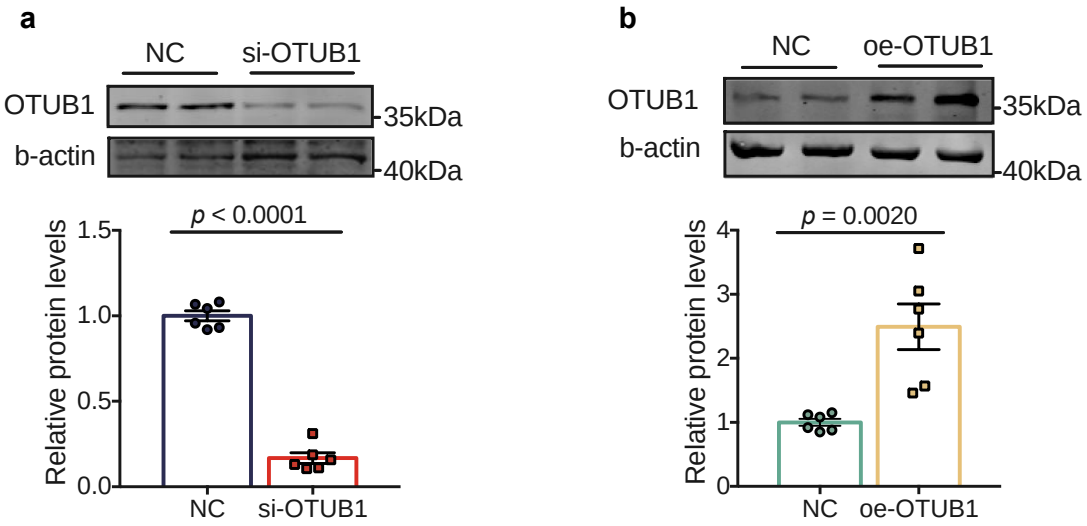
Supplementary Figure 3. Effects of knockdown or overexpression p53 on the expression of cell cycle regulators. (a) Efficiency of si-p53 in PMCFs by Western blot. n = 6 independent samples. **(b)** Efficiency of p53 overexpression plasmid in PMCFs by Western blot assay. n = 6 independent samples. **(c)** Representative images and statistical data of EdU positive cells after silence of ASPP1, and simultaneous knockdown ASPP1 and p53. n=17. Scale bar = 50 μm. Magnification 20×. **(d)** qRT-PCR was used to evaluate the expression of genes encoding cell cycle regulators in PMCFs cells with co-transfection of si-ASPP1 and si-p53. n=6 independent samples. **(e)** Representative images and statistical graphs of EdU positive cells with overexpression of ASPP1, and simultaneous overexpression ASPP1 and p53. n=17. Scale bar = 50 μm. Magnification 20×. **(f)** qRT-PCR was used to evaluate the expression of genes encoding cell cycle regulators in PMCFs cells with co-transfection of oe-ASPP1 plasmid and oe-p53 plasmid. n=6 independent samples. Data are represented as mean ± SEM. Statistics: Two tails Student's t test was used to calculate *P* values in **a** and **b**. One-way ANOVA, followed by Tukey post hoc multiple comparisons test (**c**, **d**, **e** and **f**). Source data are provided as a Source Data file.

Supplementary Figure 4



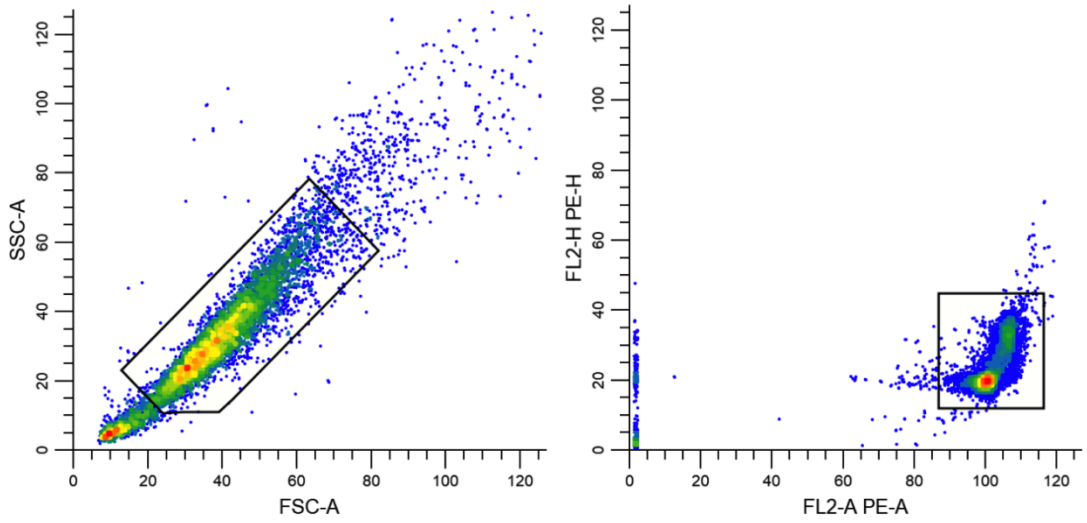
Supplementary Figure 4. The half-life of p53 following ASPP1 silencing and overexpression. (a) The half-life of p53 was prolonged with silence of ASPP1 in PMCFs. n=3 independent experiments. **(b)** The half-life of p53 was shorten with overexpression of ASPP1 in PMCFs. n=3 independent experiments. Statistics: Two tails Student's t test was used to calculate the presented *P* values in **a** and **b**. Source data are provided as a Source Data file.

Supplementary Figure 5



Supplementary Figure 5. Knockdown and overexpression efficiency of OTUB1. (a) Efficiency of si-OTUB1 in PMCFs by Western blot assay. n = 6 independent samples. **(b)** Efficiency of OTUB1 overexpression plasmid in PMCFs by Western blot assay. n = 6 independent samples. Data are represented as mean ± SEM. Statistics: Two tails Student's t test was used to calculate the presented *P* values in **a** and **b**. Source data are provided as a Source Data file.

Supplementary figure 6.



Supplementary figure6. Gating strategy of Flow Cytometry. Flow cytometry was performed to detect the transfected cardiac fibroblasts. Strategy for collecting cell cycle data after DNA staining with propidium iodide (PI). Cell debris was excluded using side scatter area (SSC-A) vs. forward scatter area (FSC-A). Ajust the PE-A and PE-H gates to exclude adherent cells from the active cell population. Creating a histogram that displays the relationship between PE-H and the number of cells. PE-A, PE-Area；PE-H，PE-Height.

Supplementary Table 1. gRNA target sequences of *ASPP1*

gRNA1 (matching forward strand of gene)	GAGTTACAGACATGTGGTGCTGG
gRNA2 (matching reverse strand of gene)	TCTAGCTTCTCTGTGGTACAGGG

Supplementary Table 2. Sequences of mouse oligonucleotide primers used for real-time quantitative PCR

ASPP1	Forward	5'-ATGCCGATGATATTAACCGTGTT-3'
	Reverse	5'-ATGTGGTCATAGGGGATGGGA-3'
p53	Forward	5'-CTCTCCCCCGCAAAAGAAAAA-3'
	Reverse	5'-CGGAACATCTCGAAGCGTTTA-3'
Col1a1	Forward	5'-TTCTCCTGGCAAAGACGGAC-3'
	Reverse	5'-CGGCCACCATCTTGAGACTT-3'
Col3a1	Forward	5'-ACGTAAGCACTGGTGGACAG-3'
	Reverse	5'-CAGGAGGGCCATAGCTGAAC-3'
Ccna2	Forward	5'-AAGAGAATGTCAACCCCGAAAAA-3'
	Reverse	5'-ACCCGTCGAGTCTTGAGCTT-3'
Ccnb1	Forward	5'-GCGTGTGCCTGTGACAGTTA-3'
	Reverse	5'-CCTAGCGTTTTTGCTTCCCTT-3'
Ccne1	Forward	5'-CTCCGACCTTTCAGTCCGC-3'
	Reverse	5'-CACAGTCTTGTCAATCTTGGCA-3'
Cdk1	Forward	5'-AGGTACTTACGGTGTGGTGTAT-3'
	Reverse	5'-CTCGCTTTCAAGTCTGATCTTCT-3'
Cdkn1a	Forward	5'-CCTGGTGATGTCCGACCTG-3'
	Reverse	5'-CCATGAGCGCATCGCAATC-3'
Postn	Forward	5'-TGGTATCAAGGTGCTATCTGCG-3'

	Reverse	5'-AATGCCCAGCGTGCCATAA-3'
Tcf21	Forward	5'-CTCCCTGAAAGTGGACTCCAA-3'
	Reverse	5'-CGGGCTTTTCTTAGTGGGC-3'
Fn1	Forward	5'-CCCAACTGGTTACCCTTCCA-3'
	Reverse	5'-GGTTGGTGATGAAGGGGGTC-3'
α-SMA	Forward	5'-GTACCACCATGTACCCAGGC-3'
	Reverse	5'-GCTGGAAGGTAGACAGCGAA-3'
β-actin	Forward	5'-CTGAGCTGCGTTTTACACCCT-3'
	Reverse	5'-CGCCTTCACCGTTCCAGTT-3'