Supplemental Materials

Table S1

Primers	sequences (5'-3')	Use
Cyto-γ-actin promoter (-773+37) (rat)	TGGAGAGCAGTCTCGCCAACTG TGTAAAGCCGGCGGCGAAGTG	Cloning
Cytoplasmic γ-actin (rat)	ATGGAAGAAGAAATCGCCGCCC GAGAGCACCGCCTGAATGGCC	Cloning/RPA
Cytoplasmic γ-actin CArG (rat)	GAAAGATCGCCATATATGGACATGTTC GAACATGTCCATATATGGCGATCTTTC	EMSA
Cytoplasmic γ-actin muCArG (rat)	GAAAGATCGTTATATATGGACATGTTC GAACATGTCCATATATAACGATCTTTC	EMSA
β-actin promoter (-739+18) (rat)	TTCTAGGTCCGGGGCAGAGG TGGACGCGGACTCGACAGTG	Cloning
β-actin promoter (rat)	ATGGATGACGATATCGCTGCGC GGTCATCTTTTCACGGTTGGCC	Cloning/RPA
β-actin CArG (rat)	CCGAAAGTTGCCTTTTATGGCTCGAGTG CACTCGAGCCATAAAAGGCAACTTTCGG	EMSA
β-actin muCArG (rat)	CCGAAAGTTGTTTTTTATGGCTCGAGTG CACTCGAGCCATAAAAAACAACTTTCGG	EMSA
SM α-actin promoter (-671+5)	ACATGCACGTGGACTGTACC GTGTCTGGGGAGGCTGAATG	Cloning
SM α-actin CArG-A (rat)	GTCTTTGCTCCTTGTTTGGGAAGCGAG CTCGCTTCCCAAACAAGGAGCAAAGAC	EMSA
SM α-actin muCArG-A (rat)	GTCTTTGCTTTTTGTTTGGGAAGCGAG CTCGCTTCCCAAACAAAAAGCAAAGAC	EMSA
SM α-actin CArG-B (rat)	GCTGAGGTCCCTATATGGTTGTGTTAG CTAACACAACCATATAGGGACCTCAGC	EMSA
SM α-actin muCArG-B (rat)	GCTGAGGTCTTTATATGGTTGTGTTAG CTAACACAACCATATAAAGACCTCAGC	EMSA
Myocardin (rat)	CAGTTACGGCTTCAACAGAGAAGG GCTTCATCTGAGCAGTTGGAATGG	mRNA splicing
Myocardin (rat)	CTGTGTGGAGTCCTCAGGTCAAACC GATGTGTTGCGGGCTCTTCAG	RT-PCR
SRF (rat)	TCTCAGGCACCATCCACCAT CCCAGCTTGCTGTCCTATCAC	RT-PCR
SM α-actin (rat/mouse)	GTGTGAAGAGGAAGACAGCAC GTGATGATGCCGTGTTCTATCG	RT-PCR
Col.1α1 (rat/mouse)	TCAGCCACCTCAAGAGAAGTC CTGCGGATGTTCTCAATCTGC	RT-PCR
GAPDH	TGCACCACCAACTGCTAAGC GGCATGGACTGTGGTCATGAG	RT-PCR
Col.1α2 promoter (mouse) (-433+55)	CACAGAGTGAAGCGGGACTG GCAATCGTGGACCGTTCC	Cloning
Col.1α1 promoter (mouse) (-479+50)	CCTCCAACAGGCACATCTGC CGAGGAGAAACTCCCGTCTGC	Cloning

Supplemental figures

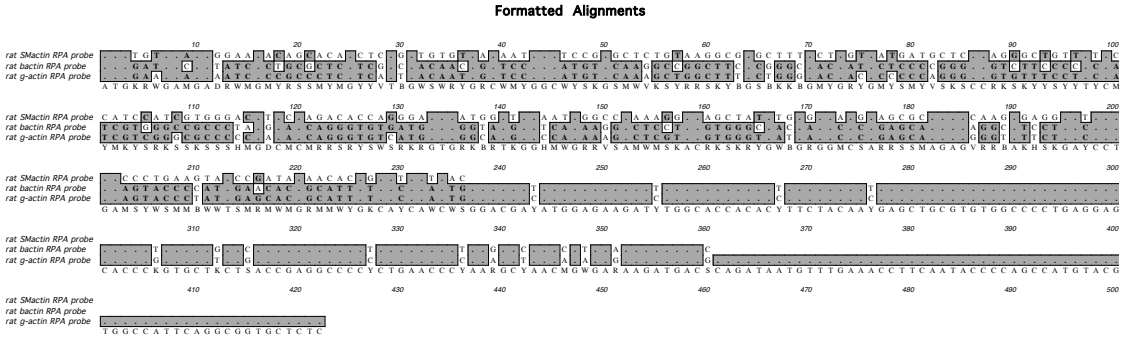

Figure S1. A formatted alignment of partial actin isoform sequences for RPA probes. Rat SM α-actin (236bp) (Genbank accession number: NM_031004), β-actin (360bp) (Genbank accession number: NM_031144.2) and cytoplasmic γ-actin (422bp) (NM_001127449) were cloned from rat hepatic myofibroblasts and ligated in to pGEM-7zf(+) vector (Promega). RPA probe sequences were shown. The mismatches were indicated by capital letters.

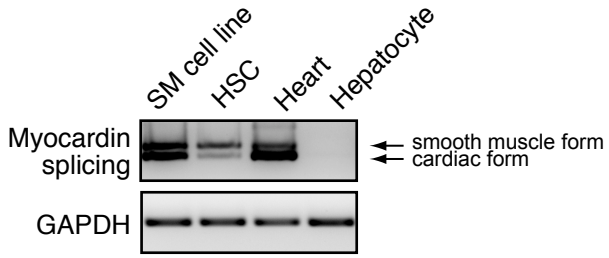

Figure S2. Hepatic myofibroblasts primarily express a smooth muscle form of myocardin. Reverse transcription PCR was performed using total RNAs from rat smooth muscle cell line, rat hepatic myofibroblasts (activated hepatic stellate cells), mouse heart tissue and rat hepatocytes. The PCR primers were designed as described before[1]. Myocardin mRNA splicing bands were indicated by arrows. GAPDH was used as control.

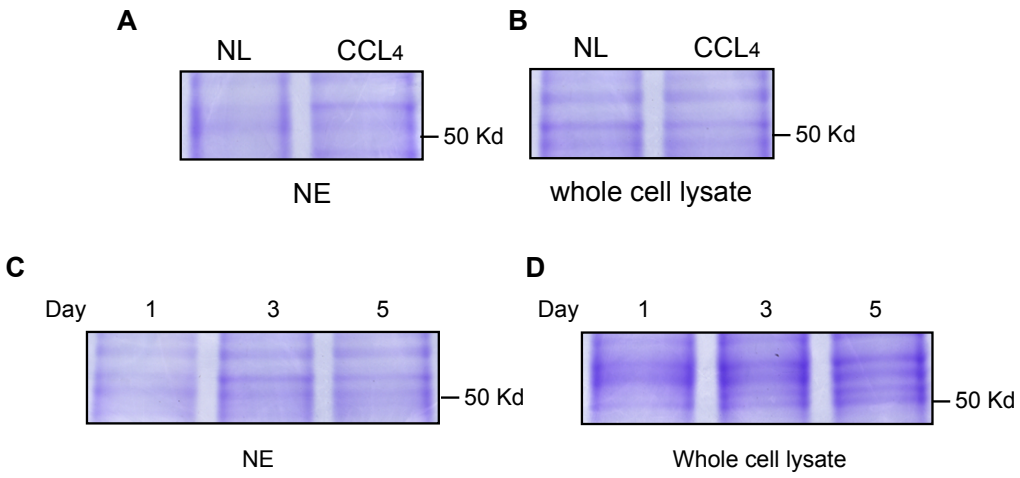

Figure S3. Coomassie blue staining used as additional immunblotting loading control. (A) 10μg of the nuclear extracts (NE) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 4–12%, Invitrogen) and then stained with R-250 coomassie blue (Biorad) according to manufacture's instruction. (B) Whole cell lysates (10μg) from hepatic stellate cells of normal and fibrotic rat liver were subjected to SDS-PAGE and coomassie blue staining as in (A). (C) HSCs from normal rat liver were cultured as indicated. 10μg nuclear extracts (NE) or whole cell lysates (D) were subjected to SDS-PAGE and coomassie blue staining. Representative images from 50-70 Kd regions were shown.

Reference:

1 Creemers EE, Sutherland LB, Oh J*, et al.* Coactivation of MEF2 by the SAP domain proteins myocardin and MASTR. *Molecular cell* 2006;23(1):83-96.