

Supplementary Information for

A role for Sfrp2 in cardiomyogenesis in vivo

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This PDF file includes: Supplementary Method, Figures S1-3, and Table S1

Supplementary Methods

RNA-seq: Cardiac tissue was harvested immediately prior to MI and 12 days after MI and Sfrp2 treatment, minced to $\sim 1\text{mm}^3$, and digested for 10 minutes at 37°C in a solution containing 200units/ml collagenase-II (Worthington), DMEM/F12 (Invitrogen), and 1xpenicillin-streptomycin (Invitrogen). Fetal bovine serum (Invitrogen) was added to a final concentration of 10% v/v to inhibit the reaction. The mixture was centrifuged at 100g for 3 minutes to remove cardiomyocytes. The cardiomyocyte fraction from the pre-MI sample was kept for RNA extraction. GFP+ cells were flow sorted and total RNA isolated using a PicoPure Arcturus kit (Invitrogen) according to the manufacturer's instructions. Complimentary cDNA was generated with an Ovation Pico WTA System V2 kit (NuGEN) which maintains the stoichiometry of the original RNA population. RNA-seq was performed with 2 lanes on a HiSeq 2000 instrument yielding a total of $>400,000,000$ PF clusters. TrimGalore was used to remove low quality sequences and Illumina sequencing adaptors. The remaining high quality sequences were mapped to the mm10 version of the mouse genome. Reads were kept for further analysis if they mapped to a single genomic location. Gene counts were compiled using the FeatureCounts tool. Cardiomyocyte genes were identified from the literature and normalized read counts (normalized to sample with the highest count) were plotted. Bioinformatic tools were used within Galaxy.org. Raw and processed data can be found in the NIH GEO database under the Accession number GSE90615.

Supplementary Figures

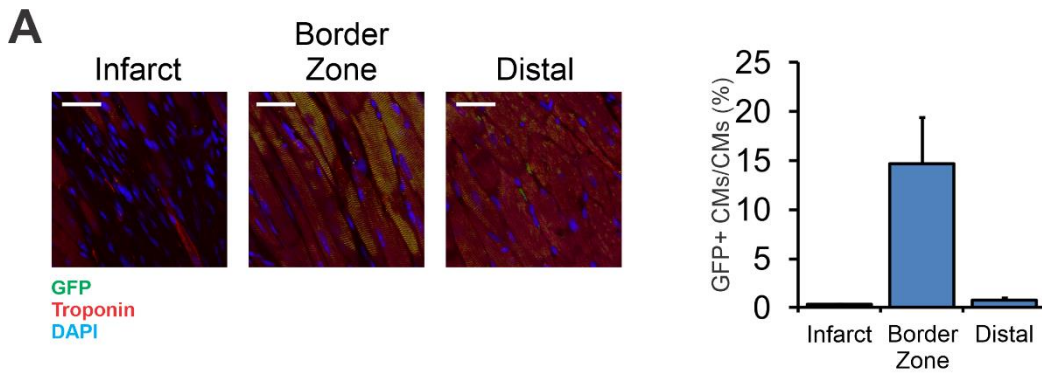
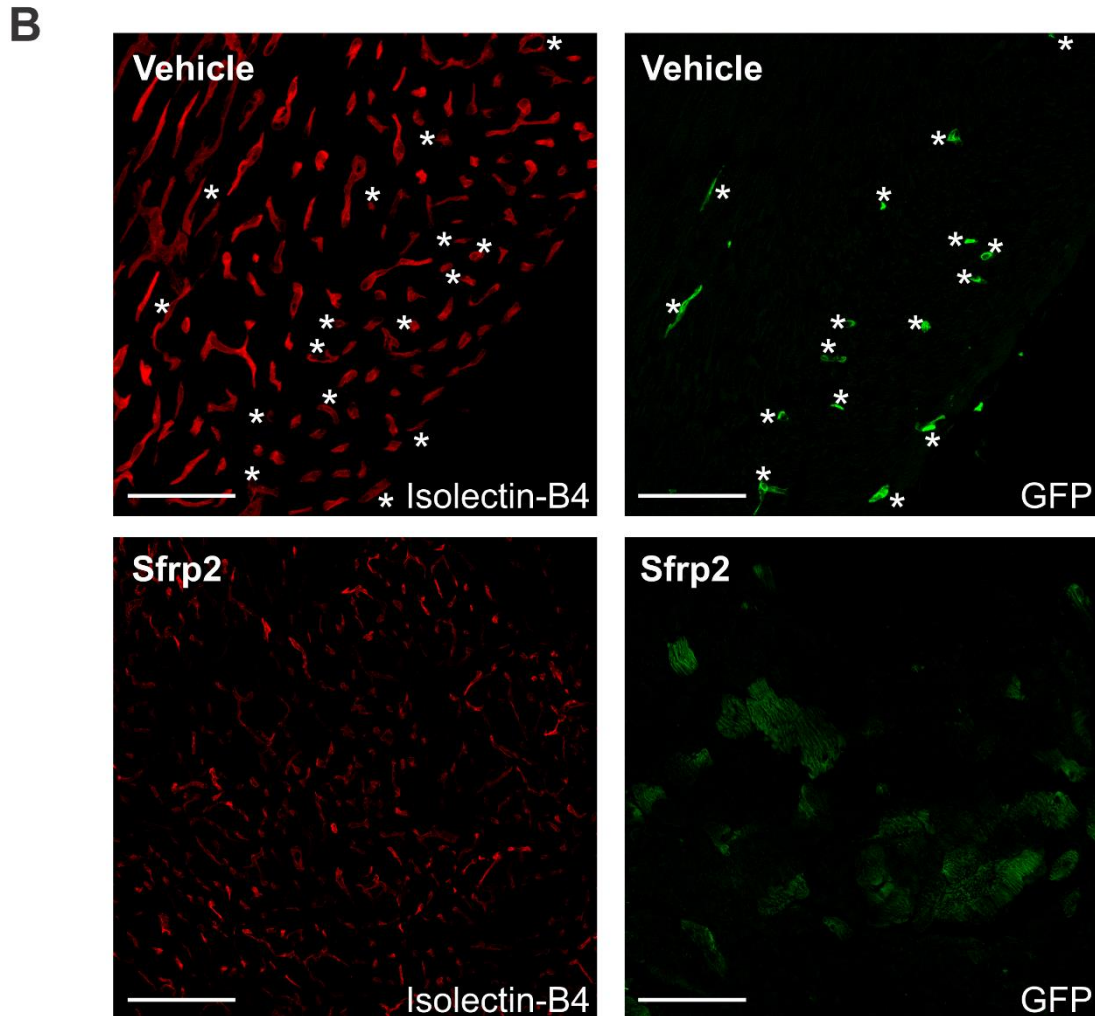


Fig. S1. Cardiomyocyte and Endothelial staining following Sfrp2 treatment. Mice (c-KitCreERT2/mTmG) were injected with tamoxifen (0.5 mg/mouse) for 14 consecutive days. Four days after tamoxifen treatment mice were subjected to myocardial infarction. Two days after injury, mice were injected with Sfrp2 (0.5 μ g) or PBS at the infarct border zone. Two months after injury, tissue sections were analyzed by immunofluorescence.



A, Expression of eGFP (cKit(+)) cells and cells derived thereof) and the cardiac marker cardiac troponin-T was analyzed by confocal microscopy.

Representative confocal images shown (scale bar 50 microns) from the infarct site, infarct border zone and a site

distal (>2 mm) from the injury zone. Quantification of Sfrp2 derived (eGFP+) cardiomyocytes in each region is expressed as a percentage of the total cardiomyocyte population. N=10. Only the Sfrp2 group is shown as there were no eGFP+ cardiomyocytes in the control group. **B,** Cardiac tissue was analyzed for co-expression of GFP and the endothelial cell specific stain Isolectin B4. Representative confocal images shown, scale bar 50 microns (vehicle) and 100 microns (Sfrp2). Individual channels are shown. Co-localization of the GFP and Isolectin-B4 signals are highlighted with an asterisk.

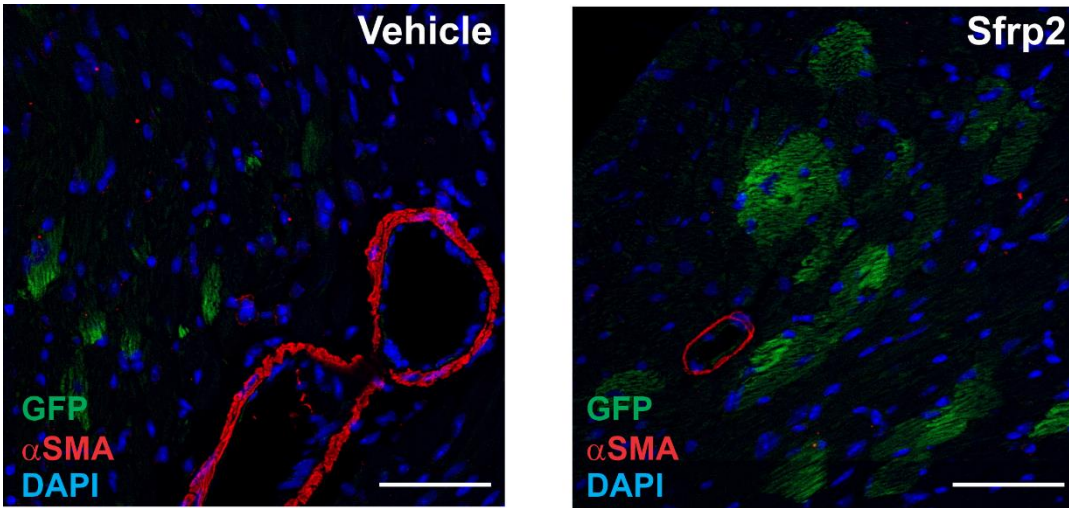


Fig. S2. Smooth muscle staining is not affected by Sfrp2. Tissue sections from vehicle and Sfrp2 treated mice were analyzed for co-expression of eGFP and the smooth muscle actin marker α SMA. N=7 (vehicle) or 10 (Sfrp2). Representative images shown. Scale bar 50 microns.

	Pre-Injury	Sfrp2	CM
Tbx5	Red	Red	Red
Gata4	Red	Red	Red
Isl1	Blue	Red	Red
Nfatc2	Blue	Red	Red
Mesp2	Blue	Red	Red
Actn2	Blue	Red	Red
Ttn	Blue	Red	Red
Tnni3	Blue	Red	Red
Myh6	Blue	Red	Red
Tnnc1	Blue	Red	Red
Tnnt2	Blue	Red	Red
Neb1	Blue	Red	Red
Actc	Blue	Red	Red
Myl2	Blue	Red	Red
Cacna1a	Blue	Red	Red
Cacna1b	Blue	Red	Red
Cacna1d	Blue	Red	Red
Cacna1e	Blue	Red	Red
Cacna1f	Blue	Red	Red
Cacna1s	Blue	Red	Red
Cacna2d4	Blue	Red	Red
Cacnb1	Blue	Red	Red
Cacnb2	Blue	Red	Red
Cacnb4	Blue	Red	Red
Cacng2	Blue	Red	Red
Cacng4	Blue	Red	Red
Cacng5	Blue	Red	Red
Fxyd3	Blue	Red	Red
Hvcn1	Blue	Red	Red
Kcna1	Blue	Red	Red
Kcna2	Blue	Red	Red
Kcnab1	Blue	Red	Red
Kcnc1	Blue	Red	Red
Kcnd1	Blue	Red	Red
Kcnd3	Blue	Red	Red
Kcne4	Blue	Red	Red
Kcnf1	Blue	Red	Red
Kcnh1	Blue	Red	Red
Kcnh2	Blue	Red	Red
Kcnh6	Blue	Red	Red
Kcnj1	Blue	Red	Red
Kcnj6	Blue	Red	Red
Kcnk10	Blue	Red	Red
Kcnk13	Blue	Red	Red
Kcnk2	Blue	Red	Red
Kcnma1	Blue	Red	Red
Kcnq2	Blue	Red	Red
Kcnq4	Blue	Red	Red
Kcnq5	Blue	Red	Red
Kcnt1	Blue	Red	Red
Kcnu1	Blue	Red	Red
Kcnv2	Blue	Red	Red
Nmur2	Blue	Red	Red
P2rx1	Blue	Red	Red
Ryr1	Blue	Red	Red
Ryr3	Blue	Red	Red
Scn11a	Blue	Red	Red
Scn1a	Blue	Red	Red
Scn2a1	Blue	Red	Red
Scn5a	Blue	Red	Red
Trpc3	Blue	Red	Red
Trpc7	Blue	Red	Red
Trpv1	Blue	Red	Red
glra1	Blue	Red	Red
clcn1	Blue	Red	Red
clcnka	Blue	Red	Red
kcnmb2	Blue	Red	Red
cnga3	Blue	Red	Red
slc25a15	Blue	Red	Red
slc34a2	Blue	Red	Red
atp2a1	Blue	Red	Red
slc1a1	Blue	Red	Red

Normalized Fold Change



Fig. S3. Sfrp2 induces the acquisition of the cardiomyocyte phenotype. Cardiomyocyte-specific gene expression levels from GFP+ cells isolated from the pre-injury heart as well as the heart following MI and Sfrp2 treatment were compared to cardiomyocytes. N=3 individual animals per group. Expression levels for each gene were averaged for each group and then normalized. Normalized read counts (normalized to group with the highest read count) are plotted.

Table S1. Echocardiographic analysis. Cardiac function was assessed by echocardiography immediately prior to injury, two weeks after injury and finally 8 weeks after injury. N=4 (vehicle) or 8 (Sfrp2). Comparisons are made between 2 and 8 weeks post-injury within the control and Sfrp2 groups. Significant changes between 2 and 8 weeks post-injury ($P < 0.05$) are marked with an asterisk.

Pre-injury	Group	Mean \pm SEM	FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVD;d (mm)	LVD:s (mm)
	Vehicle	56.0 \pm 2.1	87.6 \pm 1.6	30.9 \pm 4.5	3.8 \pm 0.7	14.0 \pm 0.8	2.8 \pm 0.2	1.2 \pm 0.1	
	Sfrp2	58.1 \pm 2.3	88.7 \pm 1.6	32.5 \pm 1.7	3.7 \pm 0.6	13.5 \pm 0.8	2.9 \pm 0.1	1.2 \pm 0.1	
2-weeks post-MI	Group	Mean \pm SEM	FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVD;d (mm)	LVD:s (mm)
	Vehicle	25.1 \pm 3.8	49.3 \pm 6.8	101.2 \pm 32.4	57.6 \pm 27.7	5.4 \pm 0.7	4.5 \pm 0.6	3.5 \pm 0.6	
	Sfrp2	18.5 \pm 3.1	37.7 \pm 5.6	98.6 \pm 19.5	67.6 \pm 16.7	4.4 \pm 0.6	4.5 \pm 0.4	3.7 \pm 0.4	
8-weeks post-MI	Group	Mean \pm SEM	FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVD;d (mm)	LVD:s (mm)
	Vehicle	24.7 \pm 5.8	47.9 \pm 9.6	100.9 \pm 26.3	59.7 \pm 26.7	6.4 \pm 1.7	4.6 \pm 0.5	3.5 \pm 0.6	
	Sfrp2	29.8 \pm 2.6 *	56.9 \pm 4.1 *	79.9 \pm 16.4	37.8 \pm 10.4 *	7.3 \pm 0.6 *	4.1 \pm 0.4 *	2.9 \pm 0.3 *	