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 ~1mm^3, and digested for 10 minutes at 37^{o}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

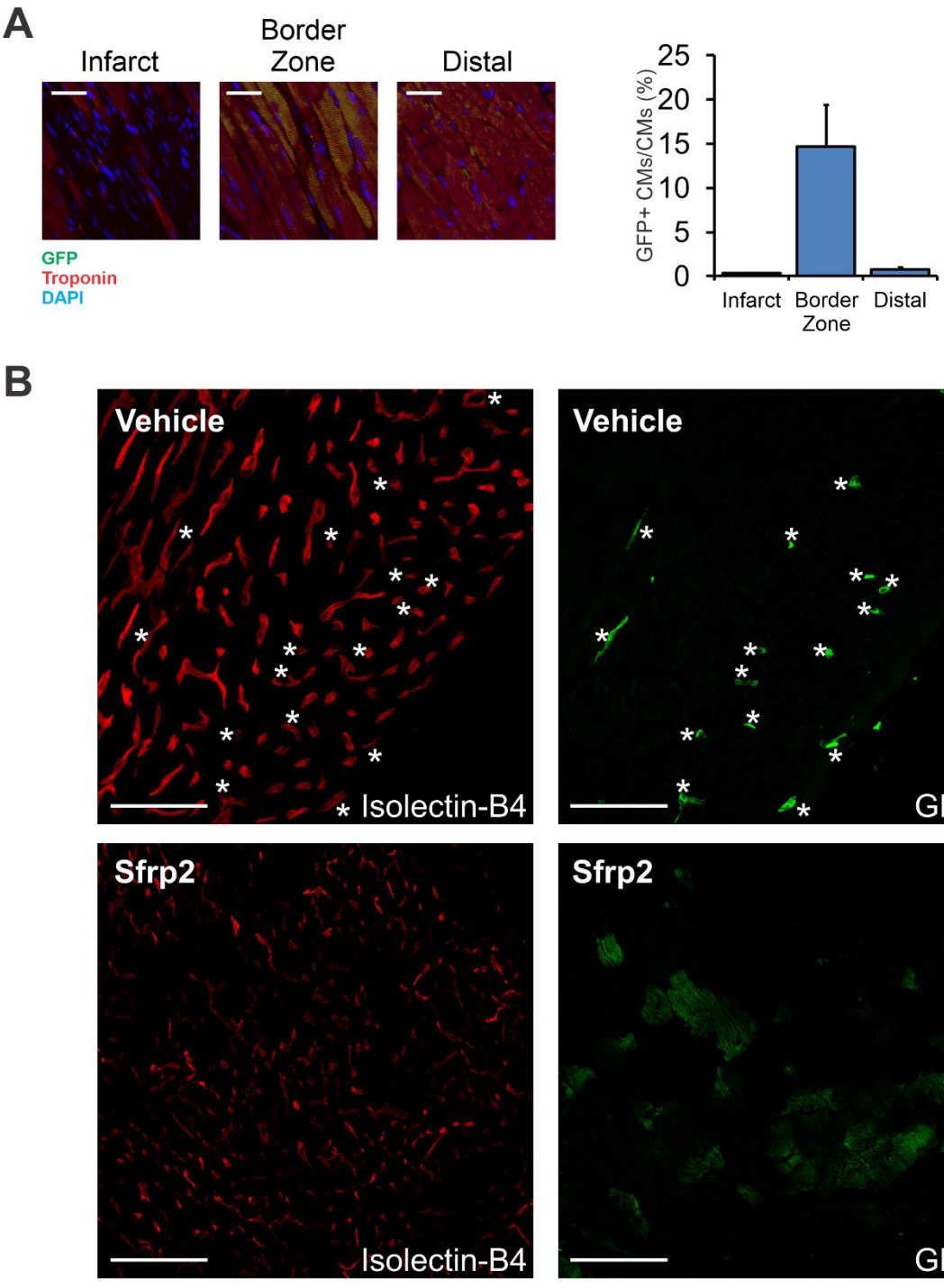

Fig. S1. Cardiomyocyte and Endothelial staining following Sfrp2 treatment. Mice (c-KitCreERT2/mTmG) were injected with tamoxifen (0.5 mg/mouse) for 14 consecutive days. Four days after tamoxifen treatment mice were subjected to myocardial infarction. Two days after injury, mice were injected with Sfrp2 (0.5 µg) 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.

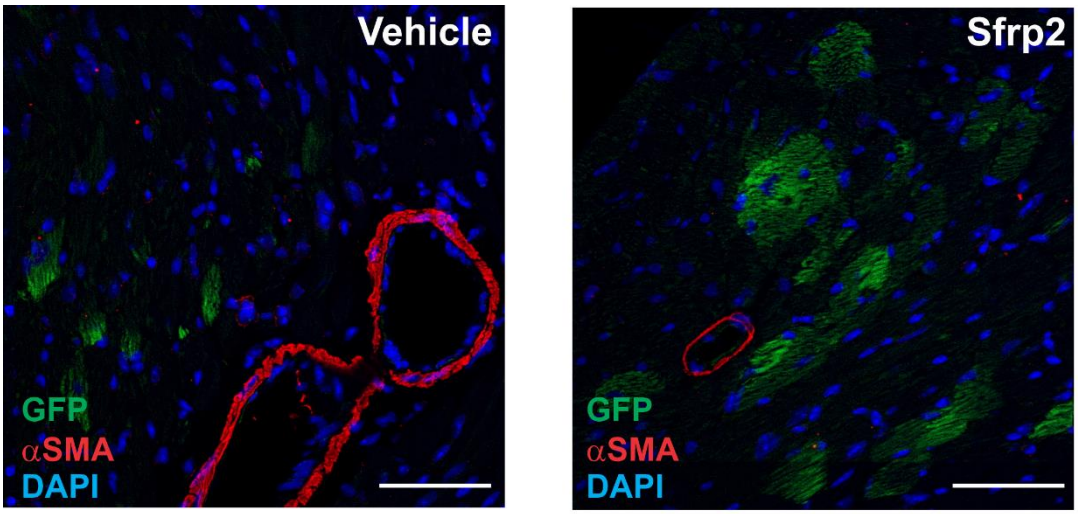

Fig. S2. Smooth muscle staining is not affected by Sfrp2. Tissue sections from vehicle and Sfrp2 treated mice were analyzed for co-expression of eGFP and the smooth muscle actin marker αSMA. N=7 (vehicle) or 10 (Sfrp2). Representative images shown. Scale bar 50 microns.

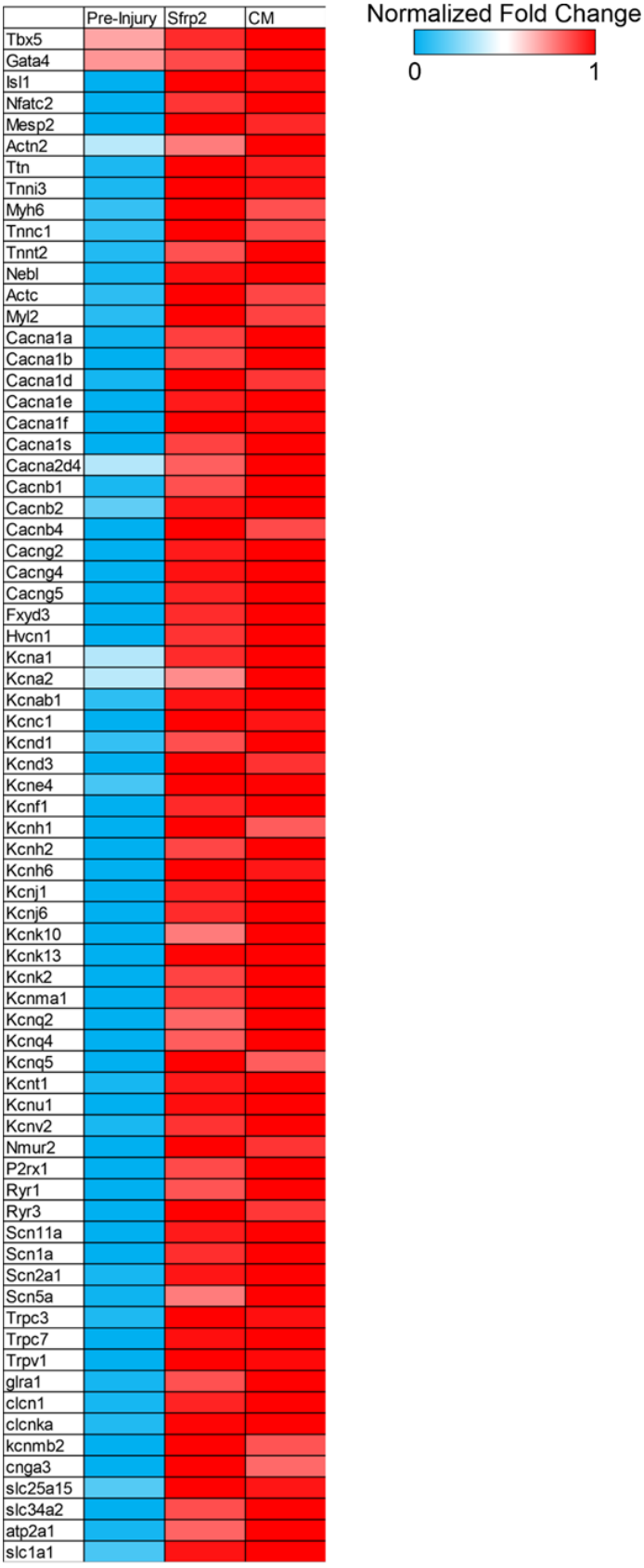

Fig. S3. Sfrp2 induces the acquisition of the cardiomyocyte phenotype. Cardiomyocyte-specific gene expression levels from GFP+ cells isolated from the pre-injury heart as well as the heart following MI and Sfrp2 treatment were compared to cardiomyocytes. N=3 individual animals per group. Expression levels 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		FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVID;d (mm)	LVID:s (mm)
	Vehicle	Mean ± SEM	56.0 ± 2.1	87.6 ± 1.6	30.9 ± 4.5	3.8 ± 0.7	14.0 ± 0.8	2.8 ± 0.2	1.2 ± 0.1
	Sfrp2	Mean ± SEM	58.1 ± 2.3	88.7 ± 1.6	32.5 ± 1.7	3.7 ± 0.6	13.5 ± 0.8	2.9 ± 0.1	1.2 ± 0.1
2-weeks post-MI	Group		FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVID;d (mm)	LVID:s (mm)
	Vehicle	Mean ± SEM	25.1 ± 3.8	49.3 ± 6.8	101.2 ± 32.4	57.6 ± 27.7	5.4 ± 0.7	4.5 ± 0.6	3.5 ± 0.6
	Sfrp2	Mean ± SEM	18.5 ± 3.1	37.7 ± 5.6	98.6 ± 19.5	67.6 ± 16.7	4.4 ± 0.6	4.5 ± 0.4	3.7 ± 0.4
8-weeks post-MI	Group		FS (%)	EF (%)	LVVol;d (ul)	LVVol:s (ul)	Contractility/s	LVID;d (mm)	LVID:s (mm)
	Vehicle	Mean ± SEM	24.7 ± 5.8	47.9 ± 9.6	100.9 ± 26.3	59.7 ± 26.7	6.4 ± 1.7	4.6 ± 0.5	3.5 ± 0.6
	Sfrp2	Mean ± SEM	29.8 ± 2.6 *	56.9 ± 4.1 *	79.9 ± 16.4	37.8 ± 10.4 *	7.3 ± 0.6 *	4.1 ± 0.4 *	2.9 ± 0.3 *