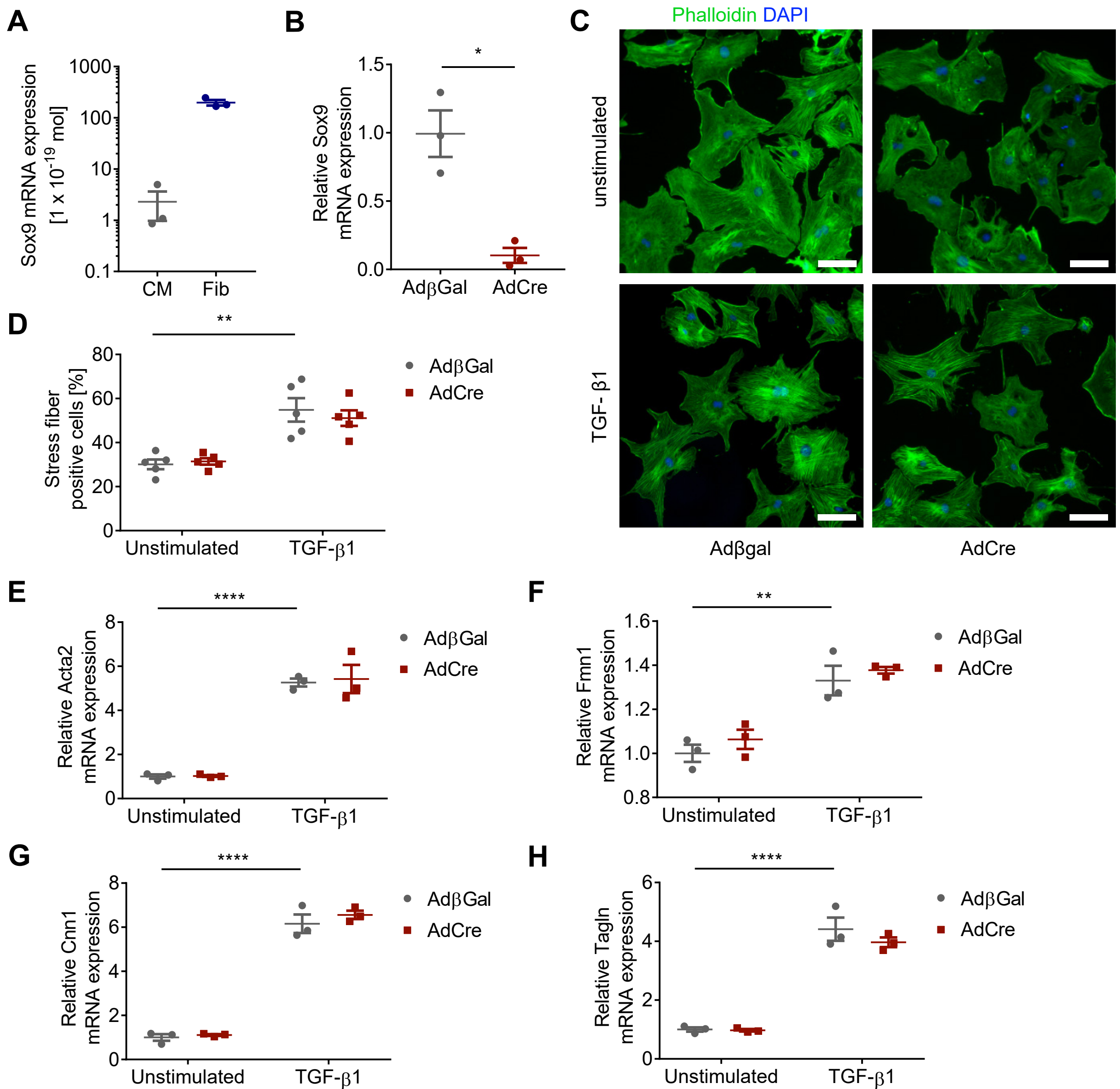
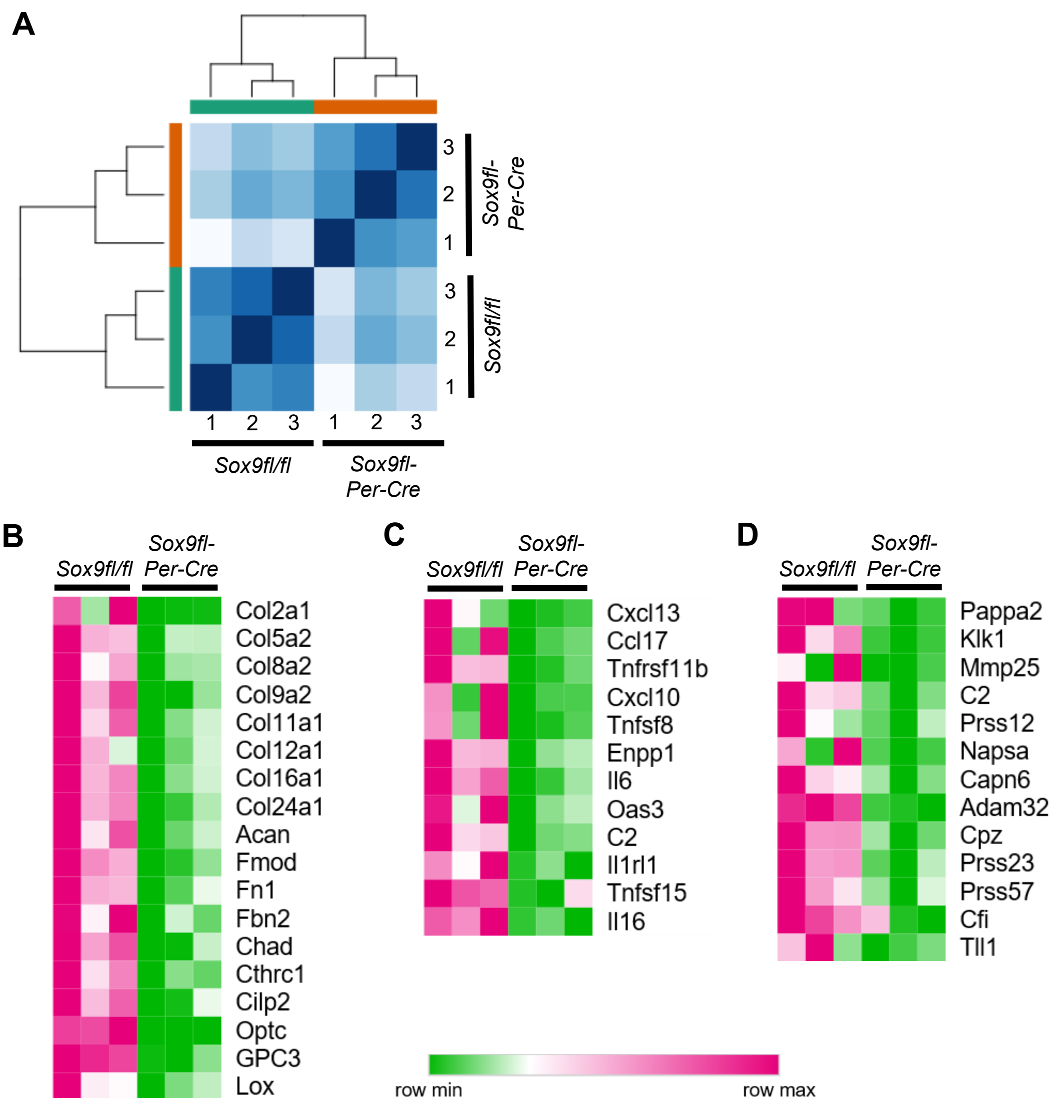
Supplemental Data



Supplemental Figure 1. Sox9 in uninjured adult cardiomyocytes and cardiac fibroblasts and in vitro loss-of-function model in isolated cardiac fibroblasts (**A**) Quantification of Sox9 mRNA expression from isolated cardiomyocytes (CM) and isolated cardiac fibroblasts (Fib) from uninjured adult wild-type mice by qPCR (**A**). Serial dilution of full-length Sox9 cDNA was used as quantification reference, n=3 samples/group.

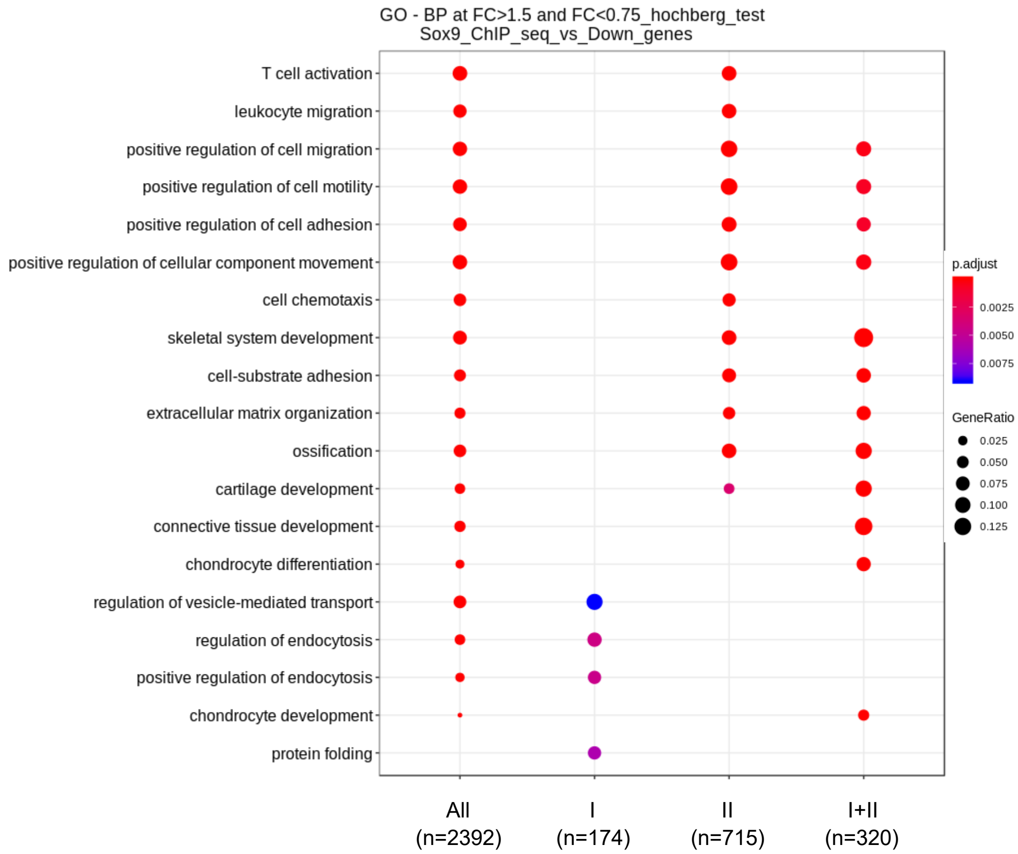
(**B-H**) Isolated cardiac fibroblasts from uninjured mouse hearts of *Sox9fl/fl* mice were infected with adenovirus expressing cre-recombinase (AdCre) or βGal as control (AdβGal) to generate in vitro deletion of *Sox9*. Efficiency of the in vitro loss-of-function model assessed on RNA level by RT-PCR for Sox9 mRNA (**B**), a 2-tailed Student's t-test was used for comparison of two groups. Fibroblasts with *Sox9* deletion or control cells were stimulated with TGF-β1 for 24h or kept in serum-free culture medium. Filamentous actin was stained with phalloidin (green) and nuclei are shown with DAPI (blue, **C**), quantification of cells that were positive for organized actin stress fibres is shown in (**D**) with n=4 biological replicates per condition and quantification of 5 images per condition. Scale bars: 100 µm.

(**E-H**) Relative mRNA expression of genes involved in myofibroblast cytoskeleton formation under baseline conditions (unstimulated) and after stimulation with TGF-β1 to induce myofibroblast formation. While significant increases of Acta2 (**E**), Fmn1 (**F**), Cnn1 (**G**) and Tagln (**H**) mRNA level were detected after TGF-β1 stimulation compared to baseline, no differences were measured between fibroblasts with *Sox9* deletion (AdCre) or control cells (AdβGal). n=3 biological replicates were measured in duplicates in each group. Data are shown as mean ±SEM. 2-way ANOVA with Sidak's multiple comparisons test, $^*p<0.05$, $^{**}p<0.01$, $^{****}p<0.0001$.



Supplemental Figure 2. Fibroblast SOX9 regulates the expression of different groups of genes in cardiac post-MI scars. (**A**) Heat map and hierarchical clustering of normalized mapped reads of RNA sequencing analysis to compare the global transcriptome in the cardiac scar 6 weeks after MI between *Sox9fl/fl* and *Sox9fl-Per-Cre* mice. (**B**-**D**) Heat maps of selected extracellular matrix (**B**), inflammatory mediator (**C**) and proteolysis (**D**) related genes significantly downregulated in the myocardial scar of *Sox9fl-Per-Cre* versus *Sox9fl/fl* mice 6 weeks after MI.

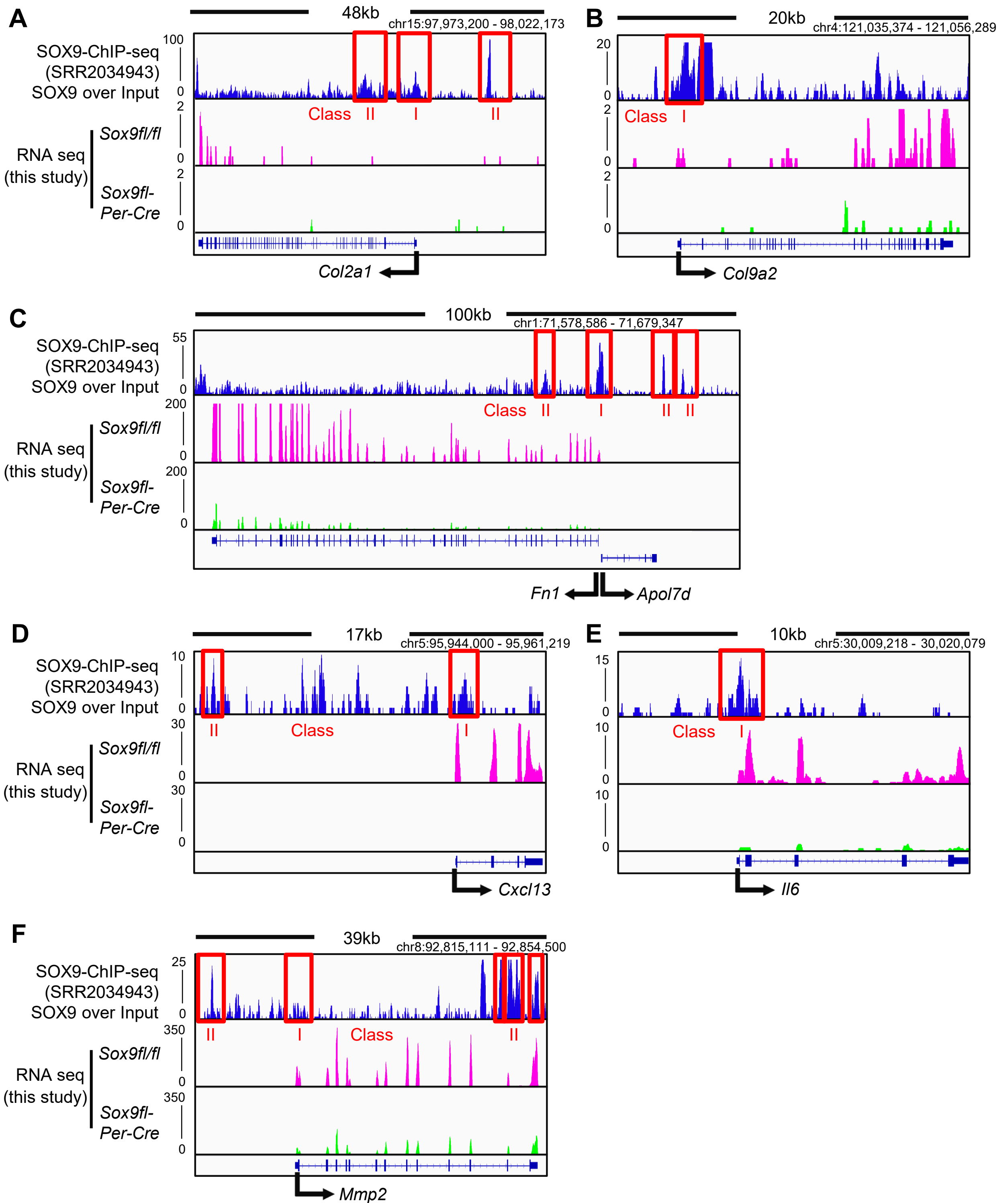
A



B

KEGG at FC>1.5 and FC<0.75_
Sox9_ChIP_seq_vs_diff_genesR
Cytokine-cytokine receptor interaction
Staphylococcus aureus infection
Cell adhesion molecules (CAMs)
Phagosome
Hematopoietic cell lineage
Complement and coagulation cascades
ECM-receptor interaction
Osteoclast differentiation
Tuberculosis
Chemokine signaling pathway
Leishmaniasis
Regulation of actin cytoskeleton
Proteoglycans in cancer
Protein digestion and absorption
Focal adhesion
PI3K-Akt signaling pathway
Rap1 signaling pathway
Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate
Axon guidance
TGF-beta signaling pathway
Platelet activation
Hippo signaling pathway
Protein processing in endoplasmic reticulum
Chronic myeloid leukemia
All (n=1120)
I (n=244)
II (n=347)
I+II (n=160)
GeneRatio
0.03
0.06
0.09
p.adjust
0.005
0.010
0.015
0.020
0.025

Supplemental Figure 3. SOX9 regulates multiple functional gene classes in part by binding to the DNA region of the respective promoter or enhancer regions. Gene ontology (GO, biological pathways, **A**) and KEGG pathway analysis (**B**) of all (All) genes with significant downregulation of at least -0.75 fold in mouse heart tissue from the scar area of *Sox9fl-Per-Cre* versus *Sox9fl/fl* mice 6 weeks after MI. These genes were intersected with SOX9 ChIP sequencing results in chondrocytes. Genes that are bound by SOX9 within the promoter region (type I binding), within enhancer regions (type II binding) as well as genes that are bound in both regions by SOX9 (type I+II binding) are shown. The number of genes identified in each category is indicated below.



Supplemental Figure 4. SOX9 is regulating the expression of extracellular matrix genes and proinflammatory genes through binding of the promotor and/or enhancer regions. Visualization with the Integrative Genomics Viewer (IGV) of enriched regions from SOX9-ChIP-seq (SRR2034943, shown as SOX9 enrichment over Input) together with RNA-seq reads from the RNA-sequencing of *Sox9fl/fl* (pink) versus *Sox9fl-Per-Cre* (green) 42 day infarct tissue from this study. Peaks in the upper lanes (blue) correspond to regions that were bound by SOX9 in the ChIP-seq of chondrocytes (SRR2034943), red boxes indicate type I (promoter region) and II (enhancer) binding. Selected extracellular matrix genes (*Col2a1* (**A**), *Col9a2* (**B**), *Fn1* (**C**)), proinflammatory genes (*Cxcl13* (**D**), *Il6* (**E**)) and proteases (*Mmp2* (**F**)) are shown.

Supplemental Table 1: Sequences of primers used in quantitative real-time PCR

Genes	Forward sequence 5' to 3'	Reverse sequence 3' to 5'
Sox9	GACTCCCCACATTCCTCCTC	CCCTCTCGCTTCAGATCAAC
Col2a1	GGCTCCCAGAACATCACCTA	CTTGCCCCACTTACCAGTGT
Col9a2	ATCGAGGAGAAATGGGTCGT	AGAAACCTGGTAGCCCCACT
Fn1	TGTGACAACTGCCGTAGACC	TGGGGTGTGGATTGACCTTG
Cxcl13	GAATCCTCGTGCCAAATGGT	GGAGCTTGGGGAGTTGAAGA
Il6	CGGCCTTCCCTACTTCACAA	TCCAGTTTGGTAGCATCCATCA
Adam32	TCAACTTCTGAGACAAGCAGT	ATTTCTCAGTGAGCCCCTGG
Mmp2	GCAGACTCCTGGAATGCCAT	TGGTGTGCAGCGATGAAGAT
Atp5b	GCCATTCCAAGTTGCTGAGG	CTTTGGCTGGAGTCCCTCAC
Aco2	CGCTGACCCCTCCGACTATA	CTGCAGCTCCTTCATCCTGT
Acaa2	GAAGGCCCTGGATCTTGACC	CCAGTAAGTGTGGGCAGTGT
Acta2	GTACCACCATGTACCCAGGC	GCTGGAAGGTAGACAGCGAA
Fmn1	AAGACAGCACCTCACTGCTC	GGACTTTCTGCAGATGGCCT
Cnn1	CGGCGTCACCTCTATGATCC	AGCCCATACACTGTCATGCC
Tagln	CTTTGGGCAGTTTGGCTGTG	TCCTCTGTTGCTGCCCATTT
Gapdh	CCGCATCTTCTTGTGCAGT	CATCACCTGGCCTACAGGAT