SUPPLEMENTAL MATERIAL

Methods

Cell culture

Primary human coronary artery smooth muscle cells (HCASMC) were obtained from Lonza and maintained in Smooth Muscle Growth Medium-2 including hEGF, insulin, hFGF-B and 5% fetal bovine serum (FBS) without antibiotics (Lonza, #CC-3182) at 37 ºC and 5% CO2. HCASMC at passage 5-9 were used for experiments. Rat aortic smooth muscle cells (A7r5) and human embryonic kidney 293 cells (HEK293) were purchased from ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (Fisher Scientific, #MT10013CV) with 10% FBS at 37 ºC and 5% CO2. A7r5 at passage 6-18 were used for experiments.

TCF21 overexpression and knockdown

Second generation lentivirus with *TCF21* cDNA cloned into pWPI (Addgene, #12254) was used for over-expression, as previously reported [1]. Briefly, the cells were cultured into 6 well plate and treated at 60% confluence with MOI of 5 for 24 hours, after which the media was replaced with non-viral media containing 5% FBS. Cells were harvested 24 hours later for downstream applications. For knockdown of *TCF21*, cells were seeded into 6 well plate (3×10^5/well) and treated with 20 nM si*TCF21* or scramble control (Origene, #SR321985) using Lipofectamine RNAiMAX (Invitrogen, #13778150) following manufacturer's protocol. Cells were collected 48 hours after transfection and analyzed.

RNA isolation and cDNA preparation

Total RNA was isolated with RNeasy mini kit (Qiagen, #74106) and cDNA was synthesized using iScript cDNA synthesis kit (Biorad, #1708891) following the manufacturer's protocol.

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qPCR) was performed using TaqMan® Primer-Probe Mix. Briefly, a 20-µl total reaction mixture containing 1 µl of cDNA, 10 µl of TaqMan® Universal Master Mix Ⅱ (Thermo Fisher Scientific, #4440048), and 1 µl of TaqMan® Primer-Probe Mix, was applied in 384-well plate and quantified on a ViiA7 Real-Time PCR system (Applied Biosystems). The thermal cycler conditions were 40 cycles of 95 ºC for 15 s and 60 ºC for 60 s after pre-incubation (50 ºC for 120 s and 95 ºC for 600 s). The relative expression was normalized to GAPDH levels. All TaqMan® probes used for qPCR were purchased from Thermo Fisher Scientific: human TCF21 (#Hs00162646_m1), human MYOCD (#Hs00538076_m1), human SRF (#Hs01065256_m1), human TAGLN (#Hs00162558_m1), human ACTA2 (Hs00426835_g1), human CNN1 (#Hs00959434_m1),

human MYH11 (#Hs00973781_m1), human GAPDH (#4310884E).

Western blot analysis

HCASMC were cultured under *TCF21* knockdown or overexpression condition as mentioned above, and then harvested with ice-cold lysis buffer (25mM Tris-HCL pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). The extracted proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membrane (Thermo Fisher Scientific, #LC2002). After blocking with 5% milk for 1 hour, the membranes were incubated with primary antibody overnight at 4℃, followed by a horse radish peroxidase-conjugated secondary antibody for 1 hour at room temperature. The signals were detected using LI-COR Odyssey imaging system, and the images were quantified by densitometry analysis using ImageJ® software. The antibodies used for Western blot are as follows: Anti-TCF21 (Abcam, # ab32981), anti-MYOCD (Sigma Aldrich, #SAB4200539), anti-SM α-actin (sigma:A5228), anti-SM22α (Abcam, #ab14106), anti-Calponin (Abcam, #ab46794), anti-SM MHC (Abcam, #ab53219), and anti-GAPDH (Sigma Aldrich, #G9545).

Dual luciferase assays

A dual Luciferase assay (Promega, #E1910) was employed to determine the luciferase and renilla activity in the transfected cells as previously reported [2]. A7r5 cells were seeded into 24 well plate (2×10^4 cells/well) in DMEM containing 10% FBS and incubated at 37 ºC and 5% CO2 overnight. Cells were transfected with luciferase reporter plasmids (pLuc-MCS (empty), pLuc-SRF, pGL3-hSM22-325, or pGL3-CNN1-luc) Renilla luciferase plasmid and cDNAs (pcDNA3.1-MYOCD and pCMV6-AC-TCF21) using Lipofectamine 2000 (Invitrogen, #11668019). Six hours after transfection, the media was changed to fresh complete media. Relative luciferase activity (firefly/Renilla luciferase ratio) was measured by SpectraMax L luminometer (Molecular Devices) 24 hours after transfection. HCASMC were plated into 24 well plate (3×10^4/well) in Smooth Muscle Growth Medium-2 (Lonza, #CC-3182) and incubated for 36 hours at 37 ºC and 5% CO2. Transfection was performed in the same manner as A7r5, followed by the measurement of luciferase activity. All experiments were conducted in triplicate and repeated at least 3 times.

The SRF enhancer reporter was constructed by amplifying the SRF intronic sequence Hg19: chr6:43141913-43142212 and this sequence cloned into the enhancer trap vector MCS-Luc. The TAGLN reporter was constructed as follows. The TAGLN enhancer was PCR amplified from human genomic DNA using the following primers: Forward ACAGTGAAGTAGGAGCAGCCGTAA and Reverse TCGCAGGAAGGAGTGAAGACTTGT. The 372 bp section includes two CArG boxes (CCAACTTGG, CCAAATATGG), separated by 9 bp. Coordinates are Hg19: chr11:117069761+117070132, corresponding to a portion of

the promoter region of TAGLN. The CNN1 reporter construct has been previously described [3].

The TAGLN enhancer was amplified by qPCR from human genomic DNA using the following primers: Forward ACAGTGAAGTAGGAGCAGCCGTAA and Reverse TCGCAGGAAGGAGTGAAGACTTGT. The 372 bp section includes two CArG boxes (CCAACTTGG, CCAAATATGG), separated by 9 bp, and was cloned into MCSluc. Coordinates are hg19: chr11: 117069761+117070132, corresponding to a portion of the promoter region of TAGLN.

The human CNN1 reporter (pGL3-CNN1-luc) included the proximal reporter and the first intron containing the CArG box in the first intron that has been shown to be necessary and sufficient for high level expression in SMC, as described [4, 5].

Co-immunoprecipitation

Co-immunoprecipitation was performed with a nuclear complex co-immunoprecipitation kit (Active Motif, #54001) according to the manufacturer's protocol. Briefly, 48 hours after transfection of plasmid DNA (pCMV3-*Myc-TCF21*, pCGN-*SRF*, and pcDNA3.1-*MYOCD*) with Lipofectamine 2000, HEK293 cells were harvested with ice-cold PBS supplemented with protease inhibitor, and then nuclear fraction was isolated with hypotonic buffer and digested with complete digestion buffer for 90 min at 4 ℃. Three hundred μg of nuclear extracts were incubated with 2 μg of each antibody (anti-MYOCD, anti-Myc tag (Abcam, #ab9106), or rabbit normal IgG (Agilent Dako, #0903)) overnight at 4 ℃ on rotator. Protein G Dynabeads (Invitrogen, #10004D) was added to the lysate and incubated for 1 hour at 4 ℃ to pulldown the protein. After washing with IP wash buffer, each bead pellet was resuspended in sample buffer and boiled at 95 ℃ for 5 min. Protein was detected by western blot as described above.

ChIP-qPCR

Chromatin immunoprecipitation (ChIP)-qPCR was performed as previously described [1]. HCASMC or HEK293 cells were fixed in 1% formaldehyde for 10 min to crosslink chromatin, followed by quenching with 125 mM glycine. Cells were washed with ice-cold PBS and resuspended in cell lysis buffer (50 mM Tris-HCl, pH 8.0, 0.5% SDS, and 5 mM EDTA). To obtain 300 to 600 bp DNA fragments, cross-linked chromatin nuclear extracts were sonicated by Sonifier® SFX250 (BRANSON) for 1-3 min. Protein G Dynabeads was added to the lysate and rotated with each antibody (anti-TCF21 antibody (Sigma, #HPA013189), anti-MYOCD (Sigma Aldrich, #SAB4200539), or anti-SRF antibody (Santa Cruz, #sc25290)) overnight at 4℃. After washing with buffer 1 (20 mM Tris pH 8, 2 mM EDTA, 150 mM NaCl, 1% Triton X100, 0.1% SDS), buffer 2 (10 mM Tris pH 8, 1 mM EDTA, 500 mM NaCl, 1% Triton X100, 0.1% SDS), buffer 3 (10 mM Tris pH 8, 1 mM EDTA, 250 mM LiCl, 1% NP40,

1% sodium deoxycholate monohydrate) and TE buffer, beads were resuspended in elution buffer (0.1 M $NaHCO_3$, 1 % SDS) and incubated for 10 min at room temperature. The supernatant was then isolated by magnetic stand and incubated at 65℃ for 6 hours. DNA was purified by DNA Clean & Concentrator-5 Kit (Zymo, #D4014) according to the manufacture's protocol. Quantitative real-time PCR reaction was performed using Power SYBR® Green Master Mix (Applied Biosystems, #4367659). qPCR values for SRF enhancer and MYOCD promoter were normalized relative to the Myogenin (MYOG) and represented as fold change compared to IgG ChIP sample. Primer sequences used for ChIP-qPCR were shown in Table S1.

Online Table I. Sequences of ChIP-qPCR primers

Gene	Primer sequences (5' – 3')
SRF	Forward: CACTGTTTGCCTTAGCAACG
	Reverse: ACCCGAAATGAGTCAACCAG
MYOCD	Forward: CAGCCCTTCCTTCTTTCCTC
	Reverse: GGGAACCGGCTCTTAACTCT
MYOG	Forward: CCTTGATGTGCAGCAACAGC
	Reverse: CCAACGCCACAGAAACCTG
ACTB	Forward: TCTCCCCTCCTTTTGCGAAA
	Reverse: CAACGCCAAAACTCTCCCTC

ChIP-seq and ATAC-seq data

Chromatin immunoprecipitation (ChIP) -sequencing for SRF was performed using HCASMC according to the procedures as previously described [2, 6] with anti-SRF antibody (Santa Cruz, #sc335). HCASMCs (passage 5-6) were cultured in the media described above ((Lonza, #CC-3182) and 10 million cells per condition were crosslinked for 10 min with 1% formaldehyde. Cells were lysed for 10 min on ice (10 mM Tris pH8.0, 10 mM NaCl, 0.2% NP-40). And then, nuclei were lysed for 10 min on ice (50 mM Tris pH 8.1, 10 mM EDTA, 1% SDS). Crosslinked chromatin was sheared for 3x5 min by sonication using Sonifier® SFX250 (BRANSON). DNA was prepared for end repair (Lucigen End-it, #ER0720) and "A" tailing (NEB Klenow, #M0212S), adaptor ligation (Promega, #M180A), and library amplification (NEB, M0531S) according to the manufacturer's protocol.

The *fastq* files of TCF21 (SRR1573744 & SRR1573745), ATAC (SRR1235287 & SRR1235291) were extracted from GEO database by *fastq-dump*. Quality control of ChIP-seq data was performed using *Fastqc*, and then low-quality bases and adaptor contamination were trimmed by *cutadapt*. Filtered reads were mapped to hg19 using *BWA backtrack* algorithm. Duplicate and unmapped reads removed by *samtools*. The two

biological replicates of SRF ChIP-seq were aligned, filtered and deduplicated individually and then the final BAM files were merged for peak calling. *macs1.4* was used for peaks calling in *--nomodel* mode with shift size 137 bp. IgG was used as control. Bigwig files were generated for University of California Santa Cruz (UCSC) Genome Browser visualization. SRF, TCF21 peaks or open chromatin regions were clustered using hierarchical algorithm and reads centering on these peaks (±2kb) were plotted with *deeptools*. We utilized the Genomic Regions Enrichment of Annotations Tool (GREAT 3.0) to analyze the detected peaks, with the parameter “basal plus extension”.

Generation and analysis of CRISPR lines

Genome editing of SRF enhancer region was performed by CRISPR-Cas9 system as previously reported [7]. In brief, HEK293 cells were seeded into 6 well plate (1×10^6 cells /well) and cultured in DMEM with 10% FBS. On the following day, cells were transfected with 2 μg sgRNA/Cas9-GFP using 9 μl Lipofectamine 2000 per well. Twenty four hours after transfection, cells were sorted using a Digital Vantage (BD Biosciences). GFP-positive cells were singly sorted into a 96-well plate and allowed to grow until clones reached about 70% confluence, around 2 weeks. All wells with colonies were marked and then split into a fresh 24-well plates. Once a majority of the wells obtained 80% confluence, cells were used for genomic DNA and total RNA extraction using Qiagen DNeasy Blood and Tissue Kit (Qiagen, #56304) or RNeasy micro kit (Qiagen, #74004), respectively according to manufacturer’s instructions. The targeted region was amplified using 50ng of gDNA, 1.25 μl of specific primers (10 μM) of SRF second intron (Forward: TGTCGGAGTCTGACAGCAGTG, Reverse: TAACAGCGCAGGCATCCCTAG), 5 μl of Q5 Reaction Buffer (NEB, #B9027S), 0.5 μl of Deoxynucleotide Solution Mix (NEB, #N0447S), 5 μl of Q5 High GC Enhancer (NEB, #B9028A), 0.25 μl of Q5 Hot Start High Fidelity DNA polymerase (NEB, #M0493S), and 11.2 μl of nuclease-free water. Cycling conditions were as follows: 98℃ for 30 sec, 35 cycles of 98℃ for 10 sec, 70℃ for 30 sec, 72℃ for 20 sec, and one cycle of 72℃ for 2 min. All wells showing a positive band at about 550 bp were Sanger sequenced with the SRF forward primer, and the sequence was analyzed for indels. cDNA synthesis, followed by qPCR was performed using the total RNA as described above.

CRISPR/Cas9 genome editing of Srf intron 2 in mice

To interrogate the function of the *Srf* intron 2 enhancer, we developed the following genome editing strategy. Using the CRISPOR (http://crispor.tefor.net/) [8] design platform, we selected a single guide RNA (sgRNA) with no predicted off-targeting in linkage disequilibrium with the on-target edits. We selected one sgRNA (GCGGCCTCCGTGCCCATATA, score=96, no predicted off-targets on Chr17) that overlaps the consensus CArG box for precision editing as we previously reported for the *Cnn1* gene [5]. The sgRNA was synthesized from a

commercial source (Synthego, Redwood City, CA) and tested for endonuclease activity with SpyCas9 (Synthego) using a PCR template encompassing the targeted sequence. Zygotes of mouse strain C57BL6/J were microinjected (nuclear) with SpyCas9 and 3pM RNP plus 10pM single-strand oligonucleotide carrying several base pair substitutions within and outside the consensus CArG box for the precision edit (Suppl. Fig. 3). Mosaic founder mice were genotyped with a duplex PCR assay we developed for the CArG edit [5] and positive mice were bred to wild type C57BL6/J mice for germline transmission of the mutation. Homozygous CArG box mutant mice were obtained through heterozygous sibling mating for downstream expression analyses. At least two founders for the deletion and CArG box mutant mouse were analyzed.

Statistical analysis

For the SRF ChIPseq analysis, peaks were called with MACS version 1.4.3 and the pval cutoff employed ($p<1e-5$) was the one benchmarked and established as default for the software as described in the original publication [9]. For the Gene Ontology analyses, the threshold of significance is provided by the EASE Score, a modified Fisher Exact test pval. $p<0.05$ was considered as enriched in the annotation categories [https://david.ncifcrf.gov/helps/functional_annotation.html#E3]. Fig. 2F shows the top 5 terms in each category ranked by pval, and all terms were shown if there were less than 5.

Otherwise, all statistical analyses were conducted using GraphPad Prism software version 6.0 (GraphPad Software). Difference between two groups were determined using a Mann-Whitney U test. Differences between multiple groups were evaluated by one-way ANOVA, followed by Dunnett's post-test or by Kruskal-Wallis test, followed by Dunn's post-test after the sample distribution was tested for normality using a D'Agostino and Pearson omnibus normality test. All data are expressed as mean ± SEM. P values < 0.05 were considered statistically significant. Where exact p-values were not determined, primary data in provided in Online Tables II, III, and IV.

No corrections for multiple testing were indicated or performed for this work.

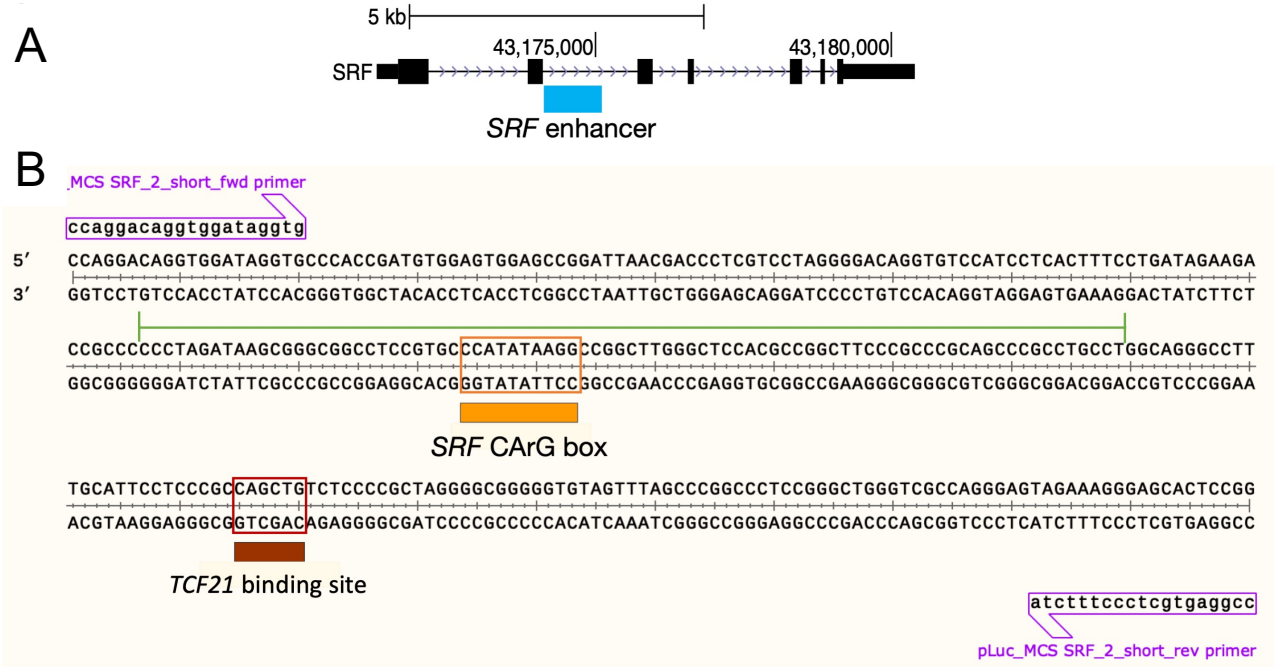
Data availability

SRF ChIP-seq data has been deposited to the Gene Expression Omnibus (GEO) database, accession number GSE124011.

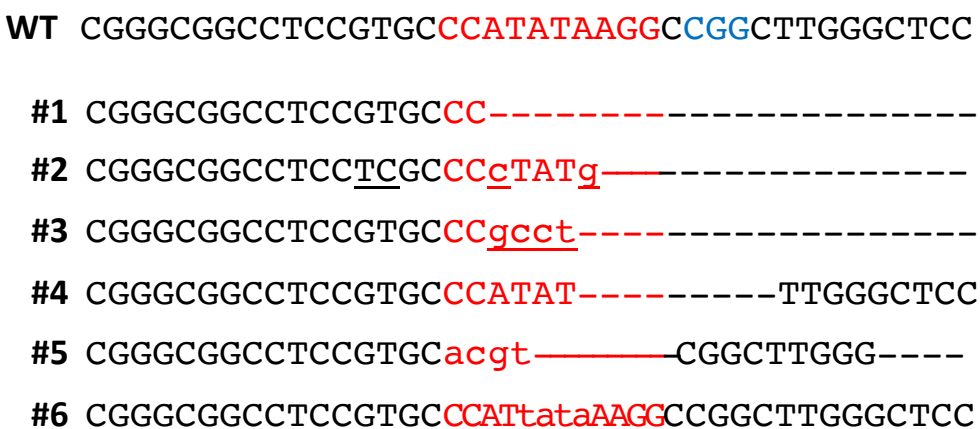
References:

1. Kim BJ, Pjanic M, Nguyen T, Miller CL, Liu B, Wang T, Sazonova O, Carcamo-Orive I, Perisic L, Maegdefessel L, Hedin U, Quertermous T. Tcf21 and the aryl-hydrocarbon receptor cooperate to activate a pro-atherosclerotic gene expression program. *PLoS Genet*. 2017;13.:1006750

2. Iyer D, Zhao Q, Wirka R, Naravane A, Nguyen T, Liu B, Nagao M, Cheng P, Miller CL, Kim JB, Pjanic M, Quertermous T. Coronary artery disease genes smad3 and tcf21 promote opposing interactive genetic programs that regulate smooth muscle cell differentiation and disease risk. *PLoS Genet*. 2018;14:e1007681
3. Miano JM, Carlson MJ, Spencer JA, Misra RP. Serum response factor-dependent regulation of the smooth muscle calponin gene. *J Biol Chem*. 2000;275:9814-9822
4. Long X, Slivano OJ, Cowan SL, Georger MA, Lee TH, Miano JM. Smooth muscle calponin: An unconventional carg-dependent gene that antagonizes neointimal formation. *Arterioscler Thromb Vasc Biol*. 2011;31:2172-2180
5. Han Y, Slivano OJ, Christie CK, Cheng AW, Miano JM. Crispr-cas9 genome editing of a single regulatory element nearly abolishes target gene expression in mice--brief report. *Arterioscler Thromb Vasc Biol*. 2015;35:312-315
6. Sazonova O, Zhao Y, Nurnberg S, Miller C, Pjanic M, Castano VG, Kim JB, Salfati EL, Kundaje AB, Bejerano G, Assimes TL, Yang X, Quertermous T. Characterization of tcf21 downstream target regions identifies a transcriptional network linking multiple independent coronary artery disease loci. *PLoS Genet*. 2015;11
7. Zhao Q, Wirka R, Nguyen T, Nagao M, Cheng P, Miller CL, Kim JB, Pjanic M, Quertermous T. Tcf21 and ap-1 interact through epigenetic modifications to regulate coronary artery disease gene expression. *Genome Med*. 2019;11:23
8. Haeussler M, Schonig K, Eckert H, Eschstruth A, Mianne J, Renaud JB, Schneider-Maunoury S, Shkumatava A, Teboul L, Kent J, Joly JS, Concordet JP. Evaluation of off-target and on-target scoring algorithms and integration into the guide rna selection tool crispor. *Genome Biol*. 2016;17:148
9. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS. Model-based analysis of chip-seq (macs). *Genome Biol*. 2008;9:R137



Online Figure I. A novel SRF enhancer in the *SRF* gene. A) The *SRF* enhancer site in the second intron of the *SRF* gene. **B)** The sequence of pLuc-*SRF*-luc. PCR primers used to amplify and clone this region of DNA are shown, as well as the canonical binding sites for SRF (CArG box) and TCF21 (E-box) which are functional in smooth muscle cells. Site directed mutagenesis was used to delete a 83 bp section (indicated by green bar) of the enhancer that includes the CArG box, with primers For GGCAGGGCCTTTGCATTC and Rev GGGCGGTCTTCTATCAGG.



Online Figure II. The genomic sequence of cloned cell lines mutated by CRISPR/Cas9 genome editing. The CArG box in the second intron of the *SRF* gene was edited in HEK293. and the genomic sequence of six different clones shown. The "CArG box" motif is highlighted in red. WT; wild type.

Sanger Sequence of Srf-Intron 2 CArG Mut Mouse Founder C4

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WT   1     ATCCTCCATTCCTGACAGGAGGCCGTCCCCCTAGATAAGCGGGCGGCCTCCGTGCCCATA  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||| |   |||
MUT 123    ATCCTCCATTCCTGACAGGAGGCCGTCCCCCTAGATAAGCGGGCGGCCTCCGCGGGGATA 182
                                                          SacII
WT   61    TAAGGCCGGTTCGGGCTCCACGCCGGCCTCCCGCCCGCAGCCCGCCTGCCTGGCAAGGCT 120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
MUT 183    TAAGGCCGGTTCGGGCTCCACGCCGGCCTCCCGCCCGCAGCCCGCCTGCCTGGCAAGGCT 242

WT   121   TTTGCATTCCTCCCGCCAGCTGTCTCCCCGCTGGGGGCGGGGGTGTAGTTTGGCCCTG    178
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
MUT 243    TTTGCATTCCTCCCGCCAGCTGTCTCCCCGCTGGGGGCGGGGGTGTAGTTTGGCCCTG    300
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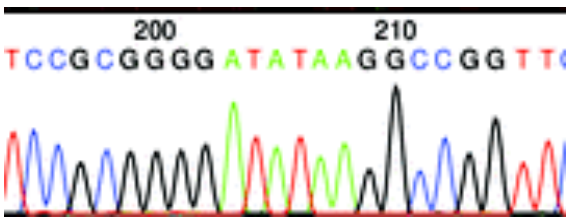
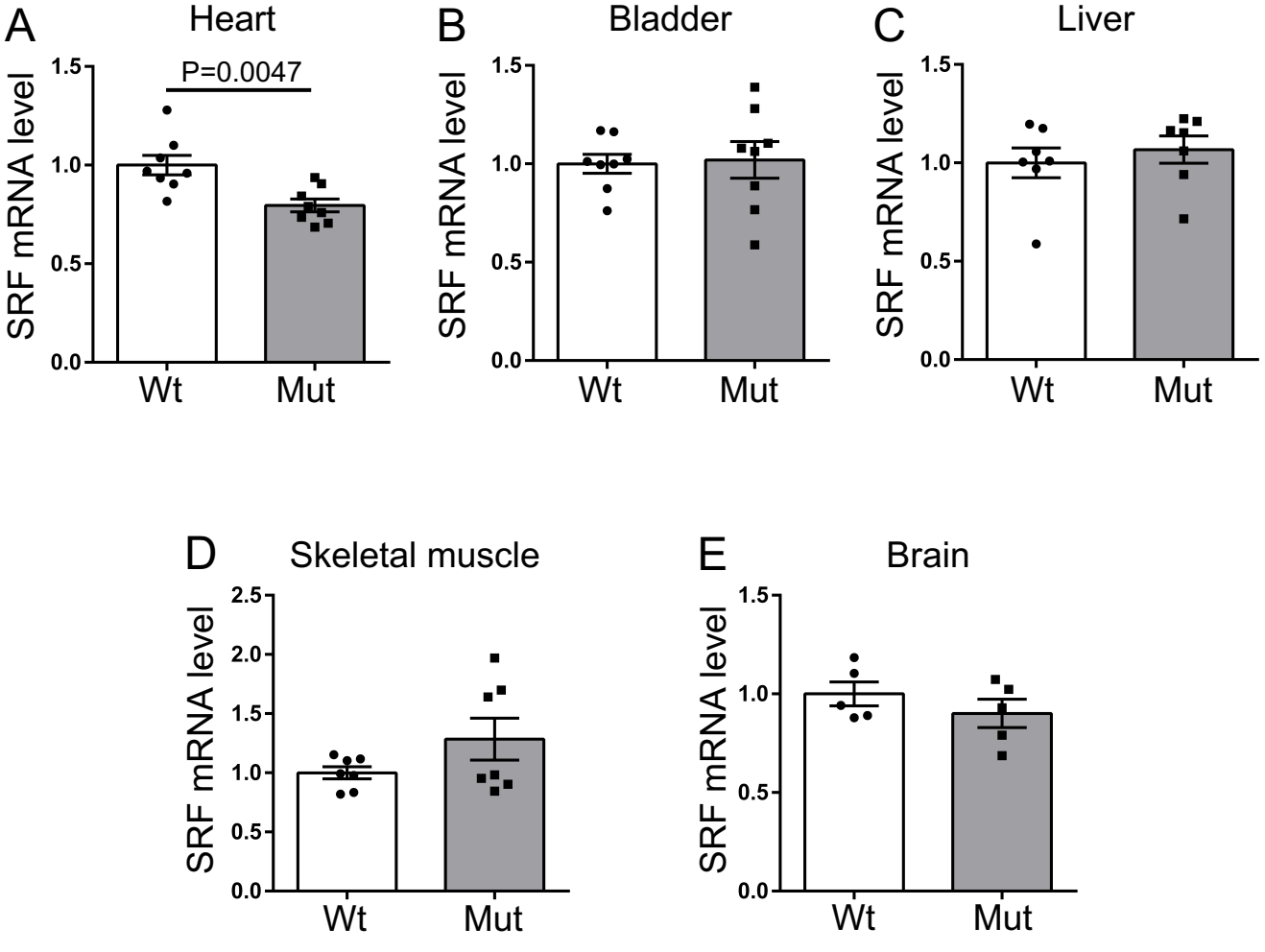
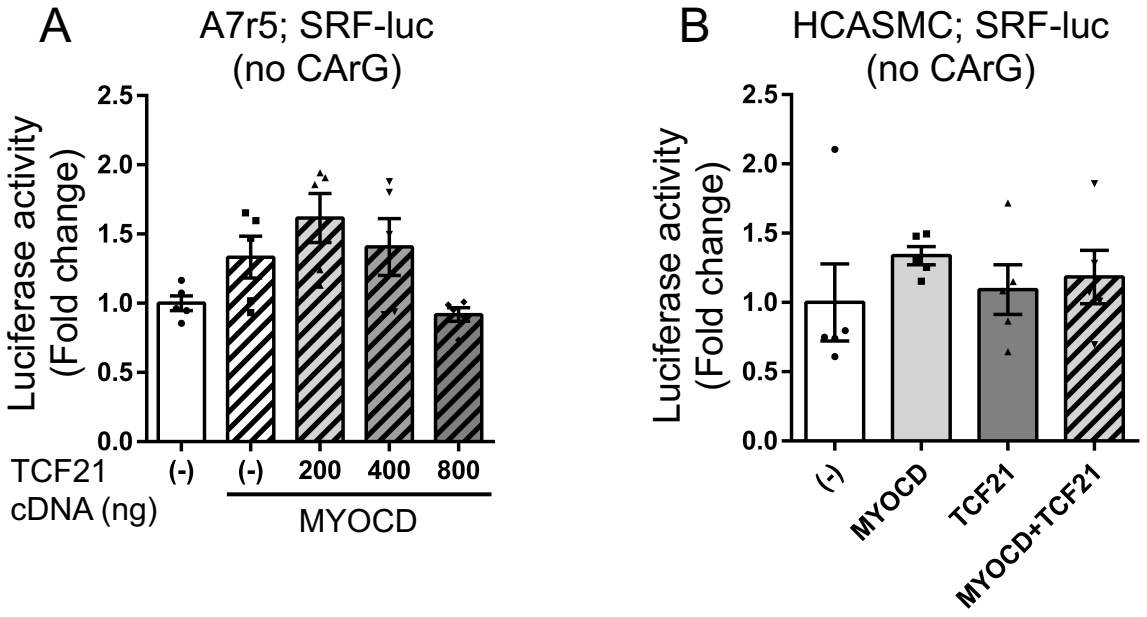


Online Figure III. The genomic sequence of mouse line C4 at the CRISPR/Cas9 genome edited CArG box in the *Srf* enhancer. The CArG box in the second intron of the murine *Srf* gene was edited by CRISPR-Cas9 editing and bred to homozygosity. Mutations at the 5' end of the CArG box are highlighted in red. The Sanger sequencing trace for the mutated region is shown below.

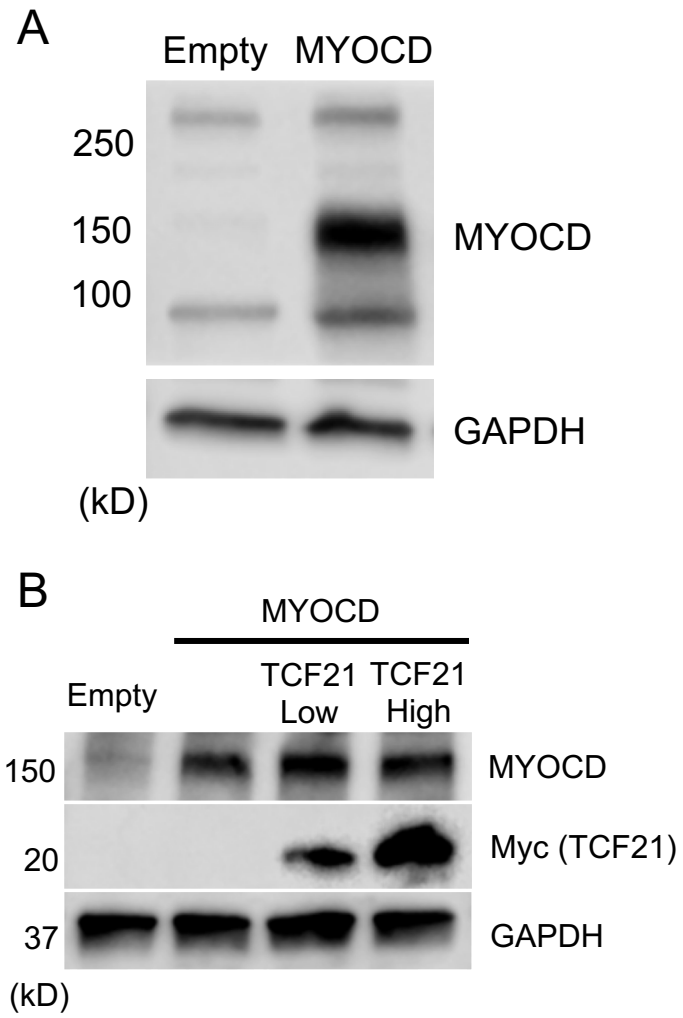


Online Figure IV. *Srf* expression in tissues from mice with the mutant CArG box at the *Srf* enhancer. A-E) qPCR analysis of *Srf* expression in wild-type (Wt) and homozygous mutant CArG box (Mut) mice in indicated tissues (n=5-8). Data analyzed by Mann-Whitney U test.

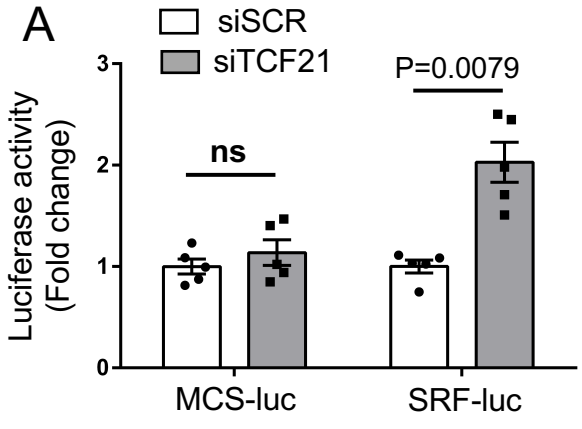
Online Figure V. TCF21 binding at the *MYOCD* promoter. A, B) The TCF21 binding region of the *MYOCD* promoter identified by TCF21 ChIP-seq, with putative binding sequences identified.



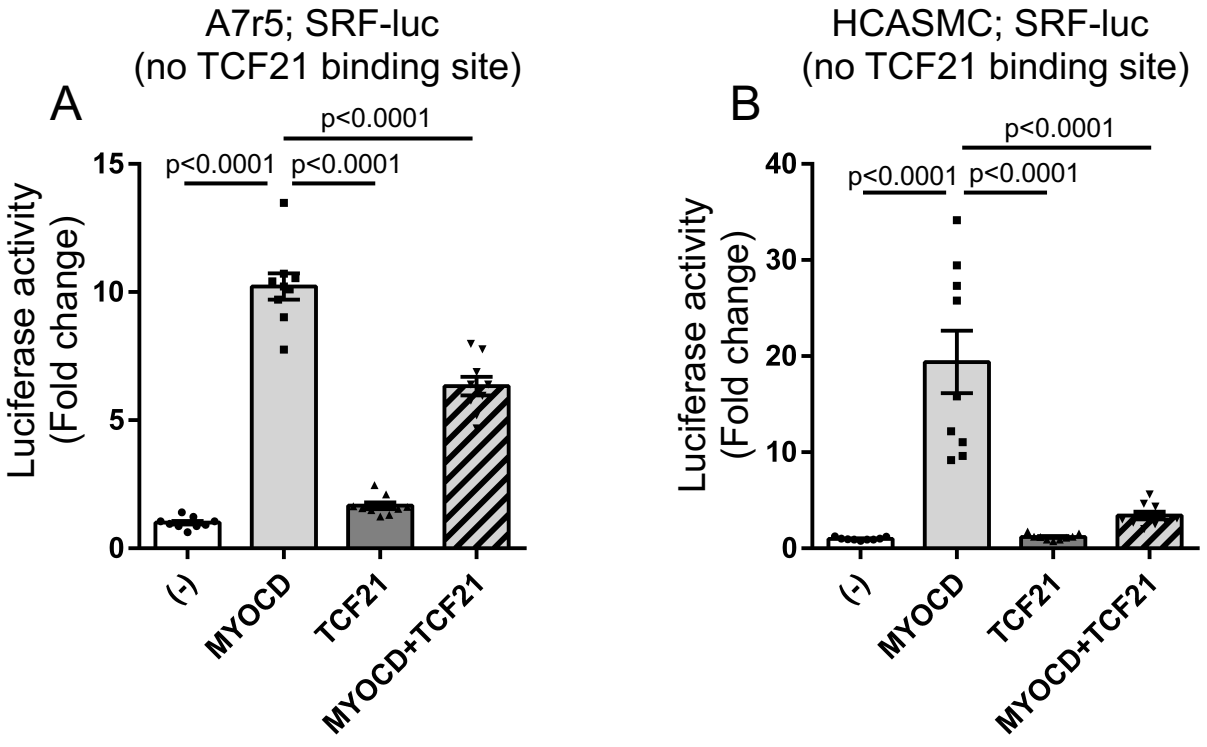
Online Figure VI. Deletion of the CArG box in the SRF enhancer abrogates the transcriptional response to TCF21. A, B) Dual luciferase assays were performed using a *SRF*-luc reporter plasmid with a deleted CArG box (*SRF*-luc "no CArG") with transfection into A7r5 (A) and HCASMC along with a *TCF21* expression construct. (B). A Renilla reporter plasmid was used as an internal control of transfection efficiency (n=5). Data analyzed by Kruskal-Wallis test, followed by Dunn's post-test.



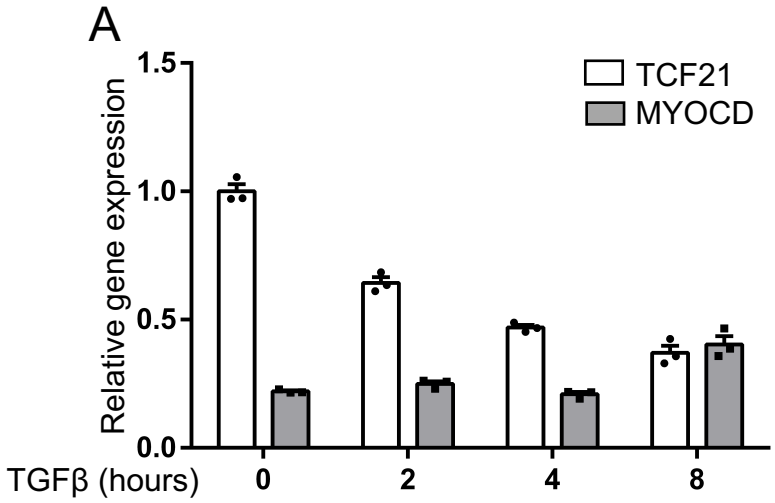
Online Figure VII. An antibody validated to specifically detect recombinant MYOCD protein showed that expression of the MYOCD protein levels in transfection reporter gene studies was unchanged. A) *MYOCD* expression plasmid was transfected into HEK293 and western blot was performed with anti-MYOCD antibody. This antibody was used for IP, and ChIP in this study. **B)** To test whether the inhibitory effect of TCF21 in reporter gene studies (Fig. 6) was caused by the suppression of *MYOCD* expression, *MYOCD* and *Myc*-tagged *TCF21* expression plasmids were co-transfected into A7r5 and western blot was performed with the antibodies as indicated. Transfection with low and high amounts of *TCF21* plasmid did not produce a decrease in *MYOCD* expression.



Online Figure VIII. Endogenous TCF21 blocks transcriptional regulation by MYOCD at the *SRF* enhancer. HCASMCs underwent *TCF21* knockdown with transfection of si*TCF21* or control siSCR regulatory RNAs, and dual luciferase assays were performed using *SRF*-luc. An empty luciferase reporter construct (MCS-luc) was used as a negative control (n=5). Data analyzed by Mann-Whitney U test.



Online Figure IX. TCF21 DNA binding is not required to block MYOCD transactivation at the *SRF* enhancer. A, B) The TCF21 DNA binding site "CAGCTG" was removed from the *SRF*-luc reporter plasmid using the following primers Q5SDM_*SRF*short_For TCTCCCCGCTAGGGGCGG and Q5SDM_*SRF*short_Rev GCGGGAGGAATGCAAAGGCC to promote site directed mutagenesis. Dual luciferase assays were performed using the modified *SRF*-luc reporter plasmid ("*SRF-luc; no* TCF21 *binding site*") with transfection into A7r5 (A) and HCASMC (B). Renilla luciferase reporter plasmid was used as an internal control of transfection efficiency (n>8). Data analyzed by one-way ANOVA, followed by Dunnett's post-test.



Online Figure X. The kinetic relationship of *TCF21* and *MYOCD* expression levels. To assess the kinetics of changes in expression of *MYOCD* and *TCF21*, HCASMC were treated with TGFβ1 (10ng/ml) for 0, 2, 4, and 8 hours after overnight serum starvation and then mRNA levels of *MYOCD* and *TCF21* genes were measured by qPCR (n=3).