

Text S1:**Supporting Materials and Methods****Quantitative Real-Time PCR**

For chromatin immunoprecipitation (ChIP) and expression analysis of HL-1 cells quantitative real-time PCR was performed using SyberGreen I PCR Master Mix (Abgene) and the ABI PRISM 7900HT Sequence Detection System according to standard protocols.

ChIP binding of Srf and histone 3 acetylation (H3ac) in mouse hearts of the three developmental stages (E18.5, P0.5, P4.5) were analyzed with TaqMan qPCR in context of a beta-side testing of the LightCycler® 1536 System from Roche Applied Science. TaqMan qPCR assays were designed using the Universal ProbeLibrary (UPL) and the ProbeFinder software which is freely accessible at www.roche-applied-science.com. Primers were designed to genomic DNA regions based on ChIP-chip/ChIP-seq data and synthesized by TIB MOLBIOL (Berlin, Germany). For the 1536-well plate format the Innovadyne™ Nanodrop™ Express pipetting robot (IDEX Health & Science LLC, Rohnert Park, CA USA) and the Velocity11 sealing machine (Agilent, Santa Clara, CA USA) were used. ChIP analysis was carried out in triplicates using the following program: Enzyme activation: 95°C for 1min; amplification (45cycles): 95°C for 1sec (ramp: 4.8°C/sec), 60°C for 30sec (ramp: 2.5°C/sec); cooling: 40°C for 30sec (ramp: 2.5°C/sec). The detection format was set to 'Mono Color Hydrolysis/UPL Probes' and the pipetting control to 'Master Control'. The qPCR reaction contained 0.8µl ChIP sample and 1.2µl mastermix containing 1x RealTime ready DNA Probes Master, 300nM primer and 400nM probe.

Chromatin Immunoprecipitation

ChIP experiments with HL-1 cells and mouse hearts were performed in biological duplicates as described [1] using 10^7 HL-1 cells or 30mg mouse heart tissue. For fixation HL-1 cells were incubated for 10min at room temperature with 1% formaldehyde. Tissue samples were minced and cross-linked for 15min at room temperature with fixation buffer (1% formaldehyde, 10mM NaCl, 100µM EDTA, 50µM EGTA, 5mM HEPES, pH 7.5). Cross-linking was terminated by addition of glycine to a final concentration of 125mM. Tissue samples were homogenized for disaggregation. Subsequently cells were incubated for 15min at 4°C with lysis buffer (50mM HEPES, pH 7.5, 140mM NaCl, 1mM EDTA, 1% Triton, 0.1% Na-deoxycholate, 0.1% SDS, 1mM PMSF, 1x Complete Protease Inhibitor Cocktail (Roche)), collected by centrifugation and homogenized using a Douncer. Nuclei were collected by centrifugation for 15min at 20.000g and 4°C and resuspended in sonification buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA and 0.5mM EGTA, 1mM PMSF, 1x Complete Protease

Inhibitor Cocktail). The chromatin was fragmented by sonification to an average size of 500bp. For immunoprecipitation buffer conditions were adjusted to the following concentrations: 140mM NaCl, 1% Triton, 0.1% SDS, 0.1% Na-deoxycholate). A fraction of material was saved as "Input". Chromatin was pre-cleared by rotation with Dynabeads Protein A/G (Invitrogen) for 1h at 4°C. Antibodies were added as given in Table S14 and incubated on a rotating wheel over night at 4°C. As negative controls rabbit and goat normal IgG were used. Protein A/G beads were added and rotation continued for 1h. The beads were washed twice with RIPA buffer A (10mM Tris-HCl, pH8.0, 140mM NaCl, 0.025% NaN₃, 1% Triton, 0.1% SDS, 1% Na-deoxycholate), once with RIPA buffer B (10mM Tris-HCl, pH8.0, 500mM NaCl, 0.025% NaN₃, 1% Triton, 0.1% SDS, 1% Na-deoxycholate), once with LiCl detergent solution (10mM Tris-HCl, pH8.0, 0.5% Na-deoxycholate, 1mM EDTA, 250mM LiCl, 0.5% Nonidet P-40) and once with TBS (20mM Tris-HCl, pH7.6, 150mM NaCl). Immunocomplexes were disrupted by eluting 10min at 65°C with 1% SDS/TE buffer (10mM Tris-HCl, pH7.6, 1mM EDTA, 1% SDS) and eluting a second time for 15min with 0.67% SDS/TE buffer (10mM Tris-HCl, pH7.6, 1mM EDTA, 0.67% SDS). Eluates were combined and reverse cross-linked by heating at 65°C over night. Subsequently DNA was treated with RNase A and Proteinase K and purified by phenol-chloroform extraction.

Linear amplification of ChIPed DNA and Input control for ChIP-chip analysis was carried out on the basis of random primer amplification developed by Bohlander *et al.* [2], which was subsequently modified for ChIP applications [3] by performing only one round of amplification with 20-22 cycles. The amplified ChIPed material and Input was combined from between two and four experiments resulting in two independent pools for each transcription factor. The enrichment of known target genes was confirmed in each separate experiment and in the two independent pools. Amplified samples were purified using Wizard SV PCR purification kits (Promega) according to the manufacturer's instructions. ChIP-chip experiments of HL-1 cells were performed on NimbleGen custom made microarrays. Samples were labeled and hybridized according to NimbleGen standard procedure.

Sample preparation for ChIP-seq of HL-1 cells was performed according the Illumina library preparation procedure. Two pooled biological replicates for Srf and H3ac were sequenced using Illumina/Solexa next-generation (single-end) sequencing technology.

ChIP after siRNA knockdown of Srf in HL-1 cells was performed using the LowCell ChIP Protein A Kit from Diagenode according to the manufacturer's instructions.

ChIP data were confirmed by quantitative real-time PCR using the SyberGreen I PCR Master Mix (Abgene) and the ABI PRISM 7900HT Sequence Detection System or using the RealTime ready DNA Probes Master with the Universal ProbeLibrary and the LightCycler®1536 (Roche). Additional results of ChIP-qPCR experiments are given in Figure S5 and primer sequences for verifications in Table S13.

ChIP-chip Data Analysis

We developed a custom two-array set (2x385K) with NimbleGen using a tiling approach, which enabled an extensive analysis of promoter and enhancer regions (10kb upstream) as well as the first exon and intron sequence of 12,625 transcripts (65% of all RefSeq promoters). We selected 89Mbp of the mouse genome related to transcripts selected based on 13 data sources (Table S1) including all expressed skeletal, smooth and cardiac muscle genes in human and mouse.

In detail, based on the annotation of Ensembl mm8 v39 for each transcript the full 2kb upstream and 100bp downstream of the annotated transcriptional start site (TSS) as well as the first exon and intron sequence was represented. Additionally, conserved non-coding blocks based on Phastcons values in the 10kb region upstream and 3kb downstream of annotated TSSs were selected (Phastcons [4] value of at least 0.2 as retrieved from UCSC Genome Browser database [5]). Conserved regions were enlarged to a minimum size of at least 1kb. Probes were designed by NimbleGen without masking of repetitive regions and probes with multiple hits in the genome were removed. The final array design (2x385k arrays) represents 12,625 TSSs of genes of the mouse genome comprising 740,000 probes with a probe length of 50-60bp and a tiling of 110bp (50-60bp gap between probes). The array design is available from ArrayExpress (accession code A-MEXP-893).

After the ChIP experiments, intensities of each channel were normalized and log-transformed using VSN [6]. Log-ratio enrichment levels for each probe were calculated by subtraction of log Cy3 (Input) from log Cy5 (ChIP sample). The data is stored in ArrayExpress database (accession code E-TABM-378). For the transcription factors the signals were smoothed by calculating a median over the probes inside a sliding window of 600bp size. To distinguish enriched probes a z-score and empirical p-value for each probe on the null hypothesis that these z-scores have a symmetric distribution with mean zero was calculated. P-values were corrected for multiple testing [7] and probes with a nominal false discovery rate of smaller than 0.1 were considered to be significantly enriched. Significant probe positions with a distance less than 210bp were combined into transcription factor binding sites. The histone binding sites were identified as described previously [8].

ChIP-seq Data Analysis

Two independent ChIP samples were profiled. DNA fragments bound by Srf or modified with H3ac in HL-1 cells were sequenced using Illumina/Solexa next-generation (single-end) sequencing technology and the Illumina Genome Analyzer. Image analyses and base calling was done using the open source Firecrest and Bustard applications (Solexa pipeline 1.4.0). Of the initial 6,967,318 and 8,364,328 sequence reads obtained in the Srf and H3ac ChIP-seq experiment, respectively, 4,543,634 (65.2%) for Srf and 6,141,144 (73.4%) for H3ac

could be mapped to the mouse reference genome (NCBI v37) using the read mapping tool RazerS [9]. Only uniquely mapped 36nt reads with at most two mismatches were retained.

We identified Srf binding sites as well as H3ac regions using a one-sample approach implemented by the CisGenome software [10], which requires no negative control. In this approach the negative binomial distribution is used as background model to simulate genome-wide random read positioning and estimates false discovery rates (FDR). For peak detection CisGenome scans the genome with a sliding window of specified length and identifies regions with a read count greater than a user-defined cutoff and FDR [10]. Overlapping peaks are merged. For our Srf ChIP-seq data we used the CisGenome software with a window size w of 100bp, a step size of 25bp for sliding, and a read count level of 10 (FDR = 1.6%). For H3ac ChIP-seq data we used a window size of 250bp, a step size of 50bp for sliding, and again a read count level of 10 (FDR = 4.7%). After peak localization the found peaks for Srf and H3ac were filtered applying boundary refinement (Srf and H3ac) and single-strand filtering (H3ac). After manual inspection of individual peaks no single-strand filtering was applied for Srf binding site detection. Finally, we could identify 2,190 and 10,486 ChIP-seq peaks for Srf and H3ac, respectively.

To investigate potential regulation of miRNAs by Srf binding events we searched for Srf ChIP-seq peaks within a genomic region of ± 10 kb from known mouse miRNAs. The miRNA annotations were obtained from miRNA database miRBase (<http://www.miRBase.org/>; release 13.0). We could identify 22 miRNAs with at least one Srf binding event (see Table S9).

Deep Sequencing of MicroRNAs in HL-1 Cells after Srf Knockdown

We performed two independent siRNA-mediated knockdowns of Srf (further named as Srf-si1 and Srf-si2) and an unspecific siRNA (siNon) in HL-1 cells. Small RNAs were isolated from total RNA and prepared for miRNA sequencing using Illumina Kit FG-102-1009 according to manufacturer's protocol. Small RNAs were sequenced using Illumina next-generation (single-end) sequencing technology on the Illumina Genome Analyzer. Image analyzes and base calling was done using the open source Firecrest and Bustard applications (Solexa pipeline 1.4.0).

Deep sequencing of the small RNA libraries produced 14,911,499 (Srf-si1), 14,518,157 (Srf-si2) and 14,742,382 (siNon) unfiltered 36bp reads. Removing redundancy, meaning that reads with an identical sequence are represented with a single FASTA entry storing the number of sequence counts, yielded 5,634,650 (Srf-si1), 5,503,661 (Srf-si2) and 5,674,429 (siNon) unique read sequences. These unique reads were mapped to the mouse reference genome (NCBI v37, mm9) using MicroRazerS [11], a read mapping tool optimized for mapping small RNAs like microRNAs. MicroRazerS searches for the longest possible

prefix-match of each read, i.e. the longest possible contiguous match starting at the first base. Hence, it is robust to possible adapter sequence at the 3' end of a read and requires no adapter trimming. Using this read aligner the parameter were set as follows: -m 20 (maximum number of best matches), -pa (purge ambiguous reads having more than 20 equally-best hits) and -sL 16 (seed length). Searching for miRNAs with a length of 19-25nt, we found a minimal seed length of 16nt to be optimal. To be robust towards possible sequencing errors we mapped reads with at most one mismatch in the seed sequence. Using these options 96.7% (5,449,988 for Srf-si1), 96.2% (5,296,564 for Srf-si2) and 96.5% (5,475,045 for siNon) of all unique sequences could be mapped to the mouse genome representing 97.3% (14,504,934 for Srf-si1), 96.8% (14,053,178 for Srf-si2) and 97.1% (14,307,881 for siNon) of the total reads, resulting in 349 (Srf-si1), 365 (Srf-si2) and 363 (siNon) known miRNAs. In total 402 miRNAs were identified, corresponding to 450 different genomic loci. To annotate the aligned sequence reads with known mouse miRNAs, we checked for overlaps with positions of miRNA stem-loop sequences (including the mature sequences) based on the miRBase database (<http://www.miRBase.org/>; release 14.0). Multi-matched reads were proportionally assigned to their loci.

Differential expression between the Srf-si1/2 and siNon libraries was tested using Fisher's exact test with FDR correction for multiple testing ($p \leq 0.05$ using the miRNA reads as sample size). Finally, 42 miRNAs (49 loci) were identified as significantly differentially expressed in both siRNA knockdown of Srf (both either up- or down-regulated) compared to negative control. In detail, we found 11 up-regulated and 38 down-regulated miRNAs after Srf knockdown in HL-1 cells (see Table S10).

For all miRNAs (in total 77) identified as significantly differentially expressed in at least one siRNA knockdown of Srf (but both either up- or downregulated) compared to negative control we did target gene predictions using the miRanda v3.0 algorithm [12]. For prediction the mature miRNA sequences and the 3'UTR sequences from all differentially expressed genes were used. To ensure a low number of false positives, the following parameter were used: score cutoff ≥ 140 ; gap open penalty -9 and gap extension penalty -4. Finally, the target prediction revealed 192 of 429 differentially expressed genes. Using a fisher exact test we found the number to be statistical significant when compared to a prediction based on all versus differentially expressed genes ($p=1.77e-5$).

Apoptosis Assay

Detection of apoptosis in RNAi treated HL-1 cells was performed by using the Annexin V-Cy3 Apoptosis Detection Kit (Biovision) according to the manufacturer's protocol. HL-1 cells grown on glass cover slips were washed with PBS 48h after knockdown and incubated with 5 μ l Annexin V-Cy3 stock solution in 500 μ l binding buffer in the dark for 5min at room

temperature. Cells were washed with PBS, DAPI counterstained and embedded in Moviol 4-88 (Roth) for examination on a LSM 510 Meta confocal microscope (Carl Zeiss). The number of apoptotic cells in 20 exemplary pictures per sample was quantified using ImageJ software (NIH).

Cell Viability Studies by Trypan Blue Assay

Cell viability of RNAi treated HL-1 cells was assessed by adding 50µl of Trypan Blue solution to 50µl of the cell suspension. After 2min, the number of living cells, which did not retain the dye was counted using a counting chamber, and was compared to the total number of cells (living + dead) to calculate the viability percentage.

1. Horak CE, Mahajan MC, Luscombe NM, Gerstein M, Weissman SM, et al. (2002) GATA-1 binding sites mapped in the beta-globin locus by using mammalian ChIP-chip analysis. *Proc Natl Acad Sci U S A* 99: 2924-2929.
2. Bohlander SK, Espinosa R, 3rd, Le Beau MM, Rowley JD, Diaz MO (1992) A method for the rapid sequence-independent amplification of microdissected chromosomal material. *Genomics* 13: 1322-1324.
3. Iyer VR, Horak CE, Scafe CS, Botstein D, Snyder M, et al. (2001) Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. *Nature* 409: 533-538.
4. Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, et al. (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res* 15: 1034-1050.
5. Kuhn RM, Karolchik D, Zweig AS, Wang T, Smith KE, et al. (2009) The UCSC Genome Browser Database: update 2009. *Nucleic Acids Res* 37: D755-761.
6. Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M (2002) Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics* 18 Suppl 1: S96-104.
7. Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A* 100: 9440-9445.
8. Fischer JJ, Toedling J, Krueger T, Schueler M, Huber W, et al. (2008) Combinatorial effects of four histone modifications in transcription and differentiation. *Genomics* 91: 41-51.
9. Weese D, Emde AK, Rausch T, Doring A, Reinert K (2009) RazerS--fast read mapping with sensitivity control. *Genome Res* 19: 1646-1654.
10. Ji H, Jiang H, Ma W, Johnson DS, Myers RM, et al. (2008) An integrated software system for analyzing ChIP-chip and ChIP-seq data. *Nat Biotechnol* 26: 1293-1300.
11. Emde AK, Grunert M, Weese D, Reinert K, Sperling SR (2010) MicroRazerS: rapid alignment of small RNA reads. *Bioinformatics* 26: 123-124.
12. John B, Enright AJ, Aravin A, Tuschl T, Sander C, et al. (2004) Human MicroRNA targets. *PLoS Biol* 2: e363.