

11 APPENDIX

11.1 Appendix A: Abbreviations

bp	Base pair	PBS	Phosphate buffered saline
BP	Biological process gene ontology	PCR	Polymerase chain reaction
BSA	Albumin from bovine serum	PMSF	Phenylmethanesulfonyl fluoride
CC	Cellular component gene ontology	Pol II	RNA polymerase II
CHD	Congenital heart disease	qPCR	Quantitative real-time PCR
ChIP	Chromatin immunoprecipitation	RNAi	RNA interference
ChIP-chip	chromatin immunoprecipitation followed by microarray analysis	RNA	Ribonucleic acid
CNB	Conserved non-coding block	RNase	Ribonuclease
DEPC	Diethyl pyrocarbonate	RT-PCR	Reverse Transcription PCR
DMEM	Dulbecco's Modified Eagle's Medium	sec	Second
DMSO	Dimethylsulfoxid	SDS	Sodiumdodecylsulfate
DNA	Deoxyribonucleic acid	siRNA	Short interference RNA
DNase	Deoxyribonuclease	TBE	Tris-borate-EDTA (buffer)
dNTP	Deoxyribonucleosidtriphosphate	TE	Tris-EDTA (buffer)
DTT	1,4-Dithiothreit	TF	Transcription factor
EDTA	Ethylenediaminetetraacetic acid	TFBM	Transcription factor binding motif
EGTA	Ethylene glycol-bis(2-aminoethylether)- <i>N,N,N',N'</i> -tetraacetic acid	TFBS	Transcription factor binding site
g	Gram	Tris	Tris(hydroxymethyl)-aminomethane
GO	Gene Ontology	TSS	transcription start site
h	Hour	U	Unit of enzyme activity
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid	UV	Ultra violet light
HRP	Horse radish peroxidase	v/v	Volume per volume
kb	Kilo base pairs	W	Watt
l	Liter	w/v	Weight per volume
m	Meter		
M	Mol/l, molar		
min	Minute		
Mol. biol.	Molecular biology grade		
NDR	Nucleosome-depleted regions		
o.n.	Over night		
<i>p</i>	<i>p</i> -value		
p.a.	pro analysis		

Prefixes of units:

f, Femto (10^{-15}); p, Pico (10^{-12}); n, Nano (10^{-9}); μ , Mikro (10^{-6}); m, Mili (10^{-3}); c, Centi (10^{-2}); d, Dezi (10^{-1}); k, Kilo (10^3); M, Mega (10^6); G, Giga (10^9); T, Tera (10^{12}); P, Peta (10^{15}).

11.2 Appendix B: The Brno Nomenclature for Histone Modifications

In this study the Brno nomenclature is used as given in the article by Bryan M. Turner²². In the following the outline is given, for details refer to the original publication.

The nomenclature starts from the left with the histone, then the residue, then the modification. In cases where the modified residue is not known, or not relevant, the modification should follow the histone, for example H4ac and H2Bar1. Multiple modifications can be accommodated by simply extending the listing (for example, H3K4me3K9acS10ph) for as long as necessary. Because each individual modified residue begins with the uppercase letter specifying the amino acid, and because the modifications themselves are all designated by lowercase letters, the use of commas or dots to separate the individual modified residues in a 'word' specifying multiple modifications is not necessary. On occasion, the presence of an unmodified residue may be an essential component of an information-bearing combination of residues; in this case the residue should be inserted without additions (for example, H3K9S10ph) to indicate H3 unmodified at Lys9 and phosphorylated at Ser10.

Table A 1. The Brno nomenclature for histone modifications.

Modifying group	Amino acid(s) modified	Level of modification	Abbreviation for modification	Examples of modified residues
Acetyl- Methyl-	Lysine	mono-	ac	H3K9ac
	Arginine	mono-	me1	H3R17me1
	Arginine	di-,symmetrical	me2s	H3R2me2s
	Arginine	di-,asymmetrical	me2a	H3R17me2a
	Lysine	mono-	me1	H3K4me1
	Lysine	di-	me2	H3K4me2
	Lysine	tri-	me3	H3K4me3
Phosphoryl-	Serine or threonine	mono-	ph	H3S10ph
Ubiquityl-	Lysine	mono-	ub1	H2BK123ub1
SUMOyl-	Lysine	mono-	su	H4K5su
ADP ribosyl-	Glutamate	mono-	ar1	H2BE2ar1
	Glutamate	poly-	arn	H2BE2arn

11.3 Appendix C: Ensembl Accessions and Description of Genes in the Text

Although efforts are being made to define unique names for genes and proteins, these are not being used consistently in the literature. Here, the following approach was used: within the running text genes and proteins were named as in the cited literature, to enable the reader to quickly find the corresponding passage in the cited report. In Table A 2 the mouse or human synonym used in the text is given, as well as the corresponding Symbols, IDs and description.

Table A 2. Symbols and Ensembl IDs of genes and proteins mentioned in the text.

Synonym used in Text	Mouse MGI Symbol	Mouse Ensembl ID	Human HGNC Symbol	Human Ensembl Gene ID	Description (Mouse)
α -cardiac actin	Actc1	ENSMUSG00000068614	ACTC1	ENSG00000159251	actin, alpha, cardiac [Source:MarkerSymbol;Acc:MGI:87905]
α MHC	Myh6	ENSMUSG00000040752	MYH6	ENSG00000197616	myosin, heavy polypeptide 6, cardiac muscle, alpha [Source:MarkerSymbol;Acc:MGI:97255]
ANF	Nppa	ENSMUSG00000041616	NPPA	ENSG00000175206	natriuretic peptide precursor type A [Source:MarkerSymbol;Acc:MGI:97367]
Ankrd1	Ankrd1	ENSMUSG00000024803	ANKRD1	ENSG00000148677	ankyrin repeat domain 1 (cardiac muscle) [Source:MarkerSymbol;Acc:MGI:1097717]
Bcl2	Bcl2	ENSMUSG00000057329	BCL2	ENSG00000171791	B-cell leukemia/lymphoma 2 [Source:MarkerSymbol;Acc:MGI:88138]
CARM1	Carm1	ENSMUSG00000032185	CARM1	ENSG00000142453	coactivator-associated arginine methyltransferase 1 [Source:MarkerSymbol;Acc:MGI:1913208]
Connexin 43	Gja1	ENSMUSG00000050953	GJA1	ENSG00000152661	gap junction membrane channel protein alpha 1 [Source:MarkerSymbol;Acc:MGI:95713]
Creb3l2	Creb3l2	ENSMUSG00000038648	CREB3L2	ENSG00000182158	cAMP responsive element binding protein 3-like 2 [Source:MarkerSymbol;Acc:MGI:2442695]
Ctgf	Ctgf	ENSMUSG00000019997	CTGF	ENSG00000118523	connective tissue growth factor [Source:MarkerSymbol;Acc:MGI:95537]
Fog-1	Zfp1	ENSMUSG00000049577	ZFPM1	ENSG00000179588	zinc finger protein, multitype 1 [Source:MarkerSymbol;Acc:MGI:1095400]
Fog-2	Zfp2	ENSMUSG00000022306	ZFPM2	ENSG00000169946	zinc finger protein, multitype 2
Foxp1	Foxp1	ENSMUSG00000030067	FOXP1	ENSG00000114861	forkhead box P1 [Source:MarkerSymbol;Acc:MGI:1914004]
Gata4	Gata4	ENSMUSG00000021944	GATA4	ENSG00000136574	GATA binding protein 4 [Source:MarkerSymbol;Acc:MGI:95664]
GATA6	Gata6	ENSMUSG00000005836	GATA6	ENSG00000141448	GATA binding protein 6 [Source:MarkerSymbol;Acc:MGI:107516]
GRIP	Ncoa2	ENSMUSG00000005886	NCOA2	ENSG00000140396	nuclear receptor coactivator 2 [Source:MarkerSymbol;Acc:MGI:1276533]
Hand2, HAND2	Hand2	ENSMUSG00000038193	HAND2	ENSG00000164107	heart and neural crest derivatives expressed transcript 2 [Source:MarkerSymbol;Acc:MGI:103580]
HDAC1	Hdac1	ENSMUSG00000028800	HDAC1	ENSG00000116478	histone deacetylase 1 [Source:MarkerSymbol;Acc:MGI:108086]
HDAC2	Hdac2	ENSMUSG00000019777	HDAC2	ENSG00000196591	histone deacetylase 2 [Source:MarkerSymbol;Acc:MGI:1097691]
HDAC3	Hdac3	ENSMUSG00000024454	HDAC3	ENSG00000171720	histone deacetylase 3 [Source:MarkerSymbol;Acc:MGI:1343091]
HDAC4	Hdac4	ENSMUSG00000026313	HDAC4	ENSG00000068024	histone deacetylase 4 [Source:MarkerSymbol;Acc:MGI:3036234]
HDAC5	Hdac5	ENSMUSG00000008855	HDAC5	ENSG00000108840	histone deacetylase 5 [Source:MarkerSymbol;Acc:MGI:1333784]
HDAC6	Hdac6	ENSMUSG00000031161	HDAC6	ENSG00000094631	histone deacetylase 6 [Source:MarkerSymbol;Acc:MGI:1333752]
HDAC7	Hdac7a	ENSMUSG00000022475	HDAC7A	ENSG00000061273	histone deacetylase 7A [Source:MarkerSymbol;Acc:MGI:1891835]
HDAC8	Hdac8	ENSMUSG00000067567	HDAC8	ENSG00000147099	histone deacetylase 8 [Source:MarkerSymbol;Acc:MGI:1917565]
HDAC9	Hdac9	ENSMUSG00000004698	HDAC9	ENSG00000048052	histone deacetylase 9 [Source:MarkerSymbol;Acc:MGI:1931221]
Hop	Hod	ENSMUSG00000059325	HOP_HUMAN	ENSG00000171476	homeobox only domain [Source:MarkerSymbol;Acc:MGI:1916782]
Hprt1	Hprt1	ENSMUSG00000025630	HPRT1	ENSG00000165704	hypoxanthine guanine phosphoribosyl transferase 1 [Source:MarkerSymbol;Acc:MGI:96217]
Jmj	Jarid2	ENSMUSG00000038518	JARID2	ENSG00000008083	jumonji, AT rich interactive domain 2 [Source:MarkerSymbol;Acc:MGI:104813]
KLF13	Klf13	ENSMUSG00000052040	KLF13	ENSG00000169926	Kruppel-like factor 13

Cardiac Transcription Networks

Lmo4	Lmo4	ENSMUSG00000028266	LMO4	ENSG00000143013	[Source:MarkerSymbol;Acc:MGI:1354948] LIM domain only 4
Lmod2	Lmod2	ENSMUSG00000029683	LMOD2	ENSG00000170807	[Source:MarkerSymbol;Acc:MGI:109360] leiomodlin 2 (cardiac)
Mef2a	Mef2a	ENSMUSG00000030557	MEF2A	ENSG00000068305	[Source:MarkerSymbol;Acc:MGI:2135672] myocyte enhancer factor 2A
Mef2b	Mef2b	ENSMUSG00000002345	MEF2B	ENSG00000064489	[Source:MarkerSymbol;Acc:MGI:99532] RIKEN cDNA 2310045N01 gene
Mef2c	Mef2c	ENSMUSG00000005583	MEF2C	ENSG00000081189	[Source:MarkerSymbol;Acc:MGI:1919618] myocyte enhancer factor 2C
Mef2d	Mef2d	ENSMUSG00000001419	MEF2D	ENSG00000116604	[Source:MarkerSymbol;Acc:MGI:99458] myocyte enhancer factor 2D
Mitf	Mitf	ENSMUSG00000035158	MITF	ENSG00000187098	[Source:MarkerSymbol;Acc:MGI:99533] microphthalmia-associated transcription factor
Myf5	Myf5	ENSMUSG00000000435	MYF5	ENSG00000111049	[Source:MarkerSymbol;Acc:MGI:104554] myogenic factor 5
Myl1	Myl1	ENSMUSG000000061816	MYL1	ENSG00000168530	[Source:MarkerSymbol;Acc:MGI:97252] myosin, light polypeptide 1
Myocd	Myocd	ENSMUSG000000020542	MYOCD	ENSG00000141052	[Source:MarkerSymbol;Acc:MGI:97269] myocardin
MyoD	Myod1	ENSMUSG000000009471	MYOD1	ENSG00000129152	[Source:MarkerSymbol;Acc:MGI:2137495] myogenic differentiation 1
Neb1	Neb1	ENSMUSG000000053702	NEBL	ENSG00000078114	[Source:MarkerSymbol;Acc:MGI:97275] nebullette [Source:MarkerSymbol;Acc:MGI:1921353]
NF-AT3	Nfatc4	ENSMUSG000000023411	NFATC4	ENSG00000100968	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
Nfib	Nfib	ENSMUSG000000052747	NFIB	ENSG00000147862	[Source:MarkerSymbol;Acc:MGI:1920431] nuclear factor I/B
Nkx2.5	Nkx2-5	ENSMUSG00000015579	NKX2-5	ENSG00000183072	[Source:MarkerSymbol;Acc:MGI:103188] NK2 transcription factor related, locus 5 (Drosophila)
p300	Ep300	ENSMUSG000000055024	EP300	ENSG00000100393	[Source:MarkerSymbol;Acc:MGI:97350] E1A binding protein p300
Ppp1r12b	Ppp1r12 b	ENSMUSG000000073557	PPP1R12B	ENSG00000077157	[Source:MarkerSymbol;Acc:MGI:1276116] protein phosphatase 1, regulatory (inhibitor) subunit 12B [Source:MarkerSymbol;Acc:MGI:1916417]
Rarb	Rarb	ENSMUSG00000017491	RARB	ENSG00000077092	retinoic acid receptor, beta
RXRA	Rxra	ENSMUSG00000015846	RXRA	ENSG00000186350	[Source:MarkerSymbol;Acc:MGI:97857] retinoid X receptor alpha
SP1	Sp1	ENSMUSG00000001280	SP1	ENSG00000185591	[Source:MarkerSymbol;Acc:MGI:98214] trans-acting transcription factor 1
Srf	Srf	ENSMUSG00000015605	SRF	ENSG00000112658	[Source:MarkerSymbol;Acc:MGI:98372] serum response factor
STAT-1	Stat1	ENSMUSG000000073685	STAT1	ENSG00000115415	[Source:MarkerSymbol;Acc:MGI:106658] signal transducer and activator of transcription 1
Tbx20, TBX20	Tbx20	ENSMUSG000000031965	TBX20	ENSG00000164532	[Source:MarkerSymbol;Acc:MGI:103063] T-box 20 [Source:MarkerSymbol;Acc:MGI:1888496]
Tbx1	Tbx1	ENSMUSG000000009097	TBX1	ENSG00000184058	T-box 1 [Source:MarkerSymbol;Acc:MGI:98493]
Tbx5, TBX5	Tbx5	ENSMUSG000000018263	TBX5	ENSG00000089225	T-box 5 [Source:MarkerSymbol;Acc:MGI:102541]
Ttn	Ttn	ENSMUSG000000047319	TTN	ENSG00000155657	titin [Source:MarkerSymbol;Acc:MGI:98864]
YY1	Yy1	ENSMUSG000000021264	YY1	ENSG00000100811	YY1 transcription factor
Zeb2	Zeb2	ENSMUSG000000026872	ZEB2	ENSG00000169554	[Source:MarkerSymbol;Acc:MGI:99150] zinc finger E-box binding homeobox 2 [Source:MarkerSymbol;Acc:MGI:1344407]

11.4 Appendix D: Antibodies

Antibodies used in Western Blot, Immunofluorescence and ChIP experiments

Primary Antibodies

Anti-acetyl-Histone H3 Antibody, rabbit polyclonal IgG	Upstate #06-599	Lot #29505
Anti-acetyl-Histone H4 antibody, rabbit antiserum	Upstate 06-866	Lot #29532
Histone H3 (dimethyl K4) antibody Rabbit polyclonal	abcam #ab7766	Lot #66726
Histone H3 (trimethyl K4) antibody Rabbit polyclonal	abcam #ab8580	Lot #77499
Normal Rabbit IgG, pre-immune serum	Santa Cruz Biotech #sc-2027	Lot #K0304
Normal Goat IgG, pre-immune serum	Santa Cruz Biotech #sc-2028	Lot #J2704
Anti-Gata4 antibody, rabbit polyclonal	Santa Cruz Biotech #sc-9053	Lot #I1906
Anti-Gata4 antibody, goat polyclonal	Santa Cruz Biotech #sc-1237	Lot #J229
Anti-Mef2	Santa Cruz Biotech #sc-313	Lot #L169
Anti-Nkx2.5	Santa Cruz Biotech #sc-14033X	Lot #H1307
Anti-Srf	Santa Cruz Biotech #sc-335	Lot #D0703
Anti- α -Tubulin	Sigma, USA #T9029	Lot #DM-A1

Secondary Antibodies

anti-rabbit IgG conjugated with HRP	Sigma, USA #A2074	Lot #032K4801
anti-goat IgG conjugated with HRP	Abcam, USA #ab6741	Lot #RG-I6
Anti-mouse IgG conjugated with Alexa Fluor 488	Invitrogen #A11029	Lot #51153A
Anti-rabbit IgG conjugated with Alexa Fluor 594	Invitrogen #A11037	Lot #49624A

11.5 Appendix E: Equipment, Reagents and Buffers

Equipment

Phase lock Gel (Heavy) 1.5/2ml tubes	Eppendorf, Germany
Neubauer Zählkammer	Carl Roth, Germany
Dounce homogenizer	Dounce, USA
Branson 250	Branson Ultrasonics, USA
Branson Tip	Branson Ultrasonics, USA
Agarose gel electrophoresis equipment	Amersham, UK
SDS-PAGE gel electrophoresis equipment	Eppendorf, Germany
Heating oven	Carl Roth, Germany
Nanodrop Spectrophotometer	Nanodrop technologies, USA
Thermocycler	PTC100, MJ Research Inc, USA
Thermomixer	Eppendorf, Germany
ABI Prism 7700	Applied Biosystems, USA
Microscopes	Carl Zeiss AG, Germany
Digital Camera	Canon, Japan
Bioanalyzer	Agilent

Reagents

Cell Culture

Cell Culture Reagents

DMSO, dimethylsulfoxid	Sigma, USA
Penicillin-Streptomycin solution, cell culture grade	Gibco, USA
PBS, cell culture grade	Sigma, USA
Cell culture flasks with filter (T75, T300)	Biochrom, Germany
Cellscraper	Biochrom, Germany
Cell culture tubes	Biochrom, Germany
Trypsin, cell culture grade	Gibco, USA

C2C12 cell culture

DMEM	Gibco, USA
FBS Lot #0055H	Biochrom, Germany
Horse serum	Biochrom, Germany
XtremeGene	Roche, Germany

HL-1 cell culture

Fetal calf serum Lot #3J0229	JRH Biosciences, USA
Water, cell culture grade	Invitrogen, USA life technologies,
Claycomb Medium	JRH Biosciences, USA
Norepinephrine	Sigma, USA,
L-Ascorbic acid Na-salt	Sigma, USA,
L-Glutamine	Gibco, USA,
Trypsine soybean inhibitor	Invitrogen, USA,
Lipofectamin 2000	Invitrogen, USA

Reagents used in Chromatin immunoprecipitation

37% Formaldehyde, mol. biol. grade	Sigma, USA
Glycogen mol. biol. grade	Roche, Germany
Dynabeads Protein G	Invitrogen, USA
Dynabeads Protein A	Invitrogen, USA
PMSF	Sigma, USA
Protease Inhibitor Cocktail, Complete mini EDTA free	Roche, Germany
Proteinase K	Sigma, USA
Triton X-100	Sigma, USA
Glycine	Merck, Germany
DTT	Sigma-Aldrich, USA
PBS	Sigma-Aldrich, USA
Na-desoxycholate	Merck, Germany
NaCl	Merck, Germany
SDS	Sigma-Aldrich, USA
Tris	Sigma-Aldrich, USA
EDTA	Sigma-Aldrich, USA
LiCl	Merck, Germany
RNase	Sigma-Aldrich, USA
Glycogen	Roche, Germany
Proteinase K	Sigma-Aldrich, USA
Chromatography Water	Merck, Germany
Phenol/Chloroform/Isoamylalcohol (25:24:1)	Roth, Germany
Chloroform	Merck, Germany
Ethanol p.a.	Merck, Germany
PMSF	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
MgCl ₂	Merck, Germany
KCl	Merck, Germany
NaN ₃	Merck, Germany
EDTA	Merck, Germany
EGTA	Merck, Germany
Nonidet P-40 IGEPAL	Sigma-Aldrich, USA

Reagents for linear DNA amplification

Sequenase buffer	Amersham, UK
BSA mol. boil grade 500µg/ml	Biolabs
DTT 0.1M, RNase free	Promega, USA
dNTPs	MPI Berlin
Taq polymerase	MPI Berlin
Sequenase T7 DNA Polymerase Version 2.0, 13U/µl	Amersham, UK
Wizard SV Gel and PCR clean-up System	Promega, USA
Trishydrochlorid	Merck, Germany
KCl	Merck, Germany
Tween20, nuclease free	Sigma-Aldrich, USA
MgCl ₂	Merck, Germany
SDS-PAGE gels	NuPAGE™ 12% Bis-Tris gels, Invitrogen, USA

Software

ABI PRISM 7900HT Sequence detection System	Applied Biosystems, USA
BioTapestry	http://www.biotapestry.org ²⁶⁷
Primer Express	Applied biosystems, USA
SDS 2.1 software	Applied biosystems, USA

Other Reagents

1 kb marker DNA Ladder	New England BioLabs, USA
100 bp marker DNA Ladder	New England BioLabs, USA
30% Hydrogen peroxide	Sigma, USA
384 clear well optical reaction plates	Applied Biosystems
Agarose	Invitrogen, USA
AMV-Reverse Transcriptase	Promega, USA
Benzonase	Roche, Germany
calf thymus histones	Worthington
DEPC water	Invitrogen, USA
DNase	Promega, USA
dNTPs	Amersham, UK
DTT 0.1M, RNase free	Promega, USA
ECL Advance detection	Amersham, UK
Ethidiumbromide solution	Sigma, USA
Optical adhesive covers	Applied Biosystems
Protein Marker: Precision Plus Protein Standard	Biorad, USA
RNase Away	Roth, Germany
RNasin Ribonuclease inhibitor	Promega, USA
RQ1 RNase-free DNase	Promega, USA
SyberGreen I master mix (ABgene)	ABgene
SybrGreen Master Mix	ABgene
Transfer membrane for Western Blots	Millipore
Trizol	Invitrogen, USA
Vectashield DAPI mounting	Vector Labs, USA
X-ray films Fuji Super RX 100NIF	Fuji, Japan

11.6 Appendix F: Buffers

Hypotonic buffer (1x):

10.0 mM HEPES buffer, pH 7.9
1.5 mM MgCl₂
10.0 mM KCl
0.5mM DTT (add directly before use)

RIPA buffer (1x):

10.0 mM Tris-HCl, pH8.0
140.0 mM NaCl
0.025% NaN₃
1.0% Triton-X 100
0.1% SDS
1.0% Na-desoxycholate

Add protease inhibitor mix directly before use.

Sonication buffer (1x):

10.0 mM Tris-HCl, pH 8.0
1.0 mM EDTA
0.5 mM EGTA

Add protease inhibitor mix directly before use.

RIPA concentrate:

0.25% Na-desoxycholate
0.25% SDS
2.5% Triton-X 100
350 mM NaCl

RIPA 500 mM NaCl buffer (1x):

10.0 mM Tris-HCl, pH8.0
500.0 mM NaCl
0.025% NaN₃
1.0% Triton-X 100
0.1% SDS
1.0% Na-desoxycholate

Li/Detergent solution (1x) :

10.0 mM Tris-HCl, pH8.0
250.0 mM LiCl
1.0 mM EDTA
0.5% IGEPAL
0.5% Na-desoxycholate

SDS/TE 1% buffer (1x):

10.0 mM Tris-HCl, pH 7.6
1.0 mM EDTA
1.0% SDS

SDS/TE 0.67% buffer (1x):

10.0 mM Tris-HCl, pH 7.6
1.0 mM EDTA
0.67% SDS

TBS (1x):

20.0 mM Tris-HCl, pH 7.6
150.0 mM NaCl

Protease inhibitor mix (100x):

Dissolve 1 tablet Roche complete protease inhibitor in
2 ml chrom. H₂O, store at -20 °C.

PCR buffer (10x):

500.0 mM KCl
150.0 mM Tris-HCl, pH 8.3
1.0% Tween 20
15.0 mM MgCl₂

C2C12 growth media:

10.0% FBS
100.0 g/ml Penicillin/Streptomycin
DMEM

C2C12 differentiation media:

2.0% Horse serum
1.0% Penicillin/Streptomycin solution
DMEM

HL-1 media:

10.0% FBS
100.0 g/ml Penicillin/Streptomycin
0.1 mM Norepinephrine (from 10 mM stock)
2.0 mM L-Glutamine (from 200 mM stock)
Protect from light, store at 4 °C

Norepinephrine [(±)-arterenol] 10 mM stock:

30.0 mM ascorbic acid in cell culture
grade H₂O
Filter sterilize using a 0.2 µm Acrodisc syringe
filter
10.0 mM Norepinephrine
Store at -20 °C

C2C12 Freezing Medium:

90.0% FBS
10.0% DMSO
Make fresh

HL-1 Freezing Medium:

95.0% FBS
5.0% DMSO
Make fresh

11.7 Appendix G: siRNAs

Name used in Text	Company	Product Name	Product ID	Entrez ID	Target MGI_Symbol	Sequence Accession	Sense Sequences
siGata4_1	Qiagen	Mm_Gata4_1	SI01009799	14463	Gata4	NM_008092	CTGGATTTAATTCGTATATAT
siGata4_3	Qiagen	Mm_Gata4_3	SI01009813	14463	Gata4	NM_008092	CACACAGAATAGCTTCATCAA
siMef2a_3	Qiagen	Mm_Mef2a_3	SI01303449	17258	Mef2a	NM_001033713 NM_194070	CACATTCTGCTGAATTATTTA
siMef2a_4	Qiagen	Mm_Mef2a_4	SI01303456	17258	Mef2a	NM_001033713 NM_194070	AAGTAATTATTAGGAATATAA
siNkx2.5_3	Qiagen	Mm_Nkx2-5_3	SI01328257	18091	Nkx2-5	NM_008700	ACCCACGCCTTTCTCAGTCAA
siNkx2.5_4	Qiagen	Mm_Nkx2-5_4	SI01328264	18091	Nkx2-5	NM_008700	CACGGGCACTTTCGACGGATT
siSrf_2	Qiagen	Mm_Srf_2	SI00217133	20807	Srf	NM_020493	CTCAATTTGCTATGAGTATTA
siSrf_3	Qiagen	Mm_Srf_3	SI00217140	20807	Srf	NM_020493	AGG GAC GGA ACC ACT TAT TTA
siNon	Qiagen	AllStars Negative Control siRNA	1027280	Non	Synthetic	Synthetic	Unknown

11.8 Appendix H: Lists of Primers

Table A 3. List of primers used for verification of microarray expression analysis, analysis of histone modifications project

All primers are exon spanning. f - forward primer; r – reverse primer

MGI Symbol	ENSEMBL Transcript ID	Primer Name	Sequence	Partner Primers	Orientation
Acta1	ENSMUST0000034453	acta1_rt_m_f	TTGTGTGTGACAACGGCTCTG	acta1_rt_m_r	f
Acta1	ENSMUST0000034453	acta1_rt_m_r	ACCCACGTAGGAGTCCTTCTGA	acta1_rt_m_f	r
Cox6a2	ENSMUST0000033049	Cox6a2-exp-f.1	CCCAGAGTTCATCCCGTATCA	Cox6a2-exp-r.1	f
Cox6a2	ENSMUST0000033049	Cox6a2-exp-r.1	TGGAAAAGCGTGTGGTTGC	Cox6a2-exp-f.1	r
Ctgf	ENSMUST0000020171	Ctgf-exp-f.1	CATCTCCACCCGAGTTACCAA	Ctgf-exp-r.1	f
Ctgf	ENSMUST0000020171	Ctgf-exp-r.1	TGTCCGGATGCACTTTTTC	Ctgf-exp-f.1	r
Hprt1	ENSMUST0000026723	hprt_m_f	AAACAATGCAAACCTTGTCTTCC	hprt_m_r	f
Hprt1	ENSMUST0000026723	hprt_m_r	GGTCCTTTTCACCAGCAAGCT	hprt_m_f	r
Lmna	ENSMUST0000029699	lmna_m_fl	CGCAACAAGTCCAACGAGG	lmna_m_rl	f
Lmna	ENSMUST0000029699	lmna_m_rl	TGGGAAGCGATAGGTCATCA	lmna_m_fl	r
Lmna	ENSMUST0000036252				
Mcm6	ENSMUST0000027601	Mcm6-exp-f.1	TCTTCCTGCCT GCCATGT	Mcm6-exp-r.1	f

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Mcm6	ENSMUST00000027601	Mcm6-exp-r.1	TCTCAGCGGTCTGTTCCCTCATC	Mcm6-exp-f.1	r
Mki67	ENSMUST00000033310	Mki67-exp-f.1	CTGTGAGGCTGAGACATGGAGA	Mki67-exp-r.1	f
Mki67	ENSMUST00000033310	Mki67-exp-r.1	TGGCTTGCTTCCATCCTCAT	Mki67-exp-f.1	r
Mylfp	ENSMUST00000032910	Mylpf-exp-f.1	AGCTCCAACGTCTTCTCCATGT	Mylpf-exp-r.1	f
Mylfp	ENSMUST00000032910	Mylpf-exp-r.1	TCGATAATGCCATCCCTGTTC	Mylpf-exp-f.1	r
Prps2	ENSMUST00000026839	Prps2-exp-f.1	AGATGCTGGAGGAGCCAAAA	Prps2-exp-r.1	f
Prps2	ENSMUST00000026839	Prps2-exp-r.1	CCATCCGGTCCACTTCATTT	Prps2-exp-f.1	r
S100a4	ENSMUST0000001046	S100a4-exp-f.1	GCTCAAGGAGCTACTGACCAGG	S100a4-exp-r.1	f
S100a4	ENSMUST0000001046	S100a4-exp-r.1	CCAAGTTGCTCATCACCTTCTG	S100a4-exp-f.1	r
Slc25a37	ENSMUST00000037064	Slc25a37-exp-f.1	CCACCCTACTCCACGATGCA	Slc25a37-exp-r.1	f
Slc25a37	ENSMUST00000037064	Slc25a37-exp-r.1	CGCCACACTGTCCGGATACA	Slc25a37-exp-f.1	r
Tbp	ENSMUST00000014911	tbp_m_fl	TGCCACACCAGCTTCTGAGA	tbp_m_r1	f
	ENSMUST00000039079				
	ENSMUST00000080441				
Tbp	ENSMUST00000014911	tbp_m_r1	GATGACTGCAGCAAATCGCTT	tbp_m_fl	r
	ENSMUST00000039079				
	ENSMUST00000080441				
Tm4sf1	ENSMUST00000029376	Tm4sf1-exp-f.1	TACGAAAACACTACGGCAAGCG	Tm4sf1-exp-r.1	f
Tm4sf1	ENSMUST00000029376	Tm4sf1-exp-r.1	CACAGTAAGCAGATCCACGAT	Tm4sf1-exp-f.1	r
Tnni1	ENSMUST00000027674	tnni1_rt_m_f	GCTCTAAGCACAAGGTGTCCAT	tnni1_rt_m_r	f
Tnni1	ENSMUST00000027674	tnni1_rt_m_r	TTCCTCCAGTCTCCTACCTCGA	tnni1_rt_m_f	r
Tnnt2	ENSMUST00000027671	Tnnt2-exp-f.1	CAGACTCTGATCGAGGCTCACT	Tnnt2-exp-r.1	f
Tnnt2	ENSMUST00000027671	Tnnt2-exp-r.1	GACGCTTTTCGATCCTGTCTT	Tnnt2-exp-f.1	r

Table A 4. List of primers used for ChIP-chip verification, analysis of histone modifications project

f - forward primer; r – reverse primer

MGI Gene Symbol	ENSMBL Closest Transcript ID	GeneBank Sequence Accession Number	Primer Name	5' to 3' Sequence	Genomic Sequence Region	Partner Primer	Orientation
Mpz	ENSMUST00000070758	AC163497	all_mr01_fl	CCACGGTTTTGAGGATTCCA	chr1:172987900-172988350	all_mr01_r1	f
Mpz	ENSMUST00000070758	AC163497	all_mr01_r1	TTCTCCCTTTGCCTTGCCA	chr1:172987900-172988350	all_mr01_fl	r
Tm9sf4	ENSMUST00000089027	AC078911	all_mr03_fl	TTAAAAACACCTCTGGCCCTG	chr2:152853750-152854300	all_mr03_r1	f
Tm9sf4	ENSMUST00000089027	AC078911	all_mr03_r1	CCTCCACTCTCATCCACAAAGA	chr2:152853750-152854300	all_mr03_fl	r
Rragc	ENSMUST00000030399	AL606962	all_mr06_fl	CAGCGATCTGCTTACGGAATTA	chr4:123418800-123419200	all_mr06_r1	f
	ENSMUST00000072215						
Rragc	ENSMUST00000030399	AL606962	all_mr06_r1	CACGTGCGAAAGGCAATTAG	chr4:123418800-123419200	all_mr06_fl	r
	ENSMUST00000072215						

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Rps11 ; Rpl13a	ENSMUST00000051978 ENSMUST00000003518 ENSMUST00000083285	AC126256	all_mr09_fl	AGCTAAATCCCCTCTCAGGCAT	chr7:44995900-44996400	all_mr09_r1	f
Rps11 ; Rpl13a	ENSMUST00000051978 ENSMUST00000003518 ENSMUST00000083285	AC126256	all_mr09_r1	AGTTCCGGAGACCCTCCAGTAA	chr7:44995900-44996400	all_mr09_fl	r
Dctn5	ENSMUST00000033156	AC122232	all_mr10_fl	ACATATGTAAACTGCCCCCGTT	chr7:121924700-121925000	all_mr10_r1	f
Dctn5	ENSMUST00000033156	AC122232	all_mr10_r1	TGTGCATTACAGACCCCACTTC	chr7:121924700-121925000	all_mr10_fl	r
Polr3b	ENSMUST00000077175	AC140333	all_mr13_fl	TAATTGCTTCACGGTGAAGTGC	chr10:84052890-84053060	all_mr13_r1	r
Polr3b	ENSMUST00000077175	AC140333	all_mr13_r1	TGCCAAAGATGTCAAGGTTTCAG	chr10:84052890-84053060	all_mr13_fl	r
Aldh3a2	ENSMUST00000066277 ENSMUST00000074127 ENSMUST00000071413	AL672172	all_mr14_fl	CACAGCCCCTCTTTACCAGAA	chr11:61082000-61082400	all_mr14_r1	f
Aldh3a2	ENSMUST00000066277 ENSMUST00000074127 ENSMUST00000071413	AL672172	all_mr14_r1	TCCAGGCATGGTAAGACCTCTA	chr11:61082000-61082400	all_mr14_fl	r
Txndc5	ENSMUST00000035988	AC154747	all_mr16_fl	TTGGATTCCACAGGCACATT	chr13:38534600-38535000	all_mr16_r1	f
Txndc5	ENSMUST00000035988	AC154747	all_mr16_r1	TGGCTGTGTTTATTGCTGAGC	chr13:38534600-38535000	all_mr16_fl	r
Sfrs2ip	ENSMUST00000047835	AC158769	all_mr19_fl	CCGCTTAGGAATGCAATGAA	chr15:96289800-96290200	all_mr19_r1	f
Sfrs2ip	ENSMUST00000047835	AC158769	all_mr19_r1	GCGAAATACTTGCACACAGGA	chr15:96289800-96290200	all_mr19_fl	r
Nhlrc2	ENSMUST00000071423	AC116849	all_mr20_fl	TTTCGGACCCTTTTGCCTC	chr19:56602500-56603200	all_mr20_r1	f
Nhlrc2	ENSMUST00000071423	AC116849	all_mr20_r1	CCTCCATGCAGCCAATTCTT	chr19:56602500-56603200	all_mr20_fl	r
1810074P20Rik	ENSMUST00000038705	AL833775	all_mr21_fl	GTTCCTCCAAACTTGATGTGA	chr4:41040964-41041134	all_mr21_r1	f
1810074P20Rik	ENSMUST00000038705	AL833775	all_mr21_r1	GCAGCGTGCTAATAGCTCTGTC	chr4:41040964-41041134	all_mr21_fl	r
Rpl21	ENSMUST00000035983	AC124828	all_mr22_fl	GGTGGTCTTCAAGTTACCCTGG	chr5:147143984-147144446	all_mr22_r1	f
Rpl21	ENSMUST00000035983	AC124828	all_mr22_r1	CCTCTTAGCAAAAGAGGCCAAA	chr5:147143984-147144446	all_mr22_fl	r
Cdh8	ENSMUST00000067860 ENSMUST00000067839 ENSMUST00000093249	AC162867	no_mr01_fl	AGGTTCCAGAGATAGGAACCCA	chr8:102300000-102303000	no_mr01_r1	f
Cdh8	ENSMUST00000067860 ENSMUST00000067839 ENSMUST00000093249	AC162867	no_mr01_r1	GGCCACCATCTGATTTAGCA	chr8:102300000-102303000	no_mr01_fl	r
Slc26a9	ENSMUST00000049027	AC161805	no_mr02_fl	CCGCTGAATGTGACCTATTGTC	chr1:133571000-133572000	no_mr02_r1	f
Slc26a9	ENSMUST00000049027	AC161805	no_mr02_r1	AAGGTCCCAAATGAACAGCC	chr1:133571000-133572000	no_mr02_fl	r
Rp2h	ENSMUST00000067979 ENSMUST00000033372	BX294384	no_mr04_fl	TCCCAGCAGCTCTTACCACATG	chrX:19531200-19532000	no_mr04_r1	f
Rp2h	ENSMUST00000067979 ENSMUST00000033372	BX294384	no_mr04_r1	TCAACCAACACTTGGATACCCA	chrX:19531200-19532000	no_mr04_fl	r
XP_916743.1	ENSMUST00000062483	AC122287	no_mr05_fl	GCACCCAGGCATTTTCTTTCA	chr1:120718500-120719500	no_mr05_r1	f
XP_916743.1	ENSMUST00000062483	AC122287	no_mr05_r1	TGTGTGTCAGTTCGGAGCTGAG	chr1:120718500-120719500	no_mr05_fl	r
Dock7	ENSMUST00000030282 ENSMUST00000097962 ENSMUST00000075836	AL935325	no_mr06_fl	TCTCCTGCCAACCTTGTGTGT	chr4:98522600-98523600	no_mr06_r1	f

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Dock7	ENSMUST00000030282	AL935325	no_mr06_rl	AATTTGGAACTTCTCCCTCCTG	chr4:98522600-98523600	no_mr06_fl	r
	ENSMUST00000097962						
	ENSMUST00000075836						
Syt6	ENSMUST00000090697	AC123057	no_mr07_fl	GCTGCTAAAGGCAGAAATGTGG	chr3:103704000-103706000	no_mr07_rl	f
	ENSMUST00000098785						
Syt6	ENSMUST00000090697	AC123057	no_mr07_rl	AATGGAAAAGGCGCTCTGG	chr3:103704000-103706000	no_mr07_fl	r
	ENSMUST00000098785						
Zswim6	ENSMUST00000052377	CT572986	no_mr08_fl	GTTTCTGGCTCCGGTTGTATTG	chr13:108908700-108910100	no_mr08_rl	f
Zswim6	ENSMUST00000052377	CT572986	no_mr08_rl	TGTGTGCAGAAGCTGACCTCT	chr13:108908700-108910100	no_mr08_fl	r
Calb2	ENSMUST00000003754	AC163615	no_mr09_fl	CATCTGATGCAATCCGCCA	chr8:113051000-113051600	no_mr09_rl	f
Calb2	ENSMUST00000003754	AC163615	no_mr09_rl	AATCTTCCCAATTCCCACA	chr8:113051000-113051600	no_mr09_fl	r
Foxj1	ENSMUST00000036215	AL645861	no_mr10_fl	AATCTCCTCTTCCCACCCAAAC	chr11:116149000-116151000	no_mr10_rl	f
	ENSMUST00000078514						
Foxj1	ENSMUST00000036215	AL645861	no_mr10_rl	CTCCTTATTCAATGCCTTTGCC	chr11:116149000-116151000	no_mr10_fl	r
	ENSMUST00000078514						

Table A 5. List of primers used for ChIP-chip verification, analysis of TF project

All primers are exon spanning. f - forward primer; r – reverse primer

MGI Symbol	ID	Primer Name	Sequence	Partner Primers	Orientation
Acta2	ENSMUSG00000035783	Vmf-f1	cagaggaatgcagtggaagaga	Vmf-r1	f
Acta2	ENSMUSG00000035783	Vmf-r1	gaagctggccgttactctaa	Vmf-f1	r
B2m	ENSMUSG00000060802	B2m-f1	tgc caa acc ctc tgt act tct	B2m-r1	f
B2m	ENSMUSG00000060802	B2m-r1	tta ggc ctc ttt gct tta cca	B2m-f1	r
Csm	ENSMUSG00000015365	Csm_f1	ccaagctctgtgcctctgacta	Csm_r1	f
Csm	ENSMUSG00000015365	Csm_r1	ggttttcattgagcactgggtt	Csm_f1	r
Ctnna3	ENSMUSG00000060843	Ctnna3-f1	gccagagattagataccacca	Ctnna3-r1	f
Ctnna3	ENSMUSG00000060843	Ctnna3-r1	ggcagtccttttagctgagca	Ctnna3-f1	r
Des	ENSMUSG00000026208	Des-f1	gtgactgaagctgtgcgtgtc	Des-r1	f
Des	ENSMUSG00000026208	Des-r1	cccagctaggaagcaaggtaca	Des-f1	r
Dpf3	ENSMUSG00000046841	Dpf3_f1	gccagagctaatgaatggg	Dpf3_r1	f
Dpf3	ENSMUSG00000046841	Dpf3_r1	cgtaggagacaacatgggaga	Dpf3_f1	r
Fos	ENSMUSG00000021250	cFos-f1	accctaagatcccaaatgtg	cFos-r1	f
Fos	ENSMUSG00000021250	cFos-r1	tcgtcaactctacccccag	cFos-f1	r
Gata4	ENSMUSG00000021944	Gata4-f1	ccgcttttgactttggccta	Gata4-r1	f
Gata4	ENSMUSG00000021944	Gata4-r1	cctcccaacaatccaaacg	Gata4-f1	r
Hand2	ENSMUSG00000038193	dHand-f1	cactcctcactgacagacca	dHand-r1	f
Hand2	ENSMUSG00000038193	dHand-r1	gccacctacagaacgctatcct	dHand-f1	r
Tbx20	ENSMUSG00000031965	Tbx20_peak1_f1	ccaaatagccctggaagtgaga	Tbx20_peak1_r1	f
Tbx20	ENSMUSG00000031965	Tbx20_peak1_r1	tgctgagccagaaaatgtctga	Tbx20_peak1_f1	r
Tbx20	ENSMUSG00000031965	Tbx20_peak2_f1	tccaagcccctttcttctcta	Tbx20_peak2_r1	f
Tbx20	ENSMUSG00000031965	Tbx20_peak2_r1	caccctaategccgactatca	Tbx20_peak2_f1	r
Tbx20	ENSMUSG00000031965	Tbx20_peak3_f1	ccctcaccgctcatctctcttt	Tbx20_peak3_r1	f
Tbx20	ENSMUSG00000031965	Tbx20_peak3_r1	aattgagactggcaagcctcc	Tbx20_peak3_f1	r
Tbx20	ENSMUSG00000031965	Tbx20_peak4_f1	gaaacgatcatcacagccaac	Tbx20_peak4_r1	f
Tbx20	ENSMUSG00000031965	Tbx20_peak4_r1	taccctgaggcgattcctctct	Tbx20_peak4_f1	r

Table A 6 . List of primers used to determine siRNA knock-down, analysis of TF project

All primers are exon spanning. f - forward primer; r – reverse primer

MGI Symbol	ID	Primer Name	Sequence	Partner Primers	Orientation
Gata4	ENSMUSG00000021944	gata4-m-exp-f.2	ctcctactccagcccctacc	gata4-m-exp-r.2	f
Gata4	ENSMUSG00000021944	gata4-m-exp-r.2	gccccacaattgacacactc	gata4-m-exp-f.2	r
Hprt1	ENSMUST00000026723	hprt_m_f	AAACAATGCAAACCTTTGCTTCC	hprt_m_r	f
Hprt1	ENSMUST00000026723	hprt_m_r	GGTCCTTTTCACCAGCAAGCT	hprt_m_f	r
Mef2a	ENSMUSG00000030557	mef2a_rt_m_f	atgggtgtgagagccctgatg	mef2a_rt_m_r	f
Mef2a	ENSMUSG00000030557	mef2a_rt_m_r	agaagtctgaggtggcaagc	mef2a_rt_m_f	r
Nkx2.5	ENSMUSG00000015579	nkx2.5-m-exp-f.3	ctccgatccatcccacttta	nkx2.5-m-exp-r.3	f
Nkx2.5	ENSMUSG00000015579	nkx2.5-m-exp-r.3	agtgtggaatccgctcgaag	nkx2.5-m-exp-f.3	r
Srf	ENSMUSG00000015605	srf_m_f1	gcttcaccagatggctgtgata	srf_m_r1	f
Srf	ENSMUSG00000015605	srf_m_r1	aataagtgggccctccttg	srf_m_f1	r
Tbx20	ENSMUSG00000031965	tbx20_m_f2	ctccaggctcactgacattga	tbx20_m_r2	f
Tbx20	ENSMUSG00000031965	tbx20_m_r2	aaggctgatcctgactctga	tbx20_m_f2	r

11.9 Appendix I: Accession IDs for Microarray Data

Histone project data was deposited at ArrayExpress:

E-TABM-182 for Histone ChIP data

E-TABM-183 for Expression array data on NimbleGen arrays

ChIP-chip and expression array data of siRNA treated HL-1 cells from the investigation of Gata4, Mef2a, Nkx2.5, and Srf will be made publicly available after publication.