Supplement: Detection of RNA-DNA binding sites in long non-coding RNAs

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1 Materials

1.1 Cardiac Differentiation

Human pluripotent stem cells (hPSCs) were differentiated into cardiomyocytes as previously reported [1]. Cells were harvested at Day 0 (undifferentiated hPSCs) and day 4 (cardiac progenitors) and lysed in TRIzol (ThermoFisher). After RNA extraction, genomic DNA was removed by DNase digestion (ThermoFisher). Digested RNA was purified by ethanol precipitation and quality checked on a Bioanalyzer (Agilent 2100). Subsequently, 1 μg of RNA was depleted of ribosomal RNA (Ribo-Zero) and used for sequencing library prep. Sequencing was performed on an Illumina HiSeq 2000 with 60Mio paired-end 100 bp reads per sample.

Single-stranded RNA-Seq was measured for ES cells (Day 0) and after 96 hours (Day 4) in triplicates and with an average depth of 145 million reads per sample. Reads were aligned to HG38 genome with STAR [2] using GENCODE version 24 [3] as reference. Cuffdiff [4] was used to find differentially expressed genes (p-value < 0.001, absolute fold-change > 2, and the sum of expression > 5). This indicated 2,101 up-regulated genes and 2,317 genes as down-regulated. Of those up-regulated genes 75 genes were annotated in GENCODE as non-coding genes. We applied TDF promoter test on these 75 up-regulated non-coding genes to study their triplex-forming potential on the promoter regions of all up-regulated or down-regulated genes (Table S8).

1.2 *GATA6-AS* RACE and ASO experiments

Rapid amplification of cDNA ends (RACE) was performed using the SMARTer®RACE 5′/3′ Kit (Clontech) following the manufacturers instructions. Specific primers used for *GATA6-AS* amplification can be found in the Table S11. PCR amplicons were cloned into plasmids and sequenced (>10 plasmid clones each) following the manufacturers protocol.

hESCs were maintained in FTDA medium [5] on matrigel-coated 6-well plates. When reaching confluency, cells were passaged by washing 1x with PBS and dissociating with Accutase (ThermoFisher #A1110501) supplemented with 10 μM ROCK-inhibitor (Y−27632, Stemgent) at 37°C for 10 minutes. The reaction was stopped with 2 volumes F12/DMEM (ThermoFisher).

300,000−350,000 cells were centrifuged at 300x g for 2 minutes, suspended in 2 ml FTDA + 10 μM ROCK-inhibitor and cultured at 37°C and 5% CO_2. Media was changed in 24 h intervals with increasing FTDA volumes until cells reached confluency again.

150,000 cells were seeded in 1ml of FTA (FTDA without Dorsomorphine) supplemented with 10 μM ROCK inhibitor. On the next day the media was changed prior to transfection (800 μl FTA). Cells were transfected with 600 ng oligos and 600 ng of a control plasmid expressing GFP using a standard FUGENE protocol (oligo sequences are provided in Table S12). 6 hours after transfection, mesoderm differentiation was induced by adding 200 μl FTA + 3.75 ng/ml BMP−4 (R&D #314−BP−010) + 1.875 μM CHIR (Axon #1386). 18 hours after mesoderm induction medium was changed to transferrin/selenium (TS) medium (KO DMEM(ThermoFisher #10829-018), 1x TS (100x: 5.5 μg/ml Transferrin (Sigma #T8158), 6.7 ng/ml Sodium Selenite (Sigma #S5261) in PBS (Millipore #BSS-1006-B)), 250 μM 2-Phospho-L-Ascorbic Acid (Sigma #49752), 1x Penicillin/Streptomycin/Glutamine (ThermoFisher #10378016)). On the next day cells were collected in TRIZOL (ThermoFisher #15596018) and RNA was isolated according to the manufacturers protocol.

Following DNaseI-treatment, 500 ng RNA was reverse transcribed using MMLV reverse transcriptase (Promega #M170B). qPCR was performed using 1:8 diluted cDNA, 2x ORA™SEE qPCR Green ROX L Mix (highQu #QPD0505) and 5 μM primers (protocol and a list of used primers are provided in Table S13 and S14).

1.3 Chromatin Oligo affinity Precipitation (ChOP)

The ChOP assay was performed as described before with some modifications [6]. Human ES cells (undifferentiated or day 4 of differentiation, 20 x 106 cells for each) were cross-linked using formaldehyde for 10 min at room temperature followed by quenching with 0.125M glycine. After centrifugation at 1000g at 40°C for 10 min, cells were resuspended in 2ml of buffer A (3mM $MgCl_2$, 10mM Tris HCl, pH 7.4, 10mM NaCl, 0.5%v/v NP-40, 0.5mM PMSF and 100 units/ml RNasin) and incubated on ice for 20 min. Nuclei were harvested by centrifugation and resuspended in 1.2ml of buffer B (50mM Tris HCl, pH 7.4, 10mM EDTA, 0.5% Triton X-100, 0.1%SDS, 0.5mM PMSF and 100 units/ml RNasin) and incubated on ice for 40 min. An equal volume of buffer (15mM Tris HCl, pH 7.4, 150mM NaCl, 1mM EDTA, 1% Triton X-100, 0.5mM PMSF and 100 units/ml RNasin) was then added and incubated on ice for 15 min. Samples were sonicated using a Bioruptor sonicator (Diagenode) for 40 cycles (30 sec on, 30 sec off at High Pulse). Ten different oligos complimentary to *GATA6-AS* RNA were pooled with a final concentration of 10μM and then used for the RNA pull down. As a control, a pool of 10 oligos in reverse orientation to the *GATA6-AS* RNA targeting oligos was used. The oligos were added to the chromatin solution along with yeast tRNA (100μg/ml), salmon sperm DNA (100μg/ml), BSA (400μg/ml). After incubation overnight at 40°C, samples were then incubated with streptavidin agarose beads for 3 hours followed by two washes of low salt buffer (20mM Tris HCl, pH7.9, 150 mM NaCl, 2mM EDTA, 0.1% SDS, 1% TritonX-100, 0.5mM PMSF and 50 units/ml RNasin), two washes of high salt buffer (20mM Tris HCl, pH7.9, 500mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100, 0.5mM PMSF and 50 units/ml RNasin) and one wash of 1x PBS. The beads were then incubated with elution buffer (1%SDS, 100 mM $NaHCO_3$) for 45 min with intermittent mixing at 65°C followed by digestion

with proteinase K (80 μg/ml) and phenol/chloroform extraction method for DNA isolation. The fold enrichment over input was calculated considering the percentage of input chromatin used for ChOP and the Ct values obtained for the target from input DNA and ChOP DNA as follows:

$$\text{Fold enrichment over input} = \% \text{ of input} \times 2^{\text{Ct(input)}-\text{Ct(ChOP)}}$$

These correspond to ChOP-PCR predictions shown in Fig. 4b. All the relevant oligo sequences are in Table S15, S16 and S17.

1.4 DBD-Capture-Seq

RNA-free genomic DNA from H1 ES cells was fragmented by sonication using Bioruptor Pico (Diagenode) to an average size of 200-300 bp. A total of 6-7 μg of fragmented DNA was incubated with 20 pmoles of biotin labeled-RNA oligonucleotides (Table S18) for 1 h at 37°C in 10 mM Tris-HCl (pH: 7.4), 50 mM KCl, 5 mM $MgCl_2$, 40U of RNasin (Promega).

RNA-DNA complexes were bound to MyOne Streptavidin C1 Dynabeads (Invitrogen) and washed three times in a buffer containing 10 mM Tris-HCl (pH: 7.4), 50 mM KCl, 5 mM MgCl2, 0.05% Tween-20. RNA-associated DNA was eluted with 25 ng/μl RNase A and 5 mU/μl of RNase I for 30 min at 37°C and recovered by phenol/chloroform extraction. Control experiments were based on the same protocol without the inclusion of an oligonucleotide. DNA libraries were prepared from two independent experiments using the NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB) and NEBNext Multiplex Oligos for Illumina (NEB) and sequenced on a NexSeq500 Illumina platform.

1.4.1 TRIPLEXES AND TDF - Implementation details

TDF is implemented in Python and based on the regulatory genomics toolbox (www.regulatory-genomics.org). TRIPLEXES is integrated directly into TDF via Python bindings. TDF is executed via the command line interface and requires as input FASTA files of lncRNAs and BED files of target regions (for the genomic region test) or a list of genes (for the promoter test).

All the results are reported in HTML interfaces. This output includes ranking of evaluated lncRNAs, listing of potential DBDs per lncRNA, and ranking of target DNA regions. TDF indicates the genes associated with the promoters or genes in the vicinity of the genomic regions, i.e., the closest upstream and downstream genes. TDF also provides sequences of all enriched DBDs, as well as BED files with the location of all DBDs and TTSs. These data allow users to explore interesting loci and triple helices in genome browsers.

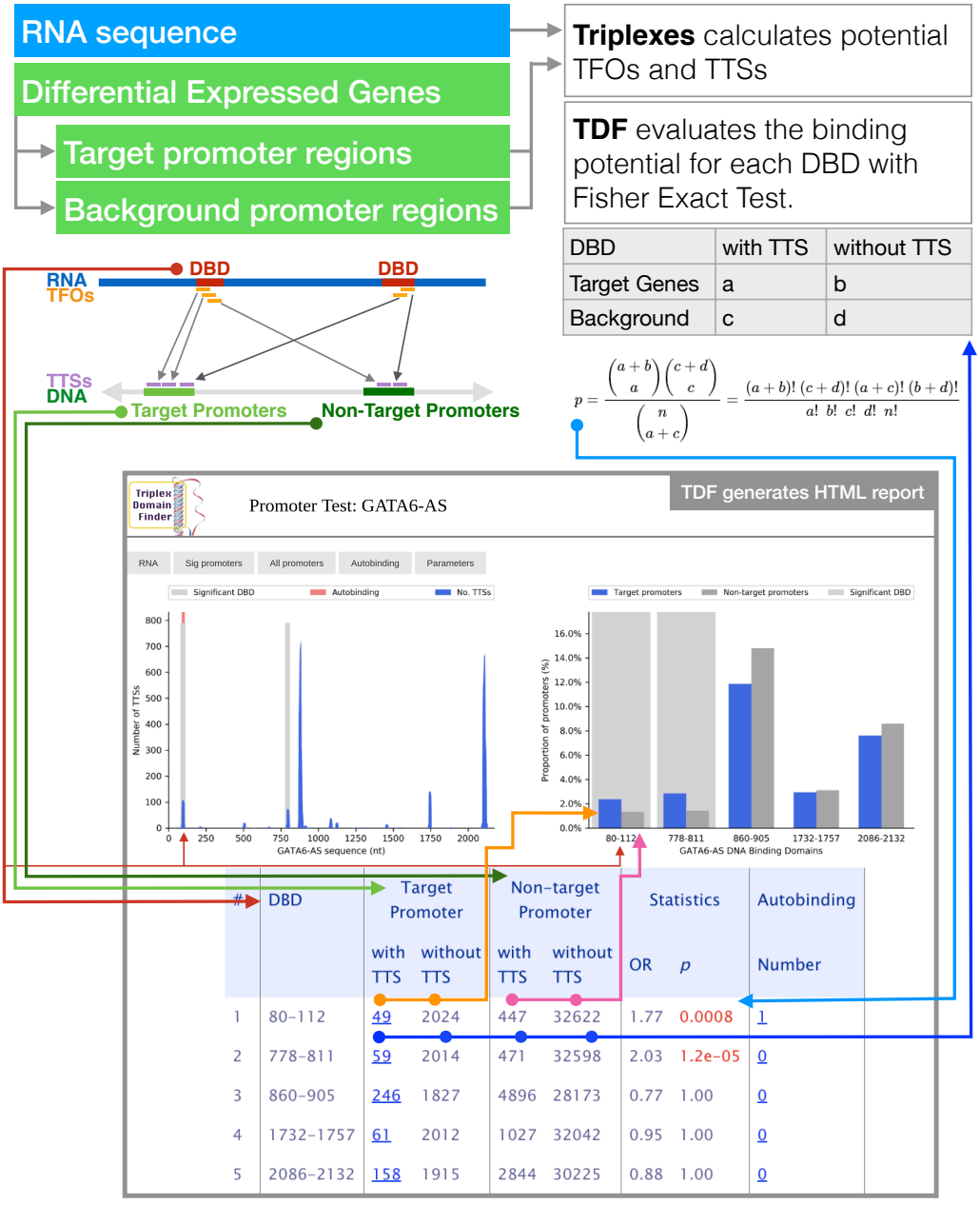

Figure S1: The scheme of TDF promoter test using *GATA6-AS* as the example. Promoter test requires RNA sequence and differentially expressed genes as the inputs. First, TDF uses TRIPLEXES to obtain all triple helices on target promoters and the non-target promoters. Next, it defines all DNA binding domains (DBD) by finding regions with contiguous TFOs (marked in red). For each DBD, TDF performs a Fisher exact test contrasting the amount of target vs. non-target promoters with at least one TTS. Arrows indicates the relation of the statistics and graphical representation of TDF report.

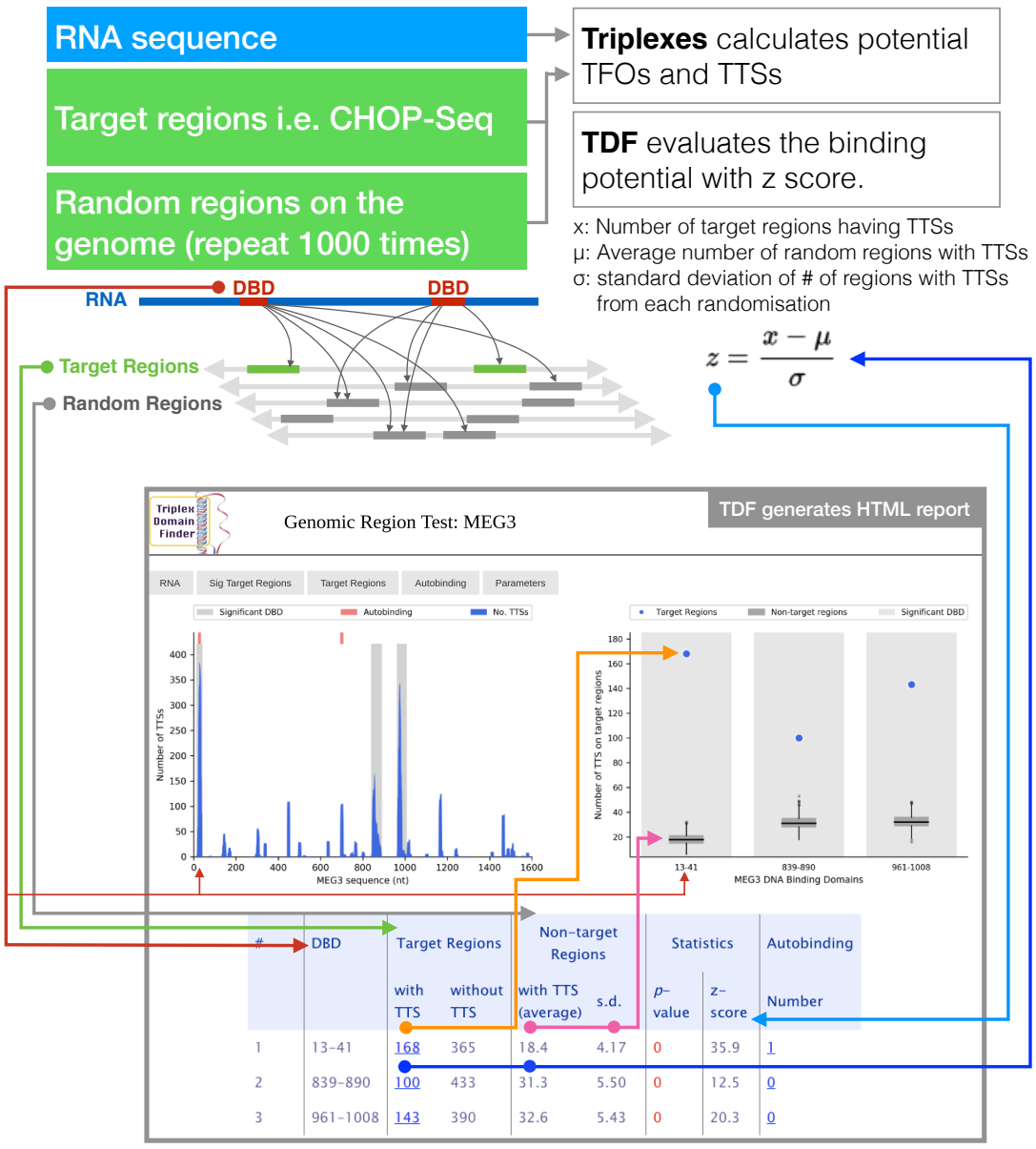

Figure S2: The scheme of TDF genomic region test using *MEG3* as the example. Genomic region test takes a RNA sequence and the target DNA regions as inputs. It generates random genomic regions with same size as the target regions (for 1,000 times). First, TRIPLEXES detects the triple helices on the target regions and all the random regions. Next, it defines all DNA binding domains (DBD) by finding regions with contiguous TFOs (marked in red) from triplexes predicted in target regions. Then it estimates an empirical *p*-value by evaluating the proportion of times a higher number of TTS is found in random regions than in the target regions. Arrows indicates the relation of the statistics and graphical representation of TDF report.

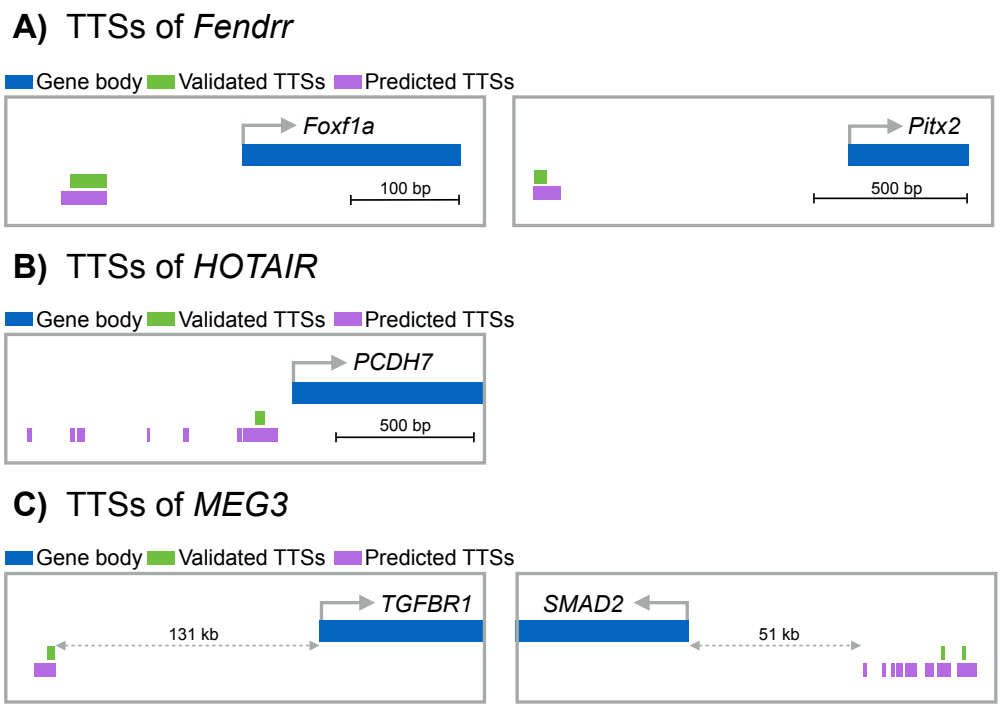

Figure S3: Locations of triple helices target sites (TTS) predicted by TDF in the targets of *Fendrr*, *HOTAIR* and *MEG3*.

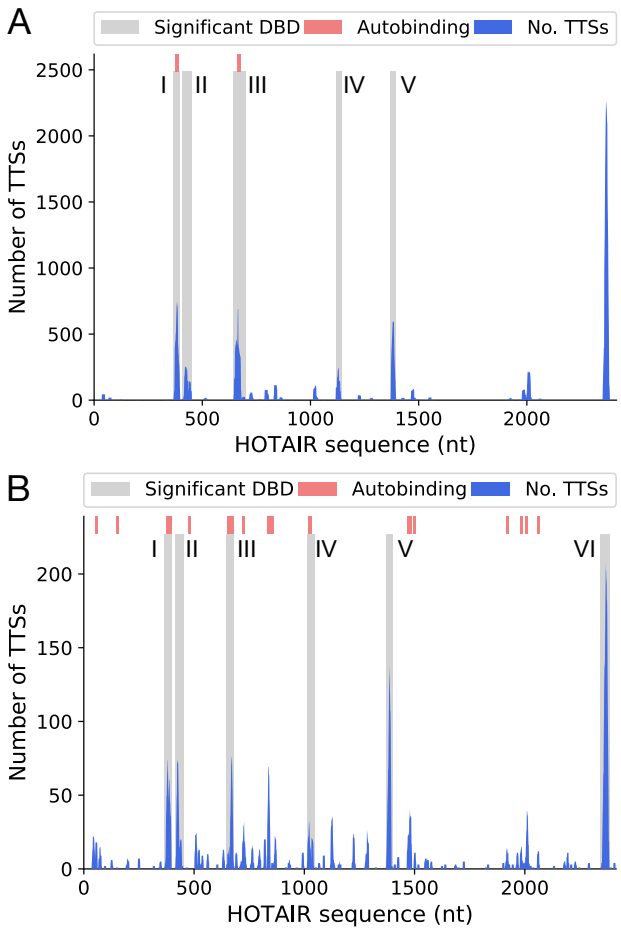

Figure S4: The coverage of triple helices (y-axis) within *HOTAIR* sequences (x-axis). **(A)** TDF promoter test on the up-regulated genes in knockdown of *HOTAIR*. Regions highlighted in gray indicate significant DBDs. Four out of five DBDs (I, III, IV and V) coincide with the ones detected with *HOTAIR* ChiRP-seq data in [7]. **(B)** TDF genomic region test on the *HOTAIR*-associated DNA regions from GRID-Seq. Regions highlighted in gray indicate significant DBDs. Five out of six DBDs (I, III, IV, V and VI) coincide with the ones detected with *HOTAIR* ChiRP-seq data.

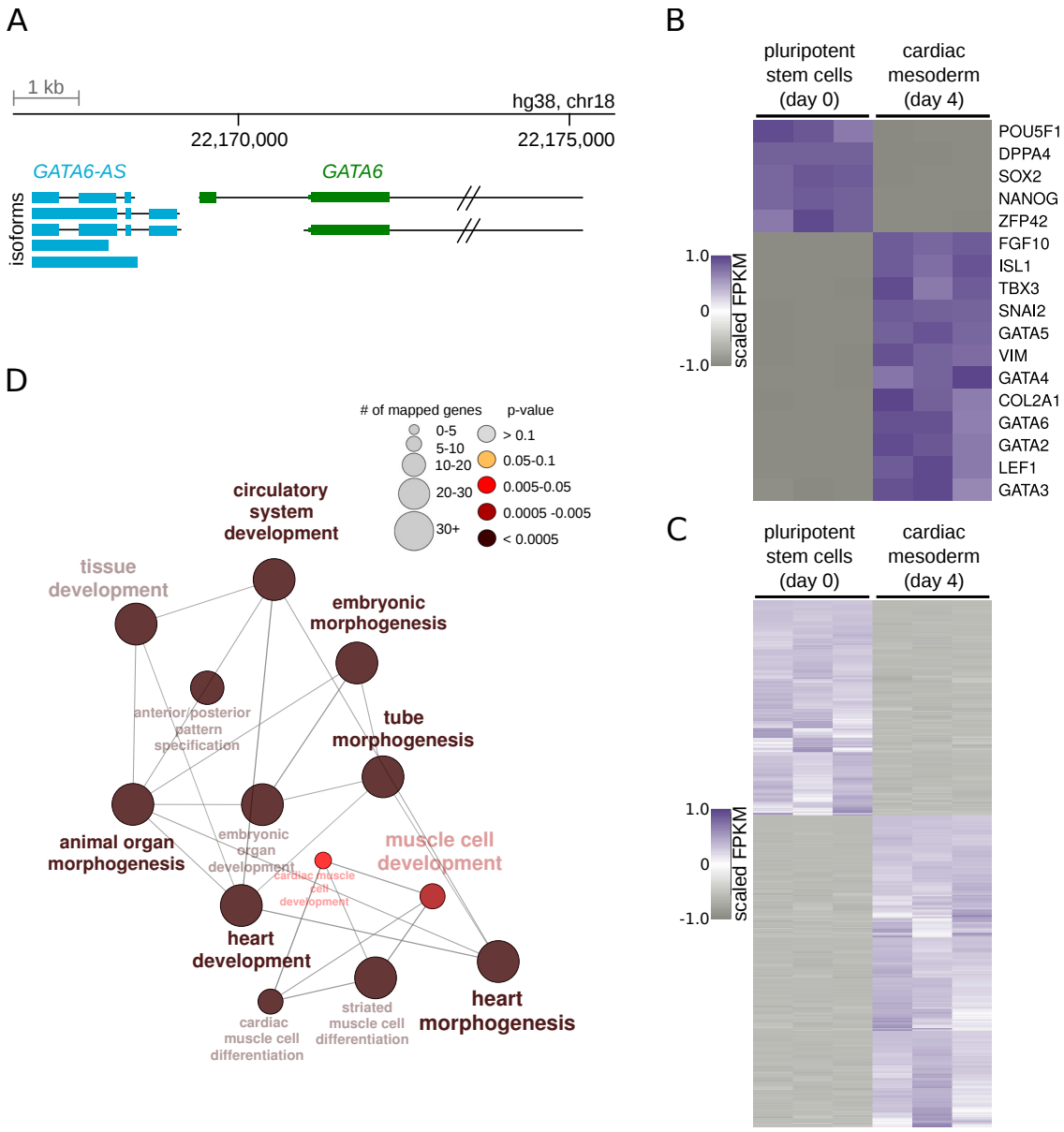

Figure S5: (A) Genome Browser view of the *GATA6-AS*/*GATA6* loci, depicting all experimentally confirmed isoforms for *GATA6-AS* (confirmed by 5′ and 3′ RACE-PCR using total RNA from day 4 cardiac mesoderm). (B) Heatmap depicting the expression profile of key markers for pluripotency and cardiac mesoderm. (C) Heatmap depicting expression profile of the differentially expressed genes between pluripotency and cardiac mesoderm (Log2FC ≥ 2 or $\leq$ -2). (D) Functional enrichment analysis using GO annotations of the differentially expressed genes.

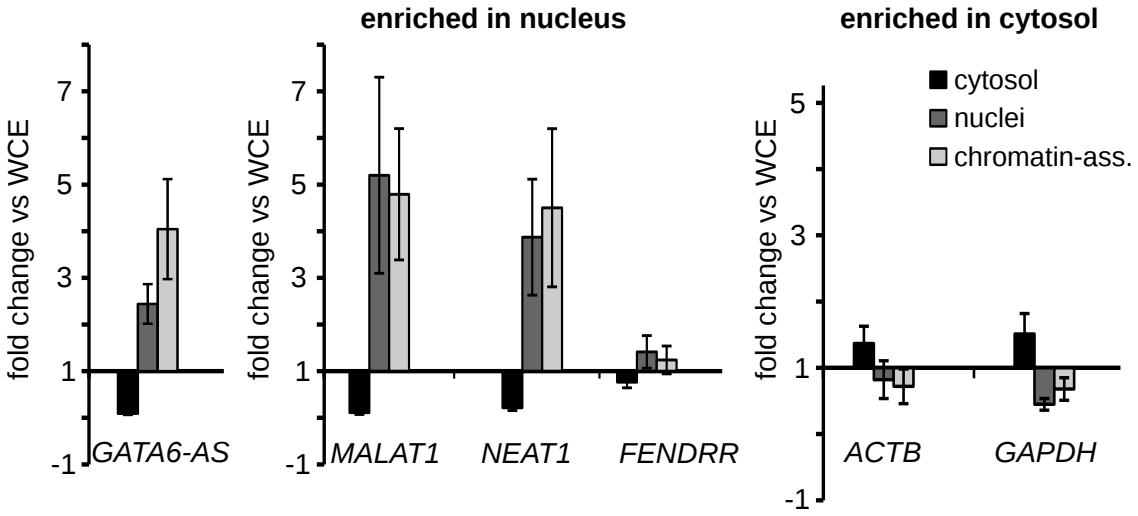

Figure S6: Bar graph representing the RT-qPCR results upon sub-cellular fractionation for *GATA6-AS*, along with nuclear enriched transcripts (*MALAT1*, *NEAT1* and *FENDRR*) and cytosol enriched transcripts (*ACTB* and *GAPDH*) of the indicated transcripts in different cellular fractions. WCE stands for whole cell extract.

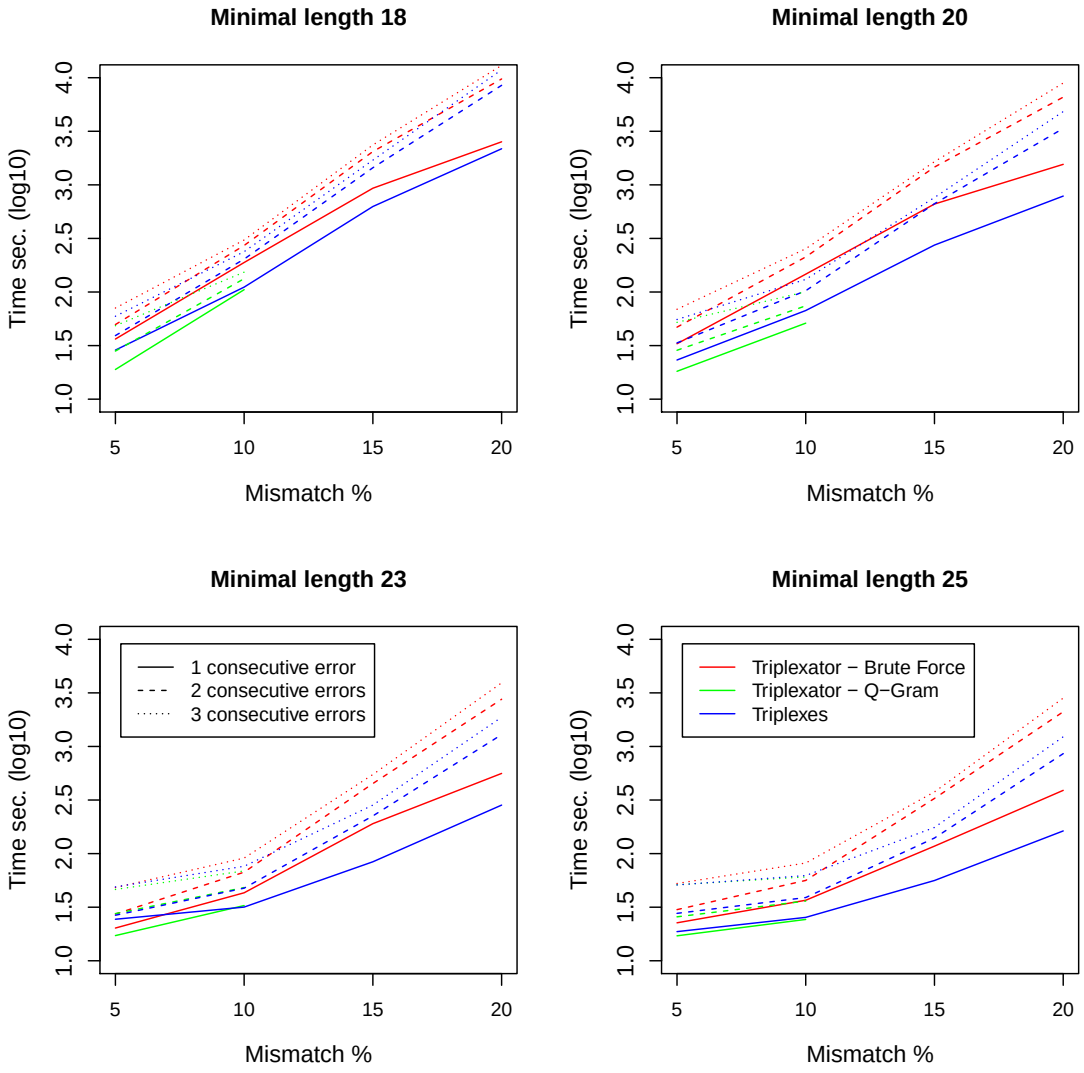

Figure S7: The time performances of TRIPLEXATOR (brute force and q-gram) and TRIPLEXES for varying minimum length, maximum mismatch rates and maximum number of consecutive errors.

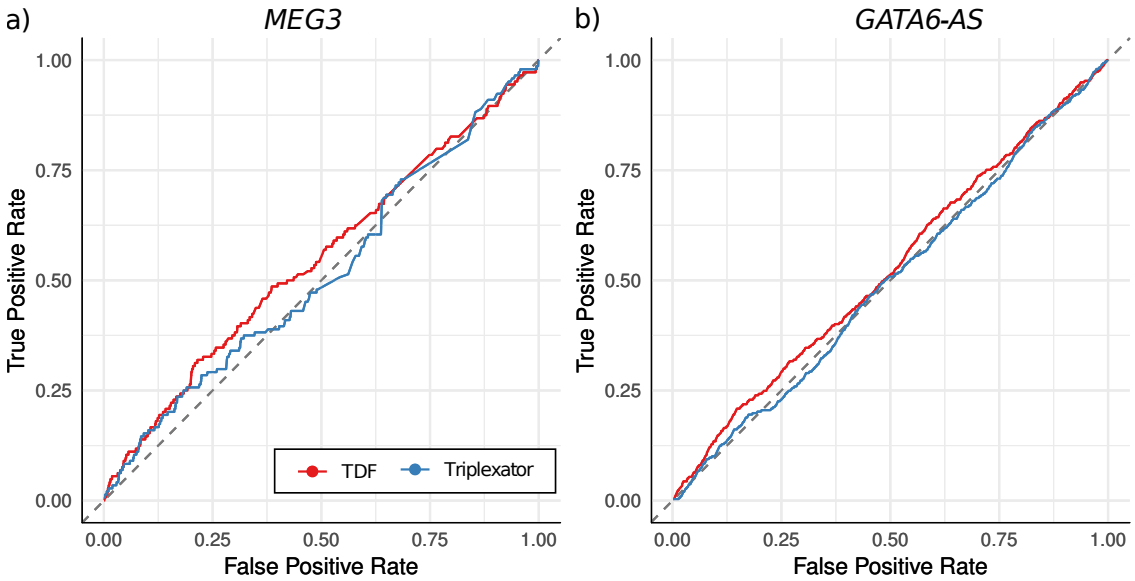

Figure S8: The Receiver Operating Characteristics (ROC) plots of comparison of TDF and TRIPLEXATOR for *MEG3* and *GATA6-AS*. TDF has an area under the curve of 0.52 for *GATA6-AS* and 0.56 for *MEG3*, while TRIPLEXATOR has AUC of 0.49 for *GATA6-AS* and 0.52 for *MEG3*.

DBD Position	Target Promoters TTS	Target Promoters no TTS	Non-Target Promoters TTS	Non-Target Promoters no TTS	Statistics Odds ratio	Statistics p-value
1502-1565	286	1091	5964	27022	1.19	0.0069

Table S1: Candidate DBDs from *Fendrr* ranked by p-values. The significant DBD is shown in bold.

NO.	TTSs counts		Sum of ranks	
1	*E130309D14Rik*	146	***Foxf1***	107
2	***Pitx2***	126	*1700123M08Rik*	133
3	*Psmg3*	124	*Lpxn*	151
4	*Cntnap2*	101	*CFTR*	156
5	*Ppapdc3*	91	*Hoxb3*	221
6	*Hmgcll1*	71	*4930570G19Rik*	224
7	*Cd79b*	40	*9330159F19Rik*	241
8	*Fam19a5*	39	*Prelp*	242
9	*D330050G23Rik*	38	*Arntl2*	249
10	*Setmar*	32	*Rnf208*	269

Table S2: Top 10 gene promoters based on different ranking strategies of *Fendrr*. The target genes which are mentioned in the main text are shown in bold.

DBD Position	Target Promoters TTS	Target Promoters no TTS	Non-Target Promoters TTS	Non-Target Promoters no TTS	Statistics Odds ratio	Statistics p-value
366-398	318	666	6426	22919	1.70	6.7e-13
1117-1143	154	830	2520	26825	1.98	5.5e-12
409-452	268	716	5982	23363	1.46	6.8e-07
1369-1396	363	621	8807	20538	1.36	6.5e-06
643-702	281	703	7107	22238	1.25	0.0019
2347-2387	612	372	17559	11786	1.10	0.10
830-847	98	886	2905	26440	1.01	0.56
1977-2027	172	812	6453	22892	0.75	1.00

Table S3: Candidate DBDs from *HOTAIR* on the up-regulated genes in knockdown experiment ranked by p-values. The significant DBDs are shown in bold.

NO.	Gene	TTSs counts	TTS coverage
1	*COL6A1*	188	0.53
2	*DCHS1*	47	0.35
3	*PAPPA*	89	0.32
4	*KLK4*	73	0.29
5	*RSPO3*	60	0.25
6	***PCDH7***	72	0.24
7	*PBX2*	52	0.23
8	*IL11*	30	0.23
9	*ADAMTS9*	41	0.22
10	*PRDM12*	26	0.21

Table S4: Top 10 gene promoters based on ranking of TTS coverage. The target gene mentioned in the main text is shown in bold.

	Target Regions		Random Regions		Statistics	
DBDs position	TTS	no TTS	TTS (average)	s.d.	p-value	z-score
13-41	168	365	17.8	4.13	0	36.4
961-1008	143	390	31.8	5.47	0	20.3
839-890	100	433	30.6	5.41	0	12.8

Table S5: Candidate *MEG3* DBDs ranked by z-score. The significant DBDs are shown in bold.

#	DBD	Target Regions		Non-target Regions		Statistics		Autobinding
		with TTS	without TTS	with TTS (average)	s.d.	p-value	z-score	Number
1	9-41 (Domain I)	2660	2340	256.2	16.4	0	146.2	1
2	1458-1498	1297	3703	67.9	8.71	0	141.1	0
3	954-1032	2931	2069	461.4	20.1	0	122.9	0
4	1159-1190	2023	2977	256.3	15.1	0	116.7	0
5	130-156	984	4016	94.4	8.12	0	109.6	0
6	629-644	859	4141	53.3	7.48	0	107.7	0
7	492-512	492	4508	19.8	4.59	0	103.0	0
8	839-890 (Domain II)	2292	2708	441.5	18.7	0	99.1	0
9	1499-1525	769	4231	55.0	7.23	0	98.8	0
10	161-179	707	4293	48.6	7.08	0	93.0	0
11	327-346	563	4437	41.5	6.08	0	85.8	0
12	284-319	1131	3869	196.1	12.8	0	72.9	0
13	442-458	1023	3977	161.7	12.3	0	70.1	0
14	737-776	962	4038	210.7	14.0	0	53.5	0
15	693-730	1587	3413	450.7	21.7	0	52.3	1
16	1091-1112	134	4866	5.82	2.64	0	48.5	0
17	1234-1254	409	4591	60.2	7.99	0	43.7	0
18	791-811	126	4874	6.63	2.82	0	42.3	0
19	517-533	72	4928	4.90	2.09	0	32.1	0
20	1280-1296	60	4940	4.24	2.09	0	26.6	0
21	1573-1588	183	4817	35.0	5.76	0	25.7	0
22	1405-1421	67	4933	10.5	3.12	0	18.1	0

Table S6: *MEG3* complete transcript on Domain I Capture-Seq peaks.

#	DBD	Target Regions		Non-target Regions		Statistics		Autobinding
		with TTS	without TTS	with TTS (average)	s.d.	p-value	z-score	Number
1	839-890 (Domain II)	1448	3552	327.8	19.7	0	57.0	0
2	1458-1498	361	4639	48.8	5.52	0	56.6	0
3	961-1032	1270	3730	343.6	17.6	0	52.7	0
4	11-41 (Domain I)	893	4107	190.8	15.0	0	46.7	1
5	1499-1522	293	4707	40.4	5.69	0	44.4	0
6	1234-1254	308	4692	42.5	6.11	0	43.5	0
7	1159-1190	764	4236	191.0	13.6	0	42.2	0
8	1405-1421	90	4910	6.94	2.28	0	36.5	0
9	629-644	241	4759	38.3	5.64	0	36.0	0
10	130-156	290	4710	69.0	7.84	0	28.2	0
11	161-179	174	4826	35.4	5.46	0	25.4	0
12	492-510	78	4922	14.2	3.74	0	17.1	0
13	290-319	320	4680	145.1	11.4	0	15.3	0
14	327-346	99	4901	29.4	5.60	0	12.4	0
15	693-730	462	4538	335.5	18.2	0	6.94	1
16	737-776	224	4776	154.4	13.2	0	5.26	0
17	442-458	169	4831	117.6	11.9	0	4.33	0

Table S7: *MEG3* complete transcript on Domain II Capture-Seq peaks.

RNA	Length	FC*	Norm TTS*	Norm DBD	Sig. DBD	Target Genes	Rank FC	Rank TTS	Rank DBD	Rank Sum
GATA6-AS	2175	6.22	740.7	2.3	2	**Up Genes**	9	1	23	1
JHDM1D-AS1	2379	3.51	3430	1.68	2	Up Genes	31	9	4	2
HOXB-AS1	1550	7.34	582.6	1.94	1	Up Genes	3	4	37	3
HOXB-AS1	1550	7.34	552.9	1.94	1	Down Genes	3	4	39	4
GATA6-AS	2175	6.22	747.6	1.38	1	Down Genes	9	23	22	5
LINC00261	5545	4.87	2409	1.08	1	Up Genes	15	35	8	6
KC6	5167	4.41	844.2	1.35	3	Up Genes	19	24	19	7
SEC24B-AS1	2572	2.72	736	1.56	1	Up Genes	39	12	24	8
LENG8-AS1	1246	1.97	687.8	1.61	1	Up Genes	69	10	27	9
PCAT7	1161	1.51	3801	1.72	1	Up Genes	101	6	3	10
ID2-AS1	2049	3.73	521.7	0.98	1	Up Genes	27	47	40	11
LENG8-AS1	1246	1.97	582.7	1.61	1	Down Genes	69	10	36	12
MALAT1	8754	3.06	301.2	1.49	1	Down Genes	37	16	64	13
ID2-AS1	2049	3.73	433.4	0.98	1	Down Genes	27	47	44	14
MALAT1	8754	3.06	317.6	1.26	5	Up Genes	37	27	60	15
NEAT1	22766	1.69	2593	1.14	13	Up Genes	85	34	6	16
HAND2-AS1	9817	7.32	261.6	0.92	5	Up Genes	5	52	71	17
LINC00890	4756	4.43	312.9	0.84	1	Up Genes	17	53	61	18
HAND2-AS1	9817	7.32	266.1	0.81	1	Down Genes	5	56	70	19
ST7-AS1	1888	1.74	1018	1.06	1	Down Genes	81	40	16	20
ST7-AS1	1888	1.74	931.1	1.06	1	Up Genes	81	40	17	21
LINC01089	4123	2.00	378.9	1.46	3	Up Genes	67	19	53	22
LINC01572	4732	1.50	651.5	1.69	3	Up Genes	103	8	29	23
LINC00890	4756	4.43	254.2	0.84	1	Down Genes	17	53	73	24
NEAT1	22766	1.69	1315	1.01	2	Down Genes	85	44	14	25
BAIAP2-AS1	4771	1.88	329.1	1.47	6	Up Genes	75	18	57	26
LINC01572	4732	1.50	588.1	1.48	1	Down Genes	103	17	35	27
MEF2C-AS1	5786	4.05	406.2	0.52	1	Down Genes	23	89	46	28
ZNF436-AS1	2653	4.35	164.3	0.75	2	Up Genes	21	65	84	29
BAIAP2-AS1	4771	1.88	284.6	1.26	5	Down Genes	75	27	68	30
ZNF436-AS1	2653	4.35	145.5	0.75	1	Down Genes	21	65	86	31
MEF2C-AS1	5786	4.05	454.4	0.35	2	Up Genes	23	107	43	32
SNAI3-AS1	5032	1.63	216.4	1.19	6	Up Genes	91	30	78	33
RNF139-AS1	6941	1.92	401.2	0.58	1	Down Genes	71	84	49	34
RNF139-AS1	6941	1.92	391.4	0.58	1	Up Genes	71	84	52	35
LINC00894	6300	3.64	127.8	0.48	3	Up Genes	29	94	88	36
SNAI3-AS1	5032	1.63	175.1	0.79	2	Down Genes	91	60	82	37
LINC00894	6300	3.64	98.7	0.32	1	Down Genes	29	108	98	38
FTX	6118	1.00	1109	0.65	1	Up Genes	147	75	15	39
CKMT2-AS1	3874	1.00	604.3	0.77	1	Up Genes	149	63	34	40
LINC01138	3930	1.24	113	1.02	2	Up Genes	117	42	91	41
PWRN1	4809	1.55	232.9	0.62	1	Down Genes	97	78	76	42
BAALC-AS1	3864	2.17	78.2	0.52	1	Up Genes	63	89	106	43
RGS5	3618	1.73	110.6	0.55	1	Up Genes	83	86	92	44
TCEB3-AS1	1342	1.63	67.1	0.75	1	Up Genes	89	65	113	45
SMC2-AS1	7616	1.48	245.7	0.53	2	Up Genes	107	88	74	46
SNHG18	1949	1.58	141.6	0.51	1	Up Genes	95	91	87	47
N4BP2L2-IT2	4889	2.20	64.6	0.41	1	Up Genes	61	98	114	48
ZNF529-AS1	1861	1.05	94	1.07	1	Up Genes	141	39	102	49
SDCBP2-AS1	4051	1.01	276.5	0.74	2	Up Genes	145	69	69	50
SDCBP2-AS1	4051	1.01	256.5	0.74	1	Down Genes	145	69	72	51
ZBTB11-AS1	2742	1.13	99.9	0.73	1	Up Genes	129	71	97	52
LINC00960	3175	1.22	70.6	0.63	1	Up Genes	121	77	109	53
A1BG-AS1	2789	1.18	124.1	0.36	1	Up Genes	125	105	89	54
DLGAP4-AS1	2425	1.10	67.2	0.41	1	Up Genes	137	98	112	55
LINC00261	5545	4.87	2226	1.08	0	Down Genes	15	35	9	56
JHDM1D-AS1	2379	3.51	2688	1.26	0	Down Genes	31	27	5	57

TMCC1-AS1	4619	3.33	4634	1.08	0	Up Genes	35	35	1	58
SEC24B-AS1	2572	2.72	793.5	1.56	0	Down Genes	39	12	20	59
TMCC1-AS1	4619	3.33	2532	1.08	0	Down Genes	35	35	7	60
KC6	5167	4.41	641.2	1.16	0	Down Genes	19	31	30	61
MANEA-AS1	2267	2.57	338.3	2.21	0	Down Genes	43	2	55	62
DICER1-AS1	3719	2.37	609	1.34	0	Up Genes	47	25	33	63
MANEA-AS1	2267	2.57	310.1	2.21	0	Up Genes	43	2	62	64
LINC01473	1444	3.37	374	1.39	0	Down Genes	33	21	54	65
PCAT7	1161	1.51	4039	1.72	0	Down Genes	101	6	2	66
DICER1-AS1	3719	2.37	577.6	1.34	0	Down Genes	47	25	38	67
LINC01473	1444	3.37	305.4	1.39	0	Up Genes	33	21	63	68
LINC01012	3486	1.61	1935	1.15	0	Up Genes	93	32	10	69
LINC01012	3486	1.61	1609	1.15	0	Down Genes	93	32	11	70
LINC01089	4123	2.00	322.6	1.46	0	Down Genes	67	19	58	71
RNU12	663	1.15	752.6	1.51	0	Down Genes	127	14	21	72
ACVR2B-AS1	2515	2.35	1574	0.4	0	Down Genes	51	102	12	73
ACVR2B-AS1	2515	2.35	1401	0.4	0	Up Genes	51	102	13	74
RNU12	663	1.15	660.6	1.51	0	Up Genes	127	14	28	75
LINC01125	6478	2.28	244.1	0.93	0	Down Genes	57	51	75	76
PPP1R26-AS1	3745	1.83	330.3	0.8	0	Down Genes	79	58	56	77
KCTD21-AS1	4090	1.44	468.5	0.98	0	Down Genes	109	47	42	78
INTS6-AS1	9842	1.91	427.6	0.61	0	Down Genes	73	81	45	79
NIFK-AS1	2546	1.54	477.6	0.79	0	Down Genes	99	60	41	80
PPP1R26-AS1	3745	1.83	290.3	0.8	0	Up Genes	79	58	66	81
KCTD21-AS1	4090	1.44	396.3	0.98	0	Up Genes	109	47	50	82
NIFK-AS1	2546	1.54	391.6	0.79	0	Up Genes	99	60	51	83
INTS6-AS1	9842	1.91	405.2	0.51	0	Up Genes	73	91	47	84
LINC01125	6478	2.28	221.5	0.62	0	Up Genes	57	78	77	85
GATA3-AS1	2212	5.56	88.2	0	0	Up Genes	13	115	103	86
PWRN1	4809	1.55	319.4	0.62	0	Up Genes	97	78	59	87
RGS5	3618	1.73	104.2	0.83	0	Down Genes	83	55	96	88
CKMT2-AS1	3874	1.00	727.4	0.77	0	Down Genes	149	63	26	89
GATA3-AS1	2212	5.56	70.1	0	0	Down Genes	13	115	110	90
PSMA3-AS1	7399	1.11	402.4	0.81	0	Down Genes	135	56	48	91
LINC01099	1587	6.91	57.3	0	0	Down Genes	7	115	117	92
PSMA3-AS1	7399	1.11	626	0.68	0	Up Genes	135	73	32	93
FTX	6118	1.00	914	0.65	0	Down Genes	147	75	18	94
LINC01099	1587	6.91	54.2	0	0	Up Genes	7	115	121	95
ZBED5-AS1	1991	1.12	294.3	1	0	Down Genes	133	45	65	96
OSER1-AS1	2180	1.18	728.9	0.46	0	Down Genes	123	95	25	97
TBX2-AS1	1200	7.46	43.3	0	0	Up Genes	1	115	128	98
LINC01440	3767	6.02	56.5	0	0	Up Genes	11	115	118	99
ZBED5-AS1	1991	1.12	288.3	1	0	Up Genes	133	45	67	100
LINC01440	3767	6.02	47.3	0.27	0	Down Genes	11	111	125	101
OSER1-AS1	2180	1.18	636.7	0.46	0	Up Genes	123	95	31	102
LINC01138	3930	1.24	105.3	1.02	0	Down Genes	117	42	94	103
TBX2-AS1	1200	7.46	23.3	0	0	Down Genes	1	115	140	104
RAB30-AS1	2424	1.85	167.9	0.41	0	Down Genes	77	98	83	105
RAB30-AS1	2424	1.85	162.5	0.41	0	Up Genes	77	98	85	106
TMEM161B-AS1	4423	1.26	199.4	0.68	0	Down Genes	113	73	80	107
SNHG18	1949	1.58	115.4	0.51	0	Down Genes	95	91	90	108
BAALC-AS1	3864	2.17	80	0.26	0	Down Genes	63	113	105	109
TCEB3-AS1	1342	1.63	44.7	0.75	0	Down Genes	89	65	127	110
LINC00648	3039	4.05	18.1	0	0	Down Genes	25	115	143	111
LINC00648	3039	4.05	17.8	0	0	Up Genes	25	115	144	112
ALOX12-AS1	3208	2.41	48.9	0	0	Up Genes	45	115	124	113
TAPT1-AS1	2758	2.69	41.3	0	0	Up Genes	41	115	131	114
N4BP2L2-IT2	4889	2.20	64.6	0.2	0	Down Genes	61	114	114	115
SMC2-AS1	7616	1.48	212.7	0.39	0	Down Genes	107	104	79	116
TMEM161B-AS1	4423	1.26	175.9	0.45	0	Up Genes	113	97	81	117

CAHM	895	2.04	69.3	0	0	Up Genes	65	115	111	118
TAPT1-AS1	2758	2.69	31.9	0	0	Down Genes	41	115	136	119
UBL7-AS1	1705	1.22	109.1	0.59	0	Up Genes	119	82	93	120
CAHM	895	2.04	60.3	0	0	Down Genes	65	115	116	121
ALOX12-AS1	3208	2.41	25.9	0	0	Down Genes	45	115	139	122
MIR210HG	2319	2.37	31.9	0	0	Up Genes	49	115	136	123
UBL7-AS1	1705	1.22	97.9	0.59	0	Down Genes	119	82	99	124
MIR210HG	2319	2.37	26.3	0	0	Down Genes	49	115	138	125
VIM-AS1	1987	2.29	33.2	0	0	Up Genes	53	115	135	126
ZBTB11-AS1	2742	1.13	80.6	0.73	0	Down Genes	129	71	104	127
PTOV1-AS1	4198	2.23	39.3	0	0	Up Genes	59	115	132	128
VIM-AS1	1987	2.29	19.1	0	0	Down Genes	53	115	142	129
MFI2-AS1	1133	2.28	14.1	0	0	Up Genes	55	115	145	130
PTOV1-AS1	4198	2.23	21	0	0	Down Genes	59	115	141	131
MFI2-AS1	1133	2.28	8.83	0	0	Down Genes	55	115	146	132
BISPR	2520	1.48	74.2	0	0	Up Genes	105	115	107	133
A1BG-AS1	2789	1.18	96.1	0.36	0	Down Genes	125	105	100	134
ZNF529-AS1	1861	1.05	74.2	0.54	0	Down Genes	141	87	107	135
FLJ20021	788	1.12	105.3	0	0	Down Genes	131	115	94	136
BISPR	2520	1.48	54	0	0	Down Genes	105	115	122	137
FLJ20021	788	1.12	95.2	0	0	Up Genes	131	115	101	138
LINC00909	3739	1.26	56.2	0	0	Up Genes	115	115	119	139
LINC00909	3739	1.26	51.6	0.27	0	Down Genes	115	111	123	140
LINC00960	3175	1.22	55.4	0.31	0	Down Genes	121	109	120	141
LINC01023	435	1.64	0	0	0	Up Genes	87	115	149	142
LINC01023	435	1.64	0	0	0	Down Genes	87	115	149	143
PAXIP1-AS1	2255	1.32	37.3	0	0	Down Genes	111	115	133	144
PAXIP1-AS1	2255	1.32	36.4	0	0	Up Genes	111	115	134	145
DLGAP4-AS1	2425	1.10	45.4	0	0	Down Genes	137	115	126	146
DHRS4-AS1	3589	1.03	41.5	0.28	0	Down Genes	143	110	130	147
DHRS4-AS1	3589	1.03	41.8	0	0	Up Genes	143	115	129	148
LINC01481	627	1.08	4.78	0	0	Up Genes	139	115	147	149
LINC01481	627	1.08	4.78	0	0	Down Genes	139	115	147	150

Table S8: TDF analyses of 75 lncRNAs on up-regulated/down-regulated genes in cardiac differentiation. LncRNAs were ranked by their expression fold change (FC), number of TTS normalized by lncRNA length (Norm TTS) and number of DBD normalized by lncRNA length (Norm DBD).

	Target Promoters		Non-Target Promoters		Statistics	
DBD Position	TTS	no TTS	TTS	no TTS	Odds ratio	p-value
80-112	49	2024	447	32622	1.77	0.0008
778-811	59	2014	471	32598	2.03	1.2e-05
860-905	246	1827	4896	28173	0.77	1.00
1732-1757	61	2012	1027	32042	0.95	1.00
2086-2132	158	1915	2844	30225	0.88	1.00

Table S9: Candidate DBDs from *GATA6-AS* on the differently expressed genes ranked by p-values. The significant DBDs are shown in bold.

#	Promoter	Gene	TTSs Count	TTS coverage	Fold Change	Sum of Ranks	Overlap Capture-Seq
1	chr10:98268249-98269250	LOXL4	11	0.0270	5.02	25	1
2	chrX:53048090-53049091	GPR173	9	0.05	2.35	34	0
3	chr2:66432451-66433452	**MEIS1**	3	0.0320	9.19	36	1
4	chr2:8677844-8678845	**ID2**	3	0.0400	3.27	46	1
5	chr10:79445626-79446627	ZCCHC24	3	0.0260	3.10	58	1
6	chr1:244047938-244048939	ZBTB18	4	0.0490	1.55	60	1
7	chr2:42047019-42048020	PKDCC	3	0.0340	2.10	66	1
8	chr1:156248116-156249117	PAQR6	10	0.0270	1.50	67	1
9	chr10:22320210-22321211	**BMI1**	2	0.0400	3.09	68	1
10	chr12:54391297-54392298	ZNF385A	4	0.0220	2.48	70	1
11	chr18:22168967-22169968	GATA6-AS1	2	0.0230	8.82	74	1
12	chr18:22168442-22169443	**GATA6**	2	0.0230	6.69	76	1
13	chr16:50245136-50246137	ADCY7	2	0.0250	3.52	78	1
14	chr18:76495189-76496190	ZNF516	2	0.0360	2.28	78	1
15	chr3:71583988-71584989	**FOXP1**	3	0.0440	1.42	80	1
16	chr7:44103360-44104361	AEBP1	5	0.0300	1.35	81	0
17	chr10:68075347-68076348	HERC4	9	0.0220	1.56	83	0
18	chr1:143729569-143730570	RNVU1-18	19	0.0260	1.22	85	0
19	chr3:50312380-50313381	NAT6	7	0.0410	1.15	85	0
20	chr2:45650344-45651345	PRKCE	2	0.0250	2.51	88	1
21	chr7:124765626-124766627	GPR37	2	0.0230	3.13	88	1
22	chr20:49568145-49569146	PTGIS	6	0.06	1.04	92	1
23	chr19:460995-461996	SHC2	2	0.0230	2.93	93	1
24	chr19:49851675-49852676	PTOV1-AS1	2	0.0250	2.23	95	0
25	chrX:54919461-54920462	TRO	3	0.0240	1.52	96	0
26	chr7:102572655-102573656	POLR2J3	10	0.0350	1.03	97	0
27	chr7:66627880-66628881	KCTD7	7	0.0210	1.40	104	0
28	chr1:23778286-23779287	TCEB3-AS1	2	0.0250	1.63	106	1
29	chr19:50568044-50569045	LRRC4B	3	0.0420	1.10	106	1
30	chr7:128032106-128033107	LRRC4	3	0.0240	1.32	109	1
31	chr9:136326475-136327476	GPSM1	3	0.0240	1.26	114	0
32	chr22:19291715-19292716	CLTCL1	2	0.0270	1.30	120	0
33	chr2:5691666-5692667	SOX11	3	0.0250	1.14	122	1
34	chr17:68601388-68602389	FAM20A	1	0.0210	5.53	132	1
35	chr11:18792720-18793721	PTPN5	2	0.0200	2.46	134	1
36	chr1:161223630-161224631	APOA2	1	0.0210	4.50	136	1
37	chr2:237626575-237627576	LRRFIP1	2	0.0250	1.14	139	0
38	chr2:168454861-168455862	CERS6	3	0.0200	1.46	141	1
39	chr12:8031702-8032703	FOXJ2	2	0.0200	1.93	145	0
40	chr1:211830771-211831772	LPGAT1	1	0.0210	3.05	146	0
41	chr18:21241241-21242242	GREB1L	1	0.0200	7.52	155	1
42	chr6:90296907-90297908	BACH2	1	0.0210	2.28	155	1
43	chr6:1388833-1389834	FOXF2	1	0.0200	6.06	157	1
44	chr14:74426101-74427102	SYNDIG1L	1	0.0220	1.68	158	0
45	chrX:103728654-103729655	GLRA4	1	0.0200	4.52	161	1
46	chr12:132686605-132687606	PMP22	1	0.0200	4.40	163	1
47	chr20:22578641-22579642	LINC00261	1	0.0180	4.87	176	1
48	chr19:40444704-40445705	SERTAD3	2	0.0190	1.25	177	1
49	chr10:117240092-117241093	SLC18A2	1	0.0200	2.29	179	1
50	chr9:128919894-128920895	PHYHD1	2	0.0200	1.15	179	1

Table S10: Targets of *GATA6-AS* predicted by TDF. Cardiac relevant genes are shown in bold.

5′ RACE Reverse: GATTACGCCAAGCTTCTGGAGGGTAAAATGCCGGGTCCC
3′ RACE Forward: GATTACGCCAAGCTTTGGAATGGAGAGGCTGGCGACTGGA
GATA6-AS chr18:22166894-22169069 strand:- ACAGTCCCGCGCCGCCGCCGCGGCGGGAGGGGCGCTCCAGACCTTGGGTCAGCACGCCCCCAGAAGGAAG GGGGAACGTGCTCTGCGCCCCCCCGCCCCCCAACCCCCGCCTAGCCCCTTCACAGCAAATGCGCTTTTTG CCCTGAAGTTGTGTGAGACCGACCTTAAACTTTCTCCGACCCTATCTCGGGATGCTACGGCTCAGGTCGT GGTGGTTTCGGGAACCTCAAGACAACATTCCCCAGCTGACCTTTGGGAACTTTAACTCGGGTCGTCATGT ACGGAAAGGTAGTCTATGGGGTACGCAGAATGGAAAAGGGCTTCCACATCAGTCGTGTCCGAGGACGCTC TCCAACTTTTGATGTCCCTGGAGAGTTTCTGATATTTCCCTGGAGAGTTTCAGGTAAGTCACAGATAAGT CACTGCGAAGAGGGGGTGTCACCTTCCCTGGACCCAGCGCTAAATGCTGCTTCCCCACCCCCACGTTCAT ATACACACCCCCTCTTCGCTCCCTCCAAACAGTTATCACAACTCTGTACAAACTGATACGGTCCCTAGCG GGCAAAAGGGACTGGAAATGAACGTCGCGGGGTTATCAGCGCCGATCTGTCAGCGGCAACTCGCGCGGCC GCAGGCTCGTCCGCTGCAGGAGCCGTGATATATGTCTCGCTCCCTTTCGCTTACAGAAAGGATTTCTTCC GACAGACGTGACCCCAGAAGGCTGCCGAGGGAGACTTTAGGACAAGAGGCGTTGTTTTTGTGAGAATGAA TTTTTATACTTTACCCCGACCCCACCCCCTACCCCCCGCCCAGGTAAATCCAAGTAAATGATTTTCTGAA GGTCGGTCTCCTAGAAAGCGTTTAGGCTCTGGTTTTTTGTTTTGTTTCTATTTTCGTGTTTTCCCAGTTA GGGAAGGAAGAGGAGCCTGTGCAGAGTTGAACTGGGGTGGAGAGGTGCCTTGTAAAGTATGGTTTCCAGA GATGGTGAGACCTCGTAGCTAAGCGTTGTTTCCTCTGCACGTAACTGTGAGTTGTGAACTTGTGGCTCCT GGGGGAGCGGGACAGGGCCTGGCAGTGCGGGGGAGGGGGCAAGGAATTCAGGAAGTTGGCGAGAATTCTT CGTTTTTCATTCTTCGTTGTAAATTGCTTCTAAACACTTCTGAATTTTAAATTGCTACCGAGAAGGTCCA GAAACCGTTCTCATCCAATTTAGTCACTTTTATTACGTTCCACTTTGCCTCCCCCAATCACTGCGGCGGC TCCCAGTTTCAGCGTGGCCGCATTTGGAAAAGGCTGAAGGTAGAAAACTCTGGAATGGAGAGGCTGGCGA CTGGAGCAGGATCCAGGGGCTTTAAATTTTAACCTGAATGCGATCGAATGCGCGACTCCCAGAAGAGATA GCCAGGCGTCCGCCCGGCCGCTCAACCCCCGCCAGGACTCGGAATGCCTTGCTTTTGGTTCTTTTCGTGA GTTCCGACCAAATGTTTAAGTGGACTGGGTTTTGCAGAAGCTCATTTGTAAGCACTGACGGAAATGGGTT GTGGGCATTTGATTTCCTTTGATTCCAACGTTTTGAAATAATGTTTCTCGGATTTGCTATTCGAAGCAAT TTTCTAAAAACTCATATCTTATTTGCTAAAAATAAACTCTTTGGCTCAGTTTGTGTCCAGGCTGTGTACG GCCTCCAACCCTGAGCCCCGAGGAGATCGAGCCCTGGCGCGAGCCTGATCTCTGTCCTGTGTGTTTCTTT GTATTTGCAAGTACAAGGATGTCTGCGTGGATATCCCTGGACACTCGATACCCCCCGGGGACCTGCTTTT CCCGCAGCTCCGCAGACGCGGCGGGAGGGCGCGCTGGCCCCGGCGCTGCTGGGACAGAGGTGAGCAGCGG GGTGGACATTGACCGATTCGACAACGTTCGGGCTCTAAGGGCTCGGGAAGGGACCCGGCATTTTACCCTC CAGATTCCCAATCTATTTTTAAAAATAATGTTCAAAACATATGGGAAAAATAAGTGATTGCTCCTTTTCA TGGTCACAGATTGTATTTATTTTTTCAGACTGGTTTTCCAACCTAACGAGGGAATGGGTAGTGAATGTTG TTGTTGTTGTTGTTGTTTATGTTGGTTAATTTCGAAATAAAACTGTTTAACTCGTACAGTTGATTCAGAC AGAAA

Table S11: Primers for GATA6-AS amplification in RACE.

ASO	sequence
GATA6-AS_ASO	C*T*T*T*T*C*C*A*A*A*T*G*C*G*G*C*C*A*C*G
ctrl-ASO	G*C*T*C*T*T*C*A*T*C*T*T*G*T*T*G*G*T*C

Table S12: Sequences of the ASOs used in this study. Asterisks represent phosphorothioate bonds.

Step	Duration	# of cycles
95°C	2:00 min	1
95°C 60°C Plateread	0:05 min 0:30 min	40
65°C RAMP 0.5°C/cycle Plateread	0:05 min	60

Table S13: ASO qPCR protocol.

qPCR primer	forward	reverse
GATA6-AS	CTGTGACCATGAAAAGGAGCAATC	CCGGCATTTTACCCTCCAGA
GATA6	TGTGCGTTCATGGAGAAGATCA	TTTGATAAGAGACCTCATGAACCGACT
MEIS1	CTAACTGACCAGCCCTCTTGG	TGTTGTCCAAGCCATCACCTT
T	CCTTGCTCACACCTGCAGTAGC	GGCCAACTGCATCATCTCCA
EOMES	GGCTGTCTCCTAGCAACTCC	GCATAATACCCTCCCATGCCT
MIXL1	CAGAACAGGCGTGCCAAGTC	TTCCAGGAGCACAGTGGTTGA
CDX2	GCCAACCTGGACTTCCTGTCA	TCTGGCTTGGATGTTACACAGACC
GATA4	AATGACTCCAGAACAACAACTGGG	CTCCCTCCAGTCCCATCAGC
RPL37A	GTGGTTCCTGCATGAAGACAGTG	TTCTGATGGCGGACTTTACCG
RPS16	ACGTGGCCCAGATTTATGCT	AGCAGGGTCCGGTCATACT

Table S14: List of qPCR primers for ASO.

Group	ChOP oligos for GATA6-AS	Sequence
Control Oligos for *GATA6* ChOP	Ctrl_GATA6-AS_1	CCGTTTTTCGCGTAAACGAC[BtnTg]
	Ctrl_GATA6-AS_2	GTTTCCAGTCGACCCCTTAC[BtnTg]
	Ctrl_GATA6-AS_3	TTTTCAACCTCTCGCAGGAG[BtnTg]
	Ctrl_GATA6-AS_4	TTTTGTTGCGGAGAACAGGA[BtnTg]
	Ctrl_GATA6-AS_5	TGTTGCGAATCGATGCTCCA[BtnTg]
	Ctrl_GATA6-AS_6	GGTTGAAGGACTTAAGGAAC[BtnTg]
	Ctrl_GATA6-AS_7	TAACCTACTCTTGCCAAAGA[BtnTg]
	Ctrl_GATA6-AS_8	TAAGGTCTCAAAAGATGGAA[BtnTg]
	Ctrl_GATA6-AS_9	TGCGTCTGTAGGAACATCTT[BtnTg]
	Ctrl_GATA6-AS_10	CTTGCAACAGCTTAGCCAGT[BtnTg]
GATA6-AS ChOP Oligos	bio_GATA6-AS_1	GGCAAAAAGCGCATTTGCTG[BtnTg]
	bio_GATA6-AS_2	CAAAGGTCAGCTGGGGAATG[BtnTg]
	bio_GATA6-AS_3	AAAAGTTGGAGAGCGTCCTC[BtnTg]
	bio_GATA6-AS_4	AAAACAACGCCTCTTGTCCT[BtnTg]
	bio_GATA6-AS_5	ACAACGCTTAGCTACGAGGT[BtnTg]
	bio_GATA6-AS_6	CCAACTTCCTGAATTCCTTG[BtnTg]
	bio_GATA6-AS_7	ATTGGATGAGAACGGTTTCT[BtnTg]
	bio_GATA6-AS_8	ATTCCAGAGTTTTCTACCTT[BtnTg]
	bio_GATA6-AS_9	ACGCAGACATCCTTGTAGAA[BtnTg]
	bio_GATA6-AS_10	GAACGTTGTCGAATCGGTCA[BtnTg]

Table S15: ChOP oligos primers. BtnTg stands for Biotin tag.

targets	region	forward	Sequence	reverse	Sequence	product size
MEIS1	chr2:66432481-66432504	MEIS1_ChOP_F1	GCCCAGCTTAGTCCTTTCCC	MEIS1_ChOP_R1	AGGCGATTTCTGTTTAATCCTTGG	125
	GtGGGGGGGtGGGGGGAtGGGGG	MEIS1_ChOP_F2	TGGCCTGAAATTCTGCTTCCA	MEIS1_ChOP_R2	TACCAGCGCCTTTATGCCTC	89
ID2	chr2:8678672-8678693	ID2_ChOP_F1	CTTCTCGGTCCTCTTTCCTTCC	ID2_ChOP_R1	AGCTGATCAATGCCTGCGA	129
	GGcGGGtGGGGGGGGGGGGGG	ID2_ChOP_F2	TGAACGCCTCGGTTGCAA	ID2_ChOP_R2	AAAGCCGGGAGAGGGAGAA	118
GATA6	chr18:22168961-22168981	GATA6_ChOP_F1	CGACCCGAGTTAAAGTTCCCA	GATA6_ChOP_R1	GAAGTTGTGTGAGACCGACCT	130
	GGGGGttGGGGGGcGGGGGG	GATA6_ChOP_F2	TGTTGTCTTGAGGTTCCCGAA	GATA6_ChOP_R2	TGCCCTGAAGTTGTGTGAGAC	99
BMI1	chr10:22321044-22321164	BMI1_ChOP_F1	ACCGACACTAATTCCCAGGC	BMI1_ChOP_R1	AAACTGACACCGGCTCCAAA	117
	GGGGcGGcGGGGGGAGGGGG	BMI1_ChOP_F2	TGGCGCTGTGGAGAAATGT	BMI1_ChOP_R2	GCCTGGGAATTAGTGTCGGT	119
	GGGGGAGGAGGGGAGGGGGG					
WNT6	chr2:218859740-218859761	WNT6_ChOP_F1	CTGCCTGACTGTCGCAACT	WNT6_ChOP_R1	GGAGAAGAAATCAGAGCCGGG	105
	GGGGGAcGGGGGGcGGGGGcG	WNT6_ChOP_F2	CTCTACTCTTCTTCCCAGCCC	WNT6_ChOP_R2	CCTCTCTCGGACTTGGAGC	80
FOXP1	chr3:71583990-71584011	FOXP1_ChOP_F1	TAAATAAGGCGCGCGAGAGA	FOXP1_ChOP_R1	GTGGATGTGACGTCAGGGAC	87
	GGAGGGAGGGGGGAGGGGGGG	FOXP1_ChOP_F2	TAGCTTCCCGGAGCCCAG	FOXP1_ChOP_R2	CTGCGGGAGGTAGAGGGT	107
HCN4	chr15:73369842-73369862	HCN4_ChOP_F1	GGTGAGTTCCTGTTCCACCC	HCN4_ChOP_R1	ACACAGTTGGATCCTTGGCG	122
	GGGGGAAGGGGGGttGGGGG	HCN4_ChOP_F2	CTCCCTGTGTTCCCATCCAC	HCN4_ChOP_R2	CTGCAGACTGGAAGGGAGTG	129
FOXP2	chr7:114085818-114085838	FOXP2_ChOP_F1	CCCTGTTTCATTTGCCAGCA	FOXP2_ChOP_R1	CAAAGCCGAGTTGTGTCTGC	85
	GAGAAGGGtGGGGGtGGGGG	FOXP2_ChOP_F2	CAAACAAGTTCTCCAGCGGG	FOXP2_ChOP_R2	ATTGTTCGGATTTGCAGCGG	81

Table S16: ChOP qPCR primers for up-regulated targets.

targets	region	forward	Sequence	reverse	Sequence	product size
E2F2	chr1:23531230-23531367	E2F2_ChOP_F1	CGATTGCAACACTAGCTTCGC	E2F2_ChOP_R1	CACTGTGAATCCCGGAACCA	126
	AGAcGGAGAAAAAAAAGAAGAAAAAAAAAAAAA AGGtGGGGGGGGGGGGGGGG	E2F2_ChOP_F2	TGGTTCCGGGATTCACAGTG	E2F2_ChOP_R2	CGCGCCAAAATGTTTCCCTT	100
MNS1	chr15:56465581-56465606	MNS1_ChOP_F1	TGCTAGGGACGATGACTGGT	MNS1_ChOP_R1	GTGAAGTGAAGGCTGCTGGA	114
	AGtGGGGGGGcGGGGGGGGGGGGG	MNS1_ChOP_F2	TCCAGCAGCCTTCACTTCAC	MNS1_ChOP_R2	GGTACCTGGACCACATAGCAG	120
TRNP1	chr1:26993578-26993600	TRNP1_ChOP_F1	CCCGTGTTCTATAAGATGCGC	TRNP1_ChOP_R1	CAGCACACCCGAACAGCTA	82
	GGGGGGGGGGGGGGGGcGGGG	TRNP1_ChOP_F2	GGCGTGCCTGTCTCTGATT	TRNP1_ChOP_R2	CACACCCGAACAGCTAGACA	121

Table S17: ChOP qPCR primers for down-regulated targets.

Name	Sequence (5′-3′)
MEG3 13-41	GACGGCGGAGAGCAGAGAGGGAGCGCGC/TEGbiotin
MEG3 839-890	CAGUCCCUUCCCACCCCUCUUGCUUGUCUACUGUCUAUUUAUUCUCCA/TEGbiotin
GATA6-AS 78-118	UGCUCUGCGCCCCCCCGCCCCCCAACCCCCGCCUAGCCCC /TEGbiotin

Table S18: RNA oligo (predicted DBD) used for DBD-Capture-Seq.

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