STRA6 Is Essential for Induction of Vascular Smooth Muscle Lineages in Human Embryonic Cardiac Outflow Tract Development

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9 **A short title:** STRA6 is essential for SMCs in cardiac OFT

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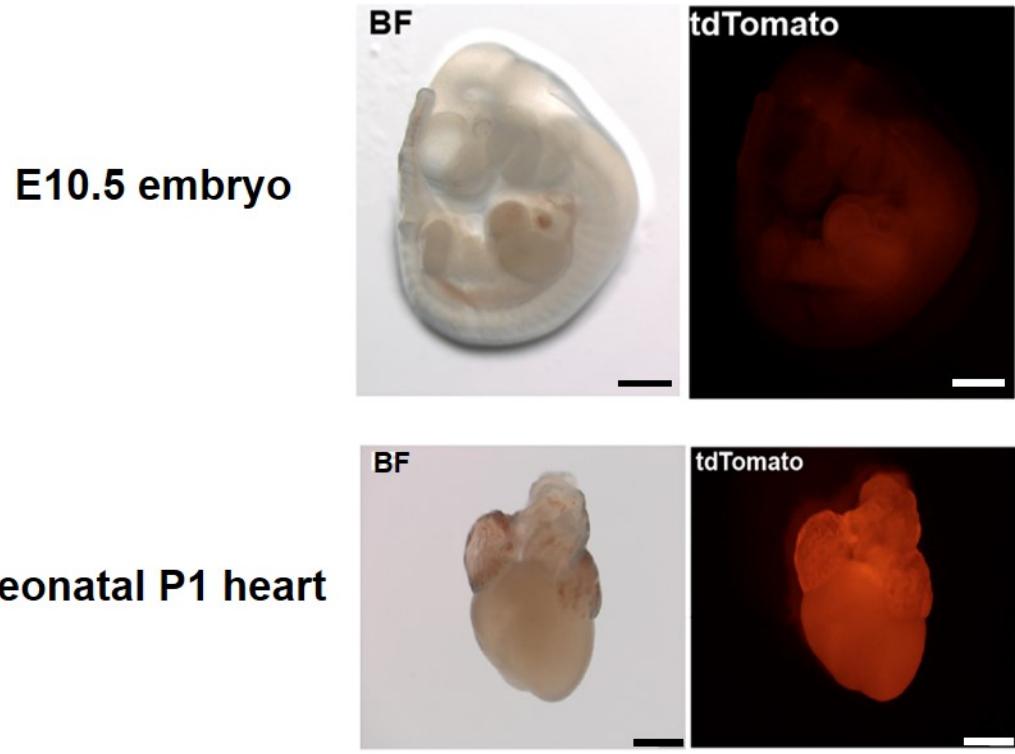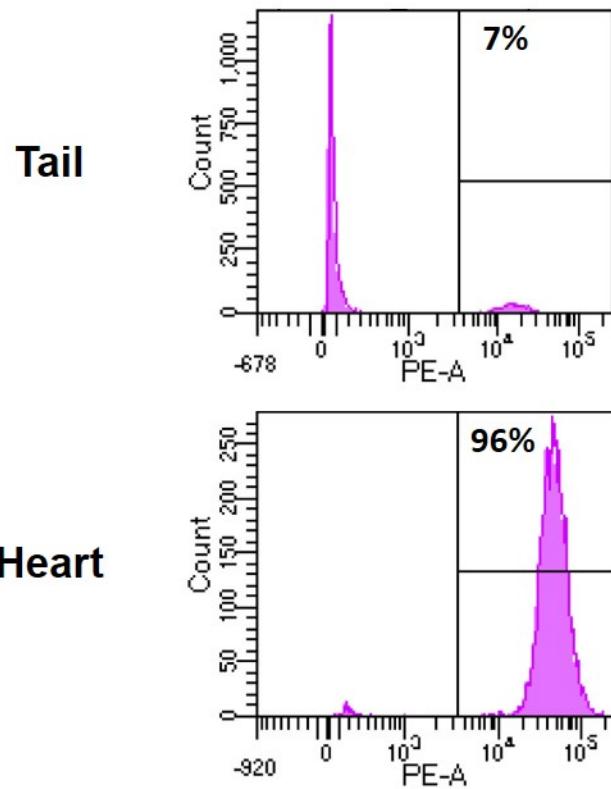
Figure S1

12   **Supplementary Figure legends**

13   **Figure S1. Mesp1⁺ lineage tracing in murine embryonic hearts.**

14   (A) Macroscopic images showing the tdTomato expression patterns in the $Mesp1^{Cre/+}$; $Rosa26^{tdTomato}$

15   embryo on E10.5 (top) and in the neonatal P1 heart (bottom) of the $Mesp1^{Cre/+}$; $Rosa26^{tdTomato}$ mouse.

16   Scale bars, 1 mm. BF, bright field. (B) Flow cytometry analysis showing the percent of Mesp1-

17   tdTomato-positive cells in the tail (top) and heart (bottom) of the $Mesp1^{Cre/+}$; $Rosa26^{tdTomato}$ mouse.

18

19

Human STRA6 locus

Figure S2

20 **Figure S2. Generation of the *STRA6*-KO hESC line by CRISPR-Cas9.**

21 The top row shows a wild-type sequence of the region in the second exon of the human *STRA6* gene.

22 The red sequence represents the target sequence of the sgRNA (*Materials and Methods*), and the blue

23 sequence represents the protospacer adjacent motif (PAM). Sequences on the bottom row are

24 highlighting frameshift mutations of the sgRNA-targeted region in both alleles of the mutated clone.

25 Within the sequences, deletions are indicated by dashed lines.

26

27

A Human *in vitro* cardiac cells' scRNA-seq

B

C
POU5F1　T　EOMES　MESP1　STRA6
MESP2　LGR5　BMP4　ISL1　MEIS2
PDGFRA　NKX2-5　TNNT2　TBX5　IRX4

Relative Expression　0 ─ Max

D
POU5F1　T　EOMES　MESP1　STRA6
MESP2　LGR5　BMP4　ISL1　MEIS2
PDGFRA　NKX2-5　TNNT2　TBX5　IRX4
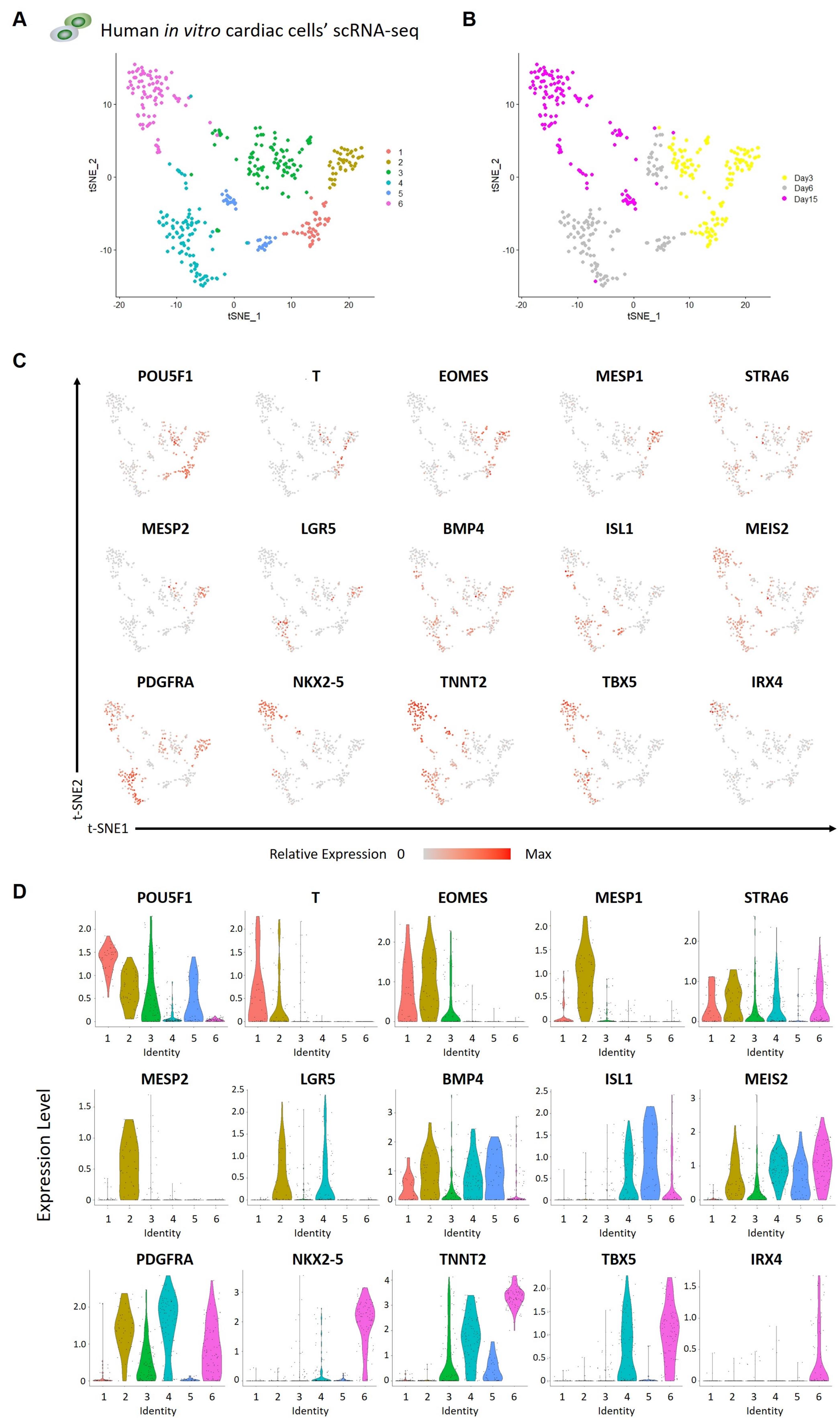
Figure S3

28   **Figure S3. Single-cell RNA-seq analysis of *in vitro* hESC-derived cells during CM differentiation.**

29   (A) The tSNE analysis using the single-cell RNA-seq dataset, which was obtained from *in vitro* hESC-

30   derived cells during CM differentiation (day 3 to 15),[16] segregated 366 individual cells into 6 clusters.

31   (B) The same tSNE plots in (A), showing days when the cells were harvested. (C) Feature plots of the

32   cardiac developmental markers, including the pluripotency/early mesoderm/SHF/pan-

33   cardiac/FHF/developing CM marker genes, respectively, with *STRA6* on the tSNE plots in (A). (D)

34   Violin plots of the same genes as in (C), in the segregated 6 clusters of the *in vitro* hESC-derived cells.

35   As shown in (C) and (D), *Mesp1* was entirely expressed in cells of the cluster #2, which was thereby

36   termed as "Mesp1+ cardiac precursors". The majority ($\approx$60%) of cells in the cluster #2 also expressed

37   *STRA6*.

38

39
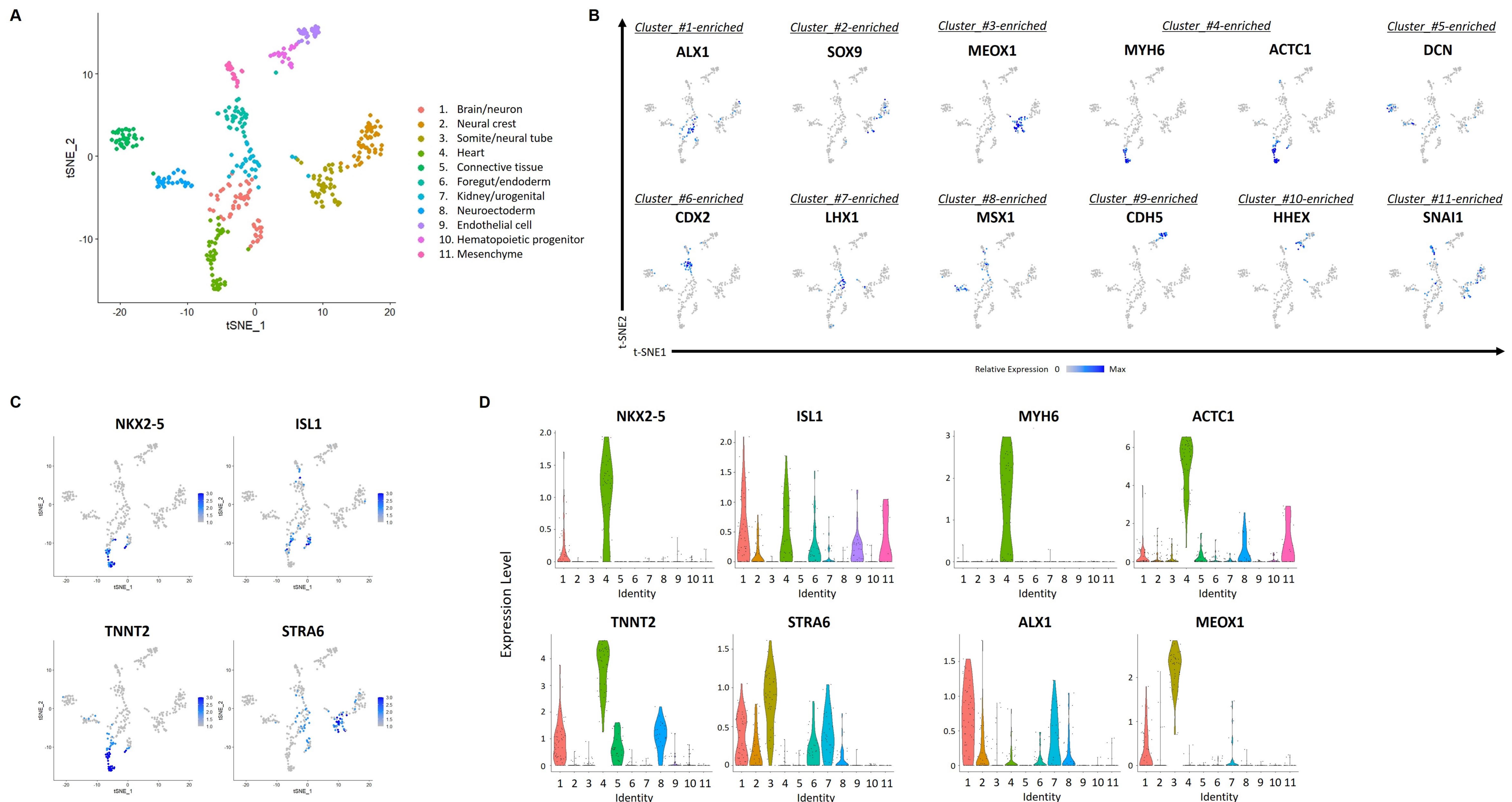
Figure S4

40   **Figure S4. Single-cell RNA-seq analysis of murine whole embryo-derived Mesp1$^+$ lineage cells at**

41   **E8.25.**

42   (A) The tSNE analysis segregated a total of 768 single Mesp1$^+$ cells, obtained from murine whole

43   embryos (*Mesp1$^{Cre/+}$; Rosa26^{tdTomato}*) at E8.25, into 11 clusters including the heart (cluster #4). (B)

44   Feature plots of each cluster-specific genes on the tSNE plots in (A). (C) Feature plots of the SHF/pan-

45   cardiac/CM marker genes with *STRA6* on the tSNE plots in (A). (D) Violin plots of the same genes as

46   in (C) and of several genes appearing in (B), in the segregated 11 clusters of the Mesp1$^+$ lineage cells

47   of murine whole embryos at E8.25.

48

49

A

Wild-type hESC : 46, XX   STRA6-KO hESC : 46, XX
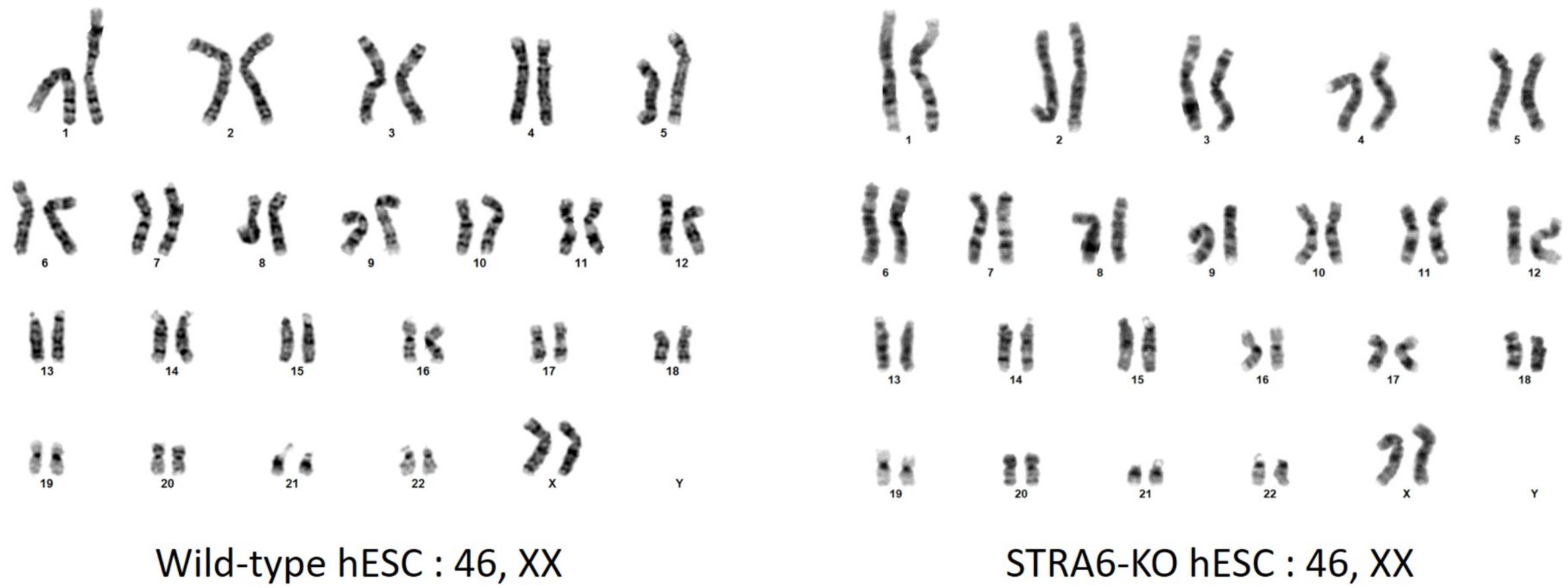
B

☐ Target sequence: **AGGGAACCAGACCTCCCCCGGGG (**chr15:74202228**)**

Location	Number of mismatches	Sequence (including mismatches)
chr10:132469417	3	CCACGGGG**tg**GG**g**CTGGTTCCCT
chr2:24203665	3	A**c**GGA**c**CCAGACCTCCCC**g**GTGG
chr2:65056892	3	CCCCGGGGGAGGTC**act**TTCCCT
chr2:109184449	3	CCC**t**GGGGGAG**c**TCTGGT**c**CCCT
chr5:10462433	3	AGGGA**g**CC**c**GACCTC**t**CCCGTGG
chr7:98223587	3	AGGGAACCAGA**g**CTCCC**tCa**AGG

Figure S5

50 **Figure S5. Karyotyping and off-target analysis of the generated *STRA6*-KO hESC line.**

51 (A) G-banding chromosomal analysis (Cell Guidance Systems) showing a normal karyotype for both

52 the WT and generated *STRA6*-KO hESC lines. (B) The off-target analysis for the sgRNA used for

53 generation of the *STRA6*-KO hESC line (Supplementary material online, *Figure S2*) found 6 putative

54 off-target sites that had the highest similarity with three base pair mismatches to the target sequence of

55 the sgRNA. The PAM of the target sequence is highlighted in blue. The mismatched nucleotides are

56 shown in red. PCR amplified these potential off-target sites using DNA extracted from the established

57 *STRA6*-KO hESC line, followed by Sanger sequencing, which showed no mutations on these regions.

58

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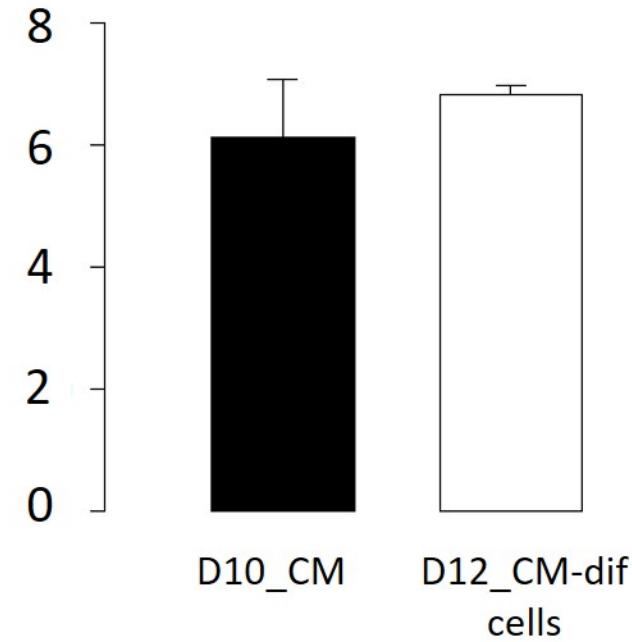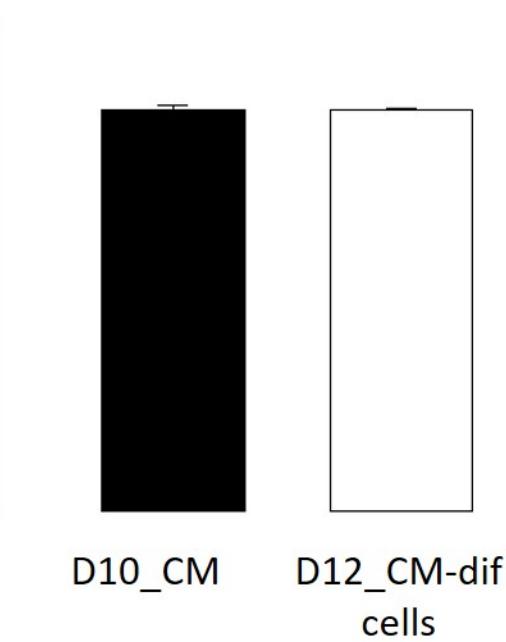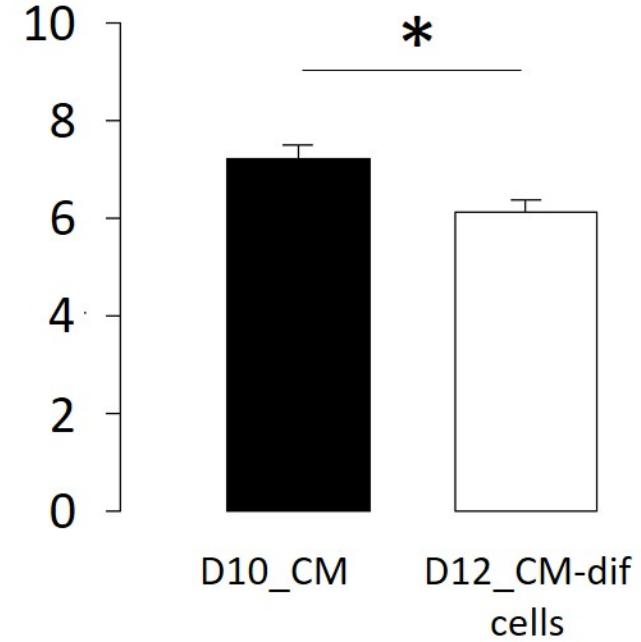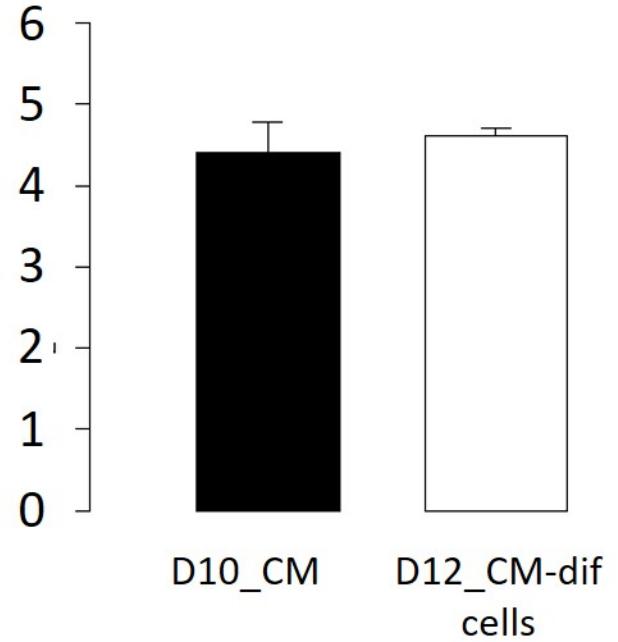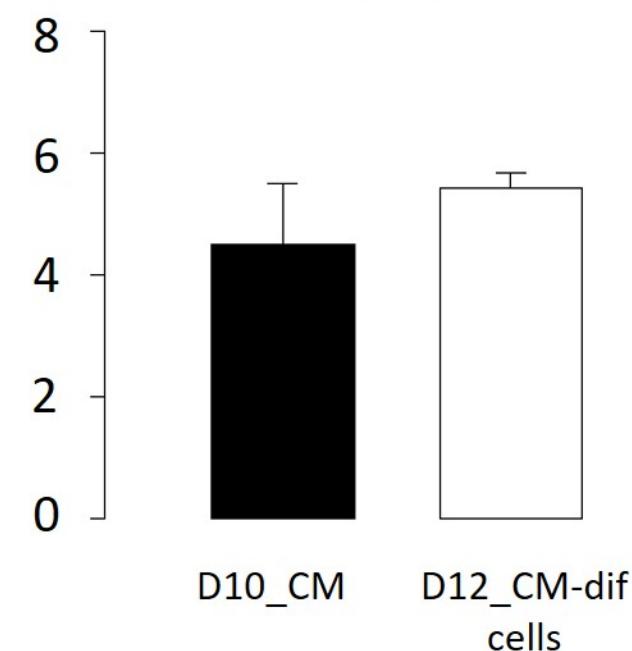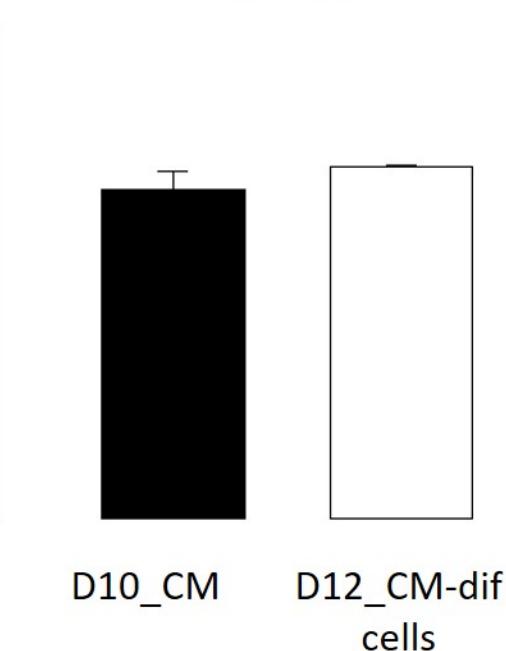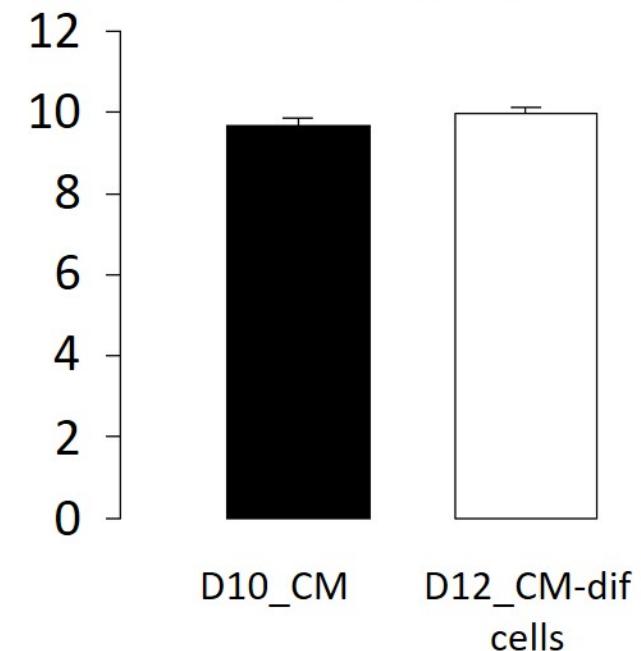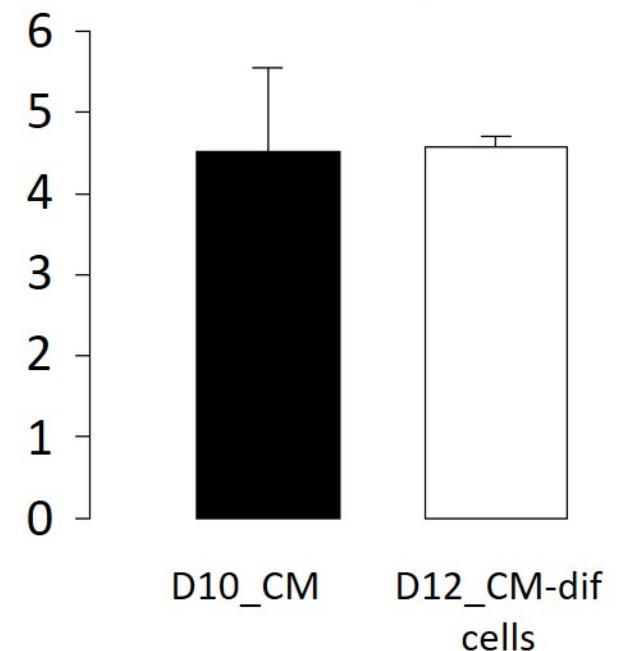
Figure S6

60 **Figure S6. Comparison of representative CM marker genes' expression between WT cells on day**

61 **12 and FACS-purified CMs on day 10 in CM differentiation.**

62 Population RNA-seq data of WT cells on day 12 in CM differentiation (around 80% $TNNT2^+$) were

63 compared with those of the previously obtained WT $ISL1^-TNNT2^+$ cells (i.e., differentiated CMs)

64 purified by FACS on day 10 in CM differentiation.[16] Comparison of representative CM marker genes'

65 expression is shown. *$P<0.05$. Differences between groups were examined with Student's *t*-test.

66

67

A

CACNA1C (Day 12) | MYL2 (Day 12) | NPPB (Day 12) | IRX5 (Day 12) | MYH7 (Day 12)

B

KCNA5 (Day 6) | ALDH1A2 (Day 6) | DHRS9 (Day 6) | PITX2 (Day 12) | MYL7 (Day 12)

C

LGR5 (Day 3) | BMP4 (Day 6) | ISL1 (Day 6) | PDGFRA (Day 6) | MEIS2 (Day 6)
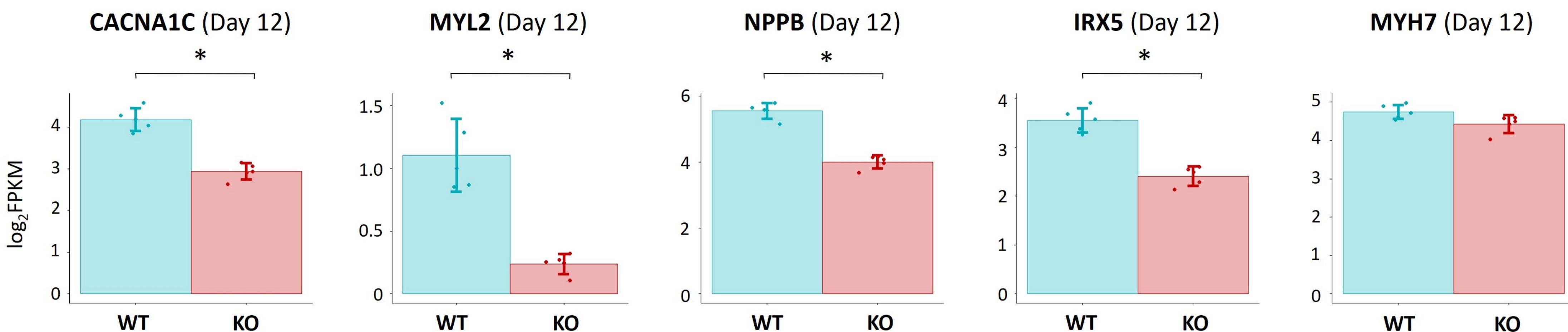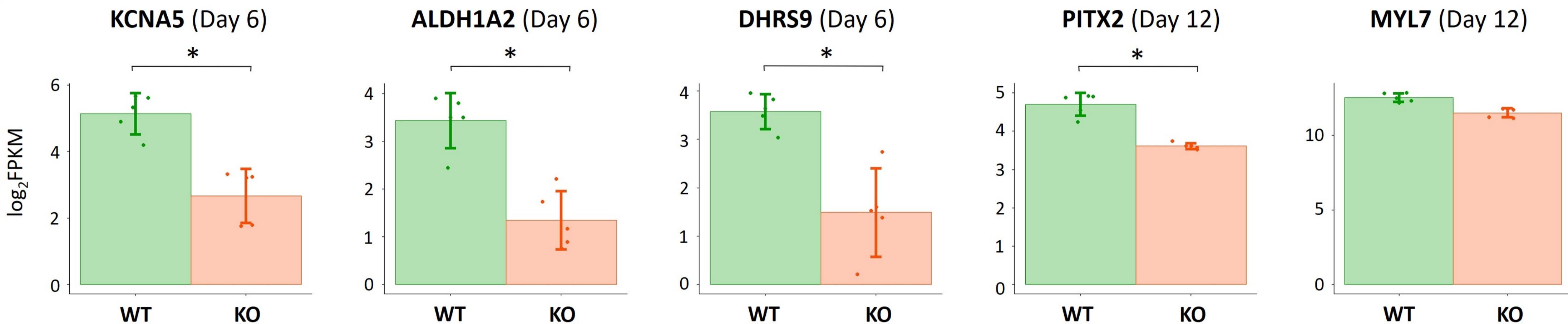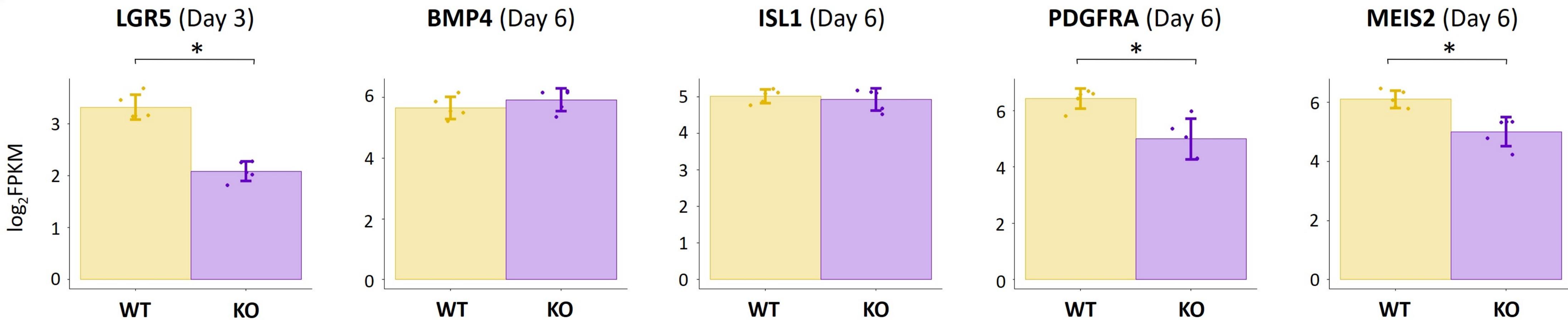
Figure S7

68   **Figure S7. Comparison of population RNA-seq data between WT and *STRA6*-KO cells during**

69   **CM differentiation.**

70   Comparison of gene expression of cardiac maturation markers (A), atrial CM markers (B), and SHF

71   markers (C) in population RNA-seq data between WT and *STRA6*-KO cells during CM differentiation.

72   *$P<0.05$. Differences between groups were examined with Student's *t*-test.

73

74

A

Day 3 | Day 6

Input | IgG | IP samples | Input | IgG | IP samples
| | 5µg | 10µg | | | 5µg | 10µg

RARα (60kDa) →

RXRα (53kDa) →

B

Day 0 | Day 3 | Day 6
WT | SKO | WT | SKO | WT | SKO

RARα (60kDa)

GAPDH (37kDa)

Day 0 | Day 3 | Day 6
WT | SKO | WT | SKO | WT | SKO

RXRα (53kDa)

GAPDH (37kDa)
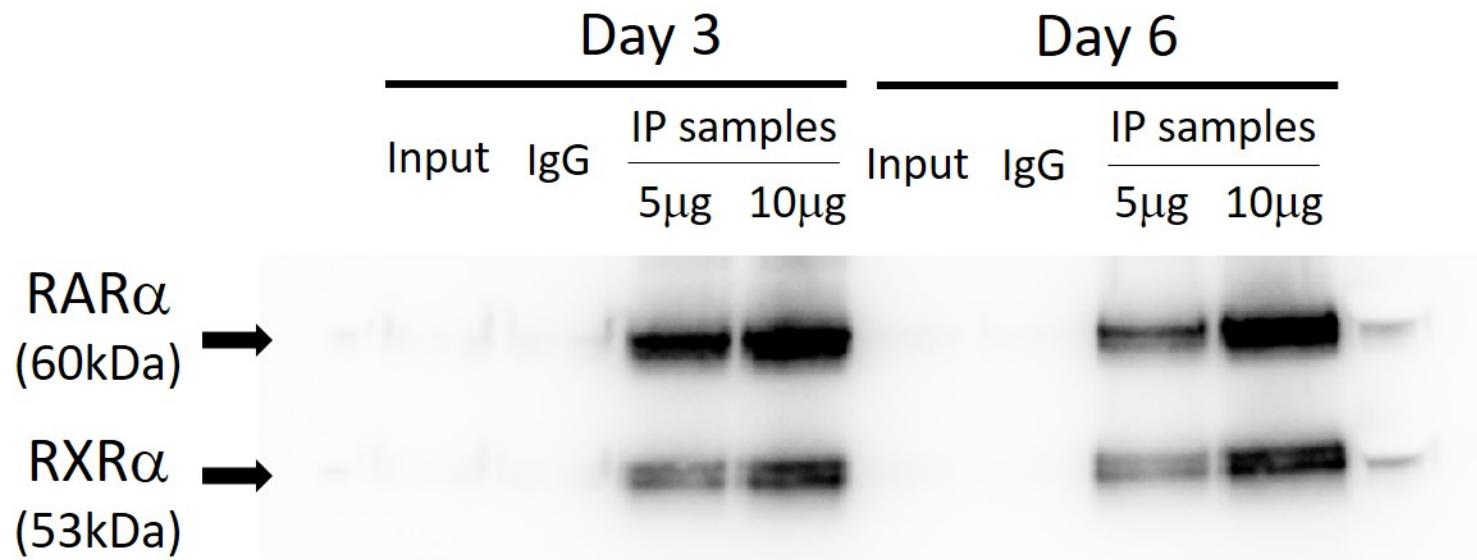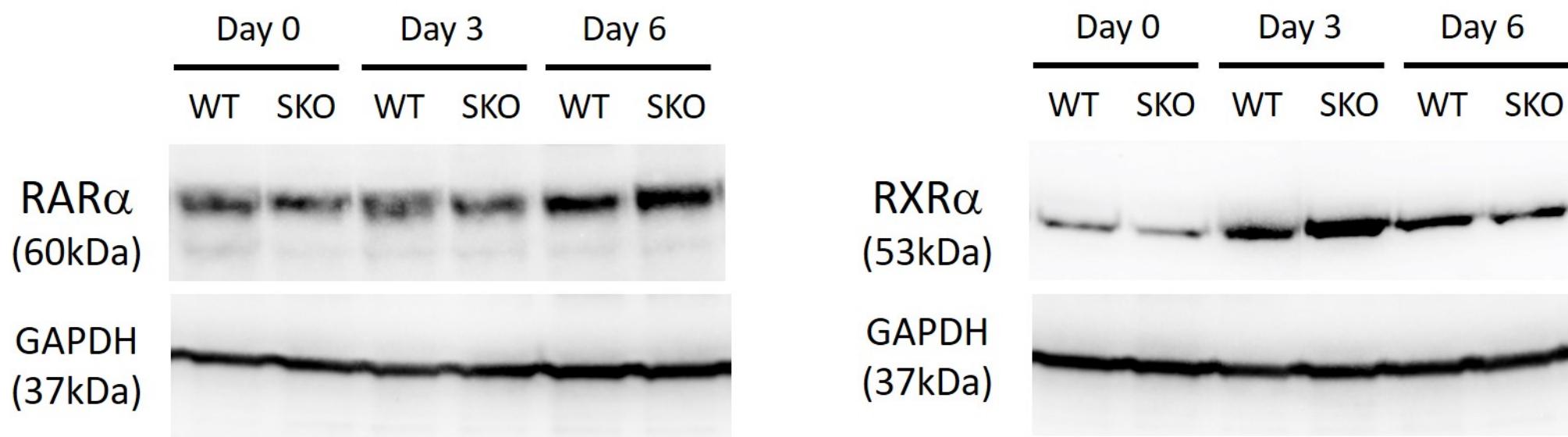
Figure S8

75 **Figure S8. Immunoblotting and immunoprecipitation for RARα and RXRα.**

76 (A) Western blotting analysis showing successful and specific protein immunoprecipitation by anti-

77 RARα and anti-RXRα antibodies using extracts derived from WT hESC-derived cells on days 3 and

78 6 in CM differentiation in the ChIP assays. IP, immunoprecipitation. Both input samples without IP

79 and normal IgG-immunoprecipitated samples were used as negative controls. (B) Comparison of

80 expression of RARα and RXRα proteins between WT and *STRA6*-KO (SKO) cells at days 0, 3 and 6

81 in hESC-CM differentiation. No significant differences were observed between the two lines. GAPDH

82 was used as a loading control.

83

84
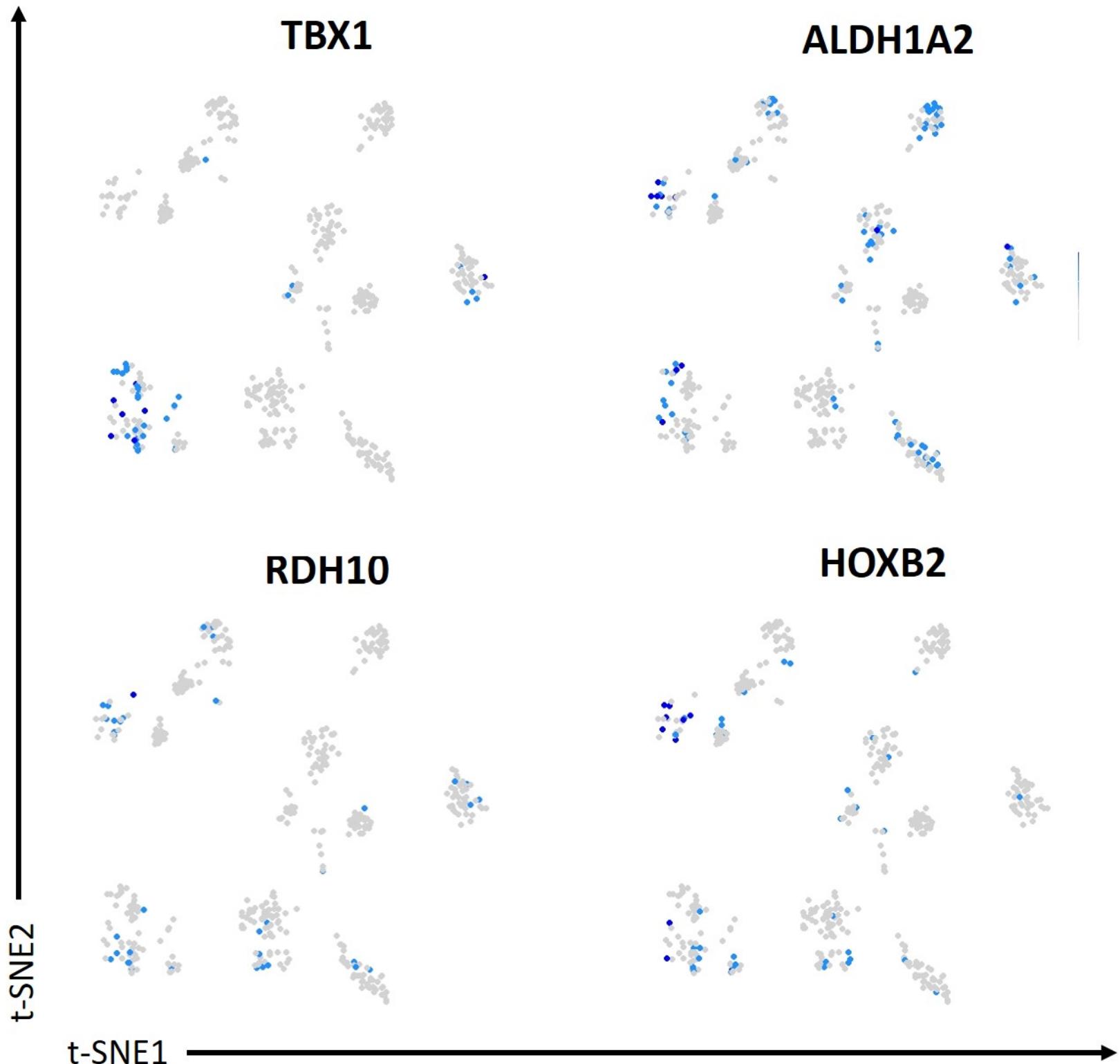
Figure S9

85   **Figure S9. Expression patterns of TBX1 and other RA signaling genes in human embryonic heart**

86   **cells.**

87   Feature plots of *TBX1* and other RA signaling genes on the tSNE plots derived from single-cell RNA-

88   seq data of human embryonic hearts (Figure 1A) are shown.

89

90

A

Human TBX1 promotor region

184 bp deletion

B
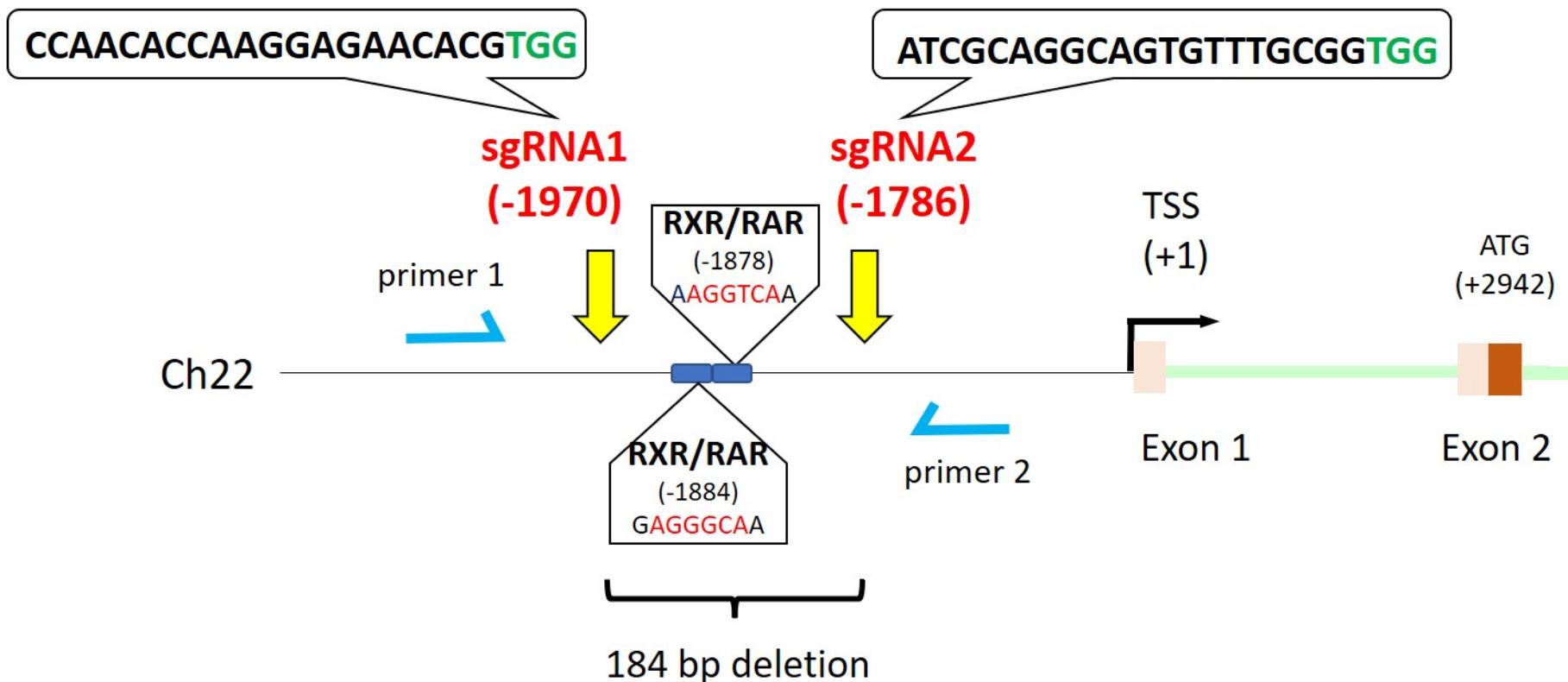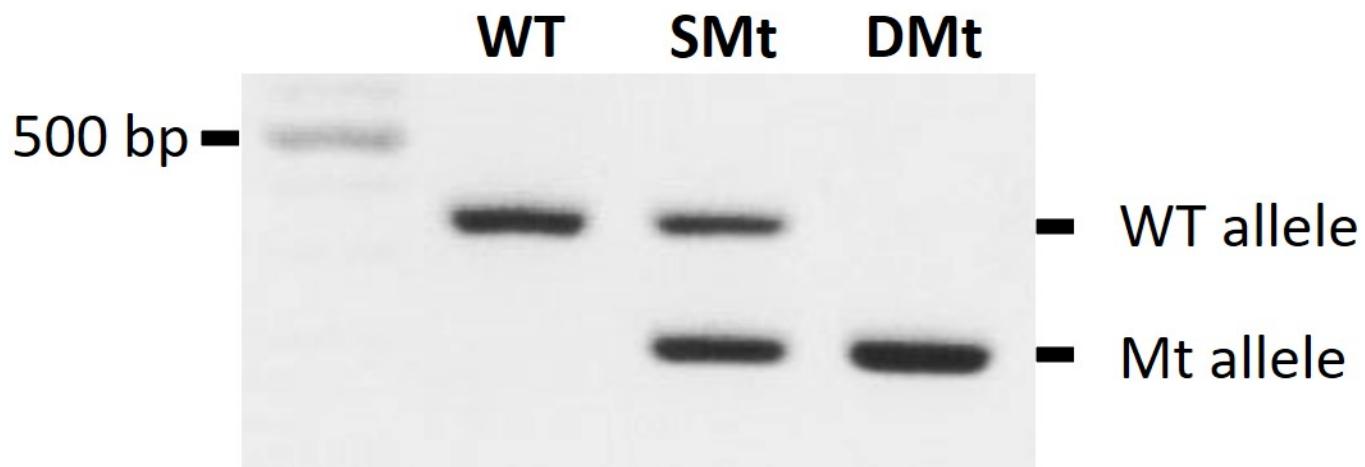
91   **Figure S10. Generation of the TBX1 promoter-mutant hESC line by CRISPR-Cas9.**

92   (A) Schema showing the strategy for deletion of a specific region (containing the identified RARE site

93   at −1,884-1,873 bp) of 1,953-1,769 bp upstream from the transcription start site (TSS) of the human

94   *TBX1* gene using the two sgRNAs and CRISPR-Cas9.   (B) Genotyping of the transduced hESC clones

95   showing single allele-mutant (SMt) and double allele-mutant (DMt) clones.

96

97
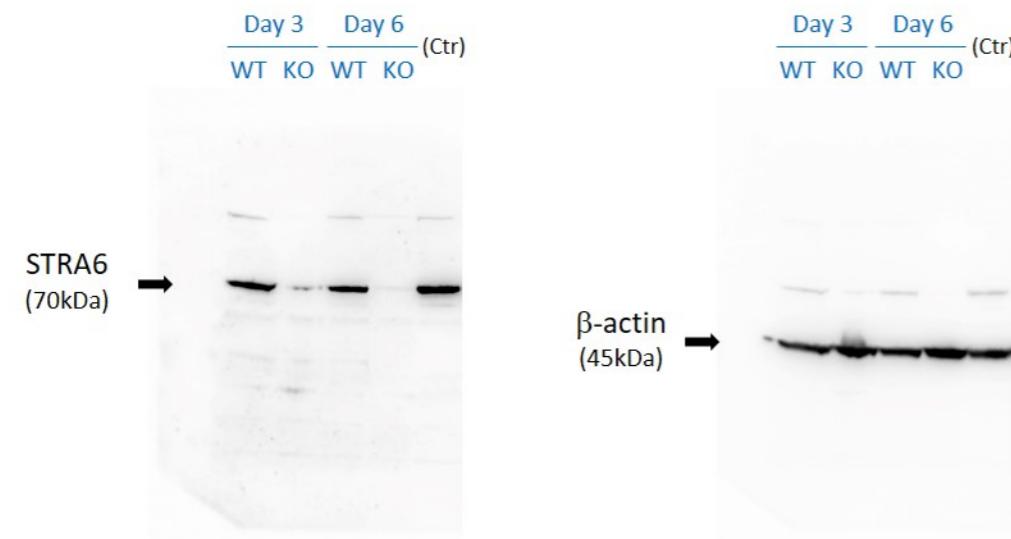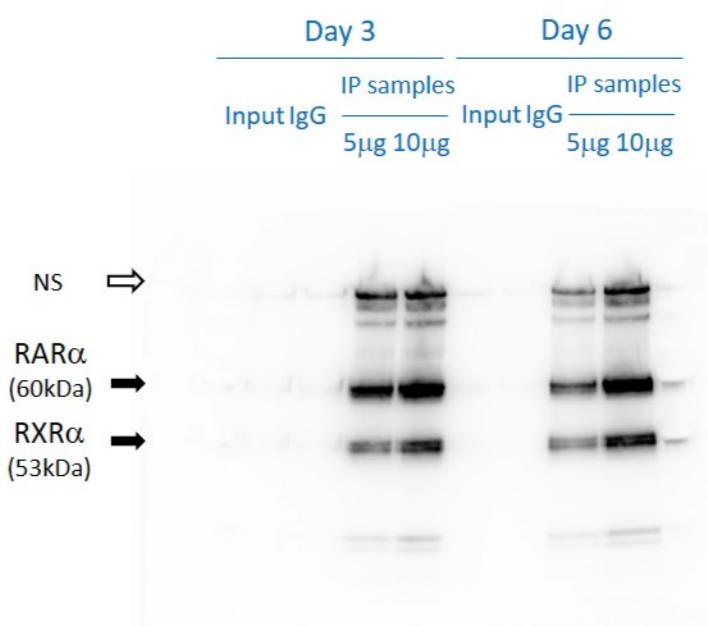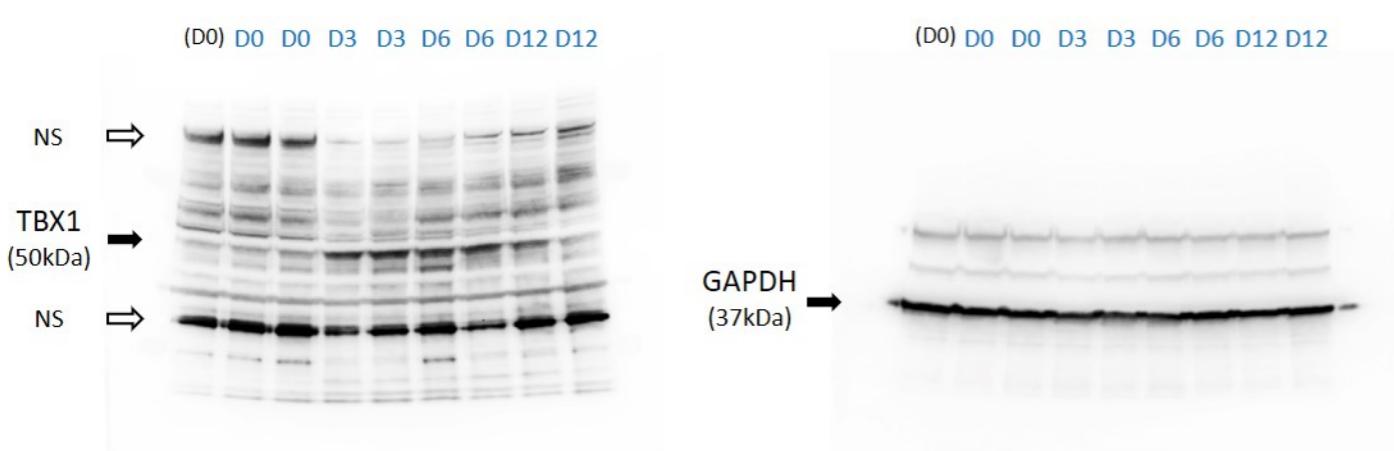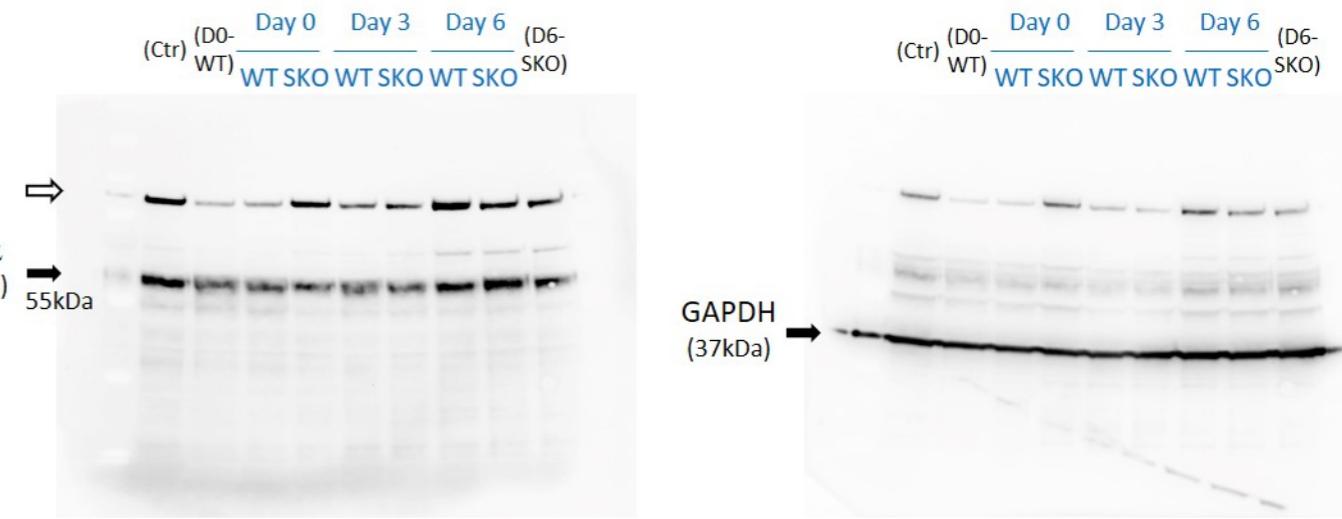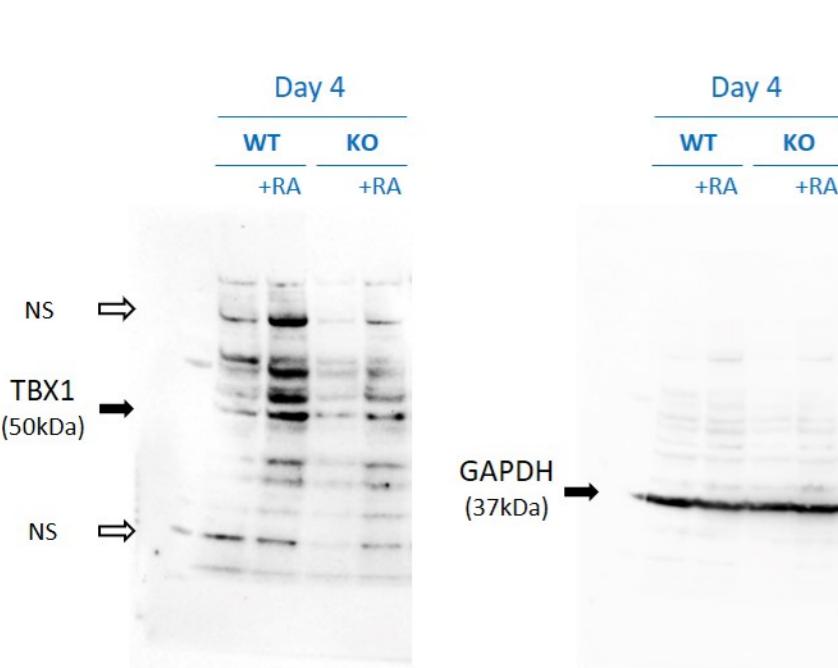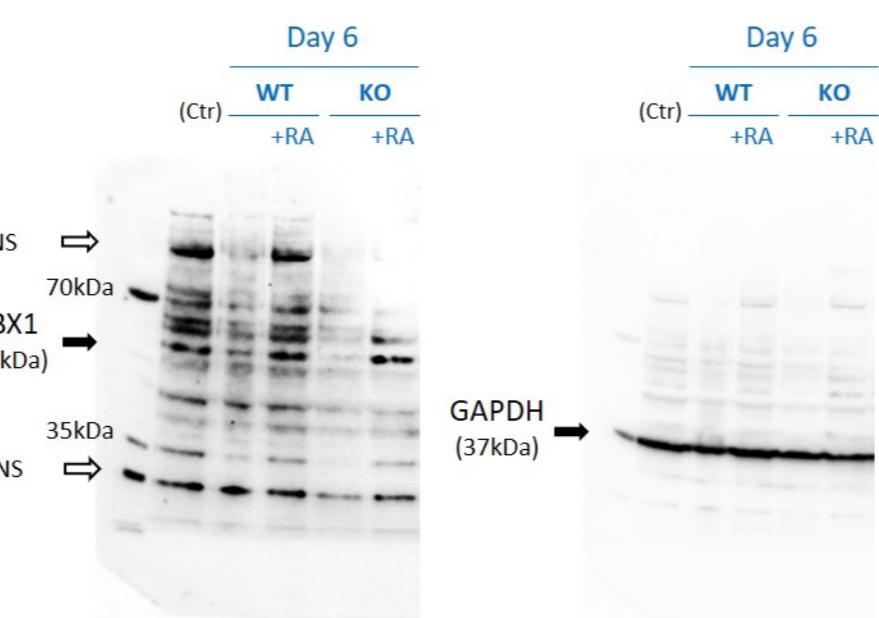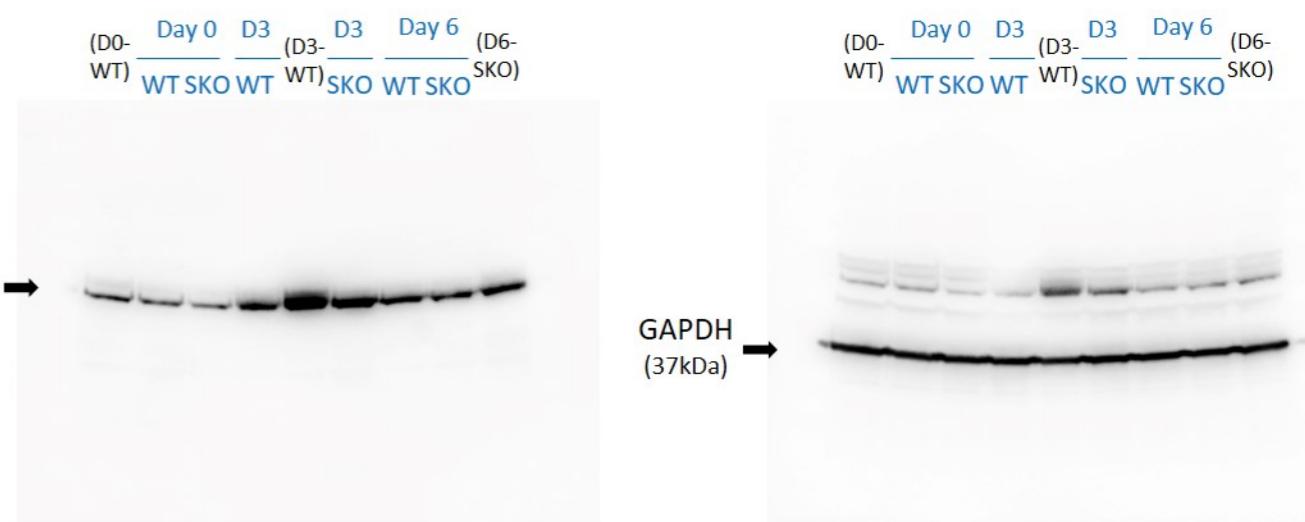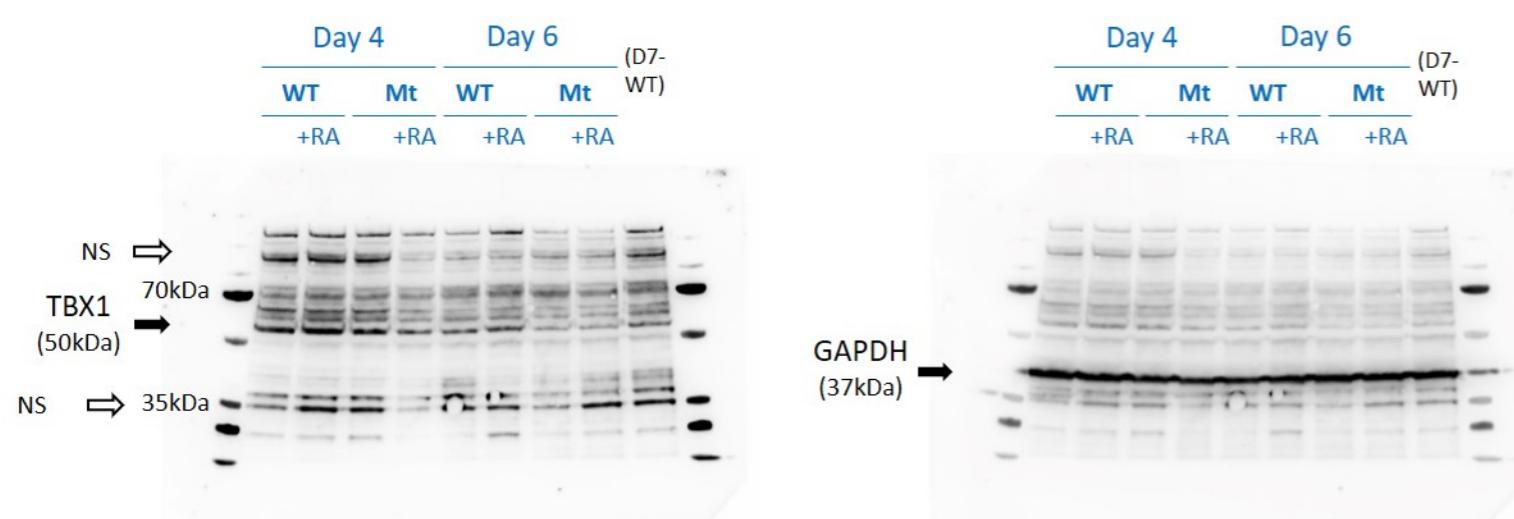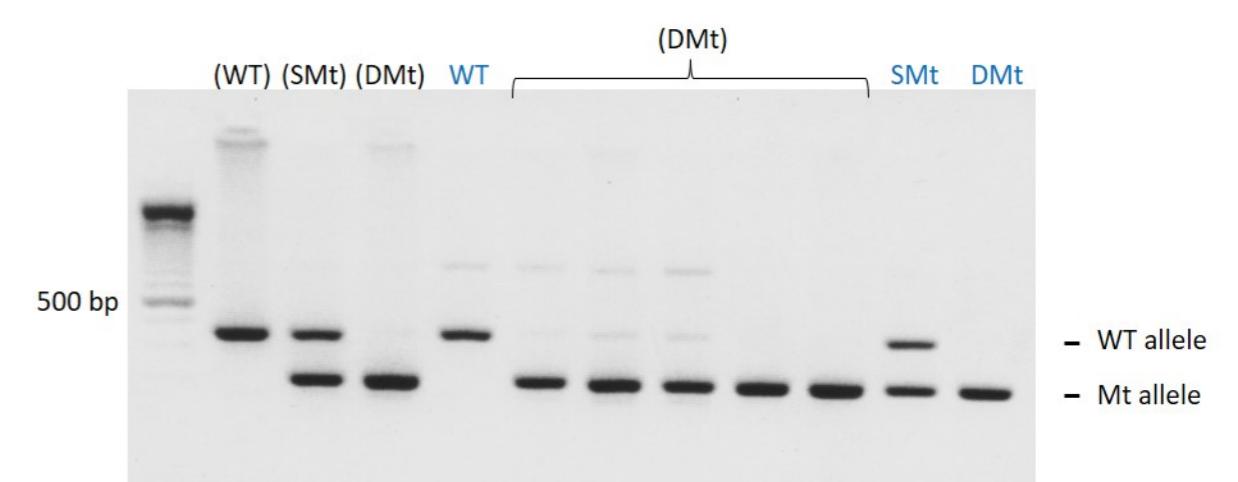
Figure S11

98 **Figure S11. Images of the complete unedited gels.**

99 Images of the complete unedited gels for each representative cropped gel within the text and figures

100 are shown. (A) is for Figure 3C; (B) for Figure 6E; (C) for Figure 6G, *left* (Day 4); (D) for Figure 6G,

101 *right* (Day 6); (E) for Figure 6I; (F) for Figure S8A; (G) for Figure S8B, *left* (RARα); (H) Figure S8B,

102 *right* (RXRα); and (I) for Figure S10B. The lanes corresponding with the lanes shown in the cropped

103 gels presented within the text and figures are highlighted in blue color. Ctr, control; D, day; NS, not

104 specific. Other abbreviations are same as those described in each of the figure legends, respectively.

105

106

Table S1. Primary antibodies used for immunostaining, flow cytometry, western blotting and ChIP experiments.

Primary Antibody	Purpose	Company	Catalog Number	Dilution
α-SMA	Immunostaining	Sigma-Aldrich	A2547	1:400
β-actin-HRP	Western blotting	Cell Signaling Technology	5125S	1:2000
CDH5	Immunostaining	R&D	AF938	1:100
GAPDH-HRP	Western blotting	Cell Signaling Technology	8884S	1:2000
ISL1	Immunostaining	DSHB	39.4D5	1:20
ISL1-PE	Flow cytometry	BD Biosciences	562747	1:100
Ki67-FITC	Flow cytometry	BD Biosciences	556026	1:20
PDGFRB-PE	Flow cytometry	BD Biosciences	558821	1:100
RARα	Western blotting ChIP	Cell Signaling Technology	62294S	1:1000 1:50
RXRα	Western blotting ChIP	Cell Signaling Technology	3085S	1:1000 1:50
SM22	Immunostaining Flow cytometry	Abcam	10135	1:100 1:100
STRA6	Immunostaining	Thermo Fisher Scientific	MA5-24206	1:40
STRA6	Flow cytometry Western blotting	Novus	NBP1-83719	1:100 1:1000
TBX1	Western blotting	Abcam	ab18530	1:1000
TNNT2	Immunostaining	Thermo Fisher Scientific	MS-295-P1	1:100
TNNT2-APC	Flow cytometry	Miltenyi Biotec	MB-130-120-403	1:100

*ChIP, chromatin immunoprecipitation

Table S2. Primers for quantitative PCR.

Gene	Forward primer	Reverse primer
ACTA1	GAGGTATCCTGACCCTGAAGTA	AAGCTCGTTGTAGAAGGTGTG
ALDH1A2	GCAGGTCACTGCACAACTA	CTCCTCCCTTTATCCCACTTTC
GAPDH	GGTGTGAACCATGAGAAGTATGA	GAGTCCTTCCACGATACCAAAG
GBX2	AAGGGAAAGACGAGTCAAAGG	CAGATTGTCATCCGAGCTGTAG
HOXA1	CAGAGGGTAGCCTTGATTTGT	GGGTAGAGATTGGGAAAGACTTAG
MEOX1	GCCCATCATAACTACCTGACTC	CCACTTCATCCTTCGGTTCT
MSX1	CAGAAGCAGTACCTGTCCATC	CTCTGCCTCTTGTAGTCTCTTTG
MSX2	CAGTACTCCTGCTCTGCTAAC	CCTCCGCCTACAGAACAAAT
MYH9	CGGACCTTCCACATCTTCTATT	TTGGACAGGAAGCGGTATTT
MYOCD	GAACTCCTGGACGTGCTTATT	CGGGAAGATCTGGGTATCTTTG
MYOD1	CCGCTTTCCTTAACCACAAATC	CCGGCTGTAGATAGCAAAGT
OSR1	CGGAACAAGATCCGGATTGA	CTGAAGGAAGGAGTAGTTGGTG
PECAM1	GCAACACAGTCCAGATAGTCGT	GACCTCAAACTGGGCATCAT
SMAD3	CCTGAGTGAAGATGGAGAAACC	GGCTGCAGGTCCAAGTTATTA
SNAI1	CAGCTATTTCAGCCTCCTGTT	CCGACAAGTGACAGCCATTA
SNAI2	CTCTCTCCTCTTTCCGGATACT	GCTTGGACTGTAGTCTTTCCTC
SOX7	CCACGTACTACAACAGCTACAG	CACTCAAGGCACAAGAAGGA
SOX9	TGACCTATCCAAGCGCATTAC	GCTTGCTCTGAAGAGGGTTTA
SOX10	AGCCCTCAGGACCCTATTAT	TCAGAGATGGCCGTGTAGA
SOX17	CAACTATCCTGACGTGTGACAG	ACCCAGGAGTCTGAGGATTT
STRA6	GAACCTCCTTTGCAGGAAGAA	GAGTGTAGATGCAGTGTCTCAAG
TAGLN	GCATCTCGGGCTTGTATGT	CTCCTGTTCTATGGCCTGTTT
TBX1	CGACAACGGCCACATTATTC	CTCGGCATATTTCTCGCTATCT
TGFB1	CGTGGAGCTGTACCAGAAATAC	CACAACTCCGGTGACATCAA
TWIST1	AGACTCTGGAGCTGGATAACT	GCCTGTCTCGCTTTCTCTTT
TWIST2	GGATGCATTCGAGTCTGTAACT	TCACTGCTGTCCCTTCTCT
ZIC1	GAGCGACAAGCCCTATCTTT	GGATTCGTGGACCTTCATGT

Table S3. List of the WT or STRA6-KO hESC-derived cardiac differentiating cells-enriched genes.

Day 3						Day 6						Day 12					
WT-enriched			KO-enriched			WT-enriched			KO-enriched			WT-enriched			KO-enriched		
Gene	LogFC	P-Value	Gene	LogFC	P-Value	Gene	LogFC	P-Value	Gene	LogFC	P-Value	Gene	LogFC	P-Value	Gene	LogFC	P-Value
FOXI2	1.58659	0.001406	NDUFA4L2	2.180206	0.000151	SLC17A8	2.489139	0.007724	MYOF	1.138333	0.002015	ACTA1	3.422072	2.59E-06	FUT3	2.813608	1.42E-08
SCGB1A1	1.584444	0.026857	PPFIA4	1.934361	0.000244	ALDH1A2	1.772209	0.008103	S100A10	1.027926	0.006054	TNC	2.370876	1.10E-07	RPS10-NUDT3	2.682573	2.06E-05
T	1.520559	0.023925	PFKFB4	1.811499	0.015405	HOXA1	1.484818	0.009704	MIR4258	0.933979	5.69E-05	TAGLN	2.217828	7.62E-05	TTR	2.28234	0.003869
TCTEX1D1	1.491943	0.009059	RP11-867G2.6	1.751092	0.009245	TLX1	1.462542	0.000122	NMRK2	0.927364	0.006566	ACTA2	2.055195	4.83E-08	LINC01363	2.240982	3.37E-08
SPOCK3	1.441055	0.000887	ASB5	1.74784	0.023841	CD82	1.381355	0.007394	CTC-550B14.7	0.908251	0.025001	TUBA1A	2.039319	1.40E-07	TFPI2	2.170877	2.00E-07
ODC1	1.336316	0.013048	SLC5A9	1.73966	0.017605	MT-TF	1.236286	0.039769	RP11-85G21.3	0.877808	0.029693	CNTN5	2.018824	8.46E-07	OR7E13P	2.163981	2.13E-07
DAND5	1.27011	0.009416	PDE4C	1.619249	0.000172	RP11-834C11.4	1.227464	0.001566	RP11-3B12.5	0.811728	0.021532	PSMD5-AS1	2.01764	0.000113	CDKN1A	2.152777	1.03E-05
CYP24A1	1.266689	0.01746	LINC011088	1.594633	0.001568	LIX1	1.211157	0.012443	MIR6807	0.805	0.010954	MFSD1	1.955004	1.67E-06	ACTN3	2.131987	5.32E-09
SPP1	1.209422	0.005319	RP11-277P12.10	1.533453	0.003682	TBX1	1.202287	0.000166	EEF1A2	0.792517	0.024609	DHFR	1.916338	6.11E-09	CTC-435M10.3	2.083333	0.028099
TPPP3	1.208138	0.010226	RP11-267L5.1	1.493404	0.00134	RPL17-C18orf32	1.177467	0.008447	DKK3	0.791302	0.01333	DHCR24	1.893371	4.11E-06	TCTEX1D1	2.044006	1.03E-05
FOXD1	1.170805	0.007798	ARHGAP40	1.485715	0.015093	HEY1	1.165475	0.032891	SNORD83A	0.774298	0.0061	ITGB1BP2	1.884873	5.40E-07	FHL2	1.992818	4.50E-05
TBX1	1.133627	0.018656	STC1	1.483094	0.009369	HOTAIRM1	1.154114	0.020935	RP11-85G21.2	0.733372	0.003657	SEC11C	1.87952	2.31E-05	ABRA	1.962749	1.38E-07

HLA-B	1.131302	0.011665	UPK1A-AS1	1.469999	0.000437	NTRK2	1.061248	0.000135	LINC01108	0.723574	0.00992	RP3-527G5.1	1.879332	0.000112	MAOA	1.935368	3.47E-06
PKIB	1.123184	0.000245	TRDN	1.459467	0.000806	PRRX2	1.058239	0.023134	ALPK3	0.715868	0.005649	TCTA	1.878493	1.95E-07	HIST1H2AC	1.92615	5.97E-05
EFCAB1	1.117372	0.002412	LINC00458	1.428268	0.005421	LINC01088	1.046111	0.016419	S100A4	0.7068	0.000426	ACAT2	1.759467	1.14E-05	RASL12	1.916309	8.30E-08
DRC1	1.099608	0.021664	RP3-467D16.3	1.404839	0.027403	AKR1B15	1.029897	0.036643	CIART	0.680766	0.009608	MYL9	1.759126	7.88E-08	MT-TF	1.867579	1.53E-05
RHBDL3	1.084739	0.01247	LINC00467	1.38738	0.000173	RP11-83C7.1	1.013563	0.000495	DNAJC15	0.674475	0.026851	MT-CYB	1.745065	2.23E-08	RP11-92G12.3	1.823371	3.95E-06
MAATS1	1.047167	0.009769	SLC2A14	1.379912	0.000243	PSMD5	0.985662	0.000777	NPY	0.668566	0.00121	PLEKHA6	1.734593	1.15E-06	PTAR1	1.799045	1.12E-06
MAPK15	1.032567	0.01583	NDRG1	1.377136	0.01435	GPC5-AS1	0.981817	0.001329	GJA4	0.663096	0.040048	PLAT	1.733906	4.31E-06	EIF4EBP3	1.771148	1.37E-07
PSMD5	1.030543	0.000683	CBR1	1.372808	0.012481	SMOC1	0.980258	0.024766	SNORD12B	0.658472	0.022009	PSMB9	1.705744	3.47E-06	PLCXD3	1.766715	3.86E-08
PKD1L1	1.006605	5.96E-05	CIART	1.36779	0.001368	NETO1	0.979023	0.043757	ZNF639	0.64847	0.000604	TP53I3	1.701381	5.52E-07	RRAD	1.742327	0.002225
RP5-857K21.11	1.006279	0.000513	SNORD3B-2	1.367004	0.014866	PLSCR5	0.97635	0.00465	MYLPF	0.647004	0.00593	MIR3164	1.659417	0.000311	N4BP2L1	1.708203	4.32E-06
PROM1	0.996095	0.042432	SNORA40	1.331588	0.02007	CDH11	0.967371	0.005443	NPAS4	0.630915	0.003993	HNRNPM	1.646681	7.92E-07	GRK5	1.695051	1.32E-06
FAM198B	0.994072	8.49E-05	AC114803.3	1.324886	0.000773	PRAC2	0.963295	0.009642	SLC2A3	0.620196	0.007562	PDLIM7	1.64576	6.80E-07	RP11-329B9.5	1.687963	4.00E-07
COL2A1	0.986222	0.029423	AC073071.1	1.317158	0.007534	SPOCK3	0.962089	0.02158	CDH2	0.613895	0.019435	OSR1	1.64331	4.91E-05	ATP1A2	1.663465	1.49E-05
CAPN9	0.978742	0.002829	CXADRP3	1.293753	0.000392	SIAH3	0.95431	0.024425	EFNA1	0.611829	0.00015	RP1-178F15.5	1.641372	0.020513	APOA2	1.662661	0.002025
NRGN	0.975037	0.012822	CD1D	1.29024	3.31E-05	RP11-977G19.10	0.920858	0.034262	ZNF790-AS1	0.609137	0.01816	MT-TE	1.62826	5.57E-05	HSPB2	1.648072	1.00E-05

RP11-288A5.2	0.958572	0.004562	PCDHGA11	1.280523	0.004407	SFRP5	0.89715	0.018115	LMO2	0.606244	0.010626	FUS	1.60938	2.08E-08	HIST1H2BD	1.599789	1.78E-05
RNASEK-C17orf49	0.939627	0.035178	CPSF1P1	1.270932	0.038796	FOXQ1	0.890868	0.008105	PTRF	0.603363	0.026123	ACTB	1.590282	8.86E-07	RP11-554A11.9	1.595001	2.28E-05
PSMD5-AS1	0.938317	0.002317	RP11-798M19.6	1.270861	0.02176	ODAM	0.888141	0.018192	TCEA3	0.602361	0.039492	SNORD14E	1.582465	0.005872	UHMK1	1.546295	1.16E-06
NECAB2	0.934283	0.003449	SLC16A3	1.270071	0.002357	CCBE1	0.885653	0.01433	SLC2A14	0.601362	0.013866	SNORD3A	1.554207	0.003548	PPP2R2B	1.542573	1.54E-05
MIR6891	0.929836	0.007355	FGF11	1.269209	0.000251	DNAH2	0.877608	0.047051	AL450992.2	0.596637	0.047558	FAM198B	1.523851	1.53E-06	CCDC169-SOHLH2	1.541899	0.047632
EML1	0.923097	0.018499	ABHD12B	1.23171	0.00616	DACH1	0.873065	0.01442	MIR548AR	0.591832	0.014087	ANAPC15	1.522927	2.45E-05	UQCRB	1.539928	3.17E-05
TMEM132E	0.918854	0.043029	SNRPCP6	1.211533	0.029072	RDH10	0.872685	0.003099	MIR22HG	0.574452	0.03793	AMH	1.521821	0.004841	AEN	1.53152	4.21E-07
SNORA73B	0.917148	0.009006	FAM162A	1.201268	0.000887	STRA6	0.870875	0.002111	PCGF5	0.571868	0.031644	TSPAN32	1.516628	6.64E-05	DDB2	1.528912	1.07E-05
RSPO3	0.914009	0.028405	ENO2	1.197504	0.000123	H2AFJ	0.86952	0.002651	LHFP	0.566689	0.00858	RAB15	1.508047	3.73E-07	RP11-247A12.2	1.52776	1.63E-06
SEMA3D	0.910333	0.003391	PLOD2	1.195219	0.001622	ATF7IP2	0.850974	0.018994	PRKAB2	0.554618	0.030902	MTG1	1.507459	1.75E-05	RP11-329B9.4	1.501472	4.07E-07
SNORD14A	0.902251	0.008353	RP11-69I8.2	1.174344	0.004625	HOXB3	0.85044	0.032274	HNF4G	0.547617	0.035243	SNORD59A	1.492518	0.000132	SNORD117	1.488355	2.58E-05
RP5-857K21.7	0.899592	0.007195	RP11-11N5.1	1.170709	0.000657	AP000275.65	0.840401	0.0055	MUC1	0.543098	0.001035	SLC38A7	1.490785	4.83E-08	EDA2R	1.472469	2.64E-06
RIPPLY3	0.891378	0.00106	TRAJ49	1.17039	0.033628	AMIGO2	0.839739	0.016493	NOVA1-AS1	0.541099	0.012004	AQP10	1.477695	2.45E-06	MYCN	1.472433	7.10E-05
WDR4	0.878018	0.002876	GATA6-AS1	1.156625	0.010571	IRX5	0.828052	0.027786	GSAP	0.526816	0.014557	SNF8	1.473856	1.28E-07	RAB25	1.465996	0.001674

ADAM28	0.876713	0.006642	BNIP3	1.156506	0.003112	PTGIS	0.824327	0.003635	MIR6895	0.526785	0.0461	ID4	1.46873	1.82E-07	TUNAR	1.463695	1.99E-07
NXPH2	0.868348	0.01836	SLC2A3	1.153905	0.000121	RALYL	0.82263	0.000749	YBX2	0.526728	0.041365	SLC1A4	1.466856	1.50E-06	ADIRF-AS1	1.458832	1.13E-05
C6orf118	0.859067	0.030385	C1orf61	1.151595	0.003507	AKR1C2	0.821443	0.015897	RWDD2B	0.517697	0.0084	SCUBE3	1.451726	3.34E-07	RASGRP1	1.444618	2.71E-06
FBLN7	0.851673	0.008889	RP1-90G24.10	1.149408	0.04807	DUSP9	0.818199	0.014819	CCDC141	0.514778	0.019694	GATA2-AS1	1.445647	1.01E-05	MT-TV	1.438587	1.75E-05
SPAG1	0.83473	0.031528	SMIM24	1.144495	0.000234	FRMD6	0.81017	0.033337	SNORD99	0.502714	0.017291	PIK3R3	1.424291	2.50E-06	ZMAT3	1.424797	5.19E-05
NDUFC2-KCTD14	0.830991	0.0364	U4	1.135268	0.006053	MAB21L2	0.804035	0.016229	CD99	0.500044	0.014185	RSPO4	1.421908	1.68E-05	ILDR2	1.409243	3.19E-05
CA2	0.822652	0.003406	MIR367	1.113387	0.041393	RP11-49K24.6	0.802626	0.007797	RCAN2	0.497535	0.04516	LANCL2	1.414772	4.08E-06	RP11-8P13.5	1.408134	2.35E-06
MUC4	0.81073	0.018031	P4HA1	1.100413	0.017835	NHS	0.798489	0.015824	LINC00240	0.482317	0.030562	FREM2	1.401502	3.65E-06	GS1-259H13.2	1.407049	9.13E-06
SOX9	0.809694	0.022351	BEX5	1.09986	0.000119	METTL7A	0.795872	0.047098	C18orf32	0.477445	0.007915	LSS	1.382376	3.73E-06	ECHDC2	1.403317	1.73E-05
CFAP45	0.807239	0.033233	SPAG4	1.099522	2.15E-05	STAR	0.792425	0.021895	RNVU1-3	0.463262	0.021473	NCOA5	1.373497	1.42E-06	PPP1R1B	1.387096	4.75E-07
AC078794.1	0.805336	0.000342	NANOGP1	1.096679	0.014329	RP11-834C11.7	0.784289	0.000827	RP4-798A10.4	0.461741	0.032443	ARSA	1.360578	0.000119	HIST1H1C	1.383838	0.000184
HSPH1	0.804621	0.025073	SERPINB9	1.08722	0.017694	RPS10-NUDT3	0.784203	0.003976	PEX19	0.461252	0.001169	MSRA	1.353167	4.11E-05	MT-TR	1.381308	0.000266
SPAG6	0.798377	0.040106	RP11-184E9.4	1.060541	0.044608	HOXB2	0.783865	0.023985	NOL4	0.460432	0.017121	GPC5-AS1	1.351155	8.79E-07	RP1-28O10.1	1.379122	0.000688
HMX1	0.797392	0.006513	BNIP3P1	1.05514	0.003274	TBX5-AS1	0.783065	0.028811	IQCA1	0.458246	0.028814	MIR324	1.347615	1.67E-05	RP11-441O15.3	1.378592	3.90E-08
HAPLN3	0.796928	0.038102	POTEC	1.051358	9.56E-05	HOXB4	0.7816	0.046904	SLFN13	0.457759	0.00563	TFRC	1.342488	4.23E-05	MIR548AR	1.377964	0.000126
C4orf48	0.788533	0.035137	RFPL2	1.04924	0.031842	FZD10-	0.779527	0.017791	PPP1R9A	0.457737	0.021655	CKMT1B	1.336781	3.70E-07	GDF15	1.363598	0.000147

						AS1											
ADAT1	0.787525	0.030488	CD177	1.044634	0.028673	SPON1	0.772356	0.035079	ARHGAP23	0.452733	0.00574	ABCA8	1.336568	1.67E-07	RASGEF1A	1.358106	2.61E-07
C3orf14	0.786821	0.017817	RP11-3B12.3	1.039449	0.042985	RP11-442N1.1	0.757447	0.029956	XXbac-B444P24.13	0.451398	0.018132	PEPD	1.331514	2.00E-06	TSPO	1.353401	2.56E-05
ARL4A	0.783169	0.033948	SLC2A1	1.035614	0.037573	CTB-12O2.1	0.754541	0.037338	SLC25A44	0.450667	0.01492	NPPB	1.31084	6.72E-05	NTRK3	1.352481	5.82E-07
RRS1	0.780458	0.022598	MIR302B	1.035282	0.0402	MMP2	0.742353	0.02766	ZNF761	0.449211	0.036758	RN7SL493P	1.306376	0.007528	MTHFD2	1.351219	1.07E-06
C9orf117	0.779384	0.024393	ZNF578	1.035125	0.000679	MCTS2P	0.731664	0.001806	TSPAN7	0.449074	0.040484	ATP6V0D1	1.305305	3.33E-07	CCND2	1.349951	1.10E-06
RARRES2	0.777041	0.038486	CTD-2562J17.6	1.022022	0.025082	MIR573	0.724791	0.010094	CDH5	0.44682	0.003379	MAMDC4	1.3043	0.000422	GNG11	1.34432	1.64E-06
CTPS1	0.773968	0.015896	NOL3	1.018167	0.002292	RP11-256I9.3	0.719387	0.009773	SHTN1	0.445798	0.006995	PFKFB3	1.299574	3.17E-06	DENND1C	1.341472	5.88E-07
ADAM11	0.772143	0.047904	NLGN4X	1.00331	0.03009	BOC	0.705537	0.023004	TRIB1	0.44449	0.002972	REC8	1.298826	9.35E-07	LYZL4	1.341245	1.89E-05
NKAIN1	0.769874	0.005472	PGK1	1.000818	0.000244	CACNG4	0.701992	0.016553	ZNF717	0.443544	0.007462	HSD17B10	1.296281	3.78E-06	RP11-329B9.3	1.336528	1.14E-06
ALKBH3	0.765659	0.002851	WNT8A	0.992221	0.043485	CSNK2B-LY6G5B-1181	0.700101	0.048522	SLC35A3	0.440486	0.001243	ZNF503-AS2	1.294322	1.73E-05	AC226119.5	1.329527	4.33E-06
TBX5	0.762626	0.006389	CTC-550B14.7	0.9777	0.001864	SNORA11	0.692829	0.01421	SLC40A1	0.440309	0.042167	LY6G5B	1.291238	5.10E-06	SLC1A5	1.321215	9.58E-07
MIR4292	0.76199	0.000239	RP11-348J12.5	0.975964	0.014574	LRP11	0.692081	0.005941	PSKH2	0.438644	0.018166	TPCN2	1.290751	2.24E-05	CD9	1.318586	0.000142
NAP1L3	0.756914	0.003139	ACTN3	0.967153	0.005276	RP11-86K22.2	0.688879	0.032785	ENOX2	0.437906	0.007701	PHYHD1	1.278421	2.88E-06	CAPN3	1.315468	2.19E-05

NKAIN4	0.756646	0.031747	SH3D21	0.95889	3.06E-05	MEIS1-AS3	0.684327	0.026692	ZSCAN1	0.434358	0.011236	TAP1	1.278048	3.90E-05	RP11-676J15.1	1.314574	0.000144
SPA17	0.754885	0.005188	RP11-20D14.6	0.954109	0.009321	DLEU1_1	0.680974	0.019468	TSEN15	0.433912	0.029918	ARHGAP1	1.276275	1.22E-05	ANGPT1	1.313351	1.06E-06
MOXD1	0.753571	0.042558	RP11-415G4.1	0.951174	0.002502	NFKBIZ	0.678584	0.025922	PIP5K1A	0.430578	0.002203	SIRPA	1.273966	2.60E-06	FAM19A4	1.312446	6.17E-05
HMGCS1	0.749984	0.020773	CYP2S1	0.949591	0.001929	NMNAT3	0.676265	0.00206	NQO1	0.427485	0.04383	CYP26A1	1.272864	0.001457	PRKX	1.310364	1.36E-05
COL26A1	0.743912	0.045183	ERVH48-1	0.947671	0.014667	APOLD1	0.670478	0.026877	TUFT1	0.420933	0.015918	MIR22HG	1.269574	2.21E-05	ELL2	1.303662	5.92E-07
SNORA65	0.74197	0.033764	C3	0.947065	0.001776	TMEM132E	0.666563	0.01687	A2M	0.417064	0.041543	TLX1	1.265343	7.96E-07	SPATA18	1.302339	5.20E-06
VWCE	0.739461	0.005978	CTD-2265O21.3	0.939329	0.000289	PITX2	0.660524	0.004469	TINAGL1	0.415807	0.020473	COMMD4	1.26182	2.12E-05	AC024592.12	1.299241	0.006951
UHRF1	0.732354	0.044181	RP11-11N5.3	0.92939	0.004843	CCDC3	0.650629	0.038242	AC010329.1	0.415535	0.024283	LINC00982	1.260686	1.55E-05	KCNIP2	1.298186	2.74E-05
SQLE	0.73197	0.014479	RP11-240B13.2	0.924836	0.004411	KAZALD1	0.650522	0.0055	MNS1	0.415046	0.007172	TMEM26	1.258106	3.22E-06	GAS5	1.276671	1.69E-06
CENPV	0.730553	0.005074	CTD-2306M5.1	0.920631	0.034244	RBM14-RBM4	0.648373	0.012348	RNF115	0.412793	0.013452	CRELD2	1.257898	9.96E-07	BTG3	1.276489	3.77E-07
RP3-467L1.6	0.726311	0.000309	MIR210HG	0.896773	0.003375	RP11-1099M24.6	0.64698	0.03869	VPS72	0.412637	0.020623	CKMT1A	1.257545	3.15E-06	LDB2	1.273284	0.000215
SNORA13	0.725034	0.032634	MAGEA4	0.89596	0.004508	PSMD5-AS1	0.64564	0.009451	RP11-567G11.1	0.412517	0.01438	CCNL2	1.251516	9.05E-05	TNFRSF10C	1.269653	8.55E-05
ERICH6-AS1	0.721158	0.032666	MID2	0.893486	0.009924	PDGFRB	0.644915	0.008671	ZDHHC23	0.412116	0.038767	RP11-2C24.9	1.248104	0.000433	CRHR1	1.263378	5.06E-07
TIGAR	0.719863	0.025721	PMF1-BGLAP	0.891609	0.02083	EBF4	0.64424	0.00553	SPRY2	0.405827	0.039791	MIR210HG	1.243419	7.25E-06	KTN1-AS1	1.24802	1.06E-05

RNU2-2P	0.715812	0.041311	RP11-268J15.5	0.886311	0.000162	SPX	0.642386	0.007041	MATR3	0.404518	0.011478	TM7SF2	1.241359	1.01E-06	GRIN2A	1.245362	1.57E-07
CCDC40	0.715441	0.00433	LRRC3-AS1	0.881634	0.025035	TFPI	0.635801	0.019927	UCHL5	0.403497	0.023078	NPHP3-ACAD11	1.241077	0.004073	SGIP1	1.242653	2.40E-05
CTD-2514C3.1	0.714719	0.020678	FAR2P2	0.877466	0.010349	LINC00890	0.635113	0.038268	GABRP	0.40277	0.009521	LIMD2	1.23632	4.23E-07	VAMP5	1.237747	1.28E-07
BZW1	0.711334	0.000623	PDK1	0.874705	0.003509	LRRC4	0.633744	0.014347	OIT3	0.401206	0.031433	ACOX2	1.232091	7.47E-06	GSDMB	1.235726	6.35E-05
RP11-395A13.2	0.710271	0.033118	VLDLR	0.869468	0.007004	SCARNA22	0.630718	0.005435	MIR6747	0.400051	0.029911	ALOX15	1.230301	0.000354	ZNF121	1.234388	3.10E-06
RP11-216L13.19	0.707229	0.003417	COL11A1	0.869251	0.007409	ANKRD19P	0.629662	0.034434	ZNF808	0.395084	0.004098	INHA	1.229246	8.88E-07	BBC3	1.228464	8.61E-06
SAMHD1	0.706262	0.001503	HHLA1	0.867535	0.021694	RP11-676J15.1	0.621875	0.017492	ITGA6	0.394791	0.033502	IDUA	1.229158	3.47E-05	DES	1.228267	4.25E-05
CHAC2	0.70417	0.031886	PLA2G2A	0.866339	0.003125	AC144522.1	0.619949	0.02361	MAP3K13	0.3943	0.009719	TNNC1	1.228866	5.72E-06	CMBL	1.223211	3.58E-06
PSPC1	0.701789	0.024502	P4HA2	0.853711	0.002803	CHURC1-FNTB	0.615064	0.006269	SNORD15B	0.393318	0.01738	RP11-561B11.2	1.227745	0.047779	PTTG1	1.222107	4.59E-05
MAPK4	0.701252	0.022459	HTR2C	0.851316	0.001547	AL139396.1	0.613555	0.007233	PCDHGA11	0.391366	0.021706	GOLGA8A	1.227246	0.000162	NPY	1.220218	0.000502
PTPN13	0.699427	0.013581	B3GNT4	0.850521	0.000559	WDR86	0.611399	0.027764	RNU2-17P	0.389702	0.049405	DERL3	1.225865	2.43E-07	ENG	1.219737	2.92E-06
PRIMA1	0.699118	0.042757	AP000688.29	0.848654	0.008386	SYT10	0.606774	0.019161	TNFSF9	0.389609	0.013215	HCN3	1.217335	2.17E-05	HMGCLL1	1.218629	3.03E-05
SNORD30	0.696473	0.044789	APOBEC3G	0.84849	0.006357	GAS2L3	0.60656	0.019589	RP11-20D14.6	0.389138	0.015528	KCNG1	1.217183	7.93E-05	ICAM5	1.212151	0.000326
TRIM17	0.695994	0.044652	RP4-590F24.2	0.847759	0.006862	ISYNA1	0.605315	0.006386	RNU6-945P	0.38409	0.022636	GATA2	1.212061	0.000284	CTB-151G24.1	1.211156	3.46E-06
SLC7A2	0.694159	0.04997	RP5-	0.845184	0.005658	CMC1	0.60418	0.005875	CERS2	0.381491	0.04019	PTOV1-	1.212034	0.000161	SNHG16	1.210769	3.57E-06

			1061H20.4						AS2					
ARRDC3	0.69206	0.035924	**AC078941.1**	0.841532	0.000236	**RNA5SP383**	0.603713	0.016564	**RP11-262I2.2**	0.381456	0.021025	**ADM**	1.206802	0.001365	**NOB1**	1.209647	3.25E-07