

Supplementary data

Supplementary Material and Methods

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated as described in the SV Total RNA Isolation System (Promega) with on-column DNase digestion. DNase-treated RNA (200 ng) was used for the first-strand cDNA synthesis by using Murine Leukaemia Virus Reverse Transcriptase and Oligo d(T)₁₆ (Applied Biosystems). One-tenth of the cDNA reaction was taken as PCR template and amplified for 25-35 cycles depending on the particular mRNA abundance with denature at 94°C for 15 seconds, annealing at 51°C to 66°C for 15 seconds according to the primers, and elongation at 72°C for 30 seconds. *GAPDH* or *TTN* was used as an internal control. The primer sequences (forward and reverse), the annealing temperatures, and the cycles used for RT-PCR analyses were listed in Table S2.

Immunocytochemical staining

Cultures at different stages were fixed with 4% paraformaldehyde (PFA, Sigma) in PBS at room temperature for 20 minutes, and then blocked with 1% BSA at 4°C overnight. The primary antibodies were used at the following dilutions: OCT4 (goat polyclonal antibody, 1:100, R&D), SOX2 (rabbit polyclonal antibody, 1:2000, Millipore), NANOG (goat polyclonal antibody, 1:200, R&D), LIN28 (goat polyclonal antibody, 1:100, R&D systems), stage-specific embryonic antigen 4 (SSEA4, mouse monoclonal IgG3, DSHB), TRA-1-60 (mouse monoclonal IgM, Millipore), α -actinin (mouse monoclonal IgG1, 1:1000, Sigma Aldrich), myosin light chain 2v (MLC2V, rabbit IgG antibody, 1:200, Proteintech, 10906-1-AP), myosin light chain 2a (MLC2A, mouse IgG2b antibody, 1:500, Synaptic Systems, 56F5), cardiac troponin T (cTNT, mouse monoclonal IgG1, 1:100, Neomarkers), smooth muscle- α -actin (α -SMA, mouse monoclonal IgG1, Sigma Aldrich), α -fetoprotein (AFP, rabbit polyclonal antibody, 1:300, Dako), and β -III-tubulin (mouse monoclonal IgG2a, 1:500, BAbCo). The primary antibodies were applied for 1 hour at 37°C or overnight at 4°C. The fluorescently labeled secondary antibodies with minimal cross reactivity were Cy3-conjugated donkey anti-goat IgG (1:600, for NANOG, OCT4, and LIN28), Cy3-conjugated goat anti-rabbit IgG (1:600, for SOX2, AFP, and MLC2V), and FITC-conjugated goat anti-mouse (1:200, for SSEA4, TRA-1-60, α -actinin, cTNT, MLC2A, α -SMA, and β -III-tubulin) from Dianova. The secondary antibodies were administered in 1% BSA for 1 hour at 37°C. Nuclei were counter-stained with 4,6-diamino-2-phenylindole (DAPI, 0.2 ng/ml, Sigma Aldrich). Samples were mounted with VectaShield mounting medium (VECTOR Laboratories). For antibodies against OCT4, SOX2 and NANOG, the samples were initially permeabilized by incubation in 0.1% Triton X-100 (Sigma Aldrich) in PBS for 10 minutes at room temperature.

Images were analyzed using a fluorescence microscope (Axiovert 200, Zeiss). Detection of the FITC fluorophore was performed by using the filter sets with excitation BP 475/40, beam splitter FT 500 and emission BP 530/50. Detection of the Cy3 fluorophore was performed by using the filter sets with excitation BP 540/25, beam splitter FT 565 and emission BP 605/55. Detection of the DAPI fluorophore was performed by using the filter sets with excitation G 365, beam splitter FT 395 and emission BP 445/50.

Bisulfite sequencing assays

The DNA methylation analysis was conducted using bisulfite sequencing assays by Epigenomics (Berlin). Briefly, genomic DNA was isolated and purified from three independent cell lines of the patient with the mutation in *RBM20* (1-RBM20-2, -3, and -7) using QIAamp DNA Mini kit (Qiagen). Human ESC line HES3 was used as positive control.

The genomic DNA was then treated with sodium bisulfite. One region (399 bp, from -213 bp to +185 bp relative to the TSS) was studied in the *OCT4* (NM_002701) promoter at the minimal promoter (MP) containing the TSS. In addition, one region (443 bp, from -378 bp to +64 bp relative to the TSS) of the *NANOG* gene (NM_024865) was studied. The following primers (forward and reverse) designed for the regions of interest were used for PCR and for sequencing: *OCT4*: 5' GAT TTG TAT TGA GGT TTT GGA 3' and 5' TCC AAA AAA ACC TTA AAA ACT T 3'; *NANOG*: 5' TAA TTT CAA ACT CCT AAC TTC AAA TAA T 3' and 5' TAA TAT GAG GTA ATT AGT TTA GTT TAG T 3'.

Spontaneous in vitro differentiation

To study the *in vitro* differentiation potential of the generated iPSC lines, spontaneous differentiation using the embryoid body (EB) method was carried out. Briefly, iPSCs were dispersed with collagenase IV into small clumps and cultured in suspension in hESC medium for 1 day. To initiate differentiation, the formed EBs were then cultured in suspension for another 7 days in differentiation medium, consisting of Iscove's modified Dullbecco's medium (Thermo Fisher Scientific), 20% FCS, L-glutamine (2 mM), 1x NEAA, and monothioglycerol (450 µM, Sigma Aldrich). EBs at day 8 were then plated on 0.1% gelatin-coated dishes and cultured in differentiation medium for another 30 days. Tissue-specific genes and proteins were analyzed in EB outgrowths at different differentiation stages by RT-PCR and Immunocytochemical staining, respectively.

Teratoma formation

To investigate the developmental potential of reprogrammed clones *in vivo*, iPSCs (about 2x 6-cm dishes at 70% confluence for each mouse) were injected subcutaneously into 8-week-old immunodeficient mice (4 mice per cell line) as described previously [1, 2]. Six to eight weeks after injection the mice were sacrificed and the resulting teratomas were examined with hematoxylin and eosin staining.

References

- [1] K. Streckfuss-Bomeke, F. Wolf, A. Azizian, M. Stauske, M. Tiburcy, S. Wagner, D. Hubscher, R. Dressel, S. Chen, J. Jende, G. Wulf, V. Lorenz, M.P. Schon, L.S. Maier, W.H. Zimmermann, G. Hasenfuss, K. Guan, Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts, *European heart journal* 34(33) (2013) 2618-29.
- [2] K. Guan, K. Nayernia, L.S. Maier, S. Wagner, R. Dressel, J.H. Lee, J. Nolte, F. Wolf, M. Li, W. Engel, G. Hasenfuss, Pluripotency of spermatogonial stem cells from adult mouse testis, *Nature* 440(7088) (2006) 1199-203.

Supplementary Movie Legends

Video S1: Differentiated beating CMs from RBM20-iPSCs.

[Video S1.wmv](#)

Video S2: A single EHM generated from RBM20-iPSC-CMs.

[Video S2.wmv](#)

Supplementary Tables

Table S1: Cells/ Cell lines used in this study.

Cells	Cell source	Sex	AA substitution
F-1-RBM20 (F-1-R)	Skin fibroblasts from the patient with the mutation in <i>RBM20</i>	F	S635A
1-RBM20-2 (1-R-2)	iPSC line 2 generated from F-1-RBM20	F	S635A
1-RBM20-3 (1-R-3)	iPSC line 3 generated from F-1-RBM20	F	S635A
1-RBM20-7 (1-R-7)	iPSC line 7 generated from F-1-RBM20	F	S635A
Control 1-1 (C1-1)	iPSC line 1 generated from fibroblast of healthy donor 1	F	-
Control 1-2 (C1-2)	iPSC line 2 generated from fibroblast of healthy donor 1	F	-
Control 2-1 (C2-1)	iPSC line 1 generated from fibroblast of healthy donor 2	M	-
Control 2-2 (C2-2)	iPSC line 2 generated from fibroblast of healthy donor 2	M	-
Control 3-1 (C3-1)	iPSC line 1 generated from fibroblast of healthy donor 3	n.d.	-

Table S2: Primers used in RT-PCR analysis.

Gene name	Product size (bp)	Forward primer sequence	Reverse primer sequence	Annealing (°C)	Cycle
GAPDH	300	AGA GGC AGG GAT GAT GTT CT	TCT GCT GAT GCC CCC ATG TT	55	34
OCT4	113	AGT TTG TGC CAG GGT TTT TG	ACT TCA CCT TCC CTC CAA CC	59	41
NANOG	194	TTT GGA AGC TGC TGG GGA AG	GAT GGG AGG AGG GGA GAG GA	64	43
LIN28	410	AGT AAG CTG CAC ATG GAA GG	ATT GTG GCT CAA TTC TGT GC	52	38
SOX2	437	ATG CAC CGC TAC GAC GTG A	CTT TTG CAC CCC TCC CAT TT	56	43
MYH6	413	GTC ATT GCT GAA ACC GAG AAT G	GCA AAG TAC TGG ATG ACA CGC T	60	40
MYH7	101	AGA CTG TCG TGG GCT TGT ATC AG	GCC TTT GCC CTT CTC AAT AGG	63	30
MLC2V	200	GGC GAG TGA ACG TGA AAA AT	CAG CAT TTC CCG AAC GTA AT	56	42
ACTN2	291	AGG AGG AAG AAT GGC CTG AT	GAT GCA GTA CTG GGC CTG AT	60	30
TNNT2	305	GAC AGA GCG GAA AAG TGG GA	TGA AGG AGG CCA GGC TCT AT	56	26
AFP	255	ACT CCA GTA AAC CCT GGT GTT G	GAA ATC TGC AAT GAC AGC CTC A	58	31
ALB	354	CCT TTG GCA CAA TGA AGT GGG TAA CC	CAG CAG TCA GCC ATT TCA CCA TAG G	62	43
TH	215	GCG GTT CAT TGG GCG CAG G	CAA ACA CCT TCA CAG CTC G	60	38
TTN-Ex49-50	71	GTA AAA AGA GCT GCC CCA GTG A	GCT AGG TGG CCC AGT GCT ACT	52	31
TTN-N2B-Ex50-219	92	CCA ATG AGT ATG GCA GTG TCA	TAC GTT CCG GAA GTA ATT TGC	52	35
TTN-N2BA-Ex50-51	84	GCC ACA GTA ACT GTG ACA GAG G	GGC TGC CTT ACC CAC AAA AG	52	31
TTN-N2BA-Ex54-55	66	GGA ATT AAT AGT TAA AGA ACC TGC CAA A	CTC CTG GTG TCA CCT GGA TCA	52	31
TTN-Ex114-122	800	GAG CCA TTT GAA CAA CCT TAT T	AGG GAC AGG TTT CTT TGG C	52	35
RBM20	416	GAT CAT CCA AAA TGC CGC CAA	AAG GAT GCA TCA CCA TGG CA	55	38
MYH7-Ex2	176	TGC TGC CCC ATA TAT ACA GCC	CCA AAG ACT GCC ATC TCC GA	55	38
FAM118A-Ex30	138	TGGCTTGATGTTTGGTAGGTC	TGCGTCTTGAGTGACGTCTT	55	31
COL14A1-Ex64	78	GTGATGGACTTCTAACATGAGATTT	AAGAAACCTGAAAACCTCAGCA	55	38
OSGEP-Ex2	92	GCCTCCCTCCTCTAATTCTCT	GTCACACAATGTGCTGTGCAT	55	34

Table S3: Comparative transcriptome analysis of RBM20- vs. control-iPSC-CMs: 161 DEGs.

Ensembl ID	Gene name	Description ¹	Chr.	Start	Stop	Biotype	Base mean ²	Base mean_control ³	Base mean_RBM20 ⁴	log2 fold change	P adjust
ENSG00000235559	NOL5BP	None	6	28751410	28751781	pseudogene	11.2	16.8	0.0	-3.83	2.9E-04
ENSG00000168878	SFTPB	surfactant protein B [Acc:10801]	2	85884437	85895864	protein_coding	39.5	58.1	2.2	-3.72	1.7E-05
ENSG00000267594	CYP4F24P	cytochrome P450, family 4, subfamily F, polypeptide 24, pseudogene [Acc:39945]	19	15870144	15890797	pseudogene	16.9	25.3	0.0	-3.32	7.4E-03
ENSG00000253563	NKX2-1-AS1	NKX2-1 antisense RNA 1 [Acc:40585]	14	36988483	36992221	antisense	16.0	23.6	0.7	-3.32	1.2E-03
ENSG00000241859	KALP	Kallmann syndrome sequence pseudogene [Acc:6214]	Y	15863536	16027704	pseudogene	13.1	19.6	0.0	-3.27	9.1E-03
ENSG00000227888	FAM66A	family with sequence similarity 66, member A [Acc:30444]	8	12219527	12268510	processed_transcript	18.8	27.7	1.1	-3.22	2.5E-03
ENSG00000180389	ATP5EP2	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit pseudogene 2 [Acc:34026]	13	28519343	28519727	protein_coding	10.3	15.2	0.7	-3.11	3.2E-03
ENSG00000224769	AC069213.1	None	3	195341818	195347104	pseudogene	36.0	51.6	4.7	-3.03	3.2E-05
ENSG00000227827	RP11-958N24.2	None	16	16450081	16471364	pseudogene	46.4	66.6	5.9	-2.77	5.5E-03
ENSG00000197550	RP11-460N11.2	None	9	69830288	69848759	pseudogene	50.4	72.0	7.4	-2.76	2.2E-03
ENSG00000099984	GSTT2	glutathione S-transferase theta 2 [Acc:4642]	22	24322339	24326106	protein_coding	72.2	102.0	12.7	-2.74	3.1E-07
ENSG00000149968	MMP3	matrix metalloproteinase 3 (stromelysin 1, progelatinase) [Acc:7173]	11	102706532	102714534	protein_coding	39.1	56.0	5.3	-2.68	1.2E-02
ENSG00000147571	CRH	corticotropin releasing hormone [Acc:2355]	8	67088620	67090960	protein_coding	52.6	73.7	10.2	-2.61	7.1E-06
ENSG00000259954	IL21R-AS1	IL21R antisense RNA 1 [Acc:27551]	16	27458990	27464714	antisense	11.2	16.0	1.7	-2.52	3.4E-02
ENSG00000164308	ERAP2	endoplasmic reticulum aminopeptidase 2 [Acc:29499]	5	96211643	96255420	protein_coding	178.7	246.1	43.9	-2.44	6.6E-24
ENSG00000223508	RPL23AP53	ribosomal protein L23a pseudogene 53 [Acc:35921]	8	163186	182231	pseudogene	44.6	62.1	9.5	-2.44	1.2E-04
ENSG00000196620	UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15 [Acc:12546]	4	69512348	69536346	protein_coding	69.0	96.9	13.3	-2.43	3.0E-03
ENSG00000270127	RP11-526I2.5	None	15	101087972	101090358	lincRNA	51.2	70.9	11.9	-2.37	3.0E-06
ENSG00000164112	TMEM155	transmembrane protein 155 [Acc:26418]	4	122680088	122686582	protein_coding	37.1	51.9	7.5	-2.36	5.5E-03
ENSG00000136352	NKX2-1	NK2 homeobox 1 [Acc:11825]	14	36985602	36990354	protein_coding	264.6	364.7	64.3	-2.32	4.8E-05
ENSG00000229415	SFTA3	surfactant associated 3 [Acc:18387]	14	36942493	36983034	protein_coding	197.1	271.8	47.8	-2.30	1.0E-04

ENSG00000139055	ERP27	endoplasmic reticulum protein 27 [Acc:26495]	12	15066969	15092016	protein_coding	133.9	184.6	32.7	-2.28	3.1E-04
ENSG00000172748	ZNF596	zinc finger protein 596 [Acc:27268]	8	182137	197342	protein_coding	106.0	144.9	28.2	-2.24	2.9E-08
ENSG00000271043	MTRNR2L2	MT-RNR2-like 2 [Acc:37156]	5	79945819	79946855	protein_coding	24.6	33.9	6.0	-2.19	1.1E-02
ENSG00000100276	RASL10A	RAS-like, family 10, member A [Acc:16954]	22	29708922	29711745	protein_coding	39.2	54.2	9.3	-2.19	1.1E-02
ENSG00000171885	AQP4	aquaporin 4 [Acc:637]	18	24432002	24445782	protein_coding	393.1	541.9	95.5	-2.12	3.5E-02
ENSG00000237039	AC018738.2	None	2	232120810	232121019	pseudogene	46.9	63.2	14.1	-2.02	7.9E-04
ENSG00000110195	FOLR1	folate receptor 1 (adult) [Acc:3791]	11	71900602	71907345	protein_coding	200.2	269.2	62.2	-1.99	9.6E-05
ENSG00000099812	MISP	mitotic spindle positioning [Acc:27000]	19	751126	764319	protein_coding	136.4	183.2	42.7	-1.95	1.8E-04
ENSG00000215513	PI4KAP1	phosphatidylinositol 4-kinase, catalytic, alpha pseudogene 1 [Acc:33576]	22	20383524	20427835	pseudogene	708.8	940.5	245.5	-1.91	1.7E-19
ENSG00000230316	FEZF1-AS1	FEZF1 antisense RNA 1 [Acc:41001]	7	121943712	121950131	antisense	76.2	101.9	24.6	-1.88	2.4E-02
ENSG00000155093	PTPRN2	protein tyrosine phosphatase, receptor type, N polypeptide 2 [Acc:9677]	7	157331750	158380480	protein_coding	250.7	333.0	86.0	-1.87	4.6E-07
ENSG00000102109	PCSK1N	proprotein convertase subtilisin/kexin type 1 inhibitor [Acc:17301]	X	48689504	48694035	protein_coding	211.4	281.6	71.0	-1.86	1.5E-03
ENSG00000114455	HHLA2	HERV-H LTR-associating 2 [Acc:4905]	3	108015376	108097132	protein_coding	62.3	83.6	19.8	-1.84	2.4E-02
ENSG00000231852	CYP21A2	cytochrome P450, family 21, subfamily A, polypeptide 2 [Acc:2600]	6	32006042	32009447	protein_coding	70.3	92.8	25.4	-1.74	5.8E-03
ENSG00000196616	ADH1B	alcohol dehydrogenase 1B (class I), beta polypeptide [Acc:250]	4	100226121	100242558	protein_coding	124.0	162.0	48.0	-1.63	1.2E-02
ENSG00000196126	HLA-DRB1	major histocompatibility complex, class II, DR beta 1 [Acc:4948]	6	32546546	32557625	protein_coding	86.9	113.1	34.6	-1.61	1.8E-02
ENSG00000185324	CDK10	cyclin-dependent kinase 10 [Acc:1770]	16	89747145	89762772	protein_coding	1027.5	1327.0	428.6	-1.59	7.0E-08
ENSG00000140961	OSGIN1	oxidative stress induced growth inhibitor 1 [Acc:30093]	16	83981887	83999937	protein_coding	63.0	81.8	25.5	-1.59	2.4E-02
ENSG00000234171	AC108488.3	None	2	3606082	3609324	antisense	101.0	129.9	43.1	-1.54	1.2E-04
ENSG00000178773	CPNE7	copine VII [Acc:2320]	16	89642176	89663654	protein_coding	78.9	101.7	33.5	-1.52	2.7E-02
ENSG00000090339	ICAM1	intercellular adhesion molecule 1 [Acc:5344]	19	10381511	10397291	protein_coding	634.7	809.3	285.6	-1.44	2.1E-03
ENSG00000170891	CYTL1	cytokine-like 1 [Acc:24435]	4	5016313	5021199	protein_coding	108.2	138.2	48.0	-1.44	8.8E-03
ENSG00000185128	TBC1D3F	TBC1 domain family, member 3F [Acc:18257]	17	36283971	36294915	protein_coding	56.7	72.2	25.6	-1.39	3.5E-02
ENSG00000105376	ICAM5	intercellular adhesion molecule 5, telencephalin [Acc:5348]	19	10400657	10407454	protein_coding	386.0	487.8	182.5	-1.38	2.8E-04
ENSG00000166979	EVA1C	eva-1 homolog C (C. elegans) [Acc:13239]	21	33784314	33887707	protein_coding	117.0	147.5	56.0	-1.32	1.9E-02

ENSG00000183578	TNFAIP8L3	tumor necrosis factor, alpha-induced protein 8-like 3 [Acc:20620]	15	51348795	51397473	protein_coding	174.8	219.6	85.3	-1.31	1.8E-02
ENSG00000100376	FAM118A	family with sequence similarity 118, member A [Acc:1313]	22	45704849	45737836	protein_coding	1302.3	1626.2	654.6	-1.30	2.7E-15
ENSG00000175084	DES	desmin [Acc:2770]	2	220283099	220291461	protein_coding	3311.8	4141.7	1651.9	-1.29	1.6E-03
ENSG00000159212	CLIC6	chloride intracellular channel 6 [Acc:2065]	21	36041688	36090525	protein_coding	661.0	827.7	327.6	-1.28	2.1E-02
ENSG00000185499	MUC1	mucin 1, cell surface associated [Acc:7508]	1	155158300	155162707	protein_coding	662.1	827.5	331.4	-1.28	2.9E-03
ENSG00000145736	GTF2H2	general transcription factor IIH, polypeptide 2, 44kDa [Acc:4656]	5	70330784	70363516	protein_coding	159.2	198.6	80.4	-1.25	1.1E-02
ENSG00000093072	CECR1	cat eye syndrome chromosome region, candidate 1 [Acc:1839]	22	17660194	17702879	protein_coding	211.3	261.7	110.6	-1.22	8.5E-04
ENSG00000223949	RP11-24J23.2	None	1	64560125	64636980	antisense	76.6	95.4	38.9	-1.22	2.6E-02
ENSG00000123610	TNFAIP6	tumor necrosis factor, alpha-induced protein 6 [Acc:11898]	2	152214106	152236560	protein_coding	143.2	177.7	74.3	-1.22	3.4E-03
ENSG00000189129	PLAC9	placenta-specific 9 [Acc:19255]	10	81891438	81905115	protein_coding	261.4	323.8	136.7	-1.22	6.4E-04
ENSG00000008517	IL32	interleukin 32 [Acc:16830]	16	3115298	3131908	protein_coding	188.3	233.1	98.8	-1.21	9.5E-03
ENSG00000187624	C17orf97	chromosome 17 open reading frame 97 [Acc:33800]	17	260118	273510	protein_coding	146.2	181.2	76.1	-1.21	9.4E-03
ENSG00000196639	HRH1	histamine receptor H1 [Acc:5182]	3	11178779	11305243	protein_coding	238.9	296.5	123.5	-1.21	9.0E-03
ENSG00000196878	LAMB3	laminin, beta 3 [Acc:6490]	1	209788215	209825811	protein_coding	944.6	1166.5	500.7	-1.17	3.2E-02
ENSG00000013588	GPRC5A	G protein-coupled receptor, family C, group 5, member A [Acc:9836]	12	13043716	13070871	protein_coding	1860.6	2282.3	1017.4	-1.14	1.3E-03
ENSG00000197632	SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2 [Acc:8584]	18	61538926	61571124	protein_coding	133.7	164.0	73.1	-1.12	1.4E-02
ENSG00000134917	ADAMTS8	ADAM metallopeptidase with thrombospondin type 1 motif, 8 [Acc:224]	11	130274820	130298888	protein_coding	873.4	1068.5	483.1	-1.11	3.2E-02
ENSG00000138587	MNS1	meiosis-specific nuclear structural 1 [Acc:29636]	15	56713742	56757335	protein_coding	111.3	135.4	63.2	-1.08	3.4E-02
ENSG00000180739	S1PR5	sphingosine-1-phosphate receptor 5 [Acc:14299]	19	10623623	10628607	protein_coding	120.6	147.1	67.7	-1.08	4.2E-02
ENSG00000146833	TRIM4	tripartite motif containing 4 [Acc:16275]	7	99474581	99517223	protein_coding	329.2	400.4	186.7	-1.07	7.3E-03
ENSG00000006327	TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A [Acc:18152]	16	3068446	3072384	protein_coding	1650.0	2006.0	937.9	-1.07	1.1E-02
ENSG00000092929	UNC13D	unc-13 homolog D (C. elegans) [Acc:23147]	17	73823306	73840798	protein_coding	263.6	315.8	159.3	-0.97	2.9E-03
ENSG00000152583	SPARCL1	SPARC-like 1 (hevin) [Acc:11220]	4	88394487	88452213	protein_coding	2042.9	2444.3	1240.1	-0.96	1.1E-02
ENSG00000013573	DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box helicase 11 [Acc:2736]	12	31226779	31257725	protein_coding	747.3	886.1	469.7	-0.90	3.8E-03

ENSG00000173715	C11orf80	chromosome 11 open reading frame 80 [Acc:26197]	11	66511922	66610987	protein_coding	249.7	295.9	157.3	-0.90	1.1E-02
ENSG00000182492	BGN	biglycan [Acc:1044]	X	152760397	152775012	protein_coding	6089.0	7212.8	3841.4	-0.90	1.1E-03
ENSG00000079257	LXN	latexin [Acc:13347]	3	158363611	158390482	protein_coding	738.6	870.6	474.5	-0.86	5.0E-03
ENSG00000123472	ATPAF1	ATP synthase mitochondrial F1 complex assembly factor 1 [Acc:18803]	1	47098409	47139539	protein_coding	1302.7	1533.3	841.7	-0.85	1.4E-03
ENSG00000147408	CSGALNACT1	chondroitin sulfate N-acetylgalactosaminyltransferase 1 [Acc:24290]	8	19261672	19615540	protein_coding	405.1	476.5	262.3	-0.84	2.5E-02
ENSG00000158292	GPR153	G protein-coupled receptor 153 [Acc:23618]	1	6307406	6321035	protein_coding	825.3	969.2	537.5	-0.84	9.1E-04
ENSG00000065534	MYLK	myosin light chain kinase [Acc:7590]	3	123328896	123603178	protein_coding	3386.2	3969.3	2220.1	-0.82	9.9E-03
ENSG00000128606	LRRC17	leucine rich repeat containing 17 [Acc:16895]	7	102553438	102585396	protein_coding	2033.4	2366.8	1366.7	-0.78	3.3E-02
ENSG00000185201	IFITM2	interferon induced transmembrane protein 2 [Acc:5413]	11	307631	315272	protein_coding	1887.8	2182.2	1298.9	-0.74	4.9E-02
ENSG00000111859	NEDD9	neural precursor cell expressed, developmentally down-regulated 9 [Acc:7733]	6	11183531	11382581	protein_coding	1323.1	1524.1	921.2	-0.72	9.5E-03
ENSG00000233098	RP11-344E13.3	None	17	20771746	20905589	antisense	709.1	814.7	497.9	-0.70	3.9E-02
ENSG00000115363	EVA1A	eva-1 homolog A (C. elegans) [Acc:25816]	2	75696428	75796848	protein_coding	232.6	266.9	164.1	-0.69	3.2E-02
ENSG00000242086	LINC00969	long intergenic non-protein coding RNA 969 [Acc:48729]	3	195384933	195460422	lincRNA	647.2	740.1	461.6	-0.68	2.8E-03
ENSG00000109586	GALNT7	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl-transferase 7 (GalNAc-T7) [Acc:4129]	4	174089904	174245118	protein_coding	991.6	851.5	1271.8	0.57	2.4E-02
ENSG00000148143	ZNF462	zinc finger protein 462 [Acc:21684]	9	109625378	109775915	protein_coding	1162.2	995.1	1496.3	0.58	1.6E-02
ENSG00000101871	MID1	midline 1 (Opitz/BBB syndrome) [Acc:7095]	X	10413350	10851773	protein_coding	2226.4	1889.9	2899.3	0.61	1.4E-04
ENSG00000102038	SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1 [Acc:11097]	X	128580480	128657477	protein_coding	5014.1	4187.9	6666.5	0.67	7.3E-04
ENSG00000123104	ITPR2	inositol 1,4,5-trisphosphate receptor, type 2 [Acc:6181]	12	26490342	26986131	protein_coding	808.5	673.1	1079.4	0.67	3.9E-02
ENSG00000144366	GULP1	GULP, engulfment adaptor PTB domain containing 1 [Acc:18649]	2	189156396	189460653	protein_coding	660.1	548.1	884.1	0.68	3.3E-02
ENSG00000180964	TCEAL8	transcription elongation factor A (SII)-like 8 [Acc:28683]	X	102507923	102510131	protein_coding	2003.9	1661.8	2688.1	0.69	7.3E-04
ENSG00000089682	RBM41	RNA binding motif protein 41 [Acc:25617]	X	106307650	106362057	protein_coding	378.2	312.6	509.3	0.69	2.1E-02
ENSG00000075651	PLD1	phospholipase D1, phosphatidylcholine-specific [Acc:9067]	3	171318195	171528740	protein_coding	558.6	462.2	751.4	0.69	2.4E-02

ENSG00000122126	OCRL	oculocerebrorenal syndrome of Lowe [Acc:8108]	X	128673826	128726538	protein_coding	1658.4	1349.2	2276.9	0.75	5.1E-04
ENSG00000147257	GPC3	glypican 3 [Acc:4451]	X	132669773	133119922	protein_coding	9121.4	7397.6	12569.1	0.75	2.4E-02
ENSG00000157600	TMEM164	transmembrane protein 164 [Acc:26217]	X	109245859	109425962	protein_coding	1129.7	913.6	1561.7	0.76	3.7E-02
ENSG00000165675	ENOX2	ecto-NOX disulfide-thiol exchanger 2 [Acc:2259]	X	129757350	130037208	protein_coding	294.3	238.2	406.5	0.76	3.4E-03
ENSG00000176593	CTD-2368P22.1	HCG1811579; Uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G5E958]	19	58513429	58522600	protein_coding	298.1	241.0	412.4	0.77	1.2E-02
ENSG00000064309	CDON	cell adhesion associated, oncogene regulated [Acc:17104]	11	125825691	125933230	protein_coding	1667.9	1347.1	2309.6	0.77	9.0E-03
ENSG00000188153	COL4A5	collagen, type IV, alpha 5 [Acc:2207]	X	107683074	107940775	protein_coding	6612.4	5327.7	9181.8	0.78	4.5E-04
ENSG00000113441	LNPEP	leucyl/cystinyl aminopeptidase [Acc:6656]	5	96271098	96373219	protein_coding	1032.8	830.3	1438.0	0.78	4.7E-03
ENSG00000066382	MPPED2	metallophosphoesterase domain containing 2 [Acc:1180]	11	30406040	30608419	protein_coding	548.3	441.2	762.7	0.78	5.4E-04
ENSG00000160352	ZNF714	zinc finger protein 714 [Acc:27124]	19	21264965	21308073	protein_coding	416.9	334.0	582.6	0.79	7.4E-03
ENSG00000182263	FIGN	fidgetin [Acc:13285]	2	164449906	164592522	protein_coding	545.2	434.6	766.5	0.81	7.4E-03
ENSG00000077616	NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2 [Acc:14526]	11	89864683	89926062	protein_coding	713.3	567.9	1004.1	0.81	1.1E-02
ENSG00000046604	DSG2	desmoglein 2 [Acc:3049]	18	29078006	29128971	protein_coding	2062.6	1637.0	2913.7	0.82	3.5E-02
ENSG00000203875	SNHG5	small nucleolar RNA host gene 5 (non-protein coding) [Acc:21026]	6	86370710	86388451	processed_transcript	1328.2	1057.6	1869.4	0.82	1.7E-05
ENSG00000134602	MST4	Serine/threonine-protein kinase MST4 [Source:UniProtKB/Swiss-Prot;Acc:Q9P289]	X	131157293	131209971	protein_coding	1249.0	989.5	1768.2	0.82	4.9E-02
ENSG00000146950	SHROOM2	shroom family member 2 [Acc:630]	X	9754496	9917483	protein_coding	470.9	372.8	667.0	0.82	2.1E-02
ENSG00000169116	PARM1	prostate androgen-regulated mucin-like protein 1 [Acc:24536]	4	75858305	75975325	protein_coding	2018.4	1595.9	2863.4	0.83	4.7E-02
ENSG00000188419	CHM	choroideremia (Rab escort protein 1) [Acc:1940]	X	85116185	85302566	protein_coding	612.4	481.6	874.0	0.85	8.5E-03
ENSG00000088305	DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta [Acc:2979]	20	31350191	31397162	protein_coding	395.6	310.0	566.9	0.86	8.2E-03
ENSG00000170542	SERPINB9	serpin peptidase inhibitor, clade B (ovalbumin), member 9 [Acc:8955]	6	2887500	2903514	protein_coding	868.1	678.0	1248.4	0.86	4.3E-02
ENSG00000257315	ZBED6	zinc finger, BED-type containing 6 [Acc:33273]	1	203765437	203769686	protein_coding	282.9	220.8	407.1	0.86	9.0E-03
ENSG00000076716	GPC4	glypican 4 [Acc:4452]	X	132434131	132549518	protein_coding	2121.5	1658.1	3048.4	0.87	2.1E-04
ENSG00000119900	OGFRL1	opioid growth factor receptor-like 1 [Acc:21378]	6	71998506	72018653	protein_coding	903.0	703.3	1302.5	0.87	1.4E-02
ENSG00000101974	ATP11C	ATPase, class VI, type 11C [Acc:13554]	X	138808505	139027435	protein_coding	770.0	599.5	1111.0	0.88	7.3E-04
ENSG00000197565	COL4A6	collagen, type IV, alpha 6 [Acc:2208]	X	107386780	107682727	protein_coding	2528.5	1960.6	3664.3	0.88	1.4E-02

ENSG00000101972	STAG2	stromal antigen 2 [Acc:11355]	X	123094062	123556514	protein_coding	2354.3	1825.5	3412.1	0.90	2.3E-07
ENSG00000133138	TBC1D8B	TBC1 domain family, member 8B (with GRAM domain) [Acc:24715]	X	106045910	106119375	protein_coding	453.2	350.1	659.5	0.90	1.8E-04
ENSG00000129682	FGF13	fibroblast growth factor 13 [Acc:3670]	X	137713735	138304939	protein_coding	664.3	504.4	984.3	0.95	9.1E-04
ENSG00000162733	DDR2	discoidin domain receptor tyrosine kinase 2 [Acc:2731]	1	162601163	162750237	protein_coding	1074.1	808.8	1604.7	0.97	1.2E-02
ENSG00000197558	SSPO	SCO-spondin homolog (Bos taurus) [Acc:21998]	7	149473131	149531068	processed_transcript	559.8	419.7	839.9	0.98	1.2E-02
ENSG00000158427	TMSB15B	thymosin beta 15B [Acc:28612]	X	103173479	103229570	protein_coding	275.1	201.3	422.7	1.04	9.9E-03
ENSG00000164318	EGFLAM	EGF-like, fibronectin type III and laminin G domains [Acc:26810]	5	38258511	38465123	protein_coding	213.4	154.7	330.8	1.05	4.5E-02
ENSG00000254995	STX16-NPEPL1	STX16-NPEPL1 readthrough (NMD candidate) [Acc:41993]	20	57226490	57290466	protein_coding	123.2	89.5	190.7	1.06	2.1E-02
ENSG00000163638	ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9 [Acc:13202]	3	64501333	64673676	protein_coding	3253.0	2364.0	5030.9	1.07	3.4E-03
ENSG00000123191	ATP7B	ATPase, Cu ⁺⁺ transporting, beta polypeptide [Acc:870]	13	52506809	52585630	protein_coding	1196.0	865.0	1857.9	1.07	2.0E-02
ENSG00000258289	CHURC1	churchill domain containing 1 [Acc:20099]	14	65381079	65411309	protein_coding	952.3	688.3	1480.2	1.09	3.6E-06
ENSG00000213468	RP11-453F18_B.1	None	X	130836678	130964671	processed_transcript	214.3	154.1	334.6	1.09	4.7E-03
ENSG00000100867	DHRS2	dehydrogenase/reductase (SDR family) member 2 [Acc:18349]	14	24099324	24114848	protein_coding	77.3	53.9	124.2	1.15	4.3E-02
ENSG00000134755	DSC2	desmocollin 2 [Acc:3036]	18	28645940	28682378	protein_coding	774.9	541.5	1241.6	1.17	6.4E-04
ENSG00000175611	LINC00476	long intergenic non-protein coding RNA 476 [Acc:27858]	9	98521513	98638259	processed_transcript	58.7	40.4	95.3	1.18	3.5E-02
ENSG00000120156	TEK	TEK tyrosine kinase, endothelial [Acc:11724]	9	27109139	27230173	protein_coding	409.1	281.7	664.0	1.20	3.7E-03
ENSG00000162998	FRZB	frizzled-related protein [Acc:3959]	2	183698002	183731890	protein_coding	1749.2	1200.4	2846.9	1.20	1.3E-02
ENSG00000230091	TMEM254-AS1	TMEM254 antisense RNA 1 [Acc:27340]	10	81806616	81838668	antisense	89.0	60.2	146.6	1.23	1.2E-02
ENSG00000187772	LIN28B	lin-28 homolog B (C. elegans) [Acc:32207]	6	105404923	105531207	protein_coding	216.9	146.4	357.8	1.24	1.1E-02
ENSG00000081479	LRP2	low density lipoprotein receptor-related protein 2 [Acc:6694]	2	169983619	170219195	protein_coding	1259.2	841.6	2094.3	1.28	1.5E-03
ENSG00000171004	HS6ST2	heparan sulfate 6-O-sulfotransferase 2 [Acc:19133]	X	131760038	132095423	protein_coding	666.2	431.9	1134.7	1.34	3.6E-03
ENSG00000250337	RP11-46C20.1	None	5	27472399	27496508	lincRNA	162.3	90.2	306.5	1.69	2.2E-04
ENSG00000090686	USP48	ubiquitin specific peptidase 48 [Acc:18533]	1	22004791	22110099	protein_coding	3478.8	1880.0	6676.5	1.75	1.2E-04

ENSG00000113209	PCDH5	protocadherin beta 5 [Acc:8690]	5	140514800	140517703	protein_coding	110.5	57.3	216.8	1.81	7.9E-04
ENSG00000226752	PSMD5-AS1	PSMD5 antisense RNA 1 (head to head) [Acc:27367]	9	123587106	123616651	antisense	326.6	169.9	640.0	1.87	1.3E-11
ENSG00000253873	PCDHGA11	protocadherin gamma subfamily A, 11 [Acc:8698]	5	140800762	140891835	protein_coding	24.8	11.3	51.7	1.97	1.2E-02
ENSG00000006747	SCIN	scinderin [Acc:21695]	7	12610203	12693228	protein_coding	50.0	20.8	108.5	2.10	2.4E-02
ENSG00000148942	SLC5A12	solute carrier family 5 (sodium/glucose cotransporter), member 12 [Acc:28750]	11	26688566	26744974	protein_coding	62.0	22.0	141.9	2.26	3.2E-02
ENSG00000260528	FAM157C	family with sequence similarity 157, member C [Acc:34081]	16	90168679	90244752	processed_transcript	25.6	9.9	57.1	2.27	3.2E-03
ENSG00000121570	DPPA4	developmental pluripotency associated 4 [Acc:19200]	3	109044988	109056419	protein_coding	109.2	42.8	241.9	2.27	1.1E-03
ENSG00000125787	GNRH2	gonadotropin-releasing hormone 2 [Acc:4420]	20	3024268	3026393	protein_coding	59.1	19.6	138.2	2.38	1.3E-02
ENSG00000259129	LINC00648	long intergenic non-protein coding RNA 648 [Acc:44302]	14	48234157	48264295	lincRNA	102.6	37.5	232.6	2.50	9.8E-09
ENSG00000166415	WDR72	WD repeat domain 72 [Acc:26790]	15	53805938	54055075	protein_coding	17.9	3.1	47.5	2.77	3.7E-02
ENSG00000180543	TSPYL5	TSPY-like 5 [Acc:29367]	8	98285717	98290176	protein_coding	105.8	28.6	260.0	2.86	1.1E-05
ENSG00000240563	L1TD1	LINE-1 type transposase domain containing 1 [Acc:25595]	1	62660503	62678000	protein_coding	155.0	38.2	388.5	3.03	8.4E-07
ENSG00000169248	CXCL11	chemokine (C-X-C motif) ligand 11 [Acc:10638]	4	76954835	76962568	protein_coding	33.9	0.9	100.0	3.09	2.6E-02
ENSG00000115112	TFCP2L1	transcription factor CP2-like 1 [Acc:17925]	2	121974163	122042783	protein_coding	20.6	3.3	55.3	3.18	1.4E-03
ENSG00000170074	FAM153A	family with sequence similarity 153, member A [Acc:29940]	5	177134982	177210399	protein_coding	16.8	1.8	46.8	3.35	2.2E-03
ENSG00000131914	LIN28A	lin-28 homolog A (C. elegans) [Acc:15986]	1	26737269	26756213	protein_coding	83.8	10.5	230.4	3.37	1.2E-03
ENSG00000180229	HERC2P3	hect domain and RLD 2 pseudogene 3 [Acc:4871]	15	20587869	20711433	pseudogene	86.5	14.9	229.8	3.61	1.6E-11
ENSG00000234665	RP11-262H14.3	None	9	66513488	66553911	lincRNA	18.9	2.0	52.5	3.74	1.2E-05
ENSG00000170373	CST1	cystatin SN [Acc:2473]	20	23728190	23731905	protein_coding	11.4	0.5	33.3	3.90	3.1E-04
ENSG00000111704	NANOG	Nanog homeobox [Acc:20857]	12	7940390	7948655	protein_coding	10.2	0.0	30.6	4.30	1.0E-04
ENSG00000188000	OR7D2	olfactory receptor, family 7, subfamily D, member 2 [Acc:8378]	19	9296279	9299493	protein_coding	12.5	0.1	37.2	5.13	4.7E-09

¹ The source of Acc numbers are from HGNC Symbol except when mentioned otherwise

² Base mean: average expression values for all the samples

³ Base mean_control: average expression values for only the control samples

⁴ Base mean_RBM20: average expression values for only the RBM20 samples

Table S4: Significantly enriched gene ontology functions and processes in DEG data sets.

GO ID	GO term	Ontology source	Term P value	Term P value corrected with Bonferroni step down	Group P value	Group P value corrected with Bonferroni step down	GO levels	Associated genes (%)	Number of genes	Associated genes found
GO:0001725	stress fiber	GO_CellularComponent-GOA_09.02.2016_16h18	7.5E-3	30.0E-3	7.5E-3	22.0E-3	[5, 6, 7, 8, 9, 10, 11]	5.00	3.0	[MYLK, TEK, UNC13D]
GO:1901880	negative regulation of protein depolymerization	GO_BiologicalProcess-GOA_09.02.2016_16h18	1.2E-3	8.7E-3	1.2E-3	7.4E-3	[5, 6, 7, 8, 9]	5.71	4.0	[FGF13, MID1, SCIN, SHROOM2]
GO:0000980	metabolism of xenobiotics by cytochrome P450	KEGG_10.02.2016	12.0E-3	25.0E-3	18.0E-3	18.0E-3	[-1]	4.11	3.0	[ADH1B, GSTT2, UGT2B15]
GO:0000982	drug metabolism	KEGG_10.02.2016	11.0E-3	33.0E-3	18.0E-3	18.0E-3	[-1]	4.35	3.0	[ADH1B, GSTT2, UGT2B15]
GO:0005146	amoebiasis	KEGG_10.02.2016	580.0E-6	7.0E-3	580.0E-6	4.1E-3	[-1]	4.95	5.0	[COL4A5, COL4A6, LAMB3, SERPINB2, SERPINB9]
GO:0005323	rheumatoid arthritis	KEGG_10.02.2016	3.1E-3	15.0E-3	3.1E-3	12.0E-3	[-1]	4.44	4.0	[HLA-DRB1, ICAM1, MMP3, TEK]
GO:0005412	arrhythmogenic right ventricular cardiomyopathy (ARVC)	KEGG_10.02.2016	13.0E-3	13.0E-3	8.1E-3	16.0E-3	[-1]	4.05	3.0	[DES, DSC2, DSG2]
GO:0014704	intercalated disc	GO_CellularComponent-GOA_09.02.2016_16h18	650.0E-6	6.5E-3	8.1E-3	16.0E-3	[4]	6.78	4.0	[DES, DSC2, DSG2, FGF13]
GO:0030203	glycosaminoglycan metabolic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	24.0E-6	390.0E-6	56.0E-6	450.0E-6	[5]	4.55	8.0	[BGN, CSGALNACT1, CYTL1, EGFLAM, FGF13, GPC3, GPC4, HS6ST2]
GO:0006024	glycosaminoglycan biosynthetic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	120.0E-6	1.7E-3	56.0E-6	450.0E-6	[5, 6]	5.22	6.0	[BGN, CSGALNACT1, CYTL1, GPC3, GPC4, HS6ST2]
GO:0006029	proteoglycan metabolic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	5.1E-6	88.0E-6	56.0E-6	450.0E-6	[5, 6]	6.93	7.0	[BGN, CSGALNACT1, CYTL1, EGFLAM, GPC3, GPC4, HS6ST2]
GO:0006027	glycosaminoglycan catabolic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	880.0E-6	7.1E-3	56.0E-6	450.0E-6	[6]	6.25	4.0	[BGN, FGF13, GPC3, GPC4]
GO:1903510	mucopolysaccharide metabolic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	280.0E-6	3.6E-3	56.0E-6	450.0E-6	[6]	4.48	6.0	[BGN, CSGALNACT1, EGFLAM, FGF13, GPC3, GPC4]
GO:0030166	proteoglycan biosynthetic process	GO_BiologicalProcess-GOA_09.02.2016_16h18	780.0E-6	7.0E-3	56.0E-6	450.0E-6	[6, 7]	6.45	4.0	[BGN, CSGALNACT1, CYTL1, HS6ST2]

GO:0043552	positive regulation of phosphatidyl-inositol 3-kinase activity	GO_BiologicalProcess-GOA_09.02.2016_16h18	1.3E-3	8.2E-3	1.3E-3	6.8E-3	[5, 6, 7, 8, 9, 10, 11]	9.09	3.0	[FGF13, TEK, TNFAIP8L3]
GO:0043200	response to amino acid	GO_BiologicalProcess-GOA_09.02.2016_16h18	24.0E-6	360.0E-6	24.0E-6	220.0E-6	[4, 5]	5.47	7.0	[COL4A6, CYP21A2, DNMT3B, FOLR1, ICAM1, MMP3, MUC1]
GO:0071230	cellular response to amino acid stimulus	GO_BiologicalProcess-GOA_09.02.2016_16h18	610.0E-6	6.7E-3	24.0E-6	220.0E-6	[5, 6]	6.90	4.0	[COL4A6, DNMT3B, FOLR1, MMP3]

The significantly enriched terms are shown for each category of DEGs.

Table S5: Comparative transcriptome analysis of RBM20- vs. control-iPSC-CMs: 34 genes with DEEs.

Gene ID	Exon ID	Dispersion	P value	P adjust	Mean base	log2 fold change	HGNC_symbol
ENSG00000035115	E032	0.31	2.3E-09	7.1E-05	16.33	-15.90	SH3YL1
ENSG00000035115	E034	0.20	1.2E-05	8.9E-02	25.65	-1.37	SH3YL1
ENSG00000035115	E033	0.23	3.1E-09	9.0E-05	21.92	-2.45	SH3YL1
ENSG00000038427	E016	0.02	5.7E-07	7.5E-03	1716.83	0.23	VCAN
ENSG00000067182	E001	0.03	6.0E-06	5.4E-02	269.36	-0.40	TNFRSF1A
ENSG00000079332	E002	0.01	2.4E-07	3.7E-03	1532.31	-0.22	SAR1A
ENSG00000084072	E027	0.53	3.8E-06	4.0E-02	32.66	1.72	PPIE
ENSG00000087245	E028	0.01	1.4E-05	9.8E-02	942.38	-0.19	MMP2
ENSG00000092054	E002	0.02	1.5E-08	3.3E-04	554.15	-0.38	MYH7
ENSG00000092094	E002	0.42	1.3E-07	2.3E-03	12.04	-14.82	OSGEP
ENSG00000100225	E017	0.03	1.4E-05	9.8E-02	191.40	0.39	FBXO7
ENSG00000100376	E030	0.20	6.7E-06	5.5E-02	47.06	-1.35	FAM118A
ENSG00000101605	E002	0.02	1.1E-06	1.3E-02	351.04	-0.36	MYOM1
ENSG00000105750	E006	0.05	6.1E-06	5.4E-02	131.21	-0.39	ZNF85
ENSG00000106631	E017	0.01	6.4E-06	5.4E-02	994.64	-0.20	MYL7
ENSG00000110367	E001	0.01	1.5E-05	9.9E-02	1213.78	-0.19	DDX6
ENSG00000113140	E001	0.02	2.7E-07	4.0E-03	6928.28	-0.23	SPARC
ENSG00000116560	E013	0.03	1.3E-05	9.5E-02	435.98	0.31	SFPQ
ENSG00000122674	E016	0.17	1.7E-07	2.8E-03	29.88	-1.73	CCZ1
ENSG00000125954	E018	0.02	0.0E+00	0.0E+00	649.82	0.56	CHURC1-FNTB
ENSG00000137154	E002	0.03	3.3E-10	1.3E-05	241.14	0.52	RPS6
ENSG00000140950	E023	0.57	4.9E-06	4.8E-02	8.76	-15.00	TLDC1
ENSG00000146197	E025	0.01	1.1E-07	1.9E-03	867.81	-0.22	SCUBE3
ENSG00000150991	E011	0.01	1.6E-08	3.4E-04	1798.80	-0.20	UBC
ENSG00000150991	E005	0.03	2.4E-10	1.0E-05	747.68	0.41	UBC
ENSG00000150991	E006	0.01	0.0E+00	0.0E+00	1134.07	0.34	UBC
ENSG00000163584	E002	0.29	0.0E+00	0.0E+00	17.17	15.95	RPL22L1
ENSG00000163584	E001	0.33	0.0E+00	0.0E+00	15.23	15.86	RPL22L1
ENSG00000163879	E008	0.15	6.4E-06	5.4E-02	35.69	0.90	DNALI1
ENSG00000180229	E053	0.82	5.5E-06	5.3E-02	6.07	-1.69	HERC2P3
ENSG00000180229	E054	0.74	3.4E-07	4.9E-03	9.09	-2.01	HERC2P3
ENSG00000180229	E059	0.76	4.4E-08	8.4E-04	6.53	-2.38	HERC2P3
ENSG00000183426	E067	0.05	1.2E-06	1.4E-02	127.39	-0.59	NPIPA1
ENSG00000183506	E016	0.49	1.6E-06	1.8E-02	10.21	-16.57	PI4KAP2
ENSG00000185070	E015	0.05	2.9E-06	3.1E-02	117.18	-0.56	FLRT2
ENSG00000187955	E064	0.02	9.2E-07	1.2E-02	476.64	-0.34	COL14A1
ENSG00000198015	E018	0.43	4.1E-12	2.8E-07	11.74	3.14	MRPL42
ENSG00000204525	E018	0.06	2.6E-11	1.5E-06	202.86	-1.16	HLA-C
ENSG00000233927	E005	0.01	3.7E-09	9.8E-05	1461.76	-0.21	RPS28
ENSG00000234741	E013	0.04	8.2E-06	6.5E-02	293.63	-0.47	GAS5
ENSG00000263753	E020	0.02	1.2E-05	9.2E-02	664.83	-0.21	LINC00667

Table S6: Significantly enriched gene ontology functions and processes in DEE data sets.

GO ID	GO term	Ontology source	Term P value	Term P value corrected with Bonferroni step down	Group P value	Group P value corrected with Bonferroni step down	GO levels	Associated genes (%)	Number of genes	Associated genes found
GO:0003229	ventricular cardiac muscle tissue development	GO_BiologicalProcess-GOA_09.02.2016_16h18	3.0E-3	9.0E-3	3.0E-3	9.0E-3	[5, 6, 7, 8, 9]	4.00	2.00	[COL14A1, MYH7]
GO:0042274	ribosomal small subunit biogenesis	GO_BiologicalProcess-GOA_09.02.2016_16h18	3.0E-3	9.0E-3	660.0E-6	3.3E-3	[4, 5]	4.00	2.00	[RPS28, RPS6]
GO:0015935	small ribosomal subunit	GO_CellularComponent-GOA_09.02.2016_16h18	250.0E-6	2.5E-3	660.0E-6	3.3E-3	[4, 5, 6, 7, 8, 9]	3.95	3.00	[MRPL42, RPS28, RPS6]
GO:0022627	cytosolic small ribosomal subunit	GO_CellularComponent-GOA_09.02.2016_16h18	2.5E-3	10.0E-3	660.0E-6	3.3E-3	[5, 6, 7, 8, 9, 10]	4.35	2.00	[RPS28, RPS6]
GO:0005416	viral myocarditis	KEGG_10.02.2016	4.1E-3	4.1E-3	4.1E-3	4.1E-3	[-1]	3.39	2.00	[HLA-C, MYH7]
GO:0010803	regulation of tumor necrosis factor-mediated signaling pathway	GO_BiologicalProcess-GOA_09.02.2016_16h18	3.5E-3	7.0E-3	3.5E-3	7.0E-3	[5, 6, 7, 8]	3.70	2.00	[TNFRSF1A, UBC]
GO:0016459	myosin complex	GO_CellularComponent-GOA_09.02.2016_16h18	200.0E-6	2.2E-3	490.0E-6	2.9E-3	[3, 4, 5, 6, 7, 8, 9]	4.29	3.00	[MYH7, MYL7, MYOM1]
GO:0031672	A band	GO_CellularComponent-GOA_09.02.2016_16h18	1.4E-3	8.9E-3	490.0E-6	2.9E-3	[3, 4, 5, 6, 7, 8, 9, 10]	5.71	2.00	[MYL7, MYOM1]
GO:0005859	muscle myosin complex	GO_CellularComponent-GOA_09.02.2016_16h18	430.0E-6	3.9E-3	490.0E-6	2.9E-3	[3, 4, 5, 6, 7, 8, 9, 10, 11]	10.53	2.00	[MYH7, MYOM1]
GO:0016460	myosin II complex	GO_CellularComponent-GOA_09.02.2016_16h18	880.0E-6	6.1E-3	490.0E-6	2.9E-3	[4, 5, 6, 7, 8, 9, 10]	7.41	2.00	[MYH7, MYOM1]
GO:0032982	myosin filament	GO_CellularComponent-GOA_09.02.2016_16h18	580.0E-6	4.6E-3	490.0E-6	2.9E-3	[4, 5, 6, 7, 8, 9, 10]	9.09	2.00	[MYH7, MYOM1]
GO:0019068	virion assembly	GO_BiologicalProcess-GOA_09.02.2016_16h18	2.2E-3	11.0E-3	2.2E-3	8.9E-3	[4, 5, 6]	4.65	2.00	[DDX6, UBC]

Supplementary Figures

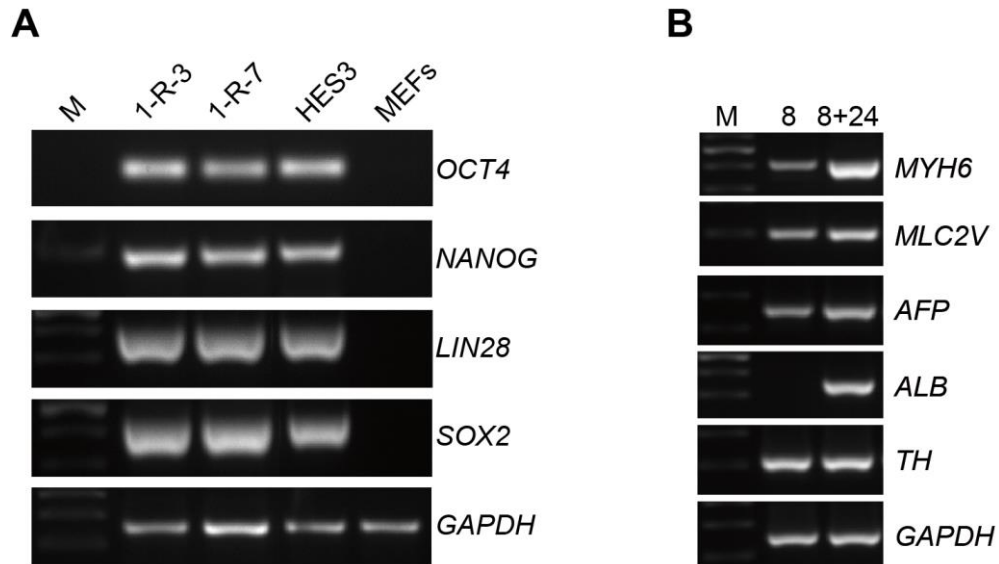


Figure S1: Proof of pluripotency. **A**, all RBM20-iPSC lines expressed endogenous pluripotency markers *OCT4*, *NANOG*, *LIN28*, and *SOX2* at higher levels than the parental fibroblasts (F-1-R) as shown by RT-PCR. *GAPDH* was used as loading control. HES3 cells and MEFs represent the positive and negative control, respectively. **B**, spontaneous differentiation capacity of RBM20-iPSC line 1-R-7. Germ layer-specific genes *MYH6*, *MLC2V* (mesoderm), *AFP*, *ALB* (endoderm), and *TH* (ectoderm) were expressed in a developmentally controlled manner during EB differentiation. *GAPDH* is included as loading control. Analyses were performed at different stages (days 8, 8+24) during differentiation of EBs after plating at day 8 (d8).

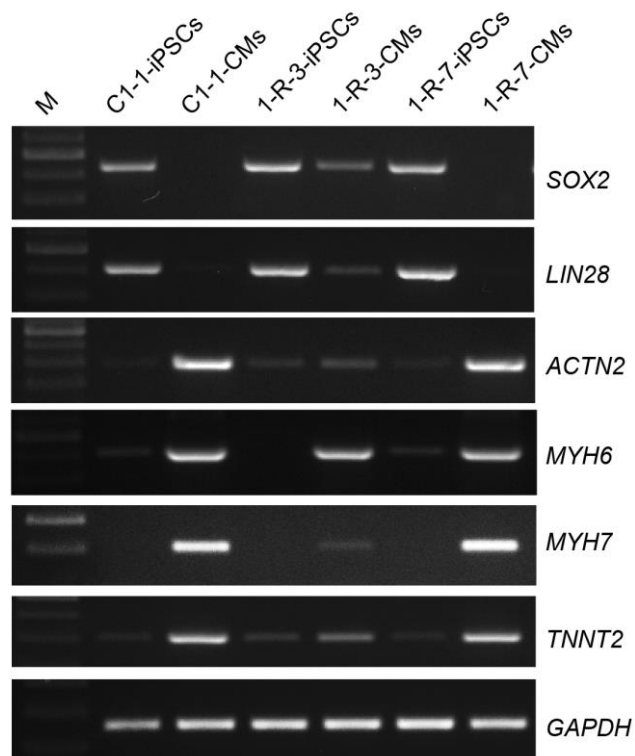


Figure S2: Expression of cardiac markers in RBM20- and control-iPSC-CMs. RT-PCR showing the expression of indicated genes in undifferentiated iPSCs and CMs differentiated from RBM20- and control-iPSCs. GAPDH was used as loading control.

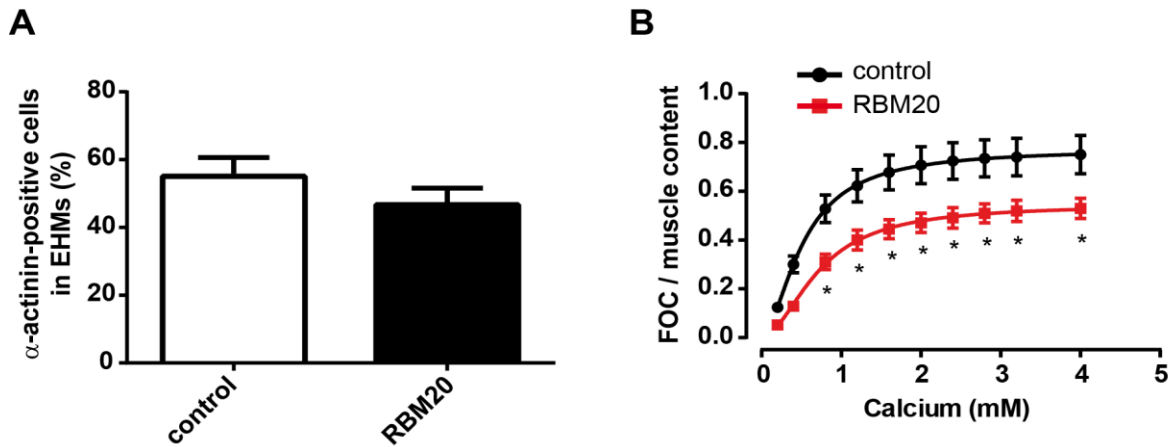


Figure S3: Functional phenotype of RBM20-EHM s. **A**, muscle contents of EHM s from RBM20- and control-iPSC-CM s as shown as the percentage of α -actinin-positive cells. **B**, force of contraction per muscle content (FOC/muscle content) of EHM s in response to increasing extracellular calcium concentration (0.2-4 mM) to assess the maximal inotropic capacity of EHM s. n=13 control-EHM s generated from 5 independent differentiation experiments with control iPSC lines C1-1 and C3-1; n=11 RBM20-EHM s generated from 3 independent differentiation experiments with RBM20-iPSC lines 1-R-2 and 1-R-3. *, $P < 0.05$ RBM20 vs. control by two-way ANOVA.

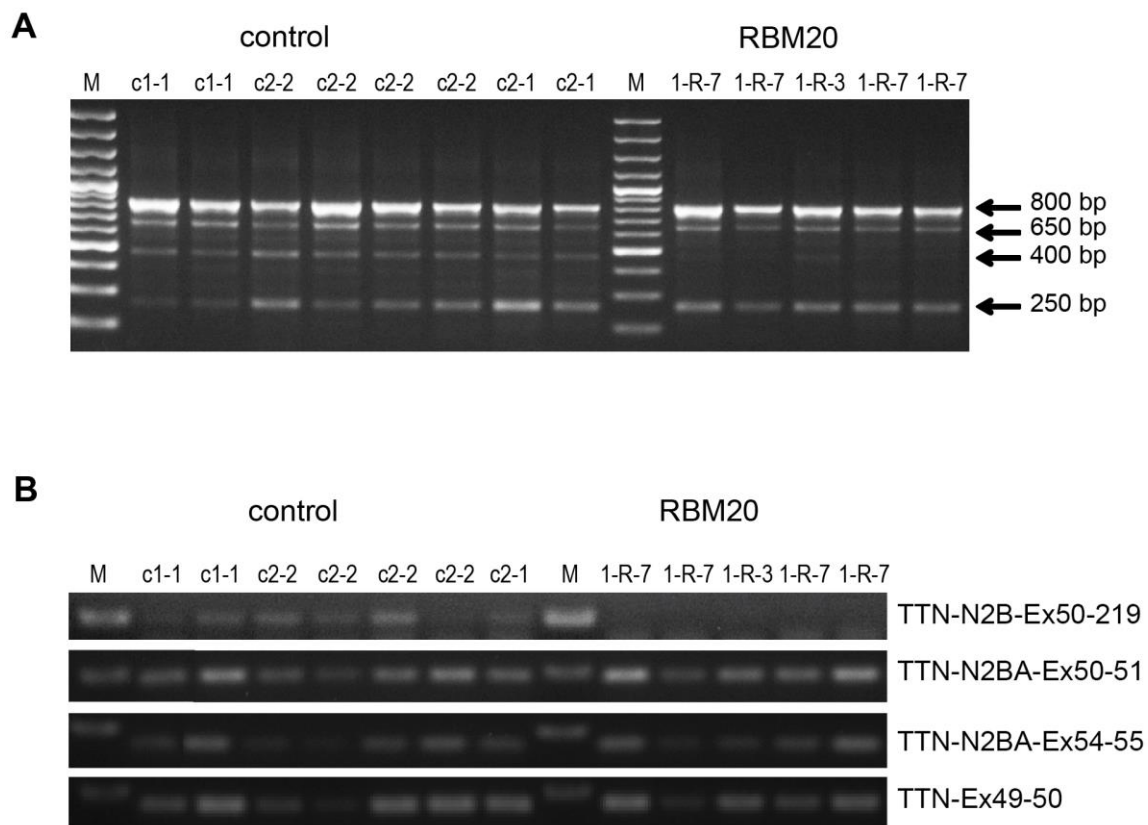


Figure S4: Semi-quantitative RT-PCR for *TTN* splicing variants in RBM20- and control-iPSC-CMs. RT-PCR of RBM20-iPSC-CMs and control-iPSC-CMs at day 60 of differentiation and relative densitometry to detect the titin splicing variants. **A**, primers were designed spanning exons 114-122 to quantify long and short *TTN* splicing isoforms with expected sizes of 250 bp, 400 bp, 650 bp, and 800 bp. The long isoform (800 bp) was used as internal control. For RBM20-iPSC-CMs, n=5 independent differentiation experiments from two cell lines 1-R-3 and 1-R-7 were studied, and for control-iPSC-CMs, n=8 independent differentiation experiments from three different control cell lines C1-1, C2-1 and C2-2 were investigated. **B**, *TTN-N2B* isoform is reduced in RBM20-iPSC-CMs in comparison to control-iPSC-CMs. Primers spanning *TTN* exons 49 and 50 were designed for amplifying total *TTN*, which was used as internal control. For expression of *TTN-N2B*, a primer pair spanning exons 50 and 219 was used. For *TTN-N2BA* variant expression, primers were designed spanning exons 50-51 and 54-55. For RBM20-iPSC-CMs, n=5 independent differentiation experiments from 2 different cell lines 1-R-3 and 1-R-7 were analyzed. For control-iPSC-CMs, n=7 independent differentiation experiments from 3 different cell lines C1-1, C2-1 and C2-2 were studied.

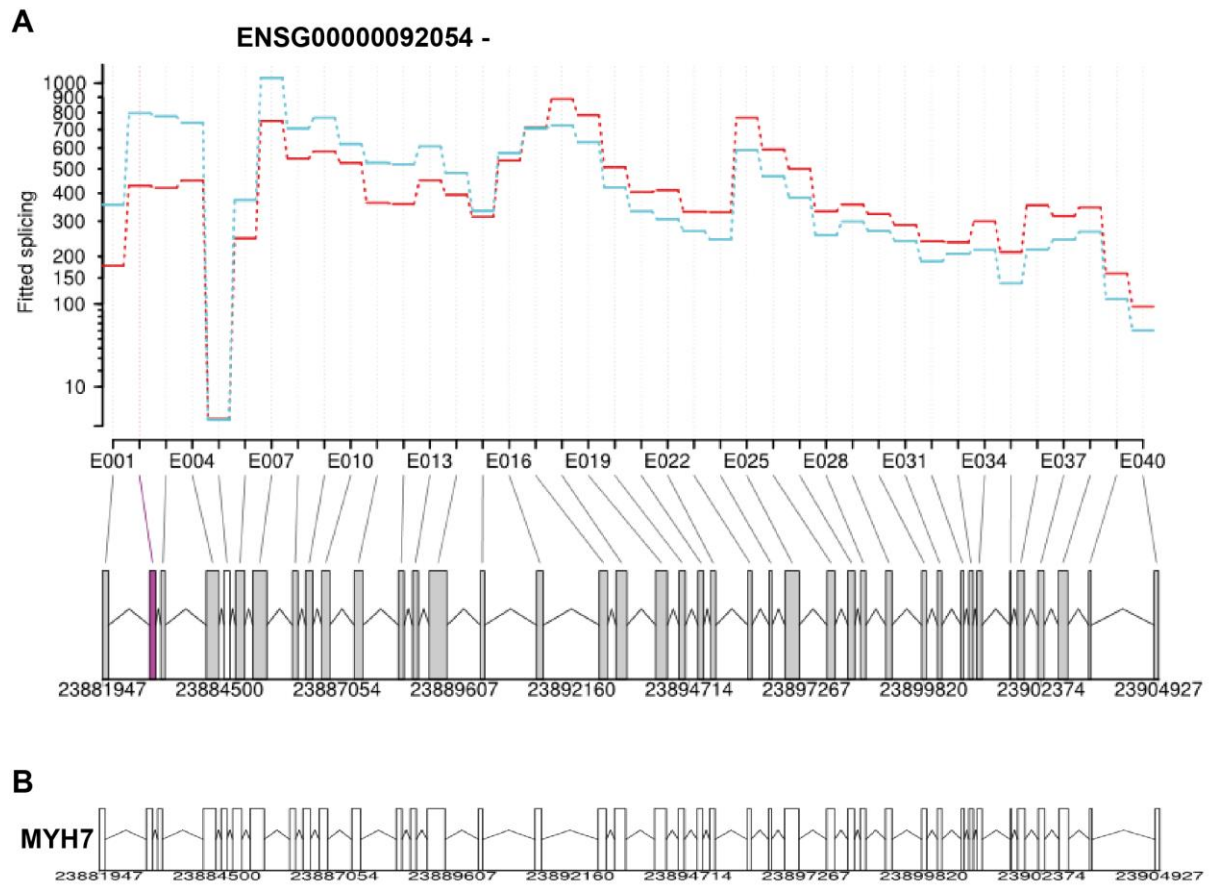


Figure S5: RBM20-dependent exon expression of the *MYH7* gene. **A**, shown is lower expression of exon 2 (pink box) in RBM20-iPSC-CMs compared to control-iPSC-CMs. Red lines: patient, blue lines: control. **B**, the only isoform of the *MYH7* gene.

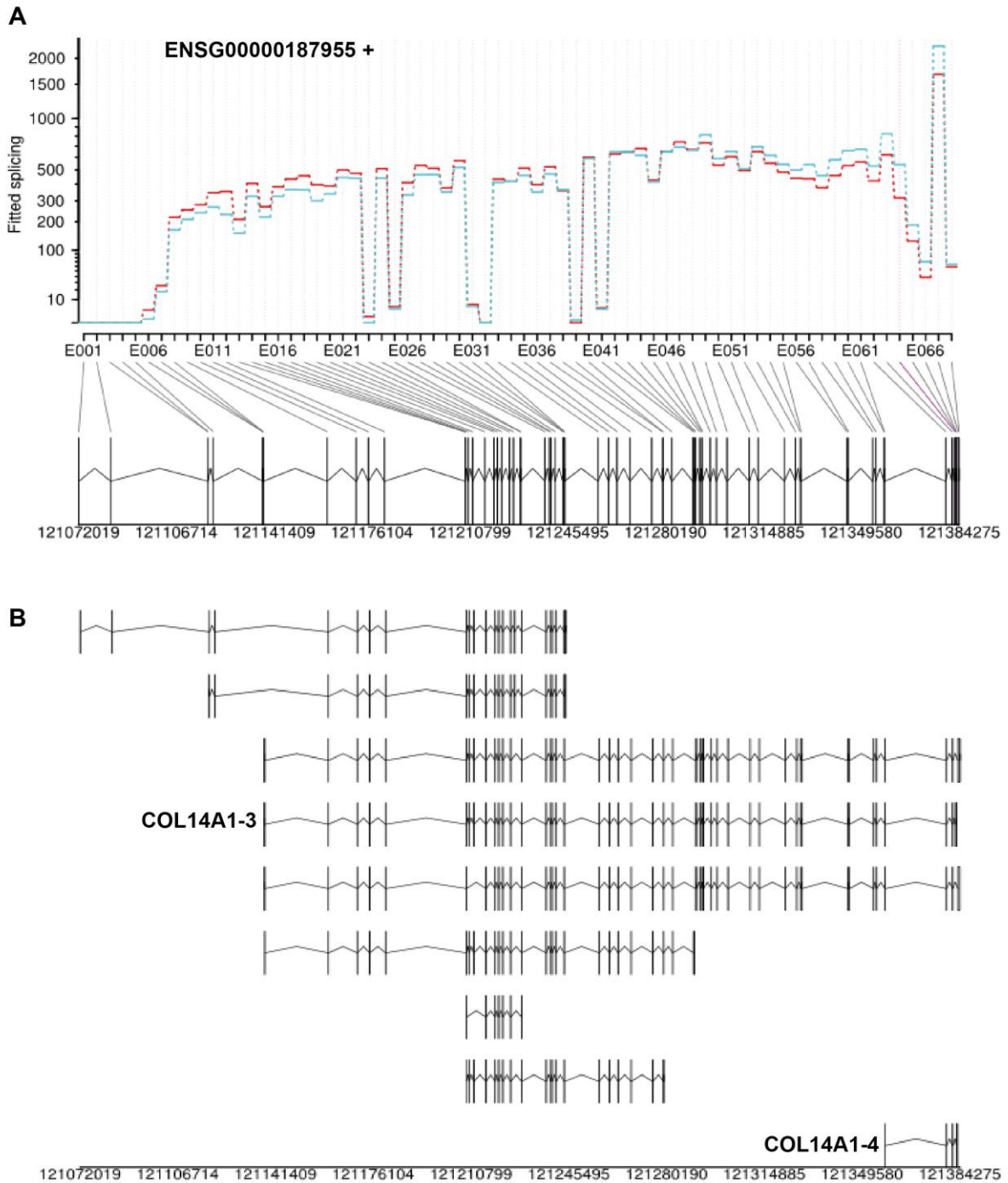


Figure S6: RBM20-dependent exon expression of the *COL14A1* gene. **A**, the gene *COL14A1* shows decreased expression of exon 64 (pink box) in RBM20-iPSC-CMs compared to control-iPSC-CMs. Red lines: patient, blue lines: control. **B**, different isoforms of the *COL14A1* gene. The decreased expression of exon 64 suggests an altered abundance of the isoforms 3 and 4 (*COL14A1-3* and *COL14A1-4*).

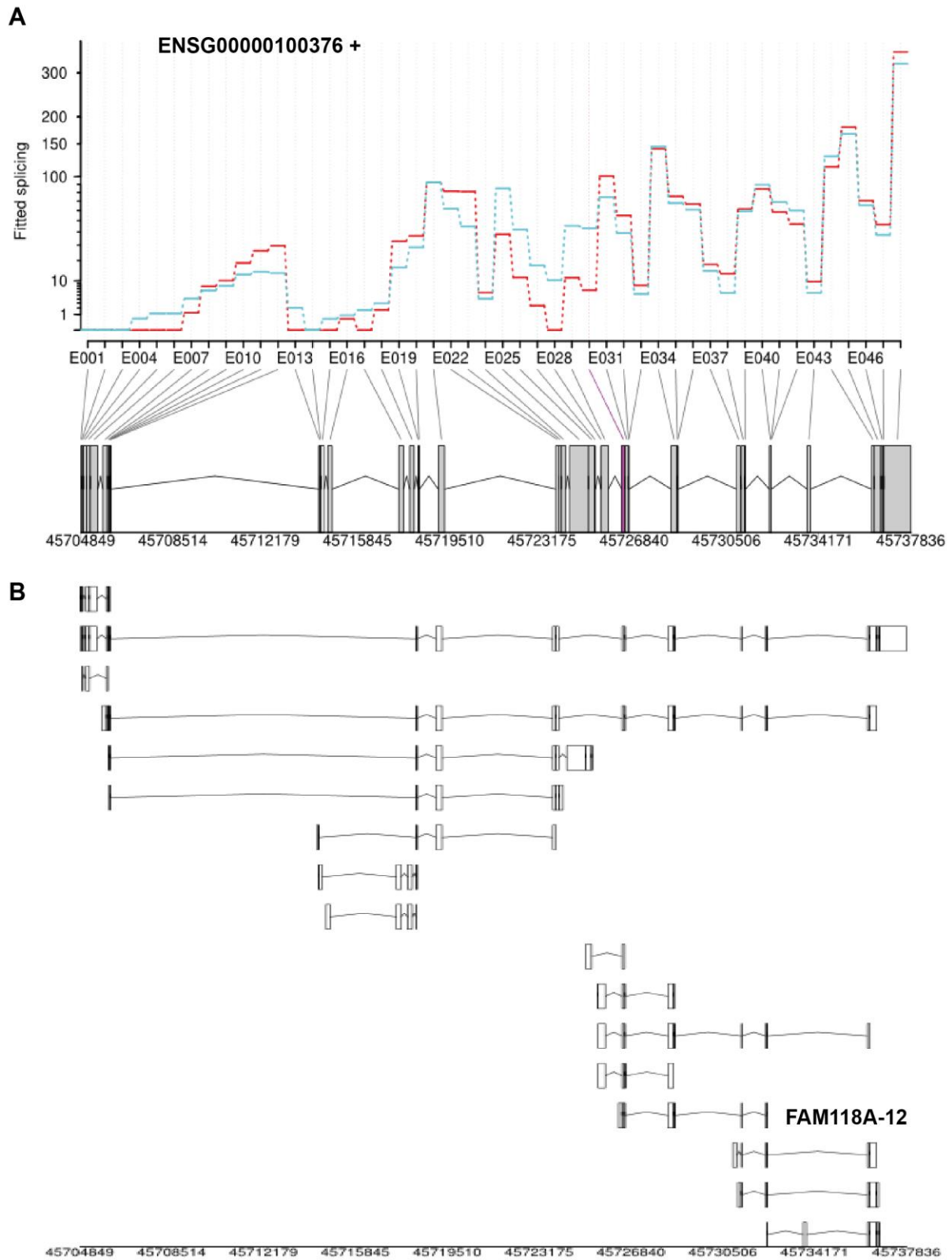


Figure S7: RBM20-dependent exon expression of the *FAM118A* gene. **A**, the gene *FAM118A* shows lower expression of exon 30 (pink box) in RBM20-iPSC-CMs. Red lines: patient, blue lines: control. **B**, different isoforms of the *FAM118A* gene. The decreased expression of exon 30 suggests lower abundance of the transcript of isoform 12 (*FAM118A-12*).

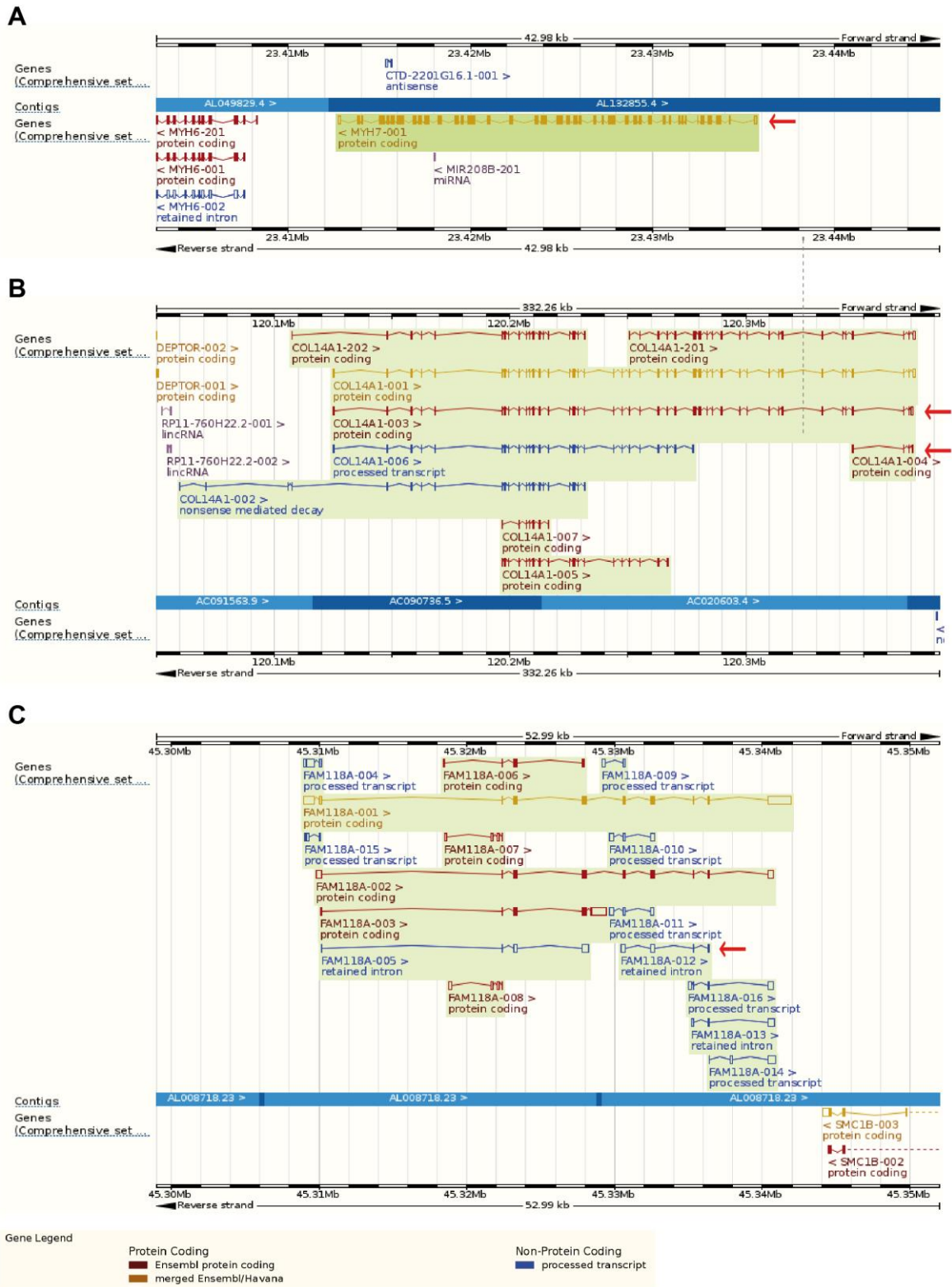


Figure S8: Genetic architecture and domain structure of *MYH7*, *COL14A1*, and *FAM118A*. The figure was downloaded from the ENSEMBL database (version e88). It shows the human genomic locus of *MYH7* (A), *COL14A1* (B), and *FAM118A* (C) and its annotated transcribed isoforms including exon structure and encoded protein domains. Isoforms 1 (*MYH7*), 12 (*FAM118A*), 3 and 4 (*COL14A1*) are marked with a red arrow. These were the isoforms of the three genes, whose expression might be influenced due to the differential exon usage in RBM20-CMs detected in RNA-seq data.