

Table S1. CNC cell markers two- or more fold upregulated in multilineage progenitors at stage 5+9d vs undifferentiated ES cells (adapted from (6))

Probe ID	FC>2	Gene	Protein	Putative role in CNC (adapted from (30))
1435176_a_at	4.4	<i>Id2</i>	Inhibitor of DNA binding 2	EMT
1447643_x_at	2.6	<i>Snai2</i>	Zinc finger protein Snai2	
1418815_at	3.7	<i>Cdh2</i>	N-cadherin	Migration
1421917_at	4.8	<i>Pdgfra</i>	Platelet derived growth factor receptor, alpha	Aortic arch remodeling
1420895_at	2.9	<i>Tgfb1</i>	Transforming growth factor, beta receptor 1	
1448460_at	2.3	<i>Acvr1</i>	Activin A receptor, type 1	
1417525_at	2.8	<i>Hand1</i>	Heart and neural crest derivatives expressed protein 1	
1422221_at	7.7	<i>Hand2</i>	Heart and neural crest derivatives expressed protein 2	Arterial tree remodeling
1448943_at	16.8	<i>Nrp1</i>	Neuropilin-1	
1421027_a_at	10.2	<i>Mef2c</i>	Myocyte enhancer factor 2C	
1451691_at	7.0	<i>Ednra</i>	Endothelin receptor type A	
1416693_at	5.3	<i>Foxc2</i>	Forkhead box C2	
1420909_at	3.5	<i>Vegfa</i>	Vascular endothelial growth factor A	
1424797_a_at	2.6	<i>Pitx2</i>	Paired-like homeodomain transcription factor 2	
1455741_a_at	2.1	<i>Ece1</i>	Endothelin converting enzyme 1	
1417649_at	6.6	<i>Cdkn1c</i>	Cyclin-dependent kinase inhibitor 1C (P57)	Myocardial development

Genes marked in bold were analyzed on transcript and protein level.

Table S2. Oligonucleotide sequences

Gene	Oligonucleotide sequence 5'>3' (forward/reverse)	T _a (°C)	No. of cycles	Amplicon size (bp)
<i>Cx43</i>	TACCACGCCACCACCGGCCCA GGCATTGGCTGTCGTCAGGGAA	52°C	40	294 bp
<i>Ece-1</i>	CTCTCTGCCTCGCTCACAC GGTGCTGGTGACGCTTCT	60°C	33	317 bp
<i>Ednra</i>	CCCTTGATTACCGCCATTGAAA TGACAACCAAGCAGAAGACAGT	60°C	35	346 bp
<i>Capdh</i>	CAGCCTCGTCCCGTAGAC	60°C	28	253 bp
<i>Hand1</i>	CGCTCCTGGAAGATGGTG AGAGGAGACGCACAGAGAGC TCCTCTCCTCTCCACGTAGC	60°C	33	410 bp
<i>Hand2</i>	GTATGGCCCTGTCCTACAGC GCCAGCAGATCCATGAGG	60°C	33	291 bp
<i>Lbx1</i>	CAGACCTCGCCTCTCTGC CTCCTCTAGGTCCCGCTTG	60°C	40	318 bp
<i>Mef2C</i>	ACTGGGAAACCCCAATCTTC ATCAGACCGCCTGTGTTACC	60°C	35	111 bp
<i>Pitx2</i>	CCCCTTCTTCAACTCCATGA CACCATGCTGGACGACATAC	60°C	37	100 bp
<i>Pax3</i>	CTGCACTCAAGGGACTCCTC GTGAAGGCGAGACGAAAAAG	60°C	35	169 bp
<i>Sema3C</i>	CCCAGATGGCACTGATCC CACACACTGCCGATCC	60°C	33	331 bp
<i>Snai1</i>	AAACCCACTCGGATGTGAAG	60°C	35	184 bp
<i>Snai2</i>	GAAGGAGTCCTGGCAGTGAG AACATTTCAACGCCTCCAAG GCCCCAAGGATGAGGAGTAT	60°C	35	231 bp

Table S3. Primary antibodies

Primary antibody	Dilution IF (WB)	Catalog number	Company
Ms anti α -actinin2	1 : 200	A7811	Sigma Aldrich, Germany
Rb anti ET-AR	1 : 50	sc-33536	Santa Cruz Biotechnology, Germany
Rb anti GAPDH	(1 : 2000)	ab36845	Abcam, UK
Rb anti HAND1	1 : 100	PA1-20319	Dianova, Germany
Rb anti HAND2	1 : 200	sc-22818	Santa Cruz Biotechnology, Germany
Rb anti MEF2C	1 : 100	ARP37342_T100	Aviva Systems Biology, USA, Germany
Ms anti Mlc2a	1 : 150	311 011	Synaptic Systems, Germany
Rb anti MYL6	1 : 100 (1 : 1000)	ab84349	Abcam, UK
Ms anti Nestin	1 : 35	MAB353	Millipore, Germany
Rb anti Pax3	1 : 600	CA1010	Calbiochem, Germany
Gt anti Pitx2	1 : 50	sc-8748	Santa Cruz Biotechnology, Germany
Ms anti α -Actin2	1 : 200	ab18147	Abcam, UK
Rb anti SNAI1	1 : 200	ab17732	Abcam, UK
Rb anti SNAI2	1 : 70	ab27568	Abcam, UK
Ms anti Titin (T12)	1 : 100		A gift from Dr. D. Fürst, Germany
Ms anti Tubb3	1 : 140	MAB1637	Millipore, Germany
Rat anti 4E9R	1 : 15 (1 : 50)		A gift from Prof. Dr. K. Ito, Japan

Ms: Mouse, Rb: Rabbit, Gt: Goat.

Table S4. Selected CNC-associated markers (adapted from (30))

Gene name	Protein name	Putative role in CNC
<i>Pax3</i>	Paired box 3	Specification, Migration
<i>Cja1</i>	Gap junction alpha-1 protein (Cx43)	Migration
<i>Snai1</i>	Zinc finger protein Snai1	Epithelial-to-mesenchymal transition
<i>Snai2</i>	Zinc finger protein Snai2	
<i>Hand1 (eHand)</i>	Heart and neural crest derivatives expressed protein 1	Arterial tree remodeling
<i>Hand2 (dHand)</i>	Heart and neural crest derivatives expressed protein 2	
<i>Mef2c</i>	Myocyte enhancer factor 2C	
<i>Ednra (EtA)</i>	Endothelin receptor type A	
<i>Pitx2</i>	Paired-like homeodomain transcription factor 2	
<i>Lbx1</i>	Ladybird homeobox 1	Myocardial development

Table S5. Proteins co-immunoprecipitating with 4E9R (Masqot ion score >100)

Analyzed gel bands	Protein hits (<i>gene name</i>)	Protein view	MW (kDa)	Sequence coverage (%)	Score	Peptides matched
1	myosin, heavy polypeptide 10, non-muscle	EDL10453.1	226.56	5	195	7
1	Myosin-10	NP_780469.1	228.85	5	195	7
1	PREDICTED: myosin-10 isoform X1	XP_006534552.1	232.32	4	195	7
1	PREDICTED: myosin-10 isoform X2	XP_017170323.1	231.21	4	195	7
1	PREDICTED: myosin-10 isoform X3	XP_017170324.1	229.97	4	195	7
2	vimentin, isoform CRA_a	EDL08053.1	46.5	18	292	6
2	vimentin	NP_035831.2	53.7	15	292	6
2	vimentin	CAA39807.1	53.7	15	292	6
2	vimentin protein	AAA40555.1	53.6	15	292	6
2	vimentin partial	CAA69019.1	51.5	16	292	6
3	alpha-actin (AA 27-375), partial	CAA27398.1	39.22	21	265	6
3	alpha-cardiac actin, partial	AAA37167.1	41.75	20	265	6
3	gamma Actin, partial	CAA31455.1	40.99	17	227	5
4	gamma-Actin, partial	CAA31455.1	40.99	22	352	8
4	alpha-actin (AA 27-375), partial	CAA27398.1	39.22	17	228	6
4	alpha-cardiac actin, partial	AAA37167.1	41.75	16	228	6
4	gamma actin-like protein	AAF08293.1	43.57	14	220	4
5	no relevant proteins reached the threshold score >100					
6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, isoform CRA_a	EDL35010.1	21.65	21	125	4
6	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial precursor	NP_079592.2	21.69	21	125	4
6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13, isoform CRA_a	EDL28764.1	13.82	26	111	4
6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13, isoform CRA_c	EDL28766.1	13.16	28	111	4
6	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	NP_075801.1	16.84	21	111	4
7	no proteins reached the threshold score >100					
8	LOC665622 protein	AAH11440.1	14.9	24	169	9
8	Hist1h2bj protein, partial	AAI15793.1	13.6	26	169	9
8	spermatid-specific, partial	AAA50377.1	15.1	24	169	9
8	histone H2B type 1-B	NP_783595.1	13.9	26	169	9
8	histone H2B type 1-K	NP_783596.1	13.9	26	169	9
8	histone H2B type 1-H	NP_835504.1	13.9	26	169	9
8	histone H2B type 1-F/J/L	NP_835505.1	13.9	26	169	9
8	histone H2B type 1-P isoform 1	NP_835509.2	15.6	23	169	9
8	H2b histone family, member A isoform 1	NP_001091448.2	14.9	24	169	9
8	histone H2B type 1-P isoform 2	NP_001277395.1	13.9	26	169	9
8	16S ribosomal protein	AAA03646.1	16.3	14	126	2
8	Rps16 protein, partial	AAH82286.1	17.5	13	126	2
8	Rps16 protein	AAH90618.1	19.3	12	126	2
8	PREDICTED: 40S ribosomal protein S16	XP_003688797.1	18.4	12	126	2
9	spermatid-specific, partial	AAA50377.1	15.1	50	285	6
9	histone H2B type 1-H	NP_835504.1	13.9	53	285	6
10	cytochrome c oxidase subunit IV	AAB02139.1	19.5	18	161	3
10	cytochrome c oxidase subunit IV isoform 1, isoform CRA_a, partial	EDL11642.1	20.7	17	161	3
10	cytochrome c oxidase subunit 4 isoform 1, mitochondrial isoform 1	NP_034071.2	23.5	15	161	3

Table S5. Continued

Analyzed gel bands	Protein hits (gene name)	Protein view	MW (kDa)	Sequence coverage (%)	Score	Peptides matched
10	cytochrome c oxidase subunit 4 isoform 1, mitochondrial isoform 2	NP_001280488.1	19.5	18	161	3
10	LOC665622 protein	AAH11440.1	14.9	24	134	3
10	Hist1h2bj protein, partial	AAI15793.1	13.6	26	134	3
10	spermatid-specific, partial	AAA50377.1	15.1	24	134	3
10	histone H2B type 1-B	NP_783595.1	13.9	26	134	3
10	histone H2B type 1-K	NP_783596.1	13.9	26	134	3
10	histone H2B type 1-H	NP_835504.1	13.9	26	134	3
10	histone H2B type 1-F/J/L	NP_835505.1	13.9	26	134	3
10	histone H2B type 1-P isoform 1	NP_835509.2	15.6	23	134	3
10	H2b histone family, member A isoform 1	NP_001091448.2	14.9	24	134	3
10	histone H2B type 1-P isoform 2	NP_001277395.1	13.9	26	134	3
11	histone protein Hist3h2bb	AAO06253.1	17.1	12	104	9
11	LOC665622 protein	AAH11440.1	14.9	14	104	9
11	Hist1h2bj protein, partial	AAI15793.1	13.5	16	104	9
11	Histone cluster 1, H2ba	AAI26924.1	14.1	15	104	9
11	spermatid-specific, partial	AAA50377.1	15.1	14	104	9
11	histone H2B type 3-A	NP_084358.1	13.9	15	104	9
11	histone H2B type 1-A	NP_783594.1	14.2	15	104	9
11	histone H2B type 1-B	NP_783595.1	13.9	15	104	9
11	histone H2B type 1-K	NP_783596.1	13.9	15	104	9
11	histone H2B type 1-H	NP_835504.1	13.9	15	104	9
11	histone H2B type 1-F/J/L	NP_835505.1	13.9	15	104	9
11	histone H2B type 1-P isoform 1	NP_835509.2	15.6	14	104	9
11	histone H2B type 2-E	NP_835586.2	13.9	15	104	9
11	H2b histone family, member A isoform 1	NP_001091448.2	14.9	14	104	9
11	histone H2B type 1-P isoform 2	NP_001277395.1	13.9	15	104	9
12	mCG140959, isoform CRA_c, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24573.1	17.42	18	151	3
12	mCG140959, isoform CRA_d, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24574.1	17.97	17	151	3
12	mCG140959, isoform CRA_e, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24575.1	18.92	16	151	3
12	mCG140959, isoform CRA_g, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24577.1	17.78	17	151	3
12	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	18	151	3
12	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	17	151	3
12	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	24	151	3
12	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	24	151	3
12	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	22	151	3
12	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	24	151	3
13	no proteins reached the threshold score >100					
14	mCG140959, isoform CRA_c, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24573.1	17.42	14	92	2
14	mCG140959, isoform CRA_d, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24574.1	17.97	14	92	2
14	mCG140959, isoform CRA_e, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24575.1	18.92	13	92	2

Table S5. Continued

Analyzed gel bands	Protein hits (<i>gene name</i>)	Protein view	MW (kDa)	Sequence coverage (%)	Score	Peptides matched
14	mCG140959, isoform CRA_g, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24577.1	17.78	14	92	2
14	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	15	92	2
14	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	14	92	2
15	no proteins reached the threshold score >100					
16	no proteins reached the threshold score >100					
17	mCG140959, isoform CRA_c, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24573.1	17.42	14	115	2
17	mCG140959, isoform CRA_d, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24574.1	17.97	14	115	2
17	mCG140959, isoform CRA_e, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24575.1	18.92	13	115	2
17	mCG140959, isoform CRA_g, partial/Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	EDL24577.1	17.78	14	115	2
17	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	15	115	2
17	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	14	115	2

Table S6. Myl6 isoforms co-immunoprecipitating with 4E9R

Analyzed gel bands	Protein hits	Protein view	MW (kDa)	Sequence coverage (%)	Score	Peptides matched
9	mCG48799	EDL05612.1	16.7	10	43	1
9	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	10	43	1
9	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	9	43	1
9	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	9	43	1
9	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	10	43	1
9	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	10	43	1
9	non-muscle myosin light chain 3, partial	AAC52278.1	15.7	11	43	1
9	mCG1031566, partial	EDK96876.1	14.9	11	43	1
9	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	10	43	1
9	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	13	43	1
9	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	13	43	1
9	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	13	43	1
	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	13	43	1
10	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	9	46	1
10	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	9	46	1
10	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	8	46	1
10	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	9	46	1
10	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	9	46	1
10	mCG1031566, partial	EDK96876.1	14.9	11	46	1
10	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	9	46	1
10	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	12	46	1
10	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	12	46	1
10	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	12	46	1
10	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	12	46	1
11	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	17	59	2
11	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	16	59	2
11	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	15	59	2
11	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	16	59	2
11	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	17	59	2
11	mCG1031566, partial	EDK96876.1	14.9	20	59	2
11	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	17	59	2
11	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	23	59	2
11	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	23	59	2
11	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	21	59	2
11	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	23	59	2
12	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	18	151	3
12	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	17	151	3
12	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	16	151	3
12	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	17	151	3
12	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	18	151	3
12	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	17	151	3
12	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	24	151	3
12	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	24	151	3
12	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	22	151	3
12	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	24	151	3
13	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	9	39	1
13	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	9	39	1
13	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	8	39	1
13	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	9	39	1
13	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	9	39	1
13	mCG1031566, partial	EDK96876.1	14.9	11	39	1

Table S6. Continued

Analyzed gel bands	Protein hits	Protein view	MW (kDa)	Sequence coverage (%)	Score	Peptides matched
13	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	9	39	1
13	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	12	39	1
13	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	12	39	1
13	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	12	39	1
13	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	12	39	1
14	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	14	92	2
14	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	14	92	2
14	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	13	92	2
14	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	14	92	2
14	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	15	92	2
14	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	14	92	2
15	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	9	42	1
15	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	9	42	1
15	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	8	42	1
15	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	9	42	1
15	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	9	42	1
15	mCG1031566, partial	EDK96876.1	14.9	11	42	1
15	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	9	42	1
15	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	12	42	1
15	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	12	42	1
15	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	12	42	1
15	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	12	42	1
16	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	8	62	1
16	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	8	62	1
16	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	7	62	1
16	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	8	62	1
16	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	8	62	1
16	mCG1031566, partial	EDK96876.1	14.9	9	62	1
16	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	8	62	1
16	myosin light polypeptide 6 isoform b	NP_001304148.1	12.93	11	62	1
16	myosin light polypeptide 6 isoform c	NP_001304149.1	12.96	11	62	1
16	PREDICTED: myosin light polypeptide 6 isoform X1	XP_006513386.1	13.67	10	62	1
16	PREDICTED: myosin light polypeptide 6-like	XP_006531628.3	12.96	11	62	1
17	mCG140959, isoform CRA_c, partial	EDL24573.1	17.42	14	115	2
17	mCG140959, isoform CRA_d, partial	EDL24574.1	17.97	14	115	2
17	mCG140959, isoform CRA_e, partial	EDL24575.1	18.92	13	115	2
17	mCG140959, isoform CRA_g, partial	EDL24577.1	17.78	14	115	2
17	myosin light polypeptide 6 isoform MLC3sm	NP_034990.1	16.95	15	115	2
17	myosin light polypeptide 6 isoform a	NP_001304146.1	17.66	14	115	2

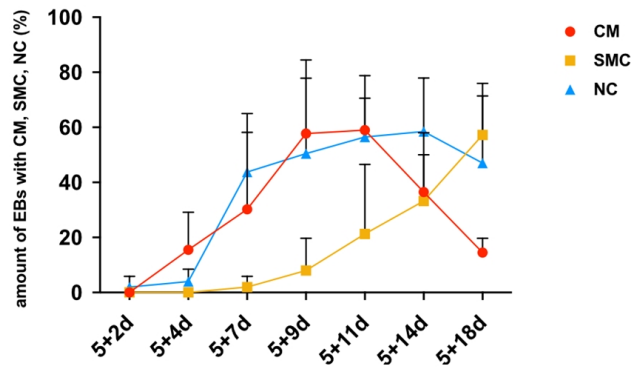


Fig. S1. Morphological assessment of ES cell-derived multilineage progeny. Differentiation potential of ES cells (n=4) into multilineage progeny was examined at several differentiation stages. Amount of EBs with respective cell types is given in percent. Values are depicted as mean with standard deviation. CM: cardiomyocytes, SMC: skeletal muscle cells, NC: neuronal cells.

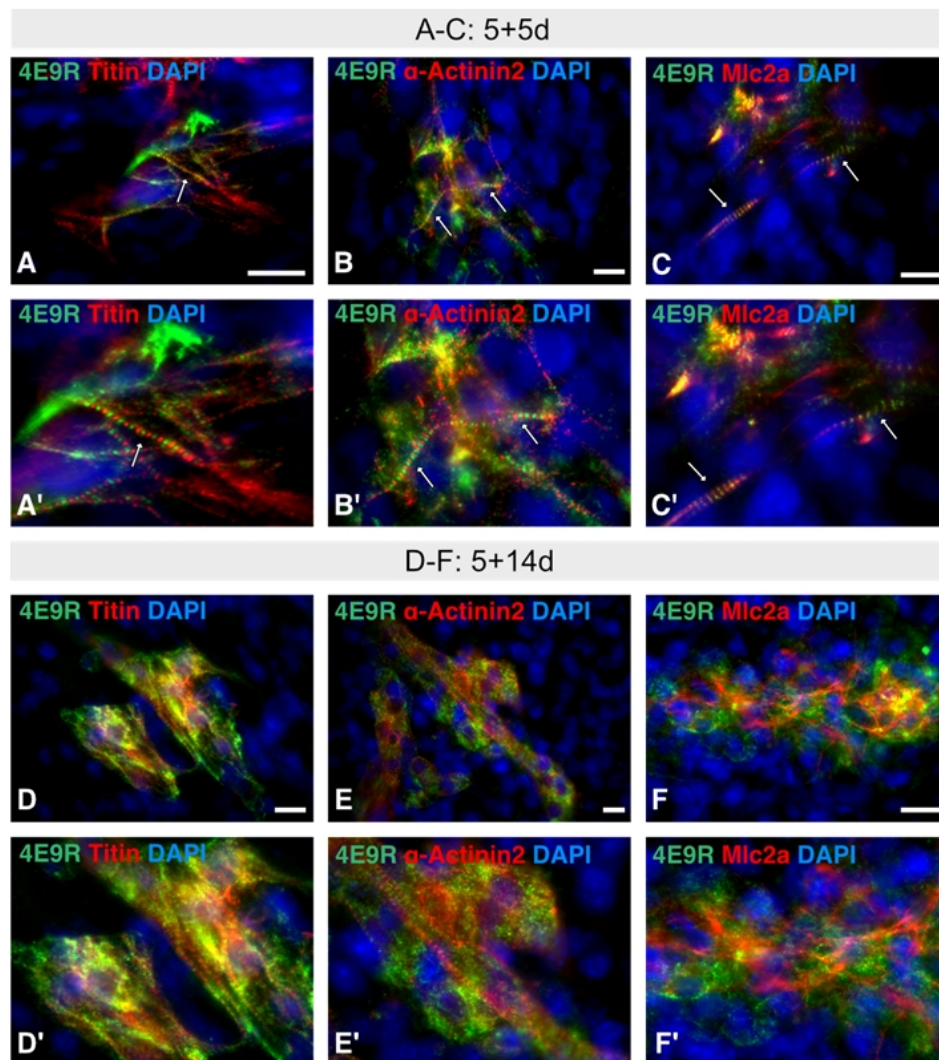


Fig. S2. Immunofluorescence analyses of early and terminally differentiated ES cell-derived cardiomyocytes using 4E9R. (A~C) ES cell-derived cardiac progeny at early differentiation stages (A: 5+5d, B: 5+5d, C: 5+4d) were co-labeled by the 4E9R antibody and sarcomere-specific markers like Titin (A, A' 5+5d) α -Actinin2 (B, B' 5+5d) and Mlc2a (C, C' 5+4d). White arrows indicate the co-labeling and the magnified area (A', B', C'). (D~F) Co-staining of 4E9R and the sarcomere-specific markers Titin (D, D' 5+14d), α -Actinin2 (E, E' 5+12d) and Mlc2a (F, F' 5+12d) in more advanced differentiation stages. (D'~F') Magnified areas. Nuclear counterstain was performed with DAPI (blue). Bars: 10 μ m (A, C, D, E), 20 μ m (B, F).