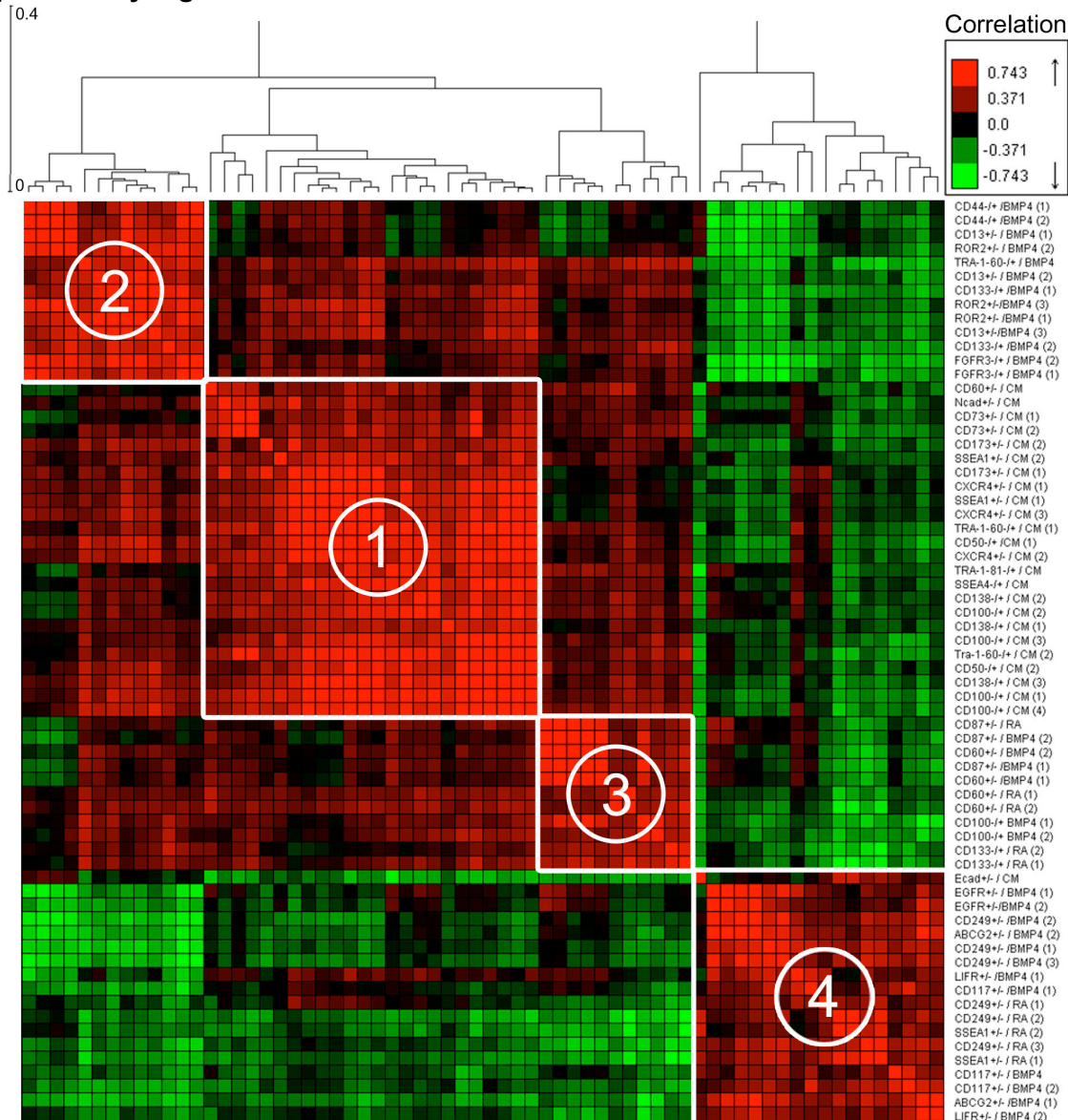


Isolation of primitive endoderm, mesoderm, vascular endothelial and trophoblast progenitors from human pluripotent stem cells

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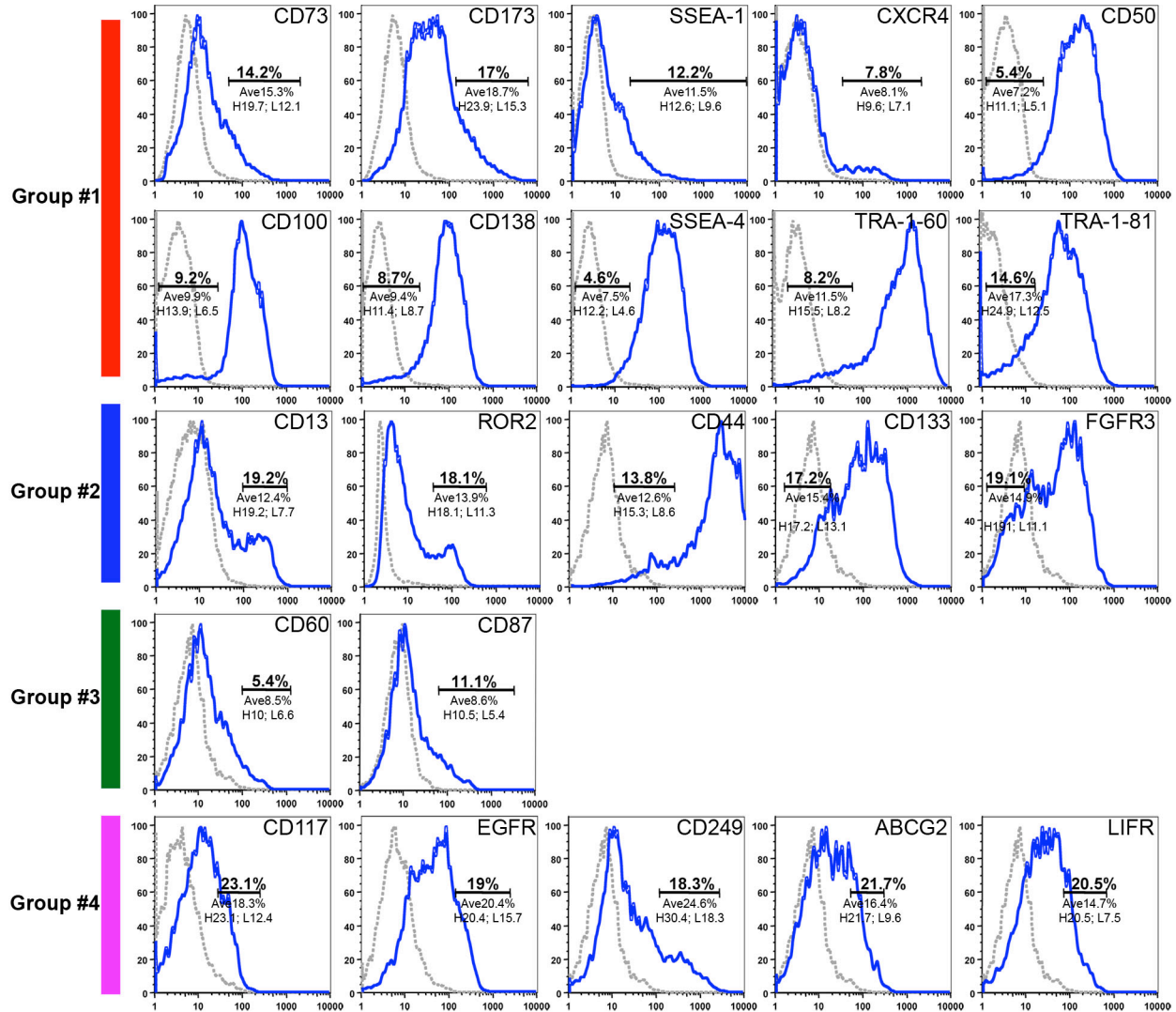
Supplementary Figure 1



Grouping of hESC-derived progenitors based on similarities in gene expression profiles

Shown is a clustered correlation matrix based on expression fold-change signatures displayed in Fig. 2. It reveals four main groups of cell subpopulations (numbered 1 to 4) corresponding to the four main types of progenitors. Each pixel displays the (Pearson) correlation between enrichment profiles of a pair of subpopulations, each sorted with a given surface marker following a given treatment (as outlined in Fig. 2). Biological repeats with same choice of marker and treatment cluster within the same progenitor groups. Red and green values correspond, respectively, to the extent of correlation and anti-correlation between expression fold-change profiles in pairs of sorted populations.

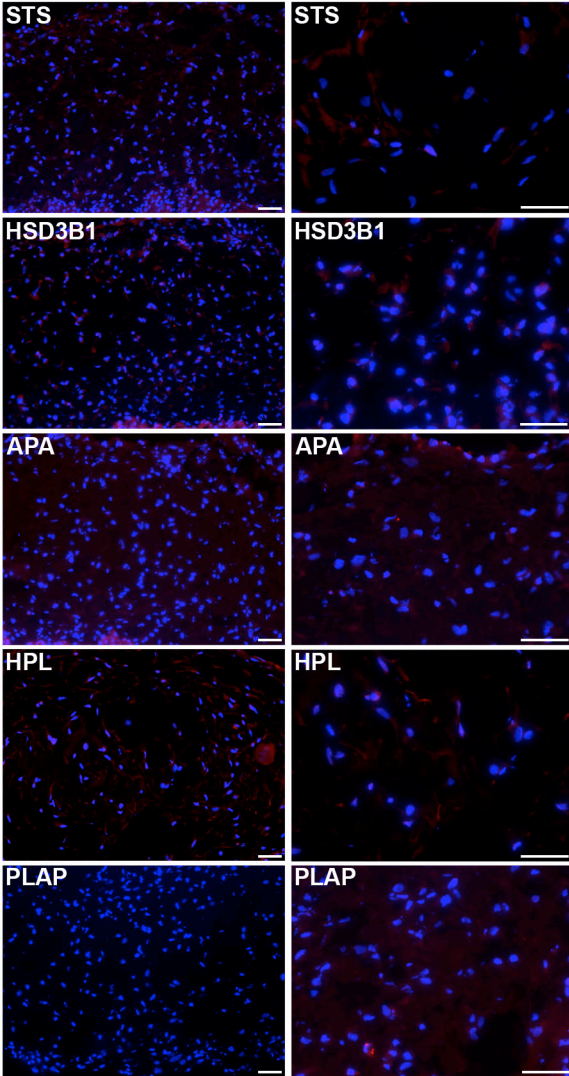
Supplementary Figure 2



Staining distributions of cell surface markers corresponding to the four main progenitor groups

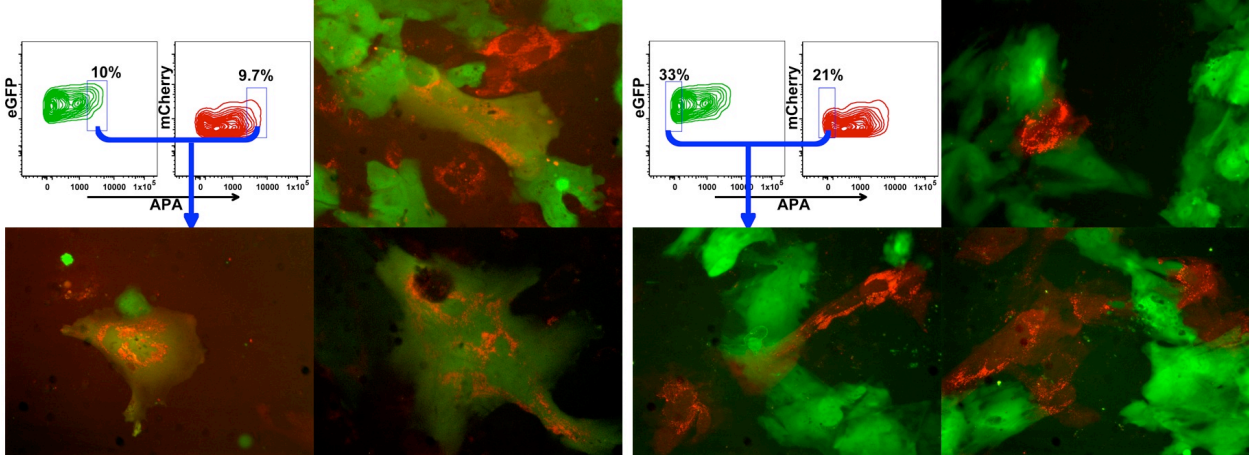
Shown are representative FACS plots of hESCs treated for 3 days with CM (Group #1) or BMP4 (Groups #2-4). The respective cell surface markers of each of the progenitor groups are noted in four panels, along with the typical gating strategies and population frequencies. Gray dotted lines represent isotype controls. Avg, L, and H correspond, respectively, to averaged, lowest, and highest progenitor frequencies measured across 3 independent experiments.

Supplementary Figure 3



8-week old grafts derived from ROR2+ cells do not exhibit staining of placental markers
Immunohistochemistry images of placental markers analyzed in grafts derived from ROR2+ cells (representing group #2 of mesoderm progenitors) demonstrating lack of staining for all the markers. Low and higher power microphotographs are shown on left and right, respectively. DAPI staining is shown in blue. Scale bar = 25 microns.

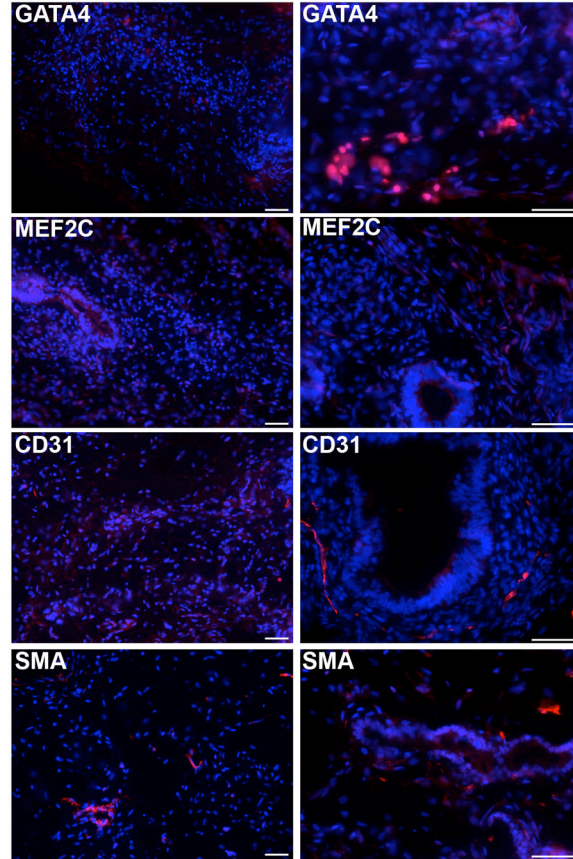
Supplementary Figure 4



Cultured APA+ cells produce syncytiotrophoblast-like cells

Left: Mixture of GFP-labeled and mCherry-labeled APA+ cells (right) were cultured for 7 days (Inset shows respective FACS plots). Right: Same for APA- cells.

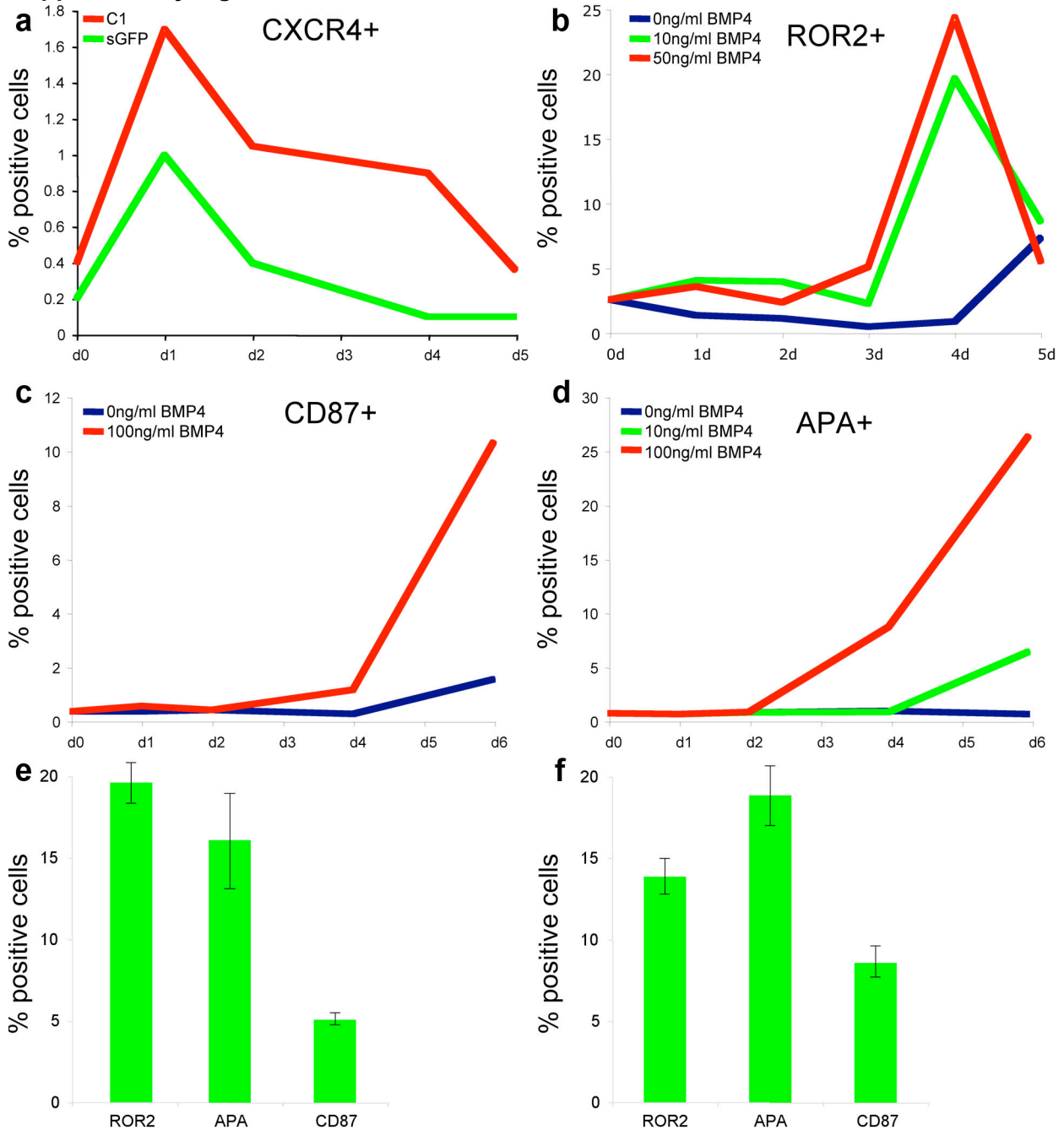
Supplementary Figure 5



8-week old grafts derived from APA+ cells exhibit little or no staining of mesoderm markers

Immunohistochemistry images of mesoderm markers analyzed on grafts derived from APA+ cells (representing group #4 of trophoblast progenitors). Infrequent or sparse staining was observed for GATA4, SMA and CD31 (PECAM1). Nuclear MEF2C signal was not detected. Low and higher power microphotographs are shown on left and right, respectively. DAPI staining is shown in blue. Scale bar = 25 microns.

Supplementary Figure 6



Emergence of progenitor populations as a function of treatment duration and variation in serum lots

(a-d) Representative frequencies of the four progenitor populations as a function of time (determined by flow cytometry; one out of three experiments shown). (a) CXCR4⁺ population peaked ~24 hrs after plating hESCs on Matrigel with CM (shown are two cell clones). (b) ROR2⁺ population peaked around day 4 of EB differentiation and decreased afterwards. (c) CD87⁺ population began accumulating in monolayer cultures around day 3 of treatment. (d) Emergence of the APA⁺ population in different dosages of BMP4. (e-f) Statistics of population frequencies for ROR2 (day 4), APA (day 4) and CD87 (day 5) following treatments using different lots of FBS (e) or using the same lot in 3 independent experiments (f). Lot statistics in (e) are based on 9 batches of FBS used for differentiation of ROR2 and APA populations, and 3 batches of FBS for CD87. Error bars represent standard errors.

Supplemental Tables

Supplementary Table 1
List of mAbs used in this study

Antigen symbol	Catalog #	Manufacturer*	Isotype
A2B5	MAB1416	R&D	Mouse IgM
ABCG2	552823	BD	Mouse IgG2b,k
ABCG2	MAB995	R&D	Mouse IgG
ACE (CD143)	MAB929	R&D	Mouse IgG1
Acetylcholine Receptor alpha	MAB398	Millipore	Mouse IgG1
Activin Receptor 1A	MAB637	R&D	Mouse IgG1
Activin Receptor 1B	MAB222	R&D	Mouse IgG1
Activin Receptor 2A/B	MAB3391	R&D	Mouse IgG1
Activin Receptor 2B	MAB3392	R&D	Mouse IgG1
ALCAM (CD166)	MAB656	R&D	Mouse IgG1
ALK 1	MAB370	R&D	Mouse IgG2a
Alzheimer Precursor Protein A4	MAB348	Millipore	Mouse IgG1
Axl	MAB154	R&D	Mouse IgG1
BACE	MAB5308	Millipore	Mouse IgG1
BAFF Receptor	MAB1755	R&D	Rat IgG2a
Beta 2 microglobulin	ab18605	Abcam	Mouse IgG2b
BMP R1B/ALK-6 (CDw293)	MAB505	R&D	Mouse IgG2a
BMP Receptor 2	MAB811	R&D	Mouse IgG2b
C Reactive Protein (CRP)	ab10026	Abcam	Mouse IgG2b
Cadherin 5 (VE-Caderin)	610251	BD	Mouse IgG1
Cadherin 6	16111	Progen	Mouse IgG
Cadherin17	MAB1072	R&D	Mouse IgG2b
CCR1	MAB145	R&D	Mouse IgG2b
CCR2	MAB150	R&D	Mouse IgG2b
CCR3	MAB155	R&D	Mouse IgG2a
CCR4	MAB1567	R&D	Mouse IgG2b
CCR5	MAB180	R&D	Mouse IgG2b
CCR6	559560	BD	Mouse IgG1, k
CCR7	MAB197	R&D	Mouse IgG2a
CCR8	MAB1429	R&D	Rat IgG2b
CCR9	MAB179	R&D	Mouse IgG2a
CD10	555373	BD	Mouse IgG1, k
CD100	555499	BD	Mouse IgG1, k
CD101	556007	BD	Mouse IgG1
CD103	550258	BD	Mouse IgG1, k
CD104 (integrin beta 4)	555721	BD	Mouse IgG1
CD105	555690	BD	Mouse IgG1, k
CD106	555645	BD	Mouse IgG1, k
CD107A	555798	BD	Mouse IgG1, k
CD107B	555803	BD	Mouse IgG1, k
CD108	ab8222	Abcam	Mouse IgM
CD109	556039	BD	Mouse IgG1
CD110 (c-Mpl, Tpo Receptor)	551312	BD	Mouse IgG1, k
CD112 (PRR2)	551056	BD	Mouse IgG1, k
CD114	554536	BD	Mouse IgG1, k

CD116	551284	BD	Rat IgG1
CD117	555713	BD	Mouse IgG1, k
CD118	RDI-PB21385	RDI	Mouse IgG2a
CD119	558395	BD	Mouse IgG2b,k
CD11a	555382	BD	Mouse IgG1, k
CD11b	555386	BD	Mouse IgG1, k
CD11c	555391	BD	Mouse IgG1, k
CD120a	552535	BD	Mouse IgG1
CD120b	552478	BD	Mouse IgG1, k
CD121a (sIL-1RI)	551388	BD	Mouse IgG1, k
CD121b	ab11869	Abcam	Mouse IgG2a
CD122	554520	BD	Mouse IgG2a, k
CD123	555642	BD	Mouse IgG1, k
CD124	IM2072	Beckman	Mouse IgG1
CD126	551462	BD	Mouse IgG1, k
CD127	552853	BD	Mouse IgG1
CD13	555393	BD	Mouse IgG1, k
CD130 (GP130)	555756	BD	Mouse IgG1, k
CD132	555896	BD	Mouse IgG2b,k
CD133/1	130-090-422	Miltenyi Biotec	Mouse IgG1
CD133/2	130-090-851	Miltenyi Biotec	Mouse IgG2b
CD134	555836	BD	Mouse IgG1, k
CD135	558995	BD	Mouse IgG1, k
CD137 (4-1BB)	555955	BD	Mouse IgG1, k
CD137 Ligand (4-1BB)	559445	BD	Mouse IgG1, k
CD138	551902	BD	Mouse IgG1, k
CD14	550376	BD	Mouse IgG2a, k
CD14	555396	BD	Mouse IgG2a, k
CD140a	556001	BD	Mouse IgG2a, k
CD140b	558820	BD	Mouse IgG2a, k
CD141	559780	BD	Mouse IgG1, k
CD142	550252	BD	Mouse IgG1, k
CD143	ab11734	Abcam	Mouse IgG1
CD144 (VE-cadherin) Cadherin 5	555661	BD	Mouse IgG1, k
CD146	550314	BD	Mouse IgG1, k
CD147	555961	BD	Mouse IgG1, k
CD15 (SSEA-1)	555400	BD	Mouse IgM, k
CD150	559591	BD	Mouse IgG1, k
CD151	556056	BD	Mouse IgG1, k
CD152	555851	BD	Mouse IgG2a, k
CD153 (CD30L)	550052	BD	Mouse IgG1, k
CD154 (CD40L)	555698	BD	Mouse IgG1, k
CD155	IM2755	Beckman	Mouse IgG1
CD157	IM2557	Beckman	Mouse IgG1
CD158a	556061	BD	Mouse IgM, k
CD158b	559783	BD	Mouse IgG2b,k
CD15S	551344	BD	Mouse IgM, k
CD16	555404	BD	Mouse IgG1, k
CD160	551887	BD	Mouse IgM, k
CD161	556079	BD	Mouse IgG1, k
CD162	556053	BD	Mouse IgG1, k

CD163	ab9246	Abcam	Mouse IgG1, k
CD164	551296	BD	Mouse IgG2a, k
CD165	559453	BD	Mouse IgG1, k
CD166	559260	BD	Mouse IgG1, k
CD169 (Sialoadhesin)	MCA1844	Serotec	Mouse IgG1
CD16b	M1390clb	RDI	Mouse IgG2b
CD171	CBL275	Millipore	Mouse IgG1
CD172a/b	552025	BD	Mouse IgG1, k
CD172b	552024	BD	Mouse IgG1, k
CD173	MCA1984	Serotec	Mouse IgG1
CD174	MCA2160	Serotec	Mouse IgM
CD175	MCA1985	Serotec	Mouse IgG1
CD176	MCA2161	Serotec	Mouse IgM
CD177	551899	BD	Mouse IgG1
CD177	ab8092	Abcam	Mouse IgG1
CD178	ab10511	Abcam	Mouse IgG1
CD179a	MCA2073	Serotec	Mouse IgM
CD18	555922	BD	Mouse IgG1, k
CD180	551890	BD	Mouse IgG1, k
CD183	557183	BD	Mouse IgG1, k
CD184	555972	BD	Mouse IgG2a, k
CD19	555410	BD	Mouse IgG1, k
CD195 (CCR5)	555991	BD	Mouse IgG2a, k
CD1A	555805	BD	Mouse IgG1, k
CD1B	555968	BD	Mouse IgG1, k
CD1C	550902	BD	Mouse IgG2a, k
CD1D	550254	BD	Mouse IgG1, k
CD2	555324	BD	Mouse IgG1, k
CD20	555621	BD	Mouse IgG2b,k
CD200	552023	BD	Mouse IgG1, k
CD200R	MCA2282	Serotec	Mouse IgG1
CD201	552500	BD	Rat IgG1, k
CD203c	IM3575	Beckman	Mouse IgG1
CD206	555953	BD	Mouse IgG1, k
CD207	IM3449	Beckman	Mouse IgG1
CD208	IM3578	Beckman	Mouse IgG1
CD209	551186	BD	Mouse IgG2b,k
CD21	555421	BD	Mouse IgG1, k
CD210 (II-10R)	556012	BD	Rat IgG2a, k
CD212	556064	BD	Mouse IgG1, k
CD22	555423	BD	Mouse IgG1, k
CD220	559954	BD	Mouse IgG1, k
CD221	556000	BD	Mouse IgG1, k
CD222	551982	BD	Mouse IgG1, k
CD223	552378	BD	Rat IgG1, k
CD226	559787	BD	Mouse IgG1, k
CD227	555925	BD	Mouse IgG1, k
CD229	552751	BD	Mouse IgG1, k
CD23	555708	BD	Mouse IgG1, k
CD231	551896	BD	Mouse IgG1, k
CD235a	555569	BD	Mouse IgG2b,k

CD236	MCA1983	Serotec	Mouse IgG1
CD236R	MCA1986	Serotec	Mouse IgG1
CD238	ab11463	Abcam	Mouse IgG1
CD239	MCA1982	Serotec	Mouse IgG2b
CD24	555426	BD	Mouse IgG2a, k
CD24	ab3274	Abcam	Mouse IgG1
CD240 DCE	MCA1981	Serotec	Mouse IgG1
CD243	ab10333	Abcam	Mouse IgG2a
CD244 (2B4)	550814	BD	Mouse IgG2a, k
CD247	ab11281	Abcam	Mouse IgG2a
CD249 Aminopeptidase A	550491	BD	Mouse IgG1, k
CD25	555430	BD	Mouse IgG1, k
CD26	555435	BD	Mouse IgG1, k
CD27	555439	BD	Mouse IgG1, k
CD28	M1650clb	RDI	Mouse IgG1
CD29 (integrin beta 1)	556048	BD	Mouse IgG2a, k
CD3	555330	BD	Mouse IgG1, k
CD30	555827	BD	Mouse IgG1, k
CD31	555444	BD	Mouse IgG1, k
CD33	555625	BD	Mouse IgG1, k
CD34	555820	BD	Mouse IgG1, k
CD35	555451	BD	Mouse IgG1, k
CD36	555453	BD	Mouse IgM, k
CD37	555456	BD	Mouse IgG1, k
CD38	555458	BD	Mouse IgG1, k
CD39	555643	BD	Mouse IgG2b,k
CD4	555344	BD	Mouse IgG1, k
CD40	555587	BD	Mouse IgG1, k
CD41A	555465	BD	Mouse IgG1, k
CD41b	555468	BD	Mouse IgG3, k
CD42a	558816	BD	Mouse IgG1, k
CD42b	555471	BD	Mouse IgG1, k
CD43	555474	BD	Mouse IgG1, k
CD44	550988	BD	Mouse IgG1, k
CD44var (v10)	MAB4079	Millipore	Mouse IgG1, k
CD45	555480	BD	Mouse IgG1, k
CD45RA	555486	BD	Mouse IgG2b,k
CD45RO	555491	BD	Mouse IgG2a, k
CD46	555948	BD	Mouse IgG2a, k
CD47	556044	BD	Mouse IgG1, k
CD48	555758	BD	Mouse IgM, k
CD49a	559594	BD	Mouse IgG1
CD49b	555497	BD	Mouse IgG1, k
CD49c	556024	BD	Mouse IgG1, k
CD49d	555502	BD	Mouse IgG1, k
CD49e	555615	BD	Mouse IgG1, k
CD49f	555734	BD	Rat IgG2a, k
CD5	555350	BD	Mouse IgG1, k
CD50	555957	BD	Mouse IgG2b,k
CD51-61	555504	BD	Mouse IgG1
CD53	555506	BD	Mouse IgG1, k

CD53	ab667	Abcam	Mouse IgG1
CD54	555510	BD	Mouse IgG1, k
CD55	555691	BD	Mouse IgG2a, k
CD56	559043	BD	Mouse IgG2b
CD57	555618	BD	Mouse IgM, k
CD58	555919	BD	Mouse IgG2a
CD58 (LFA3)	ab6127	Abcam	Mouse IgG2a
CD59	555761	BD	Mouse IgG2a, k
CD6	555356	BD	Mouse IgG1, k
CD61	555752	BD	Mouse IgG1, k
CD62b	555723	BD	Mouse IgM
CD62e	555648	BD	Mouse IgG1, k
CD62L	555542	BD	Mouse IgG1, k
CD62p	555522	BD	Mouse IgG1, k
CD63	556019	BD	Mouse IgG1, k
CD64	555525	BD	Mouse IgG1, k
CD65s	MHCD65s01	Caltag	Mouse IgM
CD66	551355	BD	Mouse IgG1, k
CD66b	555723	BD	Mouse IgM, k
CD66e	ab4451	Abcam	Mouse IgG1
CD66f	559587	BD	Mouse IgG1, k
CD67	MON1052	Caltag	Mouse IgG1
CD68	556059	BD	Mouse IgG2b,k
CD69	555529	BD	Mouse IgG1, k
CD7	555359	BD	Mouse IgG1, k
CD70	555833	BD	Mouse IgG3, k
CD71	555534	BD	Mouse IgG2a, k
CD72	555917	BD	Mouse IgG2b,k
CD73	550256	BD	Mouse IgG1, k
CD74	555538	BD	Mouse IgG2a, k
CD77	551352	BD	Mouse IgM, k
CD79a	ab3121	Abcam	Mouse IgG1, k
CD79b	ab3275	Abcam	Mouse IgG1, k
CD8	555631	BD	Mouse IgG1, k
CD80	557223	BD	Mouse IgG1, k
CD81	555675	BD	Mouse IgG1, k
CD82	CD82-BL2	RDI	Mouse IgG1
CD83	556854	BD	Mouse IgG1, k
CD84	559588	BD	Mouse IgG1, k
CD85	CD85-GHI	RDI	Mouse IgG2b
CD86	555663	BD	Mouse IgG2b,k
CD87	555767	BD	Mouse IgG1, k
CD88	550493	BD	Mouse IgG1, k
CD89	CD89-A59	RDI	Mouse IgG1, k
CD9	555370	BD	Mouse IgG1, k
CD90	555593	BD	Mouse IgG1, k
CD91	550495	BD	Mouse IgG1, k
CD92	555769	BD	Mouse IgG2b,k
CD94	555887	BD	Mouse IgG1, k
CD95	555671	BD	Mouse IgG1, k
CD97	555772	BD	Mouse IgG1, k

CD98	556074	BD	Mouse IgG1, k
CD99	555687	BD	Mouse IgG2a, k
CD99R	555817	BD	Mouse IgM, k
CDw125	555901	BD	Mouse IgG1, k
CDw128	555937	BD	Mouse IgG2b,k
CDw128b	555932	BD	Mouse IgG1
CDw131	554534	BD	Mouse IgG1, k
CDw149	CBL551	Millipore	Mouse IgG2a
CDw17	CBL463	RDI	Mouse IgM
CDw210	ab10777	Abcam	Mouse IgG1
CDw60	550812	BD	Mouse IgM, k
CDw75	ab9515	Abcam	Mouse IgM, k
CDw93	551531	BD	Mouse IgG2b,k
CNTF Receptor alpha	MAB303	R&D	Mouse IgG2b
Contactin-1	MAB904	R&D	Mouse IgG1
CRTAM	MAB1695	R&D	Mouse IgG
CTLA-4	MAB325	R&D	Mouse IgG2b
CXCR-1 (IL-8 RA)	MAB330	R&D	Mouse IgG2a
CXCR-2 (IL-8 RB)	MAB331	R&D	Mouse IgG2a
CXCR3	MAB160	R&D	Mouse IgG1
CXCR4 (fusin)	MAB171	R&D	Mouse IgG2a
CXCR-5 (BLT-1)	MAB190	R&D	Mouse IgG2b
CXCR6	MAB699	R&D	Mouse IgG2b
D6	MAB1364	R&D	Rat IgG2a
DAN	MAB955	R&D	Mouse IgG2b
DR3 (TNFRSF25)	307101	Biologend	Mouse IgG1, k
DR3 (TNFRSF25)	MAB943	R&D	Mouse IgG1
DR6 (TNFRF21)	MAB1441	R&D	Mouse IgG1
Dtk	MAB859	R&D	Mouse IgG1
E-Cadherin	13-1700	Invitrogen	Mouse IgG1
EGF-Receptor	555996	BD	Mouse IgG2b,k
EGF-Receptor	MAB1095	R&D	Mouse IgG2b
Endothelin-1 Receptor	611253	BD	Mouse IgG1
EpCAM	347197	BD	Mouse IgG1
EpCAM	14-9326-80	eBioscience	Mouse IgG1, k
EpCAM	MAB960	R&D	Mouse IgG2a
Eph B4	352900	Invitrogen	Mouse IgG1, k
Ephrin-A3	MAB359	R&D	Mouse IgG2a
Ephrin-A4	MAB369	R&D	Mouse IgG2b
Ephrin-B3	MAB395	R&D	Mouse IgG2b
Epithelial specific antigen (ESA)	CBL251	Millipore	Mouse IgG1
Epithelial specific antigen (ESA)	V1048	Biomeda	Mouse IgG1, k
ErbB2	MAB1129	R&D	Mouse IgG2b
ErbB4	MAB1131	R&D	Mouse IgG2b
FAS Ligand	556372	BD	Mouse IgG1
FGF Receptor 1 (IIIb)	MAB765	R&D	Mouse IgG1
FGF Receptor 2	MAB6843	R&D	Mouse IgG1
FGF Receptor 3	MAB766	R&D	Mouse IgG1
FGF Receptor 4	324301	Biologend	Mouse IgG1, k
FGF Receptor 4	MAB6852	R&D	Rat IgG2a
FMC7	MCA792F	Serotec	Mouse IgM

fMLP Receptor	556015	BD	Mouse IgG1, k
FMS (CD115)	556385	BD	Rat IgG2a
Frizzled-1	MAB11201	R&D	Rat IgG2a
Frizzled-3	MAB1001	R&D	Rat IgG2a
Frizzled-4	326601	Biologend	Mouse IgG1, k
Frizzled-4	MAB194	R&D	Rat IgG2a
Frizzled-7	MAB1981	R&D	Rat IgG2a
Frizzled-9	326701	Biologend	Mouse IgM, k
GABAa Receptor Alpha chain	MAB339	Millipore	Mouse IgG1
GABAa Receptor Beta chain	MAB341	Millipore	Mouse IgG1
Galactocerebroside	MAB342	Millipore	Mouse IgG3
Galectin-3	556904	BD	Mouse IgG1, k
GAP-43 (B-50)	MAB347	Millipore	Mouse IgG1
GFRa-3 (GDNF receptor alpha 3)	MAB6701	R&D	Mouse IgG1
GHR	MAB1210	R&D	Mouse IgG2b
Gi alpha-2	MAB3077	Millipore	Mouse IgG2b
GITR	MAB689	R&D	Mouse IgG1
GITR L	MAB6942	R&D	Mouse IgG1
Glucose Transporter 1 (Glut1)	MAB1418	R&D	Mouse IgG2b
Glucose Transporter 2 (Glut2)	MAB1414	R&D	Mouse IgG2a
Glucose Transporter 3 (Glut3)	MAB1415	R&D	Mouse IgG2b
Glucose Transporter 5 (Glut5)	MAB1349	R&D	Mouse IgG2a
Go alpha	MAB3073	Millipore	Mouse IgG1
GP130	MAB628	R&D	Mouse IgG1
GP230	559447	BD	Mouse IgM, k
Granzyme A	557449	BD	Mouse IgG1
Heparin Sulfate	MAB2040	Millipore	Mouse IgG1
HGF Receptor	MAB358	R&D	Mouse IgG2a
High affinity choline transporter	MAB5420	Millipore	Mouse IgG
HLA-A, B, C	311401	Biologend	Mouse IgG2a, k
HVEM (TNFRSF14)	MAB3561	R&D	Mouse IgG1
IL3R/IL5R/GMCSFR	554534	BD	Mouse IgG1
IL-4 Ra	551894	BD	Mouse IgG1, k
IL-6 R	551462	BD	Mouse IgG1, k
integrin beta 7	555943	BD	Rat IgG2a, k
Jagged-1	MAB1277	R&D	Mouse IgG2b
JAM-3	MAB1189	R&D	Mouse IgG1
LAIR-1	550810	BD	Mouse IgG1, k
Leptin Receptor	MAB867	R&D	Mouse IgG2b
LIF Receptor	559778	BD	Mouse IgG1, k
LMP-1	559898	BD	Mouse IgG2a, k
Macrophages L.S.I.	MAB1569	Millipore	Mouse IgG1
MCAM (CD146)	MAB932	R&D	Mouse IgG1
M-CSF Receptor	MAB329	R&D	Mouse IgG1
MDR (P-glycoprotein)	557001	BD	Mouse IgG2b,k
MDR1	MAB4161	Millipore	Mouse IgG2a, k
MDR1	MAB4163	Millipore	Mouse IgG2a, k
MDR1	MAB4334	Millipore	Mouse IgG2a, k
MER	MAB891	R&D	Mouse IgG2a
MSP Receptor	MAB691	R&D	Mouse IgG1
MUC2	555926	BD	Mouse IgG1, k

Mu-Calpain	550935	BD	Mouse IgG1, k
Myelin/Oligodendrocyte S.P.	MAB328	Millipore	Mouse IgM
N-Cadherin	ab19348	Abcam	Mouse IgG
NCAM-L1	MAB777	R&D	Mouse IgG1
Neuritin	MAB283	R&D	Mouse IgG2b
NGF Receptor	557194	BD	Mouse IgG1, k
NMDAR2B	MAB5220	Millipore	Mouse IgG1
NRG-1-b1/HRG-b1	MAB3771	R&D	Mouse IgG1
Oligodendrocyte marker O1	MAB1327	R&D	Mouse IgM
Oligodendrocyte marker O4	MAB1326	R&D	Mouse IgM
OV-6	MAB2020	R&D	Mouse IgG
p-Cadherin	610227	BD	Mouse IgG1
PD-L2	MAB1224	R&D	Mouse IgG2b,k
Prominin 2	MAB2024	R&D	Mouse IgG
RAGE	MAB5328	Millipore	Mouse IgG2a, k
Reck	611512	BD	Mouse IgG1
Reck	MAB1734	R&D	Mouse IgG1
Ror2	MAB2064	R&D	Mouse IgG1
Semaphorin 6A	MAB1146	R&D	Mouse IgG2b
Semaphorin 7A	MAB2068	R&D	Rat IgG2b
Siglec 6	550908	BD	Mouse IgG1, k
SSEA-1	MAB4301	Millipore	Mouse IgM
SSEA-3	MAB4303	Millipore	Rat IgM
SSEA-4	MAB4304	Millipore	Mouse IgG3
TAP2	551292	BD	Mouse IgG1
TGF beta Receptor 2	MAB241	R&D	Mouse IgG
TGF beta Receptor 3	MAB242	R&D	Mouse IgG1
Thrombopoietin Receptor	MAB10161	R&D	Mouse IgG2b
Thymus stroma	555825	BD	Mouse IgM, k
Tie-1	MAB619	R&D	Mouse IgG1
Tie-2	MAB313	R&D	Mouse IgG1
TIM 1	MAB1750	R&D	Mouse IgG
TNF Receptor 1 (TNFRSF1A)	MAB625	R&D	Mouse IgG1
TNF Receptor 2 (TNFRSF1B)	MAB726	R&D	Mouse IgG1
TNF Receptor Type II	551311	BD	Rat IgG2b, k
Toll Like Receptor 1 (TLR1)	MCA2153	Serotec	Mouse IgG1
Toll Like Receptor 2 (TLR2)	MCA2152	Serotec	Mouse IgG2a
Toll Like Receptor 3 (TLR3)	MCA2267	Serotec	Mouse IgG1
Toll Like Receptor 4 (TLR4)	551964	BD	Mouse IgG2a, k
Toll Like Receptor 5 (TLR5)	558632	BD	Mouse IgG1
Toll Like Recptor 9 (TLR9)	MCA2265	Serotec	Mouse IgG2a
TRA-1-60	MAB4360	Millipore	Mouse IgM
TRA-1-81	MAB4381	Millipore	Mouse IgM
TRAIL R2 (DR5/TNFRSF10B)	MAB631	R&D	Mouse IgG1
TRAIL R3 (DcR1/TNFRSF10C)	MAB6301	R&D	Mouse IgG1
TRAIL R4 (DcR2/TNFRSF10D)	MAB633	R&D	Mouse IgG1
TrkA Neurotrophin receptor	MAB175	R&D	Mouse IgG2a
TrkA Neurotrophin receptor	MAB1751	R&D	Mouse IgG1
Trkc	MAB3731	R&D	Mouse IgG
TROP-2	MAB650	R&D	Mouse IgG2a
TSLP Receptor	MAB981	R&D	Mouse IgG1

uPAR (CD87)	MAB807	R&D	Mouse IgG1
VCAM-1 (CD106)	BBA5	R&D	Mouse IgG1
VE cadherin	MAB938	R&D	Mouse IgG1
VEGF R1 (Flt-1)	MAB321	R&D	Mouse IgG1
VEGF R2 (KDR)	MAB3571	R&D	Mouse IgG1
VEGF R3 (Flt-4)	MAB743	R&D	Mouse IgG1

***Manufacturer list**

Abcam
 BD (BD Bioscience)
 Beckman (Beckman Coulter)
 Biologend
 Biomeda
 Caltag (Caltag Laboratories)
 Millipore
 Miltenyi Biotec
 Progen (PROGEN Biotechnik GmbH)
 R&D (R&D Systems)
 RDI (Division of Fitzgerald Industries Intl)
 Serotec (AbD Serotec)
 Invitrogen

Supplementary Table 2

Cell surface marker profile changes in response to differentiation treatments

3 days of treatment with RA and BMP4 led to changes in either most of the cells or changes that were restricted to small subsets (1-10%) of them. Markers are listed by functional category and type of change.

Functional category:	Population percentile change		Uniform change
	>10 of cells	<10 of cells	>x5 MFI
<u>Adhesion molecules</u>	CD166 CD56 (B) CD44* CD50*		CD155* CD165* LFA-3*
<u>TGF-β superfamily</u>		TGFβR2	
<u>Receptor tyrosine kinase</u>	FGF-R3*	EGF-R (B) CD117 (B)	CD220
<u>Prominins</u>	CD133*		
<u>Tetraspanins</u>			CD9*
<u>TNF family</u>	Trail R3*	NGF-R	CD30* TNF-R1* Trail R2*
<u>Cytokine receptors</u>		LIFR (B) gp130 (B)	
<u>Chemokine receptors</u>		CCR9	
<u>Transporters</u>		ABCG2 (B)	
<u>Carbohydrates</u>	CD173 SSEA1 TRA-1-60* TRA-1-81* SSEA3* SSEA4*		
<u>Wnt receptors</u>		Ror2 (B)	Frizzled 9*
<u>Semaphorin</u>	CD100*		
<u>Cadherins</u>	N* 6		
<u>Peptidases</u>	CD249	CD13 (B) CD26 (B)	BACE*
<u>Others</u>		CD60b CD87 CD107a CD107b CD138* OV6#	CD57* CD90* CD200* CD227

All markers in table indicate upregulation in differentiation except those marked by *.

* Denotes downregulation of the marker following differentiation treatment. # Indicates the existence of positive population in all differentiation conditions.

Supplementary Table 3

Cell surface markers of undifferentiated hESCs

Median fluorescence intensity (MFI) of immunostained CM-treated cells was used to categorize markers according to expression level. High, intermediate, and low expressed markers have MFI of >100, 10 to 100, or <10, respectively. Expression of previously reported hESC markers, including SSEA-4, SSEA-3, Tra-1-60 and Tra-1-81) was confirmed. Marker lists are divided to functional categories.

Functional category	Expression level		
	high>100	med 100>x>15	15>low
<u>Integrins</u>	α 2 α 3 α 5 α 6	α 1	α 4 β 1 β 4
<u>Adhesion molecules</u>	CD24 CD44* CD50* CD146 CD155 CD165 CD166 CD239 EpCAM	CD54 CD56 CD164 CD171 Jam-3 LFA-3 NCAM-L1	Contactin 1
<u>TGF-β superfamily</u>		Alk1	CD109 TGF- β R3
<u>Receptor tyrosine kinase</u>		Axl CD220 CD221 CD222 DTK FGF-R4 VEGF-R1	CD117 EGF-R ERBb2 FGF-R3 MSP-R
<u>Prominins</u>	CD133 Prominin 2		
<u>Tetraspanins</u>	CD9 CD81 CD151		CD82
<u>TNF family</u>	CD30	CD40 NGF-R TNF-R1 Trail R2 Trail R3	BAFF CD95 TNF-R2
<u>Cytokine receptors</u>		CD119 Tpo-R	LIF-R gp130
<u>Chemokine receptors</u>		CCR4	CXCR4#
<u>Transporters</u>	CD98	CD71	CD92
<u>Complement</u>	CD46 CD55 CD59		CD21
<u>Carbohydrates</u>	TRA-1-60* SSEA4*	TRA-1-81*	CD173# SSEA1#

<u>Wnt receptors</u>	SSEA3		
	Frizzled 3	Frizzled 4	Frizzled 1
	Frizzled 9		Frizzled 7
<u>Semaphorin</u>	CD100*	6A	CD108
<u>Cadherins</u>	E	N*	
		VE	
<u>Others</u>	CD57	β 2-microglobulin	BACE
	CD90	CD47	CD73#
	CD91	CD142	CD99
	CD138*	CD172a/b	CD141
	CD147	Gi-a2	PD-L2
	CD200		
	CD227#		
	HLA-A, B, C		

Positive subpopulation in CM

* Negative subpopulation in CM

Supplementary Table 4
TaqMan assays provided by Applied Biosystems

ACTB-Hs99999903_m1	HAND1-Hs00231848_m1	SNAI2-Hs00161904_m1
ACVR1B-Hs00923299_m1	HESX1-Hs00172696_m1	SOX17-Hs00751752_s1
ACVR1-Hs00153836_m1	HHEX-Hs00242160_m1	SOX2-Hs01053049_s1
AFP-Hs00173490_m1	HNF4G-Hs01071345_m1	SOX7-Hs00846731_s1
ALPL-Hs00758162_m1	HOXA11-Hs00194149_m1	STAT3-Hs00374280_m1
AMOT-Hs00611096_m1	HOXA13-Hs00426284_m1	TBX3-Hs00195612_m1
BMP2-Hs00154192_m1	HOXA1-Hs00171793_m1	TBX4-Hs00218515_m1
BMP4-Hs00370078_m1	HOXA2-Hs00534579_m1	TDGF1-Hs02339499_g1
BMPR1A-Hs01034909_g1	IFITM1-Hs01652522_g1	T-Hs00610080_m1
BMPR2-Hs00176148_m1	IFITM2-Hs00829485_sH	TWIST1-Hs00361186_m1
CDX2-Hs00230919_m1	IFITM3-Hs01635484_gH	VEGFC-Hs00153458_m1
CER1-Hs00193796_m1	IHH-Hs01081801_m1	WNT3-Hs00229135_m1
CGA-Hs00174938_m1	IRX3-Hs00735523_m1	WWP2-Hs00941266_g1
CHRD-Hs00415315_m1	ISL1-Hs00158126_m1	ZFP42-Hs01938187_s1
CSHL1-Hs00792427_g1	KIT-Hs00174029_m1	
CXCR4-Hs00237052_m1	LEFTY1-Hs00764128_s1	
DAB2-Hs01120081_m1	LEFTY2-Hs00745761_s1	
DAZL-Hs00154706_m1	LHX1-Hs00232144_m1	
DCAMKL2-Hs00382191_m1	LIN28-Hs00702808_s1	
DDX4-Hs00251859_m1	LMO2-Hs00277106_m1	
DKK1-Hs00183740_m1	MEIS2-Hs00542638_m1	
DNMT3B-Hs00171876_m1	MESP1-Hs00251489_m1	
DPPA3-Hs01931905_g1	MEST-Hs00853380_g1	
EED-Hs00243609_m1	MIXL1-Hs00430824_g1	
ELF5-Hs01063022_m1	NANOG-Hs02387400_g1	
EN2-Hs00171321_m1	NANOS1-Hs00996068_s1	
EOMES-Hs00172872_m1	NCAM2-Hs00189850_m1	
ESX1-Hs00538057_m1	NES-Hs00707120_s1	
FGF2-Hs00266645_m1	NODAL-Hs00250630_s1	
FGF4-Hs00173564_m1	NOG-Hs00271352_s1	
FGF5-Hs00170454_m1	NOTCH1-Hs00413187_m1	
FGF8-Hs00171832_m1	NR2F2-Hs00819630_m1	
FGFR2-Hs00240796_m1	OTX2-Hs00222238_m1	
FOXA1-Hs00270129_m1	PAX6-Hs00240871_m1	
FOXA2-Hs00232764_m1	PCP4-Hs01113638_m1	
FOXA3-Hs00270130_m1	PITX2-Hs00165626_m1	
FOXH1-Hs00182690_m1	POU5F1-Hs00742896_s1	
FZD8-Hs00259040_s1	POU5F1-Hs01895061_u1	
GAPDH-Hs99999905_m1	PRDM1-Hs00153357_m1	
GATA2-Hs00231119_m1	PRRX1-Hs00246567_m1	
GATA3-Hs00231122_m1	RPLP0-Hs99999902_m1	
GATA4-Hs00171403_m1	SFRP1-Hs00610060_m1	
GATA6-Hs00232018_m1	SFRP5-Hs00169366_m1	
GBX2-Hs00230965_m1	SIX3-Hs00193667_m1	
GDF3-Hs00220998_m1	SMAD1-Hs00195432_m1	
GSC-Hs00418279_m1	SMAD2-Hs00183425_m1	

* Some assays were tested individually, therefore list exceeds 96 TLDA genes

Supplementary Table 5**TaqMan assays used for single cell qRT-PCR****Analysis of sorted human CXCR4+/- cells**

*GAPDH-Hs99999905_m1
*POU5F1-Hs00742896_s1
MESP1-Hs00251489_m1
T-Hs00610075_g1
SOX17-Hs00751752_s1
FOXA2-Hs00232764_m1
PRDM1-Hs00153357_m1
EOMES-Hs00172872_m1
MIXL1-Hs00430824_g1

Analysis of sorted epiblast CXCR4+/- cells

GAPDH-Mm99999915_g1
POU5F1-Mm03053917_g1
SOX17-Mm00488363_m1
FOXA2-Mm01976556_s1
NANOG-Mm02019550_s1
GATA4-Mm00484689_m1
GATA6- Mm00802636_m1
VILLIN-Mm00494156_m1

* TaqMan assays used for "digital qRT-PCR"

Analysis of sorted human Ror2+/- cells

GAPDH-Hs99999905_m1
POU5F1-Hs00742896_s1
*MESP1-Hs00251489_m1
*T-Hs00610075_g1
SOX17-Hs00751752_s1
ROR2-Hs00171695_m1
WNT3-Hs00229135_m1
GATA4-Hs00171403_m1
MESP2-HS02742533