

Stem Cell Reports, Volume 2

Supplemental Information

Isolation of Human Induced Pluripotent Stem

Cell-Derived Dopaminergic Progenitors

by Cell Sorting for Successful Transplantation

Daisuke Doi, Bumpei Samata, Mitsuko Katsukawa, Tetsuhiro Kikuchi, Asuka Morizane, Yuichi Ono, Kiyotoshi Sekiguchi, Masato Nakagawa, Malin Parmar, and Jun Takahashi

Inventory of supplemental information

Supplemental experimental procedures

Figure S1, The dopaminergic differentiation of human iPSCs, related to Figures 1-2;

Figure S2, The purification and characterization of CORIN⁺ cells, related to Figure 2;

Figure S3, The floating spheres derived from the unsorted or the d12-CORIN⁺ cells, related to Figure 3;

Figure S4, The survival and function of DA neurons derived from the unsorted or the d12-CORIN⁺ cells *in vivo*, related to Figure 5;

Figure S5, The microarray analysis comparing human fetal VM tissue and d12-CORIN⁺ cells, related to Figure 6;

Table S1, Gene list of significantly up- and down-regulated genes of d12-CORIN⁺ and unsorted cells, related to Figure S2;

Table S2, Gene list of significantly up- and down-regulated genes of d28-CORIN⁺ and unsorted cells, related to Figure 6;

Table S3, Gene list of significantly up- and down-regulated genes of d28-CORIN⁺ cells

compared with d42-CORIN⁺ cells, related to Figure 6;
Table S4, Gene list of highly expressed genes either in d28 and d42-CORIN⁺ cells, and
human fetal VM tissue, related to Figures 6;
Table S5, List of primers for Q-PCR, related to Figures 1-2, 6, and S1-2;
Table S6, List of primary antibodies, related to Figures 1-5, S2, and S4

Figure S1

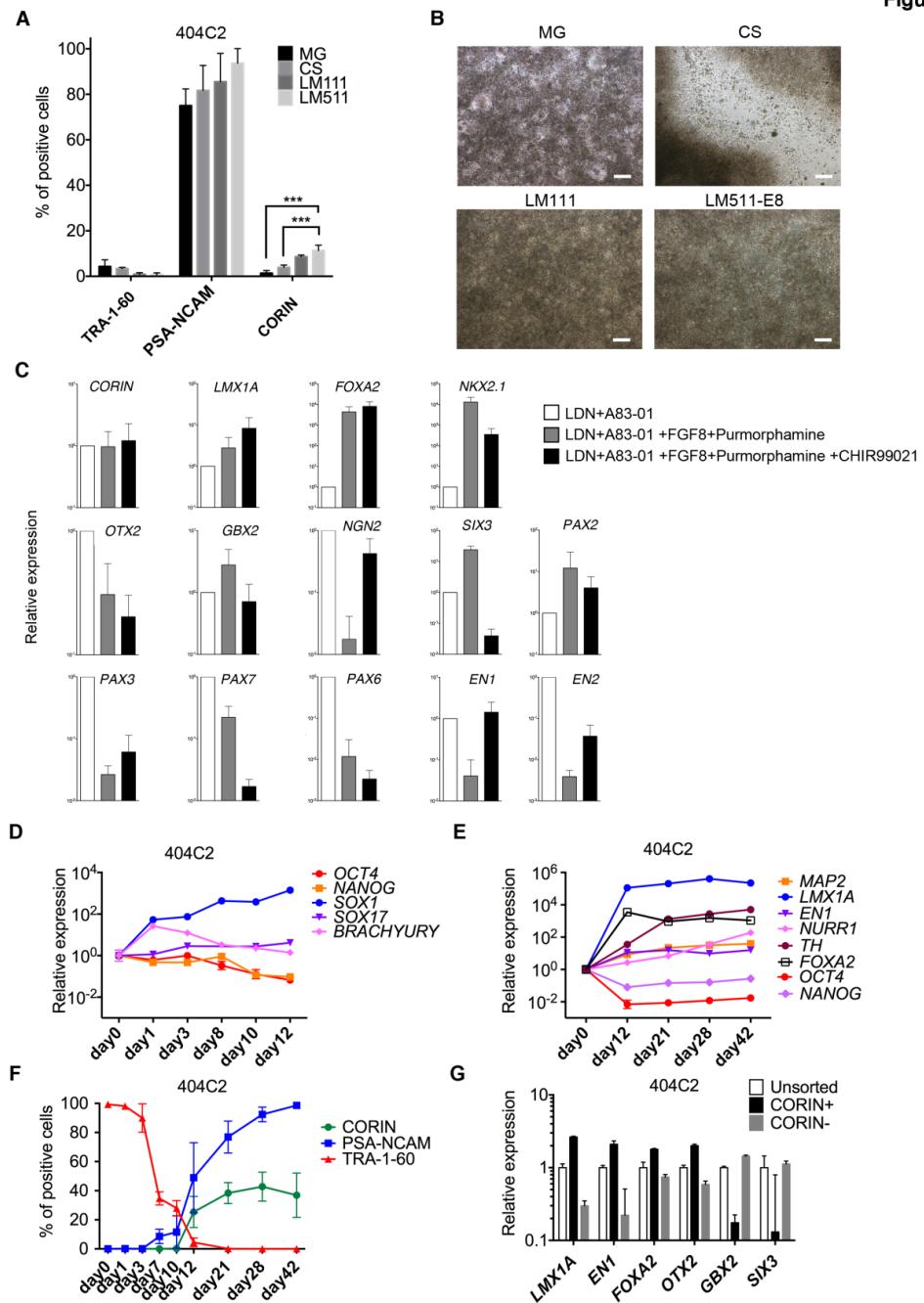


Figure S2

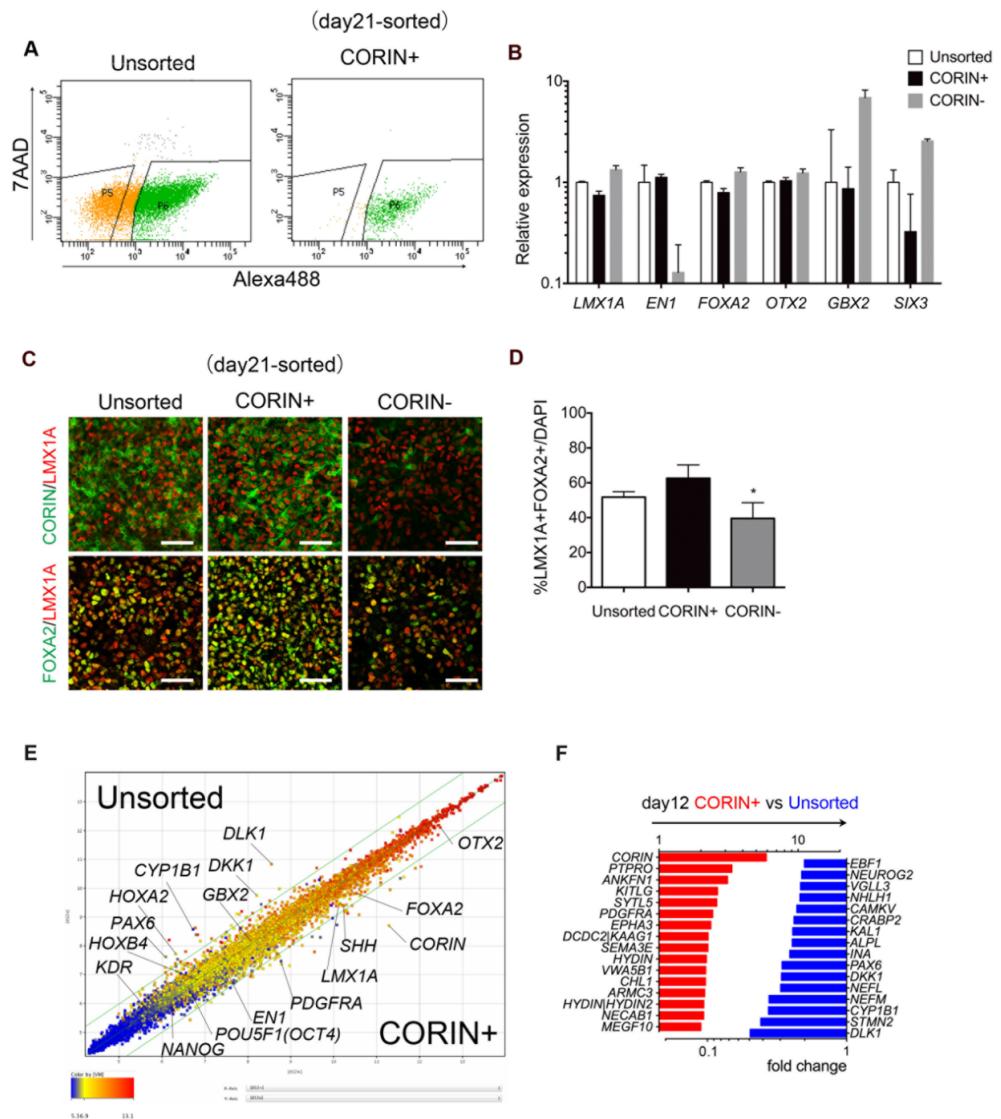


Figure S3

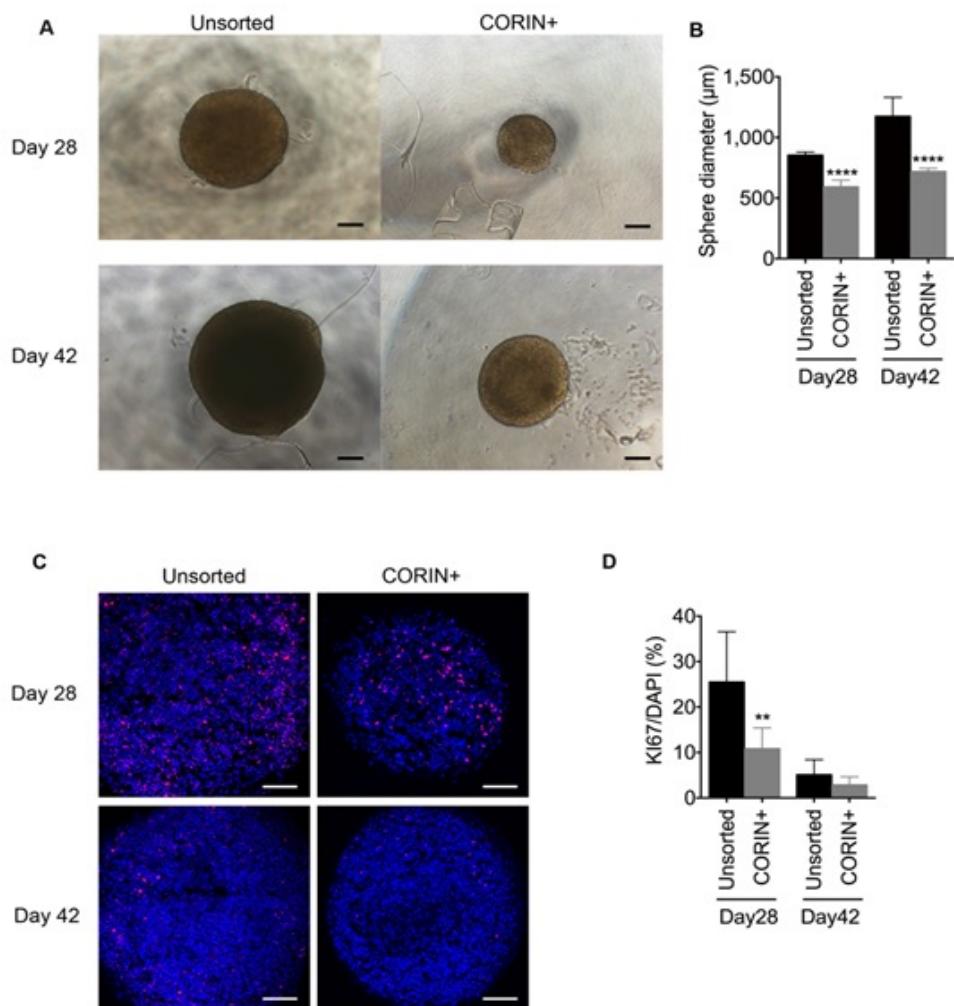


Figure S4

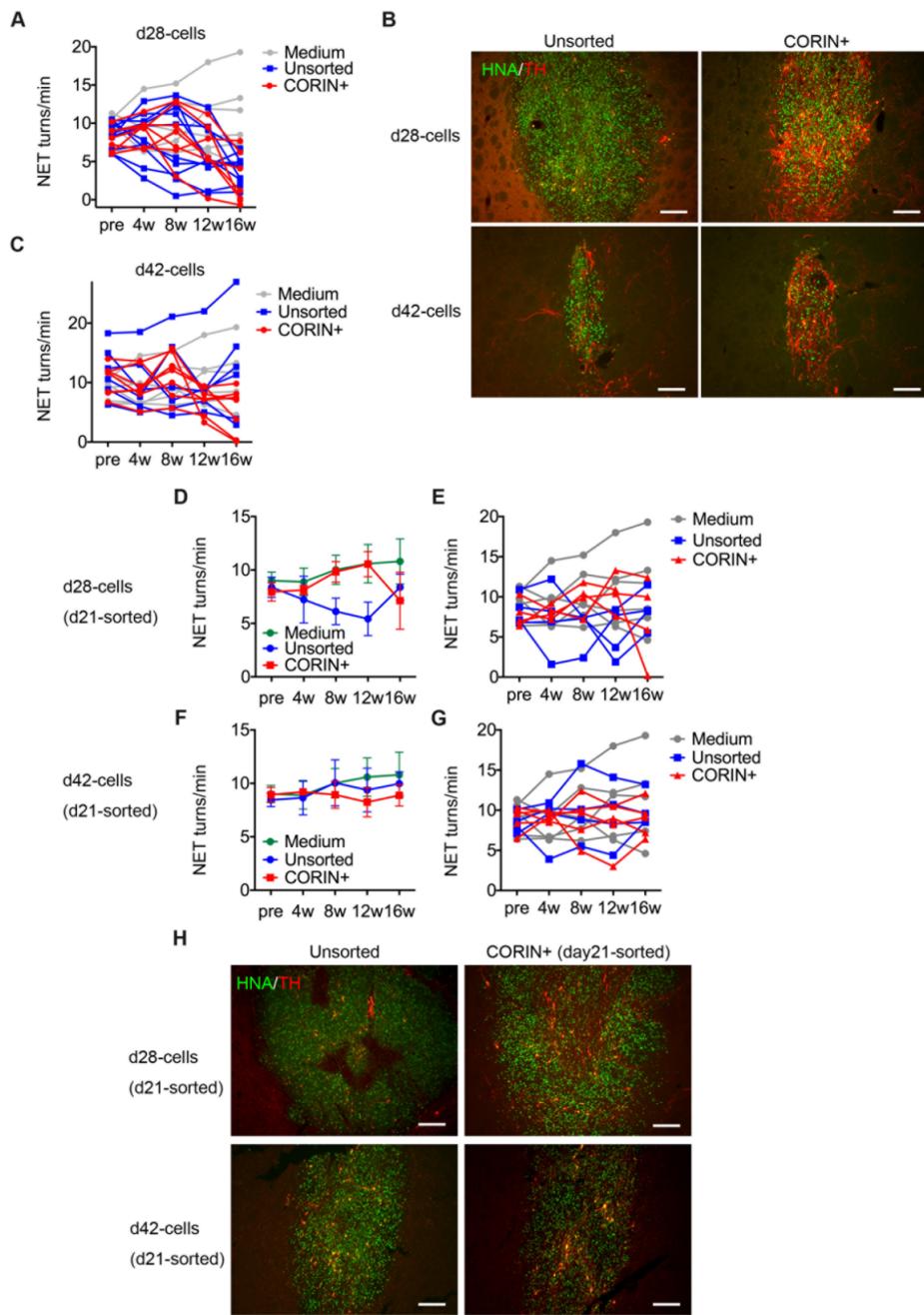
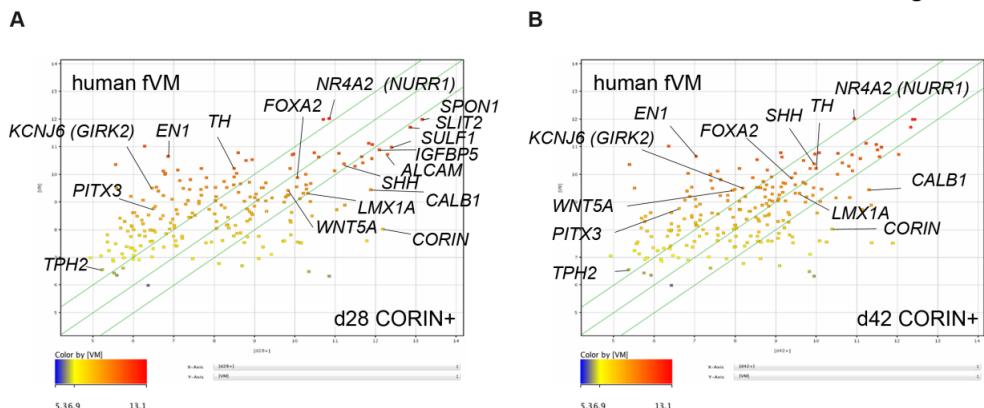


Figure S5



Supplementary Figure Legends

Figure S1

(A-C)The differentiation of human iPSCs induced by different culture conditions. **(A)** The results of the flow cytometric analysis of the differentiating cells (404C2) on Matrigel (MG), CellStart (CS), Laminin 111-E8 fragment (LM111) and Laminin 511-E8 fragment (LM511) on day 12. The values are the means \pm s.d. ***p=0.0010, ***p=0.0001 by a one-way ANOVA with Tukey's multiple comparison test (n=3 independent experiments).

(B) Phase contrast images of the differentiating cells (404C2) on different matrices on day 12. Bar = 200 μ m. **(C)** The results of the quantitative RT-PCR of the differentiating cells (836B3) on day 12. FGF8 and Purmorphamine were added from day 1 to day 7. CHIR99021 was added from day 3 to day 12. The values are the means \pm s.d. (n=3 independent experiments) **(D-G)** The differentiation of another human iPSC line (404C2) induced by attachment culture on LM511-E8. **(D, E)** The results of the quantitative RT-PCR of another human iPSC line (404C2). The expression level of undifferentiated cells (day 0) was set to 1. The values are the means \pm s.d. (n=3 independent experiments) **(F)** The results of a temporal expression analysis by flow cytometry. The values are the means \pm s.d. (n=3 independent experiments) **(G)** The results of the gene expression analysis by quantitative RT-PCR on day 12. The expression level of the unsorted cells was set to 1. The values are the means \pm s.d. (n=3 independent experiments).

Figure S2

(A-D) The purification and characterization of iPSC-derived CORIN⁺ cells on day 21. (A) Dot plots of the FACS analysis of the unsorted and the reanalyzed sorted cells. (B) The results of the quantitative RT-PCR. The expression level of the unsorted cells was set to 1. The values are the means ± s.d. (n=3 independent experiments). (C) Immunostaining for CORIN/LMX1A and FOXA2/LMX1A. Bar = 50 μm. (D) The percentages of LMX1A⁺/FOXA2⁺ cells per total cells. The values are the means ± s.d. *p = 0.0332 by a one way-ANOVA with Dunnett's multiple comparison test (n=15 totally, 5 spheres in each three independent experiment). (E-F) The results of the microarray analysis comparing the unsorted vs. CORIN+ cells on day 12. (E) Scatter plot. (F) Selected lists of differentially expressed transcripts comparing day12-CORIN+ cells versus day12-Unsorted cells.

Figure S3

(A-B) The expansion of the floating spheres derived from the unsorted or the d12-CORIN⁺ cells. (A) Phase contrast images of the spheres. Bar = 200 μm. (B) The diameter of the spheres on day 28 or day 42. The values are the means ± s.d. ****P<0.0001 by the unpaired t-test (n=30 totally, 10 spheres in each three independent experiment). (C) Double-labeled immunostaining of the spheres on day 28 and day42, for KI67 (red) and DAPI (blue). Bar=100μm. (D) The percentages of KI67 positive cells per DAPI. **p=0.0012 by the unpaired t-test (n=5, three independent experiments).

Figure S4

(**A-C**) The survival and function of DA neurons derived from the unsorted or the d12-sorted cells, which were grafted on day 28 or day 42. (**A**) The metamphetamine-induced rotation of the individual rats with the grafts of d12-sorted, d28-grafted cells. (**B**) Immunofluorescence images of the grafts derived from d12-sorted or unsorted cells stained for HNA (green) and TH (red). Bar = 200 μ m. (**C**) The metamphetamine-induced rotation of the individual rats with the grafts of d12-sorted, d42-grafted cells. (**D-H**) The survival and function of the DA neurons derived from the unsorted or the d21-sorted cells, which were grafted on day 28 (n=4, **D-E**, and **H**) or day 42 (n=4 or 5, **F-H**). The metamphetamine-induced rotation of each group (**D, F**) or the individual rats with the grafts (**E, G**). The values are the means \pm s.d. There were no significant differences. (**H**) Immunofluorescence images of the grafts derived from d21-sorted or unsorted cells stained for HNA (green) and TH (red). Bar = 200 μ m.

Figure S5

(**A-B**) The results of the microarray analysis comparing human fetal VM tissue and d12-CORIN⁺ cells cultured until day 28 (**A**) or day 42 (**B**). Only the genes whose expression levels were higher (> 2-fold) in the fetal VM compared to the fetal dorsal mesencephalon are plotted.

Table S1: Gene expression array data of significantly up-regulated and down-regulated genes of day 12-sorted and unsorted cells.

Transcripts Cluster Id	Gene symbol	Fold Change	P value
8100154	<i>CORIN</i>	5.9087105	0.001171437
7954143	<i>PTPRO</i>	3.3445656	0.005720456
8008609	<i>ANKFNI</i>	3.113136	0.023851596
7965322	<i>KITLG</i>	2.6410403	5.14E-04
8166747	<i>SYTL5</i>	2.6041105	0.020840537
8095080	<i>PDGFRA</i>	2.4332025	0.009164337
8081081	<i>EPHA3</i>	2.3614557	6.84E-04
8124196	<i>DCDC2/KAAG1</i>	2.2547202	0.042166963
8140650	<i>SEMA3E</i>	2.2485685	0.00204914
8002446	<i>HYDIN</i>	2.1930437	0.029688193
7898627	<i>VWA5B1</i>	2.1673756	0.039200813
8077270	<i>CHL1</i>	2.1607752	0.019023776
7926638	<i>ARMC3</i>	2.1327724	0.032846447
8002470	<i>HYDIN/HYDIN2</i>	2.1010761	0.019100418
8147244	<i>NECAB1</i>	2.0970535	0.002917092
8107722	<i>MEGF10</i>	2.003543	0.010272147
8115543	<i>EBF1</i>	-2.0123506	0.014606911
8102368	<i>NEUROG2</i>	-2.1043627	0.018515356
8088979	<i>VGLL3</i>	-2.143269	0.015245302
7906597	<i>NHLH1</i>	-2.1650214	0.018702803
8087530	<i>CAMKV</i>	-2.2798698	0.005241052
7921099	<i>CRABP2</i>	-2.387901	0.007732119
8171248	<i>KAL1</i>	-2.4381118	0.026552679
7898693	<i>ALPL</i>	-2.4576702	0.010827614
7930208	<i>INA</i>	-2.5598962	0.012995572
7947338	<i>PAX6</i>	-2.9077826	9.78E-04
7927631	<i>DKK1</i>	-2.938103	0.039719306

8149835	<i>NEFL</i>	-2.9777105	0.014127387
8145361	<i>NEFM</i>	-3.6068168	0.018645655
8051583	<i>CYP1B1</i>	-3.639586	0.006571199
8147030	<i>STMN2</i>	-4.1421623	0.001145821
7976783	<i>DLK1</i>	-4.9302297	6.35E-04

Table S2: Gene expression array data of significantly up- and down-regulated genes of day 28 unsorted and sorted cells.

Transcripts Cluster

Id	Gene symbol	Fold Change	P value
7922174	<i>F5</i>	10.484556	0.00196464
8083770	<i>OTOL1</i>	9.6624365	1.42E-04
7965410	<i>DCN</i>	7.884093	0.011896186
8151341	<i>TRPA1</i>	7.6784086	0.001152584
7938608	<i>SPON1</i>	7.6015487	0.016960293
8133876	<i>CD36</i>	7.270626	0.007745484
8083673	<i>IQCJ SCHIP1</i>	5.648684	0.006275537
8100154	<i>CORIN</i>	5.5950904	0.003215429
7968650	<i>C13orf36</i>	5.24648	1.71E-04
8106660	<i>RASGRF2</i>	4.8150682	0.004273195
7965403	<i>LUM</i>	4.2473927	0.005038001
8155849	<i>ANXA1</i>	4.245879	0.019833442
8131506	<i>NXPH1</i>	4.219527	0.00597194
7951565	<i>ARHGAP20</i>	4.1601496	0.002754803
8059413	<i>DOCK10</i>	4.1518664	3.33E-04
7939052	<i>FIBIN</i>	4.1420884	0.013714803
7903227	<i>PALMD</i>	4.1280413	3.67E-04
7974902	<i>RHOJ</i>	4.0372086	0.001624118
8135341	<i>FLJ23834</i>	3.9833305	0.00218651
8078397	<i>CMTM8</i>	3.9323933	0.010555872
7961540	<i>RERG</i>	3.9065168	0.035438724
8106088	<i>CARTPT</i>	3.8127122	0.033124655
8177620	<i>CARTPT</i>	3.8085206	0.03323086
8049394	<i>TRPM8</i>	3.7984648	6.74E-05
8103260	<i>DCHS2</i>	3.6421225	1.67E-04

8074606	<i>USP18 USP41</i>	3.5775964	0.002447678
8058450	<i>GPRI</i>	3.5421739	0.004501217
8016841	<i>TMEM100</i>	3.5418146	7.80E-04
8140984	<i>HEPACAM2</i>	3.4895766	0.04710368
8112971	<i>HAPLN1</i>	3.4394467	0.017581666
8076894	<i>MLC1</i>	3.4351523	0.017575841
7968678	<i>FREM2</i>	3.4215899	0.002215871
8146863	<i>SULF1</i>	3.4094722	0.013414457
7982117	<i>GABRG3</i>	3.3868933	1.08E-04
8106210	<i>RGNEF</i>	3.304427	0.005593245
8144078	<i>SHH</i>	3.3041658	0.002837645
8123744	<i>F13A1</i>	3.1656961	0.004540503
8057463	<i>CERKL</i>	3.150669	0.00264684
8082012	<i>SLC15A2</i>	3.0829906	6.18E-04
8172204	<i>MAOB</i>	3.0079098	0.018402645
8103415	<i>C4orf18</i>	2.9358177	0.007976412
8080918	<i>FAM19A1</i>	2.9149494	0.007067252
7939341	<i>CD44</i>	2.9061434	0.003175953
8156373	<i>FGD3</i>	2.9034407	9.70E-04
8081431	<i>ALCAM</i>	2.9028459	0.007804133
8129924	<i>TXLNB</i>	2.8827786	3.28E-04
8077323	<i>CNTN4</i>	2.8760664	0.02836744
7934570	<i>KCNMA1</i>	2.8699055	2.88E-04
8094743	<i>RHOH</i>	2.8629174	0.004758834
7902425	<i>ST6GALNAC3</i>	2.8499446	0.019802256
8152606	<i>SNTB1</i>	2.7894716	0.004711483
8071809	<i>GSTT2 GSTT2B</i>	2.7526436	0.002886667
8074962	<i>GSTT2 GSTT2B</i>	2.7220402	0.003455543
8115234	<i>ANXA6</i>	2.6939156	5.01E-04
7975482	<i>RGS6</i>	2.6821022	0.02144372

8091799	<i>C3orf57</i>	2.6769505	0.002148048
8070567	<i>TFF3</i>	2.6574645	0.004154954
7931977	<i>ITIH5</i>	2.654886	0.004861879
7971077	<i>POSTN</i>	2.640356	0.047866426
8070073	<i>C21orf62</i>	2.619478	0.011550013
7962327	<i>SLC2A13</i>	2.6159894	0.009402491
8004880	<i>NTNI</i>	2.6156747	0.003578502
7898627	<i>VWA5B1</i>	2.6053572	5.06E-04
8096160	<i>ARHGAP24</i>	2.5978916	0.021238877
8104930	<i>SLC1A3</i>	2.5914204	0.014512674
8094911	<i>ATP10D</i>	2.5909886	0.007341558
8059470	<i>IRSI</i>	2.5734465	0.013759398
7929732	<i>CNNM1</i>	2.5726156	0.005757415
8094574	<i>TBC1D1</i>	2.556988	0.001142018
8022655	<i>AQP4</i>	2.5565944	0.028163102
8004571	<i>EFNB3</i>	2.53976	0.007617007
8150138	<i>TEX15</i>	2.5220304	0.002017324
8077299	<i>CNTN6</i>	2.4851518	0.02012182
8146618	<i>RLBP1L1</i>	2.4758441	0.035133276
7971104	<i>TRPC4</i>	2.4724553	0.010214505
8156923	<i>RP11-35N6.1</i>	2.4619117	0.04837056
8140504	<i>MAGI2</i>	2.4571788	0.011110543
7902158	<i>TCTEX1D1</i>	2.4325416	0.033650953
8078405	<i>CMTM7</i>	2.4290893	0.014074274
8166925	<i>MAOA</i>	2.3981342	0.022063425
8008609	<i>ANKFNI</i>	2.3975136	0.004701005
8107591	<i>ZNF474</i>	2.3939266	0.014941976
8003667	<i>SERPINF1</i>	2.3871253	0.002531932
7946946	<i>TPHI</i>	2.3767436	0.014637796
8102440	<i>ARSJ</i>	2.3724394	0.006457854

8009277	<i>RGS9</i>	2.361447	0.030142432
8088560	<i>ADAMTS9</i>	2.3553352	0.006786053
8168163	<i>GDPD2</i>	2.3540742	0.001182175
8145055	<i>BMP1</i>	2.3463554	0.005386771
8127051	<i>TRAM2</i>	2.338164	0.00365277
7910111	<i>EPHX1</i>	2.3344667	0.010242027
7972177	<i>POU4F1</i>	2.313673	0.00564285
8099476	<i>PROM1</i>	2.3125432	0.023345044
8104394	<i>ADCY2</i>	2.2996483	0.001288878
8154491	<i>ADAMTSL1</i>	2.2806952	0.012762848
8055239	<i>CFC1</i>	2.2794826	0.02888268
8045198	<i>CFC1B</i>	2.2759593	0.025898548
8062427	<i>VSTM2L</i>	2.2596896	0.00186271
8149685	<i>LGI3</i>	2.239012	0.009899134
8022559	<i>ANKRD29</i>	2.2333395	0.01747538
8090214	<i>SLC12A8</i>	2.2328575	0.012348623
8113103	<i>C5orf36</i>	2.2270188	0.016636403
8113097	<i>C5orf36</i>	2.22565	0.007570411
7940323	<i>MS4A8B</i>	2.2047389	0.00314757
8100578	<i>EPHA5</i>	2.1997414	0.047808312
7971526	<i>HTR2A</i>	2.198441	0.001919438
8111887	<i>PLCXD3</i>	2.1977108	0.005123412
7904293	<i>PTGFRN</i>	2.1928596	0.00509188
8091537	<i>IGSF10</i>	2.1908095	0.016658626
8081548	<i>PVRL3</i>	2.1779037	0.001697615
7944603	<i>GRIK4</i>	2.1769218	0.005746446
7933194	<i>CXCL12</i>	2.1688948	0.001198161
8115122	<i>CAMK2A</i>	2.159405	0.024350988
8148040	<i>MAL2</i>	2.1570866	0.031561263
8082767	<i>TMEM108</i>	2.1567624	0.039613348

8169711	<i>GLUD2</i>	2.1501238	0.014982611
8051241	<i>ALK</i>	2.1448784	0.013208308
7979529	<i>KCNH5</i>	2.1385012	0.011208067
8057677	<i>SLC40A1</i>	2.130094	0.019588992
8114287	<i>SPOCK1</i>	2.1280315	0.033314068
8115875	<i>DRD1</i>	2.1213698	0.003141733
7978492	<i>HEATR5A</i>	2.1200593	0.04196309
8150419	<i>ZMAT4</i>	2.1137774	0.012065756
7950307	<i>UCP2</i>	2.1111689	0.005334504
7926506	<i>CACNB2</i>	2.1078508	0.008176062
8096335	<i>HERC6</i>	2.1024654	0.013722494
8099593	<i>KCNIP4</i>	2.1012115	0.038529713
7897966	<i>C1orf158</i>	2.097635	0.031287834
8018646	<i>FOXJ1</i>	2.0910397	0.02227275
8046815	<i>ZNF804A</i>	2.0804143	0.005424841
8141076	<i>PON2</i>	2.0797234	0.009371994
7972021	<i>TBC1D4</i>	2.0627003	0.001612802
8157922	<i>LMX1B</i>	2.0433998	0.006663436
8115756	<i>KCNMB1</i>	2.041059	0.00110294
8154512	<i>ADAMTSL1</i>	2.040122	9.77E-04
7965322	<i>KITLG</i>	2.0390725	0.02017616
8094134	<i>USP17L6P</i>	2.0383346	0.026872892
8122099	<i>ENPP1</i>	2.0132759	0.007932335
7924636	<i>TMEM63A</i>	2.0101945	0.002524594
8065344	<i>FOXA2</i>	2.0030603	0.046765033
8002403	<i>MTSS1L</i>	2.0025082	0.013355245
8161919	<i>TLE1</i>	-2.005099	3.05E-04
8114845	<i>PRELID2</i>	-2.0218534	0.006494959
8106743	<i>VCAN</i>	-2.0227196	0.002522825
7918891	<i>NHLH2</i>	-2.0330408	0.015749276

7936856	<i>CHST15</i>	-2.0386324	0.013191681
8042830	<i>MTHFD2</i>	-2.0551112	0.010851565
8145361	<i>NEFM</i>	-2.0610816	0.039704736
7960865	<i>SLC2A3</i>	-2.0629818	0.011180702
8141950	<i>RELN</i>	-2.0684507	0.015906759
8179519	<i>HLA-DPB1</i>	-2.08239	0.034210023
7939102	<i>ELP4</i>	-2.0972288	0.025683006
7956401	<i>SHMT2</i>	-2.0974576	0.006639031
7956395	<i>NXPH4</i>	-2.098063	0.007471058
8113981	<i>P4HA2</i>	-2.0993407	0.015287149
8121784	<i>FABP7</i>	-2.1035392	0.02041487
7999387	<i>EMP2</i>	-2.112551	0.009035104
7922717	<i>RGS16</i>	-2.1209857	0.009994529
8103728	<i>HMGBl2</i>	-2.132948	0.001998046
8161265	<i>IGFBPL1</i>	-2.1589382	0.022531271
8001329	<i>CBLNI</i>	-2.1598804	0.04133884
8140955	<i>CDK6</i>	-2.1889746	0.030694377
8175234	<i>GPC3</i>	-2.1954863	0.036012586
7922807	<i>GLT25D2</i>	-2.1960945	0.01720048
7957570	<i>PLXNC1</i>	-2.2013931	0.012180514
7980605	<i>KCNK10</i>	-2.210973	0.02661182
8103094	<i>NR3C2</i>	-2.2212284	0.009560429
7969613	<i>GPC6</i>	-2.2233348	0.01124238
8174351	<i>NUP62CL</i>	-2.262157	0.048474442
8150698	<i>SNAI2</i>	-2.2736783	0.047932636
7926037	<i>PFKFB3</i>	-2.2894857	0.033096205
8085475	<i>WNT7A</i>	-2.3042505	0.029058207
8086961	<i>PFKFB4</i>	-2.3127549	0.00492768
8057578	<i>CALCRL</i>	-2.3291035	0.013531334
7943984	<i>ZBTB16</i>	-2.3384817	2.38E-04

8152946	<i>KCNQ3</i>	-2.347841	0.01136561
7972567	<i>ZIC5</i>	-2.3683083	3.41E-04
8001800	<i>CDH11</i>	-2.3777068	0.035105314
8096511	<i>BMPRIB</i>	-2.3881023	0.033132683
8086185	<i>PLCD1</i>	-2.393508	0.004501432
8175269	<i>FAM122B</i>	-2.4087105	0.009665375
7934278	<i>P4HA1</i>	-2.4218552	0.022610115
8101788	<i>UNC5C</i>	-2.5003495	0.005092559
7942123	<i>CCND1</i>	-2.5150073	0.010116734
7928308	<i>DDIT4</i>	-2.5195851	0.0120356
7936734	<i>FGFR2</i>	-2.53189	0.006118621
7922994	<i>FAM5C</i>	-2.5483663	0.04084279
7961829	<i>BCAT1</i>	-2.549485	0.0295037
7972239	<i>SLTRK6</i>	-2.5549057	0.029793974
7973743	<i>BNIP3</i>	-2.5826774	0.001452661
8072926	<i>HIF0</i>	-2.6044066	0.029549332
8156770	<i>GALNT12</i>	-2.623051	0.0460108
7969815	<i>CLYBL</i>	-2.6363177	0.016043475
7921099	<i>CRABP2</i>	-2.6384778	0.001010158
7937079	<i>BNIP3</i>	-2.662988	0.002951976
8149071	<i>ANGPT2</i>	-2.6950092	0.04441186
8166469	<i>SAT1</i>	-2.7259684	0.001622605
7916493	<i>PPAP2B</i>	-2.7356348	0.002484496
7938975	<i>SLC17A6</i>	-2.7537792	0.001068092
8064978	<i>JAG1</i>	-2.7545347	0.02699388
8081001	<i>ROBO2</i>	-2.7771685	5.60E-05
8082066	<i>FAM162A</i>	-2.8157492	3.28E-04
8095110	<i>KIT</i>	-2.892008	0.023102356
7901993	<i>CACHD1</i>	-2.9385476	0.005644539
8153002	<i>NDRG1</i>	-2.9619658	0.002201587

8119898	<i>VEGFA</i>	-2.971548	0.013201094
8046078	<i>B3GALT1</i>	-2.980435	0.019020619
7897737	<i>C1orf187</i>	-3.07386	0.02668419
7939559	<i>TSPAN18</i>	-3.0823834	0.007901136
8082574	<i>TRH</i>	-3.0963366	0.023394002
7955974	<i>NEUROD4</i>	-3.1097238	0.003727583
7909064	<i>CNTN2</i>	-3.391643	0.03556373
8055465	<i>CXCR4</i>	-3.400361	0.001582446
8112053	<i>CDC20B</i>	-3.4845676	0.02614936
7969830	<i>ZIC2</i>	-3.5606766	1.66E-04
8046408	<i>PDK1</i>	-3.5723302	0.004284957
8042942	<i>HK2</i>	-3.5868874	0.002930633
7925876	<i>PFKP</i>	-3.6496274	0.00645205
8149825	<i>STC1</i>	-3.8289537	0.002052822
7915472	<i>SLC2A1</i>	-3.8315654	2.72E-04
8013660	<i>ALDOC</i>	-3.9018507	0.002411329
8102368	<i>NEUROG2</i>	-4.047714	0.012370324
7962183	<i>AK3L1</i>	-4.0585384	8.91E-05
8078286	<i>RARB</i>	-4.1241055	0.023108592
8097773	<i>MAB21L2</i>	-4.2520766	0.047678325
8174444	<i>IRS4</i>	-4.2642846	0.005260202
8088485	<i>FEZF2</i>	-4.3277993	0.04564055
8102232	<i>LEF1</i>	-6.078532	0.00354995
7947338	<i>PAX6</i>	-6.092	0.014800102

Table S3: Gene expression array data of significantly up- and down-regulated genes of sorted day 28 cells compared with day 42 cells. The genes whose expression levels were higher (> 2-fold) in the fetal VM compared to the fetal dorsal mesencephalon are selected.

Transcripts Cluster Id	Gene symbol	Fold Change	P value
8140984	<i>HEPACAM2</i>	3.9891236	0.002473559
8088180	<i>WNT5A</i>	3.7547162	6.46E-04
8100154	<i>CORIN</i>	3.4526668	0.005539011
8088560	<i>ADAMTS9</i>	3.2472312	3.84E-04
7968678	<i>FREM2</i>	3.2326806	9.51E-04
8139640	<i>DDC</i>	2.7158241	0.019403007
8144078	<i>SHH</i>	2.419754	3.56E-04
8149592	<i>SLC18A1</i>	2.351122	0.003352632
8152522	<i>ENPP2</i>	2.2268987	0.009217289
7962312	<i>ABCD2</i>	2.2089314	0.011514991
8096489	<i>PDLIM5</i>	2.0749063	8.30E-05
8083223	<i>C3orf58</i>	2.0498207	0.021859845
7933933	<i>DNAJC12</i>	-2.0034456	0.030143498
7948588	<i>SYT7</i>	-2.03904	3.21E-04
8162216	<i>SHC3</i>	-2.0780237	6.88E-04
8101757	<i>GPRIN3</i>	-2.0867722	0.022475641
8075616	<i>SYN3</i>	-2.0903604	0.004095479
7965040	<i>PHLDA1</i>	-2.1291502	0.001350848
7909789	<i>TGFB2</i>	-2.148723	0.035826754
8146403	<i>SNTG1</i>	-2.1580215	0.008335051
8134463	<i>NPTX2</i>	-2.1630187	0.011281536
7929373	<i>LGI1</i>	-2.2057762	0.027242837
7927120	<i>RET</i>	-2.226918	0.003354378

7930837	<i>SLC18A2</i>	-2.3051424	0.00456127
7920123	<i>S100A10</i>	-2.3165731	0.03491729
7951662	<i>CRYAB</i>	-2.3638616	0.001891354
8021301	<i>RAB27B</i>	-2.4572883	0.030119164
7930341	<i>SORCS3</i>	-2.474262	0.004962535
8092578	<i>ETV5</i>	-2.5377958	0.017145246
8101659	<i>SPARCL1</i>	-2.6847594	0.003016697
7945712	<i>TH</i>	-2.8422818	0.001948763
8175195	<i>HS6ST2</i>	-3.0999072	0.001448546
8098439	<i>EPCAM</i>	-3.1291287	0.023850063
8149835	<i>NEFL</i>	-3.1964908	6.38E-04
7971937	<i>KLHL1</i>	-3.2236753	0.035931613
8070279	<i>KCNJ6</i>	-3.2937126	0.002665549
8106088	<i>CARTPT</i>	-3.3537612	0.017565785
8177620	<i>CARTPT</i>	-3.3564627	0.017597582
8169598	<i>ZCCHC12</i>	-3.5249903	0.001707703
7922482	<i>TNR</i>	-3.60874	5.37E-04
8094901	<i>GABRB1</i>	-4.1370163	0.004160109
7979721	<i>C14orf83</i>	-4.500512	1.37E-04
7982102	<i>GABRA5</i>	-4.5943813	0.001458439
8023828	<i>NETO1</i>	-5.4121256	0.00112521

Table S4: Genes of highly expressed either in day 28 and day 42-sorted cells, and human ventral mesencephalon (hVM). The genes whose expression levels were higher (> 2-fold) in the fetal VM compared to the fetal dorsal mesencephalon are selected.

PLXDC2

SLIT1

SPON1

PTPRO

FOXA1

NTN1

ABCA8

CFC1B

NR4A2

IGFBP5

SULF2

ALCAM

C3orf58

ADAMTS9

SLIT2

CORIN

CHN2

SHH

SULF1

NEFL

CALB1

Table S5: Q-PCR primers. Lists of primers used for quantitative RT-PCR.

Gene	Forward	Reverse
<i>OCT3/4</i>	AGACCATCTGCCGCTTGAG	GCAAGGGCCGCAGCTT
<i>NANOG</i>	GGCTCTGTTTGCTATATCCCCTAA	CATTACGATGCAGCAAATACGAGA
<i>SOX1</i>	GCGGAGCTCGTCGCATT	GCGGTAAACAACACTACAAAAAAACTGTAA
<i>SOX17</i>	CGCTTTCATGGTGTGGGCTAAGGACG	TAGTTGGGTGGCCTGCATGTGCTG
<i>BRACHYURY</i>	TCACAAAGAGATGATGGAGGAAC	GATGAGGATTGCAAGGTGGA
<i>hGSC</i>	GAGGAGAAAGTGGAGGTCTGGTT	CTCTGATGAGGACCGCTCTG
<i>MAP2</i>	GGATCAACGGAGAGCTGAC	TCAGGACTGCTACAGCCTCA
<i>NURRI</i>	CGAAACCGAAGAGGCCACAGGA	GGTCATAGCCGGTTGGAGTCG
<i>LMX1A</i>	GATCCCTTCCGACAGGGTCTC	GGTTTCCCACCTCTGGACTGC
<i>FOXA2</i>	TTCAGGCCGGCTAACTCT	AGTCTCGACCCCCACTTGCT
<i>OTX2</i>	GATGAGGATTGCAAGGTGGA	CCCGAGCTGGAGATGTCTTC
<i>GBX2</i>	GGTAACCTCGACAAGGCGGAGG	GGTCGTCTTCCACCTTGACTCG
<i>SIX3</i>	CCGGAAGAGTTGCCATGTT	CGACTCGTGTGTTGATGGC
<i>EN1</i>	TGGGTGTACTGCACACGTTATT	GGAACCTCCGCCTGAGTCTCT
<i>TH</i>	TCATCACCTGGTCACCAAGTT	GGTCGCCGTGCCGTACT
<i>GAPDH</i>	GGTCGGAGTCAACGGATTG	TCAGCCTGACGGTGCCATG

Table S6: Primary antibodies used for immunofluorescence studies. Lists of primary antibodies used for immunofluorescence studies.

Antibody	Dilution	Supplier
Mouse anti-CORIN	1:200	Donated by the KAN laboratory
Hamster anti-LMX1A	1:200	Donated by the KAN laboratory
Rabbit anti-TH	1:400	Millipore Chemicon
Sheep anti-TH	1:200	Millipore Chemicon
Mouse anti-TUJ1	1:300	Convance Research Products
Mouse anti-NESTIN, human specific	1:100	Millipore Chemicon
Rabbit anti-AADC	1:100	Protos Biotech
Rabbit anti-GIRK2	1:200	Alomone labs
Rat anti-NURR1	1:1000	Donated by the KAN laboratory
Rabbit anti-KI67	1:1000	Novocastra
Goat anti-FOXA2	1:500	SantaCruz
Goat anti-OTX2	1:200	R&D
Rat anti-SEROTONIN	1:100	Millipore Chemicon
Rabbit anti-PITX3	1:200	Millipore Chemicon
Mouse anti-NEUN	1:100	Millipore Chemicon
Mouse anti-HNA	1:500	Millipore Chemicon