Supplementary Information

Using intracellular markers to identify a novel set of surface markers for live cell purification from a heterogeneous hIPSC culture

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Supplementary Table S1: Full list of the differentially expressed genes from comparing $LMX1^{+}/FOXA2^{+}$ to $LMX1^{-}/FOXA2^{-}$ neural progenitor cells. The microarray was done on an Affymetrix Human ST 2.0 Array.

Gene Symbol	Fold Change	Affymetrix Transcript Cluster ID
LINC00261	49.72	16917810
SPON1	17.93	16722278
CMTM8	16.23	16938647
SERPINF1	12.92	16829570
SLIT2	12.83	16965377
NTN1	12.56	16831013
HEG1	9.19	16958403
OR51F2	9.11	16721212
MYOF	8.93	16716590
CORIN	8.24	16975671
SLC7A11	8.12	16979917
TTC6	7.81	16783537
ADAMTS12	7.46	16995047
FREM2	7.34	16774130
EFEMP1	7.19	16897834
SERPINI1	7.07	16947715
FOXA1	6.27	16792181
TMEM65	6.25	17080853
SALL1	6.24	16826460
TSPAN2	5.73	16691314
PLCL1	5.64	16889160
STOX1	5.59	16705439
COLEC12	5.57	16853399
SHH	5.55	17064810
CRISPLD1	5.52	17070120
GALR1	5.38	16853210
SULF2	5.24	16919962
ALCAM	5.17	16943548
PRPS1	5.15	17106031
FREM1	5.09	17092531
TXLNB	5.07	17024265
RYR2	5.03	16679142
WNT5A	5.02	16955197
CMTM7	4.79	16938656
RAI14	4.71	16983848
PLEKHA5	4.66	16748888

PLXDC2	4.5	16703036
CCND2	4.45	16746992
TTR	4.38	16851786
NID1	4.21	16700888
DOCK10	4.18	16909081
IQGAP2	4.17	16986351
ADAMTS9	4.14	16955822
GJA1	4.14	17012148
RAB3B	4.03	16687123
CFC1	4	16902847
SEMA3E	3.98	17059292
ARMC3	3.95	16703182
PTCH1	3.9	17096091
TRAM2	3.82	17020044
EFNA5	3.77	16998682
TUBB4A	3.74	16867756
HTR2C	3.69	17106314
TCF7L2	3.64	16709333
SLC2A13	3.62	16763195
FBN1	3.58	16808793
BCAM	3.55	16863074
LRP2	3.53	16904827
PDGFD	3.52	16743816
SLIT3	3.52	17002612
FJX1	3.43	16723662
SYTL5	3.41	17102566
CADPS	3.4	16955699
GRIN2A	3.37	16823799
QKI	3.37	17014562
SSFA2	3.35	16888317
HHIP	3.35	16971102
SHC3	3.32	17095566
WLS	3.31	16688339
TSPAN6	3.31	17112607
GPR98	3.3	16987140
DSP	3.3	17004612
IGFBP5	3.29	16908197
CTGF	3.23	17023646
HECW2	3.22	16906749
RGS9	3.2	16837101
SLC44A1	3.13	17087790

PPP1R14C	3.09	17013664
FAT3	3.07	16729986
USP2	3.06	16745343
OTX2	3.05	16793329
PDZRN3	3.04	16956285
CALU	3.04	17051236
RAB11FIP4	3.03	16833000
RALGPS2	3.01	16674292
LRIG3	2.98	16766822
SEPT3	2.98	16930811
DTNA	2.93	16851866
FAM198B	2.93	16980974
TMTC2	2.92	16754808
NPC2	2.91	16794705
PAPSS2	2.9	16707009
LOC151657	2.9	16957089
C17orf58	2.89	16848062
ATXN1	2.88	17015862
MMP2	2.84	16819064
SERINC5	2.84	16997615
RFX4	2.82	16756370
LIX1	2.82	16998411
CD47	2.81	16957095
RNF220	2.79	16663899
PTPRM	2.75	16850759
LRRN2	2.74	16698407
TSPAN7	2.73	17102607
ACAA2	2.71	16855184
LDB2	2.7	16974626
ST6GALNAC3	2.69	16666326
CADPS2	2.69	17062321
CAPN6	2.66	17113362
EEF1G	2.65	17063066
ATP10D	2.58	16966514
GPC3	2.58	17114288
DGKB	2.57	17055390
SLIT1	2.56	16717032
CPAMD8	2.55	16870087
TMEM63A	2.53	16699932
CMTM6	2.53	16951917
TEX15	2.53	17076103

PXDC1	2.51	17015207
BICC1	2.49	16705089
ITPR1	2.49	16936947
PPIL6	2.49	17022406
ECE1	2.48	16682989
ATP6AP2	2.47	17102668
GSR	2.46	17076063
C1QBP	2.45	16840284
PON2	2.45	17059932
CETN2	2.45	17115077
ERMP1	2.44	17092208
GALNT2	2.43	16678579
PLTP	2.43	16919703
ULBP3	2.43	17024710
GDE1	2.42	16824555
CPD	2.42	16832768
CACNG7	2.42	16865153
DNAH7	2.41	16906656
AJUBA	2.4	16790614
KLF3	2.39	16966008
PSAP	2.38	16715241
ZC3HAV1	2.38	17063428
CPNE2	2.36	16819430
TANC2	2.36	16836868
UGDH	2.36	16975310
PTGFRN	2.35	16669180
KCNMA1	2.35	16715793
RFX2	2.35	16867613
HEPH	2.35	17104283
ACSS3	2.34	16754729
SPTSSB	2.34	16961056
NME5	2.34	17000342
CCDC47	2.33	16847605
HIPK2	2.33	17063461
LRRC6	2.33	17081323
HTRA1	2.32	16710126
GAA	2.32	16838509
ZFP36L2	2.32	16897026
ITGB8	2.32	17043982
PPP4C	2.31	16817811
KIAA1407	2.31	16957583

LPHN3	2.31	16967202
SDC2	2.31	17071144
MLEC	2.3	16757969
FZD2	2.28	16834785
NUP210	2.28	16950932
MIR218-1	2.28	16965432
PTN	2.28	17063254
NFE2L1	2.27	16835374
GALNT1	2.27	16851933
SEPT10	2.27	16901593
LAPTM4B	2.27	17071208
GANAB	2.26	16739396
MORN2	2.26	16879276
DDOST	2.25	16682842
MANBA	2.25	16978444
CELSR1	2.24	16936097
GRSF1	2.24	16976655
SPAG6	2.23	16703150
SULF1	2.23	17069816
WDR96	2.22	16718184
RPA1	2.21	16829580
SORT1	2.19	16690566
CD63	2.19	16765744
HSDL1	2.18	16828762
ADAMTS16	2.18	16982870
STON2	2.17	16795368
PCTP	2.17	16836277
VGLL3	2.17	16956532
CAP2	2.17	17005138
SPARC	2.15	17001927
NCAN	2.14	16859969
DAB2	2.14	16995645
ARRDC4	2.13	16805474
MOB1B	2.13	16967602
PTPN13	2.13	16968529
FAT1	2.13	16982269
TTC18	2.12	16715434
DNAAF1	2.12	16821437
RNF157	2.12	16849148
CDH11	2.1	16827041
CACNG4	2.1	16837172

C20orf3	2.1	16918011
TWSG1	2.09	16850896
RAD23B	2.09	17087933
NR6A1	2.09	17098305
BCOR	2.09	17110148
MACF1	2.08	16662755
ALDH18A1	2.08	16716846
PRDX3	2.08	16718922
ARPP19	2.08	16809506
RASGRF2	2.08	16986777
CHRDL1	2.07	17113346
SEL1L	2.06	16795394
HYOU1	2.05	16745186
VASN	2.05	16815509
MED12L	2.05	16946966
TJP2	2.05	17085685
ENKUR	2.04	16712587
TM9SF3	2.04	16716983
OPCML	2.04	16746290
UNC119B	2.04	16757979
CANX	2.04	16993397
DNAH5	2.04	16994434
PODXL	2.04	17062985
TRAM1	2.04	17078102
BASP1	2.03	16983451
FRRS1L	2.03	17096958
LOC339535	2.02	16701007
HYDIN	2.02	16827874
LPCAT1	2.02	16994002
SALL2	2.01	16790413
NPHP1	2.01	16901632
PDGFRA	2.01	16966809
HNRNPA1	-2.01	16751993
SLITRK1	-2.01	16780092
SNORD115-20	-2.01	16798341
COL11A1	-2.02	16690211
DCX	-2.05	17113378
VAMP1	-2.07	16760393
ARL4D	-2.08	16834621
MIR4525	-2.09	16850286
CNR1	-2.1	17021504

L1TD1	-2.11	16665439
H3F3B	-2.12	16848888
DNER	-2.13	16909319
NAV1	-2.15	16675794
DNAJB1	-2.16	16869653
SNORD115-44	-2.19	16798432
TCTA	-2.19	16940693
CA2	-2.2	17070456
HIST2H2BC	-2.22	16692624
RYR3	-2.22	16798965
PCDH15	-2.23	16714433
SNORD115-32	-2.24	16798408
DUX2	-2.27	16973271
GLUL	-2.28	16696979
MIR4294	-2.28	16714084
CDKN1A	-2.28	17008007
ERBB3	-2.29	16752397
IER5L	-2.29	17099027
ZC3H10	-2.3	16752485
RHOB	-2.3	16877555
DUX2	-2.31	16973292
RGS16	-2.35	16697018
ZIC1	-2.35	16946666
HIST1H3F	-2.36	17016400
SERTAD1	-2.37	16872443
ZIC5	-2.39	16780632
FOS	-2.41	16786587
HIST1H2BC	-2.42	17005603
EGFEM1P	-2.44	16947759
REXO1L2P	-2.46	17070478
KLF7-IT1	-2.49	16907641
RNU1-13P	-2.5	16691877
ROR1	-2.53	16665588
P2RX3	-2.54	16724818
SNAR-B1	-2.56	16874508
ANKRD37	-2.58	16972950
SNORA21	-2.6	16844046
NRP2	-2.64	16889879
NRN1	-2.68	17015324
REXO1L2P	-2.68	17070480
POU4F1	-2.69	16779990

SNORD4B	-2.7	16832568
EPHA7	-2.7	17021792
RELN	-2.71	17061298
HEXIM1	-2.74	16834921
RPS27	-2.77	16671325
SNORA42	-2.79	16694357
LOC100507303	-2.79	16823043
DUSP1	-2.79	17002846
LRRC7	-2.81	16665976
ZNF878	-2.89	16869060
ROBO2	-2.89	16942743
UNC5C	-2.9	16978054
MIR3189	-2.98	16859800
ELAVL2	-2.99	17092918
RNU11	-3.02	16661646
CNTN2	-3.03	16676405
SLC2A3	-3.03	16760868
EYA2	-3.05	16914478
RASL11B	-3.05	16966733
IER5	-3.06	16674618
MIR219-2	-3.08	17098866
NFASC	-3.09	16676355
SNAI1	-3.09	16914791
PRDM12	-3.13	17090350
SNORA45	-3.15	16721732
SGK1	-3.21	17023810
DDIT4	-3.24	16705961
LOC100132966	-3.26	16691721
NRG1	-3.3	17067696
SNORA64	-3.31	16822919
PCDH8	-3.35	16779486
SNAR-E	-3.37	16873645
MIR425	-3.37	16953809
DPYD	-3.41	16689969
STMN2	-3.44	17070249
HIST1H2AK	-3.45	17016496
BOC	-3.56	16944010
EBF1	-3.56	17002278
RPLP1	-3.6	16802553
SNORA71B	-3.64	16919223
HIST2H4B	-3.7	17005790

HSPA1B	-3.71	17030833
STARD5	-3.75	16812406
HIST1H2BN	-3.75	17005871
SMOC1	-3.82	16785897
SNORA54	-3.83	16734524
STC1	-3.84	17075553
HSPA1B	-3.87	17028007
MIR378D2	-3.89	17079181
MEIS2	-3.93	16807139
HSPA1B	-3.94	17026318
HSPA1B	-3.94	17027994
HSPA1B	-3.94	17030820
SNHG12	-4	16684178
HIST2H2BF	-4	16692603
NHLH1	-4.01	16672630
FTH1	-4.04	16739208
NABP1	-4.1	16888963
HIST2H4B	-4.15	16670377
JUN	-4.15	16687875
HIST2H4B	-4.15	16692626
HSPA1B	-4.19	17038297
EBF2	-4.21	17075628
ZIC2	-4.22	16776103
SNORA74B	-4.23	16992465
SCARNA27	-4.23	17015472
NPPC	-4.24	16909551
HSPA1B	-4.24	17006881
ADRB2	-4.31	16990848
HSPA1A	-4.41	17006863
SNORD75	-4.47	16696533
SCARNA11	-4.49	16760514
GLI3	-4.53	17056992
SST	-4.61	16962574
HSPA1B	-4.66	17038309
CXCR4	-4.68	16903140
EBF3	-4.83	16719562
METTL12	-4.85	16725901
HIST1H3J	-5.09	17016509
SNAI2	-5.1	17077004
PLK2	-5.41	16996433
REXO1L1	-6.07	17070482

PMAIP1	-6.21	16852683
GADD45B	-6.35	16856803
PCDH9	-6.42	16779667
MIR3193	-7.35	16912368
HIST1H3A	-8.06	17005532
HIST1H2BD	-9.23	17005573
LOC145845	-9.38	16807125
TFAP2B	-9.67	17009545
ID1	-9.68	16912362
ROCK1P1	-10.33	16850428
MIR3143	-11.2	17005797
HIST2H4B	-11.79	17005586
HIST1H2AH	-12.6	17005787
HIST1H2BG	-13.95	17016390
HIST1H3H	-14.12	17005862
PAX3	-17.44	16908928
HIST2H3D	-21.24	16692614

Dopaminergic Neuron Markers

Gene	fold change
FOXA2	4.99
FOXA1	6.27
LMX1A	1.97
LMX1B	1.34
OTX2	3.05
WNT5A	5.02
SHH	5.55
PTCH1	3.9

Putative Cell Surface Markers

Gene	fold change
CORIN	+8.24
CD166	+5.17
CD239 (BCAM)	+3.55
CD47	+2.81
SORT1	+2.19
CD63	+2.19
CNR1	-2.1
EPHA7	-2.7
PCDH8	-3.35
ADRB2	-4.31
CXCR4	-4.68
PCDH9	-6.42

Supplementary Table S2. Microarray analysis confirmed the fixed, sorted population was of the dopaminergic lineage by revealing upregulation of characteristic genes. Additionally, through the use of GO annotation, the microarray yielded a putative list of positive and negative surface markers for live cell purification.

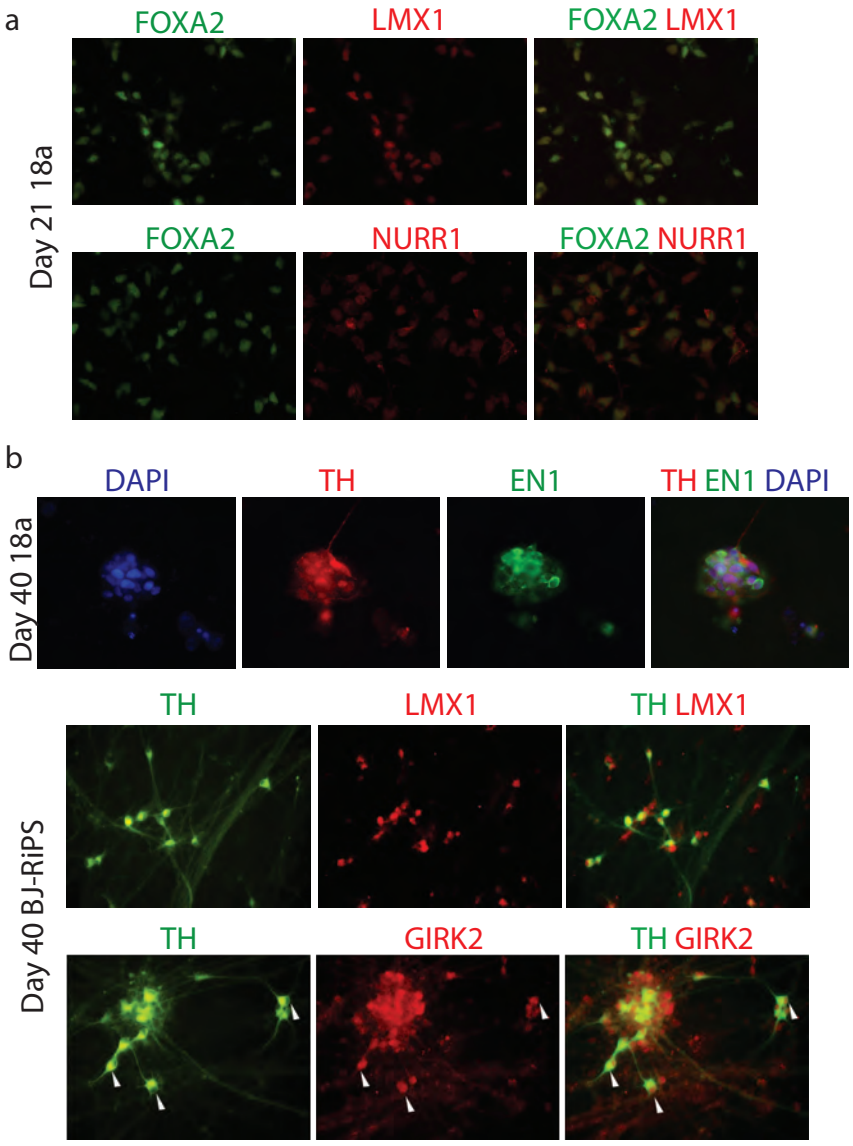

Figure S1. Additional immunostaining characterizing the iPS derived DA neurons. a. Differentiation day 21 18a cells show co-expression of LMX1/FOXA2 and FOXA2/NURR1. b. Differentiation day 40 18a cells show co-expression of TH and EN1 while day 40 BJ-RiPS show co-expression TH/LMX1 and TH/GIRK2.

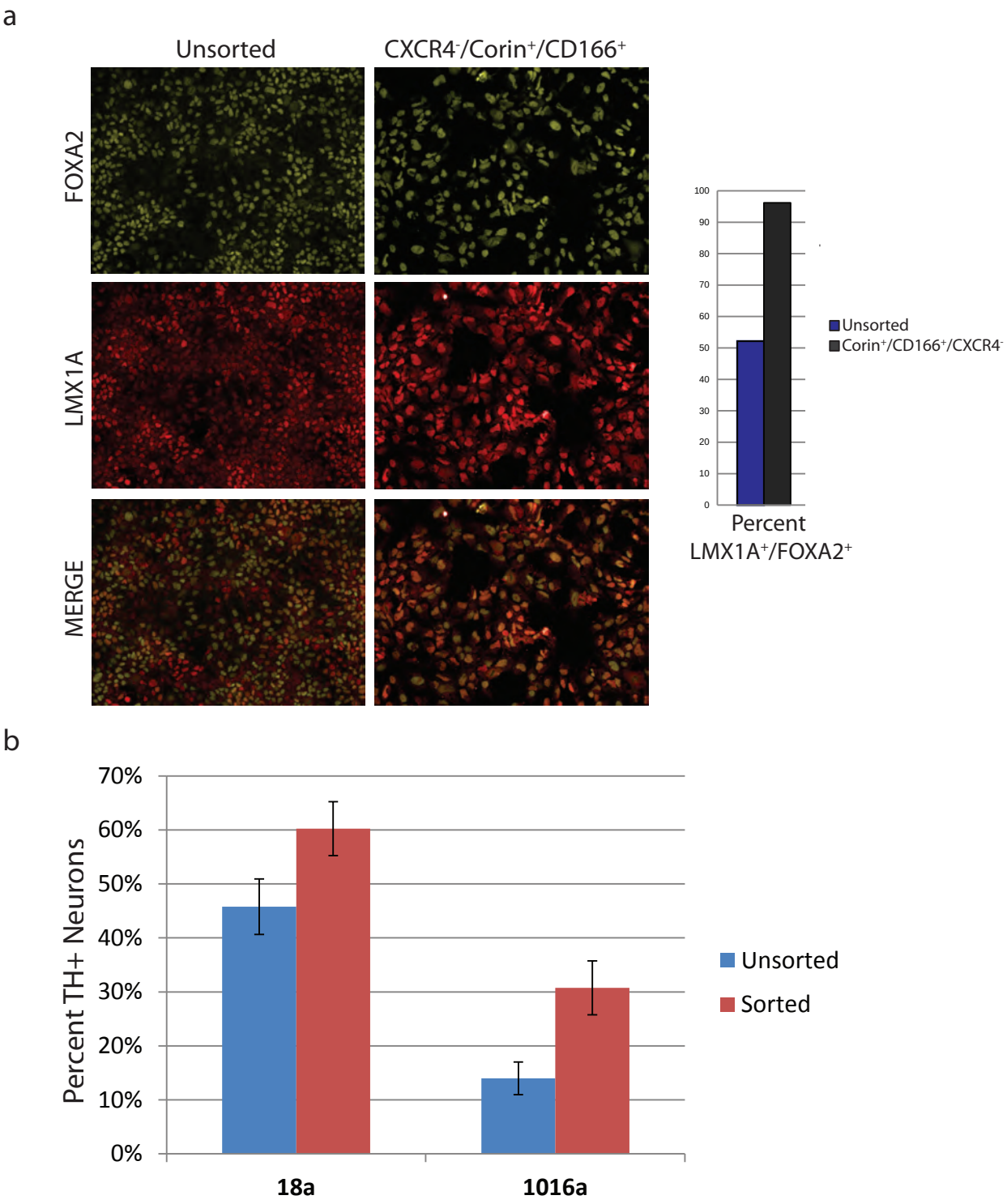

Figure S2. CXCR4$^-$/CORIN$^+$/CD166$^+$ FACS sorting enriches for the FOXA2/LMX1A double positive population and increases the differentiation efficiency to TH+ neurons. a. Day 14 BJ riPS dopaminergic progenitors were live FACS sorted for CXCR4$^-$/CORIN$^+$/CD166$^+$ and replated for 48 hours. They were then fixed and stained for FOXA2 and LMX1A. After sorting, the FOXA2$^+$/LMX1A$^+$ population is highly enriched (n=1). b. The 18a and 1016a cell lines both show increased percentages of TH+ neurons after being sorted as progenitors. n=2, error bars represent 1 standard deviation.

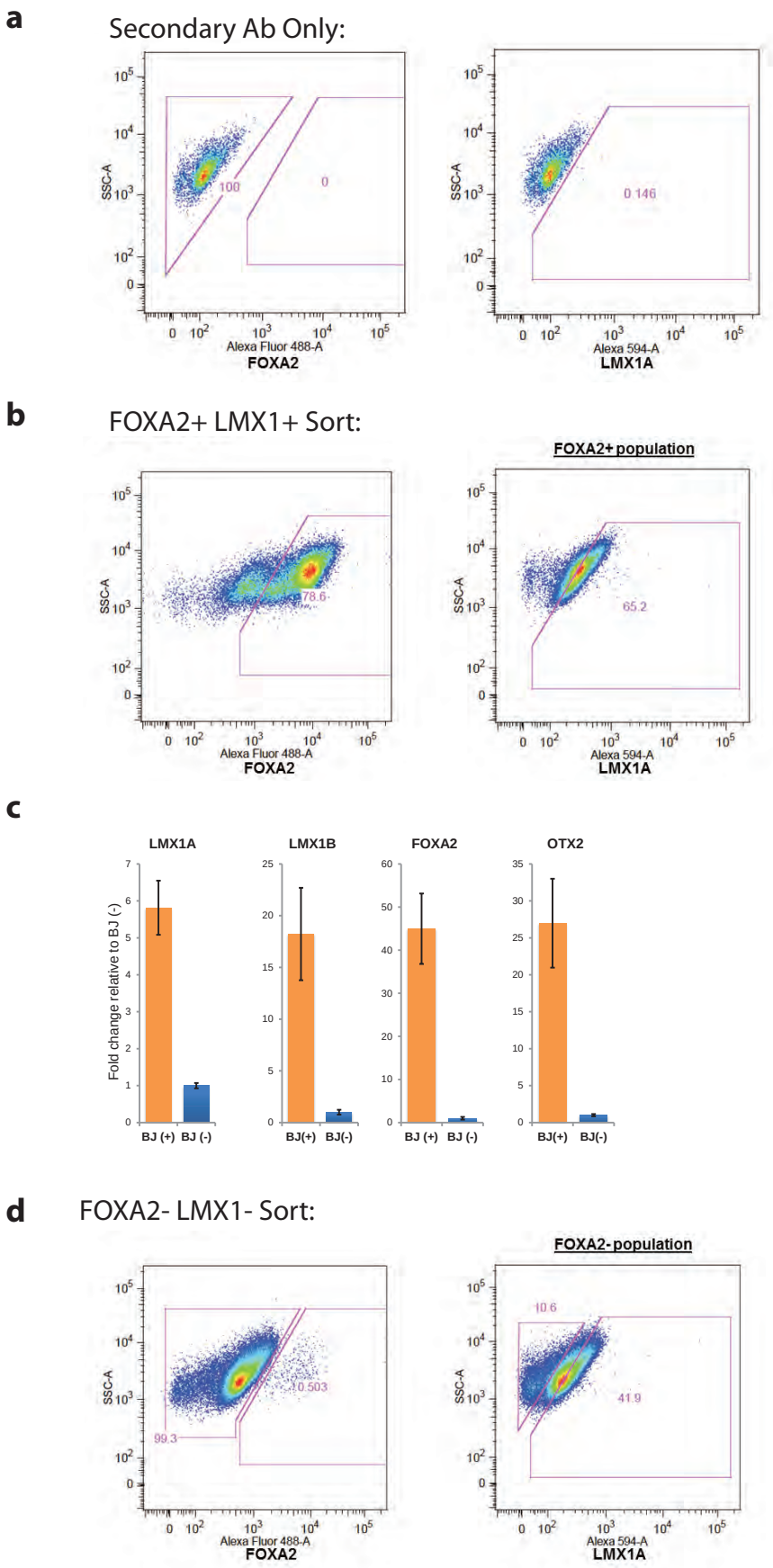

Figure S3. Representative FACS plots for collecting the LMX1+FOXA2+ and LMX1-FOXA2- populations used for microarray analysis.

a. Secondary only control plots used to set gates. b. Representative FACS plots for the LMX1A+FOXA2+ population. c. qPCR of the FOXA2+LMX1A+ sorted population (BJ+) compared to the FOXA2-LMX1A- poulation (BJ-) showing enrichment of dopaminergic progenitor markers LMX1A/B, FOXA2, and OTX2. Error bars represent standard error, qPCR normalized to GAPDH expression, n=3, performed in the BJriPS line. d. Representative FACS plots for the LMX1-FOXA2- population.

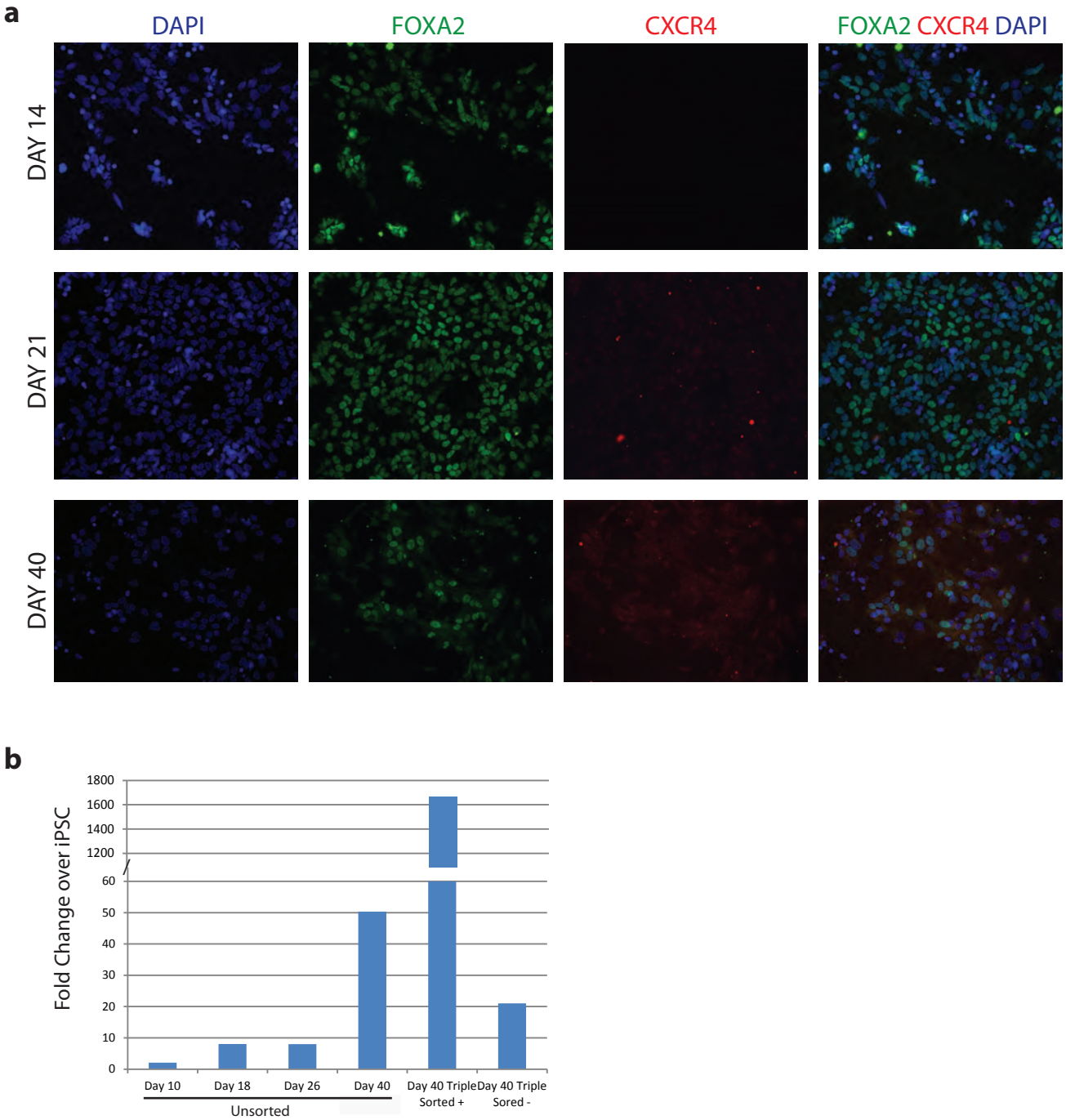

Figure S4. CXCR4 expression is low in mDA NPCs and increases over time.
a. Immunostaining of day 14, 21, and 40 BJ-RiPS differentiation. CXCR4 staining is absent in day 14 but increases to day 40. b. qPCR comparing a differentiation timeline of BJ-RiPS to the starting iPSC shows that CXCR4 expression increases over time. Additionally at day 40 the neurons derived from the CXCR4-CORIN+CD166+ NPCs, had a very high level of CXCR4 expression further illustrating their maturity and higher percentage of mDA neurons over

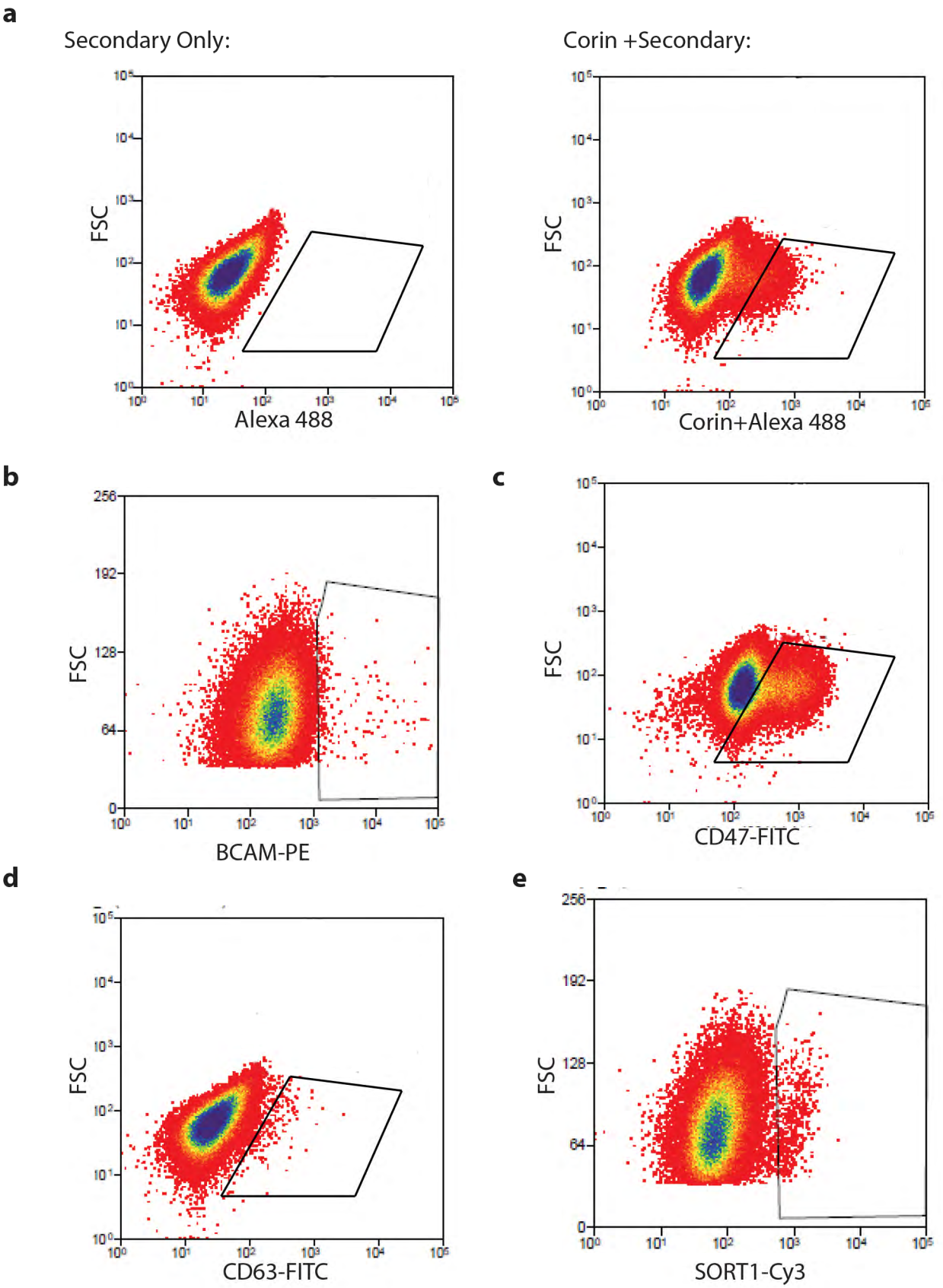

Figure S5. Representative FACS plots of secondary only control and single surface markers that did not enrich for FOXA2+ NPCs. a. Alexa 488 secondary only control (left) and with primary antibody Corin (right). b-e. FACS plots showing that the single surface markers BCAM, CD47, CD63, and SORT1 did yield labelled populations.