Figure S1

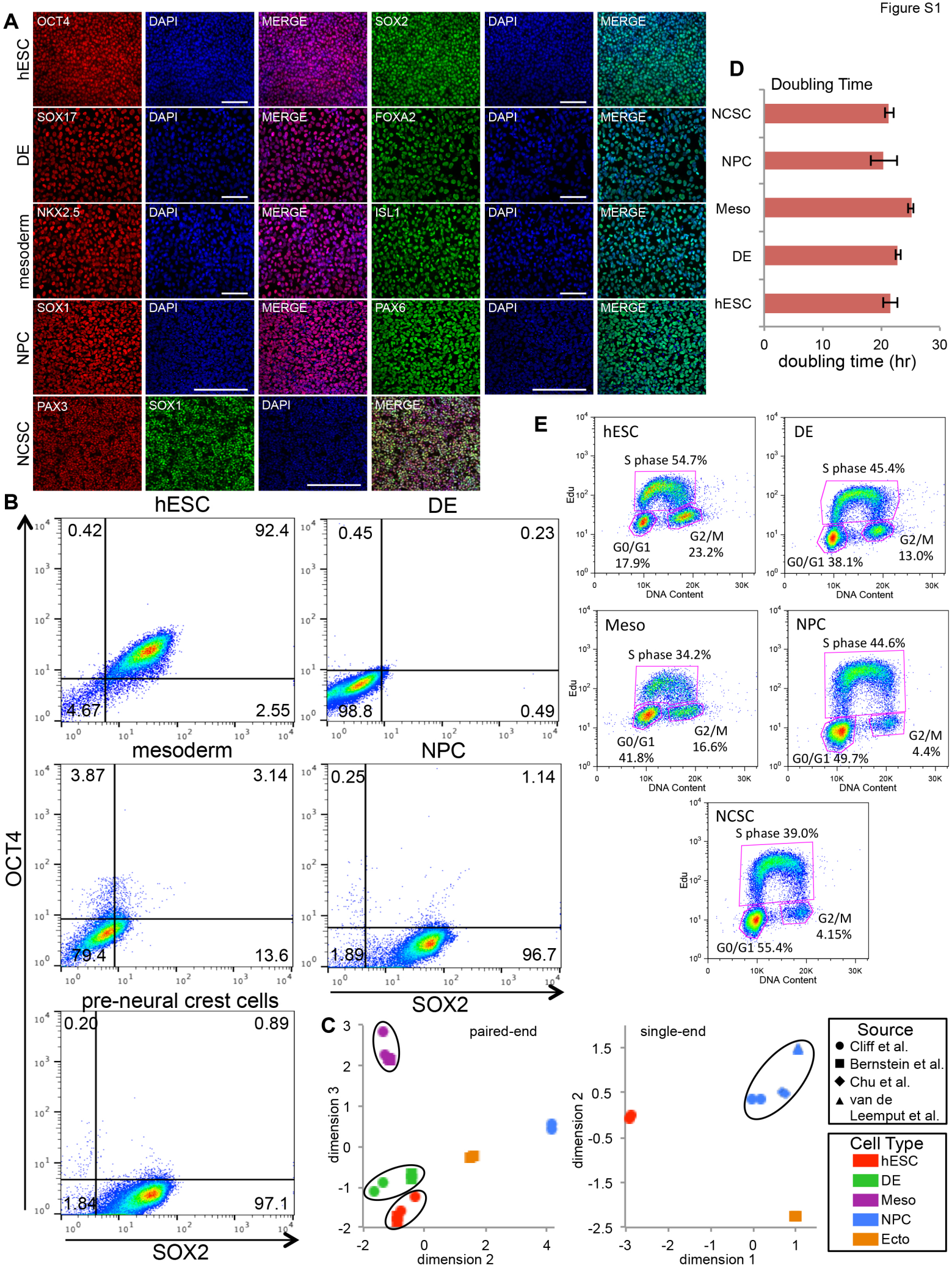
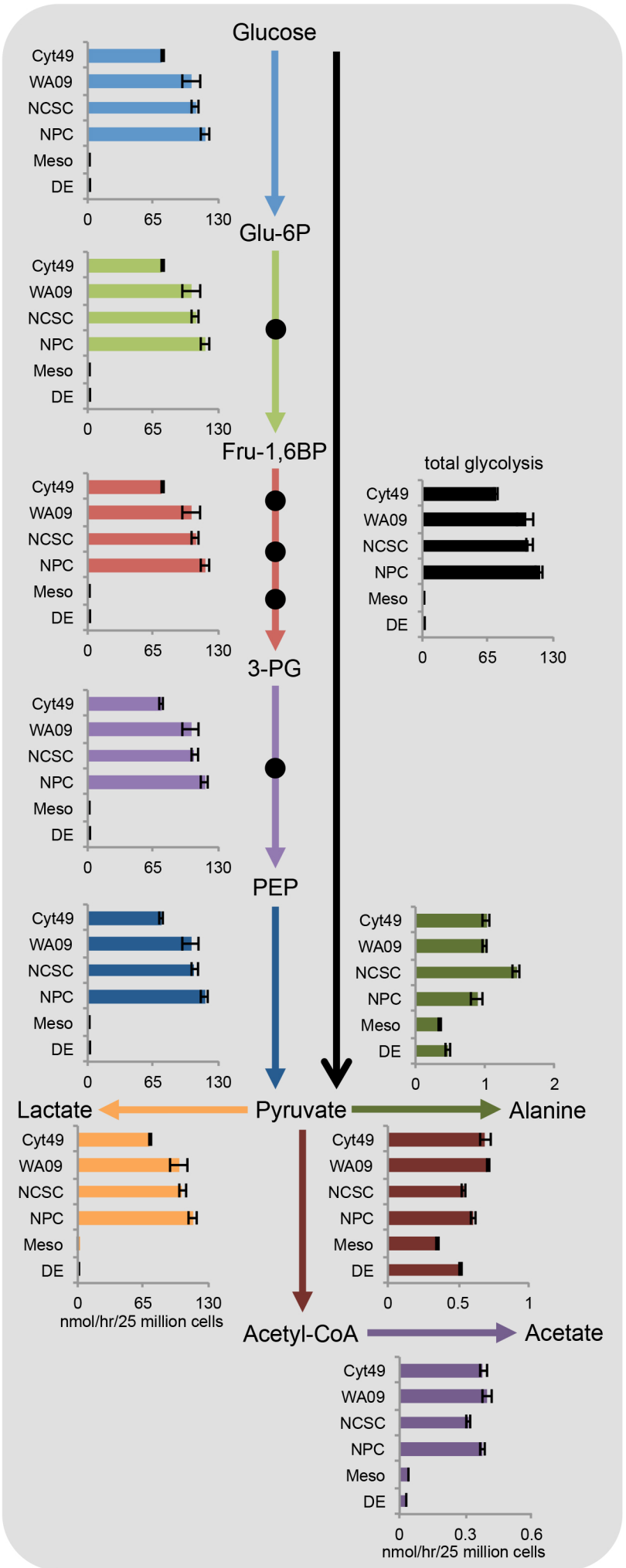
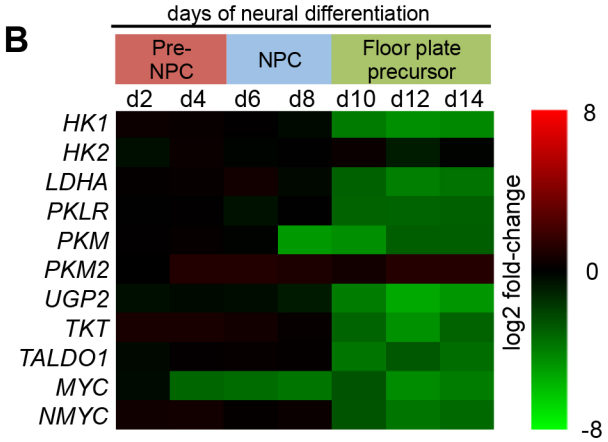
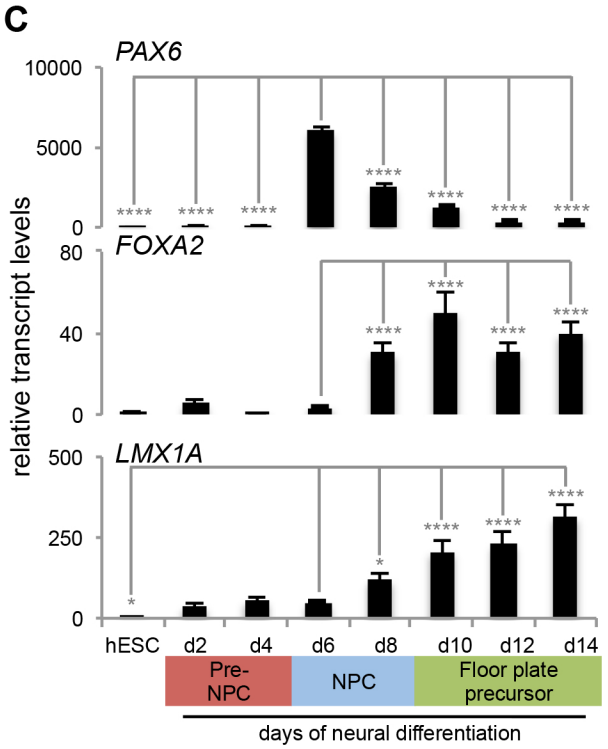

A
hESC
DE
mesoderm
NPC
NCSC
OCT4
SOX17
NKX2.5
SOX1
PAX3
SOX2
FOXA2
ISL1
PAX6
SOX1
DAPI
MERGE
D
Doubling Time
NCSC
NPC
Meso
DE
hESC
0
10
20
30
doubling time (hr)
E
hESC
S phase 54.7%
G0/G1 17.9%
G2/M 23.2%
DE
S phase 45.4%
G0/G1 38.1%
G2/M 13.0%
Meso
S phase 34.2%
G0/G1 41.8%
G2/M 16.6%
NPC
S phase 44.6%
G0/G1 49.7%
G2/M 4.4%
NCSC
S phase 39.0%
G0/G1 55.4%
G2/M 4.15%
Edu
DNA Content
B
hESC
DE
mesoderm
NPC
pre-neural crest cells
0.42
92.4
4.67
2.55
0.45
0.23
98.8
0.49
3.87
3.14
79.4
13.6
0.25
1.14
1.89
96.7
0.20
0.89
1.84
97.1
OCT4
SOX2
C
paired-end
single-end
dimension 1
dimension 2
dimension 3
Source
Cliff et al.
Bernstein et al.
Chu et al.
van de Leemput et al.
Cell Type
hESC
DE
Meso
NPC
Ecto

Figure S1. Related to Figure 1. (A) Immunofluorescence staining for markers of hESCs (OCT4, SOX2), definitive endoderm (DE) (SOX17, FOXA2), mesoderm (HNF4a, ISL1), neural progenitor cells (NPC) (SOX1, PAX6) and pre-neural crest cells (NCSC) (PAX3, SOX1). Micron bars, 100 μm. **(B)** Flow cytometry analysis of hESCs, DE, mesoderm, NPC and NCSC. Cells were probed with antibodies for OCT4 and SOX2. The percentage of positive cells is indicated in each quadrant. **(C)** Multidimensional scaling analysis of transcript levels measured by RNA-seq of cell types from this study and three equivalent, publicly available data sets. Analysis was performed on either paired-end or single-end data sets as indicated. Shapes indicate cell source and color indicates cell type. Cell types that cluster based on identity are enclosed in a black oval shape. **(D)** Doubling time calculations in hours (hr) for hESCs, DE, mesoderm (Meso), NPC and NCSC. Cells were plated and cultured under normal growth conditions and collected after 2, 3 and 4 days, for hESCs, DE and Meso, or after 4, 5 and 6 days, for NPC and NSCS. Each time point was repeated in triplicate and each replicate was counted 4 times (n=36). Error bars represent the standard deviation. **(E)** Percentage of cells in the indicated cell cycle phase for hESCs, DE, Meso, NPC and NCSC. The X-axis indicates DNA content of the cell through FxCycle dye staining and the Y-axis represents incorporation of the nucleotide analog EdU after a brief pulse period (indicative of S-phase cells).


A
Glucose
Glu-6P
Fru-1,6BP
3-PG
PEP
Pyruvate
Lactate
Alanine
Acetyl-CoA
Acetate
Cyt49
WA09
NCSC
NPC
Meso
DE
total glycolysis
nmol/hr/25 million cells


B
days of neural differentiation
Pre-NPC
NPC
Floor plate precursor
d2 d4 d6 d8 d10 d12 d14
HK1
HK2
LDHA
PKLR
PKM
PKM2
UGP2
TKT
TALDO1
MYC
NMYC
log2 fold-change
8
0
-8


C
PAX6
FOXA2
LMX1A
relative transcript levels
hESC d2 d4 d6 d8 d10 d12 d14
Pre-NPC
NPC
Floor plate precursor
days of neural differentiation

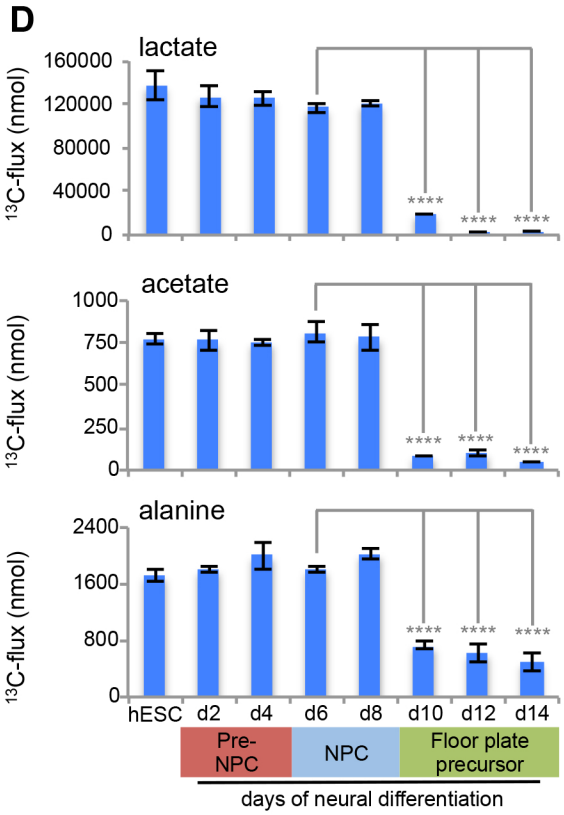

D
lactate
acetate
alanine
13C-flux (nmol)
hESC d2 d4 d6 d8 d10 d12 d14
Pre-NPC
NPC
Floor plate precursor
days of neural differentiation

Figure S2. Related to Figure 2. (A) Total ^{13}C-carbon flux through glycolytic intermediates, lactate, alanine, acetyl-CoA and acetate over 4 hr in the indicated cell types. Units are nmol/hr standardized to 25 million cells with arrow color corresponding to bar color in the plot for each reaction. Error (+/-) represents the standard deviation. All experiments were performed in biological triplicate. **(B)** qRT-PCR heat map showing relative transcript levels for MYC, NMYC and representative metabolic 'switch' transcripts (*HK1* through to *TALDO1*). Samples were analyzed at different stages of neural progenitor cell differentiation: pre-neural progenitor cells (pre-NPC) d2-5, neural progenitor cells d6-8, floor plate precursor cells d9-14. **(C)** qRT-PCR analysis of hESCs and NPC differentiation samples as in (B). Levels of *PAX6* and floor plate neuron precursor (*FOXA2*, *LMXA1*) transcripts are shown. **(D)** ^{13}C-glucose metabolic flux analysis where metabolite levels after 4 hr of labeling of hESCs and NPC differentiation samples as in (B). All experiments were performed in biological triplicate. Error bars represent the standard deviation. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$ for one-way ANOVA.

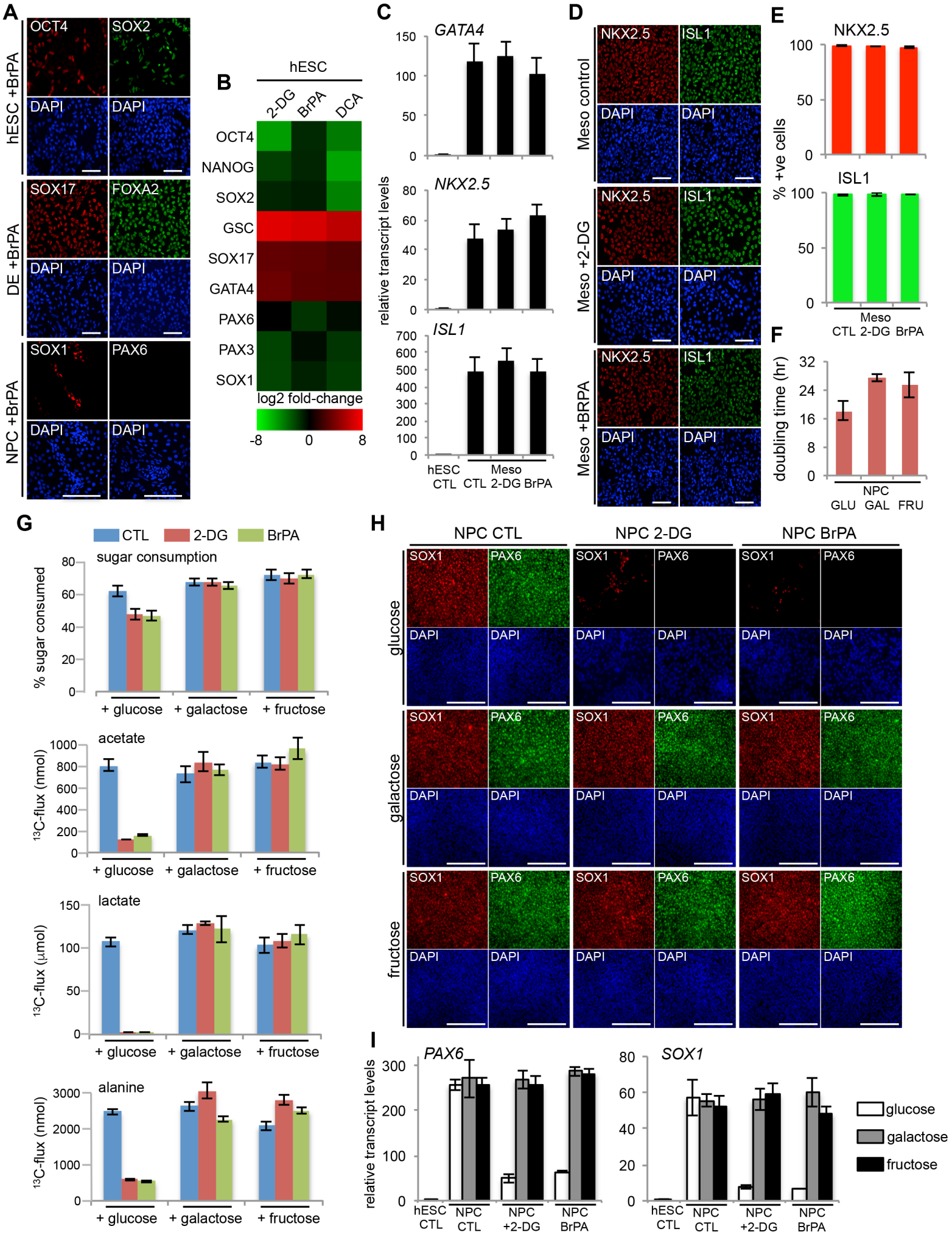

A
hESC +BrPA
DE +BrPA
NPC +BrPA
OCT4
SOX2
DAPI
SOX17
FOXA2
SOX1
PAX6
B
hESC
2-DG
BrPA
DCA
OCT4
NANOG
SOX2
GSC
SOX17
GATA4
PAX6
PAX3
SOX1
log2 fold-change
-8
0
8
C
GATA4
NKX2.5
ISL1
relative transcript levels
hESC CTL
Meso CTL
2-DG
BrPA
D
Meso control
Meso +2-DG
Meso +BRPA
NKX2.5
ISL1
DAPI
E
NKX2.5
ISL1
% +ve cells
Meso
CTL 2-DG BrPA
F
doubling time (hr)
NPC
GLU GAL FRU
G
CTL
2-DG
BrPA
sugar consumption
% sugar consumed
+ glucose
+ galactose
+ fructose
acetate
13C-flux (nmol)
lactate
13C-flux (µmol)
alanine
13C-flux (nmol)
H
NPC CTL
NPC 2-DG
NPC BrPA
glucose
galactose
fructose
SOX1
PAX6
DAPI
I
PAX6
SOX1
relative transcript levels
hESC CTL
NPC CTL
NPC +2-DG
NPC BrPA
glucose
galactose
fructose

Figure S3. Related to Figure 3. (A) Immunofluorescence staining of WAO9 hESCs and WAO9-derived definitive endoderm (DE) and neural progenitor cells (NPCs); see Figure 3B, E, and H. (B) qRT-PCR heat map analysis of pluripotency, endoderm mesoderm and ectoderm markers in WA09 hESCs treated with 2-DG, BrPA, and DCA treated cells relative to control treated cells. **(C)** Mesoderm (Meso) cells were analyzed by qRT-PCR analysis to determine transcript levels relative to WA09 hESCs and, **(D)** immunostained and probed for indicated antibodies then, **(E)** scored for the % of cells expressing NKX2.5 and ISL1. **(F-I)** Galactose and fructose rescue inhibition of hexokinase in NPCs. WA09 cells were transitioned onto glucose-free base media substituted with 17.5 mM glucose, or 17.5 mM galactose, or 17.5 mM fructose over a single passage (5 days). Cells were then maintained under these conditions for an additional passage (5 days), then differentiated to NPCs. **(F)** Doubling times in hours (hr) for NPCs cultured in glucose, galactose and fructose growth conditions. Cells were counted on day 4, 5 and 6. Each time point was repeated in biological triplicate and each replicate was counted 4 times (n=36). **(G)** Glucose-, galactose- or fructose-grown hESCs were plated and grown under NPC differentiation conditions with their respective carbon sources for 24 hr. The cells were then further differentiated for an additional 5 days in glucose, galactose or fructose-based NPC media with vector alone or with 2-DG (2.2 mM) or BrPA (17 μM). On the day of harvest, cells were labeled with ^{13}C-glucose, ^{13}C-galactose, or ^{13}C-fructose for 4 hr, as indicated. NMR-based metabolic flux analysis was standardized to 25 million cells per sample. **(H)** PAX6 and SOX1 immunofluorescence analysis on cells cultured under NPC conditions in the presence or absence of 2-DG and BrPA and, in the presence of different carbon sources. (**I)** qRT-PCR transcript analysis of *PAX6* and *SOX1* in samples corresponding to those on **(G,H).** **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$ for one-way ANOVA. Micron bars, 100 μm. See also Figures S4 and S5.

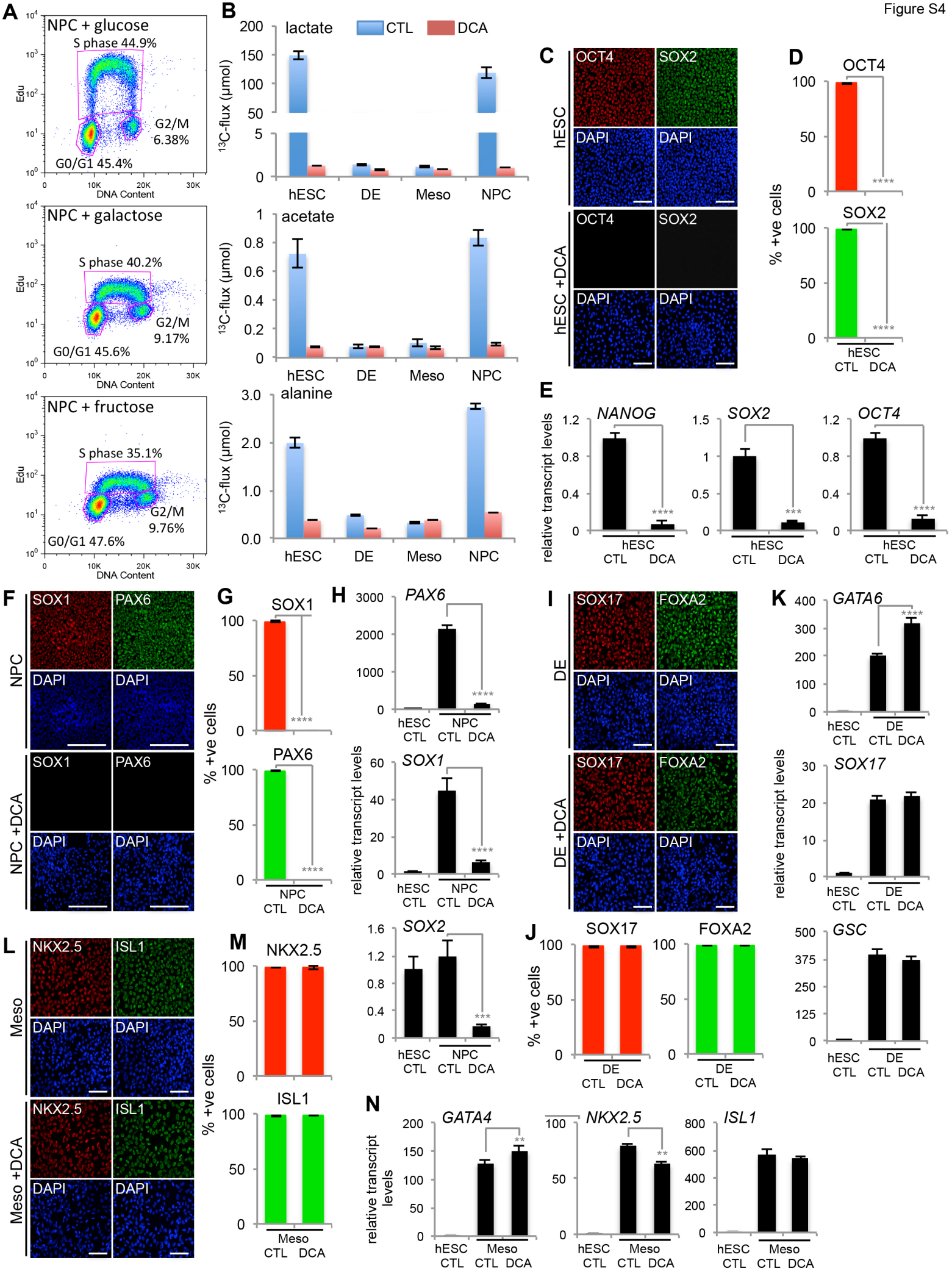

A
NPC + glucose
S phase 44.9%
G2/M 6.38%
G0/G1 45.4%
NPC + galactose
S phase 40.2%
G2/M 9.17%
G0/G1 45.6%
NPC + fructose
S phase 35.1%
G2/M 9.76%
G0/G1 47.6%
Edu
DNA Content
B
lactate
acetate
alanine
CTL
DCA
13C-flux (µmol)
hESC
DE
Meso
NPC
C
OCT4
SOX2
DAPI
hESC
hESC +DCA
D
% +ve cells
E
NANOG
SOX2
OCT4
relative transcript levels
hESC CTL DCA
F
SOX1
PAX6
NPC
NPC +DCA
G
H
PAX6
SOX1
SOX2
I
SOX17
FOXA2
DE
DE +DCA
K
GATA6
SOX17
GSC
J
L
NKX2.5
ISL1
Meso
Meso +DCA
M
N
GATA4
NKX2.5
ISL1

Figure S4. Related to Figure 3. (A) Percentage of neural progenitor cells (NPC) in different cell cycle phases when grown on glucose-free base media substituted with 17.5 mM glucose, or 17.5 mM galactose, or 17.5 mM fructose, as in Figure S3F. X-axis indicates cell DNA content by FxCycle dye staining and the Y-axis indicates cells in S-phase through incorporation of the nucleotide analog EdU, after a 2 hr pulse period. **(B)** ^{13}C-glucose metabolic flux analysis over 4 hr in hESCs, definitive endoderm, nascent mesoderm (Meso) and early ectoderm (NPC). 24 hr after plating, cells were cultured for a further 3 days (hESCs, DE and Meso) or 5 days (NPC and NCSC) in the presence of dichloroacetic acid (DCA, 2.5 mM) where indicated. Units of ^{13}C-flux are µmol, standardized to 25 million cells. Error bars represent the standard deviation. Cells were immunostained and probed for indicated antibodies, then scored for % cells expressing lineage markers and analyzed by qRT-PCR analysis to determine transcript levels in WA09 hESCs **(C-E)**, NPC **(F-H)**, DE **(I-K)** or Meso **(L-N).** **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$ for one-way ANOVA. All experiments were performed in biological triplicate. Error bars represent the standard deviation. Micron bars, 100 μm. See also Figures S3 and S5.

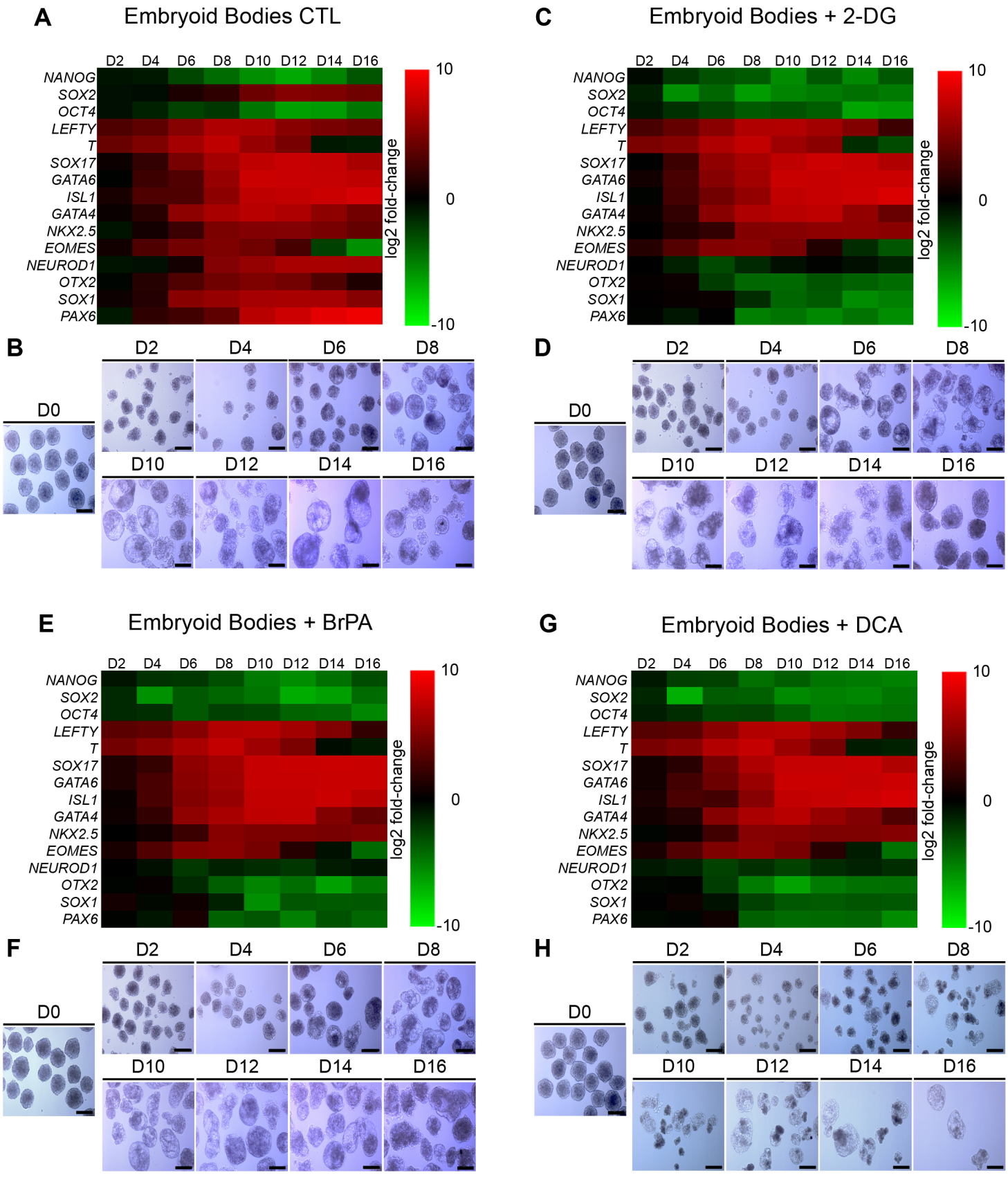

A
Embryoid Bodies CTL
C
Embryoid Bodies + 2-DG
D2 D4 D6 D8 D10 D12 D14 D16
NANOG
SOX2
OCT4
LEFTY
T
SOX17
GATA6
ISL1
GATA4
NKX2.5
EOMES
NEUROD1
OTX2
SOX1
PAX6
log2 fold-change
10
0
-10
B
D
D0
D2
D4
D6
D8
D10
D12
D14
D16
E
Embryoid Bodies + BrPA
G
Embryoid Bodies + DCA
F
H

Figure S5. Related to Figure 3. (A-H) WA09 hESCs were aggregated and cultured in 10% fetal bovine serum for 16 days to induce embryoid body differentiation as a control CTL (A-B), or in the presence of 2-deoxyglucose (2-DG, 2.2 mM)(C-D), 3-bromopyruvate (BrPA, 17μM)(E-F) or dichloroacetic acid (DCA, 2.5 mM)(G-H). (A, C, E and G) qRT-PCT heat map analysis of lineage markers analyzed every 2 days of embryoid body differentiation for the conditions indicated. (B, D, F and H) Bright field images were taken every 2 days of embryoid body differentiation for the conditions indicated. Micron bars, 200 μm See also Figures S3 and S4.

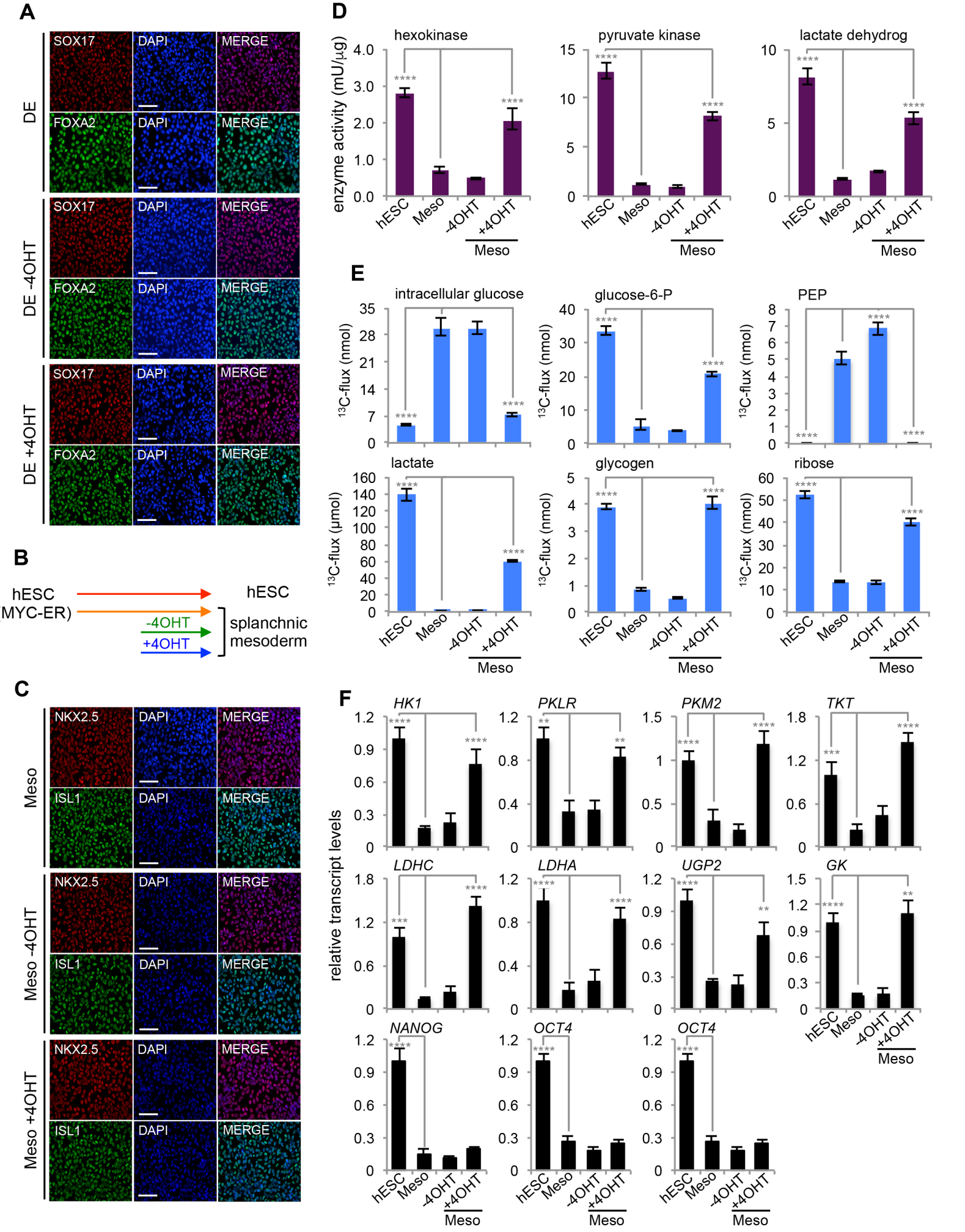

A
DE
DE -4OHT
DE +4OHT
SOX17
FOXA2
DAPI
MERGE
B
hESC
(MYC-ER)
hESC
-4OHT
+4OHT
splanchnic
mesoderm
C
Meso
Meso -4OHT
Meso +4OHT
NKX2.5
ISL1
D
hexokinase
pyruvate kinase
lactate dehydrog
enzyme activity (mU/μg)
E
intracellular glucose
glucose-6-P
PEP
lactate
glycogen
ribose
13C-flux (nmol)
13C-flux (μmol)
F
HK1
PKLR
PKM2
TKT
LDHC
LDHA
UGP2
GK
NANOG
OCT4
OCT4
relative transcript levels
hESC
Meso
-4OHT
+4OHT
Meso

Figure S6. Related to Figure 5. **(A)** Immunofluorescence analysis of WA09-derived definitive endoderm (DE) or DE derived from a WA09 line carrying a MYC-ER transgene cultured with or without 4-hydroxytamoxifen (4OHT; 1 μM), for the final 48 hours of differentiation. Cells were probed with antibodies for SOX17, FOXA2 and counterstained with DAPI. **(B)** Experimental scheme where a MYC-ER transgene is under control of 4OHT. **(C)** Immunofluorescence analysis of WA09-derived mesoderm (Meso) or Meso derived from a WA09 line carrying a MYC-ER transgene cultured with or without 4-hydroxytamoxifen (4OHT; 1 μM), for the final 48 hours of differentiation. Cells were probed with antibodies for NKX2.5, ISL1 and counterstained with DAPI. **(D)** Enzyme activity assays for hexokinase, pyruvate kinase and lactate dehydrogenase corresponding to samples described in (B). **(E)** ^{13}C-glucose NMR metabolic flux analysis following 4 hr labeling. **(F)** qRT-PCR analysis of metabolic genes for samples described in (B). **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$ for one-way ANOVA. All experiments were performed in biological triplicate. Error bars represent the standard deviation. Micron bars, 100 μm.

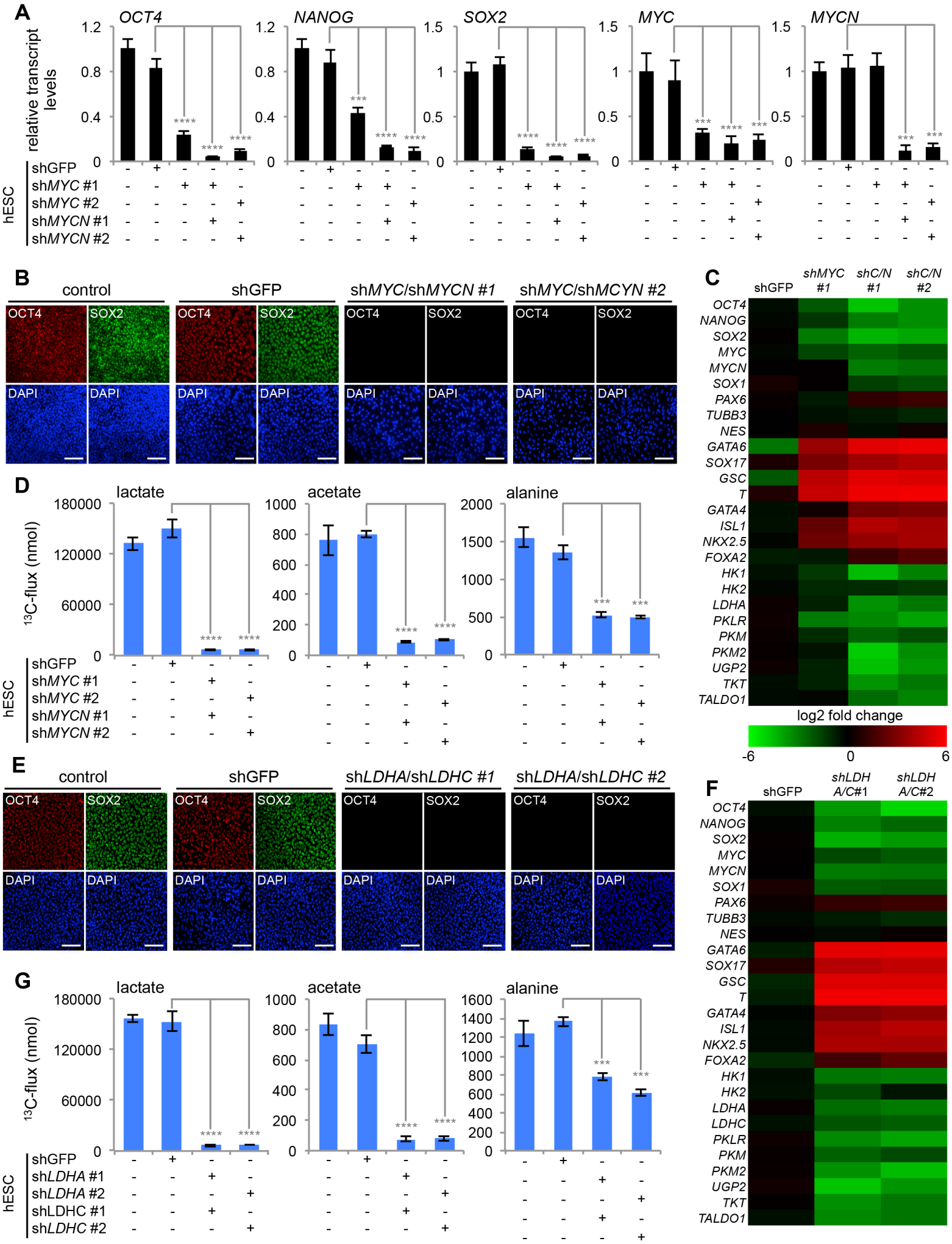

A
OCT4
NANOG
SOX2
MYC
MYCN
relative transcript levels
hESC
shGFP
shMYC #1
shMYC #2
shMYCN #1
shMYCN #2
B
control
shGFP
shMYC/shMYCN #1
shMYC/shMCYN #2
OCT4
SOX2
DAPI
C
shGFP
shMYC #1
shC/N #1
shC/N #2
OCT4
NANOG
SOX2
MYC
MYCN
SOX1
PAX6
TUBB3
NES
GATA6
SOX17
GSC
T
GATA4
ISL1
NKX2.5
FOXA2
HK1
HK2
LDHA
PKLR
PKM
PKM2
UGP2
TKT
TALDO1
log2 fold change
-6
0
6
D
lactate
acetate
alanine
13C-flux (nmol)
hESC
shGFP
shMYC #1
shMYC #2
shMYCN #1
shMYCN #2
E
control
shGFP
shLDHA/shLDHC #1
shLDHA/shLDHC #2
OCT4
SOX2
DAPI
F
shGFP
shLDH A/C#1
shLDH A/C#2
OCT4
NANOG
SOX2
MYC
MYCN
SOX1
PAX6
TUBB3
NES
GATA6
SOX17
GSC
T
GATA4
ISL1
NKX2.5
FOXA2
HK1
HK2
LDHA
LDHC
PKLR
PKM
PKM2
UGP2
TKT
TALDO1
G
lactate
acetate
alanine
13C-flux (nmol)
hESC
shGFP
shLDHA #1
shLDHA #2
shLDHC #1
shLDHC #2

Figure S7. Related to Figure 6. (A) qRT-PCR analysis of transcripts in untreated WA09 hESCs and WA09 hESCs transduced with lentivirus expressing shRNA for GFP shRNA, *MYC* shRNA#1, co-expressing *MYC* shRNA#1 and *MYCN* shRNA#1, or co-expressing *MYC* shRNA#2 and *MYCN* shRNA#2 for 3 days. **(B)** Immunofluorescence of pluripotency markers in hESCs and hESCs transduced with lentivirus expressing GFP shRNA, co-expressing *MYC* shRNA#1 and *MYCN* shRNA#1, or co-expressing *MYC* shRNA#2 and *MYCN* shRNA#2 for 3 days. Cells were probed with antibodies for OCT4 and SOX2 then counterstained with DAPI. **(C)** qRT-PCT heat map analysis of lineage marker and metabolic 'switch' transcripts of hESCs transduced with lentivirus as in (B). **(D)** ^{13}C-glucose metabolic flux analysis of hESCs transduced with lentivirus as in (B). **(E)** Immunofluorescence of pluripotency markers in hESCs and hESCs transduced with lentivirus expressing GFP shRNA, co-expressing *LDHA* shRNA#1 and *LDHC* shRNA#1, or co-expressing *LDHA* shRNA#2 and *LDHC* shRNA#2 for 3 days. Cells were probed with antibodies for OCT4 and SOX2 then counterstained with DAPI. **(F)** qRT-PCT heat map analysis of lineage marker and metabolic 'switch' transcripts of hESCs transduced with lentivirus as in (E). **(G)** ^{13}C-glucose metabolic flux analysis of hESCs transduced with lentivirus as in (E). All experiments were performed in biological triplicate. Error bars represent the standard error of the mean. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$ for one-way ANOVA. Micron bars, 100 μm.

Pluripotency Gene Expression During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
KLF4	Pluripotency	-3.173154	-4.190825	-2.277282	-2.307098
NANOG	Pluripotency	-3.331884	-3.034006	-10.782607	-9.962280
POU5F1	Pluripotency	-2.999814	-3.446101	-8.510546	-3.694330
PRDM14	Pluripotency	-2.592410	-6.367654	-7.097048	-6.195772
ZFP42	Pluripotency	-2.092724	-0.072368	-3.308495	-2.809496

Definitive Endoderm Gene Expression During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
CXCR4	Definitive Endoderm	5.668775	2.646484	3.052960	2.505940
EOMES	Definitive Endoderm	6.404072	0.183859	-6.118045	-6.108228
FOXA2	Definitive Endoderm	4.844044	-1.721897	-9.276017	-2.378229
GATA4	Definitive Endoderm	5.466895	6.488035	-5.100461	-6.321379
GSC	Definitive Endoderm	6.583340	-1.581418	1.906632	-1.010280
HNF4A	Definitive Endoderm	6.178235	0.667016	-1.064051	-4.474930
LEFTY1	Definitive Endoderm	4.312236	-4.583354	-8.338195	-8.825046
LEFTY2	Definitive Endoderm	4.375727	-2.565824	-9.714591	-10.772287
MIXL1	Definitive Endoderm	4.639764	-0.161733	-5.270483	-2.979131
SOX17	Definitive Endoderm	5.606928	0.725710	-10.278984	-10.404726

Splanchnic Mesoderm Gene Expression During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
BMP4	Splanchnic Mesoderm	0.200105	2.961441	-1.749198	-2.194169
FGF1	Splanchnic Mesoderm	0.294504	2.491767	-4.303811	1.791004
FGF10	Splanchnic Mesoderm	3.972917	7.532046	3.647088	4.870232
FLT1	Splanchnic Mesoderm	-1.343571	2.269793	-9.025288	-7.018089
FOXF1	Splanchnic Mesoderm	4.332894	12.824778	0.000000	0.000000
GATA6	Splanchnic Mesoderm	6.169624	3.589645	-4.179136	-7.020126
HAND1	Splanchnic Mesoderm	-0.979480	9.218725	-4.153407	-5.408205
HAND2	Splanchnic Mesoderm	0.911589	9.551576	0.398741	-4.912937
ISL1	Splanchnic Mesoderm	1.244146	7.035355	-8.154494	-8.067838
KDR	Splanchnic Mesoderm	-0.751757	3.049109	-3.683870	-4.006289
TBX2	Splanchnic Mesoderm	-0.001316	8.915457	-2.391443	-2.305121
TBX3	Splanchnic Mesoderm	3.369438	8.113484	-6.517687	-4.301420

Neural Progenitor Gene Expression During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
DLK1	Neural Progenitor Cell	2.493684	0.276297	9.273796	7.911424
FGF9	Neural Progenitor Cell	1.473410	2.204802	6.390610	3.973812
FOXG1	Neural Progenitor Cell	-0.623125	1.740852	7.697633	-1.611979
PAX6	Neural Progenitor Cell	3.160407	-0.216104	6.768757	1.948397
POU3F2	Neural Progenitor Cell	-1.644328	1.830727	6.637449	5.497850
SIX3	Neural Progenitor Cell	2.883278	-0.671157	8.130725	-2.156777
SOX1	Neural Progenitor Cell	-3.427894	-3.312336	4.115514	5.073688

Neural Crest Gene Expression During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
FOXB1	Neural Crest Cell	-0.507943	-3.298677	2.837312	6.329724
HOXB2	Neural Crest Cell	2.428695	7.977027	-2.399459	7.436806
ILDR2	Neural Crest Cell	-1.288922	0.022193	6.079583	7.535009
NCAM1	Neural Crest Cell	1.808666	4.147528	4.799465	3.874779
OLIG3	Neural Crest Cell	-6.443079	-8.213853	-3.521014	5.949257
PAX3	Neural Crest Cell	1.639903	6.965333	4.668740	10.106077
WNT8A	Neural Crest Cell	1.367015	0.643376	-1.415005	4.034953

Metabolic Genes That Do Not Change During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SplM**	**NPC**	**NCC**
ACSS2	Acetate Synthesis	1.487606	0.990889	-1.895270	0.278523
ACSS3	Acetate Synthesis	1.514499	0.119983	-0.244679	0.111512
AMT,NICN1	Cysteine Synthesis	-0.051919	-0.606177	-0.234320	-0.377296
CBS	Cysteine Synthesis	-0.287921	-0.579454	-1.516852	-1.433267
CTH	Cysteine Synthesis	-0.312763	-0.264083	-0.952939	-1.389898
DLAT	Acetate Synthesis	0.082696	-0.159619	-1.421213	-0.738727
DLD	Acetate Synthesis	-0.196138	-0.204578	-1.066833	-1.024097
ENO1	Glycolysis	0.757081	0.232158	-0.644414	0.661793
ENO1-AS1	Glycolysis	0.362028	-0.166477	-0.683830	-1.433399
ENO2,LRRC23	Glycolysis	-1.073721	-0.348490	0.276054	0.497133
ENO3	Glycolysis	-0.427934	-0.028448	0.719242	1.512329

Metabolic Genes That Do Not Change During Differentiation Continued

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SpIM**	**NPC**	**NCC**
ENO4	Glycolysis	1.081168	-0.190592	1.055834	-0.067863
FBP1	Glycolysis	0.318078	0.341743	-1.621244	-0.629184
FBP2	Glycolysis	0.004424	0.523966	2.197043	1.083456
G6PD	Pentose Phosphate Pathway	0.539282	0.497256	-0.795225	0.532202
GAPDH	Glycolysis	-0.138498	-0.287382	-0.442516	0.398546
GBE1	Glycogen Synthesis	0.130743	1.243874	1.093198	2.757731
GCK	Glycolysis	-0.906023	-2.469274	-5.952966	-1.946830
GCLC	Glutathione Synthesis	0.072580	-1.126622	-1.194829	-0.904549
GCLM	Glutathione Synthesis	-0.024173	1.524666	-2.432630	2.854309
GCSH	Cysteine Synthesis	0.449162	-0.352752	-0.652549	-0.132505
GFPT1	UDP-GlcNac Synthesis	-0.015703	0.137117	-1.505189	-0.713787
GFPT2	UDP-GlcNac Synthesis	-0.545241	-1.032710	-2.093027	-2.259530
GK2	Glycerol Synthesis	0.000000	3.787056	0.000000	0.000000
GK3P	Glycerol Synthesis	-0.805642	-0.672326	-0.050156	-0.346149
GK5	Glycerol Synthesis	-0.078488	-0.009743	1.314329	1.551809
GLDC	Cysteine Synthesis	-1.390186	-0.870147	-0.835756	-2.053789
GNPNAT1	UDP-GlcNac Synthesis	-0.335352	-0.962786	-1.497488	-1.074860
GPT	Alanine Synthesis	0.502870	-0.851163	-0.434115	-0.241868
GPT2	Alanine Synthesis	-0.512235	-0.264590	-0.344619	-0.747844
GSS	Glutathione Synthesis	6.750928	0.073857	-0.226385	-0.170855
GYS1	Glycogen Synthesis	0.513184	0.770647	-0.247798	-0.229391
GYS2	Glycogen Synthesis	2.011638	4.656649	-3.148524	0.590444
HK2	Glycolysis	-1.430568	-1.046071	-0.222024	0.937562
LDHAL6B	Glycolysis	4.745221	0.000000	5.522373	4.461843
LDHB	Glycolysis	-0.357299	-0.606726	-1.050780	-0.211688
MPC1	Acetate Synthesis	-0.572330	0.611876	-1.276846	0.116017
MPC1L	Acetate Synthesis	0.000000	0.000000	0.000000	0.000000
MPC2	Acetate Synthesis	0.303458	0.790915	-0.465668	-0.044965
PCK1	Glycolysis	2.085343	-2.791076	0.791856	-0.199815
PCK2	Glycolysis	-0.934111	-0.023690	-1.566246	-1.621378
PDHA1	Acetate Synthesis	0.010744	-0.034505	-1.116590	-0.352136
PDHA2	Acetate Synthesis	0.585053	0.007628	-1.009428	-0.264661
PDHB	Acetate Synthesis	0.786040	-0.498255	-1.485763	-0.974935
PDHX	Acetate Synthesis	-0.179092	-0.487517	-1.259007	-0.611034
PFKL	Glycolysis	1.075353	0.401012	0.051100	0.509836
PFKM	Glycolysis	0.049463	0.029069	0.928407	0.537045
PFKP	Glycolysis	-0.239436	1.917099	-1.153647	-0.408951
PGAM1	Glycolysis	0.181542	-0.083265	-0.404225	0.342419
PGAM1P5	Glycolysis	0.093678	-0.474565	-0.586307	0.166588
PGAM4	Glycolysis	0.163281	-0.200700	-0.319031	0.375523
PGAM5	Glycolysis	-0.316427	-0.576164	-1.141041	-0.811630
PGD	Pentose Phosphate Pathway	0.202128	-0.376871	-0.454336	0.161930
PGK1	Glycolysis	0.007423	-0.205290	-0.242146	0.946452
PGK2	Glycolysis	1.955918	0.102789	1.114214	-0.268738
PGLS	Pentose Phosphate Pathway	1.218774	1.091150	0.472890	0.413669
PGM1	Glycogen Synthesis	1.660509	-0.822978	-2.461635	-0.747500
PGM2	Glycogen Synthesis	-0.189654	0.660623	-1.693061	-0.496548
PGM2L1	Glycogen Synthesis	-0.155362	-0.694360	-0.896342	-2.151411
PGM3	Glycogen Synthesis	0.250487	0.766598	-0.629725	0.245196
PGM5	Glycogen Synthesis	2.337968	6.797313	4.359064	0.635568
PGM5-AS1	Glycogen Synthesis	0.000000	0.128476	-0.440655	0.970590
PGM5P2	Glycogen Synthesis	0.736089	2.454167	1.613584	-0.474296
PHGDH	Cysteine Synthesis	-1.204325	-0.590222	-1.000717	-0.948131
PSPH	Cysteine Synthesis	-0.089370	-0.009318	-0.201282	-0.856129
RPE	Pentose Phosphate Pathway	-0.461536	-0.496626	-1.011676	-0.688311
RPIA	Pentose Phosphate Pathway	-0.240813	-0.038142	-0.593137	-0.654932
SHMT1,TOP3A	Cysteine Synthesis	-0.747739	0.426925	-0.269473	-0.373987
SHMT2	Cysteine Synthesis	-0.747949	-0.161237	-0.370181	0.085102
SLC2A1-AS1	Glucose Transporters	-0.853790	-0.114143	1.298722	2.515161
SLC2A10	Glucose Transporters	0.783191	1.060499	0.434643	-0.115255
SLC2A11	Glucose Transporters	0.129494	0.296128	0.522482	0.596980
SLC2A12	Glucose Transporters	0.188638	-0.121903	1.673748	0.477801
SLC2A13	Glucose Transporters	0.481329	0.636043	-0.569662	0.211400
SLC2A14	Glucose Transporters	2.380354	1.372044	-0.683505	1.389201
SLC2A2	Glucose Transporters	0.000000	0.000000	0.000000	0.000000
SLC2A3	Glucose Transporters	2.822870	1.605607	-0.422098	1.654429
SLC2A4	Glucose Transporters	0.646290	-1.271195	-1.634528	1.113473
SLC2A4RG	Glucose Transporters	1.040207	1.208481	0.257236	0.300861
SLC2A5	Glucose Transporters	-0.316449	-0.103270	-5.555604	-5.459805

Metabolic Genes That Do Not Change During Differentiation Continued

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SpIM**	**NPC**	**NCC**
SLC2A6	Glucose Transporters	0.573633	0.268766	-1.893726	-0.661408
SLC2A7	Glucose Transporters	0.000000	0.000000	0.000000	0.000000
SLC2A8	Glucose Transporters	-0.054026	0.646370	-0.903229	-0.504788
SLC2A9	Glucose Transporters	1.866827	3.018429	-0.175891	-0.176386
TPI1	Glycolysis	-0.466675	-0.793688	-1.035595	-0.083561
UAP1	UDP-GlcNac Synthesis	0.230896	0.491817	-0.635158	-1.497488

Metabolic Target Genes That Do Change During Differentiation

		log2 fold change over hESCs			
Gene	**Pathway**	**DE**	**SpIM**	**NPC**	**NCC**
ACOT12	Acetate Synthesis	-6.678269	-6.668488	-2.603160	0.635880
ACSS1	Acetate Synthesis	-1.660677	-1.794854	-6.696773	0.682714
GAPDHS	Glycolysis	-2.809471	-1.540548	0.007030	0.293468
GK	Glycerol Synthesis	-2.867121	-3.321958	0.616713	0.651652
GPI	Glycolysis	-2.617558	-3.876663	-0.033789	-0.604992
HK1	Glycolysis	-2.328135	-3.141469	-0.282716	-0.059148
HK3	Glycolysis	-3.351275	-3.483042	-0.310056	-3.275697
LDHA	Glycolysis	-3.854462	-3.402367	0.375032	1.315792
LDHAL6A	Glycolysis	-6.252194	-6.231780	-2.087180	-2.053883
LDHC	Glycolysis	-3.847299	-2.715817	-0.991612	-0.935105
LDHD	Glycolysis	-3.461847	-5.882240	-1.378907	-1.513888
PGAM2	Glycolysis	-1.623741	-2.872233	0.442770	1.095669
PKLR	Glycolysis	-4.488534	-4.144053	1.474035	0.716112
PKM	Glycolysis	-2.920433	-2.379239	-0.350156	0.147448
PSAT1	Cysteine Synthesis	-2.164498	-3.961949	-0.851356	0.260383
SLC2A1	Glucose Transporters	-1.917720	-2.234013	2.057408	2.935043
TALDO1	Pentose Phosphate Pathway	-3.440604	-2.900132	0.730205	-0.049308
TKT	Pentose Phosphate Pathway	-3.406111	-2.974722	0.496059	0.258944
UGP2	Glycogen Synthesis	-3.542808	-4.455956	1.150647	-0.233097

Table S1. **Related to Figure 4**. Table showing levels of pluripotency, definitive endoderm, mesoderm and neural progenitor cell-specific transcripts, represented in Figure 4C. Metabolic transcripts, defined by gene ontology analysis, that remain constant during differentiation ('non-switch') or those that mirror metabolic switching ('switch' transcripts) are shown.

Primary Antibody	Application	Dilution or Concentration	Vendor	Product Number
CDK2	WB	1:2000	Santa Cruz Biotechnology	sc-163
FOXA2	IF	1:40	R&D Systems	AF2400
HK1	WB	1:1000	Santa Cruz Biotechnology	sc-6521
HK2	WB	1:1000	Santa Cruz Biotechnology	sc-6518
IGG	ChIP	1 ug/million cells	Abcam	ab46540
ISL1	IF	1:20	R&D systems	AF1837
LDHA	WB	1:1000	Santa Cruz Biotechnology	sc-27238
LDHC	WB	1:1000	Santa Cruz Biotechnology	sc-27230
MYC	WB	1:1000	Santa Cruz Biotechnology	sc-764
MYC	ChIP	1 ug/million cells	Abcam	ab56
MYCN	WB	1:1000	Santa Cruz Biotechnology	sc-791
MYCN	ChIP	1 ug/million cells	Santa Cruz Biotechnology	sc-53993
NKX2.5	IF	1:50	R&D Systems	MAB2444
OCT3/4	IF	1:100	Santa Cruz Biotechnology	sc-9081
OCT3/4	FLOW	1:2000	Santa Cruz Biotechnology	sc-5279
PAX6	IF	1:100	BioLegend	901301
PKLR	WB	1:1000	Cell Signaling Technology	7067S
PKM1	WB	1:1000	Cell Signaling Technology	4053S
PKM2	WB	1:1000	Santa Cruz Biotechnology	sc-133224
SOX1	IF	1:200	Abcam	ab87775
SOX17	IF	1:40	R&D systems	AF1924
SOX2	FLOW/IF	1:2000/1:200	Santa Cruz Biotechnology	sc-17320

Secondary Antibody	Application	Dilution or Concentration	Vendor	Product Number
Alexa Fluor 488 donkey anti-mouse IgG	FLOW/IF	1:400 both	Thermo Fisher	A21202
Alexa Fluor 488 donkey anti-rabbit IgG	FLOW/IF	1:400 both	Thermo Fisher	A21206
Alexa Fluor 555 donkey anti-goat IgG	IF	1:400	Thermo Fisher	A21432
Alexa Fluor 555 donkey anti-mouse IgG	IF	1:400	Thermo Fisher	A31570
Alexa Fluor 555 donkey anti-rabbit IgG	IF	1:400	Thermo Fisher	A31572
Alexa Fluor 647 donkey anti-goat IgG	FLOW/IF	1:350 both	Thermo Fisher	A21447
Goat Anti-Rabbit Immunoglobulins/HRP	WB	1:2000	Dako	P0448
Rabbit Anti-Goat Immunoglobulins/HRP	WB	1:2000	Dako	P0449
Rabbit Anti-Mouse Immunoglobulins/HRP	WB	1:2000	Dako	P0161

Table S2. **Related to Methods**. Table of primary and secondary antibodies used for immunoblots (WB), immunofluorescence (IF), quantitative ChIP (ChIP), or flow cytometry (FLOW), with dilutions or concentrations listed.

Gene	Product Number	Gene	Product Number
EOMES	Hs00172872_m1	NKX2.5	Hs00231763_m1
FOXA2	Hs00232764_m1	OTX2	Hs00222238_m1
FOXF1	Hs00230962_m1	PAX3	Hs00240950_m1
G6PC	Hs00609178_m1	PAX6	Hs00240871_m1
G6PD	Hs00166169_m1	PDK1	Hs00176853_m1
GATA4	Hs00171403_m1	PDK2	Hs00176865_m1
GATA6	Hs00232018_m1	PDK3	Hs00178440_m1
GCK	Hs01564555_m1	PDK4	Hs01037712_m1
GLCM	Hs00978072_m1	PFKFB1	Hs00159997_m1
GSC	Hs00906630_g1	PFKL	Hs00160027_m1
HAND1	Hs02330376_s1	PFKM	Hs00175997_m1
HK1	Hs00175976_m1	PKLR	Hs00176075_m1
HK2	Hs00606086_m1	PKM	Hs00987255_m1
HK3	Hs01092850_m1	PKM2	Hs00762869_s1
ISL1	Hs00158126_m1	POU5F1	Hs01895061_u1
LDHA	Hs00855332_g1	RNA 18S5	Hs03928985_g1
LDHB	Hs00600794_mH	SLC16A1	Hs00161826_m1
LDHC	Hs00255650_m1	SOX1	Hs01057642_s1
LDHD	Hs00544860_m1	SOX17	Hs00751752_s1
MIX1	Hs00430824_g1	SOX2	Hs01053049_m1
MYC	Hs00153408_m1	T	Hs00610080_m1
MYCN	Hs00232074_m1	TALDO1	Hs00997203_m1
NANOG	Hs04399610_g1	TBX20	Hs00396596_m1
NES	Hs00707120_s1	TKT	Hs01115545_m1
NEUROD1	Hs00159598_m1	TUBB3	Hs00801390_s1
NEUROG3	Hs00360700_g1	UGP2	Hs00198879_m1

Table S3. **Related to Methods**. Table of Taqman primers utilized for qRT-PCR.

Primer	Sequence 5'-3'
HK1 Left	GGAGGAGGAGGAGGAGGAG
HK1 Right	GATCATGCTGGCGGTCGG
PKLR Down1 Left	GAGTCGCGCAATGTTCATCC
PKLR Down1 Right	GGTTCTAGGGCCAGCATCTC
PKLR Down2 Left	GGAACCTGCAAAGCTCTCCA
PKLR Down2 Right	CACCTTCCCCTGAAACCCTC
LDHA Left	CTTAGACTCCCAGCGCACG
LDHA Right	CACGTGTGAGTCGGGCTG
PKM Left	CGCTGGGGACTTCTGAAGAG
PKM Right	CGTCTGGGATGCAGTGGAG
TKT Left	GCTGGTCAGGCTTGTGGTAG
TKT Right	TATCTCTGTGTGTCCGCGTG
UGP2 Left	GGGGCGAGAATATGTACGGG
UGP2 Right	GCAGCGGACACTAAGGGG
TALDO1 Left	CCCCTCGGTCTTGCTATGTC
TALDO1 Right	GTCCTCACCGTGGAAGTCG
GK Left	GCTGGATGGCTCTGCTGT
GK Right	GCAGAGGGCGATGAGACG
GPI Left	AAGCACCACTCCCGATGTG
GPI Right	TGTGGAGACTGAACCTTGCA
PSAT1 Left	CCTCCTTGGCTGACTCACC
PSAT1 Right	CTCGCGGGGACTTACTGAG
SLC2A1 Left	GTAAGGCGGGCAGGAGTC
SLC2A1 Right	AGCAGCAAGGTGAGTCGC

Table S4. **Related to Methods**. Table of primers utilized for quantitative ChIP assays.

Compound	Vendor	Product Number	Compound	Vendor	Product Number
Acetyl-CoA lithium salt	Sigma	A2181	Glycine	Sigma	G8898
Adenosine 5′-triphosphate disodium salt hydrate	Sigma	A7699	Glycogen from rabbit liver	Sigma	G8876
Alanine	Sigma	A7627	Guanosine 5′-triphosphate sodium salt hydrate	Sigma	G8877
Aracardic acid	Sigma	A7236	Lactate	Sigma	L6661
Arginine	Sigma	A5006	Malate	Sigma	O2288
Asparagine	Sigma	A0884	Nicotinamide adenine dinucleotide reduced disodim salt hydrate	Sigma	N8129
Aspartic Acid	Sigma	A7219	Phosphatidylcholine	Sigma	P3556
Cholesterol	Sigma	C8667	Phosphoenolpyruvate monopotasssium salt	appliChem	a2271
Cysteine	Sigma	C7755	Proline	Sigma	P0380
Cytidine 5′-triphosphate disodium salt	Sigma	C1506	Pyroglutamic acid	Sigma	83160
Fructose	Sigma	F3510	Pyruvate	Sigma	107360
Fructose 1,6-bisphosphate trisodium salt hydrate	Sigma	F6803	Serine	Sigma	S4500
Fumarate	Sigma	F8509	Sodium Acetate	Sigma	S5635
Galactose	Sigma	G5388	Sodium Citrate	J.T Baker	3646-01
Glucose	Sigma	G5767	Sodium palmitate	Sigma	P9767
Glucose 1-P	Sigma	G7018	Succinate	Sigma	S9512
Glucose-6-P sodium salt	Sigma	G7879	Thymidine 5′-triphosphate sodium salt	Sigma	T0251
Glutamate	Sigma	G8415	Triacylglycerol	Sigma	T5888
Glutamine	Sigma	G8540	Uridine 5′-diphosphate disodium salt hydrate	Sigma	94330
Glutathione	Sigma	G4251	Uridine 5′-diphospho-N-acetylglucosamine sodium salt	Sigma	U4375
Glycerol	J.T Baker	2136-01	Uridine 5′-diphosphogalactose disodium salt	Sigma	94333
Glycerol 2-phosphate disodium salt hydrate	Sigma	G9891	Uridine 5′-diphosphoglucose disodium salt	Sigma	94335

Table S5. **Related to Methods**. Table of compounds used for NMR standards in these studies.