# Hsa-miR-520d induces hepatoma cells to form normal liver tissues via a stemness-mediated process

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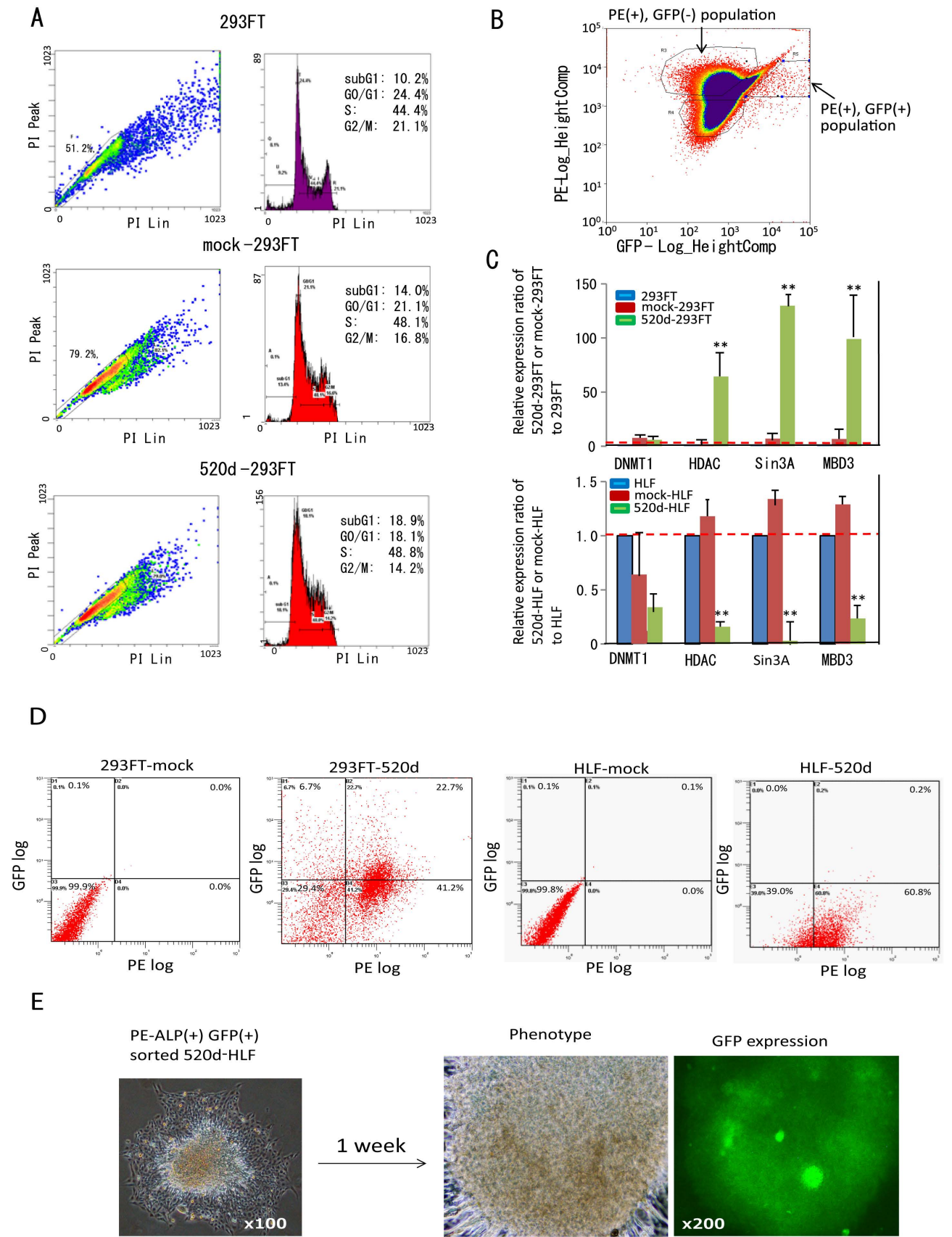
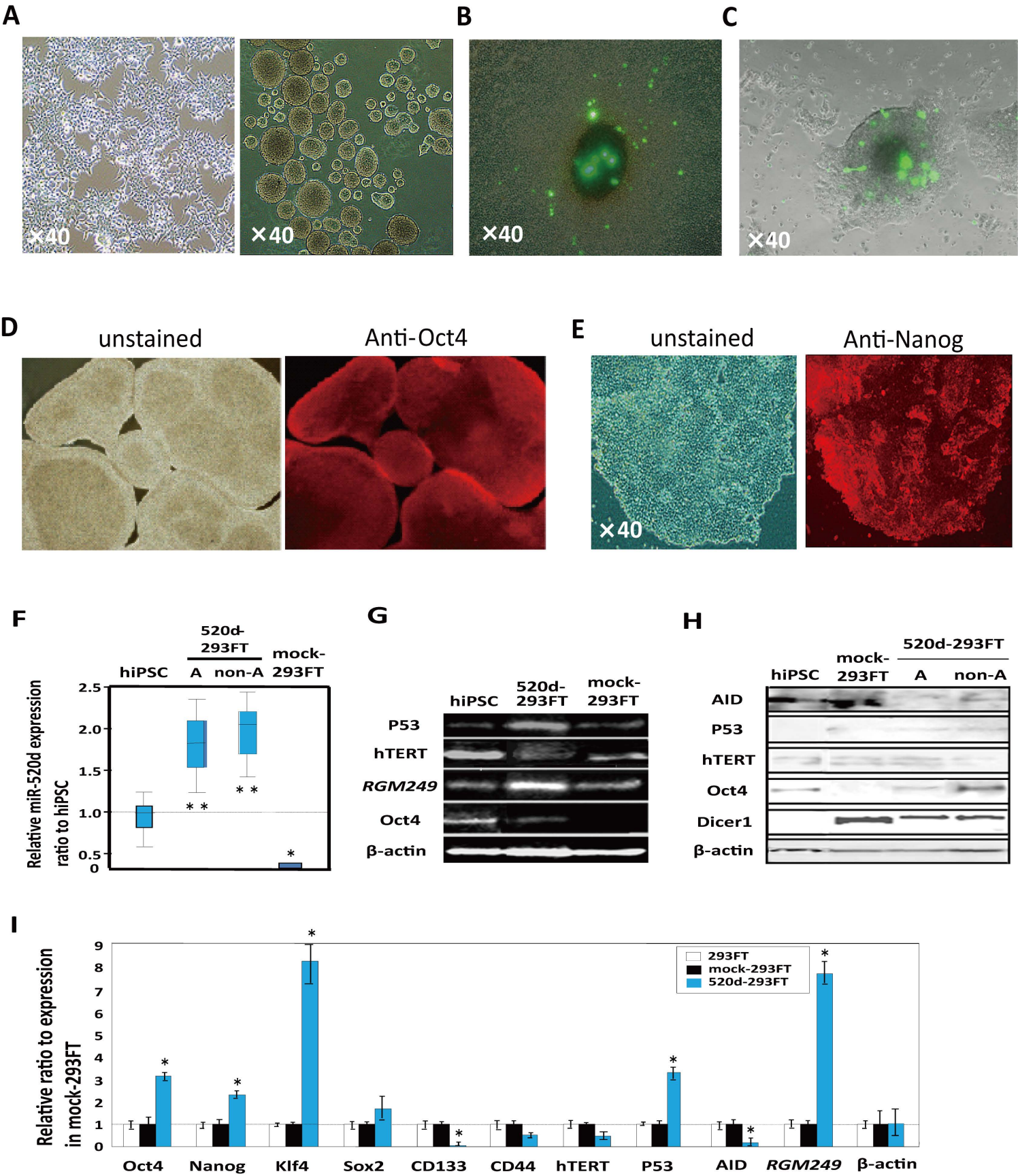
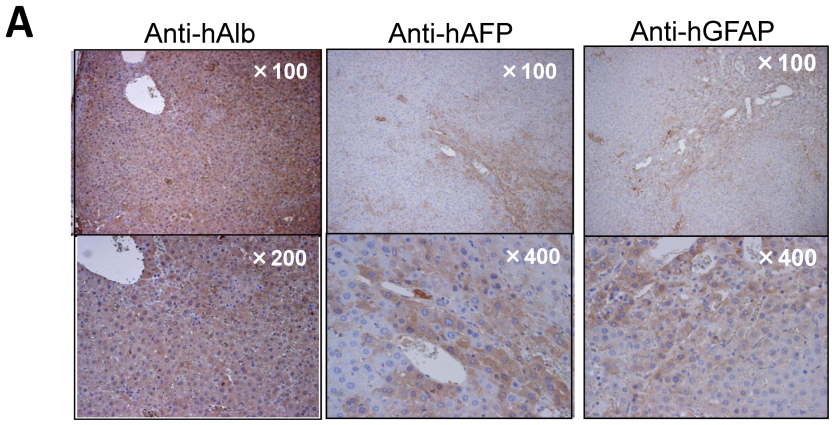
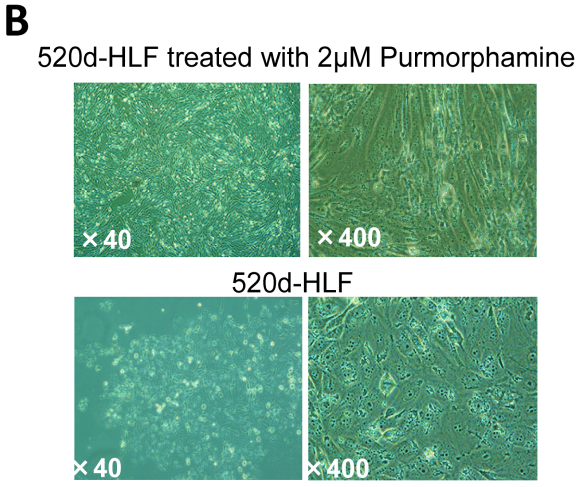
Keywords: cancer, stemness, miRNA, differentiation, iPSC

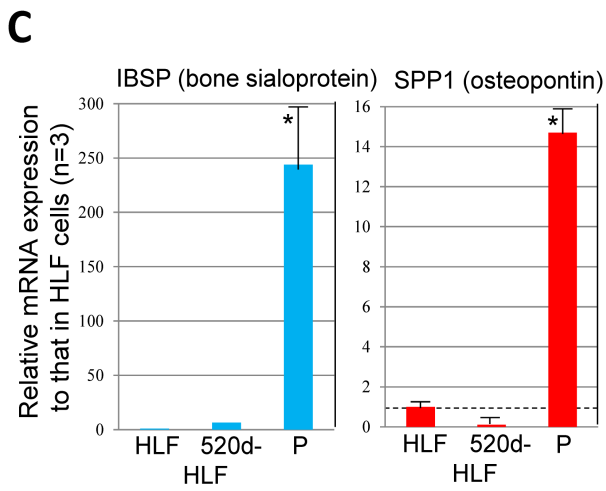
Running title: A single miRNA may revert hepatoma to pluripotent stem cells.

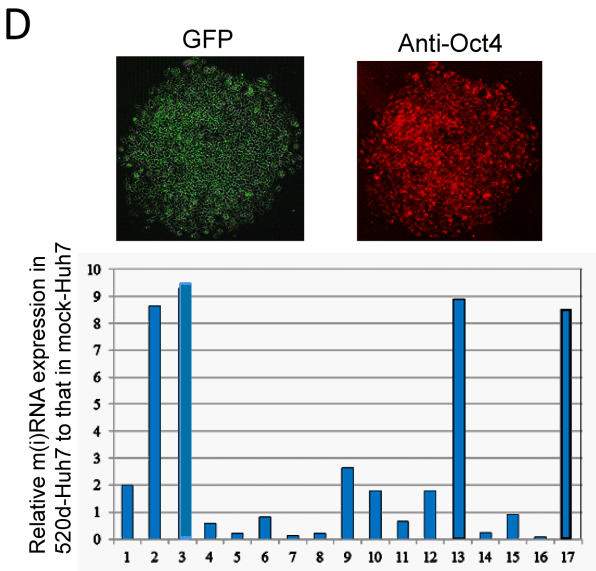

A
293FT
PI Peak
PI Lin
51.2%
subG1: 10.2%
G0/G1: 24.4%
S: 44.4%
G2/M: 21.1%
mock-293FT
79.2%
subG1: 14.0%
G0/G1: 21.1%
S: 48.1%
G2/M: 16.8%
520d-293FT
subG1: 18.9%
G0/G1: 18.1%
S: 48.8%
G2/M: 14.2%
B
PE(+), GFP(-) population
PE(+), GFP(+) population
PE-Log_HeightComp
GFP-Log_HeightComp
C
Relative expression ratio of 520d-293FT or mock-293FT to 293FT
293FT
mock-293FT
520d-293FT
DNMT1
HDAC
Sin3A
MBD3
Relative expression ratio of 520d-HLF or mock-HLF to HLF
HLF
mock-HLF
520d-HLF
D
293FT-mock
293FT-520d
HLF-mock
HLF-520d
GFP log
PE log
0.1%
99.9%
6.7%
29.4%
22.7%
41.2%
99.8%
39.0%
60.8%
E
PE-ALP(+) GFP(+)
sorted 520d-HLF
1 week
Phenotype
GFP expression
x100
x200

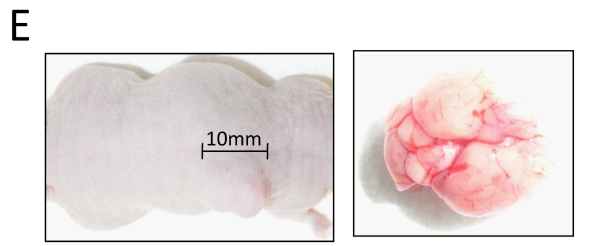

A
B
C
×40
×40
×40
×40
D
unstained
Anti-Oct4
E
unstained
Anti-Nanog
×40
F
520d-293FT
hiPSC
A
non-A
mock-293FT
Relative miR-520d expression ratio to hiPSC
2.5
2.0
1.5
1.0
0.5
0
G
hiPSC
520d-293FT
mock-293FT
P53
hTERT
RGM249
Oct4
β-actin
H
520d-293FT
hiPSC
mock-293FT
A
non-A
AID
P53
hTERT
Oct4
Dicer1
β-actin
I
293FT
mock-293FT
520d-293FT
Relative ratio to expression in mock-293FT
9
8
7
6
5
4
3
2
1
0
Oct4
Nanog
Klf4
Sox2
CD133
CD44
hTERT
P53
AID
RGM249
β-actin

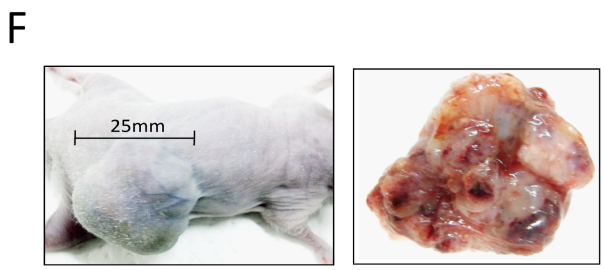

A
Anti-hAlb
Anti-hAFP
Anti-hGFAP
×100
×100
×100
×200
×400
×400

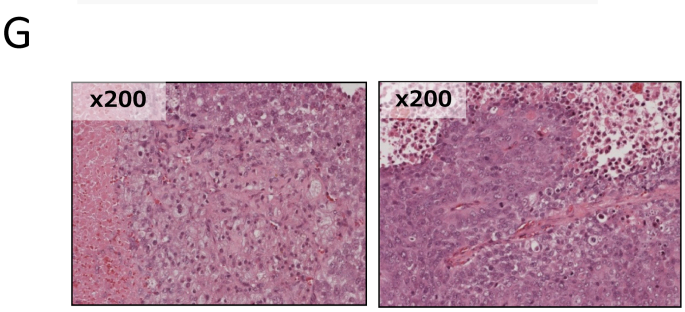

B
520d-HLF treated with 2μM Purmorphamine
×40
×400
520d-HLF
×40
×400

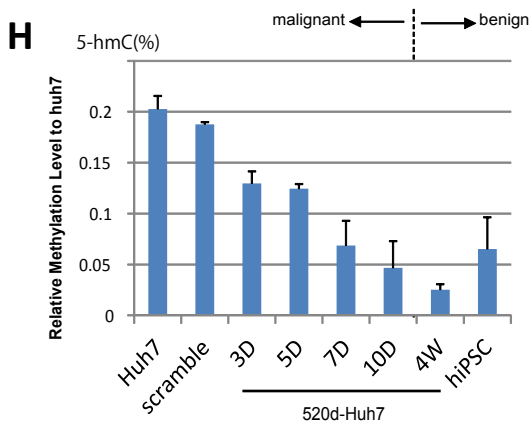

C
IBSP (bone sialoprotein)
SPP1 (osteopontin)
Relative mRNA expression to that in HLF cells (n=3)
HLF
520d-HLF
P
HLF
520d-HLF
P

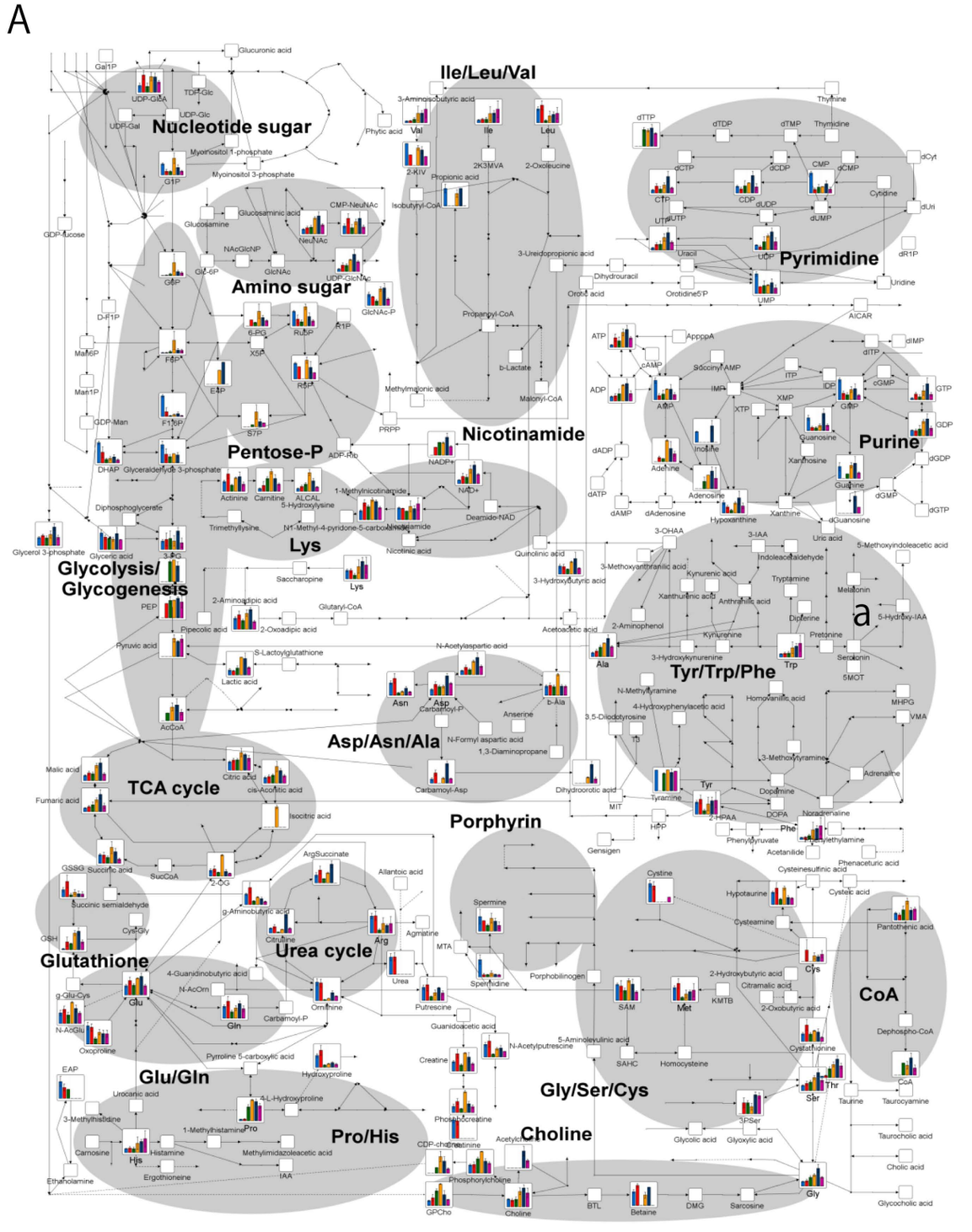

D
GFP
Anti-Oct4
Relative m(i)RNA expression in 520d-Huh7 to that in mock-Huh7

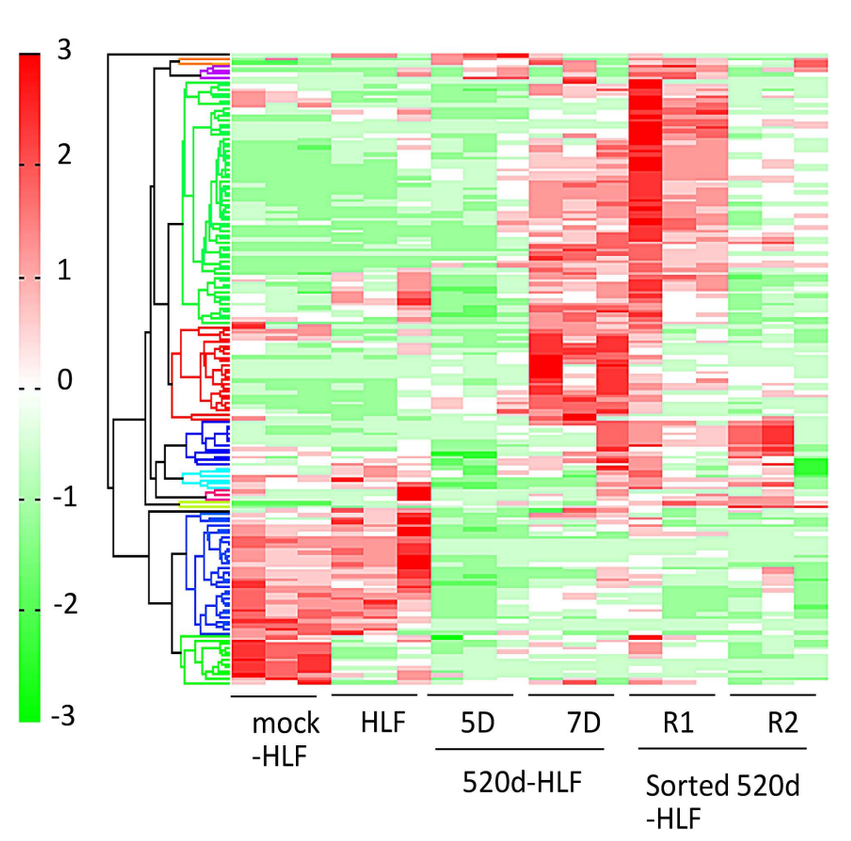

E
10mm

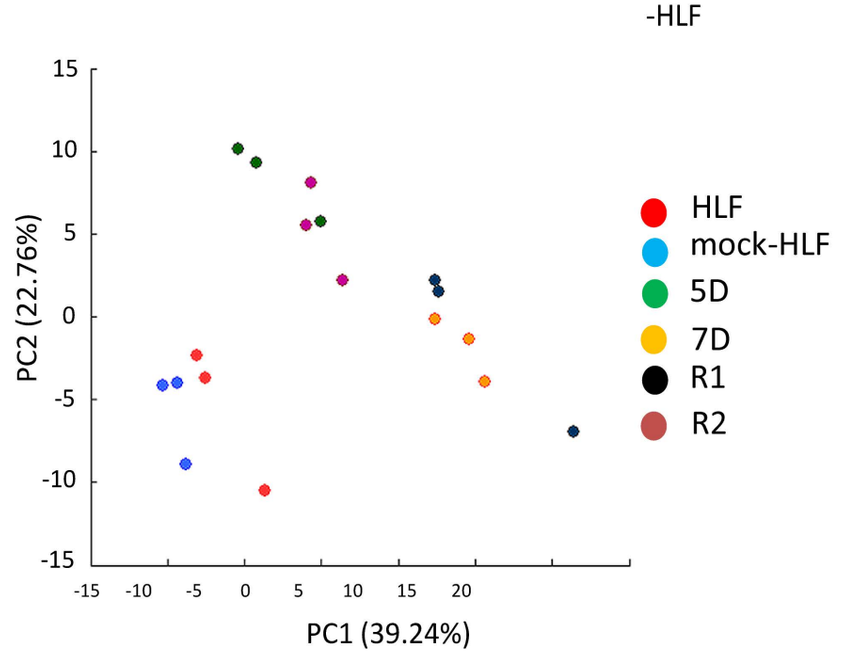

F
25mm


G
x200
x200


H
malignant
benign
5-hmC(%)
Relative Methylation Level to huh7
Huh7
scramble
3D
5D
7D
10D
4W
hiPSC
520d-Huh7


A
Nucleotide sugar
Ile/Leu/Val
Amino sugar
Pyrimidine
Nicotinamide
Pentose-P
Purine
Lys
Glycolysis/
Glycogenesis
Tyr/Trp/Phe
Asp/Asn/Ala
TCA cycle
Porphyrin
Urea cycle
Glutathione
CoA
Glu/Gln
Gly/Ser/Cys
Pro/His
Choline

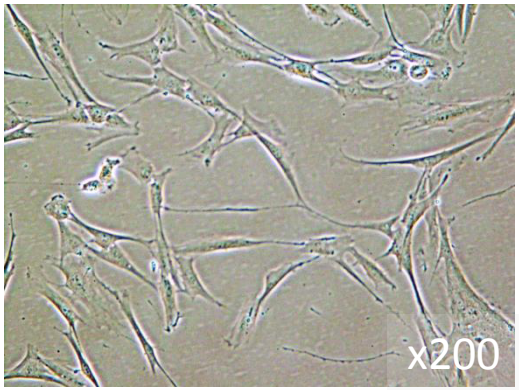

B
3
2
1
0
-1
-2
-3
mock
-HLF
HLF
5D
7D
520d-HLF
R1
R2
Sorted 520d
-HLF


C
PC2 (22.76%)
PC1 (39.24%)
HLF
mock-HLF
5D
7D
R1
R2

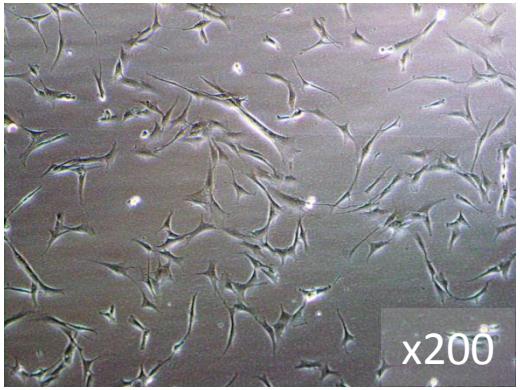

252 Primer sense (amplicon 100bp)

A

NHDF-Neo

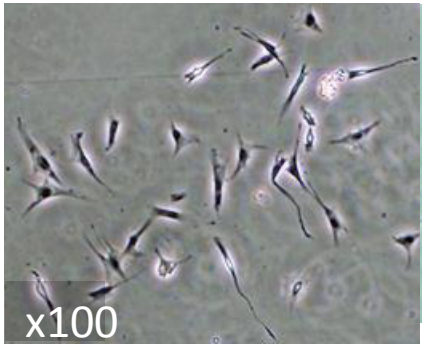



NHDF-Ad

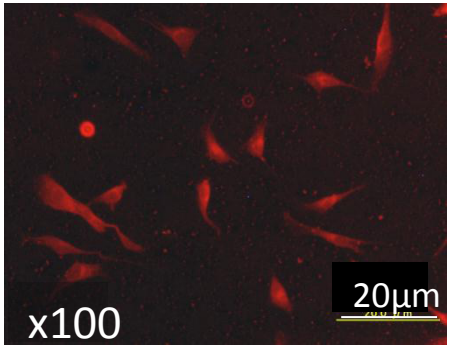



B

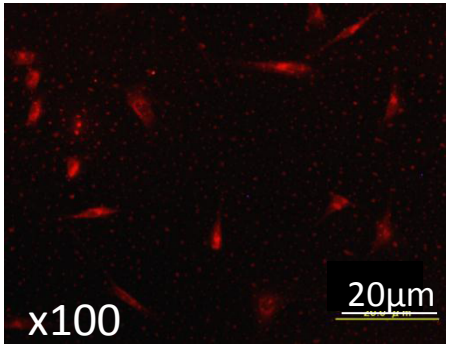
520d-NHDF-Neo




520d-NHDF-Neo Oct4

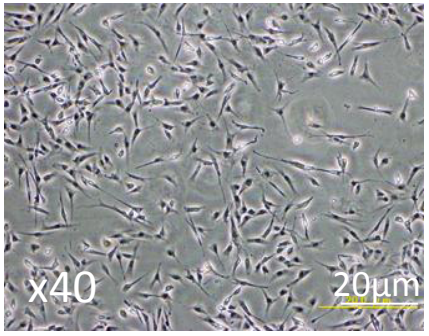



520d-NHDF-Neo Nanog

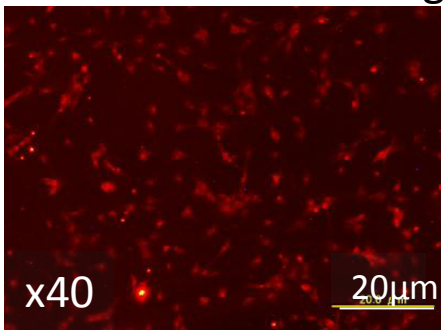



C

520d-NHDF-Ad

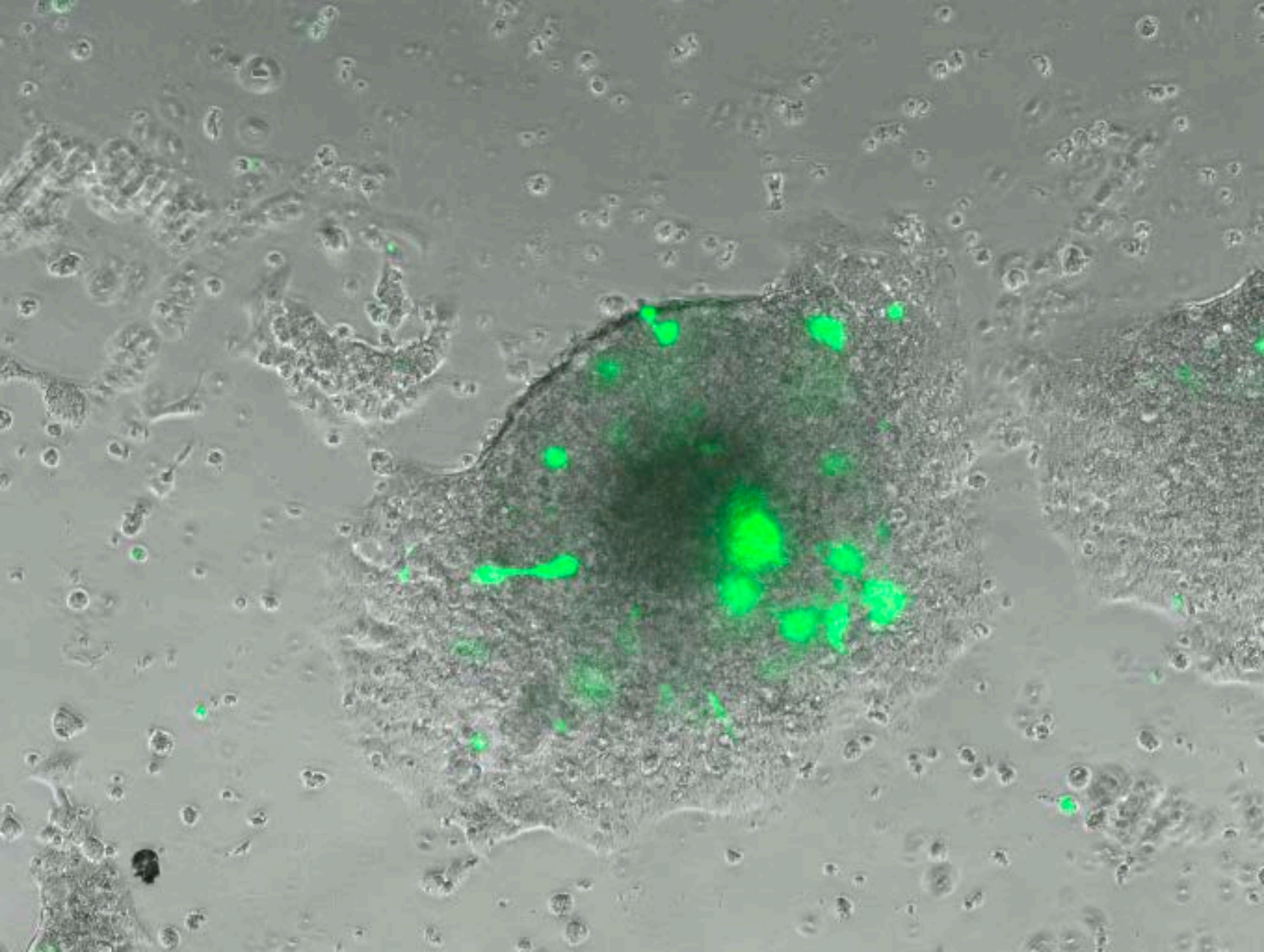


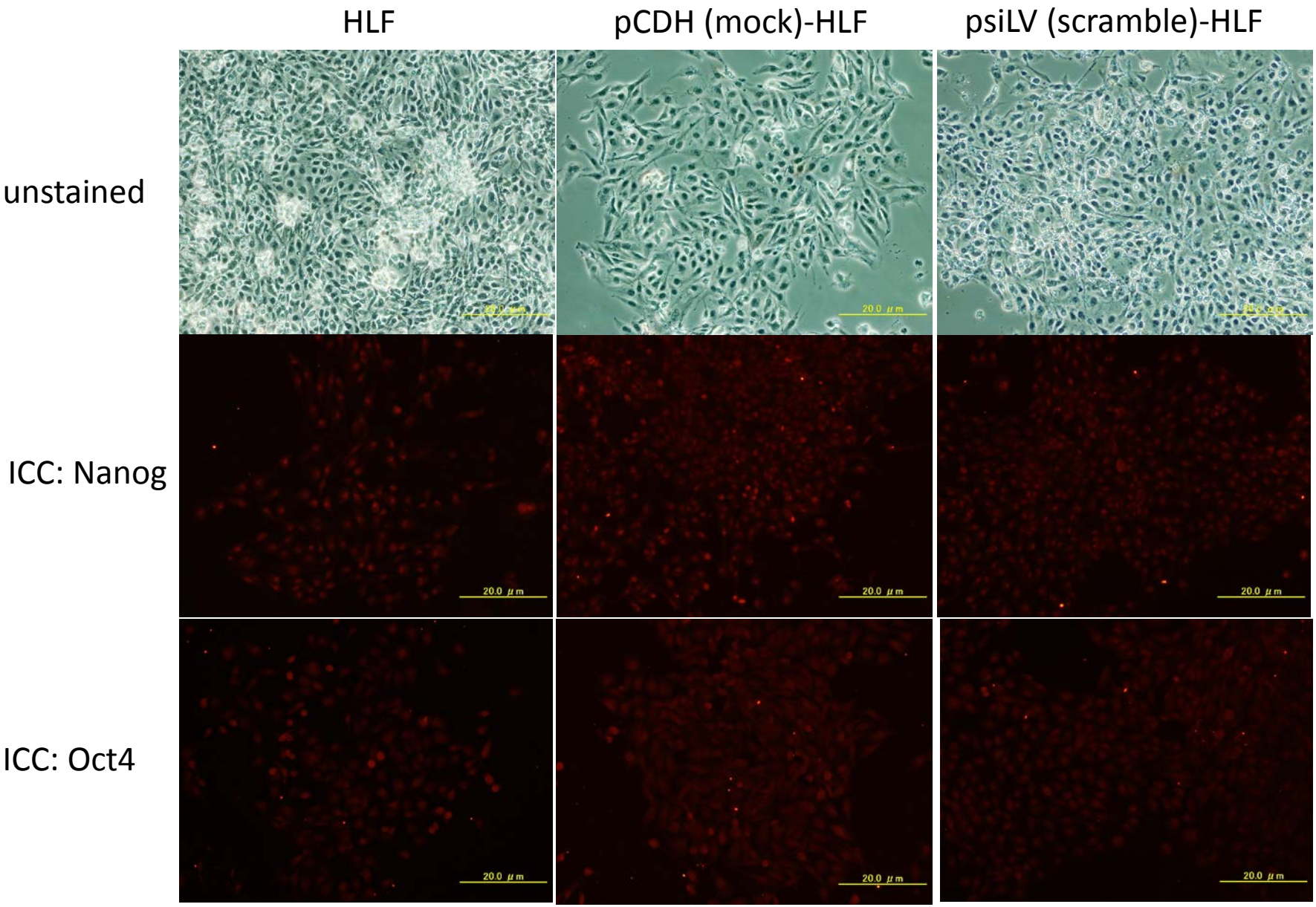

520d-NHDF-Ad Nanog

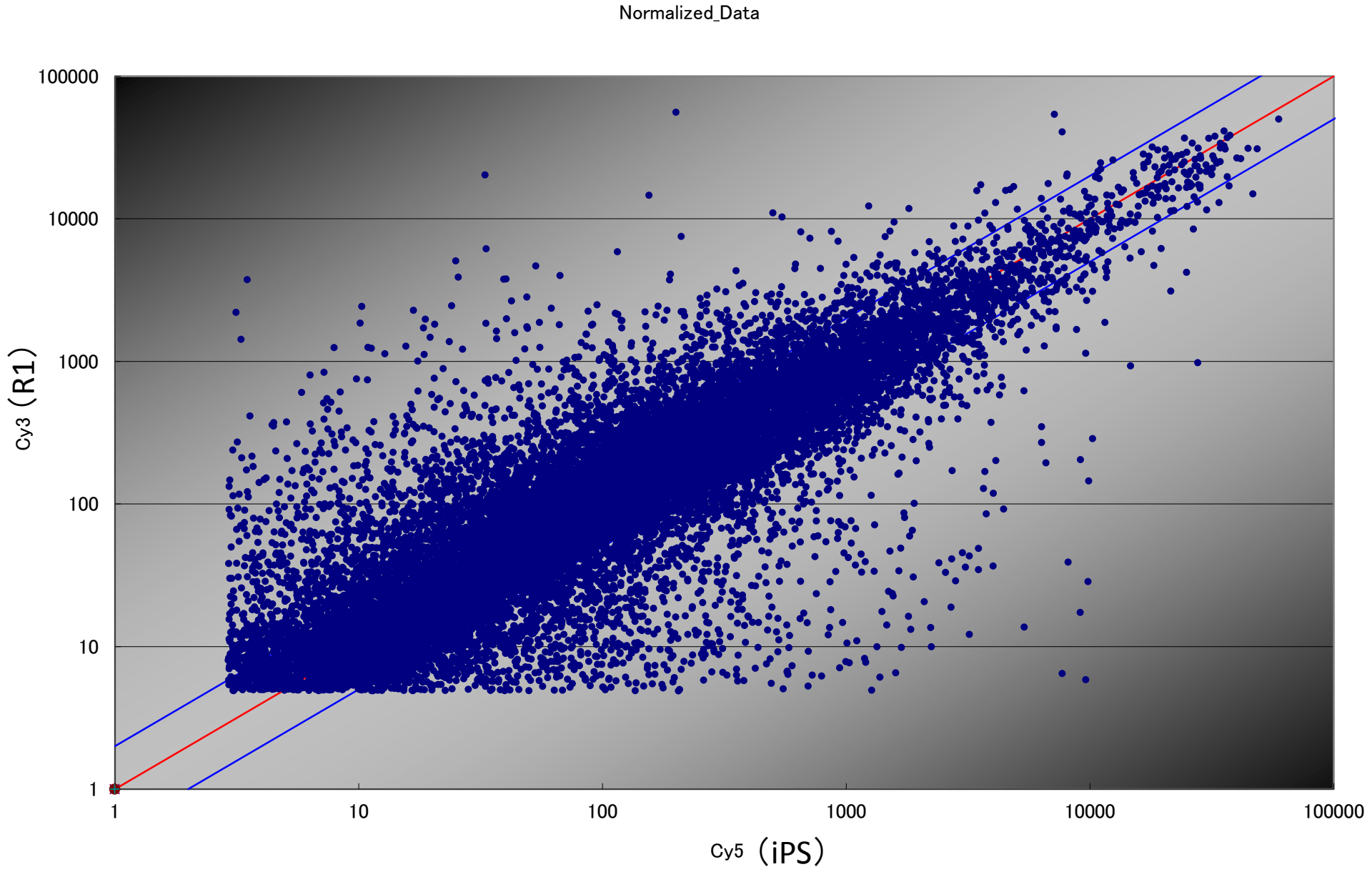



D

tumor formation 0/6




HLF
pCDH (mock)-HLF
psiLV (scramble)-HLF
unstained
ICC: Nanog
ICC: Oct4
20.0 μm
bar 20μm


Normalized_Data
100000
10000
1000
100
10
1
Cy3 (R1)
1
10
100
1000
10000
100000
Cy5 (iPS)

| symbol | description | LOG2[ratio(R1/iPS)] |
|---|---|---|
| SEMA3C | Semaphorin-3C Precursor (Semaphorin-E)(Sema E) | 10.06 |
| MAGEC2 | Melanoma-associated antigen C2 (MAGE-C2 antigen)(MAGE-E1 antigen)(Hepatocellular carcinoma-associated antigen 587)(Cancer/testis antigen 10)(CT10) | 9.45 |
| TM4SF1 | Transmembrane 4 L6 family member 1 (Tumor-associated antigen L6)(Membrane component surface marker 1)(M3S1) | 9.26 |
| RP13-36C9.3 | Cancer/testis antigen 45-3 (CT45-3) | 8.75 |
| IGFBP7 | Insulin-like growth factor-binding protein 7 Precursor (IGF-binding protein 7)(IGFBP-7)(IBP-7)(MAC25 protein)(Prostacyclin-stimulating factor)(PGI2-stimulating factor)(IGFBP-rP1) | 8.12 |
| NT5E | 5'-nucleotidase Precursor (EC 3.1.3.5)(Ecto-5'-nucleotidase)(5'-NT)(CD73 antigen) | 7.87 |
| MMP1 | Interstitial collagenase Precursor (EC 3.4.24.7)(Matrix metalloproteinase-1)(MMP-1)(Fibroblast collagenase) [Contains 22 kDa interstitial collagenase;27 kDa interstitial collagenase] | 7.65 |
| PAGE2 | G antigen family E member 2 (Prostate-associated gene 2 protein)(PAGE-2) | 7.52 |
| FILIP1 | Filamin-A-interacting protein 1 (FILIP) | 7.51 |
| ALPK2 | Alpha-protein kinase 2 (EC 2.7.11.-)(Heart alpha-protein kinase) | 7.29 |
| CCL2 | C-C motif chemokine 2 Precursor (Small-inducible cytokine A2)(Monocyte chemoattractant protein 1)(Monocyte chemotactic protein 1)(MCP-1)(Monocyte chemotactic and activating factor)(MCAF)(Monocyte secretory protein JE)(HC11) | 7.24 |
| PRAME | Melanoma antigen preferentially expressed in tumors (Preferentially expressed antigen of melanoma)(OPA-interacting protein 4)(OIP4) | 7.08 |
| IL18 | Interleukin-18 Precursor (IL-18)(Interferon-gamma-inducing factor)(IFN-gamma-inducing factor)(Interleukin-1 gamma)(IL-1 gamma)(Iboctadekin) | 6.98 |
| AC069282.6 | Putative uncharacterized protein FLJ21075 Precursor | 6.85 |
| FGB | Fibrinogen beta chain Precursor [Contains Fibrinopeptide B] | 6.84 |
| IFI44 | Interferon-induced protein 44 (p44)(Microtubule-associated protein 44) | 6.84 |
| MVP | Major vault protein (MVP)(Lung resistance-related protein) | 6.76 |
| SPANXD | Sperm protein associated with the nucleus on the X chromosome D (SPANX-D)(SPANX family member D)(Nuclear-associated protein SPAN-Xd)(Cancer/testis antigen 11.4)(CT11.4) | 6.71 |
| IFI44L | Interferon-induced protein 44-like | 6.69 |
| CXCL2 | C-X-C motif chemokine 2 Precursor (Macrophage inflammatory protein 2-alpha)(MIP2-alpha)(Growth-regulated protein beta)(Gro-beta) [Contains GRO-beta(5-73)(GRO-beta-T)(SB-251353)(Hematopoietic synergistic factor)(HSF)] | 6.66 |

| symbol | description | LOG2[ratio(Cy3/Cy5)] |
|---|---|---|
| LIN28 | Lin-28 homolog A (Zinc finger CCHC domain-containing protein 1) | -10.69 |
| TACSTD1 | Tumor-associated calcium signal transducer 1 Precursor (Major gastrointestinal tumor-associated protein GA733-2)(Epithelial cell surface antigen)(Epithelial glycoprotein)(EGP)(Adenocarcinoma-associated antigen)(KSA)(KS 1/4 antigen)(Cell surface glycoprotein Trop-1)(CD326 antigen) | -10.22 |
| EPCAM | Tumor-associated calcium signal transducer 1 Precursor (Major gastrointestinal tumor-associated protein GA733-2)(Epithelial cell surface antigen)(Epithelial glycoprotein)(EGP)(Adenocarcinoma-associated antigen)(KSA)(KS 1/4 antigen)(Cell surface glycoprote | -9.05 |
| GAL | Galanin Precursor [Contains Galanin;Galanin message-associated peptide(GMAP)] | -8.62 |
| L1TD1 | LINE-1 type transposase domain-containing protein 1 (ES cell-associated protein 11) | -8.44 |
| SERPINB9 | Serpin B9 (Cytoplasmic antiproteinase 3)(CAP-3)(CAP3)(Proteinase inhibitor 9) | -8.05 |
| FGF13 | Fibroblast growth factor 13 (FGF-13)(Fibroblast growth factor homologous factor 2)(FHF-2) | -8.02 |
| SLC16A9 | Monocarboxylate transporter 9 (MCT 9)(Solute carrier family 16 member 9) | -7.94 |
| SLC7A3 | Cationic amino acid transporter 3 (CAT-3)(Cationic amino acid transporter y+)(Solute carrier family 7 member 3) | -7.83 |
| SALL4 | Sal-like protein 4 (Zinc finger protein SALL4) | -7.82 |
| TDGF1 | Teratocarcinoma-derived growth factor 1 Precursor (Epidermal growth factor-like cripto protein CR1)(Cripto-1 growth factor)(CRGF) | -7.71 |
| CRABP1 | Cellular retinoic acid-binding protein 1 (Cellular retinoic acid-binding protein I)(CRABP-I) | -7.42 |
| LECT1 | Chondromodulin-1 Precursor (Chondromodulin-I)(ChM-I)(Leukocyte cell-derived chemotaxin 1) [Contains Chondrosurfactant protein(CH-SP)] | -7.36 |
| ZSCAN10 | Zinc finger and SCAN domain-containing protein 10 (Zinc finger protein 206) | -7.28 |
| ZFP42 | Zinc finger protein 42 homolog (Zfp-42)(Reduced expression protein 1)(REX-1)(hREX-1)(Zinc finger protein 754) | -7.18 |
| EDNRB | Endothelin B receptor Precursor (ET-B)(Endothelin receptor non-selective type) | -7.17 |
| FOXN3 | Forkhead box protein N3 (Checkpoint suppressor 1) | -7.13 |
| GPM6B | Neuronal membrane glycoprotein M6-b (M6b) | -7.08 |
| RASL11B | Ras-like protein family member 11B | -7.07 |
| PXDN | Peroxidasin homolog Precursor (EC 1.11.1.7)(Vascular peroxidase 1)(Melanoma-associated antigen MG50)(p53-responsive gene 2 protein) | -7.06 |

Table 1

Primers used in the study

| Gene or miRNA | Sense | Antisense |
|---|---|---|
| β-actin | ACCTGACTGACTACCTCATG | GCAGCCGTGGCCATCTCTTG |
| Oct4 | CGGAAAGAGAAAGCGAACCA | CGGACCACATCCTTCTCCAG |
| NANOG | CAGAAGGCCTCAGCACCTAC | ACTGGATGTTCTGGGTCTGG |
| Sox2 | CAAGATGCACAACTCGGAGA | CGGGGCCGGTATTTATAATC |
| Klf4 | AAACTGACCCTCCTCCAGGT | TGCTTTGCTCCAGGAACTTT |
| hTERT | GTGCACCAACATCTACAAGATCC | GTTCTTCCAAACTTGCTGATG |
| c-Myc | GCCAGAGGAGGAACGAGCTA | TGGACGGACAGGATGTATGC |
| p53 | GCTTCGAGATGTTCCGAGAG | TTATGGCGGGAGGTAGACTG |
| PROM1 | TGGCAACGTAGTGACTCAGG | ACAGGAAGGGAGGGAGTCAT |
| CD44 | AAGGTGGAGCAAACACAACC | GCTTTTTCTTCTGCCCACAC |
| RGM249 | TGGTACTTCACGAGGATGTGA | CCTGCCTCCTGAGTCTTCTG |
| ELAVL2 | CTGCCATGGAAACACAACTG | TTCTTCTGCCTCAATTCGCT |
| AICDA | CGTAGTGAAGAGGCGTGACA | TGTAGCGGAGGAAGAGCAAT |
| DNMT1 | GCAAGAAGTGAAGCCCGTAG | TGAACGTTAGCCTCTCCAT |
| HDAC | GCTCAGCTGGTCATTCAACA | ACTGCCTGGTTGCTTCAGTT |
| Sin3A | TTTTTATGCGACTGCACCAG | CGTTCCCATTCTCTCTCTCG |
| MBD3 | TGTCCCAGCTCCTTGAGACT | CAAACTACGCCTCCAGACC |
| SIRT1 | TCAGTGGCTGGAACAGTGAG | AGCGCCATGGAAAATGTAAC |
| miR-520d | TCTACAAAGGGAAGCCCTTTCTG | |

Supporting File Legends

**Supplementary Figure S1**

A. DNA content in 293FT, mock-293FT and 520d-293FT were assessed in approximately 20,000 collected events. GFP-positive cells in mock-293FT and 520d-293FT were sorted. Cell cycle analysis of 520d-293FT showed increases and decreases in the S and G0 phases, respectively, with synchronized and homogeneous proliferation compared with 293FT and mock-293FT, although the effect of miR-520p on G0, S phase did not appear to be significant.

B. Sorted immature populations were shown as PE positive cells or GFP (+) and ALP-PE (+) cells as arrows indicated. The cells were maintained in an immature state for two weeks after sorting. Although we found GFP (-) cells more than cells received 2% formaldehyde treatment due to the leakage of GFP during staining process, GFP (-) cells post-sorting had a similar populations to GFP (+) cells regarding gene expression and phenotype.

C. Transcriptional examination of methylation status to determine the 520d-293FT reprogramming level. DNMT1 was not significantly expressed compared with mock-293FT, although HDAC, Sin3A and MBD3 expression levels were significantly upregulated ($P < 0.01$) (top). In HLF, DNMT1 was not significantly expressed compared with mock-HLF, but HDAC, Sin3A and MBD3 levels were significantly downregulated ($P < 0.01$), unlike those in 293FT (bottom). Significant differences were not observed in expression levels between 293FT and HLF or between mock-293FT and mock-HLF, but the average relative ratio of 520d-293FT to

520d-HLF was 261.3 (range: 11.9-2164.8). Data (n = 9) were analyzed with a Mann–Whitney U test. **: $P < 0.01$.

D. FACS analysis in which mock- and 520d-293FT or mock- and 520d-HLF were compared. After 3 days, GFP positive or ALP positive cell frequencies were estimated. After one week under culture conditions to maintain an immature state, the majority of 520d-expressing 293FT and HLF cells expressed the pluripotent marker ALP (PE-labeled).

E. GFP (+) and ALP-PE (+) cells were selected and maintained in an immature state for 2 weeks after sorting. The phenotype of these cells before sorting was similar to that of iPS-like cells, and the sorted HLF continued to express GFP after sorting (left; two weeks post-sorting, right; three weeks post-sorting).

**Supplementary Figure S2**

Result of *In vitro* study and microscopic observations in miR-520d-virus-infected 293FT cells (520d-293FT) were shown.

A. Phenotypic changes in 520d-293FT were evaluated microscopically. Changes in cell morphology of 520d-293FT (right) was shown. Many non-adherent cells as well as adherent cells emerged after transfection in 12-24 hours. 293FT cells (control) were shown (left).

B. Confirmation of GFP expression in 520d-293FT that resembled a human-induced pluripotent stem cell. GFP-positive non-adherent cells were cultured in feeder cell-free ES cell medium.

C. Time-lapse observations of an induced cell with GFP expression for 12 hours (x40 magnification) to show morphology and proliferation. Observation of another cell in video mode is provided as Supplementary video 7. 520d-293FT maintained in Repro Stem medium grew up while maintaining the form of the colony unlike those cultured in DMEM. Scattered spheroid colonies were interlinked through long, branched groups of cells.

D. Immunocytochemistry in a representative round cell with an anti-Oct4 antibody and a Rhodamine Red-conjugated secondary antibody. Oct4 was strongly expressed. Unstained cells (left) and cells with Oct4 staining (right) were shown.

E. Immunocytostaining with an anti-Nanog antibody concomitantly with GFP expression. Three days to one week later, the cells formed larger colonies and maintained a Nanog-positive state under the same culture conditions.

F. The effects on miRNA expression were confirmed in 520d-293FT and the relative ratio to hiPSCs was shown. 520d-293FT stably expressed miR-520d-5p in both the adherent and non-adherent states in culture and the viral infection efficiency was greater than 99.1% according to GFP-positive cell sorting. miR-520d was significantly upregulated in 520d-293FT compared with hiPSCs. *: $P < 0.05$, **: $P < 0.01$. Data ($n = 9$) were analyzed using a Mann–Whitney U test. A: adherent cells, non-A: non-adherent spherical cells.

G. Comparison of transcriptional expression in 520d-293FT, hiPSCs or mock-293FT. RT-PCR showed that 520d-293FT showed greater upregulation of P53 and *RGM249* and weaker expression of Oct4 and hTERT than did hiPSCs. However, 520d-293FT

showed greater upregulation of Oct4 than did mock-293FT. 520d-293FT showed greater upregulation of P53, *RGM249* and Oct4 expression than 293FT.

H. Western blotting was performed in hiPSCs, mock-293FT and 520d-293FT. Western blotting showed that non-adherent cells expressed P53 and Oct4 stronger than mock-293FT. Dicer1 expression was also downregulated compared with mock-293FT. The expression of AID known as one of epigenetic markers was downregulated compared with hiPSC. A: adherent cells, non-A: non-adherent cells.

I. Relative ratio of the representative gene expression profile to that in mock-293FT by RT-PCR. Data depict the average relative ratio of 520d-293FT to mock-293FT, and a significant difference was shown, as *:$P < 0.01$ ($n = 5$). The pluripotent markers, P53 and *RGM249* were upregulated, whereas AID was downregulated. Cancer stem cell (CSC) markers (CD133 and CD44) were not upregulated.

**Supplementary Figure S3**

A. Immunohistochemical analysis of liver tissue generated from 520d-HLF cells in xenograft model. (left) Human albumin was expressed strongly in hepatocytes from liver tissue generated from 520d-HLF cells in a xenograft model. (middle-right) Human AFP or GFAP were expressed weakly in the cytoplasm of hepatocytes.

B-C. Osteoblastic differentiation from 520d-HLF cells was induced morphologically and transcriptionally. B. Morphological changes were shown in 520d-HLF that were treated with 2 μM purmorphamine (top) compared with untreated 520d-HLF cells (bottom). C. IBSP (bone sialoprotein) and SPP1 (osteopontin) were strongly expressed in 520d-HLF cells treated with 2 μM purmorphamine ($n = 4$). P:

purmorphamine-treated 520d-HLF, **: P < 0.01. Tumorigenicity of well-differentiated hepatoma cells (Huh7) received miR-520d-5p was examined.

D. Induction of pluripotency by miR-520d in Huh7. Colonies of small round cells emerged within 12 days. Both GFP (left top) and Oct4 (right top) expression were confirmed by immunocytochemistry. Average gene expression levels were examined (n = 5) and pluripotent marker gene and P53 mRNA levels were upregulated compared with mock-Huh7 (right column). Alb, c-Myc, AID and *RGM249* levels were downregulated. 1-17: Oct4, Nanog, P53, hTERT, c-Myc, PROM1, CD44, AID, HDAC, DNMT1, Sin3A, MBD3, Lin28, *RGM249*, AFP, Alb and miR-520d.

E. With the same viral vehicle titer used in the previous *in vivo* study with HLF, ten mice inoculated mock-Huh7 formed a tumor (top). The HE stain of representative tumor was shown. Well-differentiated neoplastic cells (partly squamous cell carcinoma-like cells) with substantial or alveolar arrangement were shown (bottom; x100).

F. 520d-Huh7 was cultured for one week (once per week, we infected cells with the viral construct *in vitro*), tumorigenicity was confirmed one month after inoculation.

G. Fifty percent of inoculated mice generated less-differentiated tumors one month later; the remaining mice did not generate tumors (n = 4). HE staining (x200 magnification) showed that the tumors were identical with low-differentiated hepatoma (left) and poorly-differentiated hepatoma (right).

H. Average methylation rate of Huh7 was 0.20% and the data was standardized, compared with that in Huh7. An average hmC(%) in Huh7 cells was estimated to understand general methylation level during de-differentiation process by

miR-520d-5p. Induction of hypermethylation was not induced in this cell type, but decreasing methylation level after 3D were observed, followed by the decrease less than iPS level a month later (n=3).

**Supplementary Figure S4**

A. Summarized pathway map (original summarized scheme) from HMT analysis. The result of this analysis was described in the discussion section in this text.

B. heat map (original data) obtained from metabolomic analysis. Mock-HLF and HLF (parental cells) were prominently different from the other four types of cells that expressed miR-520d-5p.

C. A principal component analysis (PCA). Mock-HLF and HLF were found to have similar patterns, and the patterns of 7D and R1 were similar. R2 appears to possess similar characteristics to those of 5D and 7D (or R1).

**Supplementary Figure S5**

The 10 predicted binding sites (shown in red letters) of miR-520d-5p in the 3'UTR (1356-3805) of ELAVL2 (1-3805) are shown. Two sites (1853-1880 and 2235-2249 in 3'UTR) were investigated with a luciferase reporter expression assay. These sites were predicted based on the four databases described above. Bases 1607-1626 of the 3'UTR were used for a sense primer sequence.

**Supplementary Figure S6**

Tumorigenicity of miR-520d-expressing fibroblasts (NHDF-Neo and -Ad) in KSN/Slc mice.

A. Parental cells [NHDF-Neo (left) and -Ad (right), x200 magnification] were infected with a miR-520d-expressing lentiviral vector and inoculated into the right hindquarters of KSN/Slc mice.

B. The fibroblast lines are represented as 520d-NHDF-Neo and 520d-NHDF-Ad. The phenotype of 520d-NHDF-Neo is shown (left; x100 magnification). Immunocytochemistry revealed the upregulation of Nanog (right) and Oct4 (middle) in 520d-NHDF-Neo (white bar = 20 μm).

C. The phenotype of 520d-NHDF-Ad is shown (left; x40 magnification). Immunocytochemistry revealed the upregulation of Nanog expression (middle; white bar = 20 μm). Average mRNA expression level of 520d-NHDF-Ad to mock-NHDF-Ad was shown.

D. The tumorigenicity of 520d-induced fibroblasts was examined in KSN/Slc mice. Neither 520d-NHDF-Neo (n = 3) nor -Ad (n = 3; right) generated tumors in mice.

**Supplementary File (video) 7**

520d-HLF cells in video mode is provided by time-lapse image.

**Supplementary File 8**

Oct4 or Nanog expression in a parental HLF, pCDH-HLF or psiLV (scrambled)-HLF was shown. pCDH and psiLV were used as controls for pMIR-520d-5p and siELAVL2 [unstained (top), ICC: Nanog (middle) and ICC: Oct4 (bottom)]. Nanog and Oct4 were

both weakly expressing the pluripotent markers. Approximately 8% of populations in HLF cells seemed to be slightly expressing both markers stronger than other populations around them. Also, Oct4 was expressed in each sample weaker than Nanog. ICC: immunocytochemistry.

**Supplementary File 9**

Normalized data between R1 and hiPSC in microarray analysis is shown as a graph.

**Supplementary File 10**

Upregulated 20 genes (more than 8 fold) were representatively shown between R1 and hiPSC for reference (n=1). R1 was a cell population formed liver tissue *in vivo*.

**Supplementary File 11**

Downregulated 20 genes (more than 8 fold) were representatively shown between R1 and hiPSC for reference (n=1).

**Supplementary Table S1**

Primers used in this study are shown.