Supplemental materials for:

Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes

Prashant Mali, Bin-Kuan Chou, Jonathan Yen, Zhaohui Ye, Jizhong Zou, Sarah Dowey, Robert A. Brodsky, Joyce E. Ohm, Wayne Yu, Stephen B. Baylin, Kosuke Yusa, Allan Bradley, David J. Meyers, Chandrani Mukherjee, Philip A. Cole and Linzhao Cheng

The references cited below are the same as in the main text, with one addition:

32. Mali P, Ye Z, Chou BK, Yen J, and Cheng L. An improved method for generating and identifying human induced pluripotent stem cells. ***Methods in Molecular Biology***. In press.

Materials and Methods

Cell culture

Human ES or iPS cells were maintained in KNOCKOUT™ D-MEM, 20% KSR, 1X NEAA, 1X L-Glutamine, 1X-Antibiotic/antimycotics, 0.1 mM β-mercaptoethanol and 10 ng/ml of basic fibroblast growth factor (bFGF, purchased from Peprotech). Mouse embryonic fibroblasts (MEFs) were used as feeder cells or as a source of conditioned media as previously described [8]. Human fibroblasts were grown in DMEM (low glucose) with Earle's Salts, 10% FBS, 1x NEAA, 1x L-Glutamine & 1x Antibiotic/Antimycotics (all from Invitrogen), with the split ratios at 1:4 and cells passaged about every 5-6 days. Addition of low levels of bFGF (1 ng/ml) was used for culture of human marrow stromal cells (also called mesenchymal stem cells, MSCs) as previously described [14].

Sources of human fibroblast cells

The human fibroblasts used for this study were IMR90 (fetal lung fibroblasts, ATCC CCL-186), human MSC1640 (passage 2) purchased from AllCells LLC, and MSCs that we established at Johns Hopkins using the published protocol [14], with approvals from Johns Hopkins internal review board for conducting laboratory research using anonymous human cells. MSCs from an adult patient with sickle cell disease (SCD) were established follows: 3 ml of bone marrow aspirate was harvested from a 39-year-old female SCD patient. Approximately 22 million mononuclear cells were purified using a Ficoll (1.077) gradient and subsequently plated into a single T-75 flask. On day 2 the floating cells were removed and the adherent cells were allowed to propagate in the same flask. By day 11, about 2 million cells were harvested in total, and plated at 5-10K/cm^2 henceforth. These adult BM-derived MSCs from a SCD patient termed as BASCs expanded robustly by about 3-fold every 4 days and growth rates were sustainable for at least 10 passages.

Retrovirus production and usage

We used retroviral vectors (pMXs-based) expressing the four standard Yamanaka 4 factors *Oct4*, *Sox2*, *Klf4* and *c-Myc*, obtained from Addgene. Consistent with other publications [2], the transgenes encoded by the mouse cDNAs appear sufficient to reprogram human cells for iPS cell derivation.

To make high-titer retroviruses, we followed the following procedure for all experiments: 8-10 million 293T cells (DMEM with 10% FBS) were plated onto 50 μg/ml poly-D-lysine coated plates. After 24 hours the plates were about 70-80% confluent, and the medium was changed to DMEM with 1% FBS (20ml per plate). The transfection cocktail per individual vector used was: 36 μl of Lipofectamine 2000 (Invitrogen), 24 μg of total DNA (3 μg VSV.G, 6 μg of retro-gag/pol, and 15 μg of retroviral vector), and 2x1.2 ml opti-MEM. The supernatant was harvested at 48 hours, fresh media was added and then

collected again at 72 hours and pooled (total 40 ml). Typical titers of un-concentrated viruses obtained using this procedure is in the range of ~1-5x10^5 transducing units/ml. Concentrate viruses for 50-100 folds using the Centricon (Plus-20, 20 ml from Millipore) with a cut-off 100,000 NMWL. Designate this final volume amount as V.

We recommend use of freshly made viruses for transduction and our recommended dosage is as follows: If V is the total amount of virus obtained per viron type per 15-cm plate (see step 1), then we recommend as a starting amount V/12 for each virus per 100K cells/10cm^2 all pooled together in a total volume of 2 ml to be transduced. The resultant ratio of retroviral viruses per cell (per each vector) is typically ~4-5. It gives at least 70-80% transduction efficiency, as measured by a compatible GFP-expressing viral vector in both IMR90 fetal fibroblasts and adult MSCs (the virus amount for the latter may need to be increased to V/8). In case transduction efficiency is too poor we recommend increasing the ratio to V/10, V/8 or V/4 of each virus type, until a positive reprogramming result is obtained.

Human iPS cell derivation using retroviral transduction

The overall reprogramming procedure is highlighted in Figure 1a. Experimental details can be found in a method paper by Mali P et al. [32]. In brief, fibroblasts are seeded at a density in the range of 50K-100K/10cm^2 and are transduced using a combination of the retroviruses as per the concentrations recommended in the retroviral usage section. The viruses are premixed with the standard fibroblast media and 6 μg/ml polybrene and the cells are incubated overnight. Following which we supplement the wells with an additional 50% by volume fibroblast media, which reduces the polybrene concentration to 4 μg/ml and incubated again overnight. Only after 48 hours the virus containing media is washed off and fresh media added. Then on day 6 the reprogramming cells are transferred onto MEF coated plates at densities typically in the range of 10K-25K/10cm^2 and after overnight attachment in native fibroblast media, the cells are finally switched to hES culture conditions starting day 7. The cells are fed fresh

medium daily or every other day, until candidate pre-iPS colonies emerge. Usually MEF conditioned media is used starting day 14. Usage of epigenetic small molecule modulators is also indicated in Figure 1a and optimal concentrations used are; RG108: 5-10 μM; BIX01294: 0.5-1 μM; VPA: 0.5-1 mM; and NaB: 0.125-0.5 mM. While careful titration is necessary to achieve maximum stimulation efficiency for a particular cell type, at 0.25 mM concentration, butyrate was found be uniformly stimulatory for nearly all cell types tested, and could also be applied for the entire duration of the reprogramming process until the day of colony picking. We also used HAT inhibitors C646 that block p300/CBP enzymatic activities and a structurally-related but inactive compound C37 [16]. Typically the small molecules are dissolved to 1000x concentration, stored at –20°C, and added fresh to the media to the final concentration desired before every change of culture. Butyrate is dissolved to a concentration of 1M, and stored at –20°C in glass vials. When troubleshooting for failure to obtain iPS cells using the above procedure the following steps must be re-evaluated (in order): first, check the titer of the un-concentrated retroviral supernatant (at least ~10^5 transducing units); second, transduction efficiency of the target cell type (at least 60-80%); third, poor quality of the pMEFs used during reprogramming; and fourth, refractory or senescent nature or late passage of the target cell type.

Human iPS cell derivation using *piggyBac* transposition

On day 0, 0.5x10^6 MSCs are electroporated using the Amaxa hMSC buffer and program U-023. The PB-OSMKL transposon and an improved *piggyBac* transposase are used in a 1:1 ratio (3μg each). This procedure typically results in about 50-60% cell death, and thus the total cells right after electroporation are split evenly into four wells of a 12-well plate (each well thus receives about 40-50K live cells). Typically transfection efficiencies for hMSCs using the above protocol and as measured by a compatible PB-CAG.EGFP based vector are about 40-60% on day 10 in the presence of the transposase. The next day i.e. day 1, the floating dead cell debris are washed off and fresh media is added, with butyrate stimulation beginning the day after i.e. on day 2. The recommended butyrate concentration is 0.125-0.25 mM

(titration is necessary for each cell type). For ease of reprogramming the addition of butyrate to the culture can be continued for the entire duration of the process right until the day of colony picking without any adverse effect on efficiency or toxicity. From days 0-6 FBS-containing MSC medium is used for culture. On day 6 the cells are either passaged onto MEF feeders with a change of medium to hES medium on day 7, or alternatively for slower growing cells (typically at later passages or for refractory cell types) they are instead not harvested and on day 7 the medium on these is directly changed to MEF conditioned hES medium (i.e. these cells remain in the 12-well plates during the entire course of reprogramming). Typically between days 8-12 several transformed epithelial colonies should become visible in the plate. In general these are mostly transient in nature, i.e., some of them tend to revert back to a fibroblast like state, while others apoptose and disappear, while some others both marginally apoptose and also simultaneously further transform to give rise to hES-like colonies that stain positive for TRA-1-60. The addition of butyrate was observed to help particularly in this further transformation of the non-hES like colonies into hES like colonies and also to prevent their reversion. Between days 16-21 several colonies with ES-like morphology should emerge and by day 24 we typically pick these colonies for further expansion.

Human iPS cell identification using TRA-1-60 live staining

Typically a large number and varying morphology of colonies are visible in different regions of the plate. However, not all epithelial-like colonies are hES like – which are characterized by a flat morphology with individual cells clearly demarcated from each other in the colonies. Moreover a lot of the non-hES-like colonies form closely resembling but not identical compact clusters of cells and are capable of sustained self-renewal and successive passaging under hES cell culture conditions. This makes identification of the successfully reprogrammed colonies a very critical step and for selecting a reprogrammed colony we thus use live staining by the TRA-1-60 antibody. The procedure is as follows: the primary and secondary antibodies are both used at a 1:200 to 1:300 dilution and are premixed together into hES cell media at 1 ml per 1 well of a 6-well plate (or 6 ml per 10-cm dish). This amount is sufficient to cover the surface of

the dishes without the need for a shaker. The plates are then directly placed into the tissue culture incubator for about 1 hour before wash off and subsequent imaging. Successful antibody staining can be detected for at least up to 24-36 hours.

Teratoma formation

Typically 12-15 wells of cells at 80-90% confluence in 6-well plates are used for the following procedure. Cells are collected into a 50 ml tube by directly scraping them in their native media using a cell-scraper. After spinning down, the cell pellet is resuspended on ice in 400 μl of a 1:1 mixture of Matrigel (BD Biosciences) and knockout DMEM and collected in an eppendorf tube and stored on ice. This volume of suspension is suitable for intra-muscular injection into the hind limb of two immuno-deficient mice (200 μl each). We prefer to use immuno-deficient mice with further reduced NK activities such as $RAG^{-/-}$ $IL2RG(\gamma_c)^{-/-}$ and $NOD/SCID/IL2RG(\gamma_c)^{-/-}$ mice. Palpable tumors can be detected as early as 4-6 weeks post injection with the improved method, but up to 3 months is also normal on rare circumstances. After sectioning, slides containing various regions of tumors were stained by H & E [8]. Complex structures with various cell types were examined at both low and high magnitude.

Immuno-staining of undifferentiated and differentiated human iPS cells

For in-situ immuno-staining, cultured hES and iPS cells were fixed with 4% para-formaldehyde in PBS for 15 min and washed with PBS for 10 min. For embryoid bodies, 14 day EBs were transferred onto gelatin coated 24-well plates for additional 2-day attachment (after mild pipetting to break the EBs into smaller pieces). The fixed sample was incubated with the following primary antibody for 2 hours at room temperature: anti-TRA-1-60 (Millipore), anti-NANOG (rabbit IgG, Peprotech), rabbit anti-AFP (1:500, DAKO), mouse-anti ß3-tubulin (1:500, Sigma), mouse-anti Smooth Muscle Actin (1:500, Millipore), and anti-CD34 (1:5, BD Biosciences). After wash with PBS, Alexa-488 or Alexa-555 conjugated goat anti-

rabbit or anti-mouse secondary antibodies (1:500, Invitrogen) were used for 1-hour incubation to visualize the cells together with DAPI nuclear staining.

Karyotyping and genomic PCR for DNA fingerprinting and bisulfite sequencing

Chromosome karyotyping (G-banding), and DNA fingerprinting using Invitrogen's MapPairs primers and PCR were described previously [8, 14]. Briefly, genomic DNA was isolated from parental fibroblasts, various hES-like (iPS) cells, H9 or H1 hES cells, using the DNAeasy kit (Qiagen). The primer set for the loci D21S2055 and D10S1214 were found to the best to distinguish the genotypes of various cells used in this study. For DNA methylation analysis, the EpiTect Bisulfite Kit (Qiagen) was used for bisulfite conversion of genomic PCR. The primers used for OCT4 are the same as in [11]. For the DPPA2 promoter the primer sequences used are: DPPA2_BS_F: GATTATAGGTATGTGTTATTATGTT, and DPPA2_BS_R: CCATTTCATTACCAT ATTTTCTTTTC.

Whole genome analysis

RNA relative expression data was generated using Agilent whole human genome 4x44K microarrays. The DNA methylation data was generated using the Infinium Human DNA Methylation27 chip. All analysis and data visualization was performed using MATLAB. K-means clustering and classical multidimensional scaling was used to categorize the genes into various clusters and analyze their ensemble dynamics.

Supplementary Tables and Figures

Table S1. Summary of derivation and characterization of 22 human iPS cell lines from mesenchymal cells. A brief summary of the origin (IMR90, MSC1640 and BASC), derivation procedure by retroviral vectors (OSKM or OSK) or the *piggyBac* plasmid (PB-OSKML), and extent of characterization of the various iPS lines described in this paper. The checked means they are satisfactory in various assays.

Figure S1. Optimization of the standard Yamanaka reprogramming protocol using the classic four factors in the pMXs based vectors. (a, b) Two aspects of this protocol were found to be crucial for successful reprogramming. First, the use of fetal bovine serum (FBS) containing media in the early stages of reprogramming significantly enhanced overall yield of reprogrammed colonies, although it also contributed to an increase in the number of transformed non-hES like colonies that appeared in the dish. Second, use of the appropriate retroviral amount for transduction was critical. At very high or low amounts, the overall efficiency of iPS derivation was low however at intermediate amounts the use of the retroviral vectors provided a wide working range for efficient reprogramming. The viral amount 'v' corresponds to the total amount of virus obtained from a single 150mm dish using standard production procedures (refer Methods Section), and the various amounts mentioned are used to transduce 50k cells seeded in a single well of a 12-well plate. Typically numerous colonies were formed and a fraction of them expressed alkaline phosphatase (AP) 10-16 days post-transduction of IMR90 cells. However we found that by day 16 not all of the AP+ colonies expressed other pluripotency-associated cell-surface markers such as TRA-1-60. The TRA-1-60+ colonies were first evident by days 10-12 post transduction and grew greater in size. The final efficiencies are measured as the number of colonies per 20k starting cells, assayed on Day 16 post transduction. **(c)** TRA-1-60+ colonies formed distinct phase bright clusters in which individual cells have a flat morphology are explicitly de-lineated from each other, a

characteristic of human embryonic stem (hES) cells, and thus use of TRA-1-60 live-staining provides a facile staining procedure for reliable identification of pre-iPS colonies.

Figure S2. Characterization of iPS cell lines derived from fetal IMR90 fibroblasts by 4 factors. Analysis of the 4-factor OSKM derived iPS line MR46 from IMR90 is provided. TRA-1-60+ colonies were picked at day 18 after transduction by OSKM and butyrate stimulation (day 2-11). After expansion the derived iPS cells express several pluripotency-associated markers: AP, TRA-1-60 and NANOG staining are shown. These also readily differentiate *in vivo* to form cystic teratomas with various cell types from all the three germ layers. Both low and high magnification images of the various sections are shown in the lower panels.

Figure S3. DNA fingerprinting and karyotyping analysis of IMR90-derived iPS lines. Analysis confirms the origin of the respective iPS lines to their parental fibroblasts counterparts and not H9 or H1 hESCs. The results from two independent loci are shown. A normal kartyotyping was found with 3 four-factor (OSKM) iPS lines MR41, MR45 & MR46, and a three-factor (OSK) iPS line MR31.

Figure S4. Great similarity of validated iPS cells to human ES cells at a global level by genome-wide signature analyses. (a) MR45 and MR46 iPS expression profiles of 25 genes as compared to those in human ES (hES) cells. The 25 genes (in an alphabetic order) were selected using the previous data on preferentially expressed genes in H9 hES cell line vs. IMR90 fibroblast cells [9]. Aligent 4x44K gene expression array is used to compare gene expression profiles of MR45 and MR46, as well as reprogramming intermediate cells, with that of H9 hES cells. At the global level, MR45 and MR46 iPS cells show a gene expression pattern similar to hES cells. Reprogramming intermediate cells including those treated with VPA (N) or sodium butyrate (N) for 4 days early (day 2-6, D6, D6V1 and D6N1) or late (day 7-12, D12, D12V2 and D12N2) were also analyzed. With few exceptions, they expressed these

hES genes at much lower levels (blue). The direct comparison in the expression between these intermediate cells and the parental IMR90 cells is shown in Figure 4. **(b)** A dendrogram (based on Pearson Correlation) showing clustering of validated iPS cells in comparison with those of their parental fibroblasts and of hES cells, based on their promoter DNA methylation profiles. Illumina's Human DNA Methylation27 Analysis (Infinium) BeadChips was used, which allows us to interrogate DNA methylation of 27,578 informative CpG sites (covering >14,000 well-annotated genes). All loci were used for clustering analysis. The Y-axis shows relative distance of each cluster. IMR90 fibroblasts (XX) and derived iPS cell lines (MR45, MR46, MR31 and MR32) are denoted in black. BASC (XX, MSC) derived iPS cell lines (MB41, MB45 and MBP5s1) are in brown. MSC1640 (XY) and derived iPS cell lines (MMW1 and MMW2) are in blue. H1 (XY) and H9 (XX) hES cell lines are in red. Similar to the whole-genome gene expression profile, DNA methylation signatures show the successfully reprogrammed iPS cells are globally similar to hES cells vs. their parental somatic cells (on the far right). More analyses are shown in Figure 4.

Figure S5. Butyrate also stimulates reprogramming efficiency in the absence of cell passaging and MEF feeders. (a) Adult MSCs were reprogrammed similar to as described in Figure 1a, however instead of being passaged onto feeders on day 6 they were directly switched to MEF condition hES media. TSA, NaB and VPA were added at varying concentrations starting on day 2 and for the whole duration of the reprogramming process with assay on day 16, by both alkaline phosphatase staining **(b)**, and count of TRA-1-60 +ve colonies **(c)**. It is evident that butyrate stimulation is significant in this scenario too with absolute efficiencies at sub mM concentrations reaching nearly 30 fold (mean ± SEM, n=3).

Figure S6. Characterization of iPS cell lines derived from adult MSC (1640) by 4 factors. Analysis (similar to Figure S2) of the 4-factor OSKM derived iPS line MMW2 from MSC1640 is provided.

Figure S7. Characterization of an additional iPS cell line MB45 from BASC by 4 factors. Analysis (similar to Figure S2) of two 4-factor OSKM derived iPS lines MB45 & MBW2 from BASC (MSC s derived from a SCD patient) is provided.

Figure S8. DNA fingerprinting and karyotyping analysis of MSC-derived iPS lines. Analysis confirms the origin of the respective iPS lines to their parental fibroblasts counterparts and not H9 or H1 hESCs. The results from two independent loci are shown. A normal kartyotype was found with 3 iPS lines with the HbS mutation MB45, MBW1 & MBW2, and one iPS line MMW2 from MSC1640.

Figure S9. Kinetics of reprogramming of human fibroblasts by the 3 factors (OSK). IMR90 cells were transduced by OSK (omitting *c-Myc* transgene) and cultured as described in Figure 1 where OSKM was used. Either valproic acid or sodium butyrate was used through out the duration of the reprogramming process starting day 2. Starting at day 12, the whole culture was stained live for TRA-1-60 (cell-surface) expression. TRA-1-60 staining faded away after 2-3 days and did not show adverse effects on cell growth. The live staining allows us to stain the same culture serially at day 20, 24 and 28. In the absence of VPA or butyrate, sizable TRA-1-60+ colonies were first spotted at day 28, similar to previous reports. Butyrate treatment gave rise to the most TRA-1-60+ colonies, which also appeared as early as day 20. Once acquiring TRA-1-60 cell surface expression, these pre-iPS colonies appear to stably express hES-like morphology and markers. TRA-1-60+ colonies picked at day 21 after OSK transduction.

Figure S10. Characterization of iPS cell line MR31 derived from IMR90 by 3 factors. Analysis (similar to Figure S2) of the 3-factor OSK derived iPS line MR31 from IMR90 fibroblasts is provided.

Cell Type	Reprogramming Factors	iPS clones	Day of colony picking	Pluripotency Markers	Finger Printing	Embryoid Body Differentiation	Teratoma	Karyotype
IMR90 (normal, fetal lung fibroblasts, XX)								
	OSKM	MR41	Day 18	√	√	√		√
	OSKM	MR42	Day 18	√	√	√		
	OSKM	MR43	Day 18	√	√	√		
	OSKM	MR44	Day 18	√	√	√		
	OSKM	MR45	Day 18	√	√	√	√	√
	OSKM	MR46	Day 18	√	√	√	√	√
	OSKM	MRW1	Day 12	√	√	√	√	mosaic
	OSK	MR31	Day 21	√	√	√	√	√
	OSK	MR32	Day 21	√	√	√	√	
	OSK	MR33	Day 21	√	√	√		
MSC1640 (normal MSCs from 24 year old, XY)								
	OSKM	MMW1	Day 12	√	√	√	√	√
	OSKM	MMW2	Day 12	√	√	√	√	√
BASC (sickle cell anemia MSCs from 39 year old, XX)								
	OSKM	MB41	Day 18	√	√	√		
	OSKM	MB45	Day 18	√	√	√	√	√
	OSKM	MB46	Day 18	√	√	√		
	OSKM	MBW1	Day 12	√	√	√	√	√
	OSKM	MBW2	Day 12	√	√	√	√	√
	PB-OSKML	MBP5s1	Day 24	√	√	√	√	√
	PB-OSKML	MBP5s2	Day 24	√	√	√	√	√
	PB-OSKML	MBP5s3	Day 24	√	√	√		
	PB-OSKML	MBP5p3	Day 24	√	√	√	√	
	PB-OSKML	MBP5p4	Day 24	√	√	√		

Table S1

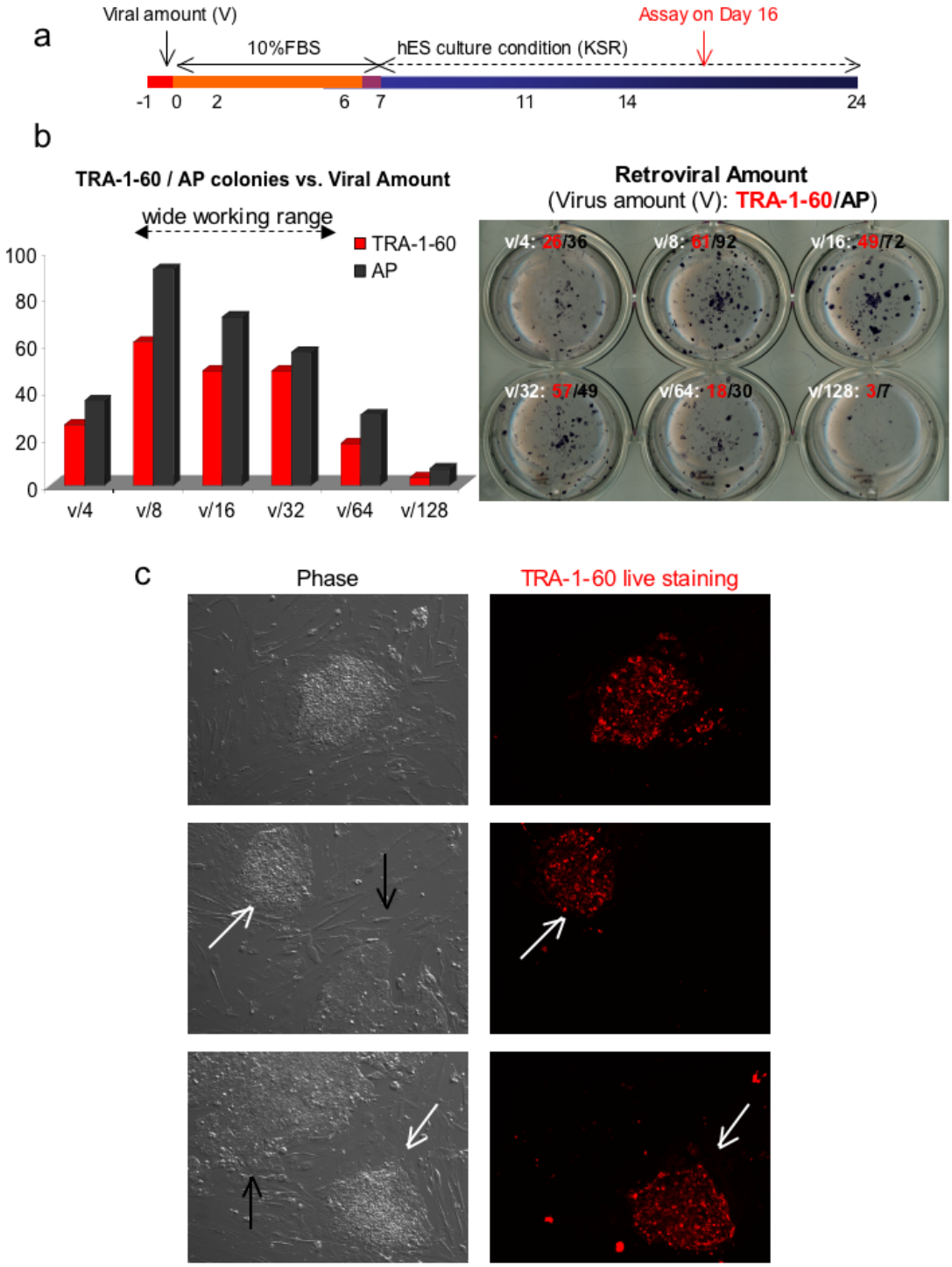

a
Viral amount (V)
10%FBS
hES culture condition (KSR)
Assay on Day 16
-1 0 2 6 7 11 14 24
b
TRA-1-60 / AP colonies vs. Viral Amount
wide working range
TRA-1-60
AP
100
80
60
40
20
0
v/4 v/8 v/16 v/32 v/64 v/128
Retroviral Amount
(Virus amount (V): TRA-1-60/AP)
v/4: 26/36
v/8: 61/92
v/16: 49/72
v/32: 57/49
v/64: 18/30
v/128: 3/7
c
Phase
TRA-1-60 live staining

Figure S1

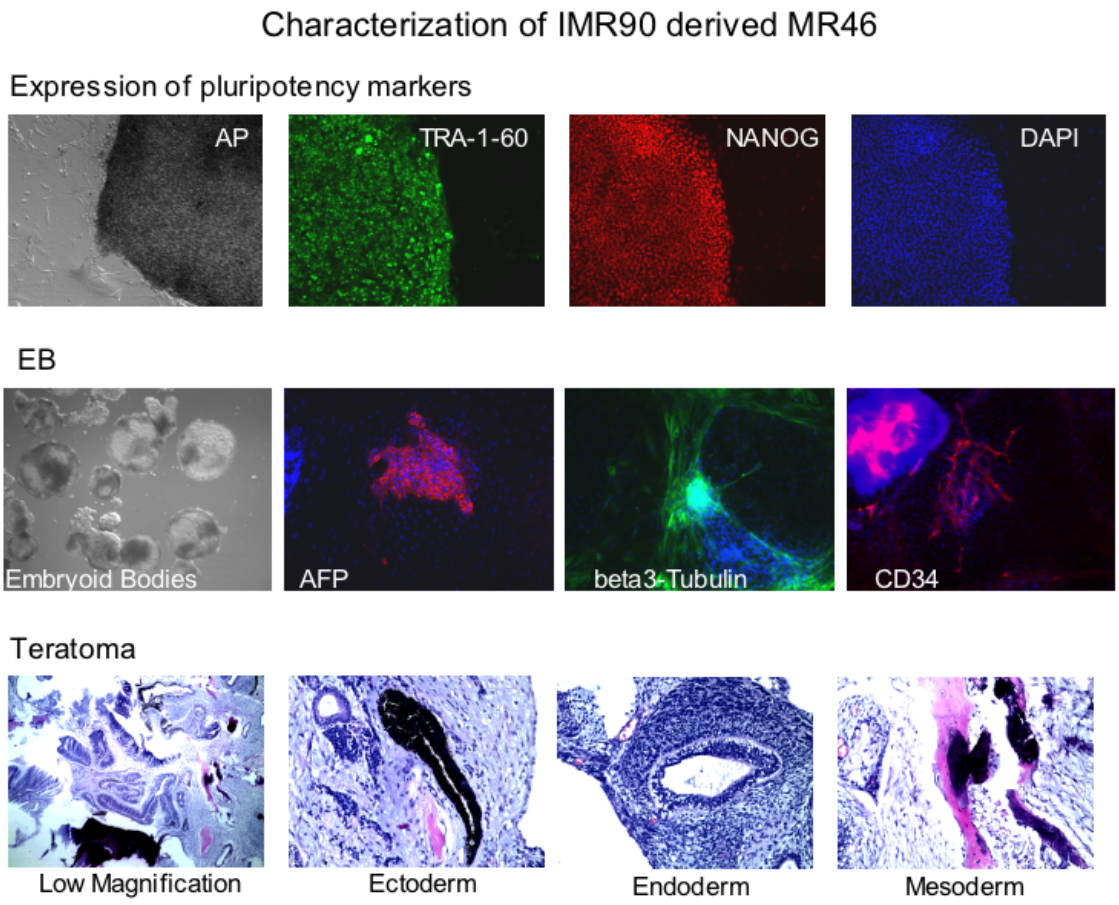
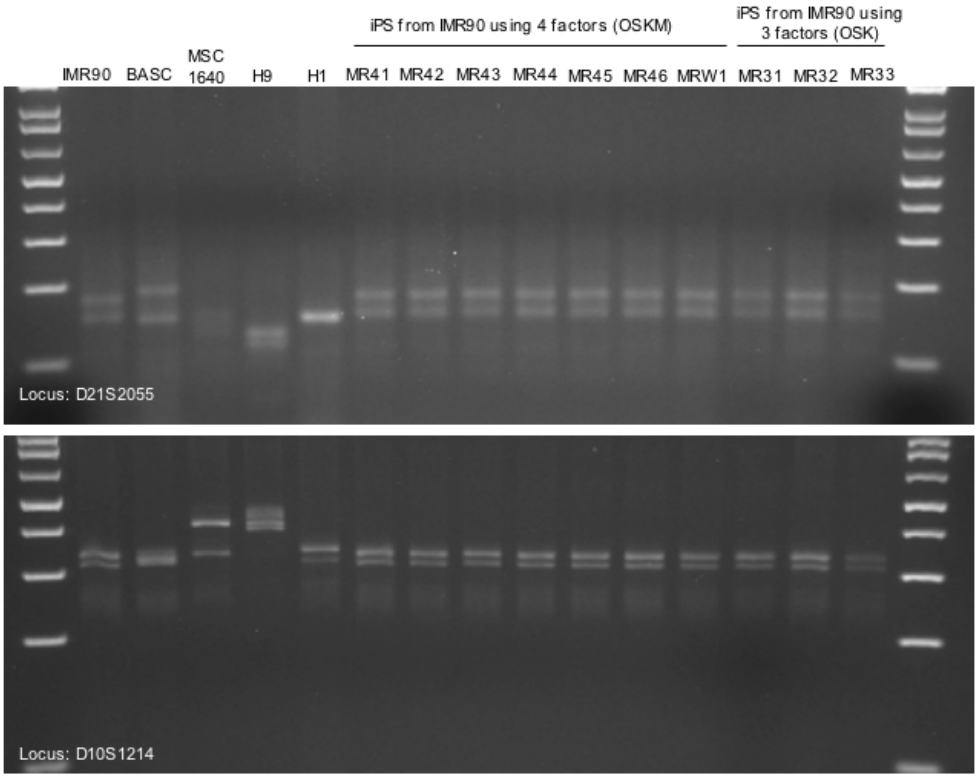

Characterization of IMR90 derived MR46
Expression of pluripotency markers
AP
TRA-1-60
NANOG
DAPI
EB
Embryoid Bodies
AFP
beta3-Tubulin
CD34
Teratoma
Low Magnification
Ectoderm
Endoderm
Mesoderm

Figure S2


iPS from IMR90 using 4 factors (OSKM)
iPS from IMR90 using 3 factors (OSK)
IMR90
BASC
MSC 1640
H9
H1
MR41
MR42
MR43
MR44
MR45
MR46
MRW1
MR31
MR32
MR33
Locus: D21S2055
Locus: D10S1214

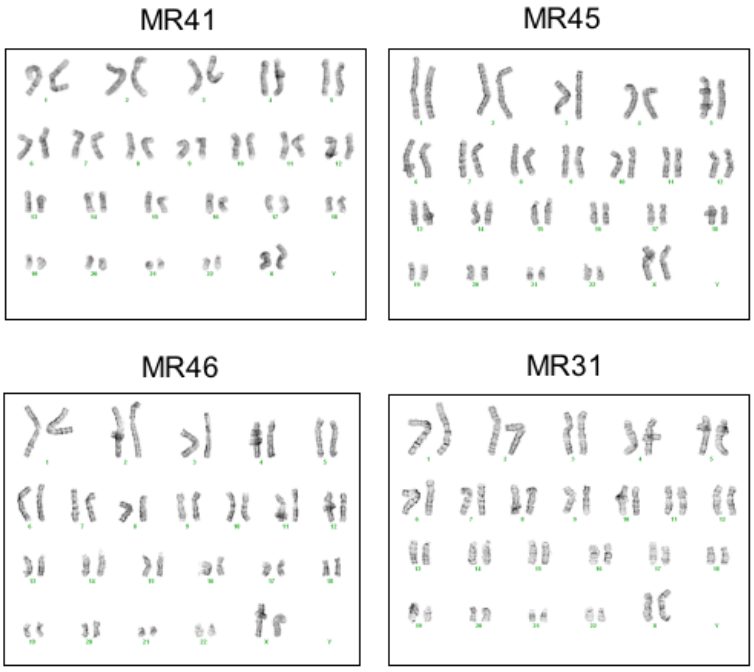

MR41
MR45
MR46
MR31

Figure S3

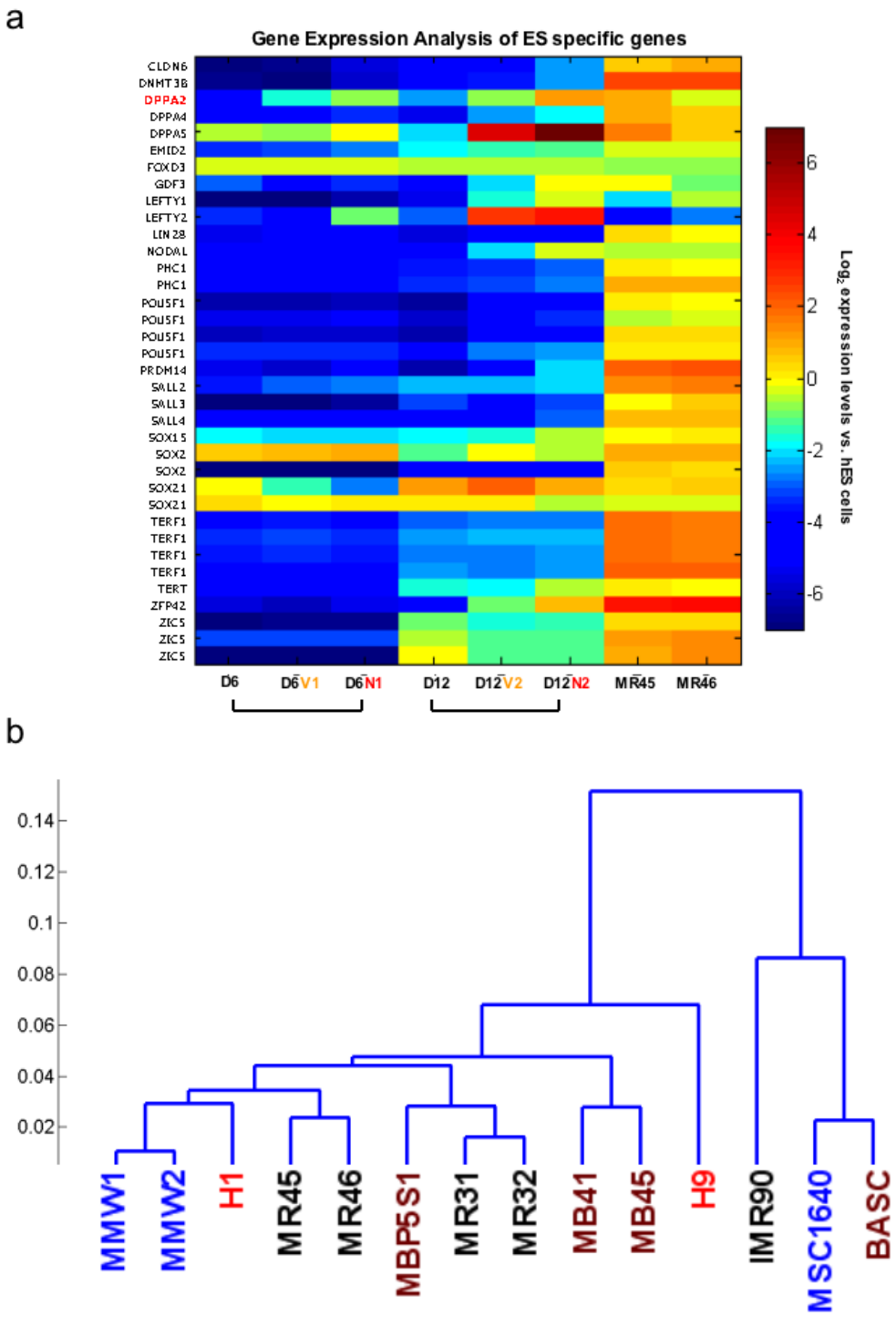

a
Gene Expression Analysis of ES specific genes
CLDN6
DNMT3B
DPPA2
DPPA4
DPPA5
EMID2
FOXD3
GDF3
LEFTY1
LEFTY2
LIN28
NODAL
PHC1
PHC1
POU5F1
POU5F1
POU5F1
POU5F1
PRDM14
SALL2
SALL3
SALL4
SOX15
SOX2
SOX2
SOX21
SOX21
TERF1
TERF1
TERF1
TERF1
TERT
ZFP42
ZIC5
ZIC5
ZIC5
D6
D6V1
D6N1
D12
D12V2
D12N2
MR45
MR46
6
4
2
0
-2
-4
-6
Log2 expression levels vs. hES cells
b
0.14
0.12
0.1
0.08
0.06
0.04
0.02
MMV1
MMV2
H1
MR45
MR46
MBP5S1
MR31
MR32
MB41
MB45
H9
IMR90
MSC1640
BASC

Figure S4

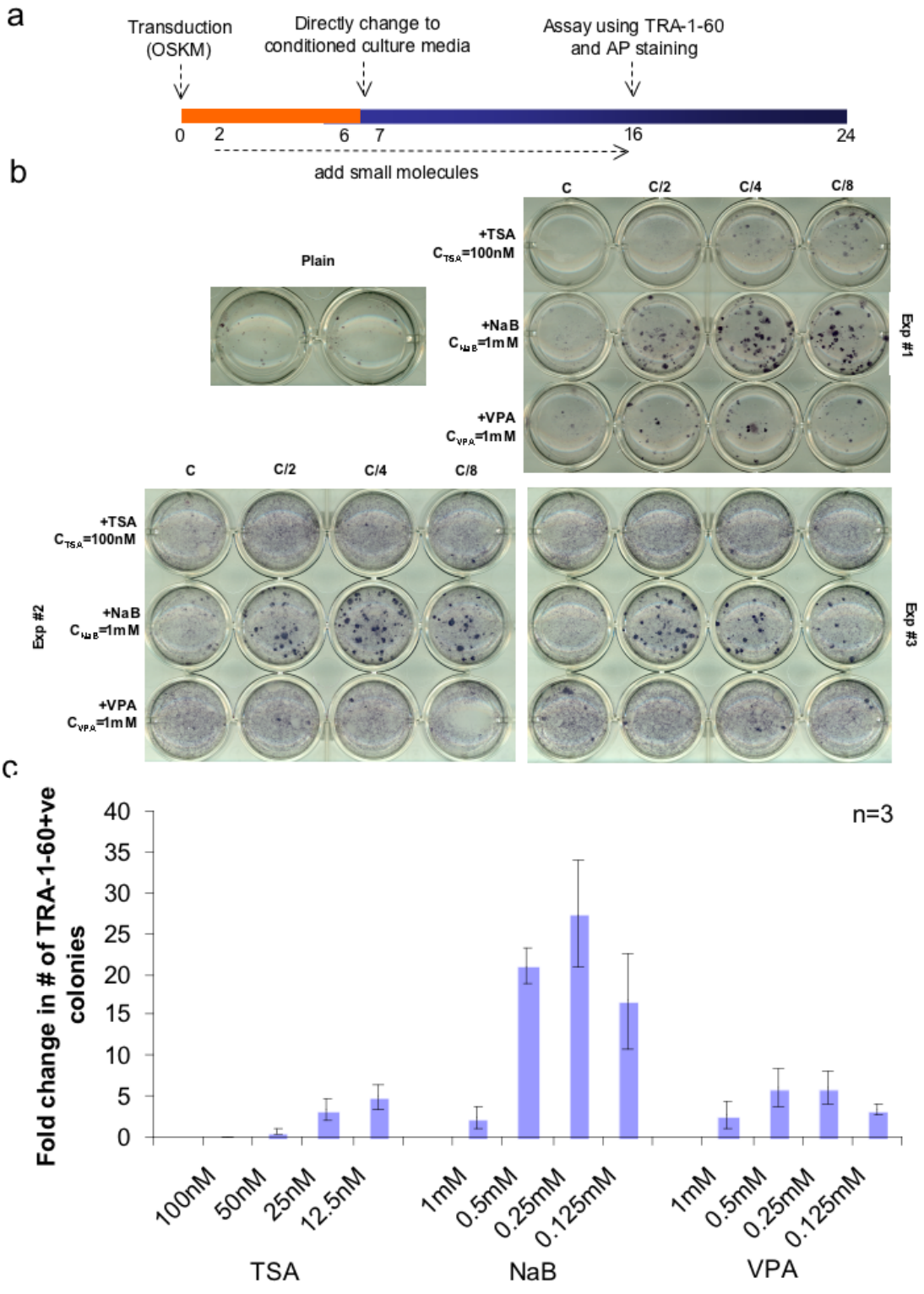

a
Transduction (OSKM)
Directly change to conditioned culture media
Assay using TRA-1-60 and AP staining
0
2
6
7
16
24
add small molecules
b
Plain
C
C/2
C/4
C/8
+TSA
C_TSA=100nM
+NaB
C_NaB=1mM
+VPA
C_VPA=1mM
Exp #1
Exp #2
Exp #3
c
Fold change in # of TRA-1-60+ve colonies
n=3
40
35
30
25
20
15
10
5
0
100nM
50nM
25nM
12.5nM
1mM
0.5mM
0.25mM
0.125mM
1mM
0.5mM
0.25mM
0.125mM
TSA
NaB
VPA

Figure S5

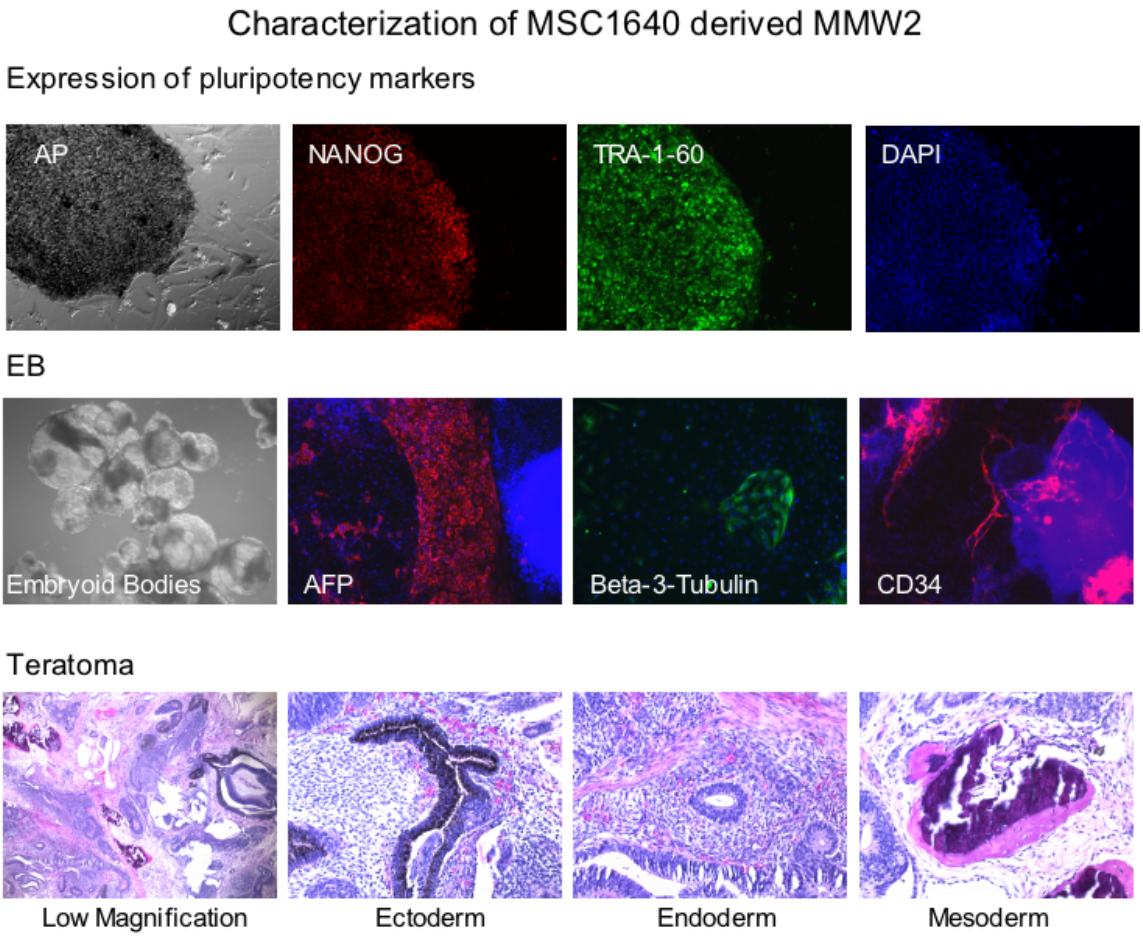

Characterization of MSC1640 derived MMW2
Expression of pluripotency markers
AP
NANOG
TRA-1-60
DAPI
EB
Embryoid Bodies
AFP
Beta-3-Tubulin
CD34
Teratoma
Low Magnification
Ectoderm
Endoderm
Mesoderm

Figure S6

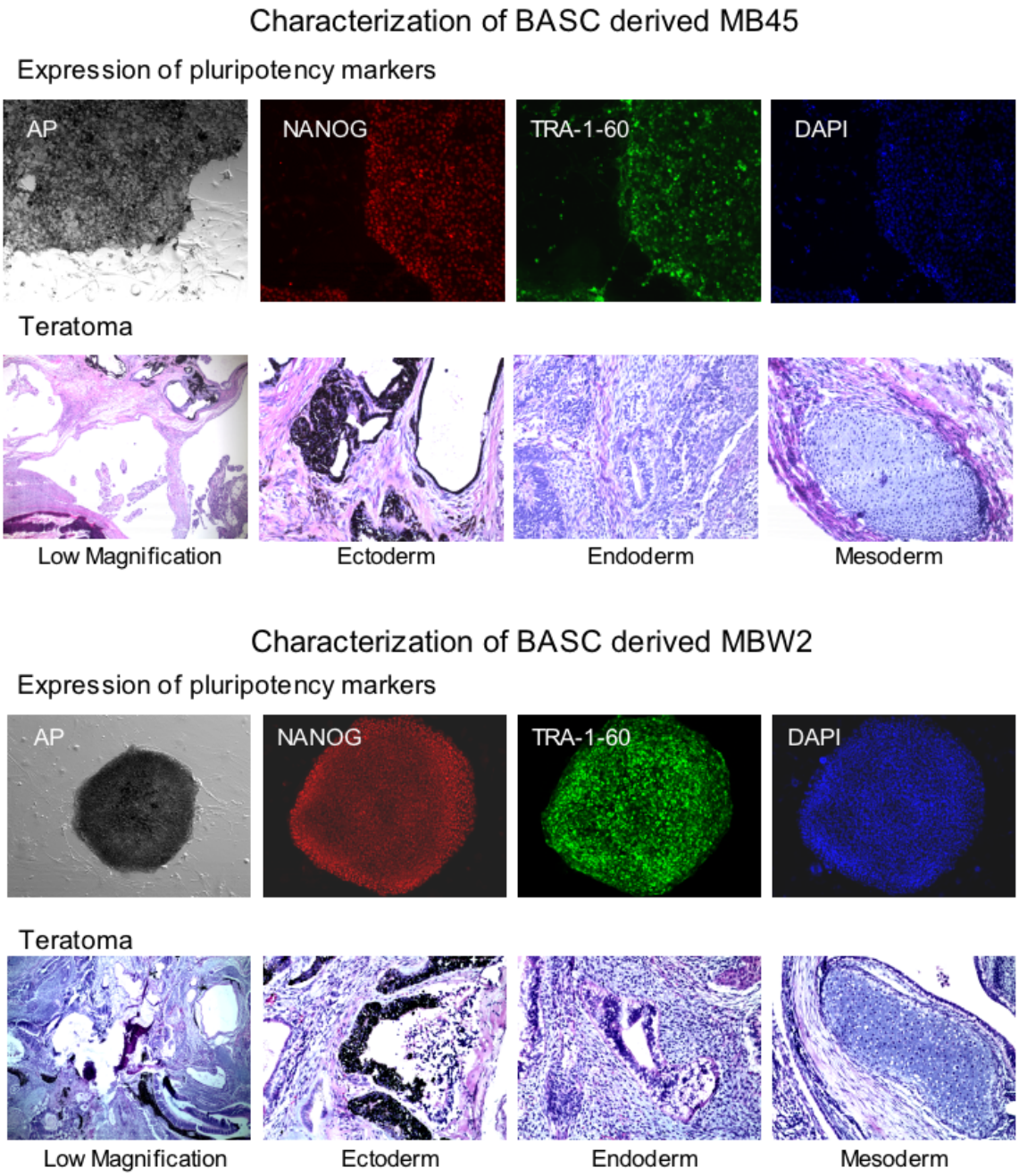
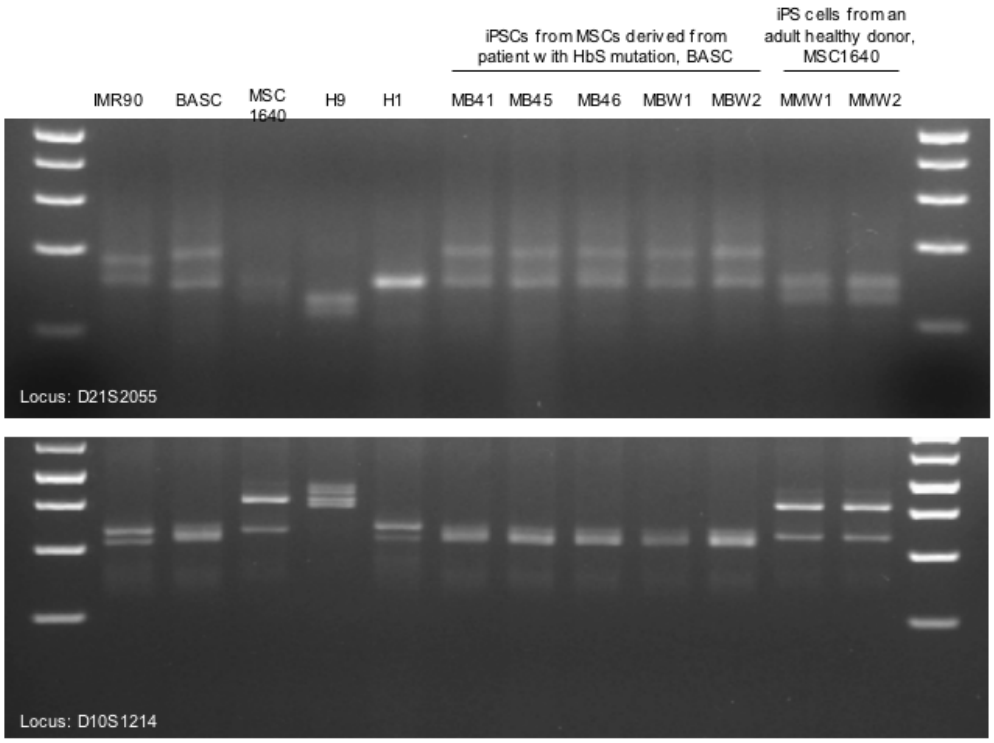

Characterization of BASC derived MB45
Expression of pluripotency markers
AP
NANOG
TRA-1-60
DAPI
Teratoma
Low Magnification
Ectoderm
Endoderm
Mesoderm
Characterization of BASC derived MBW2
Expression of pluripotency markers
AP
NANOG
TRA-1-60
DAPI
Teratoma
Low Magnification
Ectoderm
Endoderm
Mesoderm

Figure S7


iPSCs from MSCs derived from patient with HbS mutation, BASC
iPS cells from an adult healthy donor, MSC1640
IMR90
BASC
MSC 1640
H9
H1
MB41
MB45
MB46
MBW1
MBW2
MMW1
MMW2
Locus: D21S2055
Locus: D10S1214

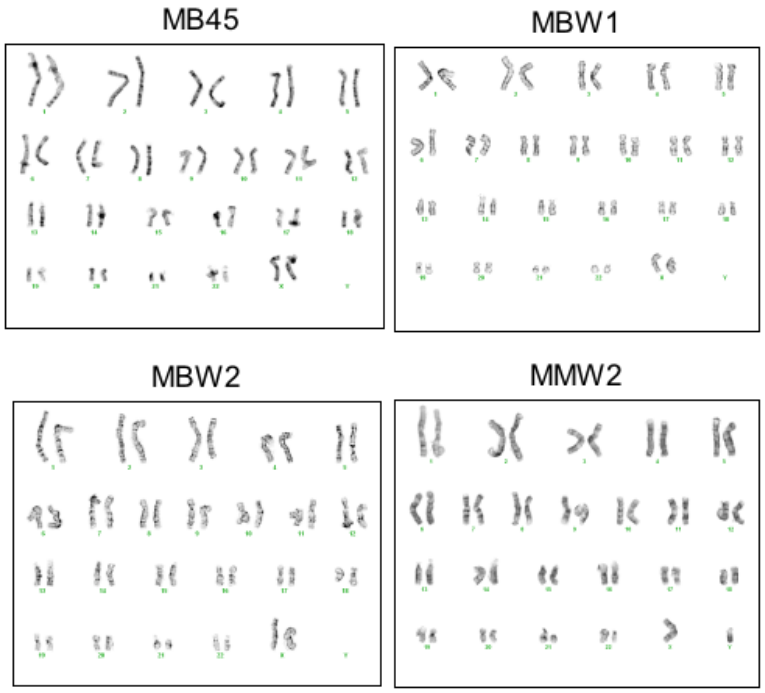

MB45
MBW1
MBW2
MMW2

Figure S8

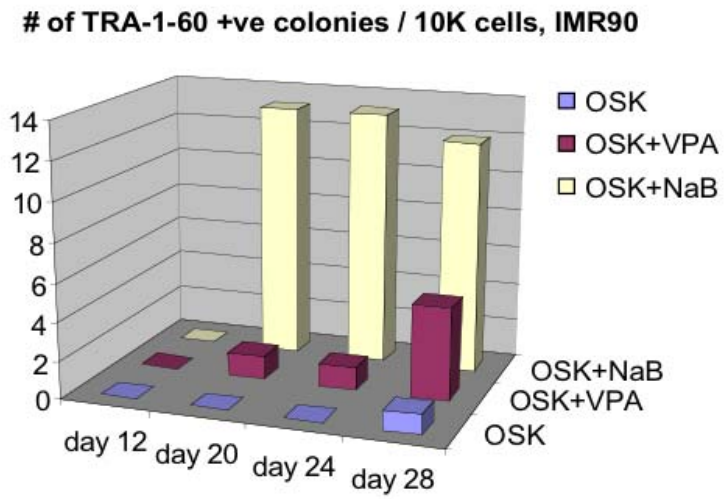

# of TRA-1-60 +ve colonies / 10K cells, IMR90
14
12
10
8
6
4
2
0
day 12
day 20
day 24
day 28
OSK
OSK+VPA
OSK+NaB
OSK+NaB
OSK+VPA
OSK

Figure S9

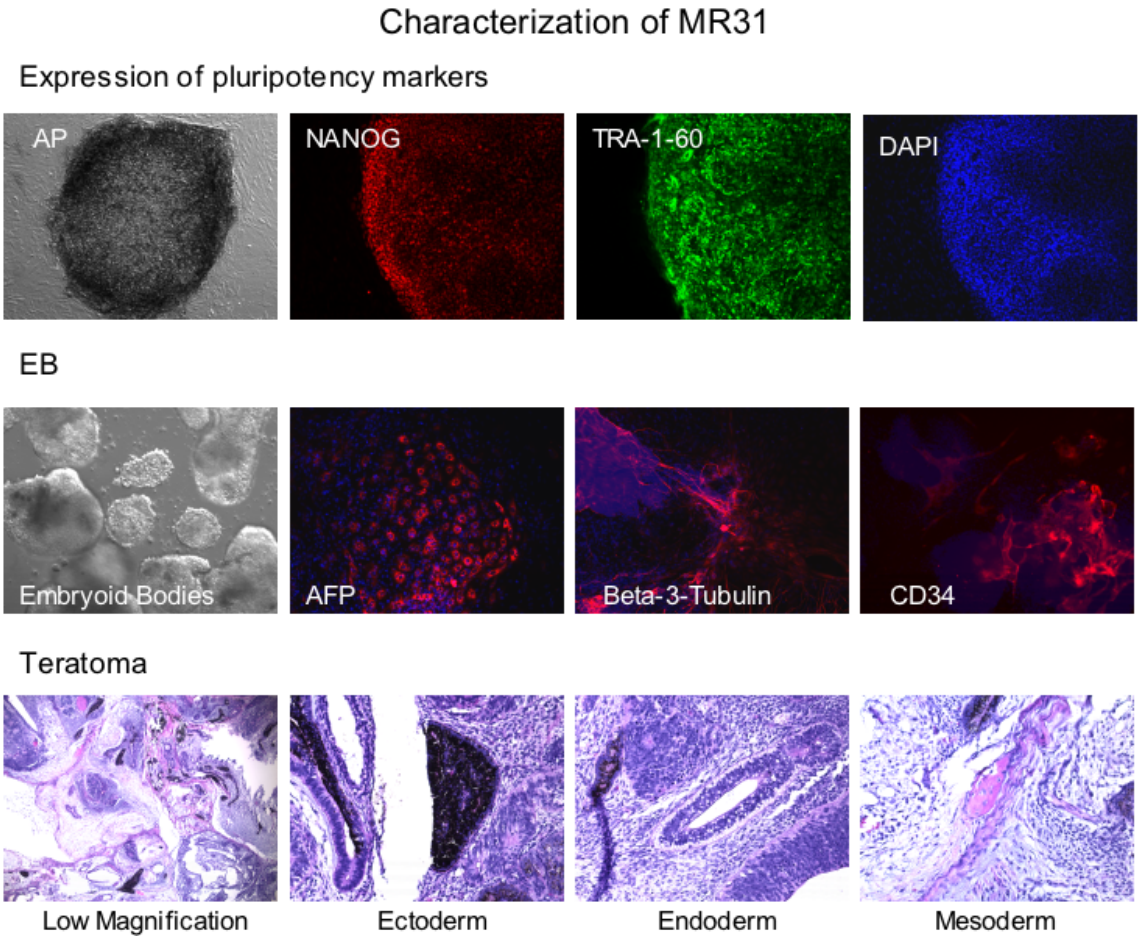

Characterization of MR31
Expression of pluripotency markers
AP
NANOG
TRA-1-60
DAPI
EB
Embryoid Bodies
AFP
Beta-3-Tubulin
CD34
Teratoma
Low Magnification
Ectoderm
Endoderm
Mesoderm

Figure S10