Supplementary Information

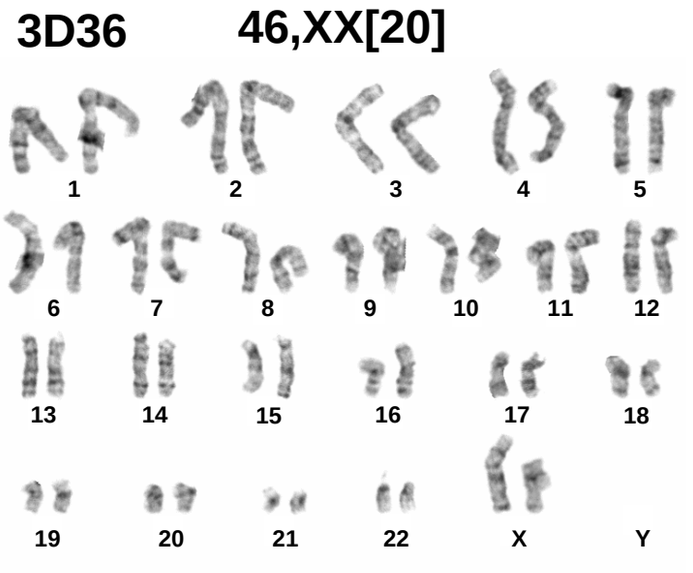
Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells

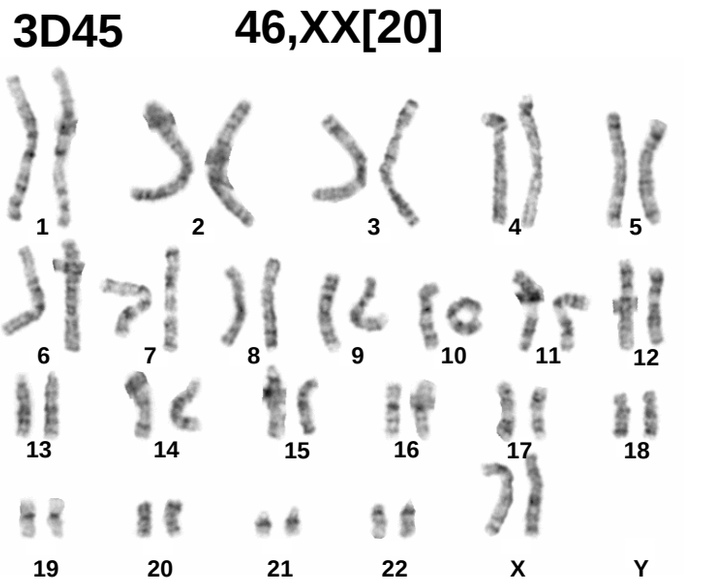
Shin-Ichi Mae, Akemi Shono, Fumihiko Shiota, Tetsuhiko Yasuno, Masatoshi Kajiwara, Nanaka Gotoda-Nishimura, Sayaka Arai, Aiko Sato-Otubo, Taro Toyoda, Kazutoshi Takahashi, Naoki Nakayama, Chad A. Cowan, Takashi Aoi, Seishi Ogawa, Andrew P. McMahon, Shinya Yamanaka & Kenji Osafune*

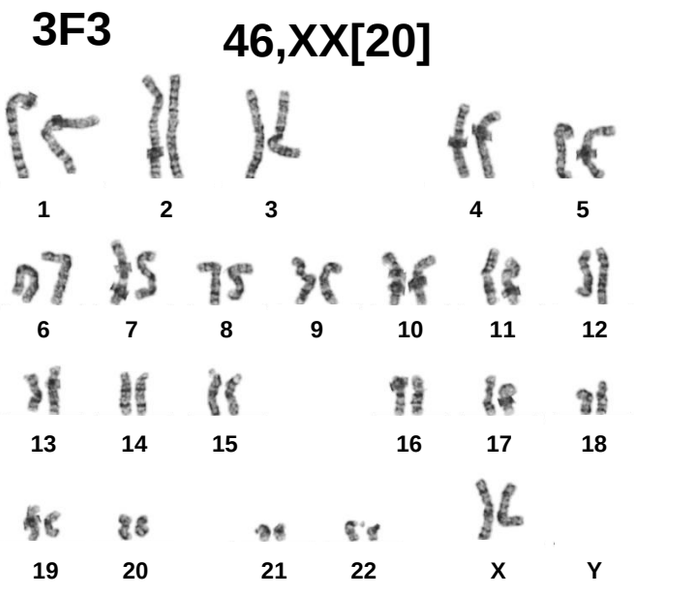
*To whom correspondence should be addressed.

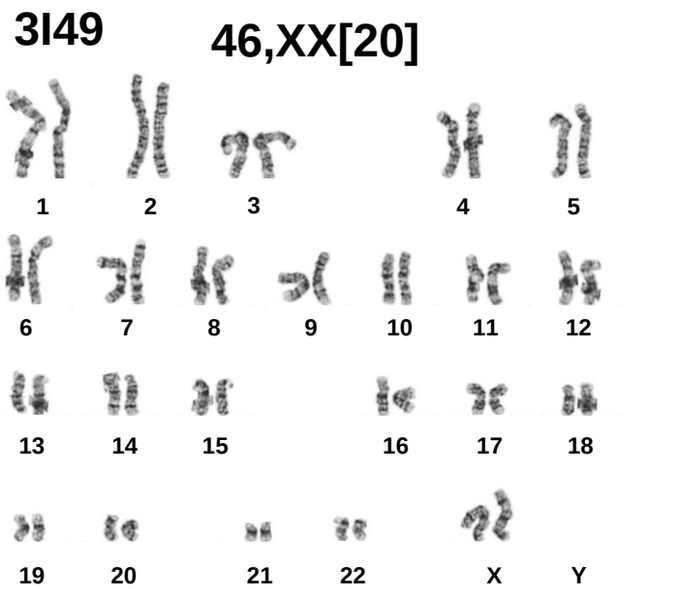
Email: osafu@cira.kyoto-u.ac.jp

Supplementary Figure S1. Normal karyotypes of OSR1-GFP reporter hiPSC lines (3D36, 3D45, 3F3 and 3I49).









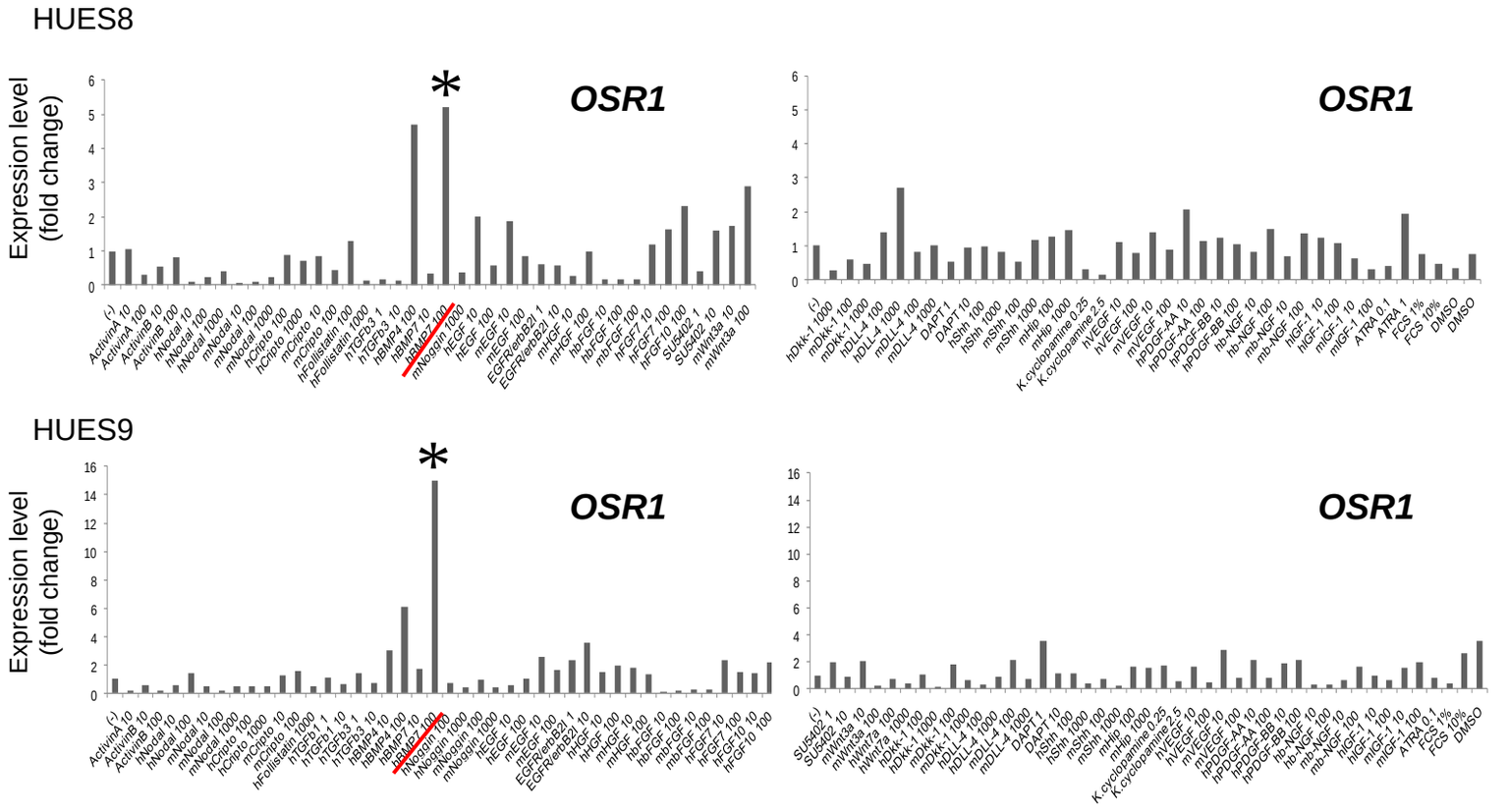
Supplementary Figure S2. Effects of around 40 different growth factors on *OSR1* expression from two hESC lines (HUES8 and 9).

a

Factor	Concentration
ActivinA	10 & 100 ng/ml
ActivinB	10 & 100 ng/ml
rhNodal	10,100,1000 ng/ml
rmNodal	10,100,1000 ng/ml
hCripto-1	0.1 & 1 mg/ml
mCripto	0.1 & 1 mg/ml
rhFollistatin	100 & 1000 ng/ml
hTGFb1	1 & 10 ng/ml
hTGFb3	1 & 10 ng/ml
hBMP4	10 & 100 ng/ml
hBMP7	10 & 100 ng/ml
hNoggin/Fc-chimera	0.1 & 1 mg/ml
mNoggin/Fc-chimera	0.1 & 1 mg/ml
hEGF	10 & 100 ng/ml
mEGF	10 & 100 ng/ml
EGFR/erbB2 inhibitor	1 & 10 mM
hHGF	10 & 100 ng/ml
mHGF	10 & 100 ng/ml
hbFGF (FGF2)	10 & 100 ng/ml
mbFGF(FGF2)	10 & 100 ng/ml
hFGF7/KGF	10 & 100 ng/ml
hFGF10	10 & 100 ng/ml

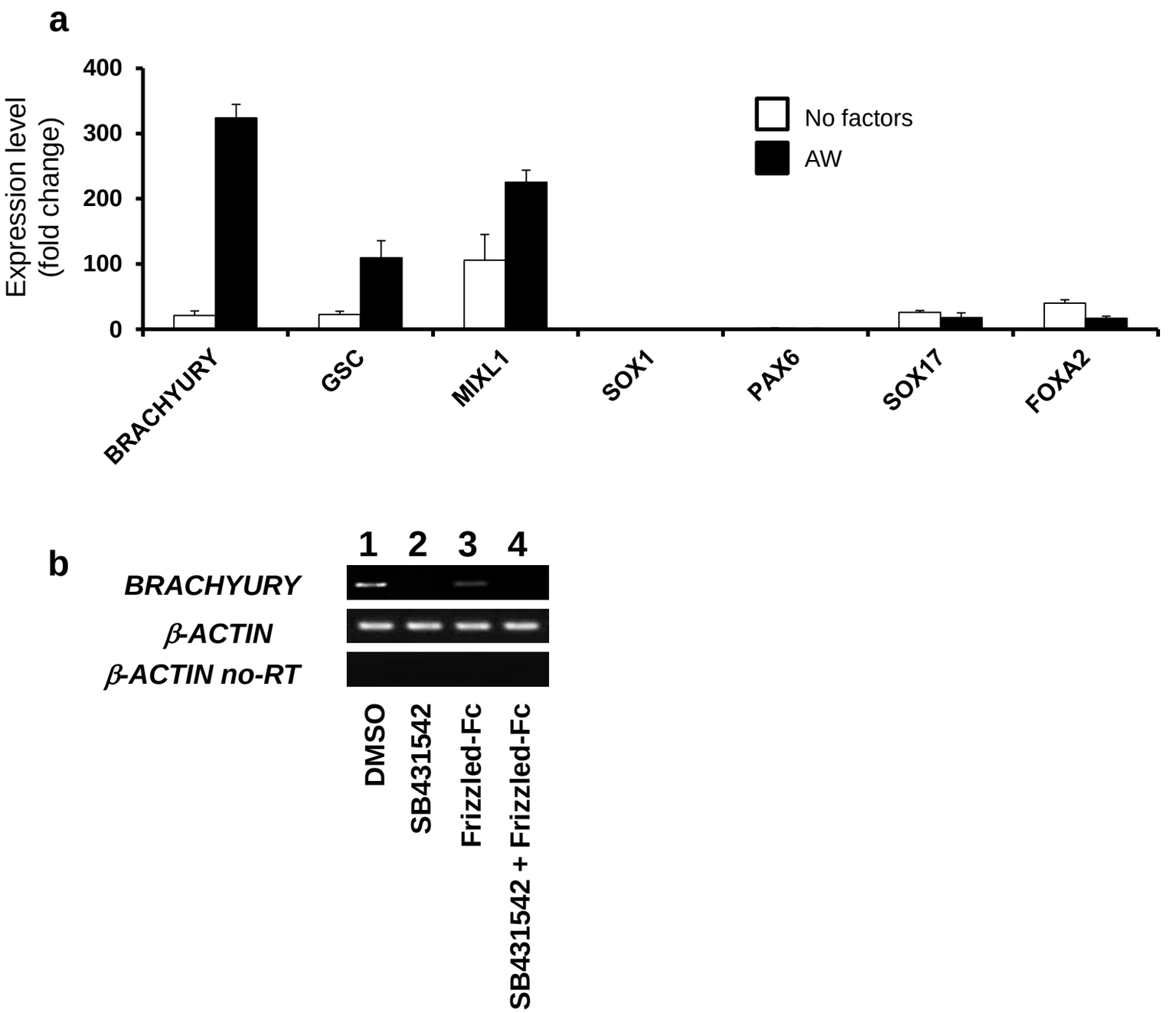
Factor	Concentration
SU5402(FGFR inhibitor)	1 & 10 mM
mWnt3a	10 & 100 ng/ml
hWnt7a	0.1 & 1 mg/ml
hDkk-1	0.1 & 1 mg/ml
mDkk-1	0.1 & 1 mg/ml
hDLL-4	0.1 & 1 mg/ml
mDLL-4	0.1 & 1 mg/ml
DAPT(Notch inhibitor)	1 & 10 mM
hShh(N-term)	0.1 & 1 mg/ml
mShh(N-term)	0.1 & 1 mg/ml
mHip	0.1 & 1 mg/ml
KAAD-cyclopamine	0.25 & 2.5 mM
hVEGF (165)	10 & 100 ng/ml
mVEGF (164)	10 & 100 ng/ml
hPDGFAA	10 & 100 ng/ml
hPDGFBB	10 & 100 ng/ml
hb-NGF	10 & 100 ng/ml
mb-NGF	10 & 100 ng/ml
hIGF-1	10 & 100 ng/ml
mIGF-1	10 & 100 ng/ml
ATRA (all-trans RA)	0.1 & 1 mM
FCS (Hyclone)	10%

b



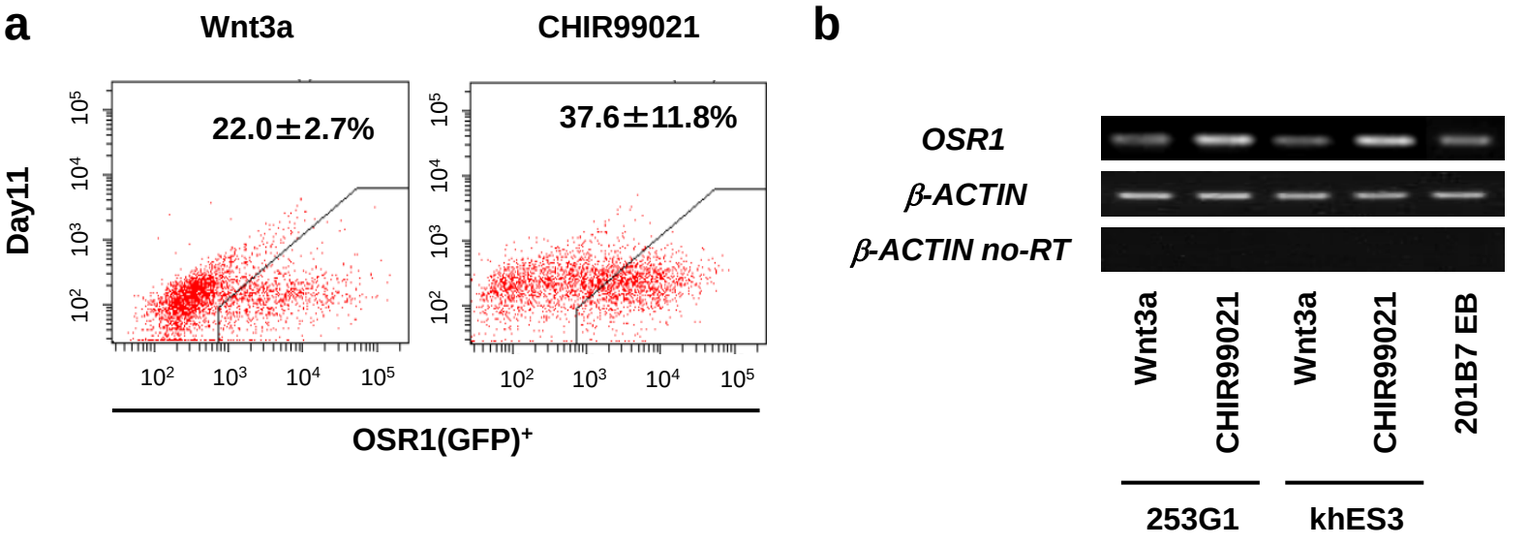
(a) A list of growth factors added to hESCs cultured on MEF feeder layer. (b) qRT-PCR analysis of the expression of *OSR1* induced by growth factors. Samples of hESCs (HUES8 or HUES9) without factors were used as a control to normalize the data. * ; 100 ng/ml hBMP7.

Supplementary Figure S3. Differentiation of hiPSCs into mesendoderm (ME).



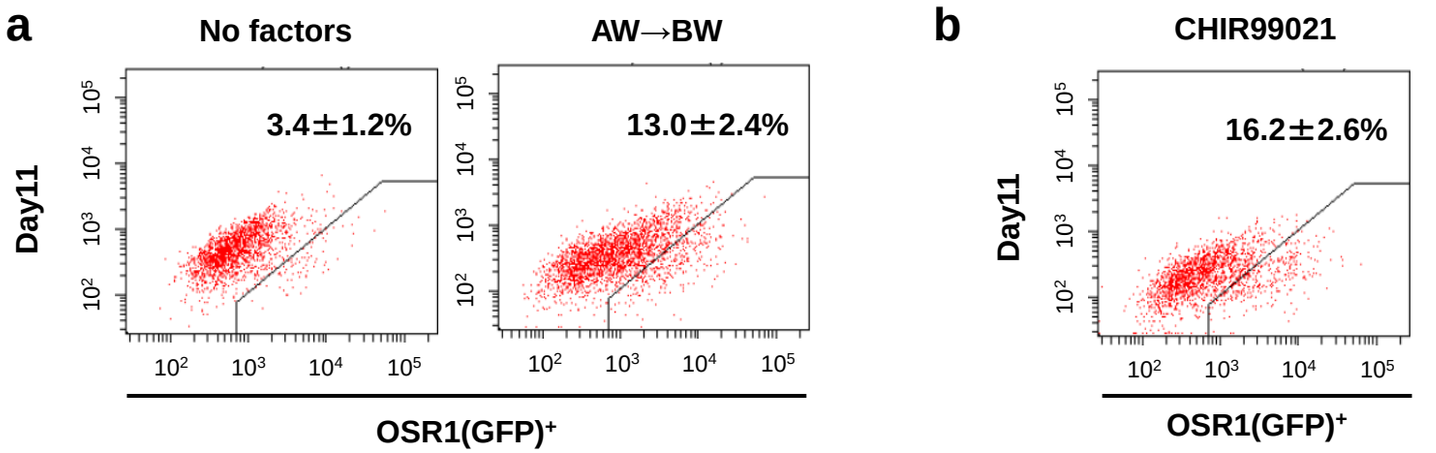
(**a**) qRT-PCR analyses of the marker gene expression for ME (*BRACHYURY, GSC, MIXL1*), ectoderm (*SOX1* and *PAX6*) and endoderm (*SOX17* and *FOXA2*) on culture day 3. White Bars : no factors; black bars (AW): 100 ng/ml activin A + 100 ng/ml Wnt3a. Each value is normalized to samples on day1 before treatments. Note that the expression of *SOX1* and *PAX6* is so low that the histogram bars are at the baseline. (**b**) The suppression of the expression of *BRACHYURY* by adding SB431542 and/or Frizzled-Fc chimeric protein to the treatment with 100 ng/ml activin A + 100 ng/ml Wnt3a, as determined by RT-PCR analyses. Lane 1: DMSO (vehicle) control; 2: 10 μM SB431542; 3: 1 μg/ml Frizzled-Fc; 4: 10 μM SB431542 + 1 μg/ml Frizzled-Fc. The data from three independent experiments are presented as the means ± SD (n=3) in **a**.

Supplementary Figure S4. Replacement of Wnt3a with a chemical compound, CHIR99021.



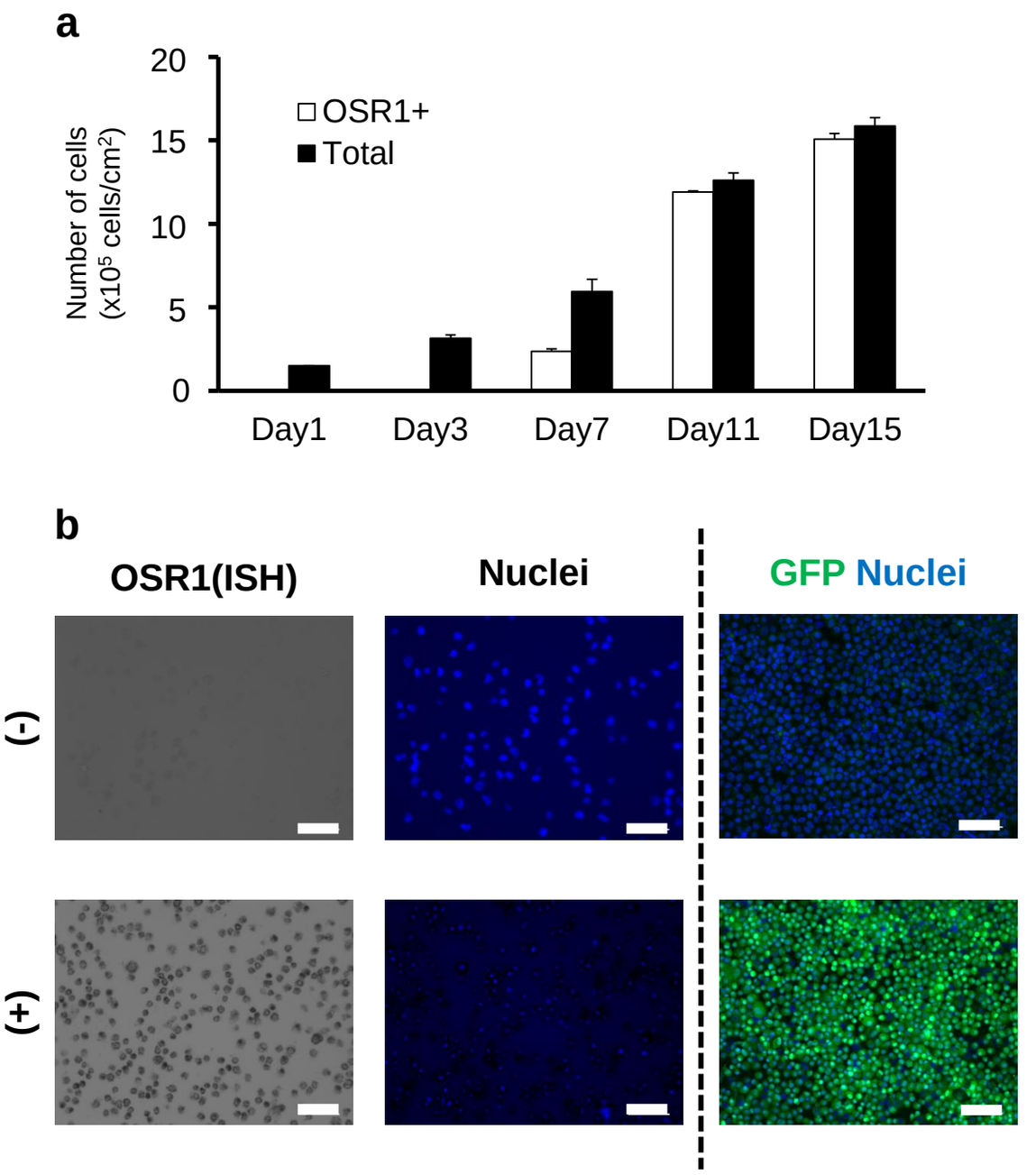
(**a**) The differentiation of OSR1(GFP)$^+$ cells on culture day 11 was analyzed by flow cytometry. The data from three independent experiments are presented as the means ± SD (n=3). (**b**) RT-PCR analyses of *OSR1* expression in a hiPSC line, 253G1, and a hESC line, khES3, on culture day 11. Wnt3a: Stage 1, 100 ng/ml activin A + 100 ng/ml Wnt3a, Stage 2, 100 ng/ml BMP7 + 100 ng/ml Wnt3a; CHIR99021: Stage 1, 100 ng/ml activin A + 1 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 1 μM CHIR99021. 201B7 EB; spontaneously differentiating EBs generated from 201B7 on day 16 of culture without any inducing factors.

Supplementary Figure S5. Differentiation of IM cells in monolayer culture formats.



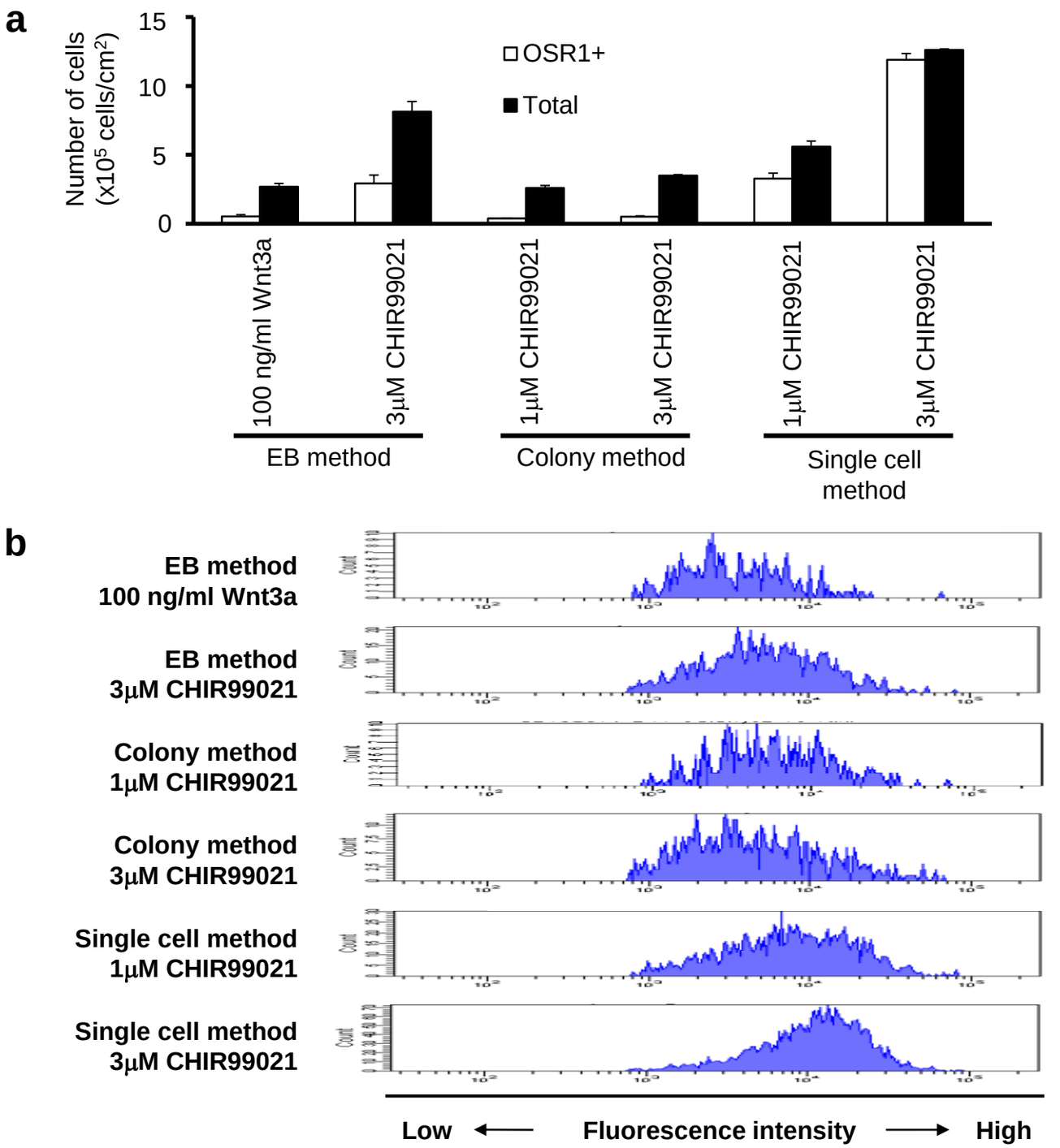
(**a**) The differentiation of OSR1(GFP)$^+$ cells on culture day 11 of monolayer culture (Colony method) was analyzed by flow cytometry. AW→BW: Stage 1, 100 ng/ml activin A + 100 ng/ml Wnt3a, Stage 2, 100 ng/ml BMP7 + 100 ng/ml Wnt3a. (**b**) Substitution of Wnt3a for CHIR99021 in the colony method. The induction rate of OSR1$^+$ cells on day 11. The data from three independent experiments are presented as the means ± SD (n=3).

Supplementary Figure S6. Highly efficient induction of OSR1^{+} cells with single cell method.



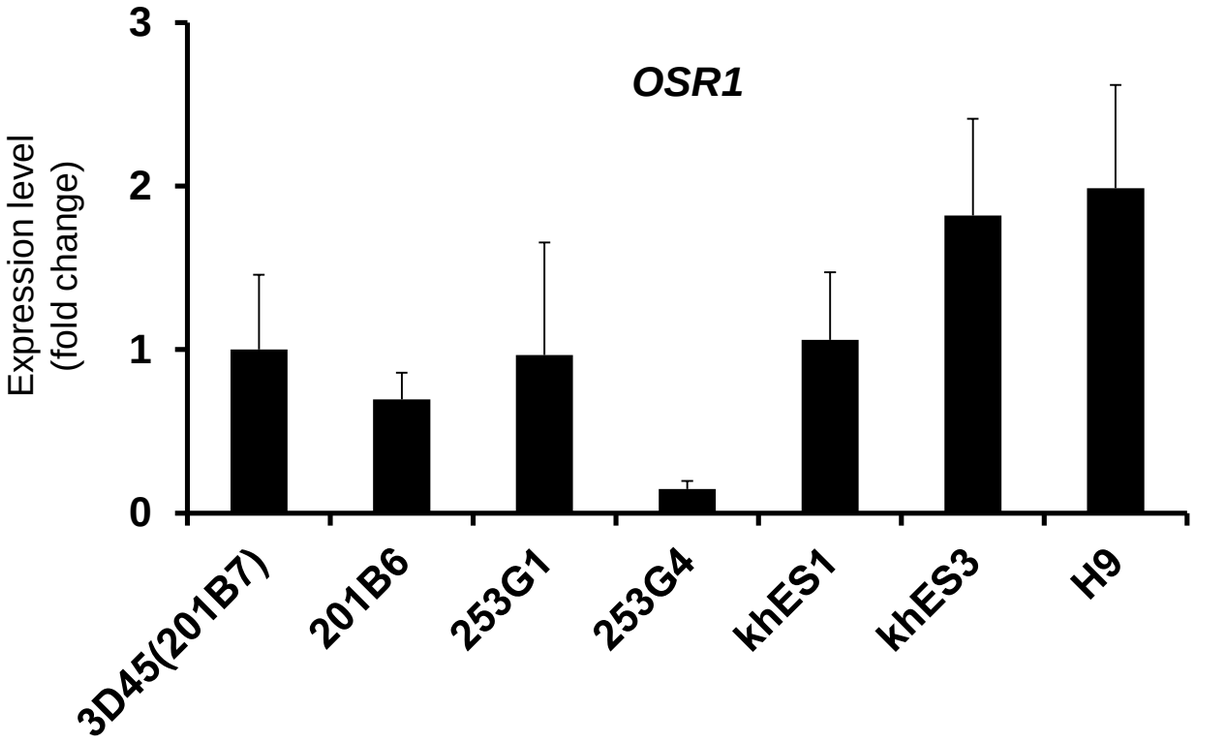
(**a**) The number of OSR1^{+}cells generated at the different time points of single cell method. The input cell number was 1.5x10^6 cells/cm^2 in all the experiments. The data from three independent experiments are presented as the means ± SD (n=3). (**b**) *In situ* hybridization analysis using *OSR1* probes and immunostaining using antibodies against GFP on differentiated cells on culture day 11 of single cell method. (-): no factors, (+): Stage 1, 100 ng/ml activin A + 3 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 3 μM CHIR99021. Differentiated cells on culture day 11 were dissociated with trypsinization and centrifuged onto a slide. Note that almost all cells treated with the single cell method express *OSR1* transcript (lower panels). Scale bars, 100 μm.

Supplementary Figure S7. Comparison of three induction protocols for OSR1$^+$ cells.



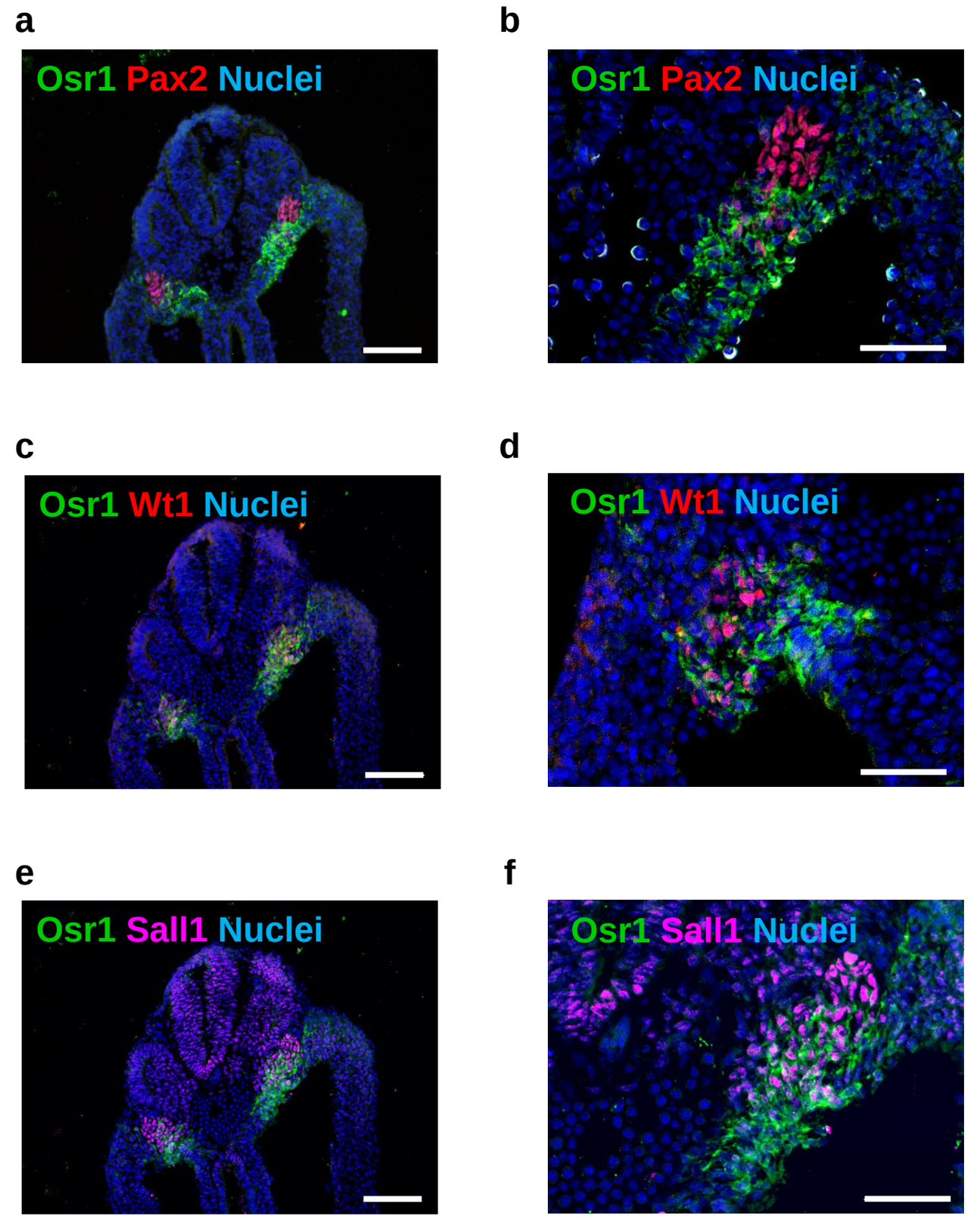
(**a**) The number of OSR1$^+$ cells generated in each protocol. The input cell number was 1.5x10^6 cells/cm^2 in all the experiments. The data from three independent experiments are presented as the means ± SD (n=3). (**b**) Fluorescence intensity of OSR1$^+$ cells differentiated by the three induction methods. Fluorescence intensity of OSR1$^+$ cells on culture day 11 of the three induction methods (EB, Colony and Single cell methods) was shown by a histogram. 100 ng/ml Wnt3a: Stage 1, 100 ng/ml activin A + 100 ng/ml Wnt3a, Stage 2, 100 ng/ml BMP7 + 100 ng/ml Wnt3a; 1μM CHIR99021: Stage 1, 100 ng/ml activin A + 1 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 1 μM CHIR99021; 3μM CHIR99021: Stage 1, 100 ng/ml activin A + 3 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 3 μM CHIR99021.

Supplementary Figure S8. *OSR1* expression in multiple hiPSC/ESC lines induced with EB method.



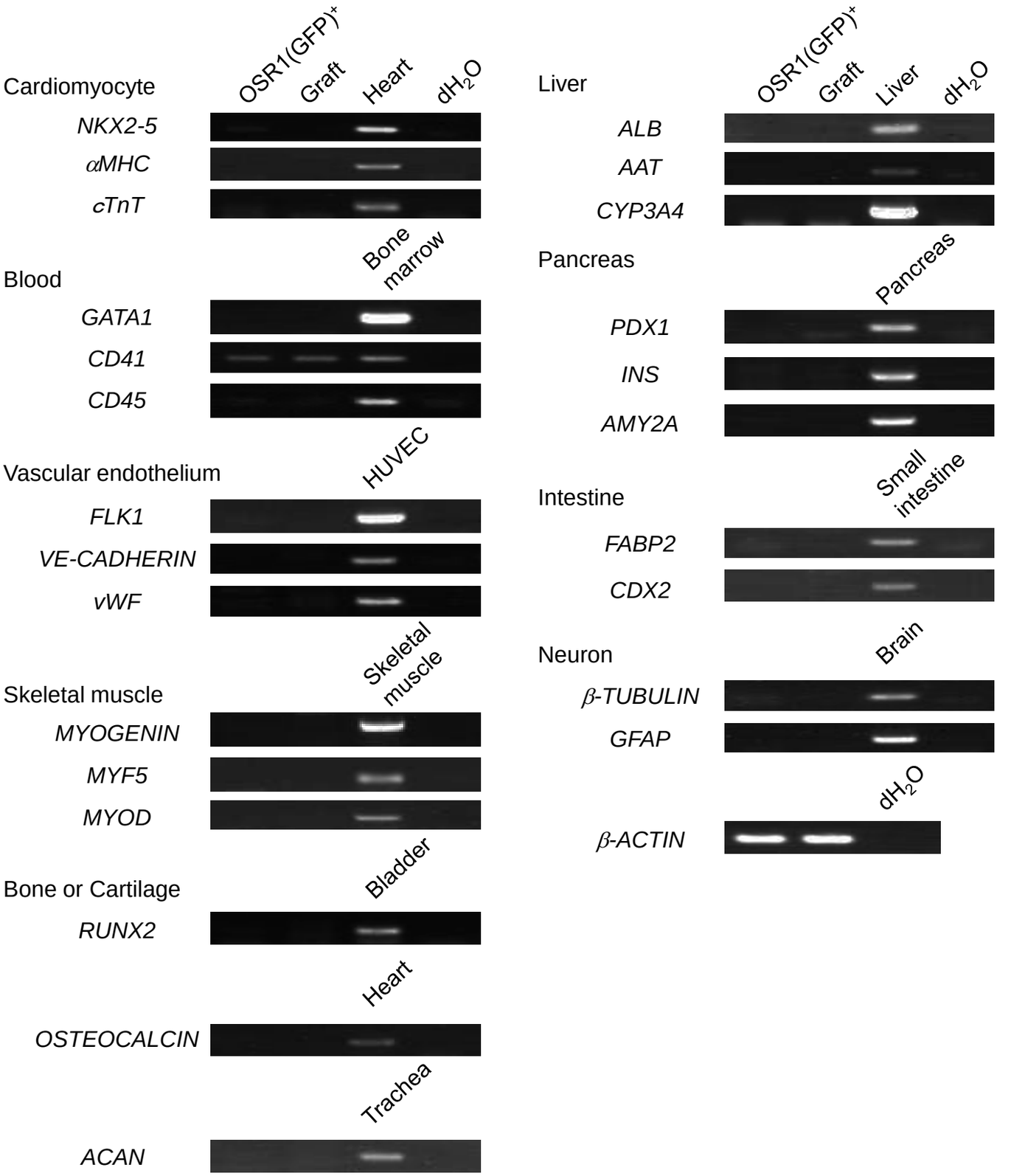
A comparison of the *OSR1* expression on culture day 11 in multiple hiPSC/ESC lines treated using the EB method. Samples of an OSR1-GFP knock-in hiPSC line, 3D45, derived from 201B7 cells were used as controls to normalize the data. The data from three independent experiments are presented as the means ± SD (n=3).

Supplementary Figure S9. Expression patterns of IM marker genes in mouse embryos.



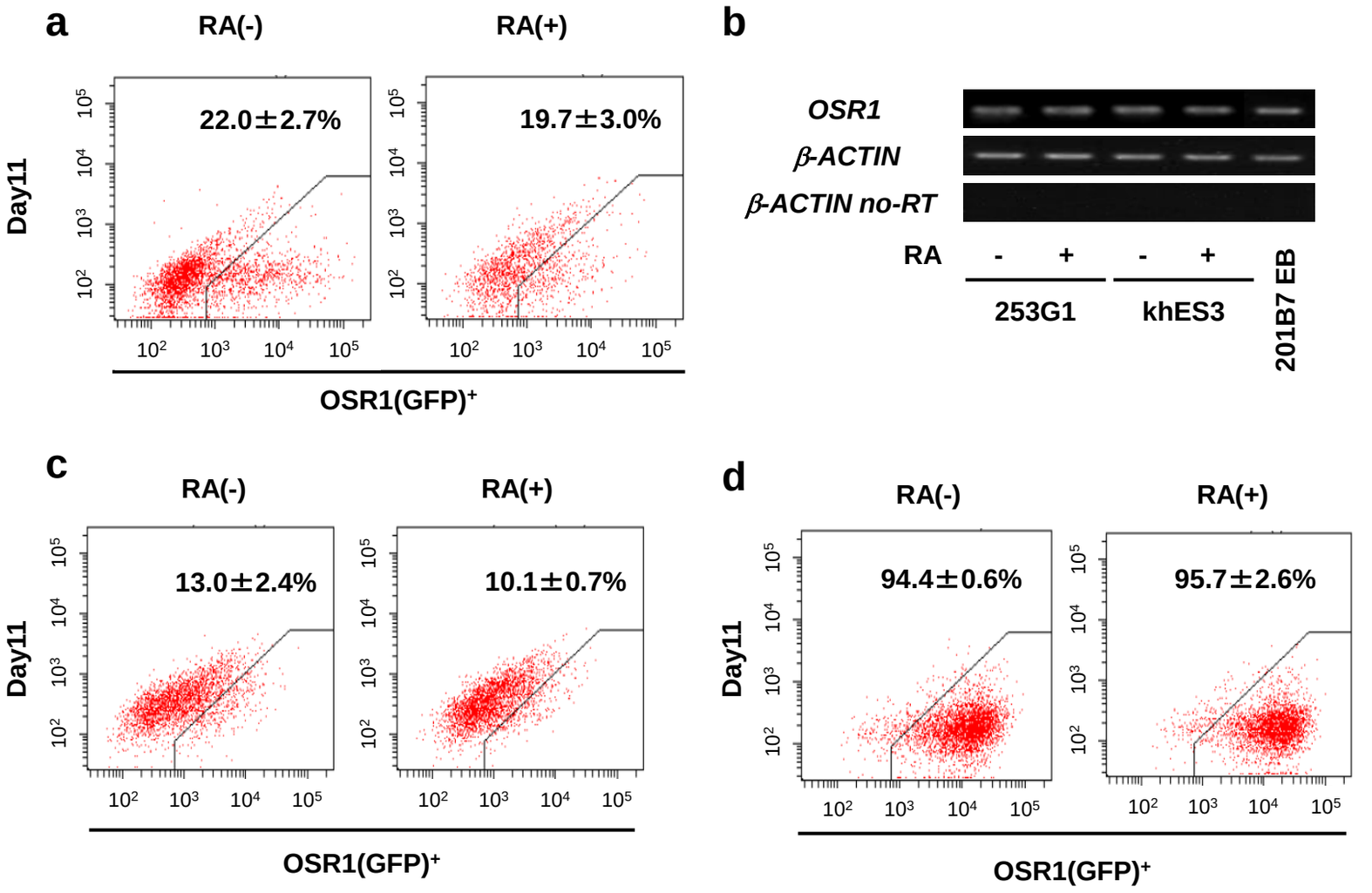
Immunostaining of the sections of embryonic day (E) 9.5 Osr1-GFP knock-in mouse embryos using antibodies against GFP and other IM markers, Pax2, Wt1 and Sall1. Scale bars, 100 μm in **a**, **c** and **e,** 50 μm in **b**, **d** and **f**.

Supplementary Figure S10. Weak or no expression of marker genes for other lineages than IM derivatives in OSR1$^+$ cells and the grafts.



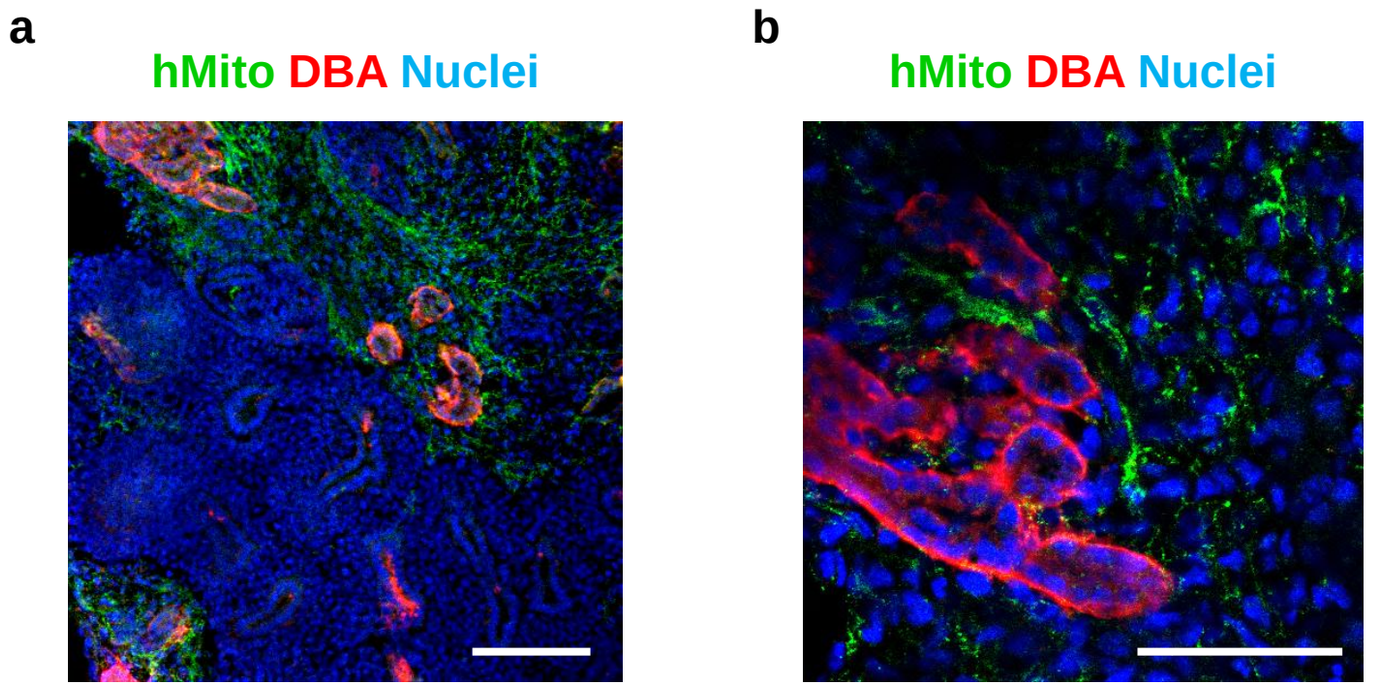
The gene expression of markers for endodermal, ectodermal or other mesodermal lineages than IM derivatives in OSR1(GFP)$^+$ cells on culture day11 and the grafts analyzed by RT-PCR.
ACAN; aggrecan, ALB; albumin, AAT; alpha-1 antitrypsin, INS; insulin, AMY2A; amylase, alpha 2A.

Supplementary Figure S11. RA addition does not provide any advantageous effects on IM induction from hiPSC/ESCs.



(**a**) The differentiation of OSR1 (GFP)$^+$ cells on culture day 11 (EB method) was analyzed by flow cytometry. RA(-): Stage 1, 100 ng/ml activin A + 100 ng/ml Wnt3a, Stage 2, 100 ng/ml BMP7 + 100 ng/ml Wnt3a; RA(+): Stage 1, 100 ng/ml activin A + 100 ng/ml Wnt3a, Stage 2, 100 ng/ml BMP7 + 100 ng/ml Wnt3a + 0.1 μM RA. (**b**) The *OSR1* expression on day 11 of differentiation culture from a hiPSC line, 253G1, and a hESC line, khES3, treated with or without RA during Stage 2 of the procedure. 201B7 EB; spontaneously differentiating EBs generated from 201B7 on day 16 of culture without any inducing factors. (**c, d**) The differentiation of OSR1(GFP)$^+$ cells at culture day11 of Colony (**c**) and Single cell (**d**) methods was analyzed by flow cytometry. RA(-): Stage 1, 100 ng/ml activin A + 3 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 3 μM CHIR99021; RA(+): Stage 1, 100 ng/ml activin A + 3 μM CHIR99021, Stage 2, 100 ng/ml BMP7 + 3 μM CHIR99021 + 0.1 μM RA. The data from three independent experiments are presented as the means ± SD (n=3) in **a**, **c** and **d**.

Supplementary Figure S12. Only a few human DBA$^+$ cells are observed in organ culture samples.



Immunostaining of histological sections of organ culture samples on day 7. Double staining with anti-human mitochondria (hMito) and the ureteric bud lineage marker, DBA. Scale bars, 100 μm in **a**, 50 μm in **b**.

Supplementary Table S1. Frequency of emergence of renal marker-positive cells differentiated from OSR1$^+$ IM cells

Marker name	Expression sites in IM derivative organs	Number of experiments performed	Percentage of wells containing the marker-positive cells (Mean ± SD)
LTL	Proximal tubule	7	2.0±1.6
AQP1		3	100.0±0.0
PNA	Glomerulus (Podocyte)	3	45.3±14.5
PODOCALYXIN		3	49.0±26.1
DBA	Nephric duct and Ureteric bud	3	93.3±3.2
HSD3β	Gonad or Adrenal cortex	4	6.0±2.8

OSR1$^+$ IM cells isolated by flow cytometry on culture day 11 were cultured on 96-well plates with BMP7 and either Wnt3a or CHIR99021 for an additional 7 days, and the number of wells that contain the marker-positive cells was counted. Each experiment used 96 wells.

Supplementary Table S2. Primer sequences

Gene name	Primer Sequence	Size (bp)
PGK-Neo check F	CTCAGTTGGAATTCCAGCAG	
PGK-Neo check R	CTGCCTGAAGGAAGGAGTAGTTGGTG	
hβ-ACTIN	CAATGTGGCCGAGGACTTTG CATTCTCCTTAGAGAGAAGTGG	126
mβ-Actin	GATCTGGCACCACACCTTCT GGGGTGTTGAAGGTCTCAAA	138
EGFP	ACGTAAACGGCCACAAGTTC AGTTCACCTTGATGCCGTTC	432
Mesendoderm		
hBRACHYURY	AATTGGTCCAGCCTTGGAAT CGTTGCTCACAGACCACA	112
hGSC	GAGGAGAAAGTGGAGGTCTGGTT CTCTGATGAGGACCGCTTCTG	72
hMIXL1	TTGGTTCAAAGCTGGACTCA CTGTCAGTCATGGCTCCTCA	107
Ectoderm		
hSOX1	CACAACTCGGAGATCAGCAA GGTACTTGTAATCCGGGTGC	133
hPAX6	GTCCATCTTTGCTTGGGAAA TAGCCAGGTTGCGAAGAACT	110
Endoderm		
hSOX17	CAGCAGAATCCAGACCTGCA GTCAGCGCCTTCCACGACT	68
hFOXA2	GGAGCGGTGAAGATGGAA TACGTGTTCATGCCGTTCAT	122
Intermediate mesoderm		
hOSR1	GCTGTCCACAAGACGCTACA CCAGAGTCAGGCTTCTGGTC	137
hPAX2	AGATTCCCAGAGTGGTGTGG GGGTATGTCTGTGTGCCTGA	264
hLIM1	TCATGCAGGTGAAGCAGTTC TCCAGGGAAGGCAAACTCTA	148
hWT1	GGCAGCACAGTGTGTGAACT CCAGGCACACCTGGTAGTTT	136
hCITED2	CACCAATGGGCTGCACCATCAC GCCGCTCGTGGCATTCATGTTG	157
hEYA1	GGACAGGCACCATACAGCTACC ATGTGCTGGATACGGTGAGCTG	189
hSALL1	AGCGAAGCCTCAACATTTCCAATCC	147

mOsr1	AATTCAAAGAACTCGGCACAGCACC GAGCGACCTTACACCTGTGA GTCTTGTGGACAGCGAGAGT	155
mPax2	GTTCCCAGTGTCTCATCCAT GGCGTTGGGTGGAAAGG	68
mLim1	CAGTGTCGCCAAAGAGAACA TGAGACGTTGGCACTTTCAG	121
mWt1	AAACCTGGAAACCTGGAAGG GGCTCCTCTCCGTCCTAACT	126
mCited2	TGCAGAAGCTCAACAACCAG CTGGTTTGTCCCGTTCATCT	107
mEya1	AATTTATGCCTGGCAACTGG CAGACCTCCCACGTTGTTTT	117
mSall1	AGCCTCAACATTTCCAATCC TGGGCATCCTTGCTCTTAGT	106
Metanephric mesenchyme		
hSIX2	AGGAAAGGGAGAACAACGAGAA GGGCTGGATGATGAGTGGT	132
hHOXD11	TGGAACGCGAGTTTTTCTTT CTGCAGACGGTCTCTGTTCA	149
Nephric duct **Ureteric bud**		
hSALL4	CAGATCCACGAGCGGACTCA CCCCGTGTGTCATGTAGTGA	109
hRET	TATCCTGGGATTCCTCCTGA TCTCCAGGTCTTTGCTGATG	166
hHOXB7	GTGGACTGTGGGTCTGGACT GAACACGCGAGTGGTAGGTT	114
Stromal progenitor		
hFOXD1	TGCGGGTCCCTCTATTTATG TAACGCCTGGACCTGAGAAT	190
Gonad **Adrenal cortex**		
hLHX9	GCGAACCTCTTTCAAGCATC TCCTTCTGAATTTGGCTCGT	164
hDAX1/NR0B1	CAAGGAGTACGCCTACCTCA GCGTCATCCTGGTGTGTTC	131
hSF1/NR5A1	CAGGAGTTTGTCTGCCTCAA GCACAGGGTGTAGTCAAGCA	126
hHSD3β2	GAGCCATTCCTGAAAAGAGC GATGAAGACTGGCACACTGG	168
hGATA4	CTGTCATCTCACTACGGGCA	132

hGATA6	GGGAGACGCATAGCCTTGT CATGACTCCAACTTCCACCT ACTTGAGCTCGCTGTTCTCG	146
Adult kidney		
hAQP1	ATTAACCCTGCTCGGTCCTT ACCCTGGAGTTGATGTCGTC	221
hPODOCALYXIN	TCATCATCACCATCGTCTGC CCACCTTCTTCTCCTGCATC	198
Epithelia		
hE-CADHERIN	CGGAGAAGAGGACCAGGACT GCCGCTTTCAGATTTTCATC	174
Cardiomyocyte		
hNKX2-5	CCCACGCCCTTCTCAGTCAA GTAGGCCTCTGGCTTGAAGG	144
hαMHC	CTGGAGGCCGAGCAGAAGCGCAACG GTCCGCCCGCTCCTCTGCCTCATCC	258
hcTnT	ATGAGCGGGAGAAGGAGCGGCAGAAC TCAATGGCCAGCACCTTCCTCCTCTC	241
Blood		
hGATA1	CAAGCTTCGTGGAACTCTCC ACTGACAATCAGGCGCTTCT	190
hCD41	ATCAGTTTGTGCTGCAGTCG TCCCCCTCTTCATCATCTTC	265
hCD45	TGTGATGCTTGTTCCCTTCA ACTGGAGTGTGGAGCAGCTT	111
Vascular endothelium		
hFLK1	TGATCGGAAATGACACTGGA CACGACTCCATGTTGGTCAC	131
hVE-CADHERIN	ACACCTCACTTCCCCATCA GACCTTGCCCACATATTCTCC	95
hvWF	GGGGTCATCTCTGGATTCAA AGGCAAACATCTCCCACAAC	111
Skeletal muscle		
hMYOGENIN	TAAGGTGTGTAAGAGGAAGTCG CCACAGACACATCTTCCACTGT	437
hMYF5	GCCTGAAGAAGGTCAACCAG GGAACTAGAAGCCCCTGGAG	461
hMYOD	CGATATACCAGGTGCTCTGAGGG GGGTGGGTTACGGTTACACCTGC	430
Bone or Cartilage		
hRUNX2	ATGCTTCATTCGCCTCACAAACAAC	286

hOSTEOCALCIN	TGAAGCGCCGGCTGGTGCTC GACTGTGACGAGTTGGCTGA CTGGAGAGGAGCAGAACTGG	119
hACAN	TGAGGAGGGCTGGAACAAGTACC GAGGTGGTAATTGCAGGGAACA	349
Liver		
hALB	CCTTTGGCACAATGAAGTGGGTAACC CAGCAGTCAGCCATTTCACCATAGG	355
hAAT	ACATTTACCCAAACTGTCCATT GCTTCAGTCCCTTTCTCGTC	183
hCYP3A4	CCTTACATATACACACCCTTTG GGTTGAAGAAGTCCTCCTAAGCT	170
Pancreas		
hPDX1	TGTTCCGAGGTAGAGGCTGT AACATAACCCGAGCACAAGG	251
hINS	GCCTTTGTGAACCAACACCT TGCTGGTTCAAGGGCTTTAT	338
hAMY2A	TGGAGAAGGATTACGTGCGT GAAGGTACGAAACCCCAACC	358
Intestine		
hFABP2	TGCAGCTCATGACAATTTGA CCCTGAGTTCAGTTCCGTCT	149
hCDX2	GCAGAGCAAAGGAGAGGAAA AAGGGCTCTGGGACACTTCT	136
Neuron		
hβ-TUBULIN	CAGATGTTCGATGCCAAGAA TGCTGTTCTTGCTCTGGATG	142
hGFAP	GGCCCGCCACTTGCAGGAGTACCAGG CTTCTGCTCGGGCCCCTCATGAGACG	328

Gene names and primer sequences (5'–3') for RT-PCR and qRT-PCR are shown.

ACAN; aggrecan, ALB; albumin, AAT; alpha-1 antitrypsin, INS; insulin, AMY2A; amylase, alpha 2A.

Supplementary Table S3. Antibodies and lectins

Antibody & Lectin	Dilution rate	Manufacturer
GFP	1 : 200	Aves Labs, Inc., GFP-1020
PAX2	1 : 100	Covance Research Products, PRB-276P
WT1	1 : 50	Santa Cruz Biotechnology, C-19
SALL1	1 : 50	Perseus Proteomics Inc., PP-K9814-001
SALL4	1 : 200	Abnova, M03
HSD3β	1 : 50	Santa Cruz Biotechnology, 37-2
GATA4	1 : 200	Santa Cruz Biotechnology, C-20
GATA6	1 : 200	Santa Cruz Biotechnology, H-92
LTL	1 : 200	Vector laboratories, B-1325
AQP1	1 : 500	MILLIPORE, AB3272
PNA	1 : 200	Vector laboratories, RL-1072
PODOCALYXIN	1 : 200	R&D SYSTEMS, MAB1658
DBA	1 : 200	Vector laboratories, FL-1031
CYTOKERATIN	1 : 200	SIGMA, C2562
αSMA	1 : 200	SIGMA, A2547
E-Cadherin	1 : 200	BD Pharmingen, 610181
Laminin	1 : 200	SIGMA, L9393
Human nuclear antigen	1 : 500	MILLIPORE, MAB1281
Human mitochondria	1 : 200	Abcam, Ab3298

Supplementary methods

Cell culture.

miPSCs (492B-4 and 20D-17) and mESCs (J1 and D3) were maintained on feeder layers of mitomycin C-treated STO cells in DMEM (Nacalai Tesque) supplemented with 15% FBS (Invitrogen), 500 U/ml penicillin/streptomycin, 0.1 mM non-essential amino acid (Invitrogen), 2 mM glutamine (Invitrogen), 0.55 mM 2-mercaptoethanol (Invitrogen) and leukemia inhibitory factor (LIF). As a source of LIF, we used a conditioned medium (1:10,000 dilution) from Plat-E cell cultures that had been transduced with a LIF-encoding vector[37]. miPSC and mESCs were passaged with enzymatic dissociation using 0.25% trypsin/EDTA (Invitrogen).

Karyotyping.

Karyotyping with a chromosomal G-band analysis of the OSR1-GFP hiPSC reporter line was carried out by the Nihon Gene Research Laboratories, Japan.

RT-PCR and real-time quantitative RT-PCR (qRT-PCR).

Total RNA was isolated from triplicate samples in three independent experiments using an RNeasy kit (Qiagen) according to the manufacturer's recommendations, followed by cDNA synthesis using standard protocols. Briefly, first-strand cDNA was synthesized from 1 μg of total RNA using ReverTra Ace (TOYOBO). The cDNA samples were subjected to PCR amplification using a thermal cycler (Veriti 96 well Thermal Cycler, Applied Biosystems). PCR was performed using the Ex-Taq PCR kit (Takara) according to the manufacturer's instructions. The PCR cycles were as follows: for *β-ACTIN*, initial denaturation at 94°C for 2.5 min, followed by 25 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 30 s, and a final extension at 72°C for 10 min. For the other genes, the cycles consisted of initial denaturation

at 94°C for 2.5 min, followed by 30-40 cycles of 94°C for 30 s, 58-62°C for 30 s, 72°C for 30 s, and final extension at 72°C for 7 min. qPCR was performed using the Step One Plus Real-Time PCR System (Applied Biosystems) and SYBR Green PCR Master Mix (Takara). Denaturation was performed at 95°C for 30 s followed by 45 cycles at 95°C for 5 s and at 60°C for 30 s. As recommended by the manufacturer, the threshold cycle method was used to analyze the data for the gene expression levels, and was calibrated to those of the housekeeping gene, *β-ACTIN*. The PCR reactions were performed in triplicate for each sample. The primer sets are shown in **Supplementary Table S2**.

Flow cytometry and cell sorting.

The cells were treated with 10 μM Y27,632 before dissociation, incubated with 0.25% trypsin/EDTA for 5 min at 37°C, and dissociated by pipetting in PBS with 2% FBS. Dead cells stained with Propidium-iodide (1 μg/ml, Wako) were excluded from the analysis. The cells were analyzed and sorted using a FACS Aria II cell sorter (BD). The isolated cells were collected into PBS with 2% FBS containing 10 μM Y27,632.

Immunostaining and lectin staining.

The cells were fixed with 4% paraformaldehyde (PFA)/PBS for 20 min at 4°C. After washing with PBS, the cells were blocked with 1% normal goat or donkey serum (Chemicon), 3% BSA (Nacalai Tesque) /PBST (PBS/0.25% Triton X-100) for 1h at room temperature. For anti-human nuclear antigen staining, fixed samples were incubated with 0.2% Tween20/PBS for 20 min at room temperature, and then blocked with 5% donkey serum/0.4% Triton X-100/PBS for 1h at room temperature. Primary antibodies were diluted in each blocking solution and incubated with samples overnight at 4°C. Secondary antibodies were incubated for 1h at room temperature. The details of antibodies and lectins used for these studies are

shown in **Supplementary Table S3**.

Supplementary Methods – Additional reference.

37. Morita, S., Kojima, T. & Kitamura, T. Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther* **7**, 1063-1066 (2000).