Supplementary information

Supplementary methods

Mouse embryonic fibroblast (MEF) isolation and culture

MEFs were isolated as previously described in [1]. Briefly, 14-days old embryos were isolated from uteri of a pregnant mouse (C57BL/6) under sterile conditions and washed in PBS containing antibiotics. The head and visceral mass were removed by surgical blade and forceps. Remaining bodies were washed with sterile PBS and transferred to a sterile dish containing Trypsin-EDTA solution (2mL per embryo) to mince into fine pieces and incubated at 37^0C for 20 minutes. 2 mL MEF medium (DMEM, 10% FBS, 1x Sodium pyruvate and Pen-strep) was added and pipetted up and down a few times. Contents were transferred into a 15 mL tube and incubated for 5 minutes. Supernatant was transferred to a fresh tube and centrifuged at 1000 rpm for 5 minutes to harvest cells. Cells were suspended in MEF medium and plated on 100 mm TC-treated dishes and grown at 37^0C with 5% CO_2. Passage 2 and passage 3 MEFs were used for total RNA and protein extraction.

Human iPSC culture

Human iPSC lines- NCRM1 and ND1.4 (RUDCR infinite biologics) were cultured in a StemFlex medium kit (Invitrogen/ A3349401) as per the described instructions. Briefly, iPSCs were treated with StemPro Accutase for 3 minutes and dissociated into small clumps by pipetting. An appropriate amount of the complete StemFlex medium was added and cells were harvested by centrifugation at 1000 rpm for 3 minutes. Cells were resuspended in the complete medium and generally split in a 1:4 ratio. Cells were plated on hESC-qualified Geltrex (Invitrogen/A1413302) pre-coated dishes in the complete StemFlex medium supplemented with 1X RevitaCell (Invitrogen/A26445) for 24-hours post-passaging and only in the complete StemFlex medium thereafter until confluency.

Alkaline phosphatase (ALP) staining

ALP activity on day 3 mESC cultures grown on 12w dish was assayed using leucocyte alkaline phosphatase staining kit (Sigma-Aldrich 85L3R-1KT procedure no.85). Briefly, spent medium was removed and cells were rinsed with PBS. Cells were fixed with 0.5 mL citrate buffered 60% acetone for 30 s and rinsed twice with distilled water. Next, 0.5 mL staining reagent (1x Fast Violet B solution & 1x Naphthol AS-MX phosphate alkaline solution in distilled water) was added and incubated at RT for 20-30 minutes. As the red color developed, staining reagent was removed and wells were rinsed with 1 mL distilled water twice and air dried. The color developed was observed under an inverted microscope with a color camera (Olympus IX81 with DP71 camera) and images were acquired using CellSens software. Integrated densities of ALP activity from control and knockdown images were calculated using ImageJ software and plotted using GraphPad Prism 8.0.

Protein extraction and western blotting

Cell pellet was suspended in RIPA buffer (10 mM Tris-Cl pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1 % SDS) supplemented with 1X Sigmafast protease inhibitor cocktail and incubated on ice for 30 minutes. Lysate was centrifuged at 12000 X g for 10 minutes and supernatant

was collected. Protein was quantified by Bradford assay reagent (Biorad) and samples were prepared for SDS-PAGE in laemmli buffer. 25 ug of each sample was loaded on 8-10% SDS-PAGE and resolved. Resolved proteins were transferred to PVDF membrane using semi-dry Transblot apparatus (Biorad) at 25 V for 75 minutes. After the transfer, the membrane was blocked in 5% BSA for 1 h and incubated with respective primary and secondary antibodies diluted in 1% BSA in TBST for 1 h at room temperature. After antibody incubation, membrane was washed 3X with TBST buffer for 10 minutes. Immobilon chemiluminescence substrate was used to develop signal and captured in iBright FL1000 instrument (ThermoFisher Scientific).

Cell cycle profiling

mESCs were dissociated with accutase and harvested on day 3. About 1 x 10^6 mESCs were fixed by adding 70% ethanol drop-wise with a high speed vortex to avoid clumps and stored at 4^0 C for at least 30 minutes. Next, cells were pelleted by centrifuging at 1200 rpm for 5 min at RT. Ethanol was removed and cells were rehydrated in PBS for 10 minutes. Cells were resuspended in 300 uL propidium iodide (PI)/ Triton X-100 staining solution (PBS with 0.1% Triton X-100, 200 µg/ mL DNase-free RNase A, 10 µg/ mL PI) and incubated for 30 min in dark. The samples were acquired in FACSCalibur instrument with a 488 nm excitation laser. The analysis of cell cycle populations was performed in BD CellQuest Pro software.

mRNA stability assay using alpha-amanitin

1.5 x 10^5 mESCs or cXEN cells were plated per well of a gelatin-coated 12w plate on the previous day. Next day, the spent medium was replaced with medium and incubated for an hour at 37^0C. Alpha-amanitin was then added at concentration of 15 ug/mL and cells were harvested in TriZol at following time points: 0 h, 2 h, 4 h, 8 h, 16 h and 24 h. RNA was isolated from each time point and cDNAs were prepared. RT- qPCR was performed to find relative abundance of *Rbm47* mRNAs by normalising to 18S rRNA at each time point. Next, exponential decay was plotted by normalizing the mRNA abundance to t= 0 h and half-life was determined using GraphPad Prism one phase decay analysis.

Supplementary figures

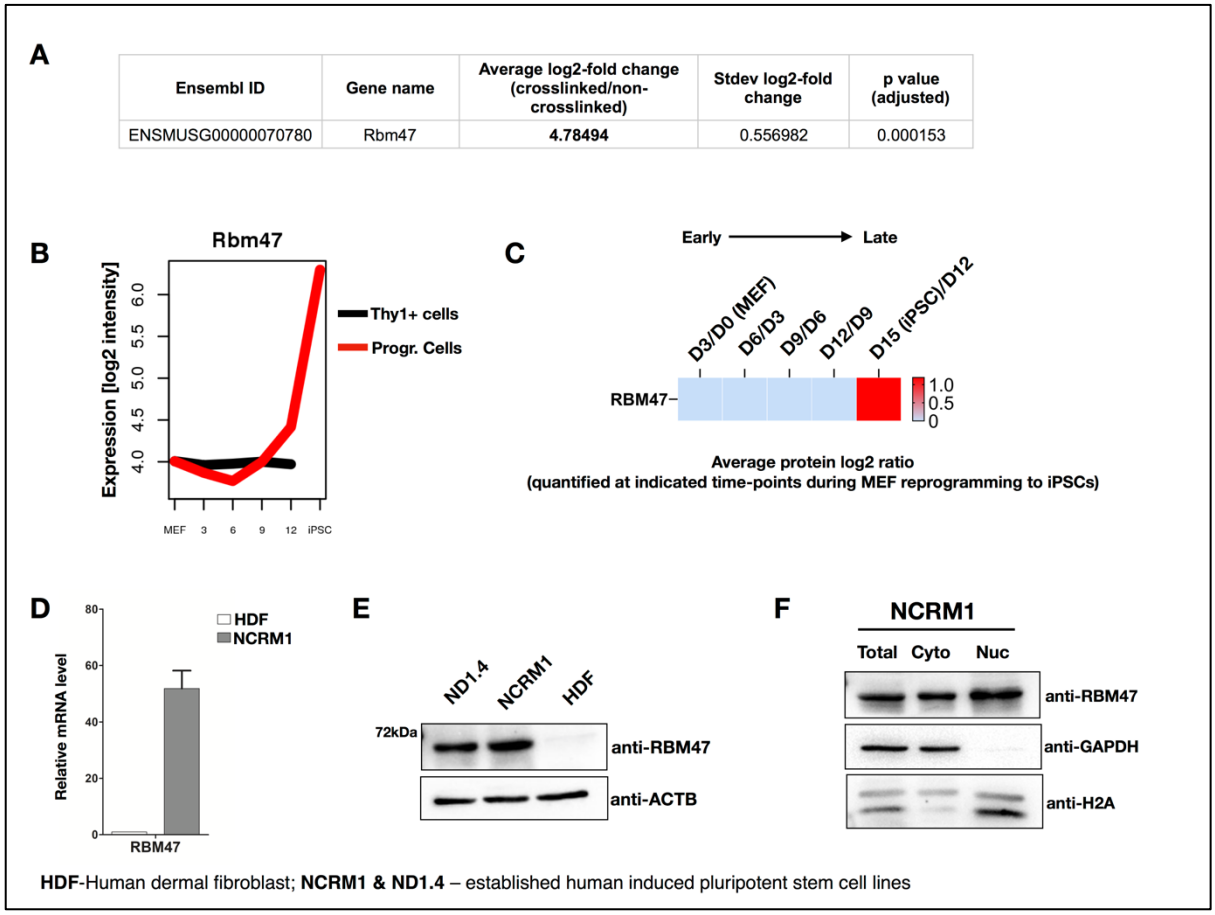

Fig S1. RBM47 protein is expressed in the late phase reprogramming and remain abundant in mouse and human PSCs. A Fold-change value of RBM47 captured in mRNA interactome of R1 mESCs [2]. **B** *Rbm47* expression profile during somatic reprogramming of MEFs from gene expression database of Polo et al. [3] **C** RBM47 protein levels during somatic cell reprogramming of MEFs from protein database of Hansson et al. [4]. **D** and **E** Relative expression of *RBM47* in human iPSCs (NCRM1 and ND1.4) and human dermal fibroblasts. **F** Nuclear and cytoplasmic fractionation of human iPSC line NCRM1 followed by western blot analysis of RBM47. GAPDH was used as a cytoplasmic marker and Histone H2A was used as a nuclear marker.

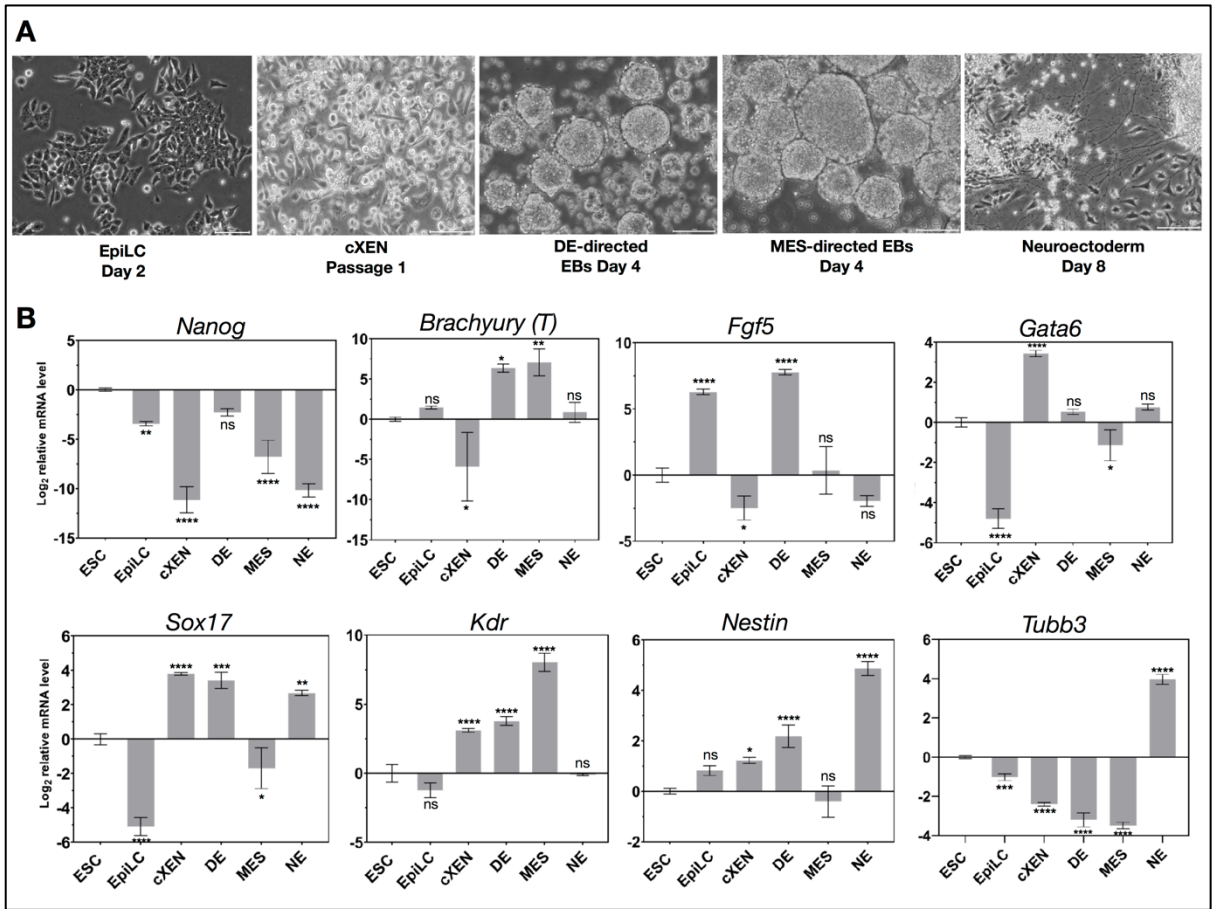

Fig S2. Lineage-specific differentiation of mESCs and characterization. **A** Phase-contrast images of differentiated mESC cultures, captured at indicated days/passages. **B** RT-qPCR profiling of lineage-specific markers. Relative mRNA levels (log2 normalized) values from three biological replicates were plotted with error bars indicating S.E.M. One-way ANOVA followed by Dunnett's multiple comparisons test was to determine statistical significance; ns- non-significant; *p<0.05; **p<0.01; ***p<0.001; **** p<0.0001. *Nanog* - pluripotency, *Fgf5* – EpiLC, *Brachyury* - mesendoderm, *Gata6* - extraembryonic endoderm, *Sox17* - endoderm and extraembryonic endoderm, *Kdr* - mesoderm, *Nestin* and *Tubb3* – neuroectoderm.

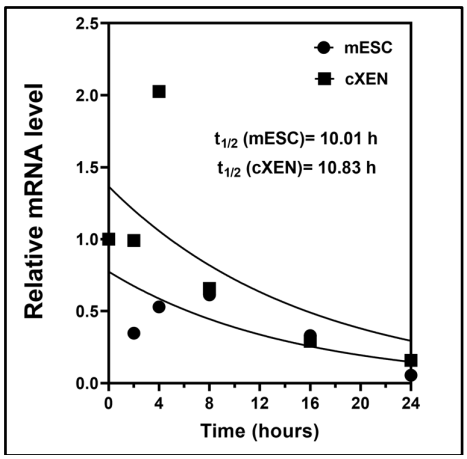

Fig S3. Determination of *Rbm47* mRNA stability in mESCs and cXEN cells. One phase decay analysis of *Rbm47* mRNA in alpha-amanitin treated mESCs and cXEN cells performed using the relative abundance of

mRNA (relative to *18S rRNA*) at indicated time points, plotted by normalizing to t= 0 h from one experiment. GraphPad Prism was used for the analysis.

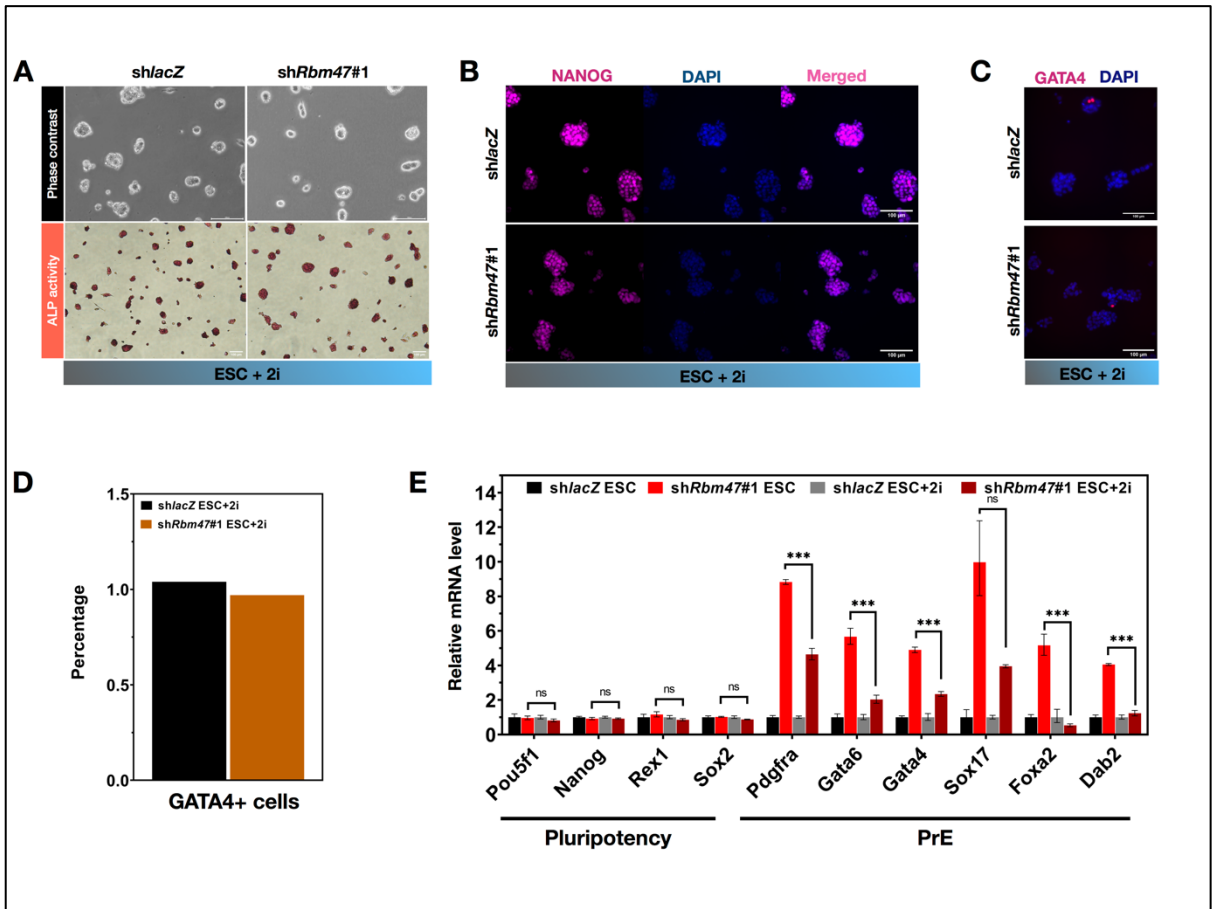

Fig S4. Culturing *Rbm47* knockdown mESCs in 2i+LIF condition brings down the PrE-like cell fraction to the control mESC level. A Phase contrast images of sh*lacZ* mESCs and sh*Rbm47* mESCs (Top). Cells were fixed and stained for alkaline phosphatase (ALP) activity (bottom) (scale-100 µm). **B** Widefield fluorescence images of mESCs stained for pluripotency marker, NANOG (scale-100 µm). **C** Widefield fluorescence images of mESCs immunostained for GATA4 (scale-100 µm). **D** Quantification of GATA4+ cell population in indicated cultures using cell counter plugin of ImageJ software. More than 1000 nuclei (DAPI) analysed in a single experiment and percentage values were plotted. **E** Relative mRNA levels of PrE markers from three biological replicates. The plotted values represent mean ± S.E.M. Unpaired student's t-test was used to determine statistical significance; ns- non-significant; *p<0.05; **p<0.01; ***p<0.001.

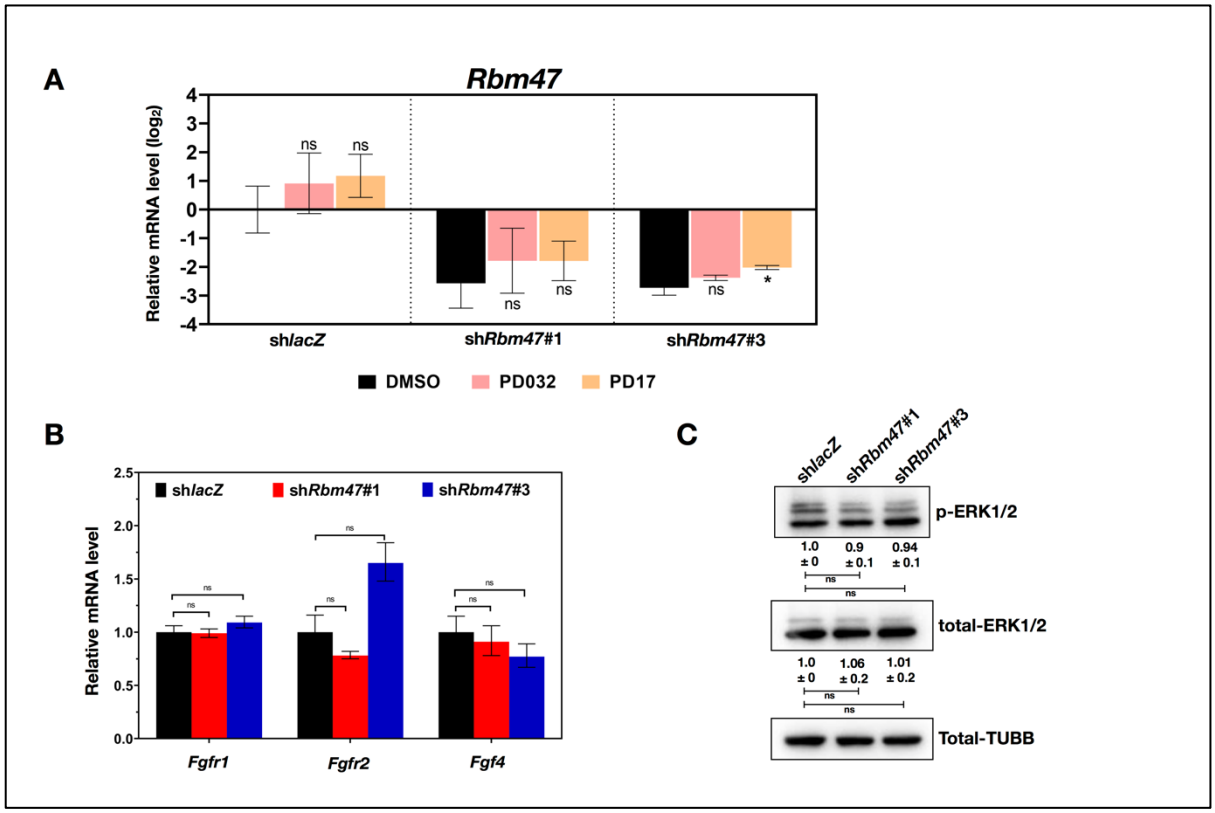

Fig S5. A Relative mRNA levels of *Rbm47* in DMSO treated, PD17 (Pan-FGFR inhibitor) treated and PD032 (MEK1/2 inhibitor) treated control and *Rbm47* knockdown mESCs (48 h treatment) from three independent experiments. Error bars indicate S.E.M. Values were normalized to DMSO treated sh*lacZ* mESCs. **B** RT-qPCR profiling of FGF receptors (*Fgfr1 and Fgfr2*) and *Fgf4* expression. The plotted values represent mean ± S.E.M. from three biological experiments. **C** Immunoblotting of total ERK1/2 and p-ERK1/2 in control and *Rbm47* cell lysates. Relative quantification is represented as mean ± S.E.M from five blotting experiments. Statistical test used for: A- ordinary one-way ANOVA followed by Dunnett's multiple comparison test, DMSO treated cells used as calibrator; B and C- unpaired student's t-test; ns- non-significant; *p<0.05.

Supplementary tables

Table S1: shRNA oligo sequences cloned in pLKO.1 TRC vector

Target gene	5'→3' Oligo sequence (target sequence in red; F-forward; R-reverse)
lacZ **(Non-targeting control)**	F: CCGGTCGTATTACAACGTCGTGACTCTCGAGAGTCACGACGTTGTAATACGATTTTTG R: AATTCAAAAATCGTATTACAACGTCGTGACTCTCGAGAGTCACGACGTTGTAATACGA
Rbm47	sh#1 F: CCGGCCCGCGTTCATACATTTCTAACTCGAGTTAGAAATGTATGAACGCGGGTTTTTG R: AATTCAAAAACCCGCGTTCATACATTTCTAACTCGAGTTAGAAATGTATGAACGCGGG
Rbm47	sh#3 F: CCGGCCGTCCAATAACTCCTGTGTACTCGAGTACACAGGAGTTATTGGACGGTTTTTG R: AATTCAAAAACCGTCCAATAACTCCTGTGTACTCGAGTACACAGGAGTTATTGGACGG

Table S2: List of primers used for RT-qPCR

Target gene	Forward (5'→3')	Reverse (5'→3')
18S rRNA (Rn18S)	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Actb	TCACTATTGGCAACGAGCG	AGGTCTTTACGGATGTCAACG
Gapdh	CCTCGTCCCGTAGACAAAATG	TGTAGTTGAGGTCAATGAAGGG
Rbm47	ACACATGATCAGCCCCATTG	GTACACAGGAGTTATTGGACGG
Pou5f1	CACTCTACTCAGTCCCTTTTCC	GTTCTCTTGTCTACCTCCCTTG
Dab2	CAATAAAGTTGGCATTCCTCAGG	GGGTTCTCGAGTGAATTGTCAG
Fgf4	TACCCCGGTATGTTCATGGC	TTACCTTCATGGTAGGCGACA
Pdgfra	GTTGCCTTACGACTCCAGATG	TCACAGCCACCTTCATTACAG
Nanog	CAAAGGATGAAGTGCAAGCG	CCAGATGCGTTCACCAGATAG
Klf4	GACTAACCGTTGGCGTGAG	CGGGTTGTTACTGCTGCAAG
Mixl1	CGTCTTCCGACAGACCATGT	GTTCTGGAACCACACCTGGAT
Esrrb	TGTGTTCCTCATCAACTGGG	CAGCTTGTCATCGTATGGGAG
Rex1	ACATCCTAACCCACGCAAAG	CATTAAGACTACCCAGCCTGAG
Sox2	CCAATCCCATCCAAATTAACGC	CTATACATGGTCCGATTCCCC
Gata6	TCTACAGCAAGATGAATGGCC	CTCACCCTCAGCATTTCTACG
Foxa2	GATGTACGAGTAGGGAGGTTTG	AACATGAACTCGATGAGCCC
Gsc	GACGAAGTACCCAGACGTG	TTCTCGGCGTTTTCTGACTC
Nodal	GTACATGTTGAGCCTCTACCG	GCCCATACCAGATCCTCTTC
Fgf5	TGACTGGAATGAGTGCATCTG	GGGTTTGGAATTTGGGTTGAG
Pax6	AGTGAATGGGCGGAGTTATG	ACTTGGACGGGAACTGACAC
Cdx2	TTAAACTCCACTGTCACCCAG	GTAGATGCTGTTCGTGGGTAG
Sox17	TTCCAAGACTTGCCTAGCATC	CTTTATGGTGTGGGCCAAAG
Afp	TGGTTACACGAGGAAAGCCC	AATGTCGGCCATTCCCTCACG
Tbx3	AGCCAACGATATCCTGAAACTG	GTGTCTCGAAAACCCTTTGC

Hnf4a	CTAACACGATGCCCTCTCAC	GCAGGAGCTTGTAGGATTCAG
Eomes	TTTCGTGGAAGTGGTTCTGG	CAGTGTTAGGAGATTCTGGGTG
Brac (T)	AGGTACCCAGCTCTAAGGAAC	CGAGGCTAGACCAGTTATCATG
Olig2	GCGAGCACCTCAAATCTAATTC	AAAAGATCATCGGGTTCTGGG
Tubb3	CGCCTTTGGACACCTATTCAG	TTCTCACACTCTTTCCGCAC
Nes	AGGGTCTACAGAGTCAGATCG	TGTCTGCAAGCGAGAGTTC
Cdh2	GGACAGCCCCTTCTCAATG	TTCTCACAGCATACACCGTG
Neurod1	GCGAGATCCCCATAGACAAC	ATAGTGAAACTGACGTGCCTC
Gata4	CTGTCATCTCACTATGGGCAC	GAGTGACAGGAGATGCATAGC
Gbx2	CTCGGAACCCCAAGATTGTC	GTTCCTTCAGGTGTTAGGGC
RBM47 (human)	AAGGATATGAACTGGTGCCG	AACATGGAATACTGAGCCCC
Map2	CAGGGCACCTATTCAGATACC	TCCTTCTCTTGTTCACCTTTCAG
Emx1	AGCGAGCCTTTGAGAAGAATC	CTGCCGTTTGTATTTTGTCCTC
Otx2	CTTCGGGTATGGACTTGCTG	CCTCATGAAGATGTCTGGGTAC
Kdr	GCAAAACACTCACCATTCCC	ACTGACAGAGGCGATGAATG
Ascl1	GTGACTGGTGTCTGAACCTAAG	CCTGTTAGTGGTTGGGAGTTAATA
Snai2	CCATTAGTGACGAAGAGGAGAG	CAGCCCAGAGAACGTAGAATAG
Twist1	CCACACCTCTGCATTCTGATAGA	CTTTCCTGTCAGTGGCTGATT
Fgfr1	AACTTGCCGTATGTCCAGATC	AGAGTCCGATAGAGTTACCCG
Fgfr2	AGGAGTTTAAGCAGGAGCATC	GTGGTAGGTGTGGTTGATGG

Table S3: Growth factors, antibodies and inhibitors

Reagent	Manufacturer/ Cat no.	Usage details
Growth factors		
Leukaemia inhibitory factor	Peprotech/AF-250-02	10 ng/mL in ESC medium
Activin A	R&D systems/338-AC-050/CF	5 ng/mL for MES 50 ng/mL for DE
BMP-4	R&D systems/314-BP-050/CF	0.25 ng/mL for MES
VEGF	Peprotech/100-20	5 ng/mL for MES
Antibodies		
Anti-NANOG	Millipore/AB5741	WB- 1:3000 | ICC- 1:100
Anti-OCT4A	Cell Signalling/2840s	WB- 1:3000 | ICC- 1:100
Anti-RBM47	Abcam/ab167164	WB- 1:8000
Anti-SOX2	Cloud clone/PAA406Hu01	WB- 1:3000 | ICC- 1:100
Anti-SSEA1	Invitrogen/MA1022	ICC- 1:100
Anti-TUBB HRP conjugated	Cell Signalling/5346	WB- 1:5000
Anti-TUBB3	Invitrogen/MA1118	WB- 1:3000 | ICC- 1:400
Anti-GATA4	Cell Signalling/36966	WB- 1:5000 | ICC- 1:800
Anti-EOMES	Abcam/ Ab23345	WB- 1:2500

Anti-ERK1/2	Santa Cruz/sc-154	WB- 1:3000
Anti-p-ERK1/2	Santa Cruz/sc-7383	WB- 1:1000
Inhibitors		
PD0325901	Sigma/PZ0162	1 μM
PD173074	Sigma/341607	100 nM
CHIR99021	Sigma/SML1046	3 μM

N2B27 medium recipe

Components	Volume in mL
DMEM/F12 21331-020	241.5
Neurobasal 21103049	241.5
50X B27 supplement 17504044	5
100X N2 supplement 17502048	2.5
B-Mercaptoethanol 55 mM	0.455
100x Glutamax	5
Pen-Strep	2.5
BSA Fraction V (25mg/mL)[#]	1
Insulin (10mg/mL)*	0.5
Insulin (10mg/mL)**	0.875

* final concentration will be 12.5 ug/mL

** Neuro-specific medium with 20 ug/mL insulin

[#] BSA was dissolved in DMEM/F12

The medium can be aliquoted and stored at -20^{0}C for 6 months. When required, thawed at 4^{0}C overnight and used. At 4^{0}C, medium stable for a month.

References:

1. Jain K, Verma PJ, Liu J (2014) Isolation and Handling of Mouse Embryonic Fibroblasts. Methods Mol Biol 1194:247–252. https://doi.org/10.1007/978-1-4939-1215-5_13
2. Kwon SC, Yi H, Eichelbaum K, et al (2013) The RNA-binding protein repertoire of embryonic stem cells. Nat Struct Mol Biol 20:1122–1130. https://doi.org/10.1038/nsmb.2638
3. Polo JM, Anderssen E, Walsh RM, et al (2012) A molecular roadmap of reprogramming somatic cells into iPS cells. Cell 151:1617–1632. https://doi.org/10.1016/j.cell.2012.11.039
4. Hansson J, Rafiee MR, Reiland S, et al (2012) Highly Coordinated Proteome Dynamics during Reprogramming of Somatic Cells to Pluripotency. Cell Rep 2:1579–1592. https://doi.org/10.1016/J.CELREP.2012.10.014/ATTACHMENT/BD2B3958-D120-410A-A770-B7621D69ED8A/MMC6.XLSX