FULL METHODS (Supplementary Material and Methods)

Human iPSC generation

Lentiviral vectors encoding human Oct4, Sox2, Klf4 and cMyc under the transcriptional control of human phosphoglycerate kinase (hPGK) promoter were constructed[13]. Overlapping PCR was used to generate bicistronic expression cassettes encoding vexGFP, mCitrine, mCherry and mCerulean linked by a P2A peptide preceded by a Gly-Ser-Gly linker to the cDNAs of human Oct4, Sox2, Klf4 and c-Myc, respectively, which were cloned in a lentiviral vector under the transcriptional control of hPGK promoter. Vector supernatants were produced as previously described[13]. Lentiviral vector supernatants were produced by triple co-transfection of the plasmid DNA encoding the vector, **pCMVΔR8.91** and **pUCMD.G** into 293T cells, filtered through a 0.22 μm pore-size cellulose acetate filter (Whatman) and concentrated by ultracentrifugation. Human FD and normal fibroblasts (GM04959, GM04589, GM04899, GM02341, GM02342, GM023036) purchased from Coriell and human fetal lung fibroblasts (MRC-5) purchased from ATCC (CCL-171) were seeded at $1.5x10^5$ cells per well of a 6-well plate in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS). The following day the fibroblasts were transduced with supernatants of the 4 lentiviral vectors in the presence of 4 μg/ml polybrene for ~16 hours. 5 days after transduction, fibroblasts were harvested by trypsinization and plated at $2x10^4$cells per 60 mm dish on a feeder layer of mitomycin C- treated mouse embryonic fibroblasts (MEFs; GlobalStem, Inc.). The next day, the medium was switched to hESC medium supplemented with 6 ng/ml FGF2 and was replaced every day thereafter. Twenty days after transduction, colonies with hESC morphology were mechanically dissociated and transferred into 24-well plates on MEFs. Cells were thereafter passaged with dispase and expanded to establish iPSC lines.

Cell culture and differentiation

Undifferentiated hESCs and iPSC lines were cultured on mitotically inactivated MEFs in a serum-replacement containing culture medium supplemented with 4 ng/ml FGF2 as used routinely for hESC cultures[15,26]. For neural induction, hESCs were plated at 5-20 x 10^3 cells on a confluent layer of irradiated (50Gy) stromal cells (MS-5) in 60-mm cell culture plates in KSR medium[26]. After 16 days in serum replacement medium, cultures were switched to N2 medium.

Medium was changed every 2–3 days, and growth factors were added at the following concentrations as described previously: 200 ng/ml SHH, 100 ng/ml FGF8, 20 ng/ml brain-derived neurotrophic factor (BDNF) (all R&D Systems), and 0.2 mM ascorbic acid (AA) (Sigma–Aldrich). Rosettes structures were harvested mechanically at day 14-20 of differentiation (termed passage 0; P0) and gently replated on 15 μg/ml polyornithine/1 μg/ml laminin (PO/Lam)-coated culture dishes in N2 medium (termed passage 1; P1). P1 cultures were supplemented with FGF2, AA, and BDNF or any alternative growth factor conditions as listed in main text. After 6-7 days of P1 culture, cells were triturated into single cells after exposure to Accutase (Innovative cell technologies, Inc., 10 min at incubator) and labeled with antibodies for flow cytometry. FACS sorting (HNK1[15]; Sigma or Forse1[14]:DSHB) was performed on a MoFlo (Dako). Sorted cells were plated on culture dishes precoated with PO/Lam and 10ng/ml fibronectin (PO/Lam/Fib). hESC-NC were maintained in N2 medium supplemented with 20ng/ml of FGF2 and 20ng/ml of EGF changed every 2-3 days and passaged every 7-8 days, as previously described. For differentiation of hESC-NCSCs towards peripheral neurons, FGF2/EGF-expanded hESC-NCSCs were subjected to mitogen withdrawal in medium supplemented with BDNF, GDNF, NGF, dbcAMP. Formation of human embryoid bodies (EBs) for hematopoietic[16] and endothelial[17,29] differentiation was performed as previously described. Intact hESC or iPSC colonies were harvested and plate in low attachment dish (Corning) with DMEM, 20 % Hyclone serum, 1% nonessential amino acids (AA), 1 mM L-glutamine (Glut), 0.1mM β-mercaptoethanol (MTG), BMP4 50 ng/mL, VEGF 50 ng/mL. In every other day, medium was changed with serum-free medium (Sigma) supplemented with Glut, AA, MTG, and following cytokines: BMP4 5 ng/mL, bFGF 10 ng/mL, VEGF 20 ng/mL, SCF 20 ng/mL, Flt3L 20 ng/mL, TPO 20 ng/mL. Cells are dissociated with Trypsin for 10 minutes in incubator. Subsequently, cells are passed through a 22G needle 3-4 times and through a 40-μm strainer. EB-derived cells were plated into semi-solid culture (StemCell Technologies) for further differentiation. For hematopoietic cell, 0.05-0.1 million cells /35mm dish were plated at day 10 of EB culture; for endothelial differentiation, 0.1-0.2 million cells/35mm dish were plated at day 5 of EB culture. Endothelial cells were obtained following seeding of the blast-like colony (at day 5-6 of methylcellulose culture) onto a matrigel-coated plate in EBM2 medium (Lonza). After 3-4 passages, hESC and iPSC-derived endothelial cells were sorted for CD144 (VE-cadherin) antibody (BD). Hematopoietic cells (at day 14 of methylcellulose culture) were harvested and

sorted for CD45 antibody (BD). For endodermal cell differentiation, semi-confluent hESC or iPSC mained feeder-free on matrigel were cultured in RPMI containing Glutamax, penicillin/streptomycin and 0.5% defined FBS (Hyclone) and recombinant human Activin A (100ng/ml, Peprotech) for 1 week. Cells were then dissociated using 0.05% trypsin/EDTA (Invitrogen), resuspended in PBS with 2% serum and sorted for CXCR4 antibody[18]. For cardiac differentiation, fully grown FD-iPSC colonies were detached by dispase and plated into ultra low attachment dishes in Knock-out DMEM supplemented with Glutamax, penicillin/streptomycin and 20% defined FBS (Hyclone) in the presence of recombinant human BMP4 (25ng/ml, R&D). Resulting embryoid body cultures were maintained for 4 days, harvested by quick spinning and plated onto gelatin-coated dishes under the same medium conditions for an additional 2 weeks. Tissues with beating foci were observed at 2 weeks of differentiation on gelatin and isolated manually for further characterization. For immunocytochemical analysis beating foci were trypsinized for 10 minutes, triturated and replated onto fibronectin-coated plates for 24 hour period. Spontaneous beating of putative cardiac tissues was monitored on an Olympus IX81 microscope under Normarski optics using a Hamamatsu ORCA CCD camera. Cells were maintained in enclosed chamber with temperature and CO_2 control (Weather Station, Precision Control) and time lapse images were assembled using a commercial software package (Slidebook). For evaluation of chemical treatment, 100μM kinetin[12] (Sigma), 12.5μg/ml d-tocotrienol[24] (Caymanchem) and 50μg/ml Epigallocatechin Gallate (EGCG)[23] (Calbiochem) were used for 24 hours for the initial test. Kinetin treatment was tested in different treatment schedules including continuous treatment from one day prior to differentiation to analysis at the neural crest stage, and treatments for either 24 hours or 5 days upon isolation of iPSC derived neural crest progeny. Migration / motility assay was performed following published protocols[30]. In brief, cells were treated with mitomycin C (2 ng/ml for neural crest cells) for 2 hours and dissociated with Accutase. A total of 100,000 cells were plated in each well of a PO/Lam/Fib-coated 24 well plate. At 24 hours of plating, each well was scratched using a 100μl pipette tip, washed and image was taken (0hr) as reference point for subsequent quantification. At 48 hours after scratching, cells were fixed by 4% paraformaldehyde and stained with DAPI for quantification of DAPI+ nuclei (48h) in the identical fields marked at day 0.

Assay for teratoma formation

For teratoma formation, 2 x 10^6 cells were re-suspended in hESC medium and injected intramuscularly into immuno-compromised *NOD/SCID IL2R gamma chain* $^{-/-}$ mice. Xenografted masses were retrieved 4-6 weeks after injection and cryo-sectioned samples were stained with hematoxylin and eosin for histological analysis and subjected to immunofluorescence for marker characteristic of tissues derived from the three main germ layers.

Immunocytochemistry and Flow cytometry

Cells were fixed in 4% paraformaldehyde, permeabilized with Triton-X100 containing buffer and stained with primary antibodies (Nanog, from R&D; Brn3a, Peripherin and CD105 from Millipore; p75, from Advanced targeting system; Paxillin, CD45, CD144, CXCR4, MASH1 and beta-catenin from BD; AP2 (3B5), Pax6, HNK1, Forse1, MF20 from DSHB; Tuj1, from Covance; α-actinin from Sigma, Collagen, from Chondrex). Appropriate Alexa Flour 488, 568 or 647 labeled secondary antibodies (Molecular Probes) and/or DAPI counter-staining was used for visualization. Cells were stained for paxillin at 1.5 hours after plating[22]. For quantification of paxillin staining, Paxillin+ puncta as defined by > 1μm diameter were quantified per individual cell. For FACS analysis, cells were dissociated with Accutase, stained with Alexa Fluor 647-conjugated anti- human SSEA-3, SSEA-4, Tra-1-81 and PE-Cy5-conjugated anti-HLA-ABC antibodies (BD Biosciences) and analyzed in a LSRII cytometer (BD Biosciences). Analysis was performed with the FlowJo software (version 8.8.4; TreeStar).

Molecular analysis

Total RNA was extracted using the RNeasy kit and DNAse I treatment (Qiagen) to avoid genomic contamination. Samples were collected and frozen for further RNA extraction. Total RNA was reverse transcribed (Superscript, Invitrogen) and approximately the equivalent of 50 ng of RNA was used for each RT-PCR reaction. The quantification of intensity of PCR bands was performed using ImageJ software (NIH). Primer sequences, cycle numbers, and annealing temperatures (**Suppl. Table 3**) correspond to those used by others previously[8,12]. Quantitative RT-PCR was performed using the Mastercycler RealPlex2 (Eppendorf) platform following the manufacturer's instruction. All results were normalized to HPRT or S18 control. For microarray analysis, total RNA was isolated from two independent sample each of undifferentiated H9, C14-iPSC, FD-iPSC#22, FD-iPSC#102 cells, and fibroblast samples (#4589 and #4959); and from

HNK1+ neural crest precursors derived from H9, C14-iPSC and FD-iPSC#22 were processed by the MSKCC Genomic core facility and hybridized on Illumina Human Ref-6v2 or Human HT-12 arrays. Normalization and model-based expression measurements were performed with using the Illumina analysis package (LUMI) available through open-source Bioconductor project (www.bioconductor.org) within the statistical programming language R (http://cran.r-project.org/). A pair-wise comparison of neural crest cell from [H9 and C14-iPSC] vs. FD-iPSC was performed using the Linear Models for Microarray Data package (LIMMA) available through Bioconductor. Genes found to have an adjusted p-value < 0.05 and a fold change greater than 2 were considered significant. Some of the descriptive microarray data analyses and presentation was performed using a commercially available software package (Partek Genomics Suite (ver. 8.4.17).) Genomic DNA (gDNA) is isolated using Qiagen kit from patient fibroblast lines and iPSC clones. For validating of sequencing, conventional PCR was used (the condition is in **Supplementary Table 3**). Microsatellite analysis of gDNA was performed at the Rockefeller genomic core facility. For bisulfite genomic sequencing, gDNA samples from FD-fibroblast and FD-iPSC were extracted. Bisulfite treatment of gDNA was carried out using a CpGenome DNA Modification Kit (Chemicon) according to the manufacturer's protocol. Converted gDNA was amplified by PCR using *NANOG* primer sets[6]. PCR products were gel purified and cloned into bacteria using TOPO TA cloning (Invitrogen). Bisulfite conversion efficiency of non-CpG cytosines ranged from 80% to 99% for individual clones for each sample.

Statistical analysis

The data were processed using Prism 4.0c software. Values are reported as means ± s. d. Comparisons among values for all groups were performed by one-way ANOVA. Bartlett's test for equal variances and Newman-Keuls Multiple Comparison Test were used to determine the level of significance.

ADDITIONAL REFERENCE FOR FULL METHODS SECTION (Supplementary Material and Methods)

30. Rodriguez,L.G., WU,X. & Guan,J.L. Wound-healing assay. *Methods Mol. Biol.* **294**, 23-29 (2005).