Supplementary information, Data S1 Materials and Methods

Materials

Sunitinib and other RTK inhibitors were purchased from SYNthesis Med Chem (Parkville, VIC, Australia) or Sigma-Aldrich (St. Louis, MO, US). PD0325901 and CHIR99021 were purchased from Selleckchem (Huston, Texas, US). Echinomycin was purchased from Biomol (Farmingdale, NY, US). Tauroursodeoxycholic acid, KC7F2 and ML 228 were purchased from TCI Chemicals (Tokyo, Japan). RepSox was purchased from Biovision (San Francisco, CA, US). Thapsigargin, tunicamycin, ionomycin, salubrinal and parnate were purchased from Sigma-Aldrich (St. Louis, MO, US). All compounds were dissolved in DMSO and used at indicated concentrations. $mVEGF_{164}$ and hPDGF-BB were purchased from CST (Danvers, MA, US).Mouse VEGFR2 neutralizing antibody DC101 were purchased from BioXcell (West Lebanon, US).

AP and immunofluorescent staining

For AP staining, mESCs and iPSCs were fixed with 4% paraformaldehyde (PFA) in PBS for 45 s, rinsed once with PBS and staining was performed using a leukocyte AP kit (Sigma-Aldrich) according to the manufacturer's protocol. For immunofluorescent staining, cells were fixed with 4% PFA for 30 min and permeated with 0.3% Triton if necessary, then incubated with primary antibodies against SSEA-1 (Santa Cruz, sc-21702), Nanog (Millipore, AB5731), Oct4 (Abcam, ab19857), Tuj1 (Covance), a-SMA (Sigma, A2547), HNF-3β (Santa Cruz, sc-377033) or PECAM (Santa Cruz, sc-376764) followed by the appropriate secondary antibodies conjugated to Alexa Fluor 555 or Alexa Fluor 488. Nuclei were counterstained with Hoechst 33342. Images were taken with an Olympus IX51inverted fluorescent microscope or an Olympus FV10i confocal microscope.

Embryonic bodies (EBs) formation and *in vitro* differentiation assay

For EB formation, 5×10^5 mESCs cultured with mES medium supplemented with LIF, sunitinib or 2i were harvested by trypsinization and re-seeded in 10 mL LIF-free mES medium in 100 mm bacterial culture dishes to generate EBs. At day 11, total

RNA was extracted from EBs to detect differentiation gene expression by qRT-PCR. All chemicals were removed during EB formation. For immunostaining of lineage markers in spontaneously differentiated cells, mESCs were trypsinized into single cell suspension and EBs were formed with the hanging drop method. For each drop, 20 μL medium containing 1×10^3 mESCs were used. After cultured in hanging drops for 2 day, EBs were reseeded in gelatin-coated 24 well plates for another 10 days and then collected for immunostaining.

Teratoma formation and Chimera generation

About 1×10^6 mESCs or iPSCs were suspended in 200 μL mES medium and injected into the thigh muscle of SCID mice. The animals were checked 2–3 times per week. Four weeks after injection, teratomas were harvested, fixed overnight with 4% PFA, embedded in paraffin, and sectioned. Sections of the teratomas were stained with hematoxylin and eosin and analyzed histologically.

Prepubertal (4-5weeks of age) donor ICR female mice were superovulated, mated overnight to intact ICR stud males and euthanized by cervical dislocation on day 3.5. Uteri were collected after euthanasia and flushed with FHM medium (Millipore) for the collection of blastocysts. Twelve-fifteen ES or iPS cells were injected into the blastocoel of each blastocyst. After injection, the blastocysts were surgically transferred into recipient female ICR mouse that were pseudopregnant by mating with vasectomized males. Recipient females carried the pups to term and nursed until weaning at three weeks. Chimeric mice were identified by coat color.

RNA interference in mES cells

For lentivirus-mediated VEGFRs knockdown, lentiviral vector FG12 (derived from pFUGW vector, Addgene) and packaging plasmids pRSV/REV, pMDLG/pRRE and pHCMVG were used. To construct the hairpin siRNA expression cassette, synthesized complementary DNA oligonucleotides were annealed and inserted immediately downstream of the U6 promoter of pBS/U6 plasmid, and the derived cassette was subcloned into FG12 vector. Recombinant lentiviruses were produced in HEK-293T cells to express shRNA against coding regions of VEGFRs. Virus that

expresses a scrambled shRNA sequence was prepared as a control. E14 cells were infected with lentiviral supernatants overnight and GFP$^+$clones were selected.RNAi sequence for *VEGFR1*: 5'-CGGAATCTTCAATCTACATAT-3', 5'-CGTGACCTTTAATCGTGCTTT-3', *VEGFR2*: 5'-AGAACACCAAAAGAGAGGAACG-3', 5'-GGGCTTTACTATTCCCAGC-3', *VEGFA-1*: 5'-TCCTGAGAAGATATTTAA-3', 5'-GCACTCCAGGGCTTCATCG-3'; *VEGFA*-2: 5'-AAAGACAGAACAAAGCCA-3', 5'-TGGCTTTGTTCTGTCTTT-3'; *PDGFB*-1: 5'-GGCAGGGTTATTTAATAT-3', 5'-ATATTAAATAACCCTGCC-3'; *PDGFB*-2: 5'-GAACCTTGTTTAATTCAT-3', 5'-ATGAATTAAACAAGGTTC-3';

Enzyme-linked immunosorbent assay (ELISA)

To detect the mVEGF-A secreted into the medium, mESCs were cultured onto gelatin-coated 6-well plates at a density of 20000 cells per well in different media (mES or N2B27, with or without LIF) for 8 days without passage. Media were changed and collected every day and stored at -80 °C. Quantikine ELISA (R&D Systems) kits were used to detect mVEGF-A in the media according to the manufacturer's protocol.

Real-time PCR

Total mRNA was isolated using TRIzol (Life Technology) and 2μg RNA were used to synthesize cDNA using PrimeScript™ RT reagent kit (Takara) according to the manufacturer's protocol. Real-time PCR was performed using JumpStart™ TaqReadyMix™ (Sigma-Aldrich) with Eva Green (Biotium) and analyzed with a Stratagene Mx3000P thermal cycler (Agilent). For semi-quantitative PCR analysis, the cDNA solution was amplified for 30 cycles at an optimal annealing temperature.

Western blot

Cells were lysed, sonicated and boiled at 95-100°C for 5 min in sample buffer (50 mM Tris-HCl, 2% w/v SDS, 10% glycerol, 1% β-mercaptoethanol, 0.01% bromophenyl blue, pH 6.8). Cell lysates were separated on SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were first incubated with blocking buffer (TBS with 0.05% Tween 20, 10% non fat milk) for 1 h

at room temperature and then incubated overnight at 4 °C in buffer containing antibody against VEGFR1 (Abcam, ab2350), VEGFR2 (CST, 55B11), Hif1α (Santa Cruz, 10790), XBP1 (Santa Cruz, 7160), CHOP (Santa Cruz, 7351), p-GSK3β (Ser9) (CST, 9336), GSK3β (CST, 9315S), p-Stat3 (Tyr705) (CST, 9138), Stat3 (CST, 9139), ERK (CST, 9102), p-ERK (CST, 4370), or GAPDH (CST, 2118). The membranes were washed thrice and incubated with proper HRP-conjugated secondary antibodies for 1 hrs. After washing, immunostaining was visualized using Western Lightning Ultra (Perkin Elmer).