

Supporting Information

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SI Methods and Materials

Materials. Antibodies: mouse anti-Oct4 (sc-5279), rabbit anti-Klf4 (sc-20691), rabbit anti-c-Myc (sc-789), goat anti-I2PP2A (E-15) (sc-5655), goat anti-CD31 (SC-31045), goat anti-VE-Cadherin (sc-6458), and rabbit anti-GAPDH (FL335, sc-25778) were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Rabbit oct4 (abcam), rabbit Nanog (abcam), Rabbit anti-Sox2 (ab59776), rabbit anti-VE-Cadherin (FITC) ab33321, mouse anti-CD31 (FITC) (ab13466), rabbit anti-IgG- FITC, mouse anti-IgG isotype control (ab37359), rabbit IgG isotype control (abcam) rabbit anti-CD31 (ab32457) (human specific), rabbit anti-CD31 (ab28364), rabbit-anti-SM22 (ab14106) and anti-mouse FLK-1 (ab2349-500) were purchased from Abcam. Rabbit anti-VE-Cadherin (human specific, 2158) was from Cell Signaling. Mouse anti-human Nestin, Mouse anti-Neuron-specific β -III Tubulin, Mouse anti-Oligodendrocyte Marker O4, Goat anti-mouse FABP-4 (962643), Mouse anti-human Osteocalcin (962645), Goat anti-human Aggrecan (962644), and anti-hVEGF (R2/KDR, FAB357F) were from R&D Systems. Mouse TRA-1-60 and Mouse TRA-1-81 (Millipore). The anti-Actin α -smooth muscle cy3 (C6198) was from SIGMA. The secondary antibodies; anti-goat Alexa488, anti-goat Alexa594, and anti-rabbit Alexa488, anti-rabbit Alexa594 were purchased from Invitrogen. The secondary antibody anti-mouse FITC, anti-goat FITC were from Dakocytomation (Glostrup, Denmark). Geneticin G-418 Sulphate (11811-023) was purchased from GIBCO. Recombinant human VEGF and PDGF-BB was purchased from R&D Systems.

Cell culture. Three human fibroblast cell lines were used in this study; CCD-1079 Sk CRL-2097 from Skin newborn (ATCC), and CCL-186 from lung 16 weeks gestation IMR-90 (ATCC), CCL-153 (ATCC). Fibroblasts were cultured on gelatine coated flasks (0.04%, Sigma) in ATCC-formulated Eagle's Minimum Essential Medium, (30-2003, ATCC) supplemented with 10% fetal bovine serum (30-2020, ATCC) and 100IU/ml penicillin and streptomycin in a humidified incubator supplemented with 5% CO₂ at 37°C. The cells were passaged every 3 days at a ratio of 1:3 to 1:6 and the medium was refreshed every 2 days.

Cell reprogramming. Fibroblasts were nucleofected with the four reprogrammed factors or control empty vector and cultured with reprogramming media comprised of Knockout DMEM (Invitrogen, SKU-10829-018), 20% Knockout Serum Replacement (Invitrogen SKU 10828-028), 10ng/ml basic fibroblast growth factor (bFGF Miltenyi Biotec, 130-093-837), 0.1mM β -mercaptoethanol and 0.1mM MEM non essential amino acids (MEM NEAA). The media were changed every day.

Microarray analysis. For the microarray analysis, fibroblasts were infected with 4 lentiviral vectors (EX-Z0092-Lv08-OCT4, EX-Z2845-Lv08-v-Myc, EX-Q0453-Lv08-KLF4, EX-T2547-Lv08-SOX2) purchased from GeneCopoeia. An empty vector (Ex-Lv08) was used as control. Samples were collected on days 4, 7, 14 and 21, RNA was extracted and a microarray analysis was performed and compared to control groups. In Brief, total RNA was extracted from each sample using RNeasy Mini Kit from Qiagen. The RNA quantity was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and RNA quality was checked using RNA6000 Nano Assay on Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA). Commercially available high-density oligonucleotide, from Illumina whole genome gene expression BeadChips (Human HT12_V3_0_R3_11283641, Illumina Inc, San Diego, California, USA), were used with 48803 probes representing 37879 human transcripts. In brief, 500ng of total RNAs were reverse transcribed to synthesize first- and second- strand complementary DNA (cDNA), followed by in vitro transcription to synthesize biotin-labeled complementary RNA (cRNA) using TotalPrep-96 RNA amplification kit from Ambion accordingly. A total of 750ng of biotin-labeled cRNA from each sample was hybridized to the HT12 BeadChip (Illumina Inc., San Diego, CA) at 58°C for 18 h. The hybridized BeadChip was washed and labeled with streptavidin-Cy3 according to the manufacture protocols. Chips were scanned with Illumina BeadScan and scanned image was imported into GenomeStudioV2010.1 (Illumina Inc) for data extraction.

SETSIP cloning and nucleofection. Full length human SETSIP cDNA fragment was obtained by RT-PCR from PiPS-ECs with the primer set shown in Supplemental Table S1 and cloned into Kpn1 and Xba1 sites of a pCMV5 plasmid defined as pCMV5-SETSIP. The construct was verified by DNA

sequencing. pCMV5-SETSIP or pCMV5 have been overexpressed in PiPS-EC cells by nucleofection and the endothelial marker expression was tested in transcriptional and protein levels.

Selection of PiPS cells. Fibroblasts were nucleofected with a polycistronic plasmid (OSKM) containing all four factors as shown above. Neomycin selection was started one day after the nucleofection up to day four where a pure population of PiPS cells has been obtained expressing an mOrange marker and the four reprogrammed genes.

Cell differentiation. PiPS cells were seeded on mouse collagen IV (BD mouse collagen IV-5 μ g/ml)-coated flasks or plates in EGM-2 media (Lonza) to induce endothelial differentiation for indicated times. The medium was refreshed every other day. PiPS cells were also differentiated into neuron cells using R&D Systems Human Stem cell Neural Kit SC011 according to the protocol provided. PiPS cells were differentiated into adipocytes, osteocytes and chondrocytes based on R&D Systems Human Mesenchymal Stem Cell Kit SC006 according to the protocol provided.

Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR. RT-PCR and real time PCR were performed as described previously (1). Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. 2 μ g RNA were reversely transcribed into cDNA with random primer by MMLV reverse transcriptase (RT) (Promega). 20-50ng cDNA (relative to RNA amount) was amplified by standard PCR with *Taq* DNA polymerase (Invitrogen) or real time PCR. PCR primers were designed using Primer Express software (Applied Biosystems). The primers' sequences are shown in Supplementary Table S1.

Immunofluorescence staining. The procedure used for immunofluorescent staining was similar to that described previously (1). Briefly, cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% Triton X-100 in PBS for 10 min and blocked in 5% swine serum in PBS for 30 min at 37°C. The cells were incubated with primary antibodies: goat anti-VE-cadherin, goat anti-CD31, goat anti-I2PP2A, rabbit anti-CD31 (human specific), rabbit anti-VE-cadherin (human specific), rabbit anti-SM22, anti-Actin α -smooth muscle cy3, mouse Nestin, mouse anti-Neuron-specific β -III Tubulin, mouse anti-Oligodendrocyte, mouse anti- Osteocalcin, goat anti-FABP-4, and goat anti-Aggrecan. The bound primary antibodies were revealed by incubation with the secondary antibodies; anti-goat Alexa488, anti-goat Alexa594, anti-rabbit Alexa488, anti-rabbit Alexa594, anti-mouse FITC, anti-goat FITC at 37°C for 30 min. Cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma), mounted in Floromount-G (Cytomation; DAKO, Glostrup, Denmark), and examined with a fluorescence microscope (Axioplan 2 imaging; Zeiss) or SP5 confocal microscope (Leica, Germany).

Immunoblotting. The method used was similar to that described previously (1). Cells were harvested and washed with cold PBS, re-suspended in lysis buffer (25mM Tris-Cl pH 7.5, 120mM NaCl, 1 mM EDTA pH 8.0, 0.5% Triton X100) supplemented with protease inhibitors (Roche) and lysed by ultrasonication (twice, 6 seconds each) (Bradson Sonifier150) to obtain whole cell lysate. The protein concentration was determined using the Biorad Protein Assay Reagent. 50 μ g of whole lysate was applied to SDS-PAGE and transferred to Hybond PVDF membrane (GE Health), followed by standard western blot procedure. The bound primary antibodies were detected by the use of horseradish peroxidase (HRP)-conjugated secondary antibody and the ECL detection system (GE Health). The band density was semiquantified by Adobe Photoshop software.

Alkaline phosphatase staining. Alkaline phosphatase staining was conducted following the manufacturer's instructions (Vector blue Alkaline phosphatase kit III, Cat. No. SK-5300).

Teratoma formation assay. PiPS cells (1×10^6) were mixed with Matrigel and subcutaneously injected into SCID mice. Eight weeks later, the plugs were harvested and sectioned for HE staining.

FACS analysis. PiPS and PiPS-EC or control cells and isotype controls were analysed with FACS in order to test the percentage of the KDR and other endothelial and pluripotent markers. Data analysis was carried out using CellQuest software (Becton Dickinson).

In vitro tube formation assay. Cell suspension containing 4×10^4 control or PiPS-ECs were placed on top of the 50 μ l/well Matrigel (10 mg/ml; BD Matrigel Basement Membrane Matrix, A6661) in 8-well chamber slides (Nunc). Rearrangement of cells and the formation of capillary-like structures were observed at 18–24 hour.

In vivo angiogenesis assay. PiPS-ECs or control cells were labelled with Molecular Probes Vybrant Cell Labelling (MP22885) before the *in vivo* angiogenesis assay to distinguish the in vitro-differentiated cells from the host cells. PiPS-ECs (1×10^6) were mixed with 50 μ l of Matrigel and injected subcutaneously into the back or flank of NOD.CB17-*Prkdc*^{scid}/NcrCrl mice. Six injections were conducted for each group. Fourteen days later, the mice were killed and the plugs were harvested, frozen in liquid nitrogen, and cryosectioned. Samples were fixed with 4% paraformaldehyde in PBS at 4°C overnight, and then HE staining was performed. Images were assessed with Axioplan 2 imaging microscope with Plan-NEOFLUAR 10 \times , NA 0.3, objective lenses, AxioCam camera, and Axiovision software (all Carl Zeiss MicroImaging, Inc.) at room temperature, and were processed with Photoshop software (Adobe).

Lentiviral particle transduction. Lentiviral particles were produced using MISSION shSETSIP plasmids DNA (ShRNA SET TRCN000063717 SIGMA) according to protocol provided and previously described (1). The shRNA Non-Targeting vector was used as a negative control. Briefly, 293T cells were transfected with the lentiviral vector and the packaging plasmids, pCMV-dR8.2 and pCMV-VSV-G (both obtained from Addgene) using Fugene 6. The supernatant containing the lentivirus was harvested 48h later, filtered, aliquoted and stored at -80°C. p24 antigen ELISA (Zeptomatrix) was used to determine the viral titre. The Transducing Unit (TU) was calculated using the conversion factor recommended by the manufacturer (10^4 physical particles per pg of p24 and 1 transducing unit per 10^3 physical particles for a VSV-G pseudotyped lentiviral vector), with 1pg of p24 antigen converted to 10 Transducing Units (TU). For lentiviral infection, PiPS-ECs were seeded overnight and the following day the cells were incubated with shSETSIP or Non Targeting control (1×10^7 TU/ml) in complete medium supplemented with 10 μ g/ml of Polybrene for 24h. Subsequently, fresh medium was added to the cells and the plates were returned to the incubator and harvested 72h later to be subjected to further analysis.

Luciferase activity assay. VE-Cadherin promoter (2000bp) region was amplified by PCR and cloned into the XhoI/HindIII sites of pGL3-Luc reporter vector, and verified by DNA sequencing. In brief, CTL or PiPS-ECs were transfected with the reporter gene (0.33 μ g/well) together with expression plasmid (0.16 μ g/well) encoded from SETSIP, or CTL and PiPS-EC were infected with SETSIP shRNA or CTL shRNA one day before the transfection. pGL3-Luc Renilla (0.1 μ g/well) was included in all transfection assay as internal control, pCMV5 was also used as mock control. Luciferase and Renilla (Promega) activity assays were detected 48hr after transfection using a standard protocol. Relative luciferase unit (RLU) was defined as the ratio of luciferase activity to Renilla activity with that of control set as 1.0.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation assay was performed with commercial kit (EZ ChIP, Millipore) according to the protocol provided, and previously described (2). In brief, PiPS-EC and control cells were treated with 1% (vol/vol) formaldehyde at room temperature for 10 min and then quenched with glycine at room temperature. The medium was removed, and cells were harvested in lysis buffer. Following a short incubation on ice, chromatin was sheared by sonication. The sheared samples were diluted into 0.9 ml ChIP Dilution Buffer and precleared with Protein G Agarose/Salmon Sperm DNA beads for 1 h. Subsequently, immunoprecipitation was conducted with similar to SET antibody (sc-5655). Normal IgG was used as a control. Immunocomplexes were collected the following morning using Protein G Agarose/Salmon Sperm DNA beads. Immunoprecipitates were pelleted by centrifugation and washed with Low-Salt Buffer, High-Salt Buffer, and Tris-EDTA buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.2) to remove any nonspecific binding. The immunoprecipitates were eluted from the beads using 200 μ l of elution buffer (100 mM NaHCO₃, 1% SDS) and the crosslink of the protein/DNA complexes were reversed by an overnight incubation of the eluted product at 65 °C. A total of 2 μ l proteinase K (10 μ g/ μ l) was subsequently added to the solution and samples were incubated at 45 °C for 1 h. DNA was then purified using the spin columns provided. Aliquots of chromatin were also analyzed before immunoprecipitation and served as an input control. VE-cadherin gene promoter sequences were amplified by PCR with primer sets listed in Table S2. The PCR products were analyzed in 2% agarose gel and images were assessed with BioSpectrum AC Imaging System and Vision- WorksLS software.

Nucleofection. Fibroblasts were nucleofected with a polycistronic plasmid (OSKM) containing all four factors (Plasmid 20866: pCAG2LMKOSimO: Sox2, Oct4, Klf4 and cMyc, Addgene), using Amaxa nucleofector (VPD-1001 NHDF Kit, LONZA), and cultured in reprogramming media for 4 days. The 4 day-reprogrammed cells were defined as partial iPS cells (PiPS).

Cell seeding and vascular bioreactor. PiPS-ECs or fibroblasts were seeded on aortic grafts, which were previously decellularized (with sodium dodecyl sulfate (SDS) at 0.075 % and washed in PBS), in a special constructed bioreactor and shear stress was applied. Briefly, a roller pump (Masterflex: Standard Drive, model 7520; Standard Pump Head, model 7018-20; Codane Tubing, Cole-Parmer UK) was used to produce mean flow in the bioreactor, the grafts were fixed between two 25 G needles after hosting them on plastic tubes fixed by 8-0 silk sutures (8-0 black virgin silk, Ethicon Inc., Johnson & Johnson, Norderstedt, Germany). The complete setup was maintained in a standard CO₂ incubator at 37 °C. The scaffolds were placed for the seeding process in a self constructed incubation chamber and preconditioned with cell culture media for 2h. Then, 5 x 10⁵ cells of PiPS-ECs or fibroblasts were seeded on the scaffolds via direct injection and allowed to seed for 12h before the initial flow was set up. Decellularized vessels have also been used as a second control. Shear stress was applied at stepwise rates ranging from 10 to 35 dynes/cm² over a period of 48h. After this time point no increase in the shear stress rate was conducted and the grafts remained under constant shear stress of 35 dynes/cm² until they were harvested by day 4. For the last 24 hours 100 Units of heparin per ml of total circulating reactor media volume (heparin sodium salt diluted, from porcine gastrointestinal mucosa, Sigma Aldrich) was added every 12h. For the double seeded PiPS cells tissue engineering vessels, decellularized vessel scaffolds were placed in a self constructed incubation chamber and preconditioned with differentiation media α -MEM, 10 % FBS and 25 ng/ μ l PDGF-BB for 2h. After this period 2 x 10⁶ PiPS cells were seeded in reprogrammed media via direct injection and allowed to seed for 12h at a continuous rotational movement before initial flow was set up. Shear stress was applied at 10 dynes/sqcm² over a period of 48h. Then, the circulating media was exchanged to EGM-2 media and a second seeding step was initiated with 1 x 10⁶ PiPS cells and after another 12h seeding period the shear stress rate was stepwise adjusted up to 35 dynes/sqcm². The grafts remained under constant shear stress of 35 dynes/sqcm² and harvested on day 5. The engineered vessels were harvested and used for further analysis in vitro or immediately grafted to animals.

Ischemia model. The ischemia model was performed as previously described (3). PiPS-ECs were injected intramuscularly into adductors of ischemic SCID mice. Fourteen days later, the hind limb blood flow and muscular neo-angiogenesis were evaluated. The tissue blood flow of both legs was sequentially assessed by a perfusion imager system (Laser Doppler Flow assessment). Hind limb muscles were harvested following in situ perfusion fixation at physiological pressure, frozen in liquid nitrogen, and cryosectioned. Sections of adductor muscles were stained with CD31 antibody and capillary density was expressed as the capillary number per mm². Human cell engraftment ability was assessed by counting cells positive for CD31 (human specific antibody) at six randomly selected microscopic fields (at x100).

En face preparation and immunofluorescence staining and frozen section staining. The procedure for en face preparation is similar to that described elsewhere (4). In short, the tissue-engineered vessels or grafts were harvested, and the samples were fixed with acetone and cut open. The vessel segments were mounted and pinned onto rubber with the lumen opened and facing up. After washing with PBS specimens were placed in a humidified chamber and blocked in 5% swine serum in PBS for 30 min at 37°C and incubated with primary antibodies rabbit anti-VE-cadherin, and rabbit anti-CD31 as described in the immunostaining section above. For frozen section staining, serial 5 μ m-thick frozen sections were cut from cryopreserved tissue blocks, fixed in a cold 1:1 acetone 10 minutes, and washed with phosphate-buffered saline (PBS) for 20 minutes, and proceed as described above.

Miller's elastin staining. To assess the elastic lamina in the tissue-engineered vessel sections, the Miller's elastin staining was applied. In brief, paraffin sections were deparaffinised and rehydrated. They were then immersed in potassium permanganate, then oxalic acid and finally rinsed in 70% ethanol. The tissue was incubated in Miller's elastin stain (BDH Laboratory Supplies, Poole UK) for 3 hrs followed by counterstaining with van Gieson solution (50% saturated picric acid solution, 0.09% acid fuchsin) for 5 min. Sections were subsequently dried, dehydrated and mounted on microscope slides. Staining was visualized as described above.

SI References

1. Margariti A, et al. (2010) Histone deacetylase 7 controls endothelial cell growth through modulation of beta-catenin. *Circ Res* 106:1202-1211.
2. Margariti A, et al. (2009) Splicing of HDAC7 modulates the SRF-myocardin complex during stem-cell differentiation towards smooth muscle cells. *J Cell Sci* 122:460-470.
3. Emanuelli C, et al. (2007) Nitropravastatin stimulates reparative neovascularisation and improves recovery from limb Ischaemia in type-1 diabetic mice. *Br J Pharmacol* 150:873-882.
4. Zeng L, et al. (2009) Sustained activation of XBP1 splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow. *Proc Natl Acad Sci U S A* 106:8326-8331.

Table S1. Primers

Name	Sequence	NCBI Reference Sequence
PHEX	5'>ACAACCTTTGCTGCCTCAATGGGAC<3' 5'>TCGTTCCCTGCATCCATCCACTCAT<3'	NM_000444.4
DMP1	5'>TGGAGATGACACCTTTGGTGACGA<3' 5'>TGGTGGTATCTTGGGCACTGTCTT<3'	NM_001079911.2
ADIPOQ	5'>ATCCAAGGCAGGAAAGGAGAACCT<3' 5'>TGGTAAAGCGAATGGGCATGTTGG<3'	NM_001177800.1
Adipsin	5'>TGATGTGCGCGGAGAGCAAT<3' 5'>TAGATCCCGGGCTTCTTGCGGTT<3'	NM_001928.2
ACVR1C	5'>TGGTTTACTGGGAAATAGCCCGGA<3' 5'>CACAACCTTTGCCACTGGTTTGGGA<3'	NM_001111031.1
NRARP	5'>GCTGTGCGCAAGGGCAACAC<3' 5'>GAACGCGGCGATGTGCAGC<3'	NM_001004354.1
Filamin	5'>CCACCGGCACTGTCTTCGC<3' 5'>AGCCACTGCCCTCAGCCCTT<3'	NM_015687.2
LIM2	5'>GCGCCTGGGGAAAGAGCAGG<3' 5'>AAGCGCCAGTCCCCAAAGCG<3'	NM_030657.3
LSS	5'>ATGCAGGGCACCAACGGCTC<3' 5'>CAGCTCCAGCAAGTGCCCCC<3'	NM_002340.5
TEK	5'>TGCATTGCCTCTGGGTGGCG<3' 5'>AGCCTGGTGAAGGCCGAGGT<3'	NM_000459.3
SPRY	5'>GCCAGCCTCCGGCAATTCCT<3' 5'>CCTCGCCCTCCTTTCGCAGC<3'	NM_032681.3
STMN2	5'>CGTGCCTCTGGCCAGGCTTT<3' 5'>CTGCGCACCTCCGCAGCAT<3'	NM_007029.2
HBA2	5'>ACGCTGGCGAGTATGGTGCG<3' 5'>GGTCACCAGCAGGCAGTGGC<3'	NM_000517.4
DNMT3L	5'>TGTGTACGGCGCCACACCTC<3' 5'>CAGAGCCCAGTGCCTGCTGC<3'	NM_013369.2
ARRDC4	5'>GGCTGCTATTCCAGCGGCGA<3' 5'>CAGGCTGAGGCGCACGTTCA<3'	NM_183376.2
Oct-01	5'>GCCTCAGCCTCCACCTCCGA<3' 5'>AGGCACCTCCAGCCGCAAAC<3'	NM_002697.2
TNC	5'>AGGCCGGTGCATTGATGGGC<3' 5'>CAGGCATGTGGGCAGGTGGG<3'	NM_002160.2
C-KIT	5'>TGAATGGCATGCTCCAATGTGTGG<3' 5'>ACATCCACTGGCAGTACAGAAGCA<3'	NM_000222
CD133	5'>TACCAAGGACAAGGCGTTCACAGA<3' 5'>GTGCAAGCTCTTCAAGGTGCTGTT<3'	NM_001145847
CD34	5'>CACTGAGCAAGATGTTGCAAGCCA<3' 5'>TCAGGAAATAGCCAGTGATGCCCA<3'	NM_001025109
KDR	5'>ATCCAGTGGGCTGATGACCAAGAA<3' 5'>ACCAGAGATTCCATGCCACTTCCA<3'	NM_002253

OCT4	5>ATGCATTCAAACCTGAGGTGCCTGC<3' 5>AACTTCACCTTCCCTCCAACCAGT<3'	NM_001173531
SOX2	5>CACATGAAGGAGCACCCGGATTAT<3' 5>GTTCATGTGCGCGTAACTGTCCAT<3'	NM_003106
KLF4	5>TGACCAGGCACTACCGTAAACACA<3' 5>TCTTCATGTGTAAGGCGAGGTGGT<3'	NM_004235
c-MYC	5>ACAGCTACGGAACCTTGTGCGTA<3' 5>GCCCAAAGTCCAATTTGAGGCAGT<3'	NM_002467
SETSIP-LOC646817	5>ATGGTCTGGTTCTTGGACTTCCCT<3' 5>AGCAGGAGGTGGTCTTGGTTTCTT<3'	XM_929774
CD31	5>GAACAGGACCGCGTTTTATCC<3' 5>ATCCGTCACGGTGACCAGTT<3'	NM_000442.4
eNOS	5>TGATGGCGAAGCGAGTGAA<3' 5>ACTCATCCATACACAGGACCCG<3'	NM_000603.4
vWF	5>TGCGAAGTACCTTGGTTACCCA<3' 5>TAATCGTCAGTACATGCCCCG<3'	NM_000552.3
CD144	5>ATGAGAATGACAATGCCCCG<3' 5>TGTCTATTGCGGAGATCTGCAG<3'	NM_001795
SETSIP-LOC646817	5>CGGTGCGGTACCCTCATGGTCTGGTTCTTG>3' 5>CGTAGCTCTAGAGTCATCTTCTCCTTCATC>3'	SETSIP CLONING
VE-Cadherin Promoter	5>CCTGAGACAGAGGAATCACTATCC>3' 5>CAGGAGAGACATAGGAGGAGCTT>3'	864-1152 bp

Table S2. Gene regulations at day 4.

	Upregulated	Downregulated
Genes involved in Differentiation	BHLHB2	CCL2
	CCND2	CEBPD
	EGR1	CTSB
	EGR2	CXCL12
	ENC1	DCN
	FABP5	EPAS1
	ID1	FAM2OC
	IGF2	GAS1
	IGFBP3	HSPA2
	IGFBP5	IL6
	IRF7	IL8
	LRRC17	LAMA4
	PNP	MMP1
	POU5F1	NFKBIA
	RGS2	PDK4
	TIMP3	SFRP1
VCAM	SOD2	
	TCF21	
	TNC	
	TNFRSF11B	
	TNFRSF12A	
	TWIST2	
Differentiation of Connective tissue	LRRC17	CCL2
	POU5F1	IL6
	RGS2	IL8
	TIMP3	LAMA4
		MMP1
		SFRP1
	SOD2	
	TNFRSF11B	
	TWIST2	
Differentiation of muscle cell lines	IGF2	CTSB
	IGFBP5	CXCL12
	TNFRSF12A	DCN
	TCF21	
Differentiation of adipocytes	RGS2	IL6
	TIMP3	LAMA4

		MMP1
		SFRP1
		SOD2
	BHLHB2	CXCL12
Differentiation of bone cell lines	CCND2	IL6
	EGR1	
	RGS2	
Differentiation of mononuclear cells		CCL2
		IL8
	IGF2	CCL2
Differentiation of osteoclast-like cells		IL8
		CXCL12
		IL6
		MMP1
		TNFRSF11B
Formation of endothelial tube	KLF2	CCL2
		CXCL12
		IL8
Adhesion of endothelial cells	EGR1	CCL2
	SERPINE1	CXCL12
	VCAN	DCN
		IL6

Genes changed during cell reprogramming are involved in cell differentiation into distinct cell lineages at day 4 of reprogramming. Data was analyzed using the Ingenuity Systems Software.

Table S3. Gene regulations at day 7

	Upregulated	Downregulated
Differentiation of memory B cells		BCL6 IL6
Differentiation of β lymphocytes	SYK	BCL6 EGR1 IL6
Differentiation of Th2 cells	LL13RA2	BCL6 IL6
Differentiation of Osteoclast Precursor cells	IL32	IL6

Genes changed during cell reprogramming are involved in cell differentiation into distinct cell lineages at day 7 of reprogramming. Data was analyzed using the Ingenuity Systems Software.

Table S4. Gene regulations at day 14

	Upregulated	Downregulated
Genes involved in Differentiation	HES5	BCL6
	IGFBP6	BDNF
	IL8	EGR1
	IL13RA2	ETS1
	MMP3	IGFBP3
	POU5F1	IL6
	STMN2	INSIG1
		NR4A2
		TSC22D3
Differentiation of stem cells	POU5F1	BDNF
		IL6
		NR4A2
		TSC22D3
Differentiation of Th17 cells		BCL6
		ETS1
		IL6
Differentiation of Dopaminergic neurons		BDNF
		NR4A2
Differentiation of memory B cells		BCL6
		IL6
Differentiation of Th2 cells		BCL6
		IL6
		IL13RA2
Differentiation of helper T lymphocytes	IL13RA2	BCL6
		ETS1
		IL6
Differentiation of Embryonic cells		BDNF
		IL6
		NR4A2
		POU5F1
Differentiation of central nervous system	IGFBP6	BDNF
		HES5
Differentiation of lymphoblastoid cell lines		IL6
Differentiation of mesenchymal stem cells		BDNF
		TSC22D3
Differentiation of plasma cells		BCL6
		IL6

Table S5. Gene regulations at day 21

	Upregulated	Downregulated
Genes involved in differentiation	CD44	BCL6
	DCN	EGR1
	FES	ETS1
	IGFBP6	INSIG1
	IL8	TNC
	IL32	TSC22D3
	IL1B	
	INSM1	
	KRT8	
	MAFB	
	MMP3	
	MMP1	
	POU5F1	
	S100A4	
	SERPINB2	
STC1		
SYK		
Differentiation of Endothelial progenitors	IL1 β	IGFBP3
Differentiation of connective tissue cells	IL8	INSIG1
	IL32	
	IL18	
	MMP3	
	MMP1	
	POU5F1	
STC1		
Differentiation of Th17 cells	IL1 β	BCL6
		ETS1
Differentiation of Oligodendrocytes		HES5
		IL1 β
Tubulation of Endothelial cell lines	FES	TGF β 1
	IL8	

Genes changed during cell reprogramming are involved in cell differentiation into distinct cell lineages at day 21 of reprogramming. Data was analyzed using the Ingenuity Systems Software.

Supplemental Figures

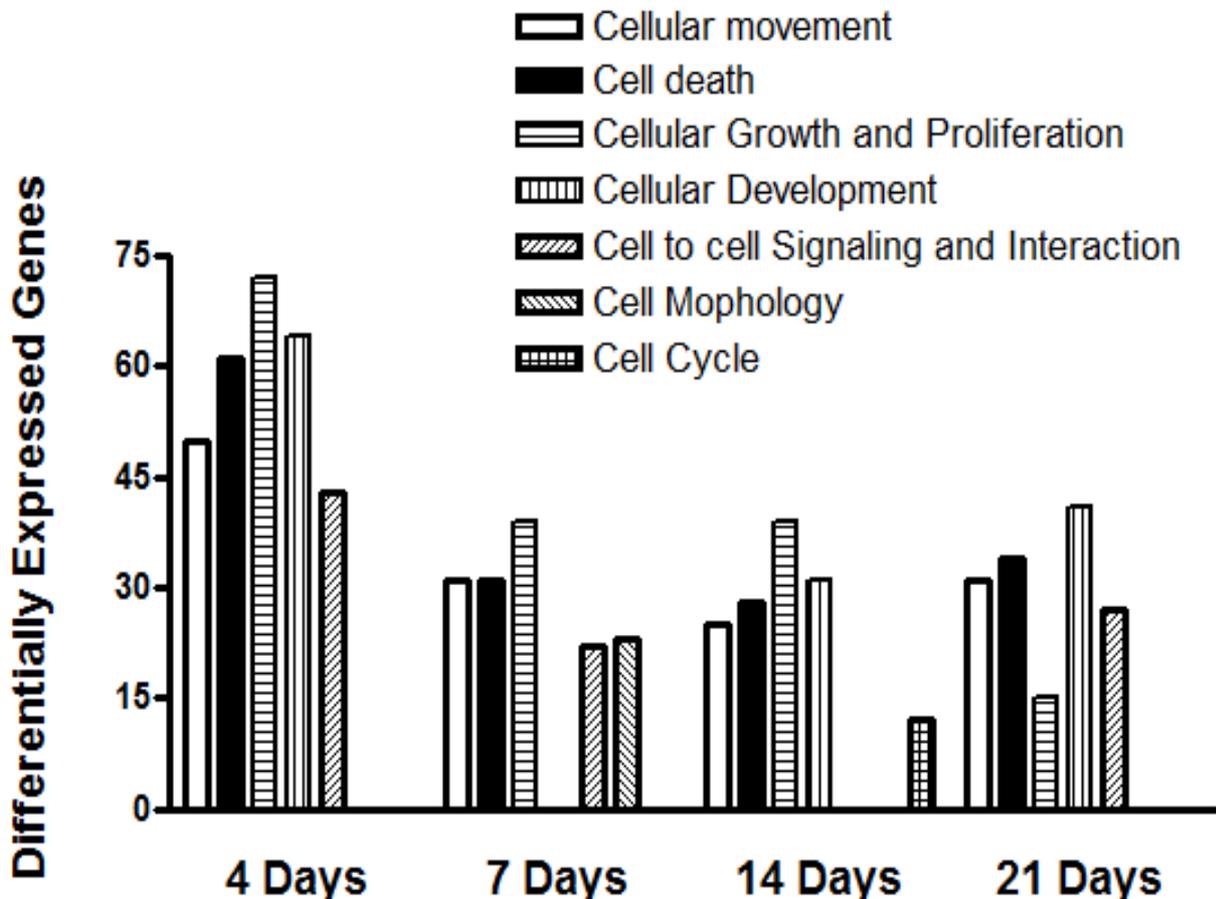


Fig. S1. Functional Classification. Human fibroblasts were virally transduced with a plasmid encoding the four transcription factors OCT4, SOX2, KLF4, c-MYC and cultured in reprogramming media. Microarray analysis was performed at days 4, 7, 14 and 21, and functional classification of the genes altered were analysed using the Ingenuity Systems Software.

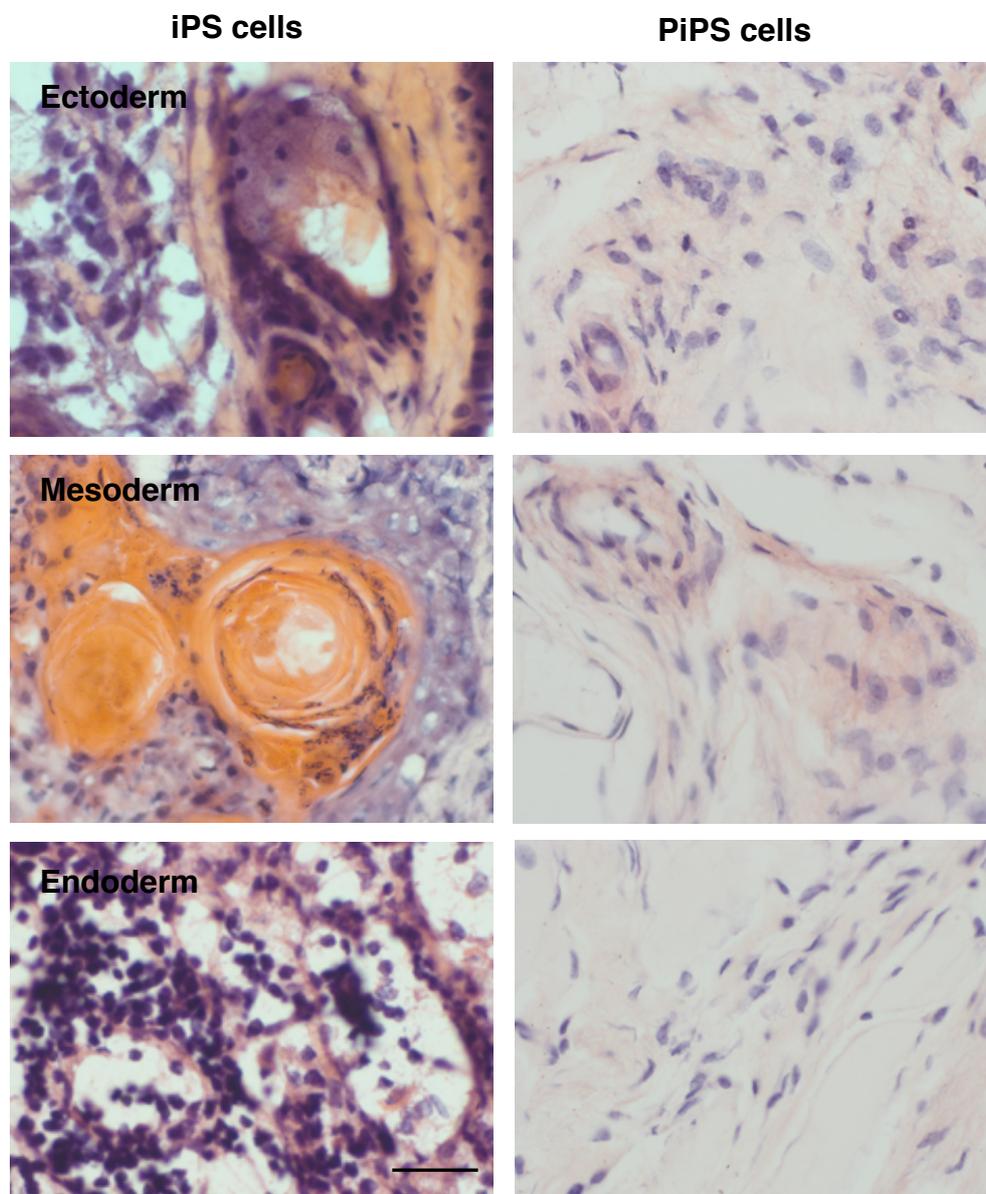


Fig. S2. Immunodeficient mouse recipients (SCID) were subcutaneously injected with human iPS cells or human PiPS cells for a Matrigel plug. Eight weeks later, the plugs were harvested, sectioned and stained with HE. Note that teratoma formation was observed in iPS cells but not in PiPS cells. iPS cells resulting teratomas demonstrate features in ectoderm, mesoderm, and endoderm (left panel). In contrast, an amorphous cellular mass was observed in PiPS cell matrigel plugs (right panel). Scale bar, 50 μ m.

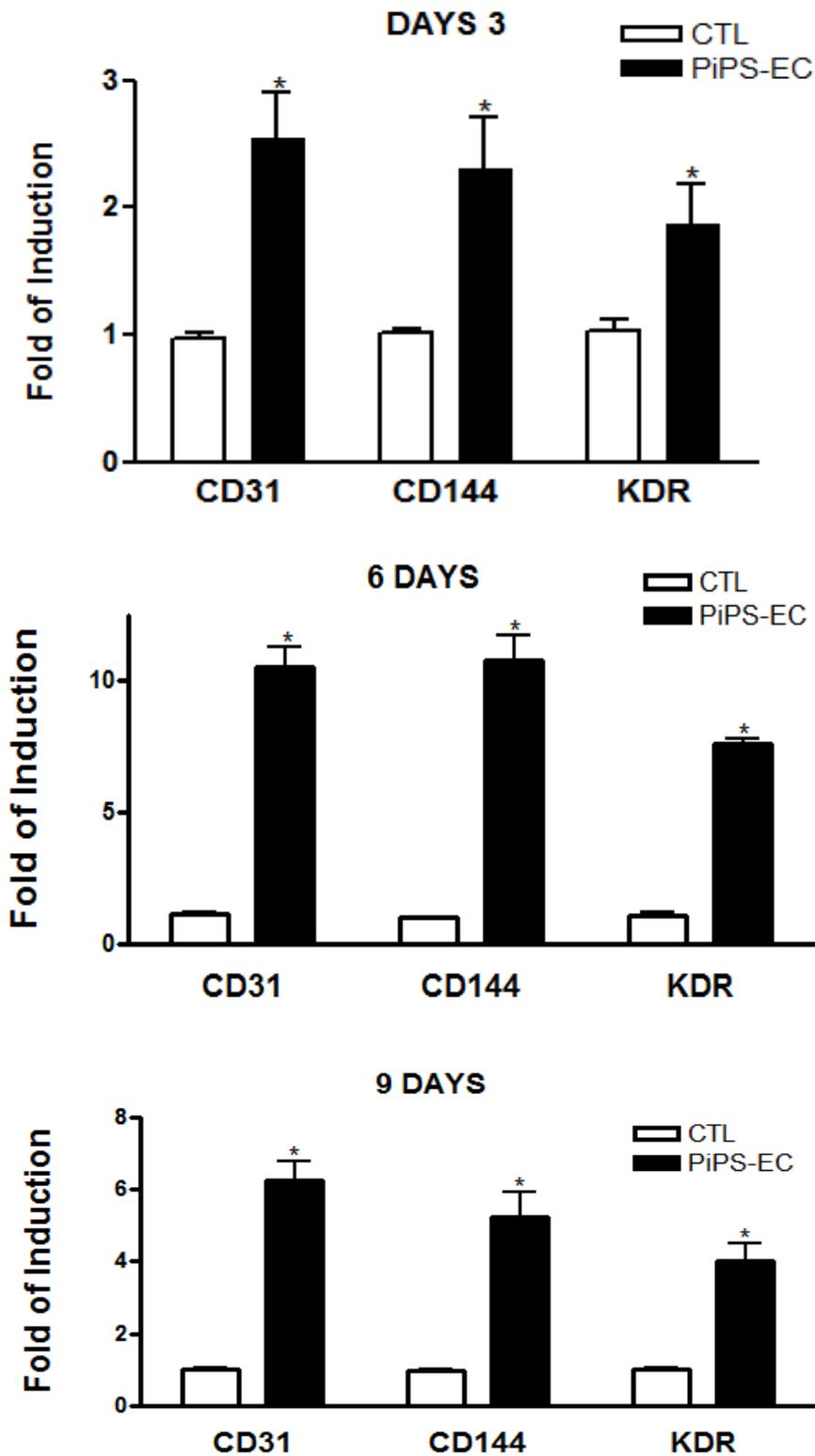
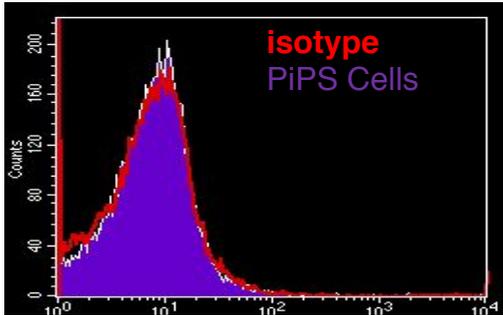


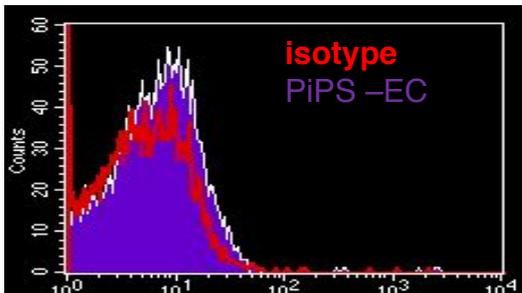
Fig. S3. Time-course of PiPS cells differentiate into endothelial cells. PiPS or control cells were seeded on collagen IV-coated plates and cultured with EGM-2 media for 3-9 days. PiPS-ECs expressed endothelial markers CD31, CD144, and KDR, (data are means \pm SEM. (n=3), *p<0.05)

A

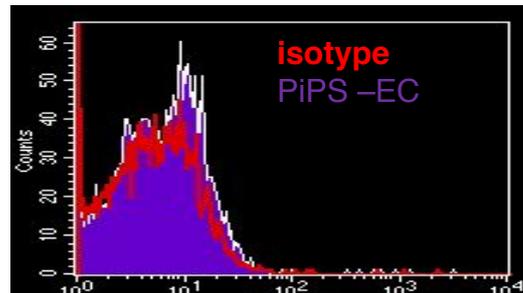


B

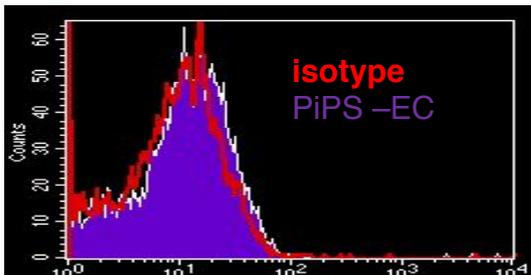
FITC-CD31



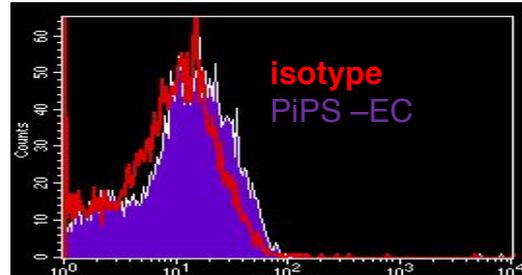
FITC-OCT4



FITC-NANOG



FITC-TRA 1-60



FITC-TRA 1-81

C

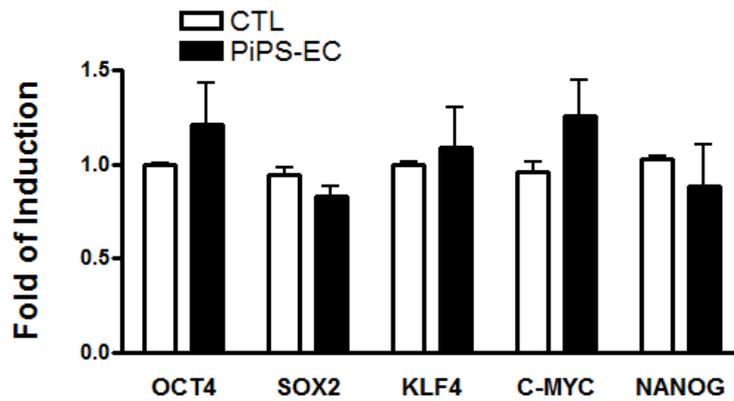


Fig. S4. PiPS-ECs did not express pluripotent markers. (A) FACS analysis for Undifferentiated PiPS cells with CD31. (B) PiPS-ECs for pluripotent markers, OCT4, NANOG, TRA1-60, TRA1-81, and isotype controls. (C) Real time PCR for OCT4, SOX2, KLF4, C-MYC, and NANOG for PiPS-ECs, (data are means \pm SEM. (n=3).

A

PREDICTED: Homo sapiens similar to SET (LOC646817), mRNA.

SETSIP

N-TERMINAL →

MVWFLDFPNS MAPKRQSPLPLQKKKPRPPPALGLEETSASAGLP

KKGEKEQQAIEHIDEVQNEIDRLNEQDSEEILKVEQKYNKLRQPFFQKRSELIAKIP

NFGVTTFVNHPQVSSLLGEEDEEALHYLTKVEVTEFEDIKSGYRIDFYFDENPYFENK

VFSKEFHLNESGDPSSKSTKIKWKSGKDVTKRSSQTQNKASRKRQHEEPESFFTWFTD

HSDAGADELEEVIKDDIWPNPLQYYLVPDMDDEEGEDDDDDDDGDEGEELEDE

GDEDEGEDEDDDEGEEGEEDEGEDD.....

B

Homo sapiens SET nuclear (SET), transcript variant 1, mRNA.

N-TERMINAL →

MAPKRQSPLPPQKKKPRPPPALGPEETSASAGLPKKGEKEQQA

IEHIDEVQNEIDRLNEQASEEILKVEQKYNKLRQPFFQKRSELIAKIPNFWVTTFVNH

PQVSALLGEEDEEALHYLTRVEVTEFEDIKSGYRIDFYFDENPYFENKVLSKEFHLNE

SGDPSSKSTEIKWKSGKDLTKRSSQTQNKASRKRQHEEPESFFTWFTDHSAGADELG

EVIKDDIWPNPLQYYLVPDMDDEEGEGEEDDDDEEEGLEDEEGDEDEGEDEDD

DEGEEGEEDEGEDD

Fig. S5. A gene defined as a similar to SET protein (SETSIP-LOC646817), was identified by microarray analysis. SETSIP gives rise to a protein containing 10 additional amino acids in the N-terminal (A) in comparison to the known SET protein (B).

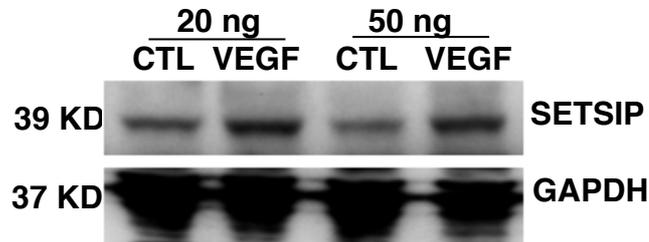


Fig. S6. VEGF induced SETSIP expression in protein level. PIPS-ECs were subjected to further treatment with 20ng/ml and 50 ng/ml of VEGF and the expression levels of SETSIP were detected.

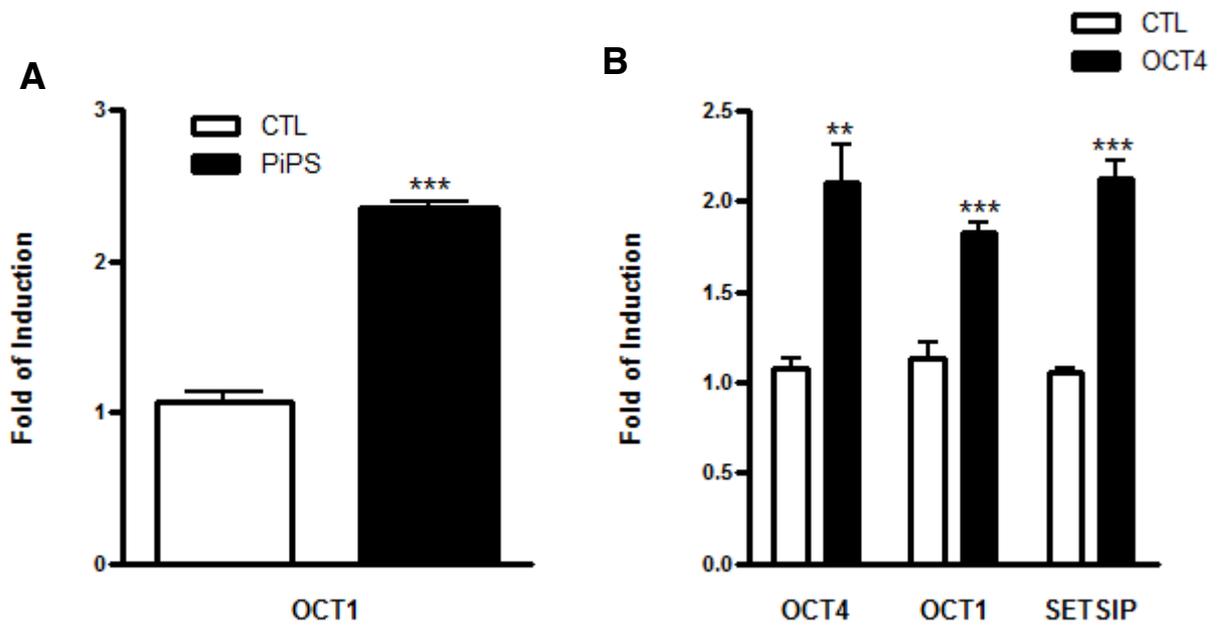


Fig. S7. OCT1 expression is induced during stages of reprogramming (data are means \pm SEM (n=3), ***p<0.001) (A), while overexpression of OCT4 in fibroblasts resulted in OCT1 and SETSIP activation (data are means \pm SEM (n=3), **p<0.01, ***p<0.001) (B).

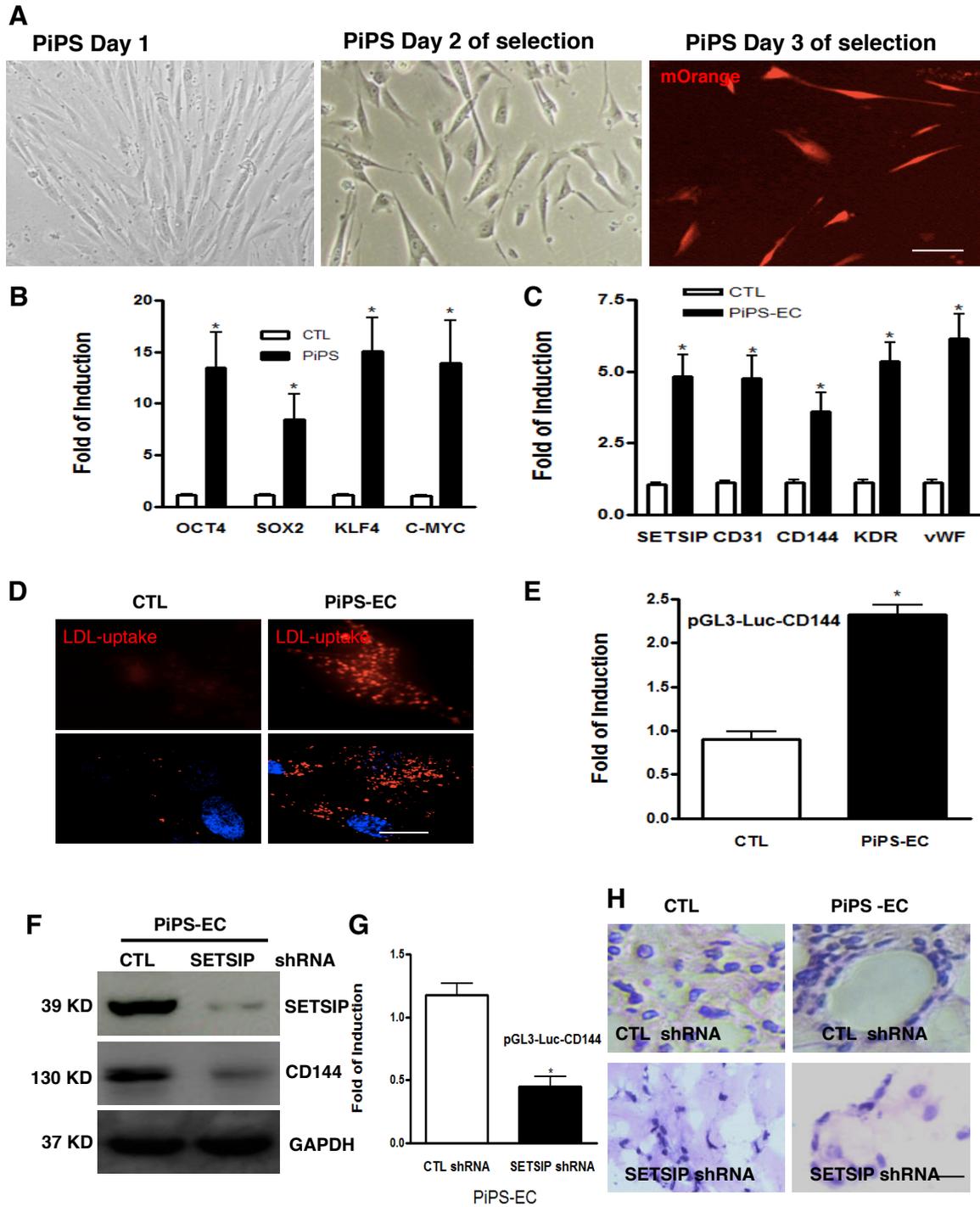
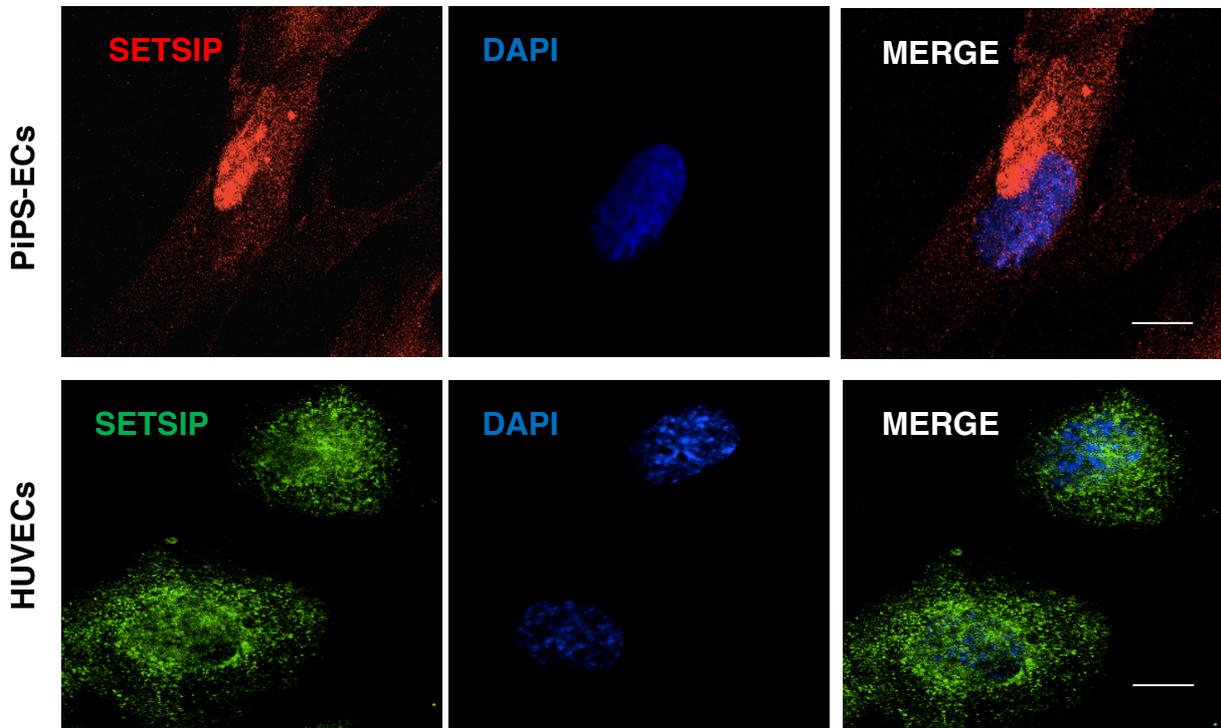


Fig. S8. A pure population of PiPS cells was selected with neomycin and differentiated into EC cells. (A) Images show one day (left panel) nucleofected human fibroblasts with a pCAG2LMKOSimO plasmid encoded the four reprogrammed factors (OCT4, SOX2, KLF4 and c-MYC), which also contains a neomycin resistant gene and a mOrange marker. Selection of PiPS cells with neomycin has started one day after the transfection and images show 2 days PiPS cells after selection (middle panel) and mOrange expression at 3 days of selection (right panel). (B) The selected PiPS cells express the four reprogrammed factors at mRNA levels (data are means \pm SEM. (n=3), *p<0.05). (C) Real time PCR assay shows that the selected PiPS cells differentiate into ECs (data are means \pm SEM. (n=3), *p<0.05). (D) PiPS-EC cells display the capacity of LDL uptake. Scale bar, 50 μ m. (Upper panel; live image, lower panel; cells were fixed and 4',6-diamidino-2-phenylindole DAPI was used and stained the cell nucleus). (E) Luciferase assays demonstrate an increased promoter activity of VE-cadherin in PiPS-EC derived from a pure population of PiPS cells in comparison to the control cells (data are means \pm SEM. (n=3), *p<0.05). (F) Knockdown of SETSIP by shRNA in PiPS-ECs resulting in suppression of VE-cadherin in the protein level. (G) Luciferase assays demonstrate a decrease in the promoter activity of VE-cadherin after knockdown of SETSIP by shRNA in PiPS-ECs (data are means \pm SEM. (n=3), *p<0.05). (H) The formation of vascular-like tubes *in vivo* in Matrigel plugs is abolished by knockdown of SETSIP using shRNA. Scale bar, 50 μ m.

A



B

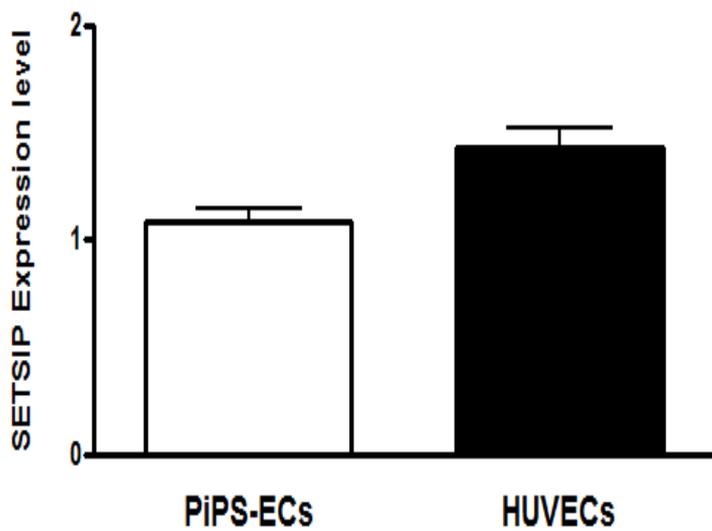


Fig. S9. SETSIP is expressed in PiPS-ECs and mature ECs. Mature ECs were isolated from human umbilical cord (HUVECs). (A) Immunostaining shows SETSIP nuclear localization in both PiPS-ECs and HUVECs, Scale bar, 50 μ m. (B) Real-time PCR data for SETSIP mRNA expression in PiPS-ECs and HUVECs (data are means \pm SEM (n=3)).

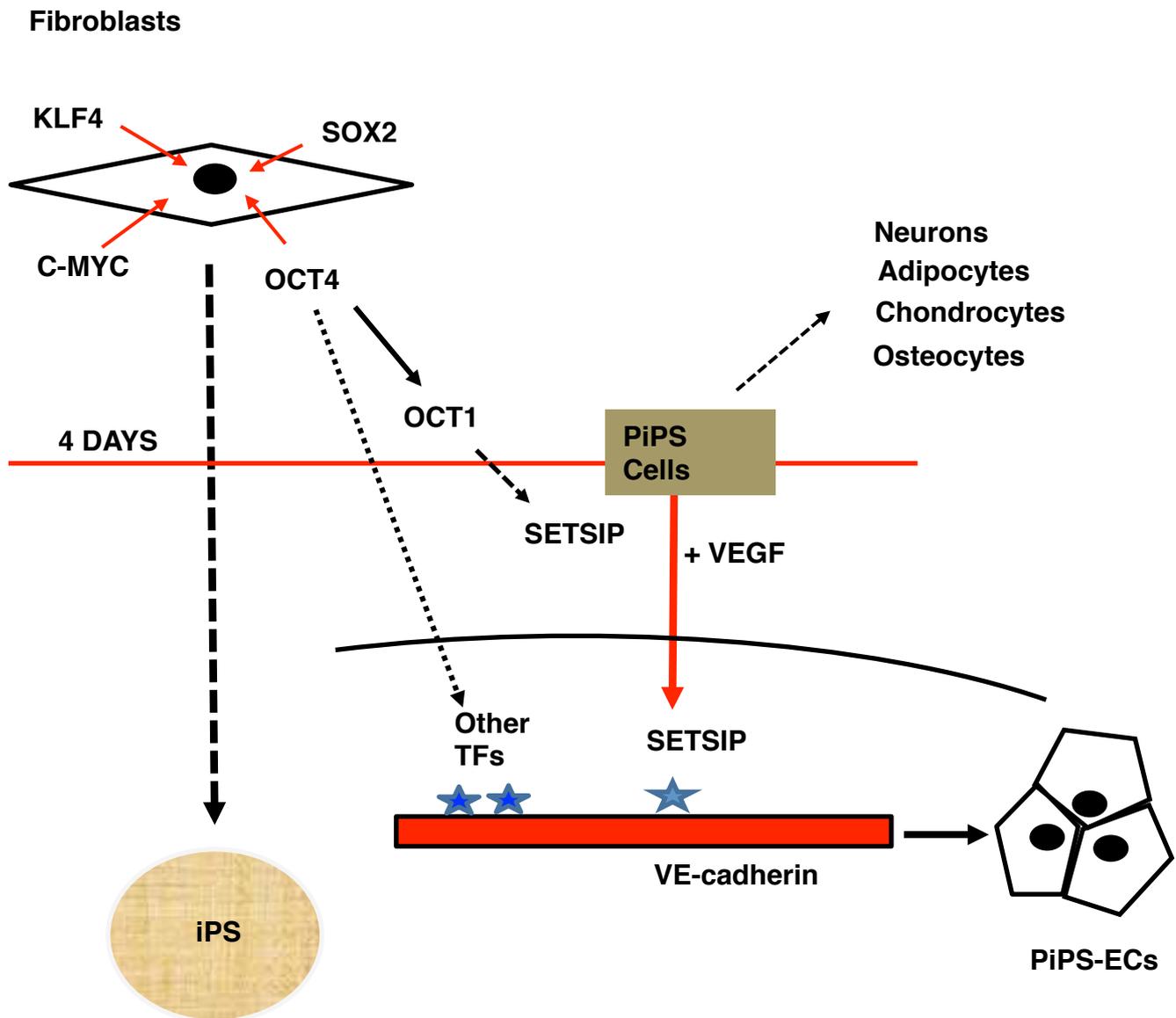


Fig. S10. Schematic diagram showing that short term reprogramming of fibroblasts with four factors generated Partial-iPS (PiPS) cells, which displayed the potential to differentiate into functionally ECs via OCT4-SETSIP and VEGF signal pathways. Preliminary data also showed that PiPS cells in response to defined media and culture conditions induced the expression of markers of other cell lineages, such as neurons, adipocytes, osteocytes, chondrocytes.