Extended Experimental Procedures

293FT cell culture and lentiviral infection

293FT cells cultured in growth medium (DMEM, 10% FBS, 500 μg/ml G418) were seeded in 96-well (primary screen) or 24-well plates (secondary and tertiary screens) at 2.2×10^4 cells or 1.32×10^5 cells per well, respectively, and incubated at 37°C in a 5% CO_2 humidified atmosphere for subsequent lentiviral infection. The RNAi Consortium (TRC) mouse kinase activity lentiviral shRNA library was purchased from ThermoScientific (RMM4957). 293FT cells were transfected with DNA from pLKO.1 plasmids expressing shRNAs targeting the mouse kinase gene family using Lipofectamine and plus according to the manufacturer's instructions. On the following day (day 2), medium was replaced with fresh 293FT medium containing 1.1% BSA, and cells were incubated at 37°C for an additional 2 days for lentivirus production. On day 4, 40 μl (96-well) or 200 μl (24-well) of lentivirus-containing medium was transferred to 4F-infected MEF plates (day 3).

Immunoblotting

On day 4 of lentiviral infection, cells were rinsed with 1X PBS, trypsinized, and harvested by centrifugation at 1,000 rpm for 5 min at 4°C. Cell pellets were resuspended in ice-cold M-PER cell lysis buffer (Thermo Scientific) with 1X protease-phosphatase inhibitor cocktail (Thermo Scientific) and incubated for 15 min at 4°C with gentle agitation. Cell lysates were then centrifuged at 14,000 *g* for 15 min at 4°C, and supernatants were transferred to cold 1.7 ml tubes. After protein concentrations were determined using DC Protein assay (Bio-Rad), 80 μg of protein was resolved on a precast 4–20% gradient SDS-PAGE gel (Lenzo), semi-dry transferred onto a PVDF membrane, and immunoblotted with the following antibodies: anti-TESK1 (Abcam, ab92707), anti-GAPDH (Abcam, ab8245), anti-cofilin (Cell Signaling, #3318), anti-phospho-cofilin (Cell Signaling, #3313S), anti-rabbit IgG-HRP (Cell Signaling, #7074), and goat anti-mouse IgG-HRP (GE Health, NA9310). Following incubation with the secondary antibody, proteins were visualized using BM Chemiluminescence Western Blotting Substrate (POD) (Roche, 11500708001). The reproducibility of lentiviral shRNA knockdown was verified multiple times (five times with three replicates).

In vitro differentiation

To induce spontaneous differentiation of iPSCs, iPSC clones that showed ESC-like proliferation and morphology were induced to from EBs using the hanging-drop method. CCE mESCs (Keller et al., 1993; Robertson et al., 1986) (StemCell Technologies) served as a control. On day 3, EBs were transferred to gelatin-coated 6-well plates and cultured with EB medium (DMEM, 15% FBS, MEM-NEAA, L-glutamine, MTG) for another 11 days. On day 14, cells were fixed with 4% paraformaldehyde (PFA) for immunostaining with the following antibodies: anti-AFP (R&D Systems, MAB1368), anti-β III tubulin (Abcam, ab7751), and anti-α-actinin (Sigma, A7811). For spontaneous differentiation of human iPSCs, hiPSC clones were cultured in hESC medium (DMEM/F12, 20% KOSR, L-glutamine, MEM-NEAA, β-mercaptoethanol, 8 ng/ml bFGF) on irradiated CF-1 feeder layers and fed every day until ready for EB formation. To initiate EB-mediated differentiation, hiPSCs were washed with 1X PBS and incubated with 1 mg/ml dispase in DMEM/F12 for 5 min at 37°C. After scraping to small clumps, hiPSC colonies were washed twice with hEB medium (DMEM/F12, 10% FBS, L-glutamine) by sedimentation and plated on 6-well ultra-low attachment plates. hEB medium was changed every other day by sedimentation. On day 7, the colonies were plated in 0.1% gelatin-coated 6-well plates and cultured for an additional 7 days. On day 14, cells were fixed with 4% PFA for immunostaining.

Immunostaining

Established iPSC clones were fixed in 4% PFA and permeabilized by 0.1% Triton X-100 in PBS. Cells were then blocked in 5% BSA in PBS containing 0.1% Triton X-100 for 1 h at room temperature. Primary antibodies anti-mNanog (Bethyl Lab, IHC00205) and anti-h/mSSEA1 (R&D Systems, MAB2156) were diluted between 1:100 and 1:400 in 2.5% BSA PBS containing 0.1% Triton X-100, according to the manufacturer's suggestion. Secondary antibody was diluted 1:400 and cells were stained for 45 min at room temperature. MEFs were plated on 0.1% gelatin-coated 12-well plates and transfected with 50 nM siRNA by Lipofectamine 2000 following the manufacturer's protocol. After 72 h, cells were fixed in 3.75% formaldehyde in PBS for 15 min on ice and permeabilized in 0.5% Triton X-100 in PBS. After blocking with 3% milk in PBS for 30 min, cells were stained for F-actin with rhodamine-conjugated phalloidin (Bitium Inc., #00027) at 1:40 dilution or with Hoechst 33342, trihydrochloride, trihydrate (Invitrogen, H3570) at 1:5,000 dilutions. To assess cytoskeletal rearrangement during reprogramming, MEFs were

plated in 12-well plates at 4×10^4 cells/well. One day later, cells were transduced with 4F virus followed by lentivirus medium containing empty vector pLKO.1 or vectors expressing shRNAs targeting mouse TESK1 or LIMK2. Lentivirus was produced in 293FT cells as described above. Cells were fixed either immediately after addition of the lentivirus (day 0) or on days 2, 4, or 6, and immunostained with rhodamine-conjugated phalloidin, as described above. For immunoblot analysis, cell lysates were prepared on days 2, 4, or 6, and blotted using phospho-cofilin and total cofilin antibodies.

Light and electron microscopy

MEFs were grown for 2–3 days on 4/2 carbon-coated finder grids (Quantifoil, GMBH). Epifluorescence images of fixed cells were acquired on an inverted light microscope (Eclipse TE 2000-U, Nikon) equipped with a manually controlled shutter, filter wheels, and a 14-bit cooled CCD camera (Orca II, Hamamatsu) controlled by MetaMorph software (Universal Imaging Corp.) by a Plan Fluor ELWD 40x/0.60 Ph2 or Plan Fluor 10x/0.30 Ph1 objective lens (Nikon). Viewing a large number of cells on a single grid, by using the grid finders, allows for localization of the exact individual cell in both light and in electron microscope imaging. Cells expressing control shRNA or shTESK1 were chemically fixed in CB containing 4% PFA, washed, and stained with aqueous 2% OsO_4 and 2% uranyl acetate. Dehydration in increasing concentrations of reagent-grade ethanol (15%, 20%, 50%, 70%, 95%, and 100%; 3 min per change) was followed by drying from liquid CO_2 by the critical-point method according to previously described methods (Anderson, 1951; Buckley and Porter, 1975). Images were obtained under low-dose conditions with a TecnaiG2 F20 microscope (FEI electron optics) equipped with FEG at 200keV. Kodak SO-163 plates were developed for 13 min by using D19 developer (Eastman Kodak Co., Rochester, NY).

Expression vectors for TESK1, COF-WT-GFP, and COF-S3A-GFP

TESK1 cDNA was cloned with or without an N-terminal HA-tag into either the pMX retroviral vector or the pCDNA3.1 vector. The PCR-amplified 1.8 kb cDNA was inserted into BamHI and XhoI restriction enzyme sites. An internal SacI restriction site in the TESK1 cDNA sequence was used to clone the entire sequence in two parts. The following primers were used for cloning:

Part 1:

HA-tagged TESK1, fwd: 5′-AATTGGATCCATGTACCTTATGATGTGCCGGATTATGCCATGGCCGGGGAACGGCCG CC-3′OR; BamH1-Tesk fwd: 5′-ATATATATGGATCCATGGCCGGGGAACGGCCGCC-3′; and TESK1, set 1, rev: 5′-GCGATGAGCTCACAGAGGACGATCCCGAAG-3′

Part 2:
TESK1, set 2, fwd: 5′-CCAGAGGTGTTGCGGGGAGAGCTGTATGAT-3′
Xho1-TESK1 Rev: 5′-AATTCTCGAGCTAAGAGCGTGCCCCAGGCAGCTG CA-3′

GFP tagged wild-type (COF-WT-GFP) and the phosphorylation site mutant COF (COF-S3A-GFP) were PCR amplified from pCDNA Cof-WT-GFP or pCDNA Cof-S3A-GFP (Delorme et al., 2007), respectively, using the primers, hsa-Hind III-Cof-WT-Fw (GATCAAGCTTATGGCCTCCGGTGTGGCTGTCTCT) or hsa-Hind III-Cof MutS3A-Fw (GATCAAGCTTATGGCCGCCGGTGTGGCTGTCTCTG) and hsa-XhoI-Cof-Rv (GATCCTCGAGCAAAGGCTTGCCCTCCAGGGAGATG). The amplified fragments were digested and cloned into pMX vector.

For overexpression of TESK1, HA-TESK1, and COF proteins, MEFs were transduced with the pMX retroviral vector as described above.

Human iPSC generation and characterization

Fibroblast culture medium: DMEM, 10% FBS, 1 mM of NEAA. iPSC culture medium: DMEM/F12, 20% Knockout^{TM} Serum Replacement, 1 mM NEAA, 5 mM β-mercaptoethanol, *1x anti-anti,* 10 ng/mL bFGF. Ectoderm differentiation media: DMEM/F12, 20% Knockout^{TM} Serum Replacement, 1 mM NEAA, 5 mM β-mercaptoethanol, and 2 μM each of dorsomorphin (iGentBio), A83-01 (Tocris), and PNU 74654 (Tocris). Mesoderm differentiation medium: DMEM/F12, 20% FBS, NEAA. Endoderm differentiation medium: DMEM/F12, 0.5% FBS, 50 ng/ml Activin A, and 100 ng/ml Wnt3A (Stem RD). For reprogramming, human foreskin fibroblast (BJ) cells (~0.7×10^6 cells) were transfected with non-targeting or TESK1 siRNA (50 pmol) together with the episomal DNA cocktail (Addgene) as described ((Okita et al., 2011; Yu et al., 2009). Transfected cells were cultured in fibroblast growth medium for 7 days, trypsinized, and re-seeded on CF1 MEF feeders (5×10^4 cells/cm^2). iPSC cells were maintained in culture media for 5 weeks, then the iPSC colonies were picked and maintained on CF1 MEF feeders for

further characterization. The iPSCs were maintained in NutriStem XF/FF culture medium (Stemgent) prior to in vitro differentiation. For ectoderm differentiation, the cells were seeded on Matrigel (BD Biosciences)-coated plates in NutriStem medium for 2–3 days until 50–60% confluent, and then treated with ectoderm differentiation medium for 5–7 days until cell clusters with a neural rosette structure were observed under microscope. For mesoderm differentiation, EBs were formed and maintained in mesoderm differentiation medium in suspension culture in ultra-low attachment plates (Corning Costa) for 6 days, and then transferred to wells coated with 0.1% gelatin (Stem Cell Technologies) in the same medium for an additional 6–8 days until the beating colonies were observed. For endoderm differentiation, the cells were treated with Accutase to yield single cells and seeded on Matrigel-coated plates at 10^4 cells/cm^2 in NutriStem medium for 2–3 days until 50–60% confluent. The cells were then treated with endoderm differentiation medium for 3–5 days before immunostaining.

For teratoma formation, iPSCs were injected into the SCID mice and the tumors were harvested eight weeks later. The tumors were embedded in paraffin, sectioned, and stained with H&E. For alkaline phosphatase staining, iPSCs were fixed with 4% PFA (Affymetrix) and stained with an AP staining kit (Vector labs) according to the manufacturer's protocol. For immunostaining, iPSCs were fixed in 4% PFA and stained with antibodies against Oct4, Sox2, Nanog, Tra-1-60, Tra-1-81 (Cell Signaling), and SSEA3 (Millipore). Ectoderm lineage cells were stained with Pax6 (Covance) and Nestin (Abcam), mesoderm lineage cells were stained with α-SMA (Sigma) and α-actinin (Sigma), endoderm cells were stained with Sox17 and FoxA2 (both R&D Systems).

References

Anderson, T.F. (1951). Techniques for the preservation of three-dimensional structure in preparing specimens. EM Trans NY Acad Sci *13*, 130-134.

Buckley, I.K., and Porter, K.R. (1975). Electron microscopy of critical point dried whole cultured cells. J Microsc *104*, 107-120.

Delorme, V., Machacek, M., DerMardirossian, C., Anderson, K.L., Wittmann, T., Hanein, D., Waterman-Storer, C., Danuser, G., and Bokoch, G.M. (2007). Cofilin activity downstream of Pak1 regulates cell protrusion efficiency by organizing lamellipodium and lamella actin networks. Developmental cell *13*, 646-662.

Keller, G., Kennedy, M., Papayannopoulou, T., and Wiles, M.V. (1993). Hematopoietic commitment during embryonic stem cell differentiation in culture. Mol Cell Biol *13*, 473-486.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K.*, et al.* (2011). A more efficient method to generate integration-free human iPS cells. Nature methods *8*, 409-412.

Robertson, E., Bradley, A., Kuehn, M., and Evans, M. (1986). Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. Nature *323*, 445-448.

Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, II, and Thomson, J.A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science *324*, 797-801.

Fig S1

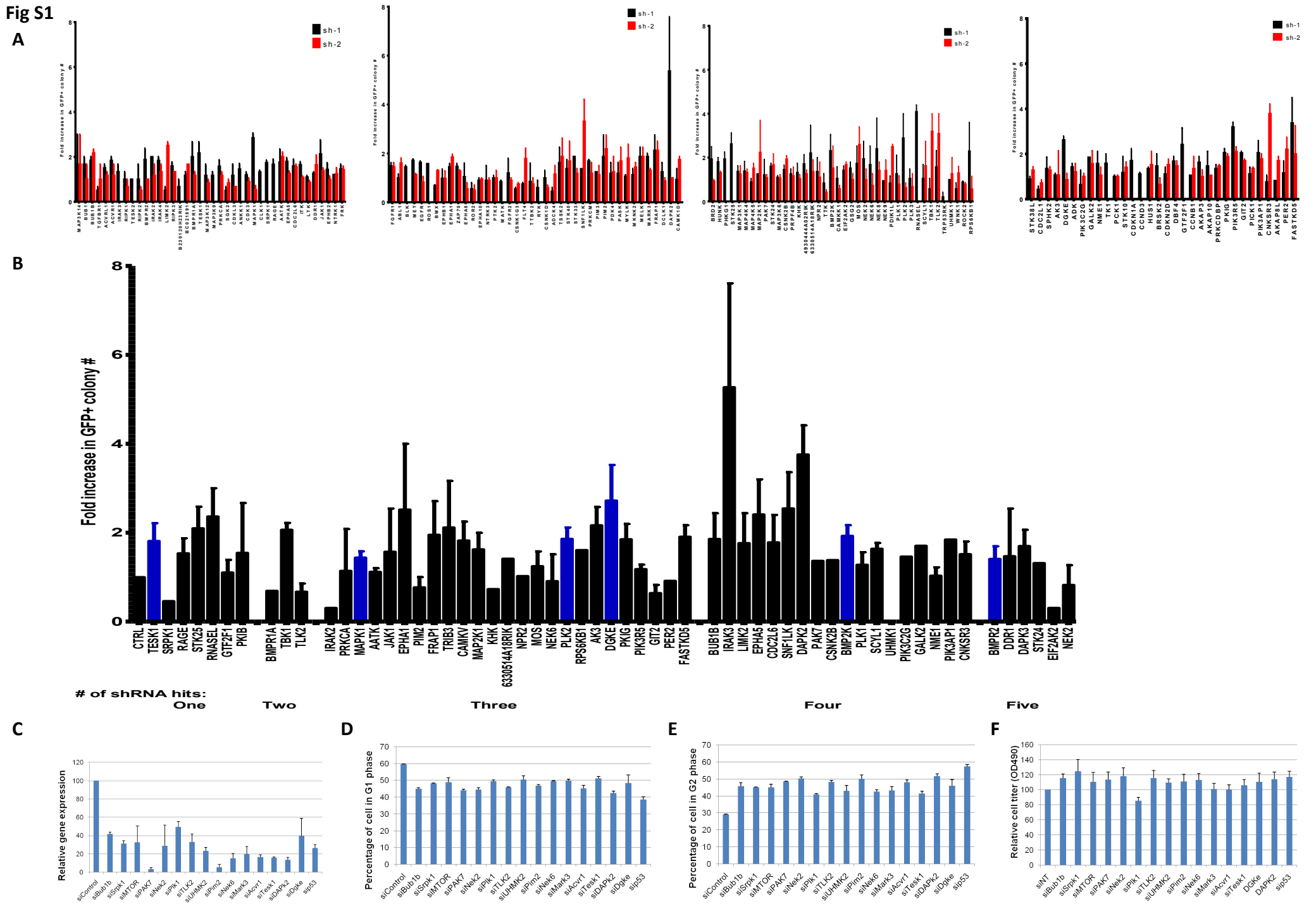

A
sh-1
sh-2
Fold increase in GFP+ colony #
B
Fold increase in GFP+ colony #
CTRL TESK1 SRPK1 RAGE STK25 RNASEL GTF2F1 PKIB BMPR1A TBK1 TLK2 IRAK2 PRKCA MAPK1 AATK JAK1 EPHA1 PIM2 FRAP1 TRIB3 CAMKV MAP2K1 KHK 6330514A18RIK NPR2 MOS NEK6 PLK2 RPS6KB1 AK3 DGKE PKIG PIK3R5 GIT2 PER2 FASTKD5 BUB1B IRAK3 LIMK2 EPHA5 CDC2L6 SNF1LK DAPK2 PAK7 CSNK2B BMP2K PLK1 SCYL1 UHMK1 PIK3C2G GALK2 NME1 PIK3AP1 CNKSR3 BMPR2 DDR1 DAPK3 STK24 EIF2AK2 NEK2
# of shRNA hits:
One
Two
Three
Four
Five
C
Relative gene expression
siControl siBub1b siSrpk1 siMTOR siPAK7 siNek2 siPlk1 siTLK2 siUHMK2 siPim2 siNek6 siMark3 siAcvr1 siTesk1 siDAPK2 siDgke sip53
D
Percentage of cell in G1 phase
siControl siBub1b siSrpk1 siMTOR siPAK7 siNek2 siPlk1 siTLK2 siUHMK2 siPim2 siNek6 siMark3 siAcvr1 siTesk1 siDAPK2 siDgke sip53
E
Percentage of cell in G2 phase
siControl siBub1b siSrpk1 siMTOR siPAK7 siNek2 siPlk1 siTLK2 siUHMK2 siPim2 siNek6 siMark3 siAcvr1 siTesk1 siDAPK2 siDgke sip53
F
Relative cell titer (OD490)
siNT siBub1b siSrpk1 siMTOR siPAK7 siNek2 siPlk1 siTLK2 siUHMK2 siPim2 siNek6 siMark3 siAcvr1 siTesk1 DGKe DAPK2 sip53

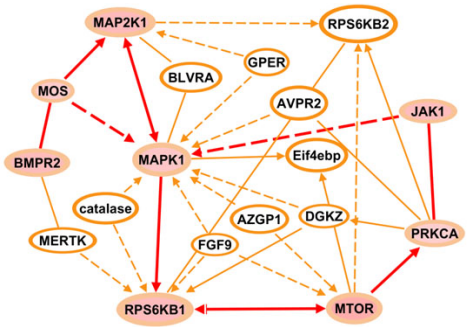
Figure S1, related to Figure 1. Identification of kinases acting as barriers for iPSC generation

Kinase genes identified as hits in the primary screen were confirmed by secondary and tertiary screens. After the primary screen (Figure 1B), kinase genes were grouped by the number of shRNAs targeting a single gene that led to a 2-fold increase in GFP+ colony numbers. Kinases targeted by at least two shRNAs (sh-1 and sh-2) were chosen as candidates for further analysis. In addition, kinases targeted with a single shRNA were included if they induced a >6-fold increase in GFP+ colony numbers. The grouping of targets based on the primary screen is labeled. From the primary screen, 153 genes were tested in the secondary screen (A) and 59 genes were tested in the tertiary (B) screen. Among the 59 selected genes, knockdown of 39 genes increased GFP+ colony numbers by ≥1.5-fold and knockdown of 20 genes increased GFP+ colony numbers at least 2.0-fold in both the secondary and tertiary screens. Blue bars in (B) indicate the six kinases selected for detailed investigation. (C) Candidate kinases were efficiently knocked down by siRNAs. Fifteen kinases that function in cell cycle and cytoskeleton formation were chosen from the tertiary screen. siRNA-mediated knockdown efficiency was confirmed by RT-qPCR on day 2 post-transfection. Results are mean ± SD from two independent experiments with duplicate wells. (D-E) Knockdown of selected kinases reduced the proportion of cells in (D) G1 phase and (E) G2 phase of the cell cycle. siRNA-transfected MEFs were stained with PI and analyzed by FACS. The percentage of cells in G1 and G2 was quantified by ModFit. p53-targeted siRNA was used as the control. (F) Knockdown of selected kinases did not affect cell proliferation. MEFs were transfected with siRNAs targeting the indicated kinases and proliferation was measured by addition of CellTiter AQueous One solution. Optical densities (OD) were read at 490 nm.

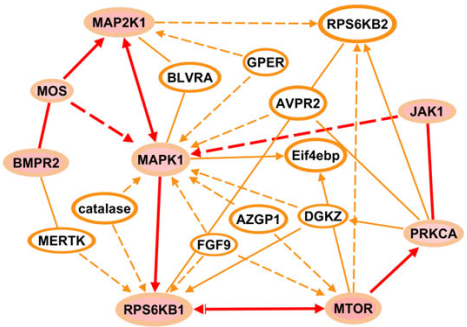
Fig S2

A

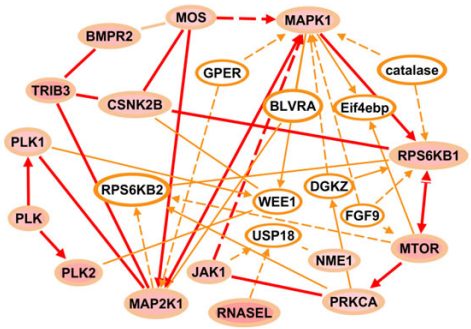
I. Amino Acid Metabolism, Post Translational Modification, Small Molecule Biochemistry



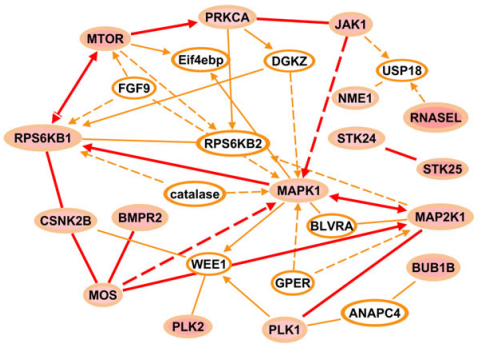
III. Cell Cycle, Cell Signaling and Cell Death



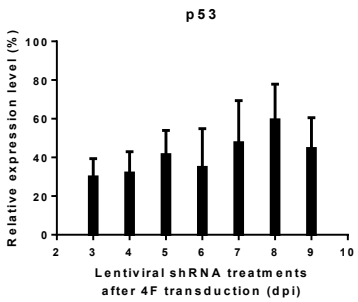
II. Gene Expression, Cellular Development

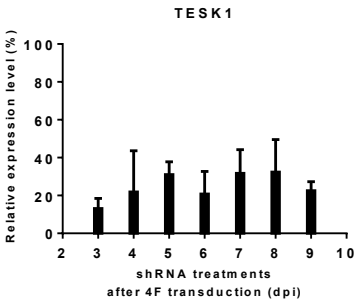


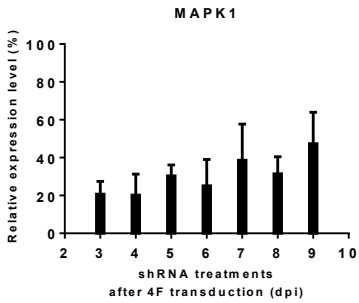
IV. Cellular Growth and Proliferation, Cancer

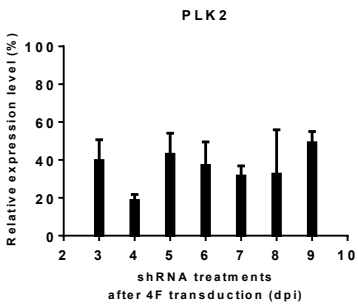


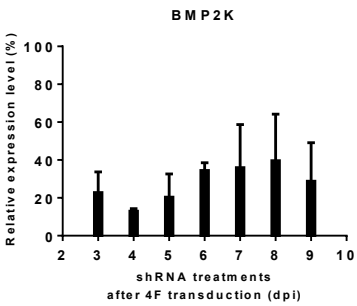
B

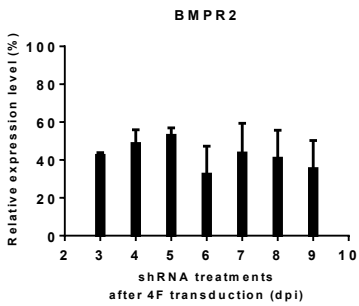


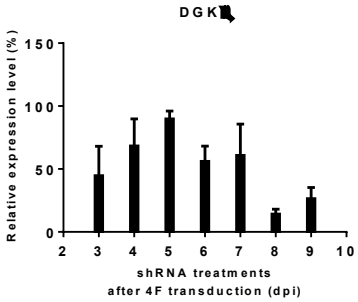


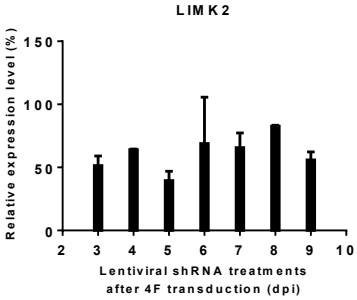












C

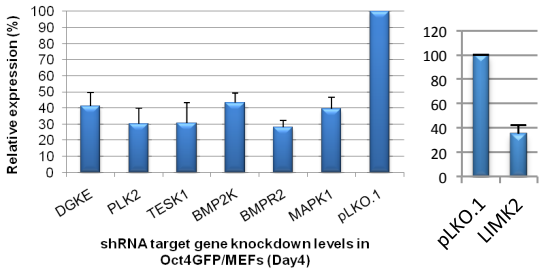


Figure S2, related to Figure 2. Bioinformatics analysis and characterization of kinase KD

(A) The 59 kinase genes confirmed by the tertiary screen and identified as barriers for reprogramming were evaluated for interactions with various biological functional networks using the Ingenuity Pathways Knowledge Base (IPKB) analysis. The top ten highly interconnected molecular functions identified from the analysis are shown as four networks. Interactions are characterized by the following arrows: direct interactions (solid lines); indirect interactions (dotted lines); activation by kinases (arrowheads); and inhibitory action (vertical lines next to arrowheads [e.g., MTOR to RPS6KB1]). Red lines show interactions between kinases identified in our functional genomics screen and the kinases are shown in pink. (B) Efficient shRNA-mediated knockdown of six kinases at different times after 4F transduction. Knockdown was determined by RT-qPCR of total RNA extracted 4 days following shRNA lentiviral transduction. Expression levels in samples treated with pLKO.1-empty vector were set as 100%. Results are mean ± SD of two independent experiments performed in triplicate. (C) Seven identified kinases were efficiently knocked down by shRNAs. Total RNA was extracted 4 days after lentiviral transduction. Expression levels in the pLKO.1 sample were set as 100%. Results are mean ± SD of three independent experiments. RNA purified from these experiments was used for examining the E-cad expression levels shown in Figure 2B for these kinases.

Fig S3

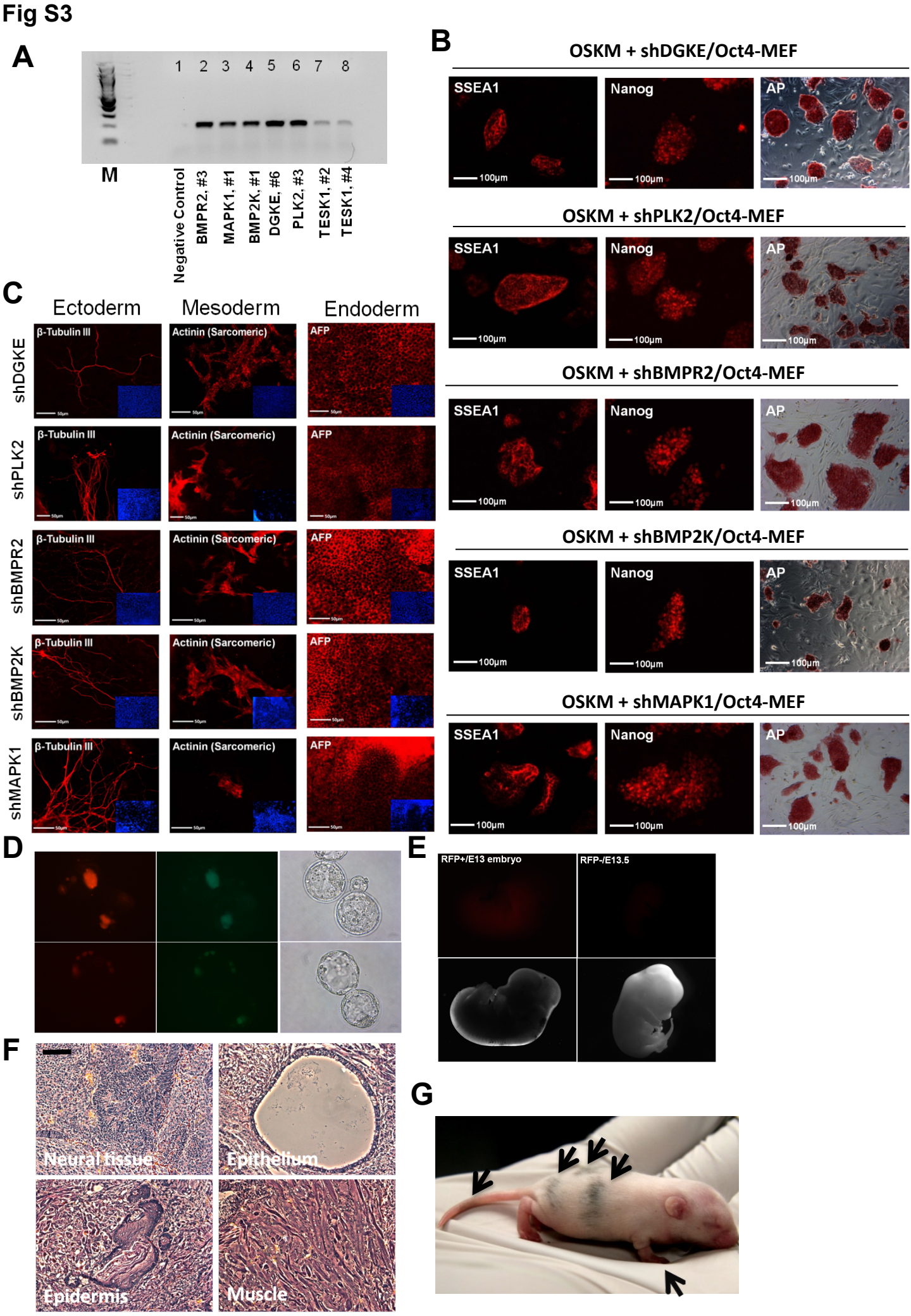

A
1 2 3 4 5 6 7 8
M
Negative Control
BMPR2, #3
MAPK1, #1
BMP2K, #1
DGKE, #6
PLK2, #3
TESK1, #2
TESK1, #4
B
OSKM + shDGKE/Oct4-MEF
OSKM + shPLK2/Oct4-MEF
OSKM + shBMPR2/Oct4-MEF
OSKM + shBMP2K/Oct4-MEF
OSKM + shMAPK1/Oct4-MEF
SSEA1
Nanog
AP
100μm
C
Ectoderm
Mesoderm
Endoderm
shDGKE
shPLK2
shBMPR2
shBMP2K
shMAPK1
β-Tubulin III
Actinin (Sarcomeric)
AFP
D
E
RFP+/E13 embryo
RFP-/E13.5
F
Neural tissue
Epithelium
Epidermis
Muscle
G

Figure S3, related to Figure 3. Characterization of kinase-knockdown iPSCs

(A) Genomic integration of shRNAs. Integration of shRNAs in genomic DNA was assessed by PCR amplification of the puromycin marker gene in the pLKO.1 vector. Genomic DNA was isolated from one clone each of five kinase-KD iPSCs (BMPR2 clone #2, MAPK1 clone #1, BMP2K clone #1, DGKε clone #6, PLK2 clone #3) and from two clones of TESK1-KD cells (clone #2 and #4, which were further characterized for teratoma formation). For the negative control, water replaced the primer set. (B) iPSCs derived from MEFs expressing kinase shRNAs express ESC pluripotent markers. GFP+ kinase-KD MEFs were cultured on feeder layers and immunostained for SSEA-1, Nanog, and AP on day 16. (C) iPSCs derived from MEFs expressing kinase shRNAs differentiate into three germ layers *in vitro*. EBs were formed using the hanging-drop method and allowed to undergo spontaneous differentiation. On day 14, differentiated cells were fixed with 4% PFA and immunostained for β-tubulin III (ectoderm), sarcomeric actinin (mesoderm), or α-fetoprotein (AFP; endoderm). DAPI as nuclear marker shows the cells in the imaged area. (D) Injected shTESK1-iPSCs integrated into the recipient embryos. Upper panel: injected iPSCs at day 0. Lower panel: injected iPSCs at day 1. shTESK1-iPSCs were labeled with constitutive RFP expression vectors before injection. (E) shTESK1-iPSCs contributed to E13.5 embryos. RFP signal indicates the contribution of injected iPSCs in recipient embryos. (F) H&E staining of teratoma derived from iPSCs. Nude mice were injected with 1.5×10^6 iPSCs and tumors were harvested ~3–4 weeks later. (G) Approximately 12–18 TESK1-knockdown iPSCs were injected into the blastocysts of albino C57BL/6 mice. The contribution from the iPSCs can be seen as the black coat color (arrow).

Fig S4

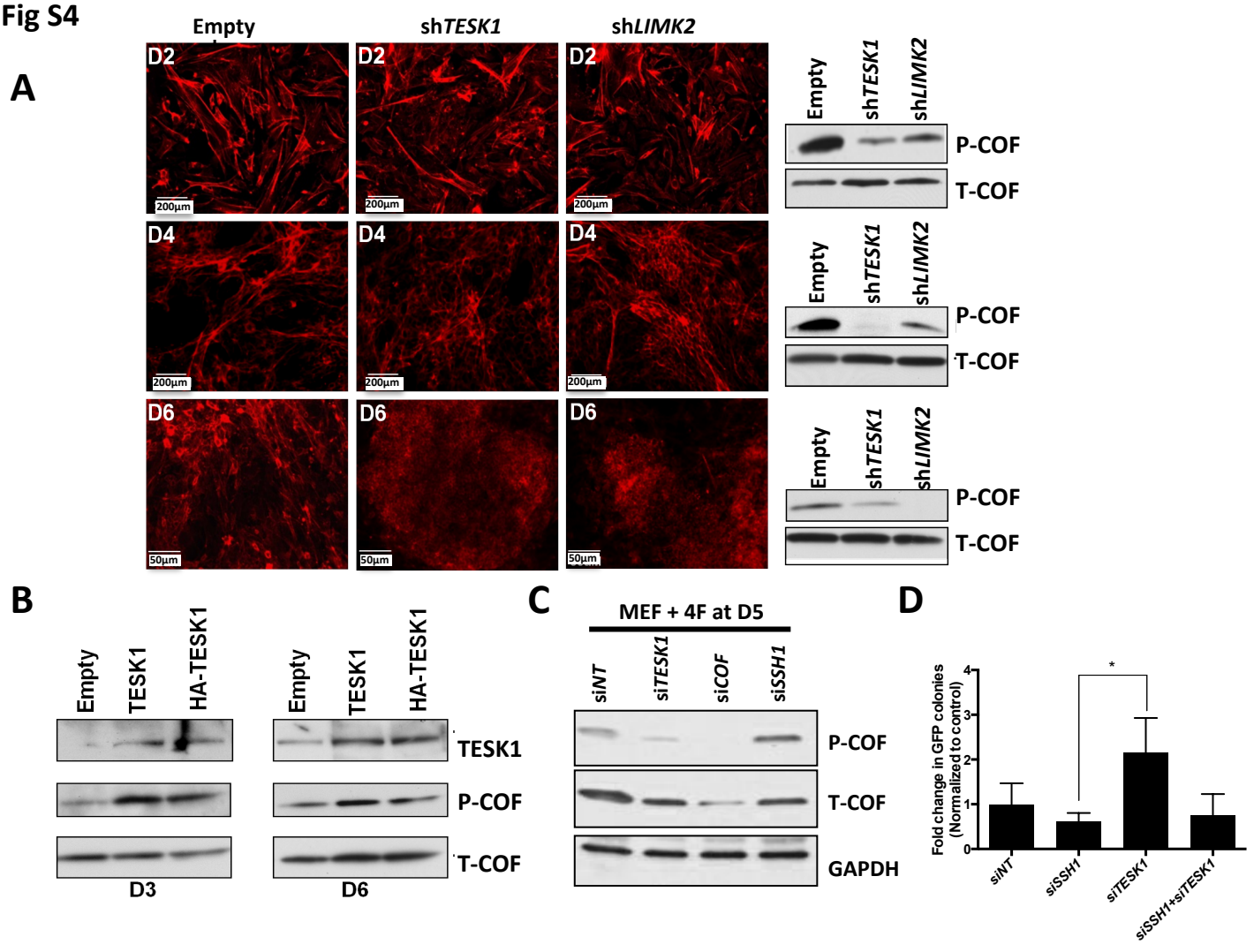
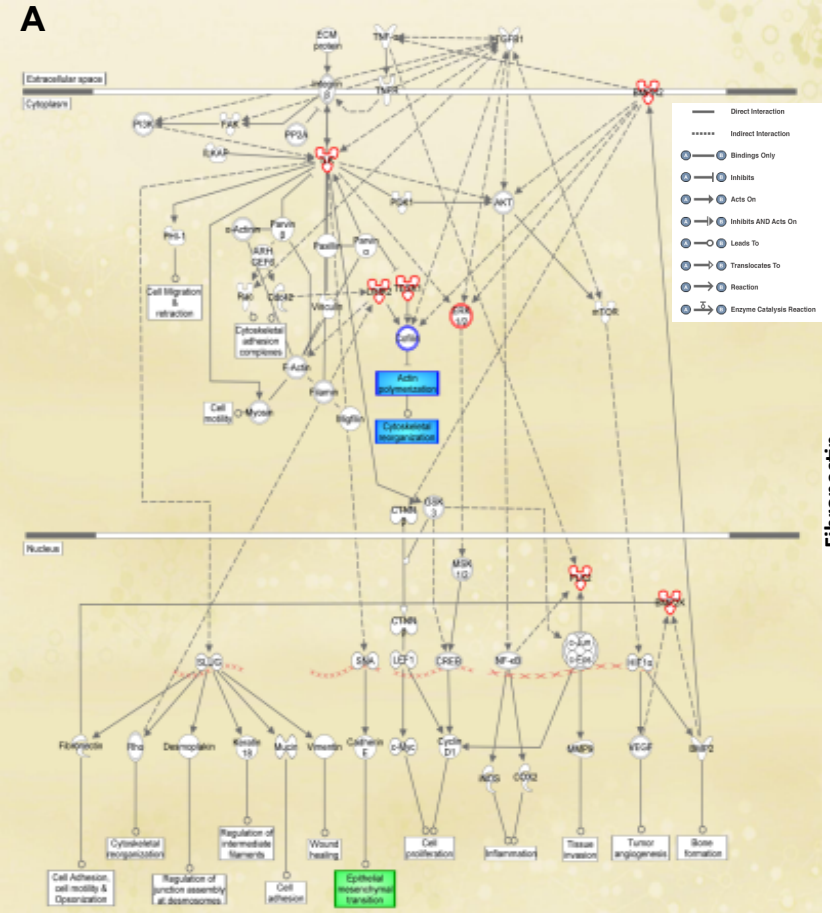
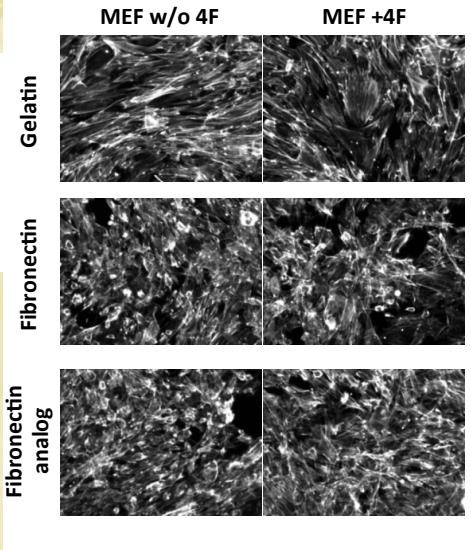

A
Empty
shTESK1
shLIMK2
D2
D4
D6
200µm
50µm
P-COF
T-COF
B
Empty
TESK1
HA-TESK1
TESK1
P-COF
T-COF
D3
D6
C
MEF + 4F at D5
siNT
siTESK1
siCOF
siSSH1
P-COF
T-COF
GAPDH
D
Fold change in GFP colonies (Normalized to control)
siNT
siSSH1
siTESK1
siSSH1+siTESK1
*
E
Control
siNT
siTESK1
siCOF
ACTIN
MYO IIb
Merge
DAPI

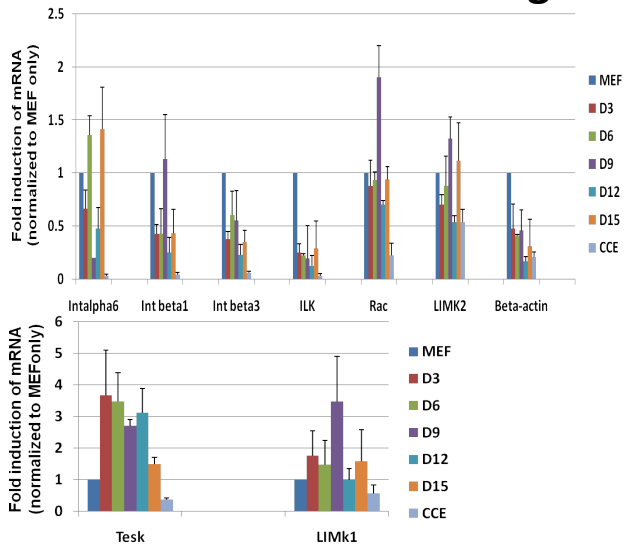
Figure S4, related to Figure 4. Mechanism of Cytoskeletal rearrangement by TESK1 and iPSC

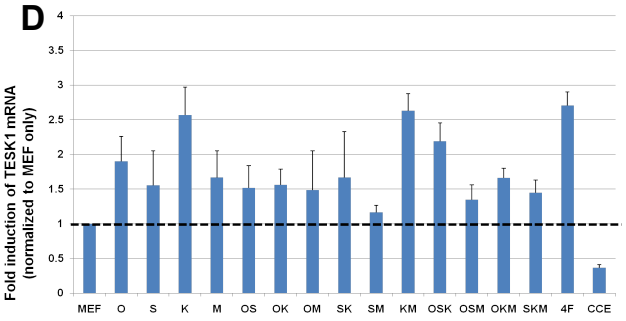
(A) Silencing of TESK1 or LIMK2 promotes cytoskeletal rearrangement during reprogramming. Visualization of actin cytoskeleton formation by rhodamine-phalloidin staining (*Left* panel) and immunoblot analysis of phosphorylated (P) and total (T) cofilin (*Right* panel). MEFs were transduced with 4F followed by sh*TESK1* or sh*LIMK2* lentivirus on day 2, 4, or 6. MEFs transduced with an empty vector served as a control. (B) TESK1 or HA-TESK1 cDNA was cloned into pMX retroviral vectors and MEFs were transduced with pMX and 4F vectors. Immunoblots of TESK1, P-COF and total COF on days 3 and 6. (C and D) Knockdown of Slingshot 1 (SSH1) inhibits iPSC generation. (C) Western blot analysis shows hyper phosphorylation of COF when SSH1 is knock down using siRNAs while knocking down TESK1 with siRNA shows hypo-phosphorylation of COF in MEFs transduced with 4F. (D) Quantification of GFP+ iPSC colonies obtained from MEFs transduced with OSKM and transfected with control siRNA or siRNAs targeting SSH1 and/or TESK1. Results are the means ± SD of three independent experiments. *p <0.05. (E) Effect of TESK1 and COF knockdown on myosin-actin binding. Confocal images of MEF cells immunostained for Myo IIb (green) and F-actin using Rhodamine phalloidin (red) are shown. MEF cells were treated with indicated siRNAs to knock down *TESK1, COF*, or si*NT* and untreated MEF cells were used as controls. In control cells MyoIIb was found on the actin cables (see the cables marked by broken white line). Similarly in si*COF* treated cells, MyoIIb was found on the actin cables (cables marked by broken lines). In si*TESK1* treated cells, MyoIIb was found dispersed without forming obvious foci on the actin cables.

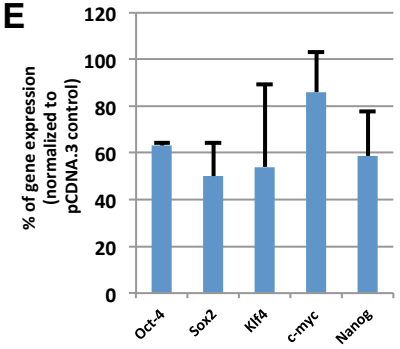
Fig S5


A
Extracellular space
Cytoplasm
Nucleus
Direct Interaction
Indirect Interaction
Bindings Only
Inhibits
Acts On
Inhibits AND Acts On
Leads To
Translocates To
Reaction
Enzyme Catalysis Reaction
Actin polymerization
Cytoskeletal reorganization
Cell Adhesion, cell motility & Organization
Regulation of junction assembly at desmosomes
Cell adhesion
Epithelial mesenchymal transition
Wound healing
Cell proliferation
Inflammation
Tissue invasion
Tumor angiogenesis
Bone formation


B
MEF w/o 4F
MEF +4F
Gelatin
Fibronectin
Fibronectin analog


C
Fold induction of mRNA (normalized to MEF only)
Intalpha6
Int beta1
Int beta3
ILK
Rac
LIMK2
Beta-actin
Fold induction of mRNA (normalized to MEFonly)
Tesk
LIMk1
MEF
D3
D6
D9
D12
D15
CCE


D
Fold induction of TESK1 mRNA (normalized to MEF only)
MEF O S K M OS OK OM SK SM KM OSK OSM OKM SKM 4F CCE


E
% of gene expression (normalized to pCDNA.3 control)
Oct-4
Sox2
Klf4
c-myc
Nanog

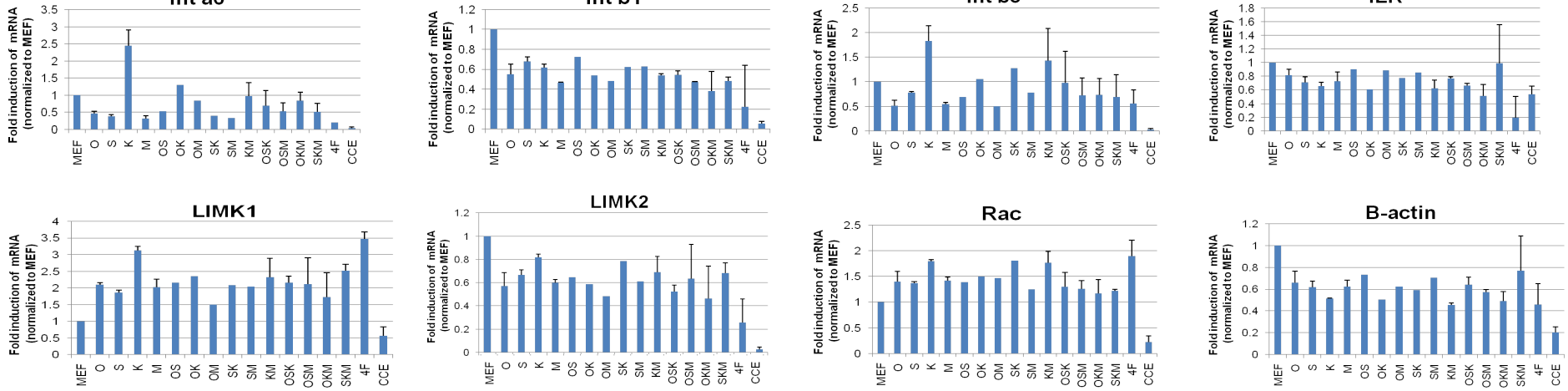

F
Int a6
Int b1
Int b3
ILK
LIMK1
LIMK2
Rac
B-actin
Fold induction of mRNA (normalized to MEF)
MEF O S K M OS OK OM SK SM KM OSK OSM OKM SKM 4F CCE

Figure S5, related to Figure 5. Reprogramming factors differentially regulate ILK pathway genes during iPSC generation

(A) Cross-talk between ILK and TGF-b signaling pathways in generation of iPSCs. Several identified kinases are involved in ILK signaling network. Kinases including TESK1, LIMK2, PLK2, BMPR2, and MAPK1 are highlighted in red. Cofilin is labeled in blue. The figure is generated by using IPA (Ingenuity Systems) and the functional interaction key is shown on the lower right corner. (B) Activation of the ILK signaling pathway results in actin remodeling. MEFs were grown on plates coated with fibronectin (Fbn) or a fibronectin analog (Fbn-Anlg) to induce the ILK signaling pathway. MEFs seeded on gelatin-coated plates served as controls. Actin filaments were visualized by rhodamine-labeled phalloidin staining of cells. (C) The expression pattern of various ILK pathway genes on different days of reprogramming was monitored by RT-qPCR analysis and compared with expression in untreated MEFs and CCE mESCs. (D) Different combinations of factors were tested for their role in regulating expression of TESK1 mRNA on 8 dpi. (E) Overexpression of HA-TESK1 in mES cells reduces the levels of pluripoency marker genes. Quantitative analysis of pluripotent marker genes upon over expression of HA-tagged TESK1. (F) Different combinations of factors were tested for their role in regulating expression of ILK pathway genes on 8 dpi.

Fig S6

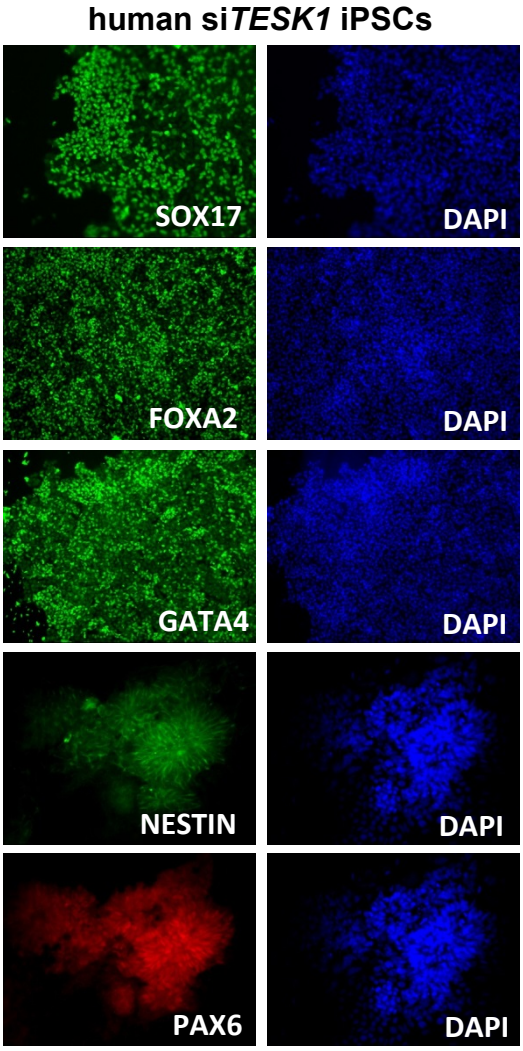

human si*TESK1* iPSCs
SOX17
DAPI
FOXA2
DAPI
GATA4
DAPI
NESTIN
DAPI
PAX6
DAPI

Figure S6, related to Figure 6. Characterization of kinase-knockdown human iPSCs

Human siTESK1-iPSCs differentiate into germ layers *in vitro*. EBs were formed using the hanging-drop method and allowed to undergo spontaneous differentiation. On day 14, differentiated cells were fixed with 4% PFA and immunostained for SOX17, FOXA2, GATA4, NESTIN, and PAX6.

SUPPLEMENTARY TABLES

Tables S1, related to Figures 1 and S1

Tables S2, related to Figures 1 and S1

Tables S3, related to Figures 1 and S1

Table S4, related to Figures 2 and S2

Table S5, related to Figure 7

Table S1, related to Figures 1 and S1. Kinases identified as primary hits

GeneSymbol	AccessionList	ClusterList
MAP3K14	NM_016896	Mm.158981
BUB1	NM_009772	Mm.2185
BUB1B	NM_009773	Mm.29133
TGFBR1	NM_009370	Mm.197552
ACVRL1	NM_009612	Mm.279542
ACVR1	NM_007394	Mm.689
RIPK3	NM_019955	Mm.46612
IRAK3	NM_028679	Mm.146194
RIPK1	NM_009068	Mm.374799
TESK2	NM_146151	Mm.425201
RIPK2	NM_138952	Mm.112765
BMPR2	NM_007561	Mm.7106
IRAK2	NM_172161	Mm.152142
IRAK4	NM_029926	Mm.422858
LIMK2	NM_010718	Mm.124176
B230120H23RIK	NM_023057	Mm.314618
BC021891	NM_145608	Mm.216458
BMPR1A	NM_009758	Mm.237825
TESK1	NM_011571	Mm.10154
MAP3K12	NM_009582	Mm.172897
MAP3K9	NM_177395	Mm.436861
PRKCA	NM_011101	Mm.222178
SGK2	NM_013731	Mm.26462
CDKL3	NM_153785	Mm.280557
ANKK1	NM_172922	Mm.119994
CDK3	NM_027165	Mm.33677
MAPK1	NM_011949	Mm.196581
CLK1	NM_009905	Mm.1761
SRPK1	NM_016795	Mm.15252
RAGE	NM_011973	Mm.140948
AATK	NM_007377	Mm.6826
EPHA5	NM_007937	Mm.137991
CDC2L6	NM_198164	Mm.200924
ITK	NM_010583	Mm.339927
LTK	NM_206941	Mm.1740
DDR1	NM_007584	Mm.5021
JAK1	NM_146145	Mm.289657
EPHB2	NM_010142	Mm.250981

GeneSymbol	AccessionList	ClusterList
NTRK1	XM_283871	Mm.80682
FRK	NM_010237	Mm.332432
FGFR1	NM_010206	Mm.265716
ABL1	NM_009594	Mm.1318
BLK	NM_007549	Mm.3962
MET	NM_008591	Mm.86844
EGFR	NM_007912	Mm.8534
ROS1	NM_011282	Mm.236163
BMX	NM_009759	Mm.504
EPHB1	NM_173447	Mm.22897
EPHA1	NM_023580	Mm.133330
ZAP70	NM_009539	Mm.8038
EPHA8	NM_007939	Mm.1390
ROR2	NM_013846	Mm.342774
EPHA10	NM_177671	Mm.171490
NTRK3	NM_008746	Mm.33496
PTK2	NM_007982	Mm.254494
MATK	NM_010768	Mm.2918
FGFR2	NM_010207	Mm.16340
CSNK1G3	NM_152809	Mm.368668
FLT4	NM_008029	Mm.3291
TTBK2	NM_080788	Mm.275698
RYK	NM_013649	Mm.335391
CSNK1D	NM_027874	Mm.216227
ADCK4	NM_133770	Mm.124728
TSSK2	NM_009436	Mm.310201
STK40	NM_028800	Mm.440269
STK33	XM_358897	Mm.389950
SNF1LK	NM_010831	Mm.290941
PRKCM	NM_008858	Mm.133719
PIM3	NM_145478	Mm.400129
PIM2	NM_138606	Mm.347478
PDK4	NM_013743	Mm.235547
PASK	NM_080850	Mm.379454
MYLK	NM_139300	Mm.33360
MKNK2	NM_021462	Mm.42126
MELK	NM_010790	Mm.268668
MARK3	NM_022801	Mm.425769

GeneSymbol	AccessionList	ClusterList
FRAP1	NM_020009	Mm.21158
DCLK1	NM_019978	Mm.458341
DAPK2	NM_010019	Mm.335252
CAMK1D	NM_177343	Mm.191949
BRD2	NM_010238	Mm.3444
HUNK	NM_015755	Mm.125874
PHKG1	NM_011079	Mm.3159
STK25	NM_021537	Mm.28761
MAP3K1	NM_011945	Mm.15918
MAP4K4	NM_008696	Mm.19073
MAP4K5	NM_024275	Mm.291936
MAP2K1	NM_008927	Mm.248907
PAK7	NM_172858	Mm.131572
STK24	NM_145465	Mm.390756
MAP3K6	NM_016693	Mm.36640
CSNK2B	NM_009975	Mm.378901
PRPF4B	NM_013830	Mm.10027
KHK	NM_008439	Mm.22451
4930444A02RIK	NM_029037	Mm.17631
6330514A18RIK	NM_183152	Mm.17613
NPR2	NM_173788	Mm.103477
TTK	NM_009445	Mm.1904
BMP2K	NM_080708	Mm.281490
CAMKK1	NM_018883	Mm.9998
EIF2AK2	NM_011163	Mm.378990
GSG2	NM_010353	Mm.42045
MOS	NM_020021	Mm.459300
NEK2	NM_010892	Mm.33773
NEK6	NM_021606	Mm.143818
NEK8	NM_080849	Mm.23788
NEK9	NM_145138	Mm.29071
PDIK1L	NM_146156	Mm.22778
PLK1	NM_011121	Mm.16525
PLK2	NM_152804	Mm.380
PLK3	NM_013807	Mm.259022
RNASEL	NM_011882	Mm.259254
SCYL1	NM_023912	Mm.276063
TBK1	NM_019786	Mm.34580

GeneSymbol	AccessionList	ClusterList
TLK2	NM_011903	Mm.126976
TRP53RK	NM_023815	Mm.330796
UHMK1	NM_010633	Mm.389214
WNK1	NM_198703	Mm.333349
ROCK2	NM_009072	Mm.276024
RPS6KB1	NM_028259	Mm.374825
STK38L	NM_172734	Mm.322121
CDC2L1	NM_007661	Mm.267410
SPHK2	NM_020011	Mm.24222
AK3	NM_021299	Mm.196067
DGKE	NM_019505	Mm.153695
ADK	NM_134079	Mm.188734
PIK3C2G	NM_011084	Mm.391538
GALK2	NM_175154	Mm.20216
NME1	NM_008704	Mm.439702
TK1	NM_009387	Mm.2661
PCK1	NM_011044	Mm.266867
STK10	NM_009288	Mm.8235
CDKN1A	NM_007669	Mm.195663
CCND3	NM_007632	Mm.246520
HUS1	NM_008316	Mm.42201
BRSK2	NM_029426	Mm.274868
CDKN2D	NM_009878	Mm.29020
DBF4	NM_013726	Mm.292470
GTF2F1	NM_133801	Mm.24632
CCNB1	NM_172301	Mm.260114
AKAP3	NM_009650	Mm.87748
AKAP10	NM_019921	Mm.274404
PRKCDBP	NM_028444	Mm.3124
PKIG	NM_011106	Mm.10091
PIK3R5	NM_177320	Mm.244960
GIT2	NM_019834	Mm.195632
PICK1	NM_008837	Mm.259464
PIK3AP1	NM_031376	Mm.222266
CNKSR3	NM_172546	Mm.37984
AKAP8L	NM_017476	Mm.281005
PER2	NM_011066	Mm.218141
FASTKD5	NM_198176	Mm.27090

Table S2, related to Figures 1 and S1. Kinases identified as confirmed hits

GeneSymbol	AccessionList	ClusterList
BUB1B	NM_009773	Mm.29133
IRAK3	NM_028679	Mm.146194
BMPR2	NM_007561	Mm.7106
IRAK2	NM_172161	Mm.152142
LIMK2	NM_010718	Mm.124176
BMPR1A	NM_009758	Mm.237825
TESK1	NM_011571	Mm.10154
PRKCA	NM_011101	Mm.222178
MAPK1	NM_011949	Mm.196581
SRPK1	NM_016795	Mm.15252
RAGE	NM_011973	Mm.140948
AATK	NM_007377	Mm.6826
EPHA5	NM_007937	Mm.137991
CDC2L6	NM_198164	Mm.200924
DDR1	NM_007584	Mm.5021
JAK1	NM_146145	Mm.289657
EPHA1	NM_023580	Mm.133330
SNF1LK	NM_010831	Mm.290941
PIM2	NM_138606	Mm.347478
FRAP1	NM_020009	Mm.21158
DAPK2	NM_010019	Mm.335252
TRIB3	NM_175093	Mm.276018
DAPK3	NM_007828	Mm.10294
CAMKV	NM_145621	Mm.274540
STK25	NM_021537	Mm.28761
MAP2K1	NM_008927	Mm.248907
PAK7	NM_172858	Mm.131572
STK24	NM_145465	Mm.390756
CSNK2B	NM_009975	Mm.378901
KHK	NM_008439	Mm.22451

GeneSymbol	AccessionList	ClusterList
6330514A18RIK	NM_183152	Mm.17613
NPR2	NM_173788	Mm.103477
BMP2K	NM_080708	Mm.281490
EIF2AK2	NM_011163	Mm.378990
MOS	NM_020021	Mm.459300
NEK2	NM_010892	Mm.33773
NEK6	NM_021606	Mm.143818
PLK1	NM_011121	Mm.16525
PLK2	NM_152804	Mm.380
RNASEL	NM_011882	Mm.259254
SCYL1	NM_023912	Mm.276063
TBK1	NM_019786	Mm.34580
TLK2	NM_011903	Mm.126976
UHMK1	NM_010633	Mm.389214
RPS6KB1	NM_028259	Mm.374825
AK3	NM_021299	Mm.196067
DGKE	NM_019505	Mm.153695
PIK3C2G	NM_011084	Mm.391538
GALK2	NM_175154	Mm.20216
NME1	NM_008704	Mm.439702
GTF2F1	NM_133801	Mm.24632
PKIG	NM_011106	Mm.10091
PIK3R5	NM_177320	Mm.244960
GIT2	NM_019834	Mm.195632
PIK3AP1	NM_031376	Mm.222266
CNKSR3	NM_172546	Mm.37984
PKIB	NM_008863	Mm.262135
PER2	NM_011066	Mm.218141
FASTKD5	NM_198176	Mm.27090

Table S3, related to Figures 1 and S1. Kinases involved in cell cycle or cytoskeleton formation

Gene name	Functions	Reference
Bub1B	Cell cycle: spindle checkpoint function, localized to the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation.	(Davenport et al., 1999; Matsuura et al., 2006)
Srpk1	Plays a central role in the regulatory network for splicing, controlling the intranuclear distribution of splicing factors in interphase cells and the reorganization of nuclear speckles during mitosis.	(Gui et al., 1994)
FRAP1/mTOR	This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex.	(Shah et al., 2001)
Nek2	Protein kinase that is involved in mitotic regulation. Integral component of the mitotic spindle-assembly checkpoint which is necessary for proper chromosome segregation during metaphase-anaphase transition.	(Chen et al., 2002; Fletcher et al., 2004; Wu et al., 2007)
PLK1	performs several important functions throughout M phase of the cell cycle, including the regulation of centrosome maturation and spindle assembly, the removal of cohesins from chromosome arms, the inactivation of APC/C inhibitors, and the regulation of mitotic exit and cytokinesis.	(Chan et al., 2008; Macurek et al., 2008; Qi et al., 2006)
TLK2	Rapidly and transiently inhibited by phosphorylation following the generation of DNA double-stranded breaks during S-phase. This is cell cycle checkpoint and ATM-pathway dependent and appears to regulate processes involved in chromatin assembly	(Sillje et al., 1999)
Uhmk1	Upon serum stimulation, phosphorylates CDKN1B/p27Kip1, thus controlling CDKN1B subcellular location and cell cycle progression in G1 phase. May be involved in trafficking and/or processing of RNA	(Crook et al., 2008)
Nek6	Activated during M phase. Required for chromosome segregation at metaphase-anaphase transition and therefore for mitotic progression. Inhibition of activity results in apoptosis.	(O'Regan and Fry, 2009)
Pim2	protein functions to prevent apoptosis and to promote cell survival	(Gong et al., 2009; Ren et al., 2010)
PAK7/PAK5	The protein encoded by this gene is a member of the PAK family of Ser/Thr protein kinases. This kinase contains a CDC42/Rac1 interactive binding (CRIB) motif, and has been shown to bind CDC42 in the presence of GTP. This kinase is associated with microtubule networks and induces microtubule stabilization. The subcellular localization of this kinase is tightly regulated during cell cycle progression.	(Cotteret et al., 2003; Matenia et al., 2005)
DAPK2	cell apoptosis, calcium calmodulin dependent protein kinase.	(Kawai et al., 1999)
DGKe	Phosphatidic acid which is produced from DAG by DGKE is important for actin cytoskeleton remodeling in plants	(Huang et al., 2006)
Mark3	A kinase of this, MARKK, is involved in microtubule and cytoskeleton remodeling and has a role to play in Tesk 1 function	(Johne et al., 2008)
Acvr1	activin receptor type 1, also known as Activin receptor-like kinase 2 or ALK2. Activin receptor-like kinase 5 or ALK 5 is a TGF-beta receptor, which is involved in Cofilin-P and eventually cytoskeleton remodeling.	(Vardouli et al., 2005)

Table S3: References

Chan, E.H., Santamaria, A., Sillje, H.H., and Nigg, E.A. (2008). Plk1 regulates mitotic Aurora A function through betaTrCP-dependent degradation of hBora. Chromosoma *117*, 457-469.

Chen, Y., Riley, D.J., Zheng, L., Chen, P.L., and Lee, W.H. (2002). Phosphorylation of the mitotic regulator protein Hec1 by Nek2 kinase is essential for faithful chromosome segregation. J Biol Chem *277*, 49408-49416.

Cotteret, S., Jaffer, Z.M., Beeser, A., and Chernoff, J. (2003). p21-Activated kinase 5 (Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. Mol Cell Biol *23*, 5526-5539.

Crook, M.F., Olive, M., Xue, H.H., Langenickel, T.H., Boehm, M., Leonard, W.J., and Nabel, E.G. (2008). GA-binding protein regulates KIS gene expression, cell migration, and cell cycle progression. FASEB J *22*, 225-235.

Davenport, J.W., Fernandes, E.R., Harris, L.D., Neale, G.A., and Goorha, R. (1999). The mouse mitotic checkpoint gene bub1b, a novel bub1 family member, is expressed in a cell cycle-dependent manner. Genomics *55*, 113-117.

Fletcher, L., Cerniglia, G.J., Nigg, E.A., Yend, T.J., and Muschel, R.J. (2004). Inhibition of centrosome separation after DNA damage: a role for Nek2. Radiat Res *162*, 128-135.

Gong, J., Wang, J., Ren, K., Liu, C., Li, B., and Shi, Y. (2009). Serine/threonine kinase Pim-2 promotes liver tumorigenesis induction through mediating survival and preventing apoptosis of liver cell. J Surg Res *153*, 17-22.

Gui, J.F., Lane, W.S., and Fu, X.D. (1994). A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature *369*, 678-682.

Huang, S., Gao, L., Blanchoin, L., and Staiger, C.J. (2006). Heterodimeric capping protein from Arabidopsis is regulated by phosphatidic acid. Mol Biol Cell *17*, 1946-1958.

Johne, C., Matenia, D., Li, X.Y., Timm, T., Balusamy, K., and Mandelkow, E.M. (2008). Spred1 and TESK1--two new interaction partners of the kinase MARKK/TAO1 that link the microtubule and actin cytoskeleton. Mol Biol Cell *19*, 1391-1403.

Kawai, T., Nomura, F., Hoshino, K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Akira, S. (1999). Death-associated protein kinase 2 is a new calcium/calmodulin-dependent protein kinase that signals apoptosis through its catalytic activity. Oncogene *18*, 3471-3480.

Macurek, L., Lindqvist, A., Lim, D., Lampson, M.A., Klompmaker, R., Freire, R., Clouin, C., Taylor, S.S., Yaffe, M.B., and Medema, R.H. (2008). Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. Nature *455*, 119-123.

Matenia, D., Griesshaber, B., Li, X.Y., Thiessen, A., Johne, C., Jiao, J., Mandelkow, E., and Mandelkow, E.M. (2005). PAK5 kinase is an inhibitor of MARK/Par-1, which leads to stable microtubules and dynamic actin. Mol Biol Cell *16*, 4410-4422.

Matsuura, S., Matsumoto, Y., Morishima, K., Izumi, H., Matsumoto, H., Ito, E., Tsutsui, K., Kobayashi, J., Tauchi, H., Kajiwara, Y.*, et al.* (2006). Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. Am J Med Genet A *140*, 358-367.

O'Regan, L., and Fry, A.M. (2009). The Nek6 and Nek7 protein kinases are required for robust mitotic spindle formation and cytokinesis. Mol Cell Biol *29*, 3975-3990.

Qi, W., Tang, Z., and Yu, H. (2006). Phosphorylation- and polo-box-dependent binding of Plk1 to Bub1 is required for the kinetochore localization of Plk1. Mol Biol Cell *17*, 3705-3716.

Ren, K., Zhang, W., Shi, Y., and Gong, J. (2010). Pim-2 activates API-5 to inhibit the apoptosis of hepatocellular carcinoma cells through NF-kappaB pathway. Pathol Oncol Res *16*, 229-237.

Shah, S.A., Potter, M.W., Ricciardi, R., Perugini, R.A., and Callery, M.P. (2001). FRAP-p70s6K signaling is required for pancreatic cancer cell proliferation. J Surg Res *97*, 123-130.

Sillje, H.H., Takahashi, K., Tanaka, K., Van Houwe, G., and Nigg, E.A. (1999). Mammalian homologues of the plant Tousled gene code for cell-cycle-regulated kinases with maximal activities linked to ongoing DNA replication. EMBO J *18*, 5691-5702.
Vardouli, L., Moustakas, A., and Stournaras, C. (2005). LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor-beta. J Biol Chem *280*, 11448-11457.
Wu, W., Baxter, J.E., Wattam, S.L., Hayward, D.G., Fardilha, M., Knebel, A., Ford, E.M., da Cruz e Silva, E.F., and Fry, A.M. (2007). Alternative splicing controls nuclear translocation of the cell cycle-regulated Nek2 kinase. J Biol Chem *282*, 26431-26440.

Table S4, related to Figures 2 and S2. Functions of six barrier genes in iPSC generation

		molecular functions[1]	microRNAs[2]
DGKE	mol func	Diacylglycerol kinase activity; ATP binding; Nucleotide and protein binding; Transferase activity	--
	role in cell	Long-term potentiation; Activation of prtein kinase C activity by G-protein coupled receptor protein signaling pathway	
PLK2	mol func	protein serine/threonine kinase activity; signal transducer activity; ATP binding, polo kinase kinase activity; nucleotide and protein binding	miR10a, miR27b, miR30a, miRn339
	role in cell	apoptosis; degradation in; survival and growth; S phase; positive regulation of I-kappaB kinase/NF-kappaB cascade	
TESK1	mol func	Protein serine/threonine kinase activity; protein tyrosine kinase activity; ATP binding; transferase activity; metal ion binding; ILK Signaling	miR127, miR196a1, miR196a2 miR196b, miRn338
	role in cell	formation; cell spreading; its protein kinase domain is most closely related to those of the LIM motif-containing protein kinases (LIMKs), expressed in testicular germ cells; outgrowth.	
BMP2K	mol func	Protein serine/threonine kinase activity; ATP binding; transferase activity; phosphatase regulator activity	--
	role in cell	BMPs play a key role in skeletal development and patterning; expression is increased during BMP-2 induced differentiation and the gene product contains a nulcear localization signal.	
BMPR2	mol func	receptor activity; transforming growth factor bata receptor activity; Protein serine/threonine kinase activity; ATP binding; transferase activity	--
	role in cell	mesoderm formation; positive regulation of endothelial cell proliferation; transmembrane receptor protein serine/threonine kinase activity; anterior/posterior pattern formation; positive regulation of pathway-restrected SMAD protein phosphorylation; negative regulation of cell growth; positive regulation of BMP signaling	
MAPK1	mol func	phosphotyrosine binding; Protein serine/threonine kinase activity; ATP binding; transferase activity; DNA binding; MAP kinase activity; MAP kinase 2 activity	miR125a, miR145, miR149 miR202, miR320a
	role in cell	nuclear translocation of MAPK; induction of apoptosis; response to stress; response to DNA damage stimulus; cell cycle; signal transduction; positive regulation of cell migration; positive regulation of cell proliferation; negative regulation of cell differentiation	

[1]Molecular functions of six kinases were determined using GO annotations: Molecular functions (mol func) and biological process (role in cell). Six kinases listed are: DGKε (NM_019505), PLK2 (NM_152804), TESK1 (NM_011571), BMP2K (NM_080708), BMPR2 (NM_007561), MAPK1 (NM_011949).

[2]MicroRNAs that are predicted to target these genes are also listed.

Table S5, related to Figure 7. Bridge proteins between barrier kinases and 4 transcription factors

Mutual Interactor	TF interacting with	Kinase interacting with	Canonical Pathways (Signal Transduction)	Canonical Pathways (Cell Communication)
Abl1	Myc	Epha1,Epha5,Mtor	ErbB	
Akt1	Myc,Sox2	Mtor,Pik3r5,Rps6kb1,Trib3	ErbB,MAPK,VEGF	Focal Adhesion,Tight Junction
Atf4	Pou5f1,Sox2	Dapk3,Trib3	MAPK	
Birc5	Myc	Bub1b,Plk1		
Bmp4	Myc,Pou5f1,Sox2	Bmpr1a,Bmpr2	TGF-β	
Cdk1	Myc	Bub1b,Map2k1,Nek2,Plk1		Gap Junction
Clock	Pou5f1,Sox2	Per2,Prkca		
Csnk2a2	Myc	Csnk2b,Per2	Wnt	Adherens Junction,Tight Junction
Ctnnb1	Myc,Klf4,Pou5f1,Sox2	Bmpr1a,Csnk2b	Wnt	Focal Adhesion,Adherens Junction,Tight Junction
Egf	Myc	Map2k1,Mapk1	ErbB,MAPK	Focal Adhesion,Gap Junction
Egfr	Myc	Jak1,Mapk1,Pik3r5	ErbB,MAPK	Focal Adhesion,Adherens Junction,Gap Junction
Erbb2	Myc	Jak1,Mapk1,Pik3r5	ErbB	Focal Adhesion,Adherens Junction
Fos	Myc,Pou5f1,Sox2	Jak1,Mapk1	MAPK	
Hras1	Myc	Map2k1,Mapk1,Mos,Mtor,Pik3r5,Prkca		
Mapk3	Myc	Dapk2,Dapk3,Map2k1,Mapk1	ErbB,MAPK,TGF-β,VEGF	Focal Adhesion,Adherens Junction,Gap Junction
Pkm2	Pou5f1	Nme1,Npr2		
Pten	Myc	Bmpr1a,Mtor,Pik3c2g,Pik3r5		Focal Adhesion,Tight Junction
Raf1	Myc	Map2k1,Mapk1,Pak7,Prkca	ErbB,MAPK,VEGF	Focal Adhesion,Gap Junction
Smad1	Pou5f1,Sox2	Bmpr1a,Bmpr2,Mapk1	TGF-β	
Smad2	Pou5f1,Sox2	Bmpr2,Mapk1	TGF-β,Wnt	Adherens Junction
Smad4	Myc	Bmpr1a,Bmpr2,Mapk1	TGF-β,Wnt	Adherens Junction
Src	Myc	Mapk1,Pik3r5,Prkca	ErbB,VEGF	Focal Adhesion,Adherens Junction,Gap Junction,Tight Junction
Stat3	Myc,Klf4,Pou5f1,Sox2	Eif2ak2,Jak1,Mapk1,Nek6		
Tnf	Myc	Irak3,Mapk1,Mtor,Pik3r5	MAPK,TGF-β	