Supporting Information

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Materials and Methods

Inhibitors and Antibodies. Rapamycin, roscovitine, and other inhibitors used for the screening experiments were purchased from EMD Bioscience. All antibodies except Cyclin G2 antibodies (1) were from commercial sources. Their sources and dilutions for Western and/or immunofluorescence analyses were listed in supporting information (SI) Table S1.

Cultivation of mESCs. E14Tg2a.4 and W4/129S6 mESC lines were plated on 6-well plates coated with 0.1% gelatin and maintained in DMEM (high glucose, GIBCO) supplemented with 15% FBS, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 10^{-6} M 2-mercaptoethanol, and 1000 U/ml LIF ("ESGRO," Chemicon). Medium was changed every other day. For rapamycin treatment, cells were incubated with 0.25% trypsin-EDTA for 6 min at 37 °C, dissociated, and plated at 1×10^5 cells/well. Cells were lysed for western analysis 6 days after rapamycin treatment (100 nM).

Western Blotting. For OCT-4 and SOX2 detection, cells (5×10^6) were lysed directly with 200 μl laemmli sample buffer (BIO-RAD). For the analysis of other proteins, lysis buffer (0.3% Triton X-100, 20 mM Tris/HCl, pH 7.5, 0.1 mM Na_3VO_4, 25 mM NaF, 25 mM β-glycerophosphate, 2 mM EGTA, 2 mM EDTA, 1 mM DTT, 0.5 mM PMSF) was used. 25 μl of each sample were analyzed by Western blot. Dilutions for various antibodies were described in Table S1. The blots were developed using Super-Signal West Pico Chemiluminescent Substrate (Pierce).

DNA Constructs. For experiments involving shRNA-mediated knockdown, we used the pSicoRGFP lentiviral vector [Addgene (2)]. For gene overexpression, we used the 2K7/Neo lentiviral vector, kindly provided by Dr. David Suter (University of Geneva Medical School, Geneva, Switzerland) (3). For the Rheb overexpression experiment, the DNA fragment encoding the human GFP-Rheb fusion protein was amplified from pEGFPN1-Rheb with the forward primer GFP-Rheb-F and reverse primer GFP-Rheb-R and was subsequently inserted into the PCR8/GW/TOPO vector (Invitrogen). Meanwhile, the EF-1α promoter, which effectively drives gene expression in hESCs, was amplified with the forward primer EF-1aPF1 and the reverse primer EF-1aPR1 and was inserted into a pENTR TOPO vector (Invitrogen). The 2 resulting entry clones containing GFP-Rheb and EF-1α promoter, respectively, were recombinated with the 2K7/Neo vector. Positive clones containing the GFP-Rheb cassette driven by the EF-1α promoter was identified by sequencing analysis. Similarly, the DNA fragment encoding GFP-PDCD4 was amplified from pEGFPC1-PDCD4 (4) with the forward primer PDCD4-F and the reverse primer PDCD4-R. The DNA fragment was then cloned into the 2K7/Neo vector with the EF-1α promoter fragment. To generate vectors for EBF2, MIXL1, and Cyclin G2 overexpression, human EBF2, MIXL1 and Cyclin G2 full-length cDNA were isolated using RT-PCR with forward primers (EBF2F, MIXL1F, and CCNG2F; Table S2) and reverse primer (EBF2R, MIXL1R, and CCNG2R; Table S2) from hESC total RNA. The isolated DNA was subsequently cloned into the 2K7/Neo vector as described earlier.

Short Hairpin (sh)RNA Design and Cloning. shRNAs for depleting human mTOR were designed by the use of Oligomaker 1.5 software as reported earlier (2). Candidate sequences were listed in Table S2. All oligos were annealed and ligated to vector pSicoR-EF-1αGFP, which was modified from pSicoRGFP by replacing the CMV promoter with the EF-1α promoter. We and others (5) found that the EF-1α promoter in lentiviral vectors was more effective in driving GFP expression than the CMV promoter. The EF-1α promoter was amplified from the 2K7 vector with the forward primer EF-1aPF1 and the reverse primer EF-1aPR1. The EF-1α promoter was then inserted into pSicoRGFP backbone by AgeI/BamHI double digestion. The right insert was confirmed by sequencing analysis.

RNA Preparation and Real-Time PCR. For real-time RT-PCR and microarray experiments, total RNA were isolated with TRIzol reagent (Invitrogen) according to the manufacturer's instructions, treated with DNase I to eliminate DNA contamination, and purified with RNeasy mini kit (QIAGEN). cDNA was synthesized from the purified RNA using Reverse Transcription System (Promega) with random primers. Real-time PCR was performed by using QuantiTech SYBR Green PCR kit (QIAGEN). The reaction mixture included SYBR Green mix (2X, 12.5 μl), 10X primer assay (2.5 μl), cDNA (2 μl), and nuclease-free water with total volume of 25 μl. The following program suggested by QIAGEN was used: 95 °C for 15 min followed by 40 cycles of 94 °C for 15 sec, 56 °C for 30 sec, and 72 °C for 34 sec. All gene-specific primers were purchased from QIAGEN except those for human SOX2 (see Table S2). ACTB gene was used as internal control to equalize cDNA loading.

Lentivirus Production and hESC Infection. All lentivirus for mTOR knockdown or gene overexpression were packaged by the use of Viralpower Lentivirus Packaging System (Invitrogen) according to the manufacturer's instructions. Briefly, HEK293T cells were plated. After reaching 70–80% confluency, cells were transfected with target plasmids (pSicoGFP or 2K7/Neo) and supermix plasmids (Invitrogen) using the Fugene 6 reagent (Roche). After over-night incubation of cells, the medium was replaced with the virus packaging medium containing DMEM, 30% FBS, 4 mM glutamine, 1 mM sodium pyruvate. Supernatant containing the lentivirus was collected 48–72 h later and was concentrated approximately 100 times with ultracentrifuge (20,000 rpm, 1 h).

To infect hESCs, lentiviruses were mixed with MEF-CM, and the mixture was incubated with H9 cells (approximately 100,000) for 12 h. A multiplicity of infection (MOI) of 100 was used. To improve the efficiency of infection, Polybrene (Sigma) was added to the infection medium to 6 μg/ml. Virus-containing MEF-CM was replaced with complete feeder-free growth medium. In experiments involving shRNA-mediated knockdown, cells were continuously cultured for 5 days. GFP-positive cells were subsequently sorted with Cytomation Plus (Dako).

To establish hESCs overexpressing GFP, GFP-Rheb, GFP-PDCD4, EBF2, MIXL1, or Cyclin G2, H9 cells were cultured on Matrigel in the presence of mTeSR medium (StemCell Technologies) (6). G418 (400 μg/ml) was added 48 h after lentiviral infection. Cells were continuously cultured for 5–7 d in G418-containing mTeSR medium. This procedure allows us to generate H9 cells that nearly homogenously express GFP or GFP-tagged proteins.

Immunofluorescence. Cells cultured under feeder-free conditions were washed once with cold PBS (PBS), fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 in PBS, and blocked with PBS containing 0.5% BSA. Primary

antibody in various dilutions (Table S1) was incubated with samples for overnight at 4 °C followed by FITC (or Alexa)-conjugated secondary antibody. Nuclei were counterstained with DAPI (Sigma). The images were collected with Axiovert 200M microscope (Zeiss).

Apoptosis Assay. To determine whether rapamycin induces apoptosis of hESCs, H9 cells with or without rapamycin treatment for 2, 4, or 6 days were dissociated into single cells with 0.25% trysin-EDTA (Invitrogen) (37 °C, 10 min). Apoptosis was measured using the Annexin-V-FITC Apoptosis Detection Kit (BD Biosciences). Briefly, cells were washed with cold PBS and then resuspended in binding buffer. 5 μl of Annexin-V-FITC and propidium iodide (PI) were incubated with cells for 20 min at RT, which were subsequently analyzed using FACSDiva (BD Biosciences). Cells that are Annexin-V-positive and PI-negative were counted as apoptotic cells.

Embryoid Body (EB) Formation Assay. H9 cells (5×10^6) were incubated with Dispase (1 mg/ml, 37 °C, 5 min), dissociated as cluster, plated onto Ultra Low Attachment 6-well plate (Corning) and then incubated with EB medium (DMEM/F12, 20% FBS, 1 mM glutamine, 1 mM 1% non-essential amino acid, and 0.1 mM β-mercaptoethanol). Medium was changed every other day. EBs were collected after 2, 4, or 6 days using low-speed centrifugation (200 rpm, 5 min) and then used for further analysis.

Reporter Assay for Wnt/β-Catenin-Mediated Transcriptional Activation. The Top-flash reporter, originally described in (7), was kindly provided by Dr. Linzhao Cheng (The Johns Hopkins University, Baltimore, MD) (8). To test its regulation by rapamycin or by Wnt3a ligand, H9 cells were cultured in 24-well plates under feeder-free conditions; 48 h after plating, cells in each well were transfected with 0.95 μg of Topflash plasmid using Fugene 6 (Roche). 0.05 μg of *Rellina* plasmid (Promega) was co-transfected as an internal control. Rapamycin and various concentrations of Wnt3a ligand were added immediately after transfection. The luciferase activity was measured by using LUMIstar plate reader (BMG Labtech) 24 h after the transfection.

Microarray and Data Assay. RNA microarray analysis was performed by the W. M. Keck Center for Comparative and Functional Genomics in the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana–Champaign, IL. RNA was prepared and hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 Expression Arrays (Affymetrix) in the Functional Genomics unit with the GeneChip Expression 3′ Amplification One-Cycle Target Labeling and Control Reagents kit, according to the manufacturer's instructions. Briefly, 4 micrograms of total RNA was spiked with 4 prokaryotic polyadenylated sense RNA controls, primed with a T7-$(dT)_{24}$ oligonucleotide containing a 5′ T7 promoter sequence, and reverse transcribed to double-stranded cDNA. After purification cDNA was in vitro transcribed and amplified, generating pseudouridine-biotinylated cRNA. The cRNA was purified and fragmented. Hybridization cocktails were prepared, adding biotinylated, fragmented controls: 4 prokaryotic antisense cRNAs and one synthetic oligonucleotide. Following a 16-h hybridization the chip was washed, stained first with streptavidin-conjugated phycoerythrin dye (Invitrogen Corporation), and the stain enhanced with biotinylated goat anti-streptavidin antibody (Vector Laboratories), then counterstained with the conjugated phycoerythrin, using a GeneChip Fluidics Station 450 and the GeneChip Operating Software version 1.4. The chips were scanned with a GeneChip Scanner model 3000 7G Plus. Files of the fluorescence signals were generated, quality control values checked, and data files transferred to the bioinformatics unit for statistical analysis.

1. Arachchige Don AS, et al. (2006) Cyclin G2 is a centrosome-associated nucleocytoplasmic shuttling protein that influences microtubule stability and induces a p53-dependent cell cycle arrest. *Exp Cell Res* 312:4181–4204.
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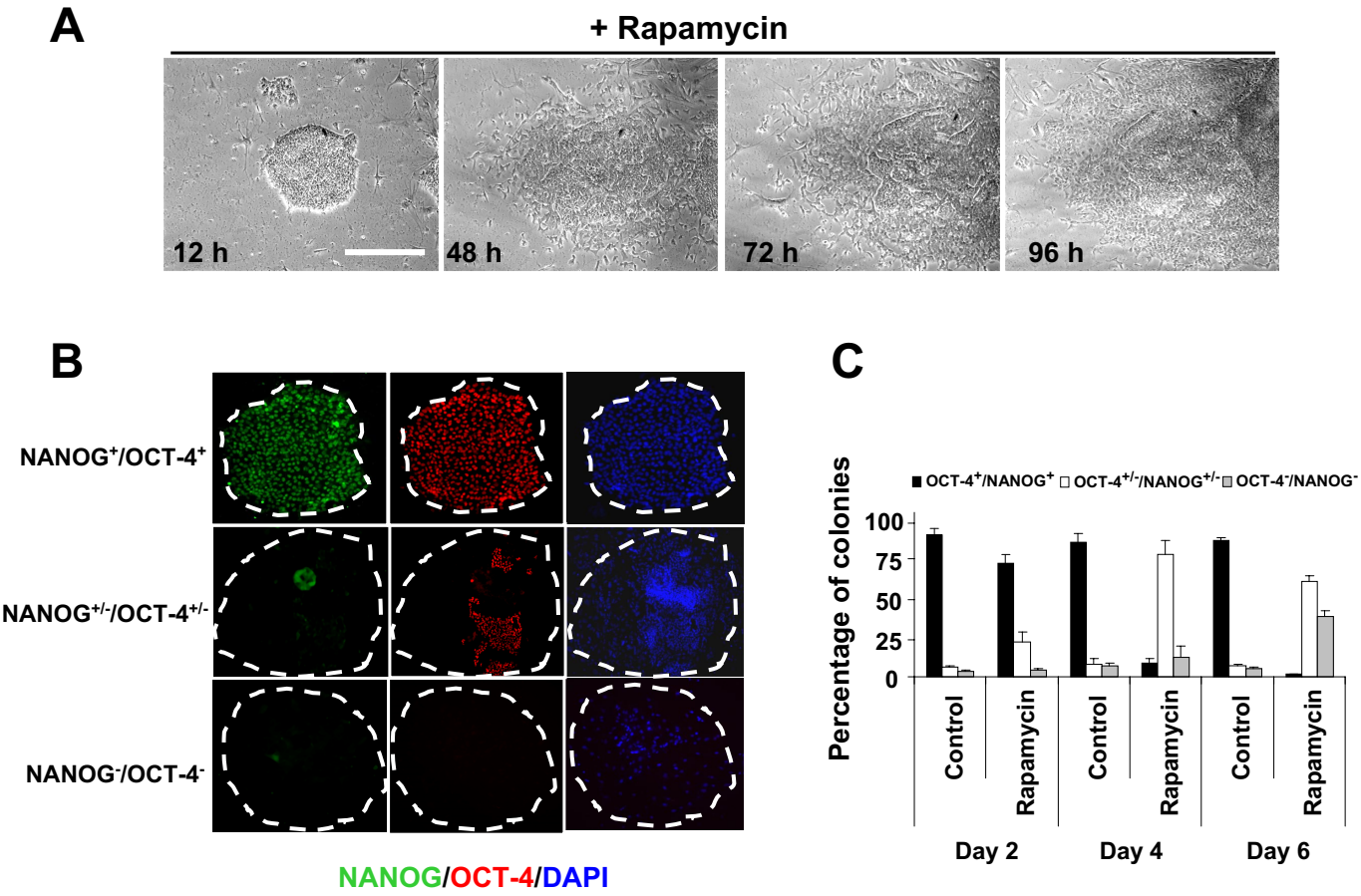
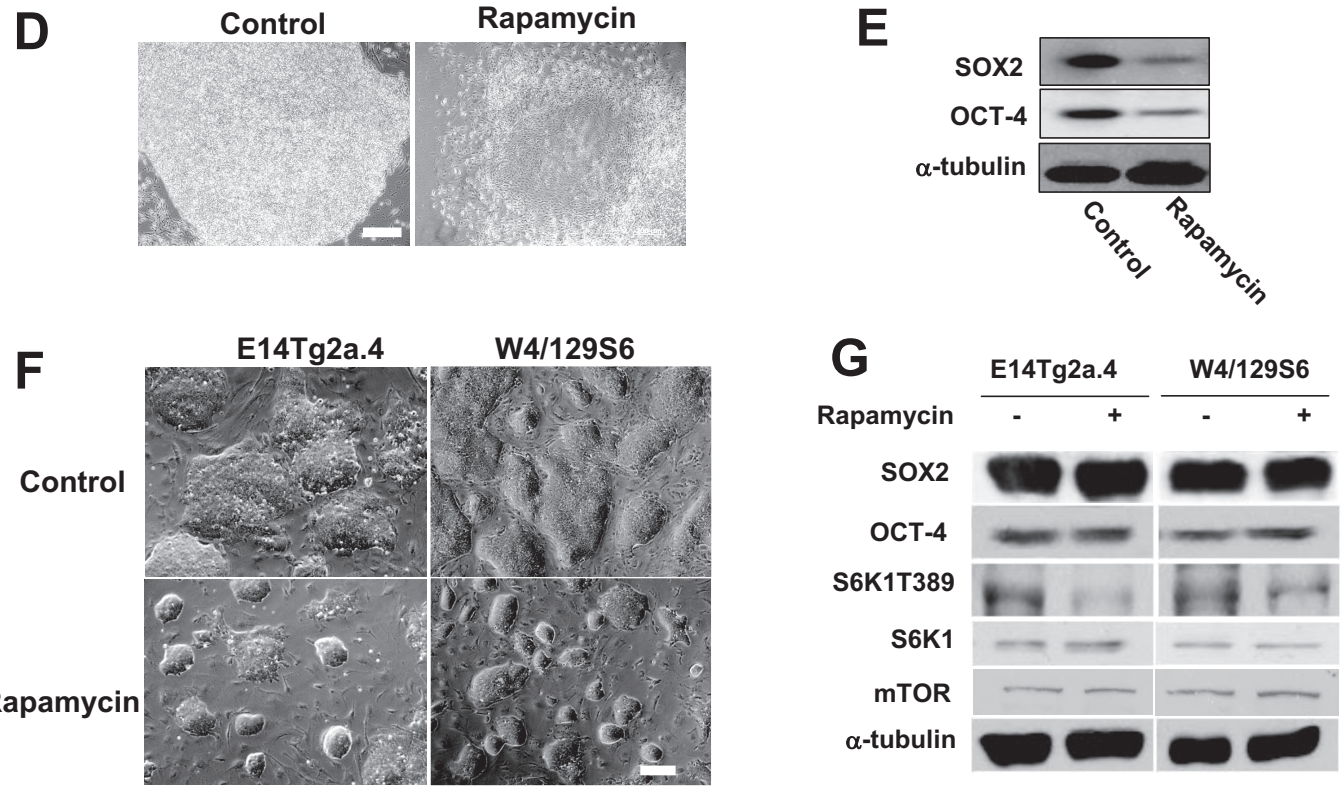


Fig. S1. (*A*) Phase-contrast images of an H9 cell colony cultured under feeder-free conditions and treated with rapamycin (100 nM) for 12, 48, 72 and 96 h; 48 h after treatment, cells began to flatten and spread out of the colonies, as indicated by the morphological changes. (Scale bar, 500 μm.) (*B*) Three types of hESC colonies common found in cultures: undifferentiated with homogenous expression of OCT-4 (green) and NANOG (red) (*Top*), partially differentiated with heterogeneous expression of OCT-4 and SOX2 (*Middle*), and fully differentiated without OCT-4 and SOX2 expression. Cell nuclei were stained with DAPI (blue). (*C*) Quantification of colonies formed by H9 cells with or without the treatment of rapamycin (100 nM) for 2, 4 and 6 days. Each bar represents the mean ± SEM (error bars) of different numbers of colonies that were analyzed in multiple experiments: 354 for control cells and 342 for rapamycin-treated cells. All values were normalized to the number (= 100%) of colonies formed by untreated control cells. (*D*) Phase-contrast images of H1 cells with or without rapamycin treatment (100 nM, 6 d). (Scale Bar, 100 μm.) (*E*) Western blot analysis of SOX2 and OCT-4 protein in H1 cells treated with or without rapamycin (100 nM, 6 days). The treatment led to 77 and 67% reductions of SOX2 and OCT-4 protein, respectively. α-tubulin was used as a loading control. A typical experiment from 5 separate experiments is shown. (*F*) Phase-contrast images of E14Tg2a.4 and W4/129S6 mESCs cultured with or without rapamycin (100 nM, 6 d). (Scale bar, 100 μm.) (*G*) Western blot of SOX2, OCT-4, S6K1T389, S6K1, and mTOR in E14Tg2a.4 and W4/129S6 cells with or without rapamycin (100 nM, 6 days). Rapamycin treatment decreased the level of S6K1T389 but had little effects on SOX2, OCT-4, S6K1, and mTOR expression. α-tubulin was a loading control. A typical experiment from 4 separate experiments is shown. (H) Phase-contrast images of NTera-2 cells cultured with or without rapamycin (100 nM) for 3 and 6 days. (I) Western blot analysis of SOX2 and OCT-4 protein in N-tera2 cells treated with or without rapamycin (100 nM, 6 days). The treatment led to 91 and 84% reductions of SOX2 and OCT-4 protein, respectively; α-tubulin was used as a loading control. A typical experiment from 4 separate experiments is shown. (*J*) Western blot of mTOR in HEK293 cells with or without shRNAs that target mTOR. Cells infected with lentivirus containing shRNA3 or shRNA4 effectively depleted mTOR; α-tubulin was a loading control. A typical experiment of 4 separate experiments is shown. (*K*) Phase contrast and fluorescent images of NTera-2 cells with or without mTOR depletion. Cells were infected with lentivirus containing shRNA3 (Fig. 1*D* in the main text) or shRNA4 (data not shown). Similar to rapamycin treatment, mTOR depletion induced differentiation morphology in NTera-2 cells. (Scale bar, 100 μm.) (*L*) Western blot analysis of mTOR, SOX2, and OCT-4 in NTera-2 cells with or without mTOR depletion. Infection of cells with shRNA3 led to 82%, 88%, and 65% reduction of mTOR, SOX2, and OCT-4 protein, respectively; α-tubulin was used as a loading control. A typical experiment from 3 separate experiments is shown.



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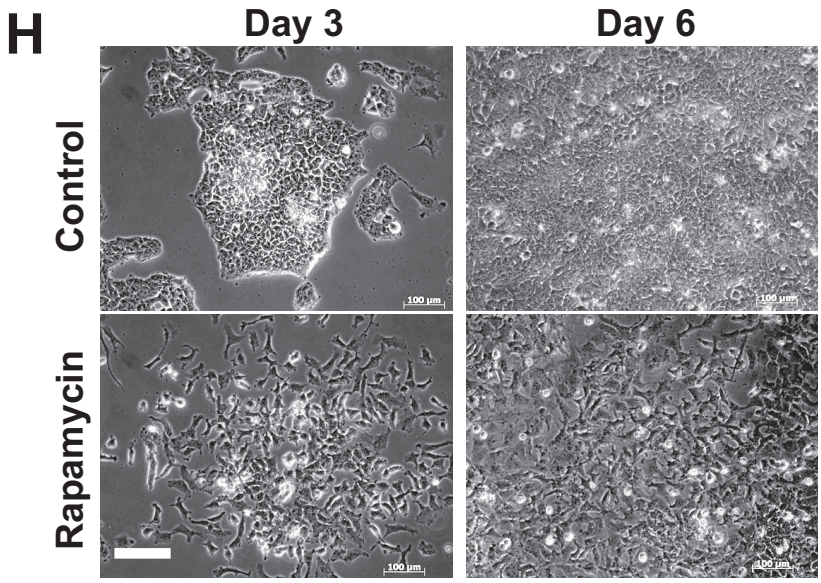

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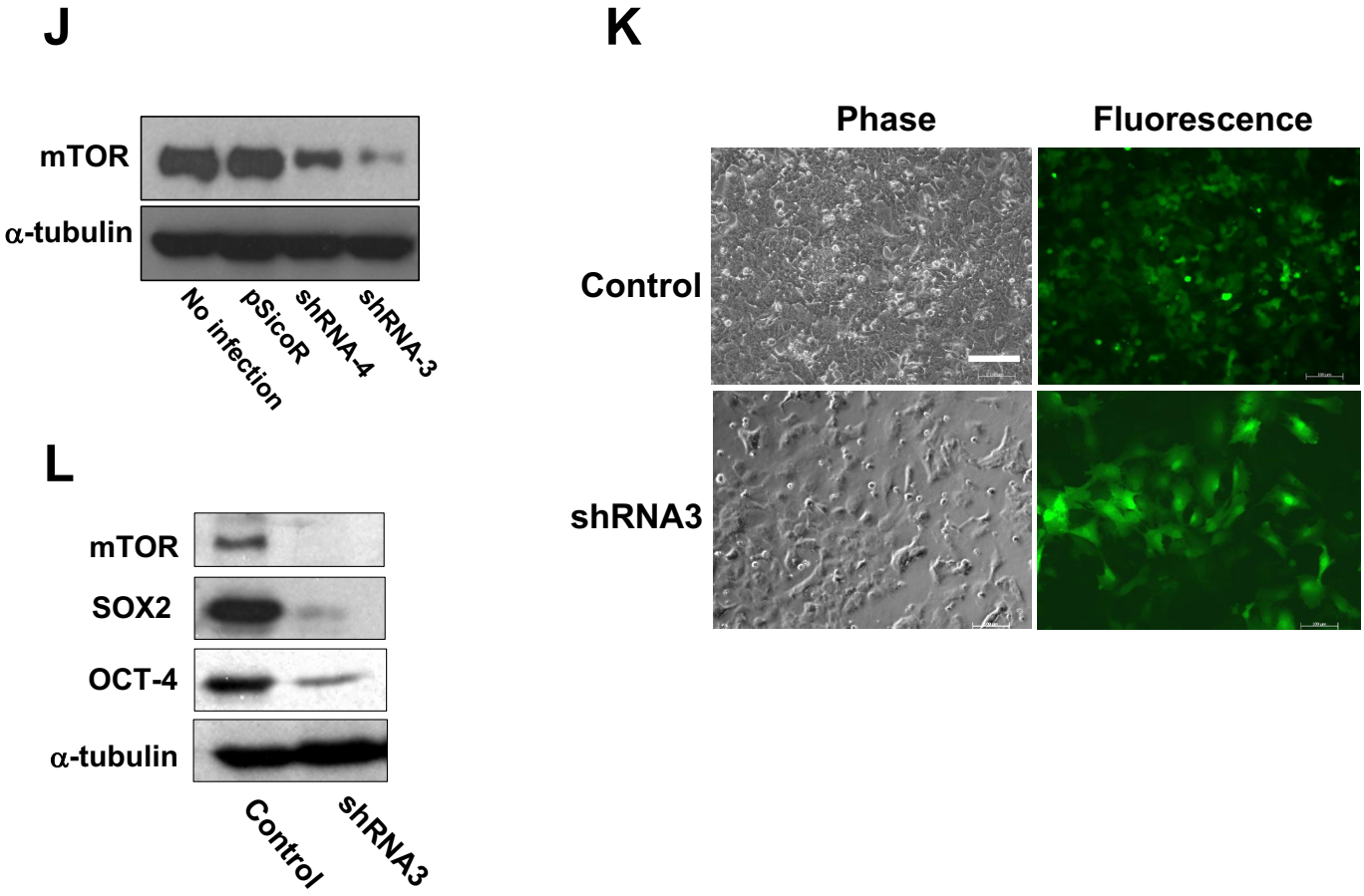

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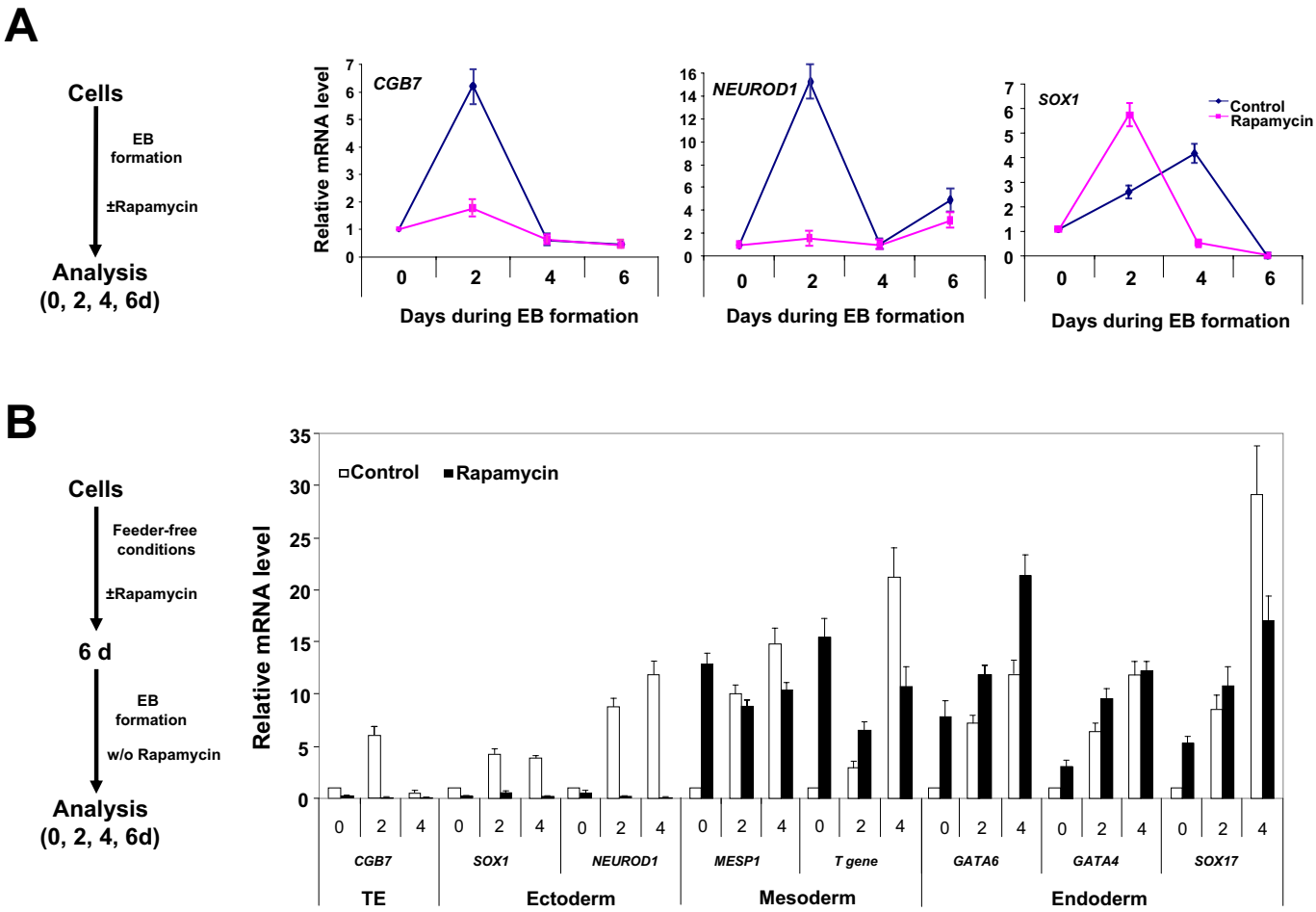
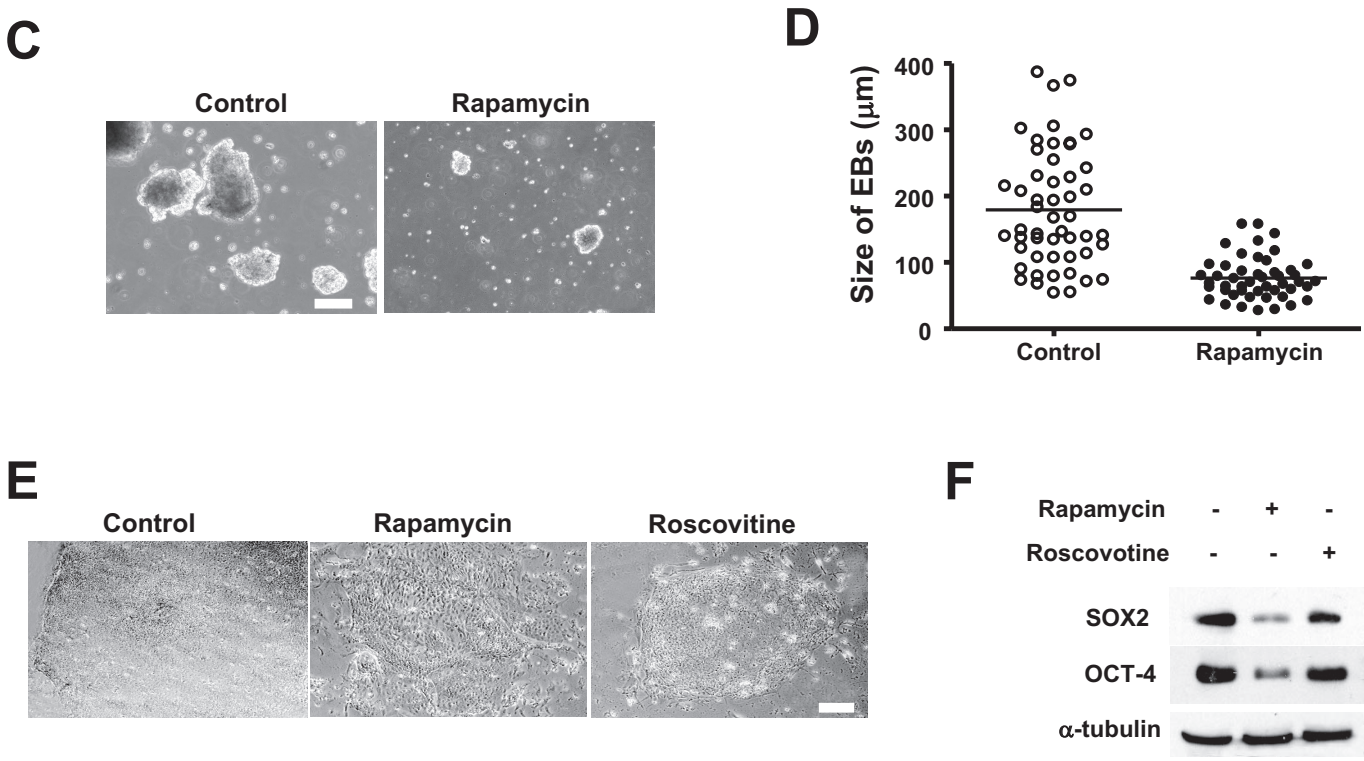


Fig. S2. (*A*) mRNA levels in control EBs or in rapamycin-treated EBs assessed by real-time PCR. Four separate experiments were conducted, and quantification of 4 replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). All values were normalized to the level (= 1) of mRNA in the control cells on day 0. *ACTB* (β-actin) was used as an internal control. Cells were induced to form EBs in the presence or absence of rapamycin (100 nM) (see diagram, *Left*). (*B*) mRNA levels in H9 cells from control EBs or from those with rapamycin pretreatment assessed by real-time PCR. Five separate experiments were conducted, and quantification of 4 replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). All values were normalized to the level (= 1) of mRNA in the control cells on day 0. *ACTB* (β-actin) was used as an internal control. H9 cells were treated with rapamycin for 6 d and then were induced to form EBs in the absence of rapamycin (see diagram, *Left*). (*C*) Phase-contrast images of EBs and (*D*) distribution of their diameters. H9 cells with or without rapamycin pretreatment (100 nM, 6 days) were induced to form EBs for 5 days, in the absence of rapamycin. In 3 separate experiments, 52 control EBs and 50 EBs from rapamycin-pretreated cells were analyzed. The sizes of EBs are 76 ± 26 μ for rapamycin pretreatment and 179 ± 58 μ for controls, respectively (means± SEM; $P < 0.001$). (Scale bar, 100 μm.) (*E*) Phase contrast images of H9 cells treated or not treated with rapamycin or roscovotine for 6 days. Roscovotine prevented hESC proliferation but failed to induce cell differentiation: the roscovotine-treated cells formed smaller but compact colonies, whereas rapamycin treatment induced cells to spread out. Note that only a portion of the control colony is shown. (Scale bar, 100 μm.) (*F*) Western blot analysis of SOX2 and OCT-4 in cells treated or not treated with rapamycin or roscovotine (6 days). Results are representative of 3 separate experiments.



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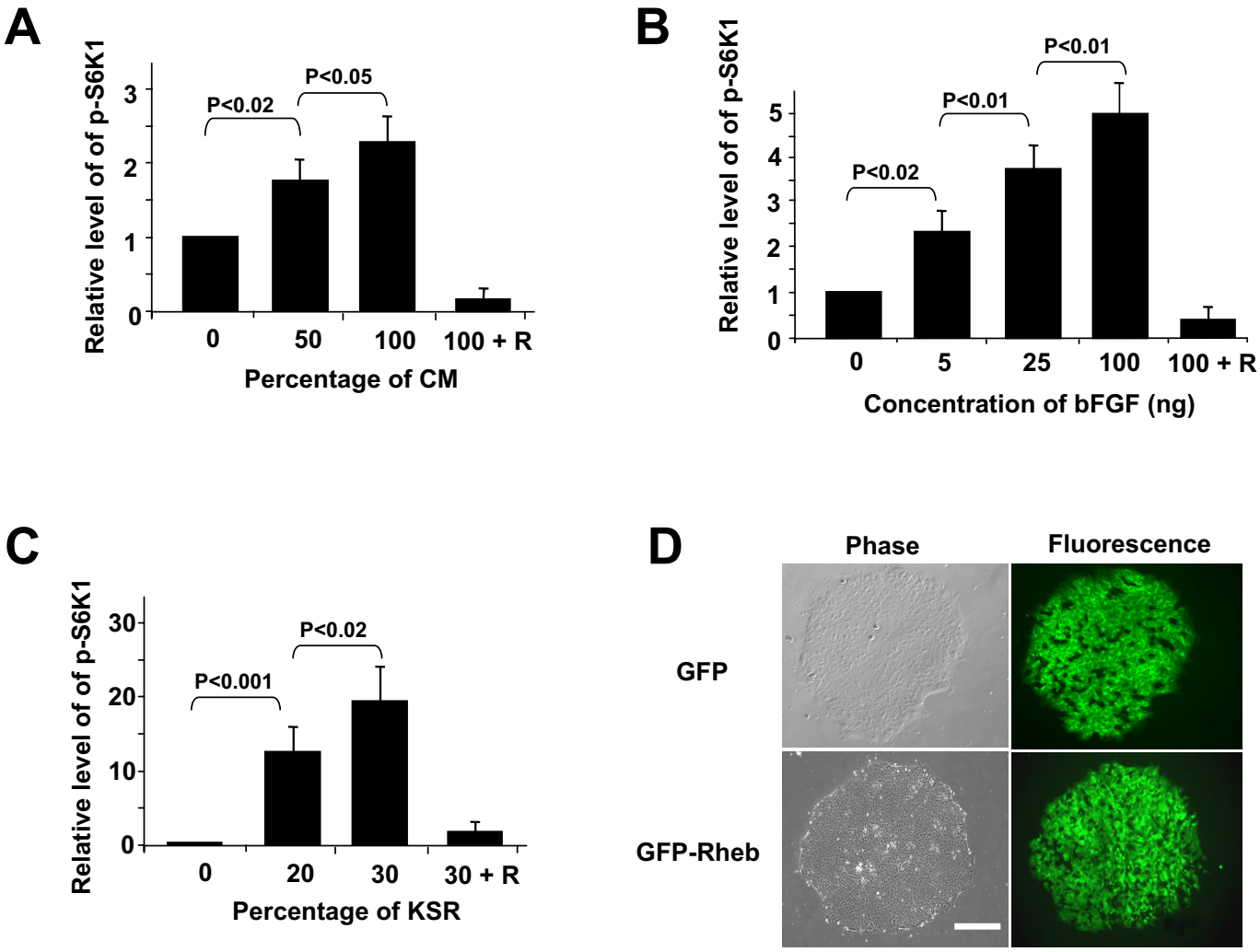

Fig. S3. (A–C) Quantification of Western blots from Fig. 3*A*. Blots from 4 separate experiments were qualified, and each bar represents the mean ± SEM (error bars). All values were normalized to the signal (= 1) detected without rapamycin. Student *t* tests compared data between experimental groups. Pairs of treatments showing a significant difference are marked. (*D*) Phase contrast and fluorescent images of H9 cells expressing GFP or GFP-Rheb. Cells were infected with lentivirus (generated from 2K7/Neo vector) containing GFP or GFP-Rheb, followed by G418 selection. This procedure leads to cells that nearly homogenously express GFP or GFP-tagged proteins. (Scale bar, 100 μm.)

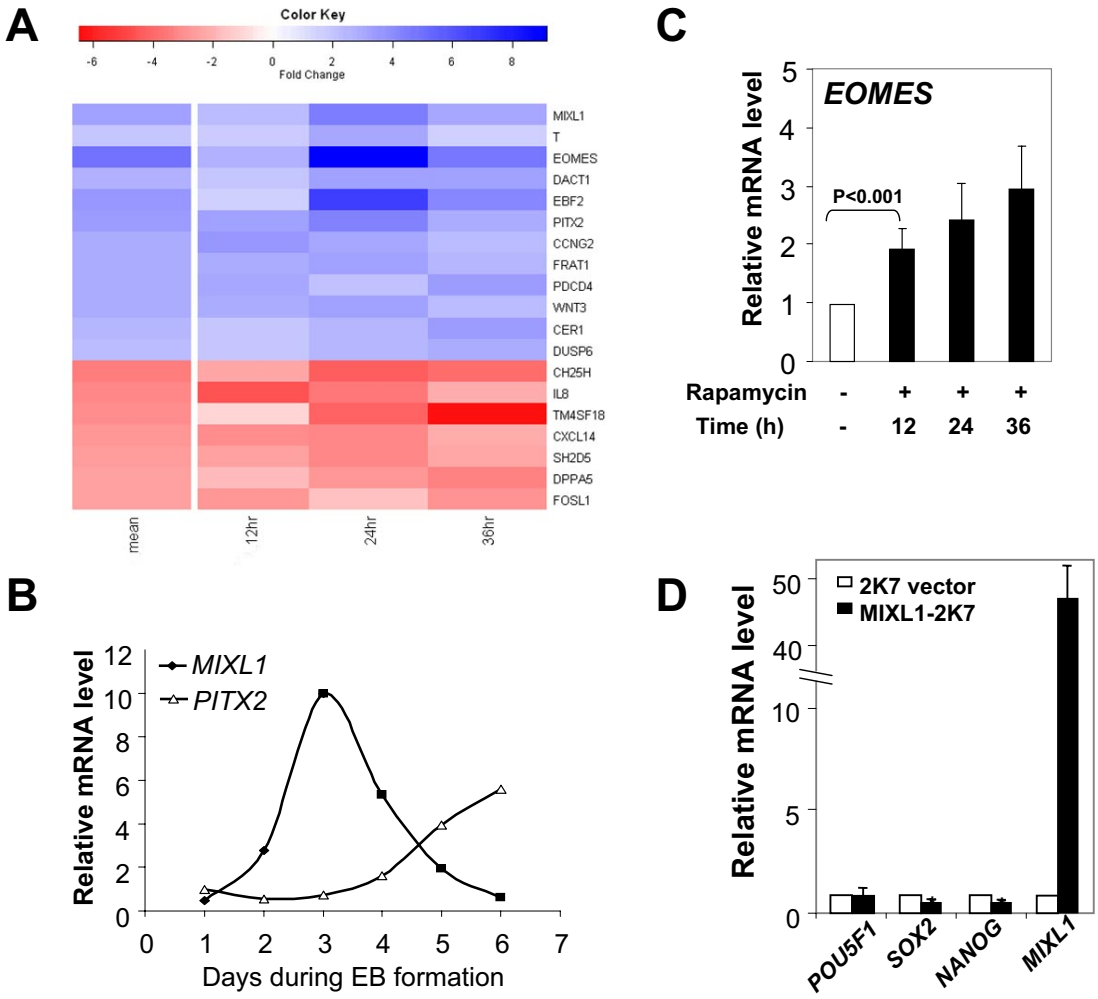

Fig. S4. (*A*) Total RNAs from H9 cells treated with or without rapamycin (100 nM) were used for microarray analysis with Affymetrix GeneChip Human Genome U133 Plus 2.0 Expression Arrays. Heatmap of expression differences for 19 genes of interest due to rapamycin treatment. The ''mean'' column is the mean difference across all time points, and each time column shows the mean difference among the 3 treatment-control replicate pairs at that time point. Directions and amounts of change are indicated by the color and intensity of the block. The heatmap was generated using the gplots package from Bioconductor. (*B*) mRNA levels of *MIXL1* and *PITX2* in H9 cells induced to form EBs as assessed by real-time PCR analysis. The *y* axis represents relative levels of mRNA with values normalized to the level (= 1) of respective genes on the first day of EB formation. A typical experiment from 4 independent experiments is shown. *ACTB* (β-actin) was used as internal control. (*C*) The mRNA levels of *EOMES* in untreated control cells (white bars) and in cells treated with rapamycin (12, 24, and 36 h; black bars) and assessed by real-time PCR analysis. Quantification of 4 separate experiments is shown. Each bar represents the mean ± SEM (error bars). Values were normalized to the level (= 1) of mRNA in the absence of rapamycin at respective time points. Student *t* tests were performed to compare data of between experimental groups. Pairs of treatments that showed a statistically significant difference are marked. ACTB (β-actin) was used as internal control. (*D*) mRNA levels in 2K7/Neo cells and in cells expressing MIXL1 assessed by real-time PCR. Each bar represents the mean± SEM (error bars). All values were normalized to the level (= 1) of mRNA in the 2K7/Neo cells. Asterisk indicates that the value for MIXL1 overexpression differs statistically from untreated cells (*, $P < 0.01$). *ACTB* (β-actin) was used as internal control. (*E*) The mRNA levels of *Wnt3* and *DACT1* in untreated control cells (white bars) and in cells treated with rapamycin (12, 24, and 36 h; black bars) and assessed by real-time PCR analysis. Quantification of 4 separate experiments is shown. Each bar represents the mean ± SEM (error bars). All values were normalized to the level (= 1) of mRNA in the absence of rapamycin at respective time points. Student *t* tests were performed to compare data of between experimental groups. Pairs of treatments that showed a statistically significant difference are marked. *ACTB* (β-actin) was used as internal control. Rapamycin treatment also up-regulated the mRNA level of FRACT1 (data not shown). (*F*) Relative luciferase activity of the Top-flash reporter plasmid in cells with and without rapamycin (100 nM, 24 h) or Wnt3a (25 and 100 ng, 24 h). The Top-flash reporter measures Wnt/β-Catenin-mediated transcriptional activation. Results from 4 separate experiments are shown as means ± SEM. All values were normalized to the level (= 1) of activity in the absence of rapamycin. Asterisks indicate that the value for cells treated with rapamycin or Wnt3a differs statistically from the control (*, $P < 0.001$; **, $P < 0.01$). *Rellina* plasmid was co-transfected with Top-flash reporter as an internal control. (*G*) mRNA levels in untreated control cells (white bars) and in cells treated with Wnt3a (100 ng, 12 h) assessed by real-time PCR. Quantification of 4 separate experiments is shown. Each bar represents the mean± SEM (error bars). All values were normalized to the level (= 1) of mRNA in the absence of Wnt3a. Asterisk indicates that the value for Wnt3a treatment differs statistically from untreated cells (*, $P < 0.01$; **, $P < 0.001$). (*H*) Phase contrast and fluorescent images of H9 cells expressing GFP or GFP-PDCD4. Note the nuclear localization of PDCD4, which is consistent with an earlier study (4). (Scale bar, 100 μm.) (*I*) Western blot analysis of PDCD4, OCT-4, and SOX2 in H9 cells overexpressing GFP and GFP-PDCD4. Endogenous PDCD4 protein (60 kDa, indicated by arrow) and GFP-Rheb (approximately 87 kDa, indicated by the arrow) were detected. Image J analysis indicated that the level of GFP-PDCD4 was 4.1 times that of the endogenous PDCD4; α-tubulin was used as loading controls. A typical experiment from 4 independent experiments is shown. H9 cells overexpressing Cyclin G2 exhibited similar results (data not shown).

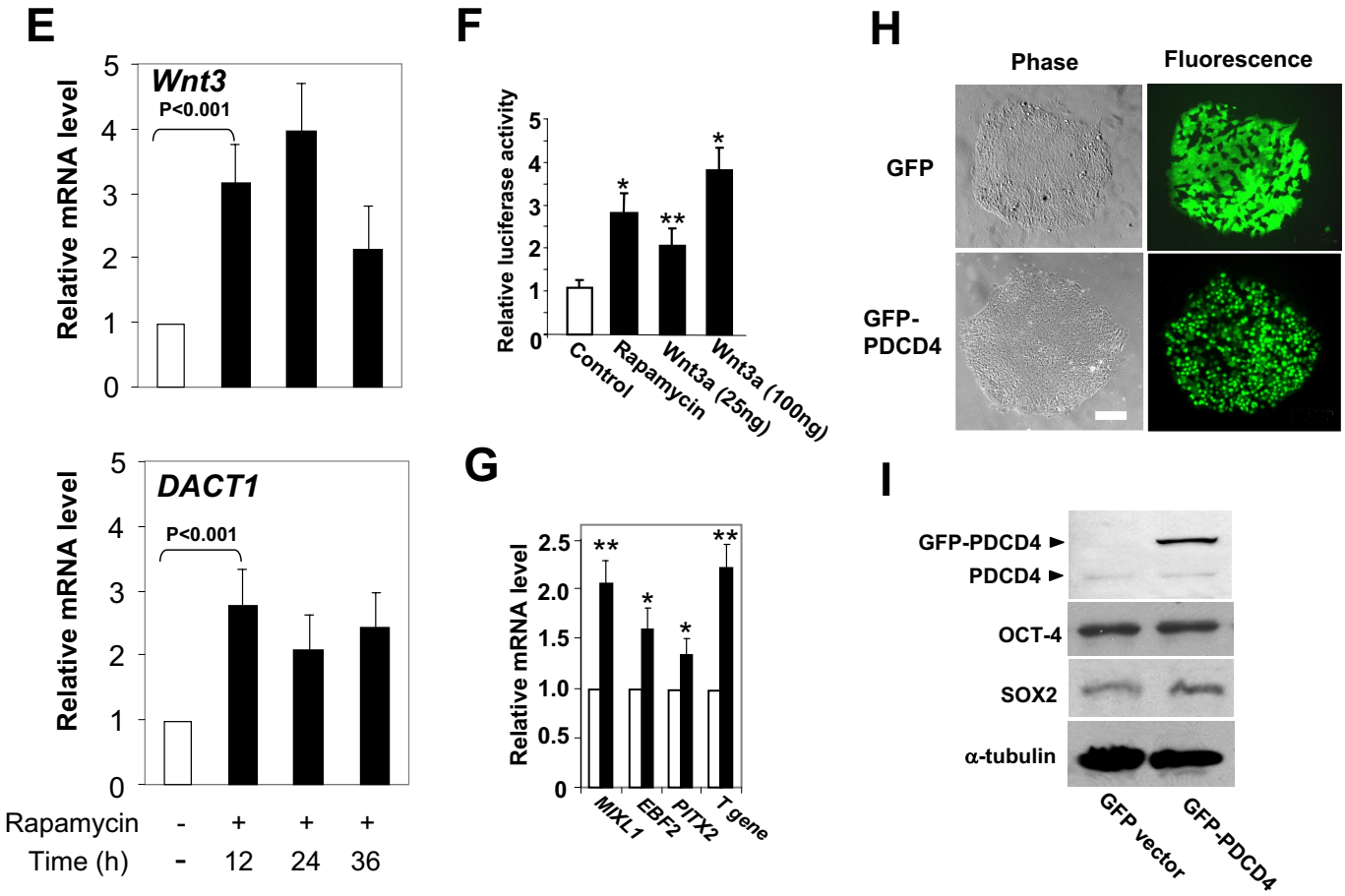

Fig. 4. (continued).

Table S1. Sources and dilutions of the antibodies

Antibody	Source	Cat. #	Dilution
OCT-3/4	Santa Cruz	SC-9081	1:500 (I) /1:2,000 (W)
SOX2	Millipore	AB5603	1:200 (I) /1:1,000 (W)
α-tubulin	Abcam	AB11304	1:10,000 (W)
NANOG	R&D System	AF1997	1:100 (I)
mTOR	Cell Signaling	#2972	1:1,000 (W)
S6K1	Cell Signaling	#9202	1:500 (W)
S6K1T389	Cell Signaling	#9205	1:500 (W)
Cyclin G2	Reference (1)	N/A	1:200 (W)
PDCD4	Abcam	Ab45263	1:500 (W)
Rheb	Santa Cruz	SC-6341	1:200 (W)

(I: immunofluorescence; W: Western blotting)

Table S2. Names and sequences of the primers

Primer name	Sequence
GFP-Rheb-F	5′-CCGGTCGCCACCATGGTGAGCAAGG-3′
GFP-Rheb-R	5′-GGACAAACCACAACTAGAATGCAGT-3′
EF-1aPF1	5′-CGCGGATCCAAGCTTTGCAAAGATGGATAAAGT-3′
EF-1aPR1	5′-GCTCCCGGGTCACGACACCTGAAATGGAA-3′
MIXL1F	5′-CCCAAGCTTCCACCATGGCCACAGCCGAGTCCCG-3′
MIXL1R	5′-GGCGAATTCTCAAAAGTTACCAAAGGCA-3′
CCNG2F	5′-CCGGAATTCGCACCATGAAGGATTTGGGGGCAGA-3′
CCNG2R	5′-CGCGGATCCCTAAGATGGAAAGCACAGTG-3′
PDCD4-F	5′-CCGGGATCCACCATGGATGTAGAAAATGAGCAGATAC-3′
PDCD4-R	5′-CCCTCGAGGTACCTCAGTAGCTCTCTGGTTTAAGACGACC-3′
hmTOR-1R	5′-/ 5Phos/TCGAGAAAAAAGTGGCTCTTGCTCATAAATCTCTTGAATTTATGAGCAAGAGCCAGCA-3′
hmTOR-1F	5′-/ 5Phos/TGCTGGCTCTTGCTCATAAATTCAAGAGATTTATGAGCAAGAGCCAGCTTTTTTC-3′
hmTOR-2R	5′-/ 5Phos/TCGAGAAAAAAGGCCTATGGTCGAGATTTATCTCTTGAATAAATCTCGACCATAGGCCA-3′
hmTOR-2F	5′-/ 5Phos/TGGCCTATGGTCGAGATTTATTCAAGAGATAAATCTCGACCATAGGCCTTTTTTC-3′
hmTOR-3R	5′-/ 5Phos/TCGAGAAAAAAGGAAGATCCTGCACATTGATCTCTTGAATCAATGTGCAGGATCTTCCA–3
hmTOR-3F	5′-/ 5Phos/TGGAAGATCCTGCACATTGATTCAAGAGATCAATGTGCAGGATCTTCCTTTTTTC-3′
hmTOR-4R	5′-/ 5Phos/TCGAGAAAAAAGGAGGCTGATGGACACAAATCTCTTGAATTTGTGTCCATCAGCCTCCA-3′
hmTOR-4F	5′-/ 5Phos/TGGAGGCTG ATGGAC ACA AATTCA AGAGATTTG TGTCCATCA GCCTCCTTTTTTC-3′
hSOX2-F	5′-AACCCCAAGATGCACAACTC-3′
hSOX2-R	5′-GCTTAGCCTCGTCGATGAAC-3′
EBF2F	5′-CCGGGATCCACCATGTTTGGAATTCAAGATAC-3′
EBF2R	5′-CTCGAGGTACCTTACATCGGGGGTACAACAA-3′