Supporting Information

10.1073/pnas.0905657107

SI Materials and Methods

Antibodies. The antibodies used for immunofluorescence or Western blotting are as follows: pErk1/2 (Cell Signaling Technology), Erk1/2 (Cell Signaling Technology), Ras (Cell Signaling Technology), pMek1/2 (Cell Signaling Technology), Mek1/2 (Cell Signaling Technology), Rcn2 (Proteintech Group), α-tubulin (Sigma), EGFP (Roche), Flag (Sigma), GATA-4 (Santa Cruz), Dab2 (BD), Nanog (Abcam for immunostaining), and His (Calbiochem). Antibodies against Oct4, Sox2, Nanog (for Western blot analysis), and serine/threonine kinase 40 (Stk40) were raised and affinity purified in our laboratory.

Plasmids. The cDNA sequence of mouse full-length Stk40 was amplified by RT-PCR using total RNA from CGR8 mouse embryonic stem cells (ESCs; Austin Smith and Ian Chambers) and cloned into pET-30a(+) (Novagen), pGEX-4T-1 (Amersham Biosciences), or pPyCAGIP vectors (Ian Chambers). Mouse full-length Rcn2 cDNA sequence was amplified by RT-PCR using total RNA from CGR8 ESCs and cloned into pGEX-4T-1 or pPyCAGIP vectors. Stk40-EGFP/pPyCAGIP or Rcn2-EGFP/pPyCAGIP was subcloned from Stk40/pEGFP-N1 or Rcn2/pEGFP-N1 (pEGFP-N1 vector was purchased from Clontech). Ras constitutively active form RasT35S [pCMV-Ras (G12V, T35S)] and dominant negative form RasS17N [pCMV-Ras (S17N); Hiroshi Koide] were subcloned into pPyCAGIP vectors. For construction of various luciferase reporter vectors, promoter 2-kb and 4-kb upstream fragments of *Stk40* were amplified using genomic DNA from CGR8 cells and cloned into pGL3-Basic vectors (Promega). The pPyCAGIP-TetR vector was previously described (1). Sequences of siRNA for *EGFP* (2), *Stk40* (gga ccc atc gga taa cta t), and *Rcn2* (gca gca gtt tgt gga gta t) were inserted into pTER+ vector (3) to generate RNAi *EGFP*, RNAi *Stk40*, or RNAi *Rcn2*, respectively. RBD (Ras binding domain; expanding residues from amino acid 51–131 on Raf-1) (4) was amplified by RT-PCR from total RNA from CGR8 cells and inserted into pGEX-4T-1 vector. The sequences of all constructs were verified by DNA sequencing.

Chromatin Immunoprecipitation Assays. CGR8 mouse ESCs were cross-linked with 1% formaldehyde, and the nuclear extracts were sonicated and incubated with control IgG or Oct4 antibody for immunoprecipitation in chromatin immunoprecipitation (ChIP) assays. The precipitated complexes were eluted and reverse cross-linked. The captured genomic DNA was obtained through ethanol precipitation and used for PCR analysis. Ten percent of total genomic DNA from the nuclear extract was used as input. The primers used are as follows: Rex1, 5′-GAG GTA CTG AGA TGT GAC TGA GTC TCA -3′ (forward) and 5′-CTC CTT GGA CCC CTC CCT TTT TAG ATG-3′ (reverse); Stk40, 5′-CTA GCC TTT TTG GAA CCC ACG AAA C-3′ (forward) and 5′-GCT GGT CTT CAT AAT GAA GTC CCA G-3′ (reverse).

Identification of *Stk40* by ChIP Assay. To identify new target genes of Oct4, affinity-purified rabbit polyclonal antibody against Oct4 was used to conduct ChIP assays using mouse ESC nuclear extract (CGR8 ESC line). The precipitated genomic DNA fragments were ligated into the pBluescript vector and subjected to DNA sequencing. The sequences were analyzed through the BLAST program, and potential target genes were identified. *Stk40* was one of the candidates.

Embryoid-Body Formation. ESCs were cultured in suspension in Petri dishes in ES growth medium without Leukemia inhibitory factor to form aggregates. At various time points, images were recorded, and the aggregates were harvested for RT-PCR, Western blot analysis, or histological analysis.

RT-PCR and qPCR Analysis. Total RNA was extracted using TRIzol reagent (Invitrogen), treated with RNase-free DNase (Promega), and transcribed into cDNA using oligo $(dT)_{15}$ and ReverTra Ace reverse transcriptase (TOYOBO) as previously described (5). For qPCR, raw data were obtained on an ABI PRISM 7900 using fluorogenic SYBR Green I double-stranded DNA-binding dye chemistry (ABI) as previously described (5). The primers used for RT-PCR and qPCR are provided in Table S3.

Western Blot Analysis. Cell extracts or samples from immunoprecipitation or GST pull-down assays were resolved on SDS/PAGE and transferred to Protran nitrocellulose membranes (Schleicher & Schuell). After blocking with 5% skim milk (5% BSA for pErk1/2 and Erk1/2) in Tris-Buffered Saline with Tween-20 (TBST) for 1 h, the membranes were incubated with primary antibodies overnight at 4°C. After washing with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Jackson) for 2 h at room temperature. Bands were detected using an ECL Western blotting detection kit (Pierce). The Western blot analysis was performed in at least three independent experiments, and representative data are shown.

Luciferase Reporter Assays. CGR8 or ZHBTc4 ESCs (1.2×10^5) were incubated in 24-well tissue-culture plates for 24 h. Tetracycline (Tc) at a concentration of 0.5 μg/mL was added to the media of ZHBTc4 ESCs to induce down-regulation of *Oct4* expression. Each reporter construct (200 ng for pGL3 Basic and equimolar amounts for the other reporters) was cotransfected with vector pRL-TK (10 ng; Promega) as an internal control using LipofectAMINE2000 (Invitrogen). Cell extracts were prepared 48 h after transfection, and luciferase activities were evaluated using a dual-luciferase assay system (Promega). The luciferase activity of each construct was calculated relative to that of control vector pGL3-Basic. All transfection experiments were conducted in duplicate at least three times. The luciferase activities are reported as means ± SD.

In Vitro Protein–DNA Binding Assays. The oligonucleotide probes were synthesized and labeled with biotin at the 5′ end of the forward oligonucleotide. The complementary reverse sequence was synthesized and annealed with the biotin-labeled oligomer in 10 mM Hepes (pH 7.8), 10 mM $MgCl_2$, and 0.1 mM EDTA by heating at 65°C for 5 min followed by gradually cooling to room temperature. Double-stranded DNA formation was verified using 3% agarose gel electrophoresis. Then, 0.5-μg biotin-labeled DNA probes were incubated with 1 mg nuclear extract from F9 EC cells at a final concentration of 1 mg/mL at 4°C overnight. The reaction mixture was centrifuged at 2000 × *g* at 4°C to remove debris. Simultaneously, the streptavidin Sepharose high performance beads (GE Healthcare) were washed three times with binding buffer. The mixture of DNA–protein complexes was added to 20 μL of 100% beads. The binding reaction was conducted for 3 h at 4°C. At the end of the reaction, the mixture was centrifuged at 500 × *g* at 4°C for 5 min. After the beads were extensively washed, the samples from the precipitates were subjected to 10% SDS/PAGE, and Oct4-binding DNA was vi-

sualized with Western blotting using the Oct4 antibody. Probe sequences used were as follows: *Stk40* (wt) probe, 5′-CTC TAA AAG TGC CTC ATT TTG CAT AAG ACA GGG CCT TAG G-3′ (forward) and 5′-CCT AAG GCC CTG TCT TAT GCA AAA TGA GGC ACT TTT AGA G-3′ (reverse); *Stk40* (mut) probe, 5′-CTC TAA AAG TGC CTC ATT GTT CCT AAG ACA GGG CCT TAG G-3′ (forward) and 5′-CCT AAG GCC CTG TCT TAG GAA CAA TGA GGC ACT TTT AGA G-3′ (reverse); *Fgf4* probe, 5′-GAA AAC TCT TTG TTT GGA TGC TAA TGG GAT ACT TAA A-3′ (forward) and 5′-TTT AAG TAT CCC ATT AGC ATC CAA ACA AAG AGT TTT C-3′ (reverse); *Utf-1* probe, 5′-GAG CCC TCA TTG TTA TGC TAG TGA AGT GCC GGC AGC A-3′ (forward) and 5′-TGC TGC CGG CAC TTC ACT AGC ATA ACA ATG AGG GCT C-3′ (reverse); negative control (NC) probe, 5′- CCA AGT ACC TGG AAC TCC GGT AGT ACC TGG AAC TCC GGA TGC -3′ (forward) and 5′- GCA TCC GGA GTT CCA GGT ACT ACC GGA GTT CCA GGT ACT TGG -3′ (reverse).

Colony-Forming Assays. E14T ESCs expressing vector or full-length Stk40 were trypsinized to obtain single-cell suspensions, and 10,000 cells were plated per 10-cm cell culture dish. After 10 days, culture plates were stained using an alkaline phosphatase substrate kit (VECTOR), and colonies were scored according to categories: pure, mixed, or fully differentiated colonies.

Chimeric Embryoid Body Formation Assays. ESCs expressing *Stk40* were labeled with DiI red fluorescent dye (Molecular Probes) and mixed with undifferentiated ESCs in an approximate ratio of 1:2–1:1. The mixture was allowed to form spheroids for 4–8 days. To trace the labeled cells, the EBs were frozen-sectioned and visualized using fluorescence microscopy.

Retinoid Acid–Induced Extraembryonic–Endoderm Differentiation in F9 Cells. A Tc-inducible *Stk40* RNAi system was constructed in F9 EC cells as described previously (1). F9 cells were plated at a density of 10,000 cells per well in 6-well plates coated with gelatin. Tc (0.5 μg/mL) was included in the medium as indicated at the same time followed by the addition of 25 nM retinoid acid (RA; an equal volume of ethanol can also be used as a negative control) after 24 h. After additional 4 days, the cells were lysed in TRIzol and subjected to RT-PCR analysis.

GST–RBD Pull-Down Assays to Detect Activated Ras (GTP-Bound). GST-RBD fusion proteins were expressed in *Escherichia coli* (BL21) and purified according to the manufacturer's instructions (GE Healthcare). Whole-cell lysate was extracted from ESCs with radio immunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM NaF, 1 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl fluoride) and normalized using a bicinchoninic acid kit (Pierce). For each reaction, an equal amount of lysate (1 mg or more supplemented with RIPA buffer to equal volumes) was incubated with 10 μg Glutathione Sepharose 4B (GE Healthcare)-bound GST-RBD recombinant protein at 4°C overnight. Subsequently, the GST beads were washed with RIPA buffer, and the precipitates were boiled in 1 × SDS loading buffer for 10 min. The supernatants were applied to SDS/PAGE. RBD pulled-down activated Ras and total Ras in the inputs were visualized by Western blot analysis with anti-Ras antibody.

Fusion Protein Expression and Affinity Purification. GST fusion proteins were expressed and purified according to the manufacturer's instructions (GE Healthcare). GST or GST-Stk40 proteins were bound to Glutathione Sepharose 4B (GE Healthcare) in equimolar amounts. Beads were incubated overnight at 4°C with the whole-cell extract from F9 EC cells. Bound proteins were washed and boiled in SDS/PAGE loading buffer. After electrophoresis, the gel was stained with Coomassie blue. Bands, present only in the GST-Stk40 with F9 whole-cell lysate column, were excised for mass spectrometric analysis (6).

GST Pull-Down and Coimmunoprecipitation Assays. GST and His fusion proteins were expressed and purified according to the manufacturer's instructions (GE Healthcare and Novagen, respectively). Subsequently, 0.5 μg of GST fusion proteins were incubated with 0.5 μg of His fusion proteins in 500 μL of tris-buffered saline-NP40 (TBS-N) [20 mM Tris-HCl (pH 7.6), 200 mM NaCl, and 0.1% Nonidet P-40] at 4°C for 2 h followed by the addition of Glutathione Sepharose 4B (GE Healthcare) for 1 h. For coimmunoprecipitation, cells were washed in the culture dish with cold PBS three times. Whole-cell lysates were prepared in Co-IP (Immunoprecipitation) buffer [50 mM Tis-HCl (pH 7.5), 150 mM NaCl, 0.5% Nonidet P-40, 2 mM EDTA, 1 mM NaF, 1mM Na_3VO_4, 10% glycerol, and 1 mM phenylmethylsulfonyl fluoride]. After vortexing and centrifugation, the supernatant was incubated with anti-Flag M2 affinity beads (Sigma) overnight at 4°C. The samples from immunoprecipitation or GST pull-down assays were studied using Western blot analysis.

Immunofluorescence Staining. ESCs were grown on glass coverslips (Fisher Scientific) and stained as previously described (6). Embryos were fixed with 4% paraformaldehyde and imbedded in optimum cutting temperature compound (Sakura Finetek) to produce frozen sections for immunostaining.

Production of Chimeric Embryos. E14Tg ESCs constitutively expressing the H2B-GFP fusion gene were used for visualizing the in vivo contribution of the injected cells. To obtain chimeric embryos, *Stk40*/E14Tg or *vector*/E14Tg control cells were injected into E3.5 B6D2F1 blastocysts, which were transferred into the uteri of day 2.5 pseudopregnant Institute of Cancer Research females. Embryos were dissected at E6.5 and E7.5, and the fluorescent signals were detected by confocal microscopy (TCS SP5; Leica). As for the chimeric embryos injected with the *Oct4* knocked down ESCs, the E14Tg ESCs were transfected with *Oct4* RNAi oligos (sequence 1 and 2 are provided in Table S4) and the control oligo for 72 h, respectively, and then, they were subjected to blastocyst injection.

Statistical Analysis. All values are shown as means ± SD of the mean. The Student's *t* test was used to determine the significance of differences in comparisons. Values of $P \leq 0.05$ were considered statistically significant.

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Oct4 Is Recruited to the *Stk40* Regulatory Region, and an Intact Octamer Motif Is Required for Oct4 Binding. An in vitro DNA-protein binding assay showed that Oct4 bound the Stk40 regulatory sequence but not a negative control probe (Fig. S1*F*). Notably, binding of Oct4 to the *Stk40* regulatory region was significantly weakened when the octamer motif was mutated, revealing that an intact octamer motif is required for Oct4 binding to the *Stk40* regulatory sequence.

Forced Expression of *Stk40* Induces ESC Differentiation into Extraembryonic–Endoderm Lineages. Transcript levels of *Coup-TF II* and *Afp* (primitive and visceral endoderm markers), *Ttr* and *ApoE* (visceral endoderm markers), *Sparc, Laminin B1*, and *tPA* (parietal endoderm markers) were markedly higher in the *Stk40*-overexpressed cells (Fig. S2*E*). This evidence supports the existence of differentiated cells at different stages of extraembryonic–endoderm (ExEn) lineage development in *Stk40*-overexpressed cells.

Cells peeling off from EBs of *Stk40*-expressing cells displayed the ExEn cell-like morphology and expressed *Gata6*, *Sox7*, and *Laminin B1* as well as Dab2 (Fig. S2 *G–I*).

Furthermore, we established a stable Tc-inducible *Stk40* RNAi system in F9 embryonal carcinoma (EC) cells, which have been often used as a model to study RA-induced ExEn differentiation (7). Knockdown of *Stk40* expression in these cells drastically decreased expression of *Dab2*, *Gata6*, and *Sox7* induced by RA (Fig. S2*L*). Overall, these findings clearly indicate that Stk40 is an important player in ExEn lineage specification during ESC differentiation.

To further define the cells injected into host blastocysts, we stained sections of chimeric embryos containing histone2B GFP-expressing cells. As shown in Fig. S2*M*, one injected *Stk40*-expressing cell (green nucleus) migrated to the ExEn layer of yolk sacs in chimera, expressing Gata4 in the nucleus of the same cell. In contrast, Oct4 was detected in the epiblast cells, interior to the injected cell. Expression of Gata4 in the *Stk40*-expressing cells proves the ExEn identity of the injected cell at a molecular level in addition to its position during early embryonic development.

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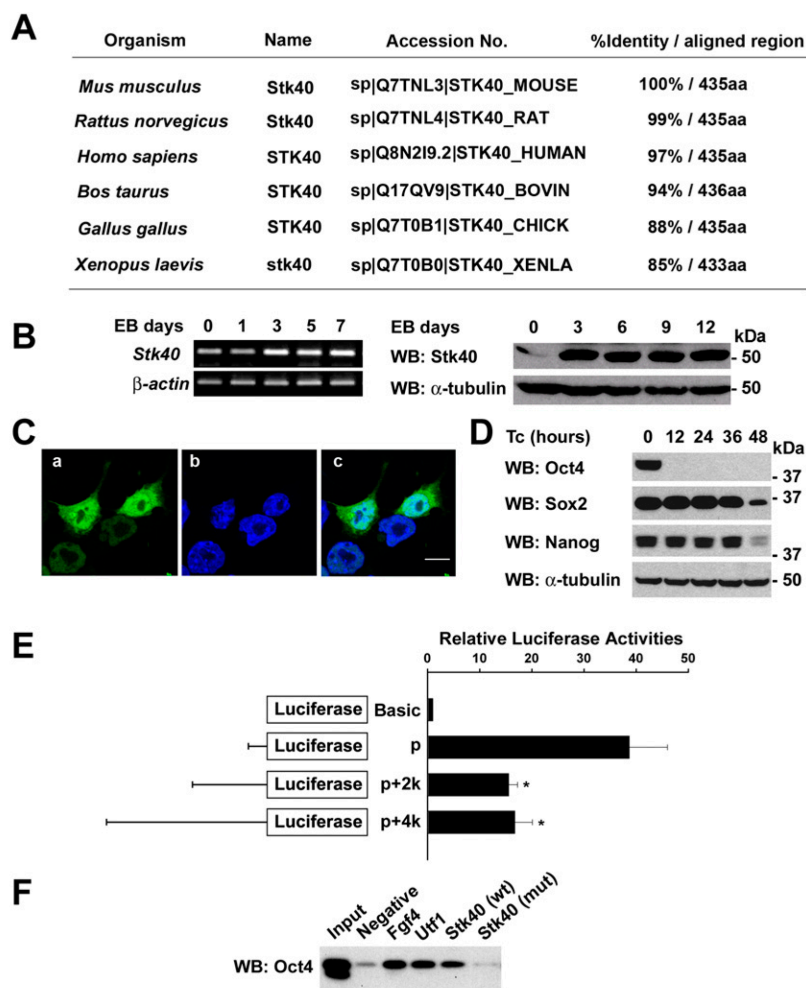

Fig. S1. (*A*) Alignment of the Stk40 orthologs across multiple organisms. (*B*) *Stk40* expression during EB formation. E14T ESCs were cultured in suspension to form EBs for the indicated time points, and Stk40 expression levels were analyzed by semiquantitative RT-PCR (*Left*) and Western blotting (*Right*). (*C*) Confocal images of Stk40–EGFP fusion protein distribution in E14T ESCs. Images were recorded from E14T ESCs transfected with *Stk40–EGFP* expression plasmid. EGFP (*a*), DAPI (*b*), and a composite image (*c*) are shown. (Scale bar: 10 μm.) (*D*) Western blot analysis of pluripotency factors Oct4, Sox2, and Nanog in ZHBTc4 ESCs treated with Tc at indicated time points. (*E*) Luciferase assays with reporter constructs containing the regulatory sequence of *Stk40* in CGR8 ESCs. Luciferase activities are shown as relative values to pGL3-Basic (Basic defined as 1). p, the reporter construct containing the putative promoter sequence of *Stk40*; p+2k, 2-kb upstream sequences of *Stk40*; and p+4k, 4-kb upstream sequences of *Stk40*. All values are shown as means ± SD of results from three independent experiments. *$P < 0.05$. (*F*) In vitro binding assays using indicated biotin-labeled probes with nuclear extract from F9 EC cells.

Fig. S2. (*A*) The morphology of different colonies overexpressing *Stk40*. (*a*) Primitive and visceral endoderm-like cells. (*b*) Mesenchymal parietal endoderm-like cells. (*c*) Epithelial parietal endoderm-like cells. (*d*) Visceral endoderm-like cells. (Scale bar: 50μm.) (*B*) Forced expression of *Stk40* results in a marked reduction

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in the number of undifferentiated colonies. Data are shown as means ± SD (n = 3). (*C*) Effect of Stk40 on expression of pluripotency markers using qPCR. Data are shown as the ratio of values for *Stk40*-expressing cells over control cells. All values are shown as means ± SD of results from three independent experiments. (*D*) Immunofluorescence staining using antibodies against Dab2, Gata4, and Nanog in *vector*- or *Stk40*-expressed E14T cells. Composite images of staining with antibodies and DAPI (blue) are shown. (Scale bar: 50 μm.) (*E*) Effect of Stk40 on expression of differentiation-related markers representing primitive, parietal, and visceral endoderm, respectively, using qPCR. Coup-TF II, Afp, primitive and visceral endoderm; Ttr, ApoE, visceral endoderm; Sparc, LamininB1, tPA, parietal endoderm. All values are shown as means ± SD of results from three independent experiments. (*F*) Increase in ExEn-like cells in EBs overexpressing *Stk40*. ESCs transfected with *vector* or *Stk40* were cultured in suspension, and images were taken at the indicated time points (Magnification: ×10). (*G*) Morphology of the EBs (*a*) and the peeling cells from the outer layer of spheres (*b*) (Magnification: a, ×10; b, ×20). (*H*) RT-PCR analysis of ExEn marker expression in the peeling cells described in (*G*). (*I*) Immunofluorescence staining of Dab2 in the peeling cells. (Scale bar: 50 μM.) (*J*) Ectoderm marker *Fgf5* (*Upper*) and mesoderm marker *Bmp4* (*Lower*) did not decrease in *Stk40*-null EBs. All values are shown as means ± SD of results from three independent experiments. (*K*) Verification of lack of Stk40 protein in EBs derived from $Stk40^{-/-}$ ESCs by Western blot analysis. (*L*) Knockdown of *Stk40* blocks *all-trans* retinoid acid-induced ExEn differentiation in F9 cells. A representative RT-PCR result of ExEn–marker expression in these cells is shown. (*M*) *Stk40*-expressing cells migrate to the ExEn layer. Confocal images of an injected GFP-positive cell and immunostaining of Gata4 and Oct4 in chimeric embryos at E6.5 from *Stk40*/E14Tg ESCs injection. (Scale bar: 75 μm.) (*Inset*) Enlarged view of the distal extraembryonic yolk sac region. Arrow indicates one injected GFP-positive cell. (Scale bar: 10 μm.) (*N*) Verification of RNAi efficiency in transient transfection of *Oct4* RNAi oligos in E14Tg cells by Western blot analysis. (*O*) Contribution of injected *Oct4* knocked down E14Tg cells to the ExEn lineage in E6.5 chimeric embryos. (Scale bar: 100 μm for control; scale bar: 250 μm for *Oct4* RNAi oligo 1 and 2.) *Oct4* RNAi oligo 1 and 2 were designed to target two independent regions of the *Oct4* coding sequence.

Fig. S3. (*A*) RT-PCR analysis of ExEn–marker expression in *vector*- or *Stk40*-expressed E14T cells treated with DMSO or U0126. (*B*) RT-PCR analysis of ExEn marker expression in E14T cells stably expressing *vector*, Ras constitutively active-form mutant *RasT35S* (*Left*), or *Stk40* or coexpressing *Stk40* and Ras dominant negative-form mutant *RasS17N* (*Right*). (*C*) Western blot analysis of pMek1/2 in E14T cells stably expressing *vector*, *Stk40*, or *RasT35S* as indicated.

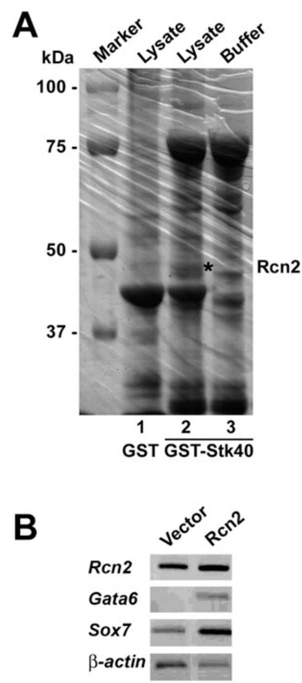

Fig. S4. (*A*) Identification of Rcn2 as a protein partner of Stk40 by GST or GST-Stk40 chromatography. Coomassie-blue staining of an SDS/PAGE gel containing the proteins purified is shown. (*B*) Overexpression of *Rcn2* induces ESC differentiation. RT-PCR analysis of ExEn marker expression in E14T cells overexpressing *vector* or *Rcn2* is shown.

Fig. S5. (*A*) Relative expression levels of *Rcn2* in different cell lines as indicated. All values are shown as means ± SD of results from three independent experiments. (*B*) Rcn2 proteins are detected in frozen sections of mouse embryos at E7.5. Immunofluorescent images of Rcn2, Gata4, and Oct4 proteins are

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shown. (Scale bar: 250 μm.) (*Inset*) Enlarged view at the distal part of ExEn layers covering the epiblast. (Scale bar: 25 μm.) (*C*) Immunofluorescent staining of Rcn2 and Oct4 in the mouse embryo at the blastocyst stage (E3.5). [Scale bar: 50 μm (*Upper*); scale bar: 25 μm (*Lower*).] (*D*) Immunofluorescent images of Rcn2 proteins in *vector*- or *Stk40*- expressed E14T cells. (Scale bar: 50 μm.) (*E*) *Stk40*-expressing cells migrate to the ExEn layer and express Rcn2. Confocal images of an injected GFP-positive cell and immunostaining of Rcn2 in chimeric embryos at E6.5 from *Stk40*/E14Tg ESCs injection. (Scale bar: 75 μm.) (*Inset*) Enlarged view of the distal extraembryonic yolk sac region. Arrow indicates one injected GFP-positive cell. (Scale bar: 10 μm.)

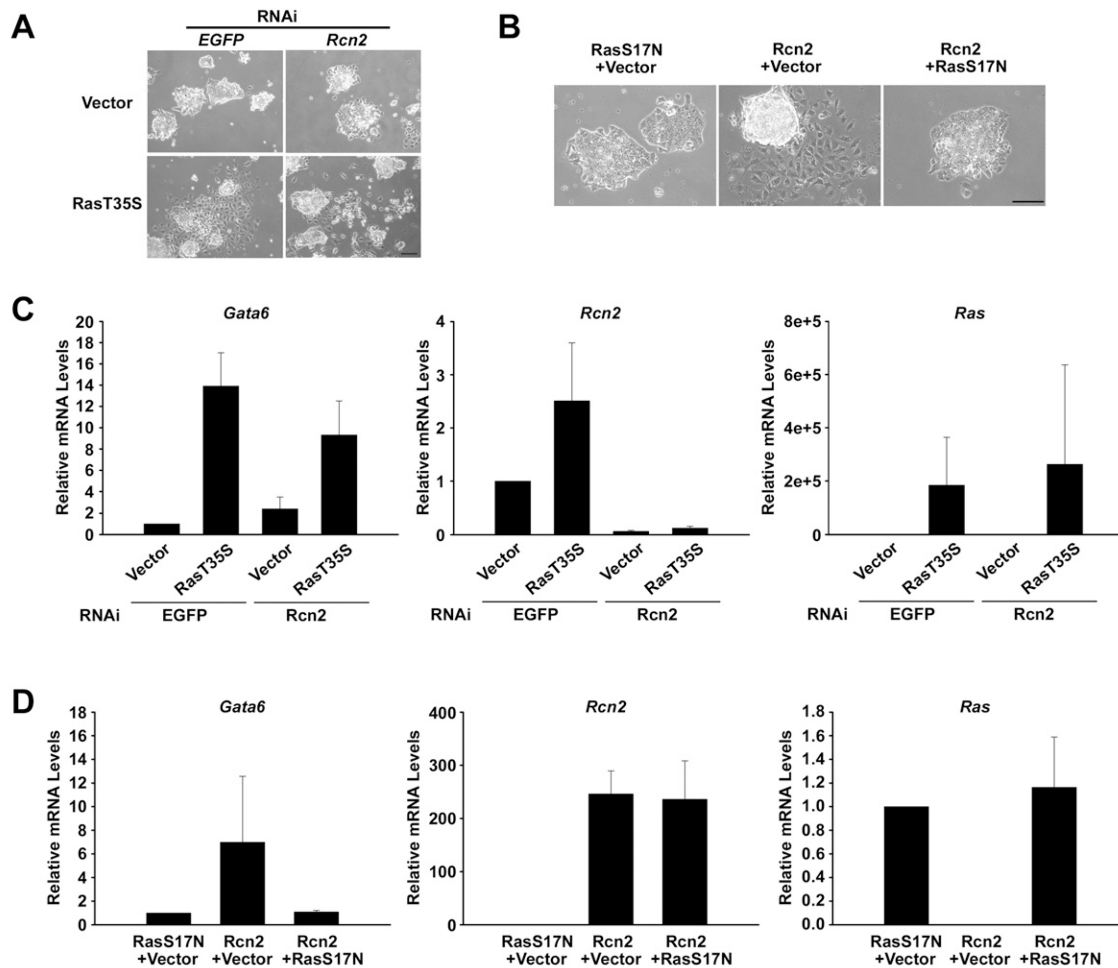

Fig. S6. (*A*) Knockdown of *Rcn2* partially blocks *RasT35S*-induced ESC differentiation. Phase–contrast images of *Vector*- or *RasT35S* overexpression in the stable *EGFP* or *Rcn2* RNAi E14T ESCs are shown. (Scale bar: 100 μm.) (*B*) *RasS17N* blocks *Rcn2*-induced ESC differentiation. Phase-contrast images of E14T ESCs overexpressing indicated genes are shown. (Scale bar: 100 μm.) (*C* and *D*) Gene expression levels in the cells described in *A* and *B* were analyzed by qPCR. All values are shown as means ± SD of results from at least three independent experiments.

Table S1. Analysis of chimeric embryos of Stk40 overexpressed E14Tg cells

Injected cell line name	Stage of embryos	Number of deciduas	Number of embryos	Number of chimeras	
				Embryonic	ExEn
Vector/E14Tg	E6.5	55	48	30	2
	E7.5	16	14	6	0
	Total	71	62 (87%)	36 (51%)	2 (3%)
Stk40/E14Tg	E6.5	86	75	0	32
	E7.5	33	30	0	5
	Total	119	105 (88%)	0	37 (31%)

Table S2. Analysis of chimeric embryos of Oct4 knocked-down E14Tg cells

Injected cell line name	Embryos with positive signals	Predominant distribution	
		Embryonic	ExEn
Negative control	13	9 (69%)	4 (31%)
Oct4 RNAi Oligo 1	41	11 (27%)	30 (73%)
Oct4 RNAi Oligo 2	40	12 (30%)	28 (70%)

Table S3. Names and sequences of the primers for RT-PCR assays

Gene name	Primer sequence
Oct4	Forward: 5′-atggcatactgtggacctca-3′ reverse: 5′-agcagcttggcaaactgttc-3′
Nanog	Forward: 5′-ctcatcaatgcctgcagtttttca-3′ reverse: 5′-ctcctcagggcccttgtcagc-3′
Rex1	Forward: 5′-acgaggtgagttttccgaac-3′ reverse: 5′-cctctgtcttctcttgcttc-3′
Gata6	Forward: 5′-gctgaacggaacgtaccacc-3′ reverse: 5′-acagtggcgtctggatggag-3′
Gata4	Forward: 5′-cctggaagacaccccaatctc-3′ reverse: 5′-aggtagtgtcccgtcccatct-3′
Sox7	Forward: 5′- gacaccttggatcagctaagcc-3′ reverse: 5′- cctccagctctatgacacactg-3′
Laminin B1	Forward: 5′-ccccaatctctgtgaaccatg-3′ reverse: 5′-gcaatttgcaccgacactga-3′
Afp	Forward: 5′-attcctcccagtgcgtgac-3′ reverse: 5′-cagcagcctgagagtccat-3′
mHox	Forward: 5′-tggcggcacaagcagacgaaag-3′ reverse: 5′-gtgaggttcacccgacgtgcgaga-3′
T (Brachyury)	Forward: 5′-ggtggcttgttcctggtgc-3′ reverse: 5′-gtaggtgggctggcgttat-3′
Bmp4	Forward: 5′-aaaagtcgccgagattcag-3′ reverse: 5′-cggtaaagatccctcatgtaa-3′
Hand1	Forward: 5′-gggctgctgaggcaactc-3′ reverse: 5′-gccaaggatgcacaagca-3′
Fgf5	Forward: 5′-aaagtcaatggctcccacgaa-3′ reverse: 5′-ggcacttgcatggagttttcc-3′
Nestin	Forward: 5′-tgagggtcaggtggttctg-3′ reverse: 5′-agagcagggagggacattc-3′
Cdx2	Forward: 5′-cagcagtccctaggaagccaa-3′ reverse: 5′-gtgtggcagccagctcactt-3′
Mash2	Forward: 5′-gggatctgcactcgaggattt-3′ reverse: 5′-cgtccatcaagcttgcattca-3′
Dab2	Forward: 5′-ggcaacaggctgaaccattagt-3′ reverse: 5′-ttggtgtcgatttcagagtttagat-3′
Ihh	Forward: 5′-acgtgcattgctctgtcaagt-3′ reverse: 5′-ctggaaagctctcagccggtt-3′
Stk40	Forward: 5′-gcaaggaatagagagccaag-3′ reverse: 5′-taccatccgaccagactctg-3′
Rcn2	Forward:5′-ggtcgttcaggcagcttcatc-3′ reverse: 5′-ctgggtcttcatttgcagttgg-3′
β–actin	Forward:5′-ttccttcttgggtatggaat-3′ reverse: 5′-gagcaatgatcttgatcttc-3′
Hras	Forward:5′-tgccatcaacaacaccaagt-3′ reverse: 5′-atctcacgcaccaacgtgta-3′

Table S4. Oct4 stealth RNAi oligo sequences

Gene name	Primer sequence
Oct4 stealth RNAi Oligo 1	Forward: ACCUUCUCCAACUUCACGGCAUUGG
	Reverse: CCAAUGCCGUGAAGUUGGAGAAGGU
Oct4 stealth RNAi Oligo 2	Forward: AUGCUAGUUCGCUUUCUCUUCCGGG
	Reverse: CCCGGAAGAGAAAGCGAACUAGCAU

Oct4 stealth RNAi oligo and stealth RNAi negative control high GC oligo (12935-400) were purchased from Invitrogen.