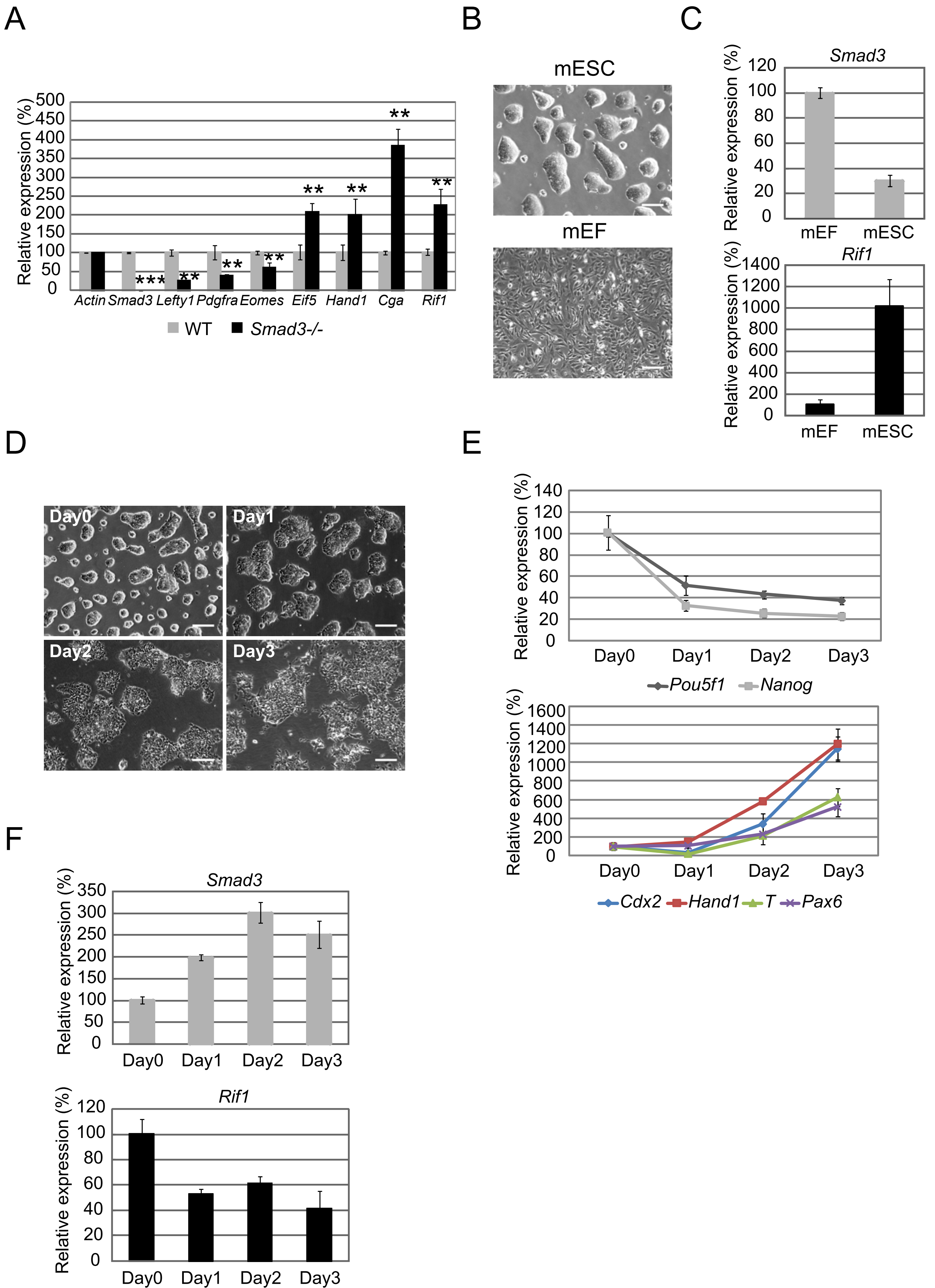


Figure S1

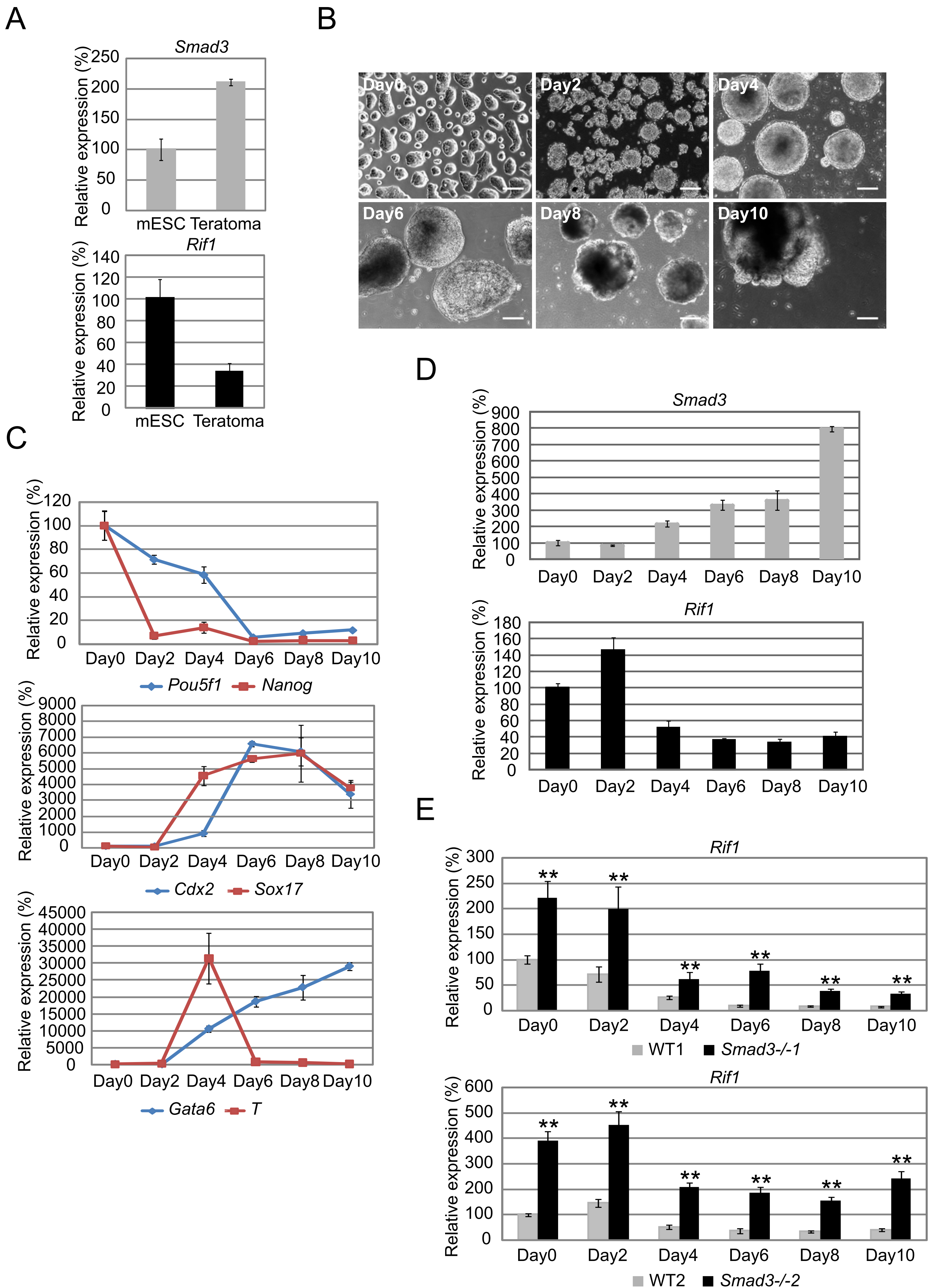
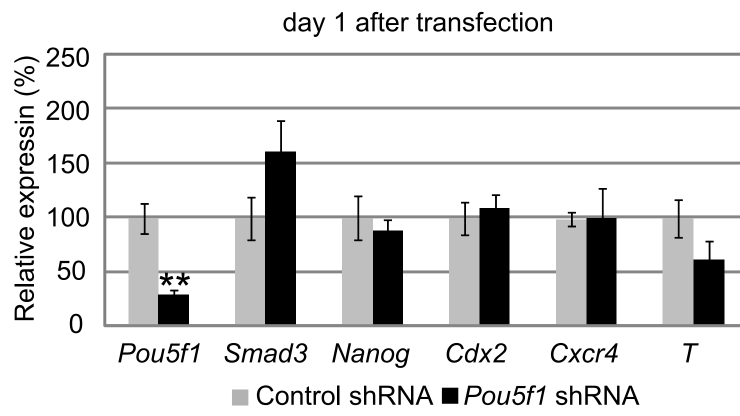


Figure S2

A



B

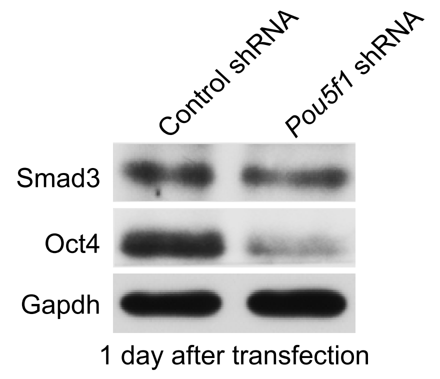
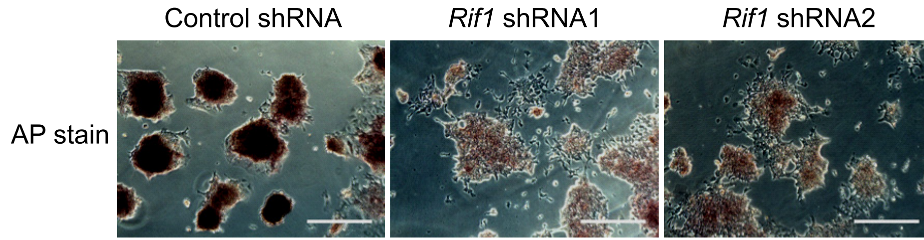
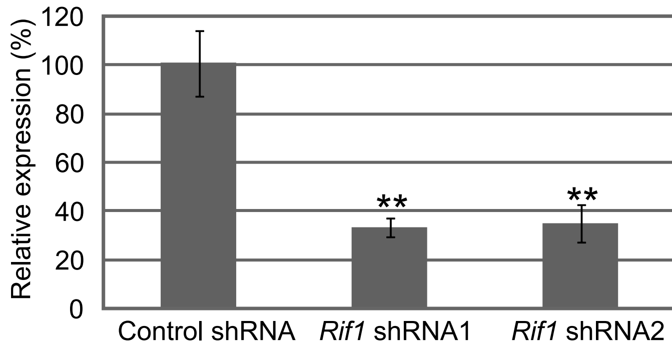


Figure S3

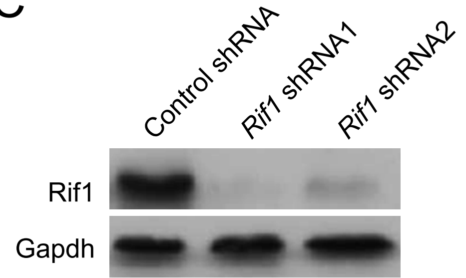
A



B



C



D

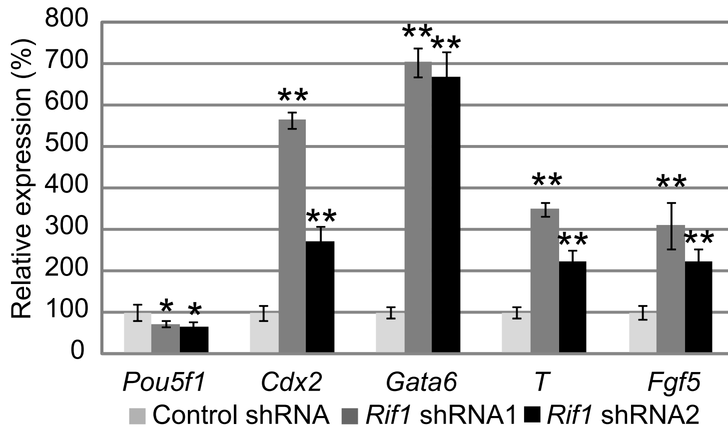
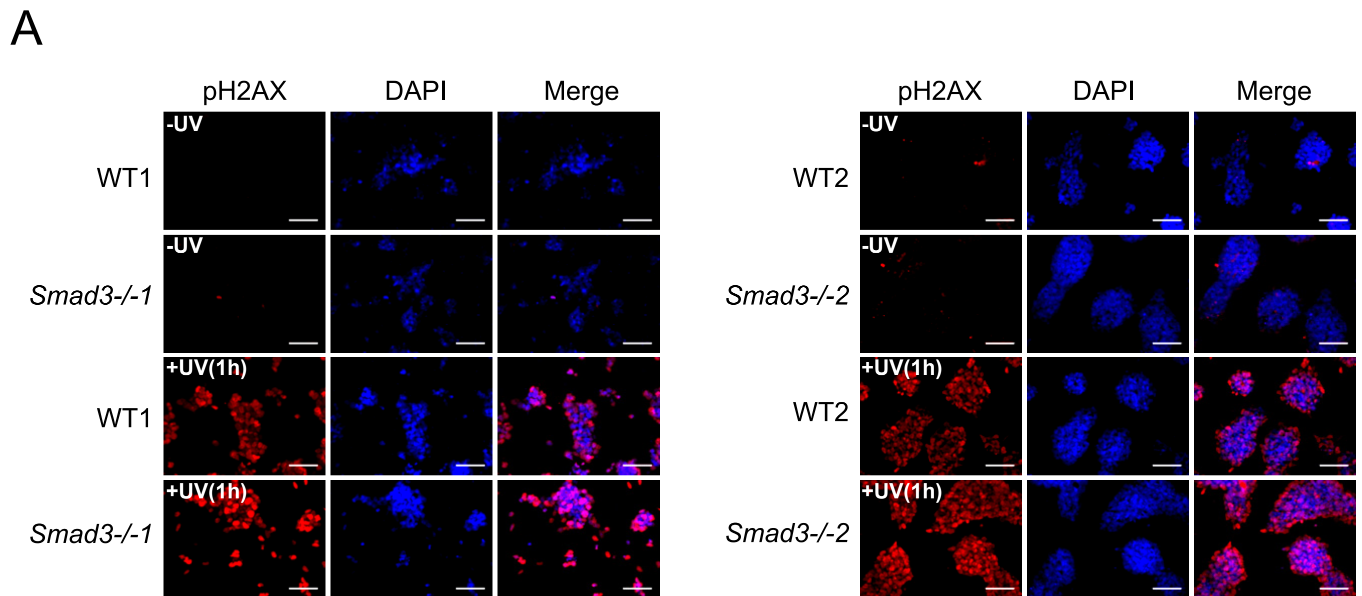


Figure S4

A



B

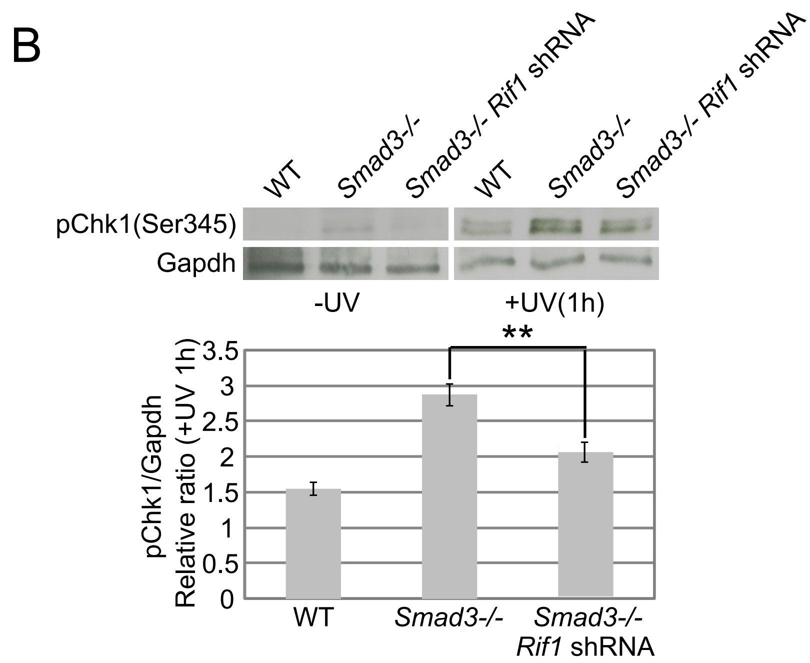


Figure S5

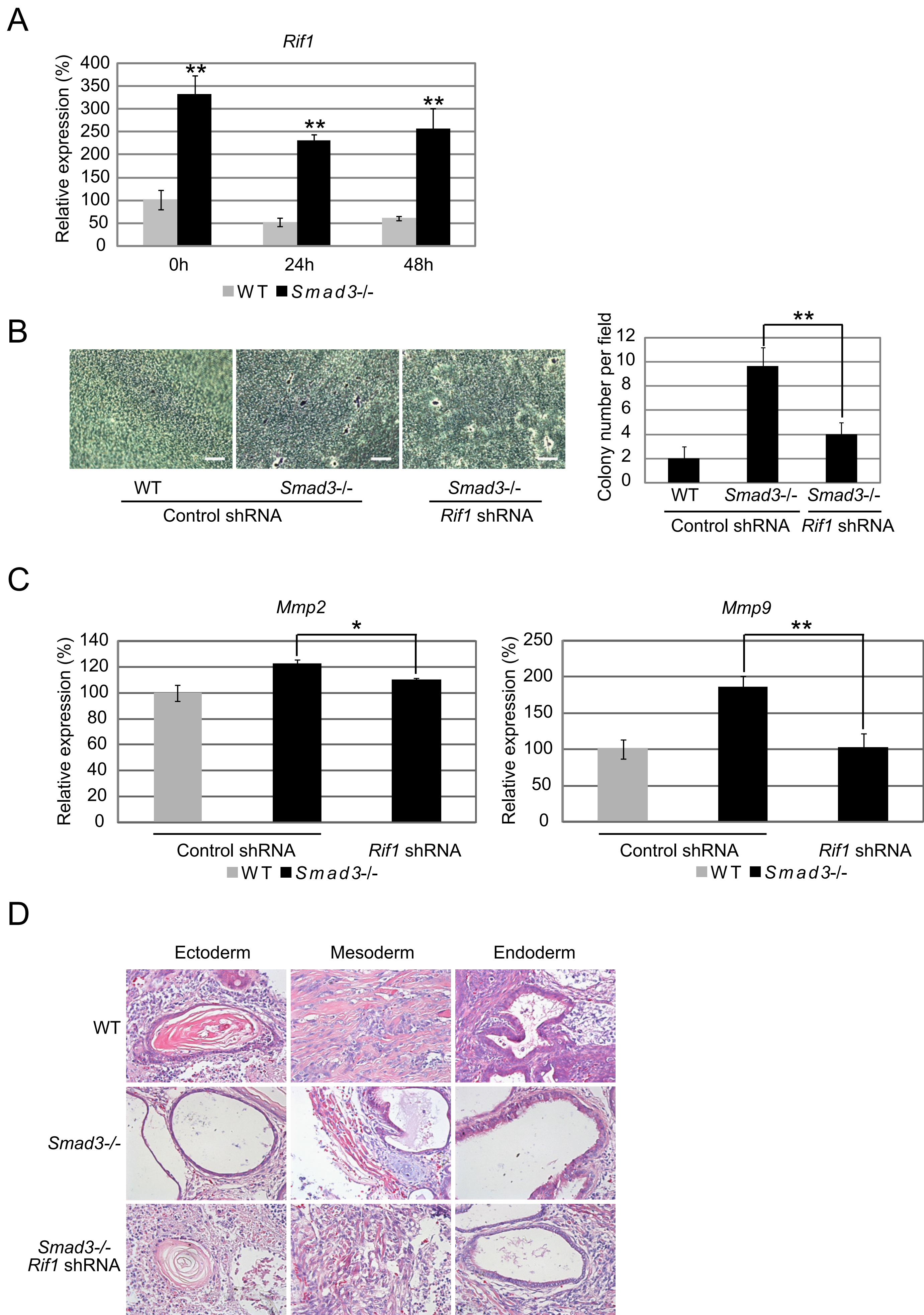
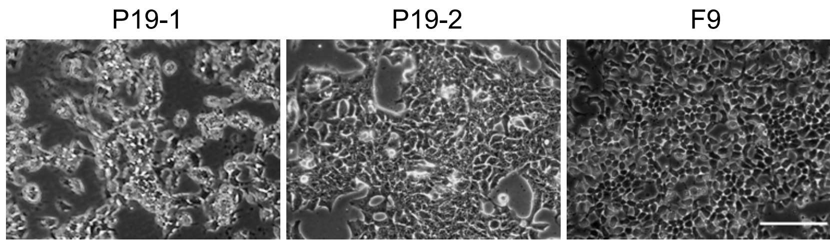


Figure S6

A



B

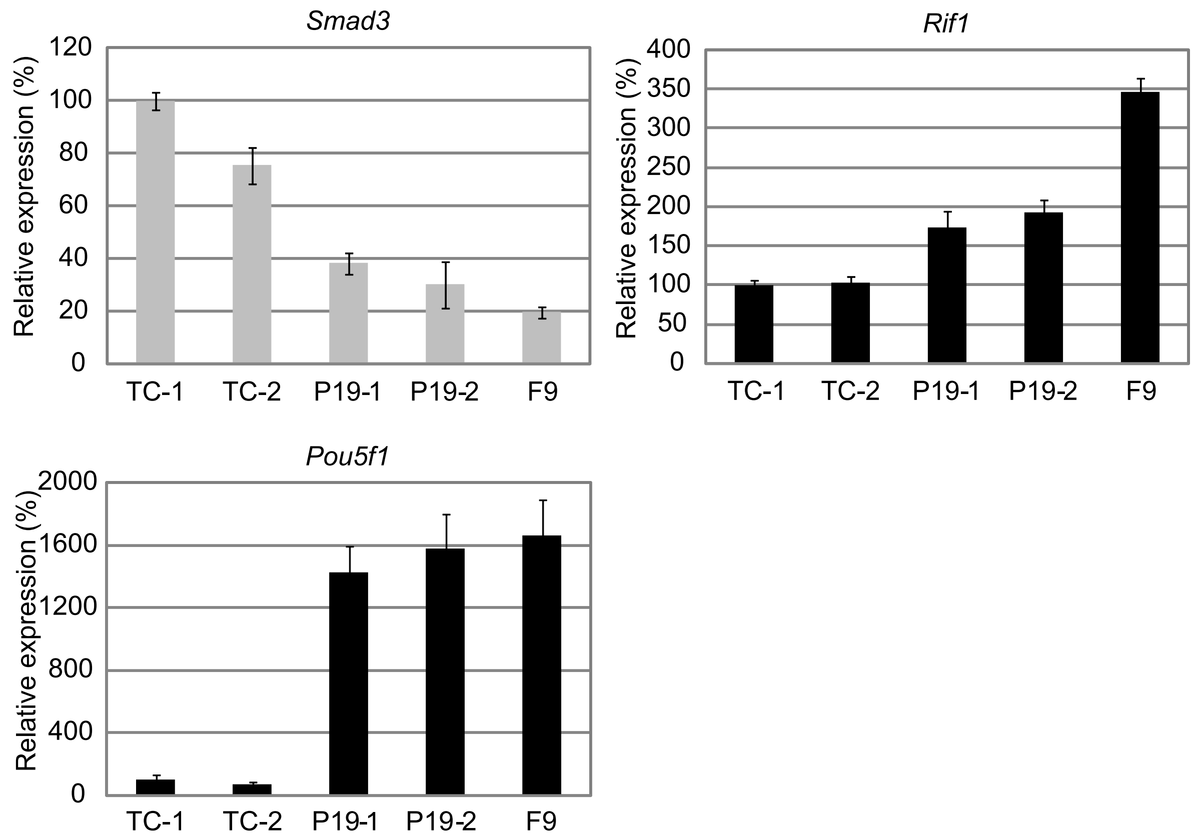


Figure S7

Table S1. Primers used in the study

Real-time PCR primers	
Actin F	5'-ACCAACTGGGACGACATGGAGA-3'
Actin R	5'-TACGACCAGAGGCATACAGGGAC-3'
Smad3 F	5'-CTGGGCCTACTGTCCAATGT-3'
Smad3 R	5'-CATCTGGGTGAGGACCTTGT-3'
Oct4 F	5'-AAGCCTGCCAGGAGCAAA-3'
Oct4 R	5'-ATCCGGCGTTATGCTGCTCT-3'
Nanog F	5'-GGCTATCTGGTGAACGCATCTGGAAG-3'
Nanog R	5'-AACTGTACGTAAGGCTGCAGAAAGTCCTC-3'
Rif1 F	5'-ACTGTCTCCACGGATGAAGA-3'
Rif1 R	5'-CAAATAGCTGGCTTCCAGTG-3'
Lefty1 F	5’-TGTGTGTGCTCTTTGCTTCC-3’
Lefty1 R	5’-GGGGATTCTGTCCTTGGTTT-3’
Lefty2 F	5'-CAGCCAGAATTTTCGAGAGGT-3'
Lefty2 R	5'-CAGTGCGATTGGAGCCATC-3'
Ccnd2 F	5’-AAGCCTGCCAGGAGCAAA-3’
Ccnd2 R	5’-ATCCGGCGTTATGCTGCTCT-3’
Cdx2 F	5'-CCTGCGACAAGGGCTTGTTTAG-3'
Cdx2 R	5'-TCCCGACTTCCCTTCACCATAC-3'
Pax6 F	5'-GCATGCAGAACAGTCACAGCGGA-3'
Pax6 R	5'-ACTCCCGTTTATACTGGGCTATTT-3'
Hand1 F	5'-GCCAAGGATGCACAAGCA-3'
Hand1 R	5'-GGGCTGCTGAGGCAACTC-3'
Fgf5	5'-GAGAGTGGTACGTGGCCCTGAACAAGAGAG-3'
Fgf5	5'-CTTCAGTCTGTACTTCACTGGGCTGGGACT-3'
T F	5'-CATCGGAACAGCTCTCCAACCTAT-3'
T R	5'-GTGGGCTGGCGTTATGACTCA-3'
Gata6 F	5'-TGCAAGATTGCATCATGACAGA-3'
Gata6 R	5'-TGACCTCAGATCAGCCACGTTA-3'
Sox17 F	5'-TTCTGTACACTTTAATGAGGCTGTTC-3'
Sox17 R	5'-TTGTGGGAAGTGGGATCAAG-3'
Cxcr4 F	5’-AGCATGACGGACAAGTACC-3’
Cxcr4 R	5’-GATGATATGGA AGCCTTACAC-3’
Mmp2 F	5’-ATGATGACATCAAGGGGATC-3’
Mmp2 R	5’-CGCCAAATAAACCGGTCCTT-3’
Mmp9 F	5’-GAGCTGTGCGTCTTCCCCTTC-3’
Mmp9 R	5’-GGAATGATCTAAGCCCAGTGC-3’

Eomes F	5'-CCTGGTGGTGTTTTGTTGTG-3'
Eomes R	5'-TTTAATAGCACCGGGCACTC-3'
Elf5 F	5'-CCCTCCTCCTCTTCAAAACC-3'
Elf5 R	5'-AAGTTGCCACAAGACCATCC-3'
Pdgfra F	5’-ACGTTCAAGACCAGCGAGTT-3’
Pdgfra R	5’-CGATCGTTTCTCCTGC CTTA-3’
CGA F	5’-GCCAGAGTGGAGAATCATAC-3’
CGA R	5’-AACTGAAGCGCGTCAGAAGT-3’
ChIP qPCR primers	
Actin F	5'-GTTACCCGGGATACTGACCT-3'
Actin R	5'-GGCACCACACCTTCTAC-3'
Lefty1 F	5'-GTAGCCAGCAGACAGGACAA-3'
Lefty1 R	5'-ATCCCCAATCCACATTCA-3'
Lefty2 F	5'-GCAATCTGCCCACTGTAAAA-3'
Lefty2 R	5'-TCGATCTTCCCAAGACTC-3'
Rif1-1 F	5'-CCAATTCTAGGCAGTTGCCT-3'
Rif1-1 R	5'-GGGAGTGTTGCTAAAGG-3'
Rif1-2 F	5'-ATCTCTGTGTTTGAGCACCC-3'
Rif1-2 R	CGTGGAATCTTTCCGTCC
shRNA sequences	
Rif1 shRNA1 F	5'-GATCCCCGAACCGTATTCAGAATCAAttcaagagaTTGATTCTGAATACGGTTCTTTTTA-3'
Rif1 shRNA1 R	5'-AGCTTAAAAAGAACCGTATTCAGAATCAAtctcttgaaTTGATTCTGAATACGGTTCGGG-3'
Rif1 shRNA2 F	5'-GATCCCCGAGTACAATAAGTGTTGATttcaagagaATCAACACTTATTGTACTCTTTTTA-3'
Rif1 shRNA2 R	5'-AGCTTAAAAAGAGTACAATAAGTGTTGATtctcttgaaATCAACACTTATTGTACTCGGG-3'
Rif1 promoter primers	
Rif1 F	5'-GTGGTCACGCGTTGTAGTTCTGAGTCTCTGG-3'
Rif1 R	5'-ACGTCACTCGAGGCTAGAGATGGGTGATGTA-3'
cDNA clone primers	
GFP F	5’-ATACCGAGATCTATGGTGAGCAAGGGCGAGGAG-3’
GFP R	5’-ATACCCCTCGAGCTATCGAGATCTGAGTCCGGAC-3’
Smad3 F	5‘-GTGGTCAGATCTATGTCGTCCATCCTGCCCT-3’
Smad3 R	5‘-ACGTCACTCGAGCTAAGACACACTGGAACAGC-3’
Pou5f1 F	5‘-GTGGTCACGCGTATGGCTGGACACCTGGCTT-3’
Pou5f1 R	5‘-ACGTCACTCGAGTCAGTTTGAATGCATGGGAG-3’
Genotyping primers	
pLvth-Rif1 shRNA F	5’-CGCTGACGTCATCAACCCGCTCCAAGGA-3’
pLvth-Rif1 shRNA R	5'-CGTATAATGTATGCTATACGAAG-3'

Supplementary figure legends

Figure S1

(A) Quantitative real-time PCR to validate the mRNA level of 8 genes randomly selected from microarray analysis in WT ES cells and *Smad3-/-* ES cells. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001). The gene expression changes of the selected genes are consistent with the microarray result.

(B) Phase contrast microscopy of mouse ES cells and embryonic fibroblasts. Scale bar equals to 200μm.

(C) mRNA expression level of *Smad3* (blue) and *Rif1* (black) was determined through real-time PCR analysis in mouse ES cells and embryonic fibroblasts (MEF), *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

(D) Phase contrast microscopy of mouse ES cell differentiated cells on withdrawing 2i and LIF from day 0 to day 3. Scale bar equals to 200μm.

(E) mRNA expression levels of pluripotency genes (*Pou5f1* and *Nanog*) and differentiation genes (*Cdx2*, *Hand1*, *T* and *Pax6*) was determined through real-time PCR analysis in mouse ES cells and ES cell differentiated cells under withdrawing 2i and LIF condition from day 0 to day 3. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

(F) mRNA expression levels of *Smad3* (blue) and *Rif1*(black) were determined through real-time PCR analysis in mouse ES cells and ES cell differentiated cells under withdrawing 2i and LIF condition. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

Figure S2

(A) mRNA expression levels of *Smad3* (blue) and *Rif1* (black) were determined through real-time PCR analysis in mouse ES cells and teratoma cells. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

(B) Phase contrast microscopy of mouse embryoid body development from day 0 to day 10. Scale bar equals to 200 μm.

(C) mRNA expression levels of pluripotency genes (*Pou5f1* and *Nanog*) and differentiation genes (*Cdx2*, *T*, *Sox17* and *Gata6*) were determined through real-time PCR analysis in mouse ES cells and mouse embryoid body development from day 0 to day 10. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

(D) mRNA expression levels of *Smad3* (blue) and *Rif1* (black) were determined through real-time PCR analysis in mouse ES cells and embryoid body cells. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

(E) Quantitative real-time PCR to examine the mRNA level of *Rif1* in WT ES cell (WT1 and WT2) and *Smad3-/-* (*Smad3-/-1* and *Smad3-/-2*) ES cell formed embryoid body from day 0 to day 10. *Actin* was analyzed as a control. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

Figure S3

(A) mRNA expression levels of *Pou5f1*, *Nanog*, *Smad3* and differentiation genes (*Cdx2*, *T* and *Cxcr4*) were determined through real-time PCR analysis in mouse ES cells transfected with pSuper shRNA control and pSuper-*Pou5f1*-shRNA plasmids and selected with 1μg/ml puromycin for 1 day. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

(B) Western blot analysis of the expression of Oct4 and Smad3 in mouse ES cells transfected with control shRNA and *Pou5f1* shRNA plasmids and selected with 1μg/ml puromycin for 1 day. Gapdh expression level was used as an internal control.

Figure S4

(A) Alkaline phosphatase stain of WT mouse ES cells transfected with pSuper control and pSuper-*Rif1*-shRNA plasmids. Scale bar equals to 200 μm.

(B) Quantitative real-time PCR to examine the mRNA level of *Rif1* in ES cells transfected with pSuper control and pSuper-*Rif1*-shRNA plasmids. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

(C) Western blot analysis of the expression of Rif1 in mouse ES cells transfected with pSuper and pSuper-*Rif1*-shRNA plasmids. Gapdh expression level was used as an internal control.

(D) mRNA levels of the pluripotency gene (*Pou5f1*) and differentiation genes (*Cdx2*, *Gata6*, *T* and *Fgf5*) were determined through real-time PCR analysis in mouse ES cells transfected with pSuper shRNA control and pSuper-*Rif1*-shRNA plasmids and selected with 1μg/ml puromycin for 3 days. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

Figure S5

(A) Immunofluorescence stain with pH2AX (Ser139) antibody in WT (WT1 and WT2) and *Smad3-/-* (*Smad3-/-1* and *Smad3-/-2*) ES cells before and after 1 hour recovery for UV (40 mJ/cm^2) irradiation treatment. Scale bar equals to 200μm.

(B) Western blot analysis the expression of pChk1 (Ser345) in WT, *Smad3-/-* and *Rif1* shRNA transduced *Smad3-/-* ES cells before and after 3 hour recovery for UV (40mJ/cm2) irradiation treatment. Gapdh expression level was used as an internal control. Densitometric analysis of the western blot data of pChk1 (Ser345) after UV irradiation treatment. The data are shown as the mean ± S.D (n=2). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

Figure S6

(A) Quantitative real-time PCR to examine the mRNA level of *Rif1* in WT and *Smad3-/-* ES cells under -2i-LIF+RA conditions from day 0 to day 2. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

(B) Transwell invasive assay for WT, *Smad3-/-* and *Smad3-/-* with *Rif1* shRNA ES cells under -2i-LIF+RA conditions. Scale bar equals to 200μm. Invasive colonies were counted for at least 3 fields individually. The data are shown as the mean ± S.D

(n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

(C) Quantitative real-time PCR to examine the mRNA levels of *Mmp2* and *Mmp9* in WT ES cells and *Smad3-/-* ES cells transfected with control shRNA and *Rif1* shRNA plasmids. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3). Statistically significant differences, calculated through student-t tests, are indicated (*, P<0.05; **, P<0.01; ***, P<0.001).

(D) Images of hematoxylin/eosin stained section of WT ES cell, control *Smad3-/-* ES cell and *Rif1* shRNA transduced *Smad3-/-* ES cell formed teratomas. All three germ layer tissues can be observed.

Figure S7

(A) Phase contrast microscopy of mouse teratocarcinoma cell lines P19-1, P19-2 and F9. Scale bar equals to 200μm.

(B) Quantitative real-time PCR to examine the mRNA levels of *Pou5f1, Smad3* and *Rif1* in mouse ESC-formed teratoma cells *in vivo* (TC-1 and TC-2) and mouse embryonic teratocarcinoma cell lines P19 (P19-1 and P19-2) and F9. *Actin* was analyzed as an internal control. The data are shown as the mean ± S.D (n=3).

Table S1 Primers used in the study