Supplementary materials

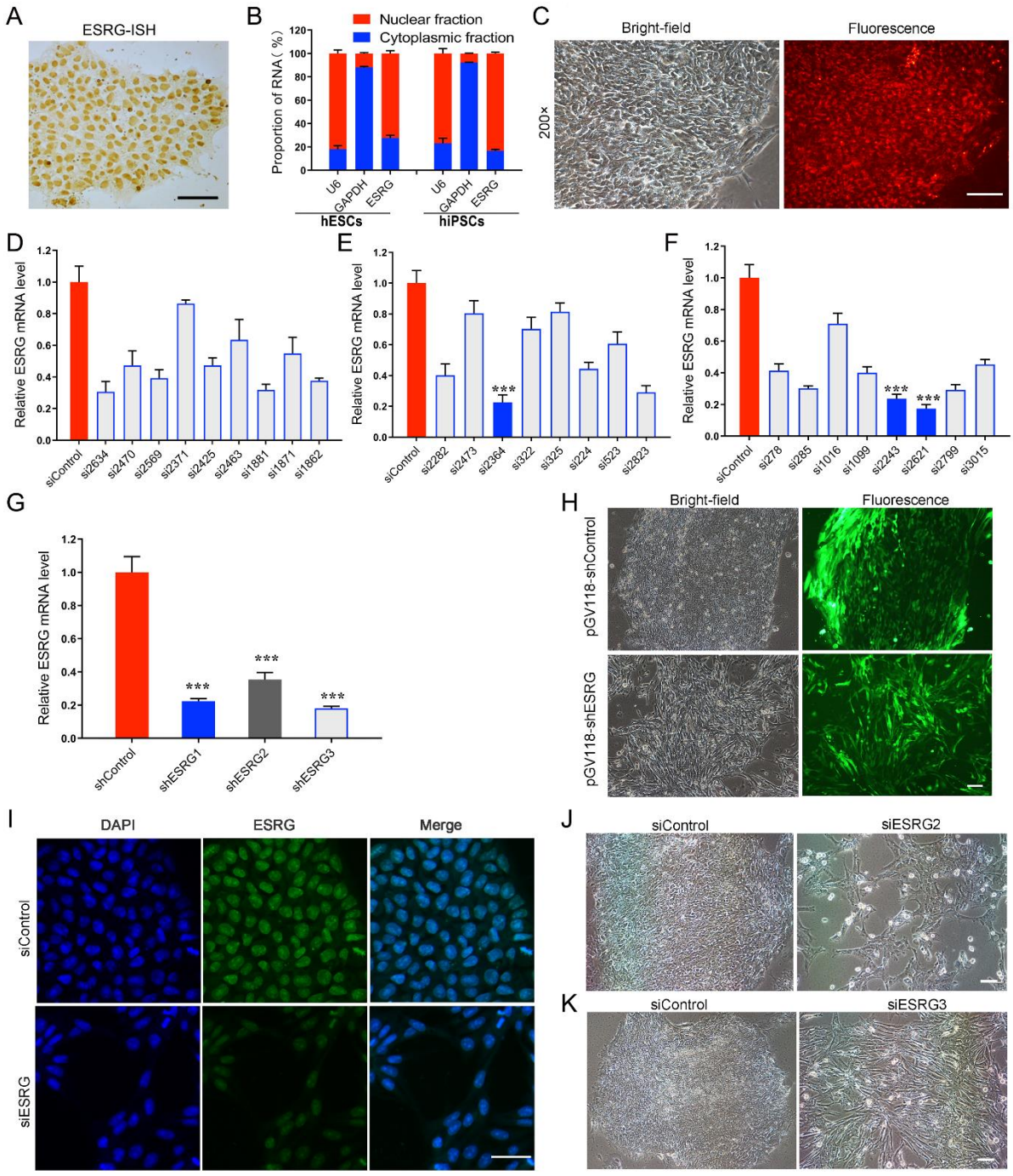

Fig. S1. The characterization of *ESRG*.

(A) *ESRG* intracellular localization was visualized in hPSCs by ISH assays. Representative image of *ESRG* (DIG) in RC1 hiPSCs is shown. Scale bar, 100 μm. **(B)** The *ESRG* expression levels in the nuclear and cytoplasmic fractions derived from H9 hESCs and RC1-iPSCs. **(C)**

Fluorescent microscope images of H9 hESCs transfected with Cy3-labeled negative siRNA. Scale bar, 100 μm. **(D-F)** QPCR analysis of the *ESRG* mRNA level in H9 hESCs transfected with twenty-five different sets of siRNAs against *ESRG*. **(G)** QPCR analysis of *ESRG* mRNA levels in H9 hESCs infected with lentivirus carrying the control vector, sh*ESRG*1, sh*ESRG*2 and sh*ESRG*3 at day 5. (**H)** Bright-field (left) and fluorescence (right) microscopy of H9 hESCs infected with the pGV118-sh*ESRG*1 (lower panel) or pGV118-shControl (upper panel) lentivirus at day 5. Green fluorescence represents cells successfully infected with lentivirus. Scale bar, 100 μm. **(I)** *ESRG* intracellular localization was visualized in H9 hESCs transfected with siControl or si*ESRG* by RNA-FISH assays. Representative images of *ESRG* (green) in H9 cells are shown. Nuclei were stained with DAPI. Scale bar, 100 μm. **(J** and **K)** Phase images of cells transfected with si*ESRG*2/si*ESRG*3 or siControl were observed by inverted phase microscopy. Scale bar, 100 μm. Data are presented as mean ± SD. ***$P < 0.001$ (two-tailed Student's *t* test).

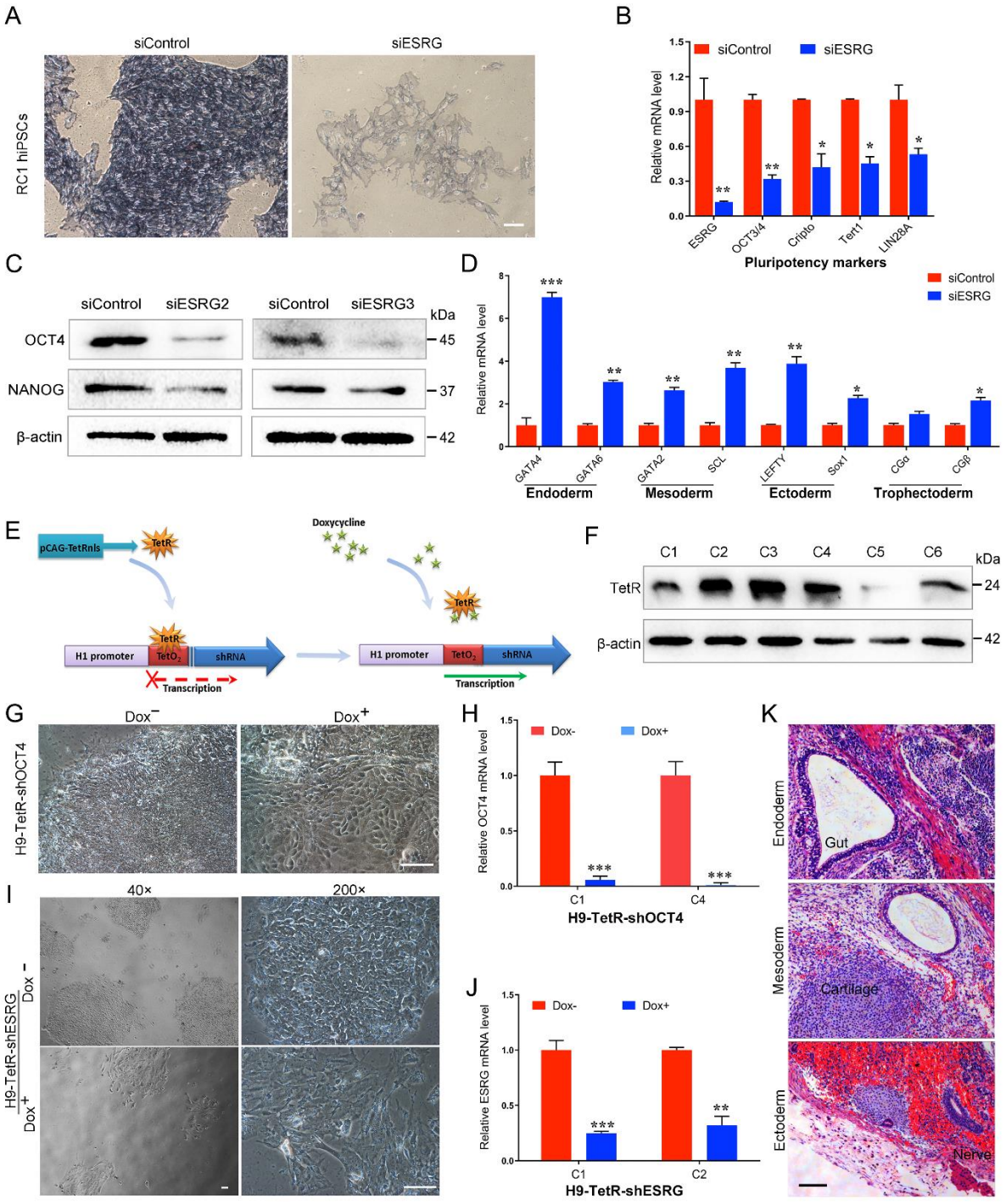

Fig. S2. *ESRG* maintains pluripotency of hPSCs.

(A) AP staining was performed in RC1 hiPSCs transfected with si*ESRG* or siControl, and the dark blue color indicated undifferentiated, AP-positive cells. Scale bar, 100 μm. **(B)** The expression of pluripotency marker genes was performed by qPCR in RC1 hiPSCs transfected with si*ESRG* or siControl. **(C)** Protein levels of OCT4 and NANOG were detected by

Western blot analysis after transfected with si*ESRG*2/si*ESRG*3 and siControl in H9 hESCs. **(D)** The expression of endoderm, mesoderm, ectoderm and trophectoderm marker genes was analyzed by qPCR in RC1 hiPSCs transfected with si*ESRG*. **(E)** Schematic diagram of the Tet-inducible shRNA interference system. Cells co-transfected with Tet repressor (TetR protein) expression vector pCAG-TetRnls and tetracycline-regulated pSUPERIOR vector can constitutively express TetR protein, which binds to the $TetO_2$ sequence in the H1 promoter of the pSUPERIOR and represses the transcription of shRNA. Upon addition of Dox, Dox binds to TetR and causes a conformational change that renders TetR unable to bind to the $TetO_2$ sequence, which allows transcription of the shRNA. **(F)** Western blot analysis of the expression of TetR protein in different H9-TetR subclones. **(G)** Phase images of H9-TetR-shOCT4 cells treated with or without Dox for 5 days. A morphological differentiation in Dox-treated H9-TetR-shOCT4 cells was observed. Scale bar, 100 μm. **(H)** QPCR analysis of OCT4 gene expression in two H9-TetR-shOCT4 subclones treated with or without Dox for 5 days. **(I)** Phase images of H9-TetR-sh*ESRG* cells treated with or without Dox for 5 days. A morphological differentiation in Dox-treated H9-TetR-sh*ESRG* cells was observed. Images were taken at 200× and 40× magnifications. Scale bar, 100 μm. **(J)** QPCR analysis of *ESRG* expression in four H9-TetR-sh*ESRG* subclones treated with or without Dox for 5 days. **(K)** HE staining of teratoma from H9-TetR-sh*ESRG* cells transplanted into NSG mice without Dox treatment. Data are presented as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ (two-tailed Student's *t* test).

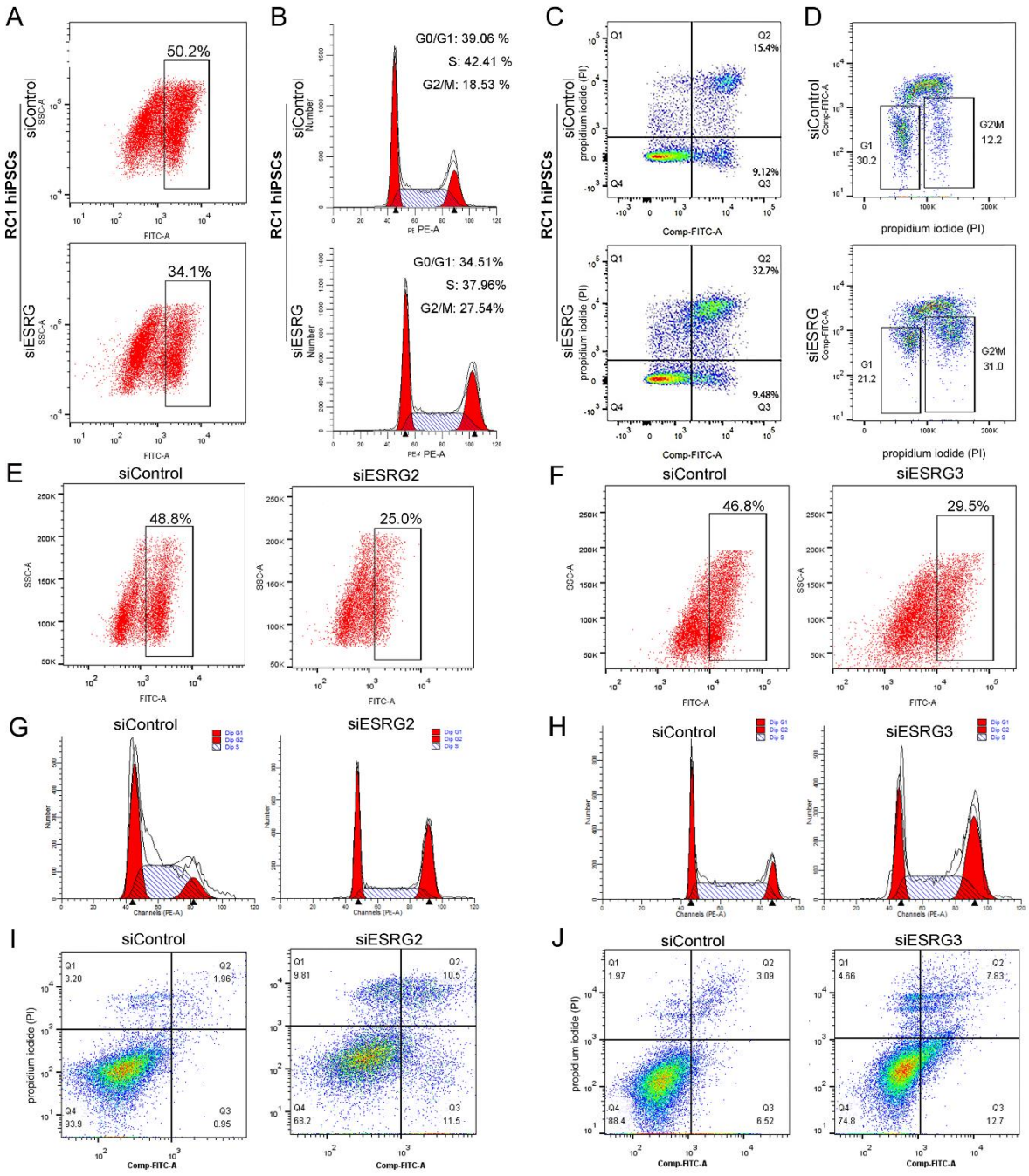

Fig. S3. *ESRG* is essential for hPSC self-renewal.

(A-C) EdU assay (A), cell cycle (B) and apoptosis assay (C) were analyzed by flow cytometry in RC1-hiPSCs transfected with si*ESRG* or siControl at 48 h after transfection**. (D)** The cell cycle distribution of H9 hESCs transfected with si*ESRG* or siControl was analyzed via flow cytometry with EdU/PI staining. **(E-J)** EdU assay (E and F), cell cycle (G and H) and apoptosis assay (I and J) were performed by flow cytometry in H9 hESCs transfected

with si*ESRG*2/si*ESRG*3 or siControl at 48 h after transfection.

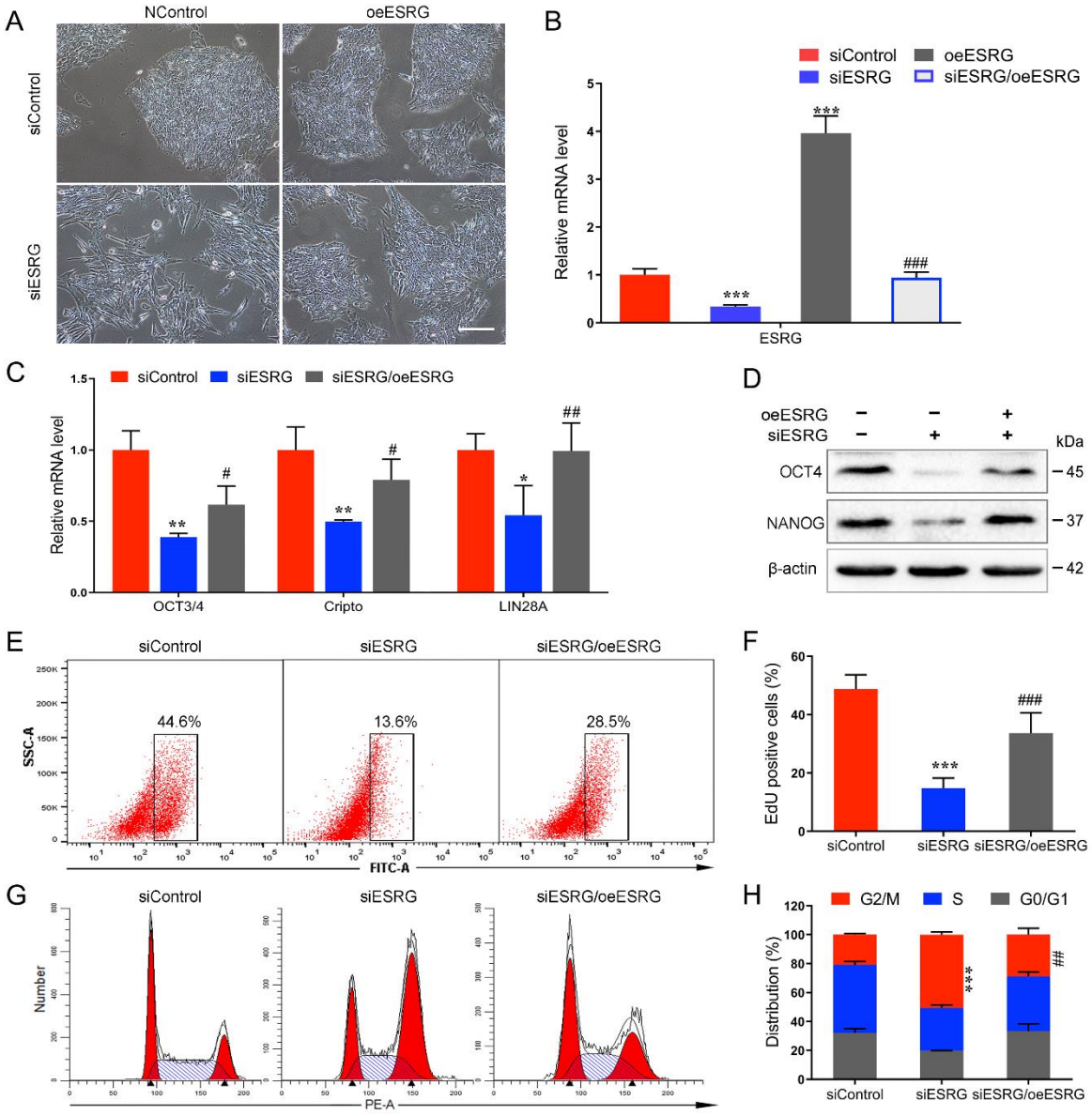

Fig. S4. Overexpression of *ESRG* rescued the morphological changes caused by *ESRG* knockdown and reversed the loss of pluripotency and the inhibition of cell proliferation.

(A) *ESRG* overexpression rescued the morphological changes induced by *ESRG* knockdown. oe, overexpression. Scale bar, 100 μm. **(B)** H9 hESCs were treated with *ESRG* adenovirus after *ESRG* knockdown, and the mRNA expression level of *ESRG* was detected by qPCR. (**C** and **D)** *ESRG* overexpression rescued the mRNA (C) and protein levels (D) of pluripotency marker genes reduced by *ESRG* knockdown. Scale bar, 100 μm. **(E-H)** *ESRG* overexpression rescued the altered cell proliferation (E) and cell cycle distribution (G) induced by *ESRG*

knockdown. The quantified analysis of EdU incorporation and cell cycle distribution are shown in (F) and (H). Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (si*ESRG* group versus siControl group) and #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 (si*ESRG*/oeMCM2 group versus si*ESRG* group) (two-tailed Student's *t* test).

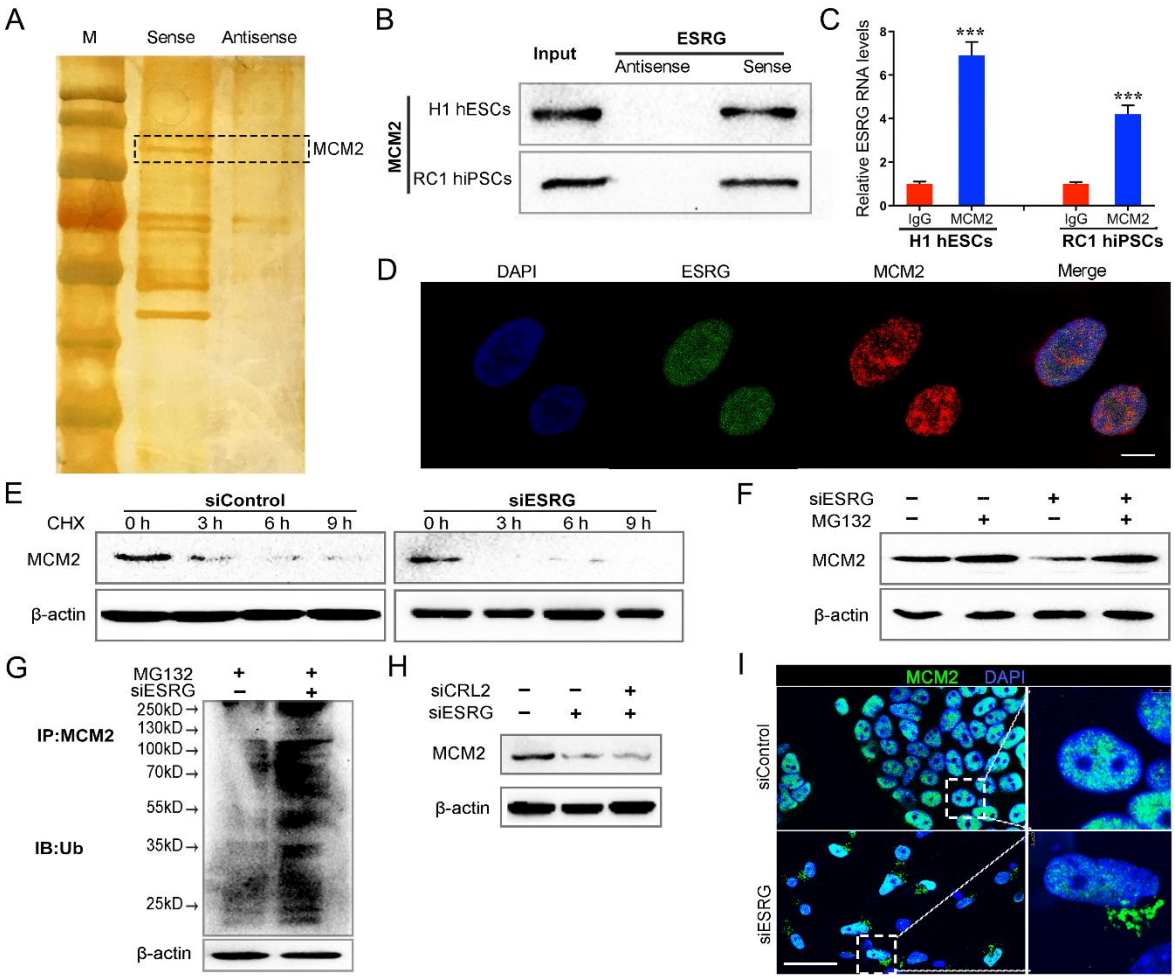

Fig. S5. *ESRG* directly binds with MCM2 in hPSCs.

(A) Biotin-RNA pulldown was performed with protein extracts of hESCs using full-length *ESRG* transcript (sense) and antisense RNA, followed by mass spectrometry. **(B** and **C)** The interaction of *ESRG* and MCM2 was detected through RNA pulldown (B) and RIP assays (C) in H1 hESCs and RC1-hiPSCs. **(D)** *ESRG* was visualized by RNA-FISH, and MCM2 by immunofluorescence staining in H9 hESCs was performed. Scale bar, 100 μm. **(E)** The expression of MCM2 in RC1-hiPSCs (siControl vs si*ESRG*) was detected by Western blot after treatment with CHX (20 μg/mL) for various time periods respectively. **(F)** RC1-hiPSCs were transfected with *ESRG* siRNA and pre-incubated with MG-132 (20 μM) for 4 h. Cell lysate was immunoblotted by anti-MCM2. **(G)** RC1-hiPSCs were pre-incubated with MG-132 (20 μM) for 4h. MCM2 was immunoprecipitated (IP) and immunoblotted (IB) by anti-Ub. The ubiquitination of MCM2 protein was detected after *ESRG* knockdown. **(H)** H9

cells were treated with CRL2 siRNA followed by *ESRG* knockdown. MCM2 protein expression was detected by Western blot. **(I)** MCM2 was visualized in RC1-hiPSCs treated with siControl and si*ESRG* by immunofluorescence staining. Scale bar, 20 μm. Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (two-tailed Student's *t* test).

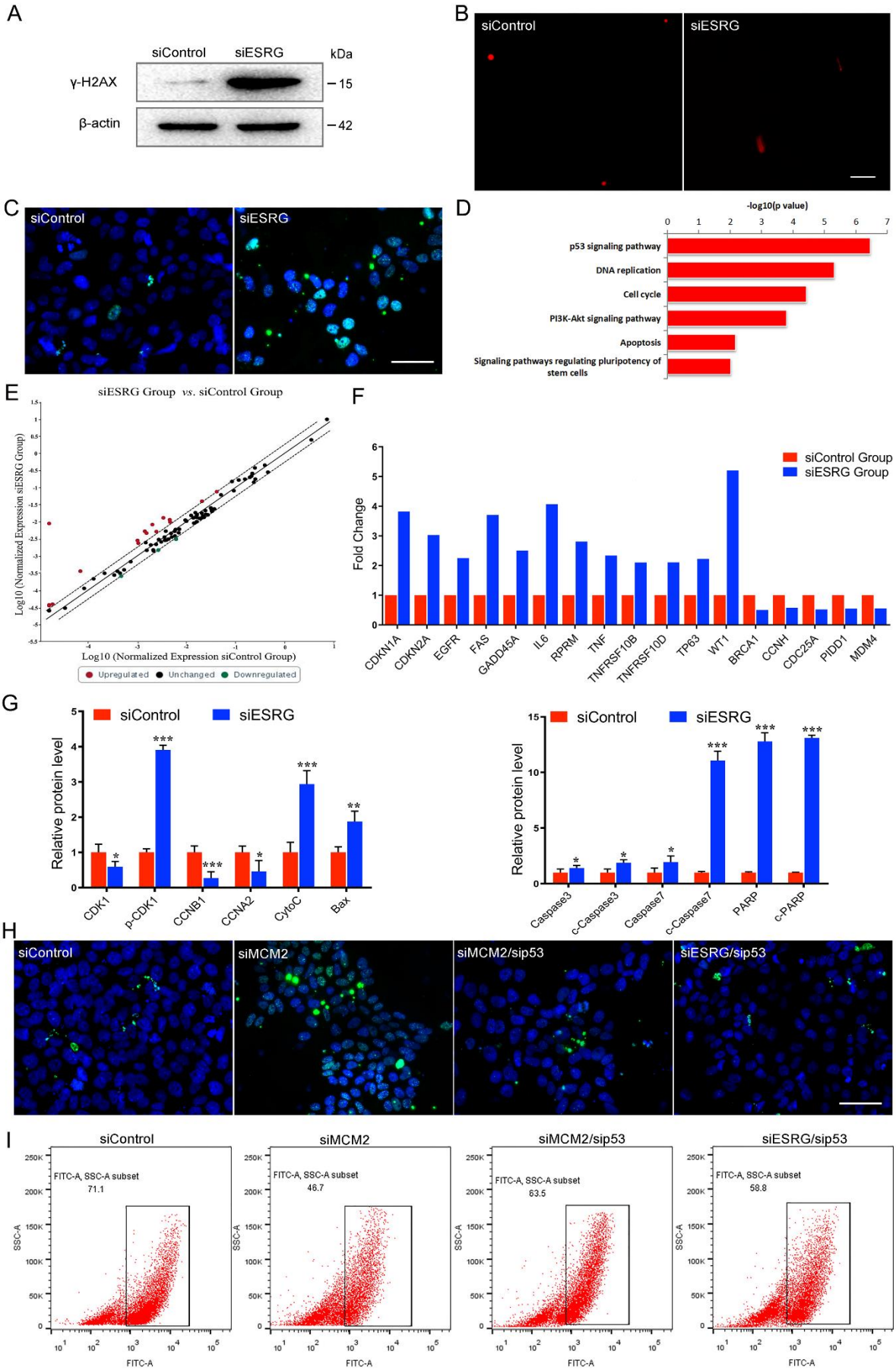
A
siControl
siESRG
kDa
γ-H2AX
15
β-actin
42
B
siControl
siESRG
C
siControl
siESRG
D
-log10(p value)
0 1 2 3 4 5 6 7
p53 signaling pathway
DNA replication
Cell cycle
PI3K-Akt signaling pathway
Apoptosis
Signaling pathways regulating pluripotency of stem cells
E
siESRG Group vs. siControl Group
Log10 (Normalized Expression siESRG Group)
Log10 (Normalized Expression siControl Group)
Upregulated
Unchanged
Downregulated
F
siControl Group
siESRG Group
Fold Change
CDKN1A
CDKN2A
EGFR
FAS
GADD45A
IL6
RPRM
TNF
TNFRSF10B
TNFRSF10D
TP63
WT1
BRCA1
CCNH
CDC25A
PIDD1
MDM4
G
siControl
siESRG
Relative protein level
CDK1
p-CDK1
CCNB1
CCNA2
CytoC
Bax
siControl
siESRG
Relative protein level
Caspase3
c-Caspase3
Caspase7
c-Caspase7
PARP
c-PARP
H
siControl
siMCM2
siMCM2/sip53
siESRG/sip53
I
siControl
FITC-A, SSC-A subset
71.1
siMCM2
FITC-A, SSC-A subset
46.7
siMCM2/sip53
FITC-A, SSC-A subset
63.5
siESRG/sip53
FITC-A, SSC-A subset
58.8
SSC-A
FITC-A

Fig. S6. *ESRG*-MCM2 functions in hPSCs by suppressing p53 signaling pathway.

(A) Western blot was performed to analyze the protein levels of γ-H2AX in RC1-hiPSCs treated with siControl and si*ESRG*. **(B)** Representative pictures of comet assay performed 48 h after RC1-hiPSCs treated with siControl and si*ESRG*. Scale bar, 200 μm. **(C)** γH2AX expression was detected by immunofluorescence staining after *ESRG* knockdown in RC1-hiPSCs. Scale bar, 100 μm. **(D)** The top KEGG pathway enrichments for differentially expressed genes between si*ESRG* and siControl by mRNA microarray. **(E)** The expression levels of 84 genes related to p53-mediated signal transduction were evaluated using a RT Profiler PCR Array in *ESRG* knockdown and control groups. Scatter plots indicate at least a two-fold up-regulated (red) or down-regulated (green) gene in the *ESRG* knockdown groups relative to the control group. **(F)** The fold changes in expression of these genes in the *ESRG* knockdown groups relative to the control group are shown in the column chart. **(G)** Quantification of proteins and statistical analyses of Fig. 6H. **(H-I)** H9 hESCs were transfected with p53 siRNA followed by MCM2/*ESRG* knockdown. γ-H2AX protein expression was detected by immunofluorescence staining (H) and EdU assay was performed by flow cytometry (I). Scale bar, 100 μm. Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (two-tailed Student's *t* test).

Table S1. RNA-pulldown identified *ESRG* binding Proteins.

Protein names	Descriptions
ACO2	Aconitate hydratase, mitochondrial
APEH	Acylamino-acid-releasing enzyme
ASNS	Asparagine synthetase [glutamine-hydrolyzing]
BSG	Basigin
CASP14	Caspase-14
CBR1	Carbonyl reductase [NADPH] 1
CBS	Cystathionine beta-synthase
CCT7	T-complex protein 1 subunit eta
CDC37	Hsp90 co-chaperone Cdc37
CSTA	Cystatin-A
EIF2S1	Eukaryotic translation initiation factor 2 subunit 1
EIF3C	Eukaryotic translation initiation factor 3 subunit C
ENO2	Gamma-enolase
FLII	Protein flightless-1 homolog
FSCN1	Fascin
GANAB	Neutral alpha-glucosidase AB
GART	Trifunctional purine biosynthetic protein adenosine-3
GMPS	GMP synthase [glutamine-hydrolyzing]
GOT1	Aspartate aminotransferase, cytoplasmic
GSPT2	Eukaryotic peptide chain release factor GTP-binding subunit ERF3B
GSTP1	Glutathione S-transferase P
H2AFJ	Histone H2A.J
HBA1	Hemoglobin subunit alpha
HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1

HSPA1L	Heat shock 70 kDa protein 1-like
HSPH1	Heat shock protein 105 kDa
IPO5	Importin-5
LAP3	Cytosol aminopeptidase
LARS	Leucine--tRNA ligase, cytoplasmic
LMNB1	Lamin-B1
LRPPRC	Leucine-rich PPR motif-containing protein, mitochondrial
MCCC1	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial
MCM2	DNA replication licensing factor MCM2
MTHFD1	C-1-tetrahydrofolate synthase, cytoplasmic
NACA	Nascent polypeptide-associated complex subunit alpha, muscle-specific form
NME2	Nucleoside diphosphate kinase B
NPM1	Nucleophosmin 1
P4HB	Protein disulfide-isomerase
PC	Pyruvate carboxylase, mitochondrial
PCMT1	Protein-L-isoaspartate(D-aspartate) O-methyltransferase
PDIA6	Protein disulfide-isomerase A6
PEBP1	Phosphatidylethanolamine-binding protein 1
PHB2	Prohibitin-2
PMPCB	Mitochondrial-processing peptidase subunit beta
PODXL	Podocalyxin
PSMB5	Proteasome subunit beta type-5
PSMD11	26S proteasome non-ATPase regulatory subunit 11
RAB8A	Ras-related protein Rab-8A
RANGAP1	Ran GTPase-activating protein 1
RRBP1	Ribosome-binding protein 1
RTN4	Reticulon-4
RUVBL1	RuvB-like 1
SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
SEPT9	Septin-9

SF1	Splicing factor 1
SF3A1	Splicing factor 3A subunit 1
SF3B3	Splicing factor 3B subunit 3
SH3GL2	Endophilin-A1
SLC3A2	4F2 cell-surface antigen heavy chain
ST13	Hsc70-interacting protein
STIP1	Stress-induced-phosphoprotein 1
TCP1	T-complex protein 1 subunit alpha
TMED10	Transmembrane emp24 domain-containing protein 10
TUBA1C	Tubulin alpha-1C chain
TXNDC5	Thioredoxin domain-containing protein 5
UBE2NL	Putative ubiquitin-conjugating enzyme E2 N-like
UBQLN2	Ubiquilin-2
UQCRC1	Cytochrome b-c1 complex subunit 1, mitochondrial
USO1	General vesicular transport factor p115
VIL1	Villin-1
WDR1	WD repeat-containing protein 1

Table S2. Gene names of enriched pathways in the differentially altered p53-target genes in hPSCs upon *ESRG* knockdown.

Term	*P*-value	Genes
p53 signaling pathway	3.48*E*-07	BID, STEAP3, ZMAT3, CHEK1, SFN, SESN2, CCNG2, CCNE2, CCNE1, CASP9, SERPINE1, RCHY1, THBS1, LOC651610, CDK1, CYCS, CDK6, ATM, CCNB1, CDKN1A, PPM1D, TNFRSF10B, RRM2, BAX, GADD45G, DDB2, MDM4, GADD45B, PERP, GADD45A
DNA replication	4.91*E*-06	SSBP1, LIG1, POLE, POLA1, POLA2, MCM2, MCM3, MCM4, RNASEH2C, MCM5, MCM6, RPA1, RFC5, POLD3, DNA2, RFC3, POLE2, RFC2, PRIM2, PCNA, FEN1
Cell cycle	3.82*E*-05	CDC14B, PRKDC, CHEK1, SFN, CCNE2, CCNE1, CDC45, ORC4L, CDKN2D, CCNA1, MYC, CUL1, STAG1, TFDP1, LOC651610, CDK1, CDC6, RBL1, ANAPC4, CDK6, ESPL1, MCM2, MCM3, MCM4, ORC1L, MCM5, CDC25A, WEE1, ATM, MCM6, CCNB1, CDKN1C, CDKN1A, CDKN1B, GADD45G, PCNA, LOC731751, ORC5L, ANAPC7, GADD45B, GADD45A

Table S3. Primer sequences for qPCR.

Genes	Sequences (5′-3′) Forward	Sequences (3′-5′) Reverse
GAPDH	GATGACATCAAGAAGGTGGTGA	GTCTACATGGCAACTGTGAGGA
ESRG	CTCCCCAGAACATCTCCAGAA	TCTGGACATTGTCCTTCCAACT
Oct3/4	TTCAGCCAAACGACCATCTG	CACGAGGGTTTCTGCTTTGC
Cripto	TACCTGGCCTTCAGAGAT	CCAGCATTTACACAGGGAACAC
FoxD3	CTAGTGAAGCCGCCTTACTCGTA	GAAGCAGTCGTTGAGTGAGAGGTT
Tert1	TGTGCACCAACATCTACAAG	GCGTTCTTGGCTTTCAGGAT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
Nanog	TGAACCTCAGCTACAAACAG	TGGTGGTAGGAAGAGTAAAG
LIN28A	CGGGCATCTGTAAGTGGTTC	CAGACCCTTGGCTGACTTCT
LIN28B	CATCTCCATGATAAACCGAGAGG	GTTACCCGTATTGACTCAAGGC
DESMIN	GCACGCCCTCCTCCTAC	GCAGCTCCACCTTCTCGT
JMJD5	GGAGCAGTTTTTGGTTCCAGG	GGCTCATTCACGATGTATTTGC
Wee1	CCTGGGTAGCTCTTTCTCG	TTGCGGAAGGTCTTGTGT
GATA4	AAGCCCAAGAACCTGAATAAATC	TGGCGTTGCTGGAGTTG
GATA6	CCATGACTCCAACTTCCACC	ACGGAGGACGTGACTTCGGC
OPN	GCCGAGGTGATAGTGTGGTT	TGAGGTGATGTCCTCGTCTG
AFP	TTGGGCTGCTCGCTATG	TTTGTAACTGTTGCTGCCTTTG
SOX17	CGCCGAGTTGAGCAAGA	TTCAGCCGCTTCACCTG
GATA2	CAGACGACAACCACCACCTTATG	TGGTCAGTGGCCTGTTAACATTG
NESTIN	TGCGGGCTACTGAAAAGTTC	TGTAGGCCCTGTTTCTCCTG
SCL	CGGCAGCGGGTTCTTTG	CCCGGCTGTTGGTGAAGATAC

HAND1	TCAGCCTTGCCCGGACTCTC	AGGTTCATGTTGGAGCGGCTAC
MSX1	CCAGAAGATGCGCTCGTCAAAG	CGGCTTACGGTTCGTCTTGTG
LEFTY	CTGGACAGGGCCGACATG	GGCCACCTCTCGGAAGCT
SOX1	ATACGTTTATTTCAGCAGCCTTAGG	TCCAGGACAAGGAAGGGTGTT
PAX6	GGCTAGCGAAAAGCAACAGA	TGGTATTCTCTCCCCCTCCT
CGα	CTTTCTGCATGTTCTCCATTC	GTGGACTCTGAGGTGACGT
CGβ	TCACCGTCAACACCACCATC	AGAGTGCACATTGACAGCTG
p53	AAGTCTGTGACTTGCACGTACTCC	GTCATGTGCTGTGACTGCTTGTAG
p21	ACCTGGAGACTCTCAGGGTCG	TTAGGGCTTCCTCTTGGAGAAGAT
GADD45A	GGATGCCCTGGAGGAAGTGCT	GGCAGGATCCTTCCATTGAGATGAATG
DR5	CTCCTGCAAATATGGACAGGACTA	TTAGCTCCACTTCACCTGAATCAC
14-3-3σ	TGACGACAAGAAGCGCATCAT	GTAGTGGAAGACGGAAAAGTTCA

Table S4. Antibodies used in this study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-OCT4	Abcam	Cat# ab181577; RRID: AB_2687916
Anti-NANOG	Abcam	Cat# ab109250; RRID: AB_10863442
Anti-gamma H2A.X(phospho S139)	Abcam	Cat# ab81299; RRID: AB_1640564
Anti-MCM2	Proteintech	Cat# 10513-1-AP; RRID: AB_2142131
Anti-Flag Tag	Sigma-Aldrich	Cat# F1804 RRID: AB_262044
Anti-p53(DO-1)	Santa Cruz Biotechnology	Cat# sc-126; RRID:AB_628082
Anti-p21	Cell Signaling Technology	Cat# 2947 RRID: AB_823586
Anti-DR5	Proteintech	Cat#15497-1-AP; RRID: AB_2240702
Anti-14-3-3 zeta/delta (D7H5)	Cell Signaling Technology	Cat# 7413; RRID: AB_10950820
Anti-FAS	Abcam	Cat# ab82419; RRID: AB_1658628

Anti-SSEA4	Abcam	Cat# ab16287; RRID: AB_778073
Anti-TRA-1-60	Santa Cruz Biotechnology	Cat# sc-21705; RRID: AB_628385
Anti-Ub(P4D1)	Santa Cruz Biotechnology	Cat# sc-8017; RRID: AB_2762364
Anti-Cleaved Caspase3 (Asp175)	Cell Signaling Technology	Cat# 9664; RRID: AB_2070042
Anti-Caspase3	Cell Signaling Technology	Cat# 9665; RRID: AB_2069872
Anti-Cleaved Caspase7 (Asp198)	Cell Signaling Technology	Cat# 8438; RRID: AB_11178377:
Anti-Caspase7	Cell Signaling Technology	Cat# 12827; RRID: AB_2687912
Anti-CDK1	Abcam	Cat# ab18; RRID: AB_2074906
Anti-CDK1 (phospho Y15)	Abcam	Cat# ab47594 RRID: AB_869073
Anti-Cleaved PARP	Cell Signaling Technology	Cat# 5625; RRID: AB_10699459
Anti- PARP	Cell Signaling Technology	Cat# 9542; RRID: AB_2160739

Anti-Phospho p53 (Ser15)	Cell Signaling Technology	Cat# 9284; RRID: AB_331464
Anti-Wee1(D10D2)	Cell Signaling Technology	Cat# 13084; RRID: AB_2713924
Anti-GADD45Aα(D17E8)	Cell Signaling Technology	Cat# 4632; RRID: AB_10544538
Anti-LIN28A	Abcam	Cat# ab46020; RRID: AB_776033
Anti-TetR	Novus Biologicals	Cat# NB600-234; RRID: AB_10001361
Anti-Histone H3	Proteintech	Cat# 17168-1-AP; RRID: AB_2716755
Anti-Cyclin B1 (CCNB1)	Cell Signaling Technology	Cat# 4138; RRID: AB_2072132
Anti-Cyclin A2 (CCNA2)	Proteintech	Cat# 18202-1-AP; RRID: AB_10597084
Anti-BAX	Proteintech	Cat# 50599-2-Ig; RRID: AB_2061561
Anti-Cytochrome C (Cyto-C)	Thermo Fisher Scientific	Cat# MA5-11674; RRID: AB_10985701
Anti-ATM	Cell Signaling Technology	Cat# 2873; RRID: AB_2062659

Anti-ATM (phospho S1981)	Abcam	Cat# ab81292; RRID: AB_1640207
Anti-TRAIP	Proteintech	Cat# 10332-1-AP; RRID: AB_10638481
Anti-GAPDH	Abcam	Cat# ab181602; RRID: AB_2630358
Anti-beta Actin antibody	Sigma	Cat# ab8227; RRID: AB_2305186
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor Plus 594	Thermo Fisher Scientific	Cat# A-21203; RRID: AB_2535789
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21206; RRID: AB_2535792