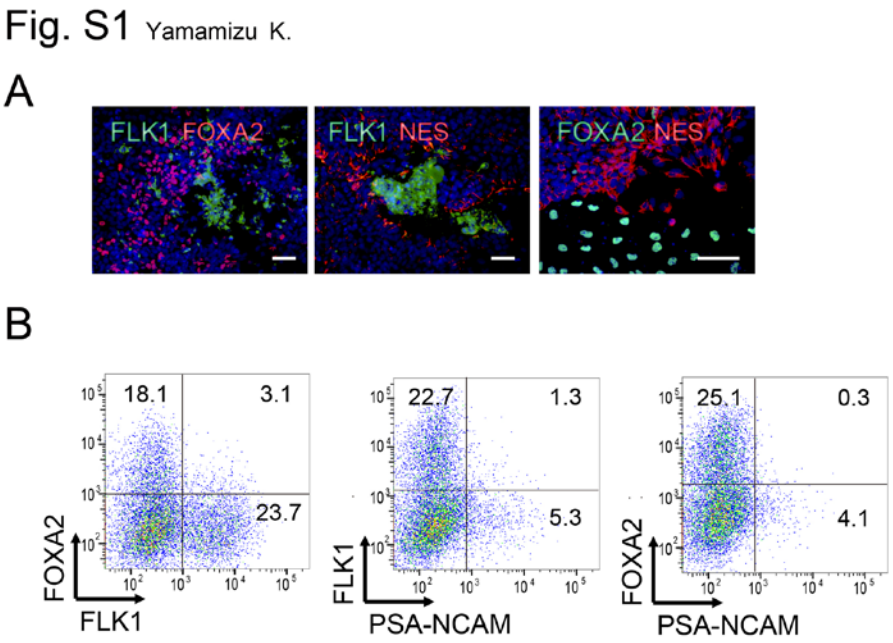

Fig. S1. Markers of different germ layers did not colocalize in the same cells. (A) Microscopic images of ESCs cultured for 5 days in the differentiation condition and stained with the combination of markers: FLK1 (mesoderm), FOXA2 (endoderm), or NES (ectoderm). Scale bar: 200 µm. (B) Examples of FACS analyses: ESCs cultured for 5 days in the differentiation condition and stained for the combination of markers: $FLK1^+$ and $FOXA2^+$, $FLK1^+$ and $PSA\text{-}NCAM^+$, or $FOXA2^+$ and $PSA\text{-}NCAM^+$.

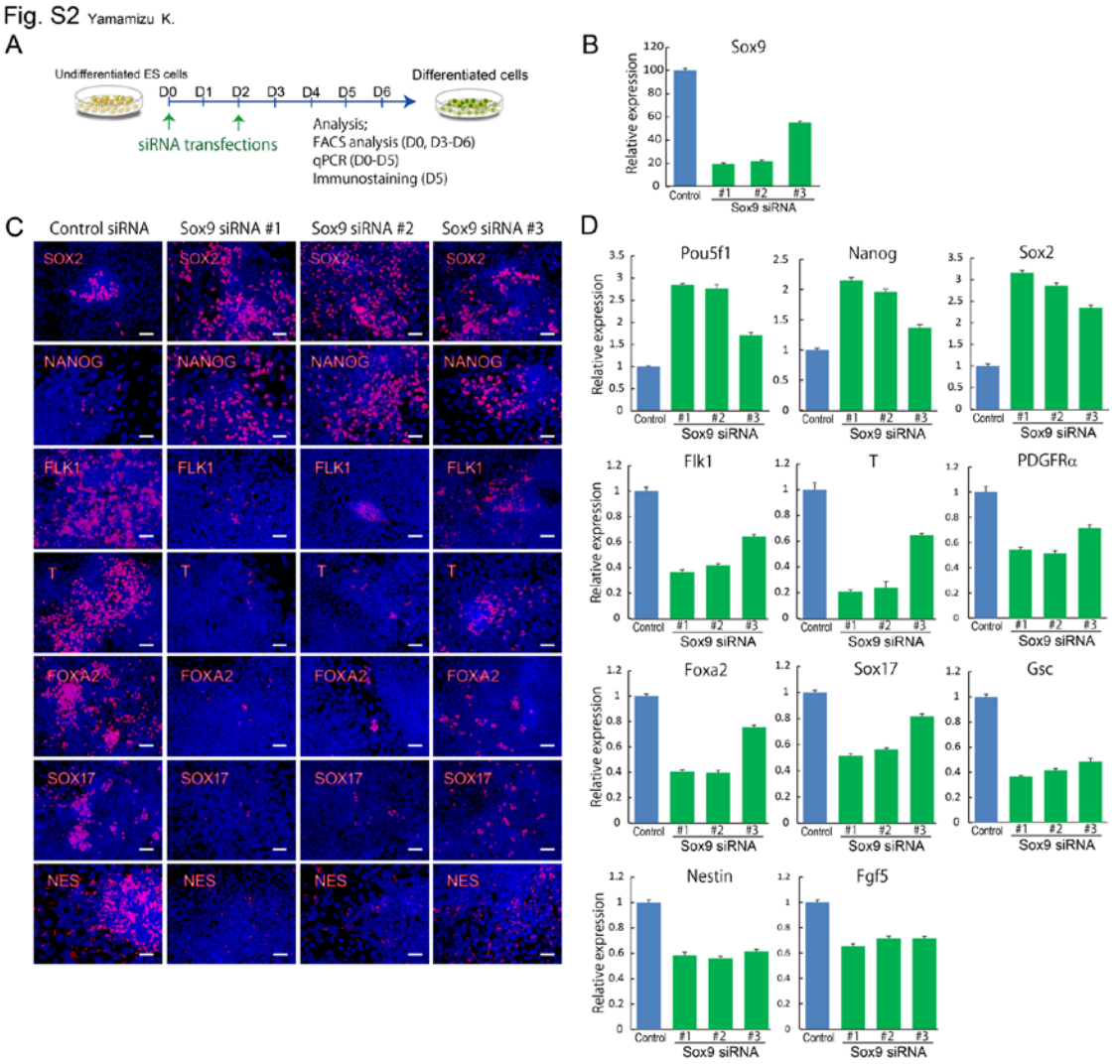

Fig. S2. Blocking the activation of endogenous *Sox9* inhibits the differentiation of ESCs into three germ layers. (A) Schematic diagram showing an experimental design. (B) qPCR analysis showing the mRNA expression of *Sox9* after treating cells with three independent siRNAs against *Sox9* or control. (C) Microscopy images of ESCs cultured for 5 days after treating with three independent siRNAs against Sox9 or control and stained with pluripotency markers (SOX2, NANOG), mesoderm markers (FLK1, T), endoderm markers (FOXA2, SOX17) and an ectoderm marker (NES). Scale bar: 200 µm. (D) qPCR analyses showing the mRNA expression of pluripotency markers (*Pou5f1*, *Nanog*, *Sox2*), mesoderm markers (*Flk1*, *T*, *PDGFRα*), endoderm markers (*Foxa2*, *Sox17*, *Gsc*) and ectoderm markers (*Nestin*, *Fgf5*) in ESCs cultured for 5 days after treating three independent siRNAs against Sox9 or control.

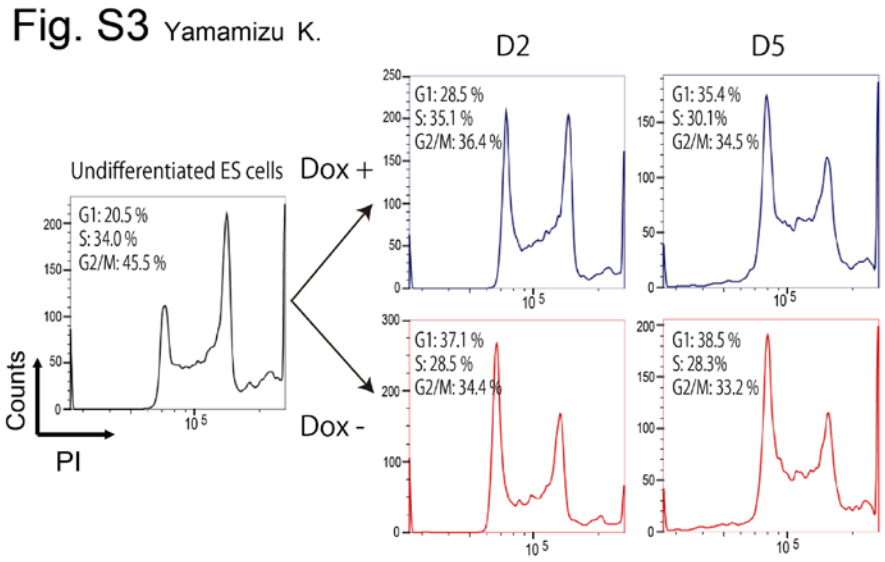

Fig. S3. Cell cycle profiles of Sox9-inducible ESCs by FACS analysis. FACS analysis showing cell cycle profiles of Sox9-inducible ESCs cultured for 2 days (D2) or 5 days (D5) in the Dox+ (control) or Dox– condition.

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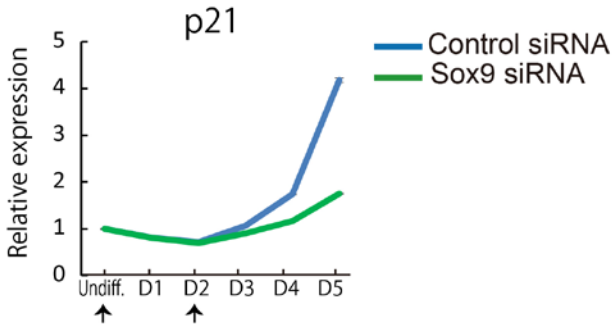

Fig. S4. Blocking the activation of endogenous Sox9 with an siRNA against Sox9 inhibits the increase of p21 expression that normally occurs from D4. qPCR analyses showing the expression changes of p21. Arrows show the timing (D0 and D2) of treatment of siRNA against Sox9 or control.

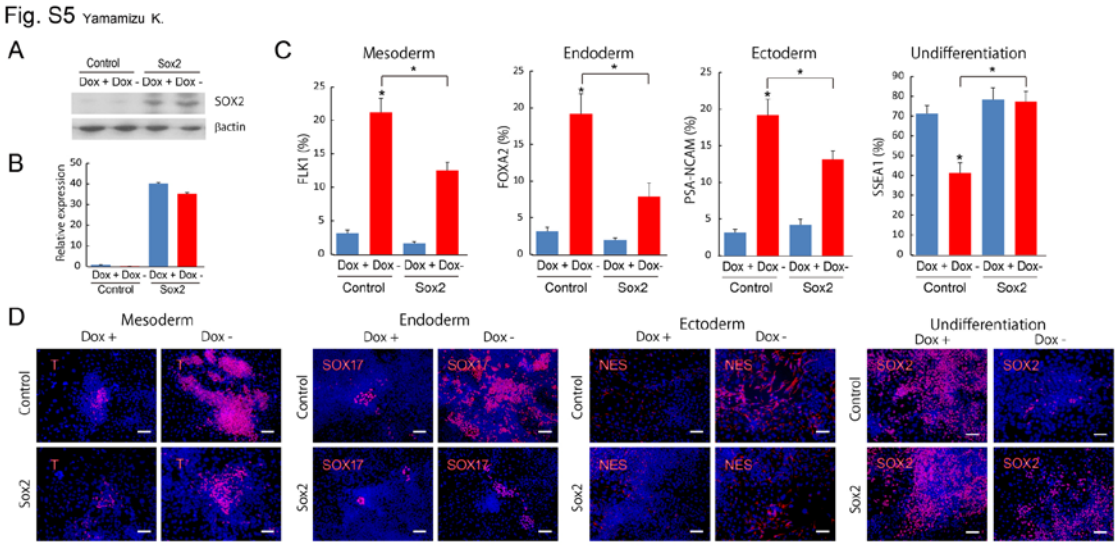

Fig. S5. Overexpression of *Sox2* prevents ESCs from differentiating into three germ layers, even when the exogenous *Sox9* was overexpressed. (A) Western blots detecting SOX2 and β-actin in Sox9-inducdible ESCs cultured for 4 days in the Dox+ (control) or Dox– condition. (B) A qPCR analysis showing the mRNA expression of *Sox2* in Sox9-inducible ESCs cultured for 4 days in the Dox+ (control) or Dox– condition after transfecting with a Sox2-expressing vector or a control vector. (C) Summary of FACS analyses: Sox9-inducible ESCs cultured for 4 days ($FLK1^+$, $FOXA2^+$ or $SSEA1^+$) or 6 days ($PSA\text{-}NCAM^+$) after transfecting with a Sox2-expressing vector or a control vector and stained for $FLK1^+$, $FOXA2^+$, $SSEA1^+$, or $PSA\text{-}NCAM^+$. (D) Microscopic images of Sox9-inducible ESCs cultured for 4 days after transfecting with a Sox2-expressing vector or a control vector and immunostained for T (mesoderm), SOX17 (endoderm), NES (ectoderm) and SOX2 (undifferentiation). Scale bar: 200 μm.

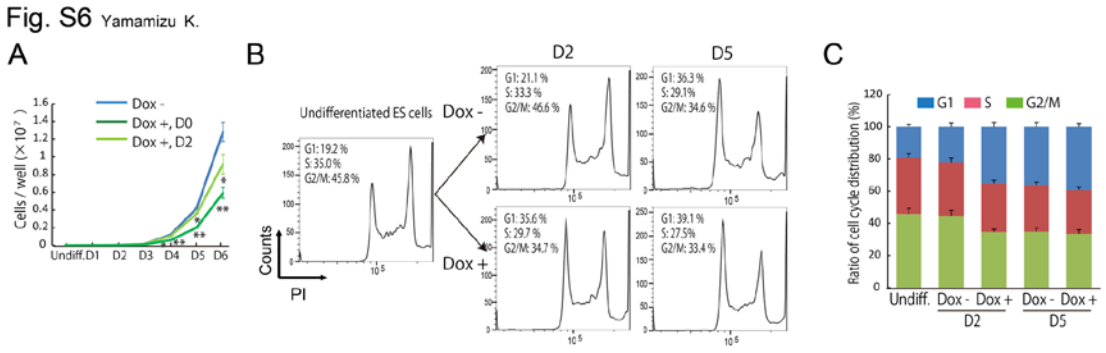

Fig. S6. Cell cycle profile of Sox2-repressible ESCs by FACS analysis. (A) Growth profiles of Sox2-repressible ESCs cultured in the Dox– (control), protocol 1 (Sox2-repressed from D0), or protocol 2 (Sox2-repressed from D2) (three independent experiments, SEM; **P*<0.05, ***P*<0.01 versus Dox+). (B,C) Cell cycle profiles of Sox2-repressible ESCs cultured for 2 days (D2) or 5 days (D5) in the Dox– (control) or Dox+ condition and analyzed by FACS (three independent experiments).

Supplementary material Table S1: Primer list for qPCR (related to Figs 2, 4-6)

Oct3/4 F	GGA CAT GAA AGC CCT GCA GAA
Oct3/4 R	GAC AGA TGG TGG TCT GGC TGA A
Nanog F	GAA TTC TGG GAA CGC CTC ATC
Nanog R	CCT TGT CAG CCT CAG GAC TTG
Sox2 F	AAC CGA TGC ACC GCT ACG A
Sox2 R	TGC TGC GAG TAG GAC ATG CTG
T F	GAA CAG CTC TCC AAC CTA TG
T R	AGA CTG GGA TAC TGG CTA GAG
Flk1 F	GGG ATG GTC CTT GCA TCA GAA
Flk1 R	ACT GGT AGC CAC TGG TCT GGT TG
PDGFRa F	CTT TGT GCC TCT CGG GAT GA
PDGFRa R	AGG TTA CTT GAG TCT CCG GAT CTG
Foxa2 F	GTC GTC CGA GCA GCA ACA TC
Foxa2 R	GGG TAG TGC ATG ACC TGT TCG TAG
Sox17 F	GGA CAC GAC TGC GGA GTG AA
Sox17 R	GGT CGG CAA CCG TCA AAT G
Gsc F	CCC GGT TCT GTA CTG GTG TC
Gsc R	AGC TGC TCA TCG GTG AAG AT
Nestin F	GGG CCA GCA CTC TTA GCT TTG ATA
Nestin R	TGA GCC TTC AGG GTG ATC CAG
Fgf5 F	AGT GTG CAG CGT CCA CAG AGA
Fgf5 R	TCC TAG TGT ATG CTT GGT GGA CAG A
Sox9 F	CTG AAG GGC TAC GAC TGG AC
Sox9 R	TAC TGG TCT GCC AGC TTC CT
p21 F	TTG CAC TCT GGT GTC TGA GC
p21 R	TCT GCG CTT GGA GTG ATA GA
p27 F	AGT CAG CGC AAG TGG AAT TT
p27 R	AGT AGA ACT CGG GCA AGC TG
p53 F	AAC CGC CGA CCT ATC CTT AC
p53 R	CTT CTG TAC GGC GGT CTC TC
Rb F	AGA GAG AAC GCC ACG AAA AA
Rb R	GAT GGC TGA TCA CTT GCA GA
GAPDH F	TGT GTC CGT CGT GGA TCT GA
GAPDH R	TTG CTG TTG AAG TCG CAG GAG

Supplementary material Table S2: Primer list for ChIP (related to Figs 6,7)

Sox2-5' UTR F	CCC ATT TAT TCC CTG ACA GC
Sox2-5' UTR R	TGT GAT TAG TTT TTG GAA AGG
Sox2-SRR2 F	ATT TAT TCA GTT CCC AGT CCA AGC
Sox2-SRR2 R	CCC TCT CCC CCC ACG C
Nanog-promoter F	CAA CTT ACT AAG GTA GCC CGA GTC TTA A
Nanog-promoter R	CCT CCA AAA GTG CGG CTT T