

### Supplemental Table 1

Primer sequences for semiquantitative RT-PCR

Gene	Primer sequence (5' to 3')	Product size
Oct3/4	F: TCACTCACATCGCCAATCAG R: CCTGTAGCCTCATACTCTTCTC	305 bp
Sox2	F: CTACAGCATGTCCTACTCGC R: CCTCCCAATTCCCTTGTATCTC	351 bp
Nanog	F: TTCAGAAATCCCTTCCCTCG R: AGTAGCAGACCCTTGTAAGC	162 bp
Pax6	F: GTACCAGTGTCTACCAGCCAATC R: ACGAGTATGAGGAGGTCTGACTG	192 bp
Nestin	F: CTGGAAGTGGCTACATACAGGAC R: AGTCTCAAGGGTATTAGGCAAGG	210 bp
Brachyury	F: GAAGTGAAGGTGGCTGTTGG R: ATTTACCTTCAGCACCGGGA	298 bp
Tbx2	F: GACATCTTGAAGCTCCCATACAG R: GCTCCTCATACAAACGGAGAGT	208 bp
Gata 4	F: GTGAGCCTGTATGTAATGCC R: CTGTGCCCATAGTGAGATGAC	274 bp
Gata 6	F: GGGAGAACTGTGACAATGAC R: ACGAACGCTTGTGAAATGTG	166 bp

## SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. CIBZ-knockdown ESCs remain undifferentiated. (A, B) Two siRNA duplexes were used to down-regulate the expression of CIBZ in ESCs. At 2 d after transfection with 50 nM *CIBZ* siRNA (siRNA-1 and -2) or 50 nM scrambled negative siRNA (NC), expression of the indicated proteins and genes was detected by Western blotting (A) and semiquantitative RT-PCR (B), respectively.  $\alpha$ -tubulin and *GAPDH* served as loading controls for immunoblotting and RT-PCR, respectively. Results shown are representative of four independent experiments. (C) Representative morphology of CIBZ knockdown and scrambled control ESCs cultured in the presence of STO feeder cells. (D) Semiquantitative RT-PCR analysis shows the expression of lineage-specific genes in CIBZ knockdown and scrambled siRNA ESCs. Results shown are representative of three independent experiments.

FIGURE S2. siRNA-mediated CIBZ knockdown in ESCs results in a reduction of cell number. Analysis of cell growth. Cells ( $4 \times 10^5$ ) were transiently transfected with *CIBZ* siRNA and scrambled siRNA and seeded into 6-well plate. Cell number was counted before and after seeding at 2 d after transfection. Results are mean  $\pm$  S.E. of three independent experiments (n=6 in total, \* $p < 0.05$ ).

FIGURE S3. siRNA-mediated CIBZ knockdown in ESCs inhibits their proliferation. (A, B) Decreased BrdU incorporation in *CIBZ* siRNA-1 or scrambled siRNA ESCs. Cells were harvested at day 2 after transfection, stained for BrdU and counterstained with DAPI. (A) Representative fluorescence microscopy images of BrdU- or DAPI-positive cells. (B) The percentage of BrdU- and DAPI-positive cells. The data shown are representative of 10 randomly chosen fields. Results are mean  $\pm$  S.E. of three independent experiments. \* $p < 0.05$ .

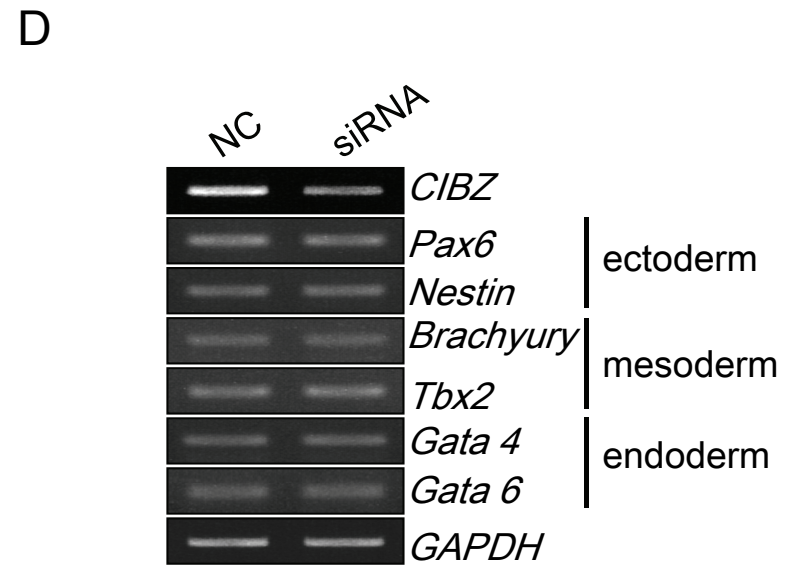
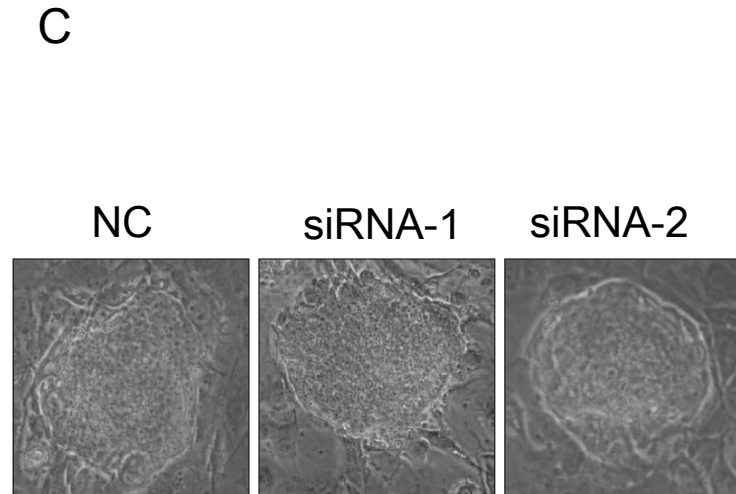
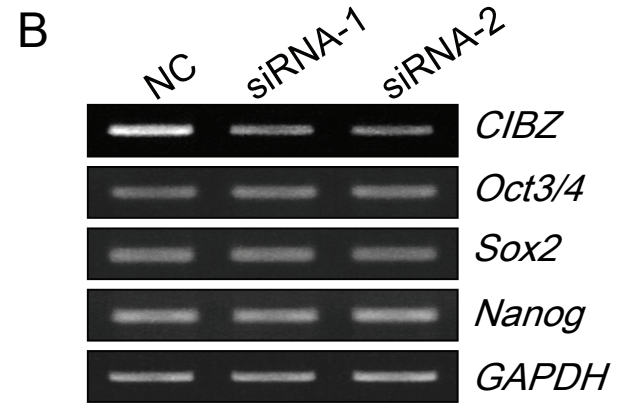
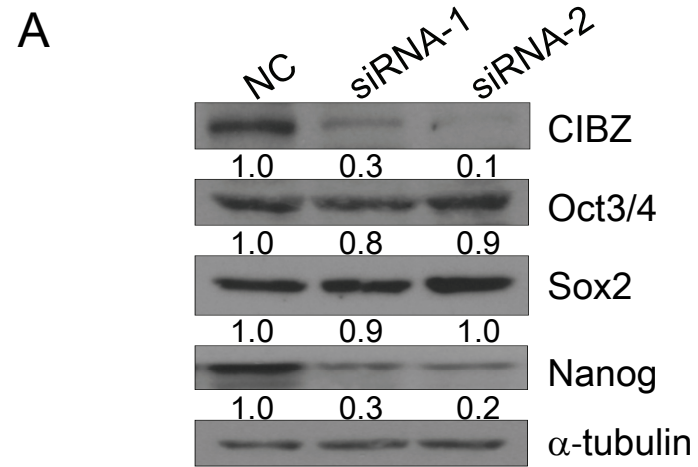
FIGURE S4. CIBZ deletion in ESCs does not induce apoptosis. (A) Western blot analysis. Total cell lysates from *CIBZ*<sup>-/-</sup> ESCs and control ESCs were isolated at day 3, and subjected to immunoblotting with the indicated antibodies. (B) Apoptosis analyzed by annexin V-FITC/PI staining. *CIBZ*<sup>-/-</sup> ESCs were stained by annexin V-FITC, counterstained by PI, and then analyzed by flow cytometry with acquisition of 20,000 events/sample. The percentages of annexin V-FITC+/PI+ cells or annexin V-FITC+/PI- cells were indicated. Data are means of three independent experiments.

FIGURE S5. CIBZ knockdown in ESCs does not induce apoptosis. (A) After transfection of *CIBZ* siRNA-1 or scrambled siRNA in ESCs for 2 d, total cell lysates were isolated and subjected to immunoblot analysis with the indicated antibodies. (B) Apoptosis analyzed by annexin V-FITC/PI staining. After transfection of *CIBZ* siRNA-1 or scrambled siRNA in ESCs for 2 d, cells were stained by annexin V-FITC, counterstained by PI and then analyzed by flow cytometry with acquisition of 20,000 events/sample. The percentages of annexin V-FITC+/PI+ cells or annexin V-FITC+/PI- cells were indicated. Data are means of three independent experiments.

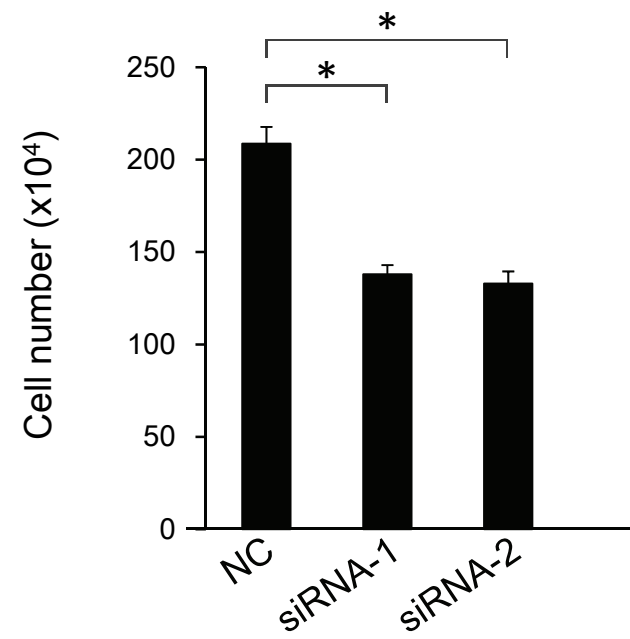
FIGURE S6. CIBZ knockdown leads delayed G1/S transition. (A, B) ESCs were transfected with *CIBZ* siRNA-1 and scrambled siRNA for 2 d. Cells were then collected and analyzed by flow cytometry. (A) Representative flow cytometry profiles show percentages in each stage of the cell cycle. (B) Graphic representation of the percentage of cells in each phase. Results are mean  $\pm$  S.E. of four independent experiments. \* $p < 0.05$ .

FIGURE S7. CIBZ knockdown results in a decreased expression of Cyclin E and Cdk2. Western blotting analysis showed the expression of the indicated proteins in *CIBZ* siRNA-1-transfected ESCs and scrambled siRNA-transfected ESCs.

# Supplemental Fig. S1

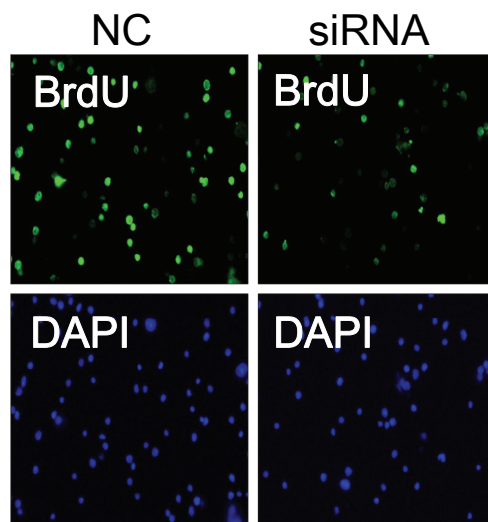


Supplemental Fig. S2

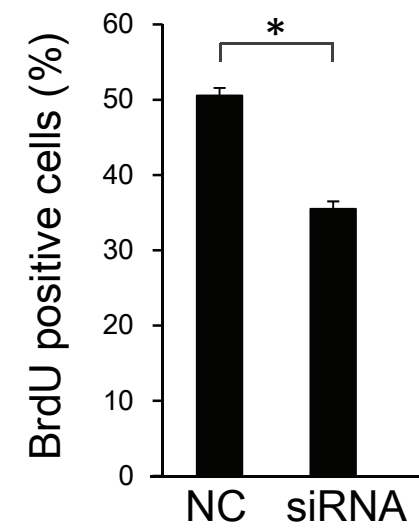


# Supplemental Fig. S3

A

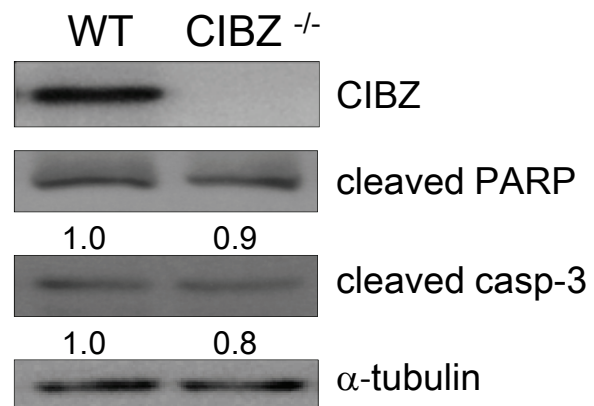


B

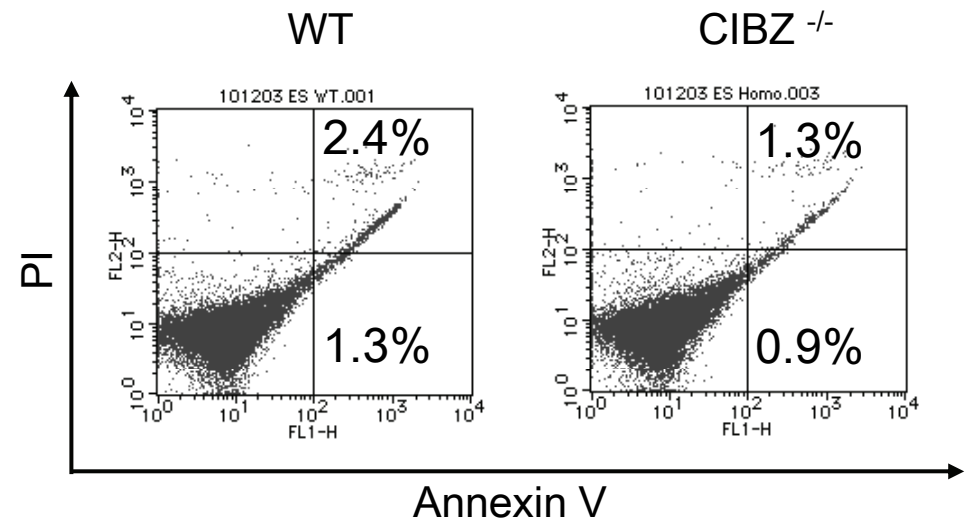


# Supplemental Fig. S4

A

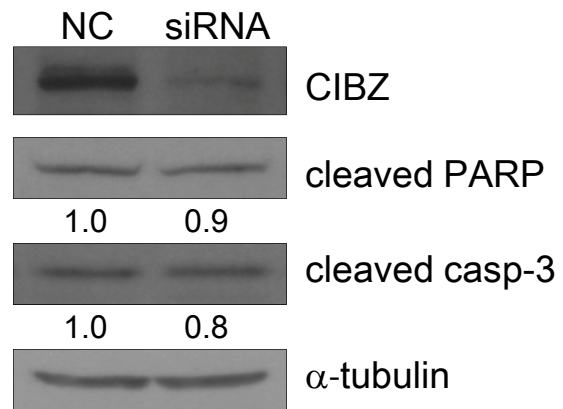


B

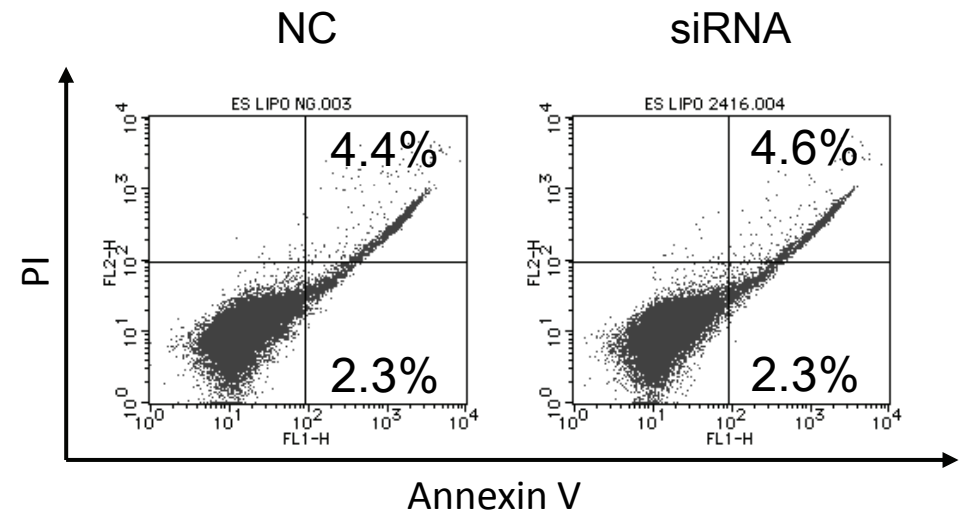


# Supplemental Fig. S5

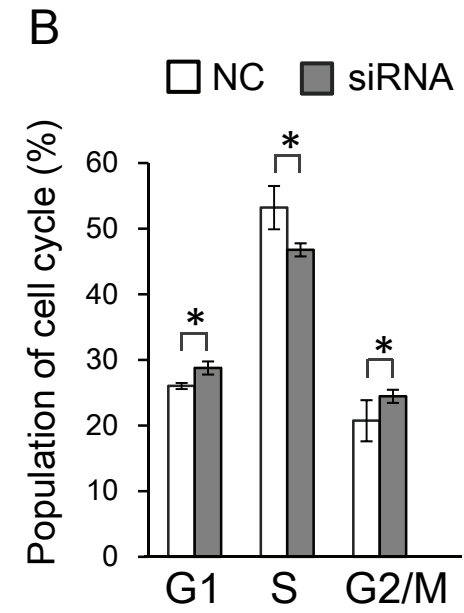
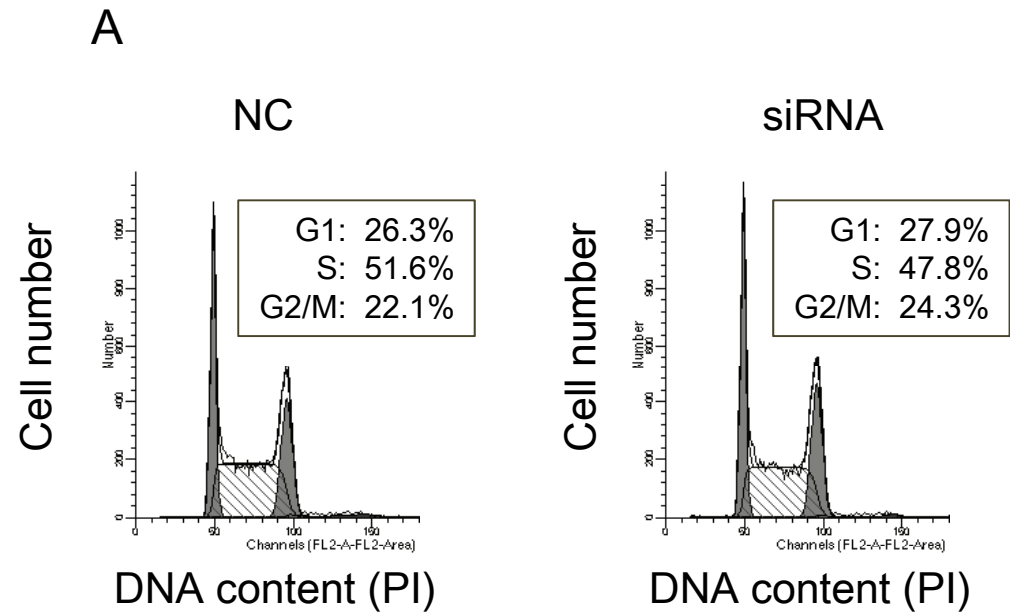
A



B



# Supplemental Fig. S6





Supplemental Fig. S7

