**Stem Cell Reports**

**Supplemental Information**

# Successful Reprogramming of Epiblast Stem Cells by Blocking Nuclear Localization of β-Catenin

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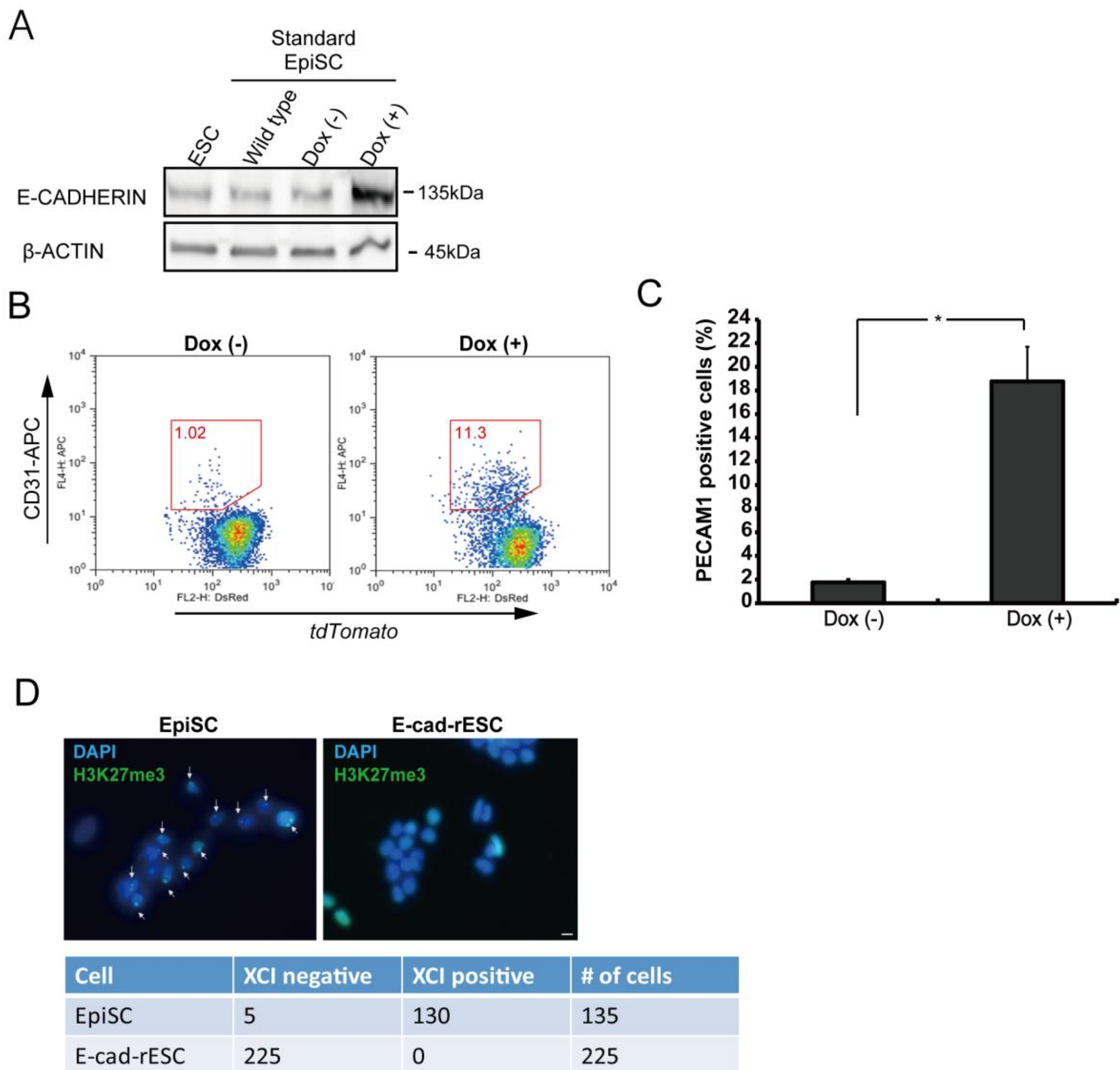



| Cell | XCI negative | XCI positive | # of cells |
|---|---|---|---|
| EpiSC | 5 | 130 | 135 |
| E-cad-rESC | 225 | 0 | 225 |

**Figure S1. *E-cadherin* overexpression in 'standard' mouse EpiSCs, related to Figure 1**

(A) Western blotting analyses for E-CADHERIN in mouse EB3DR ES cells (ESCs), 'standard' mouse epiblast stem cells (EpiSCs), and 'standard' mouse EpiSCs into which *E-cadherin* had been introduced via the tet-on system, cultured with or without doxycycline (2 ug / ml) for 2 days (Dox(+), Dox(-)). β-ACTIN was used as a loading control. (B) Shown are the percentages of CD31-expressing cells after culturing with or without doxycycline (2 ug / ml) for 7 days in the presence of LIF. (C) Proportion of PECAM1-expressing cells related to (B). Dox treatment significantly increased PECAM1-expressing cell frequency (mean ± SEM of three independent experiments, $*p < 0.05$). (D) Photomicrographs, immunocytochemical preparations (H3K27me3), 'standard' EpiSCs and E-cad-rESCs. White arrows indicate inactivated X-chromosomes (XCI).

Scale bar, 10 um.

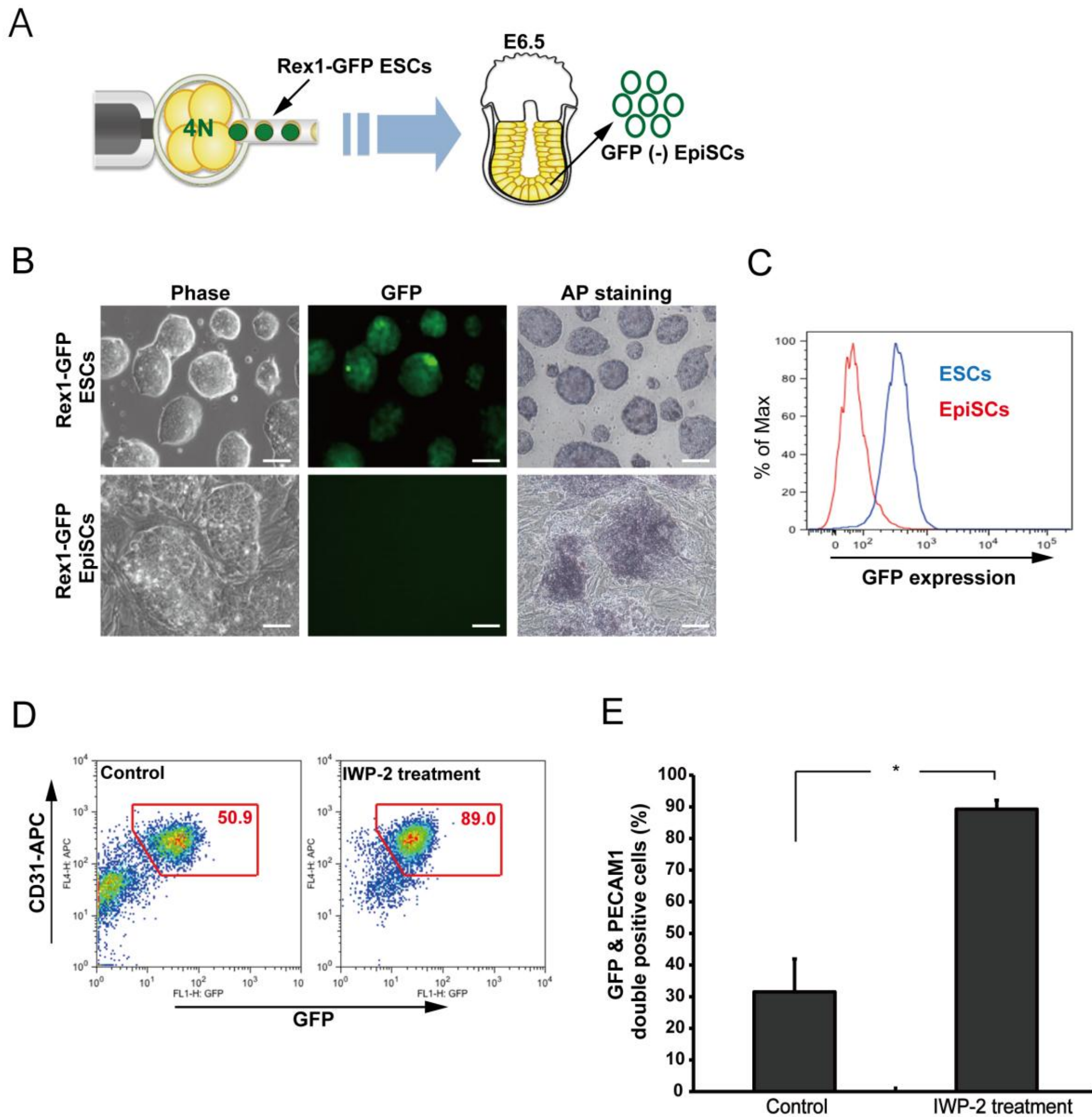
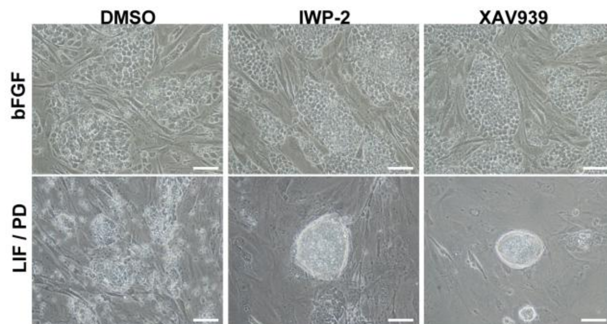



**Figure S2. IWP-2 treatment in Rex1-GFP EpiSCs, related to Figure 2**

(A) Schematic diagram of generation of mouse Rex1-GFP EpiSCs. Rex1-GFP ES cells were injected into tetraploid (4N) embryos. Rex1-GFP EpiSCs were derived from E6.5 epiblast. (B) Morphology (left) and GFP expression (center) in mouse Rex1-GFP ESCs and Rex1-GFP EpiSCs. Alkaline phosphatase (AP) staining was also performed (right). Scale bar, 50 um. (C) Flow-cytometric analysis of Rex1-GFP expression level in mouse ESCs and EpiSCs. (D) Flow-cytometric analyses in Rex1-GFP EpiSCs treated with LIF and PD (control), or LIF, PD, and IWP-2, for 7 days. The cells were stained with anti-CD31 antibody. (E) Average of Rex1-GFP and PECAM1-expressing ("double-positive") cells related to (D). Double-positive cell frequency was significantly increased by IWP-2 treatment (mean ± SEM of three independent experiments, *p <

0.05).



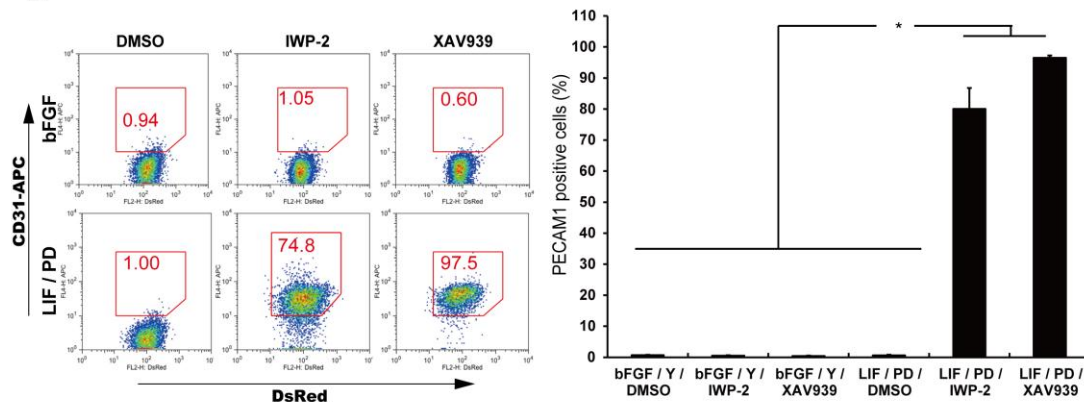
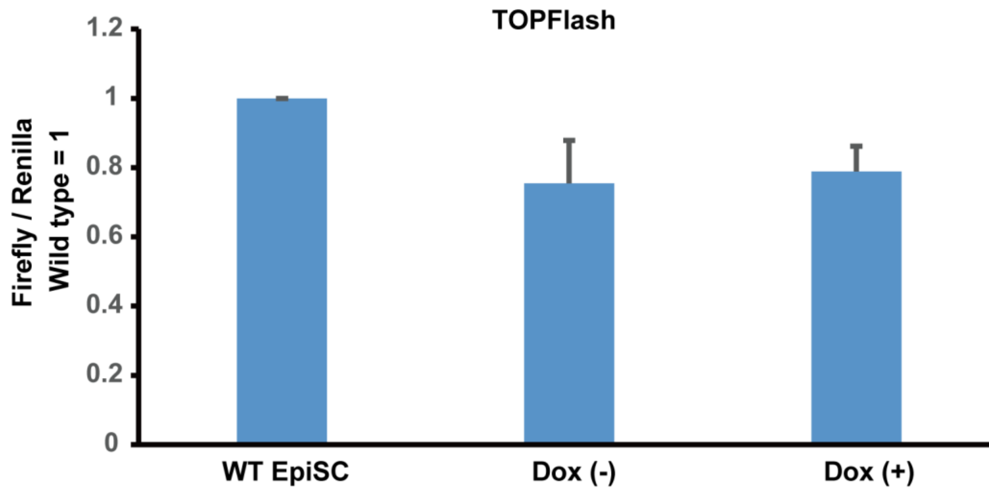



**Figure S3. XAV939 treatment in mouse EpiSCs, related to Figure 3**

(A) Appearances of chemically treated mouse EpiSCs (various conditions) for 7 days. Note that EpiSCs treated with LIF / PD and with IWP-2 or XAV formed similar naïve PSC-like colonies. Scale bar, 50 um. (B) Flow-cytometric analyses to detect CD31 expression. EpiSCs were cultured under various conditons for 7 days, then stained with anti-CD31 antibody (left panel). Average numbers of PECAM1-expressing cells were significantly increased in cells treated with LIF / PD and IWP-2 or XAV939 (right panel; mean ± SEM of three independent experiments, *$p < 0.05$). (C) Live-born chimeric mice were derived from wit-rESCs established from XAV939-treated EB3DR EpiSCs. The pups lacking DsRed expression are non-chimeric

littermates.

A




B

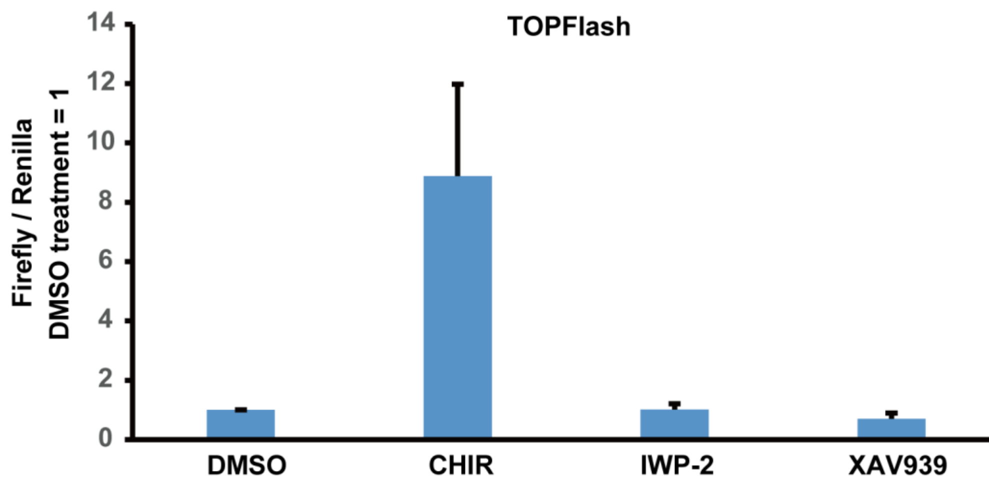



**Figure S4. TOPFlash assay for EpiSCs, related to Figure 4**

(A) TOPFlash assay for EB3DR EpiSCs, and EB3DR EpiSCs into which *E-cadherin* had been introduced via the tet-on system, cultured with or without doxycycline (2 ug / ml) for 2 days (Dox(+), Dox(-)). Data represent mean ± SEM of three independent experiments. (B) TOPFlash assay for EB3DR EpiSCs cultured with each of the inhibitors CHIR (3uM), IWP-2 (5nM), and XAV939 (10uM) for 2 days. Data represent mean ± SEM of three independent experiments.

**Table S1.**

**Primer sequences for RT-PCR, related to Figure 4**

| Gene | Forward primer (5' to 3') | Reverse primer (5' to 3') |
|---|---|---|
| *Stella* | GCAATCTTGTTCCGAGCTAG | CTGGATCGTTGTGCATCCTA |
| *Rex1* | CGTGTAACATACACCATCCG | GAAATCCTCTTCCAGAATGG |
| *Oct4* | TCTTTCCACCAGGCCCCCGGCTC | TGCGGGCGGACATGGGGAGATCC |
| *GAPDH* | TGCACCACCAACTGCTTAG | GGATGCAGGGATGATGTTC |

**Table S2.**

**Efficiency of chimera formation, related to Figures 1, 3**

| Cell type | Injected Cell line (culture condition) | Transferred embryos | Analyzed stage | Pups (Fetus) | Chimeras | Chimera rate | Associated figure |
|---|---|---|---|---|---|---|---|
| ESC | EB3DR ESC (LIF/PD/CHIR) | 48 | E13.5 embryo | 26 | 15 | 57.7 % | |
| | EB3DR ESC (LIF/PD/IWP-2) | 36 | Neonate | 15 | 7 | 46.7 % | |
| | EB3DR ESC (LIF/PD/XAV939) | 40 | Neonate | 17 | 8 | 47.1 % | |
| EpiSC | EB3DR EpiSC | 40 | E9.5 embryo | 26 | 0 | 0.0 % | |
| | E-cad OE EpiSC (Day2, bFGF) | 40 | E13.5 embryo | 22 | 0 | 0.0 % | |
| | E-cad OE EpiSC (Day2, LIF) | 40 | E13.5 embryo | 21 | 0 | 0.0 % | |
| rESC | E-cad-rESC | 44 | Neonate | 18 | 5 | 27.8 % | Figure 1I, 1J |
| | wit-rESC (IWP-2 treatment) | 14 | E13.5 embryo | 2 | 1 | 50.0 % | Figure 3B |
| | wit-rESC (IWP-2 treatment) | 40 | Neonate | 26 | 11 | 42.3 % | Figure 3C |
| | wit-rESC (XAV939 treatment) | 36 | Neonate | 16 | 5 | 31.3 % | Figure S3C |

## Supplemental Experimental Procedures

### Immunocytochemical detection of X-chromosome inactivation, related to Figure S1.

X-chromosome inactivation (XCI) was detected by staining with anti-H3K27me3 antibody (1:200 dilution; Abcam, Cambridge, UK) in accordance with the manufacturer's instructions; inactivated X-chromosomes marked.

### Generation of mouse Rex1-GFP EpiSCs by tetraploid injection, related to Figure S2.

Production of tetraploid embryos was performed as described (Nagy et al., 1993). In brief, mouse 2-cell stage embryos were collected in M2 medium (Millipore, Bedford, MA) from the oviduct of BDF1 x C57BL/6 mice 1.5 dpc. These embryos were first equilibrated in fusion medium consisting of 0.28 M mannitol (D-mannitol; Sigma-Aldrich, St. Louis, MO), 0.15 mM $MgSO_4$, and 0.01% polyvinylalcohol (Sigma-Aldrich) for 1 min and were then transferred to the fusion chamber. The blastomeres were fused by a short electric pulse (100 V for 30 usec) applied by a pulse generator (ECM 2001 Electro Cell Manipulator; BTX, San Diego, CA). The fused, tetraploid embryos were cultured in KSOM-AA medium (Millipore) for 24 h after fusion; most developed to the four-cell stage. For micromanipulation, Rex1-GFP ESCs were trypsinized and were suspended in culture medium. A piezo-driven micromanipulator (Prime Tech, Tokyo, Japan) was used under the microscope to introduce 7 Rex1-GFP ESCs per embryo into tetraploid embryos. After injection, embryos underwent follow-up culture for 1 to 2 h, and embryos were transferred into the uteri of pseudopregnant recipient ICR mice (2.5 dpc). Rex1-GFP ESC-derived epiblasts were collected at 6.5 dpc. Dissected epiblast was then plated on MEF feeder-coated dishes. Following culture with ESM plus bFGF, EpiSC colonies were observed. Medium was changed every 1 to 2 days.

### Staining for alkaline phosphatase (AP) activity, related to Figure S2.

Alkaline phosphatase (AP) activity was stained by 1-Step NBT/BCIP (Thermo) according to the manufacturer's instructions.

### TOPFlash assay, related to Figure S4.

TCF / LEF transcriptional activity was detected by TOPFlash assay. M50 Super 8x TOPFlash plasmid (Addgene plasmid 12456; Addgene, Cambridge, MA) or M51 Super 8x FOPFlash plasmid (Addgene plasmid 12457; Addgene ) were co-transfected with the Renilla expression vector (pRL-TK Vector; Promega, Madison, WI).

Luminescence measurement was performed 2 days after transfection by Dual-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. Luciferase activity was

calculated as the ratio of Firefly (FOPFlash) : Renilla to Firefly (TOPFlash) : Renilla luminescence for each well, and sample-well ratios were normalized to control-well ratios (Wild type EpiSCs or DMSO-treated EpiSCs).

### Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM). Student's two-tailed non-paired t-test was used to determine the statistical significance of differences. Significant differences were defined as $^*p < 0.05$.

## Supplemental References

Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W., and Roder, J.C. (1993). Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America *90*, 8424-8428.