Supplementary information, Data S1 Materials and Methods

Culture of embryonic stem cells (ESCs)

IVP-ES1 mouse ESCs were cultured under standard conditions according to previous reports [1, 2].

Preparation of embryoid bodies (EBs)

EBs were prepared according to the method of Zhang and Jin, 2009 [2]. Three hundred ES cells were suspended in ES cell culture medium with LIF for 3 days. EB colonies were subsequently transferred to 60 mm^2 dishes and further cultured for the indicated times in ES medium without LIF.

Western blotting

Western blotting was performed according to previous reports [3].

EBs and ES cells were lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, and 0.1% SDS). The protein concentration was measured with a BCA Assay Kit (Calbiochem). Then, the proteins were resolved by SDS-PAGE using a 15% gel. After electrophoresis, proteins were transferred onto PVDF membranes (Amersham) and hybridized with primary antibodies at the following dilutions: RNF20/BRE1 (Novus Biologicals; 1:2,000), RAD6 (Santa Cruz Biotechnology; 1:2,000), H2Bub (Medimabs; 1:2,000), H3K4me3 (Abcam, 1:2,000), Nanog (Santa Cruz Biotechnology, 1:2,000), Oct4 (Abcam, 1:2,000) and actin (Zhongshan Golden Bridge; 1:2,000). The HRP-labeled secondary antibodies (Zhongshan Golden Bridge) were all used at a dilution of 1:2,000. An ECL detection system (Amersham) was used to detect the signals on the membranes.

Constructs

Mouse pCAG-H2B-GFP was constructed by cloning the full-length H2B cDNA, which was amplified from mouse ES cells, into the pCAG-GFP vector. The pCAG-H2BK120R-GFP mutant plasmid was produced by introducing a mutation at lysine 120 using a mutation kit (TaKaRa).

Alkaline phosphatase (AP) staining

AP staining was performed according to the manufacturer's instructions (Cell Biolabs).

RT-PCR

The RT-PCR assay was performed according to previous studies [3]. ES cells (4×10^6) were lysed with TRIzol reagent (Invitrogen) to isolate total RNA according to the manufacturer's instructions. In total, 5 μg of total RNA was reverse transcribed into cDNA in a volume of 20 μL using reverse transcriptase M-MLV (TaKaRa). For each 25 μL PCR reaction, 1 μL of cDNA was used for 20-25 cycles. All of the PCR products were analyzed by electrophoresis in a 2% agarose gel, and the gel was stained with ethidium bromide and photographed.

Chromatin immunoprecipitation (ChIP)

ChIP analysis was performed according to the published protocols of Upstate and Ni et al., 2006 [3].

Refferences

1 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126:663-676.

2 Zhong X, Jin Y. Critical roles of coactivator p300 in mouse embryonic stem cell differentiation and Nanog expression. J Biol Chem 2009; 284:9168-9175.

3 Ni JQ, Liu LP, Hess D, Rietdorf J, Sun FL. Drosophila ribosomal proteins are associated with linker histone H1 and suppress gene transcription. Genes Dev 2006; 20:1959-1973.