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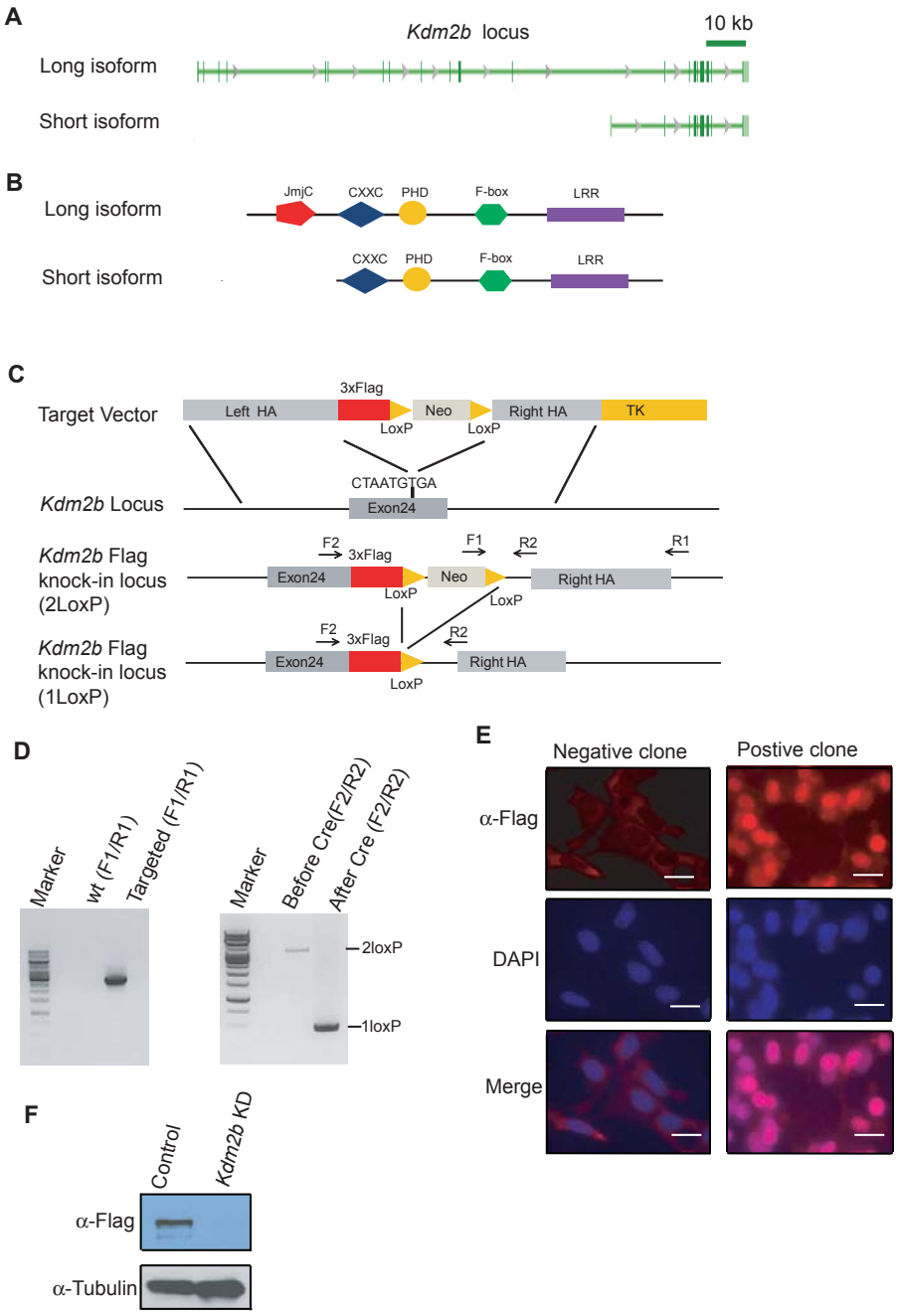



**Figure S1** Establishment of mESC line with a Flag-tag knocked into the *Kdm2b* locus. Diagrammatic illustration of the genomic structure of mouse *Kdm2b* locus. The boxes represent exons. Diagrammatic presentation of the long isoform and short isoform of Kdm2b protein with their functional domains. Schematic diagram illustrates the strategy for generating *Kdm2b* Flag-tag knock-in mESC line. The targeting vector, wild-type *Kdm2b* locus, targeted *Kdm2b* locus and targeted *Kdm2b* locus with deleted selection marker as well as the PCR primers for genotyping are indicated (HA: homologous arm; Neo: neomycin phosphotransferase; TK: thymidine kinase). Shown are the genotyping results of Kdm2b Flag-tag knock-in mESC clones. The genotyping PCR primer sets are labeled as F1, F2, R1 and R2. Immunostaining with Flag antibody revealed nuclear localization of endogenous Kdm2b-Flag fusion protein in the FLAG-tag knock-in mESC line. bars = 10μm. Western blot analysis demonstrates that Flag antibody detected the endogenous Kdm2b-Flag protein in the Flag-tag knock-in mESC line, while the signal is greatly reduced in the same mESC line with *Kdm2b* knockdown.

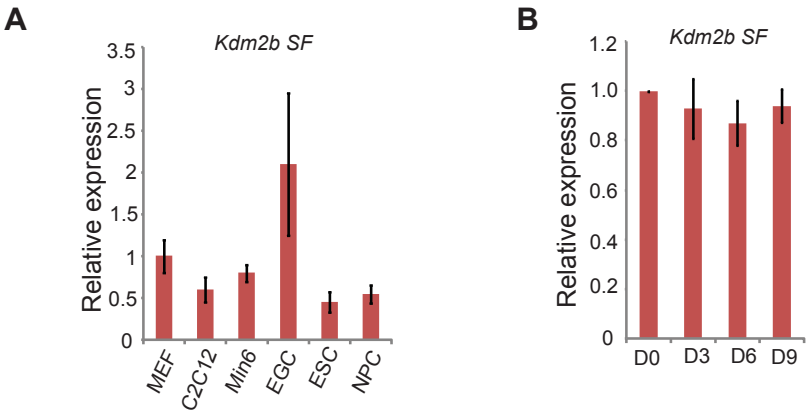



**Figure S2** Oct4 and Sox2 bind to the promoter of *Kdm2b*. Relative expression levels of *Kdm2b* short isoform in embryonic stem cells (ESC), embryonic germ cells (EGS), neural stem cells (NSC), murine embryonic fibroblasts (MEF), C2C12 myoblasts and pancreatic β cell line (Min6) measured by RT-qPCR. Values represent means ± standard deviation from three biological replicates. Relative expression levels of *Kdm2b* short isoform at different days of embryoid body differentiation measured by RT-qPCR and normalized with *Gapdh*. The mRNA level of mESCs (D0) is arbitrarily set as 1. Values represent means ± standard deviation from three biological replicates.

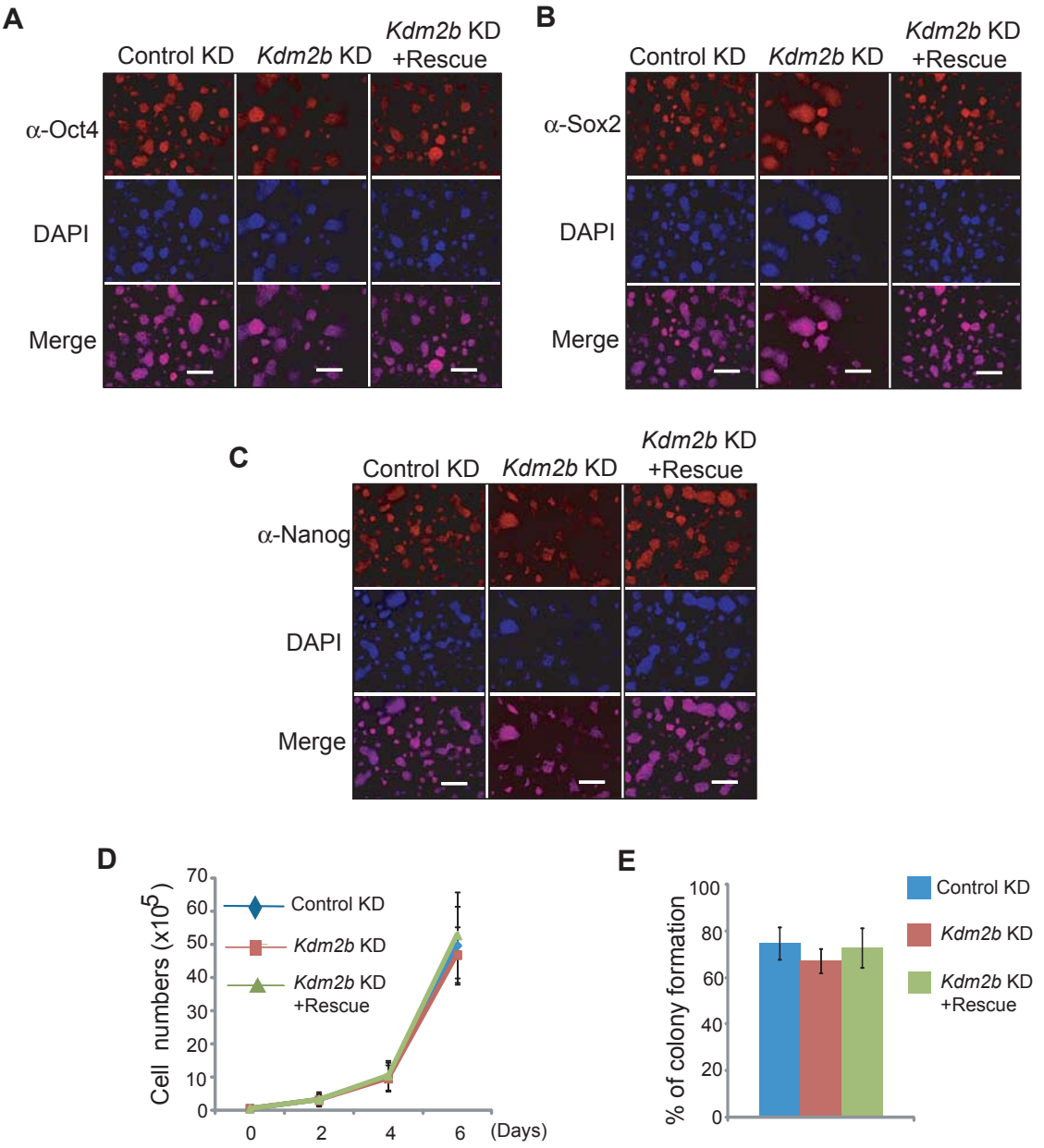



**Figure S3** Depletion of *Kdm2b* in mESCs does not affect expression of pluripotent genes, cellular proliferation and clonogenicity. **(A-C)** Immunostaining of Oct4 (A), Sox2 (B), and Nanog (C) in control, *Kdm2b* KD and *Kdm2b* rescued mESCs. bars = 100 μm. **(D)** Growth curve of control, *Kdm2b* knockdown and *Kdm2b* rescued mESCs. Values represent means ± standard deviation from three biological replicates. **(E)** Colony formation capacity of control, *Kdm2b* knockdown and *Kdm2b* rescued mESCs. Values represent means ± standard deviation from three biological replicates.

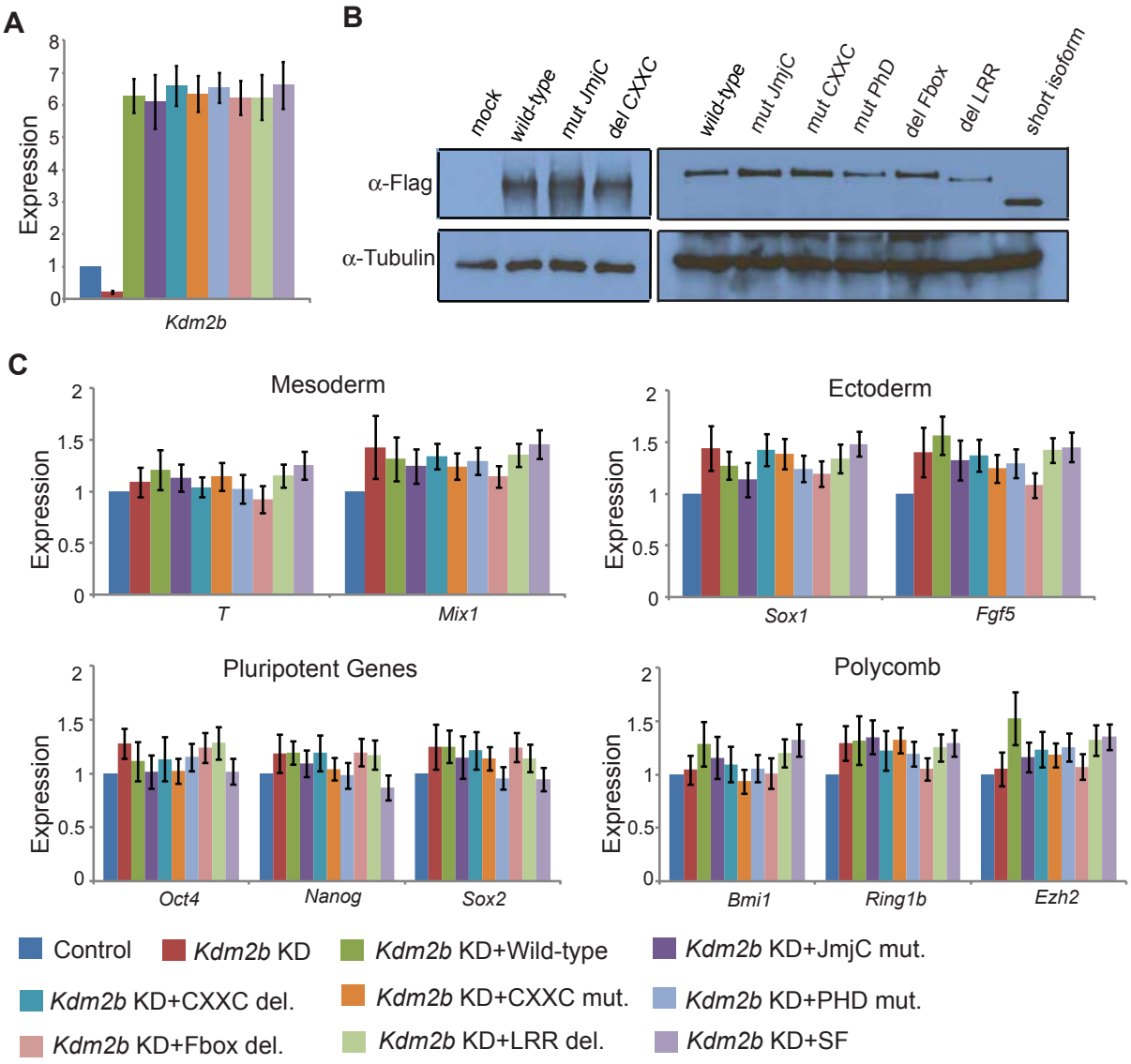



**Figure S4** Knockdown of *Kdm2b* does not affect expression of mesoderm, ectoderm, pluripotent and Polycomb genes. Relative expression levels of *Kdm2b* in the control, *Kdm2b* knockdown as well as the wild-type and various mutants rescued mESCs measured by RT-qPCR. The mRNA level of *Kdm2b* in control knockdown mESCs is arbitrarily set as 1. Values represent means ± standard deviation from three biological replicates. Western blot analyses demonstrate the expression of the exogenous wild-type, various mutants and short isoform of Kdm2b. Tubulin serves as a loading control. RT-qPCR analysis demonstrates none of the representative mesoderm, ectoderm, pluripotent and Polycomb genes are altered in the *Kdm2b* knockdown or various mutant rescued mESCs. The mRNA level of control knockdown mESCs is arbitrarily set as 1. Values represent means ± standard deviation from three biological replicates.

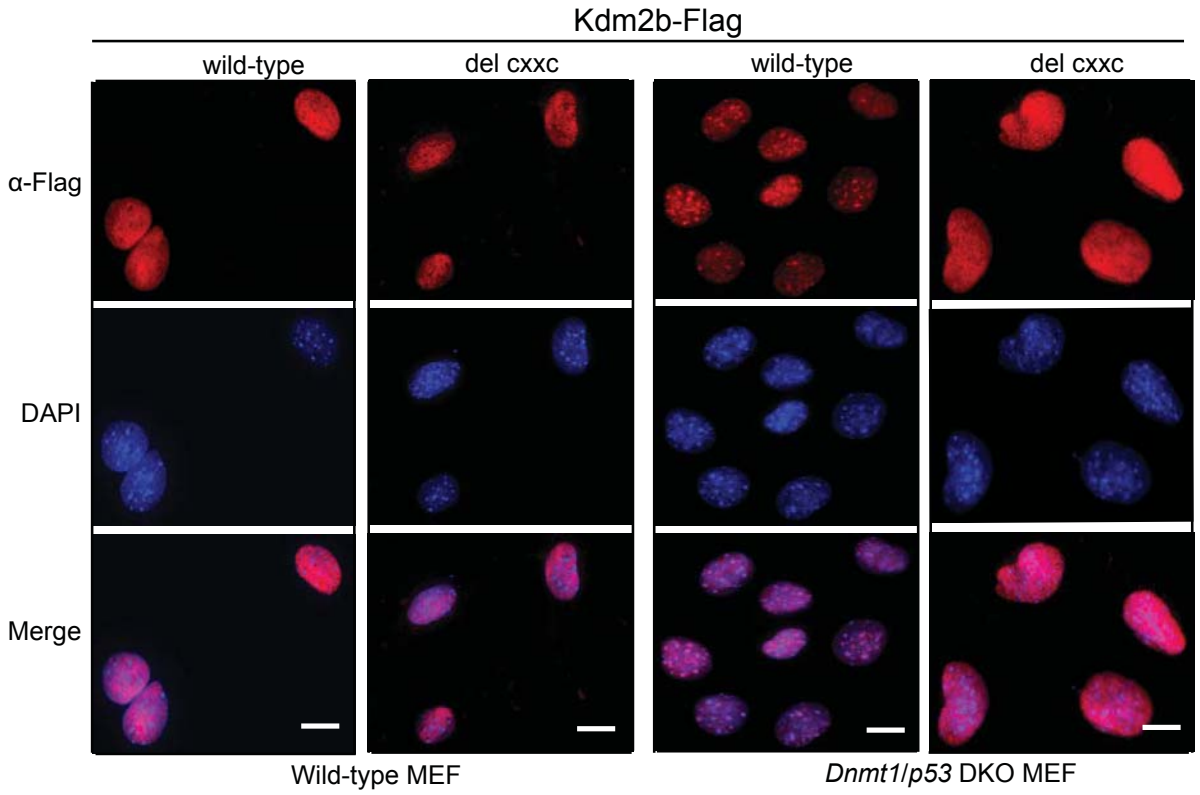
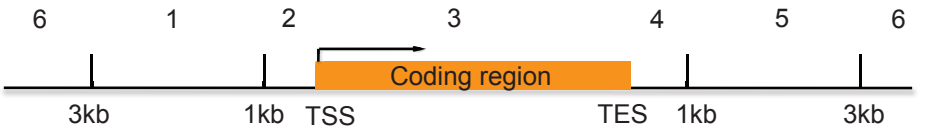
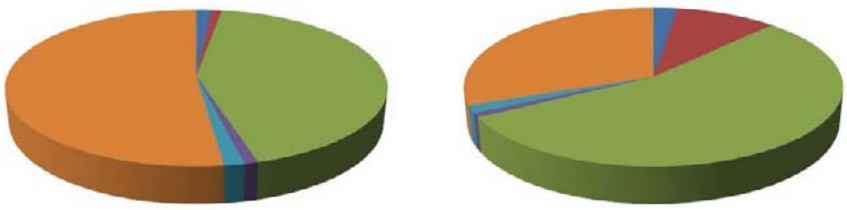



**Figure S5** Kdm2b binds to unmethylated CGIs in mESCs. Representative immunostaining images show the localization of wild-type and CXXC-ZF deletion Kdm2b mutant in the wild-type and the *Dnmt1/p53* double knockout (DKO) MEF. Note that the wild-type Kdm2b, but not the CXXC-ZF deletion mutant, is localized to DAPI heavy foci in the *Dnmt1/p53* MEFs. bars = 5μm.



Genomic Background | Kdm2b occupancy

| | Genomic Background | Kdm2b occupancy |
|---|---|---|
| 1. Distal promoter (1-3 kb) | 1.5% | 2.3% |
| 2. Proximal promoter (<1 kb) | 1% | 9.8% |
| 3. Intragenic region | 43% | 54.8% |
| -5'UTR | 0.3% | 10.2% |
| -coding region | 2% | 16.1% |
| -intron | 39.4% | 26.7% |
| -3'UTR | 1.3% | 1.8% |
| 4. Immediate downstream (<1 kb) | 1% | 0.9% |
| 5. Distal downstream (1-3 kb) | 1.5% | 1.5% |
| 6. Distal intergenic (>3 kb) | 52% | 30.7% |

**Figure S6** The genomic distribution of Kdm2b occupancy. Pie chart shows the genomic distribution of Kdm2b occupancy in mESCs. The percentage of Kdm2b occupancy at each genomic region is listed.

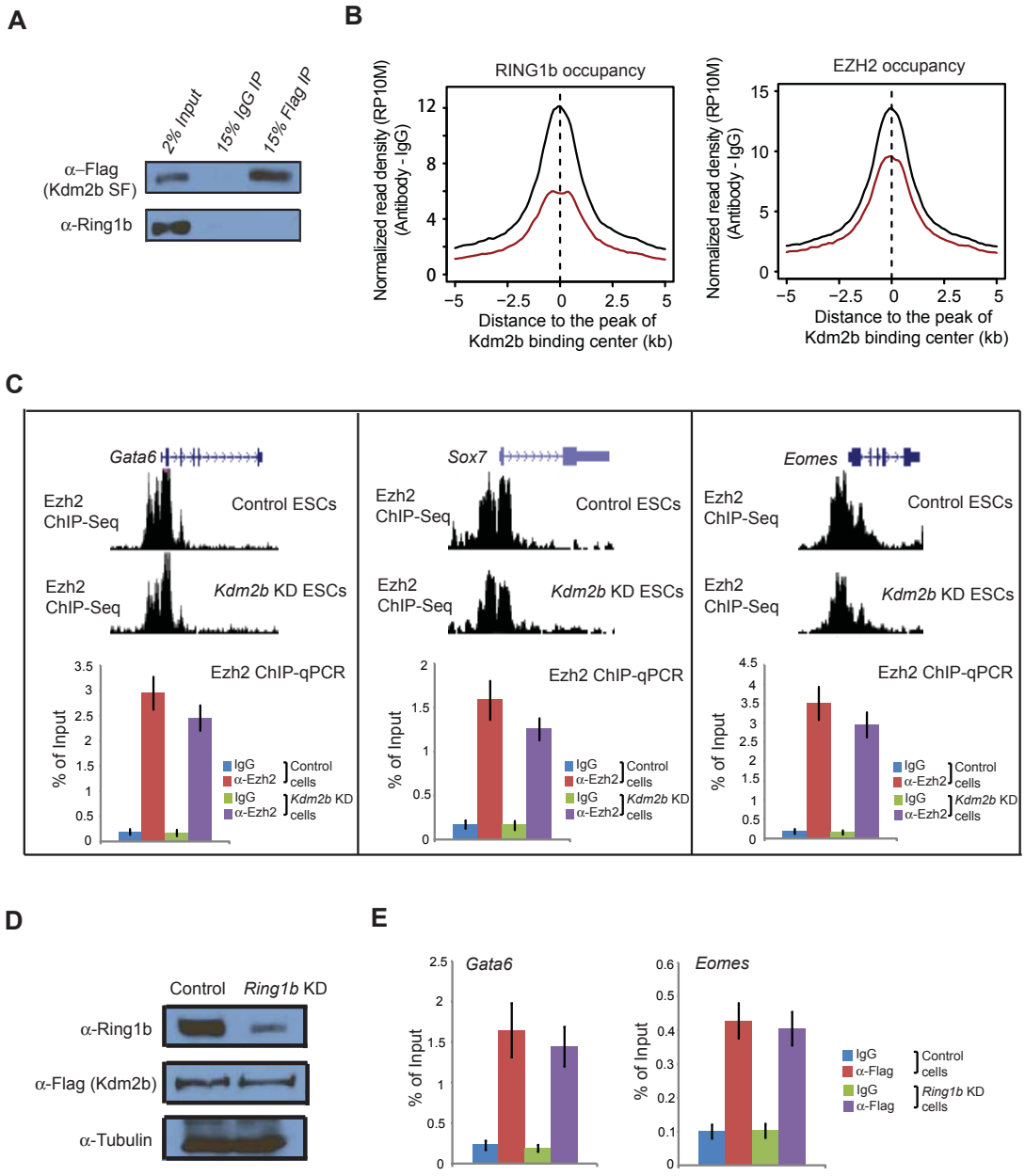



**Figure S7** Kdm2b is required for the recruitment of PRC1 to the CGIs of target genes. Co-immunoprecipitation and Western blot analyses show the short isoform of Kdm2b fails to co-immunoprecipitate with Ring1b. Genome-wide occupancy of Ring1b and Ezh2 at the Kdm2b-bound genes that do not have changed gene expression upon *Kdm2b* knockdown in control (black) and *Kdm2b* knockdown (red) mESCs. Average signal within 5 kb genomic regions flanking the center of Kdm2b peaks is shown. Shown on the top panels are the ChIP-Seq results of Ezh2 at three representative genes *Gata6*, *Sox7*, and *Eomes* in control and *Kdm2b* knockdown mESCs. Shown on the bottom panels is the relative enrichment of Ezh2 occupancy at the same sites. The enrichment of occupancy was measured by ChIP-qPCR. Values represent means ± standard deviation from three biological replicates. Western blot analyses show that Ring1b and Kdm2b protein levels at 48 hours after mESCs are transduced with control or lentiviarl *Ring1b*-shRNA vector. Tubulin serves as a loading control. Shown is the relative enrichment of Kdm2b occupancy at two representative genes *Gata6*, and *Eomes* in control and *Ring1b* knockdown mESCs. The enrichment of occupancy was measured by ChIP-qPCR. Values represent means ± standard deviation from three biological replicates.

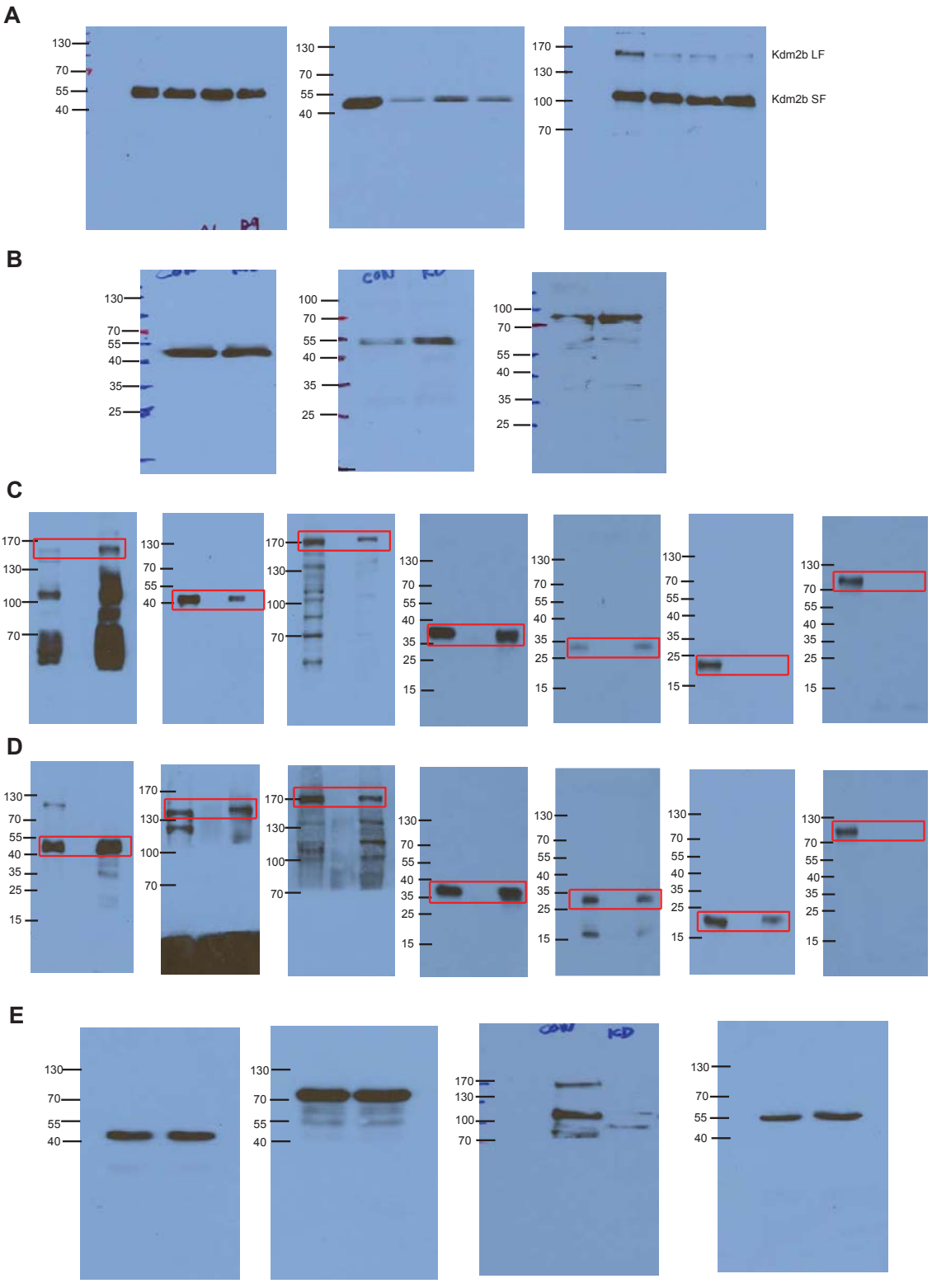



**Figure S8.** Scanned images of immunoblots. The boxed regions indicate the bands shown in the figures. (A) Fig. 1C; (B) Fig. 2F; (C) Fig. 5C; (D) Fig. 5D; (E) Fig. 7C.