PRC2 specifies ectoderm lineages and maintains pluripotency in primed but not naïve ESCs

Yongli Shan1,2,3, Zechuan Liang1,2,3, Qi Xing1,3,4, Tian Zhang1,2,3, Bo Wang1,2,3, Shulan Tian5, Wenhao Huang1,3, Yanqi Zhang1,2,3, Jiao Yao1,2,3, Yanling Zhu1,2,3, Ke Huang1,3, Yujian Liu1,3, Xiaoshan Wang1,2,3, Qianyu Chen1,3, Jian Zhang1,3, Bizhi Shang1,3, Shengbiao Li1,3, Xi Shi1,3, Baojian Liao1,3, Cong Zhang1,2,3, Keyu Lai1,3, Xiaofen Zhong1,3, Xiaodong Shu1,3, Jinyong Wang1,3, Hongjie Yao1,3, Jiekai Chen1,3, Duanqing Pei1,3 & Guangjin Pan1,3

Polycomb repressive complex 2 and the epigenetic mark that it deposits, H3K27me3, are evolutionarily conserved and play critical roles in development and cancer. However, their roles in cell fate decisions in early embryonic development remain poorly understood. Here we report that knockout of polycomb repressive complex 2 genes in human embryonic stem cells causes pluripotency loss and spontaneous differentiation toward a meso-endoderm fate, owing to de-repression of BMP signalling. Moreover, human embryonic stem cells with deletion of EZH1 or EZH2 fail to differentiate into ectoderm lineages. We further show that polycomb repressive complex 2-deficient mouse embryonic stem cells also release Bmp4 but retain their pluripotency. However, when converted into a primed state, they undergo spontaneous differentiation similar to that of hESCs. In contrast, polycomb repressive complex 2 is dispensable for pluripotency when human embryonic stem cells are converted into the naive state. Our studies reveal both lineage- and pluripotent state-specific roles of polycomb repressive complex 2 in cell fate decisions.
**Results**

**P**olycomb repressive complexes (PRCs) formed by polycomb group proteins play essential roles in development by mediating chromatin modification\(^1-3\). The polycomb repressive complex 2 (PRC2 complex) catalyzes histone H3 lysine 27 tri-methylation (H3K27me3) through its core components EZH1, EZH2, EED and SUZ12\(^4-10\). In contrast, PRC1 contains RING1A and RING1B, E3 ubiquitin ligases that mono-ubiquity-

nylate histone H2A at lysine 119 (H2AK119ub1)\(^11,12\). PRC1 and PRC2 coordinately mediate transcriptional repression through H3K27me3 modification. PRC2 is recruited to specific genomic locations and catalyzes deposition of H3K27me3, which in turn recruits PRC1, thus resulting in generation of H2AK119ub1\(^13-15\). Whole-genome studies have revealed that PRC2 and its mark H3K27me3 occupy critical developmental genes in both human and mouse embryonic stem cells (ESCs)\(^16-3\). Paradoxically, most genes occupied by H3K27me3 are also modified by H3 lysine 4 tri-methylation (H3K4me3)\(^16-18\), thus marking these loci with bivalent modifications to keep lineage genes in a poised state capable of responding rapidly to differentiation cues. Furthermore, these bivalent modifications are rapidly resolved during lineage specification to ensure the proper expression of lineage-specific genes\(^19-21\).

Loss-of-function studies on individual components of PRC2 have been performed and have been reported in Drosophila and mice\(^10,22-26\). Deletion of three core PRC2 components (Eed, Suz12 and Ezh2) in mice results in severe defect in gastrulation\(^23,25,27,28\), presumably because of the mis-expression of lineage-specific genes\(^24,25,29,30\). Interestingly, deletion of PRC2 in mouse ESCs exhibits quite different phenotypes. For example, mouse ESCs (mESCs) with Suz12, Eed or Ezh2 deletion appear to be normal with little effect on self-renewal and morphology\(^6,7,31-33\). Transcriptionally, only a small subset of PRC2 target genes are affected in those mESCs. However, EZH2\(^-/-\) human ESCs (hESCs) have severe defects in self-renewal and differentiation\(^34\). Therefore, although PRC1 and PRC2 have been proposed to play critical roles in embryonic development, their exact roles in different pluripotent states (naive vs. primed) and in the differentiation toward three germ layers remain unresolved. In this report, we generated a panel of hESC lines with deletion of EZH1/EZH2, EED and SUZ12 and found that these cells underwent spontaneous differentiation to the meso-endoderm germ layers at the expense of the neural ectoderm. Furthermore, we found that PRC2 is required for maintaining pluripotency in only the primed state but not in the naive state.

**P**RC2\(^-/-\) hESCs differentiated to default meso-endoderm fate. During gene targeting, hESCs with homozygous deletion of EED or SUZ12 or double deletion of both EZH2 and EZH1 were isolated and further cultured under defined conditions suitable for hPSCs. However, these cells subsequently underwent spontaneous differentiation, as indicated by the loss of typical hESC morphology and alkaline phosphatase (ALP) activity (Fig. 2a, Supplementary Fig. 2a). After examining the markers for the three germ layers using qRT-PCR, we found that these cell lines consistently expressed high levels of meso-endoderm genes but not neural ectoderm genes (Fig. 2b, Supplementary Fig. 2b). As controls, H1 cell-derived embryonic bodies (EBs) expressed genes corresponding to all selected lineages from the three germ layers (Fig. 2b). To further confirm the lineage fate of these differentiated cells, we performed whole-genome transcriptome analysis on EED\(^-/-\) H1, SUZ12\(^-/-\) H1 or EZH2\(^-/-\)/EZH1\(^-/-\) H1 hESCs as well as EZH1\(^-/-\) H1 and EZH2\(^-/-\) H1 hESCs. Spearman’s rank correlation analysis on the global transcriptome clearly showed a more closely related differentiation phenotype among EED\(^-/-\) H1, SUZ12\(^-/-\) H1 and EZH2\(^-/-\)/EZH1\(^-/-\) H1 hESCs, as compared with that of the WT H1 cells (Fig. 2c, Supplementary Fig. 2c). Moreover, EZH1\(^-/-\) H1 or EZH2\(^-/-\) H1 cells were similar to the undifferentiated H1 hESCs (Fig. 2c, Supplementary Fig. 2c). Furthermore, EED\(^-/-\) H1, SUZ12\(^-/-\) H1 or EZH2\(^-/-\)/EZH1\(^-/-\) H1 hESCs did not express the selected marker genes indicative of pluripotency or neural ectoderm lineage (Fig. 2d)\(^40,41\). These data suggested that disruption of PRC2 in hESCs leads to a default differentiation toward the
were electroplated into H1 or H9 human embryonic stem cells (hESCs) and selected by the corresponding drug in de
targeting vector containing puromycin or neomycin resistant cassette was constructed according to each gene. gRNA/Cas9 together with targeting vector
validated for each polycomb repressive complex 2 (PRC2) component gene showing in
shown. For
bar
NATURE COMMUNICATIONS | DOI: 10.1038/s41467-017-00668-4 ARTICLE
Fig. 1 Deletion of polycomb repressive complex 2 in human embryonic stem cells. a Overview of the gene targeting strategy. gRNA was designed and validated for each polycomb repressive complex 2 (PRC2) component gene showing in box. To delete the critical domain for each factor, a homologous
targeting vector containing puromycin or neomycin resistant cassette was constructed according to each gene. gRNA/Cas9 together with targeting vector were electroplated into H1 or H9 human embryonic stem cells (hESCs) and selected by the corresponding drug in defined condition. Positive clones were then isolated and expanded for further characterizations. b Targeting efficiencies of each gene. The functional domain that was deleted in each factor was
then isolated and expanded for further characterizations. b Targeting efficiencies of each gene. The functional domain that was deleted in each factor was shown. For SUZ12 and EED, gene targeting was performed in both H1 and H9 hESCs. c Morphology of H1 hESCs with targeted deletion of each gene. Scale bar, 200 μm. d qRT-PCR analysis on the expression level of each indicated gene in gene targeted hESCs. Wild-type H1 hESCs serve as control. Significance level was determined using unpaired two-tailed Student’s t tests. **, P < 0.01. The data represent mean ± SD from three biological repeats. e Total level of the indicated histone modification in gene targeted cells. The total histone modification level was analyzed by western-blot using the specific antibody on the whole-cell lysates from each indicated cell line. f qRT-PCR analysis on the expression level of the pluripotent genes, OCT4, SOX2, NANOG in gene targeted hESCs. Wild-type H1 hESCs serve as control. Significance level was determined using unpaired two-tailed Student’s t tests. **, P < 0.01. The data represent mean ± SD from three biological repeats. See also Supplementary Fig. 1
meso-endoderm fate. Indeed, two well-known early mesoderm markers, (CALPONIN) and endoderm (SOX17), were detected at high levels by immunostaining in $EED^{-/-}H1$, $SUZ12^{-/-}H1$ or $EZH2^{-/-}/EZH1^{-/-}H1$ hESCs but not in WT, $EZH1^{-/-}$ or $EZH2^{-/-}/EZH1^{-/-}H1$ hESCs (Fig. 2e, Supplementary Fig. 2d–f). Together, these data demonstrated that PRC2 regulates the loss of pluripotency by suppressing the meso-endoderm differentiation program.

Fig. 2 PRC2$^{-/-}$ hESCs exhibit spontaneous differentiation to meso-endoderm fate. a Morphology and alkaline phosphatase (ALP) activity staining on each indicated hESCs. Scale bar, 200 μm. b qRT-PCR analysis on the selected lineage genes in the indicated cell lines. Negative control: H1, positive control: H1 cells-derived embryonic bodies (H1-EB day 12). Significance level was determined using unpaired two-tailed Student’s t tests. **, $P < 0.01$. The data represent mean ± SD from three independent repeats. c Spearman rank correlation analysis on the whole-genome transcriptome of indicated cell lines. d Heatmap on the selected pluripotent and lineage marker genes in the indicated hESCs. We set the expression level of genes in H1 hESCs as 1 and calculated the fold change (log2) of individual gene in none of core component of PRC2 in H1 hESCs, respectively. e Immunostaining on the pluripotency and lineage markers, OCT4 (pluripotency), CALPONIN (mesoderm), SOX17 (endoderm) in the indicated cell lines. Scale bar, 100 μm. See also Supplementary Fig. 2.
**EZH1 and EZH2 specify early neural ectoderm fate.** hESCs with single deletion of EZH1 or EZH2 stayed in an undifferentiated state but had decreased levels of H3K27me3 modifications (Fig. 1c, e). Therefore, EZH1+/− or EZH2+/− H1 hESCs provide a good model to examine the role of PRC2 components in later lineage specifications. We first showed that EZH1+/− or EZH2+/− H1 hESCs cultured under defined conditions that support hPSCs retain typical pluripotency characteristics, as demonstrated by ALP staining, expression of well-known hESC markers, typical cell-cycle distribution for hESCs and low expression levels of differentiation markers (Fig. 3a–d, Supplementary Fig. 3a). To examine the differentiation potential of these cells, we injected EZH1+/− or EZH2+/− H1 hESCs into immuno-deficient mice and monitored teratoma formation. We observed normal teratoma formation for EZH1+/− or EZH2+/− H1 hESCs in all 3 injected mice. However, on the basis of H&E staining of the teratoma...
sections, we did not observe any ectoderm tissue from teratomas formed from \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 cells (Fig. 3e, Supplementary Fig. 3b). In contrast, typical mesoderm and endoderm tissues were readily detected in \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 teratomas and were similar to those in WT H1 hESC teratomas (Fig. 3e, Supplementary Fig. 3b). These data indicated that hESCs lacking \textit{EZH1} or \textit{EZH2} completely fail to specify neural ectoderm lineages in vivo. We then performed directed in vitro differentiation of \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 cells into specific lineages representing the three embryonic germ layers. For neural ectoderm, we induced neural differentiation of hESCs through a well-established and efficient protocol based on dual-SMAD inhibition\textsuperscript{40}. Through dual SMAD inhibition, WT H1 hESCs were efficiently converted into neural progenitor cells (NPCs) expressing known NPC markers and forming typical neural spheres (Fig. 3f). In contrast, \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 hESCs differentiated into only epithelial-like cells without expressing any neural lineage markers and forming neural spheres (Fig. 3f). For mesoderm, we induced differentiation of blood cells by using a published protocol with stromal cell-free condition\textsuperscript{42}. Similar percentages of CD34\textsuperscript{+} hematopoietic progenitor cells were obtained from the differentiation of both WT and \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 hESCs through cytokine treatment (Fig. 3g)\textsuperscript{42}. In addition, the known early and late mesoderm or blood lineage marker genes were also successfully induced in \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 hESCs (Fig. 3g). Then, we demonstrated very similar endoderm differentiation between WT and \textit{EZH1}\textsuperscript{−/−} or \textit{EZH2}\textsuperscript{−/−} H1 hESCs, on the basis of the expression of SOX17 and several other endoderm markers (Fig. 3b), by using a previously published protocol\textsuperscript{43, 44}. Together, our data demonstrate that \textit{EZH1} and \textit{EZH2} are required to specify the neural ectoderm lineage in hESCs but is dispensable for mesoderm or endoderm lineage.

**PRC2 deletion preferentially induces BMP signalling in hESCs.** Because the disruption of PRC2 in hESCs resulted in spontaneous differentiation, we then sought to investigate the underlying molecular events that drive differentiation. Because hESCs with PRC2 disruption (\textit{EED}\textsuperscript{−/−} H1, \textit{SUZ12}\textsuperscript{−/−} H1 or \textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−} H1 hESCs) undergo differentiation and cannot be maintained in vitro, we designed an inducible system to rescue the self-renewal capacity of hESCs with PRC2 disruption (Fig. 4a, b). We first introduced an inducible system to over-express (OE) \textit{EED} in hESCs and subsequently performed gene targeting to knockout the endogenous \textit{EED} in these cells (Fig. 4a, b, Supplementary Fig. 4a) (see “Methods” section)\textsuperscript{45}. This hESC line is referred to as H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE}. The EED level and PRC2 are maintained by DOX treatment during regular cell passaging (Fig. 4d). In the presence of DOX, H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} maintained a normal undifferentiated state and could be normally passaged under defined conditions that support iPSCs (Fig. 4c). However, they underwent gradual and spontaneous differentiation after withdrawal of DOX, despite being kept in hESC medium containing high concentrations of FGF2 to support self-renewal\textsuperscript{46, 47}. After DOX withdrawal, H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} began to morphologically differentiate at day 16 and became fully differentiated at day 20 (Fig. 4c). Moreover, the pluripotency marker genes such as 	extit{OCT4}, SOX2 and \textit{NANOG} were fully repressed at later stages of DOX withdrawal (Fig. 4e). In agreement with the data shown in Fig. 2, the meso-endoderm genes but not neural ectoderm genes were activated at later stages of DOX withdrawal (Fig. 4f). We then performed whole-genome transcriptome analysis on H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} at different time points after DOX withdrawal. Spearman’s rank correlation analysis on the whole transcriptome clearly showed a gradual differentiation of H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} after DOX withdrawal (Fig. 4g). Again, on the basis of the transcriptome data, meso-endoderm genes, but not ectoderm genes, were gradually activated during the time course of DOX withdrawal (Fig. 4h). The differentiation of H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} into the meso-endoderm fate was further confirmed by immunostaining for marker genes specific for the three embryonic germ layers (Supplementary Fig. 4b). In searching for pathways that were responsible for the differentiation caused by PRC2 disruption, we identified that TGF-β/BMP signalling factors were clearly up-regulated at very early stages of DOX withdrawal (Fig. 4i, Supplementary Fig. 4c–f). BMPs and related factors such as \textit{BMP2}, \textit{BMP4}, \textit{BMP7}, \textit{GDF6} and \textit{ID2} began to increase early at day 8 even when no obvious differentiation had been detected on the basis of morphology and the transcriptome data (Fig. 4c, g). Together, these data demonstrated that disruption of PRC2 in hESCs preferentially induces BMP signalling at early stage.

**Inhibition of BMP signalling rescues PRC2 deficiency in hESCs.** We then sought to examine whether the early induction of BMP signalling after PRC2 disruption might be the major reason for the differentiation of hESCs (Fig. 5a). To test this hypothesis, in addition to H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE}, we also prepared additional inducible systems to rescue the functional loss of other PRC2 components, for example, \textit{EZH1} or \textit{EZH2}. Similarly, we first introduced the inducible system to over-express \textit{EZH1} or \textit{EZH2} in H1-\textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−} H1 hESCs and then deleted endogenous \textit{EZH1} or \textit{EZH2} in the same cell lines (Fig. 5a, Supplementary Fig. 4a). These cells, denoted H1-\textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−}/\textit{EZH2-OE} or H1-\textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−}/\textit{EZH1-OE}, maintained a normal undifferentiated state in the presence of DOX (Fig. 5b, Supplementary Fig. 5a–d). As expected, after withdrawal of DOX, H1-\textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−}/\textit{EZH2-OE}, H1-\textit{EZH2}\textsuperscript{−/−}/\textit{EZH1}\textsuperscript{−/−}/\textit{EZH1-OE} and H1-\textit{EED}\textsuperscript{−/−}/\textit{EED-OE} all displayed complete differentiation at 20 or more days of culture (Fig. 5b). However, the differentiation was blocked by treatment with dorsomorphin.
(DM), a BMP inhibitor (Fig. 5b). As a control, another compound, SB431542, that specifically inhibits TGF-β/Nodal signalling had no effect on the differentiation process (Fig. 5b). Moreover, other reported BMP inhibitors such as DMH1 and LDN19318949, 50 also completely blocked differentiation after DOX withdrawal in H1-EZH2−/−/EZH1−/−/EZH2-OE (Supplementary Fig. 6d). Further, using qRT-PCR, immunostaining and ALP staining (Fig. 5c, d, and Supplementary Fig. 6a–f), we confirmed that whereas OCT4 was down-regulated and the mesendoderm genes SOX17 and CALPONIN were upregulated after DOX withdrawal, BMP inhibitor treatment reversed these phenotypes in all three examined hESC lines (Fig. 5c, d). These data together demonstrate that PRC2 regulates the loss of pluripotency by suppressing BMP signalling.
PRC2 is required for pluripotency in primed not naïve state. The apparent discrepancy between our results and those reported in mESCs may reflect a differential requirement of PRC2 in naïve and primed states. To test this possibility, we generated PRC2 component gene knockout cell lines in conical OG2 mESCs with GFP controlled by the Oct4 promoter (Oct4: GFP)60. Two critical PRC2 genes, Suz12 and Eed, were selected for gene targeting in mESCs (Fig. 6a, Supplementary Fig. 7a–e). As expected, and in agreement with previous reports, mESCs deficient in Suz12 (mESCs-Suz12−/−) or Eed (mESCs-Eed−/−) exhibited a normal phenotype and expressed Oct4 when they were maintained under typical conditions for mESC growth (see Methods) (Fig. 6a, Supplementary Fig. 7d) despite the upregulation of Bmp4 (Fig. 6c). However, when converted into the primed state through a well-established protocol52, mESCs-Suz12−/− and mESCs-Eed−/− but not WT mESCs displayed a gradual and spontaneous differentiation, as indicated by morphology changes and a loss of Oct4: GFP (Fig. 6a, b). The differentiation phenotype was further confirmed by the downregulation of pluripotency marker genes and upregulation of differentiation genes and TGF-β/BMPs signalling factors in mESCs-Suz12−/− and mESCs-Eed−/− (Fig. 6c, Supplementary Fig. 7f). These results suggested that PRC2 is dispensable for maintaining pluripotency in naïve but is required for primed mouse ESCs.

Additionally, hESCs are regarded as being in a primed state that can be converted to a naïve state by over-expressing NANOG and KLF2 or switching to a medium containing specific growth factors and small molecules57, 58. To test whether PRC2 functions similarly in naïve and primed states in human cells, we first knocked down EZH1 by shRNA in H1-EZH2−/− hESCs, thus resulting in an expected differentiation when the cells were in a primed state (Fig. 6d, e, Supplementary Fig. 6g, h). However, when the cells were converted to a naïve state by over-expression of NANOG/KLF2, H1-EZH2−/− hESCs became resistant to differentiation after EZH1 knockdown (Fig. 6d, e, Supplementary Fig. 6g, h)57. Furthermore, pluripotency marker genes such as OCT4, SOX2, NANOG were well maintained in the converted naïve state of H1-EZH2−/− hESCs, whereas the same genes were significantly downregulated in H1-EZH2−/− hESCs after EZH2 knockdown in an “unconverted” primed state (Fig. 6e, Supplementary Fig. 6h). These data suggested that in human pluripotent stem cells, PRC2 is also required for pluripotency in the primed state and not the naïve state. To further confirm whether this phenomenon is a general mechanism of PRC2 function in naïve pluripotency, we also examined other reported protocols to obtain hESCs in a naïve state58. As reported58, we converted WT H1 cells, H1-EED−/−EED-OE or H1-SUZ12−/−SUZ12-OE cells into naïve state by switching them to medium containing hLIF/ACTIVIN A plus 5 small molecules (5i/L/A), as demonstrated by the up-regulation of marker genes of naïve pluripotency (Fig. 6f, Supplementary Fig. 6i). In agreement with the data above, after DOX withdrawal, H1-EED−/−EED-OE or H1-SUZ12−/−SUZ12-OE maintained in a primed state underwent differentiation (Fig. 6f, Supplementary Fig. 6i). In contrast, H1-EED−/−EED-OE or H1-SUZ12−/−SUZ12-OE in naïve state maintained their undifferentiated phenotype, as indicated by morphology and marker gene expression (Fig. 6f, Supplementary Fig. 6i). Together, our data demonstrate that PRC2 is required for pluripotency in primed but not naïve ESCs.

Discussion

PRC complexes are essential regulators of cell lineage decisions during development in different species, such as Drosophila and mice59–61. At the molecular level, PRCs repress gene expression by mediating histone modifications such as H3K27me3 deposition and through other epigenetic mechanisms11, 14, 33. Mutations in each individual core component such as Eed, Suz12 and Ezh2 in mice result in early lethality due to gastrulation defects23, 25, 27. However, the molecular mechanisms underlying the gastrulation defects caused by PRC2 deficiency have not been fully elucidated. In this report, we showed that PRC2-deficient hESCs undergo spontaneous differentiation toward the meso-endoderm germ layers without neural ectoderm, thus suggesting that PRC2 is required for the specification of the ectoderm fate at early stages of differentiation. Furthermore, our data demonstrated that PRC2 is required for maintaining pluripotency in a primed state but is dispensable in the naïve state. Therefore, our analyses provide more insights into the understanding of cell fate decisions mediated by PRC complexes during lineage specification at very early stages of development (Fig. 6g).

PRC2 is part of a large network of chromatin regulators that cooperatively govern the genomic architecture. In this work, we sought to begin to understand their exact roles in the dynamic regulation of cell fate decisions. For example, the PRC2-driven H3K27me3 landscape together with H3K4me3 is considered to be critical in repressing lineage genes in a “poised” state that can respond quickly to the differentiation stimuli for lineage commitment16, 17, 60, 61. However, our data revealed a specific role of PRC2 in lineage specification as well as their differential requirement in maintaining naïve or primed state of pluripotency. Thus, it is not clear how the epigenetic state such as the “poised state” plays a critical role during cell fate decisions into specific lineages. To answer that question, a similar approach as ours could be used to investigate the mediators of H3K4me3 deposition. Given the preliminary data on the role of PRC2 in...
Fig. 5 Inhibition of BMP signalling rescues PRC2 deficiency in hESCs. a Strategy of PRC2 disruption rescue experiments. H1-EZH2−/−/EZH1−/−/EZH2-OE or H1-EZH2−/−/EZH1−/−/EZH1-OE was prepared as described in Fig. 4a, b. H1-EED−/−/EED-OE, H1-EZH2−/−/EZH1−/−/EZH1-OE and H1-EZH2−/−/EZH1−/−/EZH1-OE were cultured in defined medium in the absence of DOX, but with adding with BMP or TGF-β inhibitors (1 μM DM or 5 μM SB431542) for 20 more days. b Morphology of the indicated hESCs cultured in defined medium with indicated condition. DM while not SB431542 treatment rescued morphological change triggered by DOX withdrawal. Scale bar, 200 μm. c Expression of pluripotent genes OCT4, SOX2 and NANOG in the indicated hESCs with different treatments. Significance level was determined using unpaired two-tailed Student’s t tests. **, P < 0.01. The data represent mean ± SD from three independent repeats. d Immunostaining on the pluripotency and lineage markers, OCT4 (pluripotency), CALPONIN (mesoderm), SOX17 (endoderm) in the indicated hESCs with different treatments. Scale bar, 100 μm. See also Supplementary Figs. 5 and 6.
lineage and cell fate determination, we were particularly surprised by the degree of its specificity toward the ectoderm lineage through the suppression of the meso-endoderm. This phenotype indicates the possibility that signalling pathways and developmental factors for the meso-endoderm lineage are preferentially de-repressed after PRC2 disruption. Indeed, we showed that BMP signalling plays a critical role in this process. Mechanistically, more work is needed to further delineate PRC2 and BMP pathways.

H3K27me3 modifications were completely abolished after deletion of PRC2 components. These data indicated that the roles of PRC2 and H3K27me3 in regulating lineage specification is specific and not as broad as previously thought. Interestingly, hESCs with single deletion of EZH1 or EZH2 maintained a certain level of H3K27me3 and were in a typical undifferentiated state, in agreement with functional redundancy between EZH1 and EZH2 in catalyzing histone modifications. However, these cells were completely defective in the generation...
of neural ectoderm lineages, but the specifications of other lineages remained unaffected (Fig. 3). The detailed molecular mechanisms underlying neural ectoderm defect in H1-\(EZH2^{-/-}\) or H1-\(EED^{-/-}\) cells remain unknown. One possibility is that the decreased level of H3K27me3 in H1-\(EZH1^{-/-}\) or H1-\(EED^{-/-}\) failed to fully repress BMPs that might dominantly switch the cell fate to meso-endoderm lineages during differentiation. The detailed molecular mechanisms underlying neural ectoderm defect in H1-\(EZH1^{-/-}\) or H1-\(EED^{-/-}\) cells require further investigation.

We initially encountered an intriguing dichotomy for PRC2 disruption in hESCs and mESCs. We observed a clear differentiation phenotype in hESCs with deletion of each core component of PRC2, but mESCs-\(Suz12^{-/-}\) or mESCs-\(Eed^{-/-}\) maintained a relatively normal undifferentiated phenotype. hESCs and mESCs have been considered to represent different states of pluripotency, primed vs. naive53-55. Interestingly, after conversion into a primed state, mESCs-\(Suz12^{-/-}\) or mESCs-\(Eed^{-/-}\) clearly exhibited spontaneous differentiation, as observed in hESCs (Fig. 6)52. Moreover, disruption of PRC2 in “naïve” hESCs that were generated in vitro resulted in substantially less impairment of pluripotency (Fig. 6)57, 58. Therefore, PRC2 is differentially required for maintaining pluripotency for cells in different states, i.e., it is dispensable for the primed state but unnecessary for the naïve state. The molecular mechanisms of how PRC complexes maintain the cellular identity of naive and primed PSCs remain to be fully elucidated. Nonetheless, the differential requirement of PRC2 may serve as a molecular signature for distinguishing naive vs. primed states in hESCs.

**Methods**

**Cell culture.** Human ESC lines H1 (WiCell), H9 (WiCell) and knockout cell lines were maintained in mTeSR1 (STEMCELL Technologies) on matrigel (Corning)-coated plates. Mouse ESC cell line OG2 with GFP controlled by Oct4 promoter, was kindly provided by Dr. Jiekae Chen. OG2 mESCs and knockout mESCs based on OG2 mESCs were maintained on feeder layers in mES medium (DMEM/ high glucose (HyClone), 15% FBS ( Gibco), NEA (Gibco, 100x), Glutamax (Gibco, 100x), Sodium Pyruvate (Gibco, 100x), 1 μM PD0325901 (Selleck), 3 μM CHIR99021 (Selleck), 1000 units/mL LIF). OG2 mESCs and knockout mESCs based on OG2 mESCs were maintained on gelatin (Millipore)-coated plate in mouse N2B27 + 2iL medium (50% DMEM/High glucose (Hyclone), 50% Knockout DMEM (Gibco), N2 (Gibco, 200x) + B27 (Gibco, 100x), NEA (Gibco, 100x), Glutamax (Gibco, 100x), Sodium Pyruvate (Gibco, 100x), 1 μM PD0325901 (Selleck), 3 μM CHIR99021 (Selleck), 100 μM β-mercaptoethanol (Gibco), 1000 units/mL LIF). All cell types were maintained at 5% CO2.

**Gene knockout in human and mouse ESCS.** pX330 (Addgene) can express Cas9 protein and guide RNA. Guide RNAs (gRNAs) for \(EZH1\), \(EZH2\), \(EED\), \(SUZ12\), mouse \(Suz12\) and \(Eed\) were designed on the website (crisp.mit.edu)59. Donor DNAs of these genes containing left and right homology arms, a Loxp-flanked PGK-purycin Cassette or a Loxp-flanked PGK-neomycin cassette, and they were used for targeting. For targeting, 1 x 10^6 hESCs were electroporated with 2 μg of donor DNA and 4 μg of pX330 plasmid containing gRNA for each gene respectively. Then the electroporated hES cells were plated onto matrigel-coated six-well plates with 2 x 10^6 (10 μM, Sigma) for 1 day. Positive clones were selected by puromycin (1 μg/mL, Gibco) or G418 (100 μg/mL, Sigma) in mTeSR1 medium. Positive clones were selected by puromycin (1 μg/mL, Gibco) or G418 (100 μg/mL, Sigma) in mTeSR1 + 2iL medium. All guide RNA sequences and primer sequences are listed in Supplementary Table 1.

**Inducible system for gene knockout in hESCs.** Human ESC cell line (H1), we firstly induced an inducible over-expression (OE) system for over-expressing \(EED\) or \(SUZ12\) respectively, hereafter referred to \(H1-EED-OE\), \(H1-SUZ12-OE\). In human knockout cell line cell H1-\(EZH2^{-/-}\) or H1-\(EED^{-/-}\), we induced an inducible over-expression (OE) system for over-expressing \(EZH2\) or \(EZH1\) respectively, hereafter referred as H1-\(EZH2^{+/+}/EZH2-OE\) or H1-\(EZH1^{+/+}/EZH1-OE\). These cells were selected with 2 μg/mL doxycycline (DOX) and puromycin. And then endogenous core components of PRC2 were deleted in these inducible OE cell lines respectively. For targeting, 1 x 10^6 hESCs were electroporated with 2 μg of donor DNA and 4 μg of pX330 plasmid containing gRNA for each gene respectively. Then the electroporated hES cells were plated onto matrigel-coated 6-well plates with 2 x 10^6 (10 μM, Sigma) for 1 day. Positive clones were selected by puromycin (1 μg/mL, Gibco), and we selected positive clones using puromycin for 3 days, and then these clones were picked and cultured in mTeSR1 plus 2 μg/mL doxycycline (DOX) on matrigel-coated plates. All primer sequences are listed in Supplementary Table 2.

**PCR detection of knockout cell clones.** Genomic DNA of knockout cell clones was extracted with TIANamp Genomic DNA Kit (Tiangen) for PCR analysis. 30–50 ng of genomic DNA templates and KOD PLUS (Toyobo) were used in all PCR reactions. Primer set of each gene including F1 and R1 was used to amplify a 1.4 Kb product to identify whether random integration occurred. All primers sequences are listed in Supplementary Table 1.

**Western blot analysis.** To detect knockout efficiency of these genes and the methylation of H3K27 and H3K4, cells were lysed on ice in RIPA buffer (Beyotime). Whole-cell extracts were resolved by 10% SDS-PAGE to get knockout efficiency. Genomic DNA and 4 μg of pX330 plasmid containing gRNA for each gene. Then the electroporated hES cells were plated onto matrigel-coated 6-well plates with 2 x 10^6 (10 μM, Sigma) for 1 day. Positive clones were selected by puromycin (1 μg/mL, Gibco), and we selected positive clones using puromycin for 3 days, and then these clones were picked and cultured in mTeSR1 plus 2 μg/mL doxycycline (DOX) on matrigel-coated plates. All primer sequences are listed in Supplementary Table 3.

**Fig. 6 PRC2 is required for maintaining pluripotency in primed not naïve state.** a Conversion of WT, \(Suz12^{-/-}\), or \(Eed^{-/-}\) naïve mESCs into the primed state. WT mESCs is OG2 mESCs with GFP expression controlled by Oct4 promoter (Oct4: GFP). For conversion into the primed state, WT, \(Suz12^{-/-}\), or \(Eed^{-/-}\) naïve mESCs were treated with indicated conditions in the absence of feeder cells52. Scale bar, 100 μm. b FACs analysis on Oct4: GFP in WT, \(Suz12^{-/-}\), or \(Eed^{-/-}\) naïve mESCs at different state. c qRT-PCR analysis on Oct4, Sox2, Tcf21, and Bmp4 in WT, \(Suz12^{-/-}\), or \(Eed^{-/-}\) mESCs at different state. Significance level was determined using unpaired two-tailed Student’s t tests. \(p < 0.01\). The data represent mean ± SD from three independent repeats. d Conversion of WT or H1-\(EZH2^{-/-}\) hESCs into the naïve state. hESCs with \(NANOG/KLF2\) expression were further cultured in switched medium with indicated condition57, TFC21, a marker gene of naïve pluripotency, was detected by qRT-PCR. Significance level was determined using unpaired two-tailed Student’s t tests. \(p < 0.01\). The data represent mean ± SD from three independent repeats. Scale bar, 100 μm. e Knockdown of \(EZH1\) in primed or naïve state WT or H1-\(EZH2^{-/-}\) hESCs. OCT4, TCF21, and \(EZH1\) were examined by qRT-PCR in the indicated hESCs with \(EZH1\) knockdown. Significance level was determined using unpaired two-tailed Student’s t tests. \(p < 0.01\). The data represent mean ± SD from three independent repeats. Scale bar, 100 μm. f Conversion of WT or H1-\(EED^{-/-}\)/\(EED-OE\) hESCs into the naïve state. hESCs were further cultured in switched medium (5i/L/A) with indicated condition in the presence or absence of DOX58. Left: Morphology of H1, H1-\(EED^{-/-}\)/\(EED-OE\) with DOX or without DOX in primed or naïve state. Right: OCT4, SOX2, NANOG, and TFC21 were examined by qRT-PCR in the indicated hESCs. Significance level was determined using unpaired two-tailed Student’s t tests. \(p < 0.01\). The data represent mean ± SD from three independent repeats. Scale bar, 100 μm. g The model for the requirement of PRC2 in primed and naïve ESCS. See also Supplementary Fig. 7.
Flow cytometry analysis. The cells were trypsinized for single cells with 0.25% trypsin-EDTA (Gibco) and fixed with 1% paraformaldehyde for about 20 min at room temperature, washed twice with 2% fetal bovine serum (FBS, Natocor) in PBS. And then cells were permeabilized with 90% methanol for 30 min at 4°C. After washed, cells were incubated with primary antibodies and isotype control antibodies for 30 min at 37°C. After washed, cells were incubated with secondary antibodies for 30 min at 37°C. The cells were washed twice and resuspended in 200 μL PBS and analyzed with Accuri C6 (Becton, Dickinson and Company). The information for antibodies is listed in Supplementary Table 3.

Quantitative real-time PCR. Total RNA was extracted with Trizol (Invitrogen), and reverse transcribed with oligo dT (Takara) and RT ACE (Toyobo), and then Quantitative real-time PCR qPCR was performed with CFX96 machine (BIO-RAD) and SsoAdvanced SYBR Green Supermix (BIO-RAD) following the manufacturer’s recommendations. GAPDH was used for quantitative real-time PCR (qRT-PCR) normalization of human sample, and GAPDH was used for qRT-PCR normalization of mouse sample. All the data were measured in three repeats. All primer sequences are listed in Supplementary Table 2.

Alkaline phosphatase staining. The cells were plated on matrigel-coated 6-well plates for alkaline phosphatase (ALP) assay. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature. After washed thrice with 1 × TBST, the cells were processed with ALP buffer (Beyotime) for 5 min. And then, the sample were added with BCIP (Beyotime) and NBT (Beyotime) for 15 min. The usage of regents was followed with the manufacturer’s recommendations (Beyotime).

Immunostaining assay. Undifferentiated and differentiated ESCs were seeded onto matrigel-coated 24-well plates. Cells were fixed with 4% paraformaldehyde for 20 min at room temperature, washed thrice with PBS for 5 min each time. Cells were permeabilized with 0.3% triton X-100 (sigma) and 10% goat serum in PBS, meanwhile cells were incubated with corresponding primary antibodies overnight at 4°C. The cells were washed thrice with PBS for 5 min each time. And then, cells were incubated with secondary antibodies for 1 h at room temperature. The cells were washed thrice with PBS for 5 min each time. The cells were stained with DAPI (Sigma) for 5 min at room temperature. Images were captured with Leica DMi6000B microscope (Leica Microsystems, GmbH). The information for antibodies is listed in Supplementary Table 3.

Teratoma formation and analysis. The experiments involving animal research for teratoma formation had been reviewed and approved by IACUC at GIBH (NO. 20100102). The size of tumour growth that was acceptable for ethical approval was about 2.5 × 2.5 cm. In our experiments, EZH1−/−, EZH2−/− and wild-type H1 hES cells that were cultured on matrigel-coated 6-well plates were digested by Accutase (Sigma) for 8 min at 37°C and resuspended in 30% matrigel (Corning) in DMEM/F12 (Hyclone), and then injected subcutaneously into immuno-deficient mice. We used that the age of mice were about 4 weeks, and the sex of mice were male and female, and strain of mice were NOD-SCID mice. Teratomas were detected after 8 weeks and fixed in 4% paraformaldehyde, and then stained with hematoxylin/eosin (H&E). The size of tumour growth was about 2 × 2 cm.

Neural progenitor cells differentiation. To initiate neural differentiation, hESC were plated on matrigel-coated 12-well plates with 95–100% of cell confluence, and then these cells were cultured in N2B27 medium plus SB431542 and Dorsomorphin (50% DMEM/F12 (Hyclone), 50% Neurobasal (Gibco), N2 (Gibco, 200×), B27 (Gibco, 100×), Glutamax (Gibco, 200×), NEAA (Gibco, 200×), 5 μg/mL insulin(Gibco, 200×), 1 μg/mL heparin (Sigma), 5 μM SB431542 (Selleck), 5 μM Dorsomorphin(DM, Selleck)) 46. Every 2 days changing fresh culture medium. After 8 days, the cells were passaged on matrigel-coated 6-well plates in N2B27 medium. After 16 days, canonical neural rosettes of wild-type hESC appeared and these cells were suspended for neural sphere formation.

Hematopoietic progenitor cells differentiation. To initiate hematopoietic differentiation 47, hESC were passaged with dispase (2 mg/mL) onto matrigel-coated 12-well plates. These cells were cultured in E6 medium plus ACTIVIN A and BMP4 (DMEM/F12 (Hyclone), 64 ng/mL 1 Lascorbic acid (Sigma), NaCl (Sigma, adjusting the osmolality to 340 mOsm), ITS – (Gibco, 100×) and 50 ng/mL 1 Activin A (Sino biological, 10429-HNAB-50), 50 ng/mL 1 BMP4 (Peprotech, 120-05ET) for 2 days. And then cells were cultured in E6 medium plus 40 ng/mL 1 VEGF (Sino biological, 10088-HNAB-50) and 50 ng/mL 1 bFGF (Sino biological, 10014-HNAB-50) for next two days. For next 3 days, these cells were cultured in E6 medium plus 40 ng/mL 1 VEGF, 50 ng/mL 1 bFGF, 10 μM SB431542 (Selleck). After 7 days, these cells were collected for extracting total RNA and FACS analysis and CD34 expression. CD34 and CD45 antibody conjugation with PerCP-Cy5.5 were used according to the manufacturer’s recommendations.

Definitive endoderm cells differentiation. To initiate definitive endoderm cells (DE cells) differentiation43, 44, hESCs were cultured for 3 days in RPMI1640 (Gibco) /B27 medium (Insulin minus, Gibco) and 100 ng/mL 1 Activin A (Peprotech) on matrigel-coated 24-well plate. After 3 days, these cells were collected for extracting total RNA and immunostaining for SOX17 expression.

Conversion of primed mouse ESCs. To induce naive mouse ESCs into primed mouse ESCs (mEpESCs), conversion of WT, Suz12−/−, Eed−/− mESCs into the primed state according to our previous work 45 was performed on C57BL/6J (C57) and CBA/Ca (CBA) mice. The experimental design is listed in Supplementary Table 3. The usage of regents was followed with the manufacturer’s recommendations.

Conversion of naive hESCs. To induce primed hESCs into naive hESCs, we used methods that have been reported by Austin Smith and Rudolf Jaenisch 37, 38. At first, conversion of WT or H1 –EZH2−/− hESCs into naive state according to Austin Smith’s paper. NANOG and KLFL2 were overexpressed in hESCs though lentiviral-based inducible vectors. hESCs with NANOG/KLFL2 expression were further cultured in switch medium (human N2B27 + 2iL medium: 50% DMEM/F12 (Hyclone), 50% Neurobasal (Gibco), N2 (Gibco, 200×), B27 (Gibco, 100×), Glutamax (Gibco, 200×), NEAA (Gibco, 200×), 100 μM β-mercaptoethanol (gibcho), 1 μM CHIR99021 (Selleck), 2 μM XAV939 (Sigma), 10 ng/mL 1 Activin A (Peprotech), 10 ng/mL 1 FGZ2 (R&D systems) for 5 days. And then WT mESCs maintained typical primed state.

Conversion of naive hESCs. To induce primed hESCs into naive hESCs, we used methods that have been reported by Austin Smith and Rudolf Jaenisch 37, 38. At first, conversion of WT or H1 –EZH2−/− hESCs into naive state according to Austin Smith’s paper. NANOG and KLFL2 were overexpressed in hESCs though lentiviral-based inducible vectors. hESCs with NANOG/KLFL2 expression were further cultured in switch medium (human N2B27 + 2iL medium: 50% DMEM/F12 (Hyclone), 50% Neurobasal (Gibco), N2 (Gibco, 200×), B27 (Gibco, 100×), Glutamax (Gibco, 200×), NEAA (Gibco, 200×), 100 μM β-mercaptoethanol (gibcho), 1 μM CHIR99021 (Selleck), 2 μM XAV939 (Sigma), 10 ng/mL 1 Activin A (Peprotech), 10 ng/mL 1 FGZ2 (R&D systems) for 5 days. And then WT mESCs maintained typical primed state.

RNA-seq and spearman’s rank correlation and heatmap analysis. After the above cultured cells, WT and H1 mESCs and the target cells were collected and lysed with 400 μL Trizol (Invitrogen) 39. Total RNA was extracted using a Directzol RNA MiniPrep kit (Zymo Research) and sequencing libraries was established using a TrueSeq RNA Sample Preparation Kits v2 (48 samples) (Illumina) according to the manufacturer’s protocol. The samples were run on an NextSeq system with NextSeq 500 Mid Output Kit v2 (150 cycles). The number of raw reads mapped to human mRNA reference sequence for GRACh38/hg38 using RSEM (rsem-1.2.4) 40, Bowtie2 (v2.2.5), and normalized with EDASeq (v2.2.0) 44. Gene expression is expressed as “normalized tag count.” Other downstream analyses were performed using edgeR 45. In brief, differential expression between differentiation state and the ESC control was analyzed and visualized using the edger package 46, where raw read counts per gene were normalized using Trimmed Mean of M-values (TMM). Differentially expressed genes were extracted at the cutoff of false discovery rate (FDR) of <=0.01 and fold change of >3. We set the expression level of genes in H1 hESCs as 1 and calculated the fold change (log2) of individual gene in none of core component of PRC2 in H1 hESCs, respectively. These selected pluripotent genes and lineage genes were analyzed for heatmap, and expression level of genes in H1 hESCs were set as 1 and the fold change (log2) of individual gene were calculated in other cell lines, respectively. Spearman’s correlation coefficients were then computed with TPM values at log2 scale and all correlation coefficients among samples were represented as a heatmap in R.

Statistical analyses. In general, Results were presented as mean ± SD calculated using Microsoft Excel and GraphPad Prism at least three biological repeats. Significance was determined by Student t-test when samples was determined using unpaired two-tailed Student’s t tests. P value < 0.05 was considered statistically significant in the figures. No samples were excluded for any analysis.
References

1. Surface, L. E., Thornton, S. R. & Boyer, L. A. Polycomb group proteins set the stage for early lineage commitment. Cell Stem Cell 7, 288–298 (2010).
2. Lee, T. I. et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 125, 301–313 (2006).
3. Boyer, L. A. et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441, 349–353 (2006).
4. Di Croce, L. & Helin, K. Histone methyltransferase activity associated with a human embryonic stem cell-specific chromatin mark. EMBO J. 17, 4071–4078 (2001).
5. Boyer, L. A. et al. Polycomb complexes act redundantly to repress genomic repeats from induced pluripotent stem cells. Stem Cells 32, 543–553 (2014).
6. Shen, X. et al. EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. Mol. Cell. 32, 491–502 (2008).
7. Wang, C. et al. TGFbeta inhibition enhances the generation of hematopoietic stem cells by dual inhibition of SMAD signaling. Mol. Cell. Biol. 32, 6341–6351 (2012).
8. Shi, Y. & Schuettengruber, B. Recruitment of Polycomb group repressor complex. EMBO J. 32, 1976–1984 (2013).
9. Faust, C., Lawson, K. A., Schork, N. J., Thiel, B. & Magnuson, T. The Polycomb-group gene eed is required for normal morphogenetic movements during gastrulation in the mouse embryo. Development 125, 4495–4506 (1998).
10. Mortin-Kensicki, E. M., Faust, C., LaMantia, C. & Magnuson, T. Cell and tissue requirements for the gene eed during mouse gastrulation and organogenesis. Genesis. 31, 142–146 (2001).
11. Pasini, D., Bracken, A. P., Hansen, J. B., Capillo, M. & Helin, K. The polycomb group protein Suz12 is required for embryonic stem cell differentiation. Mol. Cell. Biol. 27, 3769–3779 (2007).
12. Chamberlain, S. J., Yee, D. & Magnuson, T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. Stem Cells 26, 1496–1505 (2008).
13. Stock, J. K. et al. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. Nat. Cell. Biol. 13, 1179–1186 (2011).
14. Stein, J. et al. Ring1-mediated ubiquitination of H2A represses poised RNA polymerase II at bivalent genes in mouse ES cells. Nat. Cell. Biol. 9, 1428–1437 (2007).
15. Wang, H. et al. Role of histone H2A ubiquitination in polycomb silencing. Nature 431, 873–878 (2004).
16. Muller, J. & Verrijzer, P. Biochemical mechanisms of gene regulation by polycomb group protein complexes. Curr. Opin. Genet. Dev. 19, 150–158 (2009).
17. Schoenfelder, S. et al. Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. Nat. Genet. 47, 1179–1186 (2015).
18. Zheng, Y. et al. Ring1 methyltransferase activity that marks chromosomal polycomb sites. Cell Stem Cell 10, 186–195 (2012).
19. Xie, W. et al. Epigenetic analysis of multilineage differentiations of human embryonic stem cells. Cell Stem Cell 1, 299–312 (2007).
20. Zhao, X. D. et al. Whole-genome mapping of histone H3 lysine 4 and 27 trimethylation reveals distinct genomic compartments in human embryonic stem cells. Stem Cell 1, 286–298 (2007).
21. Zhao, X. D. et al. Whole-genome mapping of histone H3 lysine 4 and 27 trimethylation reveals distinct genomic compartments in human embryonic stem cells. Cell Stem Cell 1, 286–298 (2007).
22. Bernstein, B. E. et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326 (2006).
23. Mikkelsen, T. S. et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448, 553–560 (2007).
24. Gifford, C. A. et al. Transcriptional and epigenetic dynamics during specification of human embryonic stem cells. Cell 153, 1149–1163 (2013).
25. Xu, W. et al. Epigenomic analysis of multilineage differentiations of human embryonic stem cells. Cell 153, 1134–1148 (2013).
26. Czerwin, B. et al. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal polycomb sites. Cell 111, 185–196 (2002).
27. Faust, C., Schumacher, A., Holdener, B. & Magnuson, T. The eed mutation disrupts anterior mesoderm production in mice. Development 121, 273–285 (1995).
28. Pasini, D., Bracken, A. P., Hansen, J. B., Denchi, E. L. & Helin, K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. EMBO J. 23, 4061–4071 (2004).
29. Nekrasov, M., Wild, B. & Muller, J. Nucleosome binding and histone methyltransferase activity of Drosophila Polycomb PRC2. EMBO Rep. 6, 348–353 (2005).
Acknowledgements

We thank the lab members in GIBH for their kindly help. This work was supported by the National Key Research and Development Program of China, Stem Cell and Translational Research (2017YFA0102601); the Frontier and Key Technology Innovation Special Grant from the Department of Science and Technology of Guangdong Province (2014B020225006, 2014B020225002, 2014B020225008, 2015B020228003, 2016B030230002, 2016B030229008); the National Basic Research Program of China, 973 Program of China (2015CB964901, 2014CB965200); the National Natural Science Foundation of China (31371514, 31500948); Cooperation Grant of Natural Science Foundation of Guangdong Province, China (2016A030313167); Science and Technology Planning Project of Guangdong Province, China (2013B030800004).

Author contributions

G.P., D.P., and Y.S.: Designed the project and wrote the manuscript. Y.S. and Z.L.: Performed most experiments and result analyses. Q.X.: Performed H1−/EZH2−/−/EZH1−/−/EZH2−/−/EZH1−/−/EZH1-OE hESCs lines and validation of other PRC2 knockout cell lines. B.W.: Performed mouse Suz12 and Eed knockout in mESCs and the induction of primed mESCs. T.Z.: Performed blood differentiation of human ESCs, W.H.: Performed neural differentiation of human ESCs, Q.Z. and Y.L.: Performed EZH1 and SUZ12 knockout in H1, Y.Z., K.H., and Y.L.: Performed induction of naive human ESCs. J.Z. and B.L.: Performed teratoma formation of human ESCs. B.S., C.Z., and K.L.: Performed validation of PRC2 knockout cell lines validation. S.L.: Performed definitive endoderm differentiation of human ESCs. S.T., X.W., Q.C., and X.S.: Performed RNA-seq and bioinformatics analysis. B.L., X.Z., X.S., J.W., H.Y., J.C.: Gave suggestions about experiments and provided some experimental materials. All authors read and approved the final manuscript.

Additional information

Supplementary Information accompanies this paper at doi:10.1038/s41467-017-00668-4.

Competing interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.