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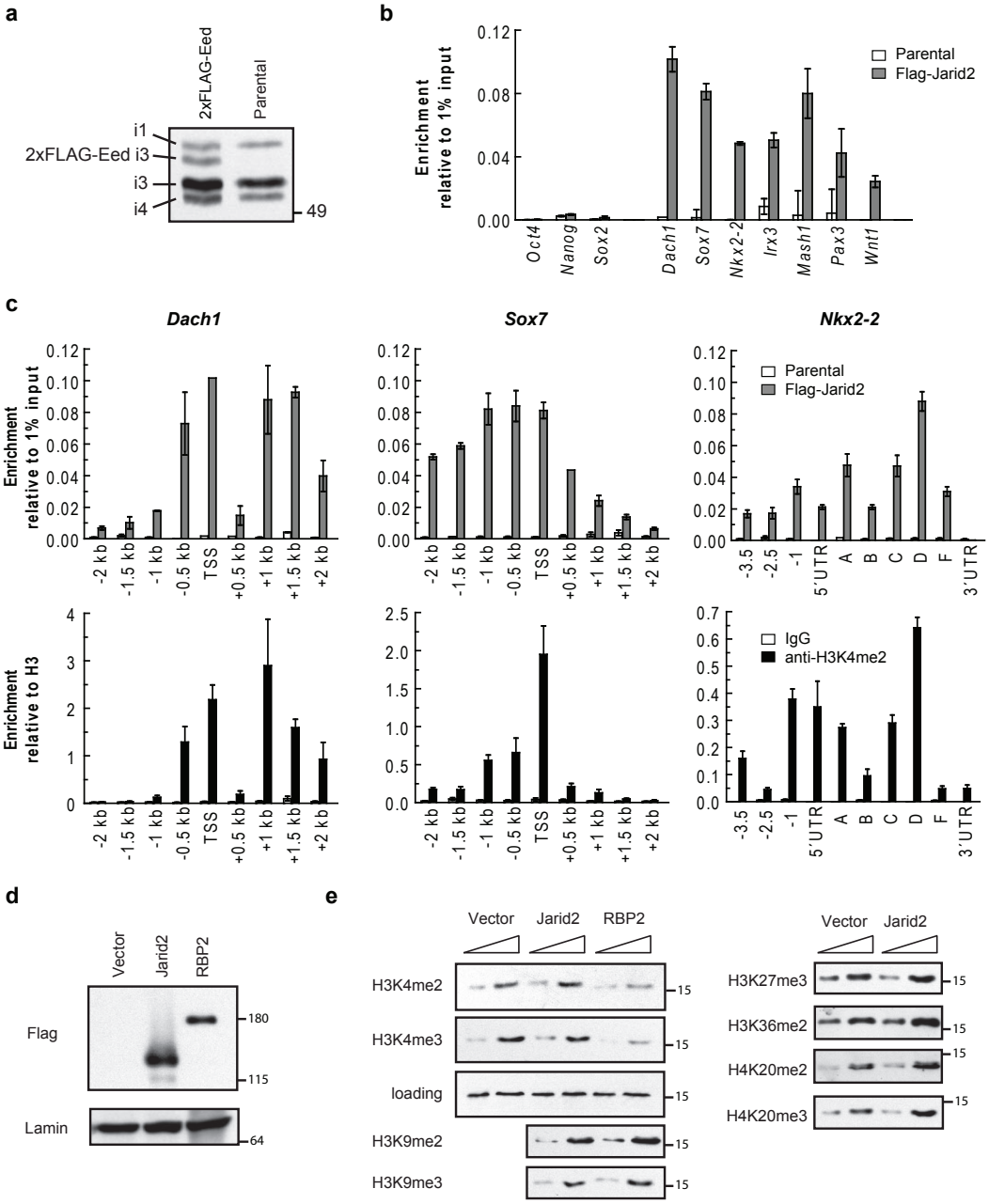
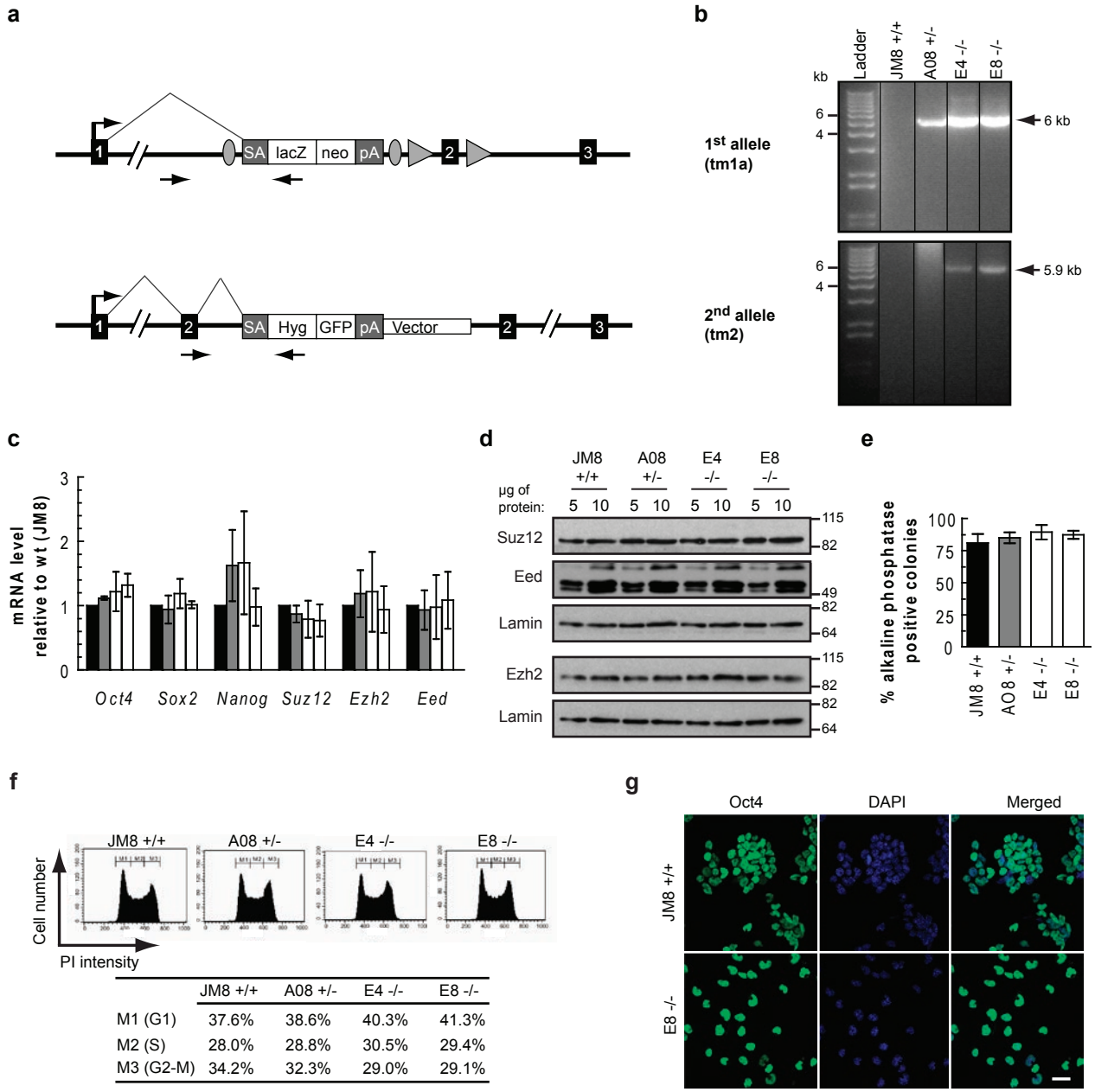


Figure S1 Jarid2 binds PRC2 target genes in ES cell. (**a**) Western blot using anti-Eed antibody of whole cell extracts from ES cells expressing 2xFLAG-Eed isoform 3 (i3) and wild-type parental ES cells. An additional band corresponding to 2xFLAG-Eed is detected. (**b**) Bar graph showing relative enrichment of Jarid2 (Flag-Jarid2) at the promoters of candidate genes identified as putative Jarid2 targets in ChIP-chip analysis. Mean ± SD of three independent experiments are shown. *Oct4*, *Nanog* and *Sox2* provide negative controls. (**c**) ChIP analysis of anti-FLAG and anti-H3K4me2 in 2xFLAG-Jarid2 expressing cells. Bar graphs of Jarid2 and H3K4me2 binding at the TSS (± 2 kb) of *Dach1* and *Sox7* and throughout the *Nkx2-2* locus, showing coincident peaks of enrichment. Mean ± SD of three independent experiments are shown. (**d**) Western blot using anti-FLAG antibodies of HEK293T cells transiently transfected with empty vector or with vectors containing 2xFLAG tagged Jarid2 or the H3K4-specific demethylase RBP2 (JARID1A). A Lamin specific antiserum was used to control for equal loading. Molecular weight is indicated in kilodaltons. (**e**) Western blot of whole cell extracts of HEK293T cells, transiently transfected with 2xFLAG-Jarid2 or 2xFLAG-hRBP2, using antibodies specific for modified histones. Adjacent lanes were loaded with 5μg and 15μg of each lysate. Molecular weight in (**a**) and (**e**) is indicated in kilodaltons.



	JM8 +/+	A08 +/-	E4 -/-	E8 -/-
M1 (G1)	37.6%	38.6%	40.3%	41.3%
M2 (S)	28.0%	28.8%	30.5%	29.4%
M3 (G2-M)	34.2%	32.3%	29.0%	29.1%

Figure S2 Generation and characterization of Jarid2-deficient ES cells. (**a**) Scheme showing *Jarid2* targeted alleles. Black boxes indicate exons, grey boxes splice acceptor sequences (SA) or polyadenylation sequences (pA), white boxes drug resistance (neomycin (neo), hygromycin (hyg)) and marker (lacZ and GFP) genes, grey spheres and triangles show Frt and LoxP sites respectively. The transcription start site in the first exon is indicated with an arrow and spliced transcripts generated from targeted alleles are marked. Opposing arrows indicate the position of primer pairs. (**b**) EtBr agarose gel showing long range PCR products of genomic DNA of Jarid2-deficient cells (A08 +/-, E4 -/- and E8 -/-) and the parental cell line (JM8) using primers indicated in (**a**). Fragment sizes are indicated in kilobases. (**c**) *Oct4*, *Sox2*, *Nanog*, *Suz12*, *Ezh2* and *Eed* transcript abundance measured by RT-qPCR in A08, E4 and E8 ES cells, relative to wild-type parental JM8. (**d**) Western blot of whole cell extracts of Jarid2-deficient and wild-type ES cells, using anti-Suz12, anti-Eed or anti-Ezh2 antibodies. Lamin was used as loading control. Molecular weight is indicated in kilodaltons. (**e**) Alkaline phosphatase (AP) assays performed five days after plating the cells at low density, where histograms show the percentage (mean ± SD) of colonies positive for AP. (**f**) FACS analysis of PI staining intensity of JM8, A08, E4 and E8 ES cell lines; table showing the percentage of cells within G1, S-phase and G2-M gates. (**g**) Confocal images showing anti-Oct4 labelling (green) and DAPI staining (blue) of wild-type JM8 and E8 -/- ES cells. Scale bar 25 µm.

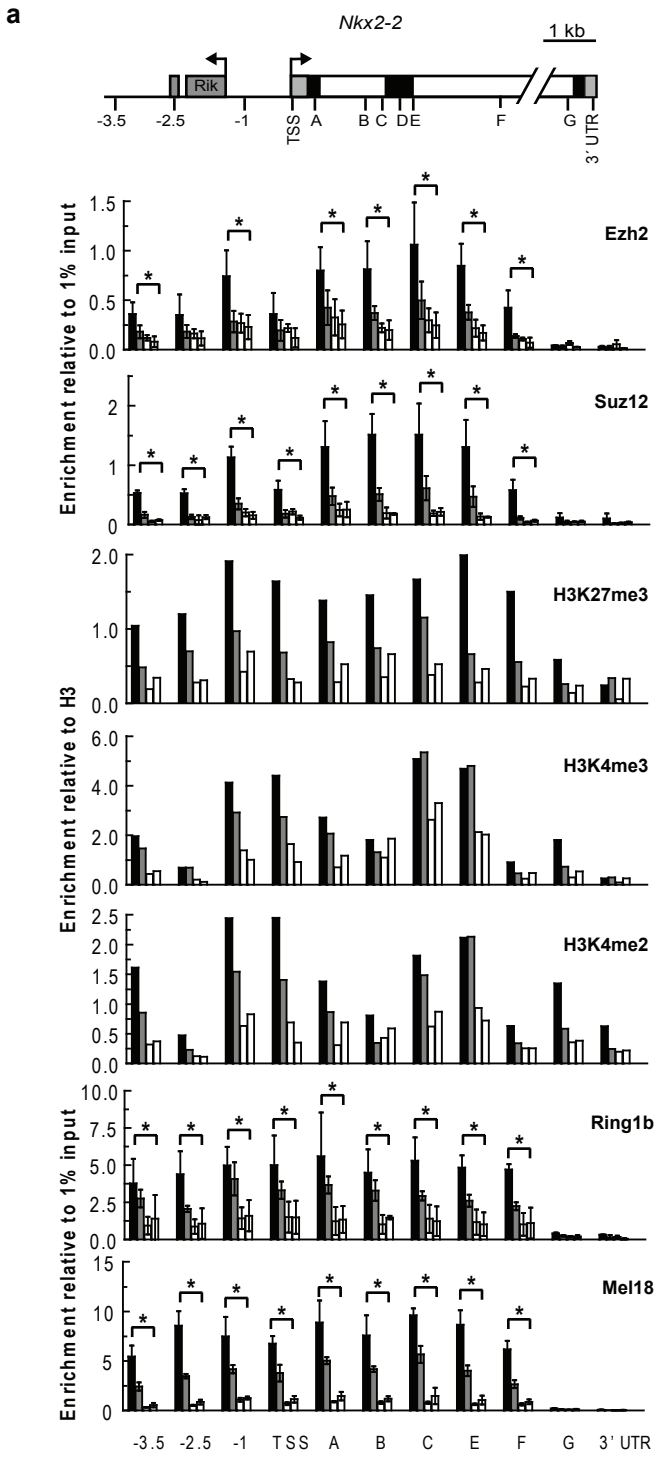


Figure S3 PRC binding and histone modifications at the *Nkx2-2* locus in wild-type versus Jarid2-deficient ES cells. (**a**) Relative abundance of polycomb proteins and histone modifications across the *Nkx2-2* locus was assessed by ChIP. Results show mean of two (H3K27me3, H3K4me3, H3K4me2) or mean ± SD of three (Ezh2, Suz12, Ring1B and Mel18) experiments. Ezh2, Suz12, Ring1B and Mel18 enrichment relative to input is shown, while abundance of H3K27me3, H3K4me3, H3K4me2 is shown relative to H3. Experiments were performed using wild-type (JM8, black), heterozygous (A08, grey) and *Jarid2* knockout ES cells (E4, E8, open bars). The location of the *Nkx2-2* TSS (arrow), a conserved region at –2.5 kb (light grey box), the coding region (8.7 kb containing three exons, black boxes), untranslated regions (light grey boxes) and the position of primer pairs are marked. Asterisks indicate statistical significant differences between JM8 wild-type and Jarid2 knockout cells ($p < 0.05$; Student´s *t*-test).

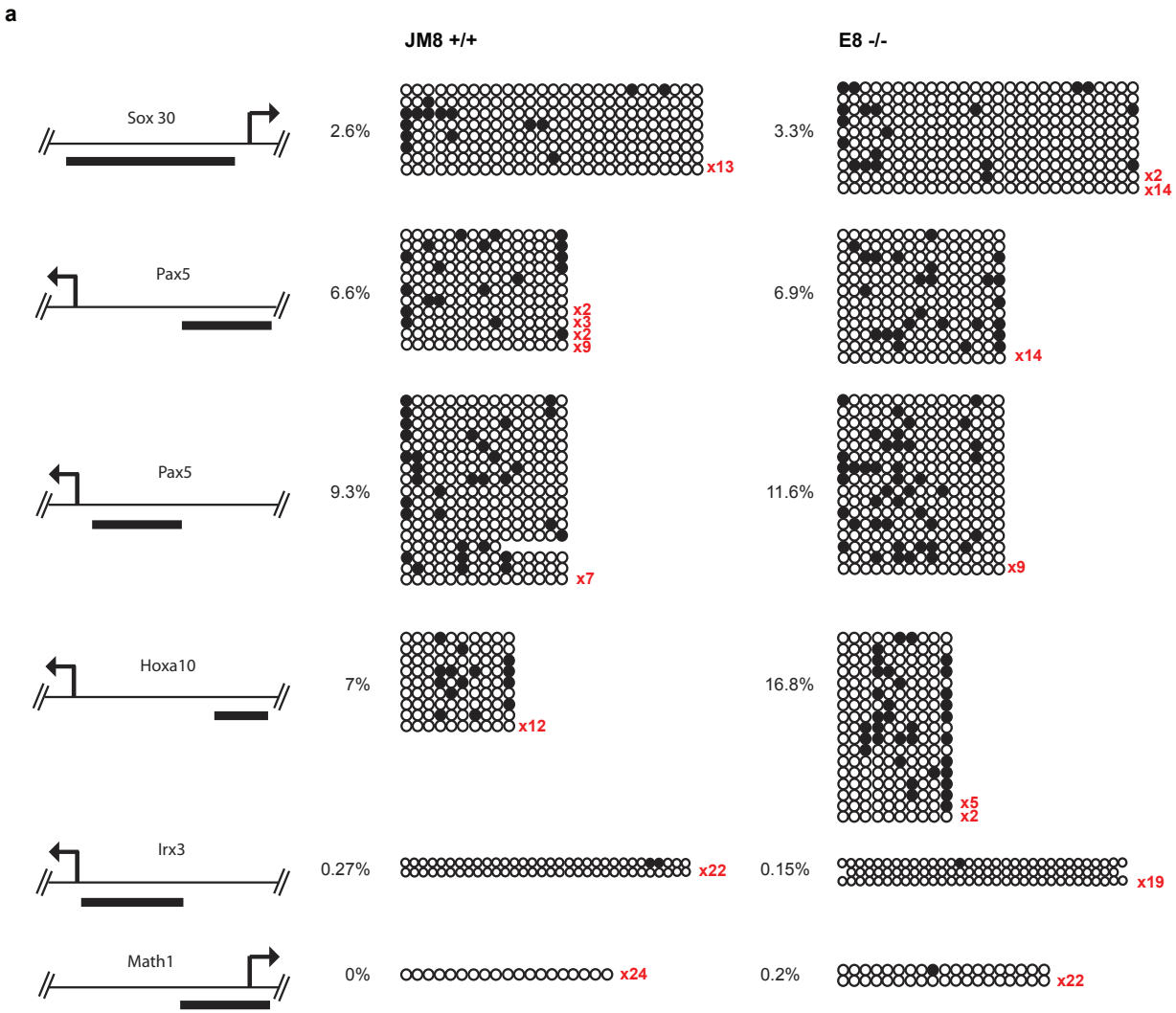


Figure S4 *Jarid2* knockout and wild-type ES cells show similar DNA methylation profiles at promoters of several bivalent genes. (**a**) Bisulfite sequencing analysis of DNA methylation at target genes in *Jarid2* knockout (E8 -/-) (right panel) and parental (JM8 +/+) (middle panel) ES cell lines are shown. The position of the sequenced region relative to the transcriptional start site (arrows) is indicated with a solid line (left panel). Open circles represent unmethylated cytosines while black closed circles represent methylated ones. Percentage of methylated cytosines is indicated.

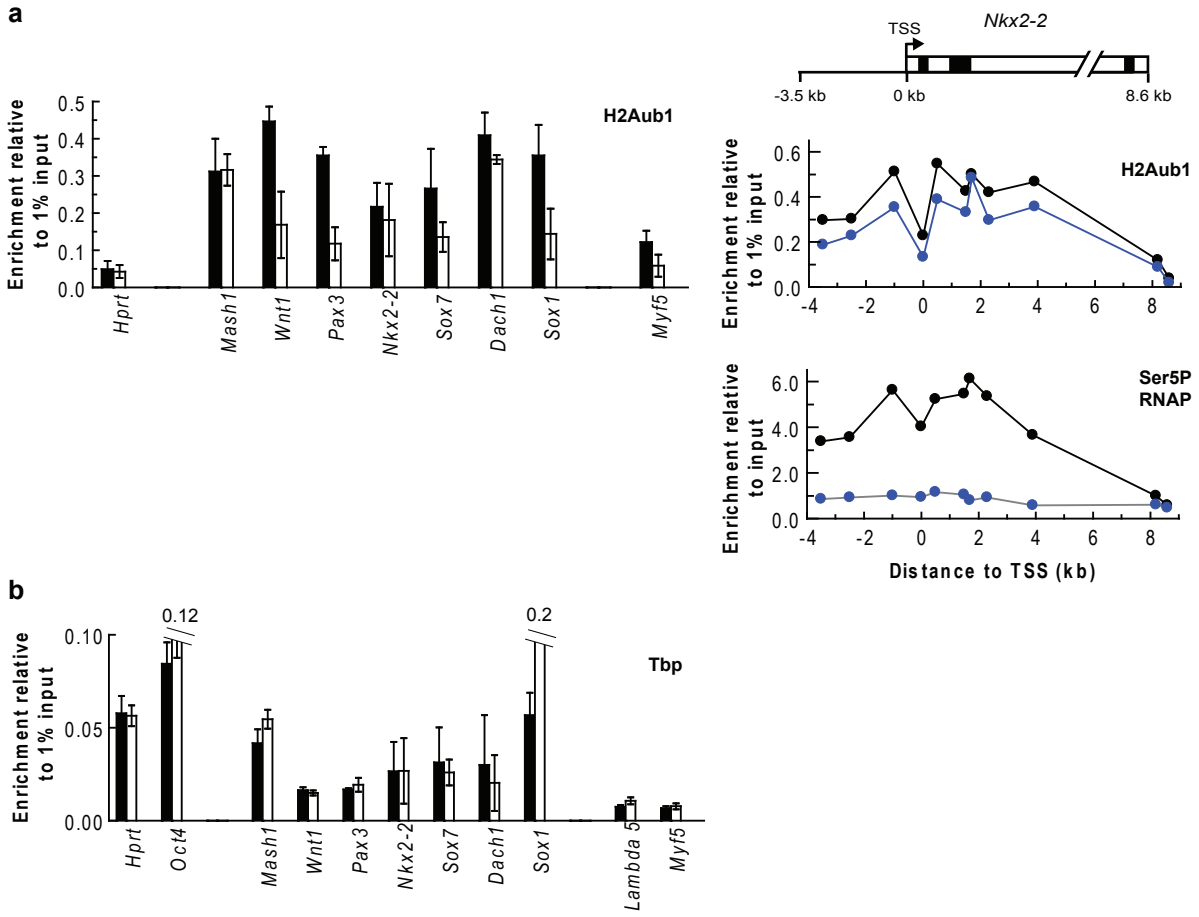


Figure S5 H2Aub1 and TBP binding at Polycomb target genes in Jarid2-deficient and wild-type ES cells. (**a**) Enrichment of monoubiquitinated H2A (H2Aub1) at the promoter regions of selected genes (left panel), or across the *Nkx2-2* locus (right panel), was assessed by ChIP. The enrichment of H2Aub1 and Ser5P-RNAP (Figure 4) at *Nkx2-2* is plotted as a function of distance to TSS. A scheme of the *Nkx2-2* locus showing TSS (arrow), three exons (black boxes) is depicted. (**b**) The recruitment of the TATA box binding protein (TBP) to the promoter regions of genes was assessed by ChIP. Results are mean ± SD of three experiments and values are expressed relative to input. Wild-type (JM8, black) and *Jarid2* knockout ES cells (E8, open bars or blue line). *Hprt, Oct4* (active genes) and *Lambda5*, *Myf5* (silent genes) provide controls.

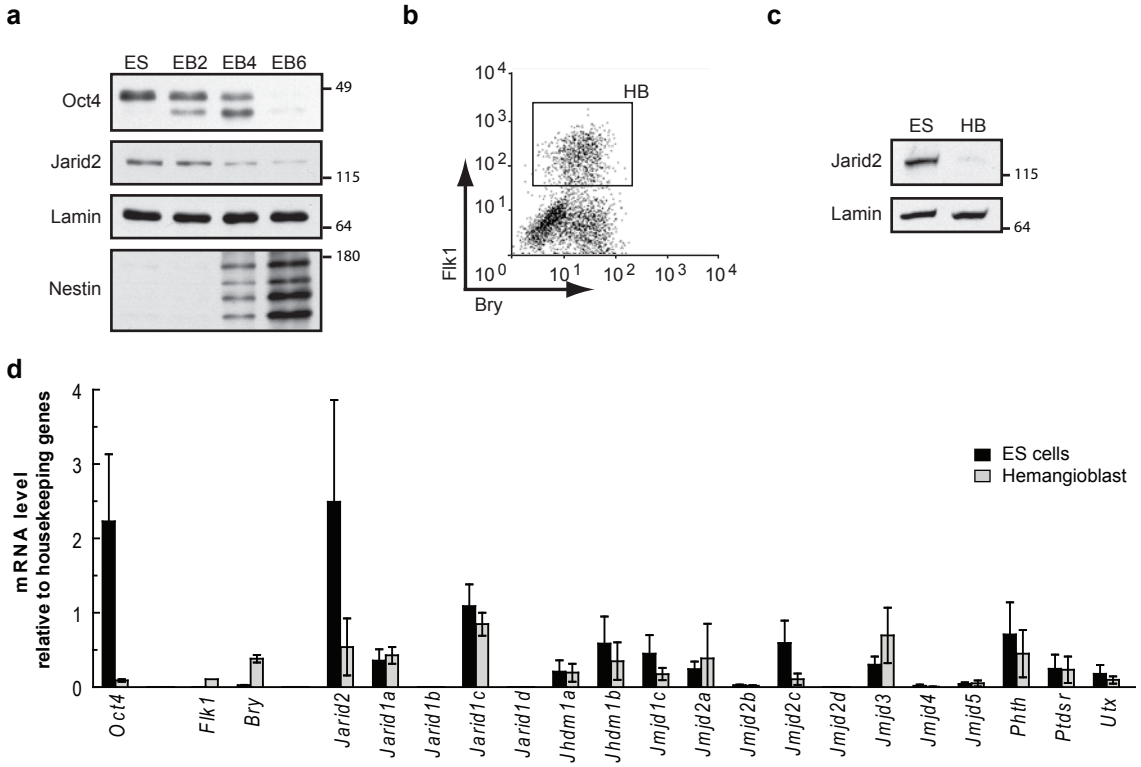


Figure S6 Jarid2 is downregulated as ES cells differentiate. (**a**) Western blot of OS25 ES cell extracts at successive times after induction of EBs in the presence of retinoic acid. Jarid2 expression is down-regulated concomitant with Oct4 loss and the up-regulation of Nestin. Lamin was used as loading control. (**b**) FACS analysis of Flk1 and Bry expression by Bry201 ES cells following differentiation towards Flk1+/Bry+ hemangioblasts (HB) at 3.75 days post induction. (**c**) Western blot analysis of whole cell extracts from undifferentiated ES cells (ES) and FACS-sorted HB samples shows that Jarid2 is down-regulated upon mesoderm differentiation. Molecular weight is indicated in kilodaltons. (**d**) Bar graph shows relative mRNA expression (measured by RT-qPCR and normalized to housekeeping genes, mean ± SEM of three experiments) of JmjC familiy members by ES cells and upon HB differentiation of Bry201. *Oct4*, *Flk1* and *Bry* provide controls.

Figure 2a

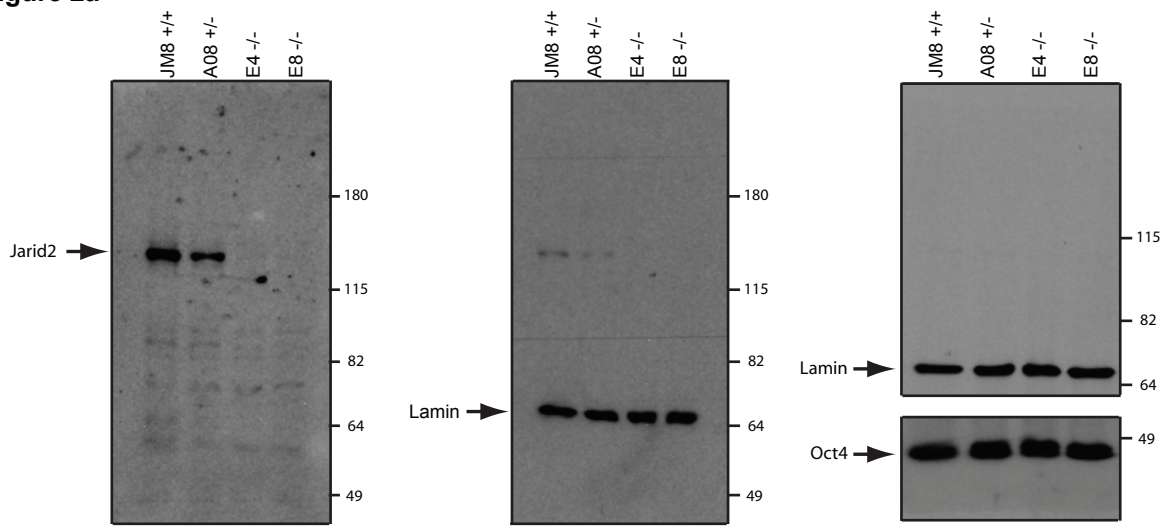


Figure S7 Uncropped versions of the western blots shown in the main figures.

Primers used for bisulfite sequencing analysis.

Gene	Forward primer	Reverse primer
Sox30	5' AGGTGTTTTTATATTTGAGAATGATTAGAA 3'	5' ATTAAAACCCTTCCAAAACCTTAACTA 3'
Pax5 RI	5' AGAATTAGGGAAAGGGTGTTAGTTT 3'	5' ACCCTATAACTCAAATTCTCCTTCC 3'
Pax5 RII	5' GTTATGTGAGATTATGTTTTGTTTT 3'	5' CTCTCTAAACCTCTTACACACCTTC 3'
Hoxa10a	5' ATATATTGAGAAGTATAAGGGTT 3'	5' TTTACAACCACATTATCACAACCATCA 3'
Irx3	5' GAGTAGAAAGGTGAGTAAAAGTAAT 3'	5' AAACCTCAAACCCCTCCTCT 3'
Math1	5' GTAAAAATTTTTTGATTGGTTTTT 3'	5' AATCTCTTCTACAAAATCTAATTTTT 3'

Table S1 Primers used for bisulfite sequencing analysis.