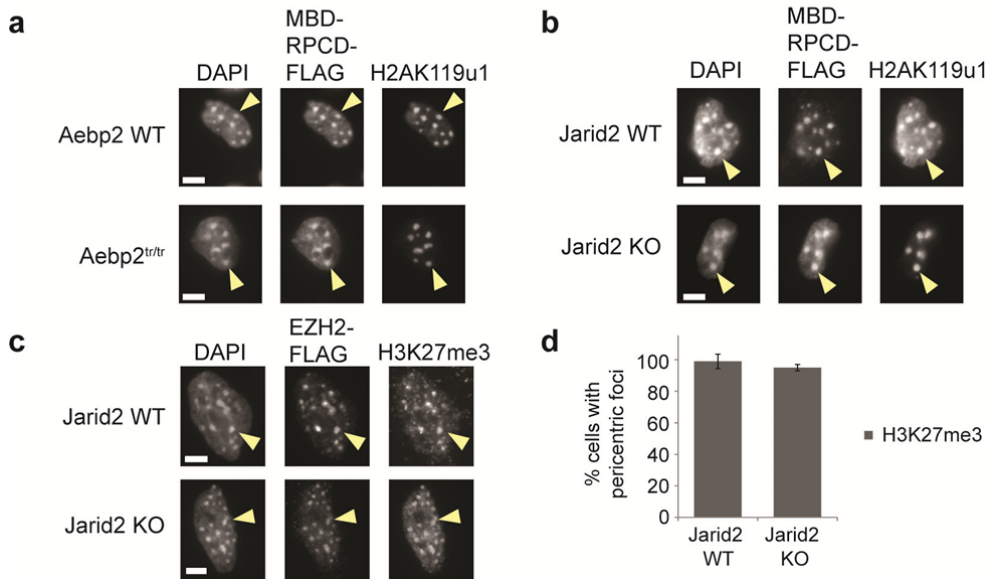


## Supplementary Figure 1



19

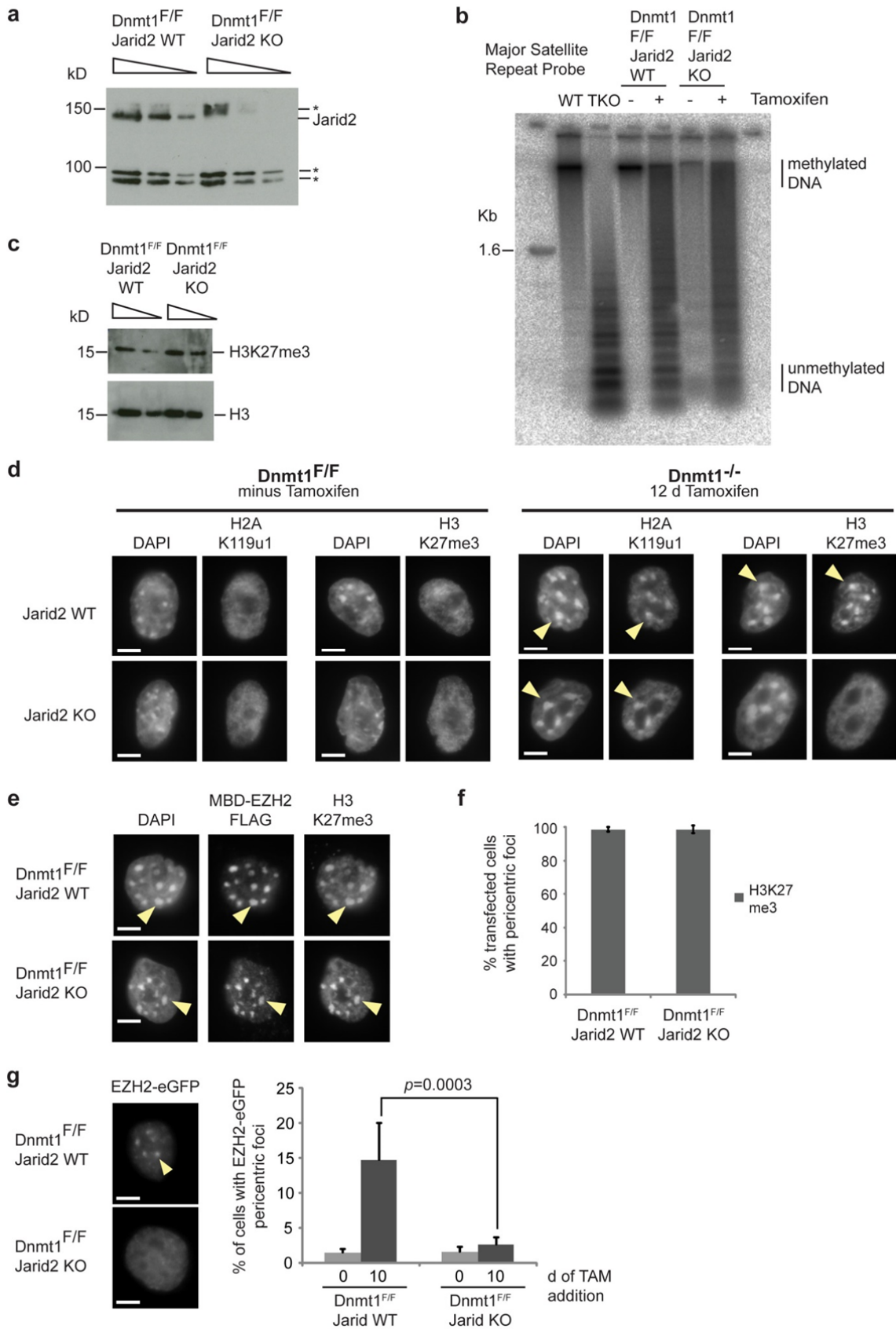
20

21 **Supplementary Figure 1: H2AK119u1 and H3K27me3 domains at pericentric**  
22 **heterochromatin in *Aebp2*<sup>tr/tr</sup>, *Jarid2* KO and complemented *Jarid2* KO mESCs.**

23 H2AK119u1 in *Aebp2*<sup>WT</sup> and *Aebp2*<sup>tr/tr</sup> (A) and *Jarid2* WT and KO (B) is observed at the  
24 pericentric heterochromatin (PCH) upon transient expression of MBD-RPCD. Arrowhead  
25 indicates a PCH focus. Scale bar is 5 μm. (C) Transient expression of MBD-Ezh2 in *Jarid2*  
26 WT and KO mESCs and immunofluorescence staining for H3K27me3. In both mESCs lines  
27 H3K27me3 is visualised at PCH (arrowhead). Scale bar is 5 μm. (D) Quantification of cells  
28 in figure (C). Error bars display standard deviation. A minimum of 300 cells were counted in  
29 3 biological repeats.

30

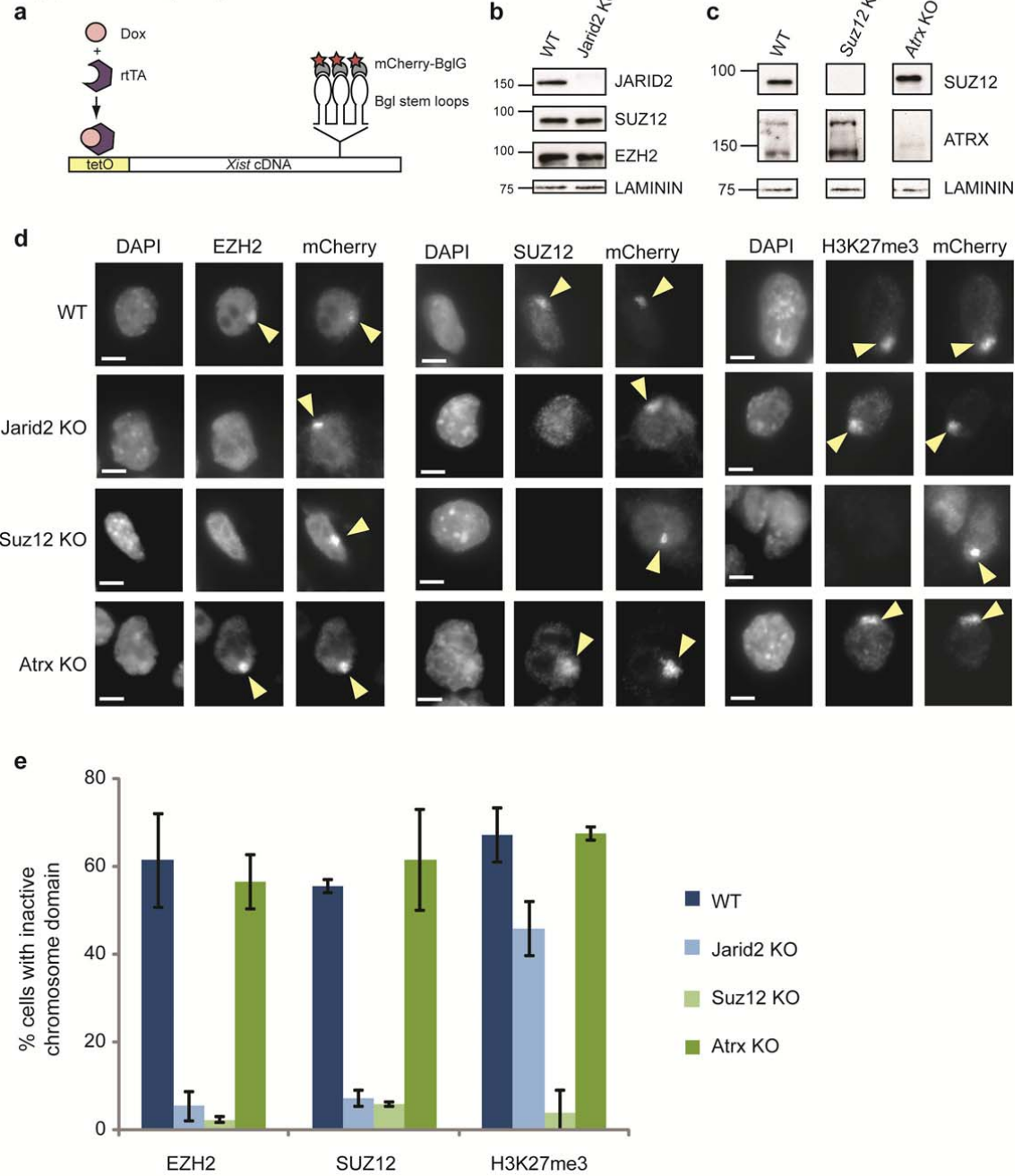
## Supplementary Figure 2



32 **Supplementary Figure 2: Characterisation of *Jarid2* KO mESCS in *Dnmt1<sup>F/F</sup>***  
33 **background.** (A) Immunoblot of whole cell extracts from mESCs showing depletion of  
34 JARID2 in the *Jarid2* KO line. Asterisks indicate species cross-reacting with the antibody.  
35 (B) Southern blot showing loss of DNA methylation at major satellite repeats upon tamoxifen  
36 induction of *Dnmt1<sup>F/F</sup>* mESCs. Genomic DNA from control mESCs :WT and TKO (*Dnmt1<sup>-/-</sup>*,  
37 *Dnmt3a<sup>-/-</sup> Dnmt3b<sup>-/-</sup>*) or test mESCs : *Dnmt1<sup>F/F</sup> Jarid2* WT or KO, plus or minus tamoxifen (to  
38 induce deletion of *Dnmt1*) was digested with the methylation-sensitive HpyCH41V and the  
39 blot was probed for major satellite repeats. (C) Immunoblot of histone extracts of *Dnmt1<sup>F/F</sup>*  
40 *Jarid2* WT or KO probed for H3 and H3K27me3. (D) Immunofluorescence staining of  
41 *Dnmt1<sup>F/F</sup> Jarid2* WT and *Dnmt<sup>F/F</sup> Jarid2* KO mESCs for H2AK119u1 and H3K27me3 either  
42 without tamoxifen induction (left) or with 12 days tamoxifen induction (right). Arrowhead  
43 indicates a pericentric heterochromatin focus. (E, F) Transient expression of MBD-Ezh2 in  
44 *Dnmt1<sup>F/F</sup> Jarid2* WT and KO mESCs and immunofluorescence staining for H3K27me3 (E),  
45 quantified in graph (F). In both mESC lines H3K27me3 is visualised at PCH (arrowhead).  
46 (G) Stable EZH2-GFP mESC lines were created in both *Dnmt1<sup>F/F</sup> Jarid2* WT and *Dnmt<sup>F/F</sup>*  
47 *Jarid2* KO backgrounds and localisation to PCH was assessed by GFP fluorescence (left)  
48 and quantified (right). P value Student's t-test, unpaired. Scale bars represent 5  $\mu$ m.  
49 Arrowheads indicate a PCH domain. In all experiments a minimum of 300 cells were  
50 counted in 3 biological repeats. Error bars indicate standard deviation.

51

**Supplementary Figure 3**



52

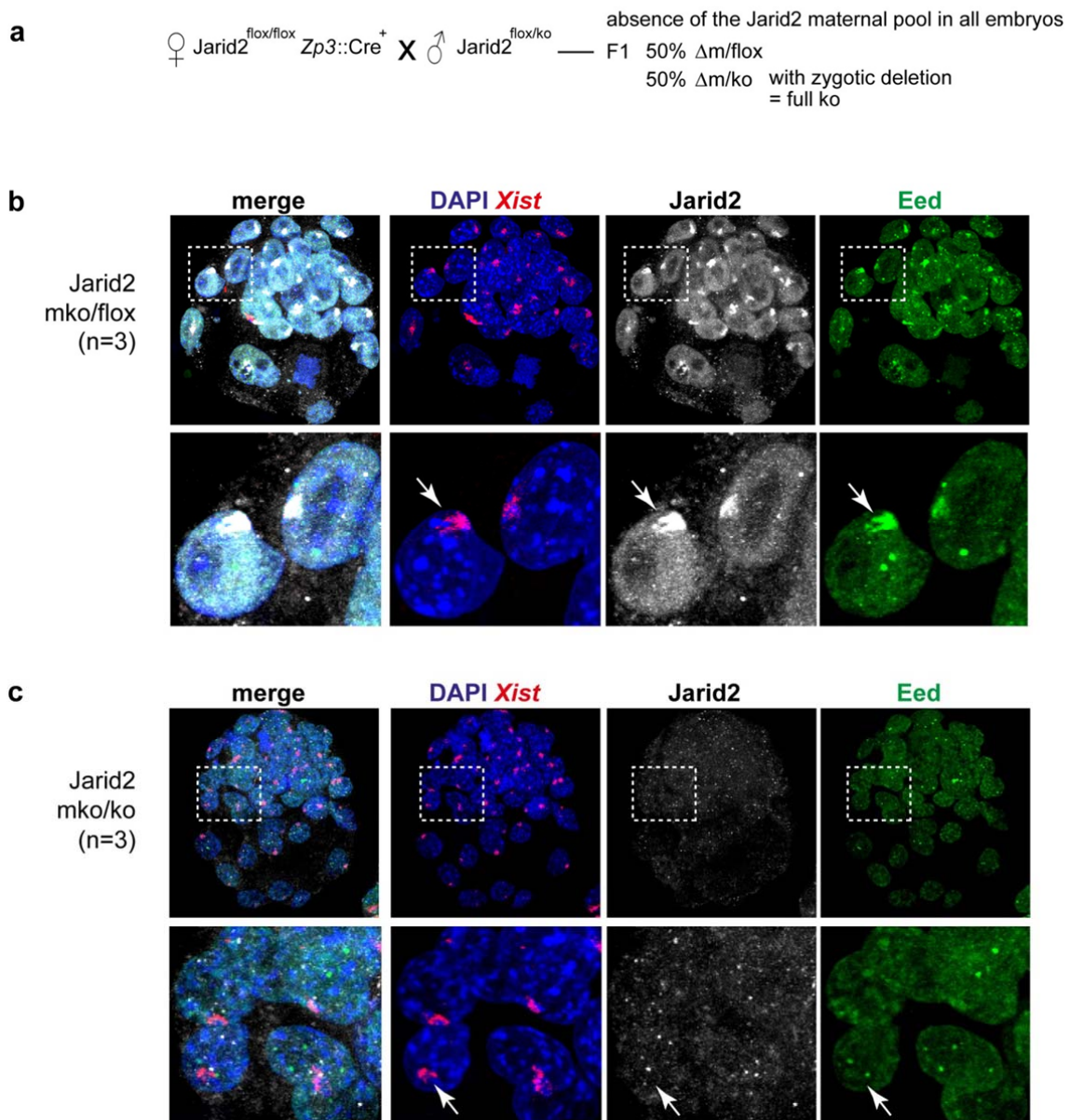
53

54 **Supplementary Figure 3: Deletion of Jarid2 diminishes Xist RNA dependent**  
 55 **recruitment of PRC2. (A)** The mESC cell line BglXist1 has a doxycyclin-inducible *Xist*  
 56 transgene which contains BglG stem loops on an autosome. The binding of Bgl-mCherry  
 57 identifies the *Xist* coated chromosome. **(B)** Immunoblot showing levels of the indicated

58 proteins in the *Jarid2* KO line. **(C)** Immunoblot showing levels of the indicated proteins in  
59 *Suz12* and *Atrx* KO cell lines. **(D)** Immunofluorescence for H3K27me3 and PRC2  
60 components EZH2 and SUZ12 in WT, *Jarid2*, *Suz12* and *Atrx* KO mESCs after induction of  
61 *Xist*. Arrowhead indicates an inactive chromosome focus. **(E)** Quantification of foci observed.  
62 A minimum of 300 cells were counted in 3 biological repeats. Error bars indicate standard  
63 deviation.

64

## Supplementary Figure 4



65

66

67 **Supplementary Figure 4: JARID2 absence disrupts EED recruitment to the inactive X**

68 **during preimplantation development. (A)** Schematic of mating scheme to generate

69 complete *Jarid2* knockout embryos. **(B)** Immunofluorescence and *Xist* RNA FISH of

70 heterozygote maternal *Jarid2* KO/paternal *Jarid2*<sup>F/F</sup> (mko/flox) embryos at late

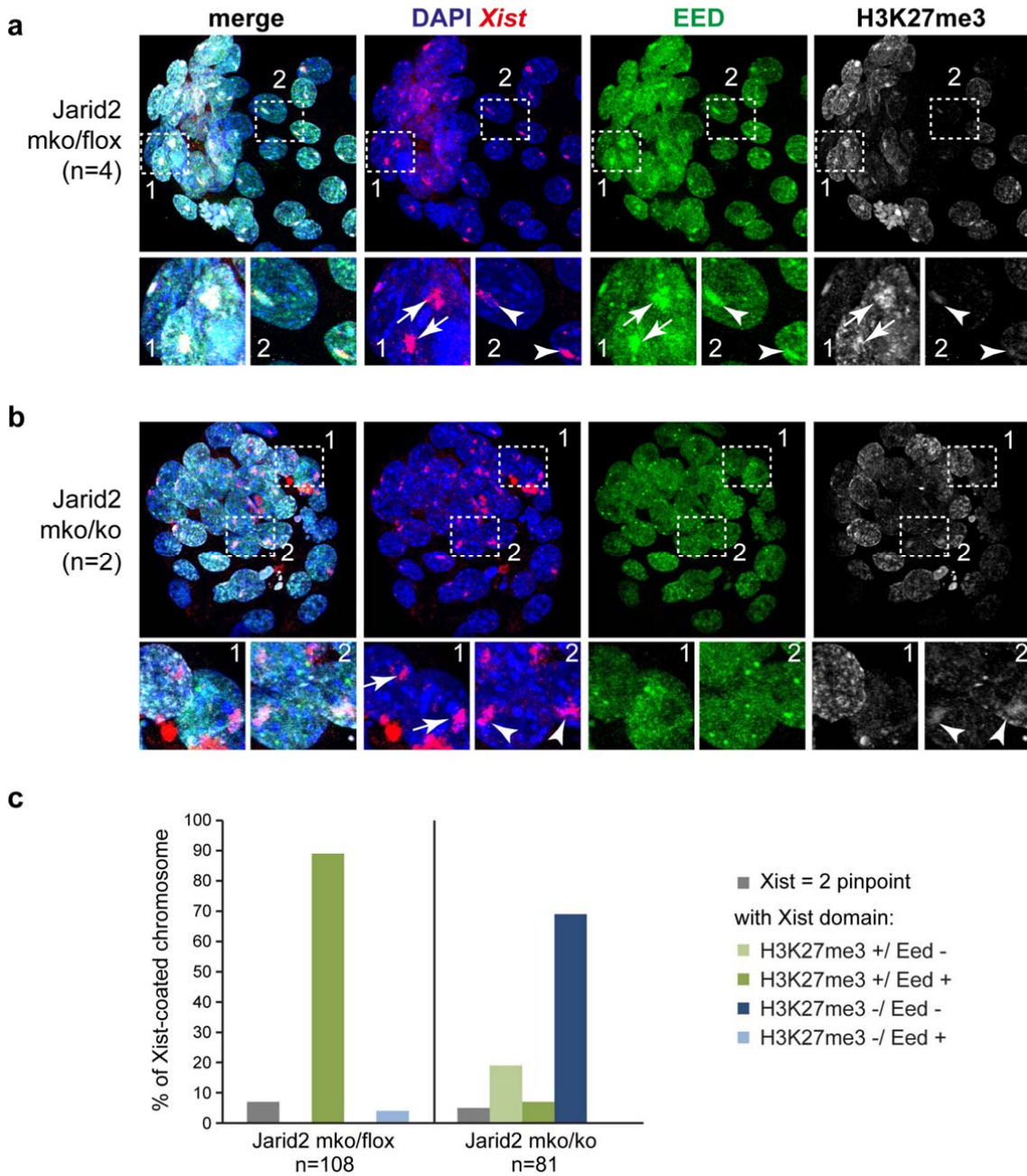
71 morula/blastocyst stage. JARID2 (grey) and EED (green) recruitment on *Xist*-coated

72 chromosomes (red; white arrow). **(C)** As **(B)**, but homozygous *Jarid2* KO embryos

73 (maternal *Jarid2* KO/paternal *Jarid2*<sup>-/-</sup> (mko/ko). In **(B)** and **(C)**, lower panel indicates zoom  
74 from the selected area in upper panel. DAPI is counterstained in blue. Scale bar is 10 μm.  
75



Supplementary Figure 5



76

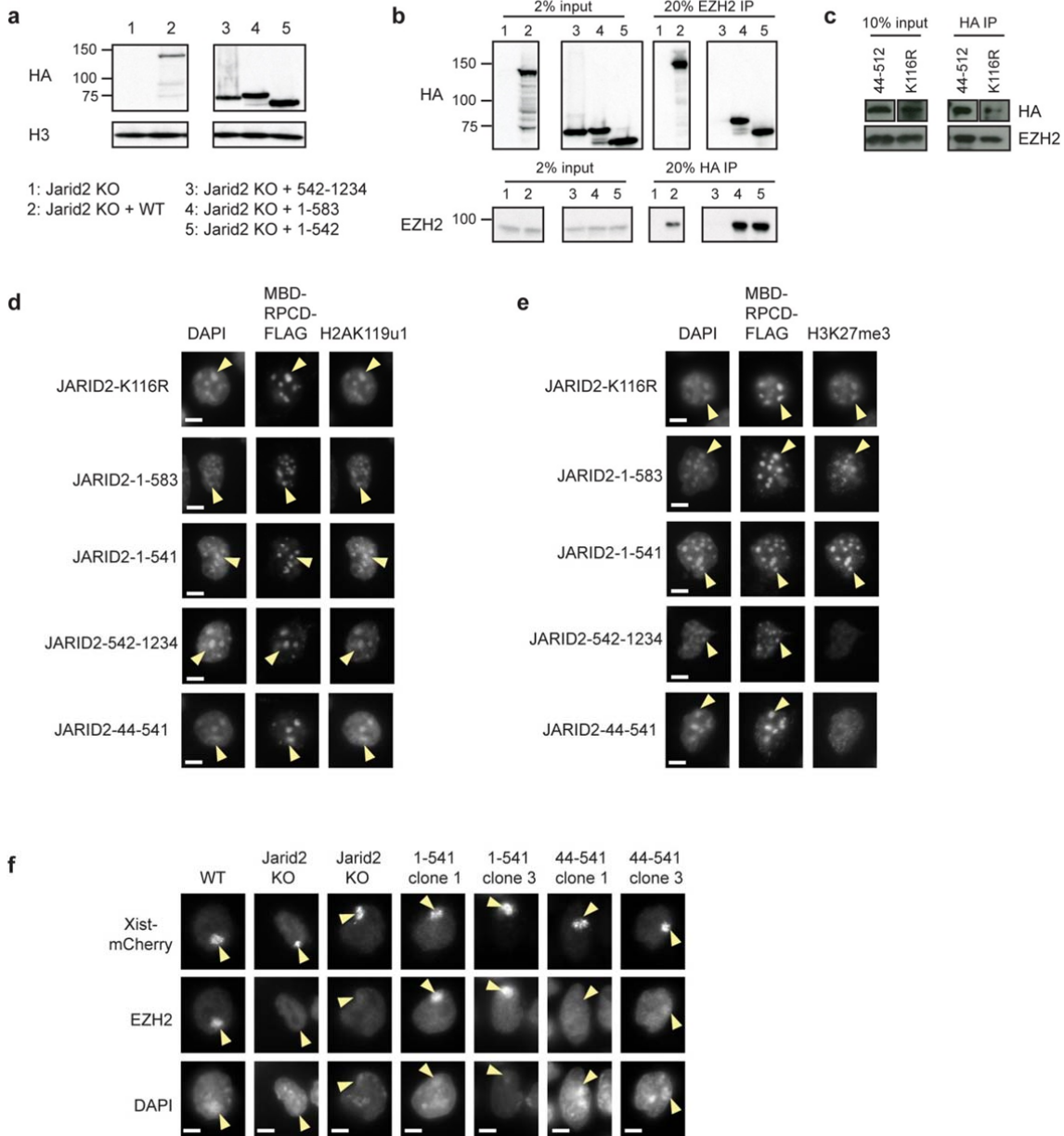
77

78 **Supplementary Figure 5: Absence of JARID2 disrupts PRC2 recruitment to the**  
 79 **inactive X during preimplantation development. (A) Immunofluorescence and *Xist* RNA**  
 80 **FISH of heterozygote maternal *Jarid2* KO/paternal *Jarid2*<sup>F/F</sup> (mko/flox) embryos at blastocyst**  
 81 **stage. EED (green) recruitment and H3K27me3 enrichment (grey) on *Xist*-coated**



82 chromosomes (red; white arrow) are shown. Lower panel indicates zoom from the selected  
83 area in upper panel. DAPI is counterstained in blue. Scale bar is 10  $\mu\text{m}$ . **(B)** As **(A)**, but with  
84 homozygous *Jarid2* KO embryos (maternal *Jarid2* KO/paternal *Jarid2*<sup>-/-</sup> (mko/ko)). **(C)** % of  
85 *Xist*-coated chromosomes counted from embryos as in **(A)** or **(B)**. n represents numbers of  
86 blastomere cells counted.  
87

**Supplementary Figure 6**



88

89

90 **Supplementary Figure 6: The N-terminus and UIM of Jarid2 complement PRC2**

91 **recruitment to H2AK119u1 chromatin. (A)** Immunoblot of whole cell extracts of

92 complemented *Jarid2* KO cell lines normalised for H3. **(B)** Immunoblot of reciprocal co-

93 immunoprecipitation of HA-tagged JARID2 fragments and EZH2. **(C)** Co-

94 immunoprecipitation of EZH2 with HA-tagged Jarid2 44-512 and Jarid2 K116R **(D)**

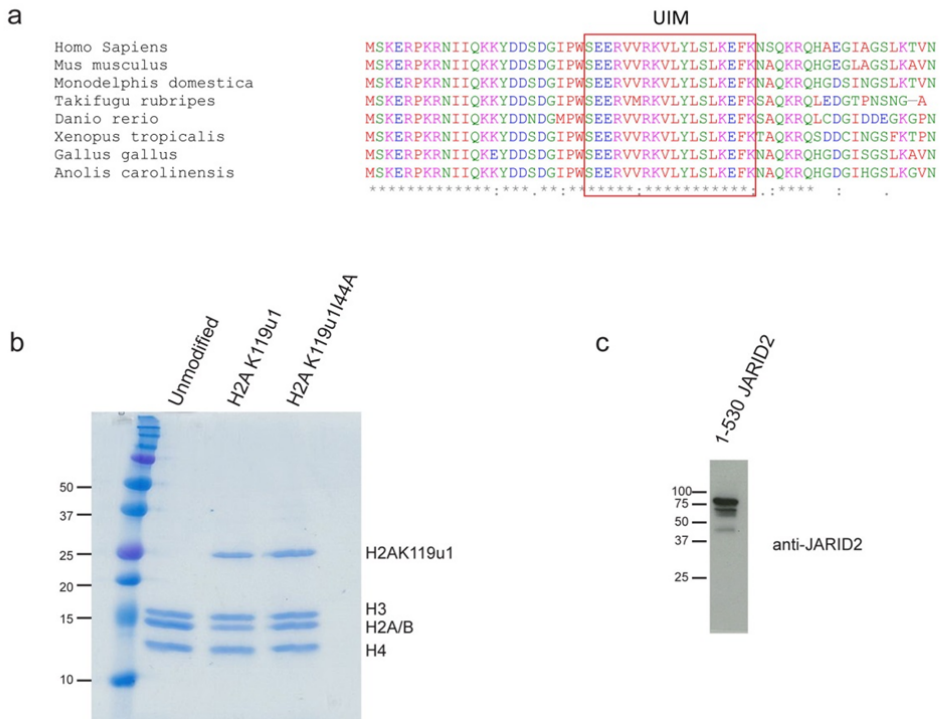
95 H2AK119u1 is observed at the PCH upon expression of MBD-RPCD in complemented

96 *Jarid2* KO mESCs. (E) H3K27me3 is observed at the PCH after complementation with  
97 deletion constructs containing the N-terminal region, but not with a construct containing the  
98 C-terminus or with the construct JARID2-44-541. Quantification is shown in Fig. 3B. Scale  
99 bar is 5  $\mu$ m. (F) Examples illustrating analysis of Xist dependent EZH2 recruitment in WT,  
100 *Jarid2* null and two independent cell lines complemented with JARID2 1-541 or JARID2 44-  
101 541. Quantification is shown in Fig. 3E. Examples for *Jarid2* null mESCs illustrate absent  
102 (left panel) or weak (right panel) EZH2 staining. Arrowheads indicate a position of Xist  
103 domain in all panels. Scale bars represent 5  $\mu$ m.  
104



106 **Supplementary Figure 7: Full Immunoblots.** (A) Complete immunoblots for the  
107 experiment shown in Supplementary Figure 6b. (B) Complete immunoblots for the  
108 experiment shown in Supplementary Figure 6c. The red boxes indicate the regions of the  
109 blots that have been cropped.  
110

## Supplementary Figure 8



111

112

113 **Supplementary Figure 8: Biochemical analysis of Jarid2 interaction of with**

114 **H2AK119u1 nucleosomes. (A) Alignment of UIMs from vertebrates. (B) Coomassie**

115 **staining of polyacrylamide gel of histone octamers reconstituted with WT H2A, H2AK119u1**

116 **and H2AK119u1I44A. Molecular weight markers are as indicated on the left. (C) Western**

117 **blot of JARID2-1-530 purified after expression in insect cells. Molecular weight markers are**

118 **as indicated on the left.**

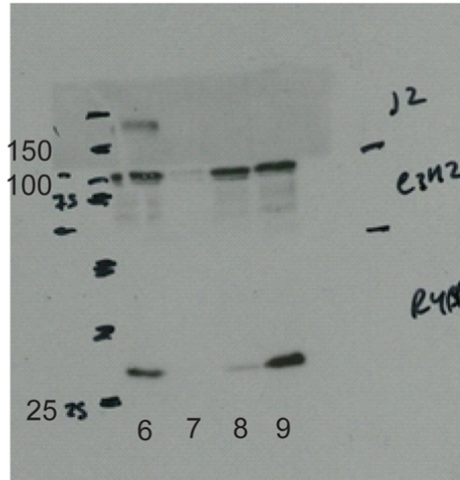
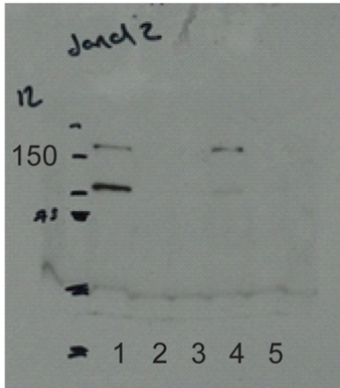
119

**Supplementary Figure 9**

WT Cells

JARID2 KO Cells

anti-JARID2

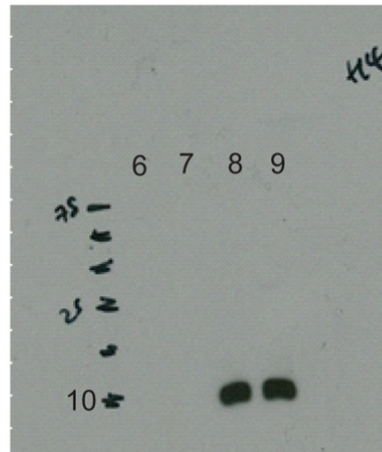
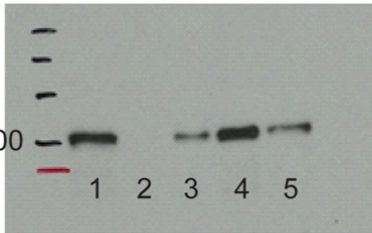


anti-JARID2

anti-EZH2

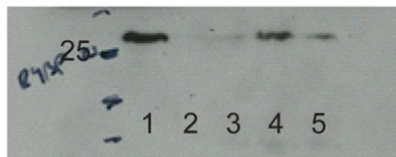
anti-RYBP

anti-EZH2

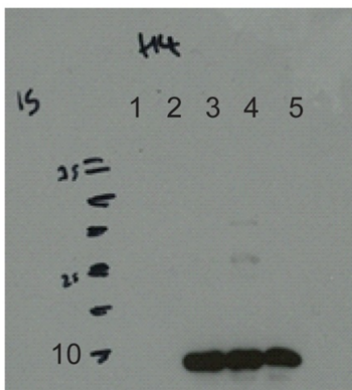


anti-H4

anti-RYBP



anti-H4



1. Input
2. Beads only
3. Unmodified Nuc
4. H2AK119u1 Nuc
5. H2AK119u1 Ub I44A Nuc
6. Input
7. Beads only
8. Unmodified Nuc
9. H2AK119u1 Nuc

120  
121

122 **Supplementary Figure 9: Full Immunoblots for the experiment shown in Figure 4a.**



123 **Supplementary Table 1.** Oligonucleotides used in this study

124

<b>Cloning primer</b>	<b>Sequence 5'- 3'</b>
J2F1	GTCGACATGAGCAAGGAAAGACCCAAGAGG
J2F121	GTCGACATGCAGCCGAATAGTCCCAGCAC
J2F542	GTCGACATGAAGGGGAGCGGCAAGTCTGGG
J2R1234	GCGGCCGCTCAGGCATAGTCAGGCACGTCATAAGGATAAAC CTTTCTCTTCTTTTTTGGTGAGGATGGGAGCCGAGATGG
J2R583	GCGGCCGCTCAGGCATAGTCAGGCACGTCATAAGGATAAAC CTTTCTCTTCTTTTTTGGCCGTA CT TCTCCACCTG
J2R541	GCGGCCGCTCAGGCATAGTCAGGCACGTCATAAGGATAAAC CTTTCTCTTCTTTTTTGGCTCTGGCTTGCCCTGTGGG
<b>sgRNA target sites</b>	<b>Sequence (PAM in bold)</b>
<i>Jarid2</i> (exon 3)	ATTTTGAAGAAGGGCCGTC <b>GAGG</b>
<i>Atrx</i> (exon 9)	ATGTCTTCTGGAACCGAGGA <b>AGG</b>
<i>Suz12</i> (exon 7)	CCAATAAGACAAGTCCCTACT <b>TGG</b>
<b>KO validation primer</b>	<b>Sequence</b>
JARID2_HRMA_F2	CCCTTTTCTCACCTGTAGGG
JARID2_HRMA_R2	GATTCACACTTGCTCCCATGT
ATRX_HRM_F3	AGCTCTCCAGTTCTGGCTCAGTC
ATRX_HRM_R3	TAGTGGGGAACATAAGGGTTCAGG
SUZ12_HRM_F2	TCAATCTTTTCTTTGCCAGGAT
SUZ12_HRM_R2	CCAATATTTTCATTGGTTTCTCC
EZH2_HRM_R2	GCAAAAGATGGAGAATGTCTAAGGA
<b>Genotyping primer</b>	<b>Sequence</b>
JD2-5ARM-F	CCTTTATCTAGAACAGTAGTTCTCAGCC
JD2-3ARM-R	CACTCACTGGGTAGATTGATTACATAC
JD2-CASS-R	AGGAATGCCCAGCCAAAATC
FLP-F	CCCATTCCATGCGGGGTATCG
FLP-R	GCATCTGGGAGATCACTGAG
CRE-F	GCCTGCATTACCGGTTCGATGCAACGA
CRE-R	GTGGCAGATGGCGCGGCAACACCATT

125