Figure S1 - Højfeldt et al.

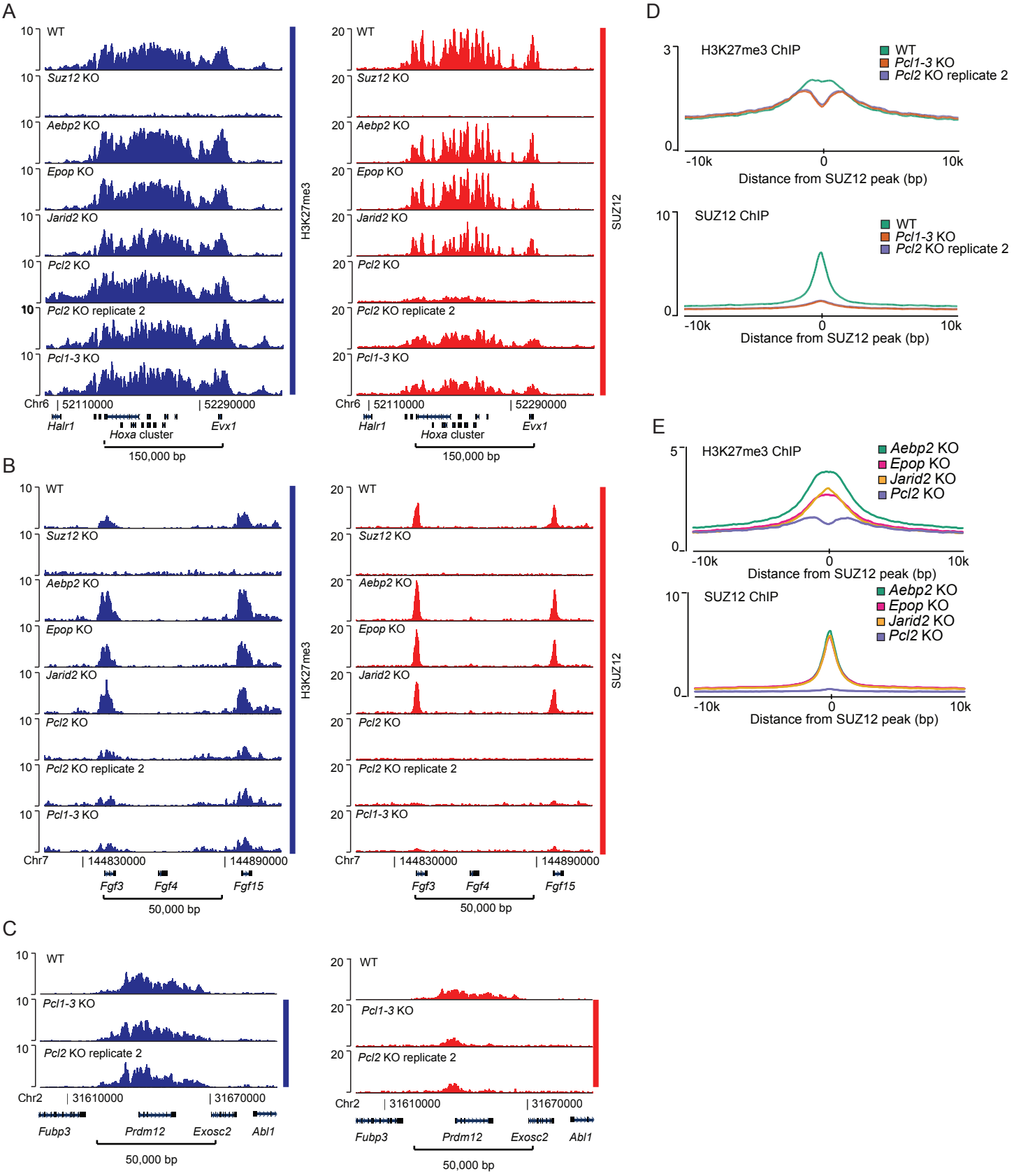


Figure S1: Individual non-core PRC2 subunits are dispensable for target site specificity. Related to Figure 1.

A) H3K27me3 and SUZ12 ChIP-seq signals (RPKM) from the indicated cell lines in a region spanning the *Hoxa* gene cluster. *Pcl2* KO replicate 2 data was generated in parallel with Pcl1-3 KO.

B) H3K27me3 and SUZ12 ChIP-seq signals (RPKM) from the indicated cell lines in a region that includes the CGI-promoter genes *Fgf3*, *Fgf15* (repressed) and *Fgf4* (active). *Pcl2* KO replicate 2 data was generated in parallel with Pcl1-3 KO.

C) H3K27me3 and SUZ12 ChIP-seq signals (RPKM) region including the PRC2 target gene *Prdm12*. *Pcl2* KO replicate 2 data was generated in parallel with Pcl1-3 KO.

D) Mean H3K27me3 (top) and SUZ12 (bottom) ChIP-seq signals (RPKM) for WT, *Pcl1-3* KO and *Pcl2* KO (replicate 2) cell lines in regions centered on 7,796 SUZ12 peak regions identified in WT mESCs.

E) Mean H3K27me3 (top) and SUZ12 (bottom) ChIP-seq signals (RPKM) for *Aebp2*, *Epop* KO, *Jarid2* KO and *Pcl2* KO cell lines in regions centered on 7,796 SUZ12 peak regions identified in WT mESCs. The preparation of these ChIP-seq data was completed separately from data summarized in Figure S1D and quantitative comparisons between the two datasets are difficult due to the variability of the method.

Figure S2 - Højfeldt et al.

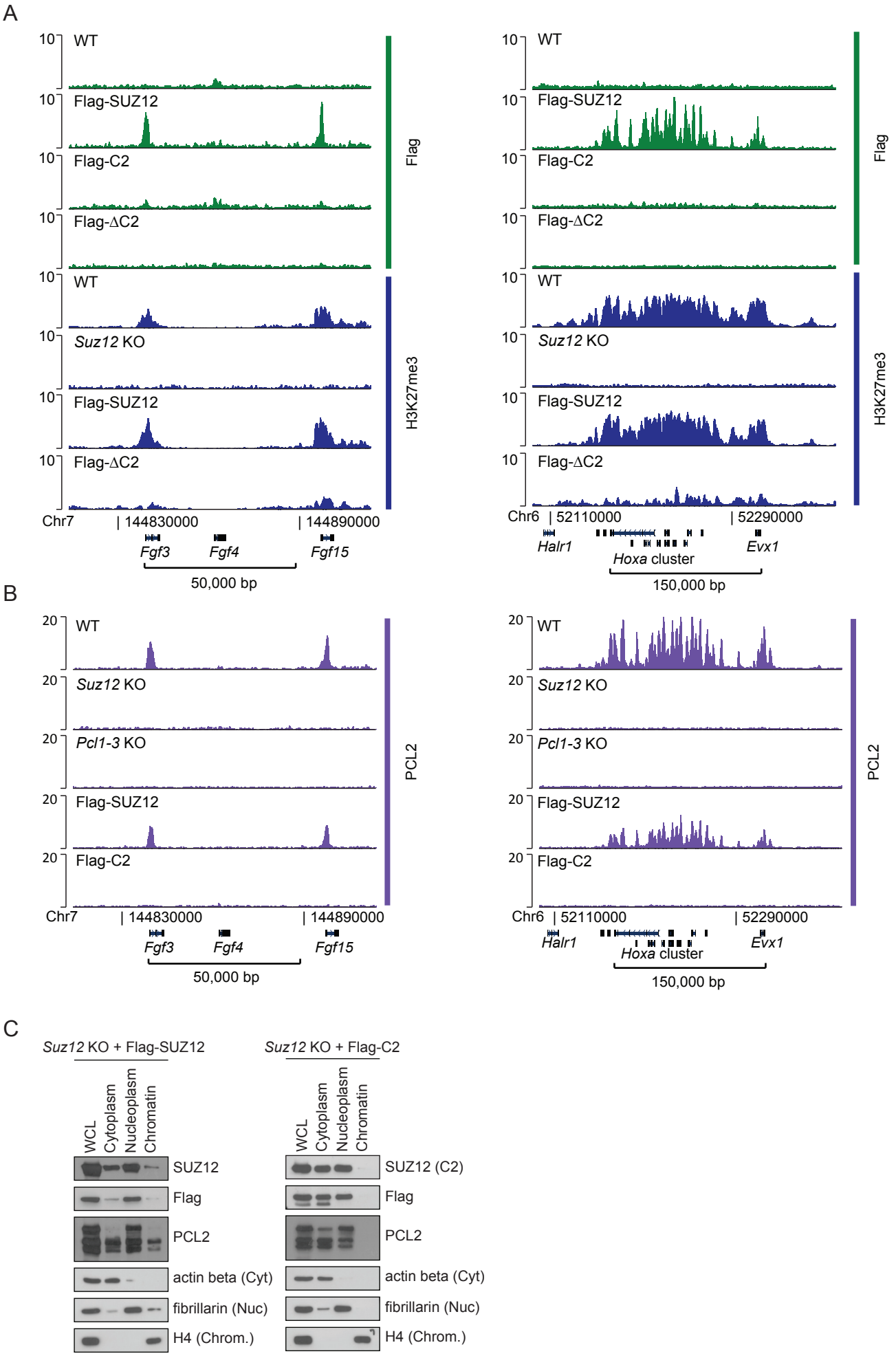


Figure S2: PCL2 binding to PRC2 target sites is dependent on SUZ12. Related to Figure 2.

A) Flag and H3K27me3 ChIP-seq signals (RPKM) from indicated cell lines within representative genomic regions.

B) PCL2 ChIP-seq signals (RPKM) from indicated cell lines within representative genomic regions.

C) Western blot for SUZ12, Flag, PCL2 and marker proteins of cellular fractions from cellular fractionation experiments using the indicated cell lines. WCL (whole-cell-lysate), cytosolic fraction, nucleoplasmic fraction, and nuclear insoluble fraction (chromatin) were loaded corresponding to equal number of cells.

Figure S3 - Højfeldt et al.

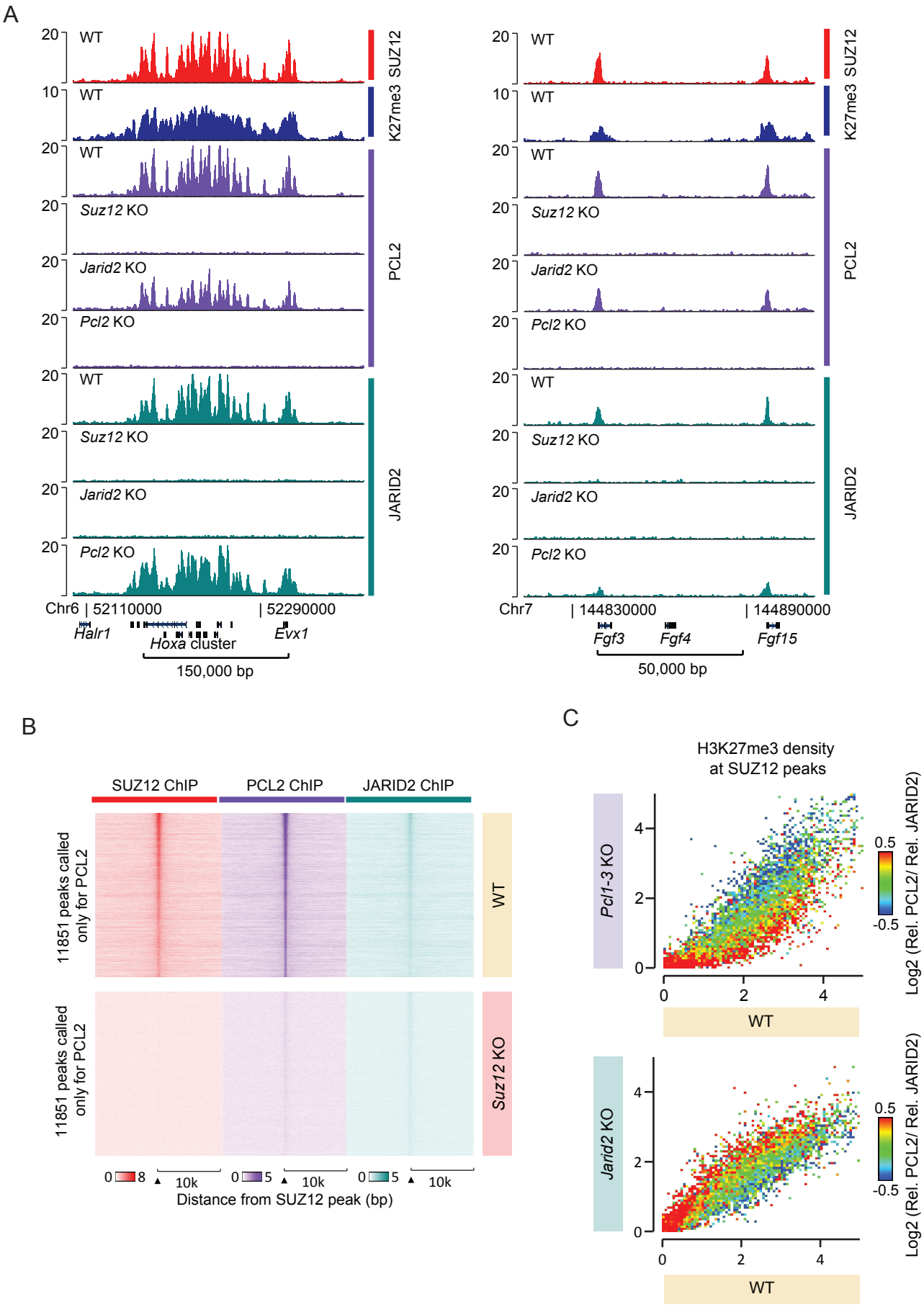


Figure S3: PRC2.1 and PRC2.2 – differential number of called peaks primarily result from differential signal-to-noise in ChIP experiments. Related to Figure 3.

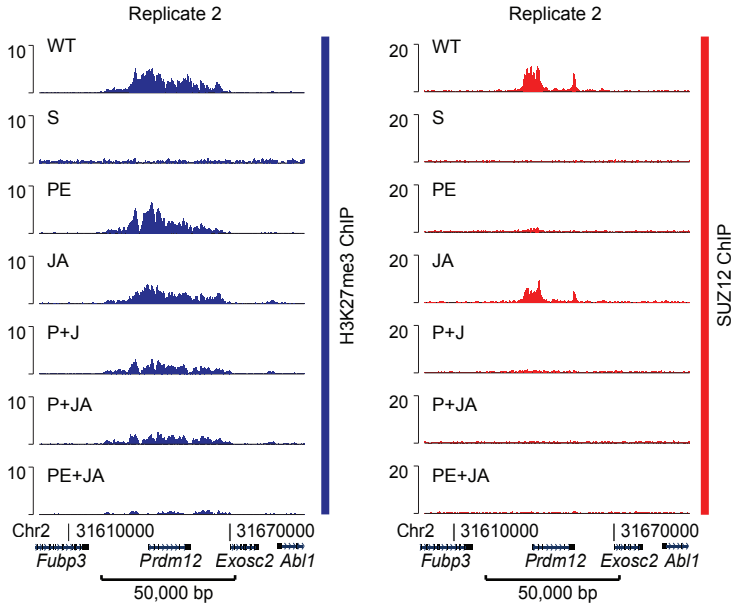
A) SUZ12, K3K27me3, PCL2 and JARID2 ChIP-seq signals (RPKM) from indicated cell lines within representative genomic regions.

B) Heatmaps of SUZ12, PCL2 and JARID2 ChIP-seq signals (RPKM) from indicated cell lines at 11,851 PCL2 peaks that were not called as SUZ12 peaks. Horizontal axis shows a 20,000 bp window centered on PCL2 peaks.

C) Scatterplots comparing H3K27me3 signals (RPKM) within each SUZ12 peak (called in WT cells) for WT mESCs (x-axis) versus *Pcl1-3* KO mESCs (y-axis top plot) or *Jarid2* KO mESCs (y-axis bottom plot). Each point in the scatterplot is pseudocolored according to the balance between relative ChIP-seq signal strength generated with PCL2 and JARID2 antibodies (PCL2 signal relative to average PCL2 signal) / (JARID2 signal relative to average JARID2 signal).

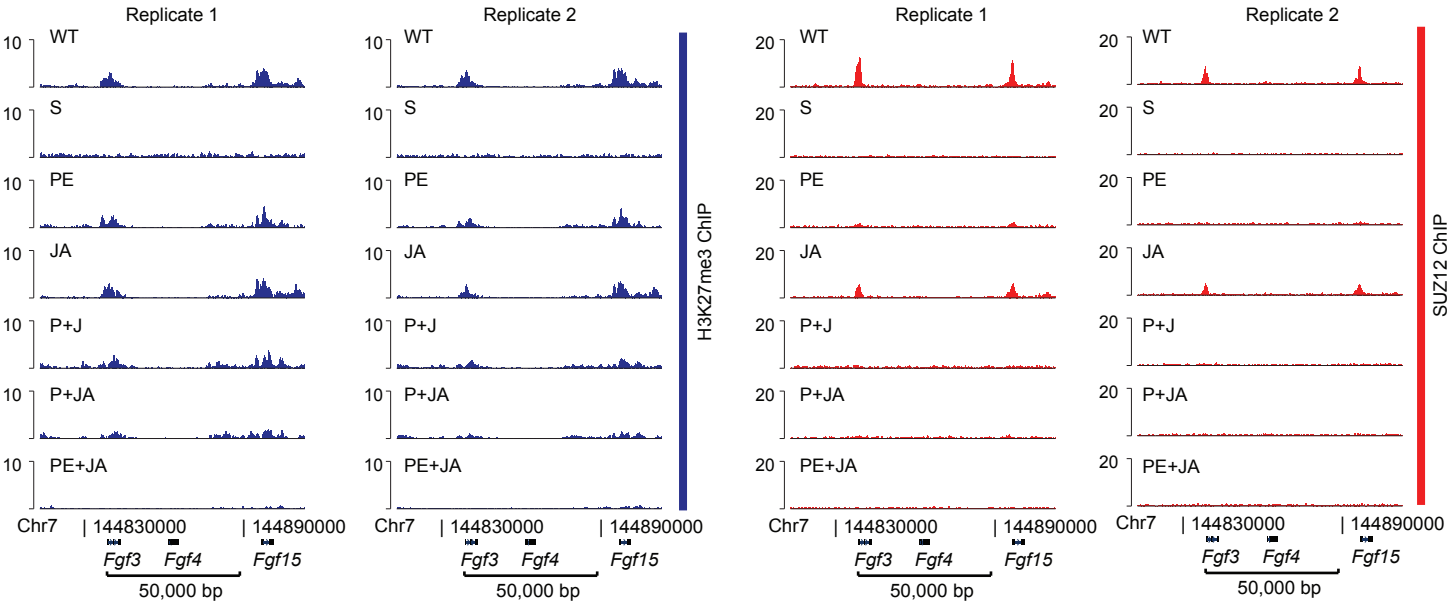
Figure S4 - Højfeldt et al.

A

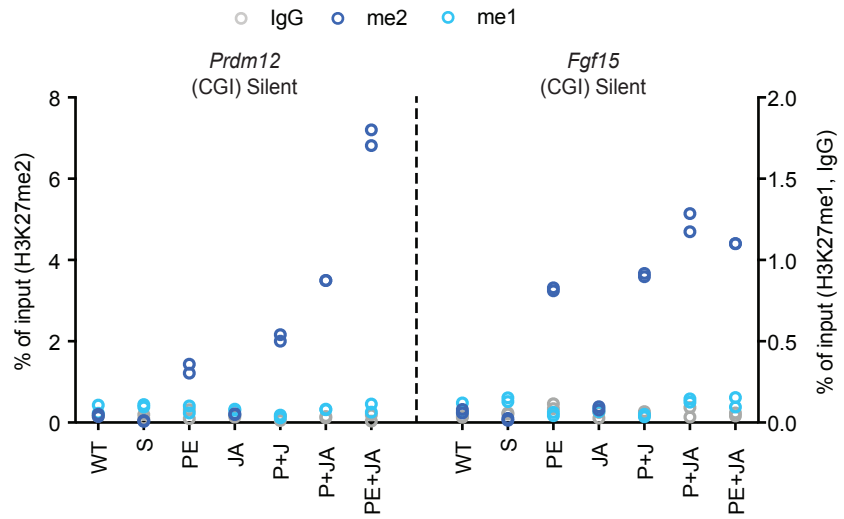


Symbol	KO combinations
S	*Suz12* KO
PE	*Pcl1-3* + *Epop* (PRC2.1) KO
JA	*Jarid2* + *Aebp2* (PRC2.2) KO
P+J	*Pcl1-3* + *Jarid2* KO
P+JA	*Pcl1-3* + *Jarid2* + *Aepb2* KO
PE+JA	*Pcl1-3* + *Epop* + *Jarid2* + *Aebp2* KO

B



C



D

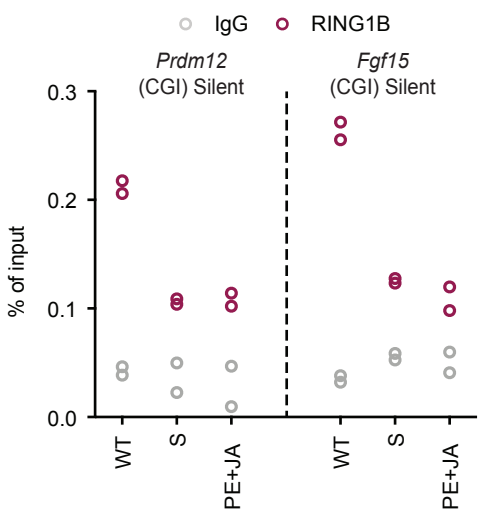


Figure S4: PRC2 non-core subunits are collectively required for PRC2 binding. Related to Figure 4.

A) H3K27me3 and SUZ12 ChIP-seq signals (RPKM) from indicated cell lines within a representative genomic region that includes the PRC2 target gene *Prdm12*. Data is from a replicate experiment (Replicate 2) of that shown in Figure 4C (Replicate 1).

B) H3K27me3 and SUZ12 ChIP-seq signals (RPKM) from indicated cell lines in a region including the *Hoxa* gene cluster for. Data from two replicate experiments are shown.

C) ChIP-qPCR signals (% of input) for H3K27me2 (left) and H3K27me1 (right) compared to IgG control (gray) in the indicated cell lines at PRC2 target sites (CGI promoters of *Prdm12* and *Fgf15*). The ChIP-qPCR values are from a single biological (n = 1) experiment with two technical replicate values shown.

D) ChIP-qPCR signals (% of input) for RING1B compared to IgG control (gray) in the indicated cell lines at PRC2 target sites (CGI promoters of *Prdm12* and *Fgf15*). The ChIP-qPCR values are from a single biological (n = 1) experiment with two technical replicate values shown.

Figure S5 - Højfeldt et al.

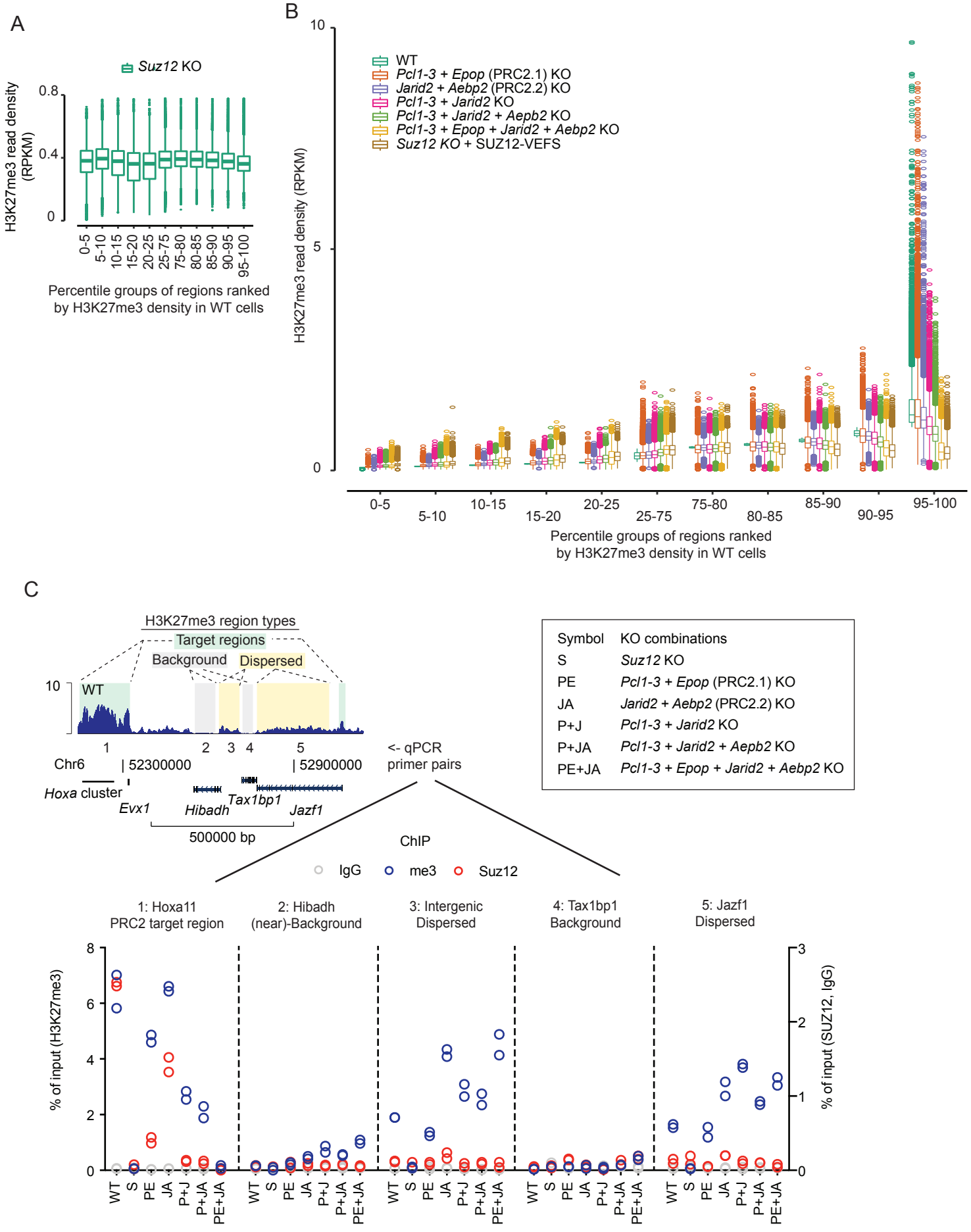


Figure S5: Dispersed and recruitment dependent populations of H3K27me3. Related to Figure 5.

A) Box plot of H3K27me3 read densities (RPKM) for *Suz12* KO cells within 5 percentile regions or the middle 50 percentile regions shown in Figure 5A. Boxes extend from first to third quartile with a band marking the median. Whiskers extend to values up to 1.5 interquartile distances from box, and all additional values outside of this range are marked with circles.

B) Box plot of same cell lines as in Figure 5B but with inclusion of all 5 percentile regions.

C) ChIP-qPCR signals (% of input) for H3K27me3 (blue, left y-axis scale), SUZ12 (red), and IgG control (gray) (right y-axis scale) in the indicated cell lines at sites indicated under ChIP-seq track (top left). Above qPCR data is indicated the type of H3K27me3 region probed by the qPCR primers (PRC2 target region, dispersed or background level). The ChIP-qPCR values are from a single biological (n = 1) experiment with two technical replicate values shown.

Figure S6 - Højfeldt et al.

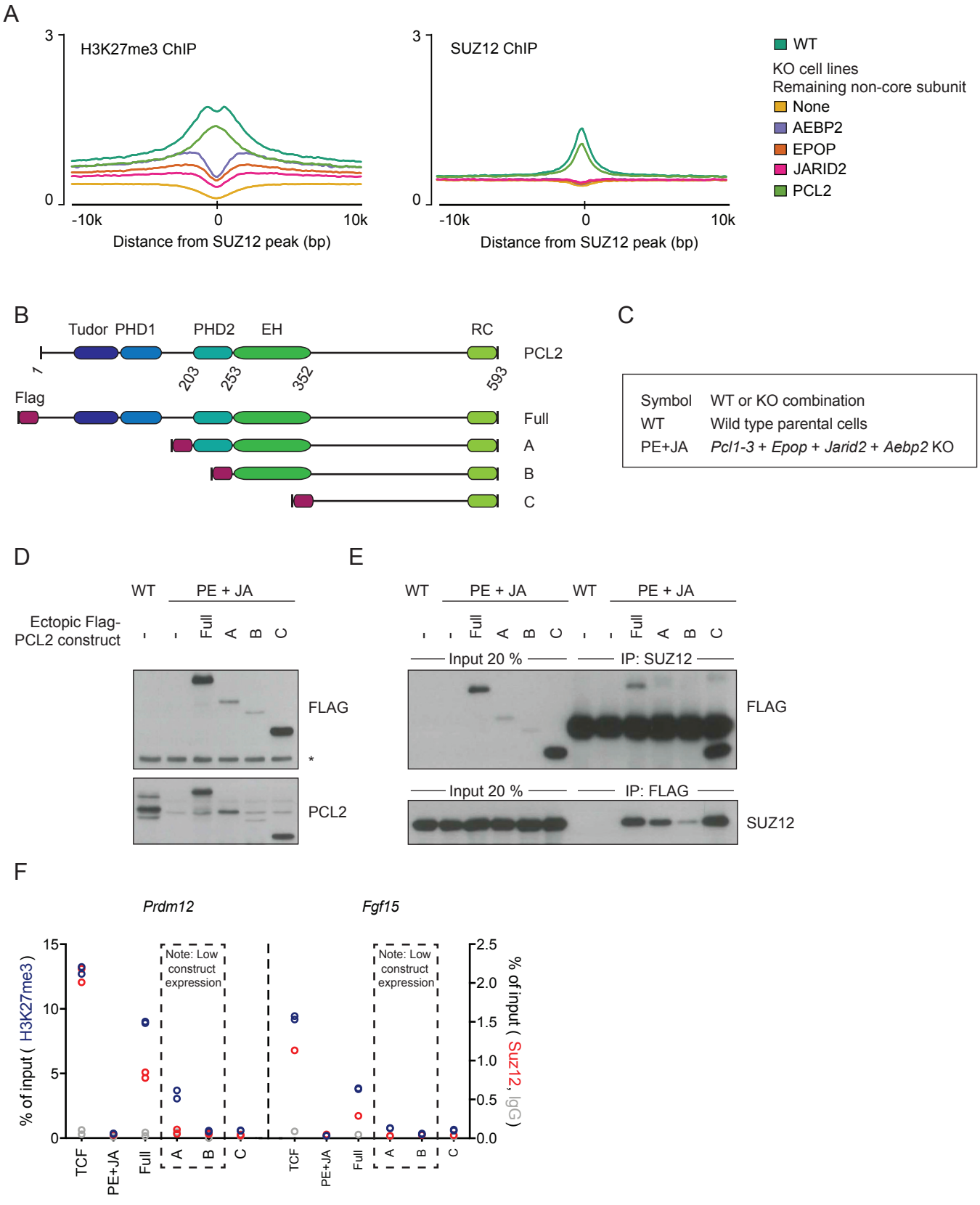


Figure S6: Each of the non-core PRC2 subunits contributes to target specific H3K27me3. Related to Figure 6.

A) Mean H3K27me3 (left) and SUZ12 (right) ChIP-seq signals (RPKM) for the indicated cell lines in regions centered on 7,796 SUZ12 peak regions identified in WT mESCs.

B) Schematic drawing of PCL2 with colored domains (Top) and of expression constructs of PCL2 fragments used in rescue experiments (Bottom). Boundaries (amino acid numbers) for the fragments used in this study are indicated.

C) Symbols for cell lines used in Figure S6D-F.

D) Western blot for PCL2 and Flag of cell extracts prepared from the indicated cell lines.

E) Western blot for PCL2 and Flag of cell extracts (input) and anti-Flag or anti-SUZ12 immunoprecipitated (IP) material from the indicated cell lines (wildtype mESC (WT) or non-core subunit knockout mESC (PE+JA) with ectopic expression of Flag-tagged PCL2 fragments). Fragment boundaries are shown in Figure S6B.

F) ChIP-qPCR signals for H3K27me3 (blue, left y-axis scale), SUZ12 (red), and IgG control (gray) (right y-axis scale) in WT and non-core subunit KO cell lines expressing PCL2 fragments. The regions probed are PRC2 target sites (CGI promoters of *Prdm12* and *Fgf15*). Data values are from a single biological (n = 1) experiment with two technical replicate values shown.

Figure S7 - Højfeldt et al.

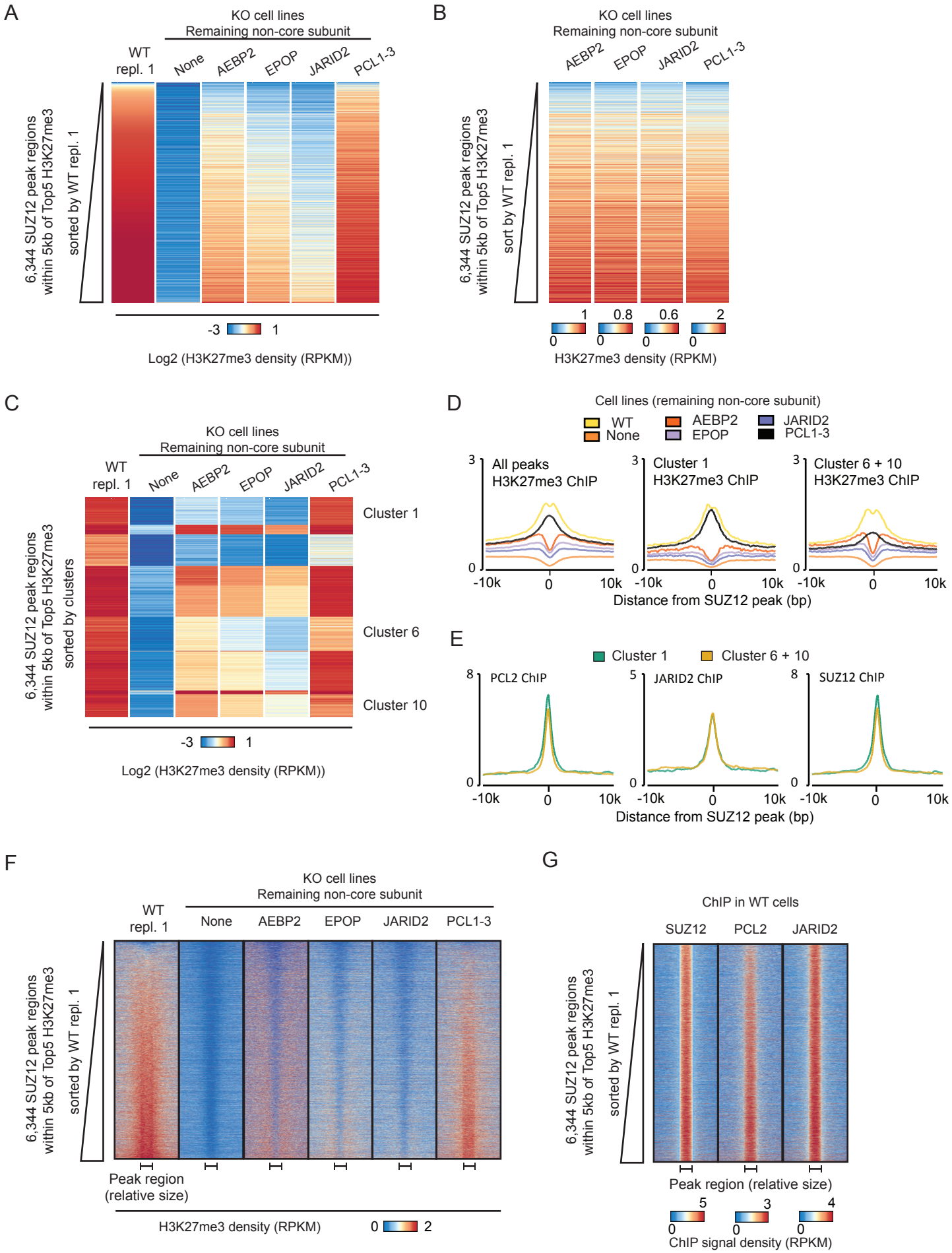


Figure S7: The non-core PRC2 subunits contribute to a shared set of regions but produce different H3K27me3 distributions within the regions. Related to Figure 6.

A) Heatmaps of the H3K27me3 ChIP-signal (RPKM) for indicated cell lines in SUZ12 peak regions (+/- 500bp) that lie within 5kb of a Top5 H3K27me3 region (Figure 5A). Lines in heatmaps are sorted by signal density in WT cells (replicate 1).

B) Heatmaps for four cell lines as in Figure S7A, but with individually scaled coloring as indicated below graph.

C) Heatmaps of the H3K27me3 ChIP-signal (RPKM) for indicated cell lines in SUZ12 peak regions (+/- 500bp) that lie within 5kb of a Top5 H3K27me3 region (Figure 5A). Lines of heatmaps are sorted by clusters identified by k-means clustering.

D) Mean H3K27me3 ChIP-seq signals (RPKM) for indicated cell lines in regions centered on SUZ12 peak regions overlapping Top5 H3K27me3 regions (right), cluster 1 (middle) and cluster 6 + 10 (right).

E) Mean PCL2 (left), JARID2 (middle) and SUZ12 (right) ChIP-seq signals (RPKM) for indicated cell lines in regions centered on SUZ12 peak regions overlapping cluster 1 (green) or cluster 6 + 10 (yellow).

F) Heatmaps of the H3K27me3 ChIP-signal (RPKM) for indicated cell lines across SUZ12 peak regions. Lines in heatmaps are sorted by signal density in WT cells (replicate 1) and are displayed with a length relative to the peak width (window is 5 peak widths wide) centered on peaks.

G) Heatmaps of the SUZ12 (left), PCL2 (middle) and JARID2 (right) ChIP-signal (RPKM) for indicated cell lines across SUZ12 peak regions. Lines in heatmaps are sorted by H3K27me3 signal density in WT cells (replicate 1) and are displayed with a length relative to the peak width (window is 5 peak widths wide) centered on peaks.

Table S1. Overview of mESC lines used in this study. Related to STAR Methods.

ID/Genotype (strain)	Short ID	Source (Reference)			
Published mESC lines					
TCF2.2 / WT (129B6F1)	WT	Transgenic core, UCPH (Martin Gonzalez et al., 2016)			
Suz12 KO	S	Helin lab (Hojfeldt et al., 2018)			
ID/Genotype	**Short ID**	**Parental line**	**Knockout strategy**	**Target exon(s)**	**gRNA sequences**
Knockout cell lines generated in this study, all derived from wildtype (TCF2.2 / WT)					
Aebp2 KO	A	WT	Frameshift	Exon 2	ACGCTGACCATCGACATGTA
Epop KO	E	WT	Frameshift	Exon1	CGAGCAGGGAGACCCCCGCG
Jarid2 KO	J	WT	Frameshift	Exon 3	ATGACAGCGATGGGATCCCG
Pcl2 KO	P2	WT	Deletion	Exon 2 Exon 15	GACGTAAAGGAGACCGCTTG CTCGTCGGGTGACGCTTGAT
Pcl1-2 KO	P21	P2	Deletion	Exon 3 Exon 15	ACTGGGAATCGTCCTCAAAC TGCTCGAAGAGTGCGGCCTG
Pcl1-3 KO	P213 / P	P21	Deletion	Exon 2 Exon 15	AGGTAATATAGCCCGTCTGT GGTCCTGAGCAGCCGGATGA
Jarid2-del KO	J-d	WT	Deletion	Exon 3 Exon 17	ATGACAGCGATGGGATCCCG TCCCCGCAAGAGAGCGACCG
Jarid2 + *Aebp2* KO	JA	J-d	Deletion	Exon 1 Exon 9	GGGTGCTGCAAACCGACCGC ACTACTGTGGCCAACCCGAA
Pcl1-3 + *Epop* KO	PE	P	Frameshift	Exon1	CGAGCAGGGAGACCCCCGCG
Pcl1-3 + *Jarid2* KO	P + J	P	Deletion	Exon 3 Exon 17	ATGACAGCGATGGGATCCCG TCCCCGCAAGAGAGCGACCG
Pcl1-3 + *Jarid2* + *Aebp2* KO	P + JA	P + J	Deletion	Exon 1 Exon 9	GGGTGCTGCAAACCGACCGC ACTACTGTGGCCAACCCGAA
Pcl1-3 + *Jarid2* + *Aebp2* + *Epop* KO	PE + JA	P + JA	Deletion	Exon 1 Exon 1	AGGGCCGCGTGACGATACCG ACTGTCATTTGAGGTCCCGC
Pcl1-3 + *Epop* + *Aebp2* KO	PE + A	PE	Deletion	Exon 1 Exon 9	GGGTGCTGCAAACCGACCGC ACTACTGTGGCCAACCCGAA
Pcl1-3 + *Epop* + *Jarid2* KO	PE + J	PE	Deletion	Exon 3 Exon 17	ATGACAGCGATGGGATCCCG TCCCCGCAAGAGAGCGACCG
Jarid2 + *Aebp2* KO + *Epop* KO	E + JA	JA	Deletion	Exon 1 Exon 1	AGGGCCGCGTGACGATACCG ACTGTCATTTGAGGTCCCGC

Table S2. Overview of antibodies used in this study. Related to STAR Methods.

Target	ID	Type	Epitope	Use	Source/Cat no.
Core PRC2					
SUZ12	D39F6	Rabbit mAb	Around aa260	WB, ChIP	Cell Signaling #3737
EZH2	BD43	Rabbit mAb	N-terminal	WB	Helin lab
EED	AA19.30	Mouse mAb		WB	Helin lab
Non-core PRC2					
RBBP4	11G10	Mouse mAb	aa1-425	WB	Abcam #230518
AEBP2	D7C6X	Rabbit mAb	Around aa1114	WB	Cell Signaling #14129
EPOP	61753	Rabbit pAb	aa59-229	WB	Active Motif #61753
JARID2	D6M9X	Rabbit mAb	Around aa345	WB, ChIP	Cell Signaling #13594
PCL2	16208-1-AP	Rabbit pAb		WB, ChIP	Protein Tech #16208-1-AP
Histone modifications					
H3K27me3	C36B11	Rabbit mAb		WB, ChIP	Cell Signaling #9733
H3K27me2	D18C8	Rabbit mAb		WB, ChIP	Cell Signaling #9728
H3K27me1	MABI0321	Mouse mAb		WB, ChIP	Active Motif #61016
H4	07-108	Rabbit pAb		WB	Millipore #07-108
Others					
Flag	M2	Mouse mAb	DYKDDDDK	IP	Sigma-Aldrich #A2220
	SAB1306078	Rabbit pAb		WB	Sigma-Aldrich #F7425
Beta actin	AB8226	Mouse mAb		WB	Abcam #ab8226
Fibrillarin	EPR10823(B)	Rabbit mAb		WB	Abcam #ab166630
RING1B	D22F2	Rabbit mAb		ChIP	Cell Signaling #5694

Table S3. Overview of primer sequences used for ChIP-qPCR. Related to STAR Methods.

Locus	Forward primer	Reverse primer
PRC2 target CGIs		
Prdm12	CACCGCAGCTTAAGGAGTGA	AGCCCAGTACTCTGTCCGAT
Fgf15	GCAGTACCTGTACTCCGCTG	GTTTTGGTCCTCCTCGCAGT
HoxA11-13	CGCCGAGGACTTGACCTTTA	CTGTCTGCTATTGGGTGGCA
CGI promoter of active gene		
Fgf4	TAGTCTCGGGGGCTGAGTAG	GAATTCCGCACCGAGAGACC
Non-CGI intra- and inter-genic regions regions		
Fubp3	GCTGGAAGTCCTCAAGCAGT	ACACTTGTTAGCGAGGGTGG
Hibadh	CAAGGTCAAGGCTGCTCGTA	TAGTCGTTTTTGTTGGCGGC
Hibadh-Tax1bp1 intergenic	GACAGCCCTTCACTGGGTTT	CTTCGGGGTGCTGACAGATT
Tax1bp1	TCTGCCATATGGGGTCTGGA	GACCATGGGGCTGGAAAAGA
Jazf1	TATGGATGGAGGGACTGGCA	GAGGCACAGATGGAGCTTGT