SUPPLEMENTARY INFORMATION:

Bivalent complexes of PRC1 with orthologs of BRD4 and MOZ/MORF target developmental genes in *Drosophila*

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Supplemental_ Table_S1.

List of PCR primers used for qPCR analysis

Name	Primer set names	Sequences (5' to 3')
C1	J312	CAATCAGCAGATTCTCCGGCT
	J313	AGCCGCACTCGCGCTTCTAC
C2	J318	GGACCTGTGCAGTTCCTCC
	J319	CCGTTATTTCTGTATCGCTGTG
R1	J473	AAAGCCAGCCACGAGGAA
	J474	CGACTGGGCAGCAGGAAT
R2	J479	CGGATGGGATGCTGAATG
	J480	GAACTGGGCACCGGGAAT
R3	J455	CCATAAGAAATGCCACTTTGC
	J456	CTCTCACTCTCTCACTGTGAT
R4	J459	TTTACTCCCAAAGCAACC
	J460	TCGGCTTCTTATTCATCT
R5	J467	TTAGGCCGAGTCGAGTGA
	J468	AACAATAATGCCGCTGAT
R6	J461	AGAAAGGACGTAACAAGC
	J462	CATCTATGGATTCGGTTTT
R7	J315	ACGGCCATTACGAACGACA
	J328	GGGCTATTCCAAGTCTGACG
R8	J471	ATCTAGCGGCATGTGGACG
	J472	GCAGATAGGCAGGTTTCGTG

C1 amplifies PKa-C1(CG4379) region, C2 amplifies Antp (CG1028) region, and R1-R8 amplify Ubx (CG10388) regions

Supplemental Figure S1. Validation of Br140 and dBRD4 BioTAP tagged protein expression in S2 cells and transgenic flies. (A) Western blot analysis of nuclear extracts from S2 cells (left panel) and embryos (right panel) expressing BioTAP-N-Br140. Fusion proteins were detected by Peroxidase Anti-peroxidase (PAP) antibody. (B) Left panel: representation of short (dBRD4 S) and long isoforms (dBRD L) of dBRD4. BD1: bromodomain 1; BD2: bromodomain 2; NET: N-terminal ET (extraterminal) domain; CTD: C-terminal domain. Right panel: western blot analysis of nuclear extracts from S2 cells expressing BioTAP-N-dBRD4 or dBRD4-C-BioTAP. Fusion proteins were detected by PAP antibody. (C) Viability rescue tests of Br140 mutants by BioTAP-N-Br140 indicates functionality of the transgenic construct.

Supplemental Figure S2. Comparison of peptide counts from BioTAP-XL pull-downs. Total peptide counts from pull-downs of Pc, and E(z) (Kang et al. 2015), and dBRD4 and Br140 (this report) compared to input chromatin proteins from S2 cells and *Drosophila* embryos. Proteins are color-coded by protein complex. See Datafile S1 for complete results.

Supplemental Figure S3. Most genes with strong occupancy by Pc are co-bound by Br140. (A) Heatmap as in Fig. 4, but showing all bound genes rather than just co-bound loci from embryo ChIP-seq enrichment patterns for BioTAP-tagged Pc, Br140, dBRD4 and indicated histone H3 modifications within ± 10 kb of TSSs of Pc-only, Br140-only, or co-bound genes. (B) Venn diagram of overlap between Pc- and Br140-bound genes in embryos using a higher threshold for Pc peak calling. (C) Heatmaps of embryo co-bound and Pc-only ChIP-seq enrichment patterns using a stringent threshold, showing that practically all strong Pc bound genes are also enriched for Br140. (D) Gene expression variability plots show higher variability in Cluster 1 (top) and Cluster 2 (bottom) co-bound genes when compared to globally expressed,

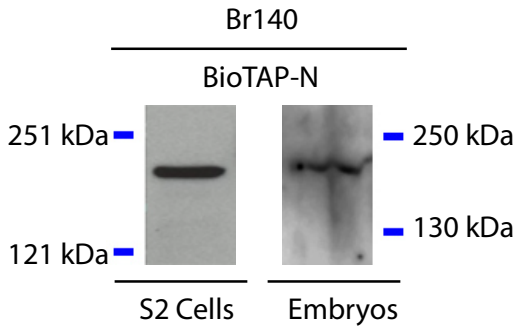
or constitutive, genes. Upper, middle, and lower lines in the boxes represent upper, median, and lower quartiles, respectively. *P*-values were determined using Wilcoxon rank-sum test.

Supplemental Figure S4. Post-translational modifications on histone tails enriched in Br140- and Pc-BioTAP-XL affinity pull-downs. (A) Schematic of experimental procedure for recovery of DNA and protein samples from BioTAP-XL affinity pull-downs. Samples intended for PTM analysis (purple arrows) were separated and underwent additional anhydride derivatization steps prior to LC-MS. (B) Relative abundance of methylation at H4K20 (specifically the 20-23 H4 peptide NH_3-K_{20}VLR-COOH) in total histones and histones enriched by BioTAP-tagged Br140 and Pc. (C) Relative abundance of combinatorial acetylation patterns at H3K18 and K23 (specifically the 18-26 H3 peptide NH_3-K_{18}QLATK_{23}AAR-COOH) in total histones and histones enriched by BioTAP-tagged Br140 and Pc. (D) Relative abundance of combinatorial modification patterns at H3K9 and K14 (specifically the 9-17 H3 peptide NH_3-K_9STGGK_{14}APR-COOH) in total histones and histones enriched by BioTAP-tagged Br140 and Pc. (E) Relative abundance of H4 acetylation levels (specifically the 4-17 H4 peptide NH_3-GK_5GGK_8GLGK_{12}GGAK_{16}R-COOH) in total histones and histones enriched by BioTAP-tagged Br140 and Pc. For clarity of the figure, only the number of acetyl groups (0, 1, 2, 3, or 4 acetyls) is plotted rather than the specific position where the acetyl group is localized. (F) Relative abundance of combinatorial modification patterns on H3K27 and K36 (specifically the 27-40 canonical H3 peptide NH_3-K_{27}SAPATGGVK_{36}KPHR-COOH) in total histones and histones enriched by BioTAP-tagged Br140 and Pc.

Supplemental Figure S5. Co-bound genes in HUES64 cells are enriched for developmental GO terms. Bubble plot showing significantly enriched GO terms from the PANTHER database for BRD1-only and co-bound genes. Significantly enriched terms after Bonferroni correction (adjusted p-value <0.01) were plotted in R. Bubble fill represents adjusted p-value and bubble size represents gene percentage in each category. The bubbles are ranked according to descending fold enrichment (observed/total number of genes per category).

Supplemental_ Fig_S1.

A



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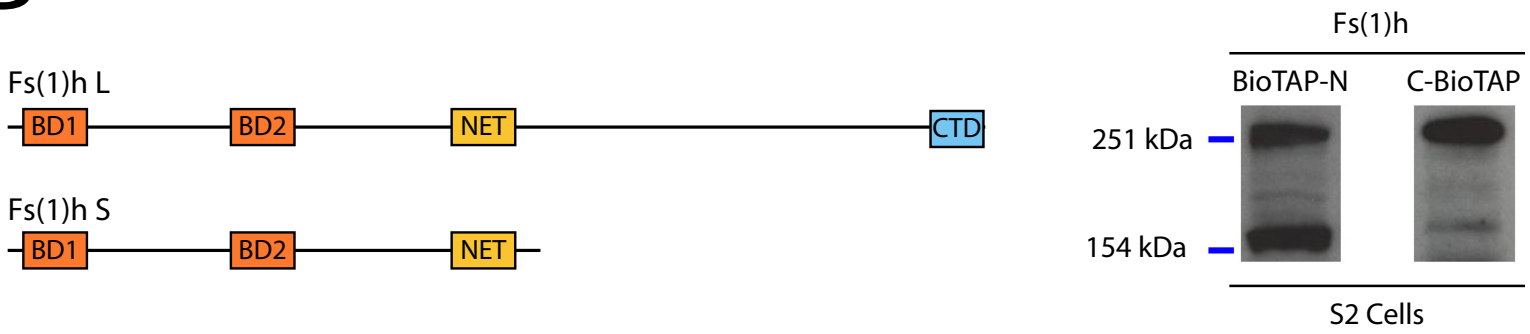


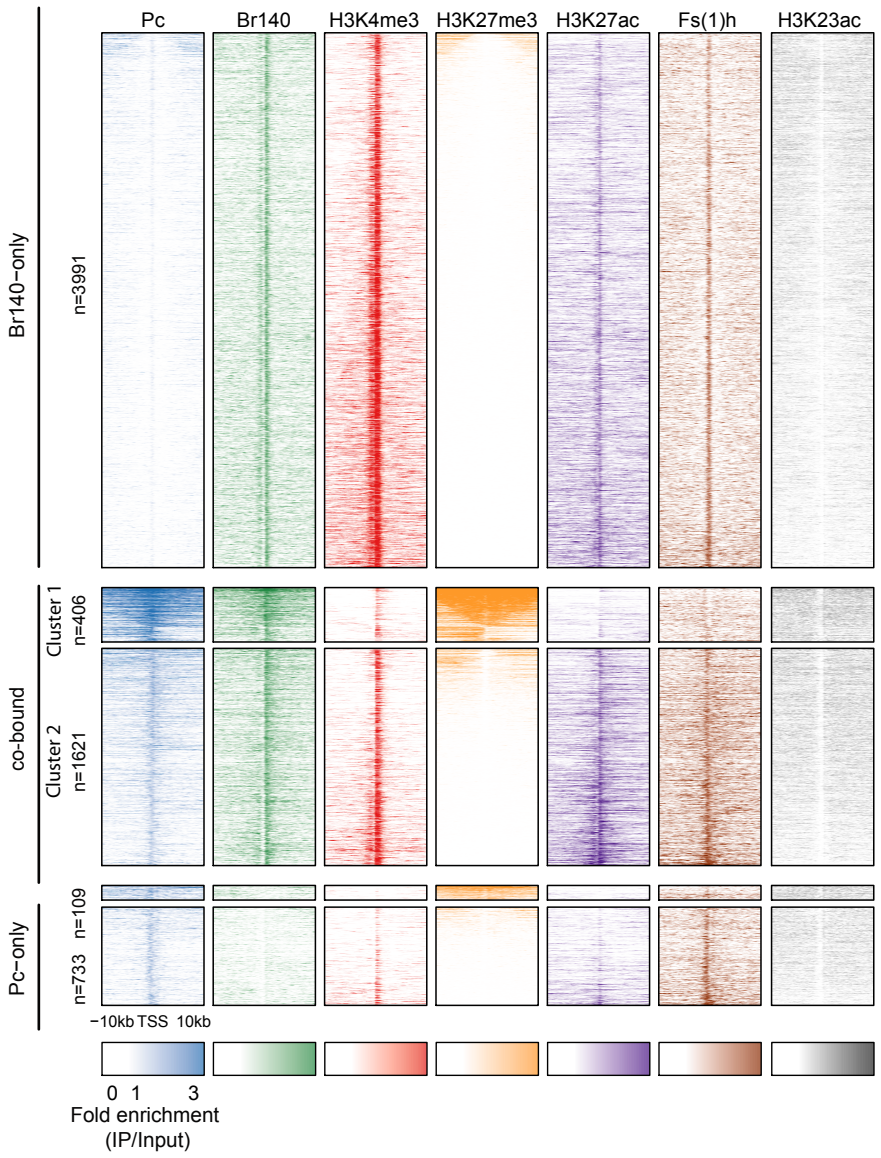
C

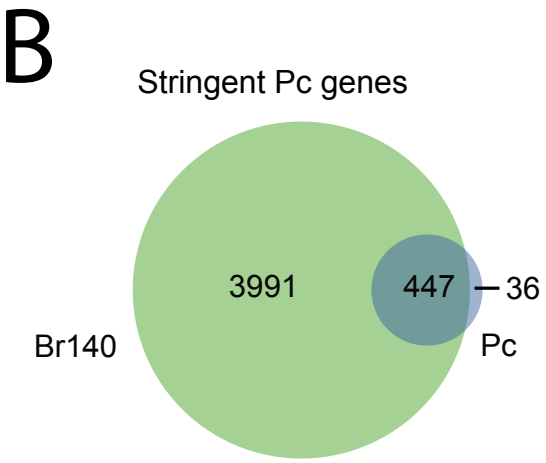
Experimental cross	Counted total # of viable F1	Genotype	Viable F1 with this genotype	Observed proportion (Expected mendelian proportion)
yw ; *Br140*^{S203}/CyO ; P{BioTAP-N-Br140}/+ X *yw* ; *Br140*^{S781}/CyO ; +/+	281	*yw* ; *Br140*^{S203}/*Br140*^{S781} ; +/+	0	0 (0.2)
		yw ; *Br140*^{S203}/*Br140*^{S781} ; P{BioTAP-N-Br140}/+	57	0.202 (0.2)
yw ; *Br140*^{S203}/CyO ; P{BioTAP-N-Br140}/+ X *yw* ; Df(2R)BSC263/CyO ; +/+	139	*yw* ; *Br140*^{S203}/Df(2R)BSC263 ; +/+	0	0 (0.2)
		yw ; *Br140*^{S203}/Df(2R)BSC263 ; P{BioTAP-N-Br140}/+	38	0.273 (0.2)

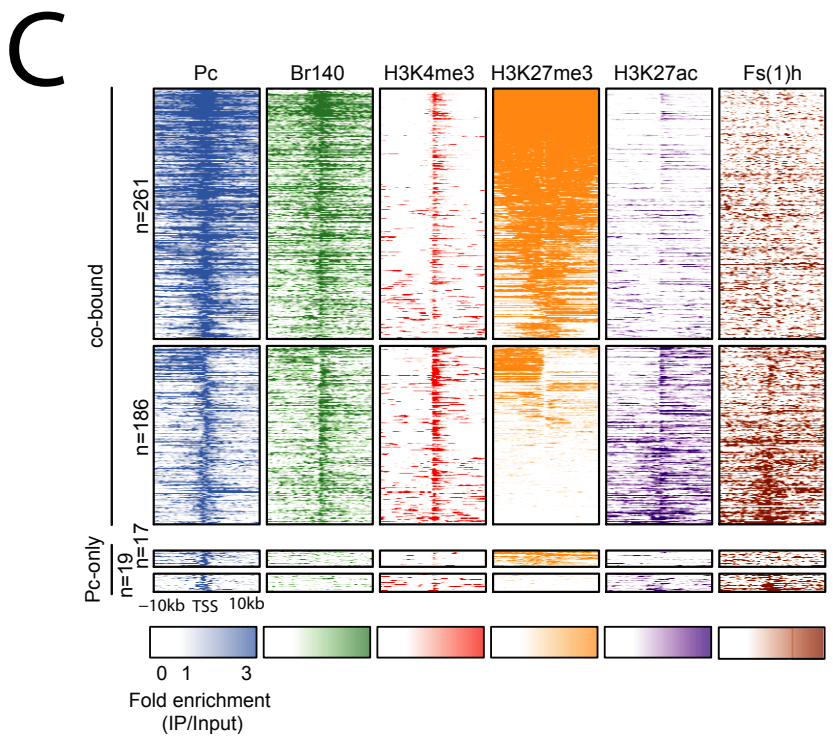
Supplemental_ Fig_S2.

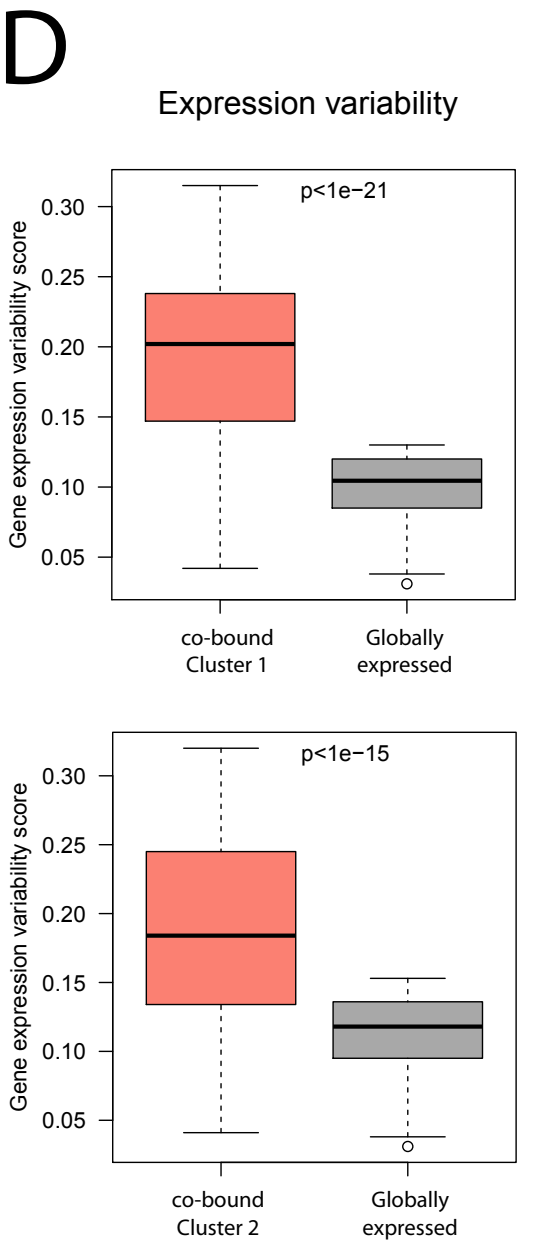
Complex	Symbol	M.W. (kDa)	Pc	E(z)	Fs(1)h		Br140		Input	
			Embryo	Embryo	S2 Cell		S2 Cell	Embryo	S2 Cell	Embryo
					Long + Short	Long				
dMOZ/MORF	Br140	157.02	40	0	7	2	210	27	0	5
	enok	254.52	34	0	6	7	130	28	1	4
	Eaf6	24.39	5	0	3	1	18	3	1	0
	Ing5	32.06	4	0	2	1	11	3	0	0
dMOZ/MORF interactor	elg1	129.29	1	0	0	1	32	16	0	5
TrxG	ash1	246.11	3	0	2	1	10	3	0	0
	Fs(1)h	205.93	61	2	476	284	9	6	7	12
PRC1	Psc	169.73	92	5	14	7	1	5	0	2
	Su(z)2	149.48	72	0	13	3	5	2	0	0
	Sce	47.23	49	2	5	3	3	1	1	2
	Pc	43.95	21	6	5	3	4	1	1	1
	ph (p+d)	167.18	66	1	6	2	2	2	0	0
	Scm	93.49	12	18	4	0	1	1	2	3
PRC2	Su(z)12	100.04	3	83	0	3	0	1	0	1
	E(z)	87.44	0	23	0	0	0	0	1	1
	esc/escl	47.96	1	42	2	0	0	0	1	1
	Pcl	114.57	1	33	0	0	1	0	0	0
	jing	181.09	0	30	0	0	0	0	0	0
	Jarid2	252.37	4	33	7	3	4	1	0	8
	Caf1-55	48.6	3	32	7	6	7	2	6	8

Supplemental_ Fig_S3.









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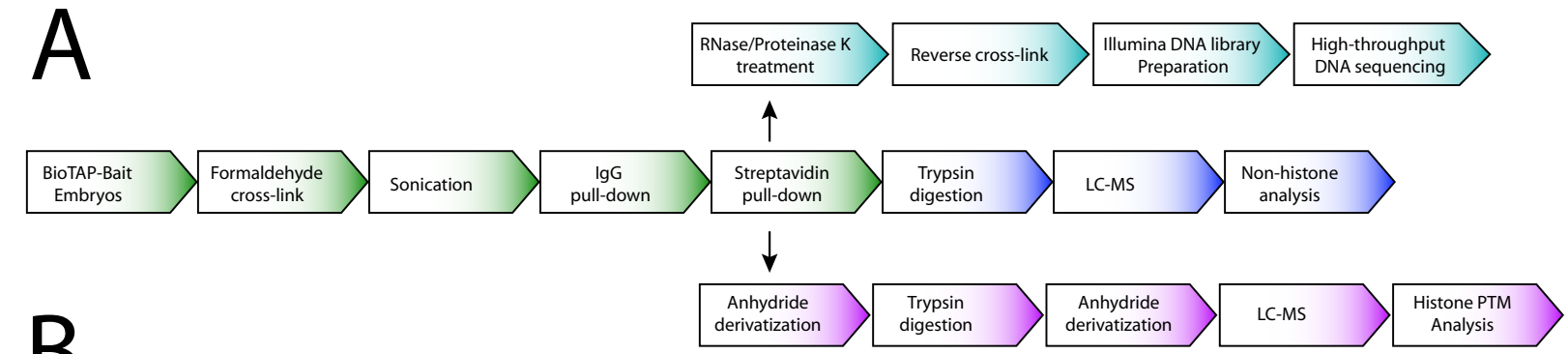

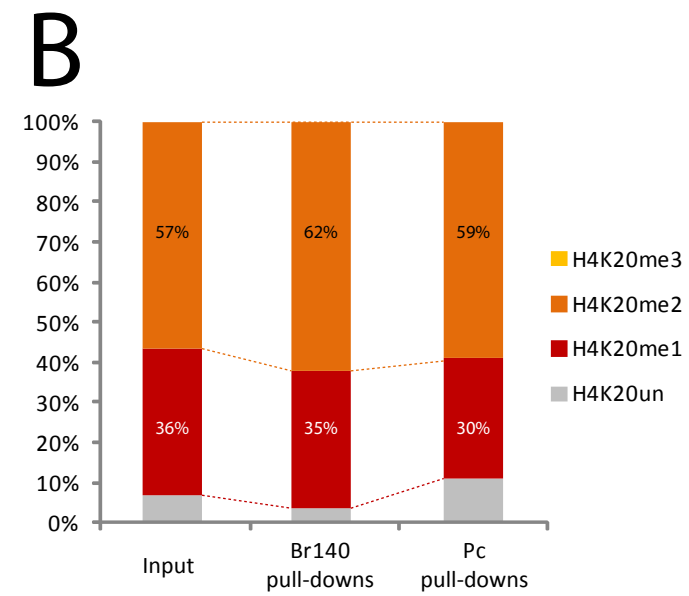

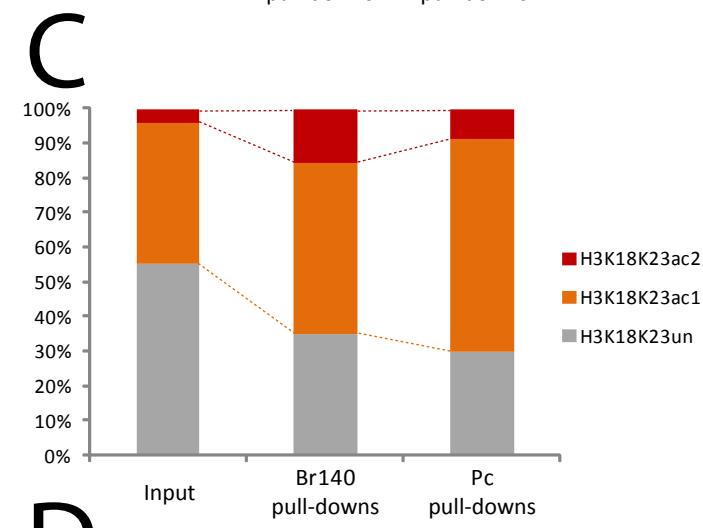

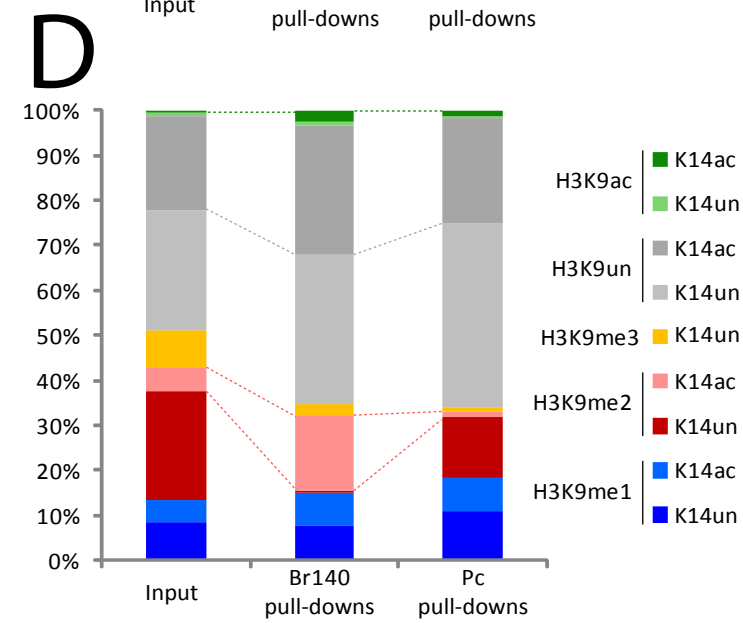

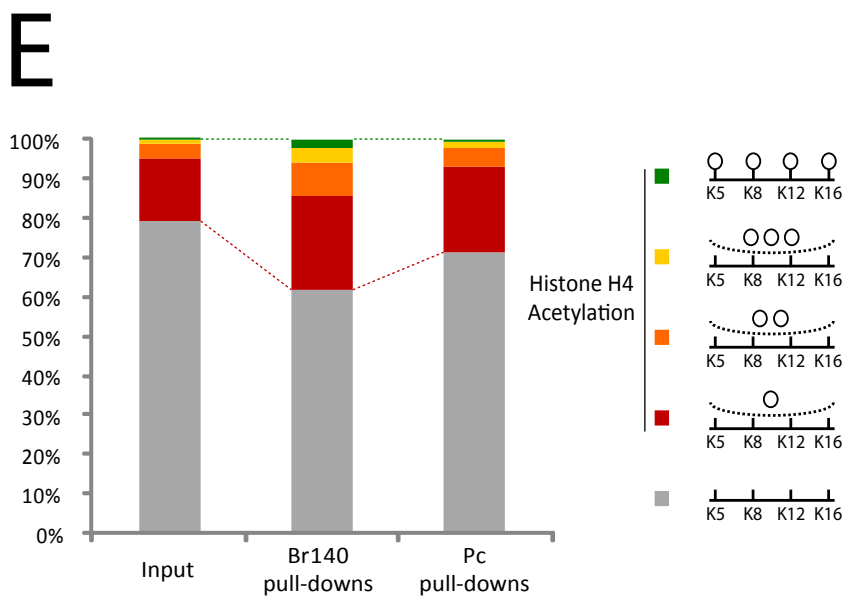

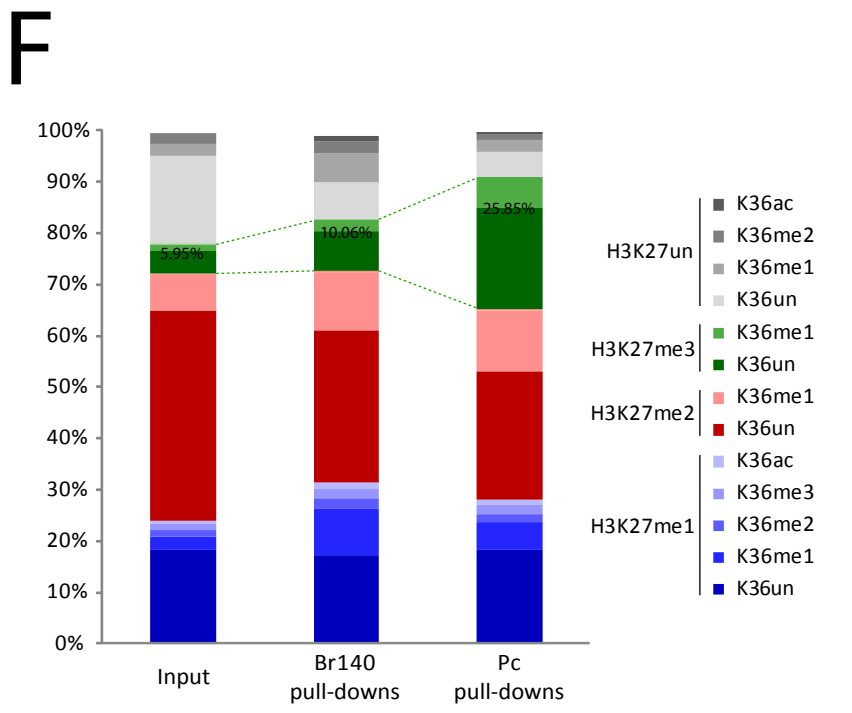

Supplemental_ Fig_S 5.