Supplemental Figure Legend

Supplementary Figure 1. BRWD2/PHIP CRISPR knockout and antibody validation

A. Design of BRWD2/PHIP CRISPR-Cas9 gene knockout strategy. CRISPR sgRNA guides were designed flanking the BRWD2/PHIP promoter and first 4 exons (region highlighted in pink). H3K4me3 and H3K27ac ChIP-seq are shown to identify the BRWD2/PHIP promoter. B. RNA-sequencing track example at the BRWD2/PHIP locus in wildtype HCT-116 and 2 independent BRWD2/PHIP knockout (ΔBRWD2/PHIP) clones. BRWD2/PHIP transcripts are undetectable in ΔBRWD2/PHIP clones whereas transcript levels of neighboring gene (HMGN3) are not altered. C. Western blotting for BRWD2/PHIP in HCT-116 wildtype and ΔBRWD2/PHIP cells. BRWD2/PHIP is undetectable in ΔBRWD2/PHIP cells using antibodies #833 (ab#833) and #834 (ab#834). Arrow to the right of the ab #834 panel indicates the specific BRWD2/PHIP band, whereas the asterisk indicates a non-specific band that migrates above BRWD2/PHIP. D. Example UCSC genome browser track of BRWD2/PHIP and H3K4me1 ChIP-seq in wildtype and ΔBRWD2/PHIP HCT-116 cells. BRWD2/PHIP ChIP-signal with both antibodies is markedly reduced in ΔBRWD2/PHIP mutant HCT-116 cells, whereas H3K4me1 signal is unchanged. E. Genome wide occupancy plot of BRWD2/PHIP and H3K4me1 ChIP-seq signal in HCT-116 wildtype and ΔBRWD2/PHIP mutant cells. F. Venn diagram of BRWD2/PHIP ChIP-seq peaks detected in HCT-116 wildtype and ΔBRWD2/PHIP cells. For antibodies #833 and #834 respectively, 31,515 and 37,972 BRWD2/PHIP peaks are detected in wildtype HCT-116 cells. In ΔBRWD2/PHIP mutant cells 29,380 (93%) and 36,557 (96%) of wildtype peaks are lost.

Supplementary Figure 2. Analysis of BRWD2/PHIP protein-protein interactions by tandem mass spectrometry

A. Table of selected MS results from the representative purification in Figure 2A. Spectral counts for two control replicates and two Flag-BRWD2/PHIP replicates are shown. Interacting proteins are broken down into six categories: CUL4-DDB1 (CRL4), COP9 Signalosome (CSN), Nedd8-binding, histones, chromatin remodelers and other proteins. Note that spectral counts for CRL4 components, in particular DDB1, are highly abundant in Flag-BRWD2/PHIP purifications suggesting a strong interaction. B. MS results from purifications of Flag-BRWD2/PHIP after treatment with combinations of MLN4924 and MG132. In the presence of MLN4924, spectral counts for Nedd8 are dramatically diminished. In addition association of CSN and the Nedd8-binding protein UBXN7 are dramatically reduced. C. Western blot of MLN4924 and MG132 treated inputs and Flag-BRWD2/PHIP immunoprecipitations. Treatment with MLN4924 converts the majority of CUL4A to the unmodified (Unmod.) non-Neddylated state. Neither inhibitor strongly diminishes BRWD2/PHIP interaction with DDB1, however, CUL4A interaction appears slightly diminished in the presence of MLN4924.

Supplementary Figure 3. BRWD2/PHIP CryptoTudor domain analysis using PHYRE2 and HHPRED secondary structure prediction

A. Diagram depicting BRWD2/PHIP domain structure (top) and a sequence alignment of the human and *Drosophila* CryptoTudor-Bromodomain module (bottom). WD repeats: green boxes, CryptoTudor domain: blue box, Bromodomains: pink boxes. B. Analysis of BRWD2/PHIP CryptoTudor domain using PHYRE2. Alignment of BRWD2/PHIP

CryptoTudor (Query sequence) and PHF20 Tudor (Template sequence) domains indicating similarity in predicted secondary structure. As indicated by the blue arrows (extended beta sheets), the BRWD2/PHIP CryptoTudor domain is predicted to adopt a 3 strand beta-barrel type conformation similar to the Tudor fold. C. Analysis of BRWD2/PHIP CryptoTudor domain using HHPRED. Sequence alignments of the BRWD2/PHIP CryptoTudor domain with the Tudor domains of PHF20, Lamin B Receptor (LBR), PHD Finger Protein 1 (PHF1) and Polycomb-Like (PCL). In all instances, BRWD2/PHIP is predicted to adopt a 3 beta-strand conformation. Blue "E"s represent regions predicted to be extended beta sheets, red "H"s represent predicted alpha-helixes.

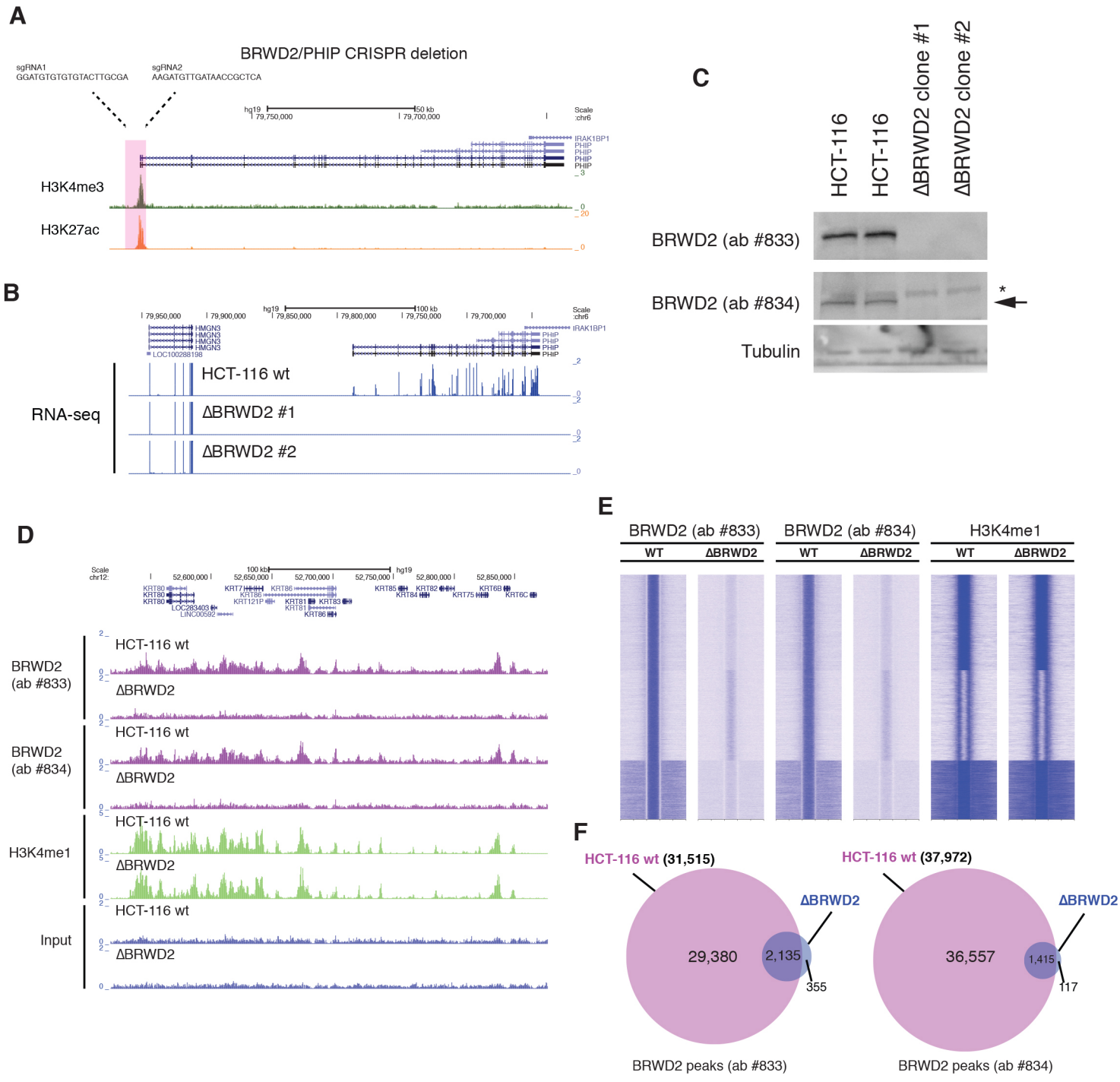
Supplemental figure 4. BRWD2/PHIP ChIP-seq in Mammalian Cells additional track examples

A. UCSC genome browser track example in HCT116 cells displaying a region upstream of the NTF3 gene where MLL1 knockout (MLL1-KO) results in increased H3K4me1 and BRWD2/PHIP occupancy (indicated by grey boxes). Some sites that gain H3K4me1 and BRWD2/PHIP in MLL1-KO cells lose occupancy of these factors in cells lacking MLL4 enzymatic activity (indicated by light blue boxes). B. Track example in v6.5 embryonic stem cells (ESCs) displaying a region near the Ier5 and Mr1 genes at which H4K4me1 and BRWD2/PHIP occupancy is dependent on MLL4 methyltransferase activity (indicated by grey boxes).

Supplemental figure 5. BRWD2/PHIP ChIP-seq in *Drosophila* additional track examples

A. UCSC genome browser track example in *Drosophila* S2 cells displaying occupancy of dBRWD3, H3K4me1, H3K4me3, H3K27ac in control and dBRWD3 RNAi treated cells. Blue highlight boxes indicate regions that lose H3K27ac signal in dBRWD3 knockdown cells. Red highlight box indicates a region where H3K27ac signal spreads in dBRWD3 RNAi treated cells. B. A. UCSC genome browser track example in *Drosophila* S2 cells displaying occupancy of dBRWD3, H3K4me1, H3K4me3, H3K27ac in control and dBRWD3 RNAi treated cells. Red highlight boxes indicate regions where H3K27ac signal spreads in dBRWD3 RNAi treated cells.

Supplementary Figure 1.



Supplementary Figure 2.

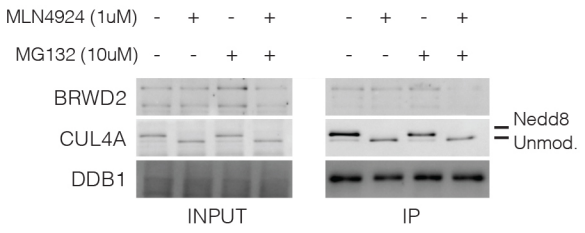
A

	Protein	Spectral Counts: Control rep1	Control rep2	Flag-BRWD2 rep1	Flag-BRWD2 rep2
CUL4-DDB1 (CRL4)	BRWD2/PH-interacting protein (PHIP)	0	0	185	316
	DNA damage-binding protein 1 (DDB1)	0	0	183	366
	Isoform 2 of Cullin-4B (CUL4B)	0	0	54	99
	Cullin-4A (CUL4A)	0	0	39	52
	NEDD8 (NEDD8)	0	0	9	9
COP9 Signalosome (CSN)	COP9 signalosome complex subunit 1 (GPS1)	0	0	9	9
	COP9 signalosome complex subunit 2 (COPS2)	0	0	10	12
	COP9 signalosome complex subunit 3 (COPS3)	0	0	8	14
	COP9 signalosome complex subunit 4 (COPS4)	0	0	21	22
	COP9 signalosome complex subunit 5 (COPS5)	0	0	6	9
	COP9 signalosome complex subunit 6 (COPS6)	0	0	4	8
	COP9 signalosome complex subunit 7a (COPS7A)	0	0	4	10
	COP9 signalosome complex subunit 8 (COPS8)	0	0	5	13
	Myeloma-overexpressed gene 2 protein (MYEOV2)	0	0	4	7
Nedd8-binding	UBX domain-containing protein 7 (UBXN7)	0	0	4	5
Histones	Histone H2B	0	0	39	54
	Histone H2A	0	0	31	38
	Histone H3.3	0	0	20	25
	Histone H3.2	0	0	18	22
	Histone H4	8	7	77	129
	Histone H2A.Z	4	2	41	31
Chromatin Remodellers	PHD finger protein 6 (PHF6)	0	0	7	20
	Remodeling and spacing factor 1 (RSF1)	0	0	12	18
	FACT complex subunit SSRP1 (SSRP1)	0	0	12	8
	FACT complex subunit SPT16 (SUPT16H)	0	0	9	22
	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 (SMARCA5)	0	0	7	12
	Chromodomain-helicase-DNA-binding protein 1 (CHD1)	0	0	6	11
	Chromodomain-helicase-DNA-binding protein 4 (CHD4)	0	0	1	3
Other proteins	Spectrin beta chain, non-erythrocytic 1 (SPTBN1)	0	0	11	8
	Nucleolar and coiled-body phosphoprotein 1 (NOLC1)	0	0	5	2
	X-ray repair cross-complementing protein 5 (XRCC5)	0	0	3	2
	Ubiquitin carboxyl-terminal hydrolase (USP7)	0	0	1	3

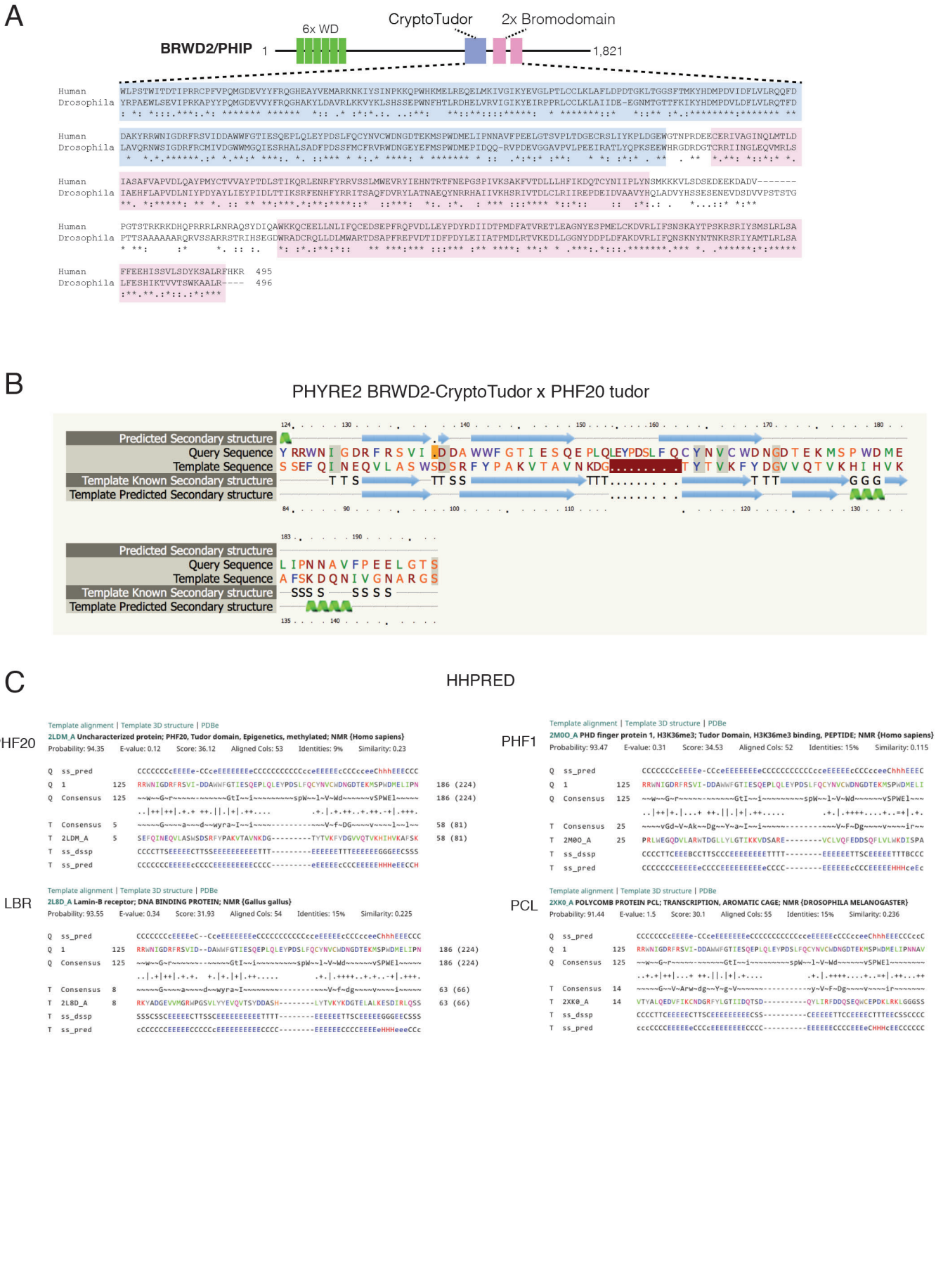
B

	Protein	Spectral Counts: DMSO	MLN4924	MG132	MLN4924 + MG132
CUL4-DDB1 (CRL4)	BRWD2/PH-interacting protein (PHIP)	160	182	252	169
	DNA damage-binding protein 1 (DDB1)	103	138	177	124
	Isoform 2 of Cullin-4B (CUL4B)	90	52	100	41
	Cullin-4A (CUL4A)	62	51	76	37
	NEDD8 (NEDD8)	23	2	19	1
COP9 Signalosome (CSN)	COP9 signalosome complex subunit 1 (GPS1)	3	0	3	0
	COP9 signalosome complex subunit 2 (COPS2)	5	0	6	0
	COP9 signalosome complex subunit 3 (COPS3)	3	0	7	0
	COP9 signalosome complex subunit 4 (COPS4)	7	0	7	0
	COP9 signalosome complex subunit 5 (COPS5)	6	0	5	0
	COP9 signalosome complex subunit 6 (COPS6)	1	0	0	0
	COP9 signalosome complex subunit 7a (COPS7A)	5	0	5	0
	COP9 signalosome complex subunit 7b (COPS7B)	1	0	1	0
	COP9 signalosome complex subunit 8 (COPS8)	5	0	3	0
	Myeloma-overexpressed gene 2 protein (MYEOV2)	1	0	2	0
Nedd8-binding	UBX domain-containing protein 7 (UBXN7)	10	0	14	1
Histones	Histone H4	38	32	42	40
	Histone H2B	23	17	28	35
	Histone H2A	20	21	32	16
	Histone H3.3	8	5	7	7
	Histone H3.2	8	5	8	7
	Histone H2A.Z	6	4	5	2
Chromatin Remodellers	PHD finger protein 6 (PHF6)	15	17	17	10
	Chromodomain-helicase-DNA-binding protein 4 (CHD4)	59	70	70	59
	Transcription activator BRG1 (SMARCA4)	17	14	13	6
	Chromodomain-helicase-DNA-binding protein 5 (CHD5)	16	16	16	9
	Protein polybromo-1 (PBRM1)	15	8	11	5
	Remodeling and spacing factor 1 (RSF1)	19	20	19	12
	FACT complex subunit SPT16 (SUPT16H)	13	18	14	12
	FACT complex subunit SSRP1 (SSRP1)	12	14	11	5

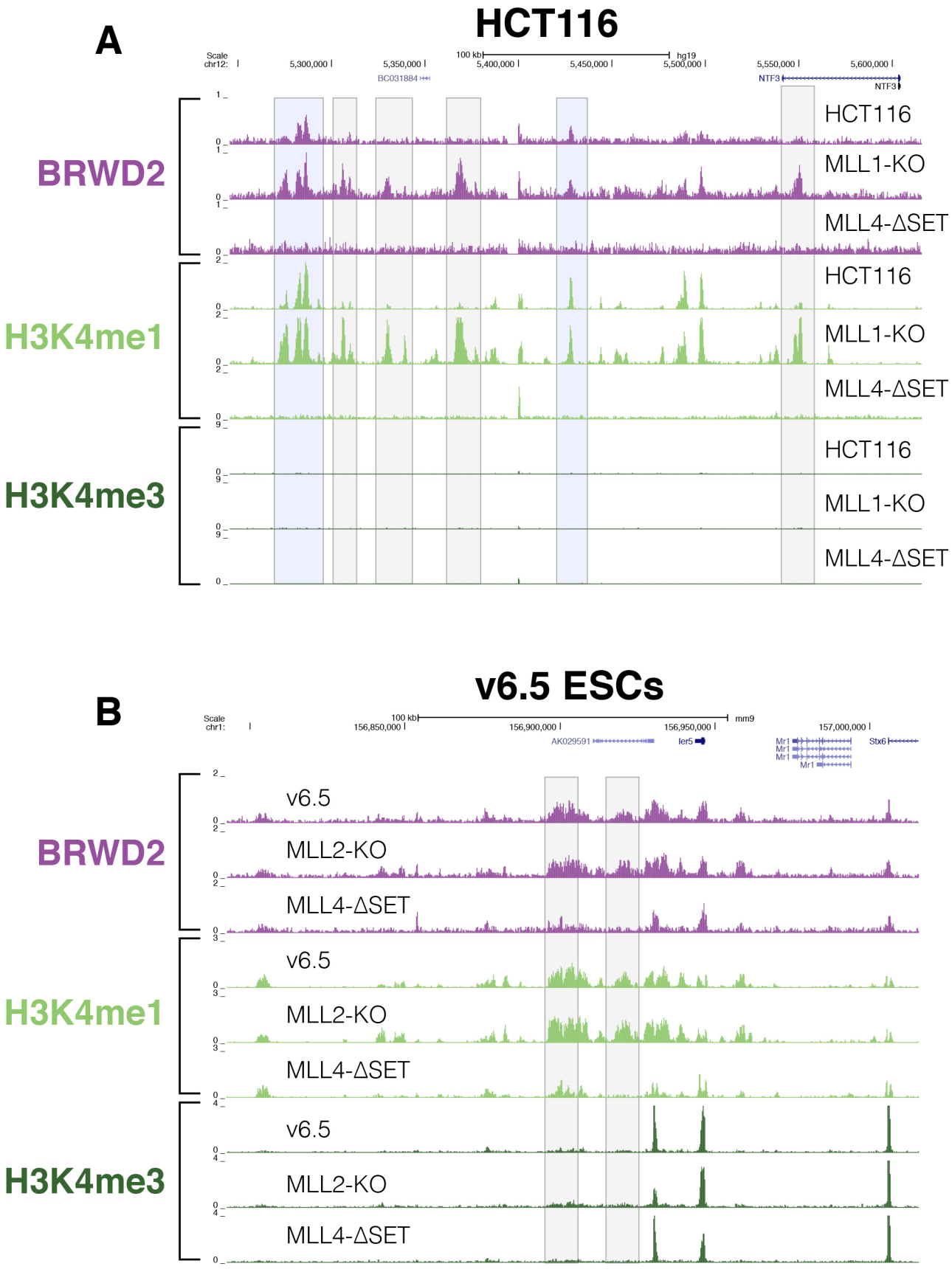
C



Supplementary Figure 3

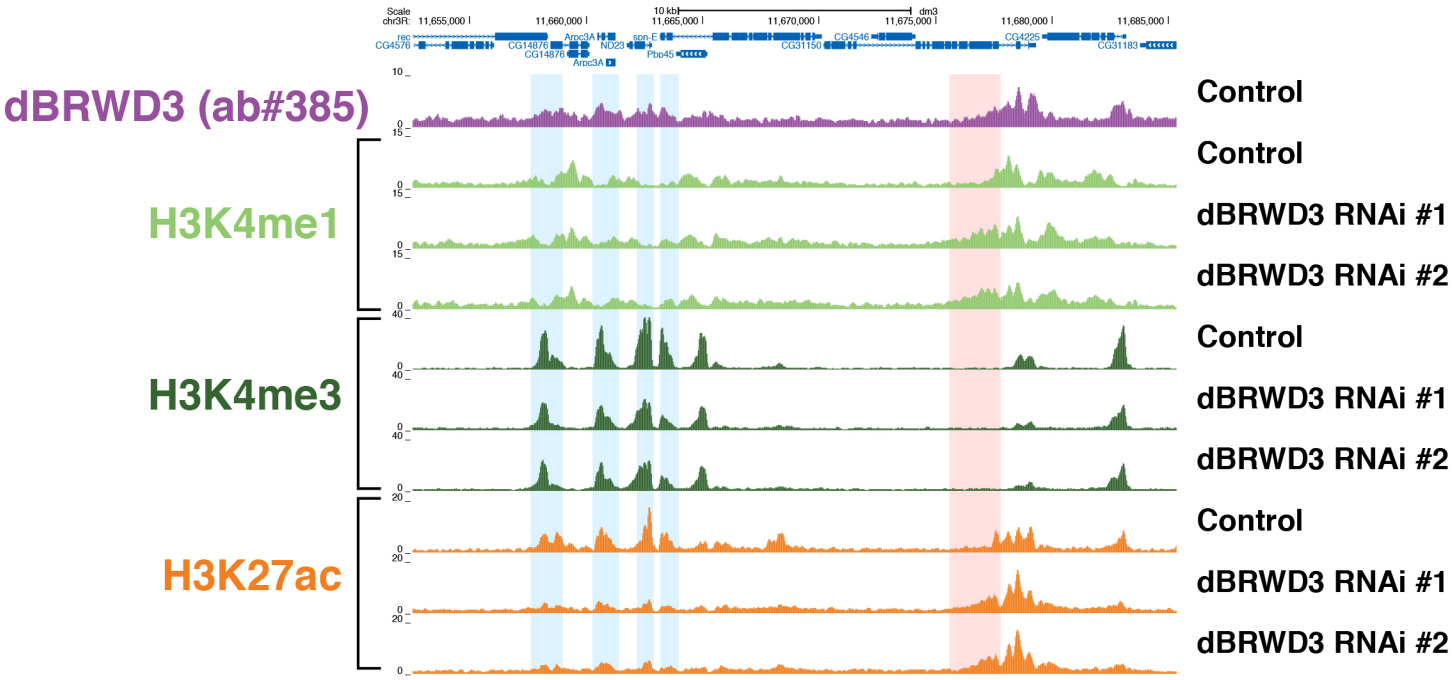


Supplementary Figure 4



Supplementary Figure 5.

A



B