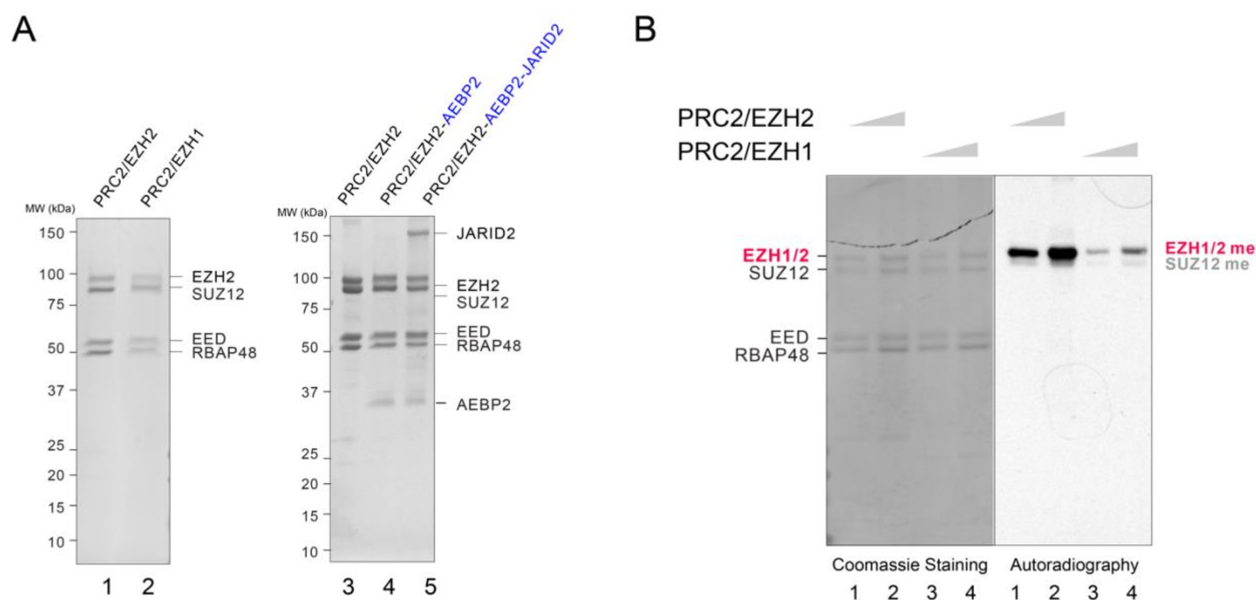
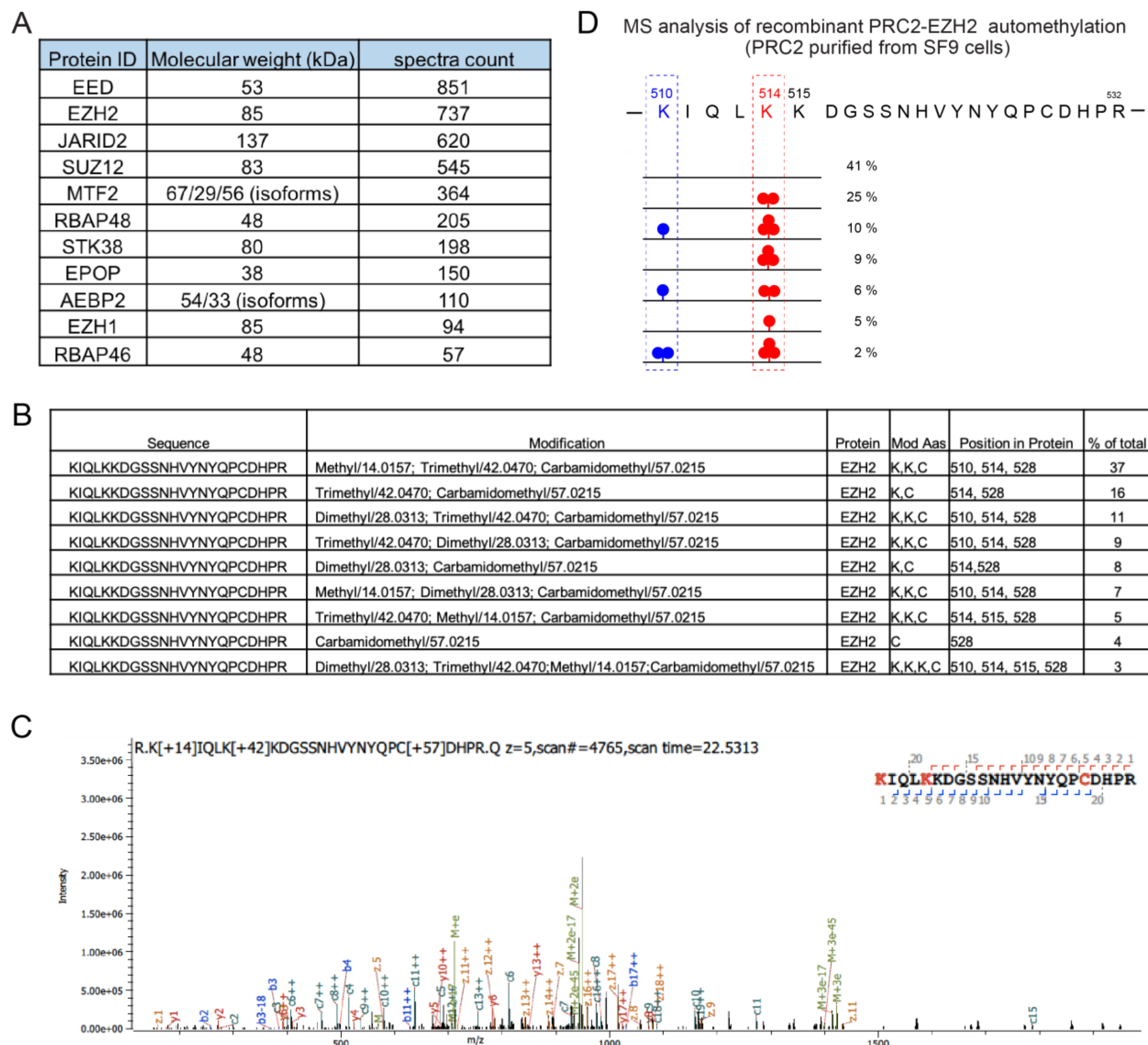


Supplemental Figure 1. Automethylation of PRC2 occurs in the presence of cofactors and with both EZH1/2 catalytic subunits.

(A) Coomassie blue staining showing recombinant PRC2 complexes containing several combinations of accessory proteins copurified from SF9 cells. The details of the purifications are described in Materials & Methods. **(B)** Methyltransferase (MT) assay showing methylation of EZH1/2 and SUZ12. MT assay containing PRC2-EZH1 or PRC2-EZH2 (30 or 60 nM) with ^{3}H-SAM (2 μM). Coomassie blue staining of SDS-PAGE gels containing nucleosomes or PRC2 components (left image) was used to visualize the relative concentration of each component present in each reaction. Autoradiography shows the levels of methylation on EZH2 and SUZ12 (right image).



A

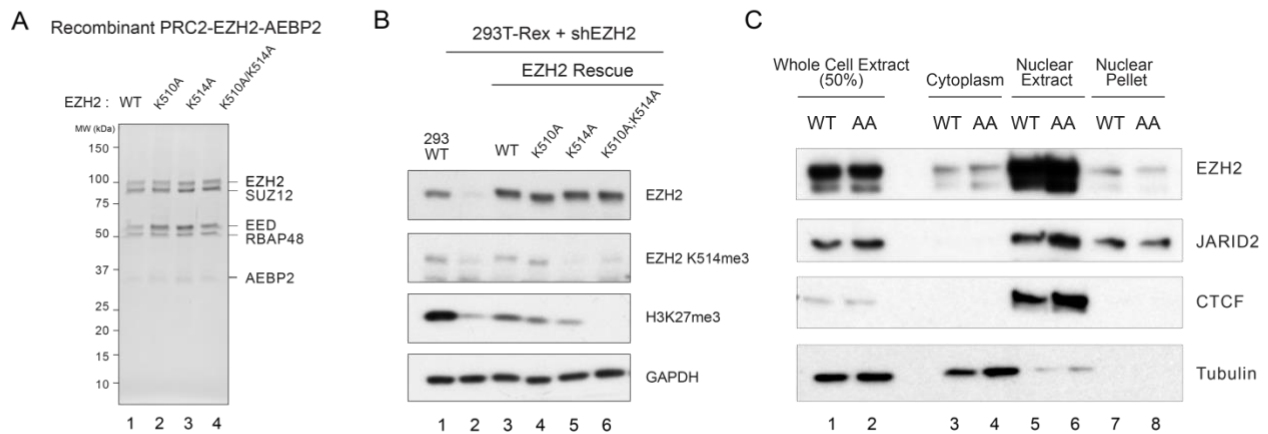
Protein ID	Molecular weight (kDa)	spectra count
EED	53	851
EZH2	85	737
JARID2	137	620
SUZ12	83	545
MTF2	67/29/56 (isoforms)	364
RBAP48	48	205
STK38	80	198
EPOP	38	150
AEBP2	54/33 (isoforms)	110
EZH1	85	94
RBAP46	48	57

B

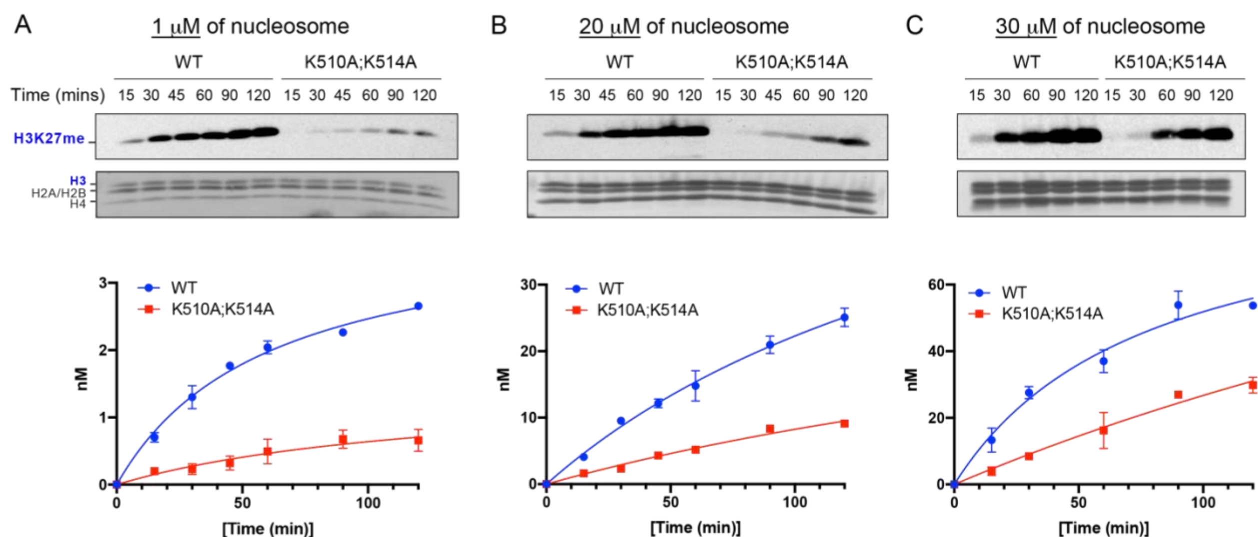
Sequence	Modification	Protein	Mod Aas	Position in Protein	% of total
KIQLKKDGSSNHVYNYQPCDHPR	Methyl/14.0157; Trimethyl/42.0470; Carbamidomethyl/57.0215	EZH2	K,K,C	510, 514, 528	37
KIQLKKDGSSNHVYNYQPCDHPR	Trimethyl/42.0470; Carbamidomethyl/57.0215	EZH2	K,C	514, 528	16
KIQLKKDGSSNHVYNYQPCDHPR	Dimethyl/28.0313; Trimethyl/42.0470; Carbamidomethyl/57.0215	EZH2	K,K,C	510, 514, 528	11
KIQLKKDGSSNHVYNYQPCDHPR	Trimethyl/42.0470; Dimethyl/28.0313; Carbamidomethyl/57.0215	EZH2	K,K,C	510, 514, 528	9
KIQLKKDGSSNHVYNYQPCDHPR	Dimethyl/28.0313; Carbamidomethyl/57.0215	EZH2	K,C	514,528	8
KIQLKKDGSSNHVYNYQPCDHPR	Methyl/14.0157; Dimethyl/28.0313; Carbamidomethyl/57.0215	EZH2	K,K,C	510, 514, 528	7
KIQLKKDGSSNHVYNYQPCDHPR	Trimethyl/42.0470; Methyl/14.0157; Carbamidomethyl/57.0215	EZH2	K,K,C	514, 515, 528	5
KIQLKKDGSSNHVYNYQPCDHPR	Carbamidomethyl/57.0215	EZH2	C	528	4
KIQLKKDGSSNHVYNYQPCDHPR	Dimethyl/28.0313; Trimethyl/42.0470;Methyl/14.0157;Carbamidomethyl/57.0215	EZH2	K,K,K,C	510, 514, 515, 528	3

Supplemental Figure 2. Mass spectrometry results showing identified proteins and EZH2 automethylation.

(A-C) PRC2 was purified through FLAG-based affinity purification from a previously constructed EED-deficient mESC line wherein FLAG-tagged EED was ectopically expressed. Purified PRC2 was digested with Arg-C protease and then subjected to mass spectrometry (MS) analysis. The details of the purification and MS analysis are described in Materials & Methods. **(A)** The chart shows the total number of spectra counted for each protein identified by MS. **(B)** Details of the methylation discovered on EZH2-K510, 514, and 515. “% of total” describes the percentage of the different peptide isoforms identified. **(C)** A representatative image of MS/MS data for the EZH2-K510me1/K514me3, the most abundant peptide. (D) MS analysis of recombinant PRC2-EZH2 methylation after Arg-C protease digestion (see Materials and Methods). Recombinant PRC2-EZH2 was purified from Sf9 insect cells. Note that these methylations occurred in Sf9 cells. Illustration of EZH2 lysine residues within amino acids 510-532 and the percentages of their mono-, di-, and tri-methylated states, as indicated.

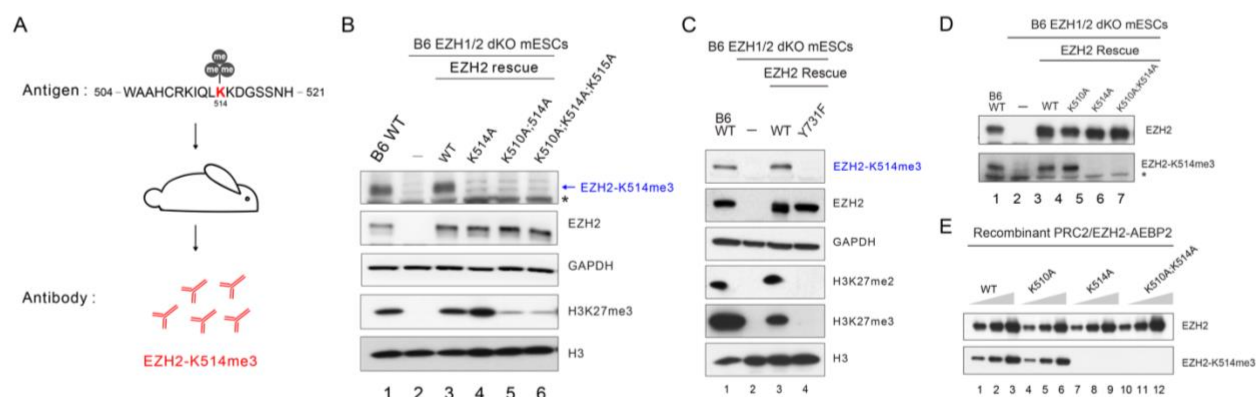


Supplemental Figure 3. Automethylation of EZH2 occurs in a variety of cell lines.
(A) Coomassie blue staining of recombinant PRC2-AEBP2 complexes containing EZH2 either wild-type or mutant in the automethylation sites. The details of the purifications are described in Materials & Methods. **(B)** Western blot of EZH2, EZH2-K514me3, H3K27me3, and GAPDH in 293T-Rex cells, including WT, EZH2 knockdown, and EZH2 rescue conditions, as indicated. **(C)** EZH2 automethylation does not influence the distribution of either EZH2 or JARID2, a non-histone substrate of PRC2. Western blot of EZH2, JARID2, CTCF (nuclear protein), and Tubulin (cytoplasmic protein). B6 EZH1/2 dKO mESC cells with either EZH2 WT (WT) or EZH2 K510A;K514A (AA) were fractionated into cytoplasm, nuclear extract and nuclear pellet. The details of cellular fractionation are described in Materials & Methods.

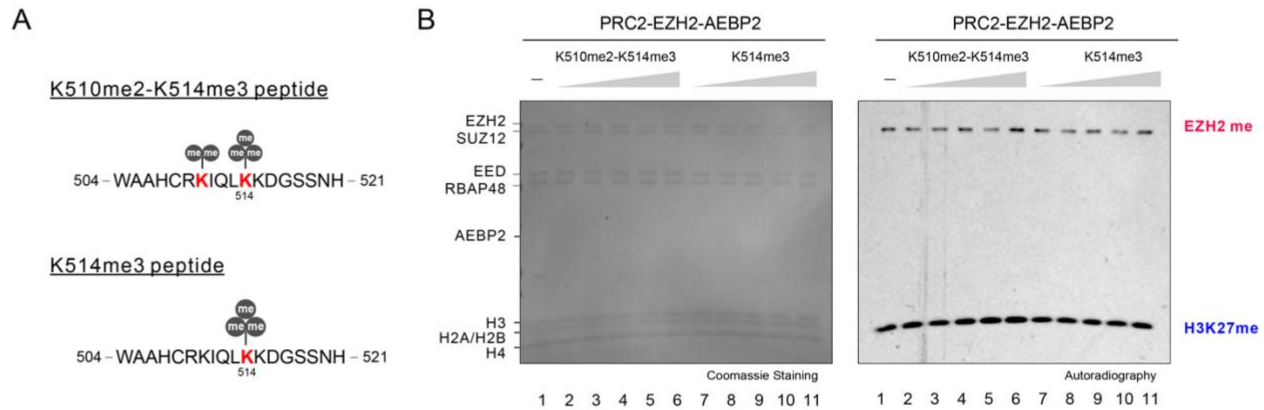


Supplemental Figure 4. Enzyme kinetic analysis of PRC2 containing WT or EZH2 automethylation mutant.

(A-C) Histone methyltransferase assays performed as a function of time using PRC2-EZH2-AEBP2 complexes containing $EZH2^{WT}$ or $EZH2^{K510A;K514A}$ (K510A;K514A) (60 nM) with either 1 μM **(A)**, 20 μM **(B)**, or 30 μM **(C)** of oligonucleosomes, 15 μM of H3K27me3, and 2.5 μM of ^{3}H-SAM. Coomassie blue staining of SDS-PAGE gels containing nucleosomes or PRC2 components was used to visualize the relative concentration of each component present in each reaction (left image). The levels of methylation on EZH2 or histone H3 are shown by autoradiography (right image).



Supplemental Figure 5. Generation and confirmation of EZH2-K514me3 specific antibody. (A) Schematic illustration showing EZH2-K514me3-containing peptide that was injected into rabbit to generate EZH2-K514me3 specific antibody. **(B and C)** Western blot of EZH2-K514me3, EZH2, GAPDH, H3K27me2, H3K27me3, and total histone H3 levels in B6 mESC cells, including WT, EZH1/2-dKO, and EZH2 rescue conditions. mESC, mouse embryonic stem cells. Asterisk (*) indicates a nonspecific band. **(D)** Western blot of EZH2 and EZH2-K514me3 in B6 mESC cells, including WT, EZH1/2-dKO, and EZH2 rescue conditions. EZH2 blot is also shown in **Figure 3B**. Asterisk (*) indicates a nonspecific band. **(E)** Western blot using anti-EZH2-K514me3 antibody and results from HMT assays performed as in **Figure 3A**, using PRC2-EZH2-AEBP2 complexes containing wild-type or mutant EZH2. The EZH2 blot is also shown in **Figure 3A**.



Supplemental Figure 6. Automethylated EZH2 peptides do not allosterically stimulate the catalytic activity of PRC2.
(A) Sequence of EZH2-K510me2-K514me3 peptide and EZH2-K514me3 peptide that were synthesized. **(B)** Methyltransferase assays using wild-type (WT) PRC2-EZH2-AEBP2 (30 nM) with increasing concentrations of EZH2-K510me2-K514me3 peptide or EZH2-K514me3 peptide (0.1, 0.3, 1, 3, 10 μM) using oligonucleosomes (300 nM) as substrate. *Left*, Coomassie blue staining of SDS-PAGE gels containing nucleosomes or PRC2 components was used to visualize the relative concentration of each component present in each reaction. *Right*, Autoradiography showing levels of EZH2 or histone H3 methylation.