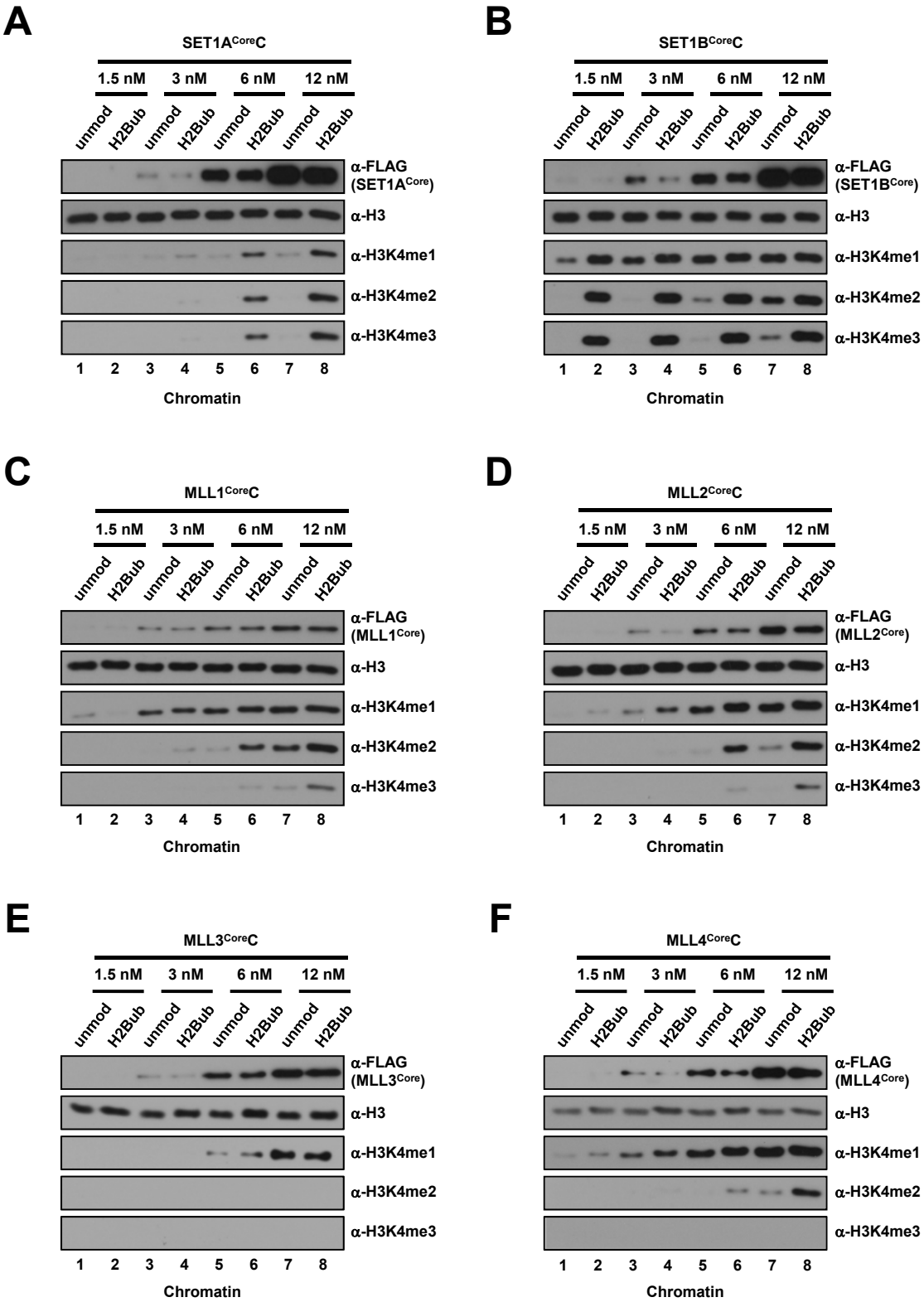
SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

Supplementary Figure S1

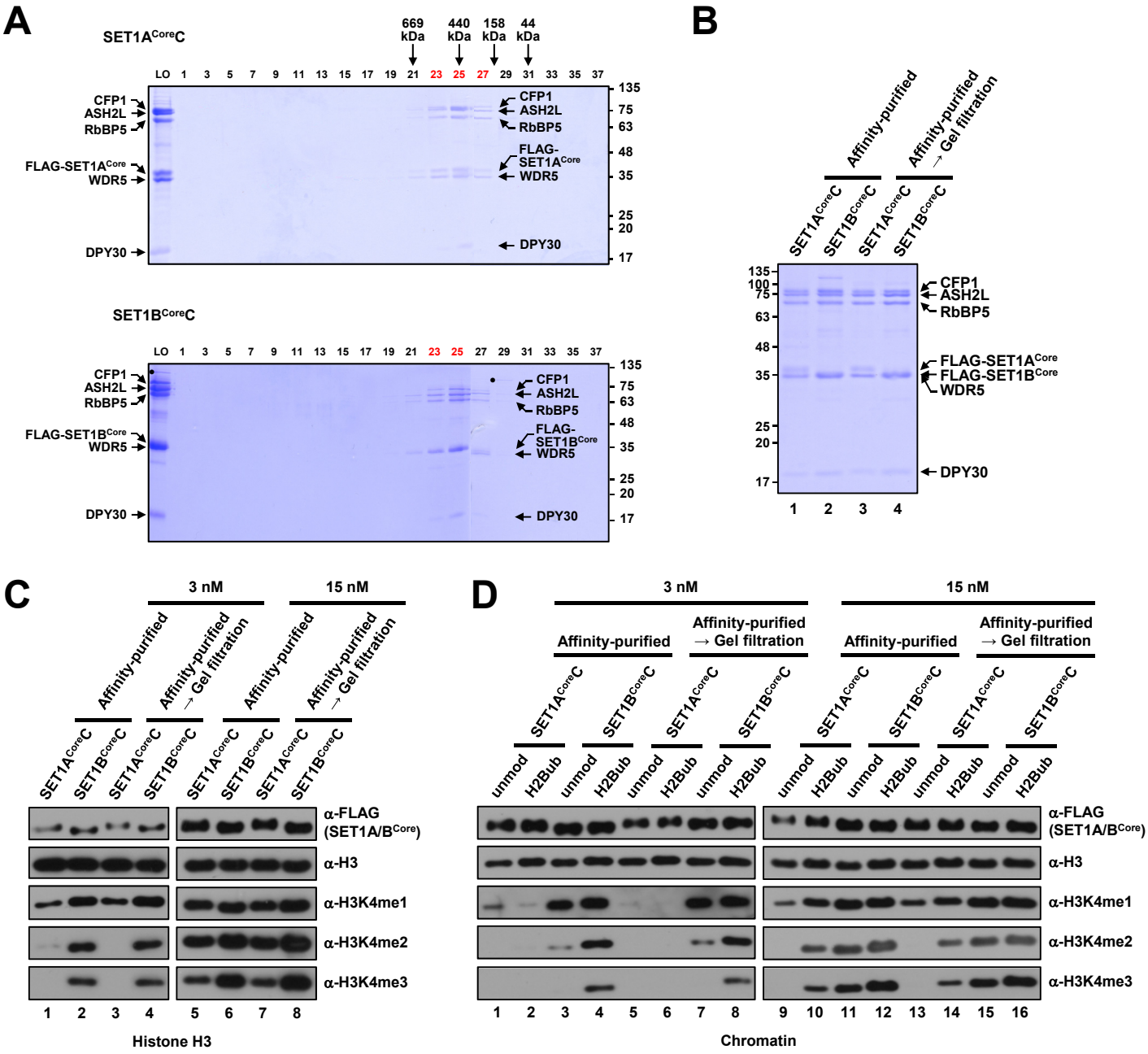


Supplementary Figure S1. H3K4 methylation activities with varying concentrations of human KMT2^{Core} complexes.

(**A**–**F**) Recombinant chromatin templates assembled with unmodified histone H2B (unmod)- or mono-ubiquitylated H2B at lysine 120 (H2Bub)-containing octamers were subjected to *in vitro* HMT assays with the indicated concentrations of purified SET1A^{Core} (A), SET1B^{Core} (B), MLL1^{Core} (C), MLL2^{Core} (D), MLL3^{Core} (E) or MLL4^{Core} (F) complexes. H3K4 methylation status was monitored by immunoblotting with the indicated antibodies in this and other figures.

Abbreviations: unmod, unmodified histone H2B; H2Bub, K120-ubiquitylated H2B; H3K4me1, K4-monomethylated H3; H3K4me2, K4-dimethylated H3; H3K4me3, K4-trimethylated H3.

Supplementary Figure S2



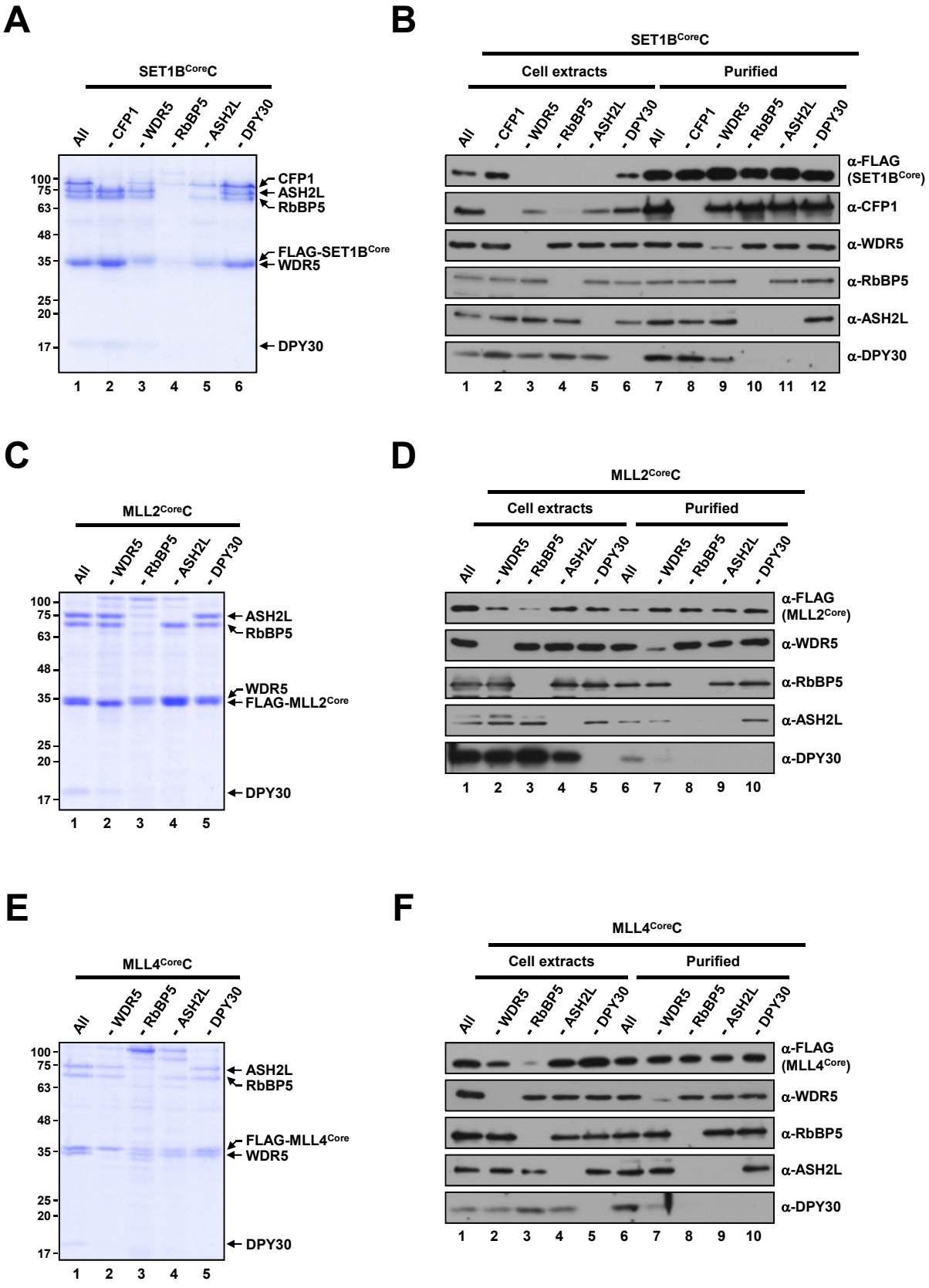
Supplementary Figure S2. Purification of human SET1A^{Core} and SET1B^{Core} complexes by gel filtration and their H3K4 methylation activities.

(**A**) SDS-PAGE/Coomassie blue staining of purified SET1A^{Core} (top) and SET1B^{Core} (bottom) complexes following Superose 6 gel filtration. Protein size markers are indicated at the top and pooled fractions are marked in red. The same column running condition was applied to all other gel filtration experiments. No protein elution in the early fractions and coelution of all complex subunits indicate that aggregated protein populations are not present in the M2 agarose affinity-purified complexes. Note that an insect protein contaminant around 100 kDa (marked by a dots) was found sometimes in the complexes prepared by single-step affinity purification and could be separated from the complex by gel filtration.

(**B**) SDS-PAGE/Coomassie blue staining of SET1A^{Core} and SET1B^{Core} complexes prepared by affinity purification alone or by further purification with gel filtration.

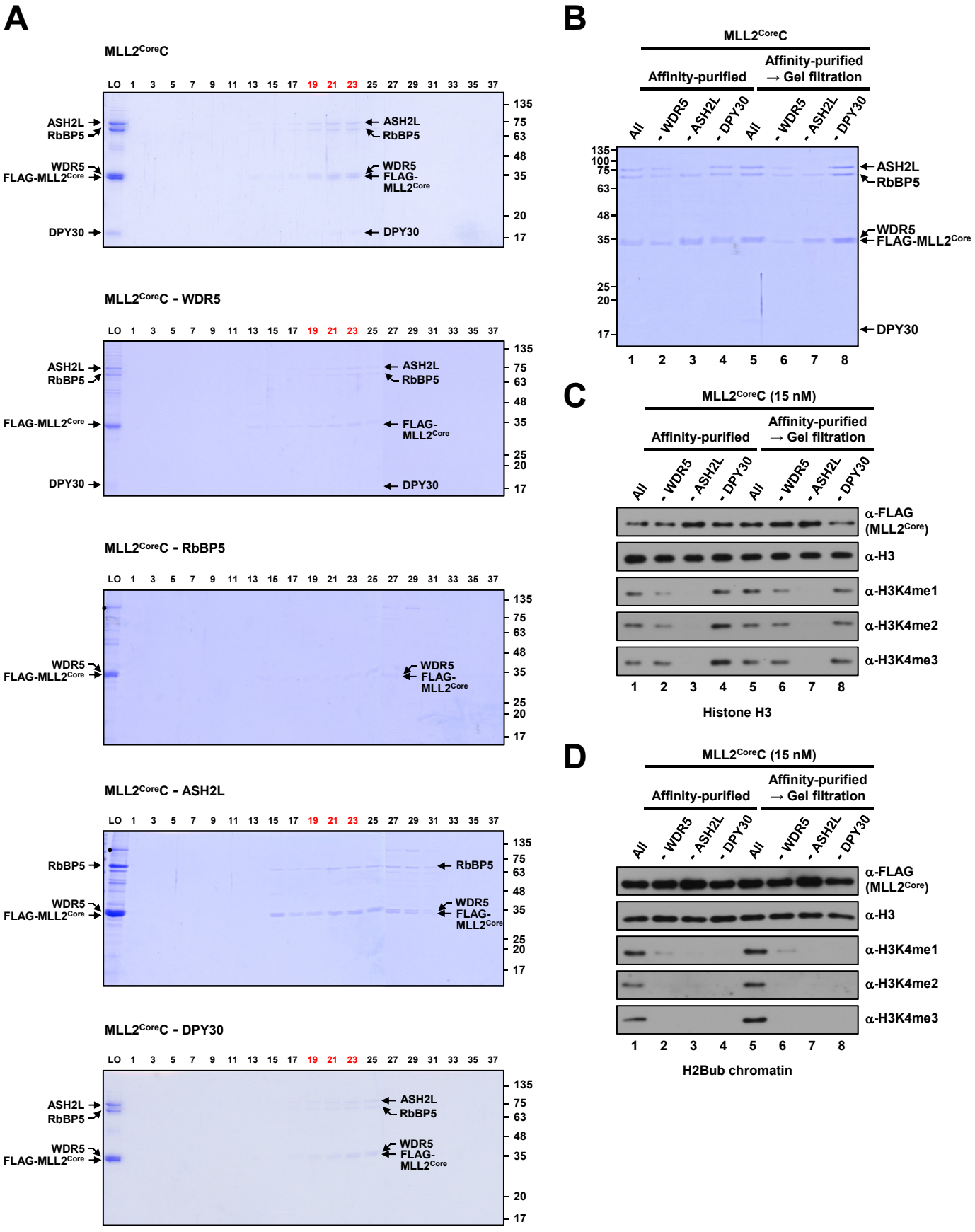
(**C** and **D**) Comparison of the H3K4 methylation activities of the affinity-purified and gel filtration-purified SET1A^{Core} and SET1B^{Core} complexes. H3 (C) and unmodified or H2Bub chromatin substrates (D) were subjected to *in vitro* HMT assays with the indicated concentrations of purified SET1A^{Core} or SET1B^{Core} complexes.

Supplementary Figure S3



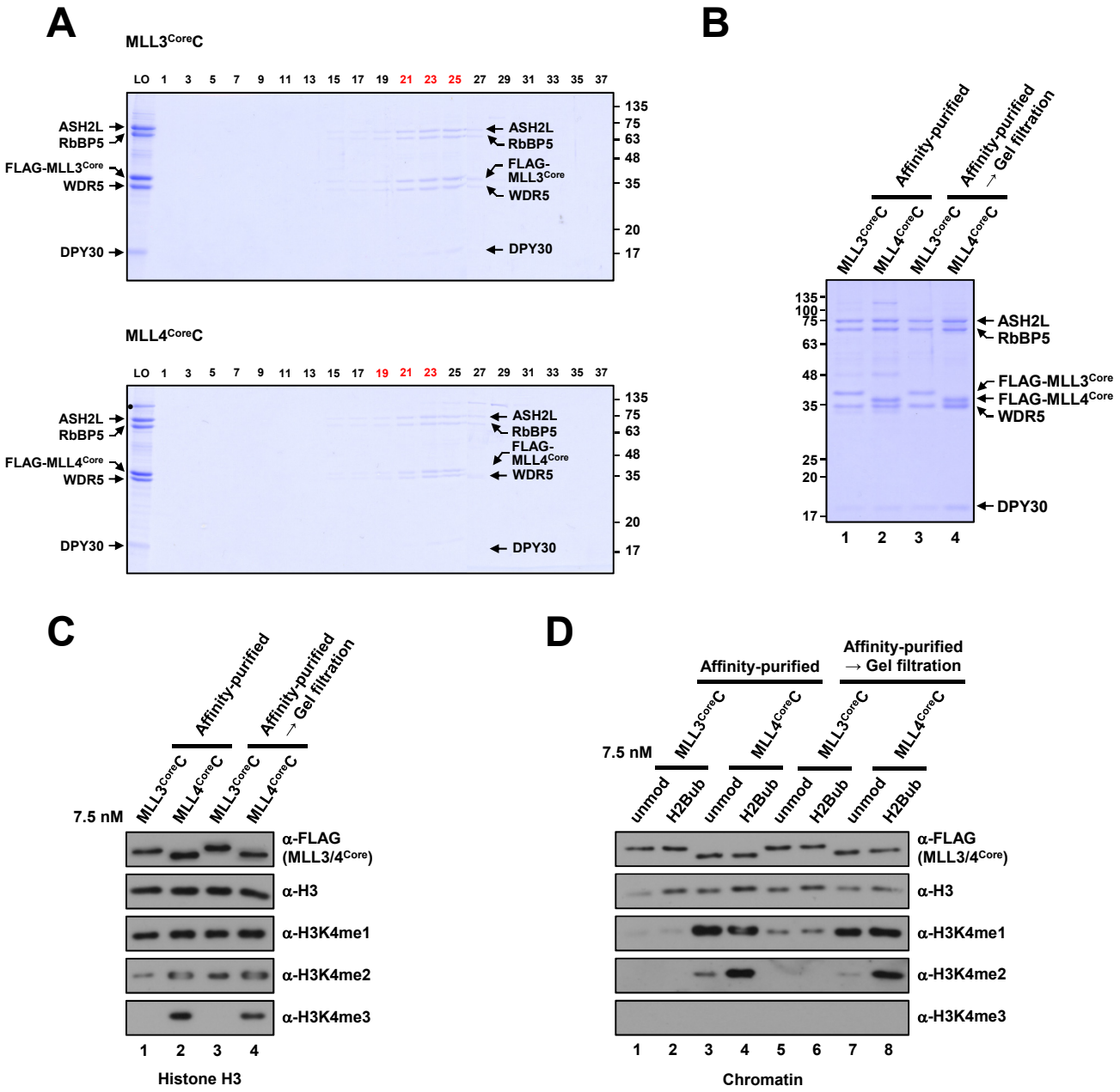
Supplementary Figure S3. Subunit compositions of purified KMT2^{Core} complexes reconstituted with baculoviruses lacking individual subunits.
(**A**, **C** and **E**) SDS-PAGE/Coomassie blue staining of purified SET1B^{Core} (A), MLL2^{Core} (C) and MLL4^{Core} (E) complexes reconstituted with baculoviruses in the absence of the indicated subunits. Sample loadings were normalized to KMT2^{Core} proteins. Note that because of inefficient complex formation, lower amounts (maximum loading volumes) of RbBP5- and ASH2L-deficient complexes (5- and 2-fold, respectively) (A) and RbBP5-deficient complex (2.5-fold) (C) relative to the corresponding intact complexes were used for SDS-PAGE.
(**B**, **D** and **F**) Immunoblot analyses of whole-cell extracts from Sf9 cells infected with baculoviruses in the absence of the indicated subunits and SET1B^{Core} (B), MLL2^{Core} (D) or MLL4^{Core} (F) complexes purified from each cell extract. Note that equal volumes of cell extracts were loaded (lanes 1-6) and purified complex loading was normalized to FLAG-KMT2^{Core} protein (lanes 7-12). The slightly fast migrating bands in anti-WDR5 immunoblots are considered an insect WDR5 homolog.

Supplementary Figure S4



Supplementary Figure S4. Purification of subunit-lacking human MLL2^{Core} complexes by gel filtration and their H3K4 methylation activities.
(**A**) SDS-PAGE/Coomassie blue staining of purified MLL2^{Core} complexes lacking the indicated subunits following Superose 6 gel filtration. Note that the RbBP5-deficient complex of which concentration is very low compared to other complexes was diluted out during gel filtration, indicating a critical role of RbBP5 for stable complex formation. This complex was excluded in the following analyses.
(**B**) SDS-PAGE/Coomassie blue staining of subunit-lacking MLL2^{Core} complexes prepared by affinity purification alone or by further purification with gel filtration.
(**C** and **D**) Comparison of the H3K4 methylation activities of the affinity-purified and gel filtration-purified subunit-lacking MLL2^{Core} complexes. H3 (C) and H2Bub chromatin substrates (D) were subjected to *in vitro* HMT assays with subunit-lacking MLL2^{Core} complexes (15 nM).

Supplementary Figure S5



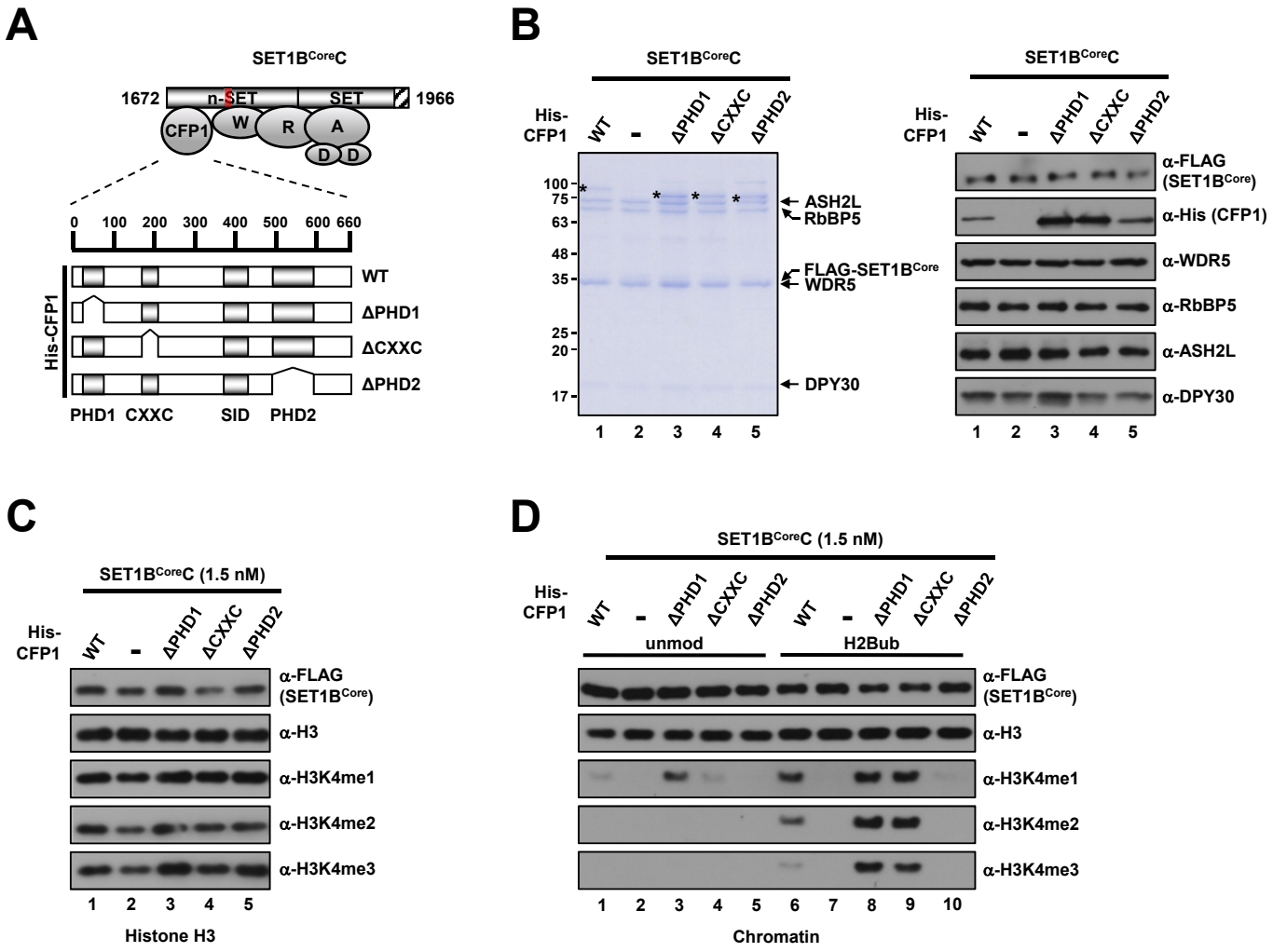
Supplementary Figure S5. Purification of human MLL3^{Core} and MLL4^{Core} complexes by gel filtration and their H3K4 methylation activities.

(**A**) SDS-PAGE/Coomassie blue staining of purified MLL3^{Core} (top) and MLL4^{Core} (bottom) complexes following Superose 6 gel filtration.

(**B**) SDS-PAGE/Coomassie blue staining of MLL3^{Core} and MLL4^{Core} complexes prepared by affinity purification alone or by further purification with gel filtration.

(**C** and **D**) Comparison of the H3K4 methylation activities of the affinity-purified and gel filtration-purified MLL3^{Core} and MLL4^{Core} complexes. H3 (C) and H2Bub chromatin substrates (D) were subjected to *in vitro* HMT assays with purified MLL3^{Core} or MLL4^{Core} complexes (7.5 nM).

Supplementary Figure S6



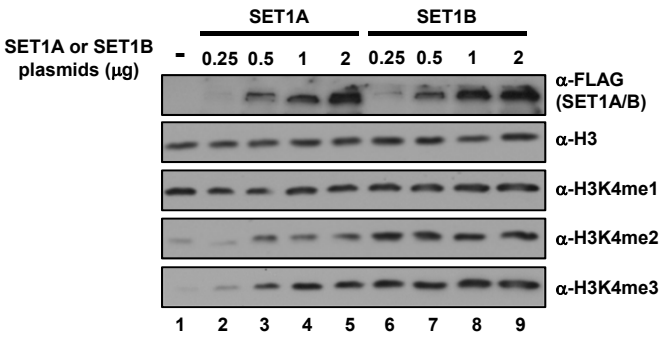
Supplementary Figure S6. Requirement of the CFP1 domain for the H3K4 methylation activity of the human SET1B^{Core} complex.

(**A**) A schematic diagram of SET1B^{Core} protein-based complexes containing wild-type (WT) or mutant CFP1. PHD1, CXXC, SID, and PHD2 domains are depicted. Abbreviation: W, WDR5; R, RbBP5; A, ASH2L; D, DPY30.

(**B**) SDS-PAGE/Coomassie blue staining (left) and immunoblot analyses (right) of purified SET1B^{Core} protein-based complexes reconstituted with baculoviruses expressing FLAG-tagged SET1B^{Core} protein, WDR5, RbBP5, ASH2L, DPY30 and His-tagged WT or mutant CFP1. His-CFP1 polypeptides are marked by asterisks.

(**C** and **D**) Requirement of the PHD2 domain of CFP1 for H2Bub-dependent H3K4 methylation activity of the SET1B^{Core} complex. H3 (C) and unmodified or H2Bub chromatin substrates (D) were subjected to *in vitro* HMT assays with the indicated SET1B^{Core} protein-based complexes (1.5 nM).

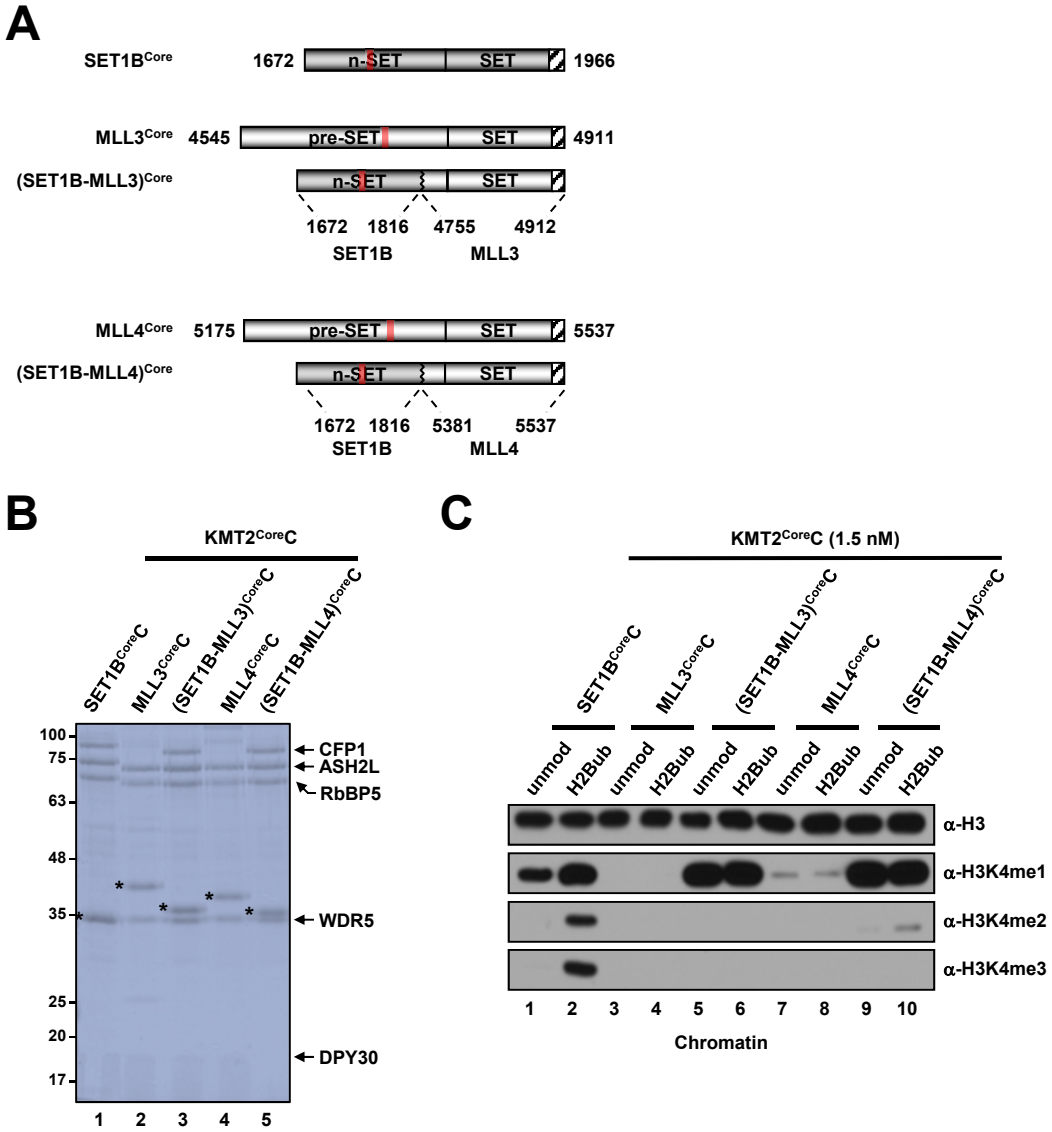
Supplementary Figure S7



Supplementary Figure S7. Effects of SET1A and SET1B overexpression on global H3K4 methylation levels in cells.

The indicated amounts of FLAG-tagged full-length human SET1A or SET1B expression plasmids were co-transfected with WDR5, RbBP5, ASH2L, DPY30, CFP1, and WDR82 expression plasmids (0.5 μg each) into about 0.5×10^6 293T cells in 6 well plates. Total cell extracts were subjected to immunoblot analyses with the indicated antibodies.

Supplementary Figure S8



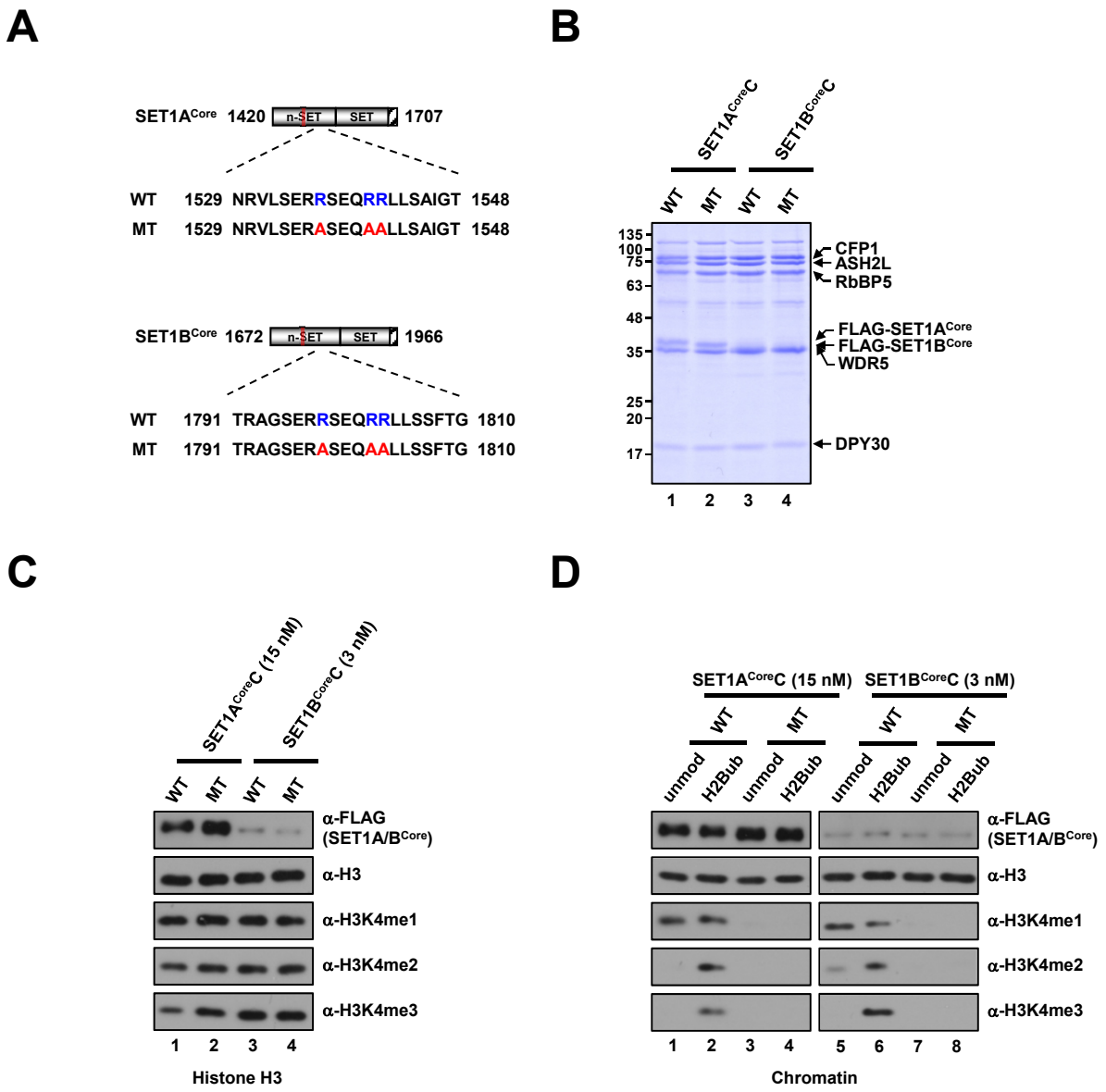
Supplementary Figure S8. H3K4 methylation activities of SET1B-MLL3 and SET1B-MLL4 fusion-containing complexes.

(**A**) A schematic diagram of KMT2-fusion proteins composed of the n-SET domain from SET1B and SET, and post-SET domains from MLL3 or MLL4, used for reconstitution of complexes.

(**B**) SDS-PAGE/Coomassie blue staining of purified KMT2-fusion complexes reconstituted with baculoviruses expressing FLAG-tagged SET1B-MLL3 or SET1B-MLL4 fusion proteins (A), WDR5, RbBP5, ASH2L, DPY30 and CFP1. Fused proteins are marked by asterisks.

(**C**) Stimulatory effects of the n-SET domain and CFP1 on H3K4 methylation activities of MLL3 and MLL4 complexes. Unmodified and H2Bub chromatin substrates were subjected to *in vitro* HMT assays with the indicated KMT2-fusion complexes (1.5 nM).

Supplementary Figure S9



Supplementary Figure S9. The RXXXRR motif is critical for the H3K4 methylation activities of human SET1A^{Core} and SET1B^{Core} complexes.

(**A**) A schematic diagram of wild-type (WT) and mutant (MT) SET1A^{Core} and SET1B^{Core} fragments used for the reconstitution of complexes. Three conserved arginine residues (blue) within the RXXXRR motif were replaced by alanine (red) in mutant proteins.

(**B**) SDS-PAGE/Coomassie blue staining of purified WT and MT SET1A^{Core} and SET1B^{Core} complexes reconstituted with baculoviruses expressing WDR5, RbBP5, ASH2L, DPY30, CFP1 and FLAG-tagged WT or MT SET1A/B^{Core} fragments.

(**C** and **D**) Requirement of the RXXXRR motif for nucleosomal H3K4 methylation activities of SET1A^{Core} and SET1B^{Core} complexes. H3 (C) and unmodified or H2Bub chromatin substrates (D) were subjected to *in vitro* HMT assays with the indicated concentrations of purified SET1A^{Core} or SET1B^{Core} complexes.