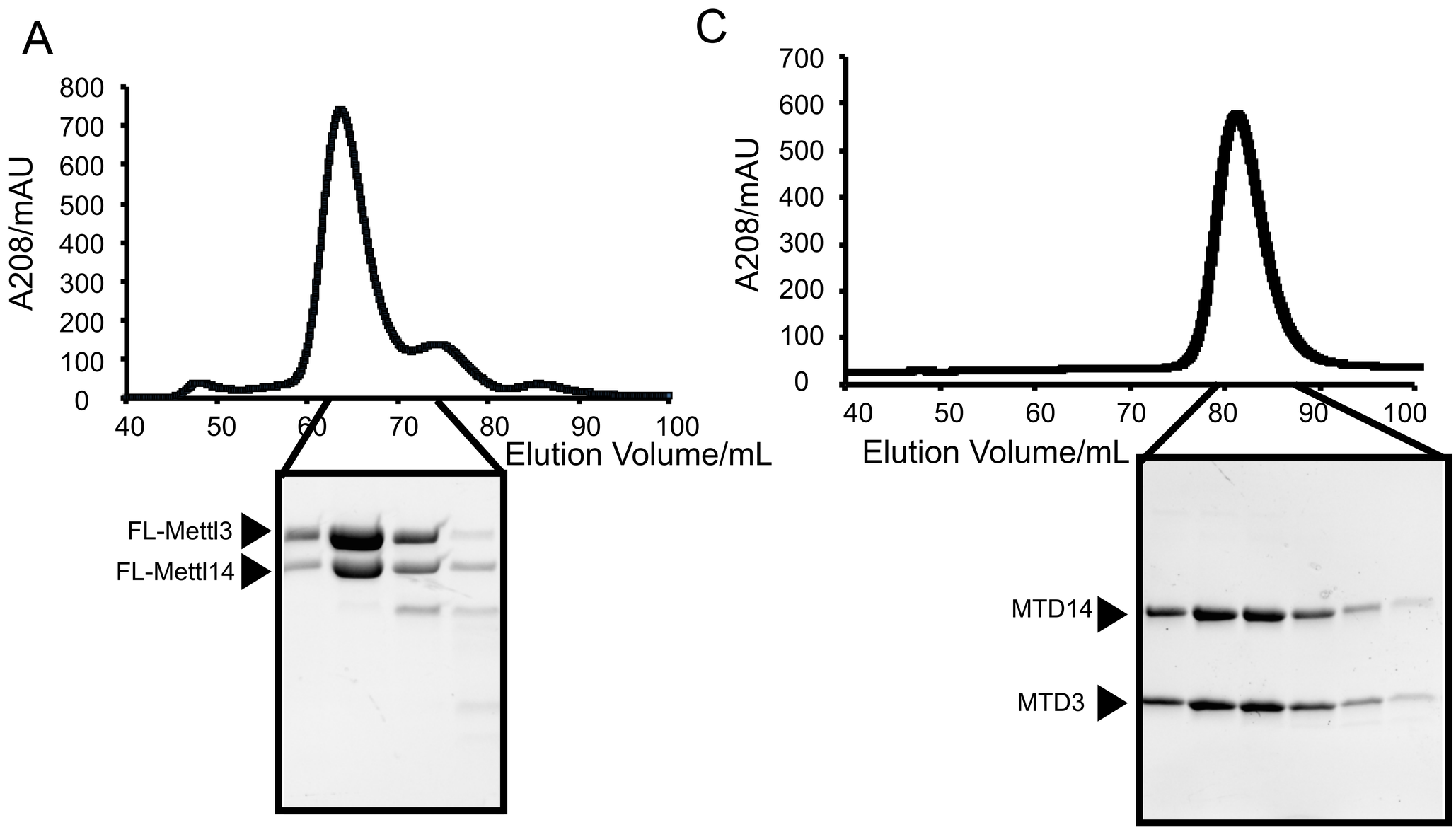
Figure S1. Related to Figure 1.



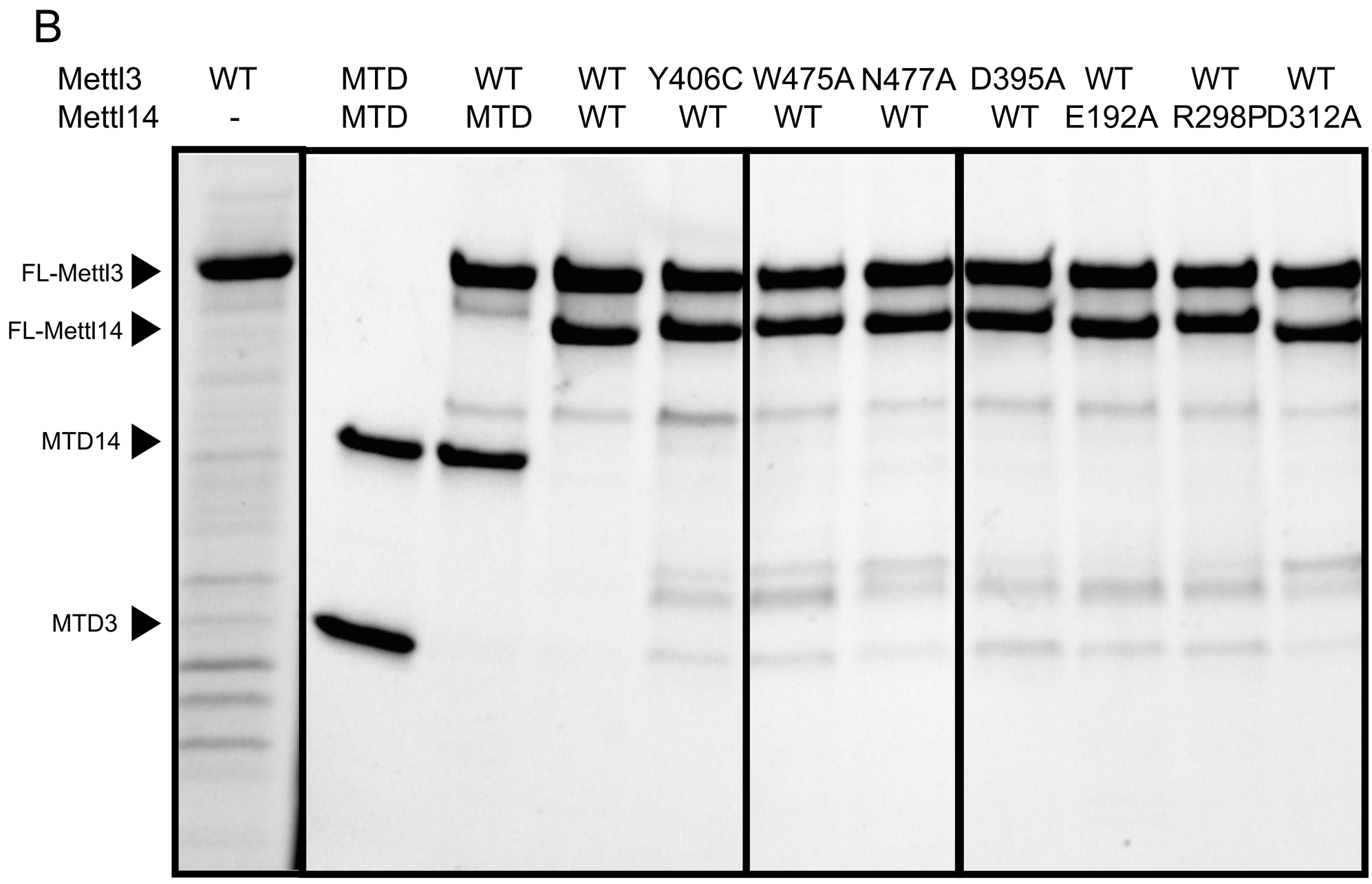


Figure S1. Protein purification of Mettl3/Mettl14 complexes, related to Figure 1.
(A) Size-exclusion chromatography (Superdex 200) of the full-length Mettl3/Mettl14 complex. The Y axis shows the absorbance at 280 nm and the X axis shows the elution volume in mLs. Peak fractions were analyzed by SDS-PAGE and visualized with Stain-Free dye (Biorad). **(B)** SDS-PAGE analysis of all purified proteins used in in vitro methylation assays, visualized with Stain-Free dye (Biorad). Black lines between sample lanes indicate non adjacent lanes in the gel. **(C)** Size-exclusion chromatography (Superdex 200) of the MTD3/MTD14 complex. The Y axis shows the absorbance at 280 nm and the X axis shows the elution volume in mLs. Peak fractions were analyzed by SDS-PAGE and visualized with Stain-Free dye (Biorad).

Figure S2. Related to Figure 2.

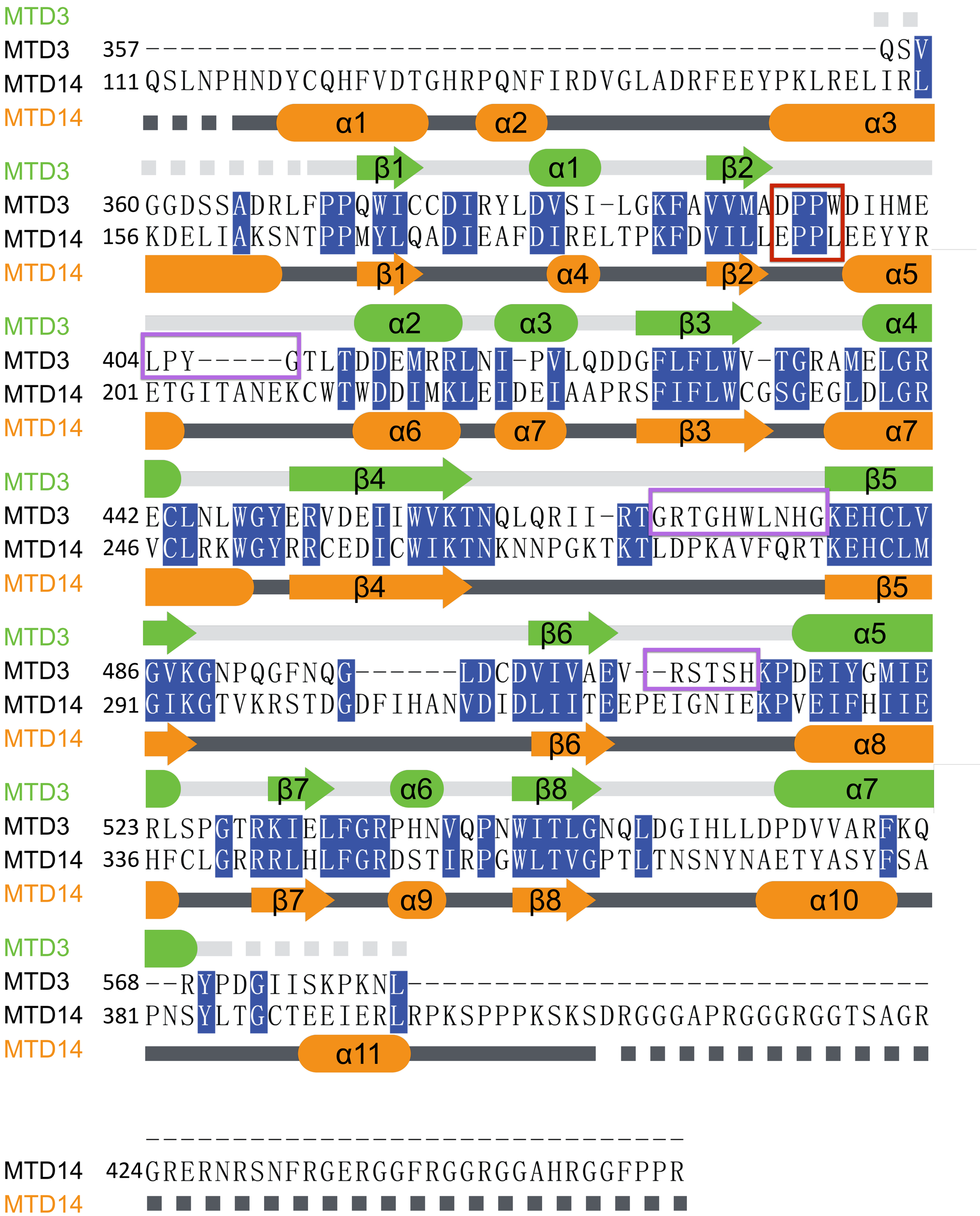


Figure S2. Sequence alignment of human MTD3 and human MTD14, related to Figure 2.
Sequence alignment of MTD3 and MTD14 with secondary structure elements assigned for MTD3 (green) and MTD14 (orange) according to the apo complex structure of MTD3/MDT14. Helices are represented by curved rectangles, beta strands by block arrows and disordered regions by dashes. Numbers listed represent the position of the residue in the full-length sequence. The catalytic motifs are highlighted with a red box and the three “fence” loops are highlighted with three purple boxes. Also see Figure 6.

Figure S3. Related to Figure 2.

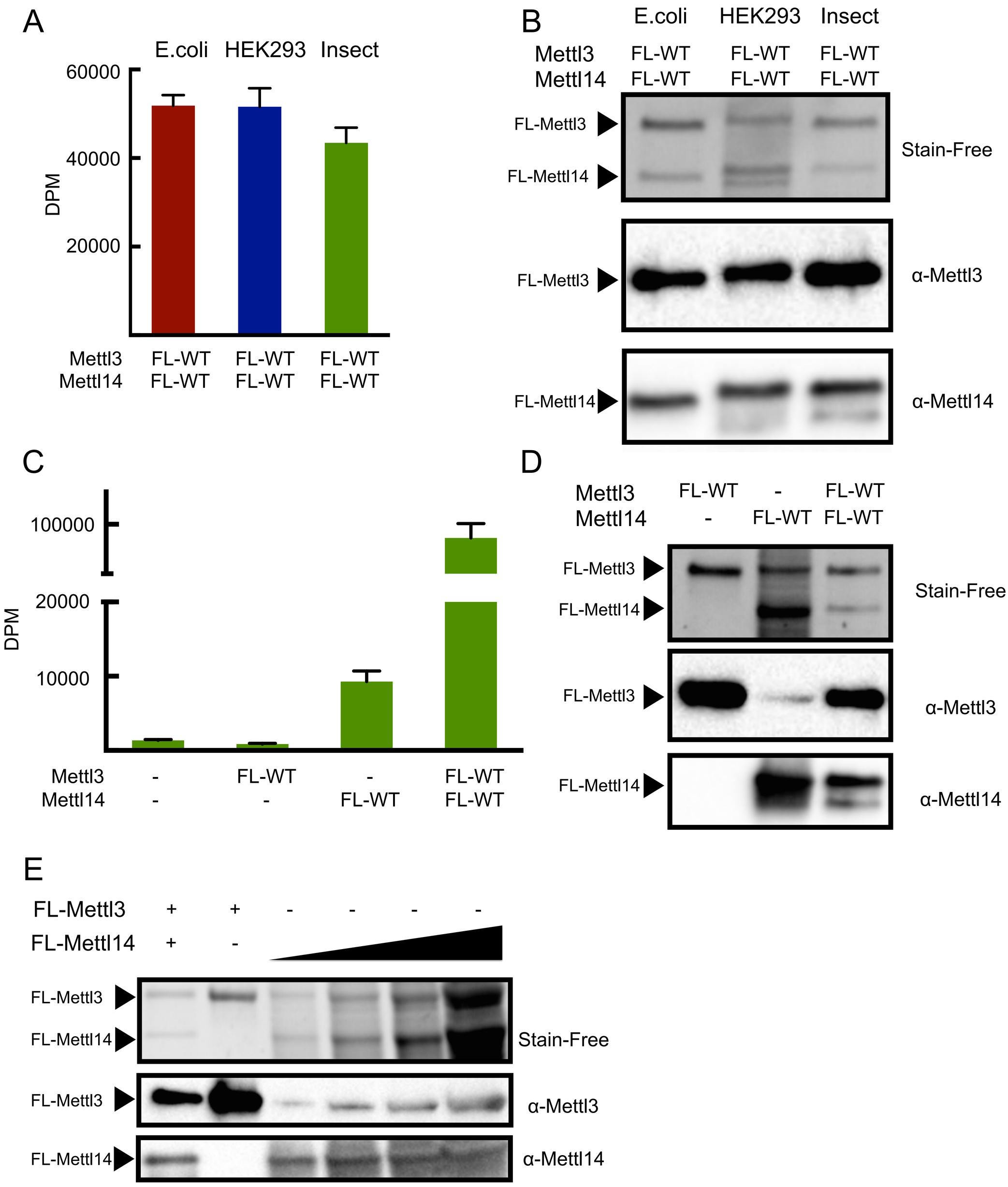
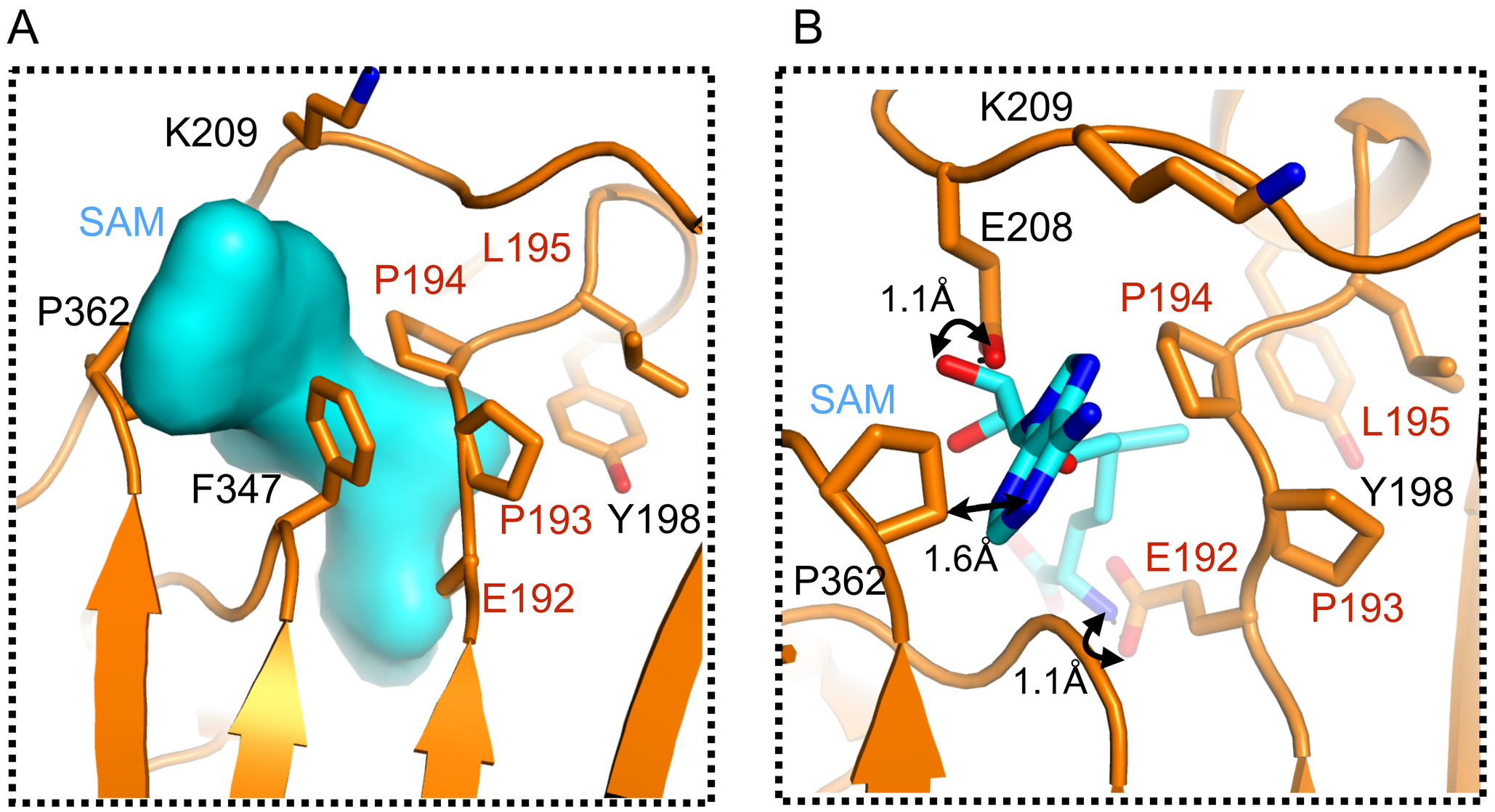


Figure S3. Comparison of methyltransferase activity of Mettl3/Mettl14 complexes from E.Coli, insect cells and mammalian cells, related to Figure 2.

(A). In vitro methyltransferase activity of the full-length Mettl3/Mettl14 complex expressed from E. Coli, insect cells and HEK293 cells. (**B).** SDS-PAGE analysis (visualized with Stain-Free dye) of the proteins used in the methylation assay in Figure S3A. (**C).** In vitro methylation activity of the full length Mettl3/Mettl14 complex and each individual protein expressed from insect cells. (**D).** SDS-PAGE analysis followed by Western Blot of the proteins used in the methylation assay in Figure S3C. (**E).** SDS-PAGE analysis of Mettl3 and Mettl14 alone, and the Mettl3/Mettl14 complex expressed from insect cells, followed by Western blot with an anti-Mettl3 antibody. Increasing the concentration of Mettl14 shows an increase in contaminating endogenous Mettl3.

Figure S4. Related to Figure 3.



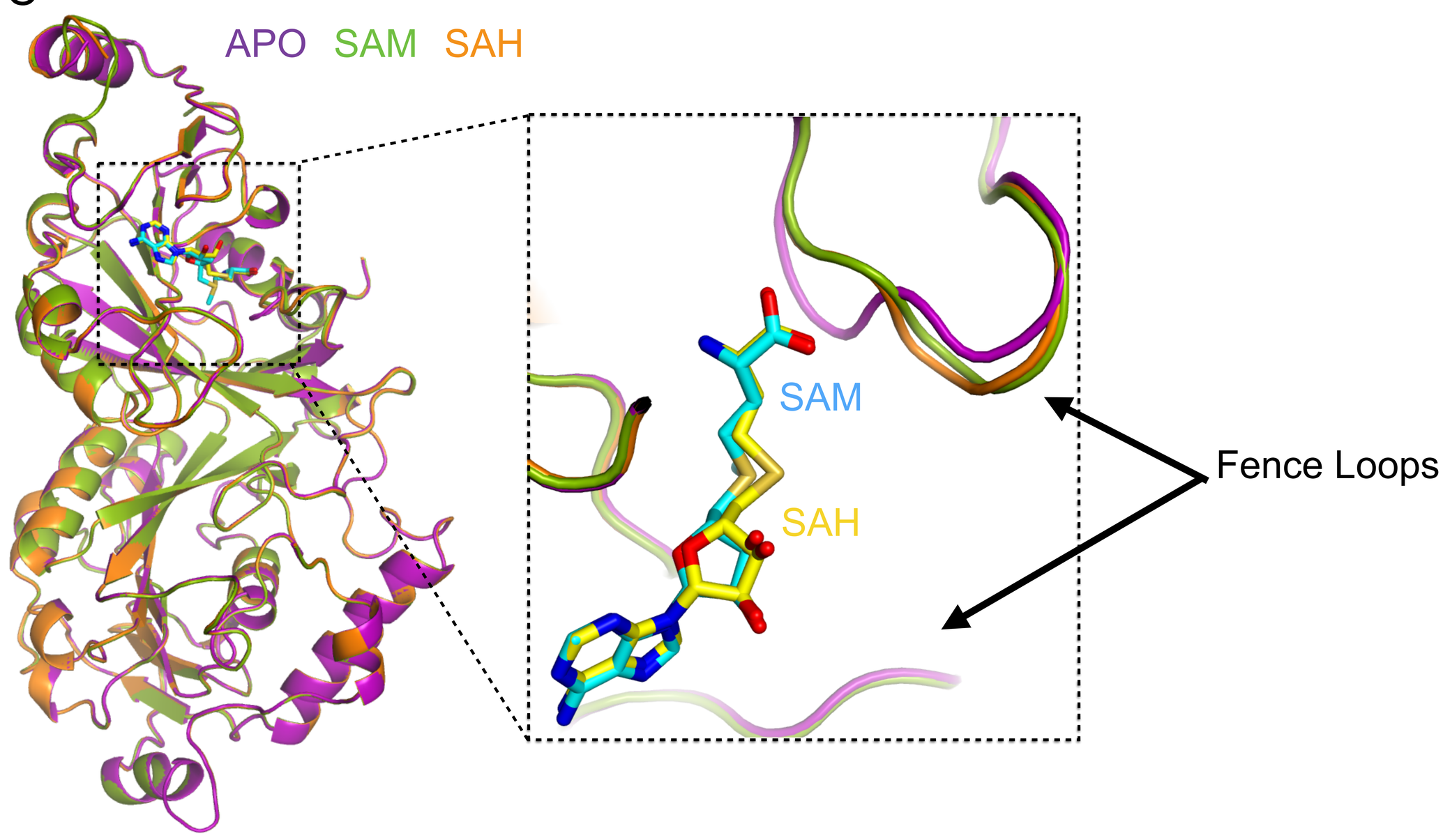
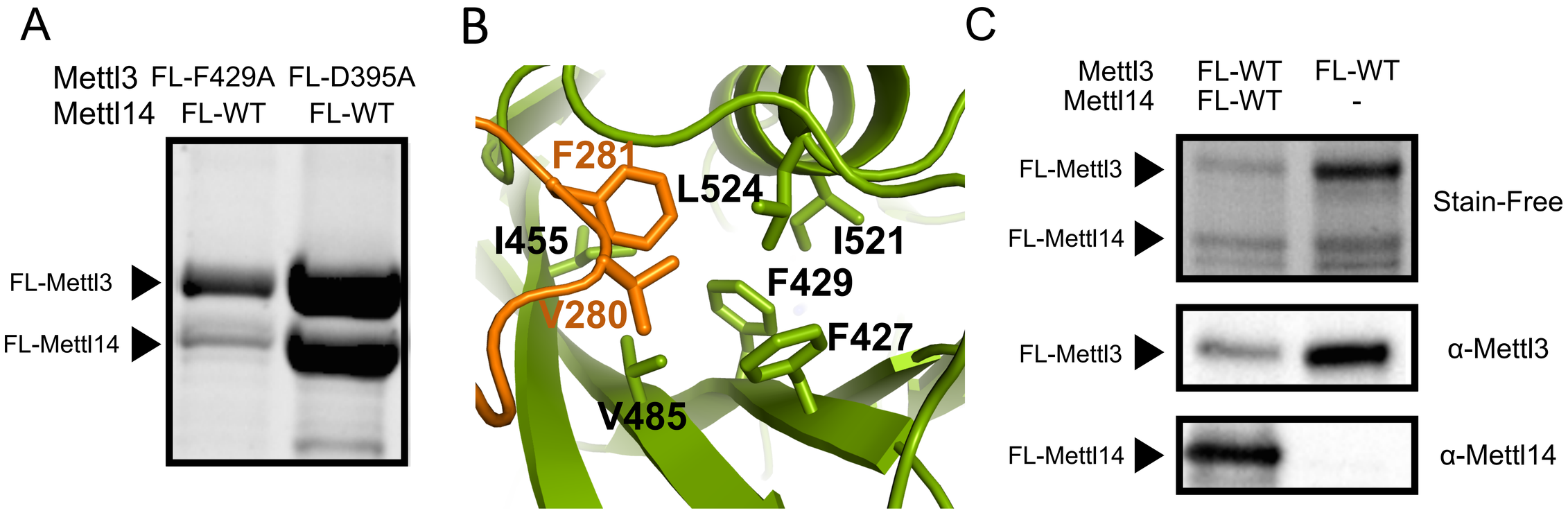
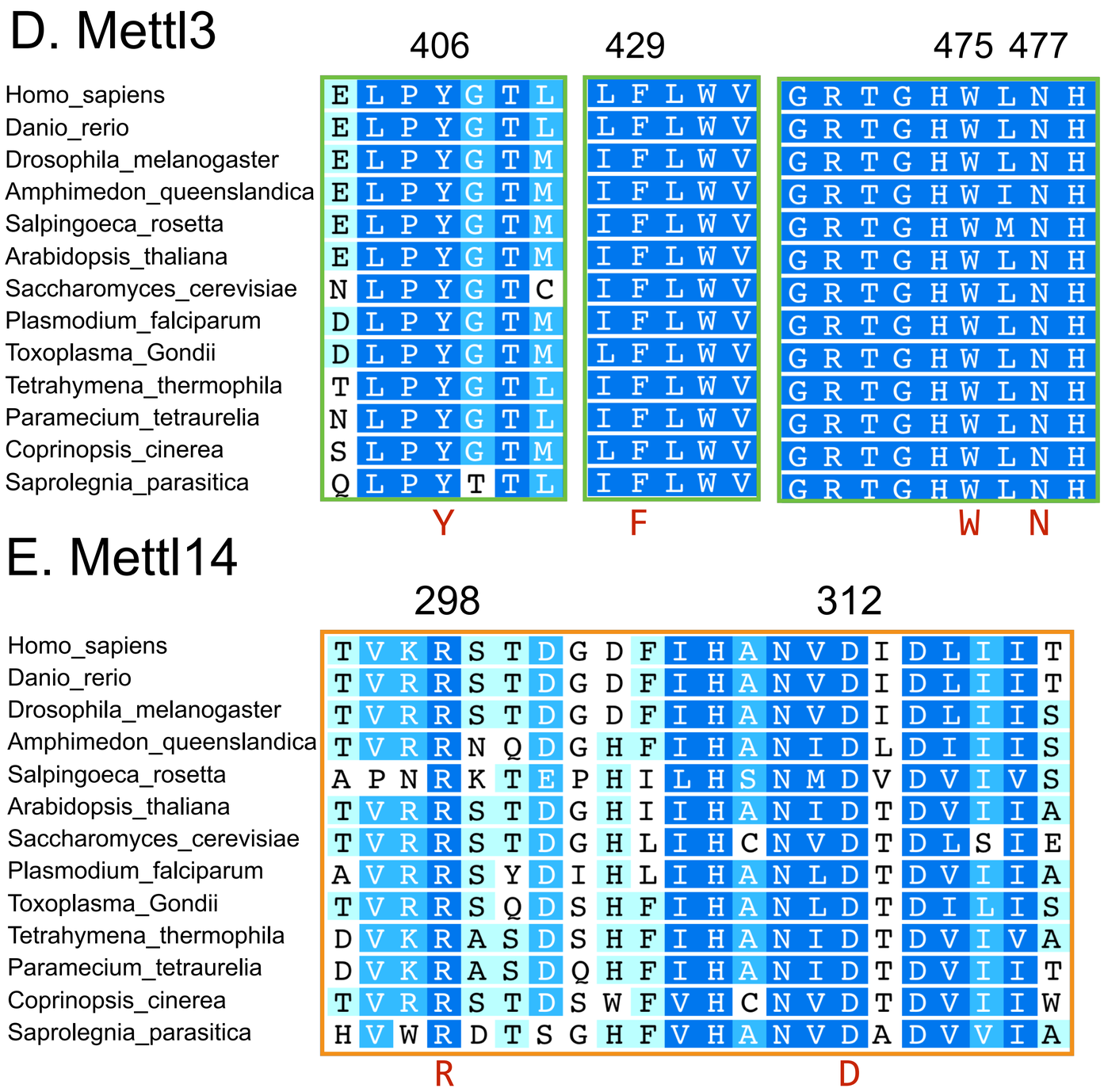


Figure S4. The SAM bound MTD3/MTD14 complex, related to Figure 3.
(A) SAM, in cyan surface representation, is modeled in the potential catalytic site of MTD14 (orange cartoon and sticks) by superimposition of SAM-bound MTD3 onto MTD14. Severe clashes are seen between SAM and the EPPL catalytic motif (labeled in red text). **(B)** SAM, in cyan stick representation, is modeled in the potential catalytic site of MTD14 (orange cartoon and sticks) by superimposition of SAM-bound MTD3 onto MTD14. The close distances between the side chains of E192, E208 and P362 from MTD14 and SAM are indicated. **(C)** Superimposition of apo (magenta), SAM-bound (green) and SAH-bound (orange) MTD3 in cartoon representation. The dashed rectangle shows a close-up view of the loop movements upon SAM binding and catalysis. “Fence” loops are marked with arrows.

Figure S5. Related to Figure 4.





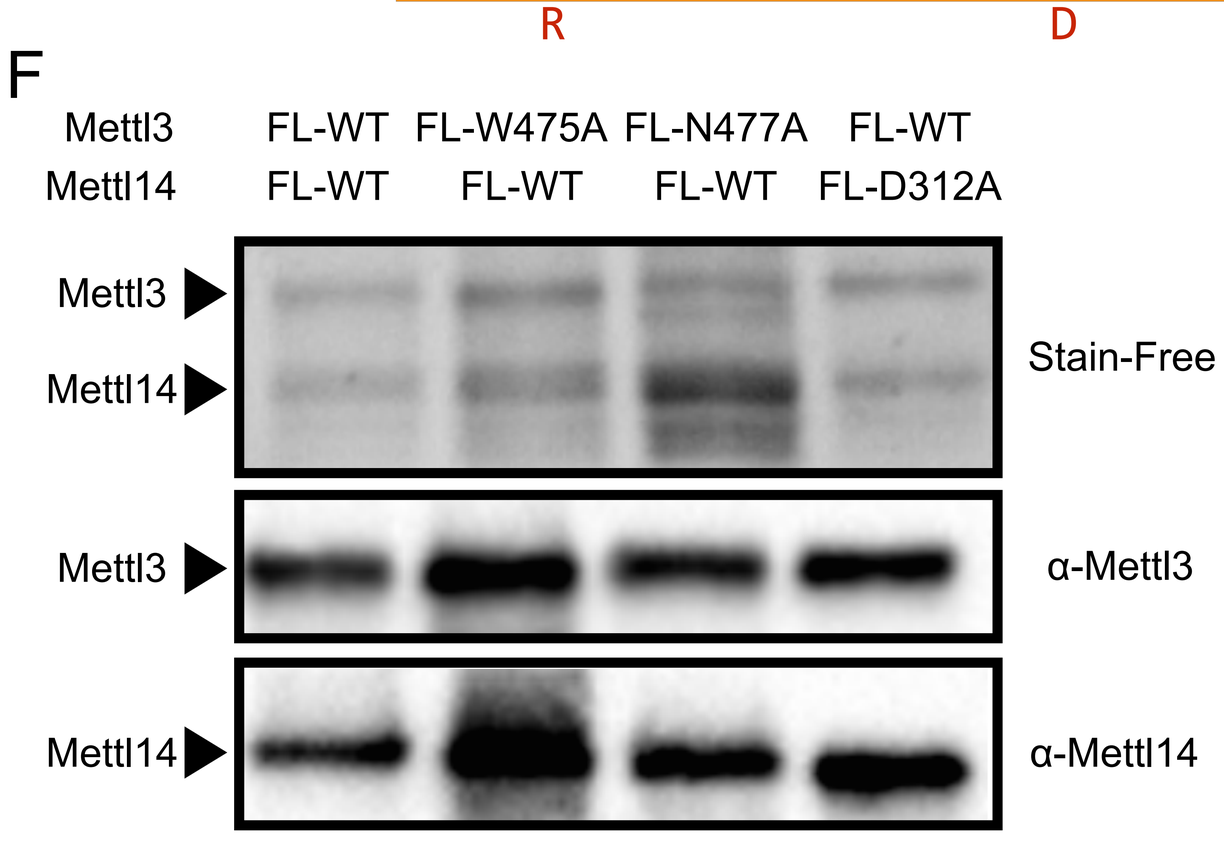


Figure S5. Specific residues involved in the heterodimer binding interface, related to Figure 4. **(A)** SDS-PAGE (visualized by Stain-Free dye) of heterodimer interface mutant F429A (MTD3) shows dramatically decreased expression and solubility compared to the MTD3 catalytic mutant D395A expressed and purified at the same scale, under the same conditions. **(B)** Views of the important residues of the MTD3 and MTD14 binding interface show hydrophobic interactions around F429A (MTD3). MTD3 residues are shown as green sticks and labeled with black text. MTD14 residues are shown as orange sticks and labeled with orange text. **(C)** SDS-PAGE analysis (visualized by Stain-Free dye) followed by Western blot of the proteins used in the in vitro methylation assay in Figure 4G. **(D)** and **(E)** Sequence alignment of Mettl3 **(D)** or Mettl14 **(E)** of fourteen different orthologs from the species marked on the left. Only the portions of each protein that contain residues discussed in text (Mettl3: Y406, F429, W475 and N477 and Mettl14, R298 and D312) are shown. Higher conservation is indicated by a darker blue background, and the lowest conservation has a white background. **(F)** SDS-PAGE analysis (visualized by Stain-Free dye) followed by Western blot of the proteins used in the in vitro methylation assay in Figure 4I.

Figure S6. Related to Figure5.

A

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1    MSDTWSSIQAHKKQLDSLRERLQRRRKQDSGHLDLRNPEAALSPTFRSDS
51   PVPTAPTSGGPKPSTASAVPELATDPELEKKLLHHLSDLALTLPTDAVSI
101  CLAISTPDAPATQDGVESLLQKFAAQELIEVKRGLLQDDAHPTLVTYADH
151  SKLSAMMGAVAEKKGPGEVAGTVTGQKRRAEQDSTTVAAFASSLVSGLNS
201  SASEPAKEPAKKSRKHAASDVDLEIESLLNQQSTKEQQSKKVSQEILELL
251  NTTTAKEQSIVEKFRSRGRAQVQEFCDYGTKEECMKASDADRPCRKLHFR
301  RIINKHTDESLGDCSFLNTCFHMDTCKYVHYEIDACMDSEAPGSKDHTPS
351  QELALTQSVGGDSSADRLFPPQWICCDIRYLDVSILGKFAVVMADPPWDI
401  HMELPYGTLTDDEMRRLNIPVLQDDGFLFLWVTGRAMELGRECLNLWGYE
451  RVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDC
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551  LDGIHLLDPDVVARFKQRYPDGIISKPKNL
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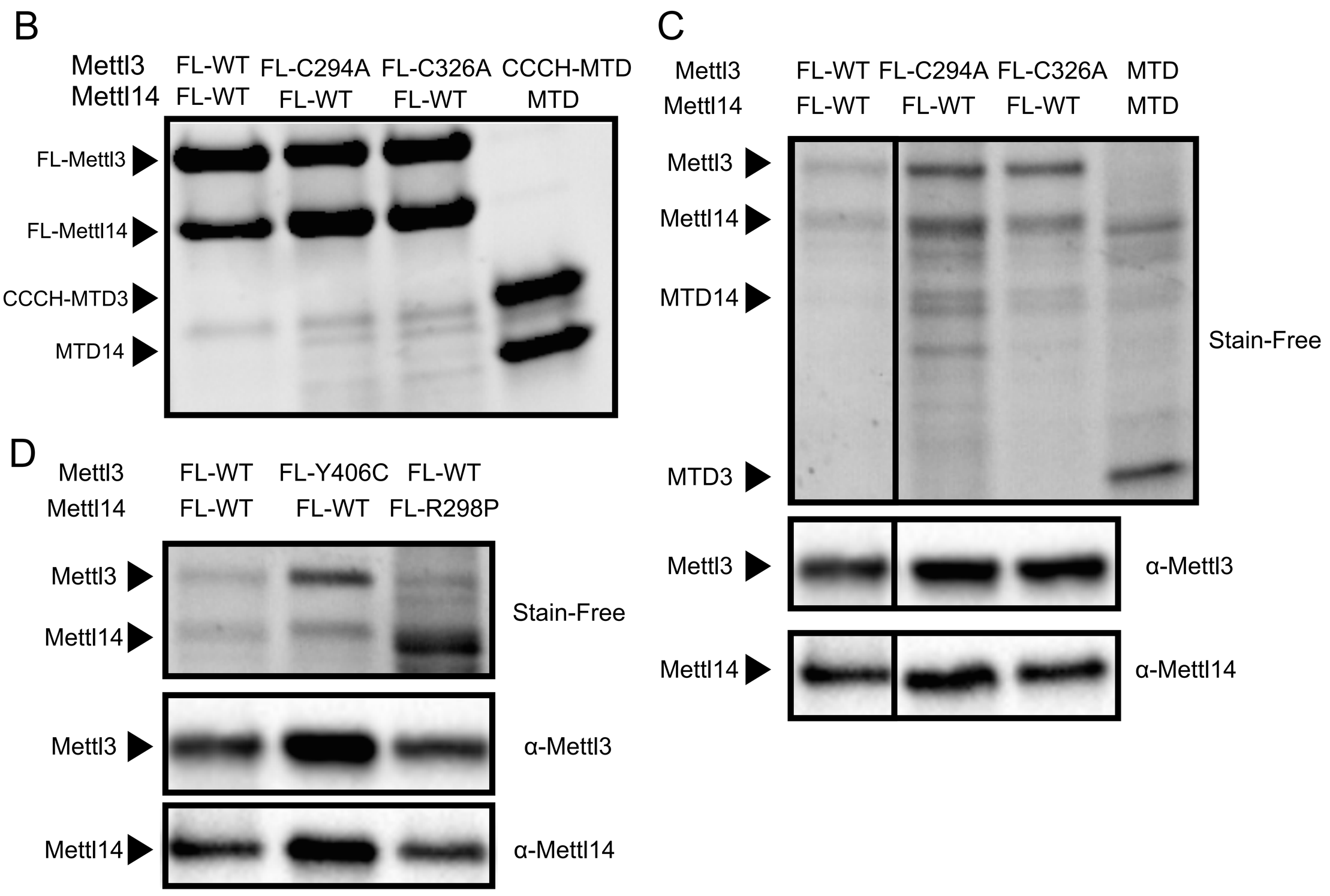

Figure S6: Mutational analysis of the zinc fingers of Mettl3, related to Figure 5.
(A) Sequence of full length Mettl3. Proposed zinc finger residues (CCCH) are highlighted in red and those underlined were mutated to alanine and analyzed in this study for *in vitro* methylation activity (Figure 5A). The catalytic domain of Mettl3 is underlined and the catalytic motif DPPW is shown in green. **(B)** SDS-PAGE analysis (visualized by Stain-Free dye) of the proteins used in the methylation assay in Figure 5A. **(C)** SDS-PAGE analysis (visualized by Stain-Free dye) followed by Western blot of the proteins used in the in vitro methylation assay in Figure 5B. The antibodies used to recognize full length Mettl3 and Mettl14 are incapable of recognizing the methyltransferase domain alone of each protein. Black lines between sample lanes indicate non adjacent lanes in the gel and blot. **(D)** SDS-PAGE analysis (visualized by Stain-Free dye) followed by Western blot of the proteins used in the in vitro methylation assay in Figure 5E.