Klare et al., http://www.jcb.org/cgi/content/full/jcb.201412028/DC1

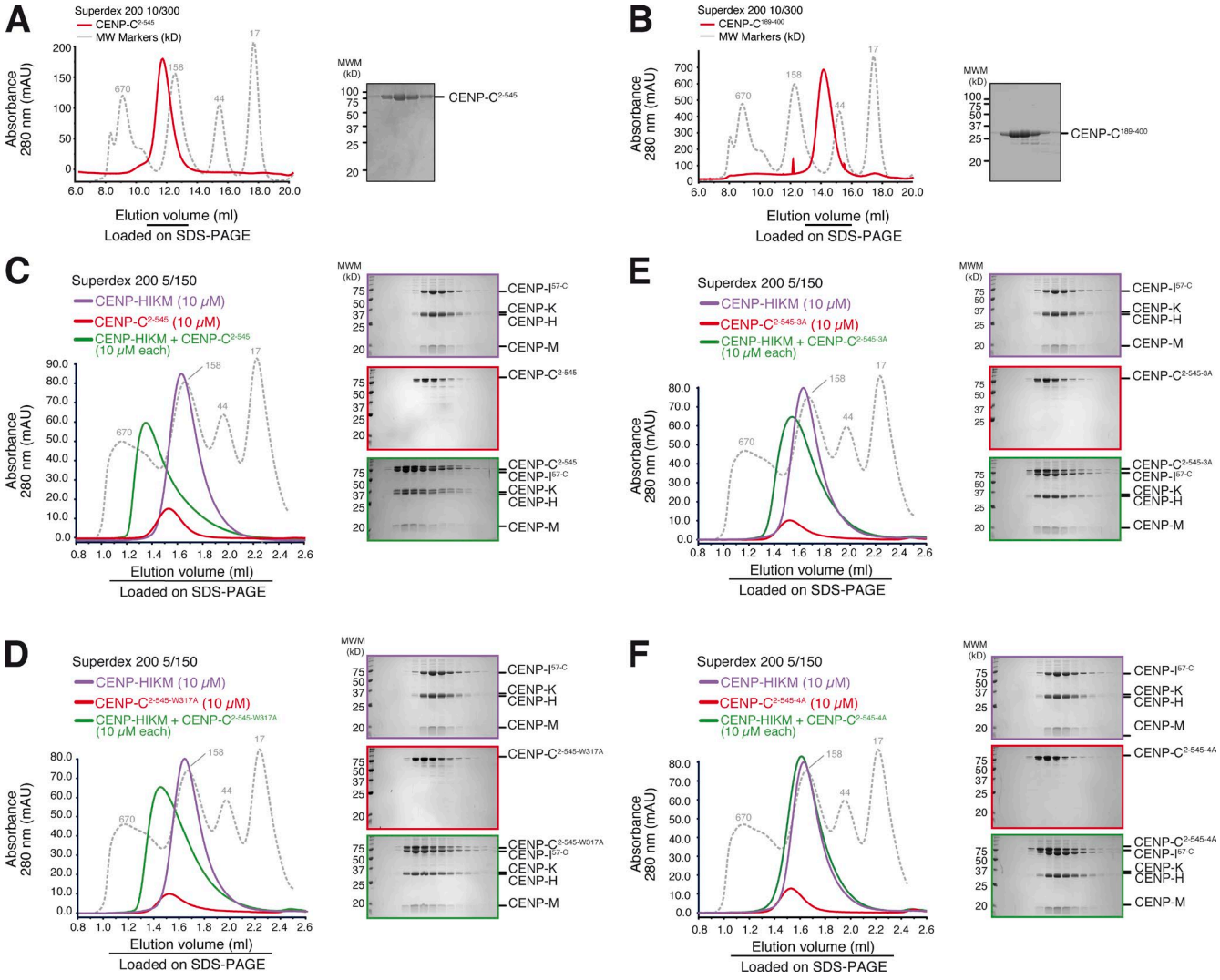

Figure S1. **Additional SEC analyses.** (A and B) Size-exclusion chromatography (SEC) elution profiles of CENP-C$^{2–545}$ (a typical outcome of at least five experiments; A) or CENP-C$^{189–400}$ (a typical outcome of at least three experiments; B). SEC separates proteins based on size and shape, and in A, the CENP-C$^{2–545}$ construct eluted significantly earlier than expected for a globular protein of equivalent molecular mass, indicative either of oligomerization or of an extended Stokes' radius typical of a predominantly unstructured protein. (C–F) Wild-type CENP-C$^{2–545}$ (~62 kD), CENP-C$^{2–545\text{-}W317A}$, CENP-C$^{2–545\text{-}3A}$, and CENP-C$^{2–545\text{-}4A}$ mutants all show the same elution volume. (C) CENP-C$^{2–545}$ and CENP-HIKM, both at 10 µM, form a stable, apparently stoichiometric complex on an analytical SEC (already shown in Fig. 1 F and depicted here again for comparison). (D and E) CENP-C$^{2–545\text{-}W317A}$ (D) or CENP-C$^{2–545\text{-}3A}$ (E) show signs of impaired binding to CENP-HIKM, with CENP-C$^{2–545\text{-}3A}$ having a more penetrant effect. (F) CENP-C$^{2–545\text{-}4A}$ is completely unable to bind CENP-HIKM. The experiments in C–F were repeated at least two times. The gray dotted lines show the elution profile of globular markers of known molecular masses, as indicated. mAU, milliabsorbance units.

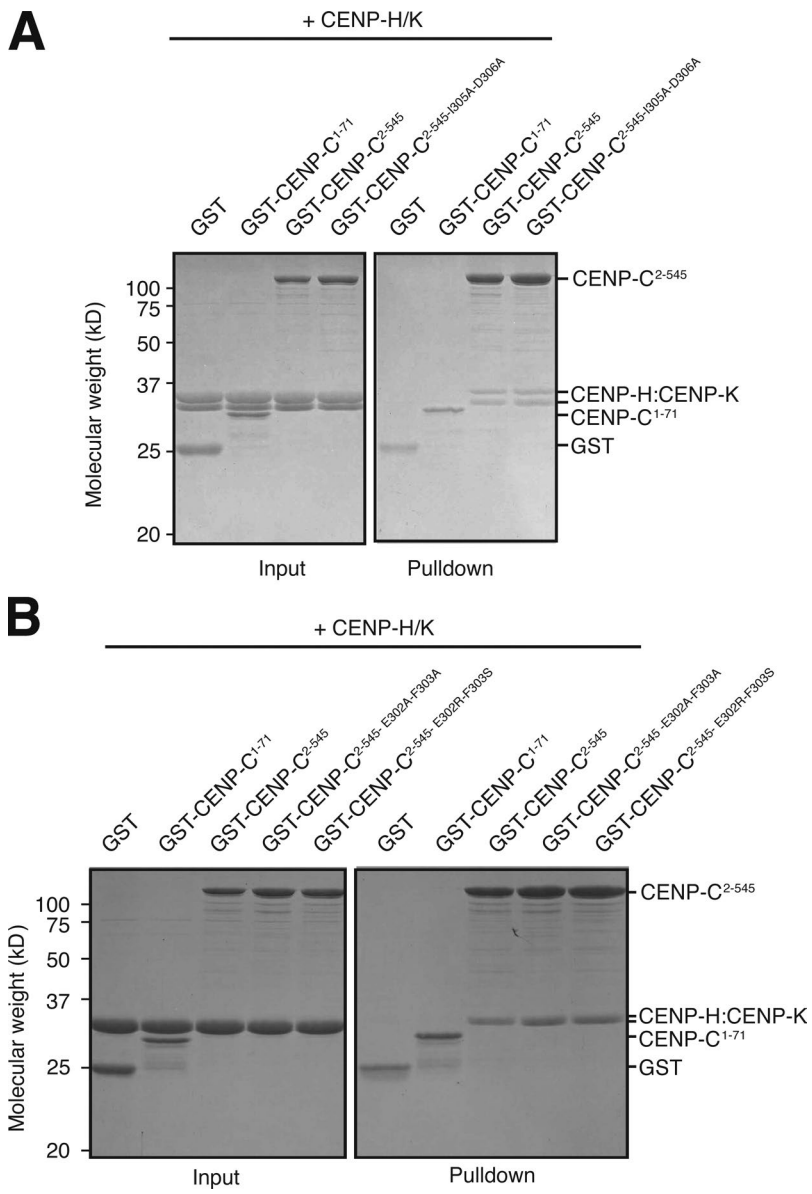

Figure S2. **Binding assays with CENP-C mutants in the Glu-Phe-Ile-Ile-Asp motif.** (A) Coomassie-stained SDS-PAGE of GST pull-down binding assays with the indicated CENP-C wild-type or mutant proteins. The lanes marked as "Input" display the content of the binding reactions. The lanes marked as "Pull-down" display bead-bound material. CENP-H–CENP-K complex binds to CENP-C regardless of the mutations introduced in the Glu-Phe-Ile-Ile-Asp motif. (B) As in A, with a different collection of mutants.

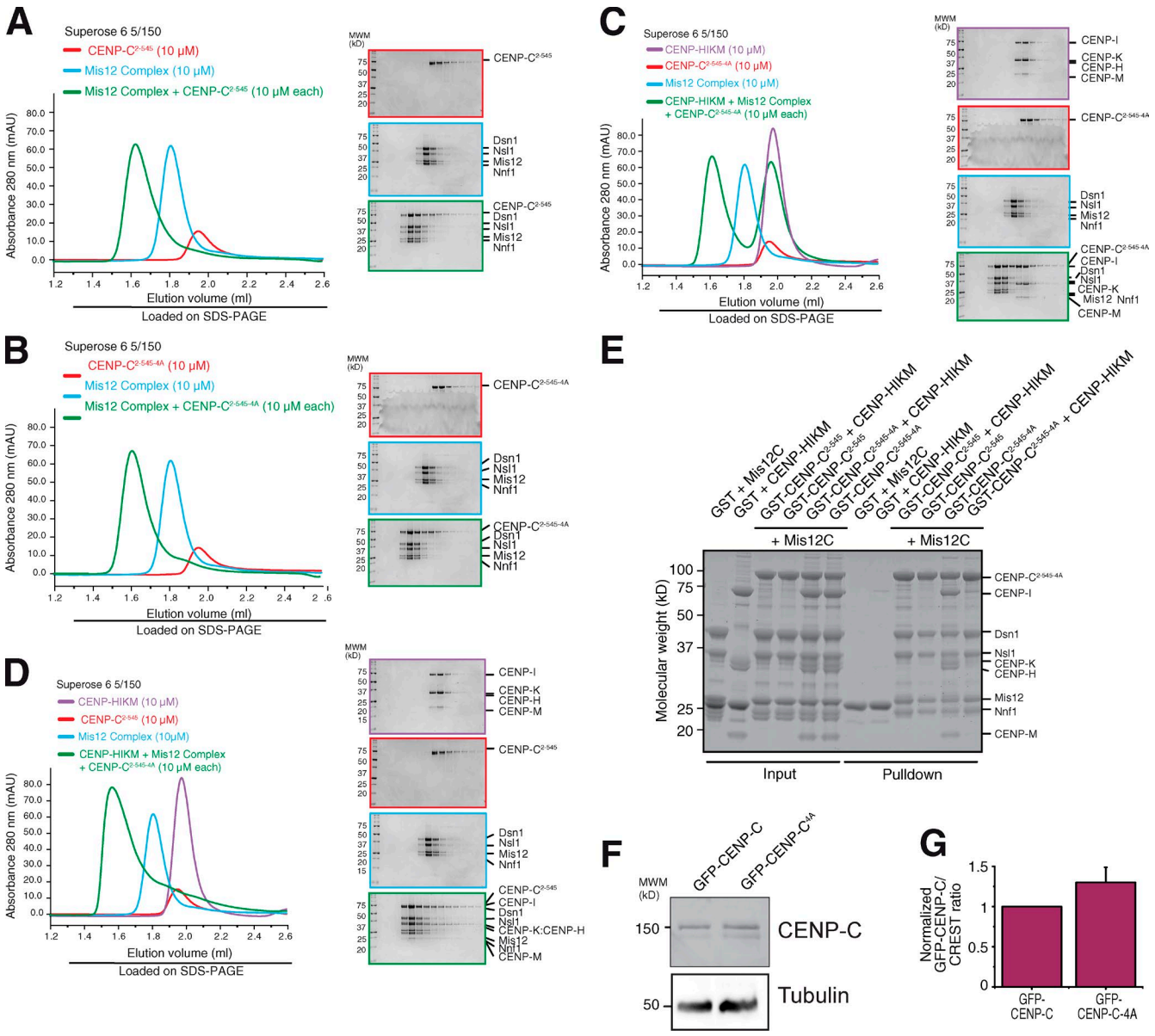

Figure S3. **Binding of CENP-C and CENP-C mutants to Mis12 and expression levels.** (A) SEC elution profile shows CENP-C$^{2–545}$ and Mis12 complex, both at 10 µM, form a stable, apparently stoichiometric complex on an analytical SEC. MWM, molecular weight marker. (B) Identical results are obtained with CENP-C$^{2–545-4A}$, indicating that the 4A mutations do not affect Mis12 binding. (C) CENP-C$^{2–545}$, Mis12 complex, and CENP-HIKM enter a stoichiometric nine-subunit complex. (D) CENP-C$^{2–545-4A}$ binds the Mis12 complex but fails to bind to CENP-HIKM, hence failing to cause a change in its elution volume. Experiments in A–D were repeated at least two times. (E) Coomassie-stained SDS-PAGE of GST pull-down binding assays with the indicated CENP-C wild-type or mutant proteins. The lanes marked as Input display the content of the binding reactions. The lanes marked as Pull-down display bead-bound material. Both CENP-C$^{2–545}$ and CENP-C$^{2–545-4A}$ mutant binds the Mis12 complex, but CENP-C$^{2–545-4A}$ fails to interact with CENP-HIKM. (F) Expression levels of the indicated GFP–CENP-C (wt and 4A mutant) rescue constructs. (G) Quantification of immunofluorescence experiment in fixed Flp-In T-REx HeLa cells expressing the indicated GFP constructs. The kinetochore levels of the indicated GFP fusion proteins were normalized to CREST. Error bars indicate SEM of GFP levels from all cells analyzed in six independent experiments. mAU, milliabsorbance units.

Table S1 contains the entire list of intra- and intermolecular cross-links and is provided online as an Excel file. A ZIP file is provided that contains custom scripts used to convert raw cross-linking data from an Excel spreadsheet to the GEXF.