Kim and Yu et al., http://www.jcb.org/cgi/content/full/jcb.201407074/DC1

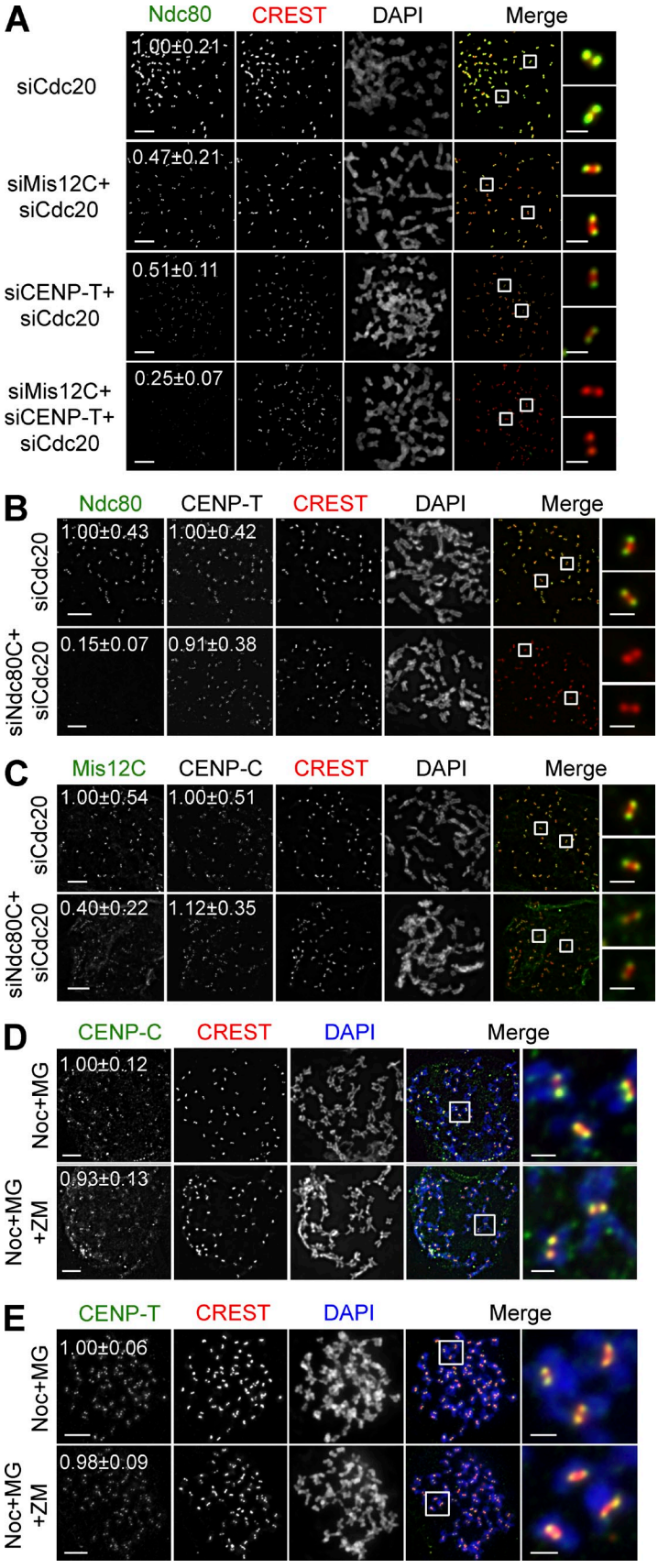

Figure S1. **Interdependency between Mis12C and Ndc80C at kinetochores.** (A–C) Mitotic HeLa cells transfected with the indicated siRNAs were stained with the indicated antibodies and DAPI. Selected channels (as labeled by the corresponding colors) were included in the merged images. Boxed regions were magnified and shown in the rightmost column. Normalized intensities (mean ± SD, $n = 400$) of kinetochore staining are shown. (D) HeLa cells were arrested in mitosis with nocodazole (Noc) and MG132 (MG), treated with ZM447439 (ZM), and stained with DAPI (blue in merge), CREST (red), and α-CENP-C (green). The boxed regions were magnified and shown in the rightmost column. The normalized intensities of the CENP-C (mean ± SD, with 40 kinetochores per cell and 19 or 20 cells per condition) were quantified and shown. (E) HeLa cells were treated as in D and stained with DAPI (blue in merge), CREST (red), and α-CENP-T (green). The boxed regions were magnified and shown in the rightmost column. The normalized intensities of the CENP-T (mean ± SD, with 40 kinetochores per cell and 30 cells per condition) were quantified and shown. The CREST and DAPI channels in E are shown in the top two panels of Fig. 2 D alongside labeling for an additional protein. Bars, 5 µm (1 µm for magnified images).

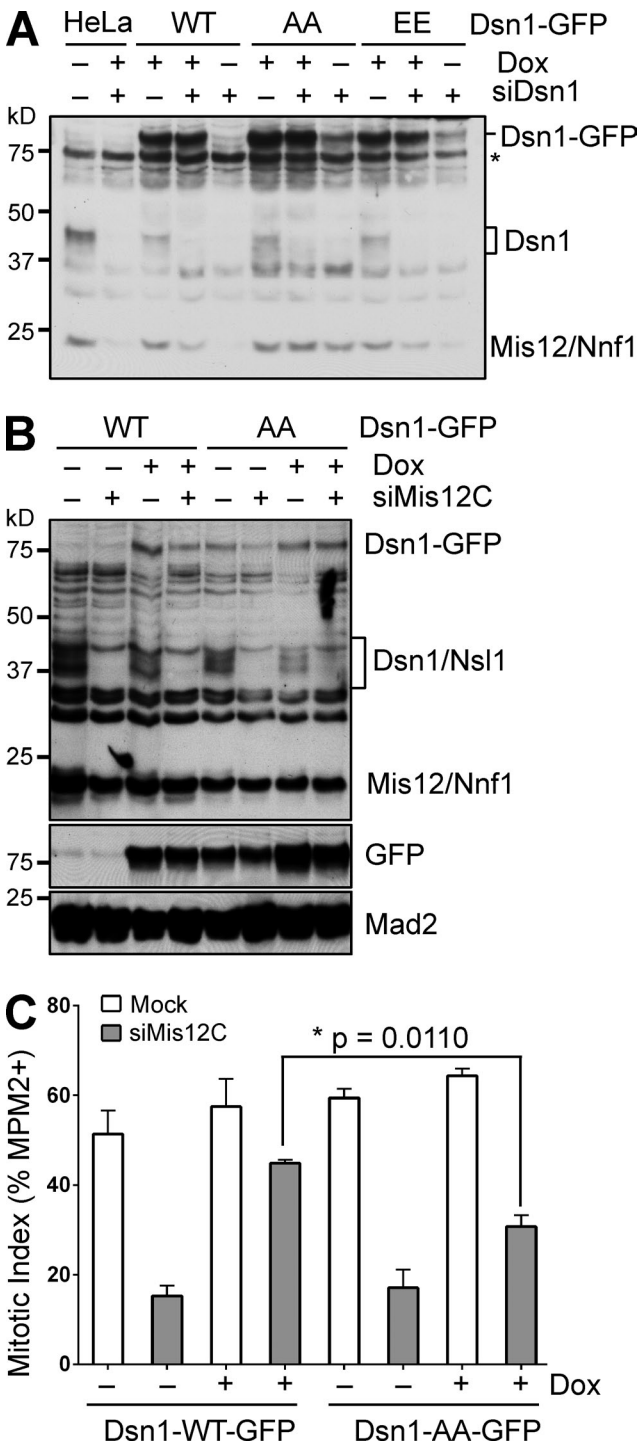

Figure S2. **Dsn1 phosphorylation contributes to spindle checkpoint signaling.** (A) HeLa Tet-On parental cells and cells stably expressing Dsn1-WT-GFP, Dsn1-S100A/S109A (AA)-GFP, or Dsn1-S100E/S109E (EE)-GFP were cultured in the absence (–) or presence (+) of doxycycline (Dox) and were either mock transfected (–) or transfected with (+) siDsn1. Cell lysates were blotted with α-Mis12C. In the absence of Dox, Dsn1-AA-GFP and Dsn1-EE-GFP lines had leaky expression. The asterisk indicates a nonspecific band that serves as a loading control. (B) HeLa Tet-On cells stably expressing Dsn1-WT-GFP or Dsn1-AA-GFP were mock transfected or transfected with siMis12C (the combination of siDsn1 and siNsl1) in the absence (–) or presence (+) of doxycycline (Dox). Cells were treated with thymidine for 14 h, and released into nocodazole-containing medium for 15 h. Lysates of these cells were blotted with the indicated antibodies. (C) The mitotic index of cells in B was determined by FACS, with means and SD (error bars) of three independent experiments shown.

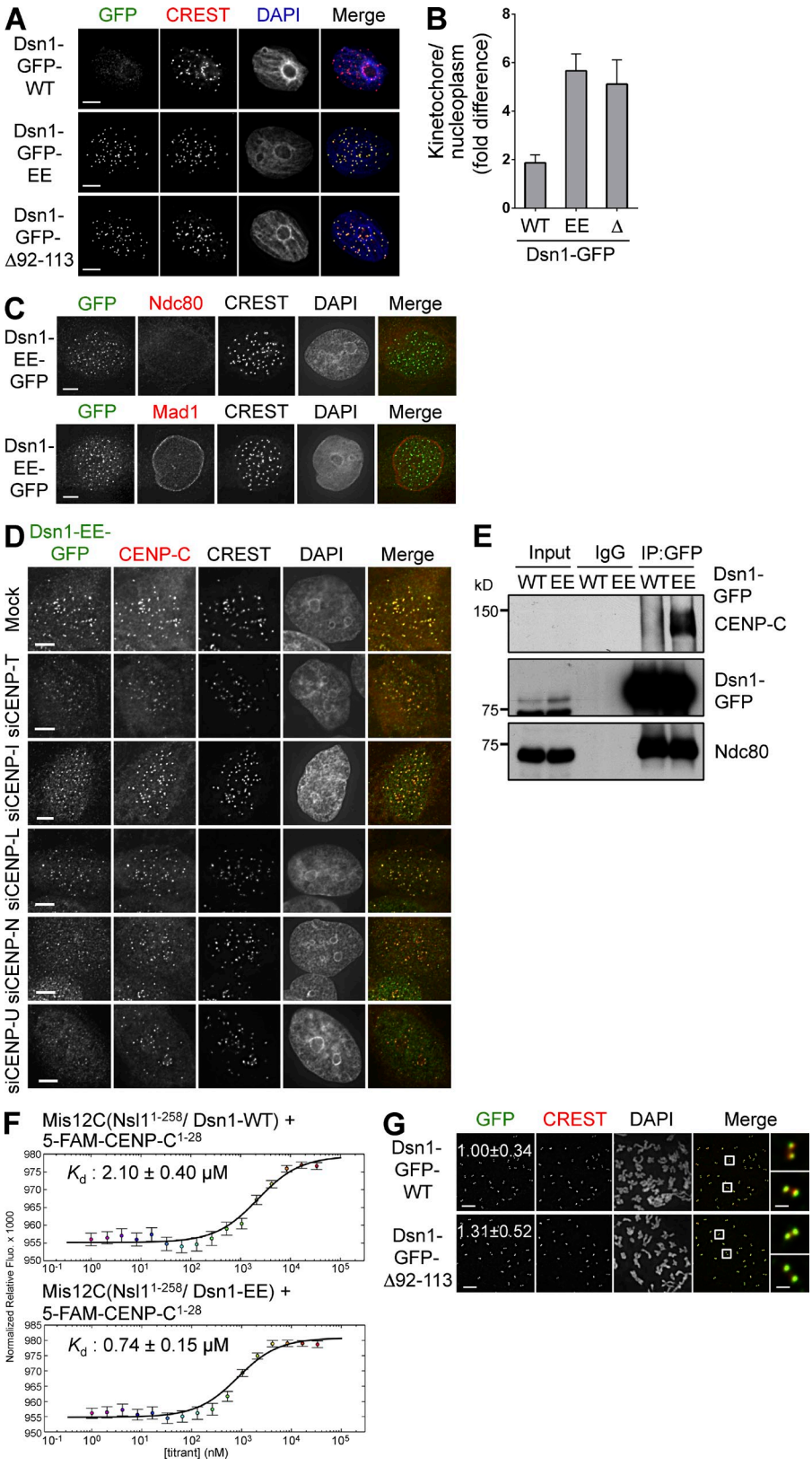

Figure S3. **Phospho-mimicking and Δ92–113 mutants of Dsn1 localize to interphase kinetochores.** (A) Interphase HeLa cells stably expressing the indicated Dsn1-GFP proteins were stained with DAPI and the indicated antibodies. (B) Quantification of the kinetochore enrichment of Dsn1-GFP proteins in A. Mean ± SD (error bars), $n = 10$. (C) Interphase cells expressing Dsn1-WT/EE-GFP were stained with the indicated antibodies and DAPI. (D) HeLa Tet-On cells stably expressing Dsn1-EE-GFP were either mock transfected or transfected with siRNAs against the indicated CENPs, arrested in thymidine, and stained with DAPI, CREST, α-GFP (green in merge), and α-CENP-C (red). (E) Lysates (Input), α-Mis12C IP (Mis12C), and IgG IP of thymidine-arrested HeLa cells expressing Dsn1-WT/EE-GFP were blotted with the indicated antibodies. (F) Microscale thermophoresis titration curves of the binding reactions between a fluorescently labeled CENP-C peptide and Mis12C containing Dsn1 WT (top) or EE (bottom). Error bars indicate SD. (G) Mitotic HeLa cells stably expressing Dsn1-WT/Δ92-113-GFP were stained with DAPI and the indicated antibodies. The relative kinetochore intensities in the GFP channel were quantified (mean ± SD, $n = 400$). Bars: (A, C, D, and G) 5 μm; (magnified images in G) 1 μm.

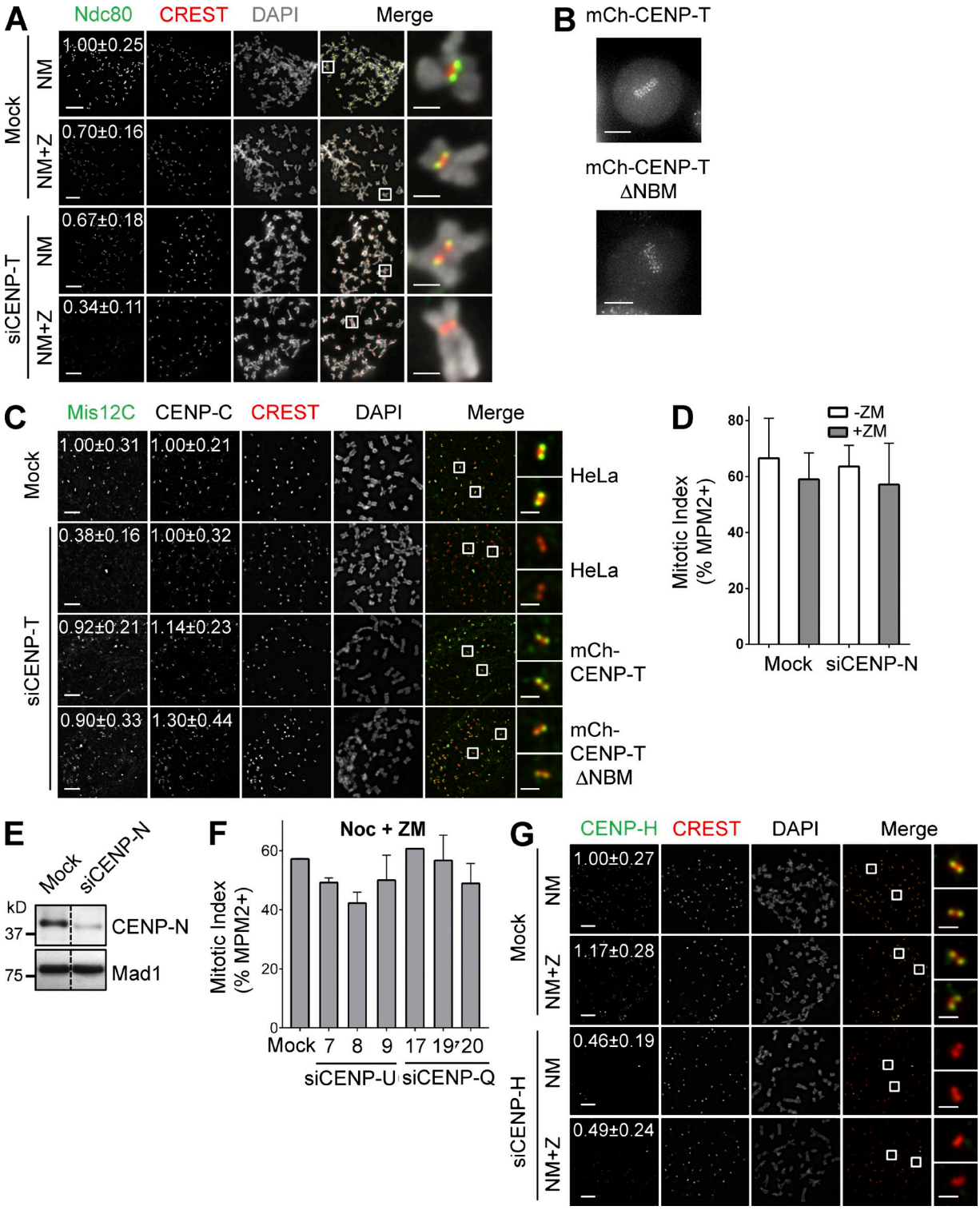

Figure S4. **Depletion of CENP-T and Aurora B inhibition compromise KMN kinetochore localization without affecting CENP-C.** (A) HeLa cells were mock transfected or transfected with siCENP-T and arrested in mitosis by nocodazole. Mitotic cells were collected by shake-off. Each sample was divided into two fresh wells. One well was incubated with MG132 (MG) for 2 h (NM) whereas the other well was treated with both MG and ZM for 2 h (NM+Z). Cells were stained with the indicated antibodies and DAPI. The boxed regions of the merged images of the selected channels were magnified and shown in the rightmost column. The relative kinetochore intensities (mean ± SD, *n* = 400) of Ndc80 were quantified and shown. (B) mCherry images of representative live metaphase HeLa cells expressing indicated CENP-T proteins. (C) Mitotic HeLa cells expressing the indicated proteins were transfected with siCENP-T, treated with nocodazole, ZM, and MG132, and stained with the indicated antibodies and DAPI. Boxed regions of merged images were magnified and shown in the rightmost column. The relative kinetochore intensities (mean ± SD, *n* = 400) of Mis12C and CENP-C were quantified and shown. (D) HeLa Tet-On cells were mock transfected or transfected with siCENP-N, treated with thymidine for 14 h, released into nocodazole-containing medium for 12 h, and treated with or without ZM for 2 h. Their mitotic index was determined by flow cytometry. Means and SD (error bars) of three experiments are shown. (E) Lysates of cells in D were blotted with the indicated antibodies. (F) HeLa Tet-On cells were mock transfected or transfected with the indicated siRNAs, treated with thymidine for 14 h, released into nocodazole-containing medium for 12 h, and treated with ZM for 2 h. Their mitotic index was determined by flow cytometry. Means and SD (error bars) of three experiments are shown. (G) HeLa Tet-On cells were mock transfected or transfected with siCENP-H and arrested in mitosis by nocodazole. Cells were further incubated with MG132 (MG) for 2 h (NM) or with both MG and ZM for 2 h (NM+Z), and stained with the indicated antibodies and DAPI. The boxed regions of the merged images with the indicated channels were magnified and shown in the rightmost column. The relative kinetochore intensities (mean ± SD, *n* = 400) of CENP-H were quantified and shown. Bars: (A, C, and G) 5 µm; (A, C, and G, enlarged panels) 1 µm; (B) 10 µm.

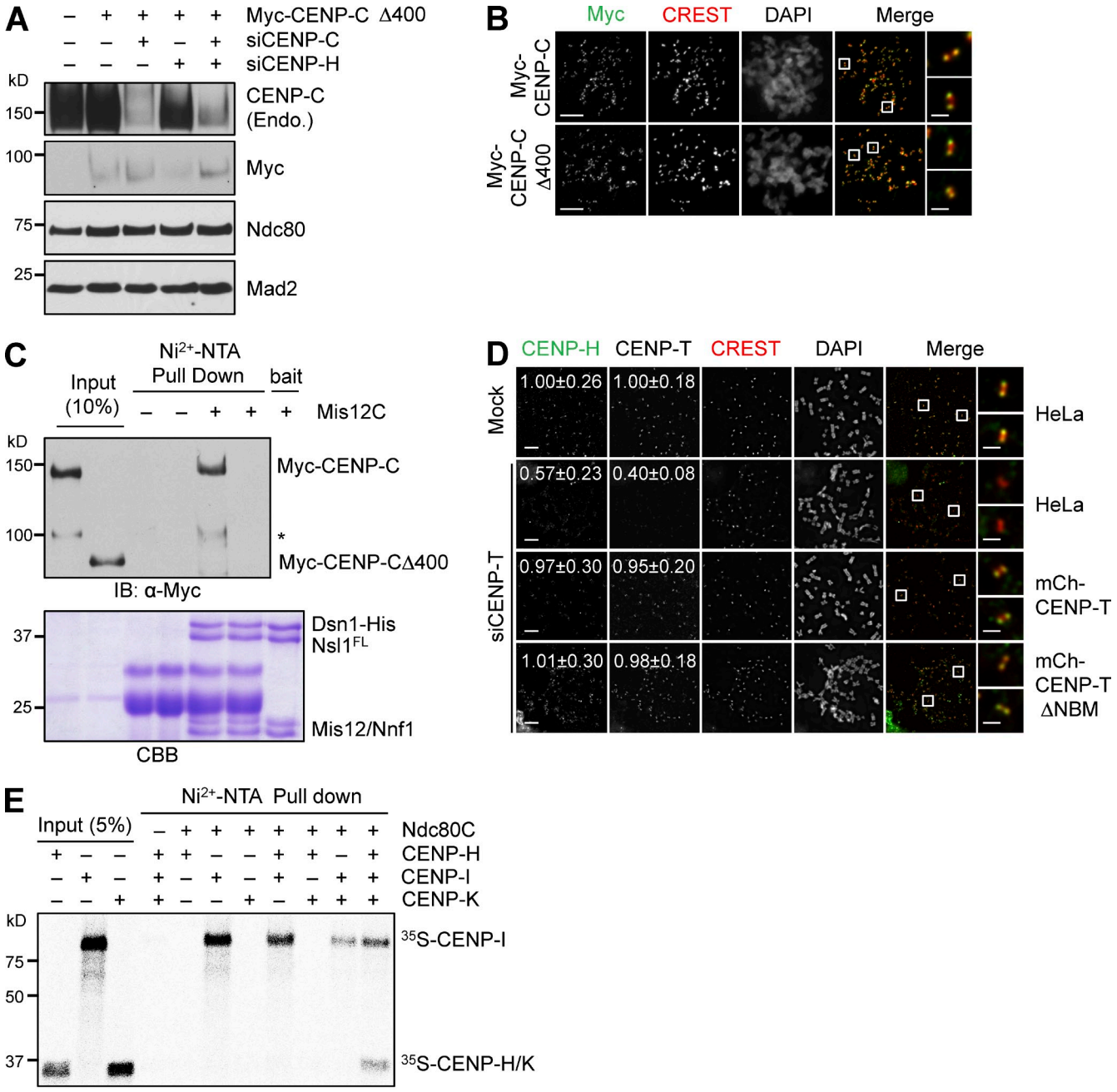

Figure S5. **CENP-T contributes to CENP-H kinetochore localization independently of its NBM.** (A) Lysates of HeLa cells transfected with the indicated plasmids and siRNAs were blotted with the indicated antibodies. (B) Mitotic HeLa cells transfected with Myc-CENP-C WT or Δ400 were stained with DAPI and the indicated antibodies. Bar, 5 μm (1 μm for magnified images). (C) In vitro binding assays between recombinant Mis12C and Myc-CENP-C WT or Δ400. The reaction mixtures were blotted with anti-Myc antibody (top) and stained with Coomassie brilliant blue (CBB; bottom). The asterisk indicates a degradation band of CENP-C. (D) Mitotic HeLa cells expressing indicated proteins were transfected with siCENP-T, treated with nocodazole, ZM, and MG132, and stained with the indicated antibodies and DAPI. Boxed regions of merged images were magnified and shown in the rightmost column. The relative kinetochore intensities (mean ± SD, n = 400) of CENP-H and CENP-T were quantified and shown. Bars, 5 μm (1 μm for magnified images). (E) Recombinant Ndc80C was immobilized on beads and incubated with ^{35}S-labeled CENP-H, -I, or -K. Bound proteins and input were separated by SDS-PAGE and analyzed with a phosphorimager.