Supplemental material JCB

Cullati et al., https://doi.org/10.1083/jcb.201512055

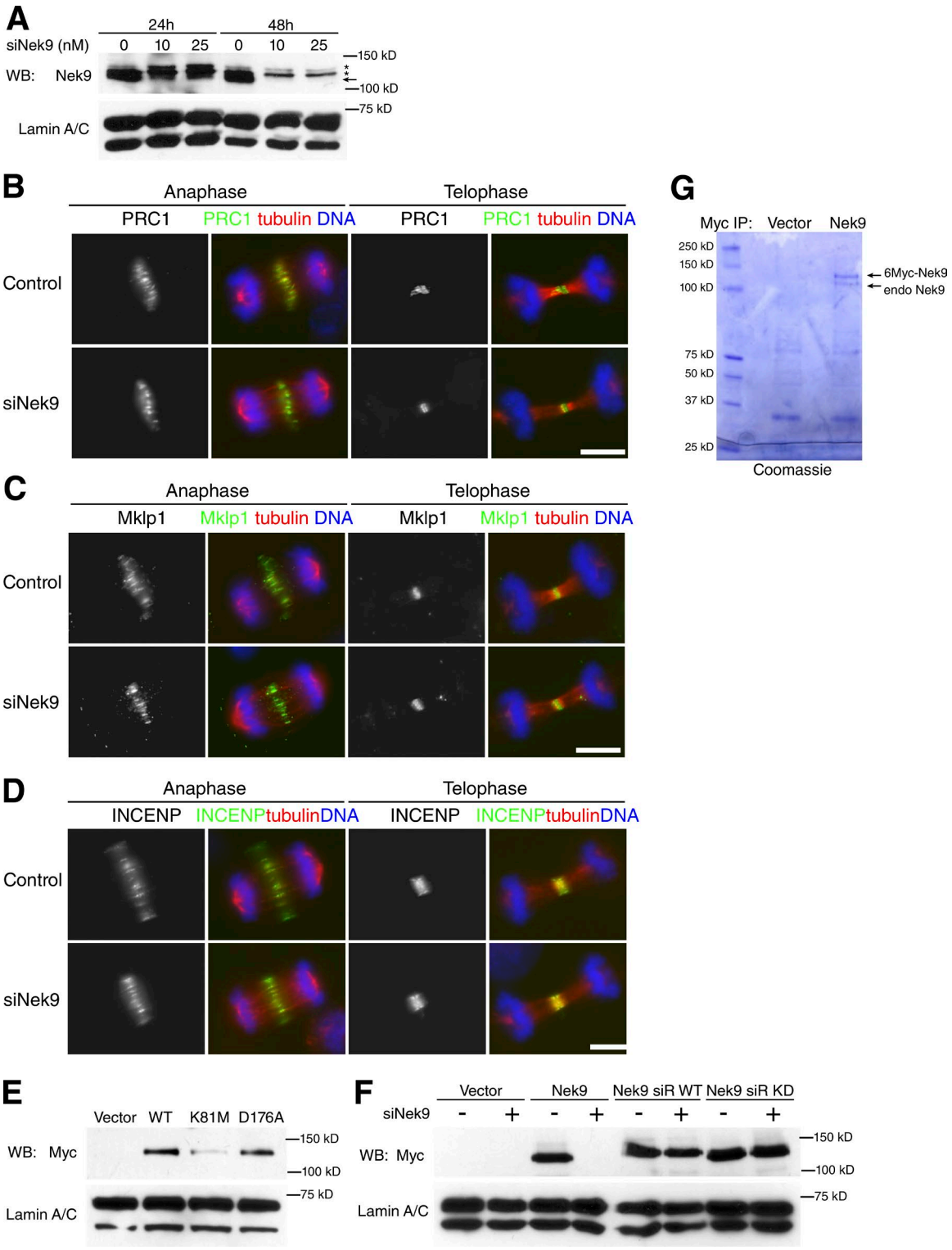

Figure S1. **Validation of Nek9 siRNAs and 6×Myc-tagged cell lines as well as Nek9 interactors with localizations unaffected by Nek9 depletion.** (A) Rabbit polyclonal antibody to Nek9 identifies a band from HeLa lysate that disappears upon treatment with Nek9 siRNA (siR). Asterisks indicate nonspecific bands; arrows indicate Nek9. WB, Western blot. (B–D) Central spindle structure is not disrupted upon depletion of Nek9, as assessed by localization of PRC1 (B), Mklp1 (C), and INCENP (D) to the central spindle in anaphase. Detergent-extracted HeLa cells stained with endogenous (endo) antibodies. Bar, 20 µm. (E) Transient transfection of 6×Myc-tagged Nek9 WT, K81M, and D176A indicated that mutation of the catalytic aspartate generated protein that was stable to WT levels, whereas the K81M mutation produced a reduced amount of protein in this system. (F) Silent mutations were introduced to make siRNA-resistant versions of WT and KD Nek9 in stable HeLa cell lines. (G) Representative Coomassie-stained SDS-PAGE gel of a 6×Myc-Nek9 immunoprecipitation (IP) indicating that microtubules do not strongly coimmunoprecipitate with Nek9.

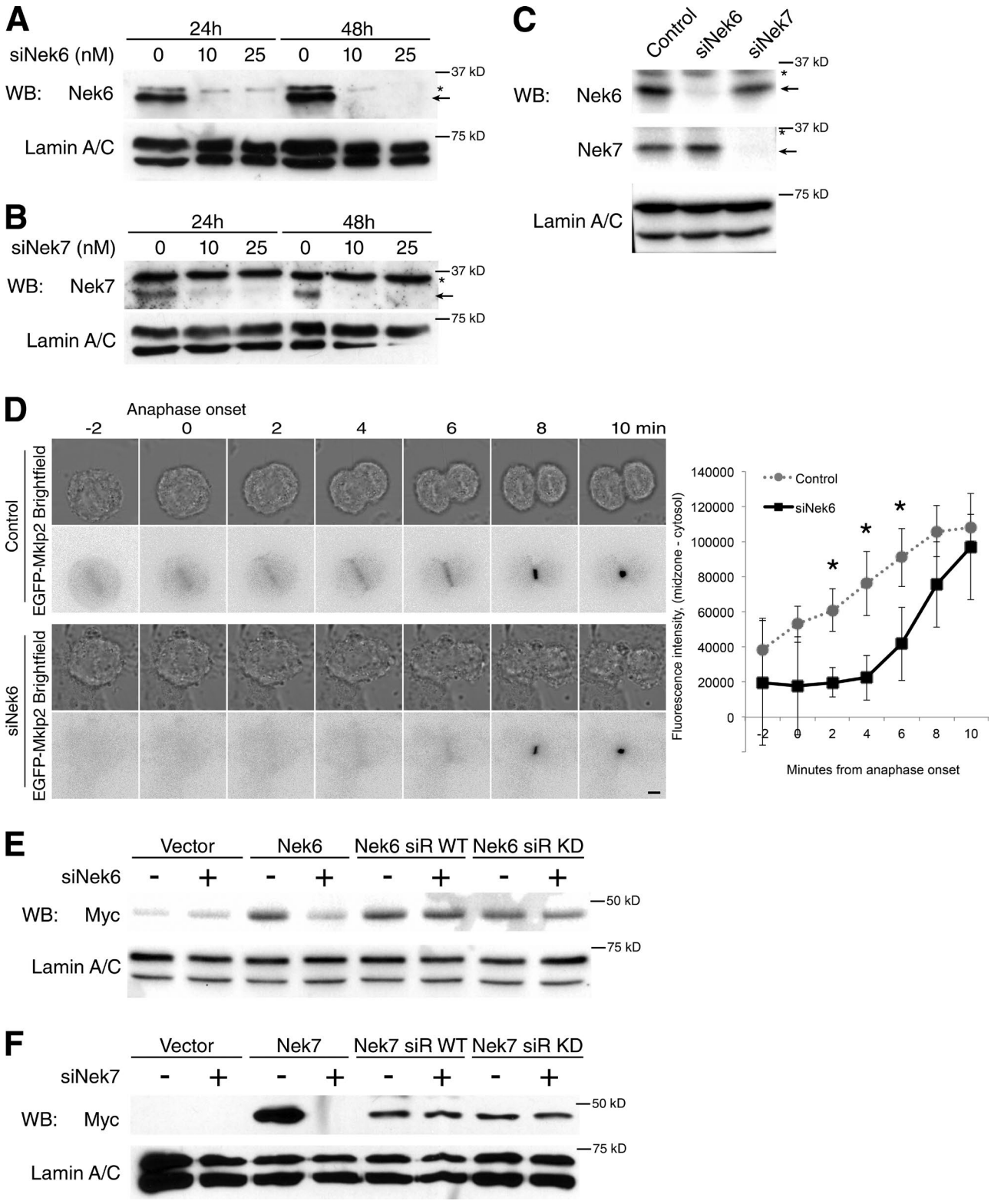

Figure S2. **Validation of Nek6 and Nek7 siRNAs and 6×Myc-tagged cell lines.** (A and B) Rabbit polyclonal antibodies to Nek6 (A) and Nek7 (B) identify bands from HeLa cell lysates that disappear upon treatment with siRNA (siR) to the target. Asterisks indicate nonspecific bands; arrows indicate Nek6 (A) or Nek7 (B). (C) The Nek7 antibody recognizes a band in lysate treated with Nek6 siRNA and vice versa, indicating that both siRNAs and antibodies are specific and selective. (D) Live-cell imaging of a stable EGFP-Mklp2 HeLa cell line indicates that under conditions of Nek6 depletion, EGFP-Mklp2 localizes to the cell midzone 4–6 min later than in control cells. $n \geq 5$ cells per condition. *, $P < 0.05$ by Student's t test; points and bar graphs show means ± SD. Bar, 10 µm. (E and F) Creation and validation of siRNA-resistant, WT, and kinase-dead 6×Myc-Nek6 (E) and -Nek7 (F) cell lines. WB, Western blot.

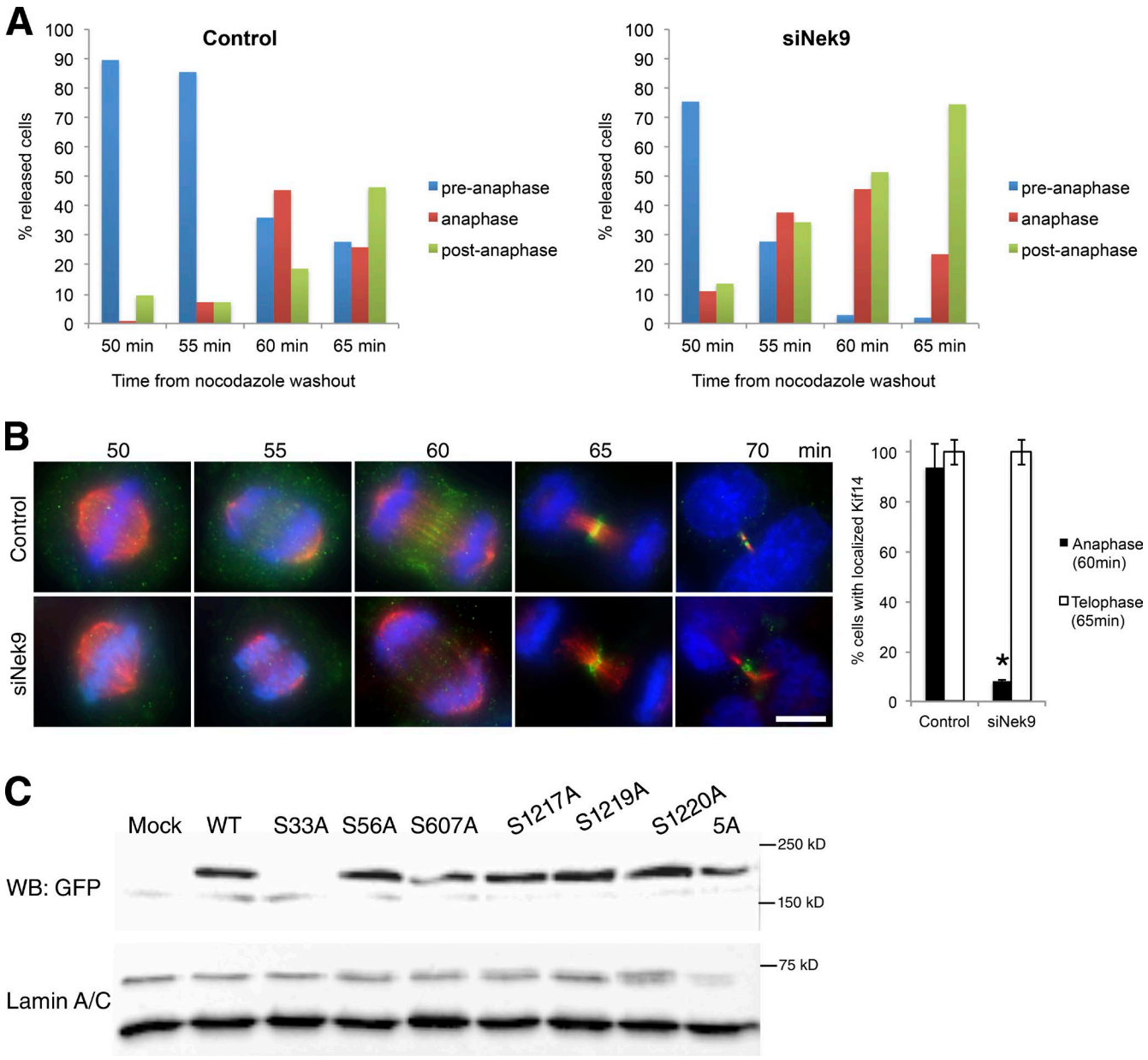

Figure S3. **Kif14 fails to localize to the anaphase midzone when Nek9 is depleted in synchronized cells.** (A and B) HeLa cells with or without siNek9 were arrested in prometaphase with 25 ng/ml nocodazole and then released. At time points 50–70 min after release, cells were fixed and stained with antibodies to tubulin and Kif14 to monitor the localization of Kif14 through the cell cycle in a synchronized population of cells. (A) The majority of released cells were in anaphase at the 60-min time point and in telophase at the 65-min time point. (B) Kif14 localization at time points after nocodazole release. The anaphase cells 60 min after release showed central spindle staining in the control group, whereas cells treated with Nek9 siRNA did not display central spindle–localized Kif14. In telophase cells at the 65-min time point, Kif14 localized to the midbody in both control and Nek9 siRNA–treated cells. HeLa cells stained with endogenous antibodies. $n > 100$ anaphase or telophase cells per condition in three independent experiments. *, $P < 0.05$ by Student's *t* test, bar graphs show means ± SD. Bar, 20 µm. (C) Expression of EGFP-Kif14 with putative Nek7 phosphorylation sites mutated to Ala and transient transfection in HeLa cells. The S33A protein was not expressed. The 5A protein includes the S56A, S607A, S1217A, S1219A, and S1220A mutations. WB, Western blot.

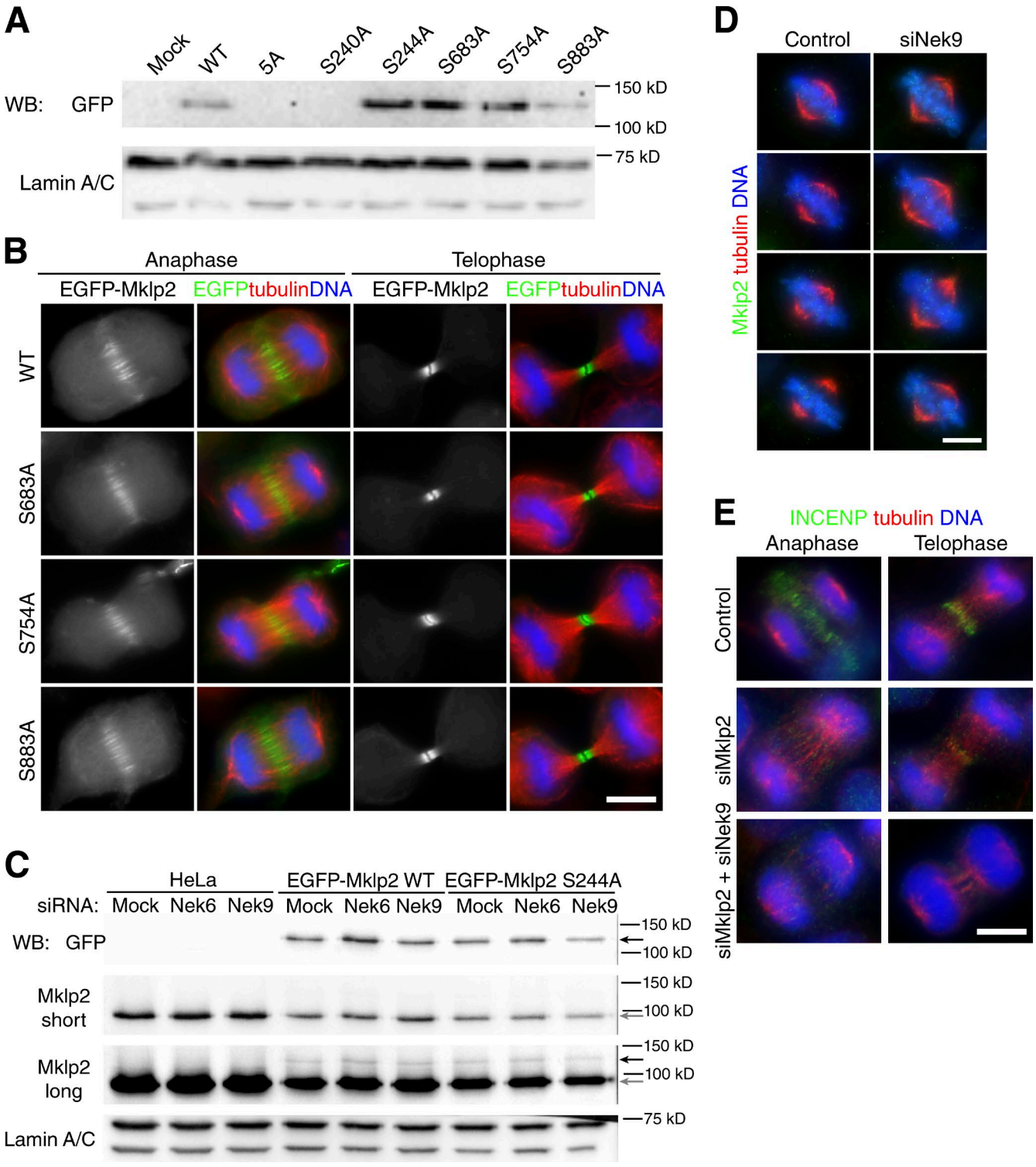

Figure S4. **Mklp2 mutants that abolish potential Nek6 phosphorylation sites.** (A) Expression of EGFP-Mklp2 with putative Nek6 phosphorylation sites mutated to Ala by transient transfection in HeLa cells. The S240A and 5A proteins were not expressed. The 5A mutant contained S240A, S244A, S683A, S754A, and S883A. (B) Localization of S683A, S754A, and S883A did not differ from WT EGFP-Mklp2 when transiently transfected in HeLa cells. (C) Treatment with Nek6 or Nek9 siRNA does not affect the levels of endogenous Mklp2 (gray arrows) or exogenous EGFP-Mklp2-WT or -S244A (black arrows). Furthermore, the level of EGFP-Mklp2-WT or -S244A overexpression in our stable cell lines was minimal. (D) In prometaphase and metaphase cells, Mklp2 displays no discrete localization, appearing in punctae throughout the spindle, chromatin, and cytosol. (E) Nek depletion does not inhibit Mklp2-dependent CPC localization.

Table S1. **siRNA sequences used in this study**

Target	Sequence (5′–3′)	Source
Nek9	UGAUUGAGCUGGAAUAUUG	Dharmacon siGENOME SMARTpool
Nek9	AGAAGGAACUGUACACUUG	Dharmacon siGENOME SMARTpool
Nek9	CCAUAAAGCUGGAAUCCUU	Dharmacon siGENOME SMARTpool
Nek9	UCACACAGCUGCUAUUGAU	Dharmacon siGENOME SMARTpool
Nek6	CAACUGAACCACCCAAAUA	Dharmacon siGENOME SMARTpool
Nek6	GCACUACUCCGAGAAGUUA	Dharmacon siGENOME SMARTpool
Nek6	UCUCGCAGAUGAUCAAGUA	Dharmacon siGENOME SMARTpool
Nek6	GAAGAUAGGCCGAGGACAG	Dharmacon siGENOME SMARTpool
Nek7	GAUACAACUUCAAAUCUGA	Dharmacon siGENOME SMARTpool
Nek7	GAUAUGGGCUAUAAUACAU	Dharmacon siGENOME SMARTpool
Nek7	GAAAAUUGGUCGCGGACAA	Dharmacon siGENOME SMARTpool
Nek7	AGACGUCACCUAUGUUUAU	Dharmacon siGENOME SMARTpool
Mklp2	AACCACCUAUGUAAUCUCAUG	Sequence from Gruneberg et al., 2006, synthesized as IDT[a] RNAi Duplex
Kif14	UUCCCGAUCUCAUUCAGUUUU	Sequence from Gruneberg et al., 2006, synthesized as IDT RNAi Duplex

[a]IDT, Integrated DNA Technologies

Table S2 is a separate Excel file containing the complete list of Nek9 interactors and quantitative data acquired by LC-MS/MS.

Reference

Gruneberg, U., R. Neef, X. Li, E.H. Chan, R.B. Chalamalasetty, E.A. Nigg, and F.A. Barr. 2006. KIF14 and citron kinase act together to promote efficient cytokinesis. *J. Cell Biol.* 172:363–372. http://dx.doi.org/10.1083/jcb.200511061