Lampert et al., http://www.jcb.org/cgi/content/full/jcb.201210091/DC1

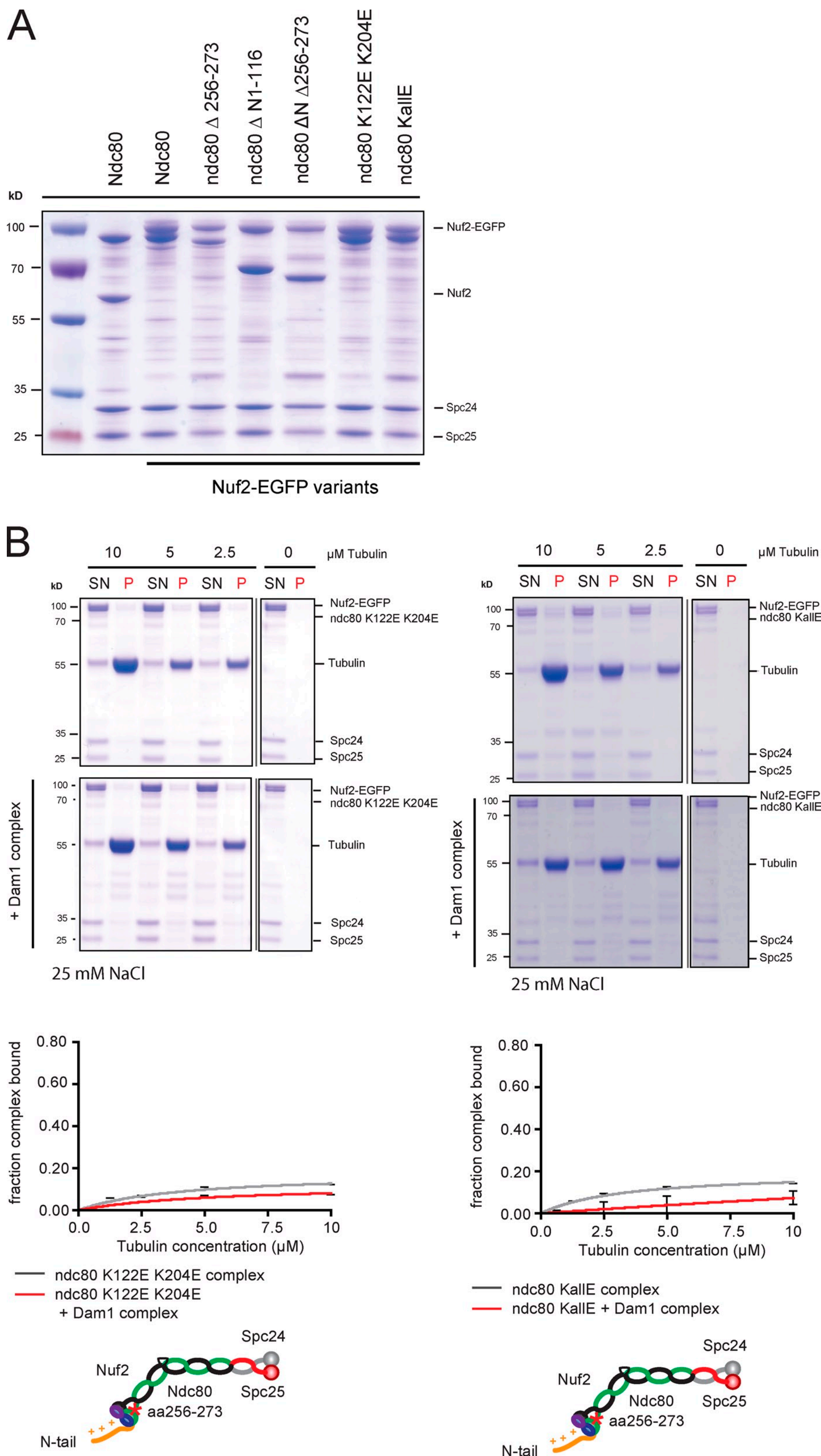

Figure S1. **Selective inhibition of the Ndc80 CH-domain eliminates the microtubule-binding ability of the complex.** (A) Coomassie-stained gel of recombinant wild-type and mutant Ndc80 complexes. (B) Coomassie-stained gels of microtubule co-pelleting assays and corresponding binding curves for the *ndc80 K122E K204E* mutant, with or without inclusion of Dam1. The Ndc80 CH-domain mutant is unable to bind microtubules in the presence or absence of the Dam1 complex, even under low salt conditions (n = 3; error bars represent standard error). Identical results were obtained with the Ndc80 CH-domain mutant where all six conserved lysine residues were replaced with glutamic acid.

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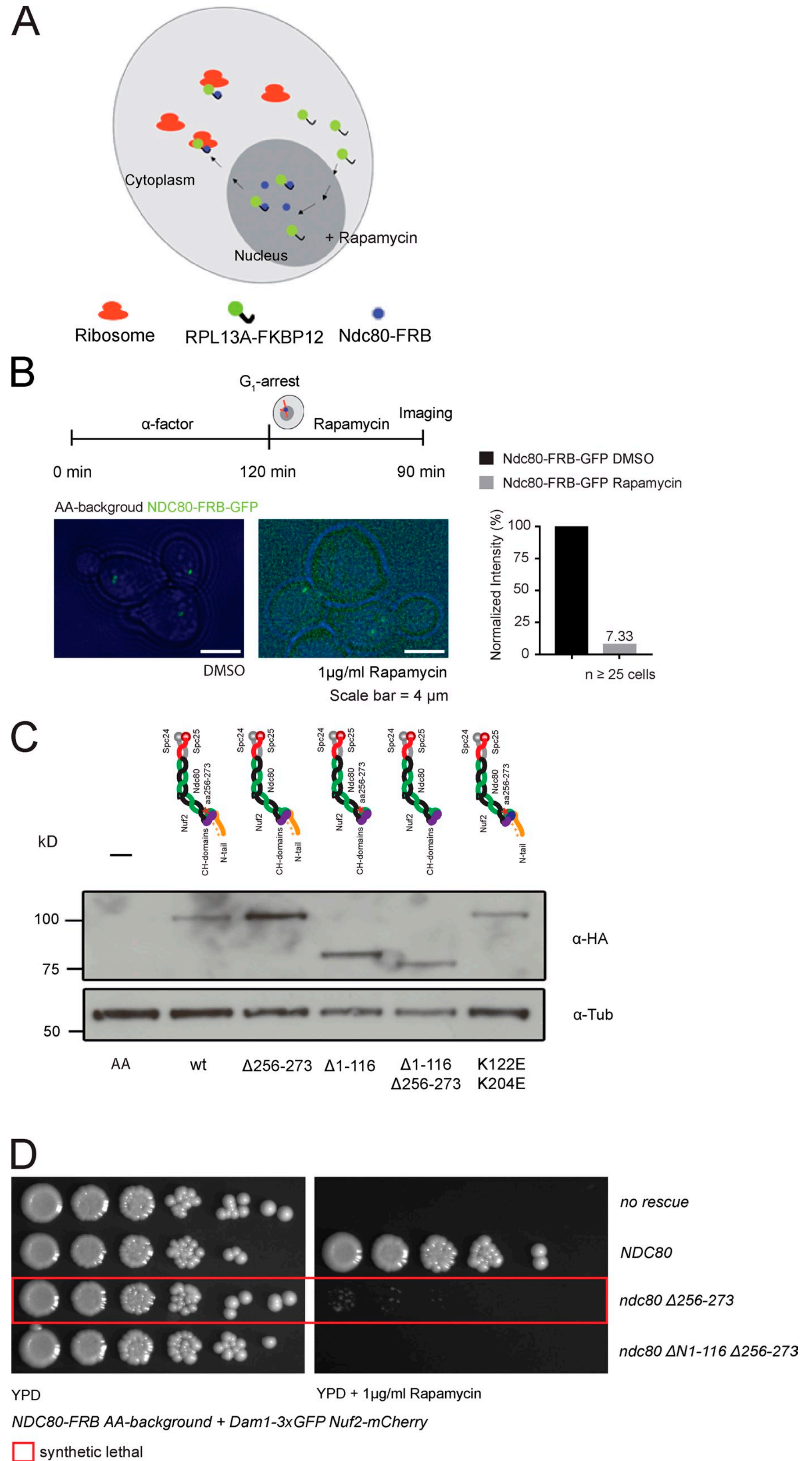
Figure S2. **Characterization of Ndc80 anchor-away phenotypes.** (A) Schematic illustration of the anchor-away approach based on Haruki et al. (2008). (B) Schematic illustration of the experimental time-course for Ndc80 anchor-away (AA) strains (top). Prolonged rapamycin exposure strongly affects Ndc80-FRB-GFP localization, whereas DMSO-treated cells show the characteristic kinetochore localization pattern ($n \geq 25$ from two independent experiments, average signal intensity [a.u.] was normalized to the average intensity of DMSO-treated control cells). Bars, 4 µm. Corresponding quantification of Ndc80-FRB-GFP kinetochore signal intensity after background subtraction (left). (C) Western blot analysis on whole-cell extracts probing the expression of mutant *ndc80-3xHA* proteins in the Ndc80-AA background. (C) Serial dilution spot assay of Dam1-3xGFP imaging strains in the Ndc80-AA background. Integration of the *ndc80 Δ256-273* allele causes synthetic lethality, presumably through negative interference by the C-terminal 3xGFP tag fused to Dam1.



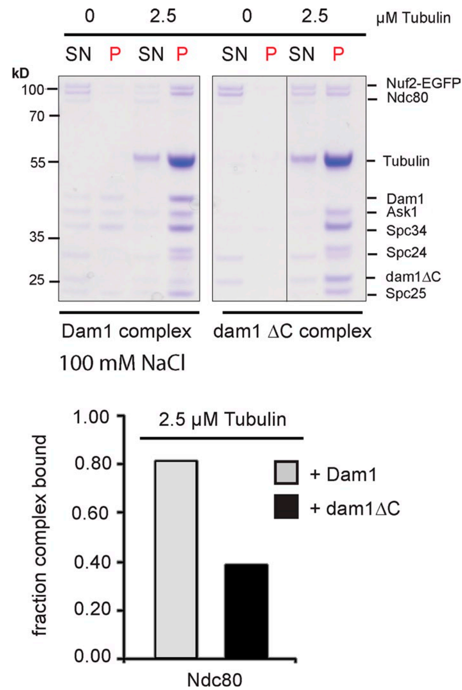
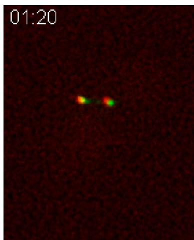
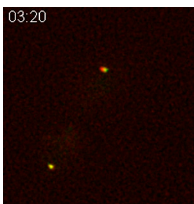


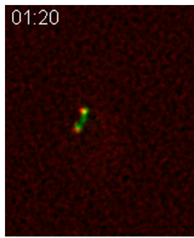
Figure S3. **The Dam1 ΔC complex shows a diminished Ndc80-recruitment activity.** (A) Representative Coomassie-stained gels (left) and corresponding quantification of a single experiment out of three repeats (right) of microtubule cosedimentation assays comparing wild-type Dam1 to the dam1 ΔC (Q205Stop) in Ndc80 complex recruitment. Although comparable amounts of the wild-type and mutant complex are bound to microtubules, lack of the C terminus of the Dam1 subunit results in lower Ndc80 enrichment in the pellet fraction. To balance the weakened intrinsic microtubule-binding activity of the Dam1 ΔC complex (Westermann et al., 2005), it was used in slightly higher concentration (0.75 μM vs. 0.5 μM) compared with wild type.



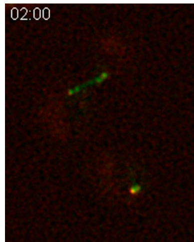
Video 1. **Time-lapse movie of a yeast cell expressing Dam1-3xGFP and Nuf2-mCherry.** The movie demonstrates typical Dam1 (green) and Nuf2 (red) localization patterns in a metaphase cell expressing Ndc80-FRB and wild-type Ndc80 in the presence of rapamycin. Frames were taken every 20 s for 5 min using a live-cell Deltavision Core Deconvolution system. This video corresponds to the experiment shown in the top left panel of Fig. 3 D.



Video 2. **Time-lapse recording of an anaphase control cell expressing Dam1-3xGFP, Nuf2-mCherry, and wild-type Ndc80 in the Ndc80-FRB background.** The fluorescent signals of Nuf2 (red) and Dam1 (green) colocalize and have been distributed equally between the mother and daughter cell. Frames were taken every 20 s for 5 min using a live-cell Deltavision Core Deconvolution system. This video corresponds to the experiment shown in the top right panel of Fig. 3 D.



Video 3. **Time-lapse movie of a metaphase cell expressing Dam1-3xGFP and Nuf2-mCherry where Ndc80-FRB is largely sequestered into the cytoplasm.** In the absence of an Ndc80 rescue allele the signal intensity of Nuf2 (red) is markedly reduced and Dam1 (green) shows an elevated association with the mitotic spindle. Time-lapse interval was 20 s and images were recorded using a live-cell Deltavision Core Deconvolution system. This video corresponds to the experiment shown in the bottom left panel of Fig. 3 D.



Video 4. **Time-lapse movie of a large-budded yeast cell expressing Dam1-3xGFP and Nuf2-mCherry where the bulk of Ndc80-FRB is mislocalized into the cytoplasm.** The cell arrests with a short spindle, reduced Nuf2 signal (red) at kinetochores, and increased levels of Dam1 (green) decorating microtubules. Frames were taken every 20 s for 5 min using a live-cell DeltaVision Core Deconvolution system. This video corresponds to the experiment shown in the bottom right panel of Fig. 3 D.

Table S1 describes yeast strains used in this study and is provided as an Excel spreadsheet.

References

Haruki, H., J. Nishikawa, and U.K. Laemmli. 2008. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. *Mol. Cell*. 31:925–932. http://dx.doi.org/10.1016/j.molcel.2008.07.020

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