Supplemental Materials

Molecular Biology of the Cell

Holland et al.

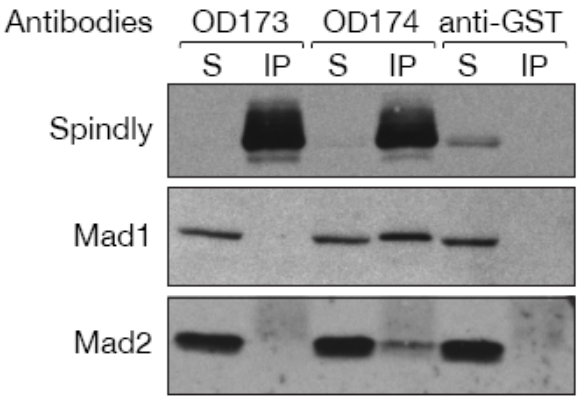

Figure S1:Spindly associates with the Mad1/Mad2 complex.

Antibody OD174 (raised against amino acids 450 - 605 of human Spindly) but not antibody OD173 (raised against amino acids 2 - 254) co-immunoprecipitates the Mad1/Mad2 complex from mitotic HeLa cell extract. Immunoblots of supernatants (S) after the immunoprecipitation reaction and immunoprecipitated fractions (IP) are shown. Anti-GST antibodies were used as a negative control. The immunoprecipitated fractions of OD173 and OD174 correspond to the fractions analyzed by mass spectrometry (Fig. 1A).

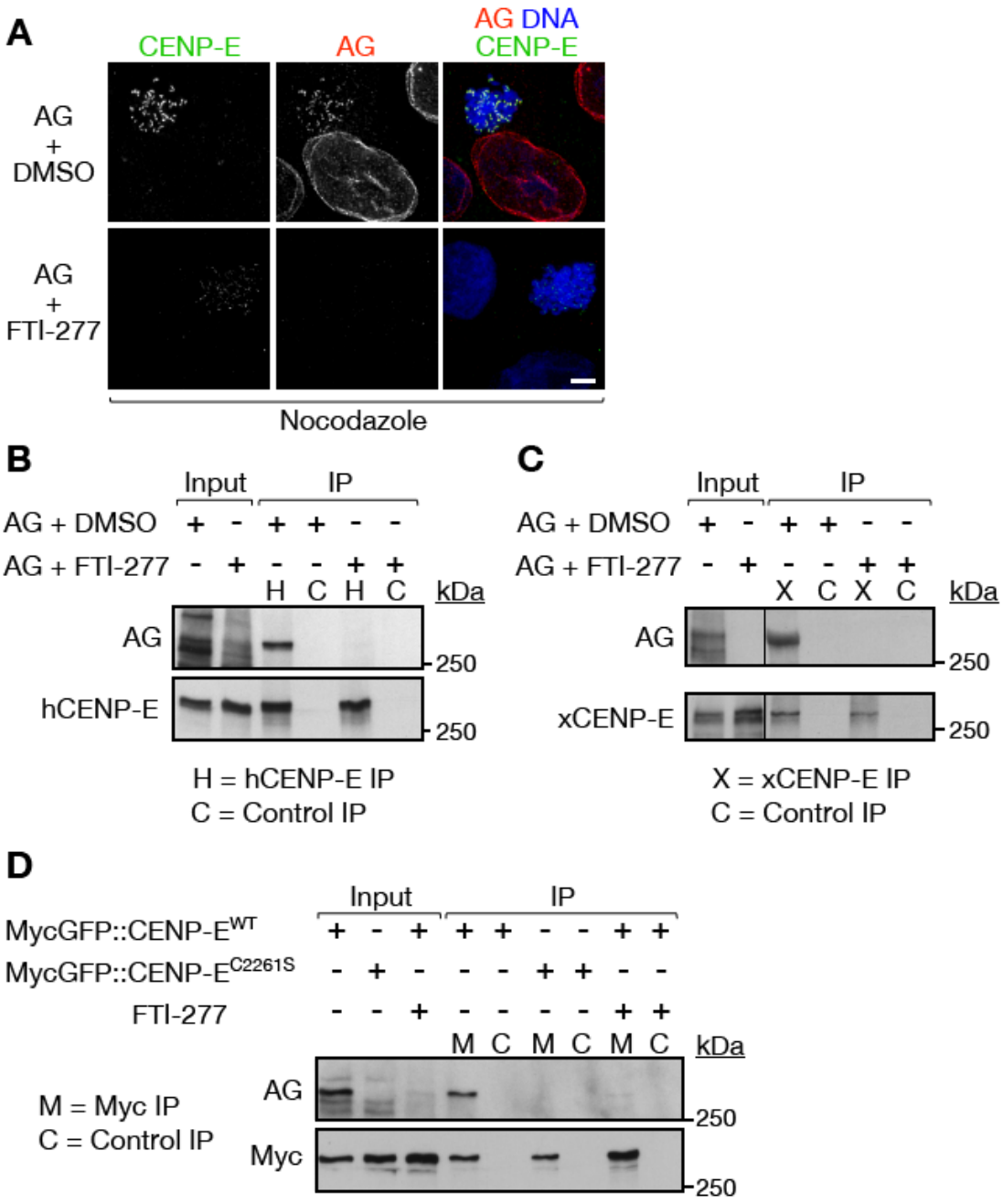

Figure S2: Human and Xenopus CENP-E are farnesylated.

(A) DLD1cells with kinetochore localization of the anti-AG signal detected by immunofluorescence. Cells were grown in the presence of AGOH with 10 μM

FTase inhibitor FTI-277 or DMSO for 48 hours before co-staining with anti-AG and anti-CENP-E antibodies. Scale bar, 5 μm.

(B) Immunoblots showing transgenic CENP-E and the corresponding AG signals in human cell lysate input fractions and fractions obtained after immunoprecipitation (IP) with anti-hCENP-E (H) or beads only controls (C).

(C) Immunoblots showing endogenous CENP-E and the corresponding AG signals in Xenopus cell lysate input fractions and fractions obtained after immunoprecipitation (IP) with anti-xCENP-E (X) or beads only controls (C).

(D) Immunoblots showing transgenic CENP-E and the corresponding AG signals in human cell lysate input fractions and fractions obtained after immunoprecipitation (IP) with anti-Myc antibody (M) or beads only controls (C).

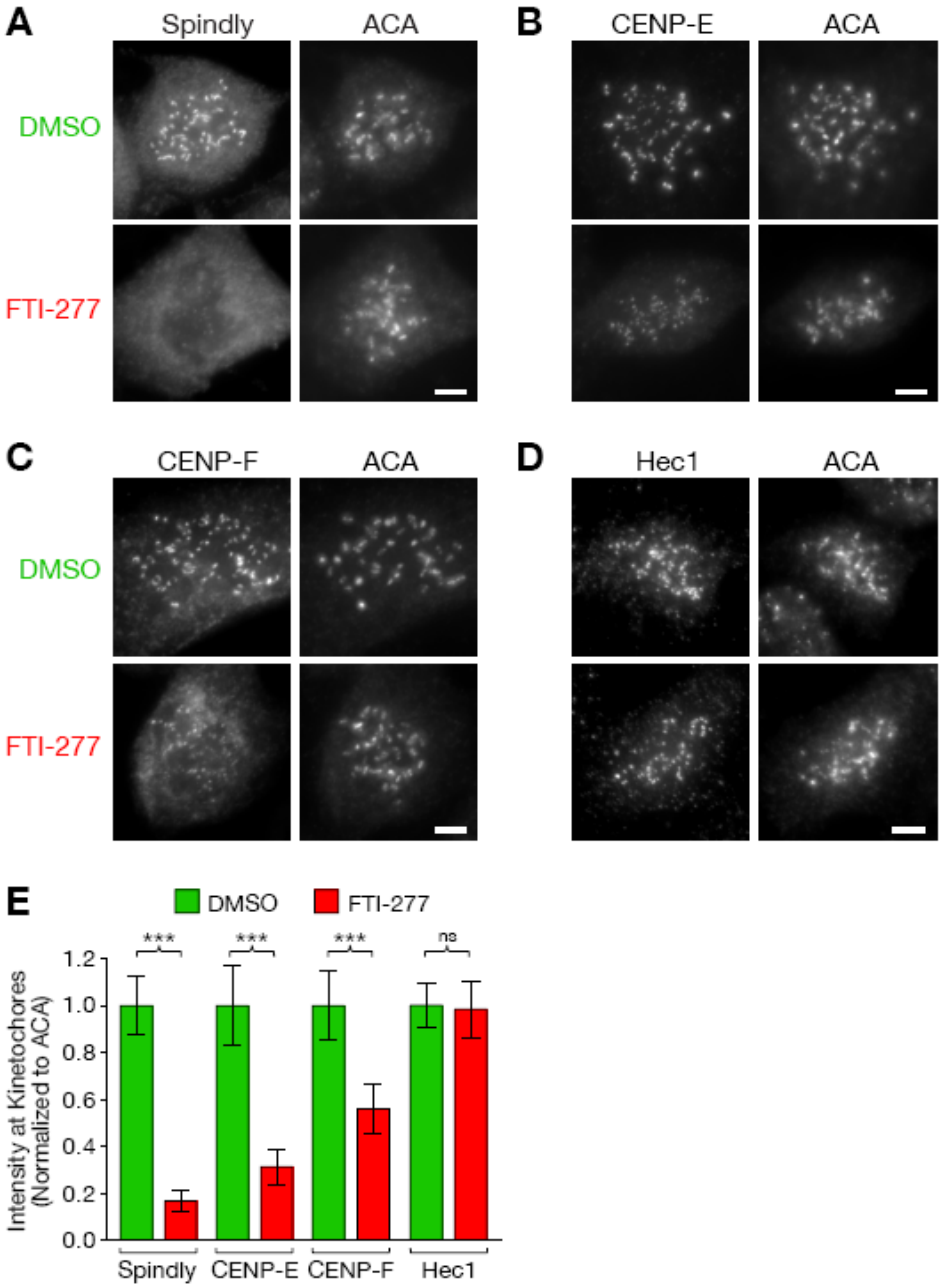

Figure S3:Kinetochore levels of Spindly, CENP-E, CENP-F, and Hec1 after FTI-277 treatment in DLD-1 cells

(A) - (D) DLD-1cells immunostained for Spindly *(A)*, CENP-E *(B)*, and CENP-F *(C)*, and Hec1 *(D)*after treatment for 48 h with 10 μM farnesyltransferase inhibitor FTI-277 or DMSO. Cells were incubated in 1 μM nocodazole for 4 h to

accumulate proteins at kinetochores and co-stained with anti-centromere antibodies (ACA). Scale bars, 5 μm.

(E) Quantification of kinetochore levels in DLD-1 cells for the conditions *(A)* - *(D)*. Normalized immunofluorescence intensity measurements are shown (a total of 50 kinetochores from 10 different cells) with error bars representing the S.E.M. with a 95 % confidence interval. The t-test was used to determine statistical significance (*** indicates $p < 0.0001$; ns = not significant).

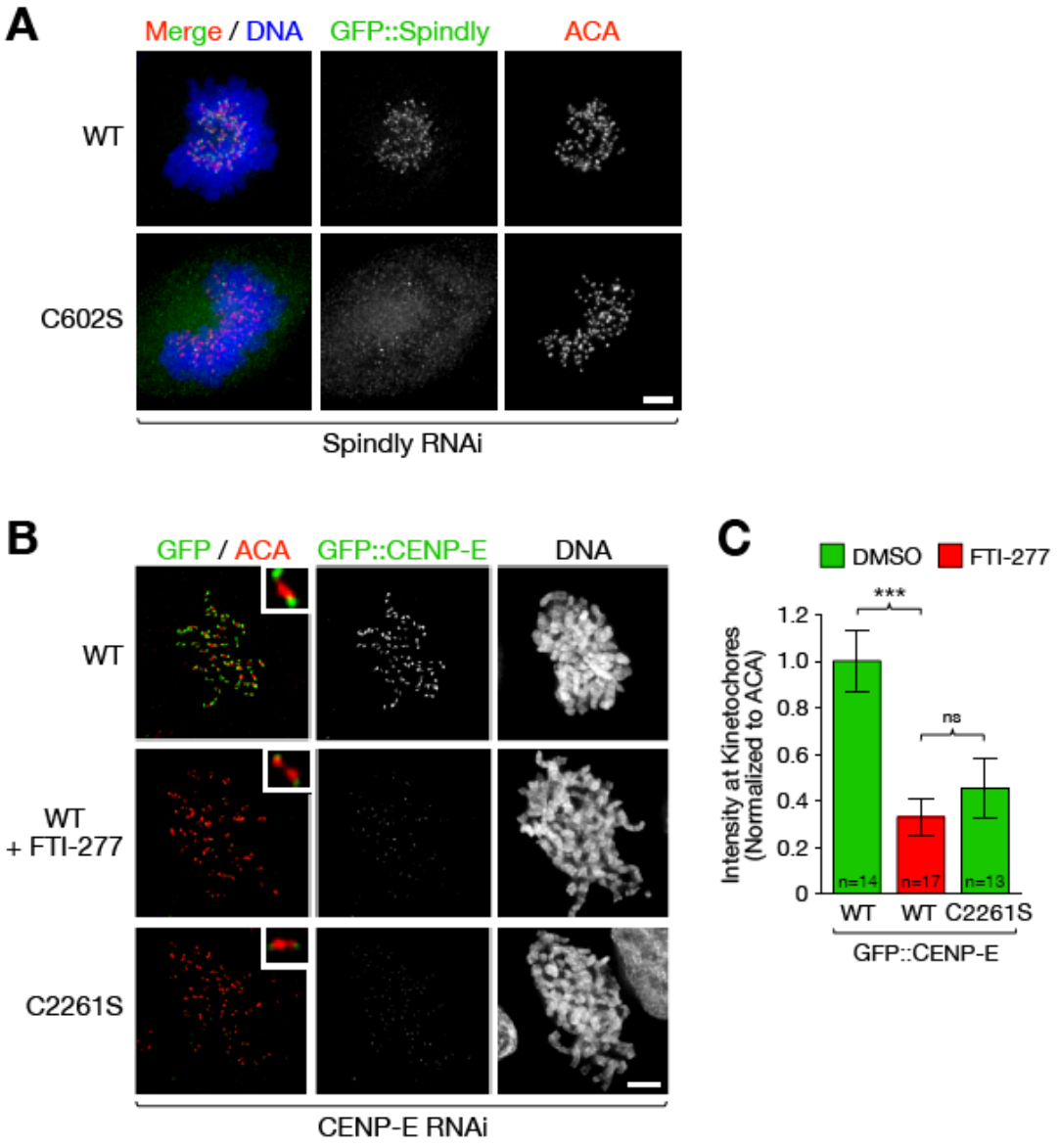

Figure S4: Non-Farnesylated Spindly and CENP-E are defective in kinetochore localization.

(A) HeLacells in early prometaphase (without nocodazole treatment) showing localization of wild-type (WT) and mutant(C602S) MycGFP::Spindly after RNAi-

mediated depletion of endogenous Spindly. Cells were immunostained with anti-GFP and anti-centromere antibodies (ACA).Scale bar, 5 μm.

(B) DLD-1cells depleted of endogenous CENP-E by RNAi showing localization of RNAi-resistant wild-type (WT) and mutant (C2261S) MycGFP::CENP-E. Cells were grown in 10 μMFTase inhibitor FTI-277 or DMSO and incubated in 1 μM nocodazole for 4 h to maximize the accumulation of the MycGFP::CENP-E at kinetochores. Scale bar, 5 μm.

(C) Quantification of WT and C2261S MycGFP::CENP-E levels at kinetochores of the conditions shown in *(B)*. Normalized immunofluorescence intensity measurements are shown. The number (n) of kinetochores measured is indicated. Error bars represent the S.E.M. with a 95 % confidence interval. The t-test was used to determine statistical significance (*** indicates $p < 0.0001$; ns = not significant).

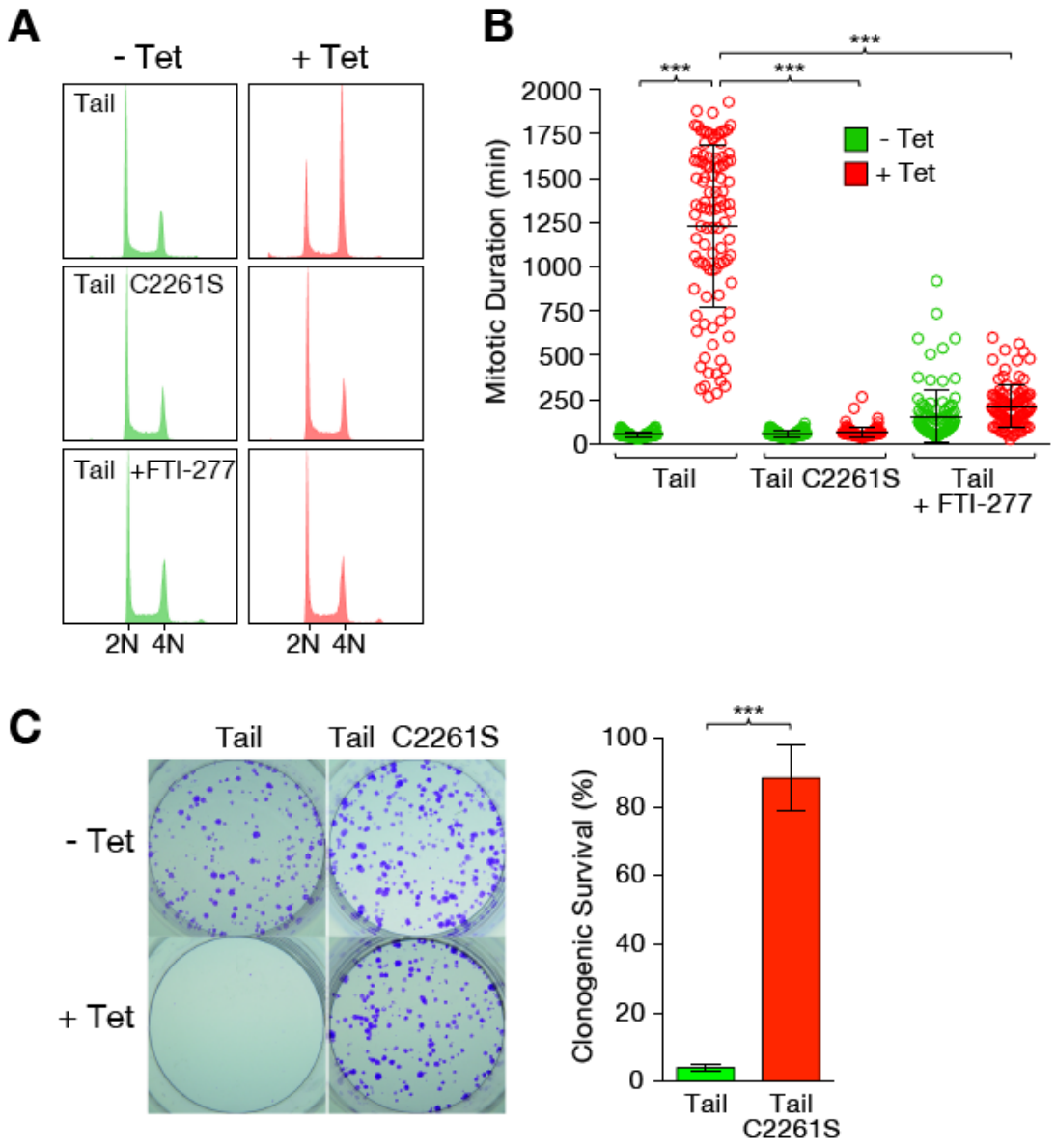

Figure S5: The toxicity of a dominant-negative CENP-E tail fragment is dependent on farnesylation.

(A) Flow cytometry analysis of DLD-1 cells expressing CENP-E tail transgenes. Cells were treated with (+Tet) or without (-Tet) tetracycline in the presence of DMSO or 10 μM FTI-277 for 24 hours.

(B) Quantification of time-lapse fluorescence microscopy experiments conducted with DLD-1 cells expressing histone H2b::mRFP with (+Tet) or without (-Tet) expression of CENP-E tail transgenes. Mitotic duration, defined as the interval between nuclear envelope breakdown and anaphase onset, is shown as scatter plots with average and standard deviation for the indicated experimental conditions.All conditions were filmed in parallel during the same imaging session and 100 cells were scored for each condition. The t-test was used to determine statistical significance (*** indicates $p < 0.0001$).

(C) Crystal violet stained colonies for indicated cell lines grown with (+Tet) or without (-Tet) expression ofCENP-E tail transgenes. Graph shows the mean of three independent experiments carried out in triplicate with error bars representingthe standard deviation.