Kaur et al., http://www.jcb.org/cgi/content/full/jcb.201412085/DC1

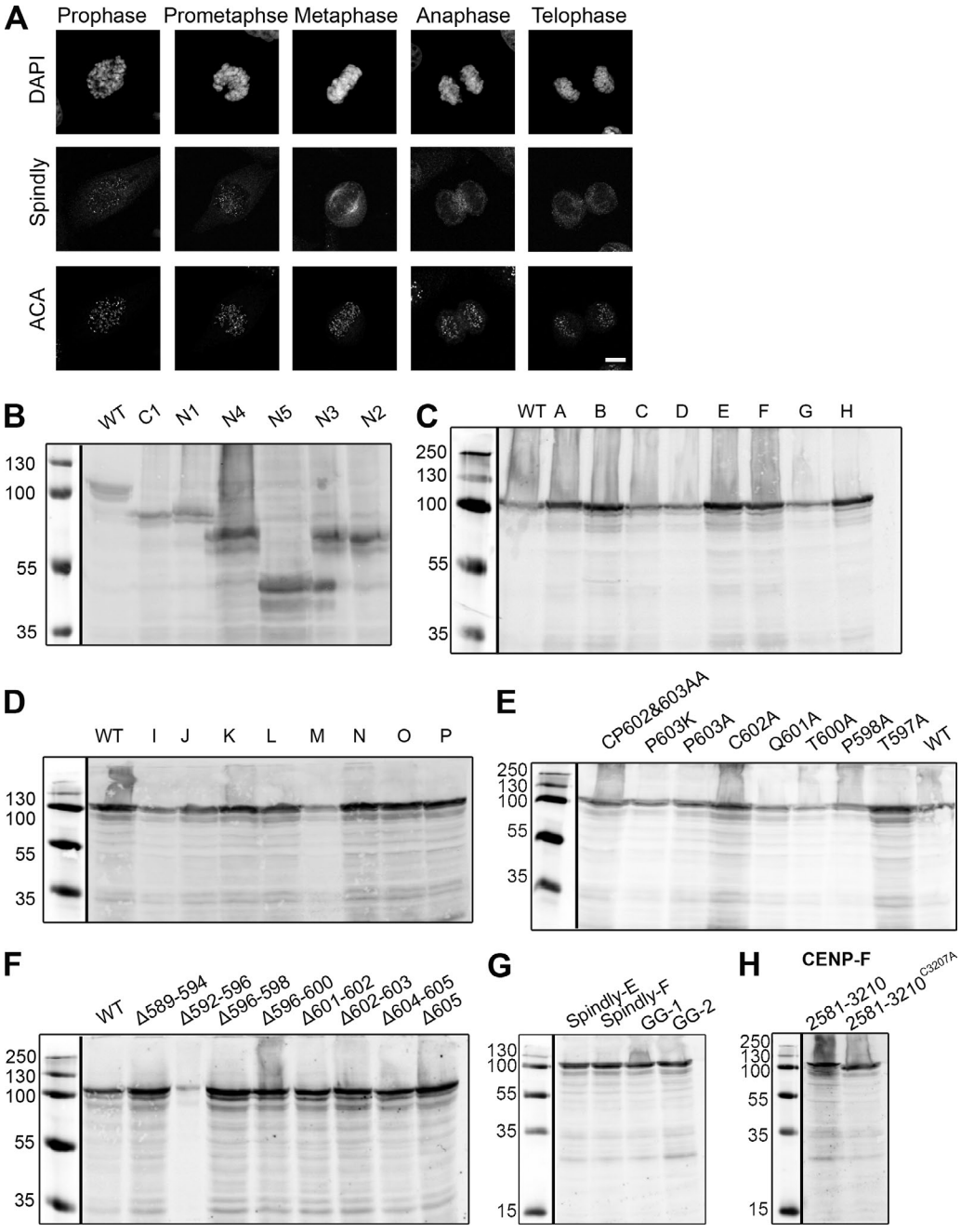


Figure S1. **Endogenous hSpindly KT localization during mitosis in HeLa cells and expression of hSpindly mutant library using western blot analysis.** (A) HeLa cells were stained with anti-hSpindly antibody, CREST antisera to immunostain centromeres (ACA), and DAPI to stain DNA. hSpindly localizes to the kinetochores at prophase and prometaphase and at spindle poles in metaphase. Bar, 5 µm. (B–H) Immunoblots showing the expression of GFP-hSpindly and GFP-hCENP-F fusion proteins transfected into HEK293 cells. GFP fusion proteins were labeled with IR800-conjugated rabbit anti-GFP antibody. Molecular mass markers detected in the same channel are shown on each blot (masses indicated in kD). All hSpindly and CENP-F mutant constructs expressed at the expected size.

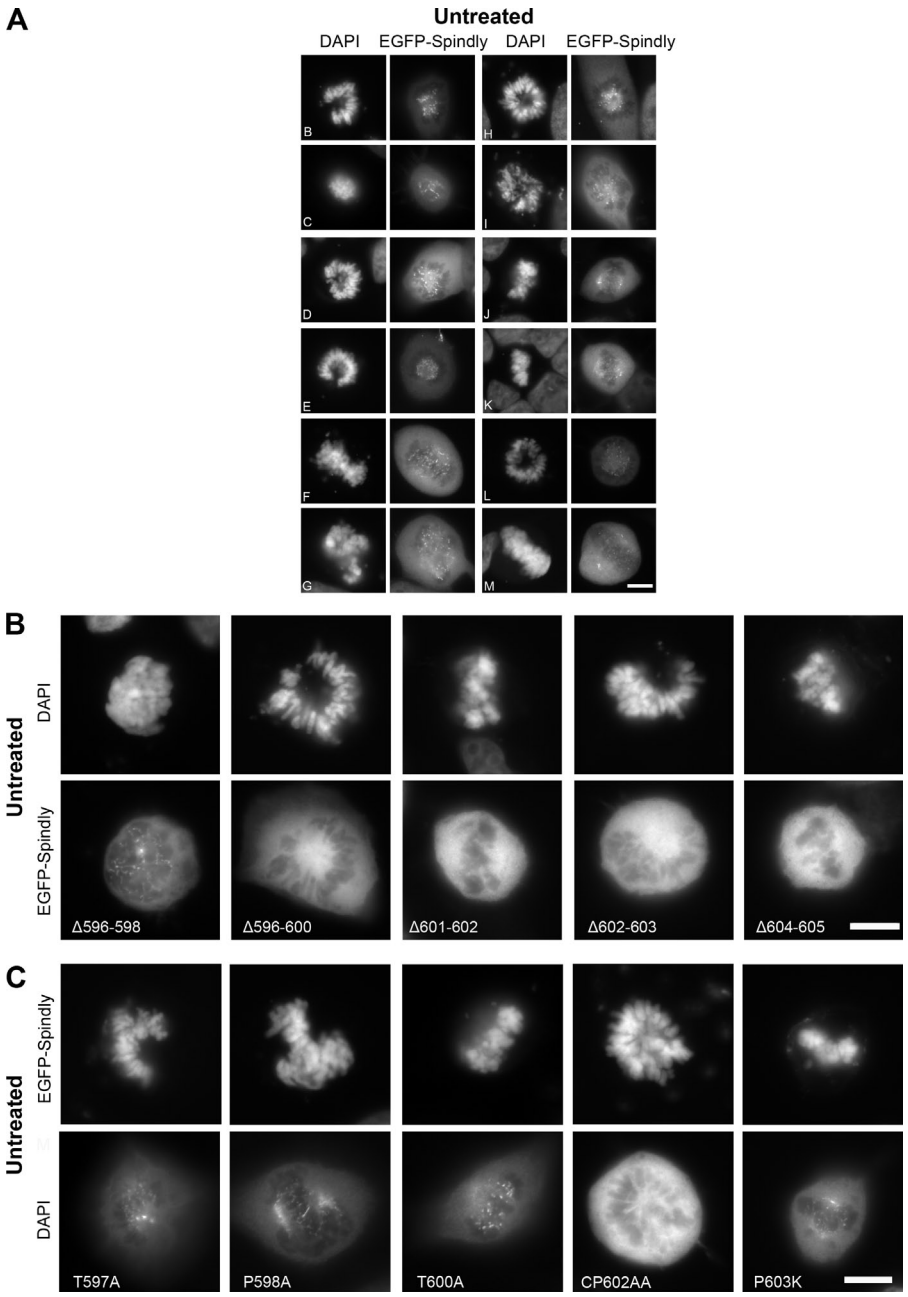


Figure S2. **HeLa cells were transfected with GFP-hSpindly mutants, fixed, stained with DAPI to visualize the DNA, and analyzed by fluorescence microscopy for kinetochore-localizing ability.** Localization results were identical with vinblastine treatment (not depicted). Positive localization is seen as double-dot staining. (A) GFP-hSpindly insertion mutants localized to kinetochores. (B) GFP-hSpindly deletion mutants did not localize to kinetochores except the Δ596–598 mutant. (C) GFP-hSpindly substitution mutants localized to kinetochores except the CP602AA mutant. Bars, 10 μm.

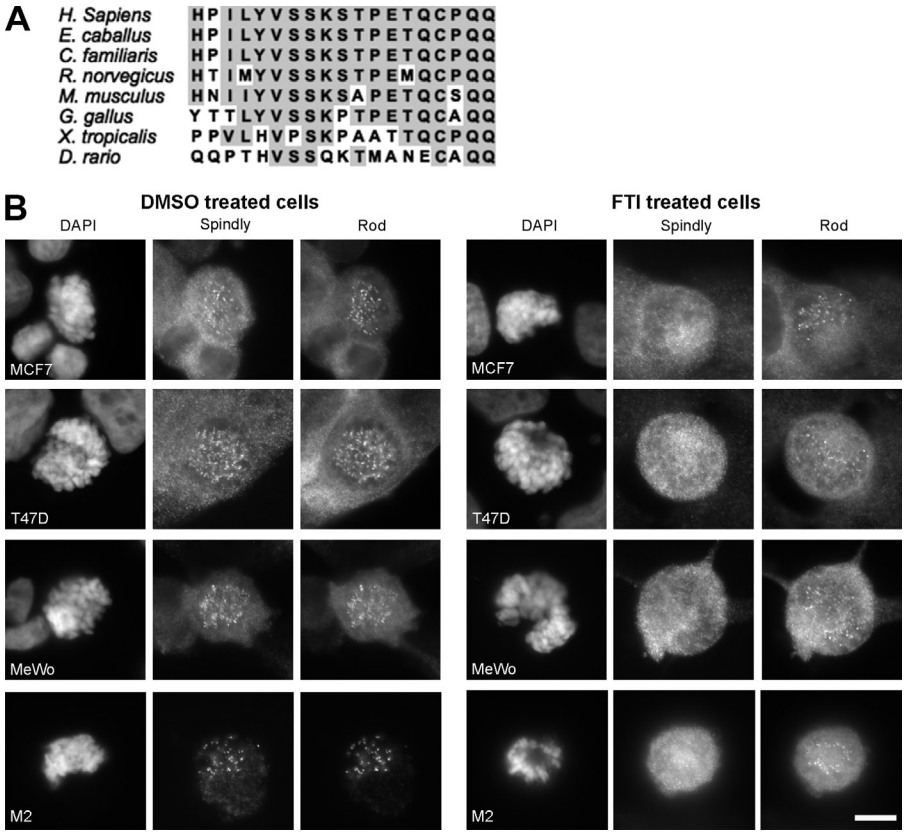


Figure S3. **Inhibition of farnesyl transferase abrogated Spindly kinetochore localization in breast cancer and melanoma cell lines.** (A) Spindly C-terminal residues are highly conserved in different species. The far C-terminal cysteine and the last two glutamine residues are conserved in all the organisms. (B) Breast cancer (MCF7 and T47D) and melanoma (MeWo and M2) cell lines were treated with FTI and DMSO for 24 h before fixation. The cells were immunostained with anti-hSpindly antibody, anti-hRod antibody, and DAPI to stain DNA. hSpindly kinetochore localization is abrogated with FTI treatment in all the cell lines without affecting Rod kinetochore localization. Bar, 10 μm.

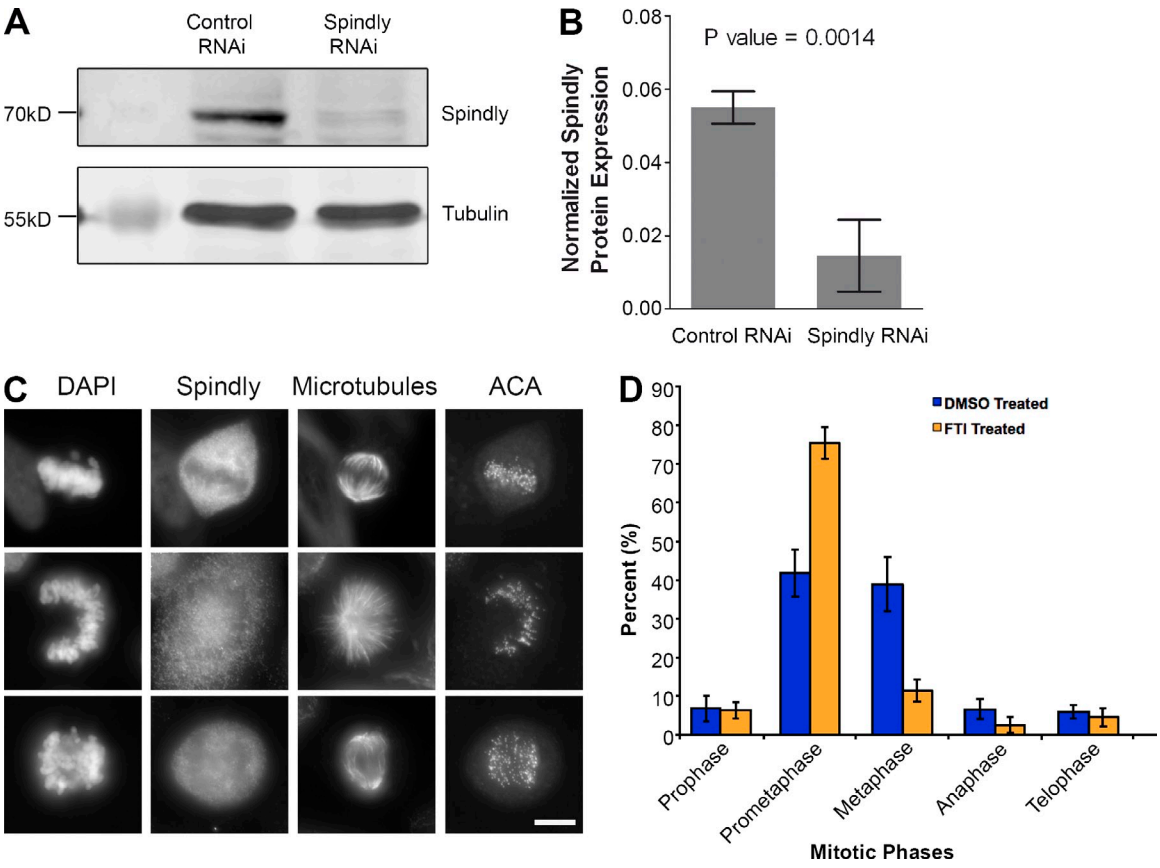
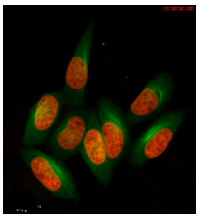
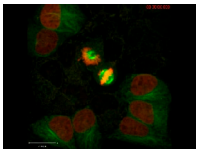


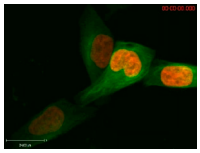
Figure S4. **Spindly is efficiently depleted by siRNA and FTI treatment results in prometaphase arrest.** (A) Immunoblots of HeLa cells transfected with either control (scrambled) or hSpindly RNAi for 24 h and harvested after 48 h of transfection. Tubulin is used as a loading control. (B) Quantitative analysis of hSpindly knockdown shows 73.5% knockdown of hSpindly protein. Bar graphs show the results of three independent experiments and error bars indicate the SD from the means. (C) Representative phenotype of FTI-treated HeLa cells showing prometaphase and metaphase phenotype. FTI treatment in HeLa cells led to prometaphase accumulation and very few metaphase cells (D). HeLa cells treated with FTI for 24 h were immunostained with anti-hSpindly, anti-tubulin, and ACA antibodies and stained with DAPI to visualize DNA. Bar, 10 µm. (D) Accumulation in prometaphase during mitosis was observed in FTI-treated cells as compared to DMSO-treated control. HeLa cells were treated with either 10 µM of FTI-L744832 or DMSO for 24 h, fixed, and immunostained for hSpindly and tubulin. DNA is visualized by DAPI staining. 100 cells were counted in separate experiments for DMSO- (n = 8) and FTI-treated cells (n = 7). Error bars are SD from the means.



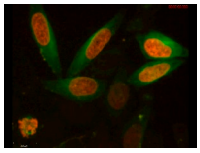
Video 1. **Mitotic timing of HeLa cells expressing GFP tubulin (green) and mCherry H2B (red) treated with solvent DMSO control for 24 h.** Time-lapse imaging was performed using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a 40× objective. Images were captured using 100-ms exposure time for GFP and Cy3 every 5 min for 8 h. Representative video shows 4-h duration of imaging. Bar, 24 µm.



Video 2. **Mitotic timing of HeLa cells expressing GFP tubulin (green) and mCherry H2B (red) treated with 10 µM of FTI L-744832 for 24 h.** Time-lapse was performed using spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a 40× objective. Images were captured using 100-ms exposure time for GFP and Cy3 every 5 min for 18 h. Representative video shows 18-h duration imaging. Bar, 24 µm.



Video 3. **Mitotic timing of HeLa cells expressing GFP tubulin (green) and mCherry H2B (red) transfected with 100 nM control (scrambled) RNAi.** Time-lapse imaging was performed using spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a 40× objective. Images were captured using 100-ms exposure time for GFP and Cy3 every 5 min for 8 h. Representative video shows 6-h duration imaging. Bar, 24 μm.



Video 4. **Mitotic timing of HeLa cells expressing GFP tubulin (green) and mCherry H2B (red) transfected with 100 nM hSpindly RNAi and imaged after 33 h of transfection.** Time-lapse imaging was performed using spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a 40× objective. Images were captured using 100-ms exposure time for GFP and Cy3 every 5 min for 16 h. Representative video shows 15-h duration imaging. Bar, 24 μm.