ONLINE SUPPLEMENTAL MATERIAL

EXPERIMENTAL PROCEDURES

Antibodies used in this study - The mouse anti-p115 antibody (7D1) was a gift from Dr. Gerry Waters (Merck, Rahway, NJ); the rabbit anti-GRASP65 was from Dr. Yanzhuang Wang (University of Michigan, Ann Arbor, MI); the mouse anti-mannosidase II (ManII; 53FC3) was provided by Dr. Brian Burke (University of Florida, Gainesville, FL). A rabbit anti-TGN38 antibody was a gift from Dr. Sharon Milgram (University of North Carolina, Chapel Hill, NC) and the mouse anti-giantin was provided by Dr. Hans Peter Hauri (University of Basel, Basel, Switzerland). In addition, the following commercially available antibodies were used: anti-GM130 (BD Transduction Laboratories); anti-Flag M2-peroxidase, anti-γ-tubulin (clone GTU-88) and anti-α-tubulin (Sigma); anti-β-actin and anti-pericentrin (PC) (Abcam, Inc.); chicken anti-GFP (Aves Lab, Inc); horseradish-peroxidase (HRP)-conjugated anti-GST(B-14) (Santa Cruz Biotechnology); HRP-conjugated anti-mouse or anti-rabbit (GE Healthcare); Alexa Fluor conjugated anti-mouse and anti-rabbit (Molecular Probes, Inc). The polyclonal rabbit anti-p115 antibody (AE800) was raised against amino acids 645-962 (Covance).

FIGURE LEGENDS

FIGURE S1. **p115 exists in a complex with γ-tubulin.** (A) Indirect immunofluorescence of U2OS cells with antibodies against pericentrin (PC; green) and p115 (red). Arrows, spindle pole-associated p115. Single section were acquired on a Nikon Eclipse TE300 inverted microscope. Scale bar, 10μm. (B) Western blot analysis of p115 – γ-tubulin binding following incubation of equimolar amounts of GST-fused p115 truncation constructs with 500μg of mitotic cytosol from NRK cells. Binding was quantified by densitometric measurement of γ-tubulin immunoreactive band intensity (ImageJ; http://rsb.info.nih.gov/ij/) and was normalized to the GST input (bottom panel and graph). Low molecular weight bands correspond to degraded GST peptides. Asterisks indicate the correct molecular weights of the purified proteins. (C) Western blot of the p115 – γ-tubulin binding following incubation of increasing amounts of GST-fused p115 truncation fragments (1X = 20pmoles) with 500μg of NRK interphase post nuclear lysate. (D) Fractionation of 293T post nuclear supernatant on a continuous 5-40% sucrose gradient shows co-sedimentation of p115 and γ-tubulin in fractions 3 and 12. Fractions 3 and 12 represent the small γTuSC and the large γTuRC complexes, respectively.

FIGURE S2. **The armadillo fold provides the minimal requirement for centrosomal localization.** (A) Cos7 cells were transfected with the indicated GFP tagged p115 constructs for 24 hours and processed for indirect immunofluorescence with a pericentrin antibody (red). The overexpressed armadillo fold domain (1-321) localizes to the centrosome. Images are projected confocal Z-series. Blue, Hoechst 33342. Scale bar, 10 μm.

FIGURE S3. **Both the *trans*- and *cis*-Golgi are disrupted under conditions of p115 depletion while the centrosome is unaffected.** Indirect immunofluorescence of HeLa cells after a 4 day treatment with either mock or a p115 siRNA. (A) Electron micrographs of interphase HeLa cells either non-silencing siRNA or p115 siRNA treated for 4 days. Three representative images of the Golgi phenotype generated by p115 knockdown are shown (b-d). (B) A HeLa GalNAcT2-GFP stable line shows disruption of both GalNAc and GM130 following p115 siRNA treatment. Mock and p115 siRNA-treated HeLa cells were prepared for immunofluorescence using either (C) a monoclonal p115 antibody and a polyclonal pericentrin (PC) antibody or (D) a polyclonal p115 antibody and a monoclonal antibody against γ-tubulin. Images are projected confocal Z-series. Arrow points to the intact centrosome. Blue, Hoechst 33342. Scale bar, 10μm.

FIGURE S4. **Larger nuclei as well as multiple aberrant spindle morphologies are generated by p115 knockdown.** (A) HeLa cells were either treated with non-silencing or p115 siRNA and processed for

indirect immunofluorescence microscopy at the end of 4 days with an antibody against p115. The nuclei were stained with Hoechst 33342 and the surface area was measured using Image J (table). (B) Indirect immunofluorescence of representative mock or siRNA-treated, mitotically synchronized HeLa cells, with an antibody against α-tubulin. Blue, Hoechst 33342. Images are projected confocal Z-sections. (C) Electron micrographs of synchronized mitotic HeLa cells either non-silencing or p115 siRNA-treated (Experimental procedures). Black line represents the metaphase plate (MP).

FIGURE S5. **Model for p115's role in Golgi biogenesis and centrosome function.** In interphase, p115 (green) may bridge an interaction between γ-tubulin (red circles) and Golgi components (GM130 - pink and giantin - purple) via its N- and C- termini, respectively. The p115-associated γ-tubulin (red circles) may be either part of (a) the centrosome (orange) and provide Golgi anchoring to the centrosomal area of the cell, or (b) it may be recruited to Golgi membranes from the soluble pool and provide nucleation sites for non-centrosomal microtubules (gray punctate lines). In mitosis, the Golgi apparatus vesiculates (black) and the majority of Golgi-associated components distribute stochastically in the cytoplasm. p115 remains associated with γ-tubulin and localizes to spindle poles. During cytokinesis, the spindle pole-associated p115 pool recruits Golgi components to the pericentrosomal area of the cell, by interactions with specific components (i.e. GM130 and giantin), to nucleate the formation of the distal (D) Golgi complex (c). Another pool of p115 may function to establish the proximal (P) Golgi apparatus through interactions with Golgi components and/ or γ-tubulin. The Golgi-nucleated microtubule cytoskeleton (punctate gray lines) is essential for Golgi structure and may play a role in post mitotic Golgi reassembly. A functional Golgi apparatus and post-Golgi trafficking may be required for delivery of membrane and proteins (gray-filled black circle, arrow) to the cytokinetic furrow (d). Alternatively, p115 may be directly involved in SNARE targeting to the furrow (e).

TIME LAPSE MOVIE 1 – **p115-depleted cells display late mitotic spindle failure.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were treated with a specific p115 siRNA for 3 days. Up to 15 fields were imaged using a temperature controlled (37°C) DeltaVision RT deconvolution system (Applied Precision Inc.) on an Olympus IX70 microscope, equipped with the softWoRx Suite software (Applied Precision, Inc.). For each field, a Z-series with 3μm spacing was acquired on a 40X oil objective, at 5min intervals for a duration of 8 hours. Z-series were projected using softWoRx Suite. Video was created in ImageJ. Only the α-tubulin-YFP channel is shown. The corresponding H2B-RFP channel is shown in Time lapse movie 2. Display rate, 10 frames/ second. See Figure 7 for related video stills.

TIME LAPSE MOVIE 2 – **p115-depleted cells display chromosome missegregation.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were mock treated for 3 days, in parallel with the p115 siRNA treatment. Up to 15 fields were imaged. For each field, a Z-series with a 3μm spacing was acquired, at 5min intervals for a duration of 8 hours. Arrow, an unequal amount of chromatin breaks off during chromosome segregation, due to late spindle failure. Only the H2B-RFP channel is shown. The corresponding α-tubulin-YFP channel is shown in Time lapse movie 1. Display rate, 10 frames/ second. See Figure 7 for related video stills.

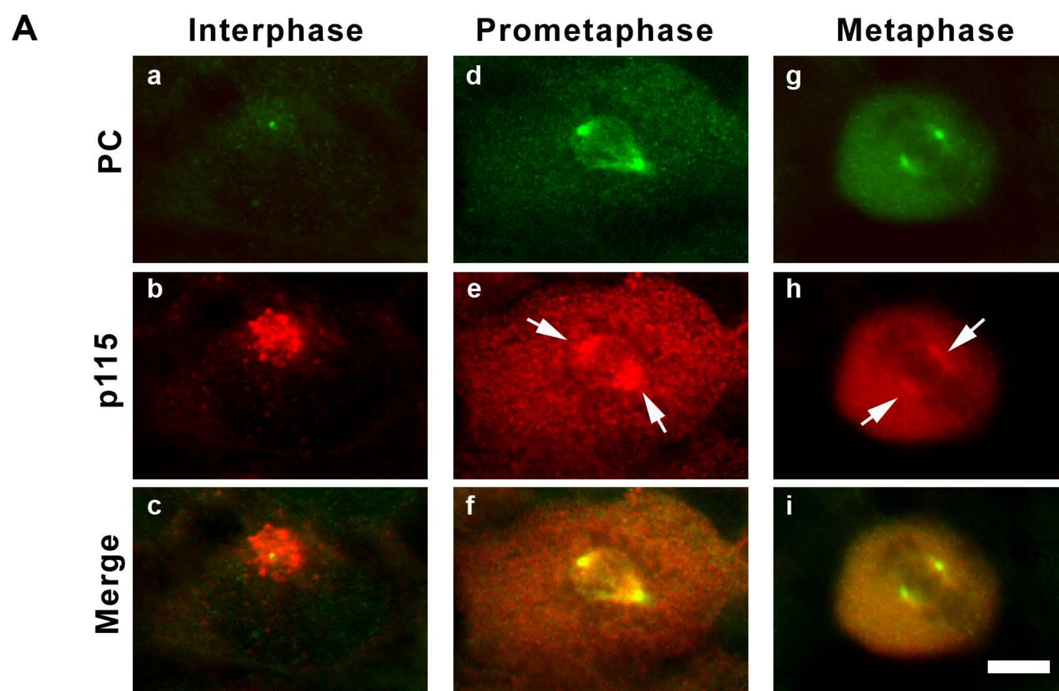
TIME LAPSE MOVIE 3 – **Mock-treated cells display normal mitotic spindle and mitotic progression.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were mock treated for 3 days, in parallel with the p115 siRNA treatment. Up to 15 fields were imaged. For each field, a Z-series with a 3μm spacing was acquired, at 5min intervals for a duration of 8 hours. Only the α-tubulin-YFP channel is shown. The corresponding H2B-RFP channel is shown in Time lapse 4. Display rate, 10 frames/ second. See Figure 7 for related video stills.

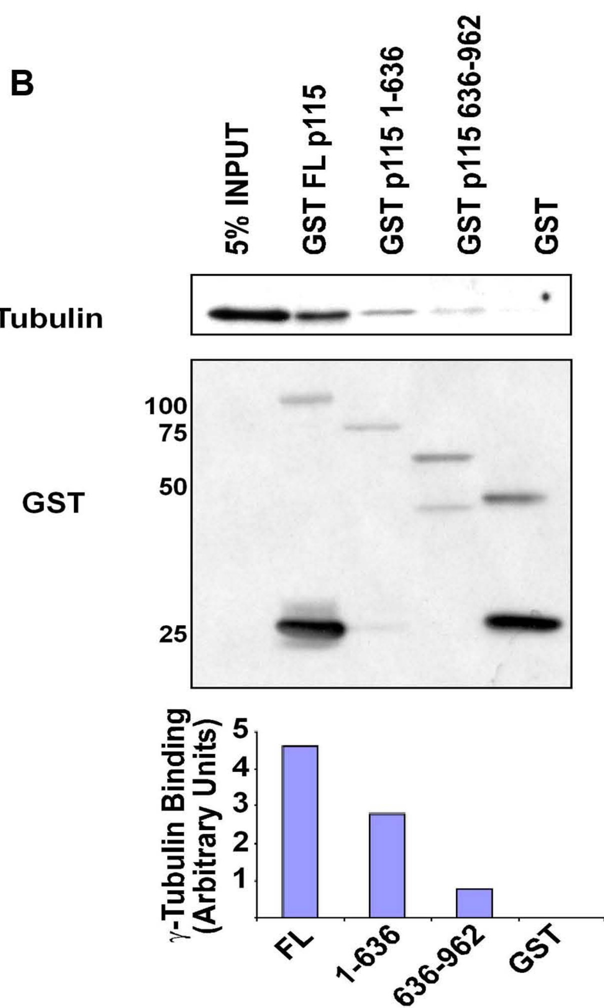
TIME LAPSE MOVIE 4 – **Mock-treated cells display normal chromosome segregation.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were mock treated for 3 days, in parallel with the p115

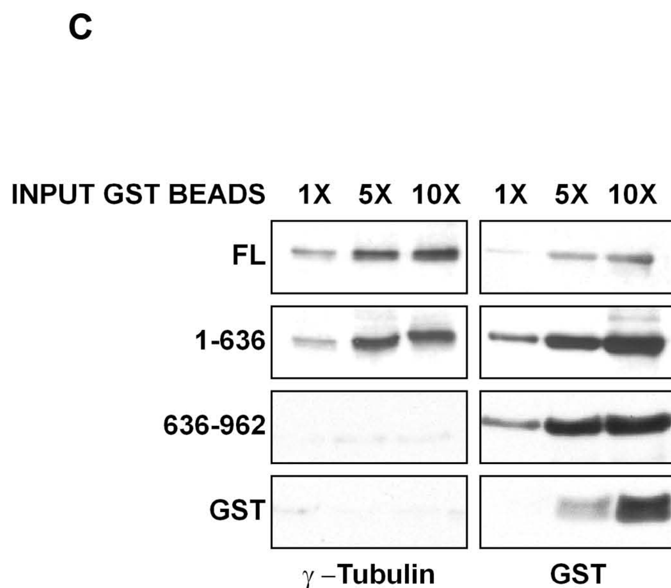
siRNA treatment. Up to 15 fields were imaged. For each field, a Z-series with a 3μm spacing was acquired at 5min intervals for a duration of 8 hours. Only the H2B-RFP channel is shown. The corresponding α-tubulin-YFP is shown in Time lapse movie 3. Display rate, 10 frames/ second. See Figure 7 for related video stills.

TIME LAPSE MOVIE 5 – **p115-depleted cells show an increased fusion phenotype.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were mock treated for 3 days. Up to 15 fields were imaged. For each field, a Z-series with a 3μm spacing was acquired at 5min intervals for a duration of 8 hours. Display rate, 10 frames/ second. See Figure 7 for related video stills.

TIME LAPSE MOVIE 6 – **Mock-treated cells display normal cytokinesis.** HeLa cells stably expressing α-tubulin-YFP and H2B-RFP were mock treated for 3 days, in parallel with the p115 siRNA treatment. Up to 15 fields were imaged. For each field, a Z-series with a 3μm spacing was acquired at 5min intervals for a duration of 8 hours. Display rate, 10 frames/ second. See Figure 7 for related video stills.







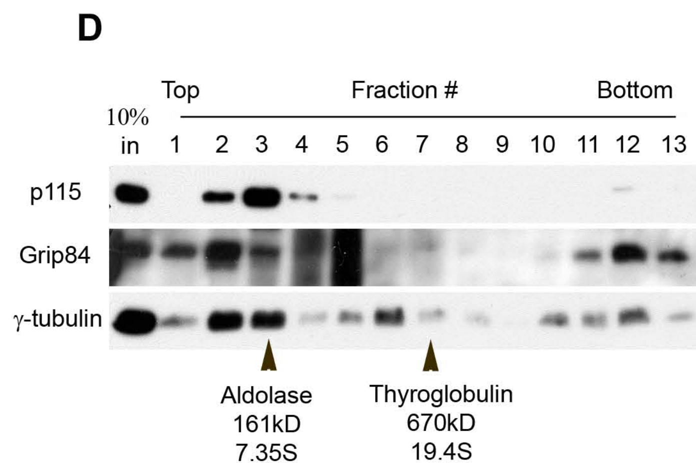


Figure S1

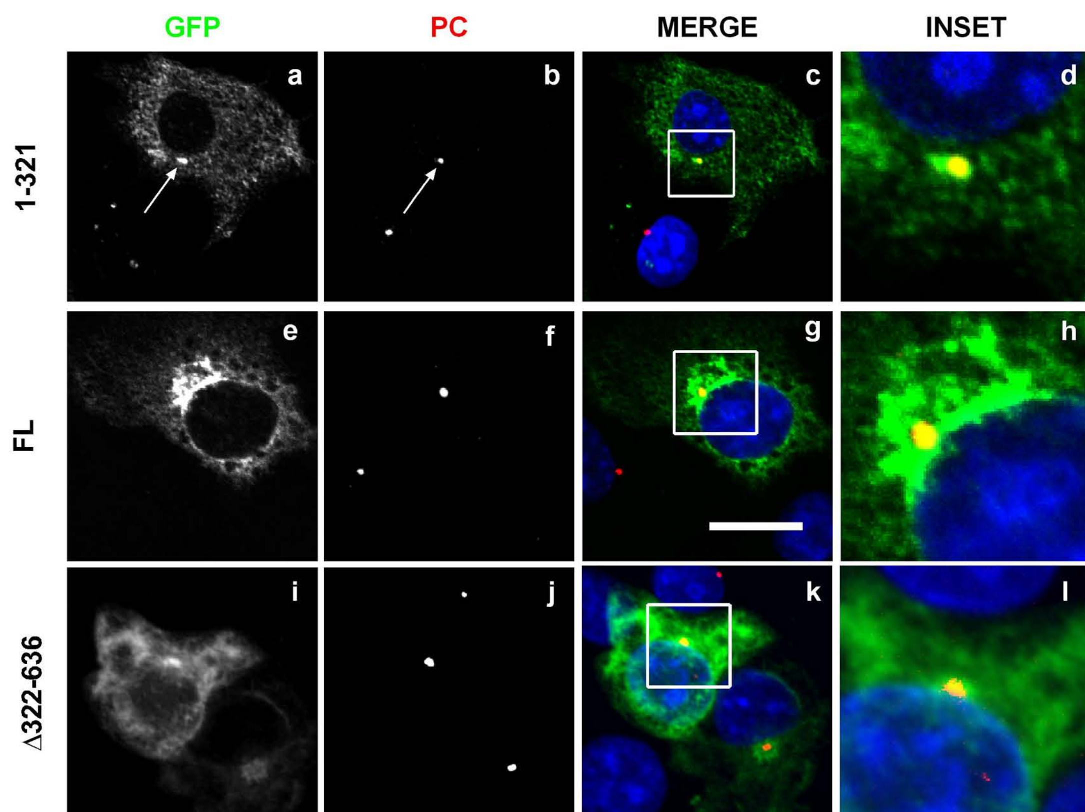


Figure S2

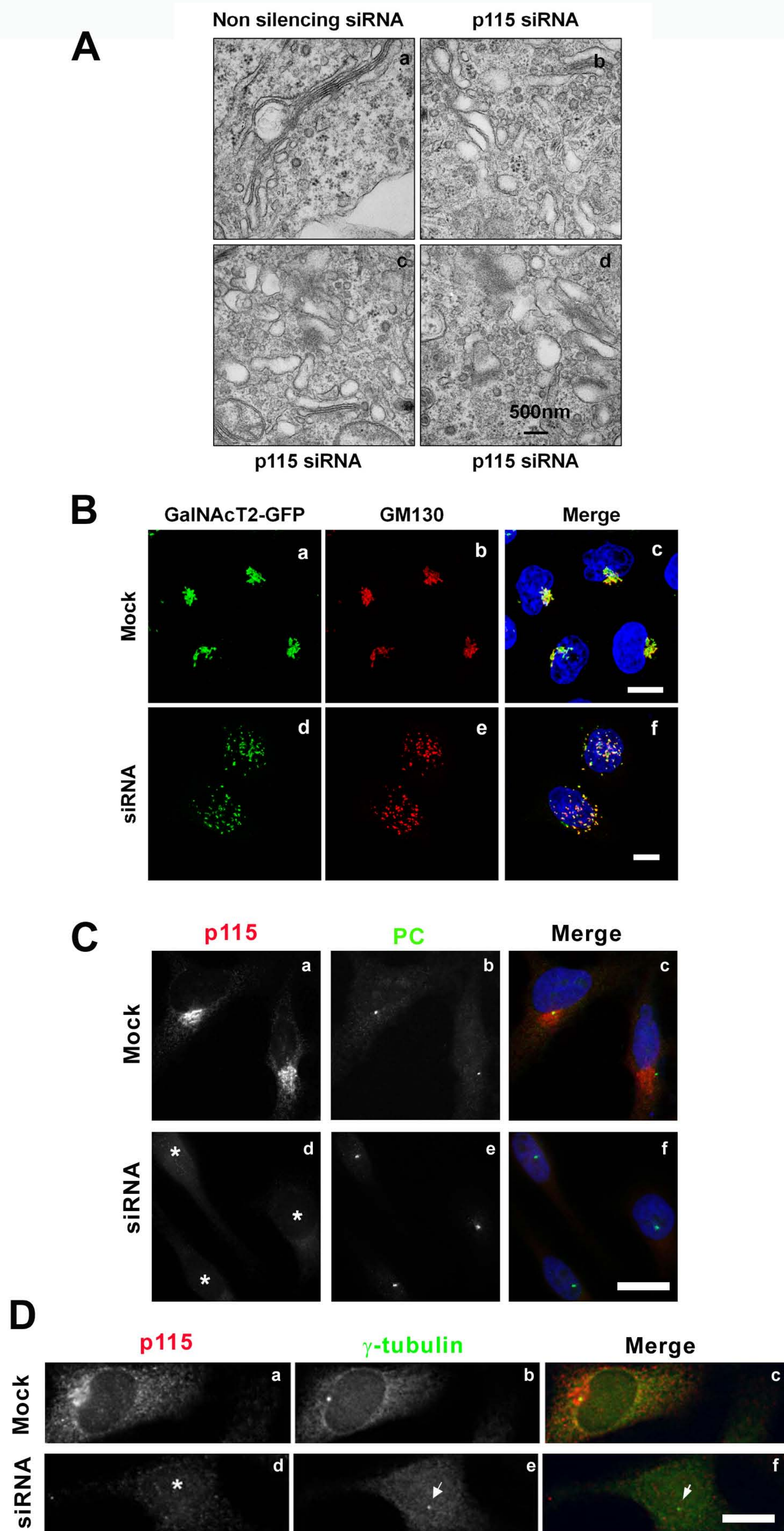
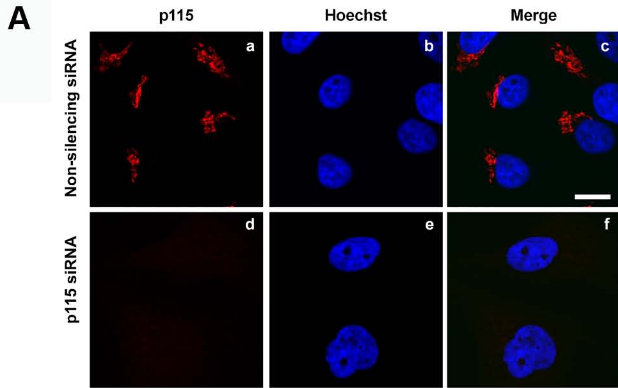
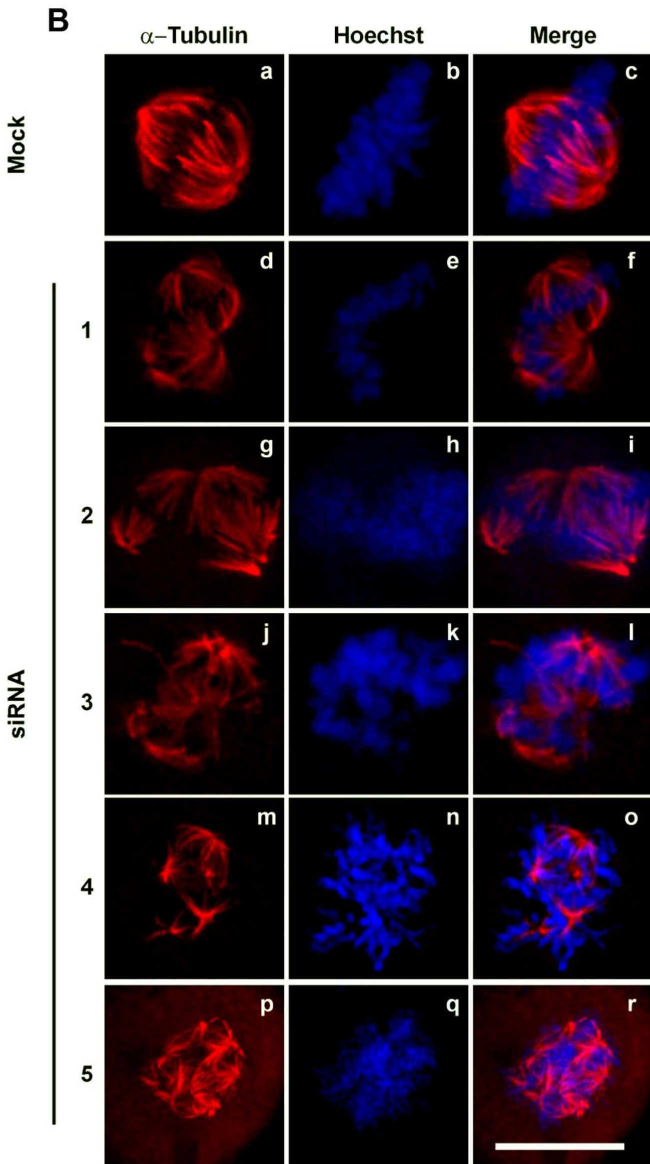


Figure S3



CELLS	AVERAGE NUCLEAR AREA (PIXELS)	NO. OF CELLS COUNTED	% CHANGE IN NUCLEAR SIZE
Non-silencing siRNA	15887	175	
siRNA	24054	200	45 +/- 5%



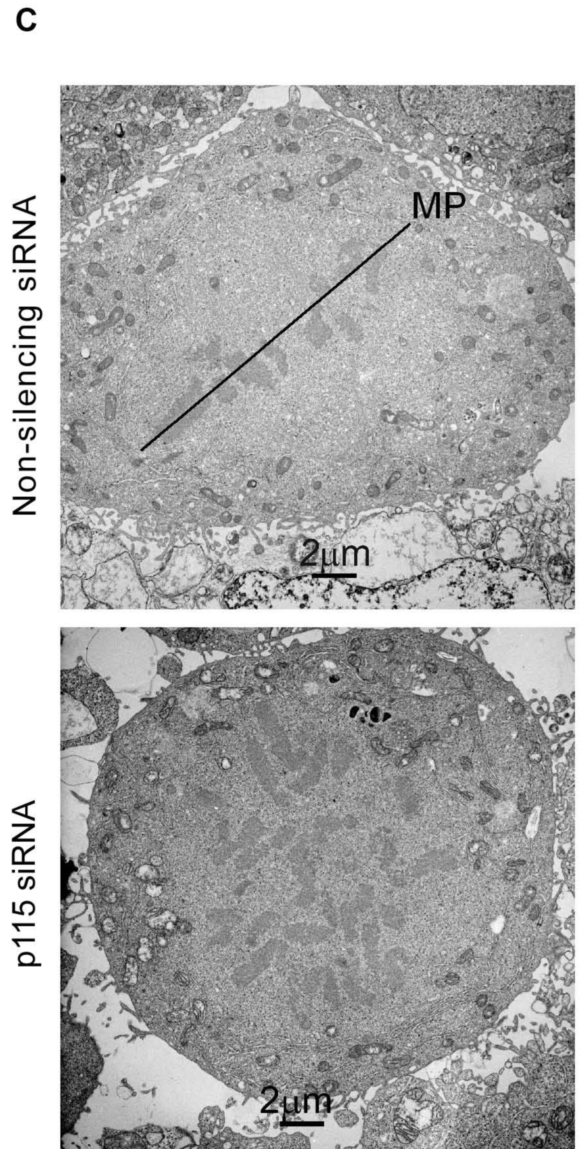
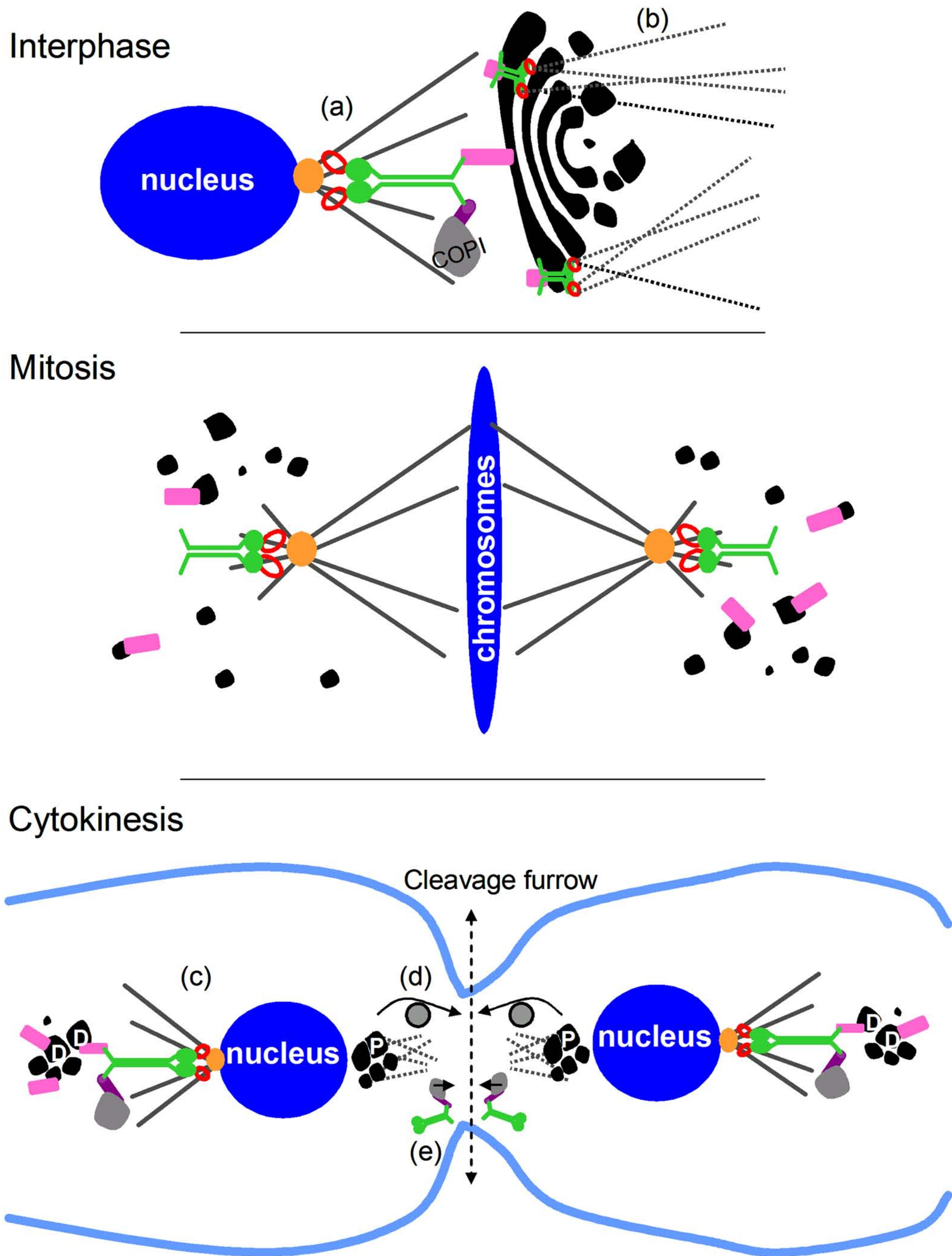


Figure S4



GM130 γ-tubulin centrosome giantin COPI vesicle

centrosomal microtubules non-centrosomal microtubules

Golgi mitotic remnants

Figure S5