

SUPPLEMENT FIGURE AND MOVIE LEGENDS.

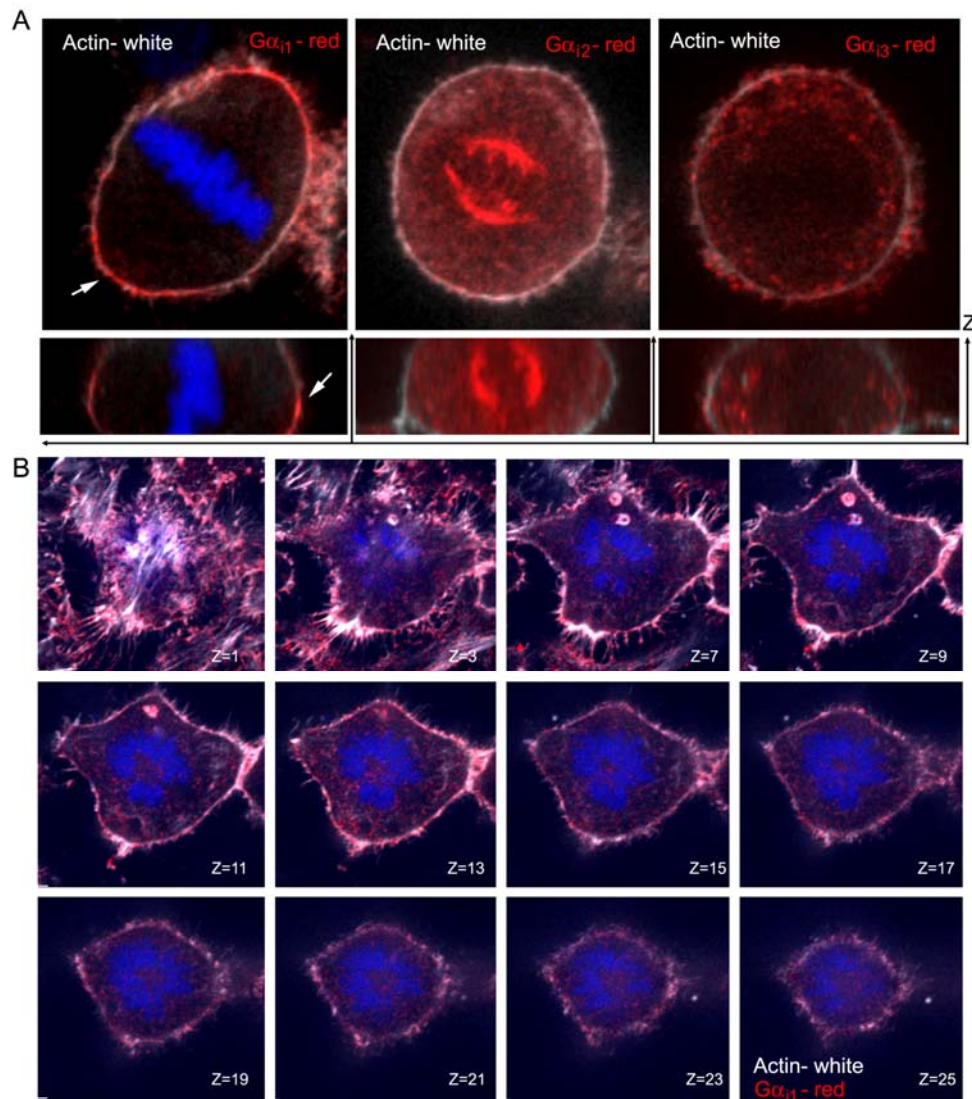


Figure S1. $G\alpha_i$ and F-actin localization. (A) $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ versus F-actin in metaphase HeLa cells. HeLa cells were fixed (paraformaldehyde) and immunostained for $G\alpha_{i1}$, $G\alpha_{i2}$, or $G\alpha_{i3}$ and stained with Alexa Fluor-488 phalloidin. A confocal image is shown along with the X-Z projection created in Imaris. DNA was stained (blue) in the first panel, but not the two other. Phalloidin staining is shown in white. (B) Co-localization of F-actin and $G\alpha_{i1}$ in a prophase HeLa cell. Shown are selected confocal images from a sequential image Z-stack (0.5 μm per slice) immunostained for F-actin (white) and $G\alpha_{i1}$ (red).

Movie 1. Co-localization of Ric-8A immunoreactivity with CENP-E. Confocal images from a z-stack acquired every 0.5 μ m between slices from fixed HeLa cells immunostained for Ric-8A (green) and CENP-E (red). DNA is blue.

Movie 2. Live cell imaging of GFP-Ric-8A expressed in HeLa cells. HeLa cells were transiently transfected with GFP-Ric-8A 48 hours previously and a mitotic cell was imaged from shortly after metaphase until cytokinesis. A single slice was acquired every minute.

Movie 3. Localization of Dynein in metaphase HeLa cells. HeLa cells growing on collagen coated dishes were fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movie 4. Localization of Dynein in metaphase HeLa cells treated with pertussis toxin. HeLa cells growing on collagen coated dishes were treated with pertussis toxin (400 ng/ml for 4 hours), fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movie 5. Localization of Dynein in metaphase HeLa cells with reduced Ric-8A expression. HeLa cells growing on collagen coated dishes were transiently transfected with a Ric-8A siRNA pool x2, fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movie 6. Localization of Dynein in metaphase HeLa cells with reduced $G\alpha_{i1-3}$ expression. HeLa cells growing on collagen coated dishes were transiently transfected with a $G\alpha_{i1-3}$ siRNA pool x2, fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movie 7. Localization of Dynein in metaphase HeLa cells with reduced Ric-8A expression. HeLa cells growing on collagen coated dishes were transiently transfected with a Ric-8A shRNA x2, fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movie 8. Localization of Dynein in metaphase HeLa cells treated with wortmannin. HeLa cells growing on collagen coated dishes were treated with wortmannin (10 nM for 2 hrs), fixed, immunostained for Dynein (green), and imaged (0.5 μ m between slices).

Movies 9 & 10. Live cell imaging of control and Ric-8 knockdown HeLa cells expressing α -tubulin GFP. Time lapse live cell images of control shRNA or Ric-8A shRNA

transiently transfected α -tubulin GFP HeLa cells are shown in maximal projection. Time of image acquisition indicated in the bottom left corner.

Movies 11 & 12. Individual HeLa cell expressing α -tubulin GFP from control shRNA or Ric-8 shRNA transfected cells. Maximum projection electronically zoomed time series of individual control and Ric-8A knockdown cell from overnight live cell imaging. Images acquired every three minutes.

Movies 13 & 14. Individual HeLa cell expressing α -tubulin GFP from control or a Ric-8 siRNA transfected cells. Electronically zoomed time series of individual control siRNA or Ric-8A siRNA transfected cells from 10 minutes of live cell imaging. Images collected every 30 seconds using a spinning disk confocal microscope with a 63X objective.