Dunsch et al., http://www.jcb.org/cgi/content/full/jcb.201202112/DC1



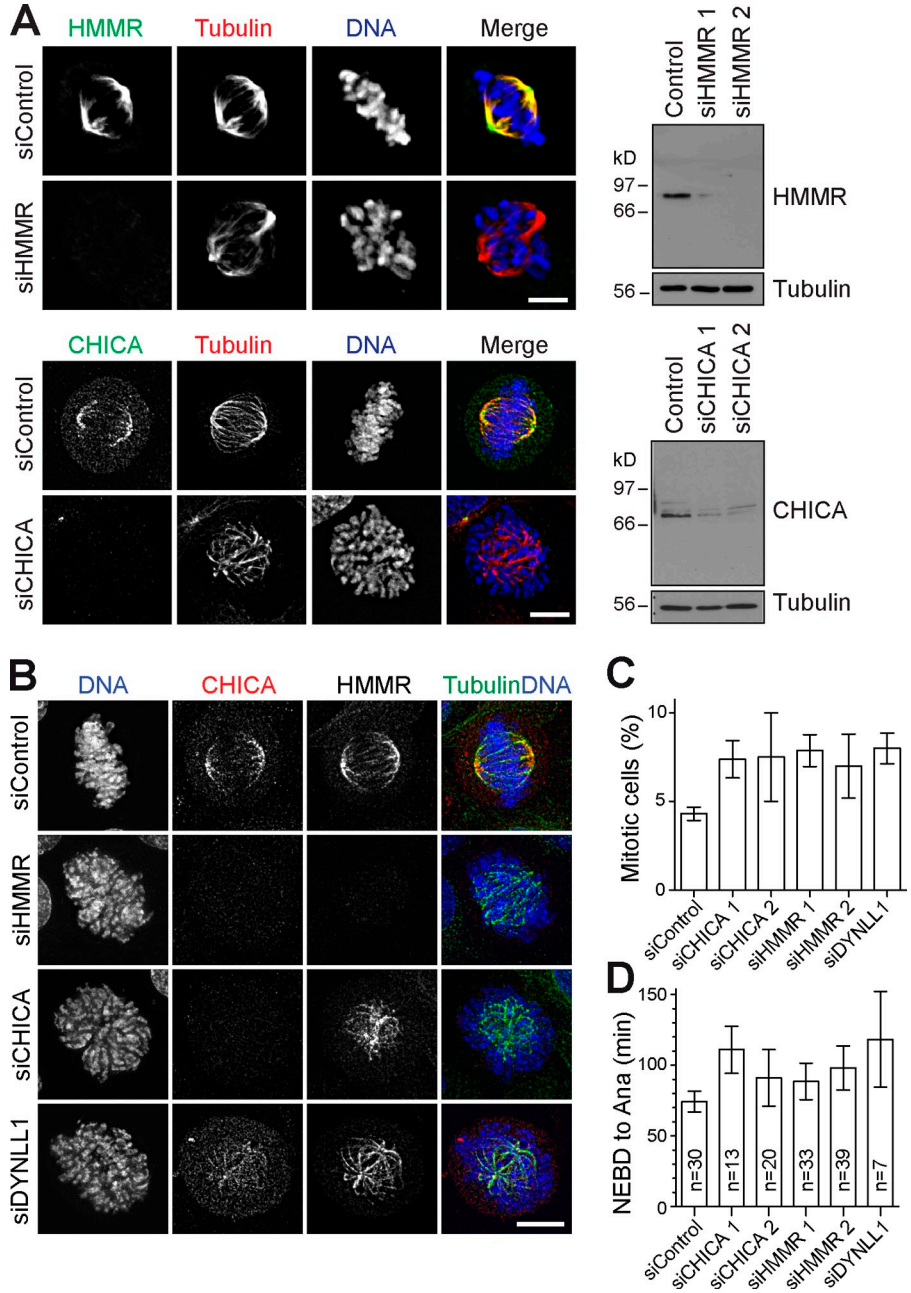



Figure S1. **Characterization of HMMR and CHICA antibodies.** (A) HeLa cells were transfected with control, or two different siRNA duplexes to HMMR or CHICA for 72 h. The cells were Western blotted to verify antibody specificity, or fixed and then stained with DAPI, tubulin, and HMMR or CHICA-C antibodies. (B) HeLa cells were transfected with control, CHICA, HMMR, or DYNLL1 siRNA duplexes for 72 h. Cells were fixed, and then stained for tubulin, CHICA, HMMR, and DAPI to detect DNA. CHICA-C antibody was used for blotting. (C) The mitotic index ($n$ = 100, three independent experiments) was counted and is plotted in the graph; error bars indicate the SEM. (D) HeLa cells stably expressing GFP-tubulin and mCherry-histone H2B were transfected with the siRNA duplexes shown in the figure for 72 h, and the full cell volume was imaged on an Ultraview spinning-disk confocal microscope every minute as the cells passed through mitosis. The time from nuclear envelope breakdown to the onset of anaphase was measured and is plotted in the graph, and error bars indicate the SEM. Bars, 10 µm.

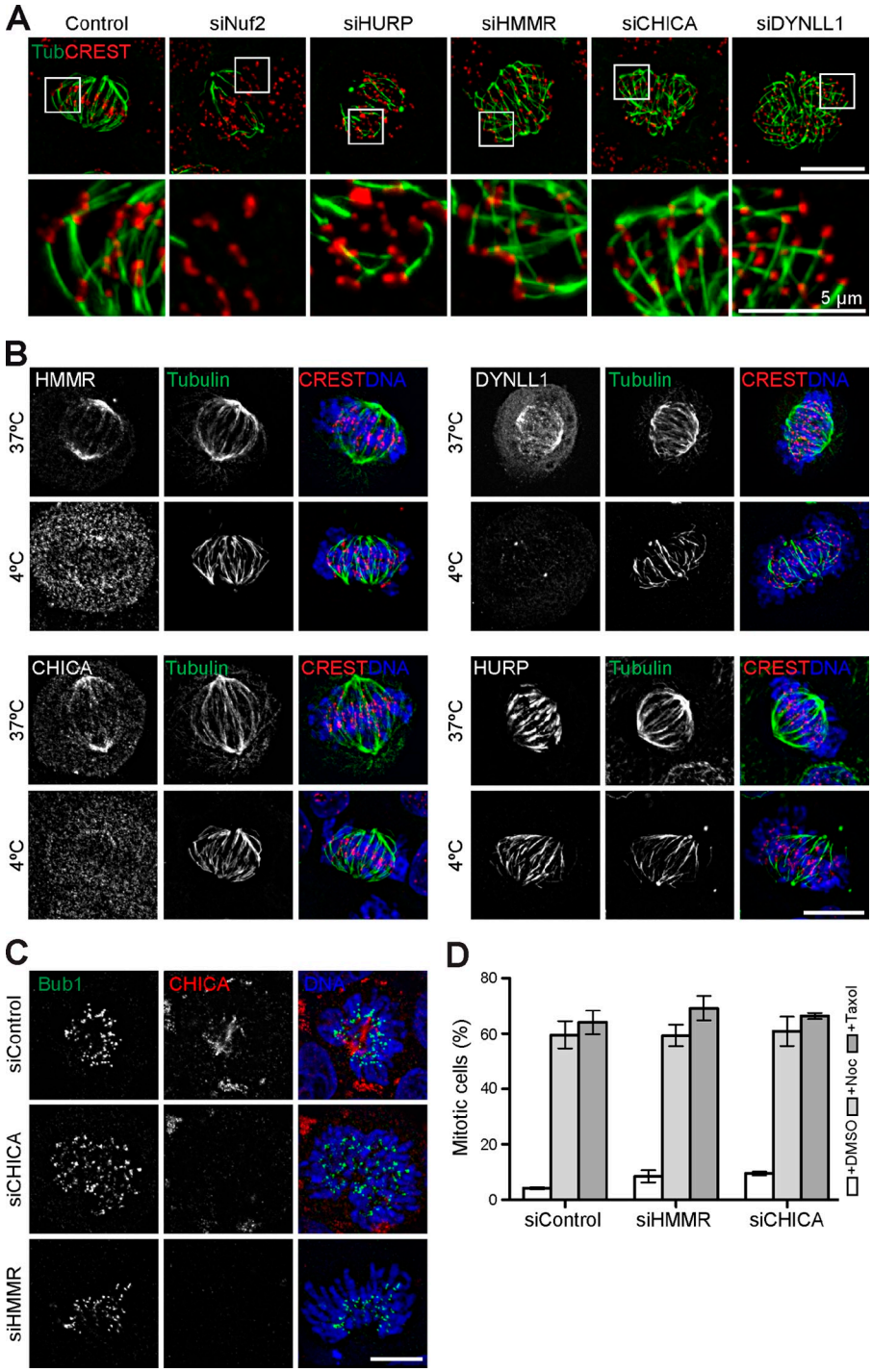



Figure S2. **CHICA, HMMR, and DYNLL1 localize to cold-sensitive microtubules, not cold-stable kinetochore fibers.** (A) HeLa cells were transfected with control, HMMR, CHICA, or DYNLL1 siRNA duplexes for 72 h, or HURP or Nuf2 siRNA duplexes for 48 h. The cells were then cold treated on ice to depolymerize nonkinetochore microtubules, fixed, and then stained for tubulin and with CREST antiserum. Bars, 10 µm (or 5 µm where indicated). Note that cells depleted of the essential kinetochore protein Nuf2 lack cold-stable kinetochore fibers. (B) HeLa cells were left untreated (37°C) or were cold treated on ice to depolymerize nonkinetochore microtubules (4°C). Cells were fixed and then stained for CHICA, HMMR, the centromere marker antiserum CREST, the kinetochore fiber protein HURP, and DAPI to detect DNA as indicated. CHICA-N antibody was used for staining. For DYNLL1, HeLa cells stably expressing GFP-DYNLL1 were used. Note that HURP staining is not lost in cold-treated cells. (C) HeLa cells transfected with control, CHICA, or HMMR duplexes for 72 h were fixed and then stained for the spindle checkpoint protein Bub1, CHICA, and DAPI to detect DNA. CHICA-N antibody was used for staining. (D) HeLa cells transfected with control, CHICA, or HMMR duplexes for 72 h were treated with DMSO, 200 ng/ml nocodazole, or 5 mM taxol for 18 h. Mitotic index was counted for $n = 100$ cells in three independent experiments and is plotted in the graph. Error bars indicate the SEM. Bar, 10 µm.

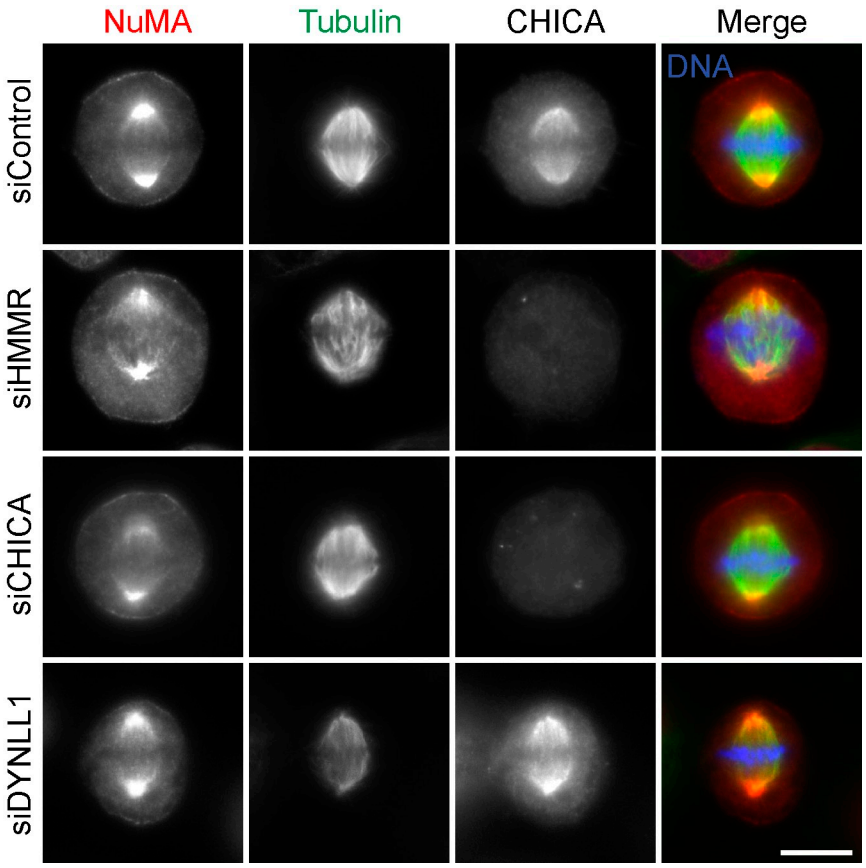
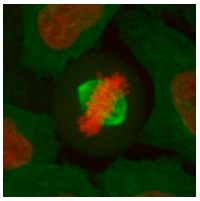
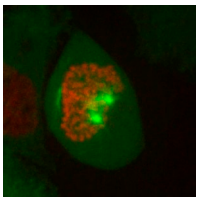


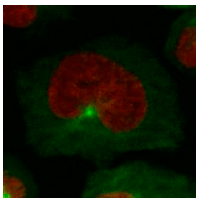

Figure S3. **Localization of the NuMa and Plk1 components of the intrinsic spindle-positioning pathway is not altered.** HeLa cells were stably transfected with control, HMMR, CHICA, or DYNLL1 siRNA duplexes for 72 h. The cells were fixed with methanol, and then stained for NuMA, tubulin, CHICA, and with DAPI to reveal DNA. CHICA-C antibody was used for staining. For ease of visualization, cells showing little alteration in spindle rotation were chosen for the CHICA, HMMR, and DYNLL1 siRNA images. Because CHICA, HMMR, and DYNLL1 cells randomly orient their spindle, by chance some will align with the coverslip. Bars, 10 µm.



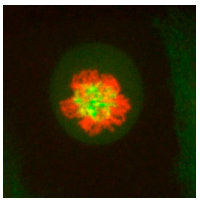
Video 1. **Spindle formation and positioning in control cells.** HeLa cells stably expressing GFP-tubulin and mCherry-histone H2B were transfected with control siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 29 planes spaced by 0.6 µm through the cell volume were collected every minute as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of tubulin (green) and histone H2B (red) are shown through time; each frame corresponds to 1 min.



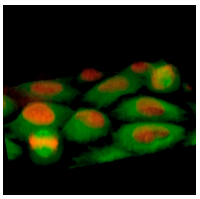
Video 2. **Spindle formation and positioning in CHICA-depleted cells.** HeLa cells stably expressing GFP-tubulin and mCherry-histone H2B were transfected with CHICA siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 29 planes spaced by 0.6 µm through the cell volume were collected every minute as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of tubulin (green) and histone H2B (red) are shown through time; each frame corresponds to 1 min.



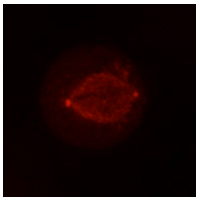
Video 3. **Spindle formation and positioning in HMMR-depleted cells.** HeLa cells stably expressing GFP-tubulin and mCherry-histone H2B were transfected with HMMR siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 29 planes spaced by 0.6 µm through the cell volume were collected every minute as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of tubulin (green) and histone H2B (red) are shown through time; each frame corresponds to 1 min.



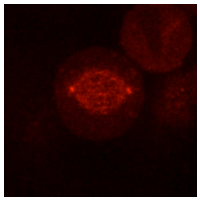
Video 4. **Spindle formation and positioning in DYNLL1-depleted cells.** HeLa cells stably expressing GFP-tubulin and mCherry-histone H2B were transfected with DYNLL1 siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 29 planes spaced by 0.6 µm through the cell volume were collected every minute as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of tubulin (green) and histone H2B (red) are shown through time; each frame corresponds to 1 min.



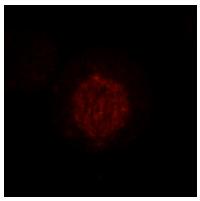
Video 5. **Rotation of the viewpoint shows that CHICA-depleted cells form bipolar spindles.** The movie shows a 3D volume render created in Volocity of a single time point from a CHICA siRNA live-cell imaging experiment, as performed in Fig. 4 B. GFP-tubulin (green) and mCherry-histone H2B (red) channels are shown. At the start of the movie, the cells are viewed from above. Note the cell at the top right has a bipolar spindle, whereas the cell at the bottom left shows the CHICA "disorganized spindle" defect. The viewpoint is then rotated to view the cells from an angle of ~30–45 degrees. This viewpoint shows that the cell at the bottom left has a bipolar spindle, contrary to the initial impression.



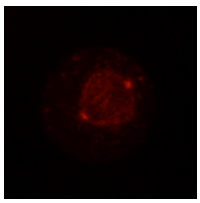
Video 6. **EB1 dynamics in control cells.** HeLa cells stably expressing mCherry-EB1 were transfected with control siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 7 planes spaced by 0.4 µm through the cell volume were collected at the maximum capture rate as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of EB1 are shown through time; each frame corresponds to 0.7 s.



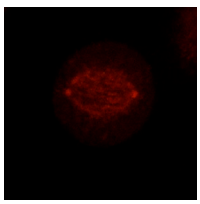
Video 7. **EB1 dynamics in CHICA-depleted cells.** HeLa cells stably expressing mCherry-EB1 were transfected with CHICA siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 7 planes spaced by 0.4 µm through the cell volume were collected at the maximum capture rate as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of EB1 are shown through time; each frame corresponds to 0.7 s.



Video 8. **EB1 dynamics in HMMR-depleted cells.** HeLa cells stably expressing mCherry-EB1 were transfected with HMMR siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 7 planes spaced by 0.4 µm through the cell volume were collected at the maximum capture rate as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of EB1 are shown through time; each frame corresponds to 0.7 s.



Video 9. **EB1 dynamics in DYNLL1-depleted cells.** HeLa cells stably expressing mCherry-EB1 were transfected with DYNLL1 siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 7 planes spaced by 0.4 µm through the cell volume were collected at the maximum capture rate as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of EB1 are shown through time; each frame corresponds to 0.7 s.



Video 10. **EB1 dynamics in CenpE-depleted cells.** HeLa cells stably expressing mCherry-EB1 were transfected with CenpE siRNA duplexes for 72 h, and were then used for time-lapse imaging. Images corresponding to 7 planes spaced by 0.4 µm through the cell volume were collected at the maximum capture rate as the cells passed through mitosis using a spinning-disk confocal microscope (UltraView Vox; PerkinElmer). Maximum intensity projections of EB1 are shown through time; each frame corresponds to 0.7 s.

Table S1. **Identification of DYNLL1-interacting spindle proteins by mass spectrometry**

| IP | Gene | Protein name | Size | Peptides | Intensity |
|---|---|---|---|---|---|
| | | | *kD* | | |
| GFP-DYNLL1 | HMMR | Hyaluronin-mediated motility receptor | 84.2 | 28 | 24,338,000 |
| GFP-DYNLL1 | DYNLL1 | Dynein light chain 1 | 10.4 | 2 | 12,885,000 |
| GFP-DYNLL1 | SPAG5 | Astrin; sperm-associated antigen 5 | 134.4 | 14 | 5,129,400 |
| GFP-DYNLL1 | FAM83D | CHICA; FAM83D | 67.6 | 9 | 5,117,900 |
| GFP-DYNLL1 | DYNC1I2 | Dynein 1 intermediate chain 2 | 71.5 | 7 | 2,798,600 |
| GFP-DYNLL1 | C15ORF23 | Kinastrin; SKAP | 35.4 | 2 | 935,910 |
| GFP-DYNLL1 | DYNC1LI1 | Dynein 1 light intermediate chain 1 | 56.6 | 3 | 751,930 |
| GFP-DYNLL1 | NEK9 | Nek9 protein kinase | 107.2 | 3 | 536,300 |
| HMMR | HMMR | Hyaluronin-mediated motility receptor | 84.2 | 16 | 42,468,000 |
| HMMR | FAM83D | CHICA; FAM83D | 67.6 | 3 | 10,339,000 |
| HMMR | DYNLL1 | Dynein light chain 1 | 10.4 | 2 | 8,286,000 |
| CHICA | HMMR | Hyaluronin-mediated motility receptor | 84.2 | 23 | 32,887,000 |
| CHICA | FAM83D | CHICA; FAM83D | 67.6 | 7 | 6,699,200 |