Lee et al., https://doi.org/10.1083/jcb.201707050

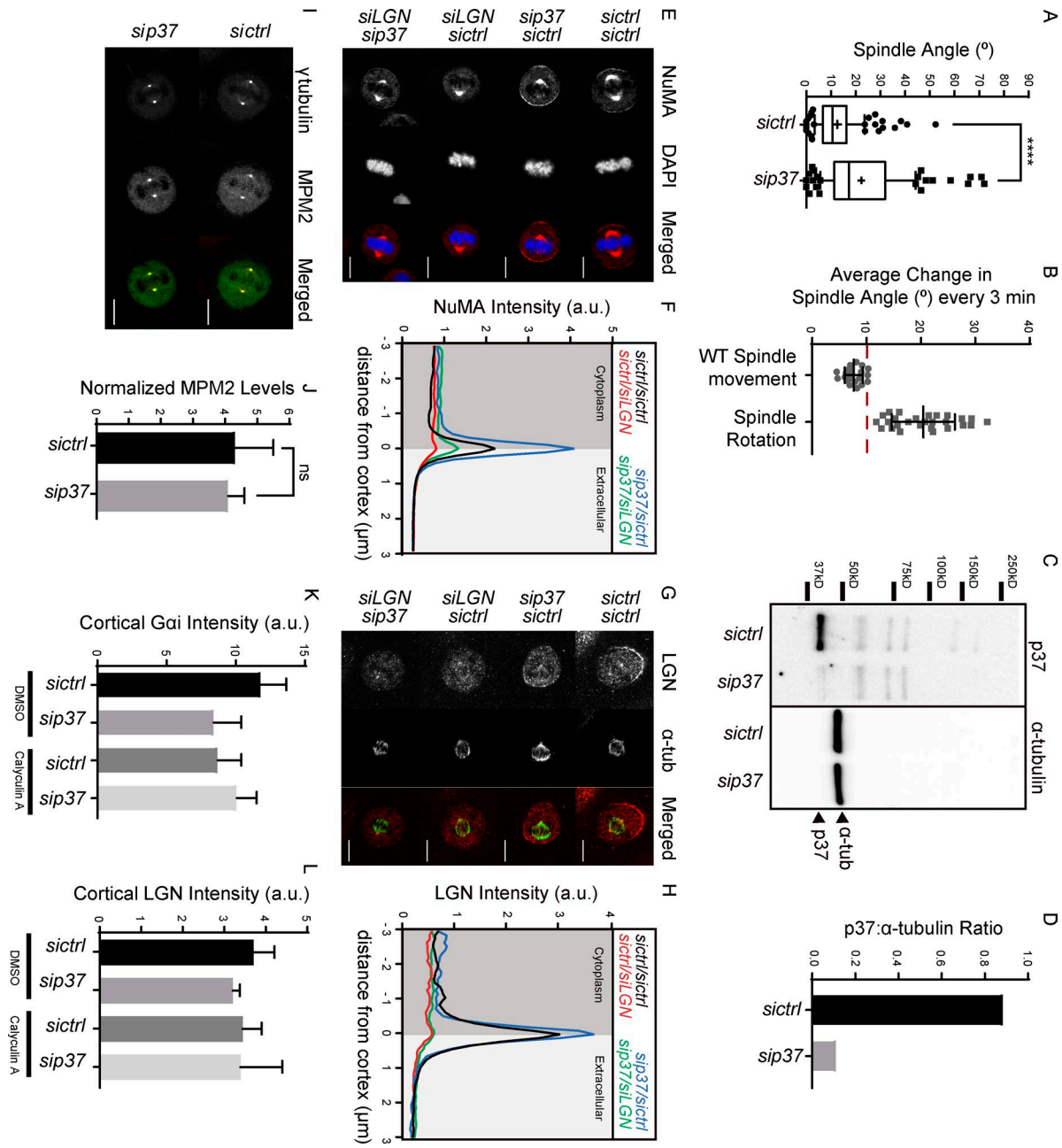

Figure S1. **Quantification of spindle angle and spindle rotation in p37-depleted cells, characterization of the p37 antibody, cortical LGN and NuMA levels in LGN and LGN/p37 depletions, MPM2 data, and levels of Gαi and LGN in control and calyculin A–treated cells. (A)** Quantification of spindle angle in *siControl* and *sip37* HeLa cells. Spindle angle quantifications were done on fixed cells immunostained with γ-tubulin as described in the Image acquisition, live-cell imaging, and image quantification section of Materials and methods and Fig. S3. $n = 4$; $n = 119–123$. ****, $P < 0.001$ in a Mann-Whitney test. **(B)** Quantification of spindle rotation speed based on live SiR-tubulin images as shown in Fig. 1 A. The dot blots show for illustration purposes the mean spindle rotations per 3-min time point of 58 p37-depleted cells from one experiment. Cells in which the spindle rotated on average >10° per 3 min were classified as having excessive spindle rotations (red line). Note the two distinct populations. **(C and D)** Western blotting of cell lysates (50 µg) from *siControl* and *sip37* cells incubated with p37 (left) and tubulin (right) antibodies (C) and quantification of the p37/α-tubulin ratio (D). **(E and F)** Confocal Images of cells with the indicated depletions stained for NuMA and DAPI (DNA; E) and corresponding line profile of cortical NuMA (F). All line profiles represent the mean intensity across the cortex. $n = 3$; $n = 74–87$. **(G and H)** Confocal images of cells with the indicated depletions stained for LGN and α-tubulin (G) and corresponding line profile of cortical LGN (H). $n = 2$; $n = 26–34$. **(I and J)** Confocal images of HeLa cells treated with *siControl* and *sip37* stained for γ-tubulin and MPM2 (I) and corresponding quantification of centrosomal MPM2 levels normalized to cytoplasmic signal (J). $n = 2$; $n = 63–66$ cells. $P = 0.9135$ in a ratio-paired *t* test. Error bars indicate SEM. Bars, 10 µm. **(K and L)** Quantification of cortical Gαi (K) and LGN (L) levels in HeLa cells with the indicated depletions and treated with DMSO (0.01%) or calyculin A (50 nM for 10 min). $n = 1–2$; $n = 20–51$. Error bars indicate SD.

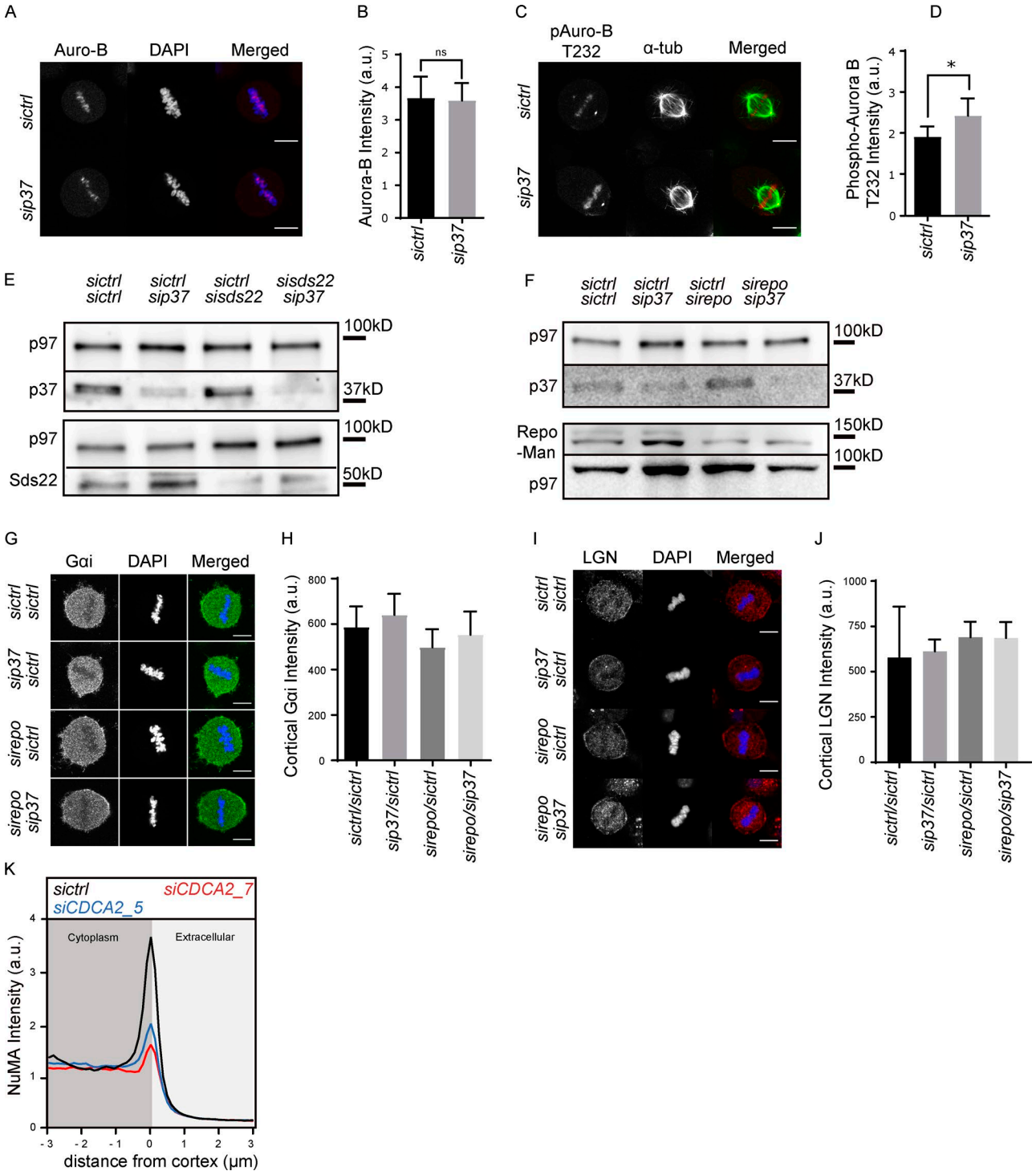

Figure S2. **Chromosomal Aurora B and phospho–T232 Aurora B levels in p37-depleted cells, Western blot of the lysates of controls and the codepletion of Sds-22 and Repo-Man with p37, corresponding quantifications and Gαi and LGN staining in the Repo-Man and Repo-Man/p37 depletions, and NuMA line profile after depletion of Repo-Man with a second independent siRNA. (A–D)** Confocal images of *siControl* and *sip37* cells stained with Aurora B antibodies and DAPI (DNA; A) or with antibodies recognizing phospho–Aurora B (pT232) and α-tubulin (C), and corresponding quantification of chromosomal Aurora B (B; $n = 2$; $n = 36–38$ cells) and phospho–Aurora B (D; $n = 4$; $n = 75–84$ cells). *, P = 0.0203 in a ratio *t* test. Error bars indicate SEM. **(E and F)** Western blots showing total levels of Sds22 and p37 (E) and Repo-Man and p37 (F) in Hela cells after the indicated depletions. 50 µg of extracts were loaded in each lane, and p97 was used as loading control. **(G and H)** Confocal images of cells with the indicated depletions stained for DAPI and Gαi (G) and corresponding quantifications (H). $n = 1$; $n = 16–24$. **(I and J)** Confocal images of cells with the indicated depletions stained for DAPI and LGN (I) and corresponding quantifications (J). $n = 2$; $n = 29–39$. Error bars indicate SD. Bars, 10 µm. **(K)** Line profile of cortical NuMA in cells treated with *siControl* and two different siRNA oligonucleotides targeting Repo-Man (siCDCA2_5 and siCDCA2_7). The line profile represents the mean intensity across the cortex. $n = 2$; $n = 25–34$.

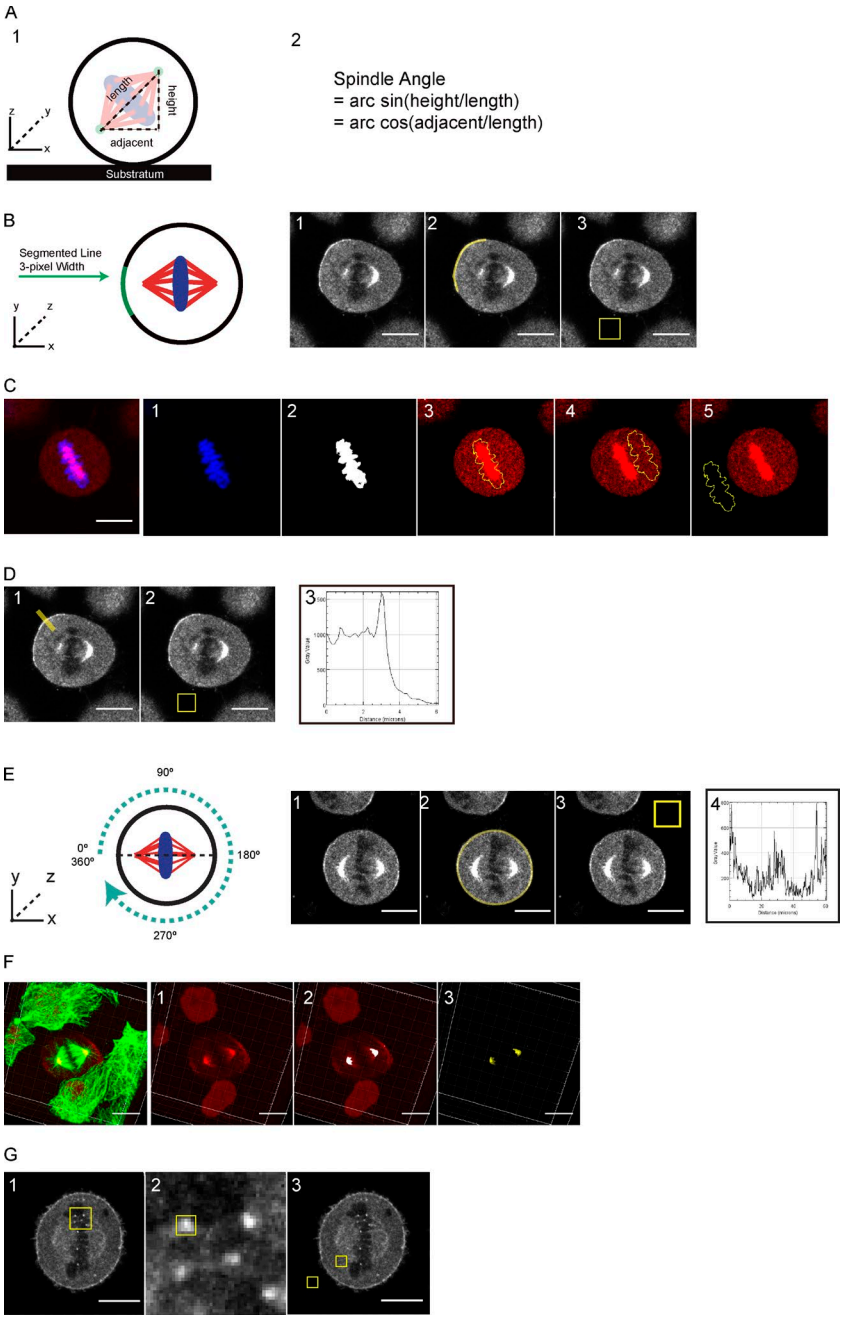

Figure S3. **Data quantification methods. (A)** Schematic representation of the method used for the quantification of the spindle angle in fixed metaphase cells stained with γ-tubulin or α-tubulin. The x, y, and z coordinates of the spindle poles were measured and used to calculate the spindle length (length) and z stack difference (height) between the two spindle poles as shown in 1. These parameters were than used to calculate the spindle angle as shown in 2 using basic trigonometry. **(B)** Schematic representation of the method used to quantify cortical intensities (left). Confocal images of NuMA staining (right) showing the quantifications steps: A 3-pixel-wide segmented line was used to select the cortical proteins as shown in steps 1 and 2 and measured for mean intensity value of fluorescent signal. A square selection was used to measure and correct for background as shown in 3. **(C)** Confocal images of cells stained with DAPI and phospho–Aurora B to represent the method used to quantify proteins localized at the metaphase plate. The DAPI signal (as shown in 1) was used to build a mask as shown in 2. The mask was used for measuring mean intensity value of fluorescent signal of chromosomal proteins (3). The same mask was used to correct for background and cytoplasmic signal (4 and 5). **(D)** Schematic explaining line profile quantification. Confocal images of cells stained with NuMA. For the line profiles shown in Figs. 1, 2, 3, 4, and 5, a 10-pixel-wide line was used to obtain the intensity profile across the cortex (1). A square selection was used to measure and correct for background (2), which resulted in the line profile shown in (3). **(E)** Schematic explaining line scan quantification of NuMA levels around the entire cortex relative to the spindle angle (x–y axis; left). A 3-pixel-wide segmented line was used to obtain the intensity profile along the entire cortex (1 and 2). A square selection was used to measure and correct for background (3), which resulted in a line profile shown in 4. **(F)** Confocal images of cells stained with NuMA to represent the method used to quantify the volume of NuMA signal at spindle poles. Thresholding was applied to the acquired images (1 and 2), and the volume was extracted using the Imaris software (3). The level of threshold was kept the same within experiments and cell to cell. **(G)** Confocal images of GFP-PP1α–expressing cells representing the method used to quantify amount of GFP-PP1α at kinetochores. A square selection with an area of 0.25 μm^2 was used to measure the intensity of GFP at the kinetochore (1 and 2). Kinetochore GFP intensity and cytoplasmic GFP intensity were corrected for background contribution (3). The relative amount of GFP at the kinetochore is presented as a ratio to cytoplasmic GFP level to normalize for GFP expression variability between cells. Bars, 10 μm.