Kushner et al., http://www.jcb.org/cgi/content/full/jcb.201311013/DC1

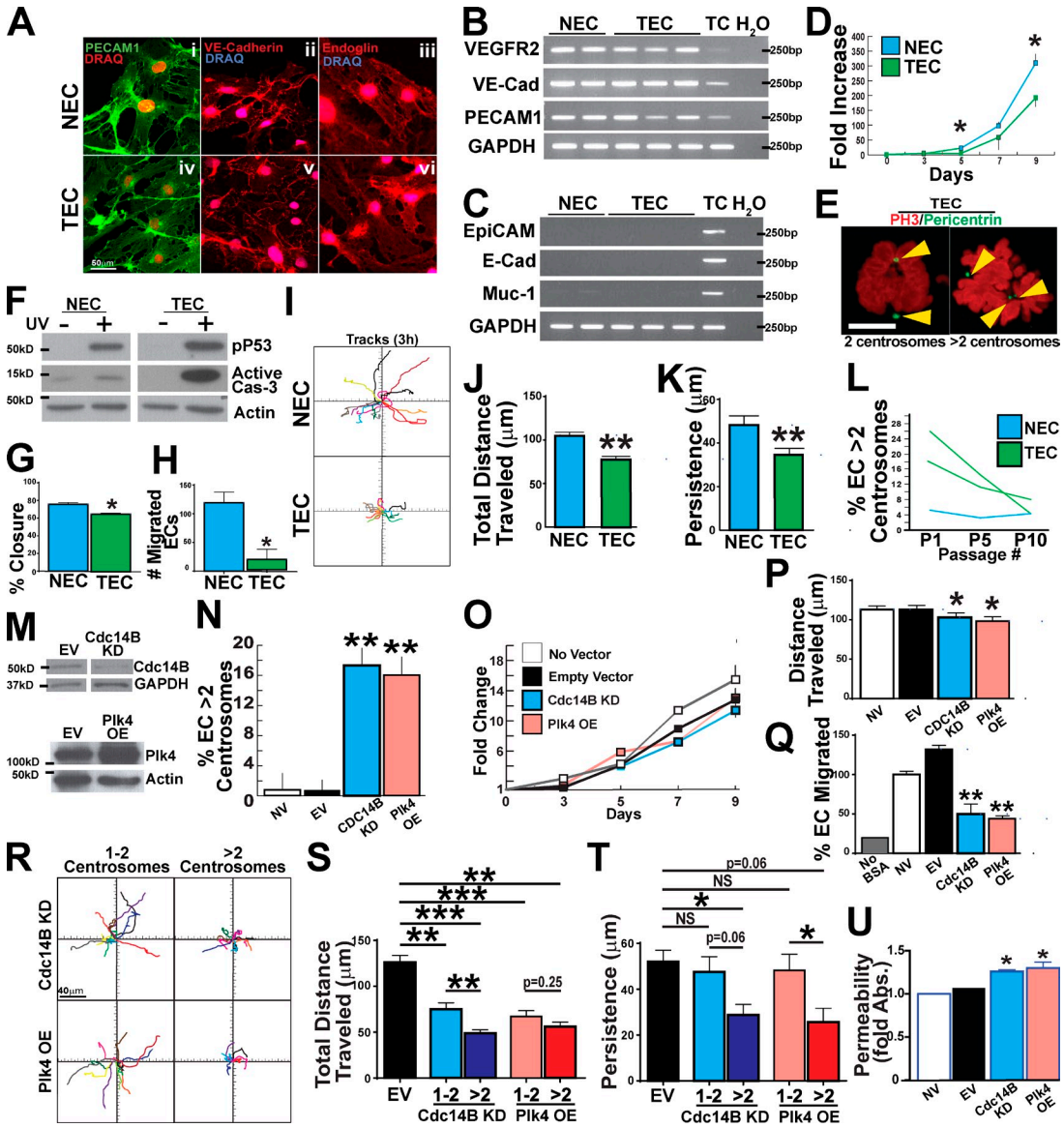


Figure S1. **Characterization of TECs and HUVECs with supernumerary centrosomes.** (A) Representative images of established normal endothelial cells (NEC) and tumor-derived endothelial cells (TEC) stained for the indicated endothelial cell markers. (B and C) RT-PCR for specific endothelial (top) and epithelial (bottom) mRNA transcripts in established NECs and TECs. Cells express vascular but not tumor cell (TC) markers. Size markers are given on the right. Cad, cadherin. (D) Growth curve of NECs and TECs. Statistical comparisons: Student's *t* test; means ± SEM. *, P < 0.05. (E) Mitotic TECs stained for phospho–histone-3 (PH3, mitotic chromosomes) and pericentrin (centrosomes). Yellow arrows, centrosomes. Image on the right shows two centrosomes clustered at spindle. Bar, 10 μm. (F) Western blot of the indicated lysates showing p53 (phospho-p53 [pP53]) and active caspase-3 (Cas-3) at baseline and after UV stimulation to activate apoptotic markers. Size markers are given on the left. (G) Mean percentage of closure in scratch wound assay at 5 h. Statistical comparisons versus NEC (Student's *t* test; means ± SEM. *, P < 0.05). (H) Mean number of migrated NECs and TECs in transwell assays. Statistical comparisons: Student's *t* test; means ± SEM. *, P < 0.05. (I) One 3-h migration track experiment of NECs and TECs, representative of three repeats. (J and K) Mean distance traveled (J) and directional persistence (K) of NECs and TECs over 3 h (NEC, *n* = 41 cells; TEC, *n* = 45 cells). Statistical comparisons: Student's *t* test; means ± SEM. **, P < 0.01. (L) Centrosome number versus passage for NECs and TECs for a single time course experiment. Note that TECs with supernumerary centrosomes are lost with passage. (M) Western blot showing knockdown of Cdc14B (top, Cdc14B KD) and overexpression of Plk4 (bottom, Plk4 OE) protein in HUVECs. Cells were infected with respective lentivirus and incubated 4 d before protein isolation. Size markers are given on the left. (N) Percentage of infected HUVECs with greater than two centrosomes (NV, no vector; EV, empty vector). Statistical comparisons versus empty vector: Student's *t* test; means ± SEM; **, P < 0.01. (O) Growth curve of the indicated groups of HUVECs. Differences were not significant between two independent experiments. Statistical comparisons: Student's *t* test; means ± SEM. (P) Mean distance traveled over 5 h of HUVECs infected with the indicated virus in scratch wound assay. Statistical comparisons versus empty vector: Student's *t* test; means ± SEM; *, P < 0.05. (Q) Mean percentage of migrated cells (relative to control) of HUVECs expressing the indicated viral constructs in a transwell assay. Statistical comparisons versus empty vector: Student's *t* test; means ± SEM; **, P < 0.01. (R) One 3-h migration track experiment of the indicated HUVECs, representative of three repeats. (S) Mean total distance traveled in migration tracking assay of HUVECs infected with the indicated lentivirus (empty vector, *n* = 27 cells; Cdc14B KD, *n* = 23 cells; Plk4 OE, *n* = 28 cells). Statistical comparisons: Student's *t* test; means ± SEM; **, P < 0.01; ***, P < 0.001. (T) Mean directional persistence in migration tracking assay of HUVEC infected with indicated lentivirus (empty vector, *n* = 27 cells; Cdc14B KD, *n* = 23 cells; Plk4 OE, *n* = 28 cells). Statistical comparisons: Student's *t* test; means ± SEM; *, P < 0.05. (U) Permeability assay, absorbance (Abs) of Evan's blue dye at 6 h (no vector, *n* = 2 wells; empty vector, *n* = 2 wells; Cdc14B KD *n* = 2 wells; Plk4 OE, *n* = 2 wells). Statistical comparisons: Student's *t* test; means ± SEM. *, P < 0.05.

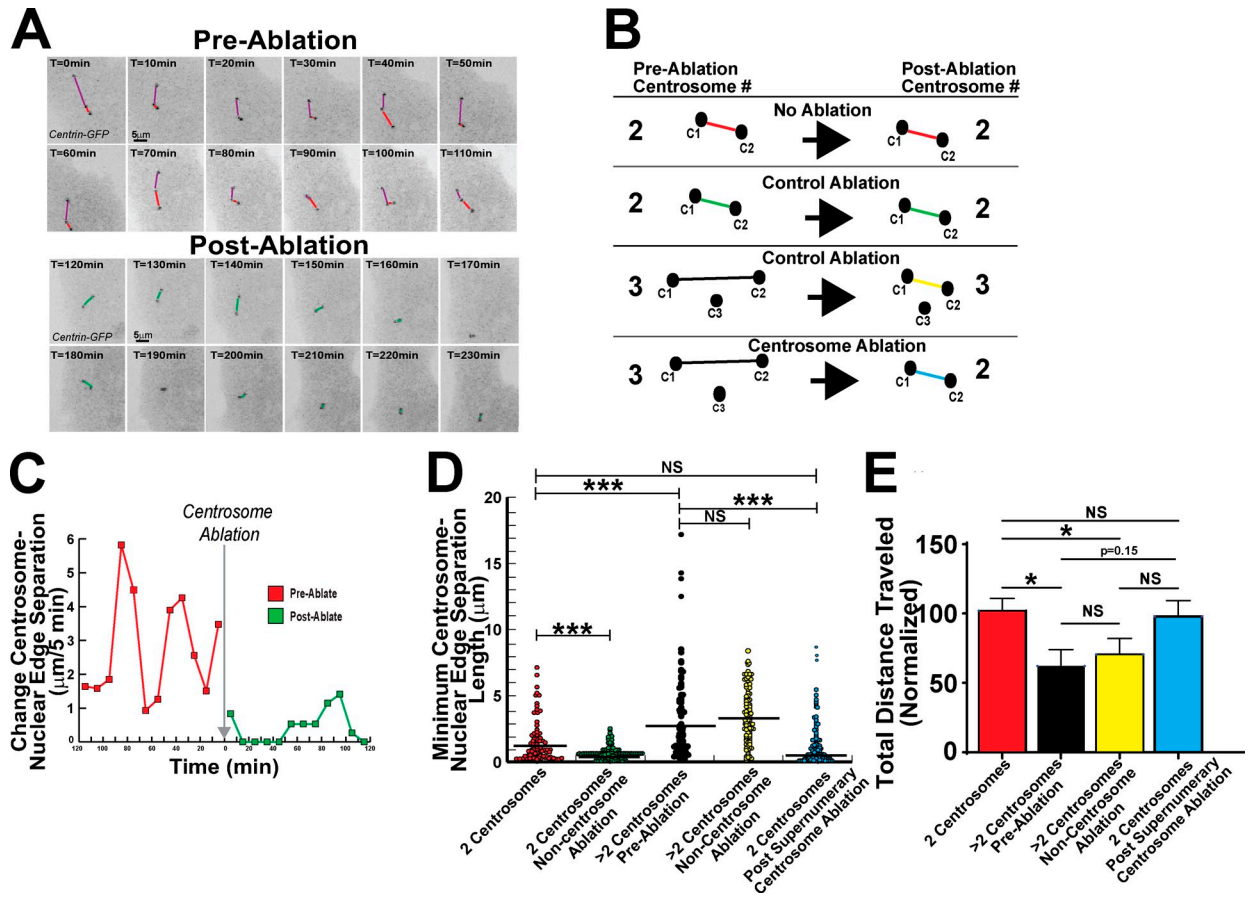


Figure S2. **Ablation of excess centrosomes rescues centrosome dynamics and endothelial cell migration.** (A) Fluorescence micrographs from live-cell imaging sequence of TECs depicting centrosome movements before (top) and after (bottom) centrosome ablation. Colored lines indicated measured distances between centrosomes as diagramed in this panel and presented in Fig. 2. (B) Diagram showing experimental design for data shown in D and E and Fig. 2 (C–E). C, centrosome. (C) Representative graph of change in centrosome–nuclear edge separation distance in TECs before and after centrosome ablation. (D) Individual centrosome–nuclear edge separation distances between the indicated groups ($n \geq 6$ cells per condition). Statistical comparisons: Student's *t* test; means ± SEM. ***, $P < 0.00001$. (E) Mean distance traveled of TECs in the indicated groups, labeled as in D ($n > 6$ cells per condition). Statistical comparisons: Student's *t* test; means ± SEM. *, $P < 0.05$.

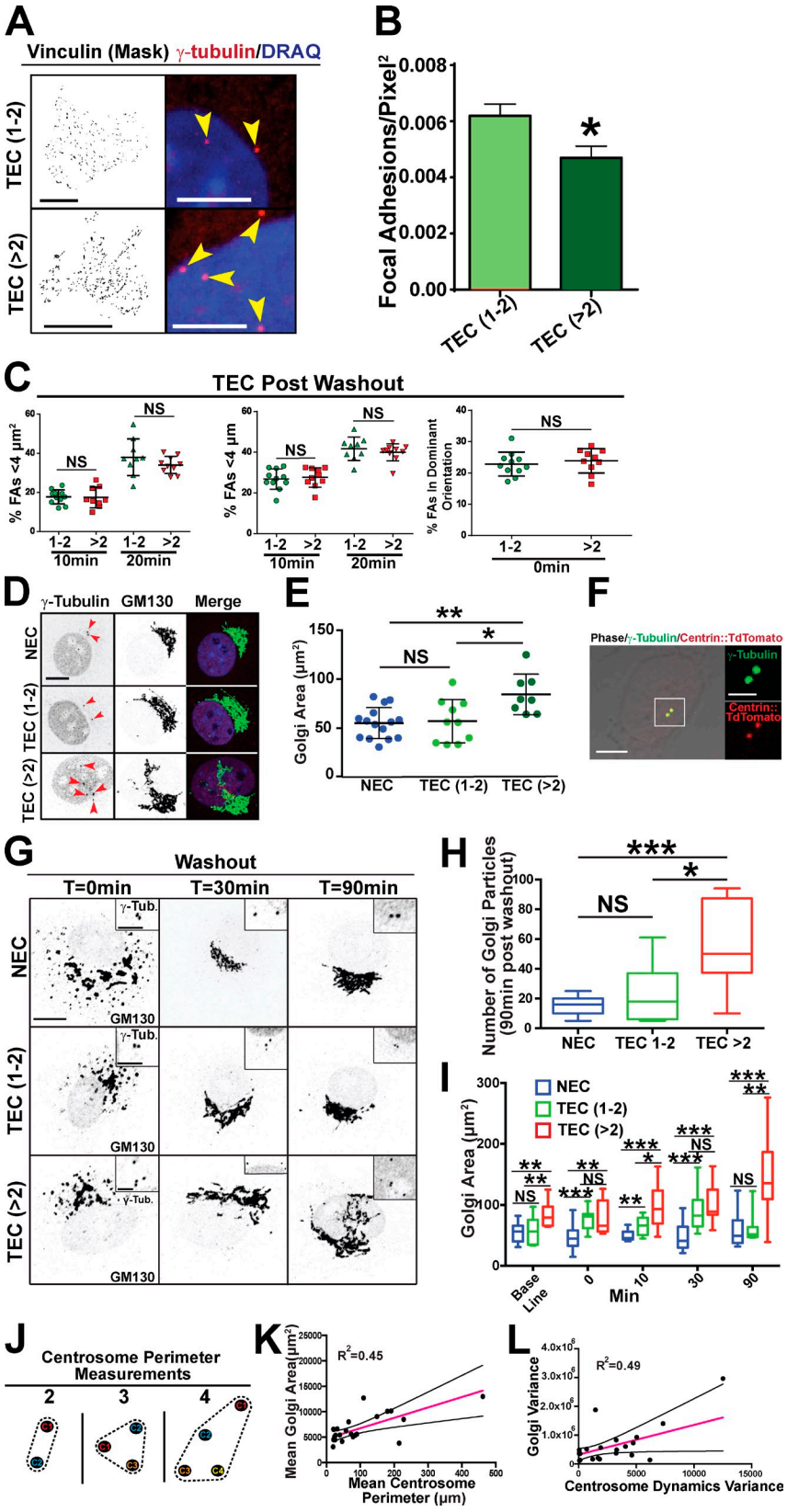


Figure S3. **Effects of excess centrosomes on FAs and Golgi.** (A) Representative image of TECs with one to two or greater than two centrosomes stained for vinculin (FAs), γ-tubulin (centrosomes), and DRAQ7 (DNA). Yellow arrows mark individual centrosomes. Bars: (left) 20 µm; (right) 10 µm. (B) Mean number of FAs normalized to cell area (TEC 1–2, n = 11; TEC >2, n = 10). Student's t test; means ± SEM; *, P < 0.05. (C) Comparison of FAs in TECs with one to two versus greater than two centrosomes after nocodazole washout at the indicated times. (left) Percentage of small FA (area); (middle) percentage of short FA (length); (right) percentage of FA in dominant quadrant. (D) NECs and TECs stained for Golgi (GM130, green), centrosomes (γ-tubulin, red), and DNA (DRAQ7, blue). Red arrows indicate individual centrosomes. Bar, 10 µm. (E) Golgi area (GM130) of NECs (n = 15) and TECs with either one to two (n = 10) or greater than two (n = 8) centrosomes. Scatter plot with mean (middle bars) and 95% confidence intervals are shown for each group. Statistical comparisons: Student's t test; *, P < 0.05; **, P < 0.01. (F) Cell expressing centrin::tdTomato and stained for γ-tubulin. White box denotes area of higher magnification. Bars: (left) 5 µm; (right) 2.5 µm. (G) Representative images of Golgi reformation after nocodazole washout between the indicated groups. Bars: (main images) 10 µm; (insets) 4 µm. (H) Box and whisker plots (middle bars, mean; boxes top and bottom quartiles with error bars [±SEMs]) of the number of individual Golgi particles in NECs or TECs 90 min after nocodazole washout (NEC, n = 13; TEC 1–2, n = 9; TEC >2, n = 9 cells). Statistical comparisons: Student's t test; *, P < 0.05; ***, P < 0.001. (I) Box and whisker plots (middle bars, mean; boxes top and bottom quartiles with error bars [±SEMs]) of Golgi area in NECs or TECs 90 min after nocodazole washout (NEC, n = 16; TEC 1–2, n = 7; TEC >2, n = 8 cells). Statistical comparisons: Student's t test; *, P < 0.05; **, P < 0.01; ***, P < 0.001. (J) Diagram of centrosome perimeter measurement. Circles represent centrosomes (C1–C4), and dotted lines indicate the length (perimeter) encapsulating all centrosomes in a given cell. (K and L) Correlations between indicated variables. Top and bottom black lines indicated 95% confidence intervals, and pink line (middle) is best-fit linear regression with indicated R^2 value (n = 22 cells for both graphs).

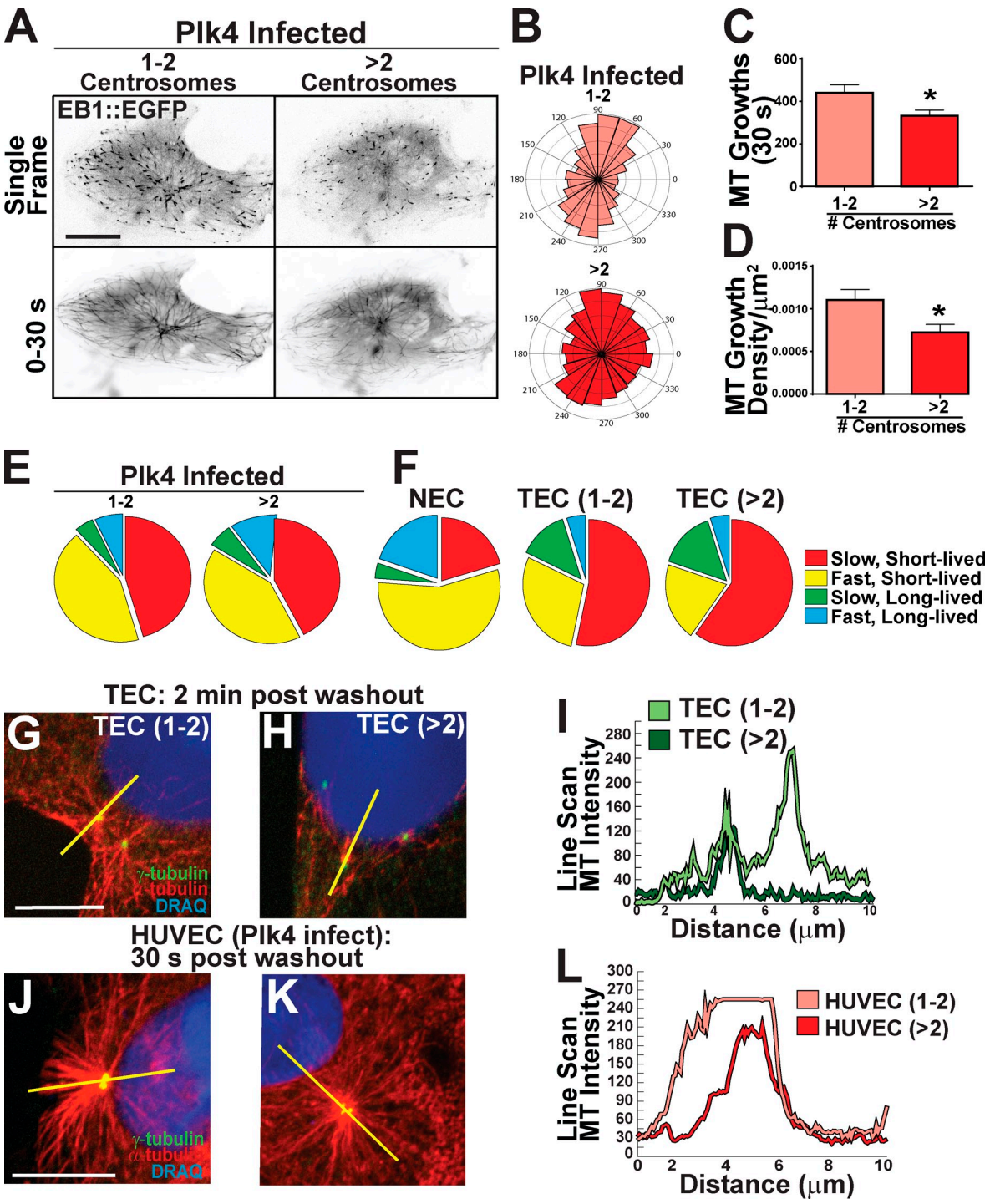


Figure S4. **Excess centrosomes reduce MT polarity and nucleations.** (A) Representative images of HUVECs expressing EB1-EGFP to mark growing MT ends and Plk4 (to induce centrosome overduplication) in the indicated groups. Bottom images are 30-s time projections. (B) Representative Rose plots showing percentage of MT growths by angle between the indicated groups. Note the more randomized distribution of MT growth angles in HUVECs with greater than two centrosomes. (C and D) Mean number of new MT growths (C) and new MT growths normalized to cell area (D) in the indicated groups. Statistics: Student's *t* test; means ± SEM. *, $P < 0.05$. (E and F) MT tip-tracking data grouped by MT growth speed and lifetime. Slow, short lived (<9 µm/min and <9.0 s); fast, short lived (>9 µm/min and <9.0 s); slow, long lived (<9 µm/min and >9.0 s); or fast, long lived (>9 µm/min and >9.0 s) are shown. (G–I) Representative images of TECs in the indicated groups after nocodazole washout stained for γ-tubulin (centrosomes), α-tubulin (MTs), and DRAQ7 (DNA). Yellow lines, MT intensity scan graphed on right (I). (J–L) Representative images of HUVECs in the indicated groups after nocodazole washout stained for γ-tubulin (centrosomes), α-tubulin (MTs), and DRAQ7 (DNA). Yellow lines, MT intensity scan graphed on right (L). Bars, 10 µm.

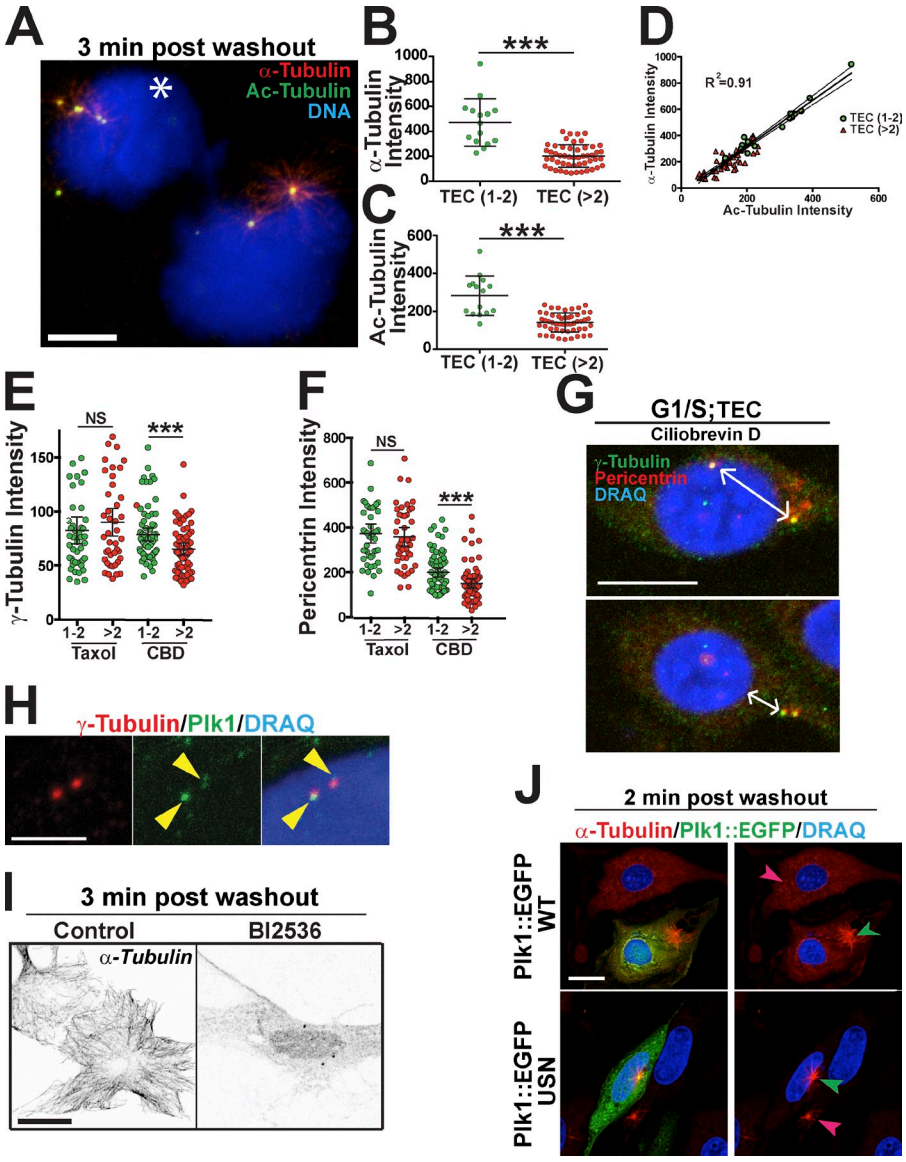
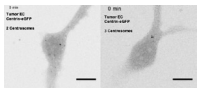
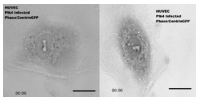


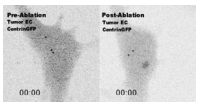
Figure S5. **Supernumerary centrosomes have reduced PCM.** (A) Representative image of a TEC after nocodazole washout stained for α-tubulin (MTs), Ac-tubulin (stabilized MTs), and DRAQ7 (DNA). White asterisk denotes cells with greater than two centrosomes. Bar, 10 µm. (B and C) Mean fluorescent intensity at the centrosome of α-tubulin (B) and Ac-tubulin (C) in TECs with one to two and greater than two centrosomes. Middle lines are means, and lines above and below are 95% confidence intervals. (TEC 1–2, *n* = 15 centrosomes; TEC >2, *n* = 51 centrosomes for both graphs). Student's *t* test; ***, P < 0.001. (D) Correlation between α-tubulin and Ac-tubulin intensities between the indicated groups (NEC, *n* = 15; TEC, *n* = 51). (E and F) Mean fluorescent intensity at the centrosome of γ-tubulin (E) and pericentrin (F) of TEC with one to two and greater than two centrosomes with the indicated treatments. (taxol: TEC 1–2, *n* = 47 centrosomes; TEC >2, *n* = 43; ciliobrevin D: TEC 1–2, *n* = 71 centrosomes; TEC >2, *n* = 65 centrosomes). Student's *t* test; ***, P < 0.001. (G) Representative images of TECs treated with ciliobrevin D (CBD) and stained for centrosomes (γ-tubulin and pericentrin) and DNA (DRAQ7). Arrows show centrosome–centrosome distance (top) and centrosome–nucleus distance (bottom) with treatment. Bar, 10 µm. (H) Representative image of a TEC stained for Plk1, centrosomes (γ-tubulin), and DNA (DRAQ7). Yellow arrows denote colocalization of Plk1 and γ-tubulin. Bar, 5 µm. (I) Representative image of a G1/S-arrested TEC stained for α-tubulin (MT) after nocodazole washout. (left) control; (right) incubation with BI2536 Plk1 inhibitor. Bar, 20 µm. (J) TECs expressing the indicated Plk1 constructs and after nocodazole washout, stained for α-tubulin (MTs) and DRAQ7 (DNA). Green arrows denote MT arrays in cells expressing Plk1, and pink arrows indicate MTs in adjacent nonexpressing cells. Right images are the same as left images without the green channel. Bar, 20 µm.



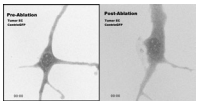
Video 1. **Migration of TECs.** Related to Fig. 1. TECs expressing centrin::EGFP to visualize centrosomes (left, two centrosomes; right, greater than two centrosomes). Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV10i; Olympus). Frames taken at 10-min intervals. Bars, 10 µm.



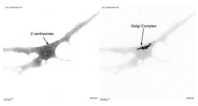
Video 2. **Migration of HUVECs with excess centrosomes.** Related to Fig. 1. HUVECs expressing Plk4 to induce centrosome overduplication and centrin::EGFP to label centrosomes (left, one to two centrosomes; right, greater than two centrosomes). Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV10i; Olympus). Time is given in minutes and seconds. Frames taken at 7-min intervals. Bars, 10 µm.



Video 3. **Centrosome ablation and change in centrosome dynamics.** Related to Fig. 2. TECs expressing centrin::EGFP to visualize centrosomes. (left) A TEC with three centrosomes, before ablation; (right) same cell with two centrosomes, after ablation. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000; Olympus). Time is given in minutes and seconds. Frames taken at 10-min intervals.

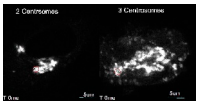


Video 4. **Centrosome ablation and change in directional migration.** Related to Fig. 2. TECs expressing centrin::EGFP to visualize centrosomes. (left) A TEC with four centrosomes, before ablation; (right) same cell with two centrosomes, after ablation. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000; Olympus). Time is given in minutes and seconds. Frames taken at 14-min intervals.

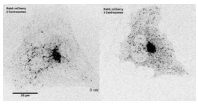


Video 5. **Golgi dynamics of TEC with two centrosomes.** Related to Fig. 3. TECs with two centrosomes expressing centrin::tdTomato (left channel) to label centrosomes and GalT::GFP (right channel) to label the Golgi. Cell were plated on poly-L-lysine to reduce migration out of frame. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (CV1000; Yokogawa Electric Corporation). Frames taken at 1.5-min intervals.

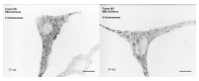
Video 6. **Golgi dynamics of TECs with greater than two centrosomes.** Related to Fig. 3. TECs with two centrosomes (cent) expressing centrin::tdTomato (left channel) to label centrosomes and GalT::GFP (right channel) to label the Golgi. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (CV1000; Yokogawa Electric Corporation). Frames taken at 1.5-min intervals.



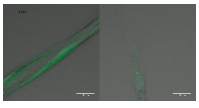
Video 7. **FRAP to determine Golgi integrity.** Related to Fig. 4. A TEC with one to two (left) or greater than two (right) centrosomes, expressing GalT::GFP (Golgi). Cells were photobleached and monitored for recovery. Red circles and arrows indicate areas of photobleaching. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1200; Olympus). Frames taken at 2-s intervals.



Video 8. **Vesicle trafficking in a TEC with and without excess centrosomes.** Related to Fig. 4. A TEC with one to two (left) or greater than two (right) centrosomes expressing Rab6::mCherry to visualize post-Golgi vesicle trafficking. Cell on right displays less localized vesicle trafficking. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1200; Olympus). Frames taken at 1-s intervals.



Video 9. **MT tip tracking of TECs.** Related to Fig. 5. A TEC with one to two (left) or greater than two (right) centrosomes expressing EB3::mCherry to visualize growing MT ends. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (Nipkow disk confocal; PerkinElmer/Yokogawa Electric Corporation). Frames taken at 1–2-s intervals. Bars, 10 µm.



Video 10. **Cell migration of HUVECs during sprouting angiogenesis.** Related to Fig. 7. HUVECs expressing centrin::GFP to mark centrosomes (left, one to two centrosomes; right, greater than two centrosomes) overlaid with phase to show overall cellular morphology, sprouting in a fibrin matrix. Images were taken by time-lapse confocal microscopy (FV1200; Olympus). Frames taken at 20-min intervals.