Supplemental Information for:

Gravin-associated kinase signaling networks coordinate γ-tubulin organization at mitotic spindle poles

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This file includes:
Figures S1-S4

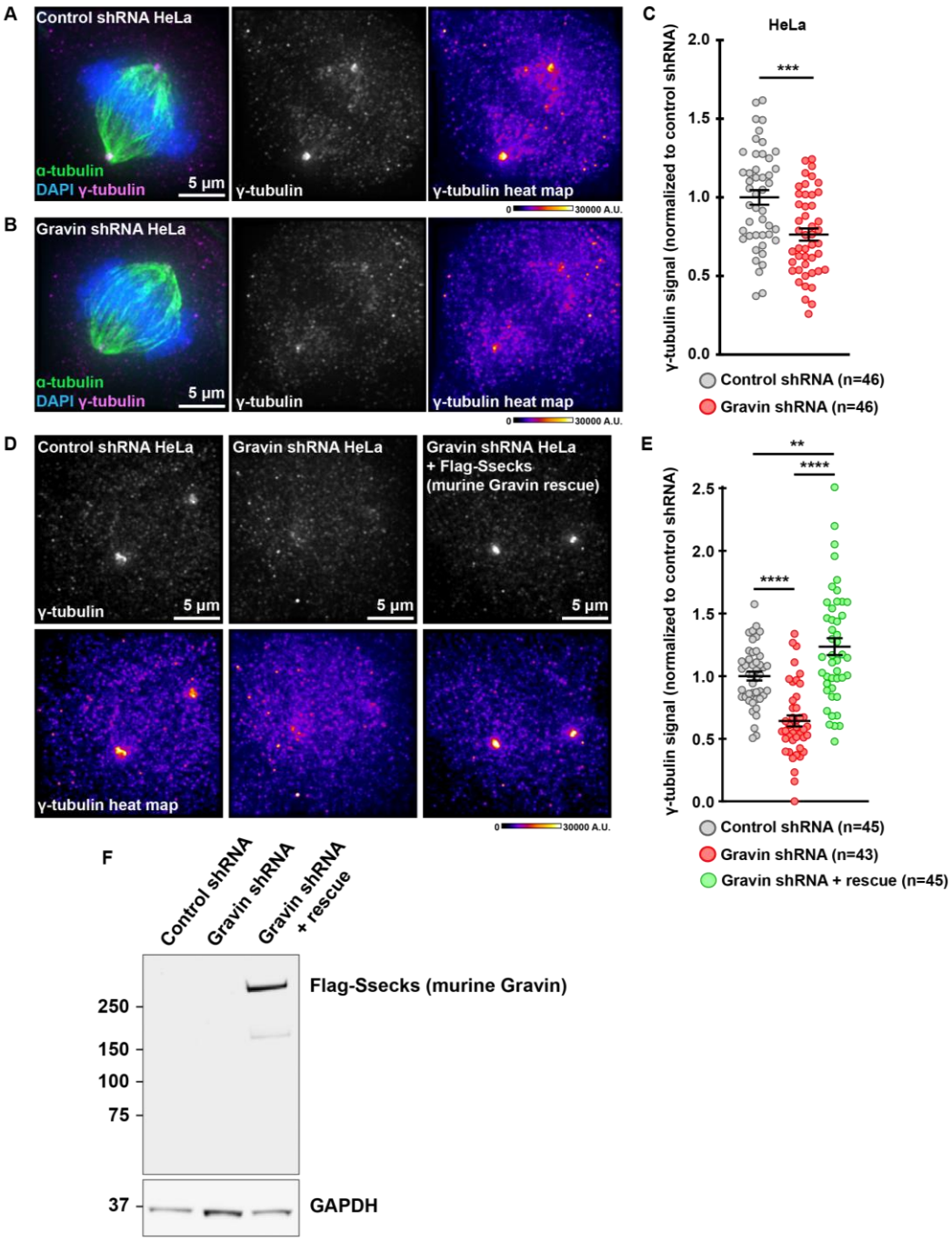

Figure S1: Loss of Gravin in HeLa cells perturbs accumulation of γ-tubulin at mitotic spindle poles. **A & B**. Immunofluorescence of representative control shRNA (**A**) and Gravin shRNA (**B**) mitotic HeLa cells. Composite images (left) show α-tubulin (green), DAPI (blue), and γ-tubulin (magenta). Distribution of γ-tubulin is represented in grayscale (middle) and with pseudo-color heat maps (right). Signal intensity scale (A.U.) is shown below. **C**. Quantification of amalgamated γ-tubulin immunofluorescence data at spindle poles in control shRNA (gray) and Gravin shRNA (red) HeLa cells; control shRNA, n=46, Gravin shRNA, n=46, ***p=0.0002. **D**. Immunofluorescence of representative mitotic HeLa cells stably expressing a control shRNA (left), Gravin shRNA (middle), or Gravin shRNA with Flag-Ssecks, a murine Gravin rescue construct (right). Distribution of γ-tubulin is represented in grayscale (top) and with pseudo-

color heat maps (bottom). Signal intensity scale (A.U.) is shown below. **E**. Quantification of amalgamated γ-tubulin immunofluorescence data at spindle poles in control shRNA (gray), Gravin shRNA (red), and Gravin shRNA + rescue (green) HeLa cells. Prior to fixation, cells were treated for 24 hr with a CDK1 inhibitor, RO3306, followed by a 25 min washout to enrich for mitotic cells; control shRNA, n=45, Gravin shRNA, n=43, ****p<0.0001; Gravin shRNA, n=43, Gravin shRNA + rescue, n=45, ****p<0.0001; control shRNA, n=45, Gravin shRNA + rescue, n=45, **p=0.0027. Points in **C** and **E** represent individual cells (n). Data in **C** and **E** are normalized to control shRNA. Cells were analyzed over three experiments (N=3). P values were calculated by unpaired two-tailed Student's t-test. Data are mean ± s.e.m. **F**. Immunoblot detection of Flag-Ssecks using an anti-Flag antibody (top) and GAPDH (bottom) in HeLa cells stably expressing a control shRNA (lane 1), Gravin shRNA (lane 2), or Gravin shRNA with the rescue construct (lane 3).

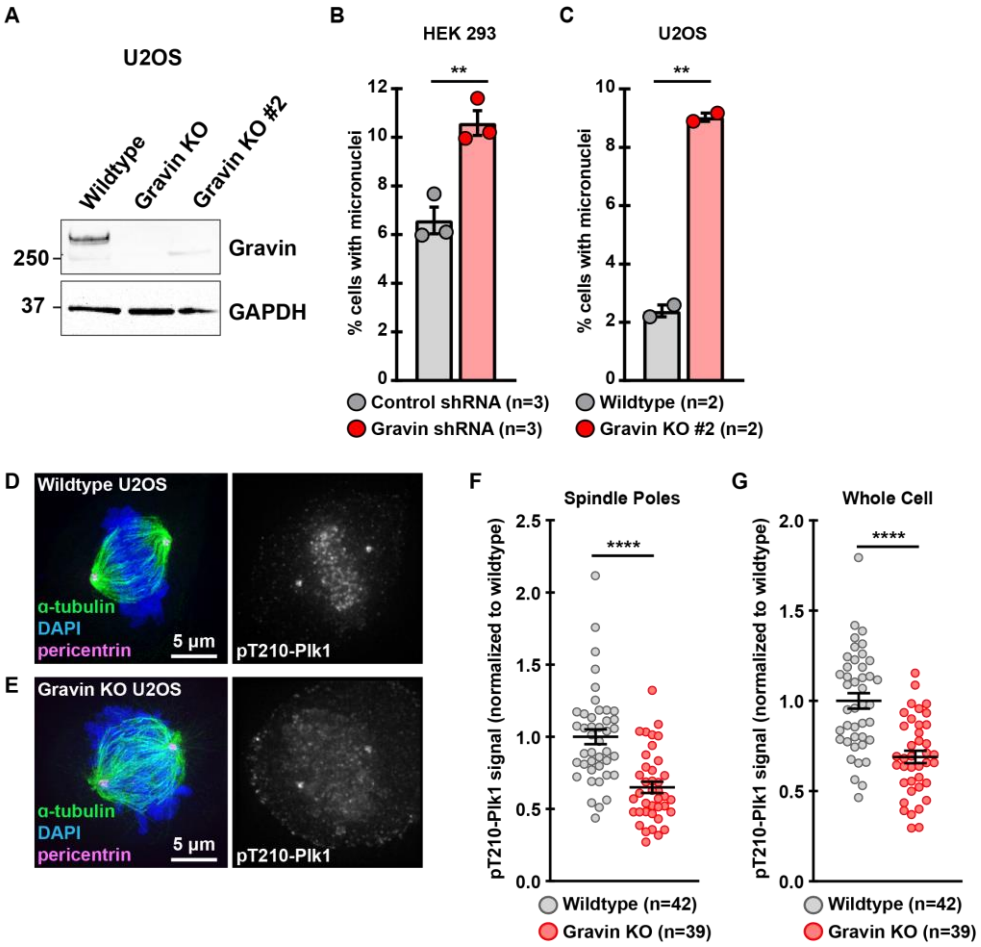

Figure S2: Further validation of Gravin knockout U2OS cells. A. Immunoblot detection of Gravin (top) using a rabbit antibody and GAPDH (bottom) in wildtype and Gravin KO clonal U2OS cells. **B & C**. Quantification representing the percent (%) of cells with micronuclei in HEK 293 (**B**) and wildtype and CRISPR/Cas9-edited U2OS clone 2 (**C**) cells. Points depict individual experiments (n); (**B**) control shRNA, n=3, Gravin shRNA, n=3, **p=0.0059; (**C**) wildtype, n=2, Gravin KO, n=2, **p=0.0014. A total of 1500 (**B**) or 1000 (**C**) cells were analyzed over three (**B**) or two (**C**) independent experiments. **D & E**. Immunofluorescence of representative wildtype (**D**) and Gravin KO (**E**) U2OS cells during mitosis. Composite SIM images (left) show α-tubulin (green), DAPI (blue), and pericentrin (magenta). Widefield images show pT210-Plk1 signal in grayscale (right). **F & G**. Quantification of amalgamated pT210-Plk1 immunofluorescence signal at spindle poles (**F**) or in the whole cell (**G**) in wildtype (gray) and Gravin KO (red) U2OS cells. Points represent individual cells (n). Data are normalized to wildtype; wildtype, n=42, Gravin KO, n=39, ****p<0.0001; Immunofluorescence experiments were conducted three times (N=3). P values were calculated by unpaired two-tailed Student's t-test. Data are mean ± s.e.m.

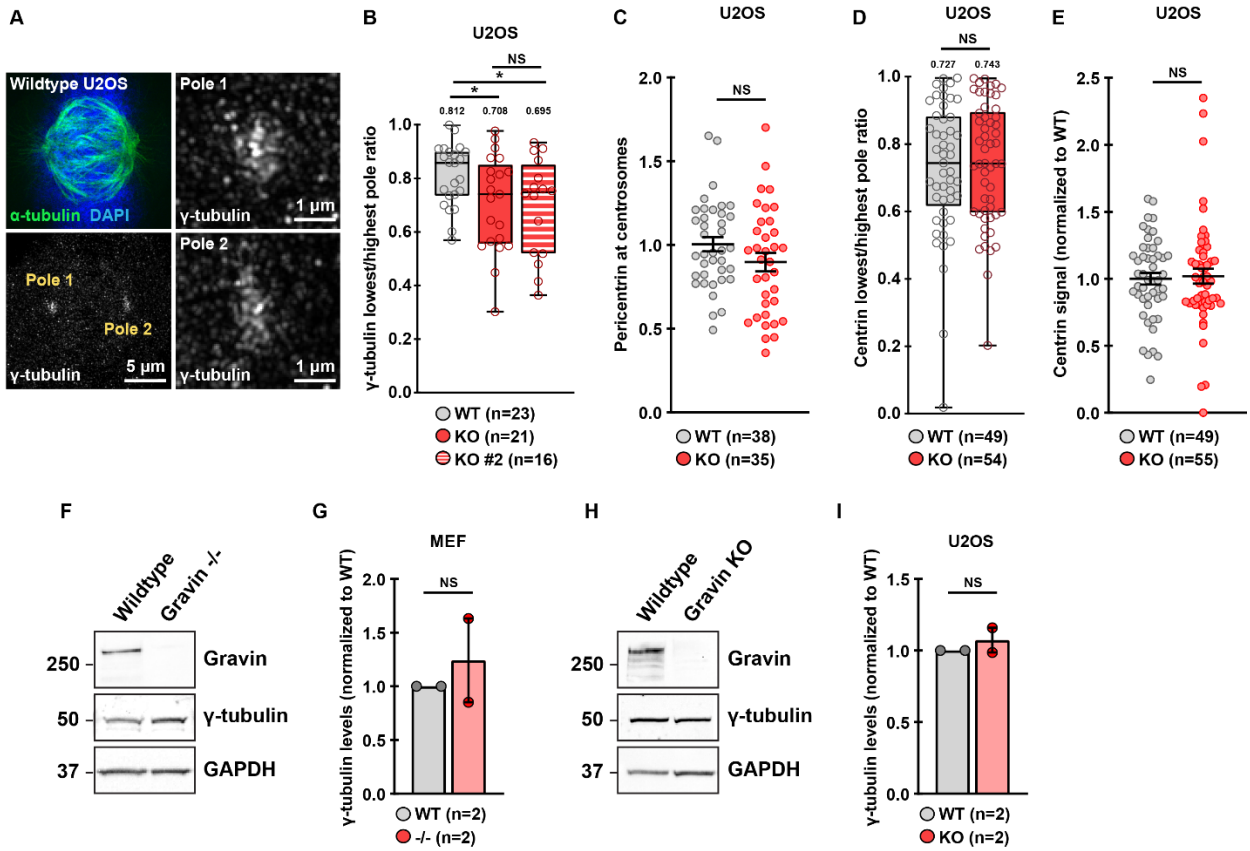

Figure S3: Pole-to-pole distribution of γ-tubulin in U2OS cells. A. SIM micrograph of a representative wildtype U2OS cell during mitosis. Composite image (top left) shows α-tubulin (green) and DAPI (blue). Grayscale image depicts γ-tubulin at both poles (bottom left). Magnified images reveal that pole 1 (top right) accumulates more γ-tubulin than pole 2 (bottom right). **B.** WT and Gravin KO conditions from Figure 3K with the addition of data for Gravin KO #2. Quantification of γ-tubulin immunofluorescence at each pole represented as box plots showing lowest/highest pole ratio in wildtype (gray), Gravin KO, (red) and Gravin KO #2 (red and white stripes) CRISPR/Cas9-edited U2OS mitotic cells. Mean values are indicated above each plot; WT, n=23, KO, n=21, *p=0.0263; WT, n=23, KO #2, n=16, *p=0.0190; KO, n=21, KO #2, n=16, p=0.8225. Experiments were conducted three times (N=3) for WT and KO and two times (N=2) for KO #2. **C**. Quantification of amalgamated pericentrin immunofluorescence data at spindle poles in wildtype (gray) and Gravin KO (red) U2OS cells. WT, n=38, KO, n=35, p=0.1256. **D**. Quantification of centrin immunofluorescence at each pole represented as box blots showing lowest/highest pole ratio in wildtype (gray) and Gravin KO (red) U2OS mitotic cells. Mean values are indicated above each plot; WT, n=49, KO, n=54, p=0.6671. **E**. Quantification of amalgamated centrin immunofluorescence data at spindle poles in wildtype (gray) and Gravin KO (red) U2OS cells. WT, n=49, KO, n=55, p=0.7883. **F-I**. Total protein levels in wildtype and Gravin null whole cell lysates. Immunoblot detection of Gravin (top), γ-tubulin (middle), and GAPDH (bottom) in wildtype and Gravin null MEF (**F**) and U2OS (**H**) cells. Quantification of γ-tubulin signal normalized to total protein levels as determined by GAPDH (**G**) or ponceau (**I**) signal; (**G**) WT, n=2, -/-, n=2, p=0.5978; (**I**) WT, n=2, KO, n=2, p=0.4847. Points in **B-E** represent individual cells (n) and points in **G** and **I** represent individual experiments (n). Data in **C, E, G** and **I** are normalized to wildtype. Cells were analyzed over three (**C-E**) or two (**G** & **I**) independent experiments (N=2-3). P values were calculated by unpaired two-tailed Student's t-test. Data are mean ± s.e.m. NS, not significant.

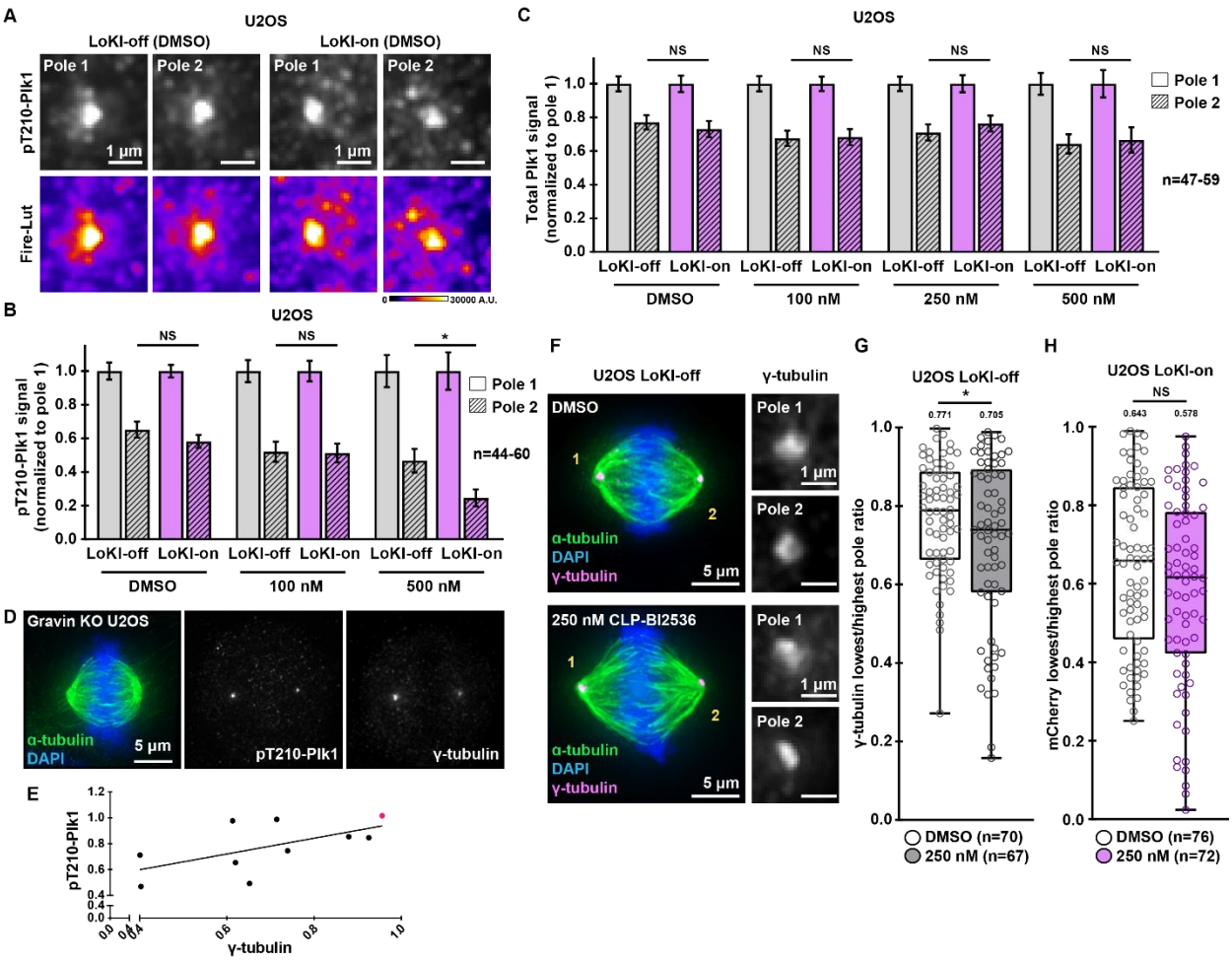

Figure S4: Plk1 inhibition promotes an asymmetric distribution of active kinase and γ-tubulin.
A. Immunofluorescence of pT210-Plk1 at individual spindle poles in representative LoKI-off (left) and LoKI-on (right) U2OS cells treated with DMSO for 4 hr. Signal of active kinase is represented in grayscale (top) and with pseudo-color heat maps (bottom). Signal intensity scale (A.U.) is shown below. **B**. Quantification of pT210-Plk1 immunofluorescence at each pole after 4 hr treatment with DMSO or CLP-BI2536; pole 2: DMSO, LoKI-off, n=44, LoKI-on, n=60, p=0.2477; 100 nM, LoKI-off, n=46, LoKI-on, n=59, p=0.9257; 500 nM, LoKI-off, n=53, LoKI-on, n=60, *p=0.0102. **C**. Quantification of total Plk1 immunofluorescence at each pole after 4 hr treatment with DMSO or CLP-BI2536; pole 2: DMSO, LoKI-off, n=52, LoKI-on, n=59, p=0.5380; 100 nM, LoKI-off, n=52, LoKI-on, n=58, p=0.9172; 250 nM, LoKI-off, n=55, LoKI-on, n=47, p=0.4275; 500 nM, LoKI-off, n=52, LoKI-on, n=52, p=0.8007. **D**. Immunofluorescence of a representative Gravin KO U2OS cell during mitosis. Composite image (left) shows α-tubulin (green) and DAPI (blue). Grayscale image depicts pT210-Plk1 (middle) and γ-tubulin at spindle poles (right). **E**. Graph depicting pT210-Plk1 lowest/highest pole ratio plotted against γ-tubulin lowest/highest pole ratio in Gravin KO U2OS cells. For all cells but one (magenta) the pole with the higher pT210-Plk1 signal is also the pole with the higher γ-tubulin signal. **F**. Immunofluorescence of representative LoKI-off mitotic cells treated with DMSO (top) or 250 nM CLP-BI2536 (bottom) for 4 hr. Composite images (left) show α-tubulin (green), DAPI (blue), and γ-tubulin (magenta). Magnified grayscale images (right) show γ-tubulin signal at individual poles. **G**. Quantification of amalgamated γ-tubulin immunofluorescence data. Data represented as box plots showing lowest/highest pole ratio for LoKI-off cells after 4 hr treatment with DMSO (white) or 250 nM CLP-BI2536 (gray) followed by a 1 hr washout. Mean values are indicated above each plot; DMSO, n=70, 250 nM, n=67, *p=0.0370. **H**.

Quantification of amalgamated mCherry immunofluorescence data. Data represented as box plots showing lowest/highest pole ratio for LoKI-on cells after 4 hr treatment with DMSO (white) or 250 nM CLP-BI2536 (purple) followed by a 1 hr washout. Mean values are indicated above each plot; DMSO, n=76, 250 nM, n=72, p=0.0846. Values depicted in **B**, **C, G** and **H** represent individual cells (n). Data in **B** and **C** are normalized to pole 1. Experiments were conducted at least three times (N=3). P values were calculated by unpaired two-tailed Student's t-test. Data are mean ± s.e.m. NS, not significant.