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Supplementary Materials for

Cell cycle–dependent centrosome clustering precedes proplatelet formation

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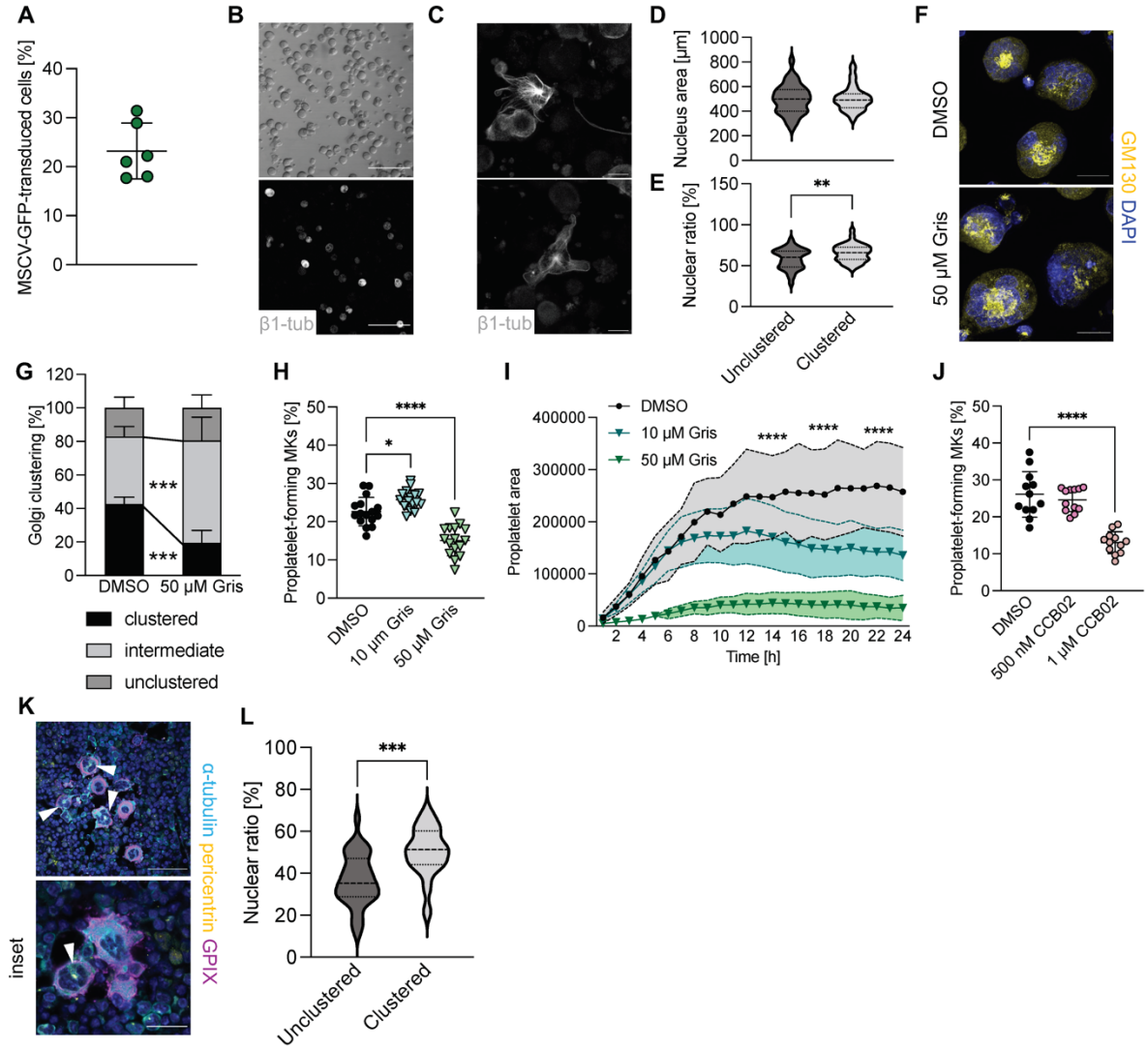

Fig S1. **(A)** Flow cytometric analysis of cells transduced with an empty MSCV-EGFP vector control. N=6. **(B)** Representative images of transduction efficiency of FLMKs transduced with MSCV-β1-tubulin-dendra2. Scale bars: 150 µm. **(C)** Representative images of MSCV-β1-tubulin-dendra2-transduced FLMKs containing clustered centrosomes during initiation of proplatelet formation. Scale bars: 20 µm. **(D, E)** Nucleus area **(D)** and relation to cell size **(E)** was analyzed in MKs with unclustered vs. clustered centrosomes. N=2. At least 50 cells were analyzed per mouse. Unpaired, two-tailed student's t-test. $P < 0.01$ **. **(F, G)** Visualization and quantification of Golgi ribbon formation in BMMKs treated with DMSO or 50 µM Gris for 6 hours. Golgi apparatus was visualized using an antibody against the Golgi membrane protein GM130. Golgi distribution was quantified manually using ImageJ Software. Scale bars: 20 µm. N=3. At least 50 cells were analyzed per mouse. Two-way ANOVA with Sidak correction for multiple comparisons. $P < 0.001$ ***. **(H, I)** Percentage of proplatelet-forming MKs at 24h and proplatelet area upon treatment with DMSO, 10 or 50 µM Gris was assessed using an automated imaging platform and custom analysis pipeline [87]. N=2; 4 technical replicates. One-way ANOVA with Sidak correction for multiple comparisons. $P < 0.0001$ ****. **(J)** Percentage of proplatelet-forming MKs at 24h upon treatment with

CCB01. One-way ANOVA with Sidak correction for multiple comparisons. $P < 0.0001$ ****. **(K)** Representative images of MKs containing clustered centrosomes in situ. Arrows point at clustered centrosomes. Scale bars: 50 µm. **(L)** DNA area in relation to cell size was analyzed in MKs with unclustered vs. clustered centrosomes in situ. N=4. At least 20 cells were analyzed per mouse. Unpaired, two-tailed student's t-test. $P < 0.001$ ***. All data are presented as mean ± SD.

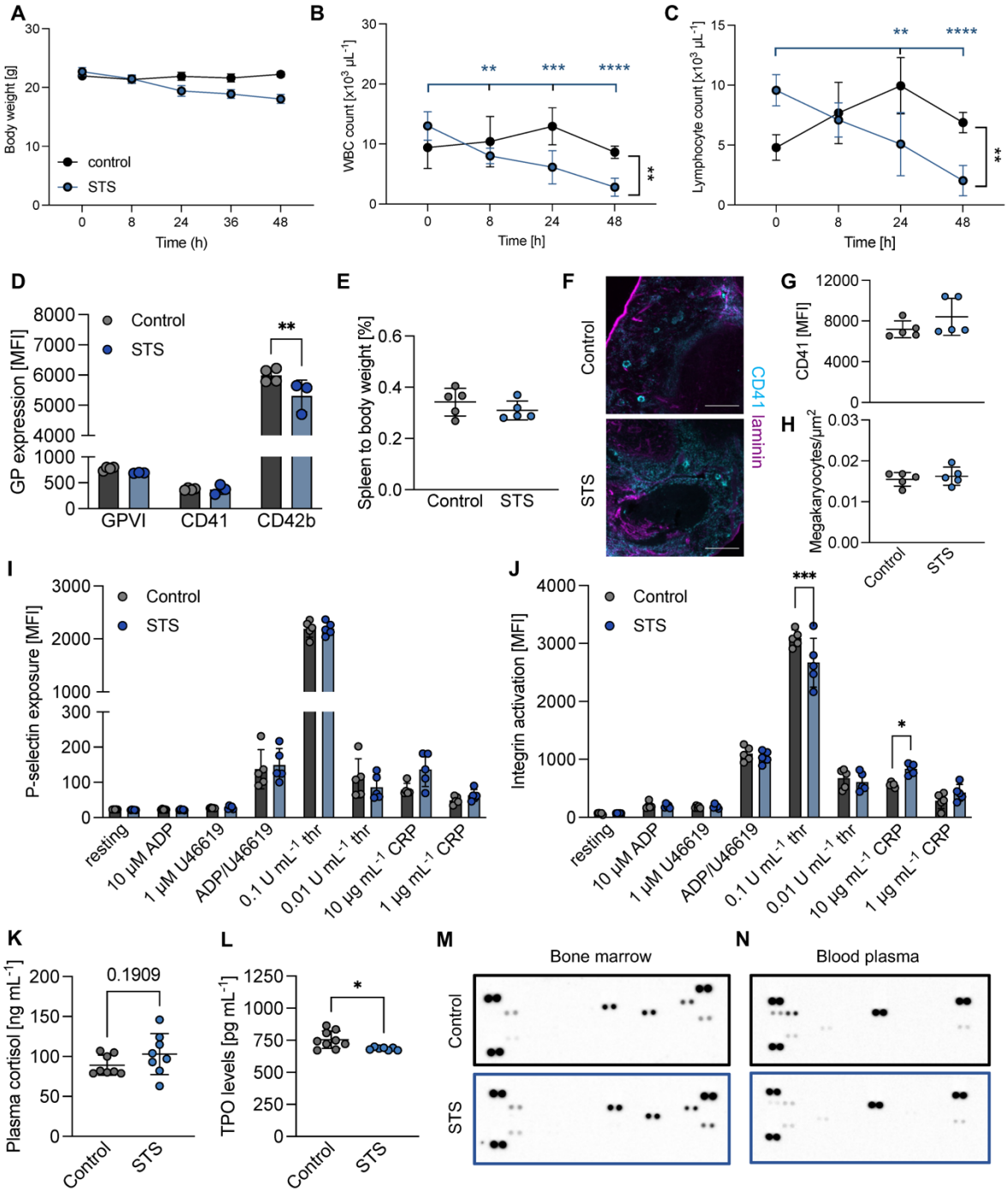

Fig S2. (A) Body weight of control and STS mice was measured at the indicated timepoints. N=5. Data are presented as mean ± SD. **(B, C)** White blood cell and lymphocyte counts of control and STS mice were assessed using an automated blood cell analyzer. N=5. One-way ANOVA with Sidak correction for multiple comparisons. P < 0.0001 ****. **(D)** Glycoprotein (GP) expression of control and STS platelets was assessed by flow cytometry. N=4. Two-way ANOVA with Sidak correction for multiple comparisons. P < 0.01 **. **(E)** Spleen weight was normalized to body weight. N=5. Data are presented as mean ± SD. **(F)** Spleens from control and STS mice were sectioned, stained for laminin and CD41 and imaged using an

automated imager. Scale bars: 100 µm. **(G, H)** CD41 MFI and MK numbers were assessed using an automated image analysis software. N=5. **(I, J)** Platelet integrin activation and P-selectin exposure were analyzed by flow cytometry upon stimulation with different agonists. N=5. Multiple student's t-tests. $P < 0.05$ *; $P < 0.001$ ***. **(K, L)** Plasma cortisol and TPO levels in control and STS mice was assessed using enzyme-based immunosorbent assays. N=9. Unpaired, two-tailed student's t-test. $P < 0.05$ *. **(M, N)** Bone marrow and blood plasma were isolated from control and STS mice and subjected to cytokine profiling. Plasma was pooled from 9 mice. All data are presented as mean ± SD.

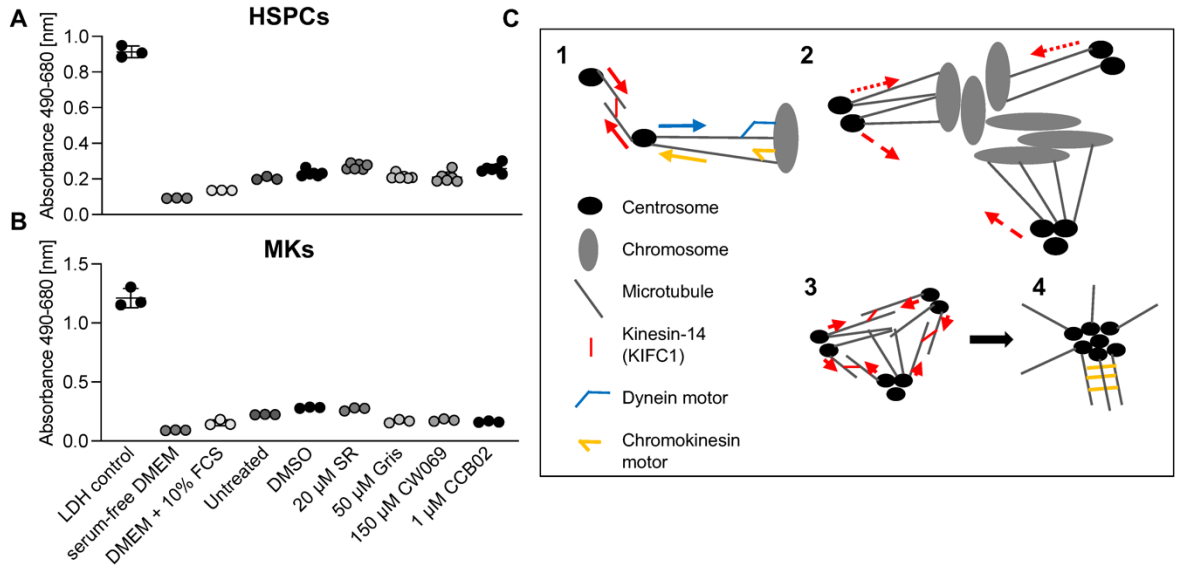

Fig S3. (A, B) Cytotoxicity of the inhibitors SR31527 (SR), CW069, griseofulvin (Gris) and CCB02 in hematopoietic stem and progenitor cells (HSPCs) cultured for 3 days **(A)** as well as mature BMMKs cultured for 24h **(B)** was assessed using an LDH cytotoxicity assay per the manufacturers' instructions. Absorbance was measured at a plate reader. **(A)** N=2; **(B)** N=1; 3 technical replicates. Data are presented as mean ± SD. **(C)** Simulation-based modeling of interactions between chromosomes and centrosomes [64, 65]: Kinesin-14 motors like KIFC1 (red) stretching from two centrosomes generate effective inter-centrosomal attraction forces (red arrows) (1). Microtubules interacting with dynein motors (blue) on the kinetochores are pulled toward the chromosomes causing the effective attraction force between the centrosome and chromosome (blue arrow). Microtubules interacting with chromokinesin motors (purple) on the chromosome arms are pushed away from the chromosomes causing the effective repulsion force between the centrosome and chromosome (purple arrow). Two opposing centrosome-chromosome forces position centrosomes at a characteristic distance from the chromosomes (2). The chromosomes aggregate to several clusters at the center, while the effective attraction clusters some of the centrosomes and localizes them around the chromosomes resulting in multipolarity. The centrosomal clusters do not aggregate further because the attraction between them is either blocked by the chromosomes in between (dotted arrows) or is too weak because of the large inter-centrosomal cluster distances (dashed arrows). Following DNA decondensation, the only remaining force is the attraction between the centrosomal clusters (3). This KIFC1-generated attraction force clusters all centrosomes together. The resulting combined microtubule aster is so dense that crosslinking action of MAPs could lead to bundling of microtubules (4), from which they are distributed to the cell cortex.

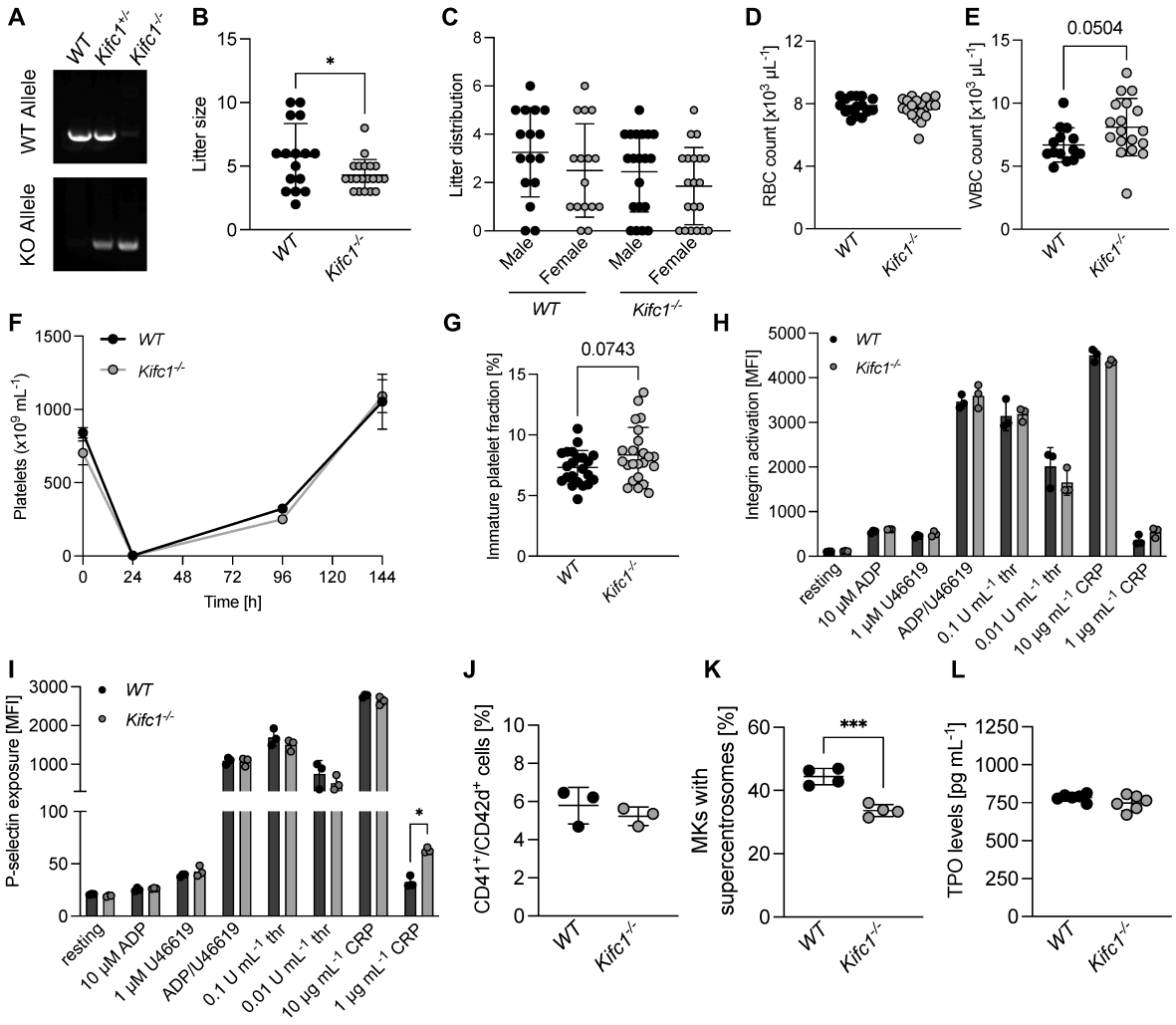

Fig S4. (A) PCR for wildtype (WT) and mutant (KO) *Kifc1* allele using DNA derived from *Kifc1*$^{-/-}$, heterozygous (*Kifc*$^{+/-}$) and *WT* mice. **(B, C)** Analysis of litter sizes **(B)** and sex distribution among the litters **(C)** of *WT* and *Kifc1*$^{-/-}$ mice. Unpaired, two-tailed student's t-test. $P < 0.05$. **(D, E)** Red and white blood cell counts in *WT* and *Kifc1*$^{-/-}$ mice were assessed using an automated blood cell analyzer. N=25. Unpaired, two-tailed student's t-test. **(F)** Platelet recovery after depletion was assessed using an automated blood cell analyzer. N=3. **(G)** Immature platelet fraction in *WT* and *Kifc1*$^{-/-}$ mice was assessed using an automated blood cell analyzer. N=25. Unpaired, two-tailed student's t-test. **(H, I)** Activation of platelet $\alpha_{IIb}\beta_3$ integrins and exposure of P-selectin were analyzed by flow cytometry upon stimulation with different agonists. N=4. Unpaired, two-tailed student's t-test. $P < 0.05$. **(J)** Percentage of MKs expressing CD41 and CD42d after 4 days of culturing was assessed by flow cytometry. N=3. **K)** Number of MKs containing clustered centrosomes was quantified by immunofluorescence stainings for pericentrin and α-tubulin. N=4. Unpaired, two-tailed student's t-test. $P < 0.001$ ***. **(L)** TPO levels in plasma derived from WT or *Kifc1*$^{-/-}$ mice were analyzed using an enzyme-linked immunosorbent assay. N=6. All data are presented as mean ± SD.

Table S1.

Gene	Protein	Method	Function
Tpx2	Microtubule Nucleation Factor	Ubiquitin Pulldown & Polysome Profiling [48]	Spindle assembly factor required for normal assembly of mitotic spindles. Mediates AURKA localization to spindle microtubules [89].
Aurka	Aurora Kinase A	Polysome Profiling [48]	Associates with the centrosome and the spindle microtubules during mitosis and plays a critical role in various mitotic events [90].
Aurkb	Aurora Kinase B	Polysome Profiling [48]	Kinase component of the chromosomal passenger complex (CPC), a key regulator of mitosis [90].
Plk1, 4	Polo-like Kinase 1, 4	Polysome Profiling [48]	Regulates centrosome maturation, spindle assembly, and cytokinesis. Contributes to the regulation of AURKA function [91].
Kif11	Kinesin Family Member 11	Polysome Profiling [48]	Motor protein required for establishing a bipolar spindle during mitosis [92].
Kif2a	Kinesin Family Member 2a	Ubiquitin Pulldown	Plus-end-directed microtubule-dependent motor required for progression through mitosis [93].
StarD9	STAR Related Lipid Transfer Domain Containing 9	Ubiquitin Pulldown	Microtubule-dependent motor protein required for spindle pole assembly during mitosis and to stabilize the pericentriolar material (PCM) [94].
CEP192	Centrosomal Protein 192	Ubiquitin Pulldown	Required for mitotic centrosome and spindle assembly. Appears to be a major regulator of PCM recruitment and centrosome maturation [95].

Table S1. Cell cycle proteins identified by polysome profiling [48] and ubiquitin pulldown.

Movie S1. Timelapse of MSCV-Centrin2-GFP-transduced FLMKs imaged every 10 min for 2 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Scale bar: 5 µm.

Movie S2. Timelapse of MSCV-FUCCI-transduced FLMKs imaged every hour for 24 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Scale bar: 30 µm.

Movie S3. Timelapse of MSCV-FUCCI-transduced FLMKs imaged every hour for 24 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Video shows MKs transitioning to proplatelet formation. Scale bar: 60 µm.

Movie S4. Timelapse of MSCV-β1-tubulin-dendra2-transduced FLMKs imaged every hour for 24 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Video shows MKs prior to proplatelet formation. Scale bar: 50 µm.

Movie S5. Timelapse of MSCV-β1-tubulin-dendra2-transduced FLMKs imaged every hour for 24 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Video shows MKs during proplatelet formation. Scale bar: 50 µm.

Movie S6, 7. Timelapse of MSCV-β1-tubulin-dendra2-transduced FLMKs imaged every hour for 24 hours at a Yokogawa spinning disk confocal/inverted Nikon Ti fluorescence microscope with incubation enclosure (20x objective). Video shows MKs transitioning to proplatelet formation. Scale bar: 5 µm.

Movie S8. Timelapse of FLMKs transduced with SFV-EB3-GFP visualizing EB3 comets emanating from clustered centrosomes towards the cell cortex [7]. MKs were imaged every 5 sec at a Zeiss Axiovert 200 microscope (63x objective).

Movie S9. Proplatelet formation of DMSO-treated FLMKs was imaged using the Incucyte imaging system over 24 hours. Scale bar: 150 µm.

Movie S10. Proplatelet formation of FLMKs treated with 40 µm SR31527 was imaged using the Incucyte imaging system over 24 hours. Scale bar: 150 µm.

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