**Supplemental information**

**Article title:** Microtubule sliding drives proplatelet elongation and is dependent on cytoplasmic dynein

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**Supplemental Method:**

**Isolation of human MKs.** Human umbilical cord blood was collected with the Institutional Review Board approval from healthy full term neonates (38–42 weeks gestation) at Brigham and Women’s Hospital Labor and Delivery. CD34+ cells were then isolated using a positive magnetic selection system (Miltenyi Biotec, Cambridge, MA). CD34+ cells were plated in 24-well plates at 1 x 10⁵ cells/mL, and were cultured in serum-free medium with rTPO (50 ng/mL; PeproTech, Rocky Hill, NJ), with twice weekly medium changes for 14 days, as previously described.¹ Live cell number was quantified twice weekly by staining with 0.4% Trypan blue.

**Reference:**

Supplemental Figures:

Supplemental Figure 1. Primary mouse fetal liver cell-derived megakaryocytes elongate and retract proplatelets under static tissue culture.
Representative DIC microscopy time lapse of (A) proplatelet elongation and (B) proplatelet retraction. Scale bars are 25 µm. (C) Determination of proplatelet length over time under static condition. Each color represents one proplatelet. (D) Distribution of unidirectional rates of proplatelet extension/retraction in culture. Data are derived from events in (C). (E) Determination of proplatelet length over time under physiological shear stress. Pause phases under physiological shear stress are reduced as compared to number of pause phases under static conditions in (C). Note different time scale: (C) x-axis in minutes, (E) x-axis in seconds.
Supplemental Figure 2. Schematic representation of FRAP and FLAC.

(A) Region of interest (ROI) of MK expressing β1-tubulin-Dendra2 is photoconverted with a 405 nm laser. Photoconverted spot is followed over time in relation to cell movement. (B, top) ROI of MK expressing β1-tubulin-Dendra2 is photobleached with a 488 nm laser and fluorescence intensity recovers after photobleaching. (B, bottom) Representative cell with
Supplemental Figure 3. Quantitative immunofluorescence microscopy of released proplatelets from primary mouse fetal liver cell-derived megakaryocytes in static tissue culture.

(A) Released proplatelets were probed for β1-tubulin and objects were thresholded on a binary mask to quantify perimeter and number. (B) Individual objects were plotted as perimeter (µm, x-axis) versus object count (y-axis). A minimum perimeter for barbell-proplatelet/preplatelets (30 µm), and long proplatelets (240 µm) was used to classify intermediate stages in platelet production and quantify proplatelet extension in static tissue.
Supplemental Figure 4. Released proplatelets in static tissue culture continue to elongate over time.

(A) Representative distribution of platelet and released proplatelet perimeter for a single static tissue culture over 9 hour incubation period. (B) Quantification of platelets, barbell proplatelets/preplatelets and long proplatelets over time. Analyses were performed for at least five independent samples. Error bars represent one standard deviation about the mean. Statistical significance was established using a one-tailed Student's t-test for paired samples (**P<0.01).
Supplemental Figure 5. Released proplatelets in static tissue culture continue to retract over time after Na$_3$V0$_4$ or EHNA addition.

Representative time lapse DIC microscopy of proplatelet retraction following 1 mM Na$_3$V0$_4$ and ENHA addition. Scale bars are 25 µm.
<table>
<thead>
<tr>
<th></th>
<th>Control (static culture / physiological shear stress)</th>
<th>Nocodazole (100 nM) (static culture / physiological shear stress)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ [sec]</td>
<td>55 / 6</td>
<td>30 / 8</td>
</tr>
<tr>
<td>Plateau [%]</td>
<td>72.0 / 48.4</td>
<td>61.3 / 58.6</td>
</tr>
<tr>
<td>Plateau [sec]</td>
<td>255 / 30</td>
<td>215 / 28</td>
</tr>
</tbody>
</table>

**Supplemental Figure 6.** Comparison of FRAP values derived from control and 100 nM nocodazole-treated samples (static culture / physiological shear stress).
Supplemental Figure 7. Strongly decreased FRAP in proplatelets treated with taxol and Na₃V₀₄ in the platelet bioreactor.

Cells were preincubated with 10 µM taxol or 1 mM Na₃V₀₄ and fluorescence intensity in photobleached ROI was measured. Na₃V₀₄ treatment to control: **P<0.01 or *P<0.05 except t = 8 and 26 seconds; Taxol treatment to control: **P<0.01 or *P<0.05 for all time points.
Supplemental Figure 8. Primary human cord blood-derived MKs elongate and retract proplatelets under continuous unidirectional physiological shear stress.

Rate of proplatelet elongation/retraction is comparable and predictive of bidirectional microtubule sliding independent of direction of shear stress. White and black arrows denote two different proplatelets. Scale bars are 50 µm.
Supplemental Videos:

**Video 1.** Primary mouse megakaryocyte proplatelet production in static culture. Scale bar is 25 µm. Time bar is hh:mm:ss.

**Video 2.** Primary mouse megakaryocyte proplatelet retraction in static culture. Scale bar is 25 µm. Time bar is hh:mm:ss.

**Video 3.** Primary mouse megakaryocyte released proplatelet elongation and retraction in static culture. Scale bar is 25 µm. Time bar is hh:mm:ss.

**Video 4.** Proplatelets can slide into the proplatelet tip. Video of FLAC microscopy performed with MKs expressing β1-tubulin-Dendra2. Two ROIs were photoconverted with a 405 nm laser for 150 milliseconds. Video: 46 minutes. Interval: 1.378 seconds.

**Video 5.** Primary mouse released proplatelet retraction in static culture following addition of 1 mM Sodium Orthovanadate. Scale bar is 25 µm. Time bar is hh:mm:ss.

**Video 6.** Primary mouse released proplatelet retraction in static culture following addition of 1 mM EHNA. Scale bar is 25 µm. Time bar is hh:mm:ss.

**Video 7.** Normal FRAP in control proplatelet. Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI was photobleached with a 488 nm laser for 9 seconds. Video: 300 seconds. Interval: 5 seconds. Visual field: 29.38 µm x 29.38 µm.

**Video 8.** Normal FRAP in 100 nM nocodazole treated proplatelet. Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI was photobleached

**Video 9. Abolished FRAP in 1 mM EHNA treated proplatelet.** Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI was photobleached with a 488 nm laser for 9 seconds. Video: 300 seconds. Interval: 5 seconds. Visual field: 38.83 µm x 38.83 µm.

**Video 10. Abolished FRAP in 100 µM Ciliobrevin D treated proplatelet.** Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI was photobleached with a 488 nm laser for 9 seconds. Video: 300 seconds. Interval: 5 seconds. Visual field: 38.83 µm x 38.83 µm.

**Video 11. Normal FRAP in 10 µM taxol treated proplatelet.** Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI was photobleached with a 488 nm laser for 9 seconds. Video: 300 seconds. Interval: 5 seconds. Visual field: 38.79 µm x 38.79 µm.

**Video 12. Primary mouse megakaryocytes under continuous unidirectional shear stress.** Scale bar is 50 µm. Time bar is hh:mm:ss.

**Video 13. Primary mouse megakaryocytes under continuous unidirectional shear stress following addition of 1 mM Nocodazole.** Scale bar is 50 µm. Time bar is hh:mm:ss.

**Video 14. Primary mouse megakaryocytes under continuous unidirectional shear stress following addition of 1 mM EHNA.** Scale bar is 50 µm. Time bar is hh:mm:ss.
Video 15. Normal FRAP in control proplatelet in platelet bioreactor. Video of FRAP microscopy performed with MKs expressing β1-tubulin-Dendra2. ROI is photobleached (zoom in) with a 488 nm laser for 4 seconds. Video: 46.27 seconds. Interval: 0.661 seconds. Visual field: 112.59 μm x 112.59 μm.

Video 16. Primary human cord blood megakaryocyte proplatelet retraction in static culture. Scale bar is 25 μm. Time bar is hh:mm:ss.

Video 17. Primary human cord blood megakaryocyte proplatelet retraction under continuous unidirectional shear stress. Scale bar is 50 μm. Time bar is hh:mm:ss.