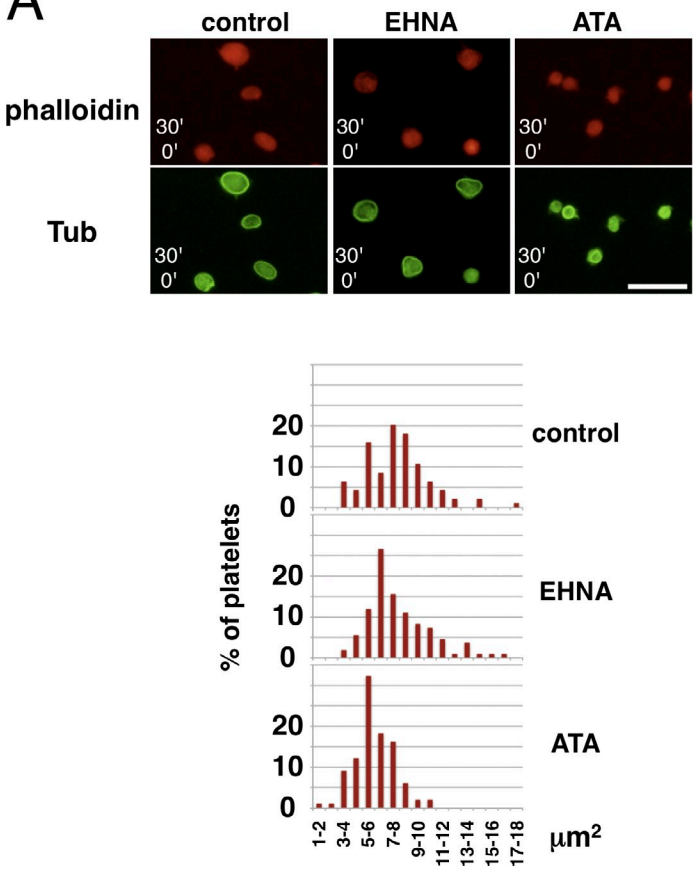
Diagouraga et al., http://www.jcb.org/cgi/content/full/jcb.201306085/DC1

A



B

	round/ stellate	completely spread
Chariot assay		
control IgG	14.9 % ± 5.6	7.8 % ± 4.7
anti-dynein	66.7 % ± 4.7	0 %
anti-kinesin	22.1 % ± 8.2	14.2 % ± 2.8
Inhibitor treatment		
control	34.1 % ± 3.9	12.2 % ± 3.6
EHNA	86.1 % ± 2.3	0 %
ATA	16 % ± 9	38.1 % ± 9.1

Figure S1. **Quantification of microtubule motor inhibitor treatments.** (A) Platelets shown in Fig. 1 B (top) had been double stained for tubulin (Tub) and phalloidin–rhodamine. Platelet size has been estimated using the phalloidin staining. The histogram depicts the percentage of platelets present in different size categories as indicated on the x axis. 200 platelets were counted for each condition of a typical experiment repeated four times. Bar, 10 µm. (B) Quantification of platelet spreading assays after EHNA and ATA inhibition (as shown in Fig. 1 B, bottom) as well as after introduction of function-blocking dynein and kinesin antibodies (as shown in Fig. 1 C).

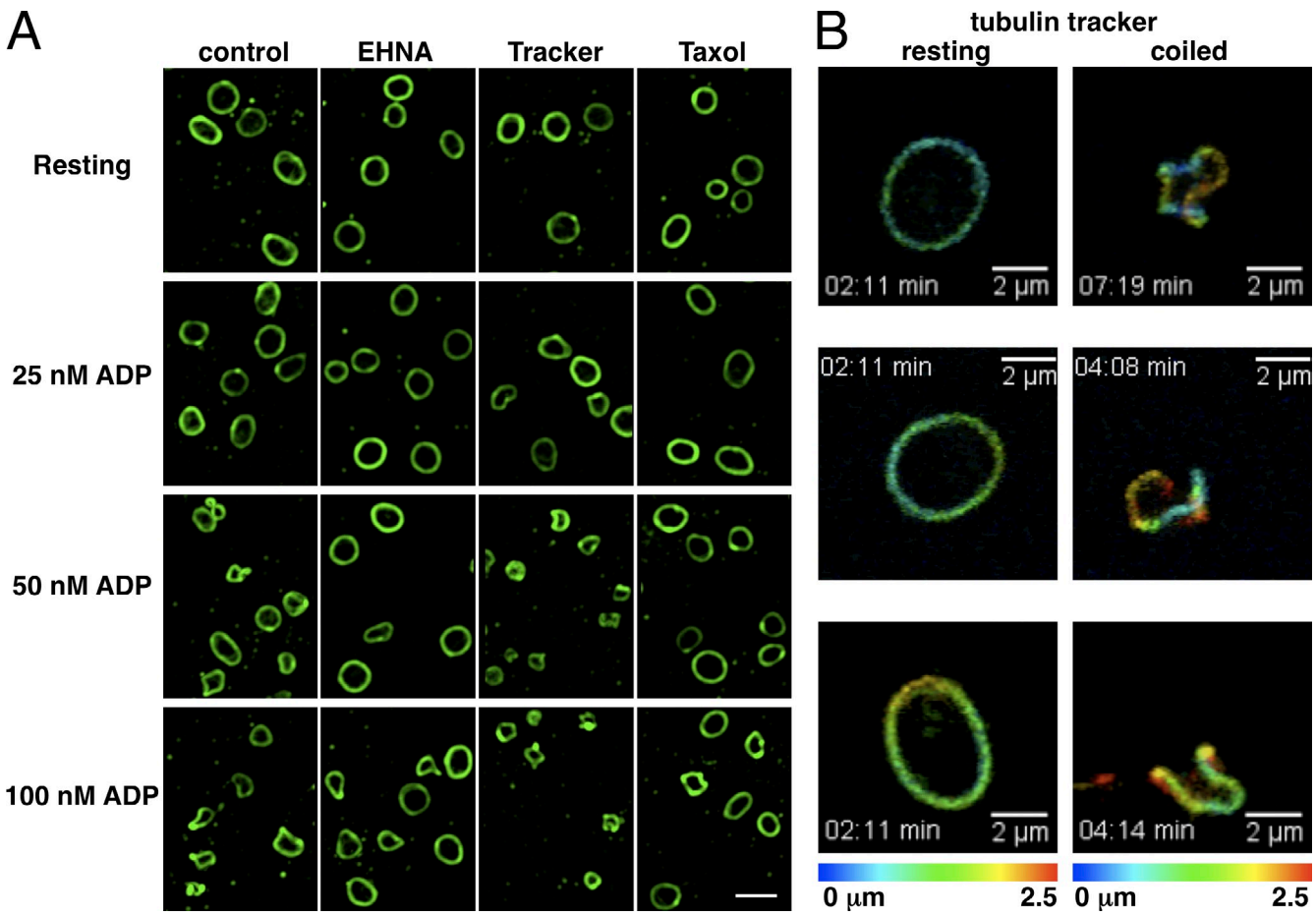


Figure S2. **Characterization of MB coiling conditions.** (A) Resting platelets in PRP from buffy coats were diluted in PBS, 16 × 10^6/ml, and preincubated with or without EHNA (1-mM final), tracker stain (1 µl/ml), or taxol (25-µM final) for 45 min at RT before platelet activation for 60 s with ADP at 25-, 50-, and 100-nM final concentrations. Platelets are then fixed and stained for α-tubulin. Bar, 5 µm. (B) Time-lapse videos of 3D reconstructions of confocal stacks of microtubule tracker-stained platelets. MB coiling is induced by activation of actomyosin contraction using calyculin (17-nM final). Shown are time points for resting and coiled MBs. 3D projections are depth color coded as indicated. See also Video 10.

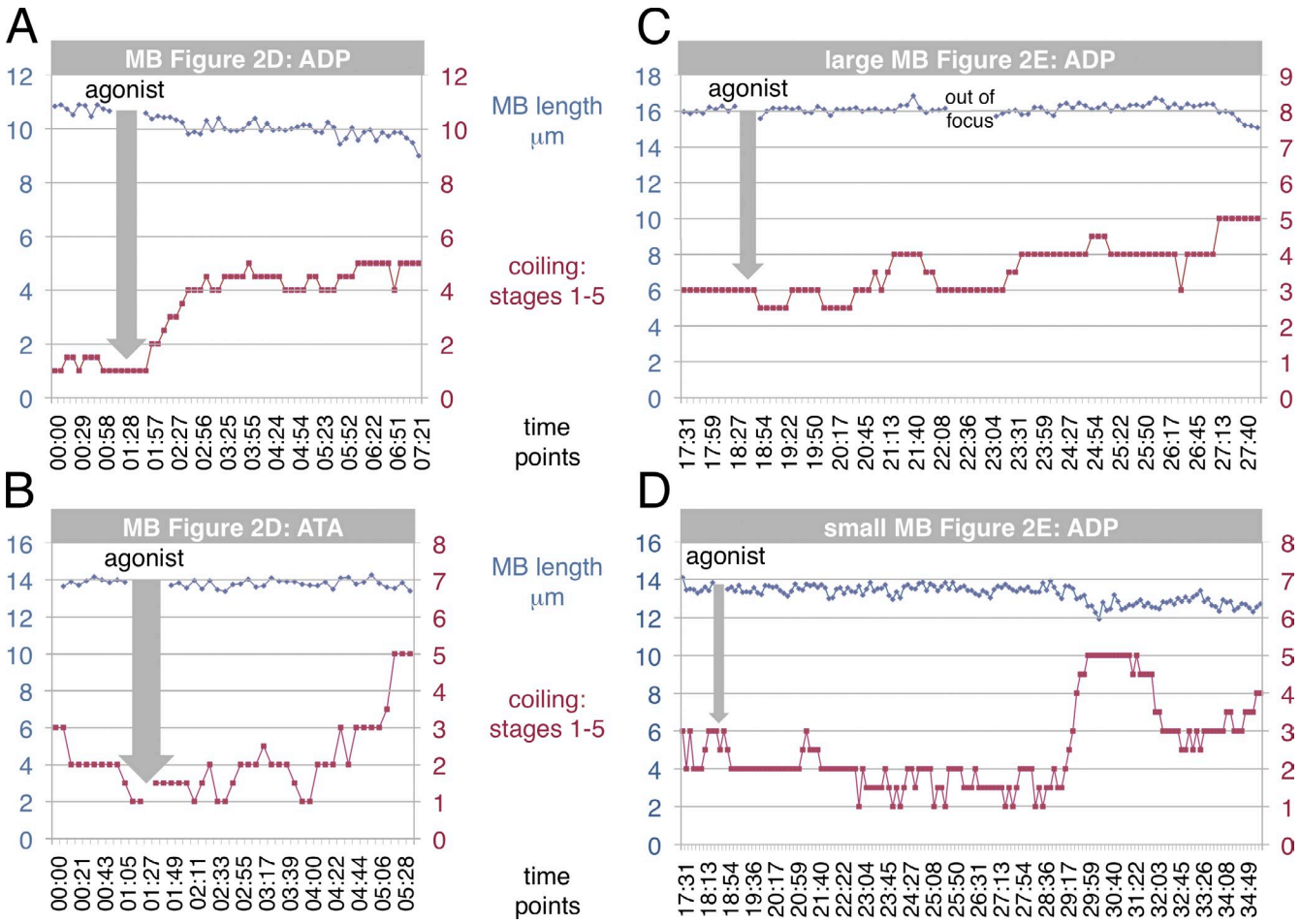
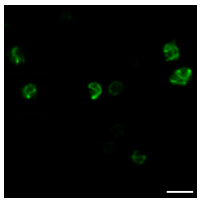
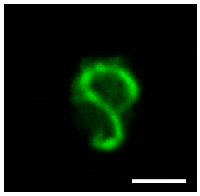


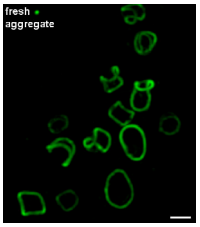
Figure S3. **MB length measurements.** The MB length of four platelets shown in the videos associated with Fig. 2 (D and E) was measured at successive time points of the time-lapse videos (in micrometers) and compared with the degree of coiling (stages 1–5). Time points are given in minutes and seconds.



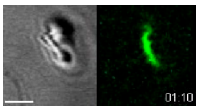
Video 1. **MBs coil after kinesin inhibition.** Related to Fig. 1 D. Resting platelets in PRP from buffy coats were diluted in PBS, 2.5 × 10^6/ml, and incubated in suspension with 10 µM ATA (kinesin inhibitor) for 3 min at RT and then fixed in suspension, centrifuged onto glass coverslips, and stained with an anti–acetylated tubulin antibody. Shown is a 360° rotation of a 3D reconstruction of confocal z-stack images acquired with a laser-scanning confocal microscope (LSM 510). Bar, 5 µm.



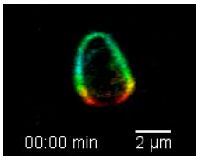
Video 2. **Transiently activated platelets have acetylated, coiled MBs.** Related to Fig. 2 A. Transiently activated platelets present in PRP prepared from freshly drawn blood, fixed, and stained with an anti–acetylated tubulin antibody. Three representative examples of platelets are shown by 360° rotations after 3D reconstruction of confocal z-stack images acquired with a laser-scanning confocal microscope (LSM 510). Bars, 2 µm.



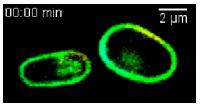
Video 3. **Platelets activated by different stimuli have coiled MBs.** Related to Fig. 2 B. Left images show transiently activated platelets present in PRP prepared from freshly drawn blood: either single platelets or platelets forming a rarely observed small aggregate or platelets of the same PRP, which have regained the resting state after a recovery period (120 min at RT with gentle agitation). Right images show platelets present in PRP prepared from buffy coats activated for 60 s with the following agonists: 6.25 µM arachidonic acid (AA), 25 nM ADP, and 0.01 U/ml thrombin (Th). Please note that to be able to observe MB coiling, agonist concentrations have to be adjusted for each experiment because of interdonor variations and depending on the PRP preparation (resting platelets present in PRP prepared from buffy coats are less reactive compared with resting platelets prepared from freshly drawn blood after a recovery period). Platelets were fixed in suspension and stained with the mouse monoclonal anti–α-tubulin antibody. Shown are 360° rotations of 3D reconstructions of confocal z-stack images acquired with a laser-scanning confocal microscope (LSM 710). Bars, 2 µm.



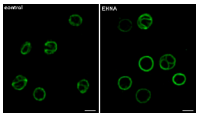
Video 4. **MB coiling during platelet spreading.** Related to Fig. 2 C. Time-lapse video microscopy of a microtubule tracker-stained human platelet spreading on a glass surface. Transmission (left) and fluorescence (right) images are taken simultaneously every 5 s for 19 min and 30 s using a laser-scanning confocal microscope (LSM 510). Bar, 2 µm.



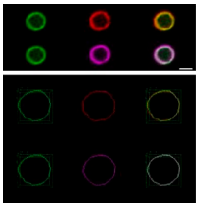
Video 5. **MB coiling triggered by different agonists or kinesin inhibition.** Related to Fig. 2 D. Time-lapse videos after 3D reconstructions of confocal stacks of microtubule tracker-stained platelets activated with thrombin (0.017-U/ml final) and ADP (135-nM final) or treated with ATA (6.7-mM final). 3D reconstitutions are depth color coded as indicated. Stacks were acquired every 10.5 s for 26 min and 5 s for thrombin activation, every 7.23 s for 7 min and 21 s for ADP activation, and every 7.3 s for 8 min and 16 s for the ATA treatment using a laser-scanning confocal microscope (LSM 710 ConfoCor 3).



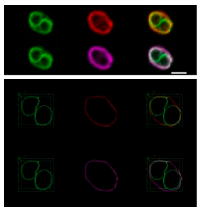
Video 6. **MB coiling after platelet activation with ADP.** Related to Fig. 2 E. Time-lapse video of 3D reconstructions of confocal stacks of microtubule tracker-stained platelets activated with ADP (270-nM final). 3D reconstitutions are depth color coded as indicated. Shown are two different view angles (0 and 160° rotation) to observe microtubules short cutting the coiled bundle. Stacks were acquired every 6.91 s for 57 min and 33 s using a laser-scanning confocal microscope (LSM 710 ConfoCor 3).



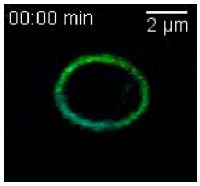
Video 7. **Transiently activated platelets treated with the dynein inhibitor.** Related to Fig. 3 B. Platelets prepared from freshly drawn blood with coiling MBs were diluted in PBS, 2.5 × 10^6/ml, and incubated without inhibitor or with the dynein inhibitor EHNA (1-mM final) for 10 min at RT. Platelets were then fixed and stained with the monoclonal mouse anti–α-tubulin antibody. Shown are 360° rotations of 3D reconstructions of confocal z-stack images acquired with a laser-scanning confocal microscope (LSM 710). Bars, 2 µm.



Video 8. **Colocalization of microtubule subpopulations in differently coiled MBs.** Related to Fig. 4 (A–C). Shown are 360° rotations of 3D reconstructions of confocal z-stack images of platelets triple immunostained with antibodies against acetylated α-tubulin (green), tyrosinated α-tubulin (red), and total α-tubulin (magenta). Individual stainings as well as merges are shown in the top images. Bars, 2 µm. The bottom images are generated from the different stainings after thresholding and skeletonizing of the MB. Images were acquired with a laser-scanning confocal microscope (LSM 710). (A) Resting platelet (stage 1; MB of 9.1 µm). (B) Platelet after 10 µM ATA (kinesin inhibitor) treatment for 3 min (beginning of coiling, stage 3; MB of 9 µm). (C) Platelet present in PRP from freshly drawn blood (coiled MB, stage 4; MB of 13.4 µm).



Video 9. **Colocalization of microtubule subpopulations in differently coiled MBs.** Related to Fig. 4 (D–F). Shown are 360° rotations of 3D reconstructions of confocal z-stack images of platelets triple immunostained with antibodies against acetylated α-tubulin (green), tyrosinated α-tubulin (red), and total α-tubulin (magenta). Individual stainings as well as merges are shown in the top images. Bars, 2 µm. The bottom images are generated from the different stainings after thresholding and skeletonizing of the MB. Images were acquired with a laser-scanning confocal microscope (LSM 710). (D) Platelet after 5 µM ADP activation for 10 s (strongly coiled MB, stage 5; the coiled MB is 13.3 µm, and the smaller ring is 10.4 µm). (E) Platelet after 5 µM ADP activation for 10 s (strongly coiled MB, stage 5; the coiled MB is 13.6 µm, and the smaller ring is 9 µm). (F) Platelet after 5 µM ADP activation for 10 s (strongly coiled MB, stage 5; the coiled MB is 13.4 µm, and the smaller ring is 7.8 µm). Please note that there may be two reasons for the absence or faint staining of microtubule subpopulations by the general anti–α-tubulin antibody. First, antibodies directed against acetylated and tyrosinated tubulin have a very high affinity for the individual modification and will give a strong signal even when only very few microtubules are present, whereas a higher number of microtubules may be necessary to obtain a strong signal with the general tubulin antibody. Second, there might also be an effect of steric hindrance between the mouse monoclonal anti–acetylated tubulin and the rabbit monoclonal anti–α-tubulin antibody because their epitopes are in relative close proximity.



Video 10. **MB coiling induced by activation of actomyosin contraction.** Related to Fig S2 B. Time-lapse videos of 3D reconstructions of confocal stacks of microtubule tracker-stained platelets. MB coiling is induced by addition of calyculin (17-nM final). 3D reconstitutions are depth color coded as indicated. Stacks were acquired every 7.26 s for 13 min and 11 s (top), every 7.23 s for 10 min and 29 s (middle), and every 7.13 s for 6 min and 25 s (bottom) using a laser-scanning confocal microscope (LSM 710 ConfoCor 3).

A source code for a custom ImageJ macro for Marginal Band 3D Analysis, with a toolset for image processing, 3D viewing, skeletonization of MBs, manual skeleton editing, and MB length measurements is provided as a text file.

A source code for a custom ImageJ macro for 3D Project Color Depth Coding, with a tool for depth color coding of 3D confocal time-lapse series and visualization at different view angles is provided as a text file.