Supplemental material

Braun et al., http://www.jcb.org/cgi/content/full/jcb.201401063/DC1



Figure S1. Summary of MT growth and cell behavior for all MT populations and experimental groups analyzed. (A and B) Analysis of MT growth dynamics in HUVECs. Comparison of mean MT growth speed (A) and mean MT growth excursion lifetime (B) from mApple EB3 tracks using plusTipTracker software under the conditions described in Figs. 1–7 of the main text. (C–E) Analysis of branching morphology and cell migration in HUVECs. HUVECs were plated on 0.7-kPa fibronectin-coupled polyacrylamide substrates to induce cell branching (C and D) or on coverslips and subjected to monolayer wounding to induce directional migration (E). Quantification of HUVEC branch number (C), branch length (D), and directionality of cell migration (E) shown under the conditions described in Figs. 1, 5, and 7. *, P < 0.001; **, P < 0.05.

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Figure S2. Summary of MT growth excursions for all leading and trailing edge MT populations and experimental groups analyzed. (A and B) Analysis of MT growth dynamics in the leading and trailing edges of HUVECs migrating at the edge of a monolayer wound. Comparison of mean MT growth speed (A) and mean MT growth excursion lifetime (B) from mApple EB3 tracks using plusTipTracker software under the conditions described in Figs. 1–7 of the main text. *, P < 0.001



Video 1. **MCAK tracks with growing EB3-labeled MT plus ends.** Live-cell imaging of GFP-MCAK (left) and mApple-EB3 (middle) and overlay (right) reveals that MCAK (green) tracks with EB3 (red) on growing MT plus ends at the leading edge of wound-edge HUVECs. Images were acquired by time-lapse spinning disk confocal microscopy (Yokogawa CSU-X1 spinning disk [Andor Technology]; coupled to a TiE microscope [Nikon]). Frames were taken at 2-s intervals for 2 min using a 500-ms exposure for the 488-nm (green) and a 600-ms exposure for the 560-nm (red) excitation. Bar, 2 µm.



Video 2. **Aurora A tracks with MCAK-labeled MT plus ends.** Live-cell imaging of Em–Aurora A (left) and mCherry-MCAK (middle) and overlay (right) reveal that Em–Aurora A (green) tracks with a proportion of mCherry-MCAK (red) on growing MT plus ends at the leading edge of wound-edge HUVECs. In addition to growing MCAK-labeled MT plus ends, Em–Aurora A labeling is also visible on filaments behind the growing MCAK-labeled MT plus ends. Images were acquired by time-lapse spinning disk confocal microscopy (Yokogawa CSU-X1 spinning disk [Andor Technology]; coupled to a TiE microscope [Nikon]). Frames were taken at 2-s intervals for 2 min using a 500-ms exposure for the 488-nm (green) and a 600-ms exposure for the 560-nm (red) excitation. Bar, 2 µm.



Video 3. Aurora A association with MTs is enhanced by Rac1 activation. Live-cell imaging of Em-Aurora A (left) and mApple-Tubulin (middle) and overlay (right) reveal that Em-Aurora A (green) association with MTs (red) is enhanced at the leading edge of wound-edge HUVECs in response to CA-Rac1 (red). Images were acquired by time-lapse spinning disk confocal microscopy (Yokogawa CSU-X1 spinning disk [Andor Technology]; coupled to a TiE microscope [Nikon]). Frames were taken at 2-s intervals for 2 min using a 500-ms exposure for the 488-nm (green) and a 600-ms exposure for the 560-nm (red) excitation. Bar, 2 µm.



Video 4. **Aurora A association with MTs is reduced by Rac1 inactivation.** Live-cell imaging of Em–Aurora A (left) and mApple-Tubulin (middle) and overlay (right) reveal that Em–Aurora A (green) association with MTs (red) is reduced at the leading edge of wound-edge HUVECs in response to DN-Rac1 (red). Images were acquired by time-lapse spinning disk confocal microscopy (Yokogawa CSU-X1 spinning disk [Andor Technology]; coupled to a TiE microscope [Nikon]). Frames were taken at 2-s intervals for 2 min using a 500-ms exposure for the 488-nm (green) and a 600-ms exposure for the 560-nm (red) excitation. Bar, 2 µm.