Myers et al., http://www.jcb.org/cgi/content/full/jcb.201006009/DC1

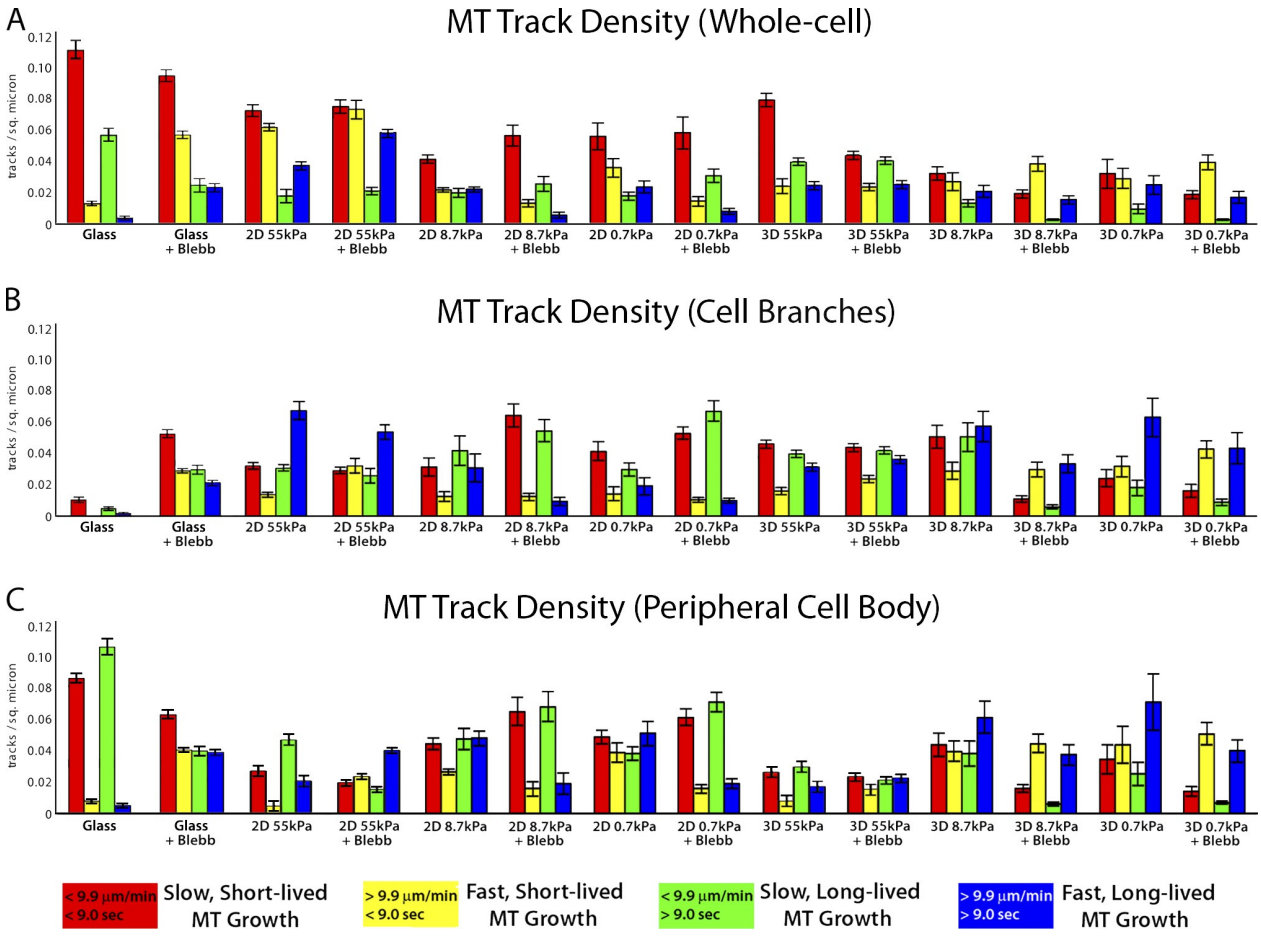


Figure S1. **Whole cell and regional MT track density.** The density of MTs growth excursions tracked in HUVECs expressing fluorescent EB3 by plusTip-Tracker Software for whole cells (A), within cell branches (B), or within the peripheral cell body (C). A color code key for the classification of MT growth excursion subpopulations is shown below. Error bars indicate standard error of the mean.

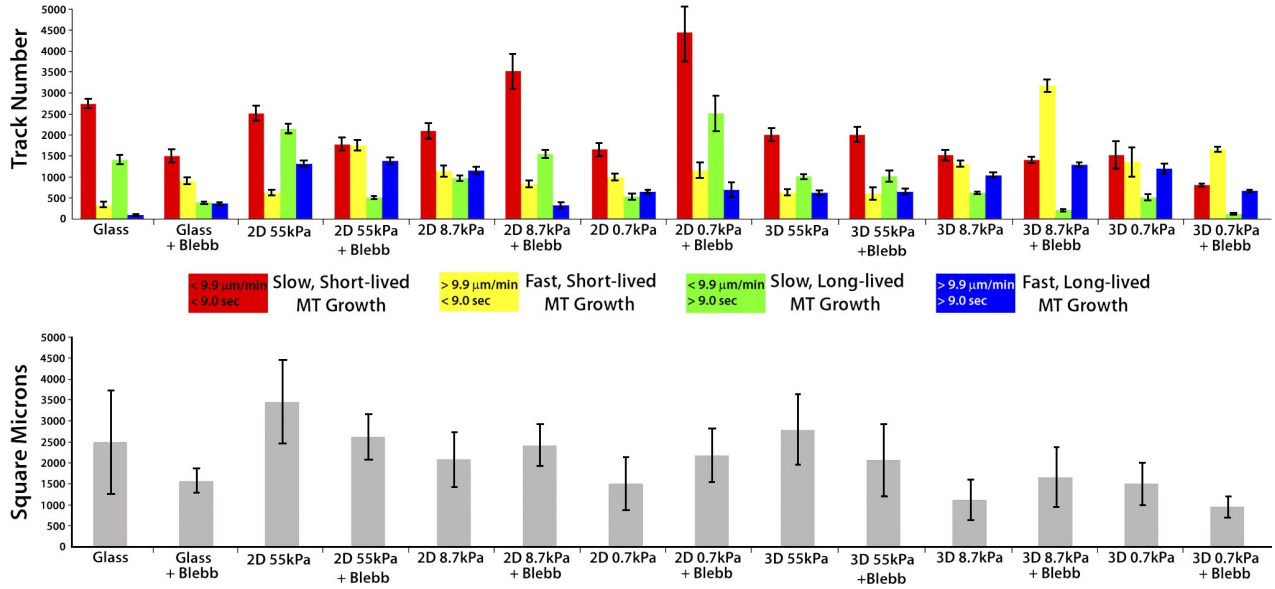
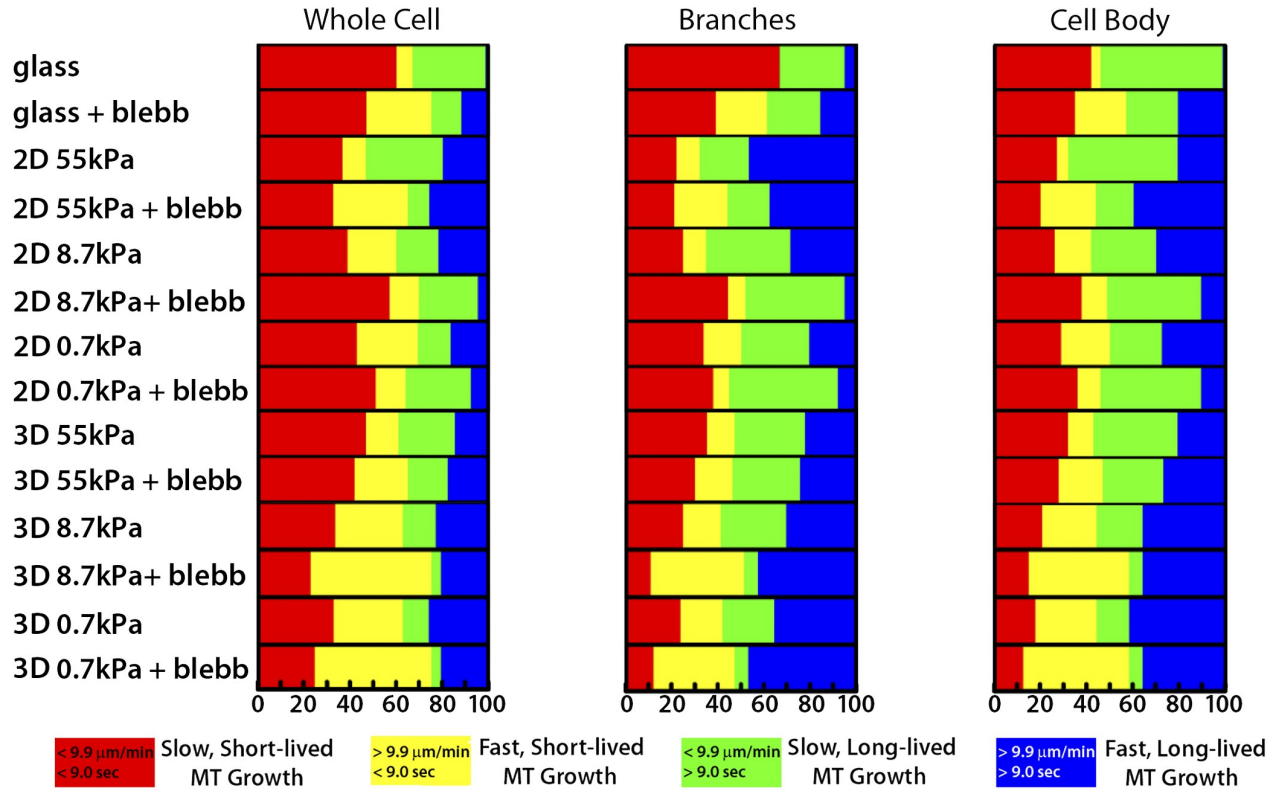


Figure S2. **Number of fluorescent EB3 tracks per cell correlates with cell area.** Number of tracks per cell for each of the four classes of MT growth dynamics (a color code key for the classification of MT growth excursion subpopulations is shown below) for each ECM condition (±Blebb, top), and the mean cell area for each ECM condition (±Blebb, bottom).



	Whole Cell						Branches						Cell Body					
	N_t	N_C	%R	%Y	%G	%B	N_t	N_C	%R	%Y	%G	%B	N_t	N_C	%R	%Y	%G	%B
glass	4623	13	60	7	31	2	18	4	66	0	28	6	2285	13	42	4	52	2
glass + blebb	3235	8	47	28	13	12	251	13	39	22	23	16	1029	8	35	22	22	21
2D 55kPa	6614	10	37	10	33	20	94	2	22	10	21	47	3131	10	27	5	47	21
2D 55kPa + blebb	5438	10	33	32	9	26	521	12	21	23	18	38	2306	10	20	24	16	40
2D 8.7kPa	5417	9	39	21	18	22	301	9	25	10	36	29	2661	9	26	16	28	30
2D 8.7kPa + blebb	6253	8	57	13	25	5	1765	44	44	8	42	6	1745	8	38	11	40	11
2D 0.7kPa	3874	8	43	26	14	17	173	10	34	16	29	21	1888	8	29	21	22	28
2D 0.7kPa + blebb	8903	10	51	13	28	8	1657	57	38	7	46	9	2435	10	36	10	43	11
3D 55kPa	4265	9	47	14	24	15	350	12	35	12	30	23	1896	9	32	11	36	21
3D 55kPa + blebb	4311	12	42	23	17	18	585	40	30	16	29	25	1985	12	28	19	26	27
3D 8.7kPa	4604	11	34	29	14	23	644	18	25	16	28	31	2178	11	21	23	20	36
3D 8.7kPa + blebb	6234	17	23	52	4	21	709	41	11	40	6	43	2234	17	15	43	6	36
3D 0.7kPa	4644	10	33	30	11	26	956	33	24	18	22	36	1969	10	18	26	14	42
3D 0.7kPa + blebb	3392	14	25	50	4	21	460	28	12	35	6	47	1332	14	13	45	6	36

Figure S3. **Summary of MT growth excursion class proportions for all MT populations analyzed.** Bar graphs (top) depict the percentages of the population of MTs analyzed that were categorized according to the color scheme (middle). The cell regions (branches and cell body) are described in Results. Glass, collagen coated glass; 2D, collagen coupled to PA; 3D, collagen sandwich gel of 55 kPa, 8.7 kPa, or 0.7 kPa ± 20 μM Blebbistatin. %R, %Y, %G, and %B (bottom) indicate the percentages of red (slow, short-lived), yellow (fast, short-lived), green (slow, long-lived), and blue (fast, long-lived) tracks. Nc, number of cells or regions; Nt, number of MTs tracked.

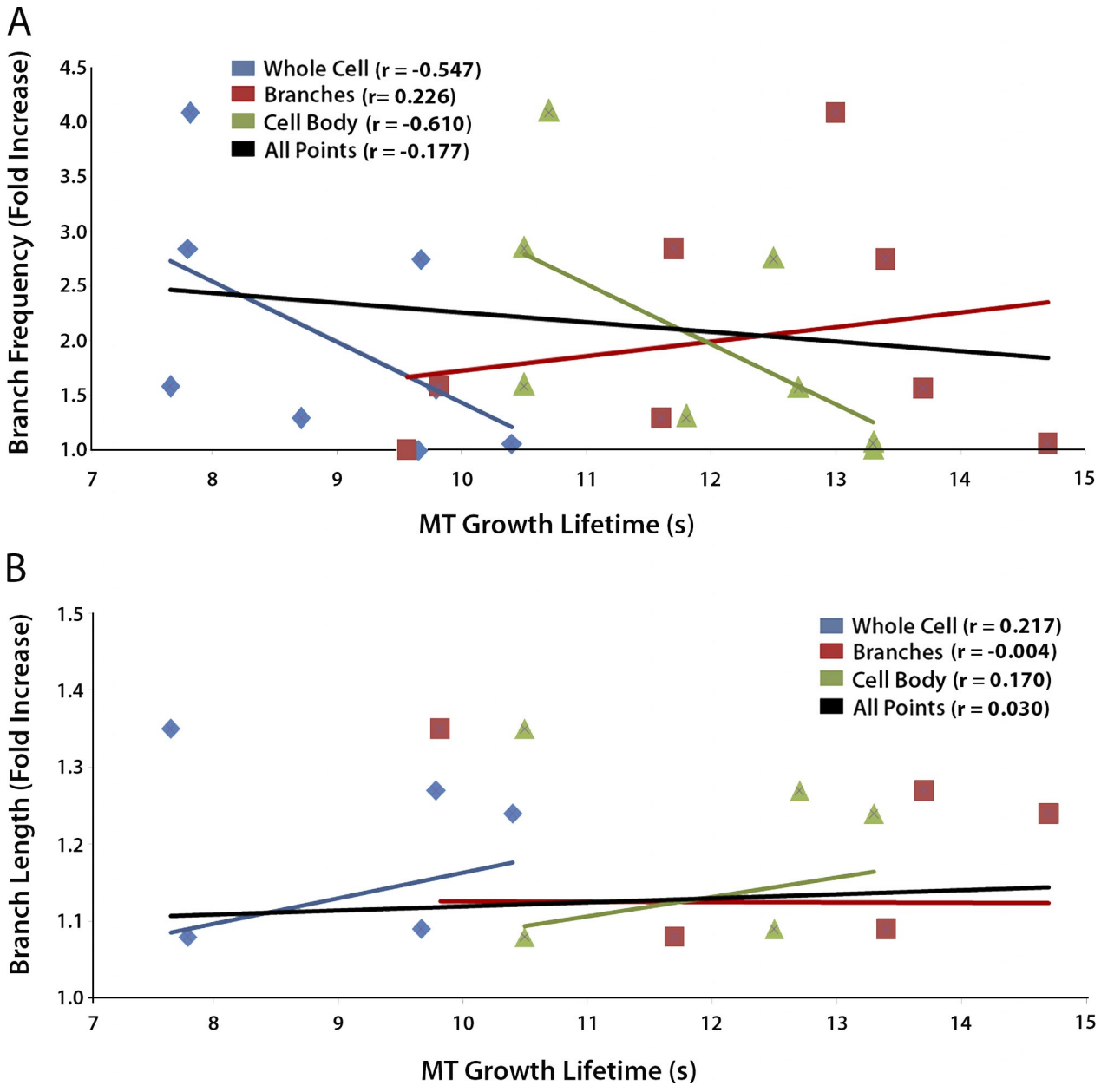
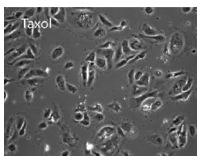


Figure S4. **Correlation analysis for MT growth lifetimes and EC branching morphogenesis.** Relationships between branch frequency (A) or branch length (B) and MT growth lifetime (whole cell; frequency vs. lifetime: r = −0.547; length vs. lifetime: r = 0.217) reveal that growth lifetimes are not correlated with EC branching morphogenesis. r, Pearson correlation coefficient.

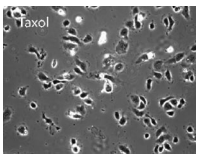
Table S1. **Summary of MT growth dynamics**

ECM condition	*n*	Mean speed	Mean lifetime
		μm/min	*s*
Whole cell			
Glass	4,623	5.02 ± 0.254	9.65 ± 0.596
Glass + Blebb	3,235	9.10 ± 0.323	7.66 ± 0.354
2D 55kPa	6,614	7.73 ± 0.200	14.2 ± 0.556
2D 55kpa + Blebb	5,438	11.6 ± 0.322	9.59 ± 0.401
2D 8.7kPa	5,417	8.82 ± 0.222	10.4 ± 0.394
2D 8.7kPa + Blebb	6,253	6.73 ± 0.166	8.67 ± 0.287
2D 0.7kPa	3,874	8.90 ± 0.282	8.71 ± 0.366
2D 0.7kPa + Blebb	8,903	6.71 ± 0.151	9.97 ± 0.328
3D 55kPa	4,265	7.82 ± 0.504	10.4 ± 0.504
3D 55kPa + Blebb	4,265	9.06 ± 0.441	9.45 ± 0.441
3D 8.7kPa	4,604	10.7 ± 0.409	9.79 ± 0.476
3D 8.7kPa + Blebb	6,234	14.0 ± 0.480	7.80 ± 0.387
3D 0.7kPa	4,644	11.7 ± 0.463	9.67 ± 0.460
3D 0.7kPa + Blebb	3,392	14.8 ± 0.668	7.83 ± 0.510
Branches			
Glass	18	5.10 ± 1.08	9.56 ± 1.17
Glass + Blebb	251	8.62 ± 0.299	9.82 ± 0.394
2D 55kPa	94	10.2 ± 0.377	15.0 ± 0.989
2D 55kpa + Blebb	521	11.9 ± 0.235	8.35 ± 0.197
2D 8.7kPa	301	8.42 ± 0.200	14.7 ± 0.551
2D 8.7kPa + Blebb	1,765	6.48 ± 0.073	11.4 ± 0.165
2D 0.7kPa	173	8.52 ± 0.344	11.6 ± 0.488
2D 0.7kPa + Blebb	1,657	6.54 ± 0.076	12.8 ± 0.203
3D 55kPa	350	8.75 ± 0.240	13.4 ± 0.497
3D 55kPa + Blebb	585	9.05 ± 0.180	12.9 ±0.369
3D 8.7kPa	644	9.30 ± 0.207	13.7 ± 0.353
3D 8.7kPa + Blebb	709	15.2 ± 0.233	11.7 ± 0.267
3D 0.7kPa	956	11.2 ± 0.220	13.4 ± 0.270
3D 0.7kPa + Blebb	460	17.1 ± 0.353	13.0 ± 0.410
Periphery			
Glass	2,285	4.93 ± 0.237	13.3 ± 0.747
Glass + Blebb	1,029	9.39 ± 0.535	10.5 ± 0.744
2D 55kPa	3,131	7.58 ± 0.261	17.1 ± 0.818
2D 55kpa + Blebb	2,306	12.4 ± 0.449	13.2 ± 0.679
2D 8.7kPa	2,661	9.14 ± 0.296	13.2 ± 0.629
2D 8.7kPa + Blebb	1,745	7.20 ± 0.323	12.2 ±0.749
2D 0.7kPa	1,888	9.55 ± 0.425	11.8 ± 0.667
2D 0.7kPa + Blebb	2,435	6.87 ± 0.225	13.2 ± 0.698
3D 55kPa	1,896	8.19 ± 0.364	13.7 ± 1.22
3D 55kPa + Blebb	1,985	9.59 ± 0.380	12.4 ±1.08
3D 8.7kPa	2,178	11.5 ± 0.523	12.7 ± 0.673
3D 8.7kPa + Blebb	2,234	14.5 ± 0.519	10.5 ± 0.506
3D 0.7kPa	1,969	13.5 ± 0.613	12.5 ± 0.678
3D 0.7kPa + Blebb	1,332	16.0 ± 0.789	10.7 ± 0.722

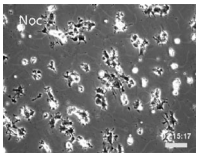
Shown are MT growth speeds and MT growth lifetimes in HUVECs under all experimental conditions. HUVECS were cultured on 2D ECMs (2D) or in 3D collagen-PA-glass sandwich cultures (3D). MT growth parameters were calculated for the entire cell area (whole cell) and subcellular regions (cell branches [Branches] vs. peripheral cell body [Periphery]). ECM condition refers to collagen-coated glass, collagen coupled to PA of defined shear modulus with (+ Blebb) or without treatment with 20 μM blebbistatin, or a 3D collagen gel coupled to PA of a defined shear modulus with (+ Blebb) or without treatment with 20 μM blebbistatin. Mean values reported are ± standard error of the mean. *n*, number of tracks.



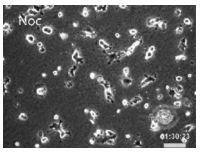
Video 1. **HUVEC migration on glass.** Montage of time-lapse movies of HUVECs treated with either DMSO control (left), 20 μM Taxol (middle), or 20 μM nocodazole (right) were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 20× 0.45 NA objective lens (Nikon). Images were acquired for 15 h (with a frame taken every 15 min) beginning 60 min after addition of the drug. Bar, 50 μm.



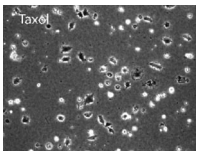
Video 2. **HUVEC migration on 8.7 kPa 2D PA substrates.** Montage of time-lapse movies of HUVECs treated with either DMSO control (left), 20 µM Taxol (middle), or 20 µM nocodazole (right) were acquired a spinning disc confocal microscope (TE2000; Nikon) and a 20× 0.45 NA objective lens (Nikon). Images were acquired for 15 h (with a frame taken every 15 min) beginning 60 min after addition of the drug. Bar, 50 µm.



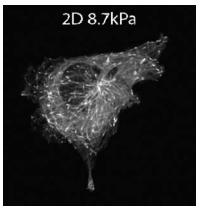
Video 3. **HUVEC migration on 0.7 kPa 2D PA substrates.** Montage of time-lapse movies of HUVECs treated with either DMSO control (left), 20 µM Taxol (middle), or 20 µM nocodazole (right) were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 20× 0.45 NA objective lens (Nikon). Images were acquired for 15 h (with a frame taken every 15 min) beginning 60 min after addition of the drug. Bar, 50 µm.



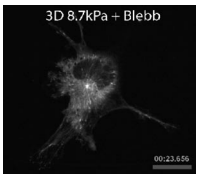
Video 4. **HUVEC migration on 8.7 kPa 3D sandwich gels.** Montage of time-lapse movies of HUVECs treated with either DMSO control (left), 20 µM Taxol (middle), or 20 µM nocodazole (right) were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 20× 0.45 NA objective lens (Nikon). Images were acquired for 15 h (with a frame rate taken every 15 min) beginning 60 min after addition of the drug. Bar, 50 µm.



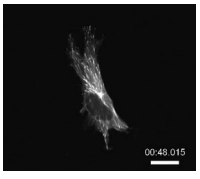
Video 5. **HUVEC migration on 0.7 kPa 3D sandwich gels.** Montage of time-lapse movies of HUVECs treated with either DMSO control (left), 20 µM Taxol (middle), or 20 µM nocodazole (right) were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 20× 0.45 NA objective lens (Nikon). Images were acquired for 15 h (with a frame taken every 15 min) beginning 60 min after addition of the drug. Bar, 50 µm.



Video 6. **GFP-EB3 dynamics in HUVECs on 8.7 kPa 2D PA substrates and 8.7kPa 3D sandwich gels.** Time-lapse images of HUVECs transiently transfected with GFP-EB3 to label polymerizing MT plus ends were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 60× water immersion 1.20 NA objective lens (Nikon). Images were acquired for 1 min, 10 s (frame rate = 2 s). Bar, 20 µm.



Video 7. **GFP-EB3 dynamics in HUVECs on 0.7 kPa 3D sandwich gels and 8.7 kPa + blebbistatin 3D sandwich gels.** Time-lapse images of HUVECs transiently transfected with GFP-EB3 to label polymerizing MT plus ends were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 60× water immersion 1.20 NA objective lens (Nikon). Images were acquired for 1 min, 11 s (with a frame taken every 2 s). Bar, 20 µm.



Video 8. **Long-term imaging of branching morphogenesis and GFP-EB3 dynamics.** Time-lapse images of a HUVEC expressing GFP-EB3 migrating in a 0.7 kPa 3D sandwich gel were acquired using a spinning disc confocal microscope (TE2000; Nikon) and a 60× water immersion 1.20 NA objective lens (Nikon). Image series were acquired at 4-s intervals for 1 min, and this cycle was repeasted at 4-min intervals to reduce photobleaching. Total imaging time was ∼32 min. Bar, 20 µm.