Supplemental material

Chikina et al., https://doi.org/10.1083/jcb.201806075

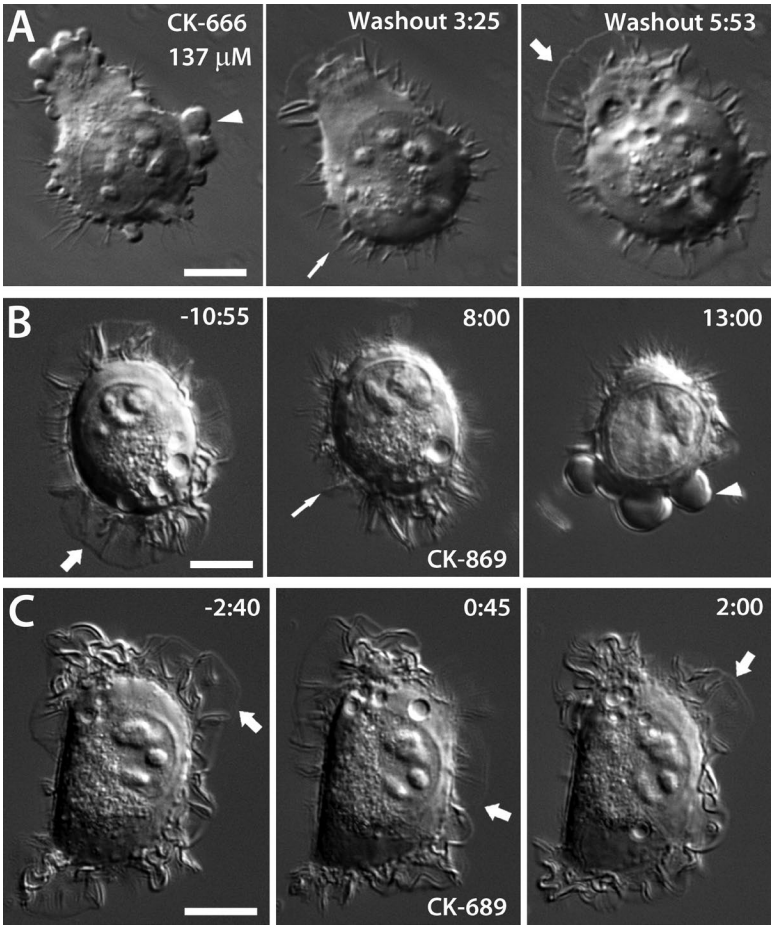


Figure S1. **Protrusive activity of HT1080 cells in different conditions. (A)** Restoration of lamellipodia after washout of CK-666 occurs through an intermediate stage of filopodia formation. Left: Blebbing cell (the same as shown in Fig. 1 B) in the presence of 137 µM CK-666. Middle: Formation of filopodia shortly after CK-666 washout. Right: Formation of lamellipodia after full recovery. **(B)** Induction of blebbing through an intermediate stage of filopodia formation in an HT1080 cell treated with CK-869 (25 µM). **(C)** Lamellipodial protrusion in an HT1080 cell is not affected by treatment with 100 µM CK-689 (inactive compound). Time is shown in minutes:seconds relative to the time of washout (A) or addition of the drug (B and C). Thick arrows indicate lamellipodia, thin arrows indicate filopodia, and arrowheads indicate blebs. Scale bars: 10 µm.

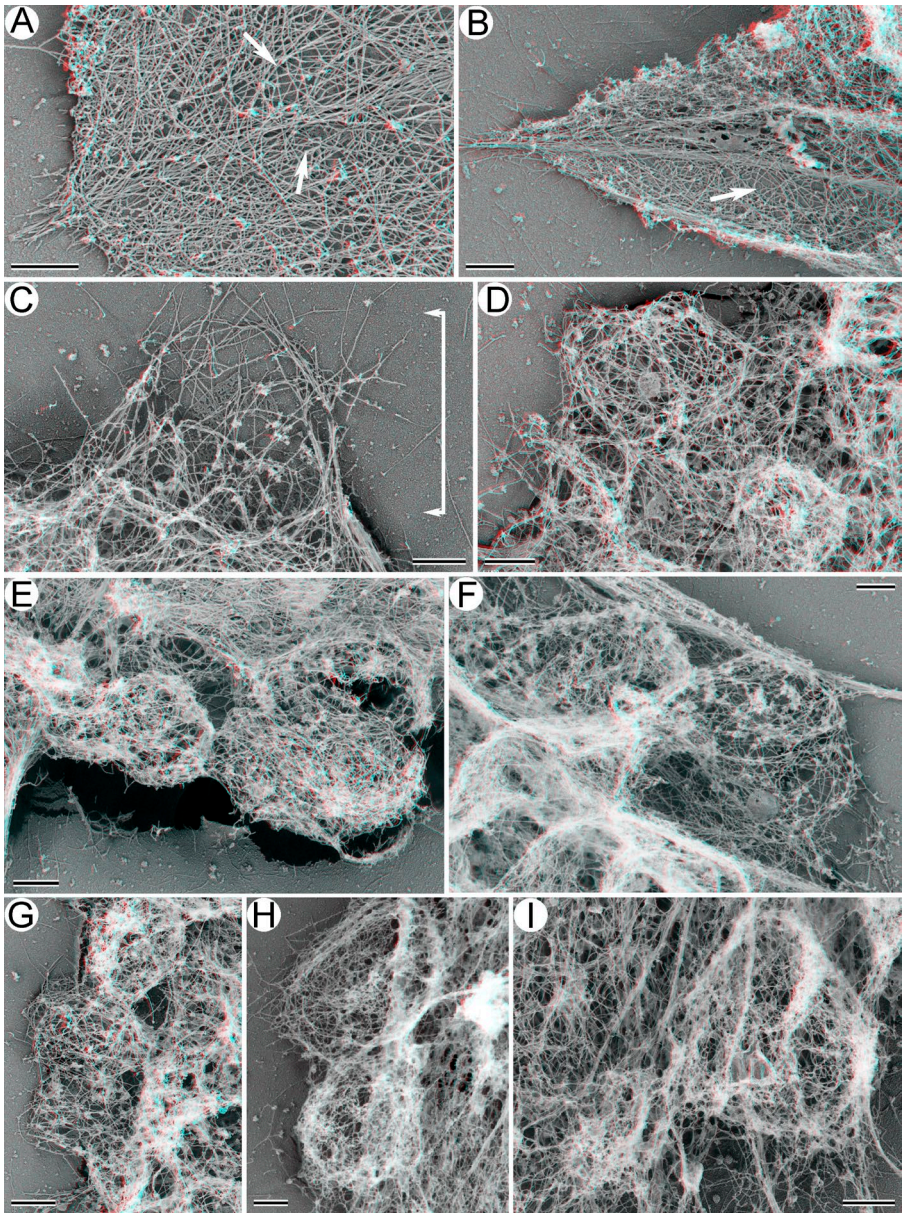


Figure S2. **Additional PREM examples of protrusive structures in HT1080 cells shown as anaglyph images.** Related to Fig. 2. **(A and B)** Filopodial bundles within lamellipodia of untreated cells. Cytoskeleton density is lower near the internal parts of the bundles (arrows). **(C–I)** Different types of blebs in cells treated with 200 µM CK-666, including a bleb with low cytoskeleton density at the tip (C; approximate bleb boundaries are indicated by the bracket), blebs with relatively uniform cytoskeleton density (D, upper bleb; F, right bleb; and G, lower and middle blebs), blebs with increased cytoskeleton density at the bleb margin (E; left bleb; F, left bleb; and H, both blebs), and blebs with an irregular shape and dense cytoskeleton (D, lower bleb; E, right bleb; G, upper bleb; and I, both blebs). **(D)** Bleb marked by an arrowhead in Fig. 2 E. Anaglyph images were prepared from stereo pairs taken at ±10°. Use red-cyan anaglyph glasses with red filter over the right eye to view 3D organization. Scale bars: 500 nm.

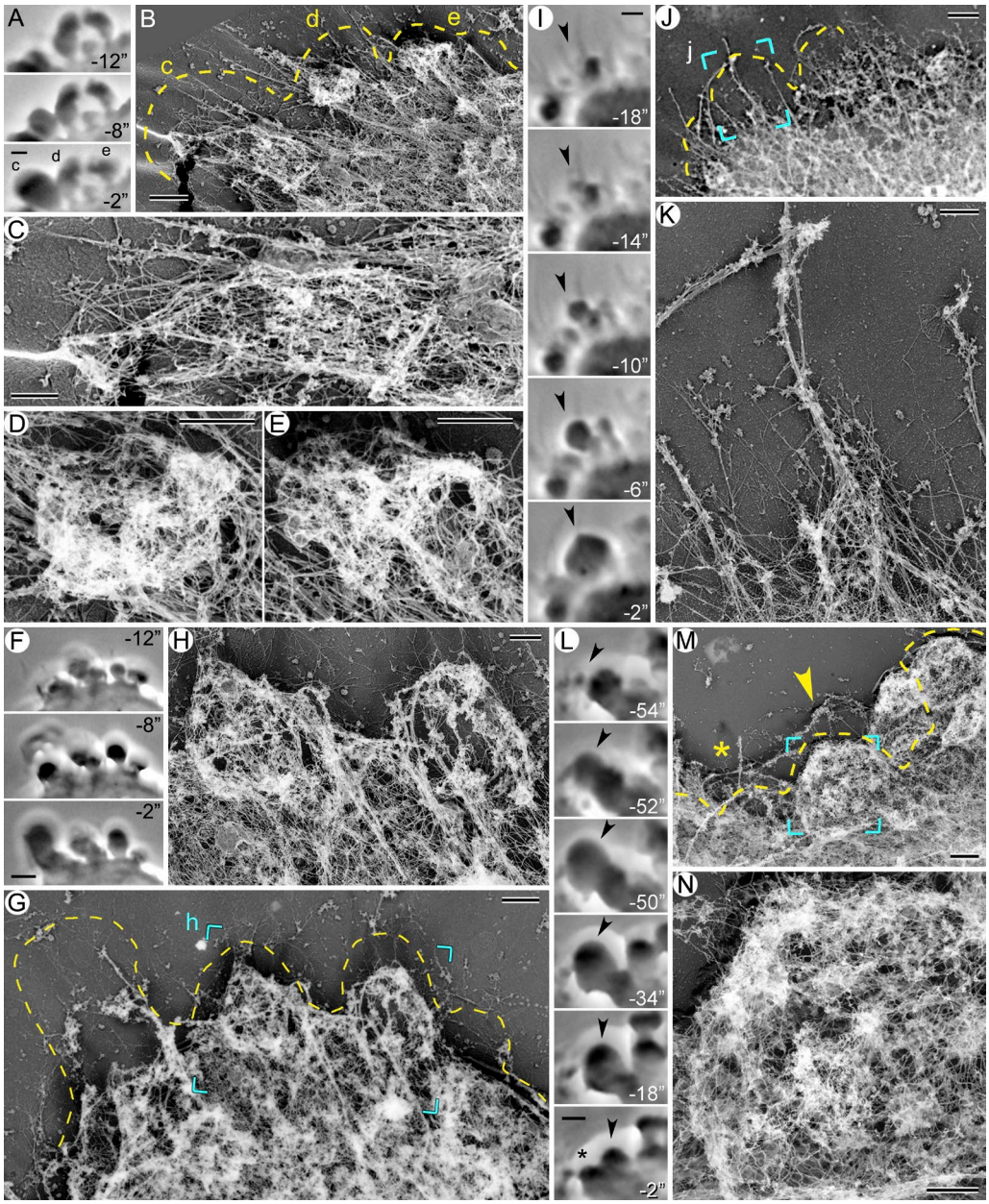


Figure S3. **Additional examples of correlative PREM of blebs in HT1080 cells treated with 200 µM CK-666.** Related to Fig. 3. **(A–E)** Example from cell #1 in Fig. 3 (lower left corner of the cell). **(A)** Phase-contrast frames showing formation of a nascent bleb (c) and two retracting blebs (d and e). **(B)** Correlative PREM image of the region shown in A. Yellow dashed line marks the cell edge contour in the corresponding phase-contrast image. **(C–E)** PREM images of individual blebs marked by corresponding letters. **(C)** Expanding bleb contains sparse cytoskeleton mostly consisting of actin filaments entering from the cell body. **(D and E)** Retracting blebs have crumpled shape and contain dense networks of actin filaments. **(F and G)** Example from cell #1 in Fig. 3 (upper right corner). **(F)** Phase-contrast frames showing formation of a nascent bleb (left), a retracting bleb (middle) and a bleb that apparently just finished expanding (right). **(G)** Correlative PREM image of the region shown in F. Yellow dashed line marks the cell edge contour in the corresponding phase-contrast image. The expanding bleb is nearly empty (left), although the material inside might represent shrunk cortex. **(H)** PREM image of the bracketed area in G. The retracting bleb (left) has prominent cortical layer, as well as internal cytoskeletal network. The bleb at the stage of late expansion (right) has a sparser and more uniform cytoskeleton. **(I and K)** Example from cell #2 in Fig. 3 (an adjacent bleb). **(I)** Phase-contrast frames showing formation of a nascent bleb (arrowhead) between filopodia (top). At the end of the sequence, the bleb overlaps with the right filopodium. **(J)** Correlative PREM image of the region shown in I together with the adjacent area. Dashed line marks the cell edge contour in the corresponding phase-contrast image. **(K)** PREM image of the bracketed area in J. The expanding bleb is nearly empty, except for a few filaments entering from the cell and the preexisting filopodium. **(L–N)** Example from cell #4 (not shown in Fig. 3). **(L)** Phase-contrast frames showing formation of a bleb at 54 s before extraction (arrowhead). The bleb underwent fast expansion for 4 s, entered an extended stationary/slow retraction phase, and then switched to faster retraction at ∼20 s before extraction. A new bleb (asterisk) was formed 16 s before extraction. **(M)** Correlative PREM image of the region shown in L. Dashed line marks the cell edge contour in the corresponding phase-contrast image. One bleb appeared to be masked by phase halo but visible in the PREM image. The retracting bleb (arrowhead) has a dense cytoskeleton enriched at the periphery, but it still retains a roundish shape. The recently formed bleb (asterisk) has sparse and nearly even cytoskeleton. **(N)** PREM image of the bracketed area in M showing the retracting bleb. Time in A, F, I, and L is shown in seconds before extraction. Scale bars: 2 µm (A, I, J, and L), 1 µm (B, G, and M), 500 nm (C–E, H, K, and N), and 5 µm (F).

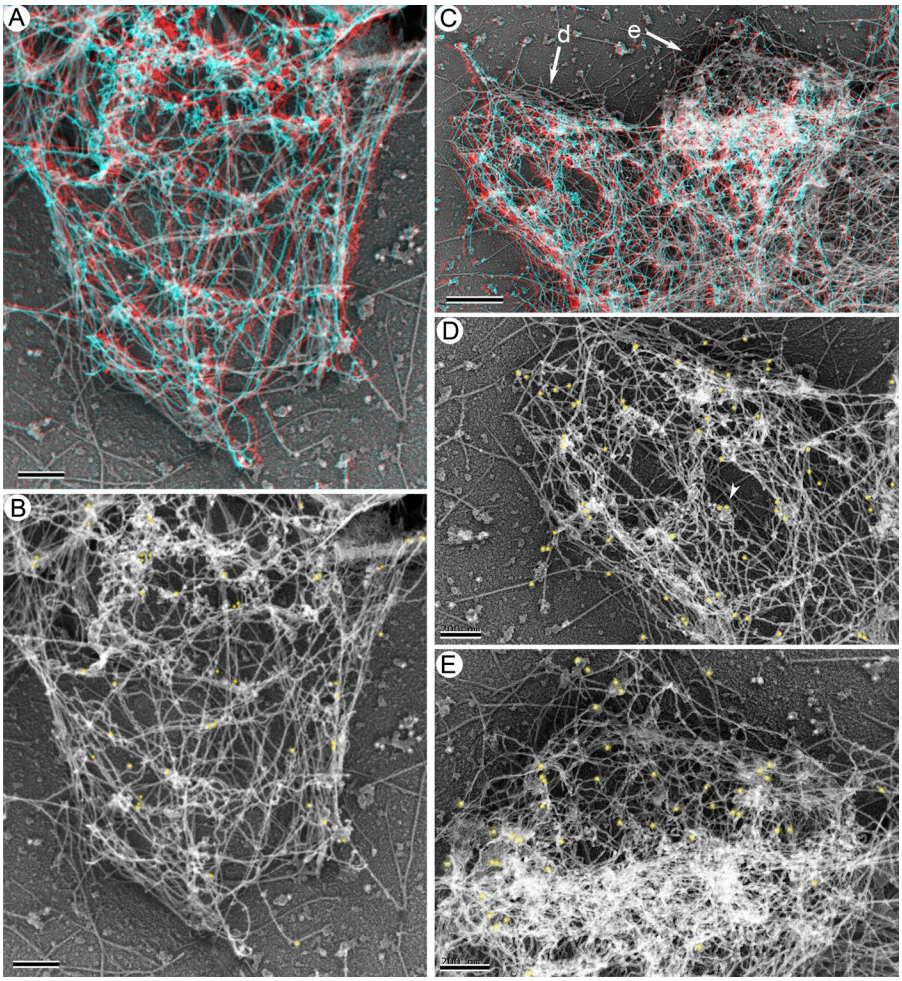
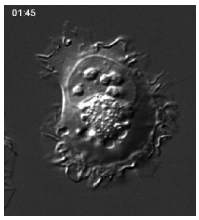
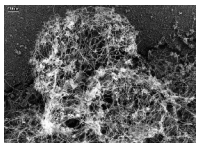


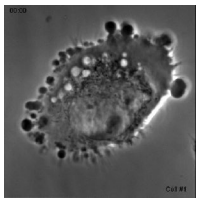
Figure S4. **NMII is associated with the bleb cytoskeleton in different types of blebs.** Immunogold PREM of blebbing HT1080 cells treated with 200 µM CK-666. **(A and B)** An early-stage bleb shown as an anaglyph stereo image (A) and as 2D image with NMII immunogold pseudocolored yellow (B). **(C)** Anaglyph images of two blebs at the cell periphery, showing a middle-stage bleb (d arrow) and late-stage bleb (e arrow). **(D and E)** Enlarged 2D images of the blebs from C. Immunogold particles are pseudocolored yellow. Scale bars: 200 nm (A, B, D, and E) and 500 nm (C).



Video 1. **Protrusive activity of the HT1080 cell shown in Fig. 1 in control conditions and after treatment with increasing concentrations of CK-666.** Lamellipodia and ruffles are first replaced with filopodia and then with blebs, which typically emerge from the sites at the filopodial bases. Time is shown in minutes:seconds.



Video 2. **Animated tilt series of PREM images of the blebs shown in Fig. 2 G taken at −10, 0, and +10 degrees.** Playback speed is 12 frames/s.



Video 3. **Blebbing activity of cell #1 used for correlative PREM in Fig. 3.** Extracted cell is shown at the end of the sequence. Time is shown in minutes:seconds.