Nightingale et al., http://www.jcb.org/cgi/content/full/jcb.201011119/DC1

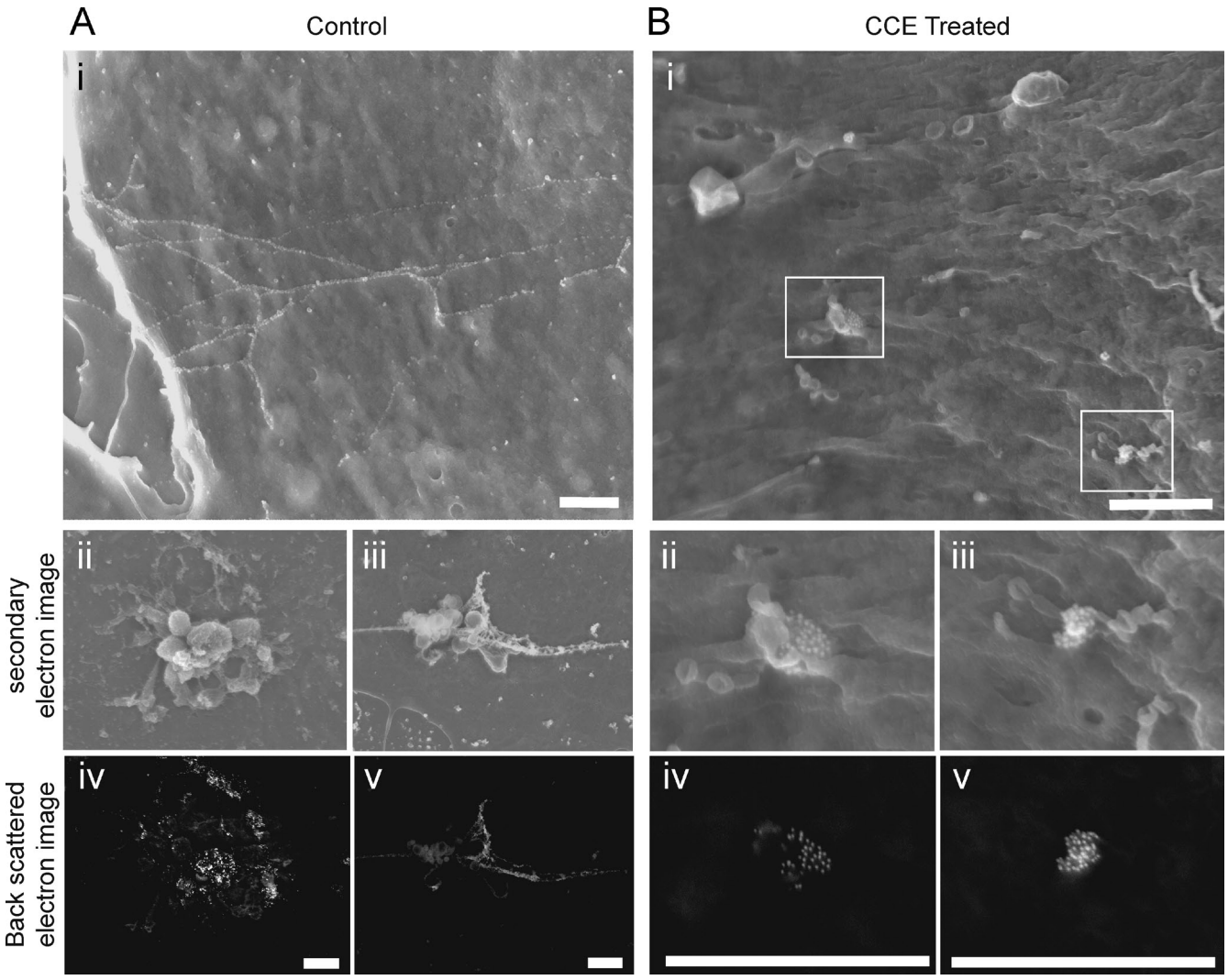


Figure S1. **Scanning EM characterization of WPB exocytosis.** (A and B) HUVECs were stimulated with 100 ng/ml PMA for 10 min (A) or pretreated with 1 µM CCE for 15 min before PMA stimulation (B). (A) Scanning EM of PMA-stimulated HUVECs show a network of VWF strings (i) and marked membrane ruffling (ii and iii) that appear to be sites of WPB exocytosis and initial VWF string release. (iv and v) These strings and sites are extensively labeled with anti-VWF/gold. (B, i) Cytochalasin E (CCE)–treated cells show a marked absence of VWF strings. Instead, only small patches of intense gold labeling are present with no membrane ruffling, indicating the WPBs have fused but failed to release VWF as strings. (ii and iii) Magnified regions of the areas described by the white boxes. (iv and v) Backscatter images of gold labeling. Bars, 1 µm.

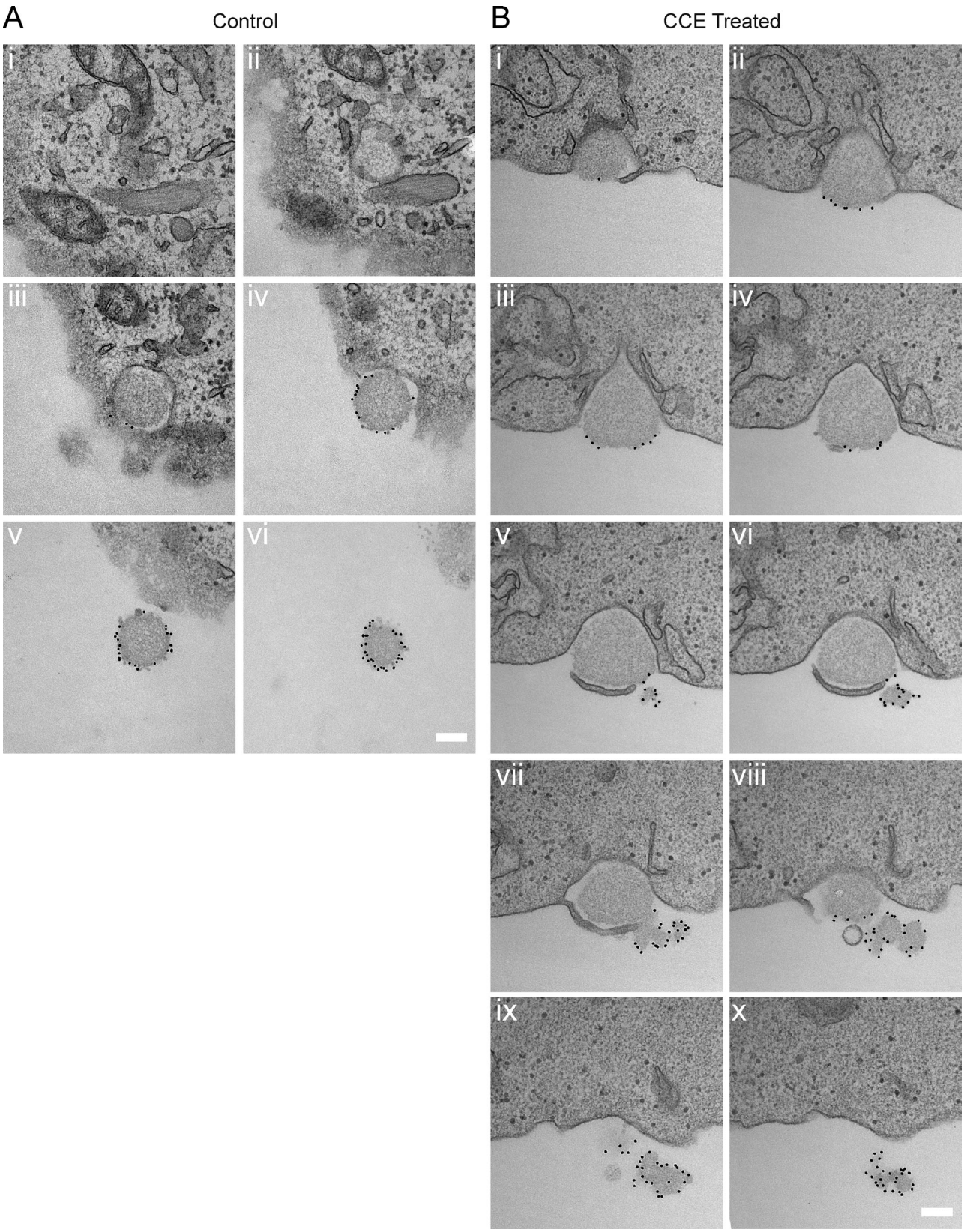


Figure S2. **Complete set of serial transmission electron microscope sections of exocytic events from a control and a CCE-treated HUVEC.** (A, i–vi) Serial TEM sections of an exocytic event from a control HUVEC. (B, i–x) Serial TEM sections of an exocytic event from a CCE-treated HUVEC. vi is the highest section in A, and x is the highest section in B. Bars, 200 nm.

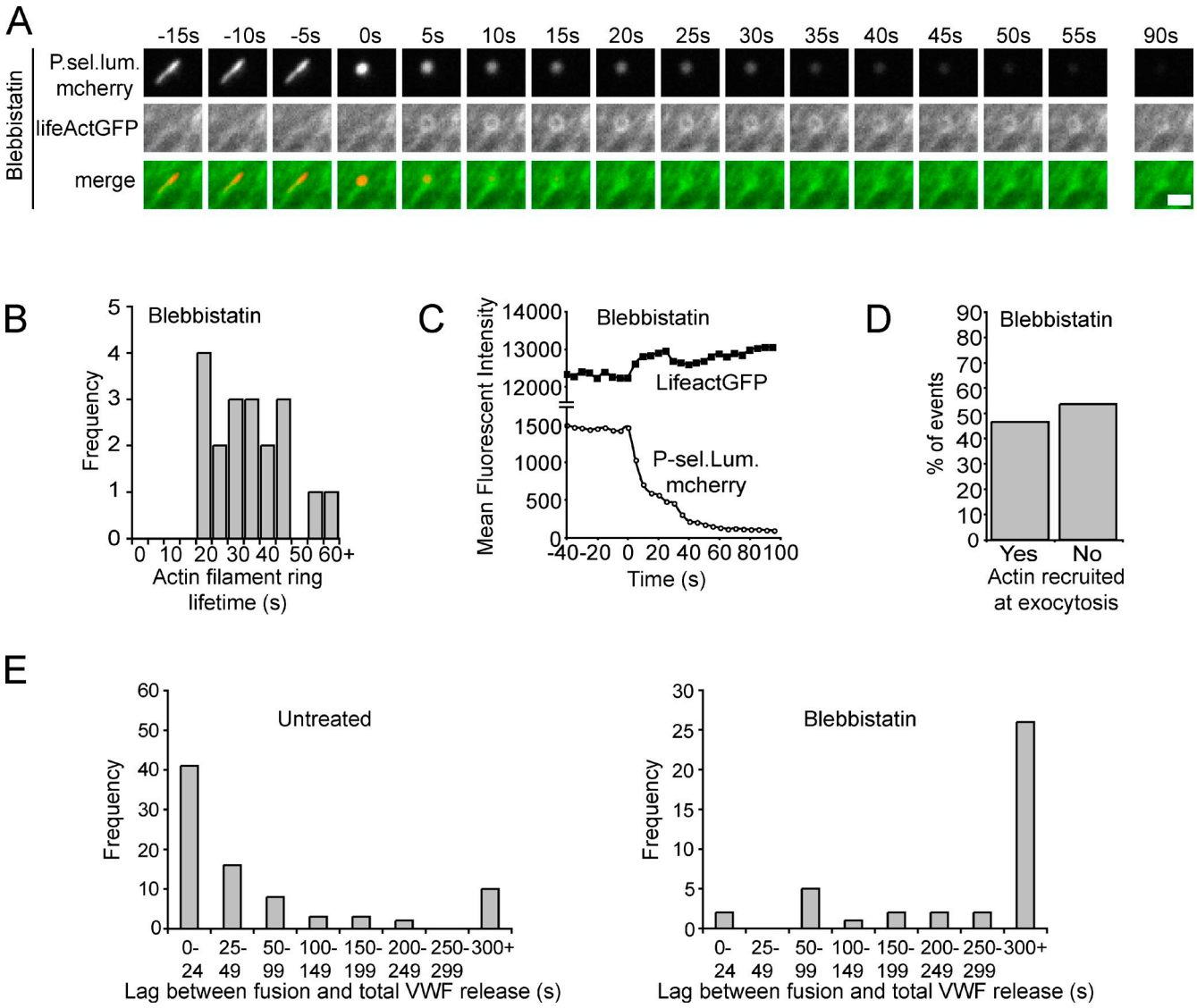
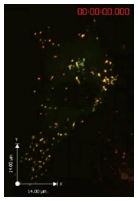
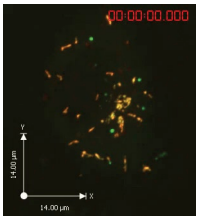


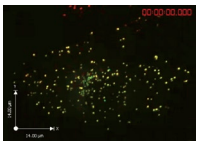
Figure S3. **Myosin II activity is required for the release of VWF contents (with blue light illumination).** (A–E) HUVECs coexpressing GFP-VWF and the mCherry–P-selectinLum (P.sel.lum.mcherry) domain were stimulated with 100 ng/ml PMA for 10 min with (A–E) or without (E) a 5-min preincubation with 25 µM blebbistatin. Fusion is assigned to 0 s. (A) HUVECs coexpressing mCherry–P-selectinLum and Lifeact-GFP were imaged after a 5-min preincubation with 25 µM blebbistatin. Cells were stimulated with 100 ng/ml PMA (in the presence of blebbistatin) for 10 min. Still images are shown from a video of individual WPB fusion and blebbistatin-inhibited release of VWF in live cells. Bar, 2 µm. (B) Actin filament ring total lifetime, defined as total time to reach peak fluorescence intensity and subsequent decay of signal, in blebbistatin-treated cells (plotted from 19 actin-positive fusion events in eight cells). (C) Quantification of intensity of fluorescence of mCherry–P-selectinLum and Lifeact-GFP for the individual organelle shown in A. (D) Quantification of WPB fusion events in which Lifeact-GFP was recruited (plotted as the percentage of 53 total fusion events in eight cells) to WPBs in the presence of blebbistatin. (E) Lag between WPB fusion and full release of GFP-VWF for individual WPBs in live PMA-stimulated cells. 300+ s indicates that VWF failed to release during 600 s of time-lapse filming (untreated: 83 fusion events in 11 cells; blebbistatin treated: 40 fusion events in five cells). Note that blocking myosin II has no effect on the fusion of WPBs yet blocks subsequent secretion of VWF from the same fused organelle and extends the lifetime of the actin filament ring.



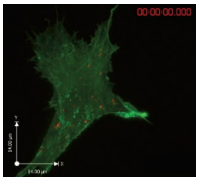
Video 1. **Unstimulated endothelial cell.** HUVECs coexpressing GFP-VWF (green) and the mCherry–P-selectinLum domain (red) were visualized by spinning-disk confocal microscopy (UltraVIEW VoX). Z stacks of 12 0.4-µm slices were acquired every 5 s for 10 min, and the image shown is a maximum intensity projection. The video is shown at seven frames per second.



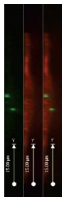
Video 2. **PMA-stimulated endothelial cell.** HUVECs coexpressing GFP-VWF (green) and the mCherry–P-selectinLum domain (red) were stimulated with 100 ng/ml PMA and visualized by spinning-disk confocal microscopy (UltraVIEW VoX). Z stacks of 13 0.5-µm slices were acquired every 5 s for 10 min, and the image shown is a maximum intensity projection. The video is shown at seven frames per second. Note fusion events typified by loss of mCherry signal and subsequent exocytosis of GFP-VWF.



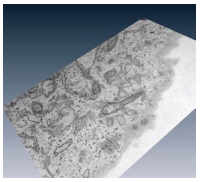
Video 3. **The effect of CCE pretreatment on exocytosis from a PMA-stimulated endothelial cell.** HUVECs coexpressing GFP-VWF (green) and the mCherry–P-selectinLum domain (red) were pretreated with 1 µM CCE for 15 min before stimulation with 100 ng/ml PMA and visualization by spinning-disk confocal microscopy (UltraVIEW VoX). Z stacks of 14 0.5-µm slices were acquired every 5 s for 10 min, and the image shown is a maximum intensity projection. The video is shown at seven frames per second. Note the increased incidence of fusion, but GFP-VWF fails to fully exit the cell.



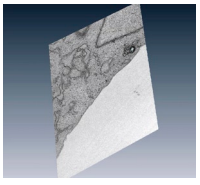
Video 4. **Actin recruitment after WPB fusion.** A HUVEC coexpressing Lifeact-GFP (green) and the mCherry–P-selectinLum domain (red) were stimulated with 100 ng/ml PMA and visualized by spinning-disk confocal microscopy (UltraVIEW VoX). Z stacks of nine 0.5-µm slices were acquired every 2 s for 5 min, and the image shown is a maximum intensity projection. The video is shown at seven frames per second. Multiple fusion events are visible, which coincide with recruitment of actin. Arrowheads highlight this process and appear adjacent to the exocytic site just before exocytosis and disappear after actin remodeling has completed.



Video 5. **Fused WPBs translocate out of the plane of the plasma membrane during WPB release of contents.** HUVEC coexpressing Lifeact-Ruby (red) and GFP-VWF (green) were stimulated with 100 ng/ml PMA and visualized by spinning-disk confocal microscopy (UltraVIEW VoX). Z stacks of 14 0.5-µm slices were acquired every 5 s for 10 min, and the image shown is a yz slice through an exocytosing WPB. The video is shown at seven frames per second. Note the recruitment of actin after fusion and the subsequent translocation (in z) and diffusion of VWF coincident with actin loss.



Video 6. **3D reconstruction of a WPB undergoing exocytosis.** HUVECs were stimulated with 100 ng/ml PMA for 10 min and then fixed and labeled for VWF (15-nm gold). 70-nm serial sections were prepared and imaged by TEM (Tecnai G2 Spirit). The serial sections (shown in Fig. S2 A, i–vi) were aligned and reconstructed into a 3D projection using Amira 5.2.2 software. For the 3D projection, the VWF is highlighted in green, whereas the rest of the cell is purple.



Video 7. **3D reconstruction of a WPB undergoing exocytosis after CCE treatment.** HUVECs were pretreated with 1 µM CCE for 15 min and then stimulated with 100 ng/ml PMA (in the presence of CCE) for 10 min before fixing and labeling for VWF (15-nm gold). 70-nm serial sections were prepared and imaged by TEM (Tecnai G2 Spirit). The serial sections (shown in Fig. S2 B, i–x) were aligned and reconstructed into a 3D projection using Amira 5.2.2 software. For the 3D projection, the VWF is highlighted in green, whereas the rest of the cell is purple.