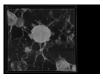
Supplemental material

JCB

Gabel et al., http://www.jcb.org/cgi/content/full/jcb.201412030/DC1

Unstimulated Stimulated Latrunculin B

Figure S1. Distribution of GM1 and actin on plasma membrane sheets visualized by immunogold labeling and electron microscopy at lower magnification (70,000x). Membrane sheets were prepared from untreated cells or cells stimulated with 20 µM nicotine in the presence of biotinylated cholera toxin to detect external GM1. Cells were treated with 50 µM latrunculin B when indicated. Membranes were then incubated with anti-actin antibodies revealed with rabbit antibodies coupled to 10 nm gold particles and streptavidin coupled to 6 nm gold particles to reveal cholera toxin/GM1. Bars, 200 nm.



Video 1. Cortical actin network surrounding the secretory granule docked at the plasma membrane of a nicotine-stimulated chromaffin cell. This 3D representation of docked granule presented in Fig. 2 was obtained from electron tomography acquisition of cytoplasmic face-up membrane sheets of stimulated cell, and shows an organized meshwork of actin filaments connecting the docked granule to the plasma membrane.



Video 2. **Disorganization and thickening of the cortical actin meshwork associated to the docked granule of a WA-treated cell.** This 3D representation of docked granules presented in Fig. 6, obtained from electron tomography image analysis of a nicotine-stimulated chromaffin cell after WA treatment, shows an increase in thickness of the actin coat and bundles connecting secretory granules to the plasma membrane.