Investigation of Ebola VP40 Assembly and Oligomerization in Live Cells Using Number & Brightness Analysis

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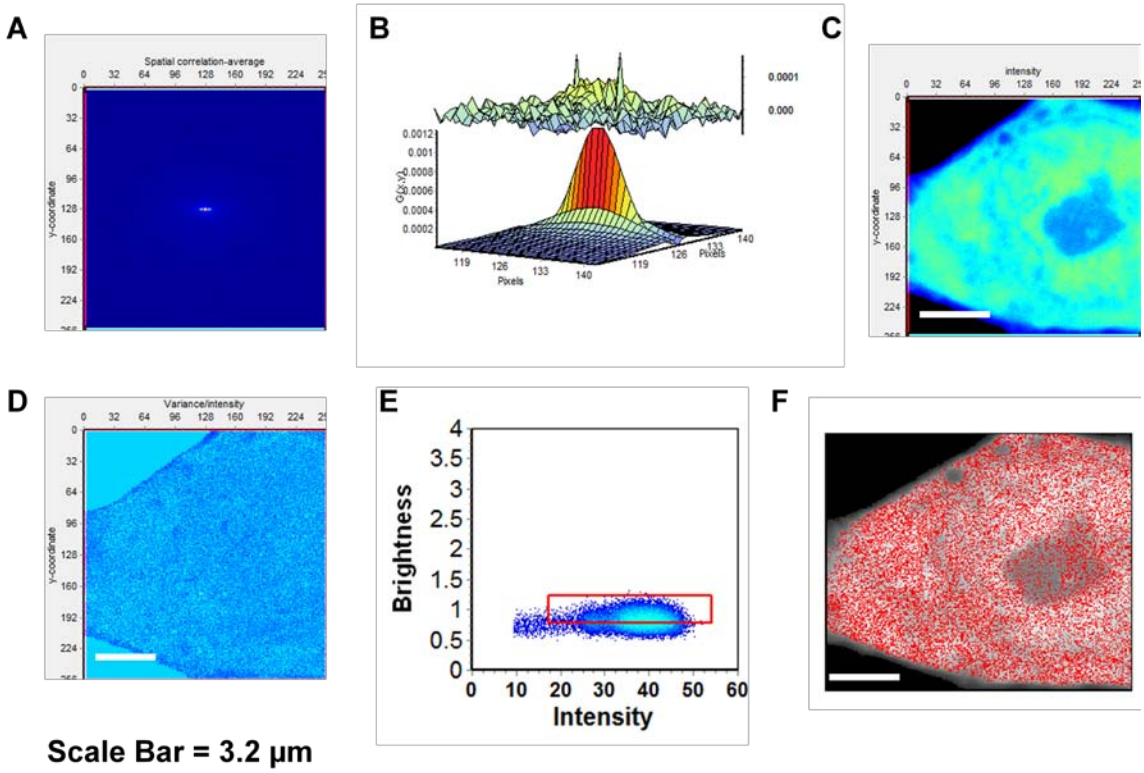
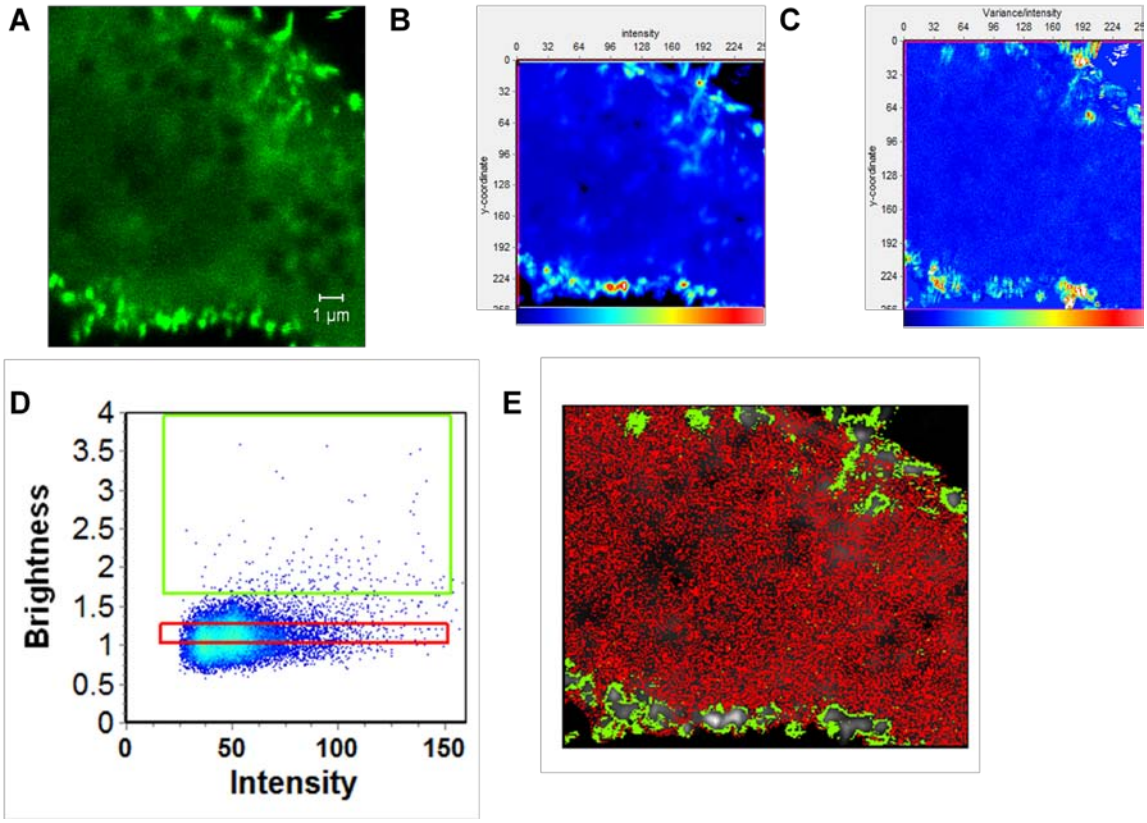
SUPPLEMENTAL MATERIAL

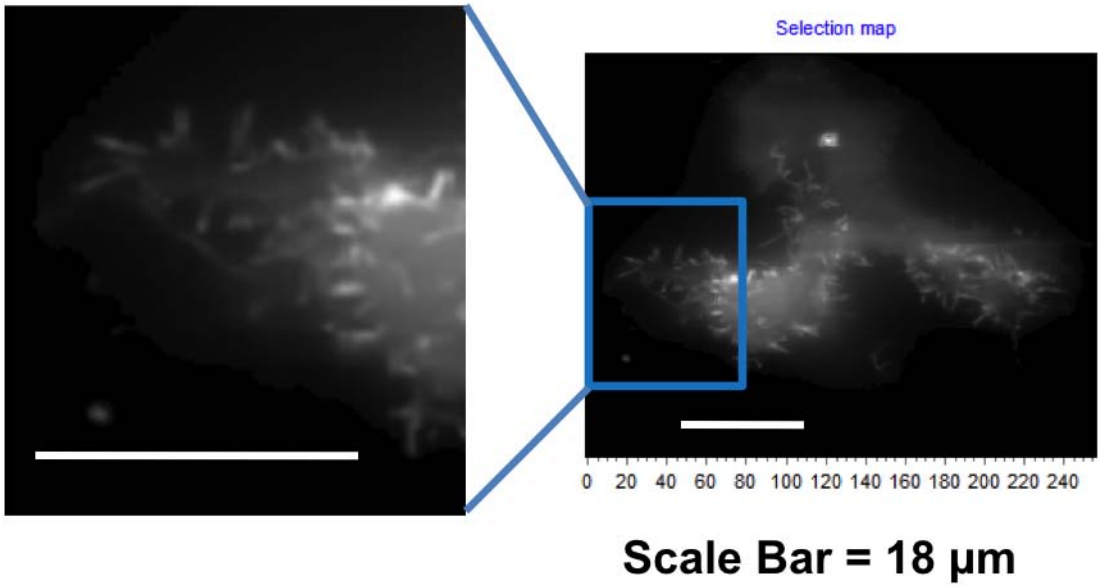
Supplemental Figure 1. **RICS and N&B analysis of EGFP with confocal microscopy**. HEK293T cells were transfected with plasmid expressing EGFP using standard transfection protocols. Cells were imaged 24 hours post-transfection. RICS data was acquired as described in materials and methods. (*A*) Spatial correlation map (x and y planes) of a HEK293T cell expressing free EGFP. (*B*) The correlation function from S1A was fit (lower surface) using a single species diffusion model to yield the diffusion coefficient ($7.7 \pm 1\ \mu m^2\ s^{-1}$) (fit shown as the lower surface and the residuals as the upper surface in S1B). The ability to determine the diffusion coefficient in different regions of the cell allowed for comparison of VP40 diffusion at the plasma membrane and in the cytoplasm. (*C*) Intensity image of HEK293T cell expressing EGFP. (*D*) Image of the same cell exhibiting uniform brightness distribution of EGFP. (*E*) Brightness versus Intensity plot of monomeric (red box) EGFP. (*F*) Image of the same cell showing selected brightness of monomers in red as determined from the red box in E. White scale bar = 3.2 μM.



Supplemental Figure 2. **RICS and N&B analysis of VP40 with confocal microscopy**. HEK293T cells were transfected with plasmids expressing either EGFP or EGFP-VP40 using standard transfection protocols. Cells were imaged 24 hrs post-transfection with RICS data acquired as described in materials and methods. (*A*) Confocal image of HEK293T cell expressing EGFP-VP40 demonstrating enrichment of EGFP-VP40 on the plasma membrane. White scale bar = 1 μM. (*B*) Fluorescence intensity image of the cell showing most intensity at bud sites at the plasma membrane. (*C*) Image of the same cell showing brightness intensity distribution indicating EGFP-VP40 may be clustered at the plasma membrane. (*D*) Brightness versus intensity plot where the red box represents monomers and the green box oligomers (hexamers and larger). (*E*) Image of the same cell depicting monomers (red) and selected oligomers of hexamers and greater (green).



Supplemental Figure 3. **Visualization of Membrane Protrusion Sites.** Membrane protrusion sites from the plasma membrane enriched with EGFP-VP40 were visually inspected using TIRF microscopy. This also allowed for spatial resolution of VP40 brightness and visualization of enrichment of EGFP-VP40 in these membrane protrusion sites. Note that the oligomerization deficient mutation W95A/E160A-VP40 (VP40 WE-A) lacked detectable membrane protrusion sites (See Fig. S4). White scale bar = 18 μM.



Supplemental Figure 4. **RICS and N&B analysis of the VP40 WE-A Mutant.** (*A*) TIRF intensity image of a HEK293T cell transfected with plasmid expressing EGFP-VP40 WE-A. (*B*) Brightness image of the same cell shown in *A* indicates a lack of VP40 WE-A enrichment in membrane protrusions as well as a lack of VP40 WE-A clustering. (*C*) Brightness vs. Intensity map of VP40 WE-A showing monomers (red box) and trimers (blue box). (*D*) Image of the cell with selected pixels from *C* showing monomers (red) and trimers (blue). (*E*) Brightness vs. Intensity map of VP40 WE-A showing hexamers (red box) and octamers (blue box), which are not significantly detected in the brightness versus intensity plot. (*F*) Image of the cell with selected pixels from *E* showing hexamers (red) and octamers (blue) demonstrating a significant lack of detectable oligomers and membrane protrusion sites for this mutation. White scale bar = 18 µM.