Supplementary Information (SI) for Nanoscale Horizons.
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Supplementary Materials for

Actin-Dependent Regulation of RSV F-Mediated Cell-cell Fusion Revealed by Visualizing Its Spatiotemporal Dynamics

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This PDF file includes:

Supplementary Text
Figs. S1 to S6
Movie S1 to S3

Supplementary Text

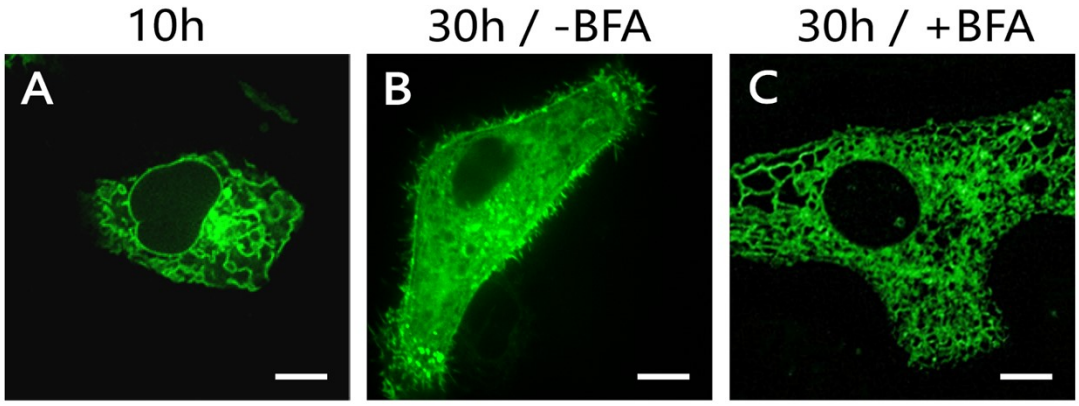

Figure S1: Effect of BFA on RSV F transport from the Golgi to the PM. The cells expressing GFP-RSV F for 10 h were chased to 30 h with or without BFA treatment at 37 °C. (A) Representative confocal images of cells expressing GFP-RSV F for 10 h. (B) A representative confocal image of cells expressing GFP-RSV F for 30 h without BFA. (C) A representative confocal image of cells expressing GFP-RSV F for 10 h followed by 20 h with BFA treatment. Scale bars = 10 μm.

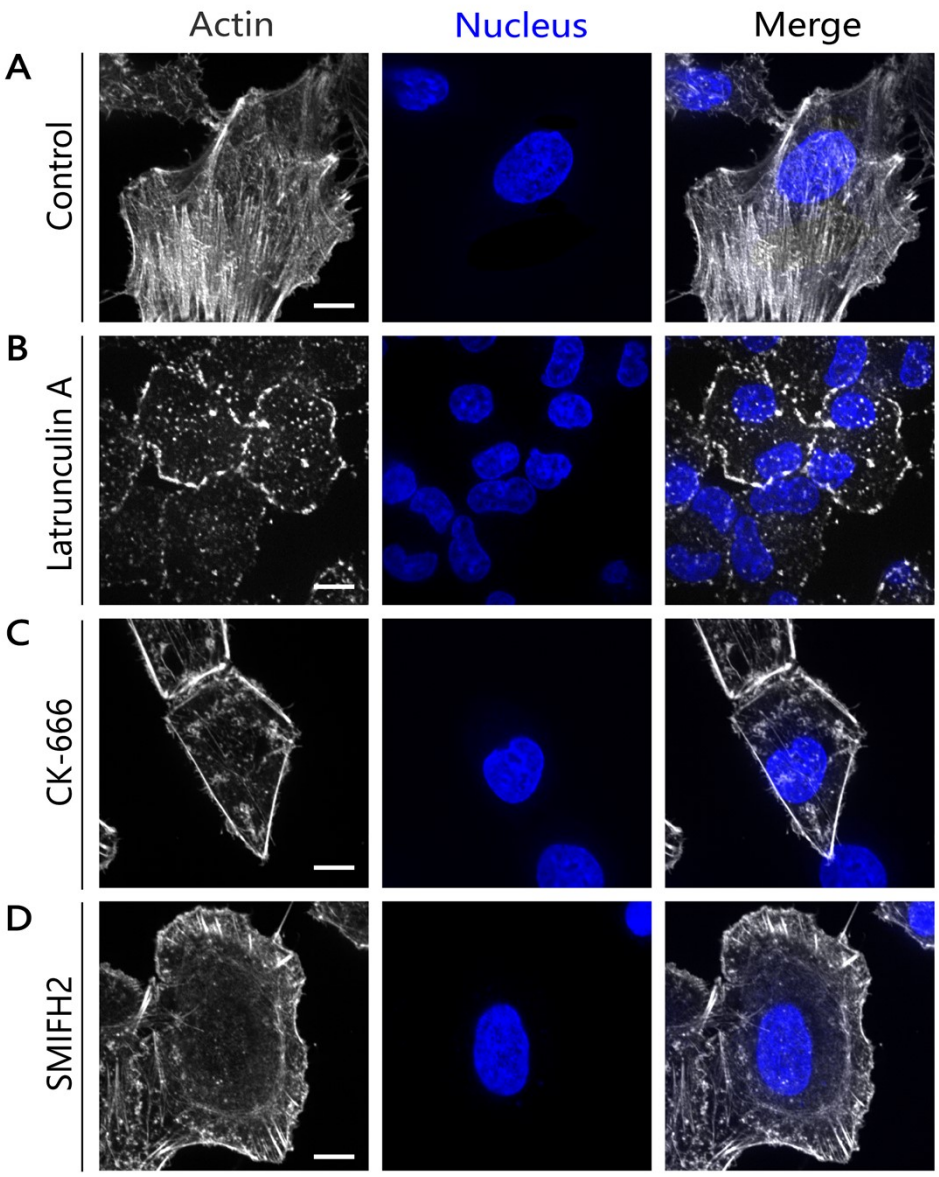

Figure S2: Effect visualization of small-molecule drugs targeting distinct actin cytoskeleton components. A549 cells were treated with cytoskeletal drugs for 24 h: (A) control cells (no treatment); (B–D) drug-treated groups: (B) 250 nM Latrunculin A, (C) 50 μM CK-666, (D) 10 μM SMIFH2. After treatment, cells were fixed with 4% paraformaldehyde, then stained with Alexa Fluor 647-conjugated phalloidin and Hoechst 33342. Scale bars = 10 μm.

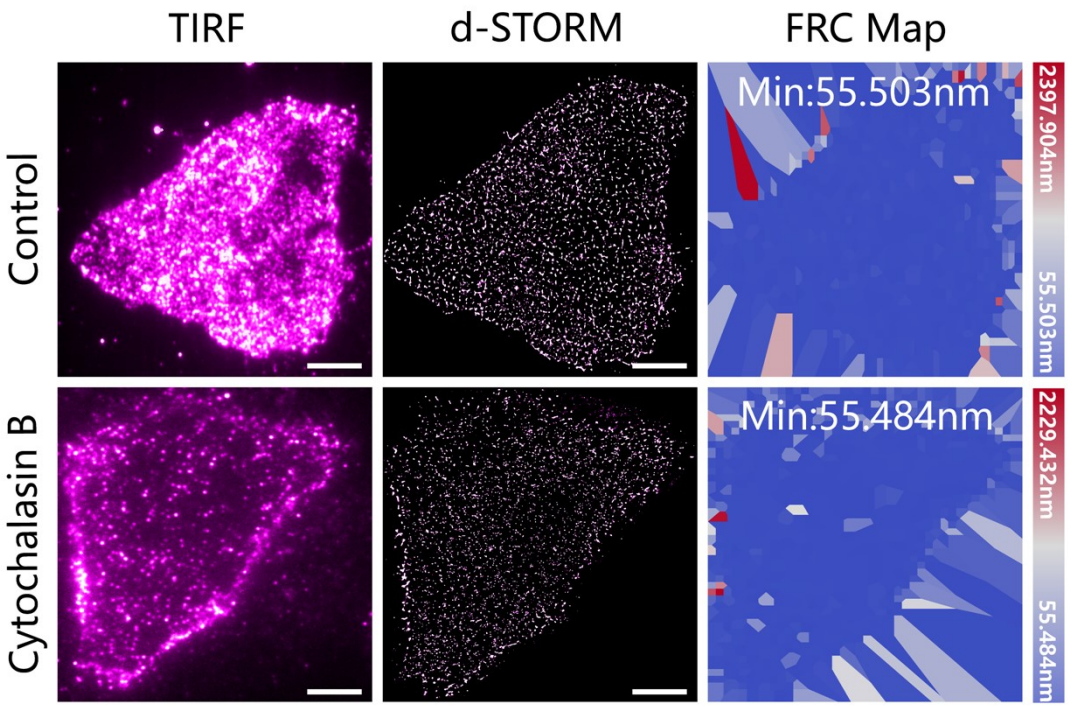

Figure S3: Recognition of RSV F antibodies on cell membranes. Conventional TIRF images, corresponding dSTORM images, and FRC maps of RSV F labelled with Cy5-conjugated antibodies, respectively on the control and CB treatment group. Scale bars = 10 μm.

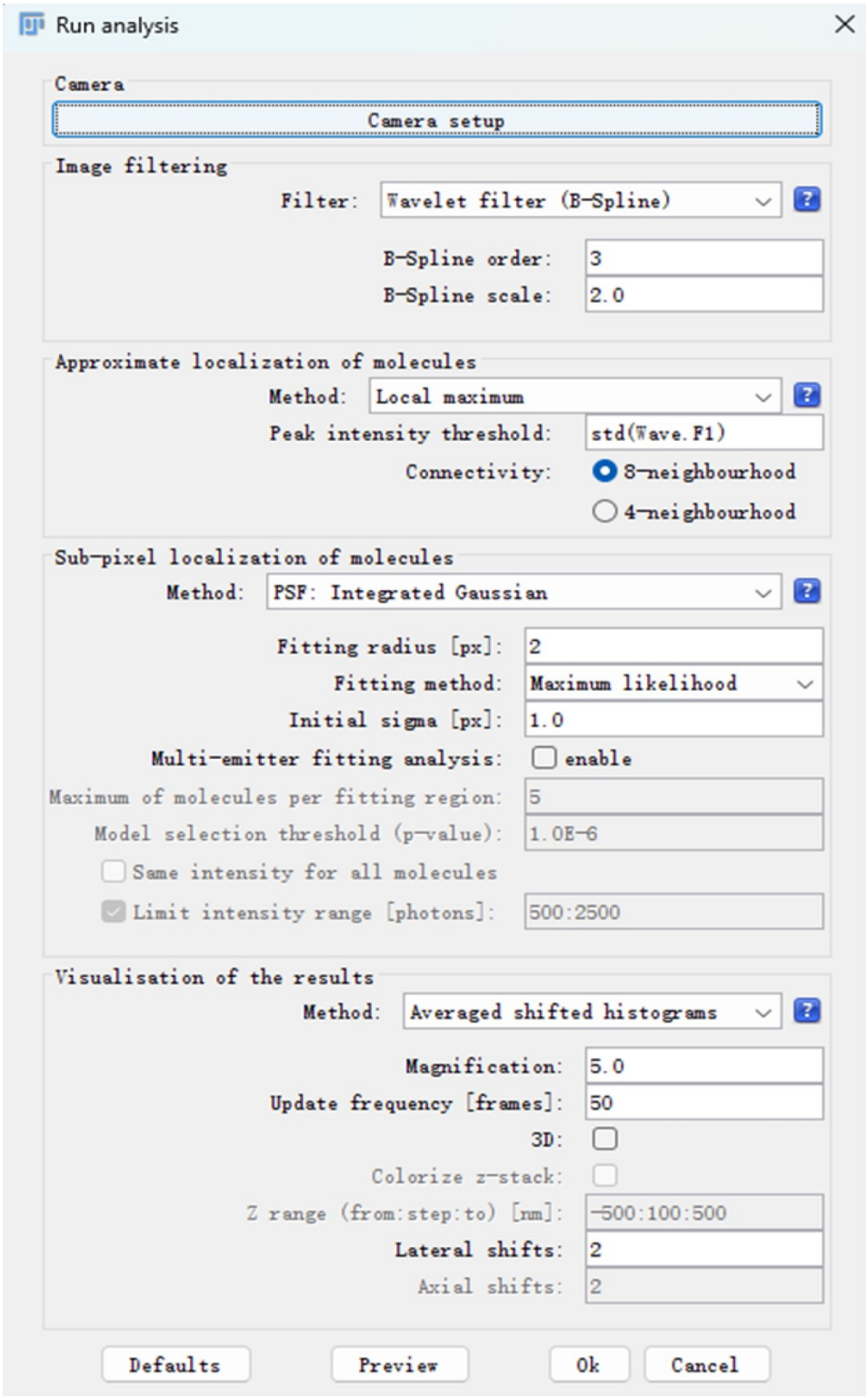

Figure S4: The parameter settings utilized for data processing and image reconstruction with the ThunderSTORM plug-in in ImageJ.

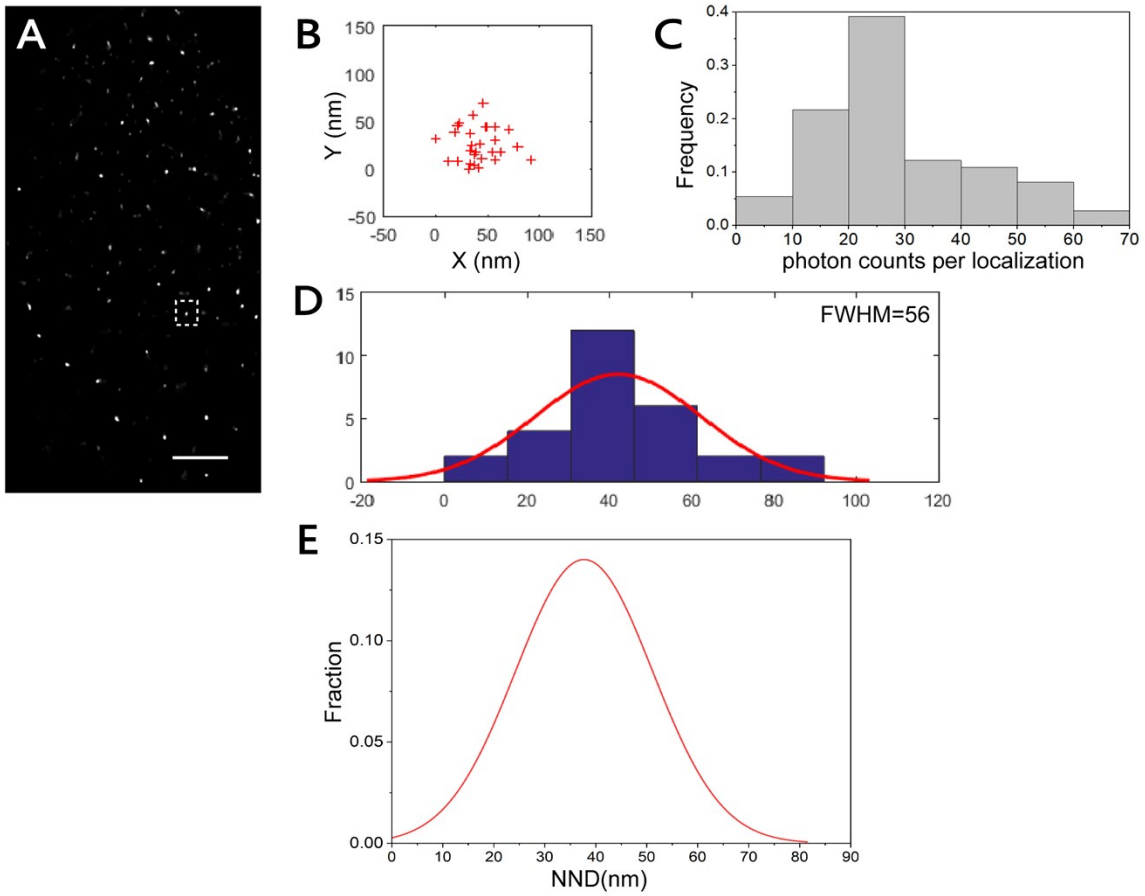

Figure S5: Measurement of localization accuracy for a single RSV F-fluorescent secondary antibody. (A) d-STORM image of the RSV F-fluorescent secondary antibody. Scale bar = 10 μm. (B) Repeated localization of a single RSV F-fluorescent secondary antibody in the boxed region of A. (C) The distribution of photon counts per localization. Data are obtained from over 60 regions across at least three independent experiments. (D) Two-dimensional localization distribution of a single RSV F-fluorescent secondary antibody. The average localization count per single RSV F-fluorescent secondary antibody is 27 with a FWHM of 56 nm. (E) Average normalized RSV F-fluorescent secondary antibody NND is 38 nm.

Movie S1 (separate file). The entire secretion process of A549 cells expressing RSV F-GFP. Time interval is 30 min. Scale bars = 10 μm.

Movie S2 (separate file). HEp-2 cells expressing GFP-RSV F induced syncytia formation was tracked using a real-time imaging. Time interval is 0.2 min. Scale bars = 10 μm.

Movie S3 (separate file). HEp-2 cells expressing GFP-RSV F induced fusion pores formation was tracked using a real-time imaging. Scale bars = 10 μm. Time interval is 1 min.