Supporting Information

Dynamic cell entry pathway of respiratory syncytial virus revealed

by tracking the quantum dots-labeled single virus

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Movie S2. Tracking the intercellular trafficking of QD-RSV in HEp-2 cells.



Fig. S1 Characterization of SA-QD. (A) Fluorescence spectra of SA-QD. (B) TEM images of SA-QD.



Fig. S2 Fluorescence colocalization imaging between immunofluorescence of RSV G protein and QD fluorescence. (A) Fluorescence imaging of QD-RSV infected cells at 0 min, 60 min and 120 min post-infection. Cells were inoculated with biotin-RSV and SA-QD (red) at 4 °C, then shifted to 37 °C for different time. After being fixed and permeabilized, the cells were stained with mouse monoclonal antibody against RSV G protein (Anti-G) and DyLight 488-conjugated goat anti-mouse IgG (green). (B) Three-dimension view and orthogonal slice views of QD-RSV infected cells at 120 min p. i.. The nucleuses of cells were stained with Hoechst 33258.



Fig. S3 Snapshots of time-lapse imaging of QD-RSV entry. HEp-2 cell were infected with QD-RSV and imaged from 0 to 20 min with time interval of 2 min. Yellow line shows the cell boundary. Scale bar, $10 \mu m$.



Fig. S4 Definition of the region of interest. The cell peripheral region was defined as the region inside the cell extending for approximately 2 μ m from the inner side of the plasma membrane. The perinuclear region was referred to the region just around the nucleus with an average band width of 1 μ m. Cytoplasmic region was between the peripheral and perinuclear region. Scale bar, 10 μ m.



Fig. S5 Colocalization of QDs-RSV (red) and lysosome (green) at 2 h, 4 h and 11 h p. i.. HEp-2 cells were infected with biotin-RSV at 4 °C for 30 min and incubated with SA-QD at 4 °C for 10 min. The cells were then shifted to 37 °C for 2 h, 4 h and 11 h. Cells were treated with BODIPY® FL Histamine for labeling lysosome. The nucleuses of cells were stained with Hoechst 33258.



Fig. S6 Immunofluorescence imaging of unlabeled RSV in infected cells at 0 min, 60 min and 120 min post-infection. Cells were inoculated with RSV at 4 °C, then shifted to 37 °C for different time. After being fixed and permeabilized, the cells were stained with mouse monoclonal antibody against RSV G protein and DyLight 488-conjugated goat anti-mouse IgG (green).



Fig. S7 Maximum instantaneous velocity of QD-RSV traveling in the peripheral region (n=17) and in the cytoplasmic region (n=11). Thick bars represent the mean values.