Supplementary Material

Supplementary Figures

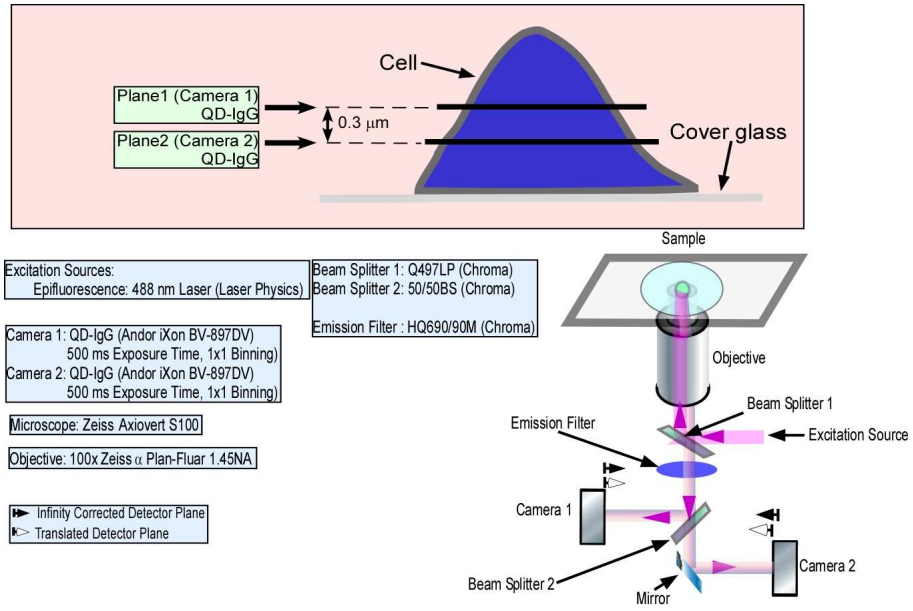

Figure S1: Two plane MUM setup. The schematic shows the emission light path of a MUM setup built on a Zeiss Axiovert S100 microscope that has a bottom port. The setup is used to acquire images of the stationary QD sample 1 by simultaneously imaging two distinct focal planes within the specimen. The schematic also shows the various optical filters used in the emission light path and the camera acquisition parameters such as exposure time and binning. The cell is epi-illuminated with a 488 nm laser. Camera 1 and camera 2 capture the QD-IgG fluorescence signal from inside the cell. The QD images acquired in the two cameras are then used in MUMLA to deduce the z-position. The top cartoon diagram shows a cell that is on the cover glass and the relative spacing between the different focal planes that are imaged.

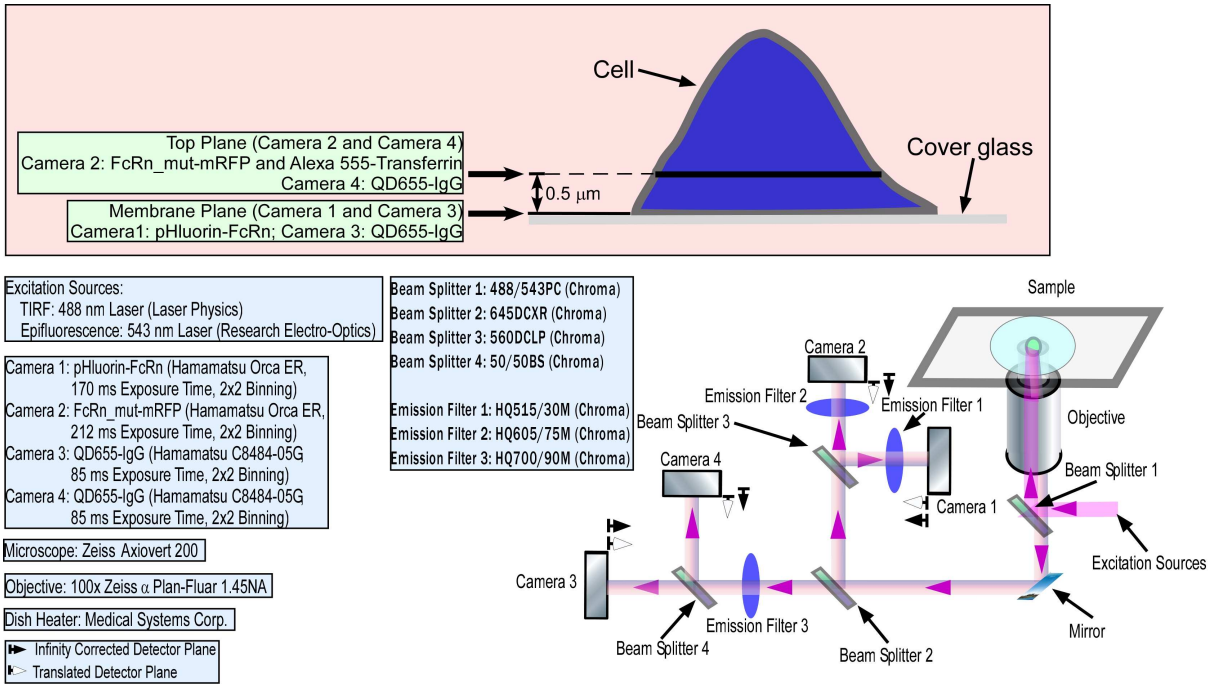

Figure S2: Four plane MUM setup. The schematic shows the emission light path of a MUM setup built on a Zeiss Axiovert 200 microscope that has a side port. The setup is used to image the trafficking of fluorescently labeled FcRn and IgG across two distinct focal planes within the cell. The schematic also shows the various optical filters used in the emission light path and the camera acquisition parameters such as exposure time and binning. The top cartoon diagram shows a cell that is on the cover glass and the relative spacing between the different focal planes that are imaged. The membrane plane is illuminated with a 488 nm laser through TIRF excitation, while the higher plane is epi-illuminated with a 543 nm laser. Both laser lines continuously illuminate the sample during the imaging experiment. A 50:50 beamsplitter is used to separate the emission of the QDs into two light paths. One of the light paths is projected onto Camera 3 to provide a focused image of the QDs at the plasma membrane, whereas the other light path is projected onto Camera 4 to provide a focused image of the QDs 0.5 μm higher into the cell. These images are then used in MUMLA to deduce the z-position of the QD. The FcRn signal from the membrane plane and the FcRn and transferrin signals from the higher plane are also simultaneously captured on two other separate cameras (camera 1 and 2). Specifically, pHluorin-FcRn signal is captured at the membrane plane and FcRn_mut-mRFP and Alexa Fluor 555-labeled Transferrin fluorescence signals are captured at the higher plane.

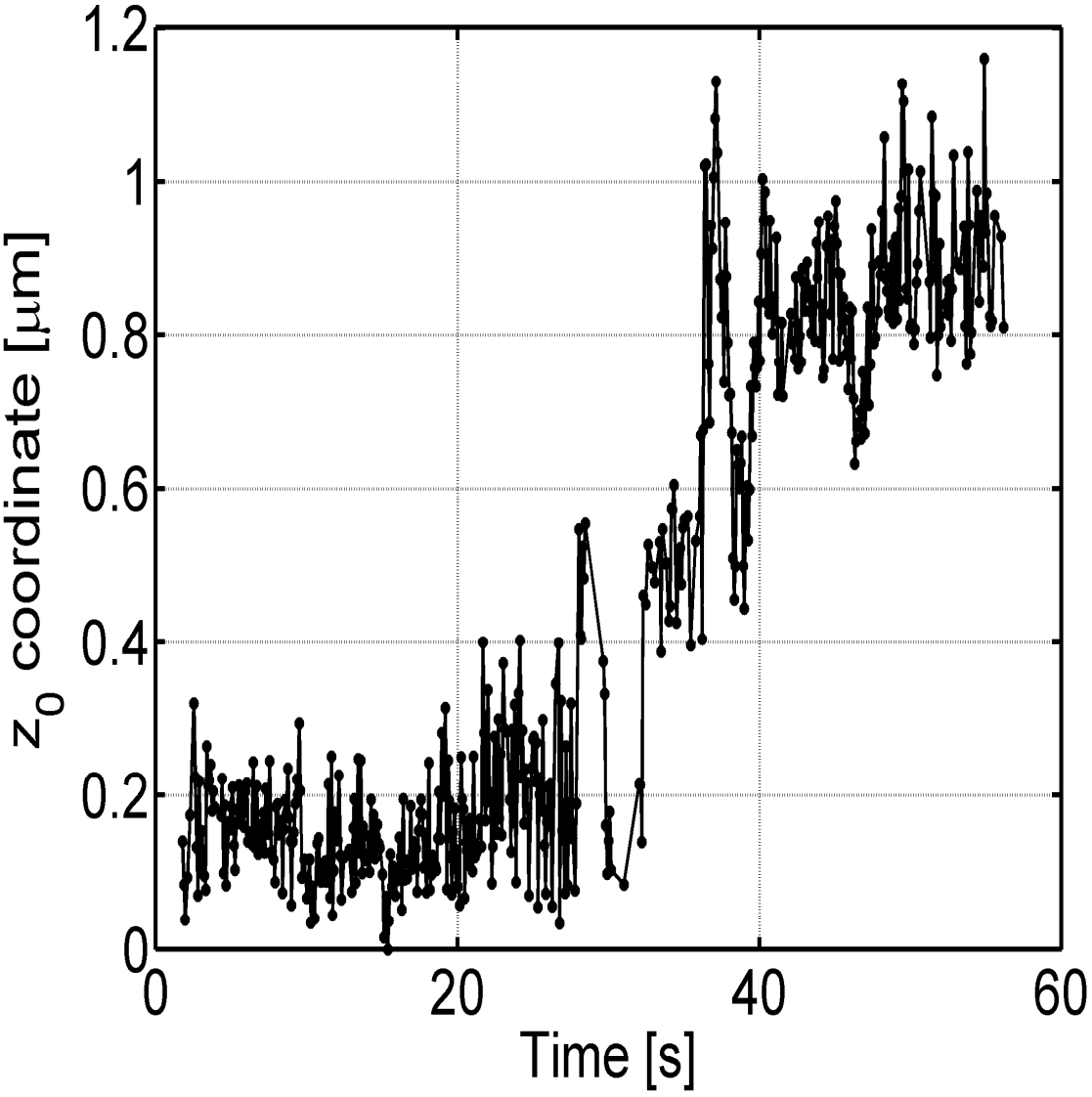

Figure S3: The plot shows the z-position of the QD-IgG molecule as a function of time for the data shown in Figure 5.

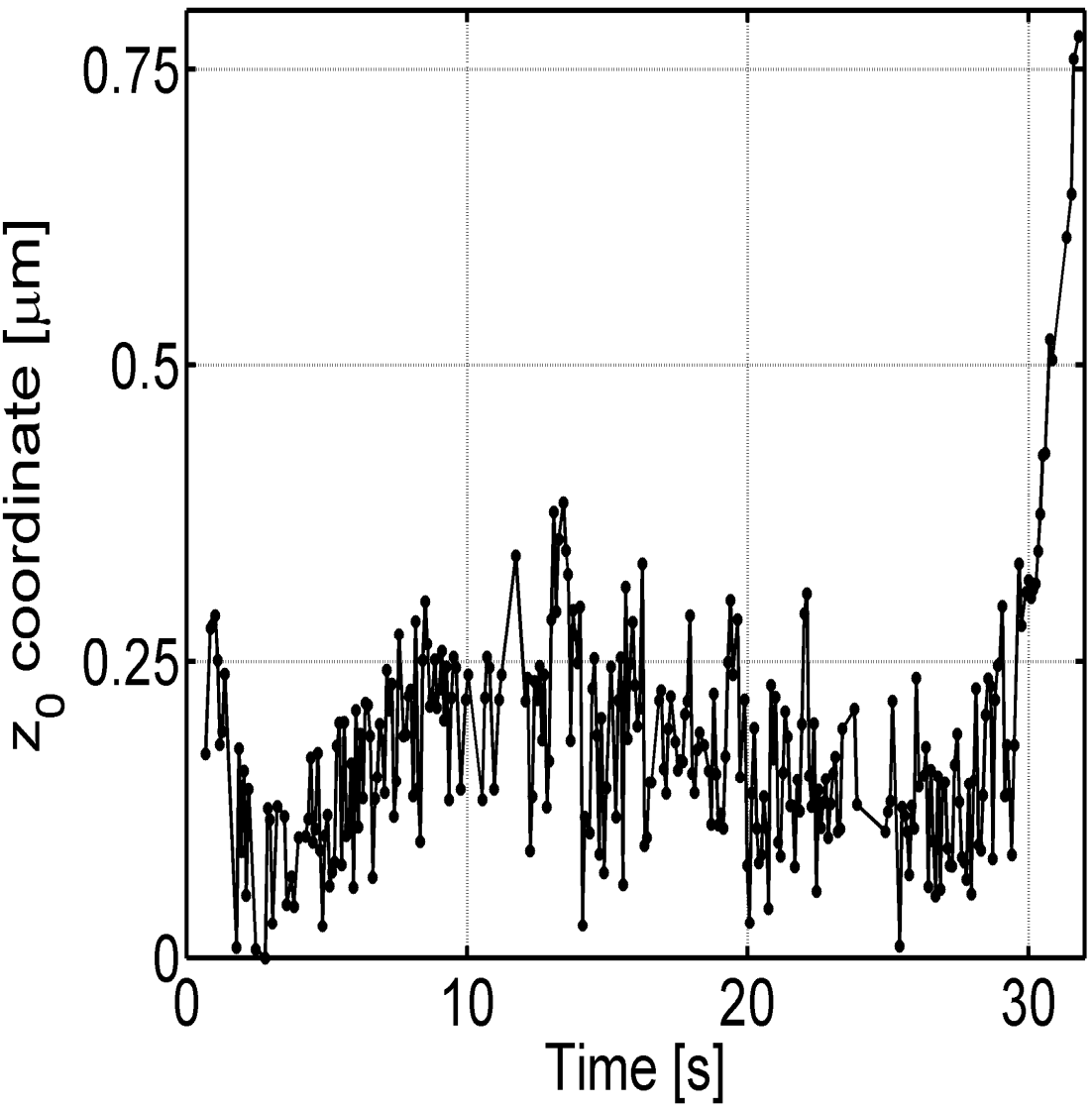

Figure S4: The plot shows the z-position of the QD-IgG molecule as a function of time for the data shown in Figure 6.

Movie Legends

Movie 1. Complex 3D trafficking itinerary of a QD-IgG molecule undergoing endocytosis. The movie corresponds to Figure 5 and shows the top plane (upper row) and the membrane plane (lower row) images of FcRn (first column) and IgG (second column) along with their overlay (third column). The QD-IgG molecule of interest is indicated by a white arrow in the IgG channel and in the overlay. Due to QD blinking, some of the frames will not have the arrow. Movie plays at two times the acquisition speed. (Bar = 1 μm.)

Movie 2. Endocytosed QD-IgG molecule moves directly to the sorting endosome. The movie corresponds to Figure 6 and shows the top plane (upper row) and the membrane plane (lower row) images of FcRn (first column) and IgG (second column) along with their overlay (third column). The QD-IgG molecule of interest is indicated by a white arrow in the IgG channel and in the overlay. Due to QD blinking, some of the frames will not have the arrow. Movie plays at two times the acquisition speed. (Bar = 1 μm.)