

Supplementary Information

Movie 1 Dynamics of MRV virion uptake in nonpolarized MDCK cells. Cells expressing AP2-GFP (green) were seeded 1 day before transfection with a plasmid encoding CLCa-TOM (red). After one additional day, Alexa647-labeled virions (blue) were added to the cells, and the 2D time series from a single optical was acquired for 300 s at 2-s intervals using 100 ms exposures by live-cell spinning-disk confocal microscopy. Numerous examples of clathrin/AP2-associated uptake events for single virions are visualized.

Movie 2 Dynamics of MRV virion uptake at the apical surface of polarized MDCK cells. Cells expressing AP2-GFP (green) were seeded 3-5 days before the experiment to allow polarization. Alexa647-labeled virions (red) were added to the cells, and the 2D time series was acquired for 160 s at 2-s intervals using 100 ms exposures by live-cell spinning-disk confocal microscopy. The complete AP2-associated uptake event for a single virion is visualized, from virion attachment and capture in a coated pit to directional movement after coated-vesicle budding and uncoating.

Movie 3 Dynamics of MRV virion uptake at the apical surface of polarized MDCK cells. Cells expressing AP2-GFP (green) were seeded 3-5 days before the experiment to allow polarization. Alexa647-labeled virions (red) were added to the cells, and the 3D time series was acquired for 240 s at 2-s intervals using 100 ms exposures by live-cell 4D spinning-disk confocal microscopy. Each frame in the movie is the maximum-intensity Z-projection from three serial optical sections spaced 0.5 μm apart. Several examples of AP2-associated uptake events for single virions are visualized.

Movie 4 Dynamics of MRV ISVP uptake at the apical surface of polarized MDCK cells. Cells expressing AP2-GFP (green) were seeded 3-5 days before the experiment to allow polarization. Alexa647-labeled ISVPs (red) were added to the cells, and the 3D time series was acquired for 200 s at 2-s intervals using 100 ms exposures by live-cell 4D spinning-disk confocal microscopy. Each frame in the movie is the maximum-intensity Z-projection from four serial optical sections spaced 0.5 μm apart. Several examples of AP2-associated uptake events for single ISVPs are visualized.

Movie 5 Dynamics of MRV $\mu 1(N42A)$ -ISVPs uptake at the apical surface of polarized MDCK cells. Cells expressing AP2-GFP (green) were seeded 3-5 days before the experiment to allow polarization. Alexa647-labeled $\mu 1(N42A)$ -ISVPs (red) were added to the cells, and the 3D time series was acquired for 200 s at 2-s intervals using 100 ms exposures by live-cell 4D spinning-disk confocal microscopy. Each frame in the movie is the maximum-intensity Z-projection from four serial optical sections spaced 0.5 μm apart. Several examples of AP2-associated uptake events for single ISVPs are visualized.