

Supplementary Figure 2. The relative displacements of CCPs from their associated viruses. Black and red symbols are the displacements in the *x* and *y* directions in units of camera pixels (95 nm/pixel), respectively. We used least-squares fitting to determine the location of any EYFPlabeled clathrin structures near the virus prior to its stage II movement. In each frame, we considered a 4 μ m² area centered on the virus peak. The EYFP signal in this region was fit to a Gaussian function $I(x, y) = I_0 + Ae^{-[(x-x_0)^2 + (y-y_0)^2]/2\sigma^2}$, where I_0 is the average background fluorescence level, A is the amplitude of the peak, x_0 and y_0 are the center coordinates, and σ is the width. I_0, A, x_0 , and y_0 were treated as free fitting parameters, while σ was fixed to be the experimentally measured width of a diffraction-limited spot. The error bars are determined from the variance matrix obtained by the least-squares fitting. Visual inspection of the movies shows that we are able track the peak as soon as a clathrin structure discernable from the background appears in the 4 μ m² area. In 96% of the trajectories, the clathrin peak position (x_0 , y_0) starts centered on the virus to within 1 pixel, as indicated by examples shown in a-e. Among these, only 2% of viruses seem to land on a pre-existing CCP. The rest show the appearance of a clathrin spot after viral binding. The EYFP-clathrin intensity gradually increased and then rapidly disappeared before the virus started stage II movement. After the clathrin-structure has

disappeared, tracking is no longer possible, as indicated by the large error bars appearing at the end of the plots. In the remaining 4% of the trajectories, the initial clathrin peak position is separated from that of the virus (i.e. $x_0 \neq 0$ and $y_0 \neq 0$) and the separation in general decreases with time until the virus joins the clathrin structure, as shown in **f**. This quantitative analysis confirms the results of our visual inspection and indicates *de novo* formation of CCPs at the site of bound viruses.