

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 ( $95 \mathrm{~nm} /$ 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 $4 \mu \mathrm{~m}^{2}$ area centered on the virus peak. The EYFP signal in this region was fit to a Gaussian function $I(x, y)=I_{0}+A e^{-\left[\left(x-x_{0}\right)^{2}+\left(y-y_{0}\right)^{2}\right] / 2 \sigma^{2}}$, 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 $\sigma$ is the width. $I_{0}, A, x_{0}$, and $y_{0}$ were treated as free fitting parameters, while $\sigma$ 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 $4 \mu \mathrm{~m}^{2}$ area. In $96 \%$ of the trajectories, the clathrin peak position $\left(x_{0}, y_{0}\right)$ 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 $\mathbf{f}$. This quantitative analysis confirms the results of our visual inspection and indicates de novo formation of CCPs at the site of bound viruses.

