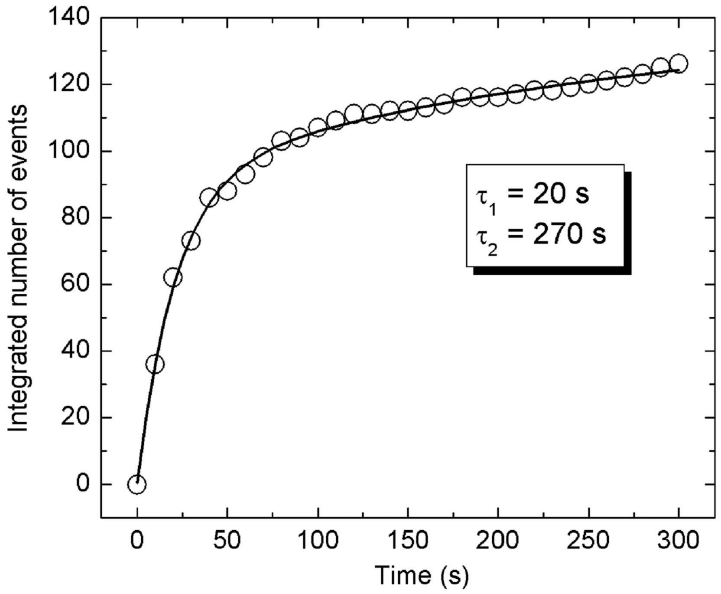

Supplementary Figure 3. An integrated histogram of the relative time between the last CCV uncoating event prior to stage II viral movement and the onset of stage II movement. Circles indicate the number of events with the relative time shorter than value indicated on the horizontal axis. The solid curve is a fit to a double exponential decay with time constants 20 s and 270 s. These data clearly show a biphasic behaviour: while the majority of viruses started their stage II movement relatively rapidly after uncoating with a time constant of 20 s, the rest remained in the cell periphery for several minutes before stage II movement. The former population of viruses most likely stays inside the cell during the brief period between clathrin uncoating and stage II movement, as both recycling and endocytosis typical take a few minutes or longer. However, the relatively long lag between clathrin uncoating and stage II movement for the latter population leaves open the possibility that these viruses may have entered the cell via a CCP, then recycled back to the cell surface and re-entered via a clathrin-independent mechanism. We thus re-evaluate the partition ratio of viruses between the clathrin-dependent and -independent pathways by classifying endocytosis based on the internalization event immediately preceding stage II movement. This is reasonable as viral fusion was not observed without microtubule-dependent movement. In the extreme case that all of the viruses under the second phase were recycled and re-entered the cell without using a CCP, the fraction of viruses that enter via the clathrin-independent pathway would be increased from the observed 35% to 50%. The true fraction is most likely between these values.