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2 **Legends for Supplementary Movies**

3 **MOVIE S1. 3D reconstruction of CT.C357S puncta associated with a mitochondrial tubule.** The
4 data used to create Fig.7E was animated to highlight the arrangement of the UL16 mutant (green) on
5 and around a mitochondrial tubule (red).

6 **MOVIE S2. CT.C357S positive punctate structures overlap with the sites where mitochondrial**
7 **division occurred in Vero cells.** Live Vero cells expressing CT.C357S-GFP (green puncta) were
8 labeled with MitoTracker Red to stain mitochondria, and time-lapse fluorescent imaging was performed
9 in a single plane. Images were captured every second for 7 minutes, and an event from the first 60 sec
10 of the movie is shown at 6 frames per second. Still frames of this movie are shown in Fig.7G.

11 **MOVIE S3. Repeated contacts between UL16-GFP puncta and mitochondria in infected cells.**
12 Vero cells infected with HSV.UL16-GFP were stained with MitoTracker Red to visualize mitochondria at
13 about 12 h post infection. Movies of at least 6 different whole cells were captured in single planes at 2
14 frames per second for 5 minutes. Multiple small areas of the whole cell recordings were analyzed for
15 UL16-GFP-mitochondria interactions. This movie is of the region marked with a box in Fig. 9C, which
16 shows $t=0$. Only the first 60 seconds (played at 6 frames per second) of the recording is shown. Still
17 frames are shown in Fig. 9D. Note the repeated contacts that the large green punctum makes with
18 mitochondria (red). The transient contacts of mitochondria and UL16-GFP appear as yellow region
19 (overlapping green and red pixels) in the movie. The shown movie has been edited to enhance
20 brightness and contrast using Volocity for easy visualization of the UL16-GFP and mitochondria
21 contacts.

22 **MOVIE S4. Contacts between UL16-GFP puncta and mitochondria in the absence of capsids.**
23 Vero cells infected with an UL18-null virus that expresses UL16-GFP were stained for mitochondria,
24 and were subjected to live cell recording. Images were acquired every second for 5 minutes in single
25 plane. At least 6 different whole cells were imaged. Recordings were analyzed by dividing each cell into
26 multiple regions of interest (ROI), and the first 53 seconds of one of these is shown at 6 frames per
27 second. Regions that turn yellow in the movie at various times represent the contact-points between

- 1 UL16-GFP positive puncta and mitochondria. Acquired movies were edited to enhance the brightness
- 2 and contrast of the objects.