Figure S1. **Fluorescent protein gene constructs for recombinant virus generation.** (A) The triple-mCherry–vp39 chloramphenicol acetyltransferase (CAT) locus, used to generate the 3mC virus, consists of the viral promoter of the vp39 gene (black arrow) followed by the vp39 ORF (blue arrow) fused in frame to three mCherry ORFs (red arrows) and a CAT cassette (black). (B) The triple-mCherry–vp39 gentamycin resistance (GentR) locus, used to generate the 3mC I358A virus, contains the same elements as in A, except a gentamycin resistance cassette was substituted for the CAT cassette because CAT had already been inserted during the introduction of the I358A mutation (Goley et al., 2006). (C) The ie-1 GFP gentamycin resistance locus, used to construct the ie-1 GFP virus, consists of the baculovirus hr5 enhancer and ie-1 promoter (Jarvis et al., 1996) followed by EGFP and a gentamycin resistance cassette. (D) The transfer plasmid, used to introduce the aforementioned loci into the WOBpos bacmid, contains flanking viral sequences (light blue) on each side of the aforementioned loci for homologous recombination with viral genomic sequences. For the native promoter triple-mCherry–vp39 CAT locus, the flanking sequences are a truncated kanamycin resistance cassette and a truncated polyhedrin ORF. For the ie-1 GFP locus, the flanking sequences are a truncated kanamycin resistance cassette and a truncated mini–F replicon.
Figure S2. Growth curves of fluorescent protein-expressing viruses. One-step viral growth curves (performed in triplicate), comparing the growth of WOBpos (WT; black), 3mC (WOBpos expressing VP39-mCherry; red), and ie-1 GFP (WOBpos expressing GFP under the control of the ie-1 promoter; green). For growth curves, Sf9 cells were inoculated at an MOI of 10, aliquots of culture media were collected at 12, 18, 24, 36, and 48 hpi, and virus was subsequently titered to determine plaque-forming units (PFU) per milliliter. Error bars indicate mean ± SD.
Figure S3. Images of AcMNPV accumulation at nuclear pores and the cell surface. (A–C) Deconvolved images representing lower magnification versions of the images shown in Fig. 3. Colors indicate virus (red; 3mC), actin (green; FITC-phalloidin), and nuclear pores (blue; anti–nuclear pore immunofluorescence). (A) Virus colocalizing with a nuclear pore. (B) Virus adjacent to a nuclear pore. (C) Virus on the nuclear membrane in the space between pores. Bars, 2 µm. (D) Virus accumulates in cell surface spikes in the presence of aphidicolin. Virus (red; anti-capsid immunofluorescence), actin (green; FITC-phalloidin), and DNA (blue; DAPI) in a High Five cell infected with WT AcMNPV in the presence of 5 µg/ml aphidicolin at 4 hpi. Bar, 5 µm. Inset shows a higher magnification view of the boxed region. Bar, 2 µm.
Video 1. **AcMNPV actin-based motility.** AcMNPV 3mC virus (red) is shown in a High Five cell expressing EGFP-actin (green). The cells were imaged starting at 22 min after infection with an MOI of ~200 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 7.3 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 10 µm.

Video 2. **Comparison of the motility of AcMNPV WT and I358A mutant.** AcMNPV 3mC (red; left) or 3mC I358A (red; right) nucleocapsids are shown in High Five cells expressing EGFP-actin (green). The cells were imaged starting at 70 min after infection for 3mC and 90 min after infection for 3mC I358A with an MOI of ~200 (3mC) or ~20 (3mC I358A) and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 4.2 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 3. **I358A actin-based motility.** AcMNPV 3mC I358A virus (red) is shown in a High Five cell expressing EGFP-actin (green). The cells were imaged starting at 35 min after infection with an MOI of ~20 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 2.4 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 4. **Comparison of the movement tracks of AcMNPV WT and I358A mutant.** Tracks of AcMNPV 3mC (left) and 3mC I358A (right) nucleocapsid movements in infected High Five cells are shown in cartoon form. The initial position of each virus is marked with a colored dot, and the movement path is indicated by a colored line. The cells were imaged starting at 57 min after infection for 3mC and 29 min after infection for 3mC I358A with an MOI of ~200 (3mC) or ~20 (3mC I358A) and 2 d after transfection with the plasmid expressing EGFP-actin. Frames represent 5-s intervals and are displayed at 10 frames/s. Bars, 10 µm.

Video 5. **Viral collision with the nuclear periphery.** AcMNPV 3mC nucleocapsids (red) are shown colliding with the nucleus in High Five cells expressing EGFP-actin (green). The yellow circle highlights a virus colliding with the nucleus, although other collisions are also apparent. Some collisions occur out of plane with the apparent nuclear border (i.e., we have not observed actin tails within the nucleus). The cells were imaged starting at 5 min after infection with an MOI of ~200 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 2.3 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 6. **Viral collision with the nuclear periphery.** AcMNPV 3mC nucleocapsids (red) are shown colliding with the nucleus in High Five cells expressing EGFP-actin (green). Some collisions occur out of plane with the apparent nuclear border (i.e., we have not observed actin tails within the nucleus). The cells were imaged starting at 5 min after infection with an MOI of ~200 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 5.3 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.
Video 7. **Viral collision with the nuclear periphery.** EGFP-actin is shown in High Five cells 5 min after infection with an MOI of ~200 and 2 d after transfection with the EGFP-actin-expressing plasmid. The yellow circle highlights a virus colliding with the nucleus, although other collisions are also apparent. After collisions, virus-associated actin comet tails persist, resembling corkscrews that radiate from the nuclear periphery. Images were captured every 5 s for 13.3 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 8. **Viral entry into the nucleus.** AcMNPV 3mC nucleocapsids (red) are shown separating from their actin comets and entering the nucleus in High Five cells expressing EGFP-actin (green). After nucleocapsid entry into the nucleus, actin polymerization ceases. The cell was imaged starting at 33 min after infection with an MOI of ~200 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 4.2 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 9. **I358A mutant virus association with the nuclear periphery.** An AcMNPV 3mC I358A nucleocapsid (red) is shown with a virus-associated actin corkscrew structure radiating from the nuclear periphery in High Five cells expressing EGFP-actin (green). The cells were imaged starting at 74 min after infection with an MOI of ~20 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 4.2 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

Video 10. **Viruses moving into cell surface spikes.** AcMNPV 3mC virus (red) is shown moving into surface spikes in a High Five cell expressing EGFP-actin (green). This video is a magnified and cropped view from Video 1. The cells were imaged starting at 22 min after infection with an MOI of ~200 and 2 d after transfection with the plasmid expressing EGFP-actin. Images were captured every 5 s for 2.3 min by time-lapse epifluorescence microscopy and are displayed at 10 frames/s. Bar, 5 µm.

References

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