Supporting Information

Bohannon et al. 10.1073/pnas.1221896110

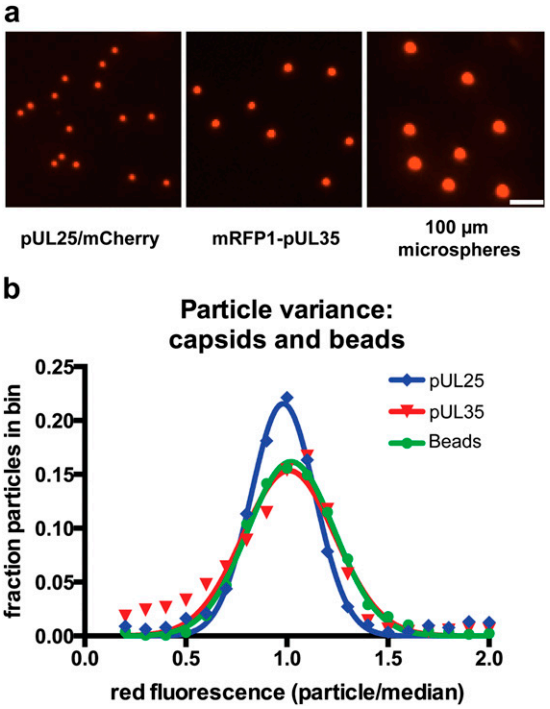

Fig. S1. Capsids emit diffraction-limited projections similar to equivalently sized microspheres. (*A*) Images of 1.5-s exposures of capsids incorporating either pUL25/mCherry (1) or mRFP1-pUL35 protein tags, or 0.1 μm TetraSpeck Microspheres (Life Technologies cat. T7279). Images are equivalently scaled to show relative fluorescence intensities, which appear as larger areas of diffraction despite all particles sharing similar physical size. (Scale bar, 3 μm.) (*B*) Histogram of red-fluorescent particle intensities of pUL25/mCherry, mRFP1-pUL35, or 0.1-μm microspheres (beads). Each particle intensity was divided by the sample median, plotted as a histogram, and fit to a Gaussian curve by nonlinear regression. More than 1,200 virions or beads were analyzed for each sample. The Gaussian model aligns with the expected outcome of imaging particles with little fluorescence variability, with deviation coming from slight differences in light path, focal plane, and photobleaching during focusing.

1. Bohannon KP, Sollars PJ, Pickard GE, Smith GA (2012) Fusion of a fluorescent protein to the pUL25 minor capsid protein of pseudorabies virus allows live-cell capsid imaging with negligible impact on infection. *J Gen Virol* 93(Pt 1):124–129.

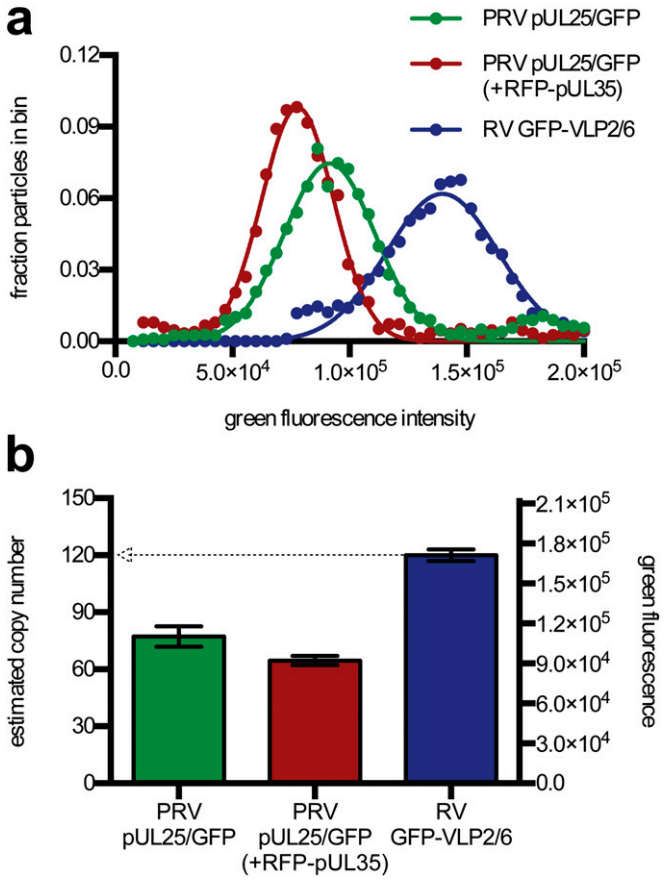

Fig. S2. Copy number of pUL25 based on a rotavirus (RV) virus like particle (VLP) standard. (*A*) Histograms of green fluorescence intensity of diffraction-limited green particles. Bins sizes were selected using the Freedman–Diaconis rule for pseudorabies virus (PRV) pUL25/GFP. Nonlinear regression was used to fit Gaussian curves to the data, where all data fit with $R^2 > 0.95$. A peak of very dim debris-like particles (mode = 1.6×10^4 arbitrary green fluorescence units) was removed from the RV GFP-VLP2/6 distribution by setting a threshold at 7.5×10^4 arbitrary green fluorescence units. (*B*) Bar graph depicting green fluorescence intensities (right axis) of PRV virions and RV VLPs. Error bars show SEM from three independent experiments. Copy numbers (left axis) were determined by setting the average of RV GFP-VLP2/6–120 copies, which was previously determined by cryo-electron microscopy (cryo-EM) reconstruction.

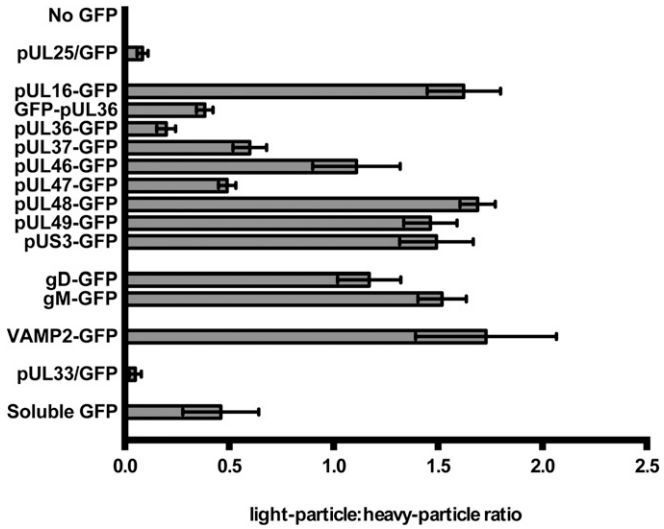

Fig. S3. Relative light (L)- and heavy (H)-particle production. H-particles were identified as in Figs. 2 and 3. L-particles were defined as green fluorescent puncta that were brighter than a threshold approximating five molecules of GFP and lacked a red-fluorescent capsid. Error bars represent SEM for $n \geq 3$ independent experiments.

Table S1. Strains used in this study

Strain	mRFP1*	GFP	GFP fusion site	Titer (pfu/mL)	Source
PRV-GS847	RFP-pUL35	—	—	5.4×10^8	(1)
PRV-GS909	RFP-pUL35	GFP-pUL36	N terminus	5.1×10^8	(1)
PRV-GS1022	RFP-pUL35	pUL37-GFP	C terminus	4.7×10^8	(1)
PRV-GS1046	RFP-pUL35	pUL48-GFP	C terminus	6.7×10^8	(1)
PRV-GS1178	RFP-pUL35	GFP-gM	N terminus	1.1×10^8	Present study
PRV-GS1236	RFP-pUL35	GFP-gD	N terminus	2.1×10^8	(2)
PRV-GS1504	RFP-pUL35	US3-GFP	C terminus	1.0×10^8	(3)
PRV-GS1903	RFP-pUL35	pUL36-GFP	C terminus	2.2×10^8	(4)
PRV-GS2484	RFP-pUL35	CMV > GFP†	Soluble	2.1×10^8	(5)
PRV-GS2795	RFP-pUL35	CMV > VAMP2-GFP†	C terminus	0.8×10^8	(6)
PRV-GS3081	RFP-pUL35	pUL16-GFP	C terminus	1.2×10^8	Present study
PRV-GS3171	RFP-pUL35	pUL25/GFP	Amino acids 42–43	3.1×10^8	Present study
PRV-GS4078	RFP-pUL35	pUL49-GFP	C terminus	3.6×10^8	Present study
PRV-GS4127	RFP-pUL35	pUL46-GFP	C terminus	2.3×10^8	Present study
PRV-GS4379	—	pUL25/GFP	Amino acids 42–43	9.1×10^8	(5)
PRV-GS4750	RFP-pUL35	pUL33/GFP	Amino acids 33–34	6.8×10^8	Present study
PRV-GS4848	RFP-GFP-pUL35	RFP-GFP-pUL35	N terminus	0.8×10^8	Present study
PRV-GS4938	RFP-pUL35	pUL47-GFP	C terminus	3.7×10^8	Present study

*mRFP1 was fused to the amino terminus of pUL35.
†CMV expression cassettes were inserted into the US4 gene.

1. Luxton GWG, et al. (2005) Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins. *Proc Natl Acad Sci USA* 102(16):5832–5837.
2. Antinone SE, Smith GA (2006) Two modes of herpesvirus trafficking in neurons: Membrane acquisition directs motion. *J Virol* 80(22):11235–11240.
3. Coller KE, Smith GA (2008) Two viral kinases are required for sustained long distance axon transport of a neuroinvasive herpesvirus. *Traffic* 9(9):1458–1470.
4. Leelawong M, Lee JI, Smith GA (2012) Nuclear egress of pseudorabies virus capsids is enhanced by a subspecies of the large tegument protein that is lost upon cytoplasmic maturation. *J Virol* 86(11):6303–6314.
5. Bohannon KP, Sollars PJ, Pickard GE, Smith GA (2012) Fusion of a fluorescent protein to the pUL25 minor capsid protein of pseudorabies virus allows live-cell capsid imaging with negligible impact on infection. *J Gen Virol* 93(Pt 1):124–129.
6. Antinone SE, Zaichick SV, Smith GA (2010) Resolving the assembly state of herpes simplex virus during axon transport by live-cell imaging. *J Virol* 84(24):13019–13030.

Table S2. Primers used to construct PRV recombinants unique to this study

Strain	PCR template	Primer pairs
GS1178	pGreenFKF	5′-CACCCTTATCTTCGCCTCGCTCCCGGAAATGGGGTTCCCGGTGAGCAAGGGCGAGG
		5′-TGTCGTAGTCGGCCACGGTCGCGTAAAAGCACGGGAACCCTGAACCGCCCGAGAAGTTCC
GS3081	pEP-EGFP-in	5′-CCCCGCCCGCGCCATCCCCGAGCTAATAAACGATTATGTGAGCAAGGGCGAG,
		5′-GCCACAATACAAACGCAAGTACCCATTTTTTTCATTTCACTTGTACAGCTCGTC
GS3171	pEP-EGFP-in	5′-CTGGGCCCGCCCGGGCTTCAGCGAGGGCCTCGACGCGCGCGTGAGCAAGGGCGAGGAG,
		5′-CGGCCGCGCGGCGGCGCGCGTTCGCGTGCGCCAGCGCGAGCTTGTACAGCTCGTCCATGC
GS4078	pEP-EGFP-in	5′-GGACGAGAGCACCCCCGGGCGGAAGGGAAAAGTGTATAAAGTGAGCAAGGGCGAGGAG,
		5′-CAGCGGACGACTGAGCGGGGTGCCATTTGCAACGCCTTTACTTGTACAGCTCGTCCATGC
GS4127	pEP-EGFP-in	5′-GCCGCCCCGGGGCCGCTAACCCGCCTCTGCCTCCGCCATGGTGAGCAAGGGCGAGGAG,
		5′-CCTTCCACGAAGCGCGGCGCGTTCCTCGGGCGCGGCGGAGCTTGTACAGCTCGTCCATGC
GS4750	pEP-EGFP-in	5′-CTGCGCGACTTCGACGTGGACTTTCTCGAGGCCAACTACGTGAGCAAGGGCGAGGAG,
		5′-CATCACGTCCTCGAACCAGACGCGCACCCGCGGGGGCAGCTTGTACAGCTCGTCCATGC
GS4848	pEP-EGFP-in	5′-GGAACAGTACGAGCGCGCCGAGGGCCGCCACTCCACCGGCGGAGGTGGCGTGAGCAAGGGCGAGGAG,
		5′-TCTGCGCGGTGATCGTCCGGGGATTGTTCGGGTCGAAGGACTTGTACAGCTCGTCCATGCCG
GS4938	pEP-EGFP-in	5′-CCGAGCGCATTTATCGGCGCGCCGGCCGCCCCGGGGCCGCGGTGGCGGAGGTGGTGTGAGCAAGGGCGAGGAG
		5′-CTCGGGCGCGGCGGAGCATGGCGGAGGCAGAGGCGGGTTACTTGTACAGCTCGTCCATGC

Underlined sequences share homology to template plasmid.