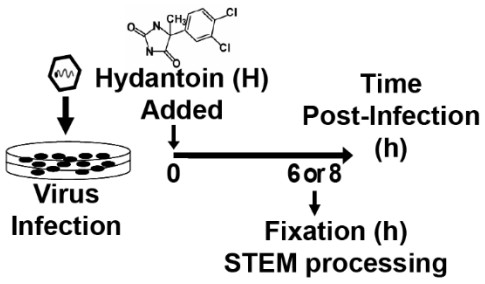
A



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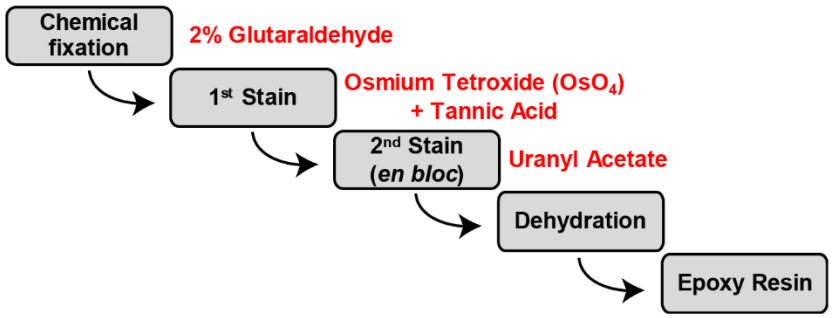

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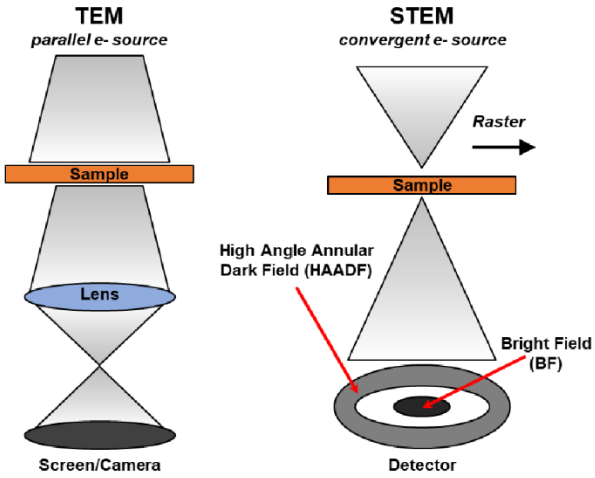
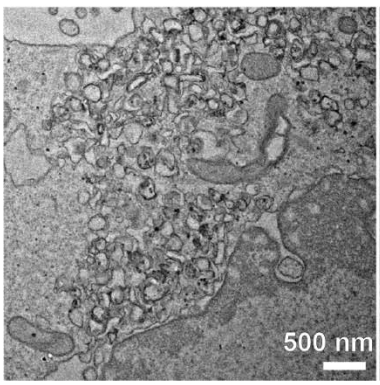
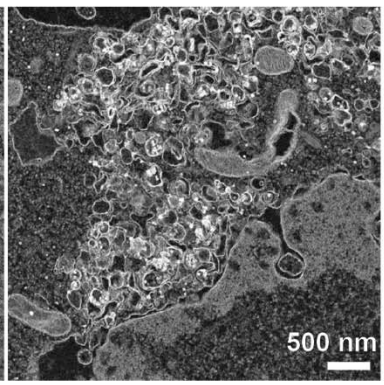


D

	Transmission Electron Microscopy (TEM)	Scanning Transmission Electron Microscopy (STEM)
Mass contrast	Poor	Optimal
Sample thickness capability	Thinner	Thicker
Chromatic aberration	High	None
Beam Damage	High	Low
Elemental mapping capability	N/A	+
Contrast/resolution trade-off	High	None
De-focus	High	None

E





F

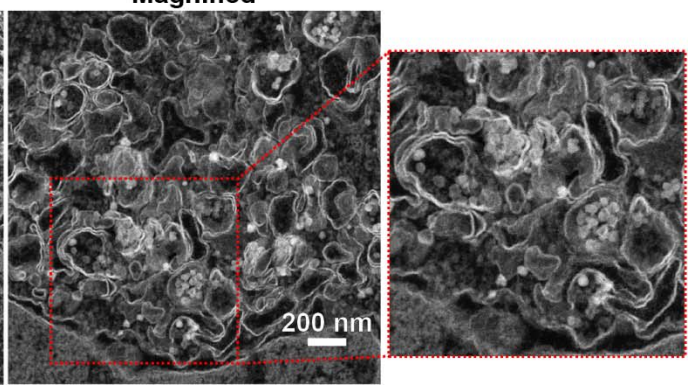
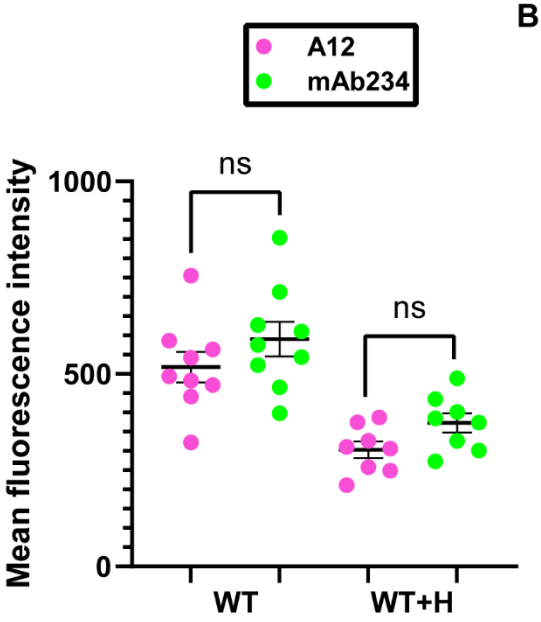


Figure S1. An alternate imaging approach: Scanning transmission electron microscopy (STEM).

(A) Cell lysate preparation for STEM. Infection of HeLa cell monolayers was carried out in the presence or absence of hydantoin. A cell suspension is then prepared using trypsin to release the monolayer at the stated time points 6 or 8- hours post-infection. Cells are gently pelleted, fixed, and processed as described in panel **(B)**. **(B) Cell microsection preparation for STEM.** Cell pellets were subjected to chemical fixation using 2% glutaraldehyde. An initial stain was performed using osmium tetroxide, followed by tannic acid treatment. A second *en bloc* stain was completed using uranyl acetate. Cell pellets were then dehydrated and embedded in an epoxy resin. Thin microsections were then collected and placed on a carbon-coated grid, where a third and final on-grid stain was performed. **(C) Schematic of TEM and STEM microscopy**. TEM is set up much like light microscopy but uses electrons and electromagnetic lenses instead of light. Briefly, the beam hits the sample, electrons are scattered, and the lens forms an image projected to the camera. STEM is entirely different. The beam is converged to a single point, then rastered across the sample, and a detector collects the resulting scattered electrons. In short, TEM contrast comes from unscattered electrons. In STEM, contrast comes from scattered electrons. **(D) Advantages and disadvantages of TEM and STEM imaging.** This table discusses the advantages and disadvantages of Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) to provide some perspective on the factors influencing the contrast gains obtained when imaging biological samples using STEM. In short, we enumerate several advantages of using STEM imaging in the ultrastructural analysis of biological samples, such as membrane derangements in infected cells. **(E) TEM and STEM imaging mode comparison.** HAADF-STEM (High Angle Annular Dark Field - Scanning Transmission Electron Microscopy) imaging of WT PV-infected HeLa cells. HeLa cells were infected with WT PV at an MOI of 10 and then fixed in glutaraldehyde 6 hours post-infection (hpi). Fixed samples were dehydrated, stained, embedded, and sectioned in thin micrographs for imaging as described in panels **(A)** and **(B)**. Images were collected using a Thermo Scientific Talos F200X G2 (S)TEM operated at 200 kV and a beam current of approximately 0.12 nA. The contrast is also reversed when compared to TEM, with the vacuum appearing dark. WT infection induces virus-containing double membranous vesicles and multi-vesicular amphisome-like vesicles with virions in the intra-luminal vesicles. Large outer vesicles with intra-luminal vesicles (100-300 nm diameter) contain ~30 nm particles inside. Double membrane vesicles are located at sites where vesicular-tubular clusters are observed in TEM mode. **(F) STEM imaging of WT PV-infected HeLa cells (magnified)**. In this magnified view, we look closely at observed structures in panel **(E)**. Large outer vesicles with intra-luminal vesicles (100-300 nm diameter) contain ~30 nm particles inside. Double membrane vesicles are located at sites where vesicular-tubular clusters are observed in TEM mode. 30 nm virus particles observed inside of intra-luminal vesicles. Close-up view of an intra-luminal vesicle that contains 30 nm particles.

A



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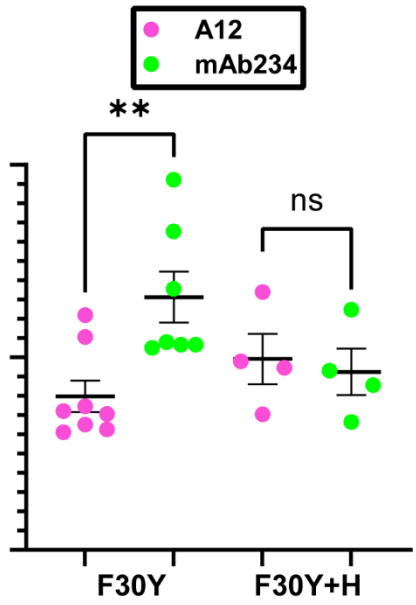

C

	A12 vs mAb234			
	WT	WT+H	F30Y	F30Y+H
P value	0.2431 (ns)	0.0527 (ns)	0.0047 (**)	0.7197 (ns)
Mean A12	517.6	302.7	399.1	496.1
Mean mAb234	590.1	373.0	656.6	462.5
Dif. of means	72.48 ± 59.80	70.23 ± 33.18	257.5 ± 75.61	-33.57 ± 89.21
95% C.I.	-54.29 to 199.2	-0.9366 to 141.4	94.14 to 420.8	-251.9 to 184.7
R squared	0.08409	0.2424	0.4715	0.02305

	A12			
	WT vs WT+H	WT vs F30Y	WT vs F30Y	WT vs F30Y+H
P value	0.0003 (***)	0.2178 (ns)	0.0548 (ns)	0.7745 (ns)
Mean A12	517.6	399.1	517.6	517.6
Mean mAb234	302.7	496.1	399.1	496.1
Dif. of means	214.8 ± 46.65	96.99 ± 73.75	118.5 ± 56.90	-21.50 ± 73.22
95% C.I.	115.4 to 314.3	-67.34 to 261.3	-2.792 to 239.8	-182.7 to 139.7
R squared	0.5857	0.1474	0.2243	0.007780

	mAb234			
	WT vs WT+H	WT vs F30Y	WT vs F30Y	WT vs F30Y+H
P value	0.0010 (**)	0.0835 (ns)	0.4038 (ns)	0.1343 (ns)
Mean A12	590.1	656.6	590.1	590.1
Mean mAb234	373.0	462.5	656.6	462.5
Dif. of means	-217.1 ± 53.36	-194.1 ± 99.75	66.53 ± 77.28	-127.6 ± 78.91
95% C.I.	-330.8 to -103.3	-419.7 to 31.57	-99.23 to 232.3	-301.2 to 46.13
R squared	0.5246	0.2961	0.05027	0.1919

Figure S2. A12 and mAb234 fluorescence intensity analysis of PV-infected cells

(A) Confocal immunofluorescence imaging intensity measurements of A12 and MAb234 in WT PV-infected HeLa cells. Images illustrate intensity measurements of whole cells in representative immunofluorescence image fields of WT-infected HeLa cells (MOI of 10) in the presence and absence of hydantoin, as described in **Fig 2C**. Mean fluorescence intensity is plotted on the y-axis, and the conditions on the x-axis. **(B) Confocal immunofluorescence imaging intensity**

measurements of A12 and MAb234 in F30Y PV-infected HeLa cells. Images illustrate intensity measurements of whole cells in representative immunofluorescence image fields of F30Y-infected HeLa cells (MOI of 10) in the presence and absence of hydantoin, as described in **Fig 2C**. Mean fluorescence intensity is plotted on the y-axis, and the conditions on the x-axis. **(C) Statistical analysis on fluorescence intensity measurements.** An unpaired student t-test analysis was performed to compare the intensity measurements of WT and F30Y-infected cells in the described conditions. A p-value lower than 0.005 was considered significant with a 95% confidence interval.