Novel labeling strategy for automated detection of single virus fusion and assessment of HIV-1 protease activity in single virions

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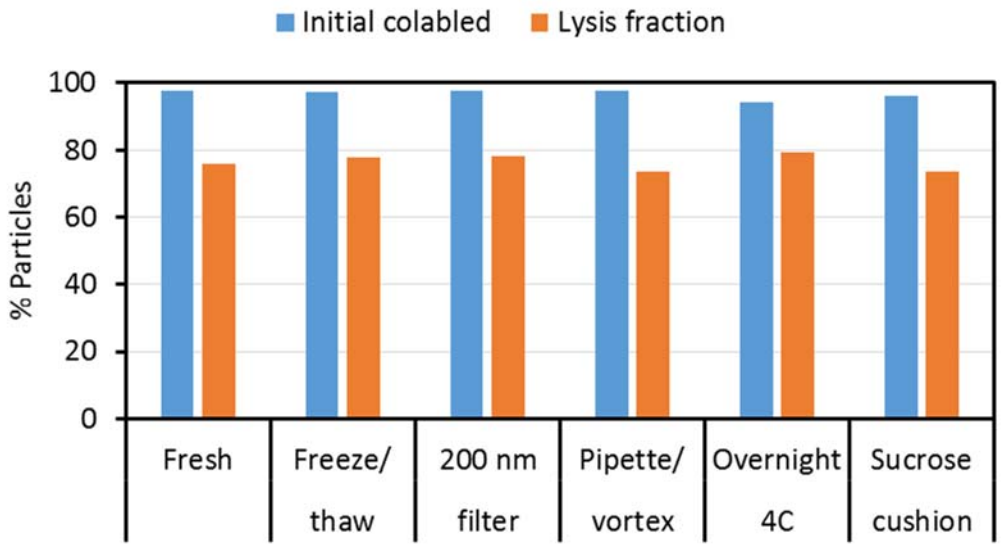
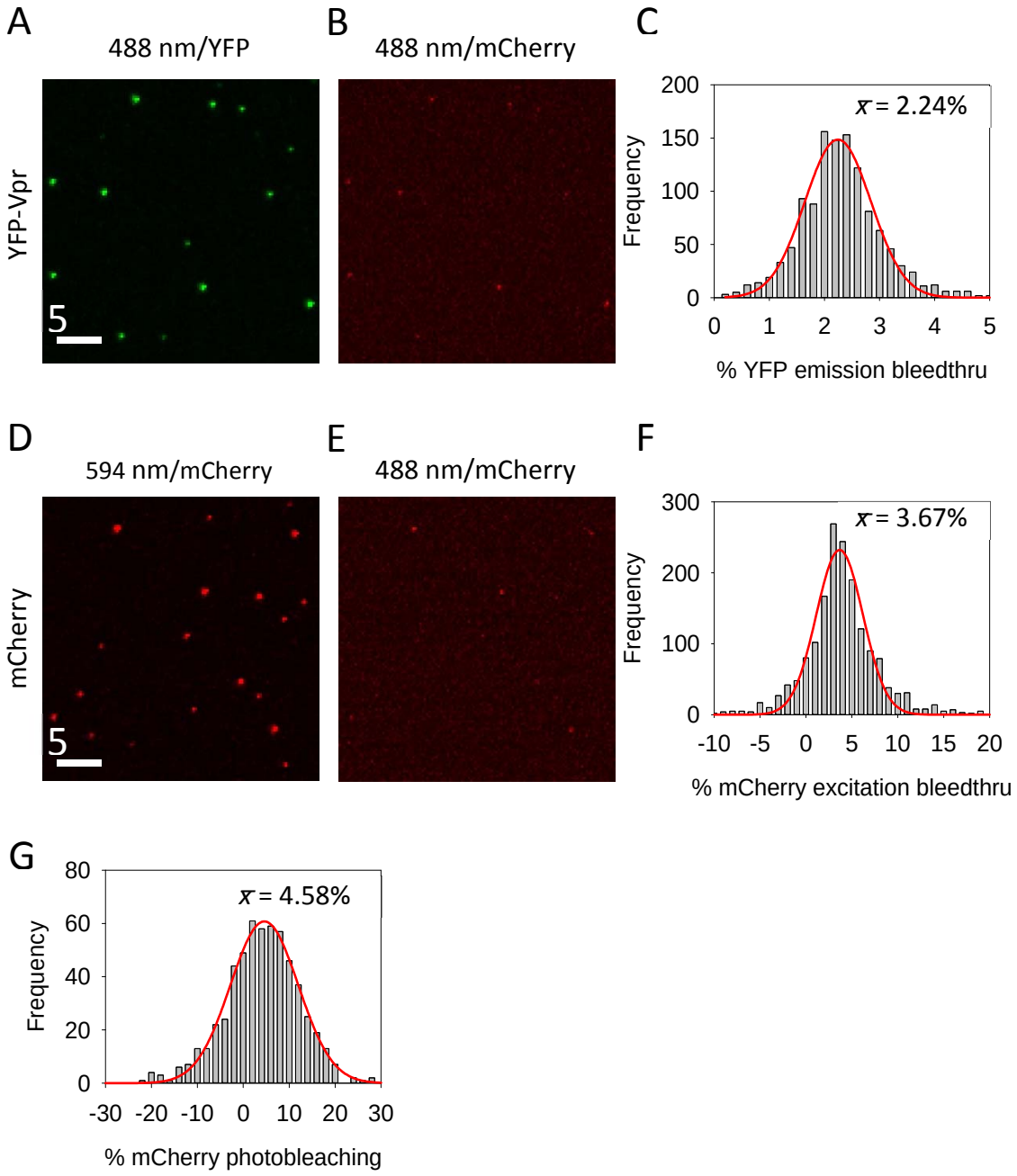
List of attached files:

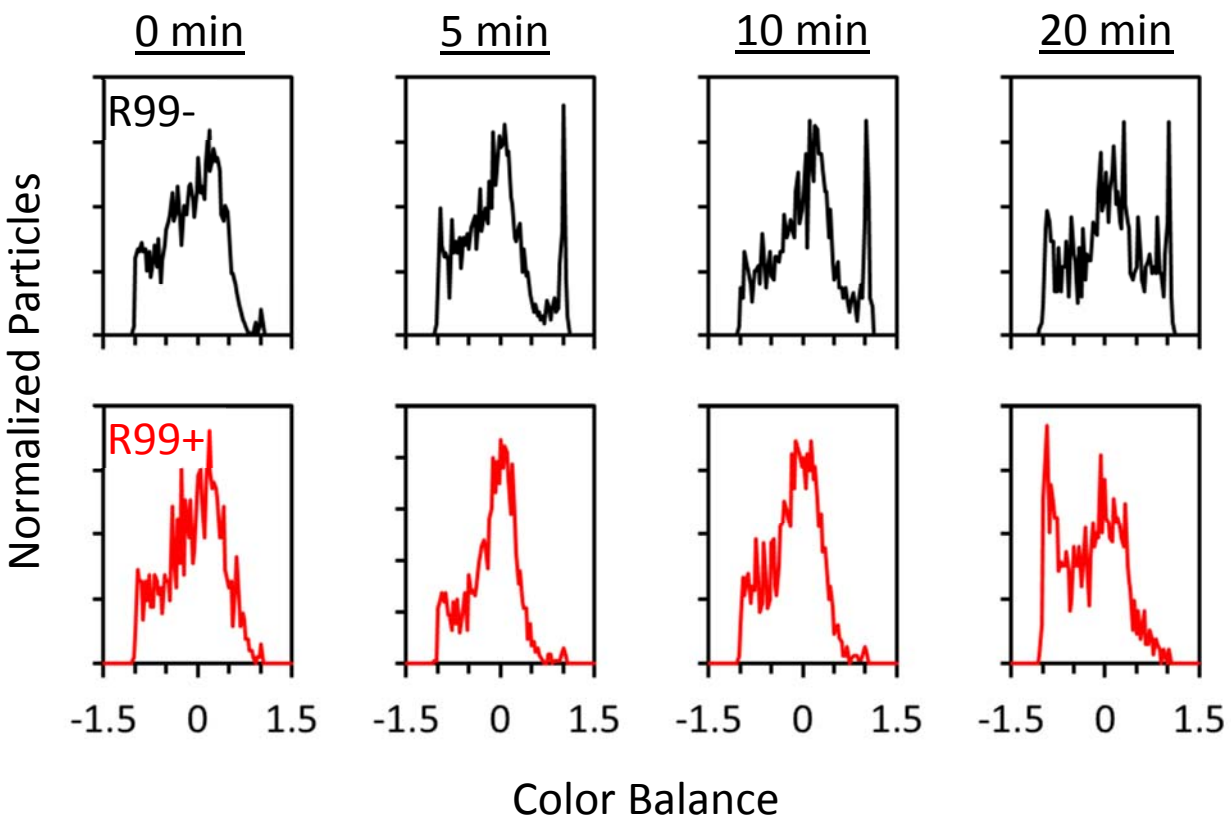
Supplemental Figures and Legends



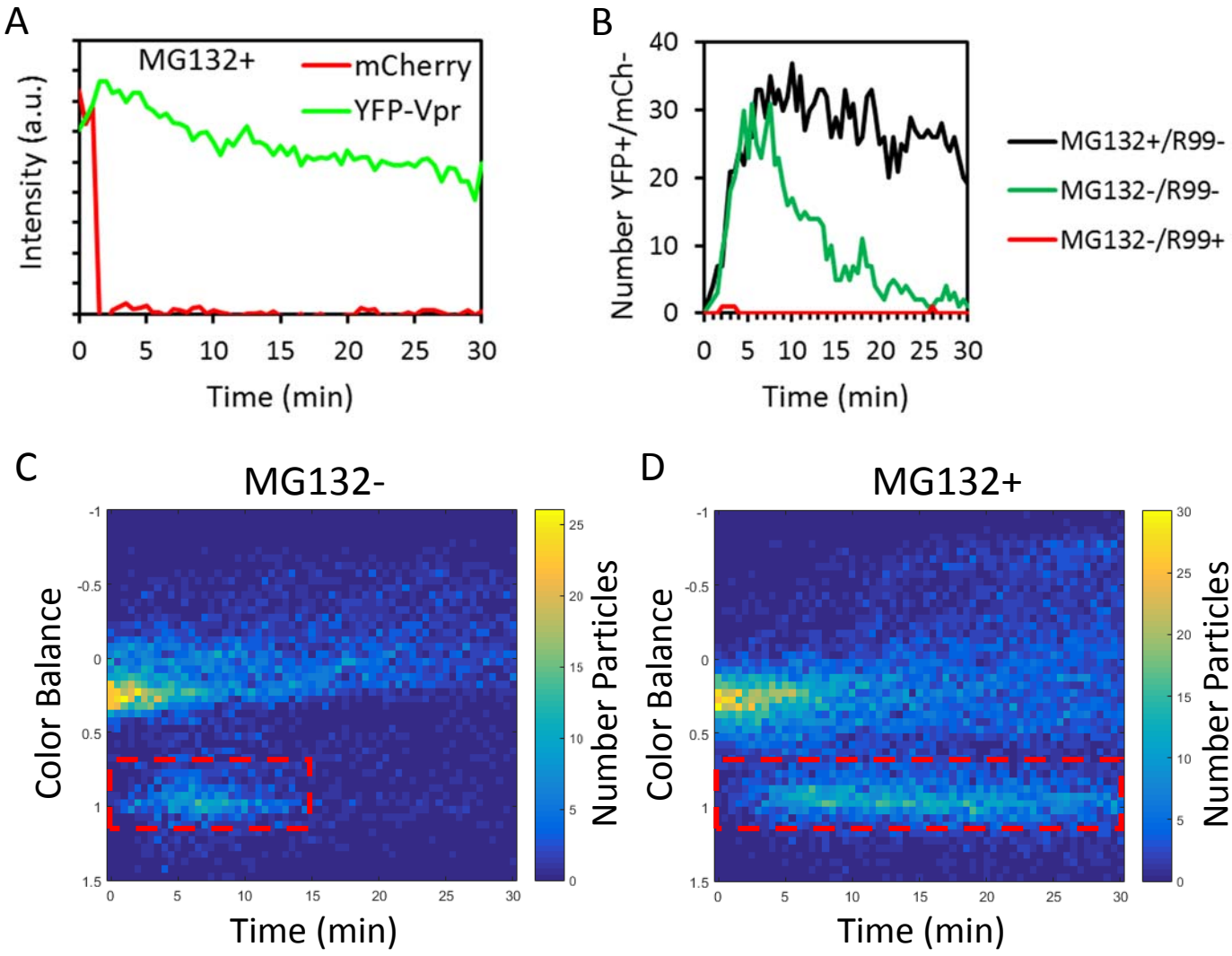
Supplemental Figure 1. Handling of virus preps labeled with mCherry-2xCL-YFP-Vpr does not release content marker. HXB2 pseudotyped virus stocks labeled with the bi-functional marker mCherry-2xCL-YFP-Vpr were produced and subjected to various conditions. Pseudoviruses were then immobilized and imaged on cover glass before and after the addition of 0.1 mg/ml saponin to mildly permeabilize the viral membrane and release mCherry. Fractions of co-labeled YFP+ particles and co-labeled particles with releasable content were assessed. Fresh stock was compared to stocks that had been freeze/thawed, passed through 200 nm syringe filter, pipetted repeatedly and vortexed, incubated overnight at 4^{o}C or concentrated through a sucrose cushion.



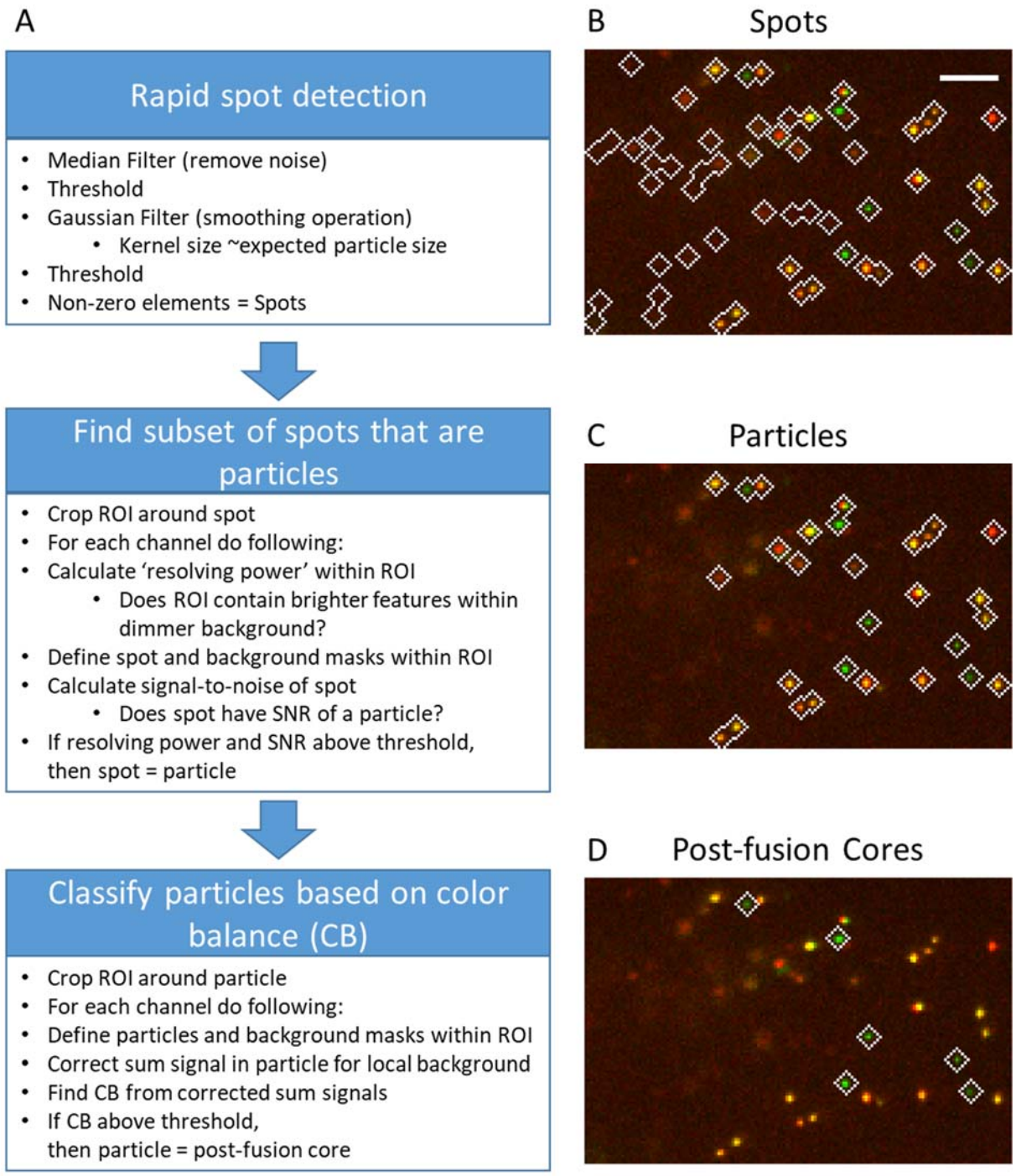
Supplemental Figure 2. Measurement of stimulated-emission FRET first-order cross-talk parameters and photobleaching during FRET measurement. Virus single-labeled for YFP-Vpr (A-C) or Gag-imCherry (D-G) were immobilized on coverslips and imaged by sequentially exciting with 488 nm and 594 nm light, while detecting in both YFP and mCherry emission bands. YFP-only (A) and mCherry-only (D) particles were detected in 488 nm/YFP and 594 nm/mCherry channels, respectively. YFP excitation (B, C) and mCherry emission (D, E) bleedthrough contributions to sensitized-emission FRET intensity measurement were detected in the 488 nm/mCherry channel and mean bleedthrough contribution was characterized by Gaussian fit of single-particle intensities. The Gag-imCherry sample was imaged twice to measure photobleaching in mCherry channel, which was estimated by Gaussian fit to fractional loss in intensity across single-particles (G).



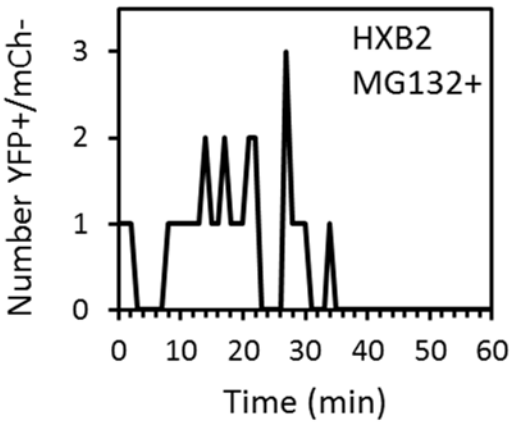
Supplemental Figure 3. Detection of post-fusion viral cores in fixed-cells. ASLV pseudoviruses labeled with the bi-functional marker were pre-bound to TVA950-expressing CV1 cells in the cold in the absence or in the presence of 50 µg/ml ASLV fusion inhibitor R99. Virus fusion was initiated by shifting to 37°C for short intervals after which cells were immediately fixed and imaged. Plots show the distribution of single-particle color balance (CB) at 0, 5, 10 or 20 min time points. The appearance of the peak at CB=1 indicates an accumulation of YFP-only particles that have shed the mCherry content marker.



Supplemental Figure 4. MG132 treatment delays post-fusion loss of YFP-Vpr signal. ASLV pseudoviruses labeled with the bi-functional marker were pre-bound to TVA950-expressing CV1 cells in the cold and temperature was shifted to 37°C by addition of pre-warmed buffer immediately prior to imaging. Cells were untreated or pre-incubated for 30 min with 20 μM MG132 and imaged in the presence of the same MG132 concentration. Additionally, in parallel with MG132- samples, fusion inhibitor R99 was used as a negative control. (A) Intensity traces from single-particle tracking of virus in cells treated with MG132 shows a very gradual post-fusion decay of the YFP-Vpr signal within the 30 min imaging window. (B-D) Automated detection of post-fusion (YFP+/mCherry-) viral cores (B) and time course of single-particle Color Balance distributions (C-D) in live cells in the absence or in the presence of R99 or MG132, as indicated. The panels show the persistence of particles with high color balance with MG132 treatment (red boxes).



Supplemental Figure 5. Algorithm for computer-automated detection of post-fusion cores in live cell experiments. (A) High-level description of primary steps in automated detection of post-fusion cores. (B-D) Representative images demonstrate identification of spots (B), classification of subset of spots as particles (C), and detection of YFP-only post-fusion cores (D). White outlines annotate objects detected at each step and scale bar is 5 μm.



Supplemental Figure 6. Computer-automated detection of post-fusion cores for virus particles pseudotyped with inefficient HIV-1 envelope glycoprotein. HXB2 pseudoviruses labeled with the bi-functional marker were pre-bound to CD4/CXCR4-expressing CV1 cells in the cold and temperature was shifted to 37ºC by addition of pre-warmed buffer immediately prior to imaging. Computer-automated detection yielded small numbers of post-fusion cores that could not be meaningfully separated from the background.

Movie Legends

Supplemental Movie 1. Post-fusion YFP-Vpr signal loss in untreated cells. ASLV pseudoviruses labeled with the bi-functional marker were pre-bound to TVA950-expressing CV1 cells in the cold, and temperature was shifted to 37^{o}C by addition of pre-warmed buffer immediately prior to imaging. After viral fusion, which manifests as loss of mCherry content marker, core-associated YFP-Vpr signal is lost within 10 min. Scale bar is 1 μm.

Supplemental Movie 2. MG132 treatment delays post-fusion loss of YFP-Vpr signal. ASLV pseudoviruses labeled with the bi-functional marker were pre-bound to TVA950-expressing CV1 cells treated with 20 μM MG132 in the cold and temperature was shifted to 37^{o}C by addition of pre-warmed buffer immediately prior to imaging. After viral fusion, which manifests as loss of mCherry content marker, core-associated YFP-Vpr signal is still detected at the end of the 30 min imaging window. Scale bar is 1 μm.

Supplemental Movie 3. Computer-automated detection of post-fusion virus cores in live cells. ASLV pseudoviruses labeled with the bi-functional marker, mCherry-2xCL-YFP-Vpr, were pre-bound in the cold to TVA950-expressing CV1 cells pre-treated with 20 μM MG132 and temperature was shifted to 37^{o}C by addition of pre-warmed buffer immediately prior to imaging. Viral fusion manifests as loss of mCherry content marker, and MG132 treatment prolongs detection of post-fusion YFP-Vpr signal. Imaging in high signal-to-noise regime enabled computer-automated detection of YFP-only post-fusion cores (white outlines). Scale bar is 5 μm.