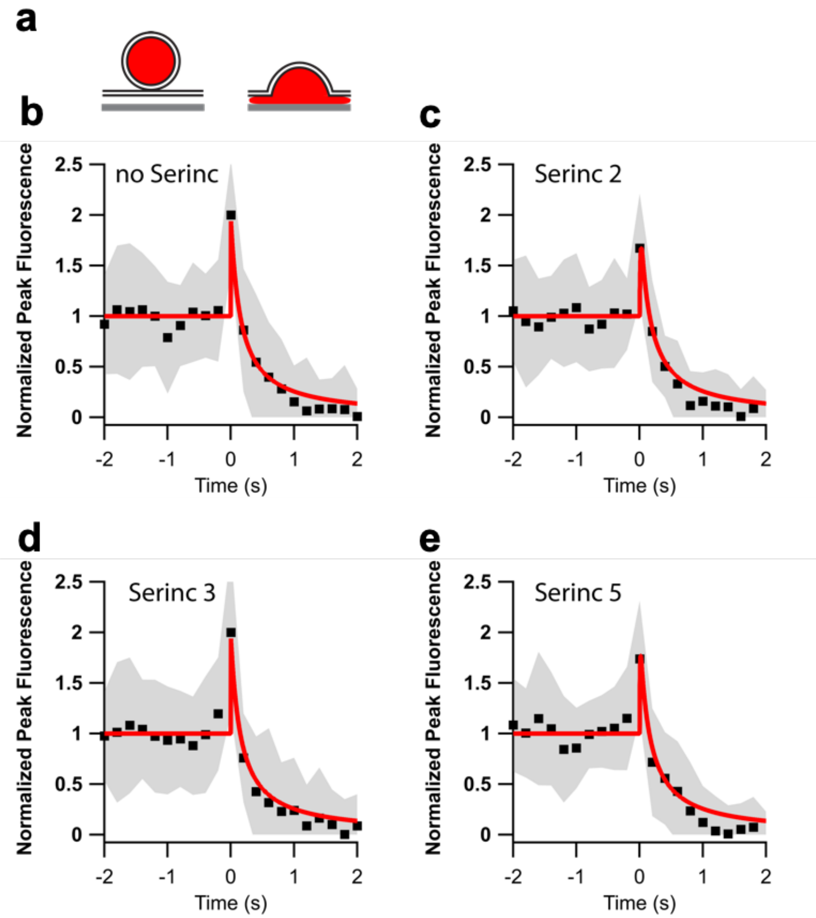
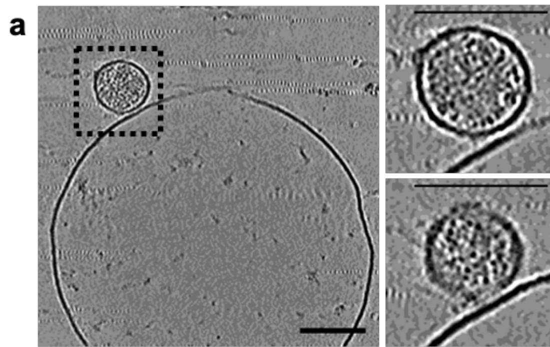
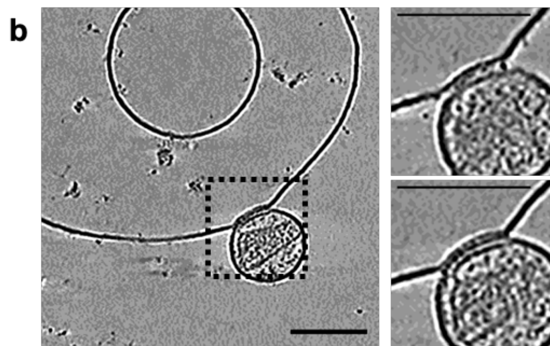
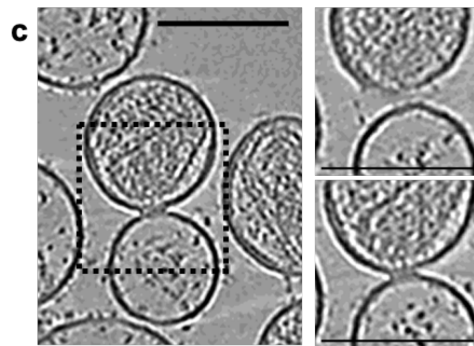


Fig. S1. Model of mCherry content release from HIV pseudoviruses during fusion. (a) Cartoon of pseudovirus mCherry content release model. We assume the mCherry content is freely mobile within the virus and is released into the aqueous cleft between SPPM and quartz slide at the onset of fusion pore opening with a specific release rate k_r. Within the cleft, mCherry diffuses in 2-dimensions with a characteristic diffusion coefficient D (0.1 $\mu m^2/s$). Other numerical parameters of the model include the radius of the viral particle R (55 nm) and the characteristic penetration depth d_p of the evanescent field at the quartz/water interface. The release rate k_r, was adjusted for each type of pseudovirus (no Serinc: (1000 s^{-1}, Serinc 2: 100 s^{-1}, Serinc 3: 1000 s^{-1}, Serinc 5: 200 s^{-1}). The model is a simplified, one step release model, based on the more complex release model of secretory vesicles described in detail in Kreutzberger et al., 2017 (b-e). Average content release traces of HIV mCherry pseudoviruses with model calculations based on the parameters above. Individual fusion events for each virus were aligned to the peak at the onset of fusion and intensities were normalized to the "binding state" before fusion. Aligned and normalized traces were averaged and the mean (black squares) and standard deviation (grey shaded area) were plotted. (b) Serinc-lacking pseudovirus content release. Average represents 35 individual fusion events. (c) Serinc2 pseudovirus content release. Average represents 32 individual fusion events. (d) Serinc3 pseudovirus content release. Average represents 32 individual fusion events. (e) Serinc5 pseudovirus content release. Average represents 39 individual fusion events.







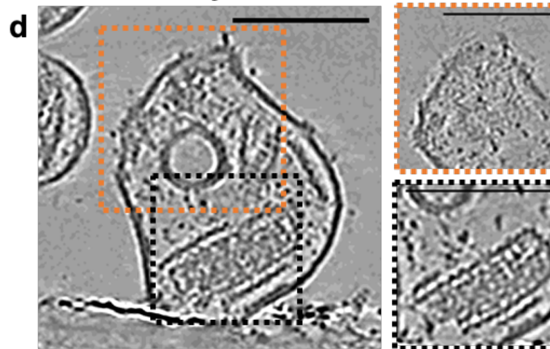
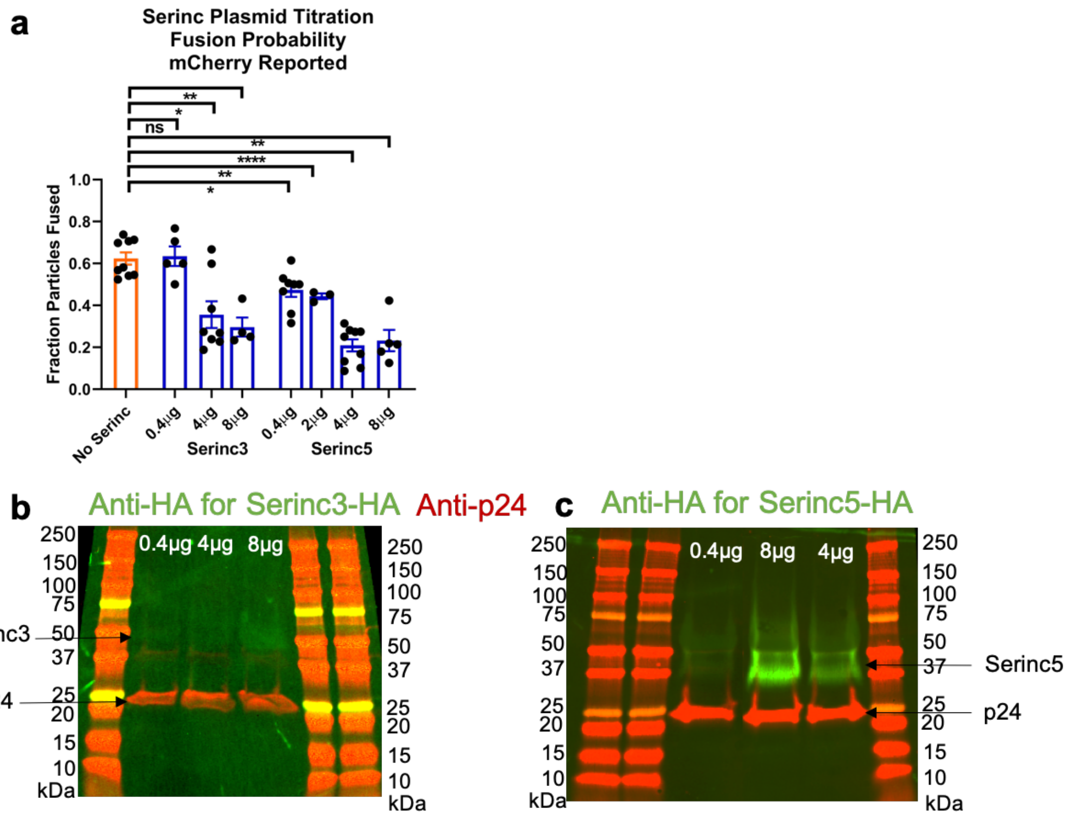


Fig. S2. Additional examples of HIV pseudovirus-bleb fusion intermediates. All samples were supplemented with IP6. Tomograms were all prepared with additional IP6 and are shown with cryoCARE denoising to enhance contrast for display. Scale bars are 100nm. (a) Example of receptor mediated binding (b) example of hemifusion with a wider area of contact (c) example of hemifusion with a narrower contact (d) Early fusion where a viral capsid and an enclosed vesicle can be seen. Many blebs are multilamellar (like the bleb in panel b) whereas viruses are never multilamellar. Additional views of the boxed areas at different slices in the z direction are shown to the right. The orange boxed inset shows that the internal vesicle is closed and does not extend as far out as the outer vesicle.



33

34 **Fig. S3. Titration of Serinc3 and Serinc5's viral incorporation and inhibition of viral**
35 **content release.** (a) Fraction of bound HIV pseudovirus particles that underwent fusion, as
36 reported by loss of mCherry content marker. Each point represents a separately prepared bilayer.
37 No Serinc and 4 µg data reproduced from Figure 3b for comparison. All other data collected
38 from 3 experiments and 1 preparation of HIV pseudovirus. (b,c) The pBJ5 Serinc plasmids are
39 expressed as C-terminal fusion proteins with an HA tag which was used for detection of Serinc
40 incorporation in HIV pseudoviruses by western blot (green channel). The concentration of p24 in
41 each preparation was measured by ELISA and a roughly normalized amount of pseudovirus was
42 loaded. p24 was detected by mouse anti-p24 (red channel). The expected molecular weights are
43 53kDa and 47kDa for Serinc3 and Serinc5 respectively. (b) Titration Serinc3 incorporation into
44 HIV pseudoviruses. The mass of pBJ5-Serinc3-HA plasmid transfected per 10 cm^2 plate is
45 indicated for each lane. (c) Titration Serinc5 incorporation into HIV pseudoviruses. The mass of
46 pBJ5-Serinc5-HA plasmid transfected per 10 cm^2 plate is indicated for each lane. The density of
47 Serinc5 bands is consistently higher than the density of Serinc3 bands for an equivalent amount
48 of virus (see Supplemental Figure 7).
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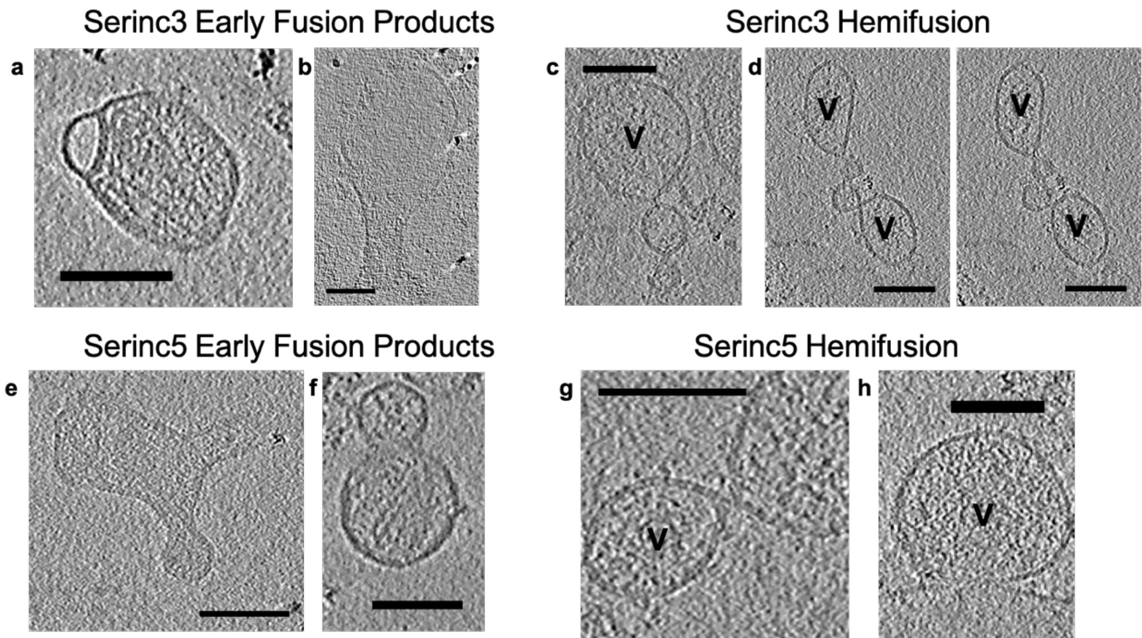
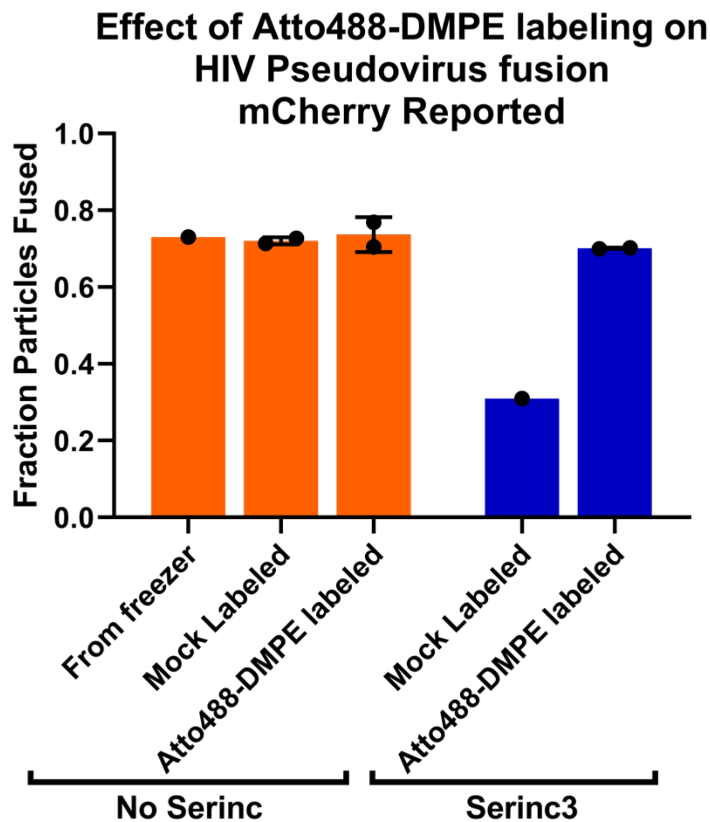
50

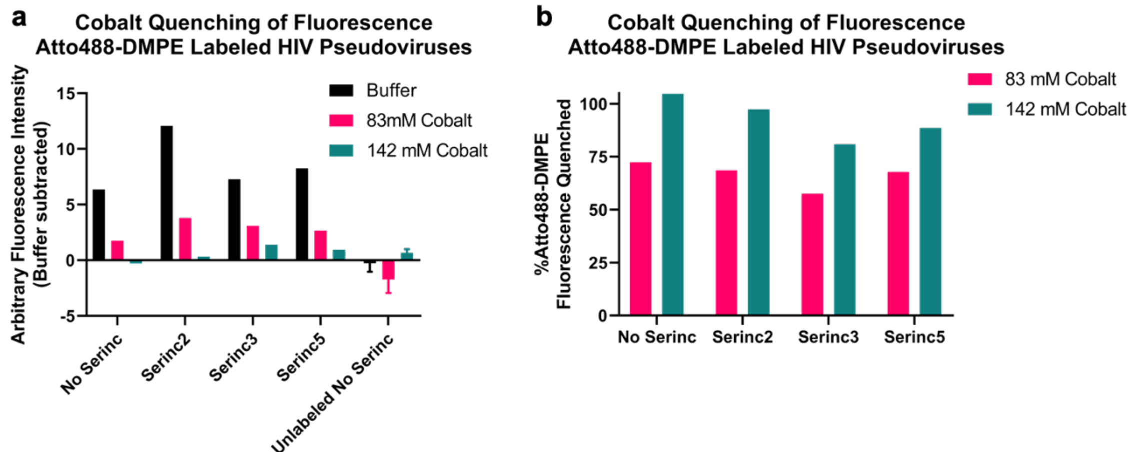
51
52



53 **Fig. S4. Additional examples of tomograms of Serinc3- and Serinc5-disrupted HIV fusion**
54 **events.** All images are single z-slices through tomograms of HIV pseudoviruses mixed with
55 CD4/CCR5-containing blebs and warmed to 37°C for 30 seconds before freezing. All scale bars
56 are 100 nm. "v" designates a viral particle. (a) Serinc3 HIV pseudovirus mixed with blebs.
57 Defocus = -10 µm. (b) Serinc3 HIV pseudovirus mixed with blebs. Defocus = -4 µm. (c) Serinc3
58 HIV pseudovirus mixed with blebs. Defocus = -4 µm. (d) Serinc3 HIV pseudovirus mixed with
59 blebs. Defocus = -4 µm. (e) Serinc5 HIV pseudovirus mixed with blebs. Defocus = -4 µm. (f)
60 Serinc3 HIV pseudovirus mixed with blebs. Defocus = -10 µm. (g) Serinc5 HIV pseudovirus
61 mixed with blebs. Defocus = -4 µm. (h) Serinc5 HIV pseudovirus mixed with blebs. Defocus = -
62 4 µm.
63



64
65 **Fig. S5. Atto488-DMPE labeling does not affect fusion of Serinc-lacking particles, but**
66 **increases fusion of Serinc3-containing particles.** Fraction of bound HIV pseudovirus particles
67 that underwent fusion, as reported by loss of mCherry content marker. Each point represents a
68 separately prepared SPPM. Data are paired repeats from a single experiment. The same
69 preparation of HIV pseudovirus was used for each set of conditions (Serinc-lacking or Serinc3,
70 respectively). The virus for the "No Serinc from freezer" condition was thawed immediately
71 before the experiment, diluted in buffer, and added directly to the SPPM without modification.
72 The mock labeled conditions were treated with the same buffers, incubations, and centrifugations
73 alongside the Atto488-DMPE labeled pseudovirus but without the addition of the lipid. Error
74 bars are SD.
75

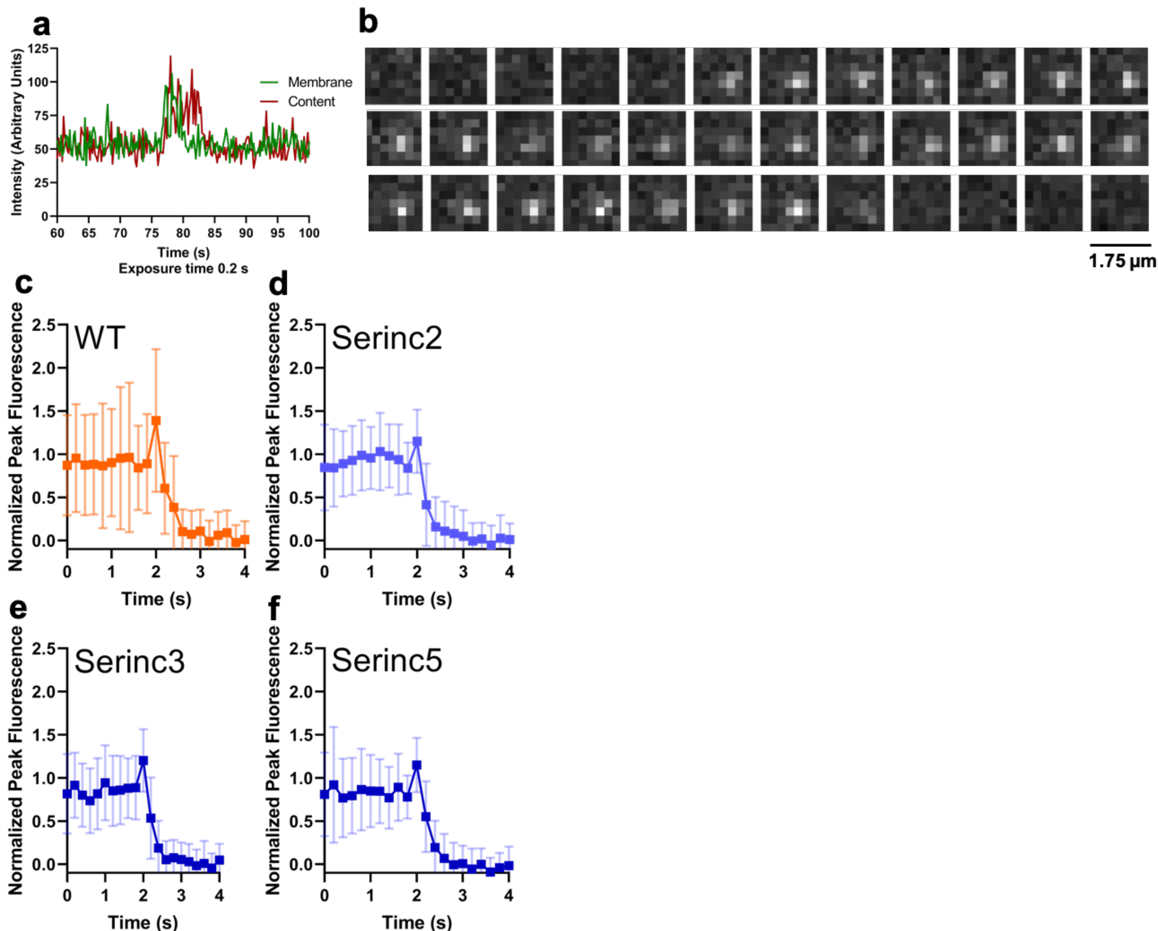


76

77 **Fig. S6. Atto488-DMPE is mostly confined to the outer leaflet of the viral membrane.** HIV
78 pseudoviruses were labeled with Atto488-DMPE as described in the Methods section and
79 fluorescence was measured in a plate reader (Exc 488nm, Em 514nm, Cutoff 505nm) before and
80 after addition of cobalt chloride to quench solvent accessible fluorophores. (a) Arbitrary
81 fluorescence intensity (buffer fluorescence subtracted) of HIV pseudoviruses before and after
82 cobalt addition. Data are from one experiment. Error bars show SD for 2 replicates of unlabeled
83 No Serinc virus (b) The same data as in panel a represented as percent of original fluorescence
84 remaining after cobalt addition.

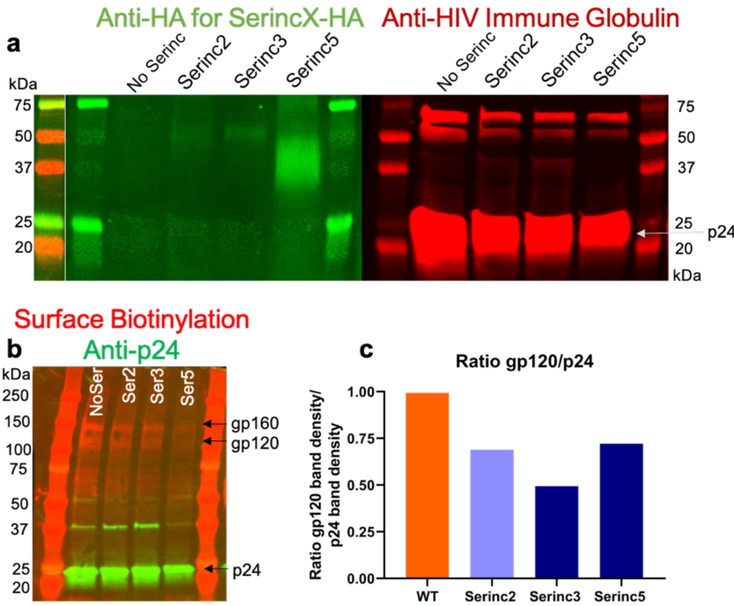
85

86



87 **Fig. S7. Average lipid mixing traces of HIV mCherry pseudoviruses labeled with Atto488-**
88 **DMPE.** (a) Fluorescence intensity of a dual labeled (mCherry and Atto488-DMPE) HIV
89 pseudovirus particle fusing with an SPPM. (b) Example micrographs of the membrane label for
90 the particle shown in panel a. Each box represents the same region separated in time by 0.2
91 seconds. (c-f) Individual events for each virus were aligned and normalized in the same manner
92 as the content release traces where events were aligned to peak at the onset of fusion and
93 intensities were normalized such that "binding" before fusion was set to one and "baseline" after
94 fusion was set to zero. Aligned and normalized traces were averaged together and the mean
95 (squares) and SD were plotted. Dissipation of the Atto488-DMPE after lipid mixing would be
96 expected to occur quickly via 2-dimensional diffusion of labeled lipids within the larger SPPM.
97 The smaller peaks we observe could be due to changes in the direction of the fluorescence dipole
98 as the spherical viral membrane collapses into the planar SPPM, as described by (Kiessling et al.,
99 2010). Averaged lipid mixing trace of Atto488-DMPE labeled (c) Serinc-lacking (d) Serinc2 (e)
100 Serinc3 (d) Serinc5 mCherry pseudoviruses.
101

102



103 **Fig. S8. Protein incorporation into HIV pseudoviruses and effects on HIV pseudovirus**
104 **fusion.** (a) The pBJ5 Serinc plasmids are expressed as C-terminal fusion proteins with an HA tag
105 which was used for detection of Serinc incorporation in HIV pseudoviruses by western blot
106 (green channel). The concentration of p24 in each preparation was measured by ELISA and a
107 normalized amount of pseudovirus was loaded. p24 was detected by HIV Immune Globulin (red
108 channel) and used for normalization. Expected molecular weights of Serincs: Serinc2 51kDa,
109 Serinc3 53kDa, Serinc5 47kDa. (b) To assess surface glycoprotein incorporation, an equal
110 amount of virus as measured by p24 ELISA was surface biotinylated by sulfo-NHS-succinimide
111 ester before western blotting and detection by streptavidin-IR680 (red channel). (c)
112 Quantification of the ratio of the gp120 and p24 bands shown in b.

113

114 **Movie S1 (separate file). Z-slices through tomograms of HIV pseudovirus and blebs treated**
115 **with fusion inhibitor, T20.** The video shows slices through the 3D volume of the same
116 tomogram as Figure 2b, moving from the bottom to the top. The pseudovirus and bleb mixture
117 was treated with 135 ng/mL T20. Tomogram is shown with non-anisotropic diffusion filtering to
118 enhance contrast for display. Scale bars are 100 nm.

119

120 **Movie S2 (separate file). Z-slices through tomograms of HIV pseudovirus and blebs**
121 **showing receptor mediated binding.** The video shows slices through the 3D volume of the
122 same tomogram as Figure 2c, moving from the bottom to the top. The pseudovirus and bleb
123 mixture was warmed to 37°C for 10 seconds before freezing. Tomogram is shown with
124 cryoCARE denoising to enhance contrast for display. Scale bars are 100 nm.

125

126 **Movie S3 (separate file). Z-slices through tomograms of HIV pseudovirus and blebs**
127 **showing hemifusion.** The video shows slices through the 3D volume of the same tomogram as
128 Figure 2d, moving from the bottom to the top. The pseudovirus and bleb mixture was warmed to
129 37°C for 10 seconds before freezing. Tomogram is shown with cryoCARE denoising to enhance
130 contrast for display. Scale bars are 100 nm.

131

132 **Movie S4 (separate file). Z-slices through tomograms of Serinc2-containing HIV**
133 **pseudovirus and blebs.** The video shows slices through the 3D volume of the same tomogram
134 as Figure 3g, moving from the bottom to the top. The pseudovirus and bleb mixture was warmed
135 to 37°C for 30 seconds before freezing. Tomogram is shown with cryoCARE denoising to
136 enhance contrast for display. Scale bars are 100 nm.

137

138 **Movie S5 (separate file). Z-slices through tomograms of Serinc3-containing HIV**
139 **pseudovirus and blebs.** The video shows slices through the 3D volume of the same tomogram
140 as Figure 3i, moving from the bottom to the top. The pseudovirus and bleb mixture was warmed
141 to 37°C for 30 seconds before freezing. Tomogram is shown with cryoCARE denoising to
142 enhance contrast for display. Scale bars are 100 nm.

143

144 **Movie S6 (separate file). Z-slices through tomograms of Serinc5-containing HIV**
145 **pseudovirus and blebs.** The video shows slices through the 3D volume of the same tomogram
146 as Figure 3k, moving from the bottom to the top. The pseudovirus and bleb mixture was warmed
147 to 37°C for 30 seconds before freezing. Tomogram is shown with cryoCARE denoising to
148 enhance contrast for display. Scale bars are 100 nm**.**

149