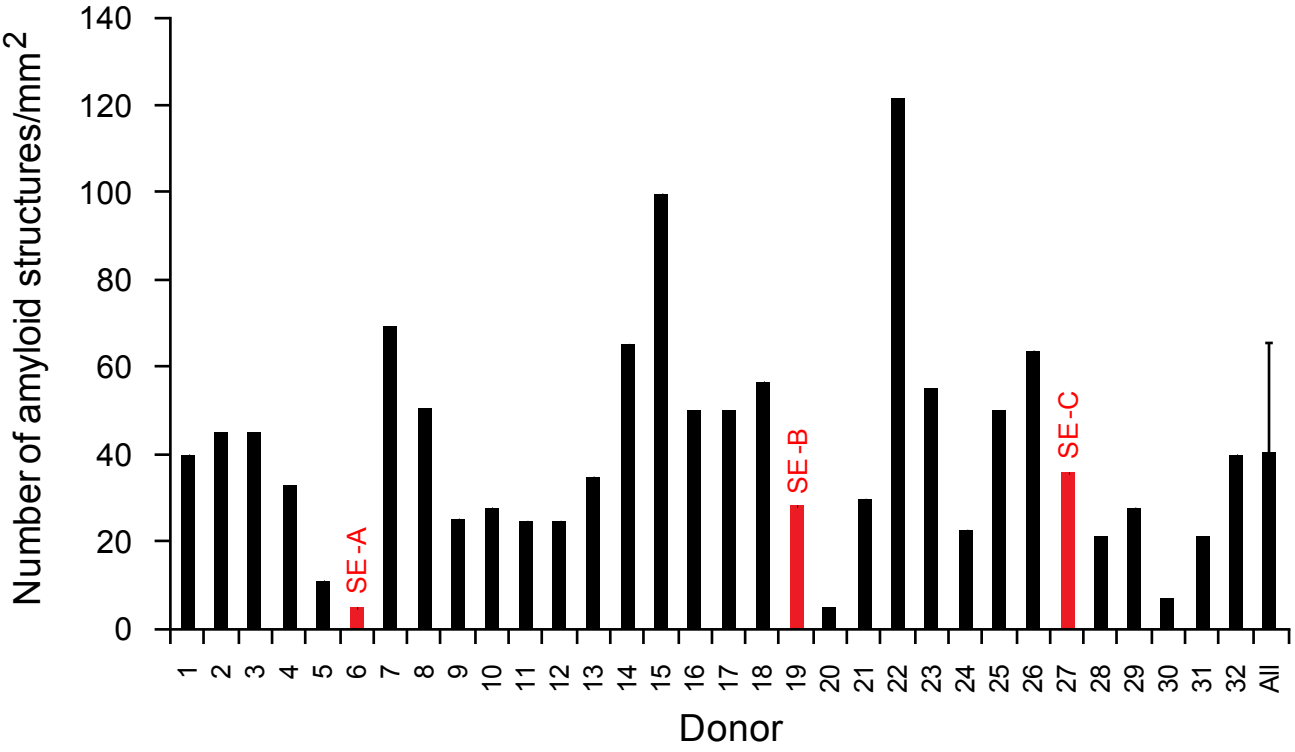
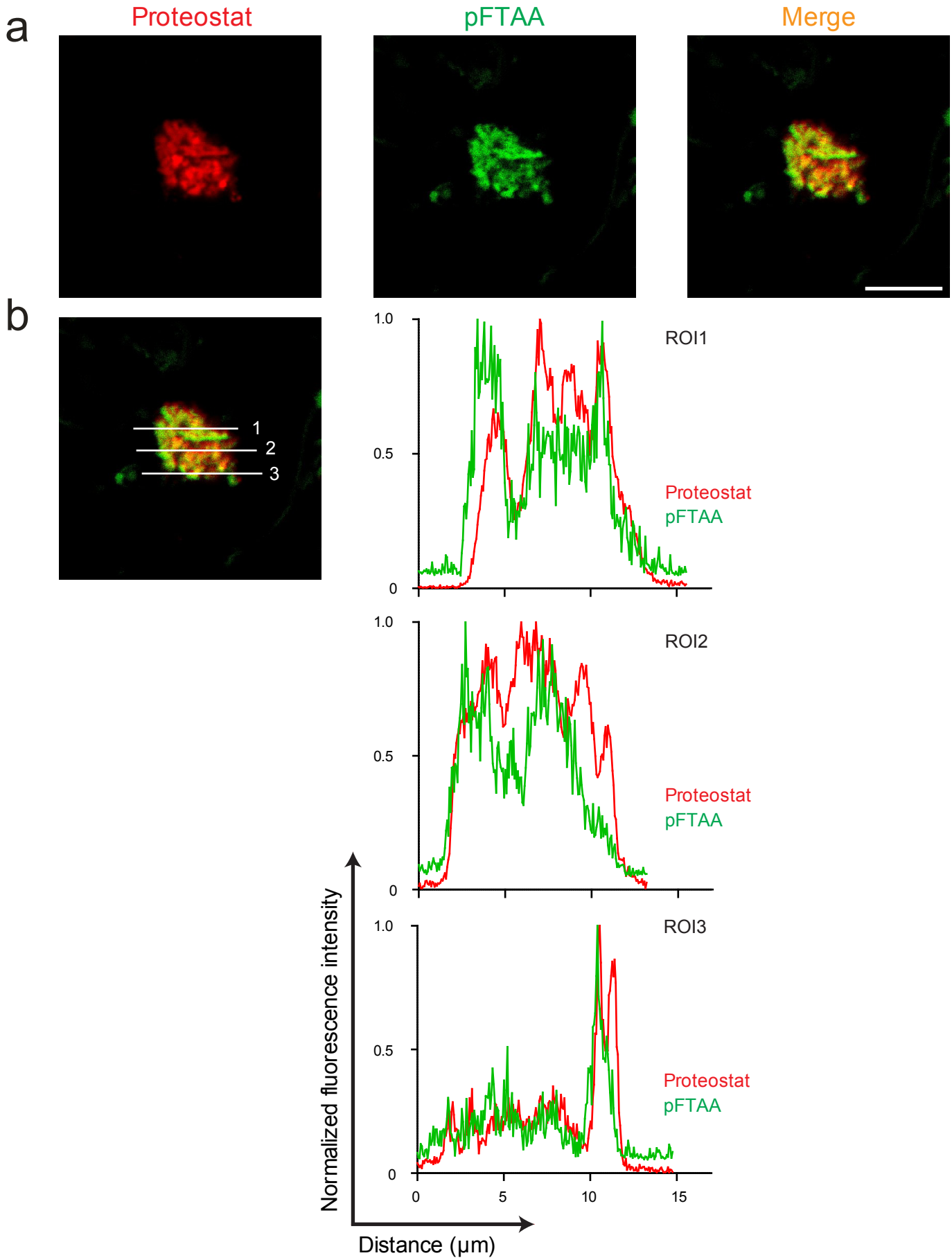
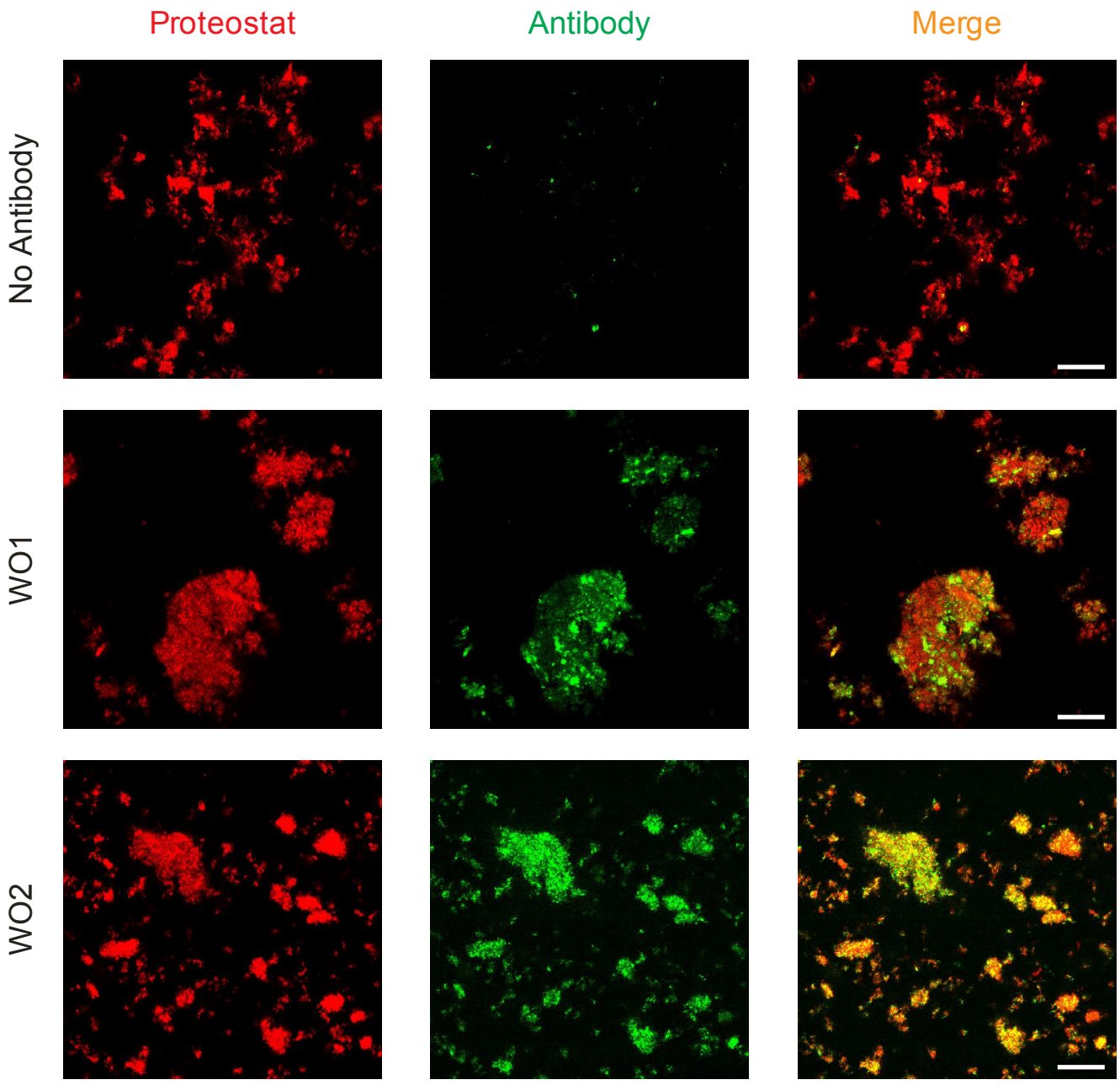


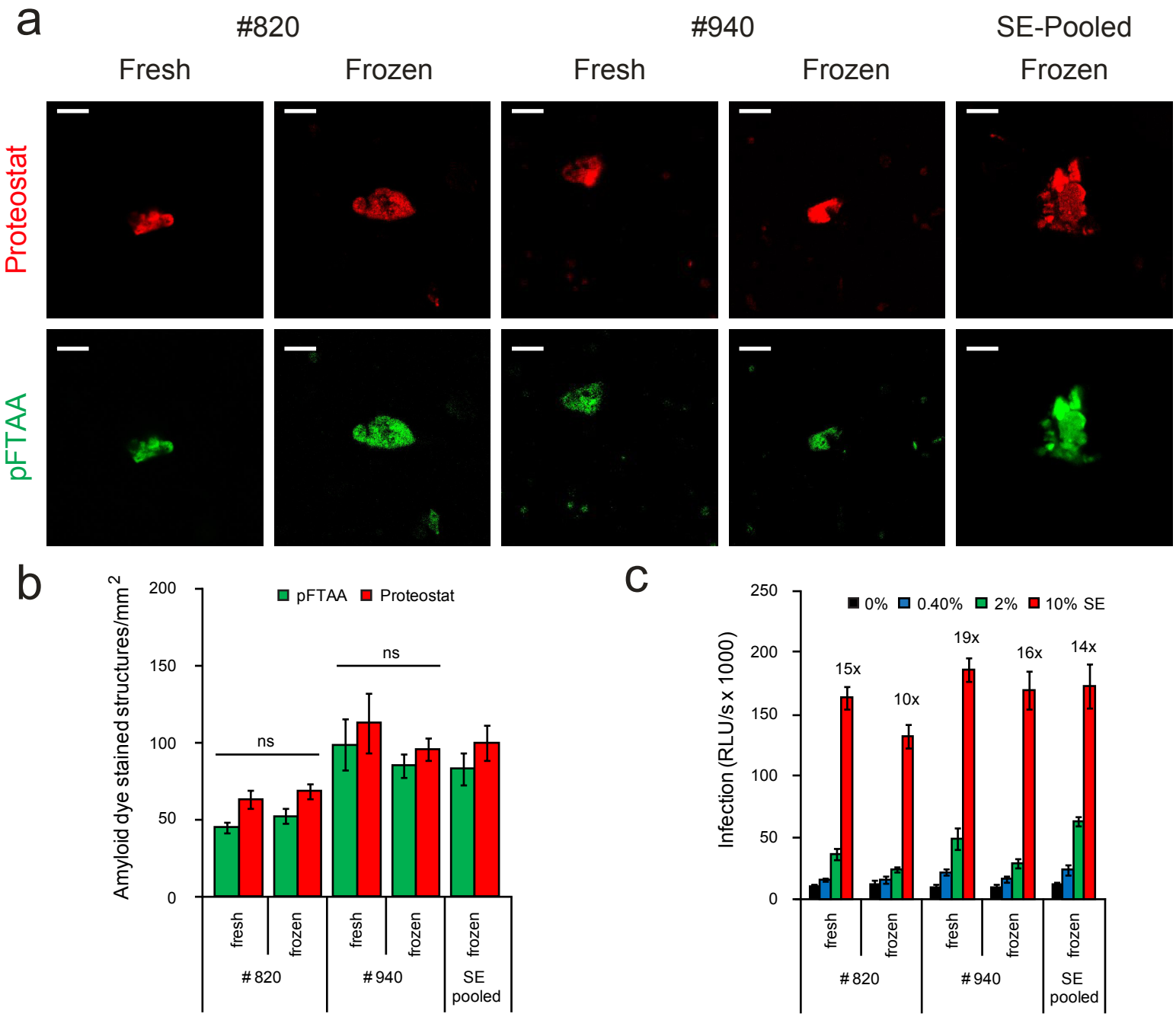
Supplementary Figure 1. Quantification of Proteostat-stained structures in semen derived from individual donors. Liquefied ejaculates were stained with the amyloid-specific dye Proteostat at room temperature for 15-20 min and imaged using a LSM710 confocal microscope. From every sample, 5 or more images were acquired from different regions, and fluorescent structures larger than 2 x 2 µm were analyzed by FIJI software.



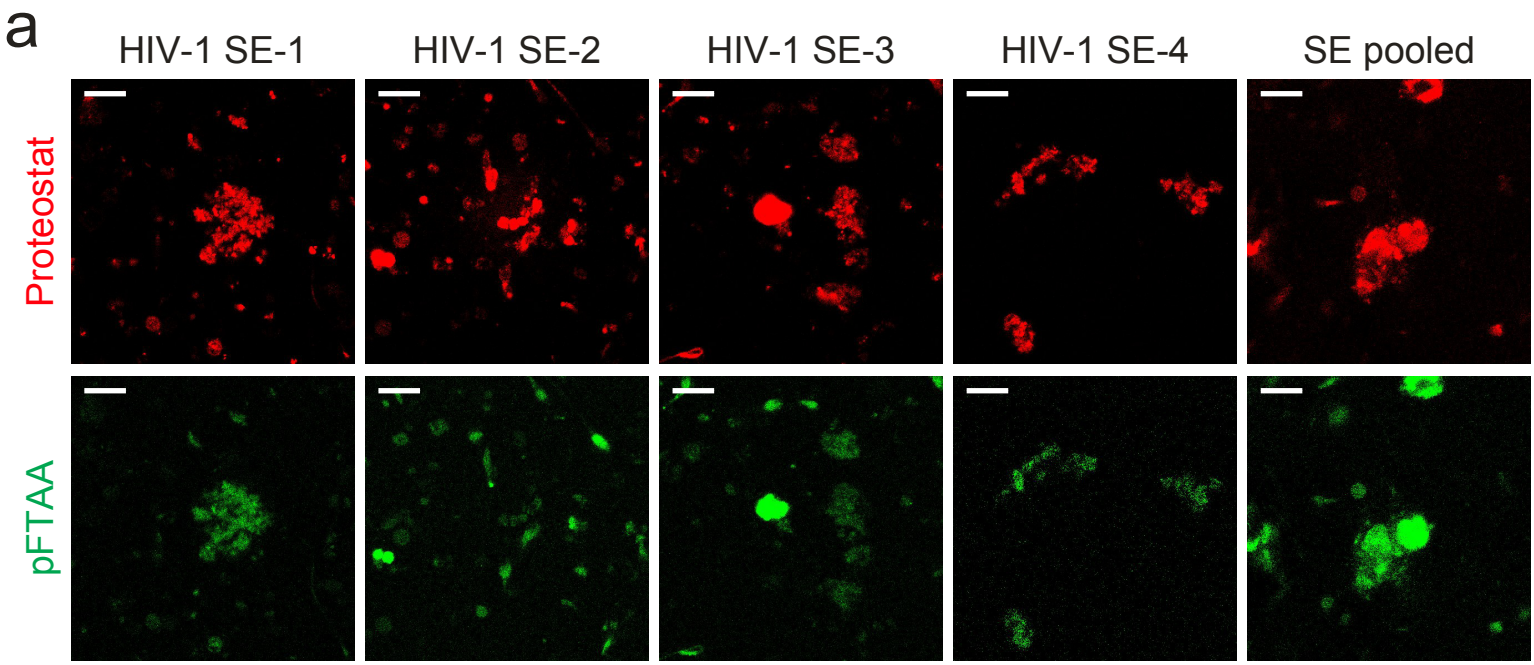
Supplementary Figure 2. Proteostat and pFTAA probes allow detection of similar structures in semen. (a) Confocal microscopy images showing colocalization of amyloid-specific probes Proteostat dye and pFTAA in semen. Scale bar = 20 µm. (b) Plot profiles showing the normalized fluorescence intensities for the two dyes along three different regions of interest (ROIs).

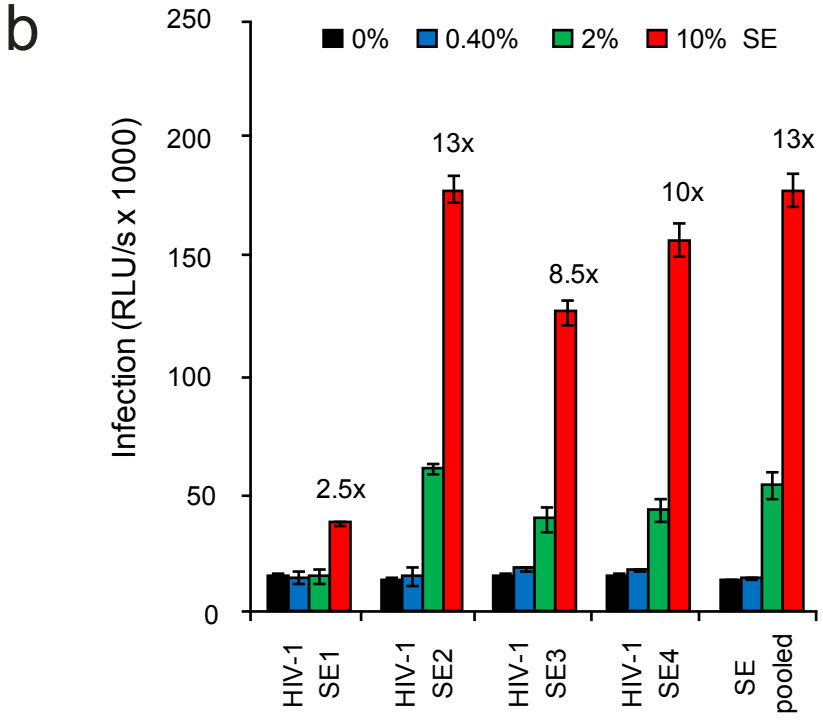


Supplementary Figure 3. Amyloid-specific antibodies recognize in vitro generated SEVI amyloid fibrils. 100 μg/ml SEVI amyloid fibrils were mock-treated or treated with amyloid-specific WO1 or WO2 antibodies. Amyloid/antibody complexes were then pelleted, washed and detected using Alexa488-coupled secondary antibody (green). All samples were then co-stained with Proteostat dye (red). Scale bar = 20 μm.

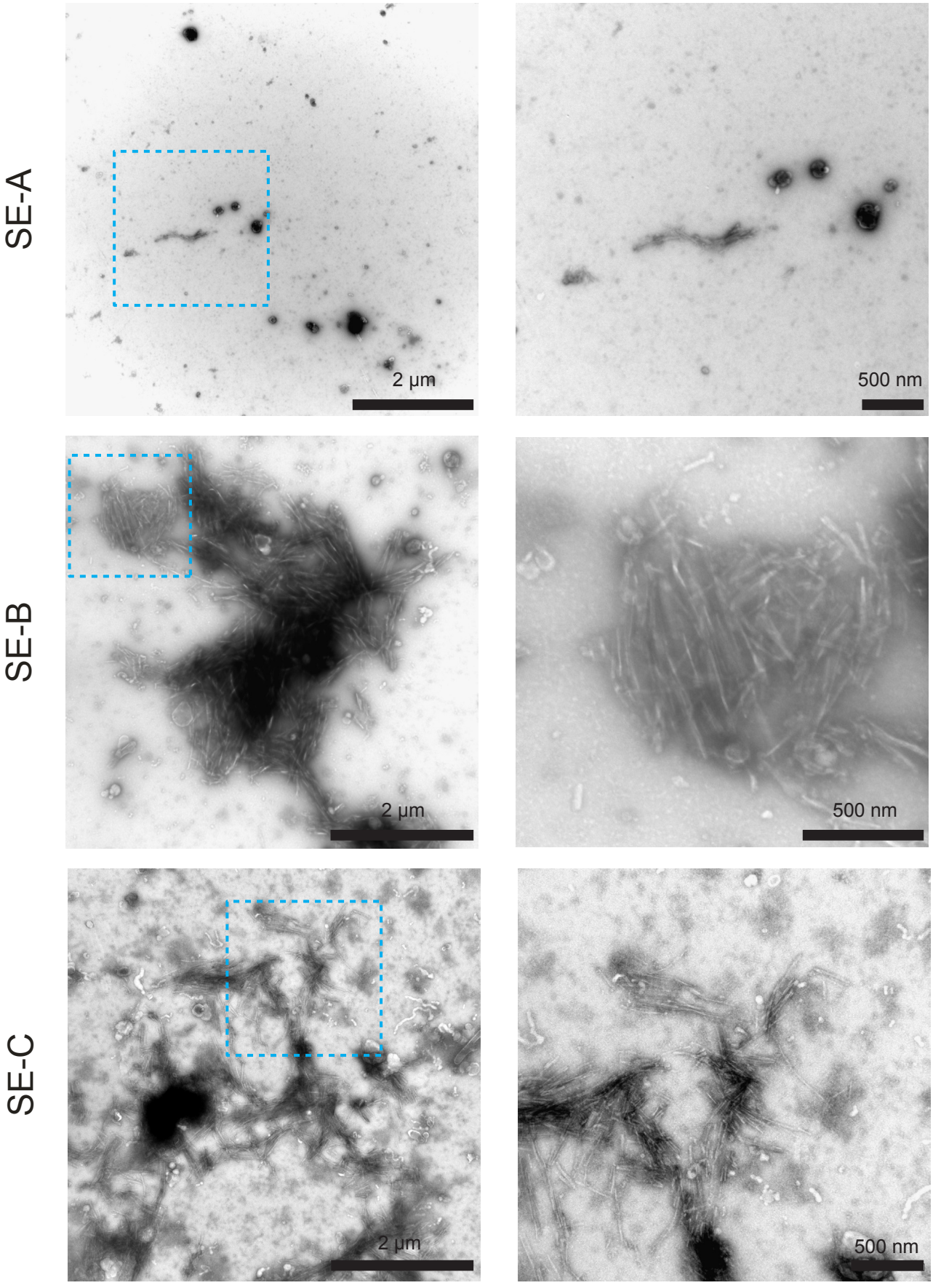


Supplementary Figure 4. Detection of endogenous amyloid in fresh and frozen/thawed semen. Ejaculates 820 and 940 were allowed to liquefy for 30 min at room temperature. Thereafter, 200 µl aliquots were either stored at 4 °C for 30 min or frozen at -80 °C for 30 min. (a) Fresh and rapidly thawed samples were stained in parallel with amyloid-specific probes Proteostat (red) or pFTAA (green) at room temperature for 15-20 min and imaged using a LSM710 confocal microscope. Scale bar = 10 µm. (b) Numbers of Proteostat- and pFTAA-stained structures in fresh and frozen semen. Data are shown as mean number of amyloid stained structures obtained from 5 - 10 images ± standard error of the mean. (c) Effect of fresh vs. frozen semen on HIV infection. R5-tropic HIV-1 was exposed to indicated concentrations of semen (%), and then added to TZM-bl cells. After 2 h, semen-virion mixtures were replaced with fresh medium. HIV-1 infection rates were measured three days later by quantifying ß-galactosidase activities in cell lysates. Values shown are average values derived from triplicate infection ± standard deviation. The numbers above the columns give the n-fold enhancement of infection observed after treatment with 10 % semen relative to infection with mock-treated virus. Pooled and previously frozen semen served as control.

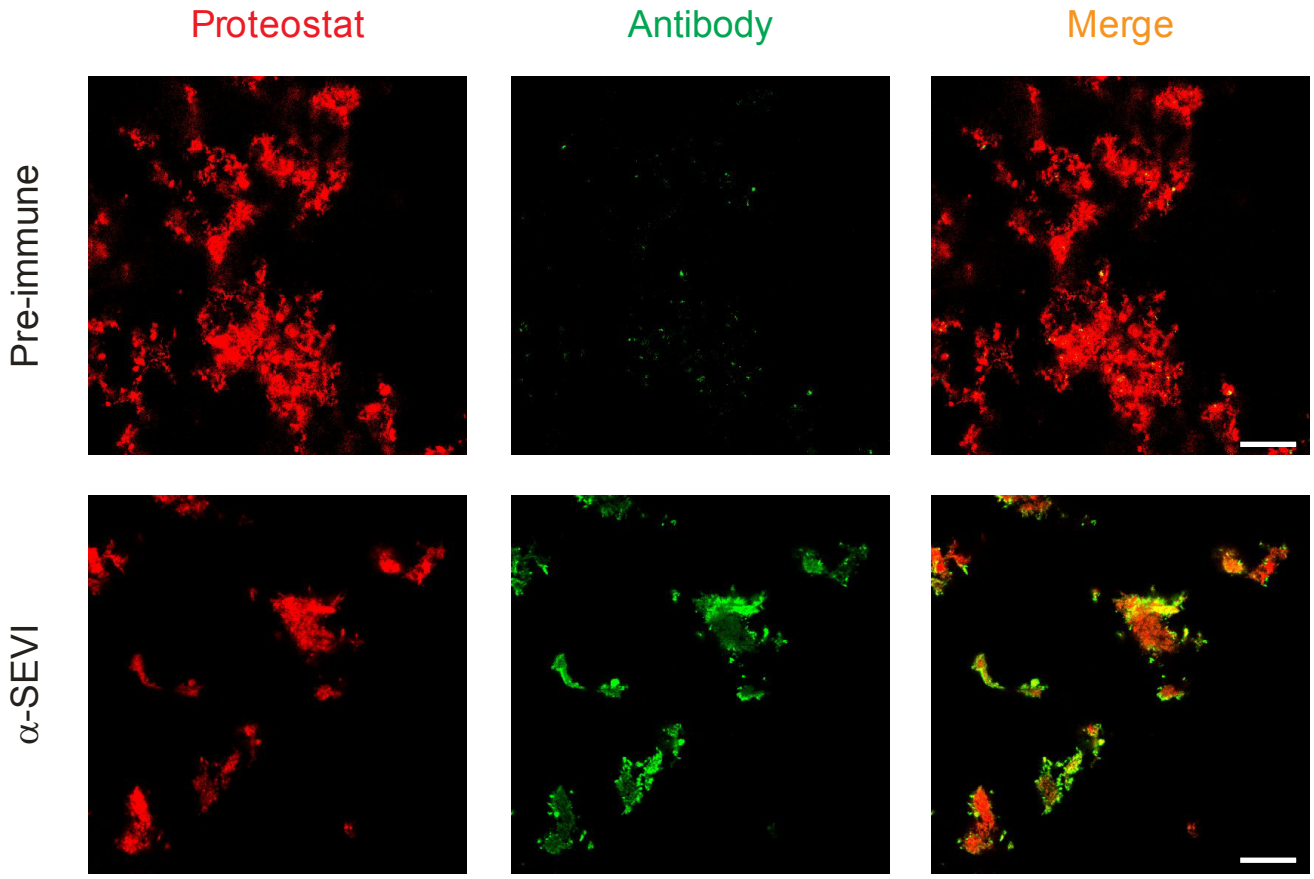




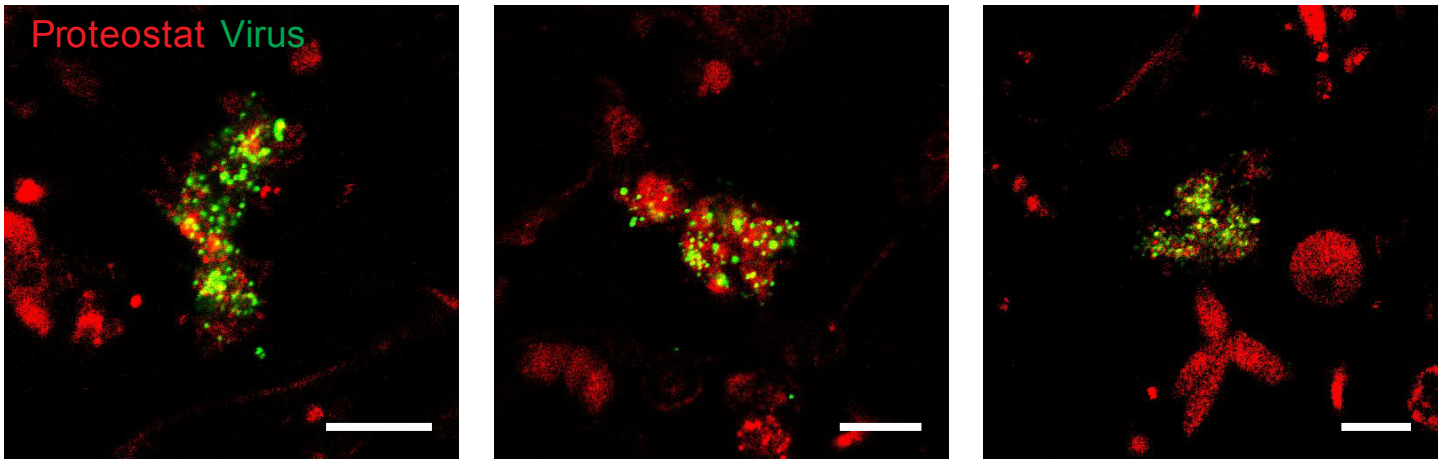
Supplementary Figure 5. Detection of endogenous amyloid in semen from HIV-1 infected individuals. (a) Samples were treated with 2 % paraformaldhyde for 30 min, followed by staining with the amyloid-specific dyes Proteostat (red) and pFTAA (green) at room temperature for 15- 20 min and imaged using a LSM710 confocal microscope. Scale bar = 10 µm. (b) Effect of analyzed HIV-1 semen samples and pooled semen from seronegative donors on HIV infection. R5-tropic HIV-1 was exposed to the indicated concentrations of semen (%), and then added to TZM-bl cells. After 2 h, semen-virion mixtures were replaced with fresh medium. HIV-1 infection rates were measured three days later by quantifying ß-galactosidase activities in cell lysates. Values shown are average values derived from triplicate infection ± standard deviation. The numbers above the columns give the n-fold enhancement of infection observed after treatment with 10 % semen relative to infection with mock-treated virus.



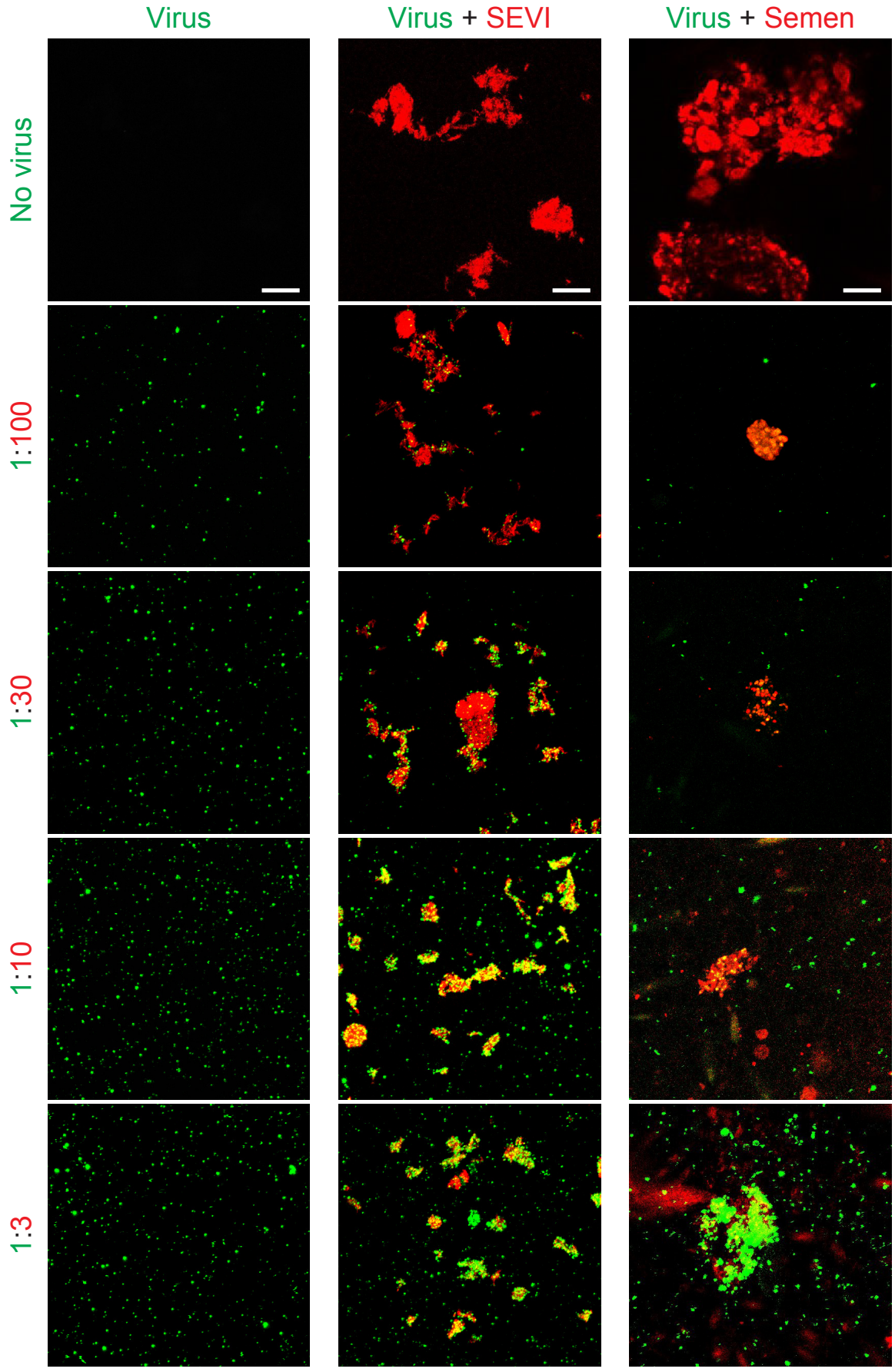
Supplementary Figure 6. TEM images of semen samples A, B and C.



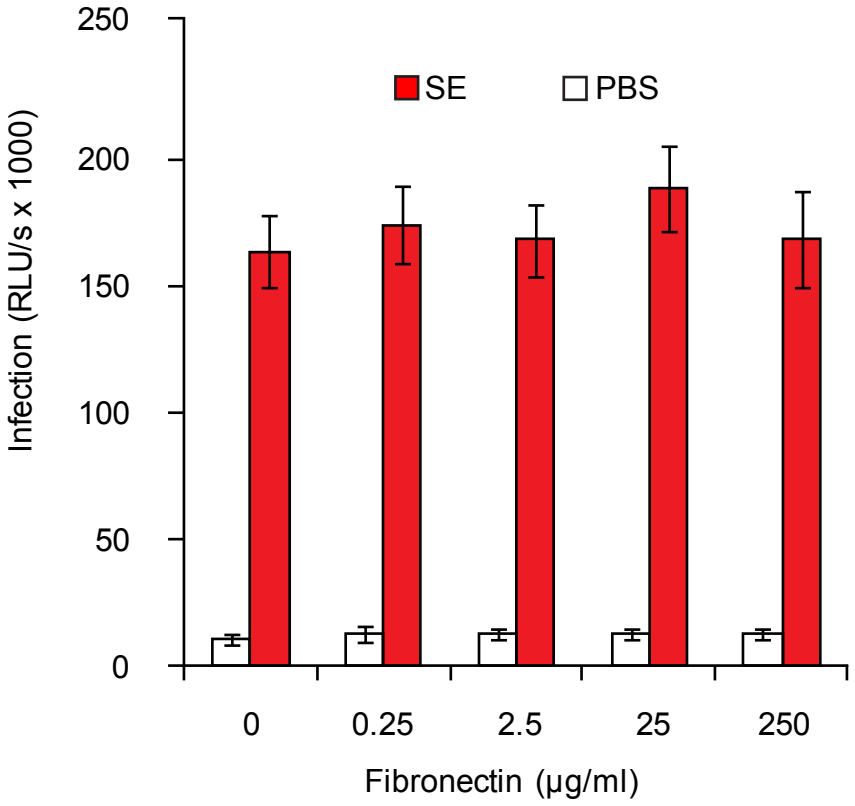
Supplementary Figure 7. An anti-SEVI antiserum recognizes in vitro generated SEVI fibrils. 100 μg/ml SEVI amyloid fibrils were incubated with a pre-immune or anti-SEVI antiserum. Amyloid/antibody complexes were then pelleted, washed and detected using Alexa488-coupled secondary antibody (green). All samples were subsequently costained with Proteostat dye (red). Scale bar = 20 μm.



Supplementary Figure 8. Not all seminal amyloid interact with retroviral particles. Shown are images derived from three independent ejaculates stained with Proteostat dye (red) and incubated with EYFP-labeled virions (green). Images were acquired 15 min later on a laser scanning confocal microscope. Scale bar = 10 μm.



Supplementary Figure 9. Interaction of amyloid with increasing doses of retroviral particles. EYFP-labeled virions (green) were mixed with Proteostat-stained SEVI (100 µg/ml) or semen (100%) (red) and incubated for 15 min. Z-Stacks with an increment of 0.5 µm were acquired on a laser scanning confocal microscope. Shown here are the maximum projections from representative confocal Z-stack images. Final dilutions of virions with SEVI or semen in a volume of 100 µl are indicated on the left. Scale bar = 10 µm.



Supplementary Figure 10. Fibronectin does not affect semen-mediated HIV infectivity enhancement. R5-tropic HIV-1 was treated with indicated concentrations of fibronectin for 5 min. Thereafter, virus/fibronectin solutions were incubated with 10% semen or PBS and used to infect TZM-bl cells. Infection rates were determined 3 days later. Shown are average values derived from triplicate infection ± standard deviation. .