Supplementary Information:

Stimulated Emission Depletion Nanoscopy Reveals Time-Course of Human Immunodeficiency Virus Proteolytic Maturation

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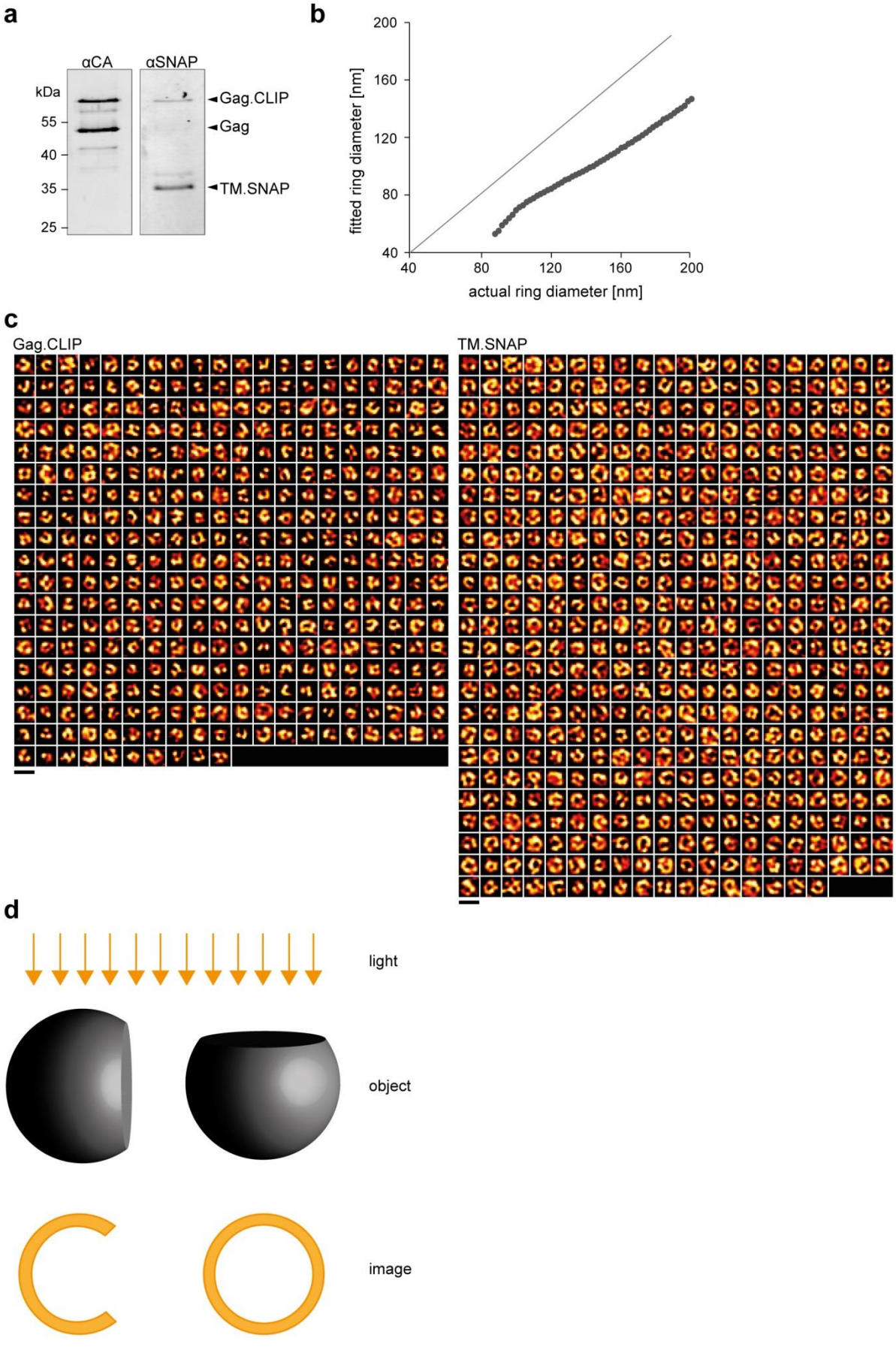
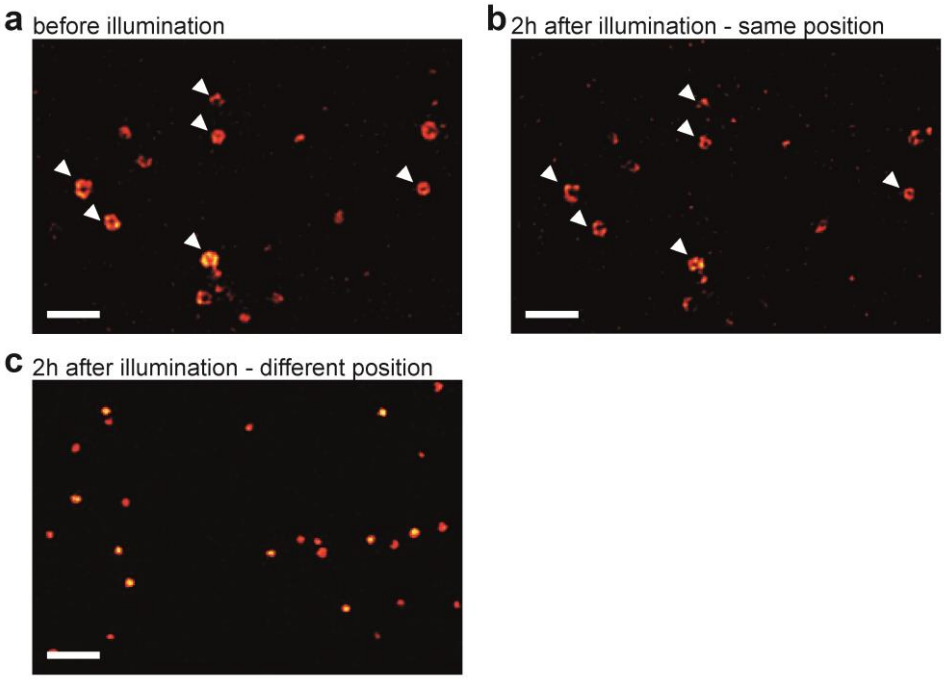

a
αCA
αSNAP
kDa
55
40
35
25
Gag.CLIP
Gag
TM.SNAP
b
fitted ring diameter [nm]
actual ring diameter [nm]
200
160
120
80
40
40
80
120
160
200
c
Gag.CLIP
TM.SNAP
d
light
object
image

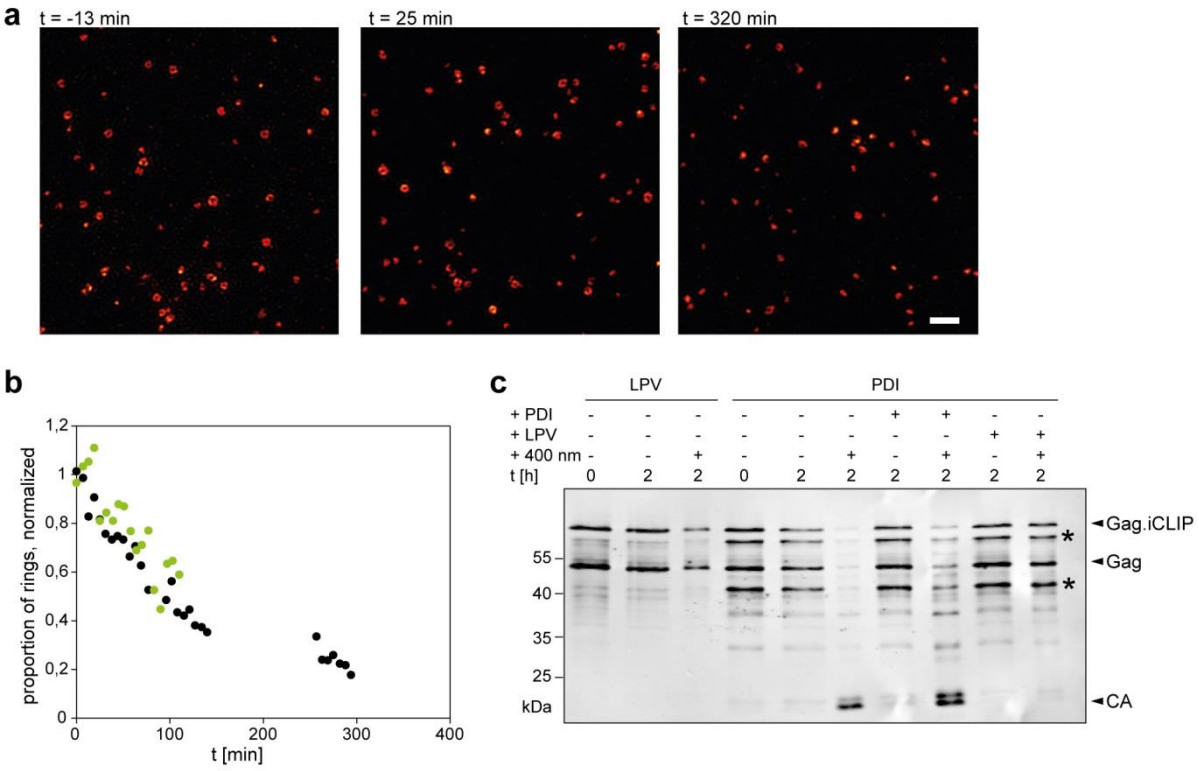
Supplementary Figure S1

(a) Immunoblot of particles shown in Figure 1b, c, f detected with the indicated antisera. **(b)** Ring diameters obtained by a 2D projection of a hollow sphere and subsequent convolution are plotted against the actual diameter of the sphere (grey dots). The solid grey line represents identical values of measured ring diameters and actual sphere diameters to guide the eye. **(c)** Gallery of all detected ring-like structure for SiR-CLIP and SiR-SNAP-stained samples used for analysis shown in Figures 1d, e. Scale bars are 205 nm. **(d)** Illustration of the 2D projection of Gag spheres by the microscope for two different particle orientations.



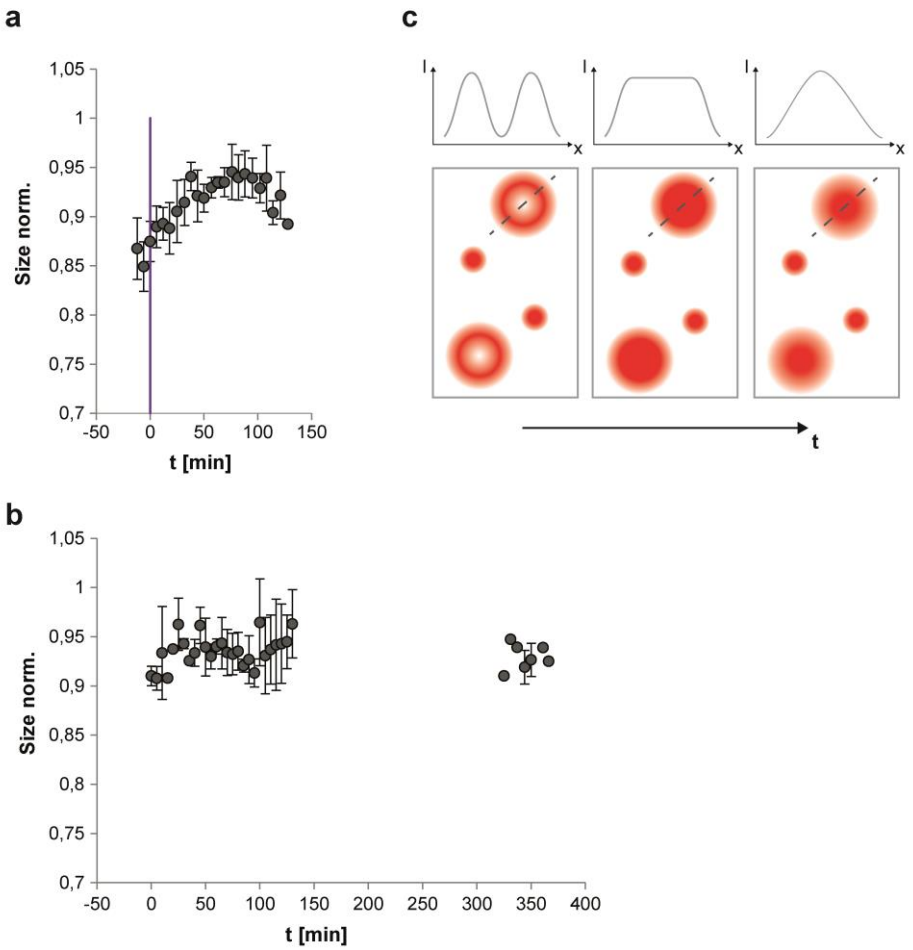
Supplementary Figure S2

Deconvolved STED images of purified particles containing PDI before 400 nm illumination **(a)** and 2 h after illumination **(b, c)**. **(b)** The same field of view as in (a) was imaged. Ring-like particles previously imaged by STED mostly retained their morphology (arrowheads). **(c)** Different field of view on the same sample. Scale bars: 500 nm.



Supplementary Figure S3

(a) Deconvolved images acquired 13 min before illumination and 25 min or 320 min after illumination. Images correspond to data shown in Figure 4b (light blue dots). Scale bar: 500 nm. **(b)** HIV^{iCLIP} particles produced in the presence of 2 µM PDI imaged in PR buffer over time with the STED microscope without 400 nm illumination as in Figure 5. The graph shows the proportion of rings detected over time, normalized to the proportion determined at the start of the experiment. Differently colored symbols represent two independent measurements. **(c)** Immunoblot analysis of samples shown in Figure 4d. Virus-specific proteins were detected using polyclonal antiserum raised against HIV-1 CA. The molecular masses in kDa of standard proteins are indicated on the left. Asterisks indicate proteolysis intermediates (MA-CA or MA-iCLIP-CA) resulting from incomplete inhibition of processing by PDI.



Supplementary Figure S4

Apparent particle diameters observed over time (a,b) Mean sizes of all spots fitted by a 2D Gaussian plotted over time from the experiment shown in main Figure 4. (a) PDI treated particles (b) control particles produced in the presence of a stable PR inhibitor. Mean values of ~1,200 spots per time-point and standard deviation of means from five independent experiments are shown. **(c)** Model of changes in fluorescence distribution during HIV-1 proteolytic maturation. The Gaussian spots arising from the ring-like structures by partial cleavage and CLIP redistribution appear flat and larger than the small 2D Gaussian spots detected in the initial preparation, which presumably represent background staining. The appearance of virion-sized 2D Gaussian spots thus results in an increase of the mean diameter of the 2D Gaussian spot population. Complete cleavage and redistribution of the CLIP-tag from Gag then results in more compact fluorescent spots, leading to a decrease in mean spot diameter over time. The mean particle size increase and decrease are thus both attributed to changes of fluorophore distribution inside the virions.

Movie S1

STED data shown in Figure 4a rendered as a time-lapse movie. The violet box at time point “0:00” indicates the time of illumination with 400 nm. Scale bar: 100 nm.