Super-Resolution Fluorescence Imaging Reveals That Serine Incorporator Protein 5 Inhibits Human Immunodeficiency Virus Fusion by Disrupting Envelope Glycoprotein Clusters

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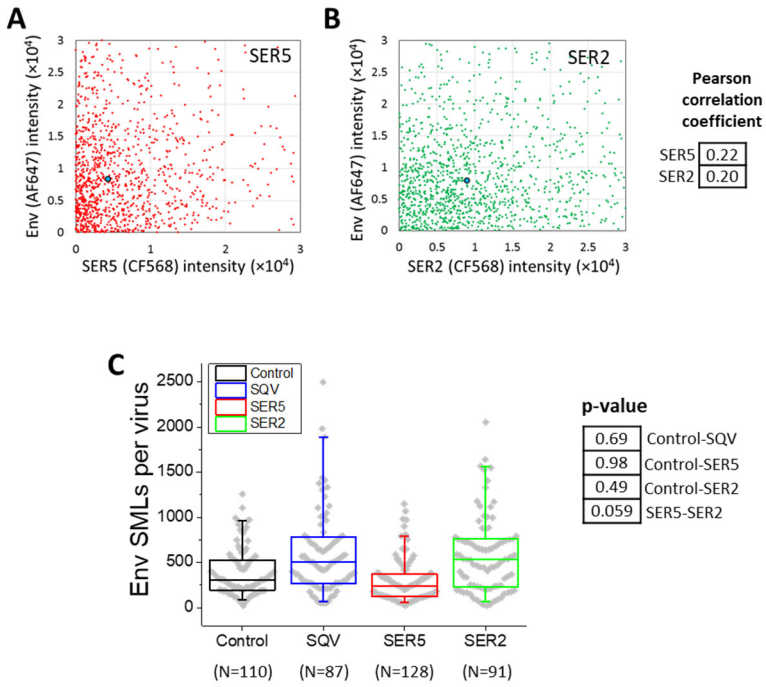
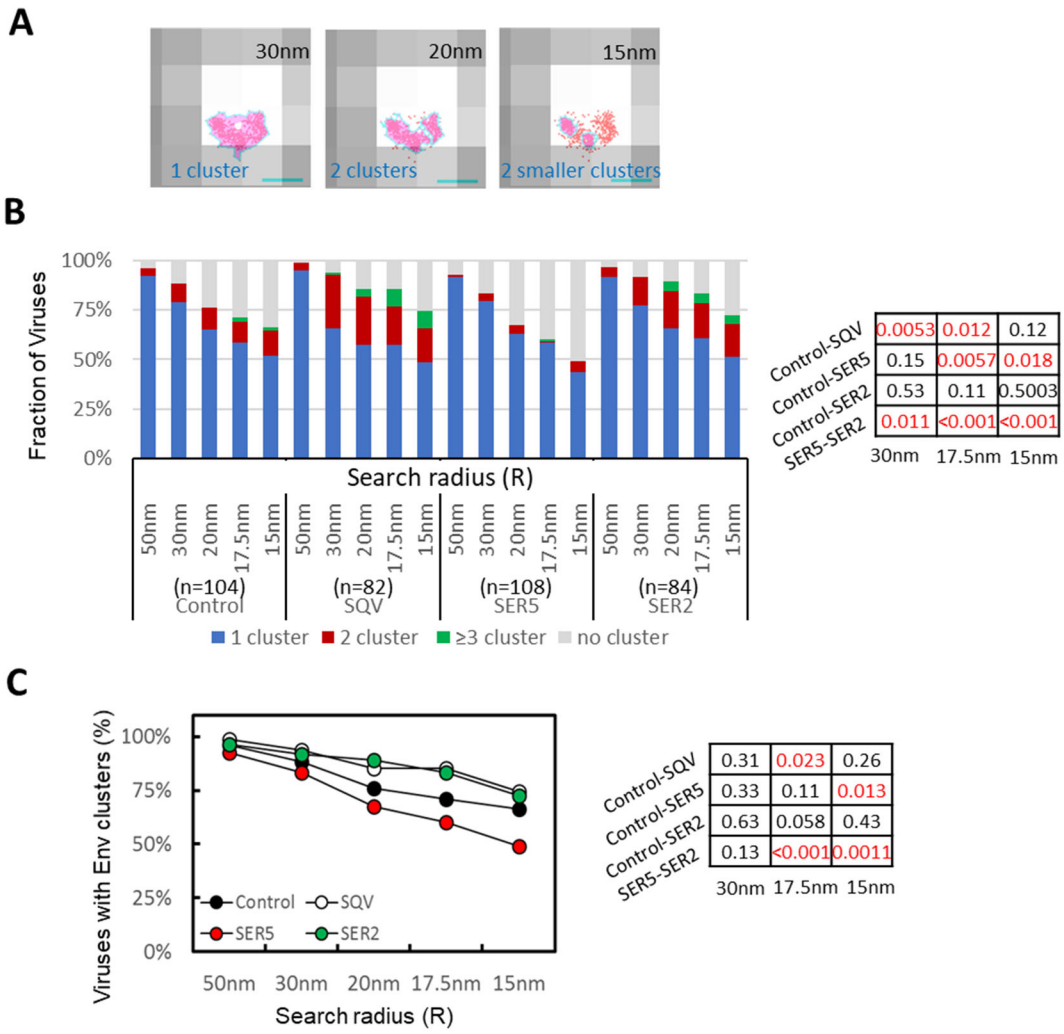
Supporting information

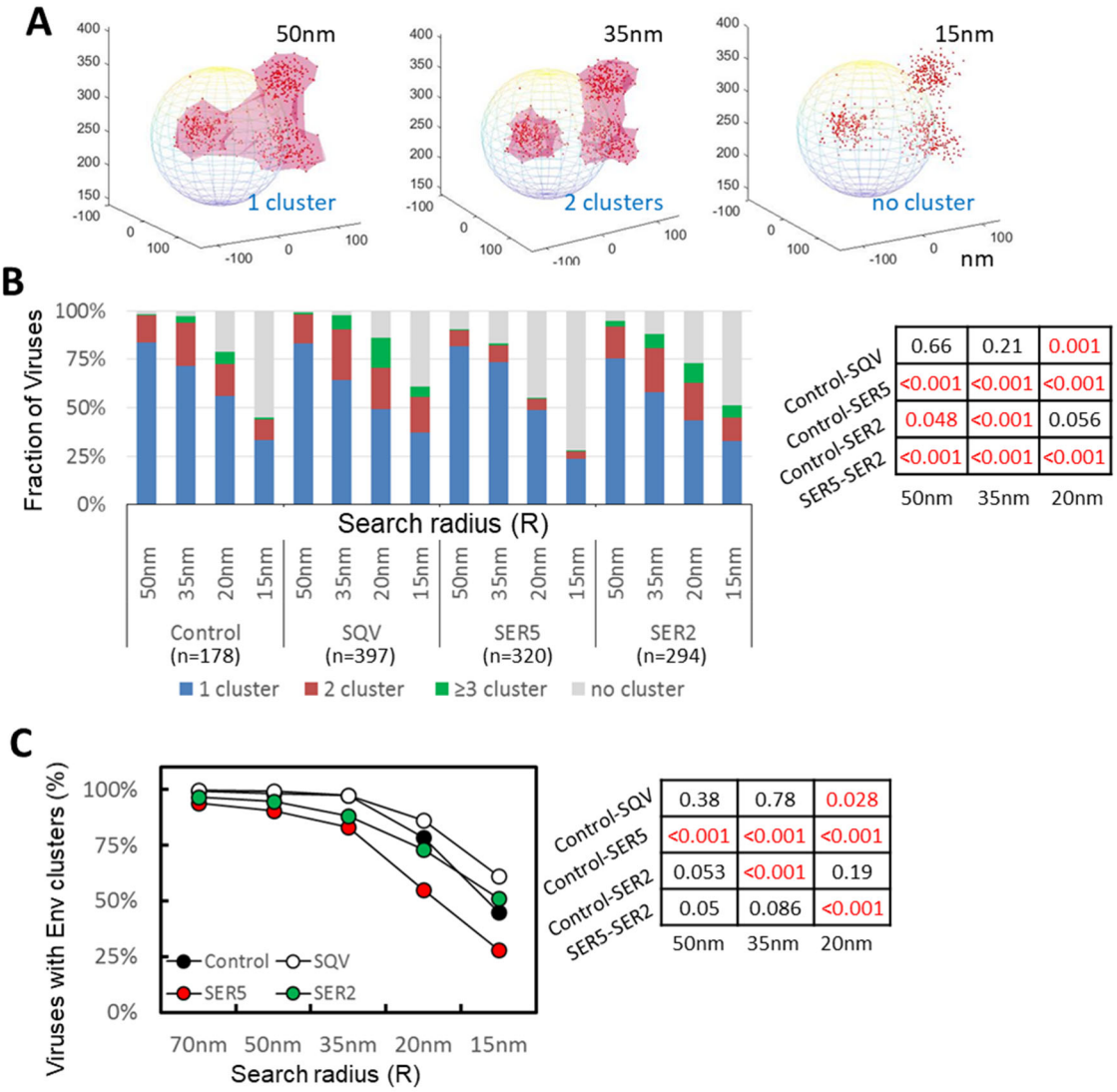
Supplemental Figures



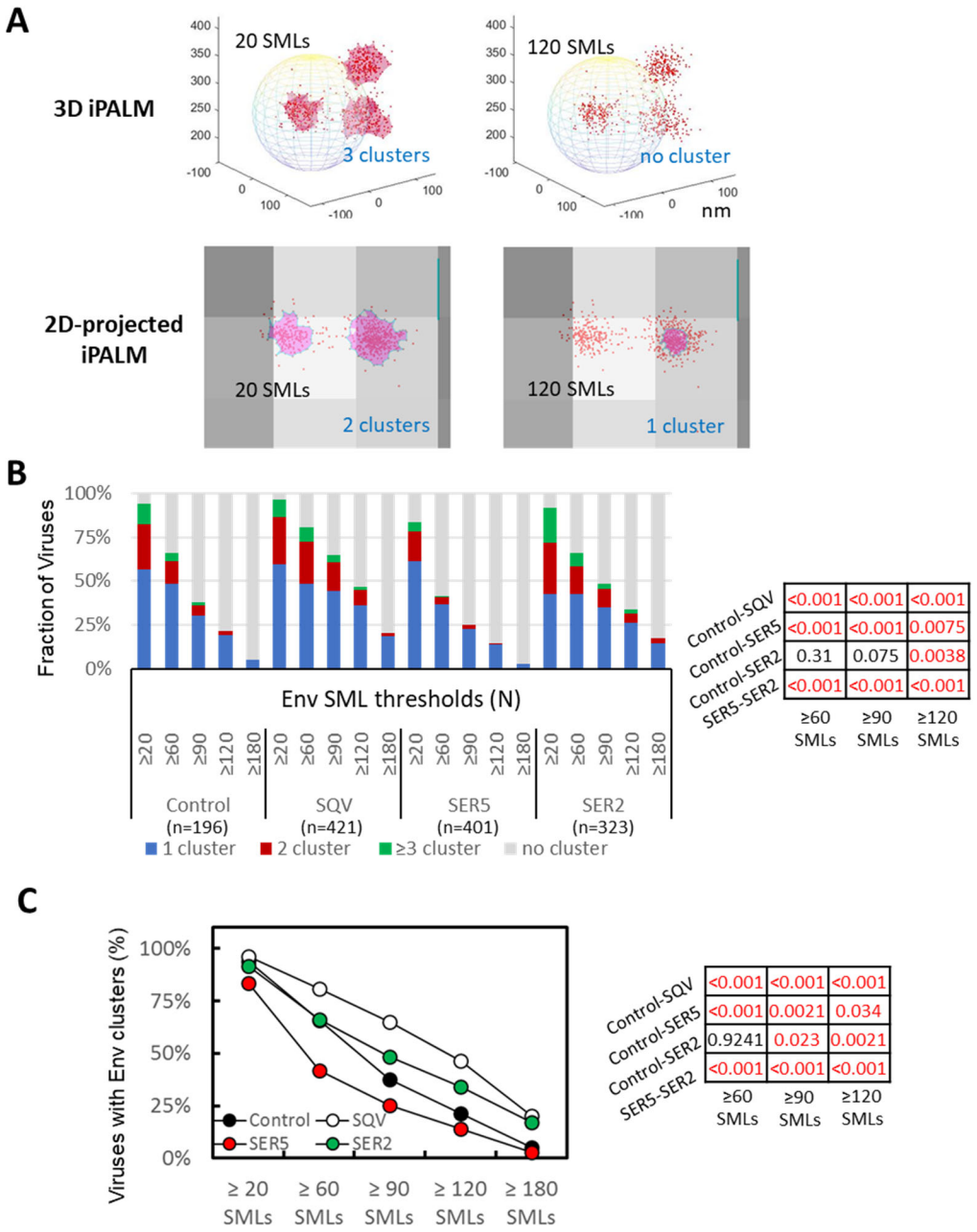
Supp. Fig. S1. Single virus immunofluorescence analyses of Env and SERINC incorporation for virus panel A. (A, B) No significant correlation is observed between Env and SERINC incorporation (Pearson correlation < 0.3). Blue dot is median (50%) of the intensity. (C) Single molecule localization (SML) distributions per virion for this virus panel obtained by dSTORM. No significant differences were observed between control, SQV, SER5 and SER2 pseudoviruses ($p>0.05$), using a two-sample Kolmogorov–Smirnov test after optimal binning of data (see Methods). Box plot includes 1st, 2nd, and 3rd quantiles. Whiskers are 5% and 95% value.



Supp. Fig. S2. DBSCAN Env cluster analysis using varied search distance R. (A) Illustration of single Env molecule localizations (SMLs, red dots) obtained by 2D dSTORM and overlaid onto a diffraction-limited GFP-Vpr spot. Env clusters for different search radii, as indicated, and a fixed minimum localizations of 90 are colored light magenta area with cyan contours. Scale bar: 100 nm. (B) Cluster analysis for a panel of HIV-1 pseudoviruses consisting of control and immature (SQV) particles, as well as particles containing SER2 and SER5, using different DBSCAN searching distances (50, 30, 20, 17.5, 15 nm), as indicated, and SML threshold of 90. Pseudoviruses with less than 90 Env localizations were excluded from analysis. The results are categorized into no cluster, 1, 2, and ≥3 clusters per virion. (C) Same as in B, but plotted for two categories of viruses – with and without Env clusters. Statistical comparison for panels B and C is done using Fisher's Exact Test and shown on the right.

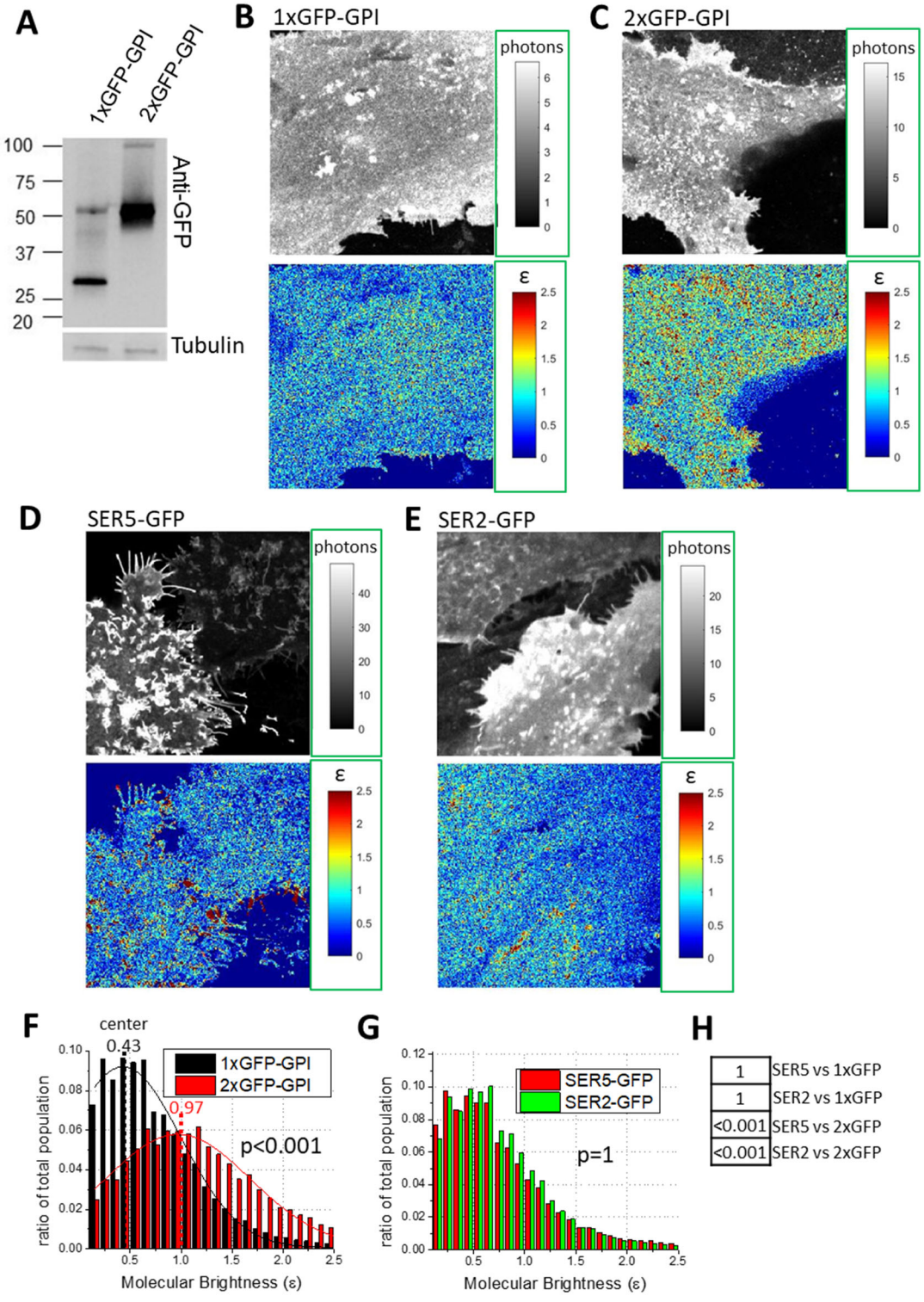


Supp. Fig. S3. DBSCAN analysis of Env distribution obtained by 3D iPALM. (A) Illustration of single Env molecule localizations (SMLs, red dots) and clustering in 3D overlaid onto an idealized viral particle shown as a sphere with radius of 100 nm located by diffraction-limited spot corresponding to a GFP-Vpr labeled virion. Dependence of Env clustering (light red area) on the DBSCAN searching distance (50, 35, 20 nm), using the same minimum localizations parameter N=60. X, Y, Z axes are in nm. (B) A panel of 4 viruses analyzed in Fig. 4C is similarly categorized into no cluster, 1, 2, and ≥3 clusters per virion and plotted as staggered bars for searching distances 50, 35, 20, 15 nm, using the same minimum 60 Env SMLs. Pseudoviruses with less than 60 Env localizations were excluded from analysis. (C) Relative fractions of pseudoviruses containing or lacking Env clusters (2 categories) as a function of searching distance for a minimum of 60 SMLs for searching distances 50, 35, 20, and 15 nm. Statistical comparison for panels B and C is done using Fisher's Exact Test and shown on the right.



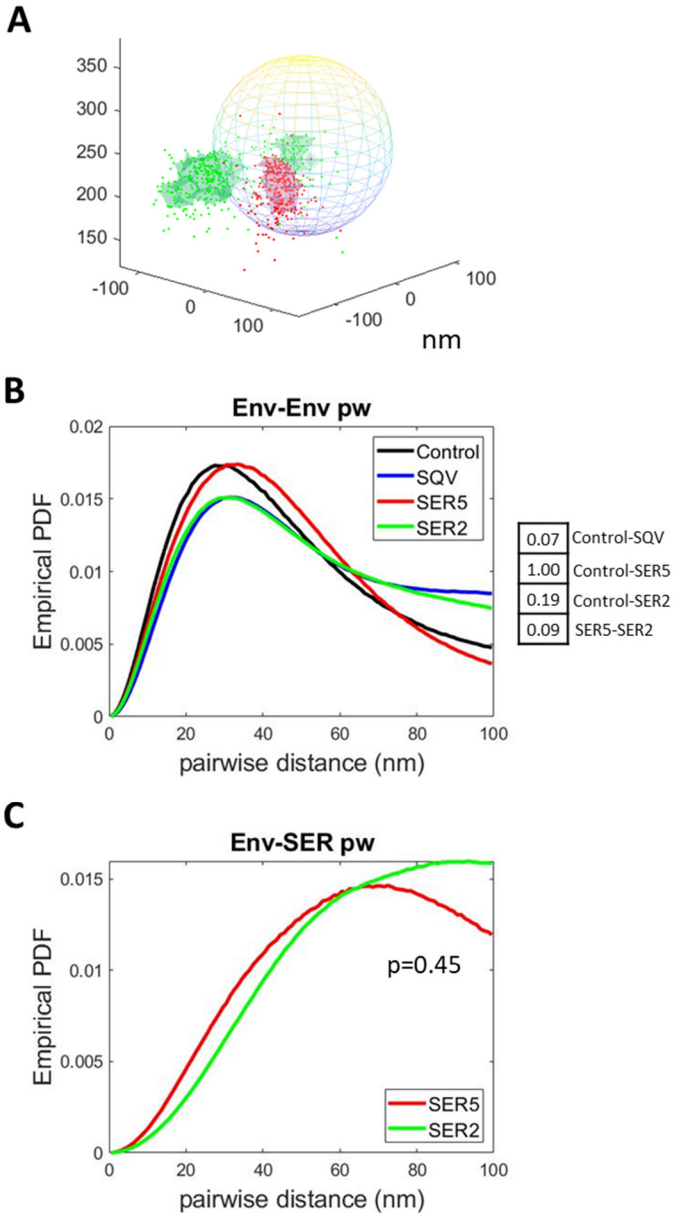
Supp. Fig. S4. HIV-1 Env cluster analysis of 2D-projected iPALM data. (A) *Top*: Illustration of single Env molecule localizations (SMLs, red dots) and cluster analysis in 3D using different DBSCAN SML number threshold parameters. SMLs are overlaid onto an idealized viral particle. *Bottom*: 2D projections of 3D iPALM data overlaid on a diffraction-limited GFP-Vpr spot. Dependence of Env clustering (light magenta area) in 3D and in 2D-projections of iPALM data on the DBSCAN SML threshold of 20 and 120 and searching distance of 20 nm (3D data) 15 nm (2D data). X, Y, Z axes are in nm. (B) Analysis of Env clustering using 3D iPALM data projected on a single plane for a panel of HIV-1 pseudoviruses using varied DBSCAN SML thresholds, as indicated, and the same search radius R=15 nm. The results are categorized into virions with no clusters, 1, 2, and ≥3 clusters. (C) Same is in A, but plotted for 2 categories of viruses (containing

or lacking Env clusters) as a function of SML threshold. Statistical analysis of DBSCAN-based Env clustering data carried out using Fisher's Exact Test is shown on the right in panels B and C.

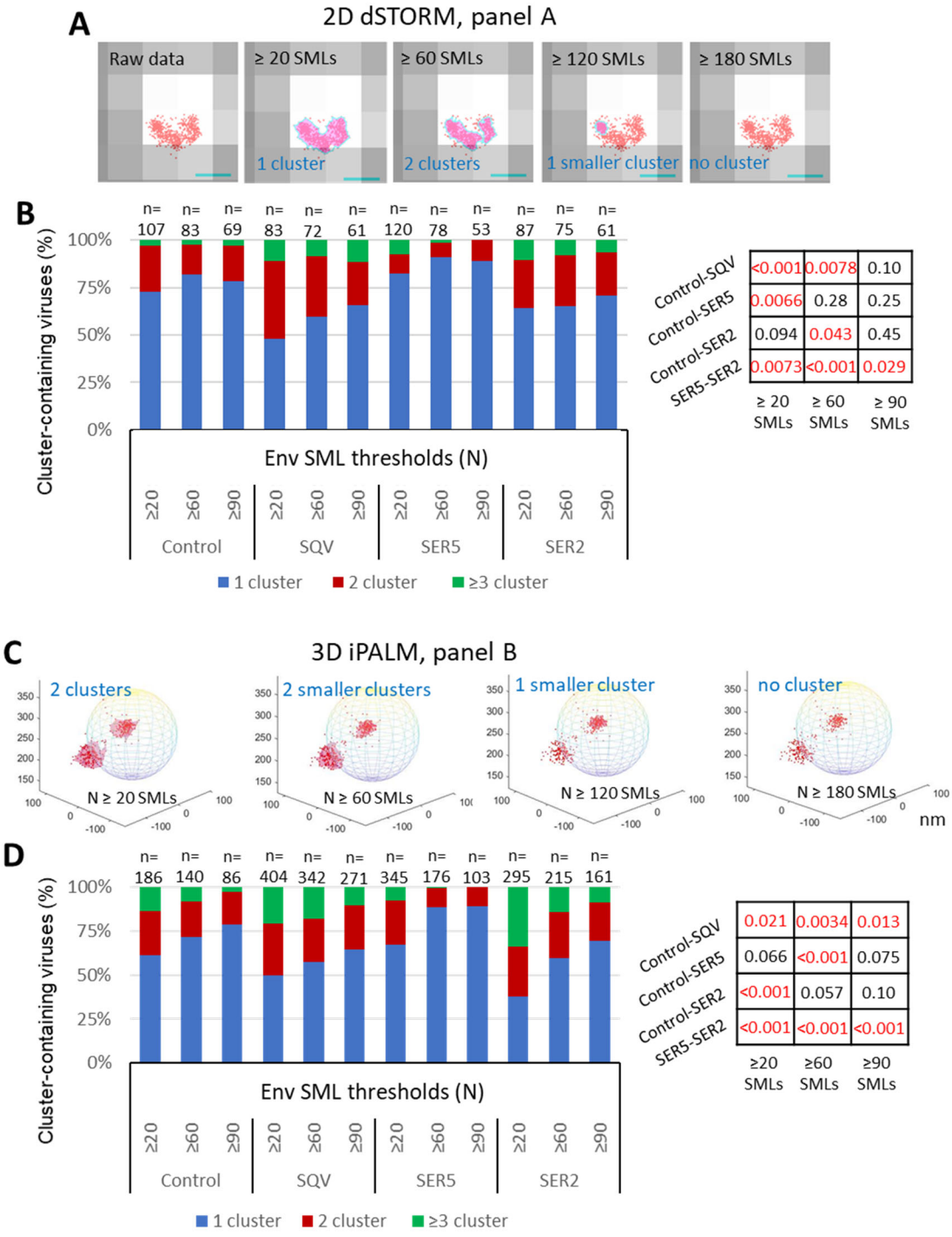


Supp. Fig. S5. Lack of significant dimerization of SER5-GFP or SER2-GFP in the plasma membrane. (A) Western blot analysis of 1xGFP-GPI and 2xGFP-GPI proteins expressed in HeLa cells. (B, C) Average fluorescence (Top) and Number and Brightness (N&B) analysis (Bottom)

were carried out using live HeLa cells transfected with 1xGFP-GPI (B) and 2xGFP-GPI (C). (E, F) Average fluorescence (Top) and N&B analysis (Bottom) were carried out in live HeLa cells transfected with SER5-GFP (E) and SER2- GFP (F). Less than 1% of outlier pixels representing motion artifacts due to filopodia movement and endosome trafficking were excluded from analysis. (F) Histograms showing ~2-fold molecular brightness (ε) differences between 1xGFP-GPI and 2xGFP-GPI based upon Gaussian fitting ($p<0.001$). (G) No differences between SER5-GFP and SER2-GFP. (H) Comparison of molecular brightness (ε) between the pooled data of SER5-GFP ($n_{cells}=16$), SER2-GFP ($n_{cells}=15$), 1xGFP-GPI ($n_{cells}=19$), and 2xGFP-GPI ($n_{cells}=9$) shows SER5 and SER2 N&B similar to 1xGFP-GPI and different from 2xGFP-GPI by two-sample Kolmogorov–Smirnov test after optimal binning of data (see Methods).



Supp. Fig. S6. Pairwise distance analysis for Env-Env, SER-SER, and Env-SER localizations by 2-color iPALM. (A) An example of 3D Env (red) and SER5 (green) co-distribution on a virion. Probability density function (PDF) for pairwise SML distance distributions per virus calculated for Env-Env (B), and Env-SERINC (C) is plotted. None of the distributions is significantly different (p-values > 0.05). Data are compared by two-sample Kolmogorov–Smirnov test after optimal binning.



	≥ 20 SMLs	≥ 60 SMLs	≥ 90 SMLs
Control-SQV	<0.001	0.0078	0.10
Control-SER5	0.0066	0.28	0.25
Control-SER2	0.094	0.043	0.45
SER5-SER2	0.0073	<0.001	0.029

	≥20 SMLs	≥60 SMLs	≥90 SMLs
Control-SQV	0.021	0.0034	0.013
Control-SER5	0.066	<0.001	0.075
Control-SER2	<0.001	0.057	0.10
SER5-SER2	<0.001	<0.001	<0.001

Supp. Fig. S7. Analysis of Env clustering after excluding particles that did not contain clusters. Replotting DBSCAN analysis results of 2D dSTORM from Fig. 2 (A, B) and 3D iPALM from Fig. 4 (C, D) for varied SML number thresholds, after excluding the viruses lacking clusters. Statistical comparison for panels B and D is done using Fisher's Exact Test and shown on the right.