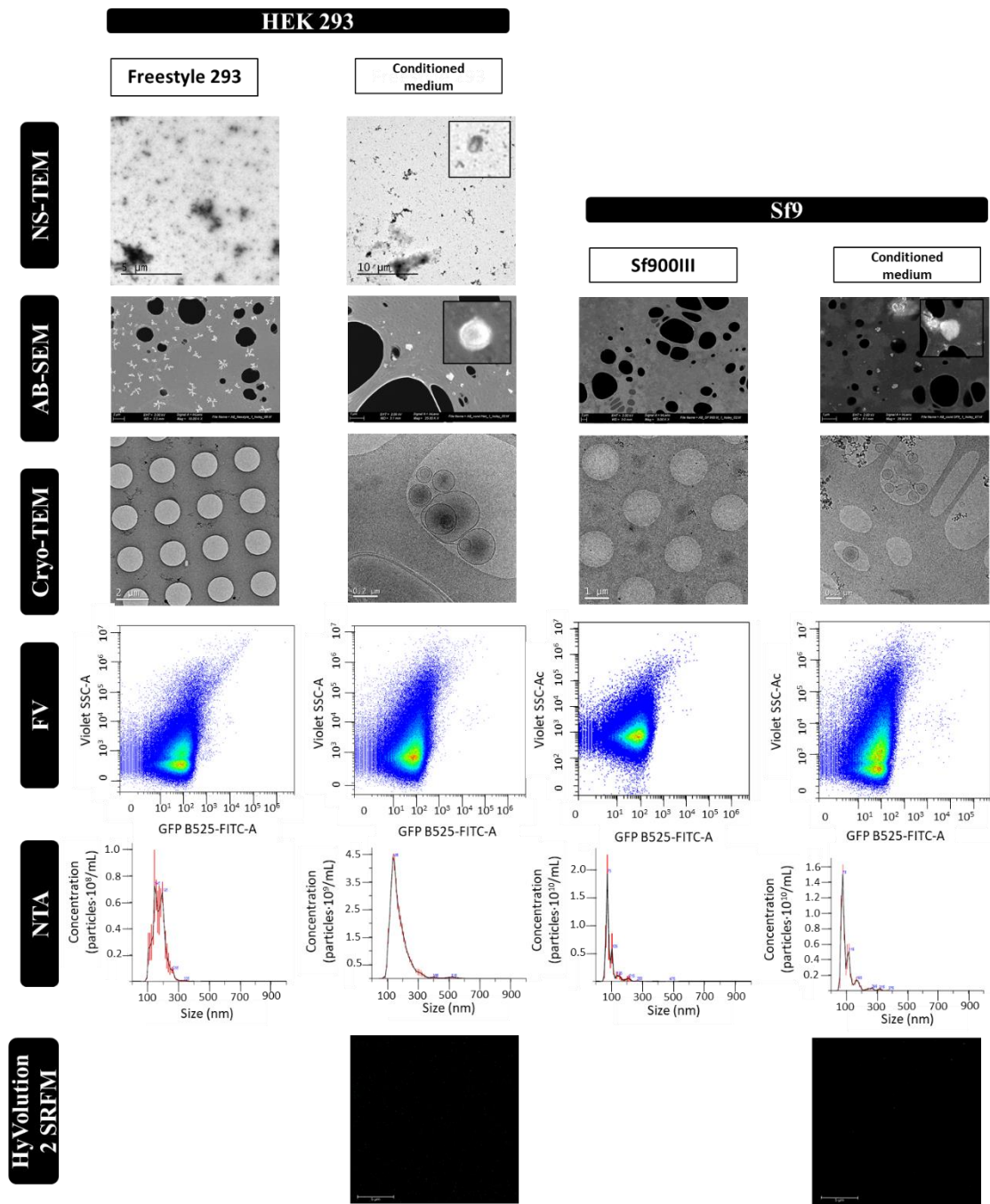


1 SUPPLEMENTARY MATERIALS

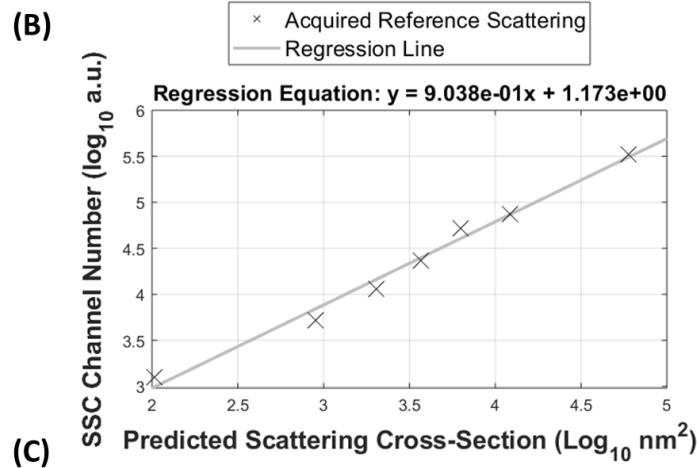
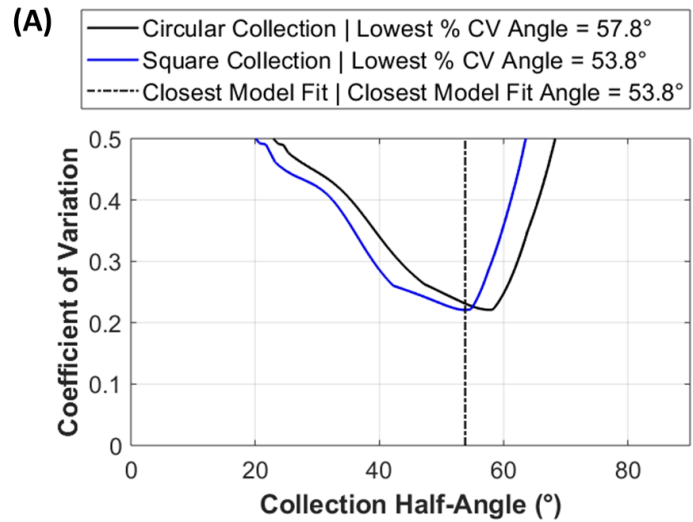


2

3 S1. Negative control analyses: cell culture and conditioned media controls. FreeStyle 293 (HEK  
 4 293) and Sf900III (Sf9) media were assessed by NS-TEM, AB-SEM, cryo-TEM, NTA, flow  
 5 virometry and SRFM. Analyses were performed by applying the same sample preparation  
 6 protocol and equipment set up that used for VLP samples. In NS-TEM, AB-SEM and cryo-TEM  
 7 analyses of FreeStyle medium, the presence of salts was detected while low electrodense  
 8 aggregates were observed in the Sf900III medium by cryo-TEM. In EM micrographs of both  
 9 conditioned media, the presence of EVs was detected. NTA analyses of FreeStyle and Sf900III  
 10 media, and conditioned media resulted in a mean nanoparticle diameter of  $174 \pm 39$  nm  
 11 (FreeStyle),  $174 \pm 65$  nm (conditioned FreeStyle),  $92.7 \pm 74.6$  nm (Sf900III),  $101 \pm 47$  nm  
 12 (conditioned Sf900III) with a respective concentration of  $0.6 \pm 0.1$  E+9,  $17.6 \pm 0.9$  E+9,  $24.4 \pm 1.1$

13 E+9 and  $27.3 \pm 1.5E+9$  diffracting particles/ml. Conditioned media analyses are adapted from  
 14 [24] and FreeStyle medium results from [31]. Flow virometry analyses of FreeStyle medium,  
 15 conditioned FreeStyle, Sf900III medium and conditioned Sf900III yielded a nanoparticle  
 16 concentration of  $0.1 E+9$ ,  $0.2 E+9$ ,  $0.1 E+9$ ,  $0.8 E+9$  of EVs, respectively. No particles were detected  
 17 by HyVolution2 SRFM analysis of FreeStyle and Sf900III conditioned media samples due to the  
 18 absence of HIV-1 Gag-eGFP VLPs, as expected. This data was adapted from [24].

19



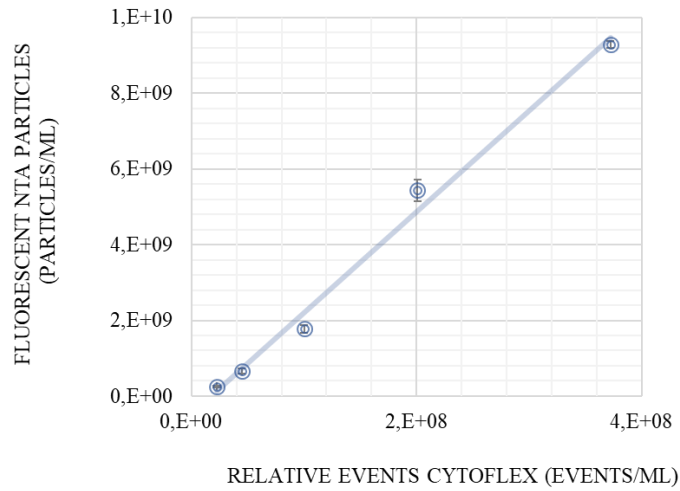
(C)

Bead Diameter (nm)	Median V-SSC (a.u.)	rCV (%)
100	1246.4	18.9
160	5248.8	10.3
200	11542.3	3.5
240	23266.4	6.5
300	51993.8	2.6
500	75025.8	3.8
900	328552.7	2.4

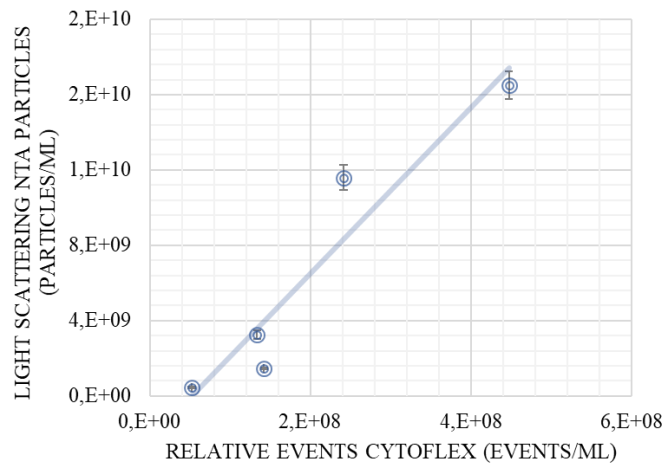
20

21 **S2.** Mie correlation for EV analysis with FCM<sub>PASS</sub> software. (A) Collection half-angle plot. (B)  
 22 Lineal regression SSC vs. predicted scattering cross-section plot. (C) Megamix beads  
 23 information acquired with the CytoFlex LX and introduced in the FCM<sub>PASS</sub> software.

**(A) HIV-1 Gag-eGFP VLPs**



**(B) EVs**



24

25 **S3.** Correlation between flow virometry and NTA of nanoparticles produced in HEK 293 cells.  
26 (A) HIV-1 Gag-eGFP VLPs (fluorescent particles) measured with NTA using the fluorescence  
27 filter and the population corresponding to VLPs in flow virometry are represented. Regression:  
28 (Fluorescent particles/mL) = 26,67 (events /mL) - 5E+08;  $R^2 = 0,9909$ . (B) EVs measured with light  
29 scattering by NTA and the population corresponding to EVs in flow virometry are represented.  
30 Regression: (Light Scattering particles/mL) = 44,18 (events /mL) - 2E+09;  $R^2 = 0,9082$ .

31

32