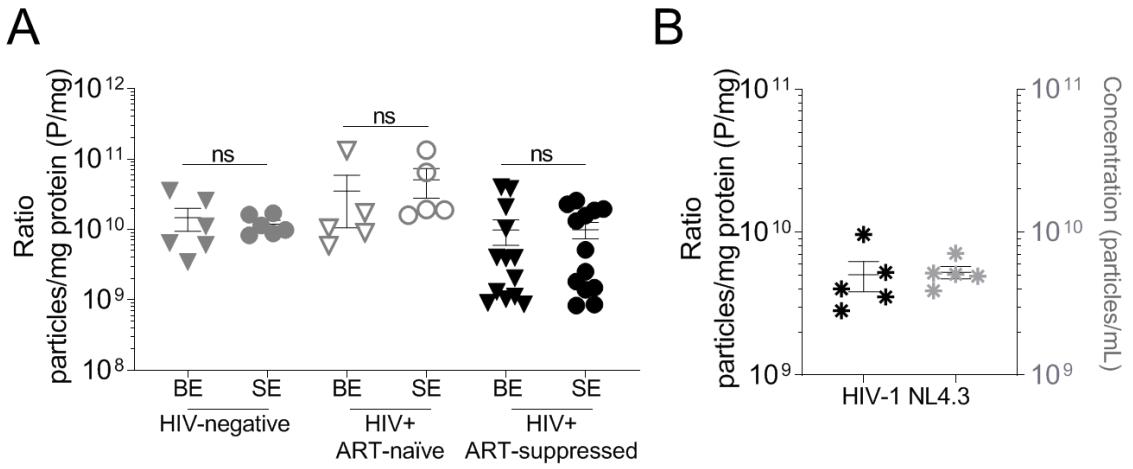
Demographic Characteristics	
Demographic factors[a]	
Age, years (range)	27 (22.75–31.25)
Weight, kg (range)	67.4 (60.4–76.8)
BMI[a] (range)	24.1 (21.6–26.9)
Time on ART, years (range)	>5 (0.17–>5)
CD4 count, cells/mm^3	583 (361–1303)
Race	
White	7
African American	4
Asian American	2
American Indian	0
More than one race	0

[a]Data are median value (interquartile range) or number of subjects. Body mass index (BMI) is calculated as the weight in kilograms divided by the height in square meters.

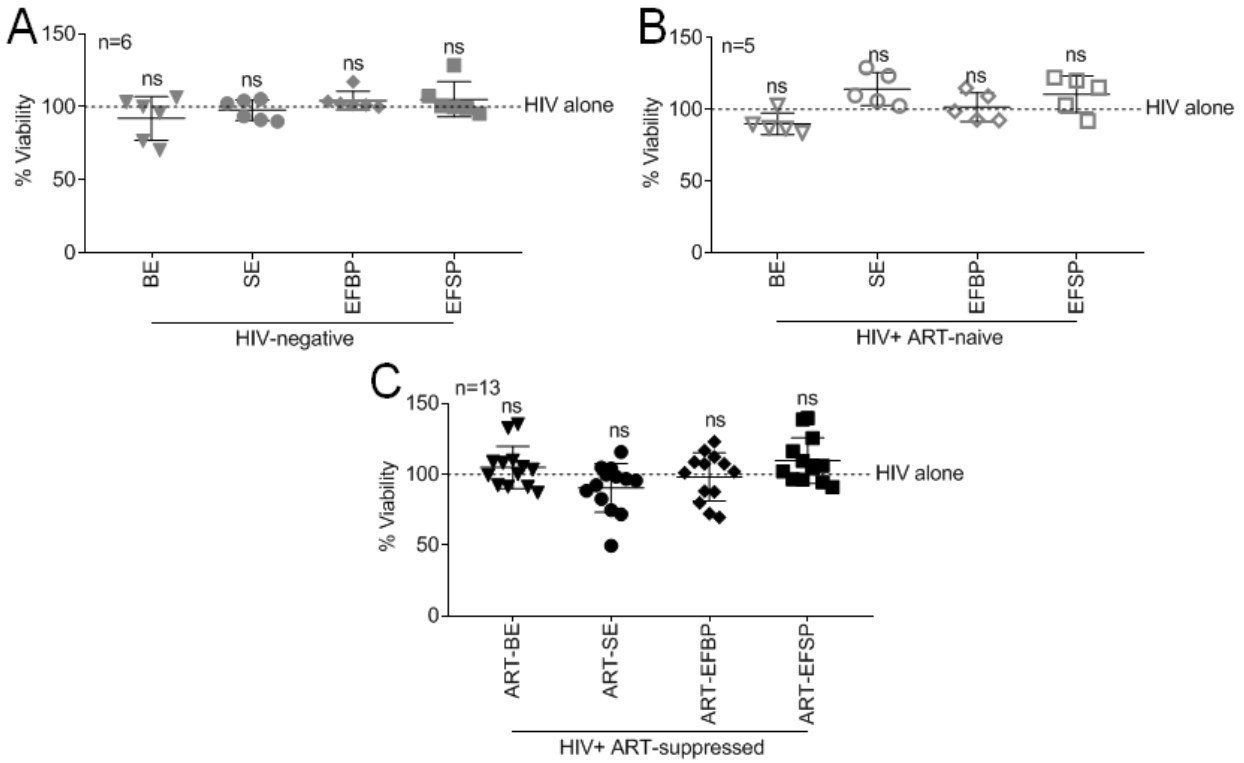
Supplemental Table 1: HIV-infected ART-suppressed donor demographics.

	Donor 1: Tenofovir (TAF), emtricitabine, dolutegravir ARV Concentration (ng/ml)				Donor 2: Tenofovir (TAF), emtricitabine, dolutegravir ARV Concentration (ng/ml)			
ARV	EV-Free Blood Plasma (EFBP)	Blood EVs (BE)	EV-Free Seminal Plasma (EFSP)	Semen EVs (SE)	EV-Free Blood Plasma (EFBP)	Blood EVs (BE)	EV-Free SP (EFSP)	Semen EVs (SE)
TFV-TAF	17.16	4.28	221.4	46.47	8.484	1.847	115.4	14.42
TFV-TDF	BLQ	BLQ	0.1099	BLQ	BLQ	BLQ	0.1338	BLQ
FTC	1379	388.6	1650	357.5	692.5	146.7	5199	711.2
FTC-TP	0.1860	0.4263	0.1141	BLQ	BLQ	BLQ	0.7078	0.1178
DTG	3286	962.9	68.55	35.12	3252	724.9	134.3	62.83
	Donor 3: Tenofovir (TDF), emtricitabine, efavirenz ARV Concentration (ng/ml)				**Donor 4: Tenofovir (TDF), emtricitabine, efavirenz ARV Concentration (ng/ml)**			
ARV	EFBP	BE	EFSP	SE	EFBP	BE	EFSP	SE
TFV-TDF	42.38	10.87	738.2	122.9	84.26	18.21	LIP	76.18
TFV-DP	BLQ	0.1030	0.3652	BLQ	BLQ	0.3302	LIP	BLQ
FTC	242.4	62.24	2169	346.5	184.5	39.70	LIP	119.7
FTC-TP	BLQ	BLQ	0.2594	0.2632	BLQ	BLQ	LIP	BLQ
EFV	1110	737.0	42.64	58.09	550.7	390.8	LIP	52.63

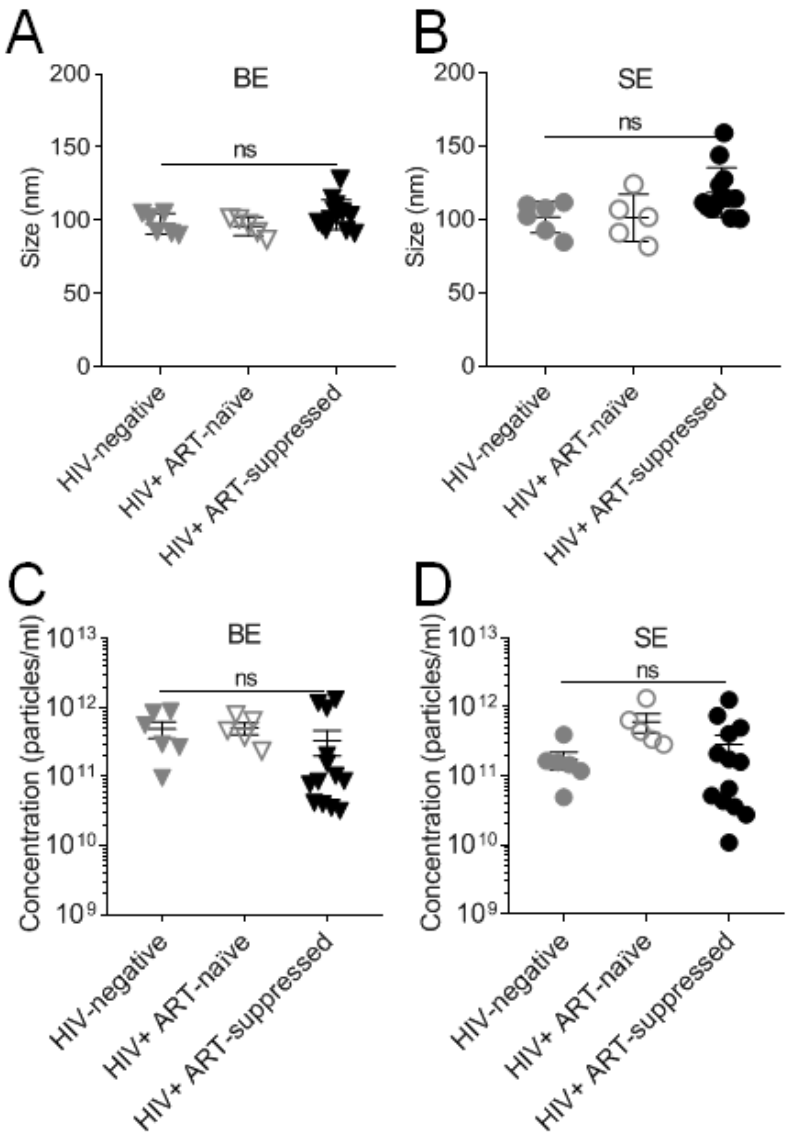
Supplemental Table 2: ARV concentrations in extracellular vesicle-free plasma and extracellular vesicles from HIV-infected ART-suppressed donor blood and semen. EV=extracellular vesicle. TFV=tenofovir; TAF=tenofovir alafenamide; TDF=tenofovir disoproxil fumarate; TFVdp=tenofovir diphosphate; FTC=emtricitabine; FTCtp=emtricitabine triphosphate; DTG=dolutegravir; EFV=efavirenz. BLQ=below quantitation. LIP=lost in processing



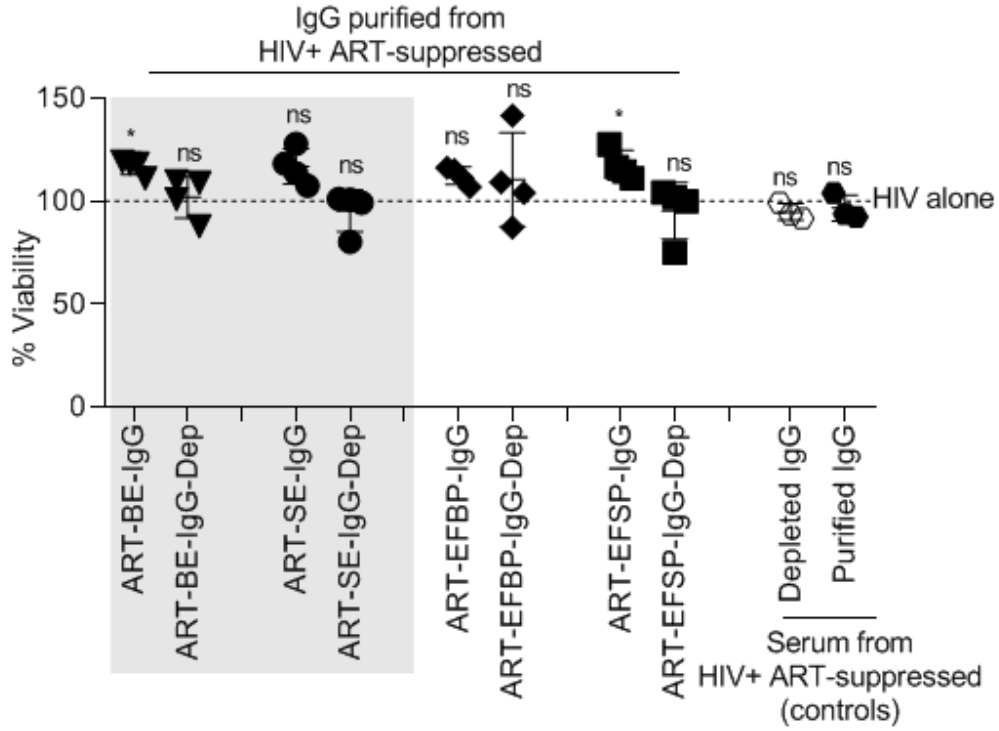
Supplemental Figure 1: Donor-derived blood and semen extracellular vesicles contain similar concentrations of particles per mg of protein. (A) BE and SE were isolated from HIV-negative (n=6), HIV-positive ART-naïve (n=5), and HIV-positive ART-suppressed (n=13) donors. (B) HIV-1 NL4.3 virus preps (n=5) were produced by pNL4.3 293T transfection. Concentrations were calculated as the ratio of particles per mg of protein or virus culture and grouped by donor cohort. Particle numbers were determined by ZetaView nanoparticle tracking analysis (NTA), and protein by Nanodrop absorbance at 280 nm. Significance was determined by student's t test. Error bars are SD of biological replicates. ns= not significant.



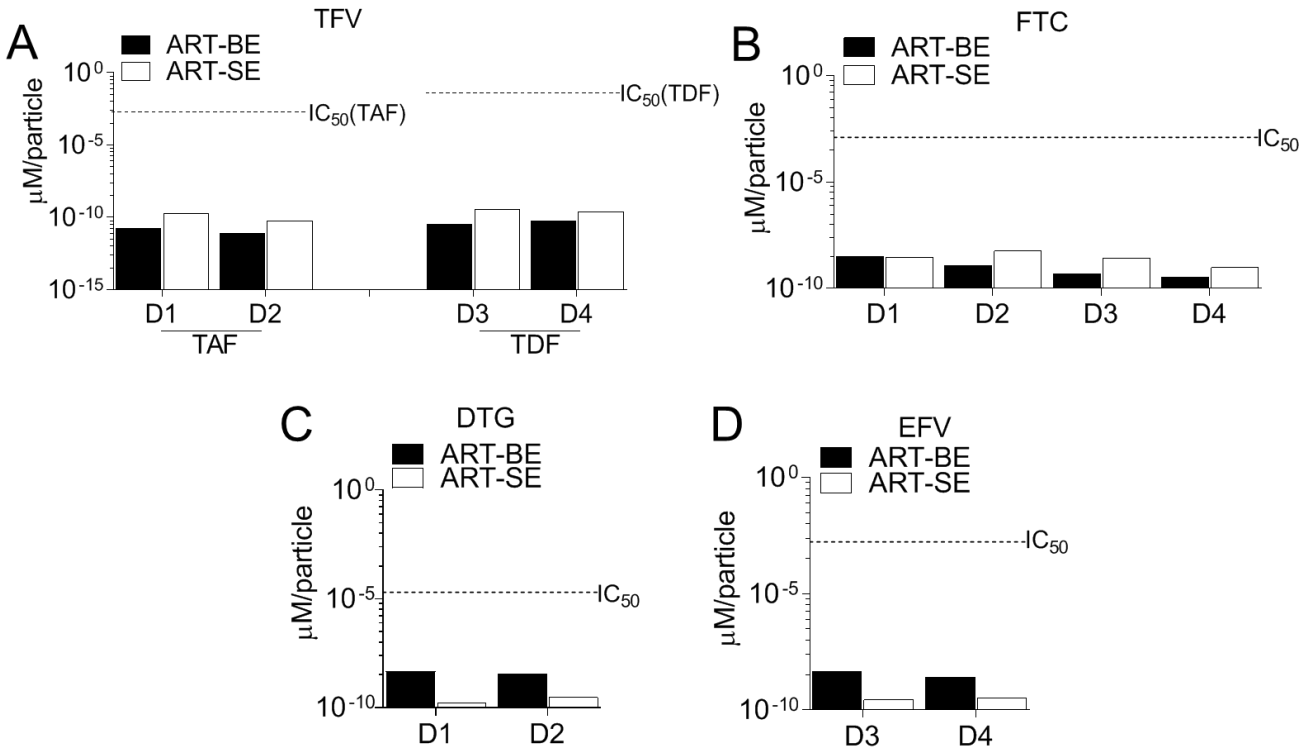
Supplemental Figure 2: Donor-derived extracellular vesicles and extracellular vesicle-free plasma do not reduce cell viability. (A-C) Vehicle PBS or 100 µg/ml BE, SE, EFBP, and EFSP isolated from HIV-negative (n=6), HIV-positive ART-naïve (n=5), and HIV-positive ART-suppressed (n=13) donors were added simultaneously with 100,000RLU of HIV-1 NL4.3 virus to TZM-bl indicator cells for 24 h. TZM-bl viability was measured by MTT assay. Vehicle treated cells viability are set as reference at 100% (broken line). (A) TZM-bl cell viability of HIV-1 treated with BE, SE, EFBP, and EFSP from HIV-negative donors. (B) TZM-bl cell viability of HIV-1 treated with BE, SE, EFBP, and EFSP from HIV-positive ART-naïve donors. (C) TZM-bl cell viability of HIV-1 treated with BE, SE, EFBP, and EFSP from HIV-positive ART-suppressed donors. Statistics was determined by comparing infectivity values from all donors to vehicle control for each treatment. Significance was determined by student's t test. Error bars are SD of biological replicates from the mean of triplicate measurements. ns= not significant.



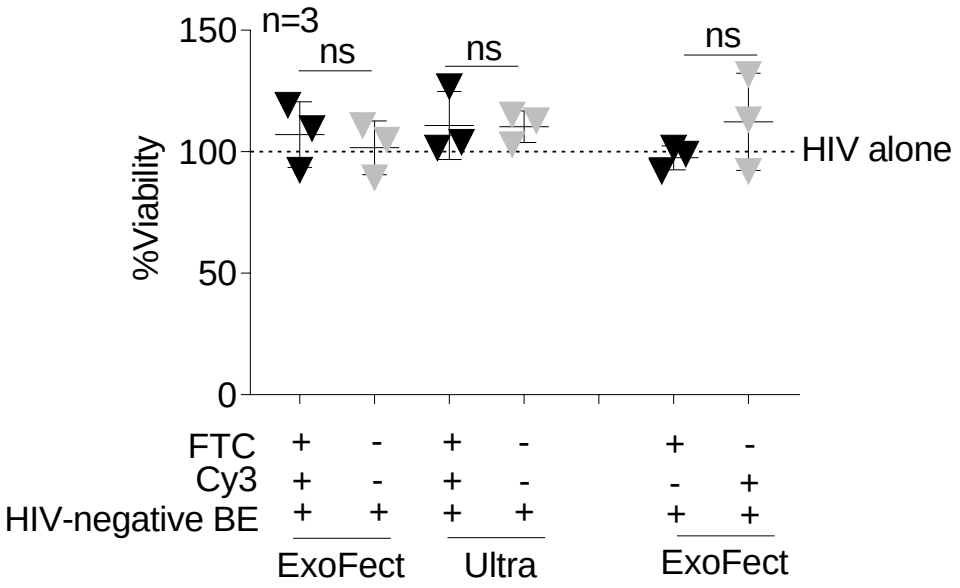
Supplemental Figure 3: Donor HIV and ART status does not affect extracellular vesicle size or concentration. BE and SE were isolated from HIV-negative (n=6), HIV-positive ART-naïve (n=5), and HIV-positive ART-suppressed (n=13) donors. (A-B) mean size (nm) of BE (A) and SE (B) grouped by donor cohort. (C-D) particle concentration per mL of plasma of BE (C) and SE (D) grouped by donor cohort. Size and concentration measurements were determined by ZetaView nanoparticle tracking analysis (NTA). Significance was determined by one-way ANOVA. Error bars are SD of biological replicates. ns= not significant.



Supplemental Figure 4: HIV-1-specific IgG does not reduce cell viability. A series of immuno-capture techniques purified IgG from HIV-infected ART-suppressed extracellular vesicle-free plasma and extracellular vesicles (n=4). Viability was determined by incubation of 100,000RLU HIV-1 NL4.3 virus with purified and depleted IgG fractions, IgG controls, or vehicle PBS on TZM-bl cells for 24 h. Viability was measured by MTT assay. Vehicle treated cells are set as reference at 100% (broken line). Statistics was determined by comparing viability values from all donors to vehicle control for each IgG fraction. Significance was determined by student's t test. *=P<0.05, **=P<0.01, ***=P<0.001. Error bars are SD of biological replicates from the mean of triplicate measurements. ns= not significant.



Supplemental Figure 5: ARV drug concentrations per extracellular vesicle particle from HIV-infected ART-suppressed donors. EVs were isolated from HIV-positive ART-suppressed donors' plasma (ART-BE) and semen (ART-SE). Drug concentrations were measured by LC-MS/MS from 4 donors with similar drug regimen. ARVs are denoted as (A) TFV (tenofovir), (B) FTC (emtricitabine), (C) DTG (dolutegravir), (D) EFV (efavirenz). Broken lines indicate FDA-half maximal inhibitory concentrations (IC_{50}) for HIV-1. Particle numbers were determined by ZetaView nanoparticle tracking analysis (NTA), and protein by Nanodrop absorbance at 280nm. Estimations of µM ARV/particle were calculated from particles/mg and µM ARV/50µg EV.



Supplemental Figure 6: BE functionalized with ART do not alter cell viability. 200 µg BE from HIV-negative donors (n=3) were loaded with 50 µg Cy3 labeled emtricitabine (FTC) by two methods (ExoFect vs. Ultra). Viability was determined by incubation of 100,000RLU HIV-1 NL4.3 virus in the presence of 100 µg/ml FTC-loaded BE, BE-loading controls, or vehicle PBS control on TZM-bl indicator cells for 24 h. Viability was measured by MTT assay. Vehicle treated cells are set as reference at 100% (broken line). Statistics was determined by comparing FTC-loaded BE to relevant controls. Significance was determined by student's t test. Error bars are SD of biological replicates from the mean of triplicate measurements. ns= not significant.