SUPPLEMENTAL MATERIAL
Figure S1. Donor characteristics in relation to CD14+ tetherin, CD14+ MVs, tissue factor+ (TF+) MVs, and CD14+TF+ microvesicles (MVs) levels.
(A-D) Sex, age, and ethnicity for all donors, and for HIV+ donors viral loads (VL), CD4+ T cell count, and type of treatment (including combinations of nucleoside reverse transcriptase inhibitors (NRTIs) with protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) or integrase strand transfer inhibitors (INSTIs)), were compared in CD14+ tetherin expression (A), circulating CD14+ MV (B), circulating TF+ MV (C), and circulating CD14+ TF+ MV(D) levels. Comparing between sexes differences for CD14+ and CD14+ TF+ MVs, the Mann-Whitney test was used; comparing differences between sexes for tetherin and TF+ MVs, unpaired t test was used. To evaluate CD14+ MVs, TF+ MVs and CD14+ TF+ MVs in correlation to age, Pearson correlation analysis was used. To evaluate tetherin in correlation to age, Spearman correlation analysis was used. For all comparisons between ethnicity and types of treatment, a one-way ANOVA test with Tukey’s multiple comparisons was performed. For tetherin expression, TF+ MVs, and CD14+ TF+ MVs comparing different viral loads, unpaired t test was used. For CD14+ MVs in comparing viral load, Mann-Whitney test was used. To evaluate all CD4 cells counts in correlation to either tetherin or MV levels, Pearson correlation analysis was used.
Figure S2. Validation of Accuri quantification of CD14+ MVs.

(A) Plasma was collected from consented donors and was spun to remove platelets and cell debris. Each sample was stained with two different antibodies, both against CD14, but with different epitopes and fluorophores. The fold change (FC) over the average was taken for each donor and correlated against the different antibodies. Spearman correlation analysis was performed to evaluate the correlation between the fold change of MVs using the FITC CD14+ antibody and the PE CD14+ antibody. (N=29). (B) Plasma from the same donors were stained CD14 with FITC and analyzed by NS300 and NTA or stained for CD14 conjugated to PE followed by quantification on the BD Accuri C6. Spearman correlation analysis was performed to evaluate the correlation between MVs quantified by the NS300 and MVs quantified by the BD Accuri C6. (N=28).
Figure S3. Integrase inhibitor effect on monocyte tissue factor (TF) expression and microvesicle (MV) release.

Primary monocytes were isolated from HIV- donors and exposed for 18-24 hours to the integrase inhibitor raltegravir (RAL, 5 μM) alone or in combination with common nucleoside reverse transcriptase inhibitors tenofovir disoproxil fumarate (TDF, 5 μM) or emtricitabine (FTC, 5 μM). Dimethyl sulfoxide (DMSO) served as a vehicle control. (A) Monocytes were then fixed and stained. TF expression was quantified by flow cytometry. (B) Media was then collected, centrifuged to remove cell debris, and analyzed for MV content using NTA. (N=3 biological replicates averaged from 1-2 technical replicates).
Figure S4. Super resolution microscopy of tetherin on the surface of monocytes.

Primary monocytes were isolated from HIV-donors and fixed in PFA. Cells were then labeled for tetherin (green) and Cell Mask Red was used to mark the cell membrane (red). White boxes inset in images on left represent areas shown enlarged on right.
Figure S5. Tetherin expression on Tat-treated monocytes.

(A) Primary monocytes were exposed to Tat (500 nM) for 4 hours (4 HR Tat). Quantification of tetherin expression on cells was measured by flow cytometry (N=3 biological replicates averaged from 2-3 technical replicates) and compared using a paired t test. (B) Primary monocytes treated Tat for 18 hours and fixed. Tetherin expression was quantified by flow cytometry and expression between concentrations was compared using a one-way ANOVA test with Dunnett’s multiple comparisons test. (N=4 biological replicates averaged from 2-3 technical replicates).
Figure S6. Size analysis of in vitro particles from Tat-treated monocytes.

(A) Primary monocytes treated with the HIV Tat (500 nM) and media was collected for particle analysis using the NS500 and NanoSight Tracking Analysis. Represented plots of particles for non-treated (NT) and Tat-treated (Tat) are displayed with the blue line delineating the 200 nm size. (B) The mean and mode of particles greater than 200 nm was calculated for each sample and compared using paired t test.
To characterize MVs released by primary monocytes in cell culture, cells were harvested from a healthy donor and incubated in media overnight. The media was then centrifuged to remove cells and stained with calcein green to observe if particles were membrane bound. Additionally, MVs were isolated by high speed centrifugation, resuspending in Annexin V binding buffer, and stained for Annexin V and CD14. All MVs were quantified using flow cytometry. Media isolated from cells contained significantly higher amounts of MVs than that observed in media alone. (N=3 technical replicates). All experiments were compared using an unpaired t test.
Primary monocytes were isolated and infected with lentiviruses to over express tetherin. The vectors used were empty vector (Vector) or wild-type tetherin (WT). Transfected monocytes were either left untreated or treated with the viral protein Tat (100nM) for 1 hour followed by fixation and preparation for scanning electron microscopy (SEM) as outlined in the method section. In left corner of image, black bars denote 1μM and red bars denote 2μM.
Aged- and sex-matched wild-type (WT) and KO over the age of 8 weeks were bled retro-orbitally. Blood was then fixed and stained for CD41 to identify platelets and CD62P to mark activation. Samples were run through flow cytometry and the percent of platelets expressing CD62P was measured and compared using unpaired t test.