Supplemental Materials and Methods

Platelet Isolation and Cryopreservation

Vacutainers were centrifuged at 250 x g for 15 minutes, then the top 75% of the platelet rich plasma (PRP) layer was transferred into a 50mL conical tube with PGI2. This step was repeated with a 10 minute centrifugation. PRP was centrifuged at 1000 x g for 10 minutes, the plasma was removed and the pellet was resuspended in Tyrode's salt solution (Millipore-Sigma), mixed with ACD (Millipore-Sigma), and PGI2, then centrifuged as in the previous step. Finally, platelets were resuspended in Tyrode's solution. An aliquot of platelets was fixed in 2% paraformaldehyde (PFA) and counted (VetScan HM5, or Scil Vet ABC Hematology™ Analyzer).

Samples were cryopreserved by aliquoting plasma into cryovials and freezing at a controlled rate using a Nalgene Mr.Frosty freezing container prior to storage in liquid nitrogen. Platelets were isolated and counted as described above. For imaging experiments platelets were fixed in 2% PFA for 20 minutes, then washed with PBS.

Transmission Electron Microscopy

Platelets were fixed in 0.1 M phosphate-buffered 2% glutaraldehyde overnight at 4°C, washed in buffer, postfixed in 0.1 M phosphate buffered 1% osmium tetroxide for 30 minutes, washed again, and trapped in 2% agarose. The agarose pellets were dehydrated through a graded series of ethanol to 100% (x3), transitioned into propylene oxide, and infiltrated with a mixture of epon/araldite epoxy resin, embedded, and polymerized for 2 days at 65°C. One-micron sections were stained with Toluidine blue for light microscopy identification of cells. An ultramicrotome and diamond knife were used to thin section the epoxy embedded cells at 70 nm, sections were then placed onto formvar/carbon coated slot grids. The grids were stained with aqueous uranyl acetate and lead citrate and photographed using a Hitachi 7650 transmission electron microscope with an attached Gatan 11-megapixel digital camera.

Reverse Transcriptase - Polymerase Chain Reaction (RT- PCR)

RNA was extracted from platelets incubated with HIV by using TRIzol™ Reagent (Invitrogen), following manufacturer's instructions. RNA was reverse transcribed using an iScript™ cDNA Synthesis kit (Bio Rad), and non-quantitative PCR was performed on the resulting cDNA using Platinum™ Taq DNA Polymerase kit (Invitrogen) following the manufacturer's instructions and probing for HIV tat or GAPDH using the following primers: HIV-1 tat forward: 5'- GGAATTCACCATGGAGCCAGTTCCT -3', HIV-1 tat reverse: 5'- CGGCATCCCTATTCCTTCGGGCCTGT -3', GAPDH forward: 5'- CCTGCACCACCAACTGCTTA -3', GAPDH reverse 5'- CCATCACGCCACAGTTTCC -3' (IDT). The resulting PCR products were resolved on a 2% agarose gel made from UltraPure™ Agarose (Invitrogen™).

For quantitative RT-PCR (qRT-PCR) analyses, RNA was extracted from platelets incubated with HIV using TRIzol™ Reagent (Invitrogen), following the manufacturer's instructions and cDNA was synthesized using an iScript™ cDNA Synthesis Kit (Bio Rad). qRT-PCR was performed using Primetime Gene Expression Master Mix and the following primers and probes (supplied by Integrated DNA Technologies) [31,32]: HIV-1 gag forward: 5'- CATGTTTTCAGCATTATCAGAAGGA -3', HIV-1 gag reverse: 5'- TGCTTGATGTCCCCCCACT -3', HIV-1 gag Probe: 5'- /56-FAM/CCACCCCAC/ZEN/AAGATTTAAACACCATGCTAA /3IABkFQ/ -3'. Samples were run in triplicate on 96-well low-profile PCR plates (Bio Rad) using a CFX-Connect Real-Time PCR Detection System (Bio-Rad). For each experiment, RNA was extracted from the same amount of viral stock used to inoculate the platelets. Each condition was normalized to the input and is represented as fold change.

T Cell Isolation and Culture

Whole blood was layered over Histopaque® (Millipore Sigma) and centrifuged at 2000 rpm for 25 minutes. The buffy coat layer, was collected and suspended in 5mL RPMI 1640

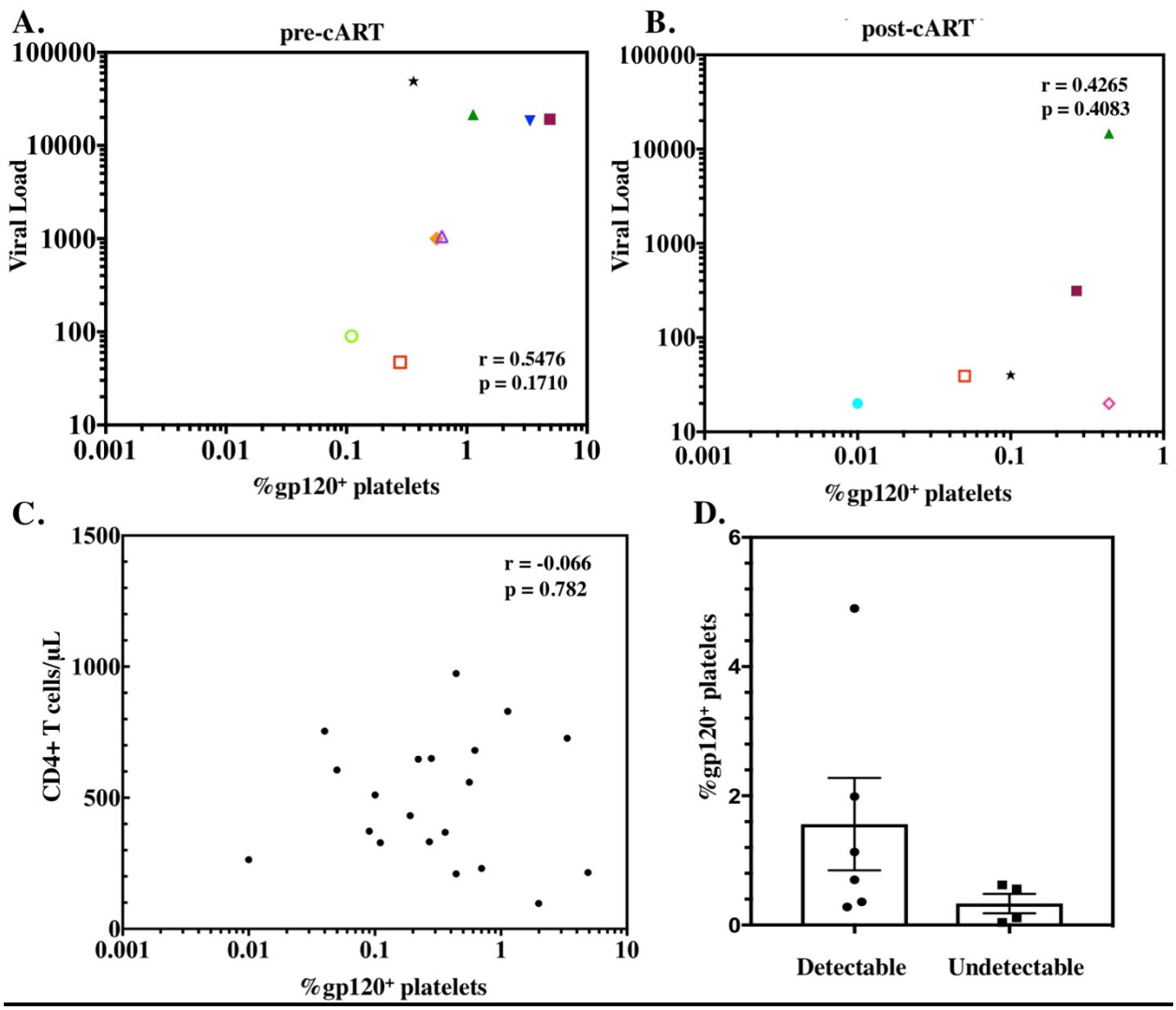
containing 2% FBS. CD4+ T cells were isolated using MACS CD4+ Microbeads (Miltenyi Biotec) as per the manufacturer's instructions. Isolated CD4+ T cells were stimulated with IL-2 (Gibco™) and CD3/28 Dynabeads™ (Gibco™) according to manufacturer's instructions. Cells were cultured in RPMI 1640 supplemented with 10% FBS at a concentration of 1 x 10^6 cells/mL. Cells were split to 1.5 x 10^6 cells/mL, and the Dynabeads were removed after 4 days.

Flow Cytometry

Platelets were fixed in 2% PFA and stained with antibodies against CD61-AF647 and CD62P-FITC. 1 x 10^7 cells were acquired on an Accuri C6 flow cytometer and were analyzed for CD61+ CD62P mean fluorescence intensity. CLEC-2 (Novus Biologicals) and DC-SIGN (BD Biosciences) antibodies were used in conjunction with CD61-AF647, and analyzed for CD61+ CLEC2/DC-SIGN mean fluorescent intensity.

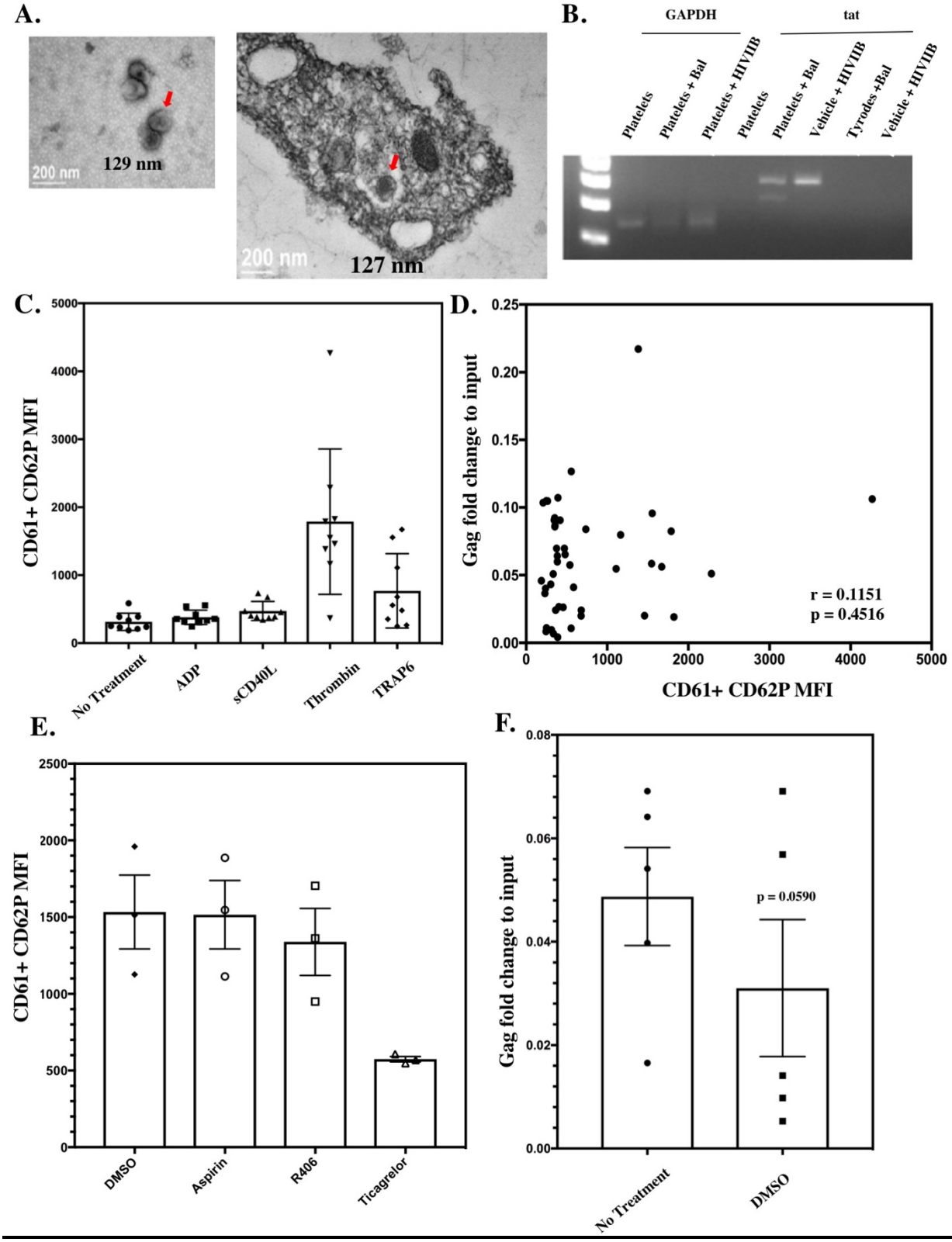
CD4+ T cells were fixed as described above then stained for CD4-FITC and CD61-AF647. All antibodies were obtained from Biolegend unless otherwise stated.

Supplemental Figures



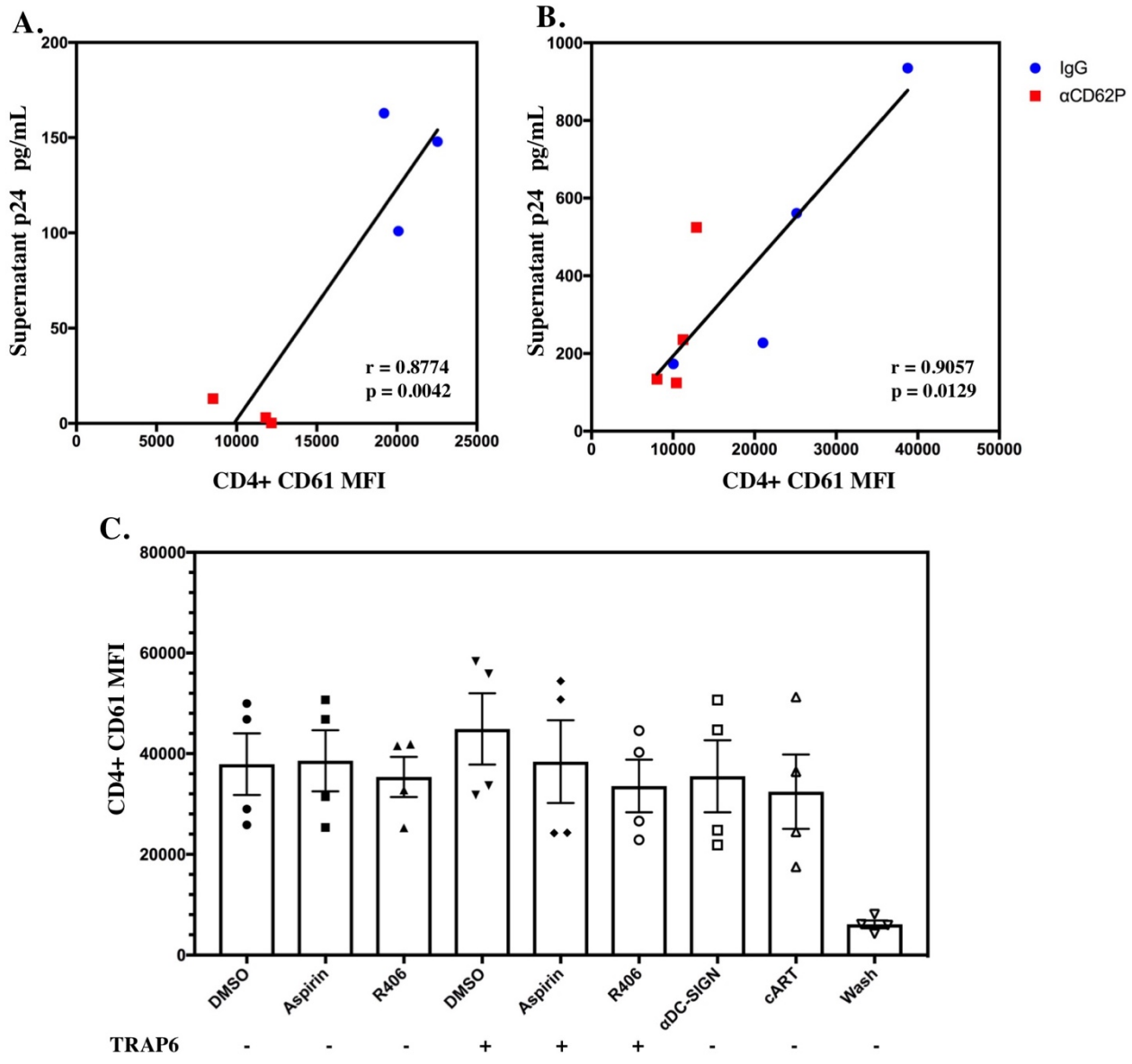
Supplemental Figure 1

(A) Correlation between viral load %gp120+ platelets in cART naïve & 3 months post cART samples. (D) Correlation between CD4+ T cell count and %gp120+ platelets was examined in all matched samples from persons infected with HIV. Correlation was calculated by nonparametric Spearman correlation. (E) %gp120+ platelets in cART naïve patient samples from individuals who had a detectable viral loads and those who had undetectable viral loads after 3 months of cART.


A.
200 nm
129 nm
200 nm
127 nm
B.
GAPDH
tat
Platelets
Platelets + Bal
Platelets + HIVIIB
Platelets
Platelets + Bal
Vehicle + HIVIIB
Tyrodes +Bal
Vehicle + HIVIIB
C.
CD61+ CD62P MFI
5000
4000
3000
2000
1000
0
No Treatment
ADP
sCD40L
Thrombin
TRAP6
D.
Gag fold change to input
0.25
0.20
0.15
0.10
0.05
0.00
0
1000
2000
3000
4000
5000
CD61+ CD62P MFI
r = 0.1151
p = 0.4516
E.
CD61+ CD62P MFI
2500
2000
1500
1000
500
0
DMSO
Aspirin
R406
Ticagrelor
F.
Gag fold change to input
0.08
0.06
0.04
0.02
0.00
p = 0.0590
No Treatment
DMSO

Supplemental Figure 2

(A) Transmission electron microscopy was performed on HIV-1 Bal (left), and platelets from HIV-negative donors incubated with 2.25 ng of HIV-1 Bal for 1 hour prior to washing and fixation (right). (B) RT-PCR for GAPDH mRNA and HIV Tat genomic RNA was performed on platelets incubated with HIV-1 Bal (R-5 tropic) or HIV-1 IIIB (X4-tropic) viruses. Virus in Tyrode's solution without platelets was used as a negative control. (C) Platelet activation was measured by mean fluorescent intensity of CD62P on CD61+ cells following exposure to indicated agonists. (D) Viral RNA levels (the amount of gag genomic RNA expressed as a ratio of the amount of input genomic RNA from the viral stock) were correlated with platelet activation levels (measured as in panel C) using a nonparametric Spearman correlation. (E) Platelets were pre-treated with 0.5% DMSO prior to incubation with HIV-1 Bal, the amount of gag genomic RNA associated with platelets was measured, and normalized to the amount of input viral stock. (F) Platelet activation was measured as described above, following exposure to platelet activation inhibitors and subsequent treatment with TRAP6.



Supplemental Figure 3

(A) HIV-1 p24 antigen levels were measured in cell-free supernatants from cocultures of differentiated CD4+ T cells and platelets from viremic cART-naïve donors, at 72 hours following treatment with IgG or αCD62P. Data were correlated with levels of platelet-T cell complex formation as measured by the mean fluorescent intensity of CD61 in CD4+ cells (as outlined in Figure 3A&B). (B) Correlation between HIV-1 p24 antigen levels in cell free

supernatants from platelet-T cell cocultures (established after *in vitro* incubation of platelets with HIV-1). (C) Platelets from HIV-negative donors were pre-treated with Aspirin, R406, αDC-SIGN antibodies or DMSO control prior to treatment with TRAP6 or vehicle control and subsequent incubation with HIV-1 IIIB prior to coculture with differentiated CD+ T-cells. Platelet–T cell complex formation was analyzed by flow cytometry as described previously.