**SUPPLEMENTAL MATERIAL**

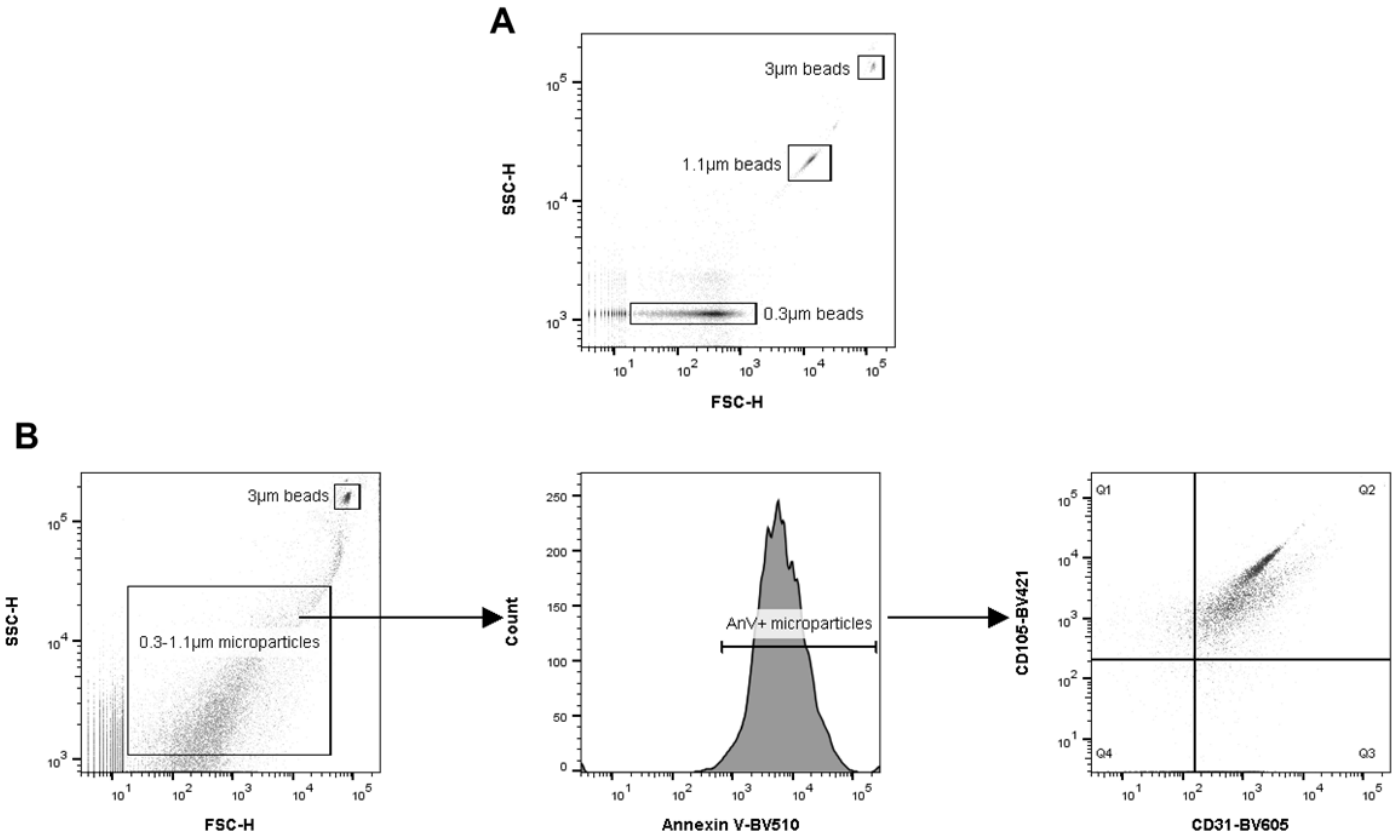
## Supplemental Methods

*Flow cytometry antibodies*

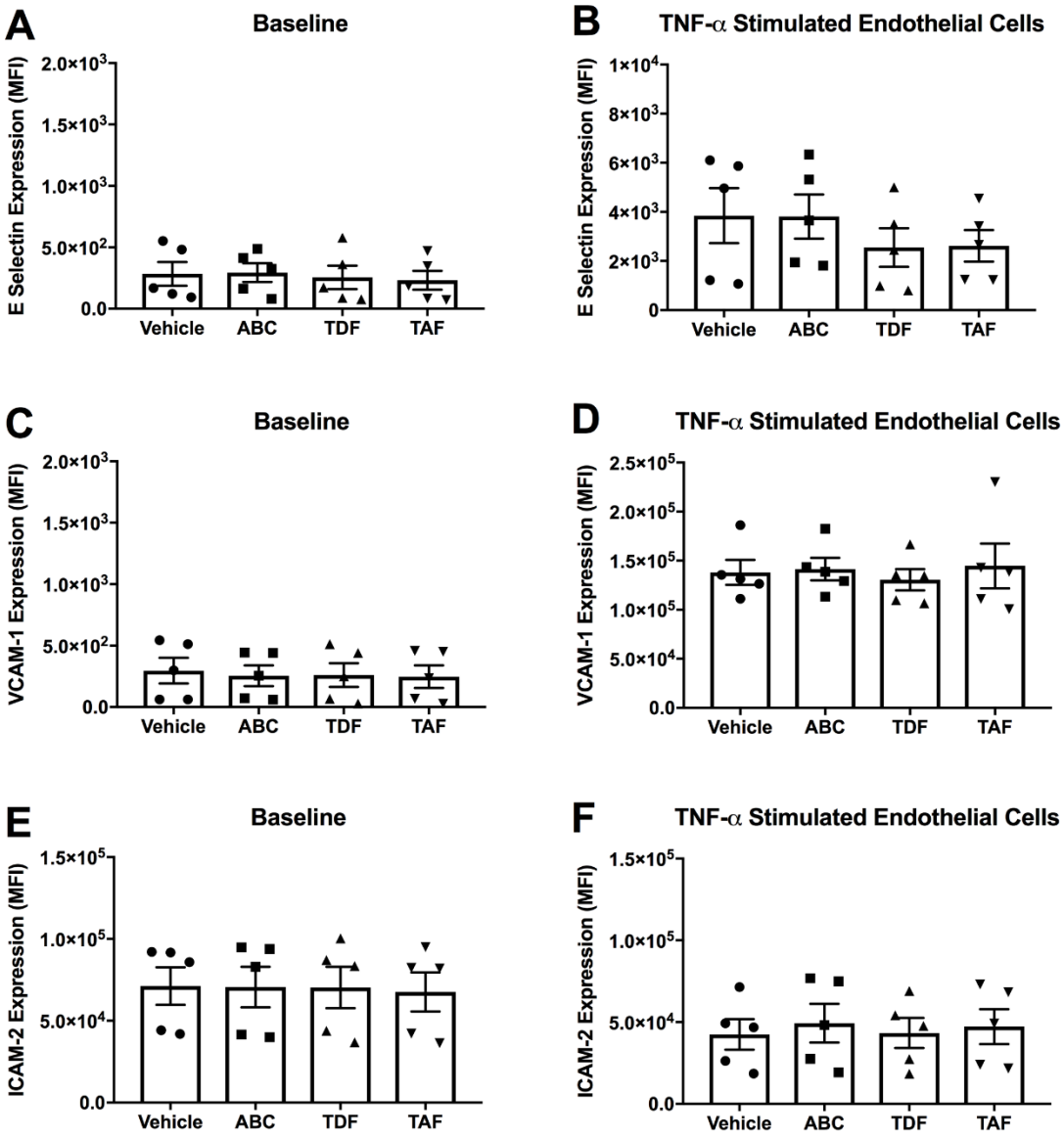
For endothelial cell and EMP analysis, the following antibodies were used: BV510-conjugated anti-AnV (#640937); BV605-conjugated anti-human CD31 (WM59, #303122); BV421-conjugated anti-human CD105 (SN6h, #800510); APC-conjugated anti-human CD62E (HAE-1f, #336012); PE/Cy7-conjugated anti-human CD54 (HA58, #353116); FITC-conjugated anti-human CD102 (CBR-IC2/2, 328507), PE-conjugated anti-human CD106 (STA, #305806); PE-conjugated anti-human CD142 (NY2, #365206); APC-conjugated anti-human CD39 (A1, #328210); PE-conjugated anti-human CD73 (AD2, #344004). The following isotype controls were used: APC-conjugated mouse IgG1,κ (MOPC-21, #400122); BV421-conjugated mouse IgG1,κ (MOPC-21, #400158); BV605-conjugated mouse IgG1,κ (MOPC-21, #400162); FITC-conjugated mouse IgG2a,κ (MOPC-173, #400210), PE-conjugated mouse IgG1,κ (MOPC-21, #400112); PE/Cy7-conjugated mouse IgG1,κ (MOPC-21, #400126). All endothelial and EMP flow cytometry antibodies were purchased from BioLegend UK Ltd.

Real-time platelet activation studies were conducted using: FITC-conjugated anti-human PAC-1 (PAC-1, #340507); APC-conjugated anti-human CD62P (Ak-4, #550888); PE-conjugated anti-human CD63 (H5C6, #556020). All platelet activation antibodies were purchased from BD Biosciences.

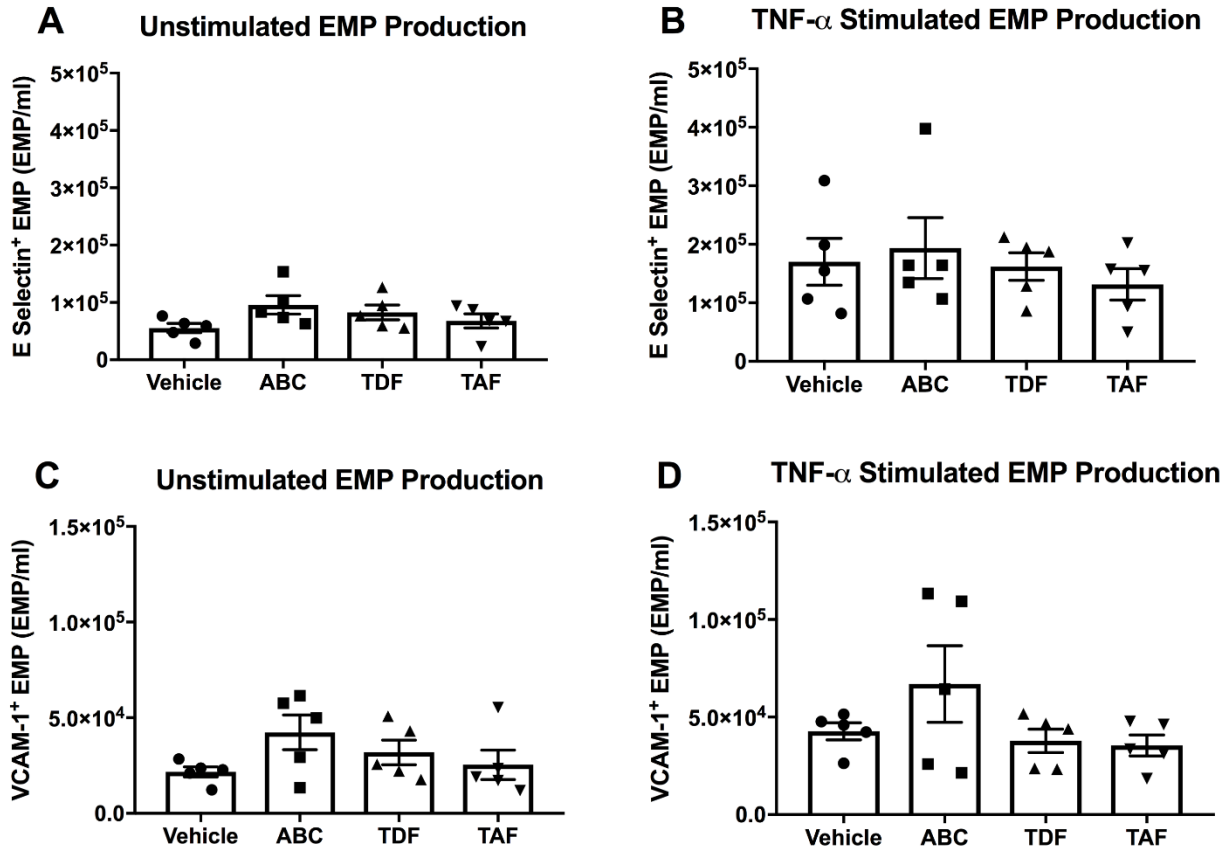
**Supplemental Figures and Figure Legends**




**Online Figure I. Endothelial microparticle (EMP) gating strategy overview. A,** An initial gate was made using 0.3µm and 1.1µm latex beads to ensure only particles of the right size were considered. 3µm latex beads were also added to allow for EMP enumeration. **B,** Particles between 0.3-1.1µm were then assessed for Annexin V (AnV) binding. $AnV^+$ particles were then assessed for CD31 and CD105 positivity, with particles expression both CD31 and CD105 being considered as EMP. Further characterisation examined these $AnV^+CD31^+CD105^+$ EMP for the presence of additional cell markers.

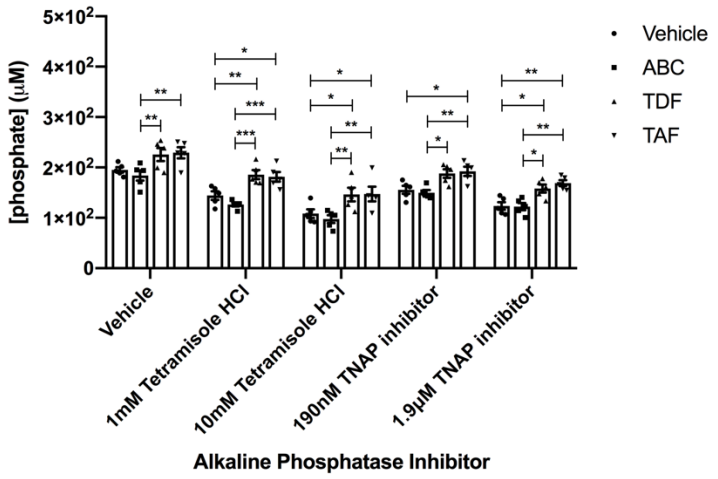



**Online Figure II. Antiretroviral treatment does not affect E-selectin, vascular cell adhesion molecule (VCAM)-1 or intercellular adhesion molecule (ICAM)-2 expression in cultured human umbilical cord vein endothelial cells (HUVEC). A,** Baseline E-selectin expression was not affected by abacavir sulphate (ABC), tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) treatment. **B,** E-selectin expression was not modulated by ABC, TDF or TAF following TNF-α stimulation (10ng/ml, 4h). **C,** Basal VCAM-1 expression was unaffected by antiretroviral drug treatment. **D,** ABC, TDF or TAF treatment did not alter VCAM-1 expression in response to TNF-α stimulation (10ng/ml, 6h). **E,** Resting ICAM-2 expression was comparable between antiretroviral-treated HUVEC. **F,** ICAM-2 expression following TNF-α stimulation (10ng/ml 24h) was similar between vehicle-, ABC-, TDF- and TAF-treated HUVEC. Data presented as mean ±SEM from 5 independent experiments. One-way ANOVA with a Tukey's multiple comparison test was used for analysis.




**Online Figure III. Abacavir sulphate (ABC), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) do not affected E-selectin$^+$ or VCAM-1$^+$ endothelial microparticle (EMP) production.** **A,** Antiretroviral treatment did not affect E-selectin$^+$ EMP production by unstimulated human umbilical cord vein endothelial cells (HUVEC). **B,** There were no differences in the number of E-selectin$^+$ EMP generated by HUVEC following TNF-α stimulation (10ng/ml, 4h). **C,** VCAM-1$^+$ EMP generation was not affected in unstimulated HUVEC by ABC, TDF or TAF. **D,** Vehicle-, ABC-, TDF- and TAF-treated HUVEC produced comparable numbers of VCAM-1$^+$ EMP following TNF-α stimulation (10ng/ml, 4h). Data presented as mean ±SEM from 5 independent experiments. One-way ANOVA with a Tukey's multiple comparison test was used for analysis.

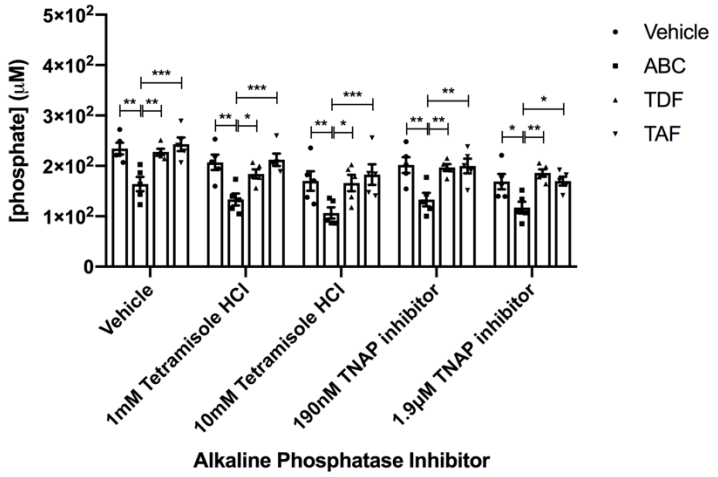
A




B

| Comparison | p value | | | | |
|---|---|---|---|---|---|
| | Vehicle | 1mM Tetramisole HCl | 10mM Tetramisole HCl | 190nM TNAP inhibitor | 1.9μM TNAP inhibitor |
| Vehicle vs ABC | ns (0.8173) | ns (0.5179) | ns (0.8326) | ns (0.9684) | ns (0.9997) |
| Vehicle vs TDF | ns (0.0956) | 0.0095 | 0.0211 | ns (0.0650) | 0.0444 |
| Vehicle vs TAF | ns (0.0523) | 0.0265 | 0.0194 | 0.0299 | 0.0043 |
| ABC vs TDF | 0.0097 | <0.0001 | 0.0016 | 0.0200 | 0.0347 |
| ABC vs TAF | 0.0044 | 0.0003 | 0.0014 | 0.0082 | 0.0032 |
| TDF vs TAF | ns (0.9940) | ns (0.9834) | ns (>0.9999) | ns (0.9898) | ns (0.8447) |

C

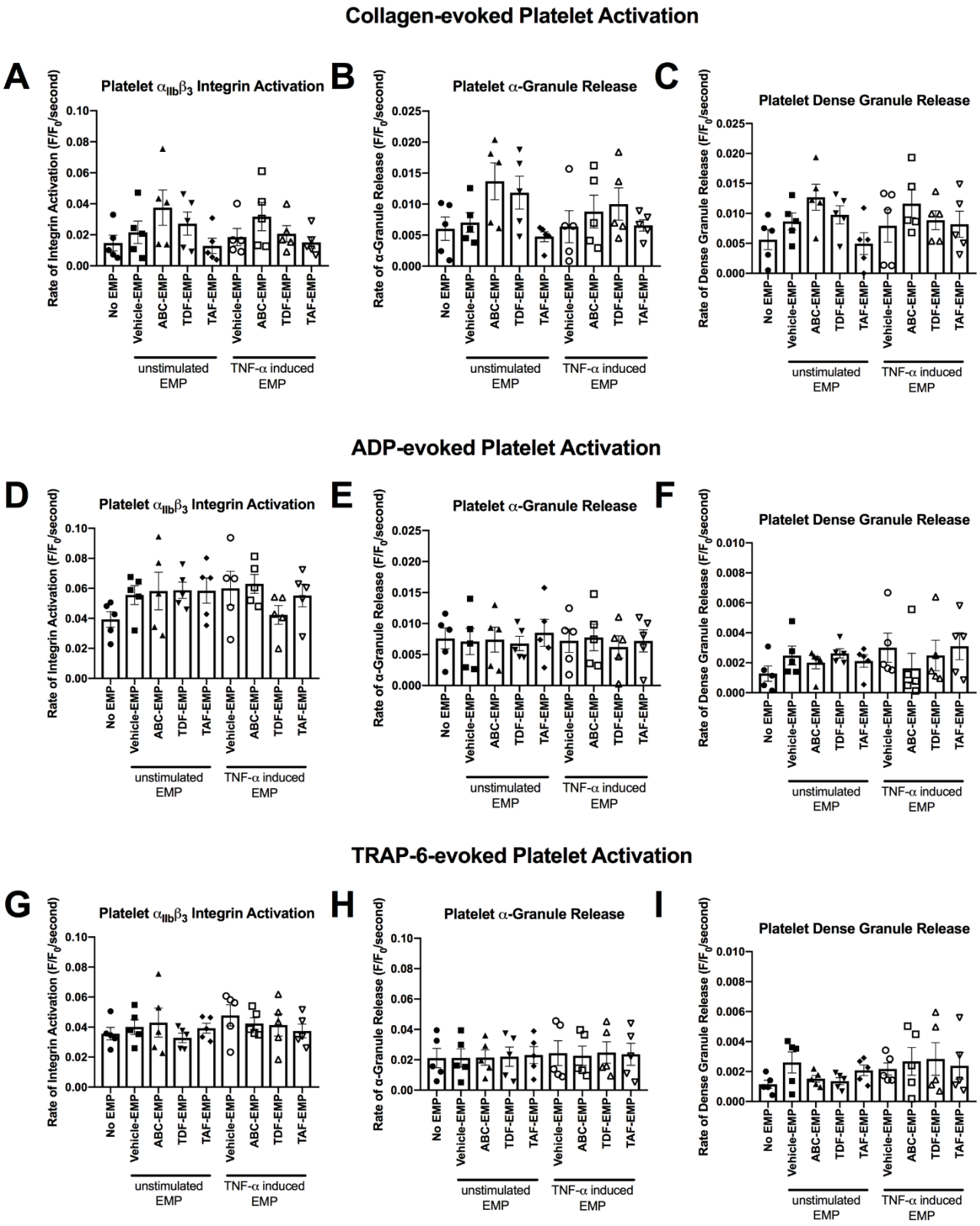



D

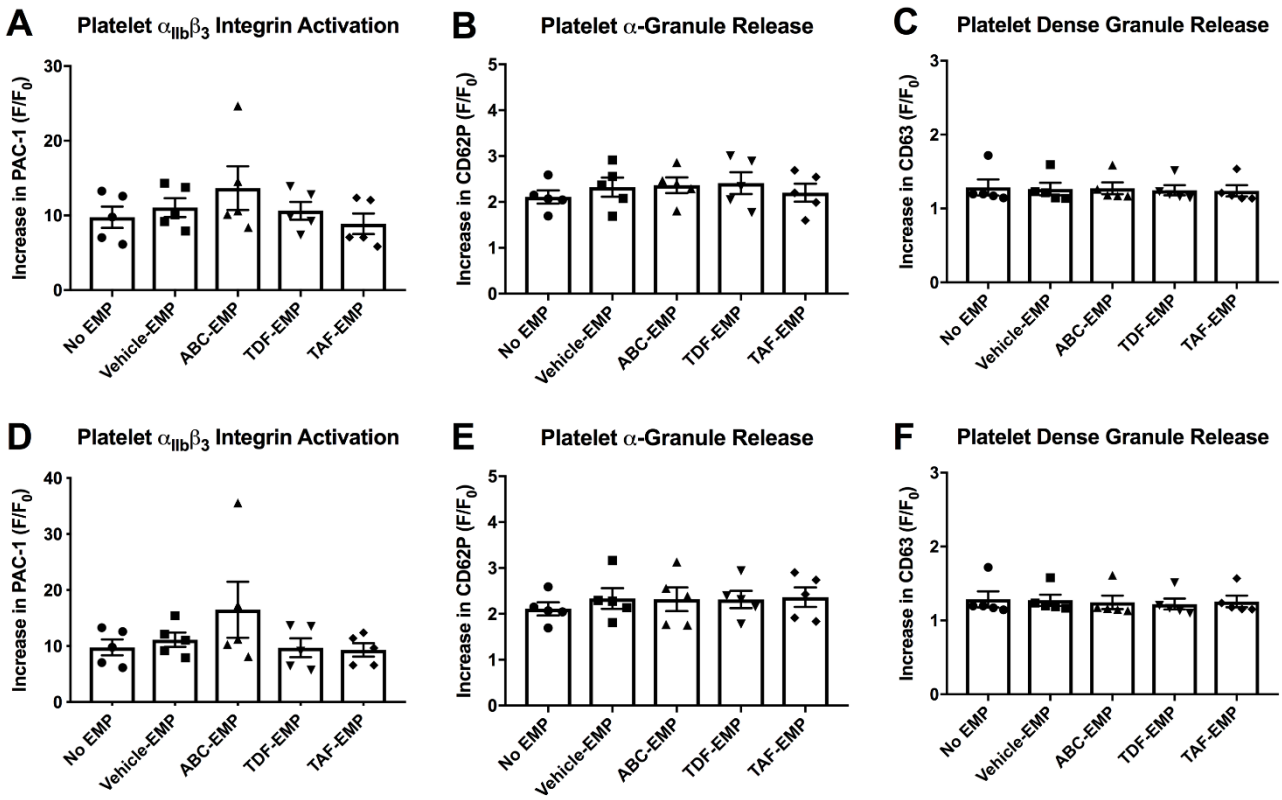
| Comparison | p value | | | | |
|---|---|---|---|---|---|
| | Vehicle | 1mM Tetramisole HCl | 10mM Tetramisole HCl | 190nM TNAP inhibitor | 1.9μM TNAP inhibitor |
| Vehicle vs ABC | 0.0018 | 0.0010 | 0.0060 | 0.0025 | 0.0373 |
| Vehicle vs TDF | ns (0.9840) | ns (0.6180) | ns (0.9962) | ns (0.9932) | ns (0.7999) |
| Vehicle vs TAF | ns (0.9678) | ns (0.9923) | ns (0.9101) | ns (0.9996) | ns (>0.9999) |
| ABC vs TDF | 0.0058 | 0.0115 | 0.0115 | 0.0059 | 0.0026 |
| ABC vs TAF | 0.0004 | 0.0007 | 0.0007 | 0.0035 | 0.0328 |
| TDF vs TAF | ns (0.8462) | ns (0.8131) | ns (0.8131) | ns (0.9983) | ns (0.8254) |

**Online Figure IV. Alkaline phosphatase inhibition does not mitigate antiretroviral-induced changes in free phosphate generation.** Human umbilical cord vein endothelial cells (HUVEC) or human coronary artery endothelial cells (HCAEC) were treated with abacavir sulphate (ABC), tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF). Prior to experimentation, cells were spiked with either IC50 or 10x IC50 concentrations of tetramisole hydrochloride (1mM or 10mM) or tissue-nonspecific alkaline phosphatase (TNAP) inhibitor (190nM or 1.9μM) for 30min. Phosphate generation experiments were conducted in the continued presence of inhibitors. **A,** In the absence of any inhibitors, TDF- and TAF-treated HUVEC had greater phosphate concentrations compared to ABC. This difference was consistent in the presence of both inhibitors at either concentration. **B,** Table showing p values from analysis of HUVEC ectonucleotidase activity in the absence or presence of alkaline phosphatase inhibitors. **C,** ABC-treated HCAEC displayed reduced ectonucleotidase activity compared to vehicle-, TDF- and TAF-treated HCAEC. The presence of either inhibitor reduced phosphate concentrations, however the ABC-induced effect remained under all conditions. **D,** Table showing p values from analysis of HUVEC ectonucleotidase activity in the absence or presence of alkaline

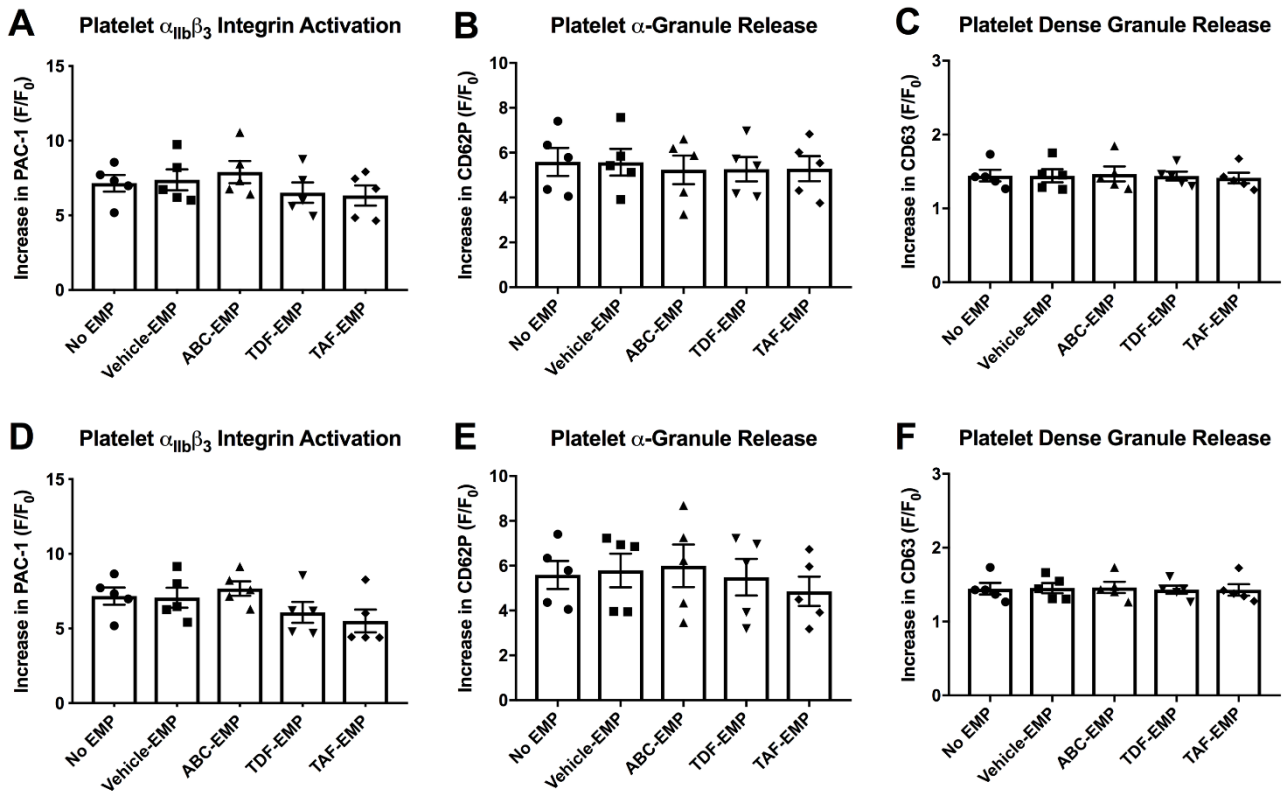
phosphatase inhibitors. Data presented as mean ±SEM from 5 independent experiments. Two-way ANOVA with a Tukey's multiple comparison test was used for analysis. *$p<0.05$, **$p<0.01$, ***$p<0.001$.


Collagen-evoked Platelet Activation
A
Platelet $\alpha_{IIb}\beta_3$ Integrin Activation
Rate of Integrin Activation (F/F$_0$/second)
B
Platelet α-Granule Release
Rate of α-Granule Release (F/F$_0$/second)
C
Platelet Dense Granule Release
Rate of Dense Granule Release (F/F$_0$/second)
No EMP
Vehicle-EMP
ABC-EMP
TDF-EMP
TAF-EMP
unstimulated EMP
TNF-α induced EMP
ADP-evoked Platelet Activation
D
E
F
TRAP-6-evoked Platelet Activation
G
H
I

**Online Figure V. Endothelial microparticles (EMP) do not affect the rate of platelet activation.** EMP generated from human umbilical cord vein endothelial cells (HUVEC) in the absence or presence of TNF-α (10ng/ml, 4h) were pre-incubated with platelets (50,000 EMP/ml) for 30min. The slope of the linear range of platelet activation traces were calculated to determine the rate of platelet activation. Following collagen stimulation (10μg/ml), we did not see any differences between EMP sources on the rate of **A,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **B,** α-granule release or **C,** dense granule release. In response to ADP stimulation (10μM), we did not observe any differences between EMP sources on **D,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **E,** α-granule release or **F,** dense granule release. Finally, following thrombin receptor activator peptide 6 (TRAP-6) stimulation (10μM), no differences were seen on **G,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **H,** α-granule release or **I,** dense granule release. Data presented as mean ±SEM from 5 independent experiments. One-way ANOVA with a Tukey's multiple comparison test was used for analysis.




**Online Figure VI. Endothelial microparticles (EMP) do not affect ADP-evoked platelet activation.** EMP generated from human umbilical cord vein endothelial cells (HUVEC) in the absence of additional stimulus were pre-incubated with platelets (50,000 EMP/ml) for 30min and then stimulated with 10μM ADP. There was no effect of any EMP source upon **A,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **B,** α-granule release or **C,** dense granule release. Next, EMP generated from TNF-α stimulated (10ng/ml, 4h) HUVEC were tested. Again, no affect was seen on **D,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **E,** α-granule release or **F,** dense granule release. Data presented as mean ±SEM from 5 independent experiments. One-way ANOVA with a Tukey's multiple comparison test was used for analysis.




**Online Figure VII. Thrombin receptor activator peptide 6 (TRAP-6)-evoked platelet activation is not affected by endothelial microparticles (EMP).** EMP generated from human umbilical cord vein endothelial cells (HUVEC) in the absence of additional stimulus were pre-incubated with platelets (50,000 EMP/ml) for 30min and then stimulated with 10μM TRAP-6. There was no effect of any EMP source upon **A,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **B,** α-granule release or **C,** dense granule release. Next, EMP generated from TNF-α stimulated (10ng/ml, 4h) HUVEC were tested. Again, no affect was seen on **D,** platelet $\alpha_{IIb}\beta_3$ integrin activation, **E,** α-granule release or **F,** dense granule release. Data presented as mean ±SEM from 5 independent experiments. One-way ANOVA with a Tukey's multiple comparison test was used for analysis.