Supplemental Appendix

Supplemental Methods

Quantitative real-time PCR (qPCR)

Total RNA was isolated using the Direct-zol™ RNA MiniPrep. One µg of total RNA was reverse-transcribed with iScript™ Reverse Transcription Supermix (Bio-Rad Laboratories), according to manufacturer' protocol. Up to one 100 ng of cDNA was used for the reaction mixture and amplification of the genes of interest was performed using IDT primers (IDT) and SsoFast EvaGreen Supermix (Bio-Rad) on an iCycler Real-Time Detection System (Eppendorf). Quantitative normalization of complementary DNA in each sample was performed using RNA18S5 as an internal control. The human primer sequences are reported in Suppl Table 1. Polymerase chain reaction assays were conducted in duplicate wells for each sample. Baseline values of amplification plots were set automatically and threshold values were kept constant to obtain normalized cycle times and linear regression data. Relative quantification was performed using delta Ct method. The following human primer sequences were used for *IL8*: 5'-TTT CTG CAG CTC TGT GTG AA -3'and 5'-CTC AGC CCT CTT CAA AAA CTT -3; *ICAM-1*: 5'-GAGCTTCGTGTCCTGTATGG -3 and 5'- CAGTCACTGATTCCCCGATG -3; *MCP-1*: 5'-ACT CAC CTC TTC AGA ACG AAT TG -3' and 5'-GCA GAT TCT TGG GTT GTG GA -3; *IL-6*: 5'-CCC AAA GAA GCT GTG ATC TTC A -3' and 5'-CCA TCT TTG GAA GGT TCA GGT TG -3; *ABCC4:* 5'-GCATGACTTGGACACGGTAA-3' and 5'-GTC TCA TCC CGT TAG CAA GAG-3; *RNA18S5*: 5'-GGA CAT CTA AGG GCA TCA CAG-3' and 5'-GAG ACT CTG GCA TGC TAA CTAG-3.'

Western blot

ABCC4 protein was separated by SDS 7.5% PAGE and detected with the following anti-ABCC4 antibody (1:500, overnight 4°C, Abcam). Vasodilator-stimulated phosphoprotein (VASP) and Phospho-VASP (ser 157) loaded onto a SDS 12% PAGE and visualized with the following primary antibodies (1:1000, overnight 4 °C, Cell Signaling).

For ABCC4 protein quantification, 30 μg of protein were separated by SDS 7.5% PAGE, transferred onto a nitrocellulose membrane, and then blocked in 5% non-fat milk/Tris-buffered saline–0.1% Tween-20 (TBS-Tween-20) for 1 hr at RT. The blots were incubated with primary antibodies diluted in TBS-Tween-20: anti-ABCC4 (1:500, overnight 4°C, Abcam) and anti-GAPDH (1:500, 1 h RT, Santa Cruz Biotechnology). The membranes were washed in TBS–Tween-20 and incubated for 1 hr at RT with secondary antibodies: anti-mouse IgG (1:2000, Santa Cruz Biotechnology) for ABCC4 or anti-rabbit IgG (1:5000, Santa Cruz Biotechnology) for GAPDH. Immunoreactive proteins were visualized by an ECL detection system (Thermoscientific). Optical density was measured by ImageJ software (National Institutes of Health).

Forty ug of protein were loaded onto a SDS 12% PAGE for VASP and Phospho-VASP (ser 157) expression (1:1000, overnight 4 °C, Cell Signaling).

Flow Cytometry

To asses P-selectin and PAC-1 expression, citrated whole blood was incubated with CD42b-APC (platelets) and CD62P-FITC (P-selectin) or PAC-1-FITC for 30 min in the dark at room temperature (RT). P-selectin and PAC-1 expression was recorded as mean florescence intensity

(MFI) of 10,000 CD42b+ events. All antibodies were purchased from BD Biosciences and flow cytometry was performed on an Accuri C6 flow cytometer (BD Biosciences).

c-AMP secretion

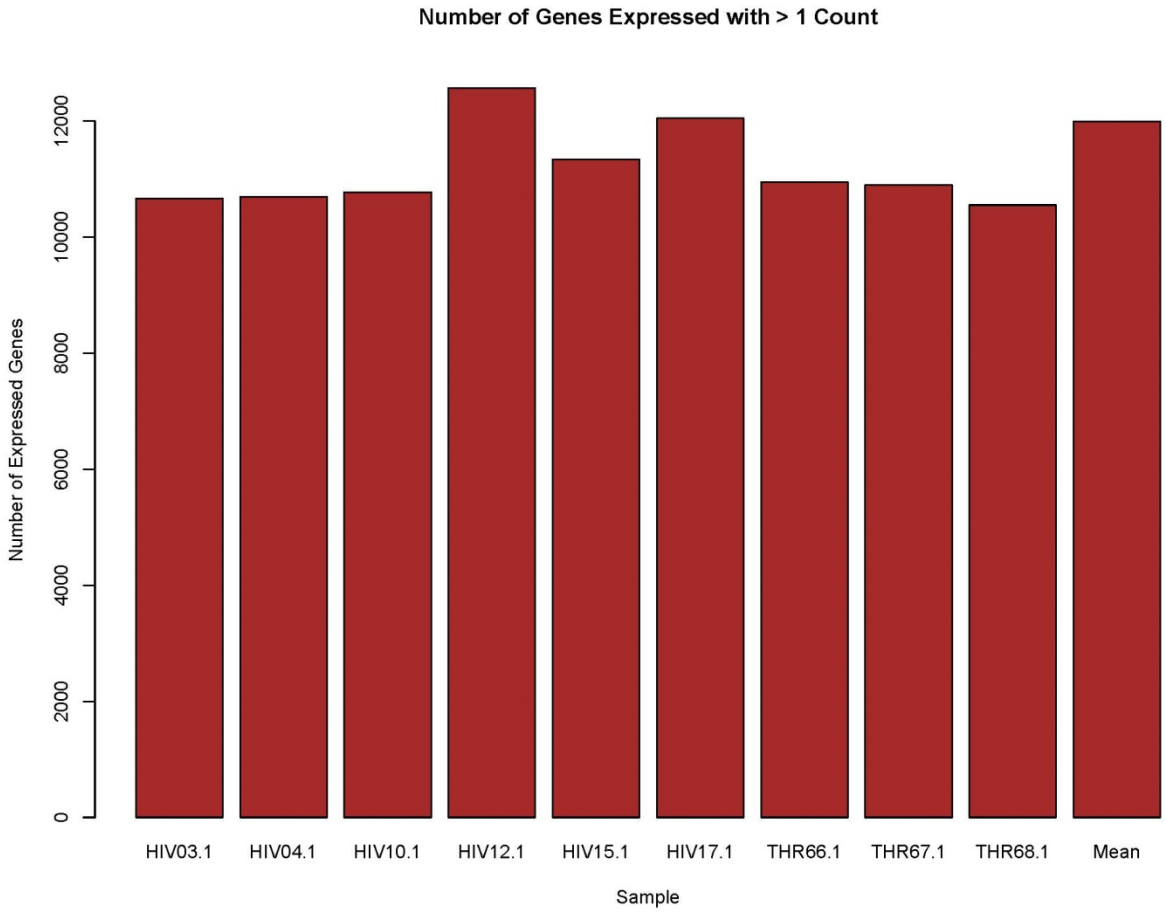
Levels of c-AMP were assessed with cAMP Parameter Assay Kit (R&D Systems) on platelet supernatants after activation with thrombin (0.05U/mL).

Supplemental Table 1. Current drug regimen of the HIV cohort

Characteristics	HIV (N=55)
Drugs currently taking	
NRTI (#yes)	53
NNRTI (#yes)	18
PI (#yes)	29
II (#yes)	17
Aspirin (# yes)	0
NSAID (# yes)	0
Statin (# yes)	9
ART regimen	
Abacavir (#yes)	13
Zidovudine (#yes)	3
Lamivudine (#yes)	15
Lopinavir (#yes)	1
Ritonavir (#yes)	27
Tenofovir (#yes)	46
Emtricitabine (#yes)	37
Efavirenz (#yes)	8
Rilpivirine (#yes)	5
Elvitegravir (#yes)	5
Cobicistat (#yes)	5
Dolutegravir (#yes)	1
Darunavir (#yes)	9
Raltegravir (#yes)	11
Atazanavir (#yes)	12
Etravirine (#yes)	3
Amprenavir (#yes)	2
Nevirapine (#yes)	2
Indinavir (#yes)	1
Saquinavir (#yes)	1

Supplemental Figure 1.

Depicts the number of reads aligned (per sample) against the hg19 utilizing the STAR/2.5.1 aligner.



Supplemental Table S2. Differentially expressed transcripts in HIV vs control cohort; p-value<0.01.

Row.names	log2FoldChange	Fold change	p-value
ABCC4	1.794547659	3.469066891	1.96E-11
CPXM2	-1.949669118	0.258875597	1.71E-06
NOMO1	-1.547326877	0.342143424	4.25E-06
METTL7A	1.285226603	2.437203307	1.20E-05
ST3GAL1	1.061979922	2.087794799	3.80E-05
FLJ90757	1.200847954	2.298747418	0.000403121
LOC100128252	-1.213080106	0.431346721	0.000415064
MAP2	1.272899052	2.416466597	0.000458628
NID1	1.215128114	2.321613983	0.000650823
IER3	1.423988998	2.683263988	0.000660924
MFN2	1.185287207	2.274086619	0.0007077
MESTIT1	1.372302548	2.588834157	0.00085954
ZNF347	-1.154313896	0.4492798	0.000876436
APBB1IP	-1.330288949	0.397688583	0.00109127
LOC729178	-0.93375458	0.523494186	0.001114143
IRX3	1.317415652	2.492192748	0.00112386
ZNF83	-1.191257256	0.437921062	0.001256982
GPX7	-1.296410632	0.407137882	0.001848307
SC5DL	-1.067651529	0.477095	0.002425478
RNPEPL1	1.152635646	2.223196774	0.002685136
UBE2C	1.00890557	2.012383925	0.003114383
DYNLL1	-0.805918863	0.571997655	0.003261116
CTSS	-1.033911872	0.488384097	0.003306439
DOCK2	-1.171967938	0.443815532	0.003371093
FBXO40	1.081043546	2.115565783	0.003551921
SF1	0.92274358	1.895716962	0.003717472
ABTB1	0.720367255	1.647601397	0.003865617
MAP1B	-1.059936877	0.479653046	0.003947135
GPR1	-1.087891442	0.470448452	0.003990886
ZNF37A	1.058214104	2.082352205	0.004197095
SIRPB1	1.198930107	2.295693607	0.004246913
HIST1H4C	0.849145482	1.801433608	0.004406643
LOC283089	-0.751580167	0.593952652	0.004685624
WLS	-1.084073007	0.471695255	0.004855257
BLM	1.165667645	2.243370094	0.004972031
DCTN5	0.941742466	1.920846809	0.005209501

TMEM8A	1.042065264	2.059173317	0.005356393
PRDM7	-1.134715384	0.455424754	0.005500989
KLRC1	-0.967822655	0.511277111	0.005717164
SNX29	0.8597296	1.814698156	0.005897047
ROGDI	1.158117808	2.231660871	0.005912227
CD164	-0.862977013	0.549816835	0.005936619
BTBD6	0.942408966	1.921734412	0.006135678
STAT1	1.056386747	2.079716311	0.006155263
TIMP3	1.146119626	2.213178211	0.006382295
SNHG3	-0.835412715	0.560422695	0.006474205
CCDC103	-0.937245998	0.522228827	0.006703492
TARS	1.04095083	2.057583288	0.006733018
HBB	-0.874447692	0.545462646	0.006764142
CARHSP1	1.025315144	2.035403951	0.006808728
PKP2	1.017564534	2.024498444	0.007405012
DHX38	-1.00714974	0.497528219	0.007663716
WBSCR22	-0.962571809	0.51314135	0.007752517
COL17A1	1.101778852	2.146191562	0.007763912
TPCN1	0.913095782	1.883081945	0.007879226
ADAT1	0.866845325	1.8236708	0.00814798
RHOF	0.900149458	1.866259311	0.008188136
WRB	0.836741777	1.786012003	0.008255766
HERC6	-0.885745916	0.54120763	0.008318494
RPIA	-0.945390672	0.519288913	0.00832936
TRHDE	1.095885561	2.137442427	0.008475277
TCTEX1D1	0.911738643	1.881311368	0.008485131
DYNLT3	0.83046453	1.778257848	0.008578458
PPM1M	0.919269983	1.891158107	0.008595175
GDAP1	0.973287779	1.963309717	0.008635952
MDM1	0.654889061	1.574494875	0.009009868
CD36	0.832455106	1.780713113	0.009027608
CCL21	-0.90063471	0.535651021	0.009319974
HMGB3	1.064025025	2.090756467	0.00945412
BPIFB3	-0.864827077	0.54911222	0.009497734
LRRC16A	0.750704084	1.682613803	0.009672541
LOXL3	0.980531641	1.973192408	0.00973769
CTSD	0.737490905	1.66727364	0.009947505

Supplemental Table 3. GO enrichment analysis of differentially expressed transcripts between the two cohorts (p-value<0.01). See excel document.

Supplemental Table 4. Univariate association of ABCC4 gene expression with demographics and clinical variables in subjects with HIV infection.

β=beta coefficient; S.E=standard error.

Univariate	β (S.E.)	p value
Age (per year)	-0.03 (0.03)	0.24
BMI (per unit [kg/m2] change)	-0.06 (0.04)	0.16
CD4 Count	0.0001 (0.0007)	0.87
Years HIV Duration	-0.048 (0.0322)	0.14
Platelet Count	-0.001 (0.003)	0.70
Male Sex	-0.98 (0.44)	0.02
White race	0.64 (0.6)	0.29
Non-Hispanic Ethnicity	1.57 (0.64)	0.01
Smoking Status		0.81
current	-0.06 (0.72)	
past	-0.34 (0.72)	
Hypertension	-0.43 (0.5)	0.39
Diabetes Mellitus	1.14 (0.72)	0.11
Hepatitis A	-0.36 (0.64)	0.58
Hepatitis C	0.12 (0.68)	0.86
AIDS	0.27 (0.46)	0.56
Statin	0.49 (0.64)	0.44
ART		
NRTI	0.4 (1.13)	0.72
NNRTI	-0.44 (0.47)	0.35
PI	-0.38 (0.45)	0.40
II	0.62 (0.49)	0.21

Supplemental Table 5. ABCC4 is significantly increased in HIV subjects (versus controls) after multivariable adjustment

β=beta coefficient; S.E=standard error.

	β (S.E.)	p value
Unadjusted	-2.42 (0.74)	0.001
Adjusted by age	-1.88 (0.76)	0.01
Adjusted by age, sex, race, ethnicity	-2.53 (0.74)	0.001
Adjusted by age, sex, race, ethnicity, BMI, smoking status	-2.91 (0.86)	0.001

Supplemental Figure 2. Validation of ABCC4 gene expression

ABCC4 expression by qPCR in the RNA-Seq cohort (HIV=6) and (C=3). ABCC4 mRNA fold-change of HIV platelets vs control platelets by qPCR (2.3 fold change; $p<0.05$) were compared to RNA-Seq analysis (3.5 fold change; $p<0.0001$).

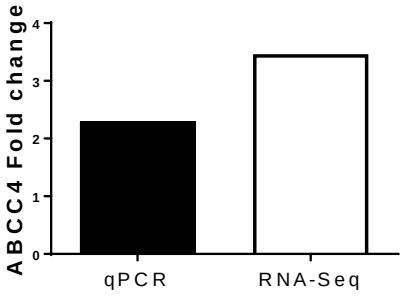
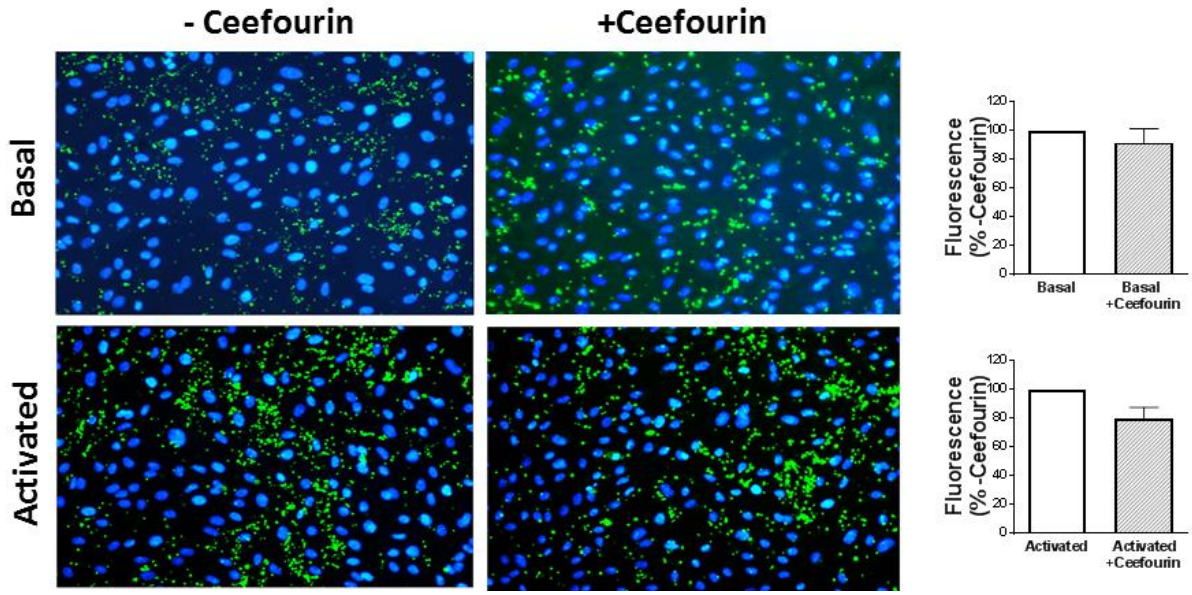


Figure S2

Supplemental Figure 3. The selective ABCC4 inhibitor has no effects on platelet adhesion to endothelial cells in healthy controls

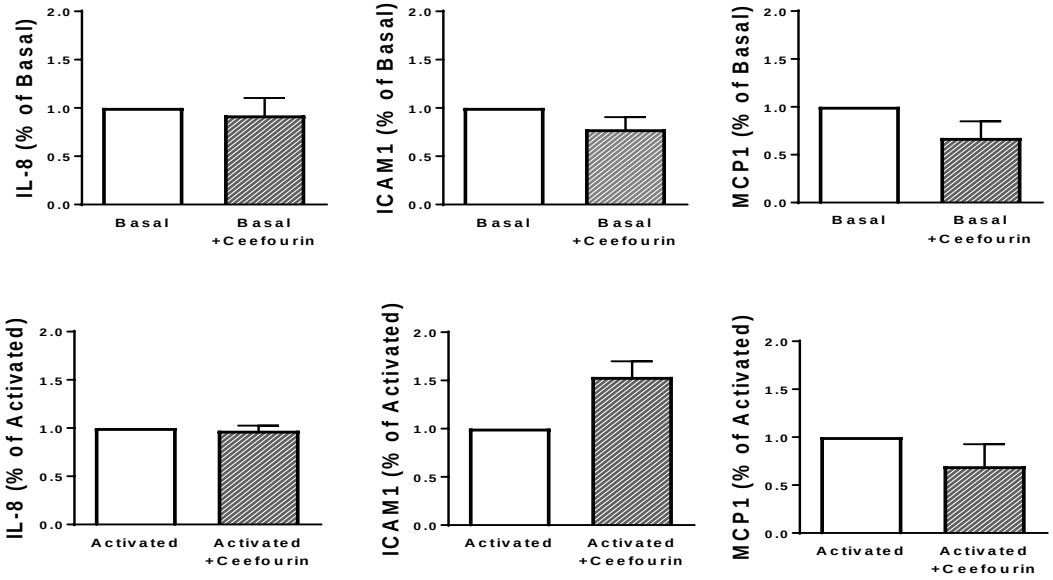
Stained platelets (green) from healthy individuals (n=3) were co-incubated with HUVECs and left untreated (Untreated) or treated with Ceefourin2 (Ceefourin, 10µM) for 30 min. Thrombin (0.05 U/ml, 5 min) was then added (Activated). HUVECs nuclei are stained with DAPI (blue). Values are represented as percentage of respective controls.



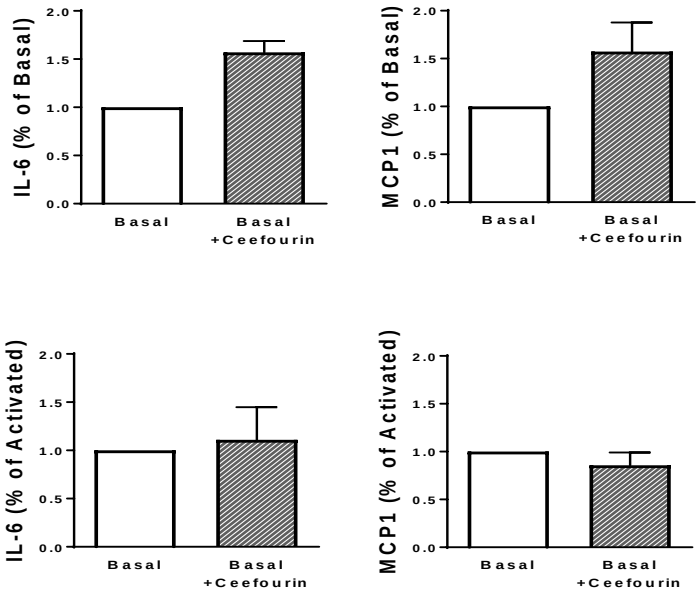
Supplemental Figure 4. ABCC4 inhibition in healthy subjects does not affect platelet effector cell function

(A) Transcriptional analysis of IL-8, ICAM-1 and MCP1 in HUVECs co-cultured with untreated or pre-treated (Ceefourin, 10μM) platelets isolated from healthy (n=4) subjects. Platelets were then left untreated (Basal) or stimulated with thrombin (0.05U/mL, Activated). **(B)** Co-culture experiment of untreated or Ceefourin pre-treated platelets from controls (n=4) with THP-1 at basal or following activation with thrombin (0.25U/mL). Gene expression of THP-1 activation markers IL-6 and MCP-1 was assessed. All results were normalized on 18S5 RNA. Values represent fold change after normalization to basal or activated condition.

A


IL-8 (% of Basal)
ICAM1 (% of Basal)
MCP1 (% of Basal)
IL-8 (% of Activated)
ICAM1 (% of Activated)
MCP1 (% of Activated)
2.0
1.5
1.0
0.5
0.0
Basal
Basal +Ceefourin
Activated
Activated +Ceefourin

B


IL-6 (% of Basal)
MCP1 (% of Basal)
IL-6 (% of Activated)
MCP1 (% of Activated)
2.0
1.5
1.0
0.5
0.0
Basal
Basal +Ceefourin