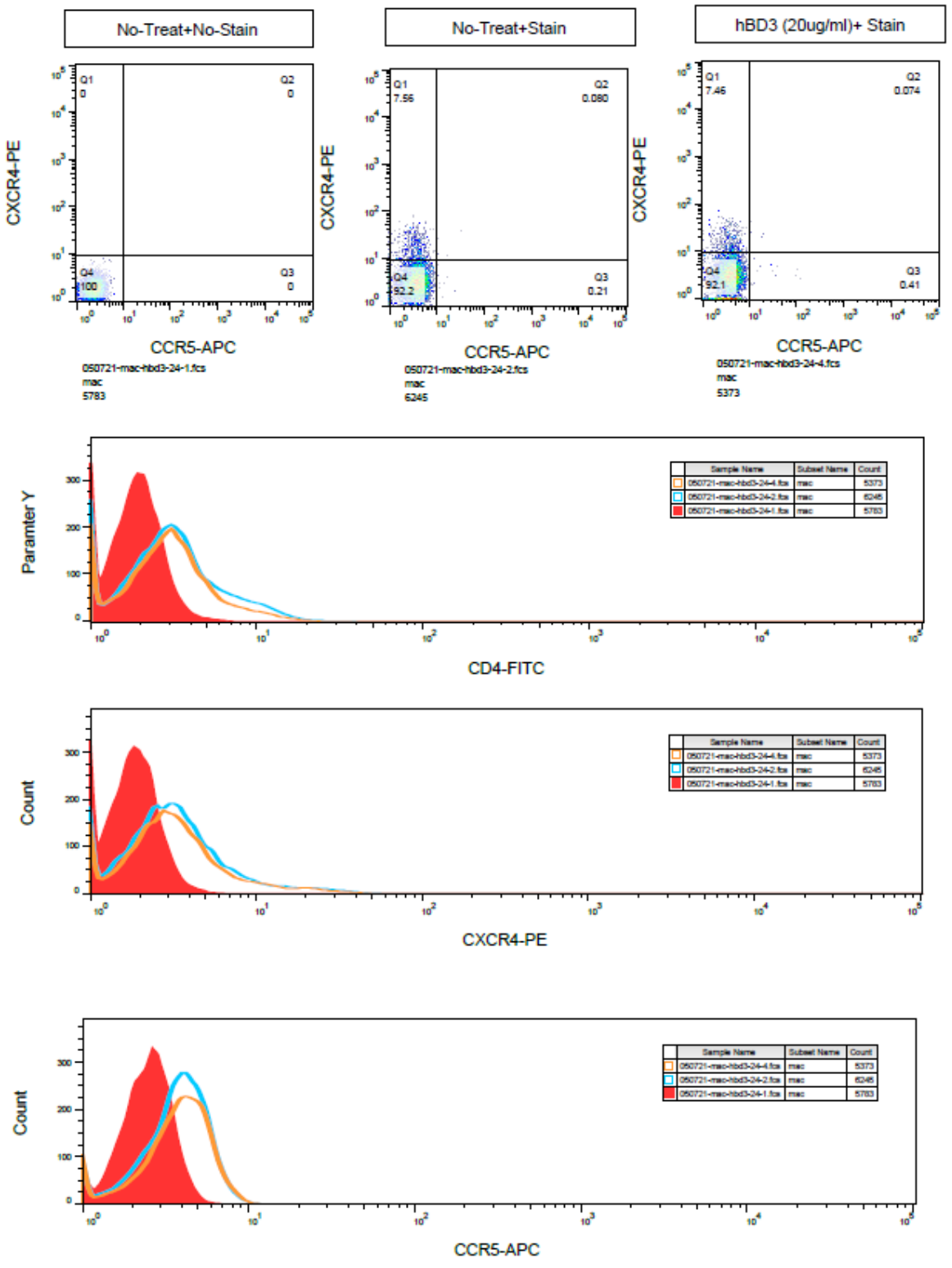
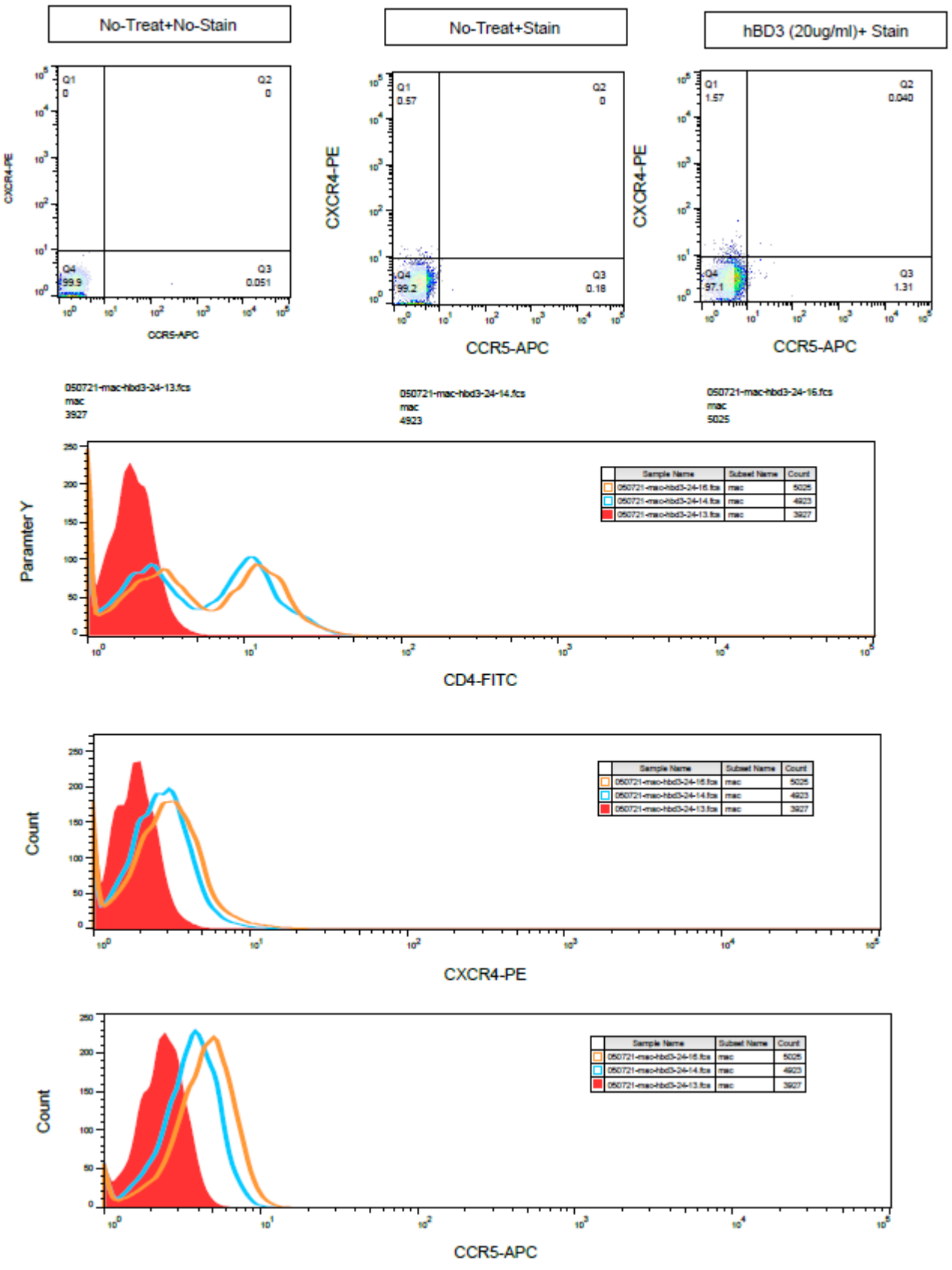
A

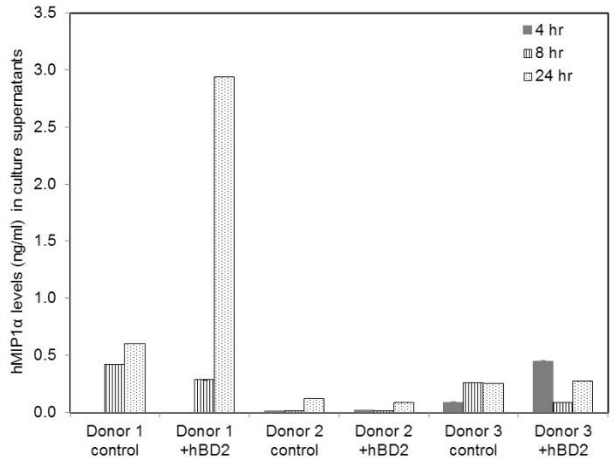

No-Treat+No-Stain
No-Treat+Stain
hBD3 (20ug/ml)+ Stain
CXCR4-PE
CCR5-APC
Q1
Q2
Q3
Q4
050721-mac-hbd3-24-1.fcs
mac
5783
050721-mac-hbd3-24-2.fcs
mac
6245
050721-mac-hbd3-24-4.fcs
mac
5373
Paramter Y
CD4-FITC
Count
Sample Name
Subset Name
Count

B

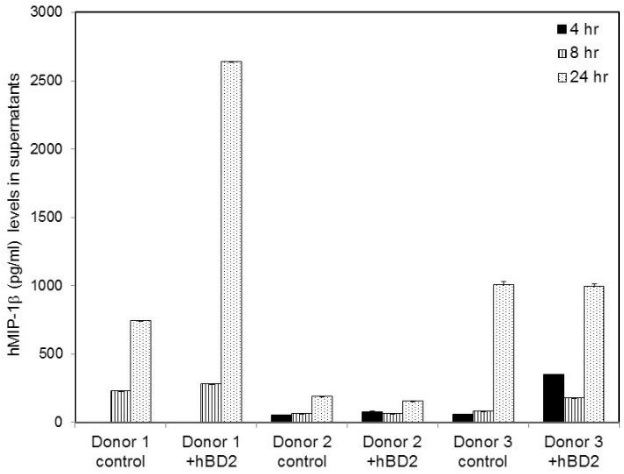

No-Treat+No-Stain
No-Treat+Stain
hBD3 (20ug/ml)+ Stain
CXCR4-PE
CCR5-APC
Q1 0
Q2 0
Q4 99.9
Q3 0.051
Q1 0.57
Q2 0
Q4 99.2
Q3 0.18
Q1 1.57
Q2 0.040
Q4 97.1
Q3 1.31
050721-mac-hbd3-24-13.fcs
mac
3927
050721-mac-hbd3-24-14.fcs
mac
4923
050721-mac-hbd3-24-16.fcs
mac
5025
Paramter Y
CD4-FITC
Count
CXCR4-PE
Count
CCR5-APC

Supplemental Figure S1. hBD3 does not induce modulation of HIV receptors on the surface of MDM. MDMs were treated with hBD3 (4.7uM); 1, 2, 3 and 24 hours after treatment cells were analyzed for CD4, CCR5, CXCR4 expression by flow cytometry. Shown are the results of a representative experiment from one of 2 donors, 1 hour (A) and 24 hours (B) after treatment. In red, unstained cells. In blue, cells stained with the antibody as indicated below the graph; in orange, hBD2-treated cells.

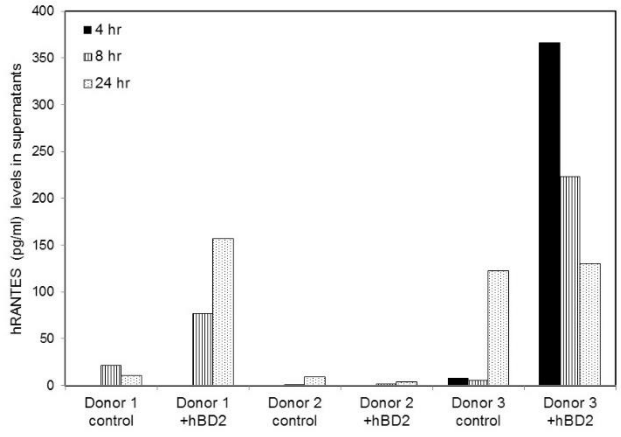
A


hMIP1α levels (ng/ml) in culture supernatants
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4 hr
8 hr
24 hr
Donor 1 control
Donor 1 +hBD2
Donor 2 control
Donor 2 +hBD2
Donor 3 control
Donor 3 +hBD2

B

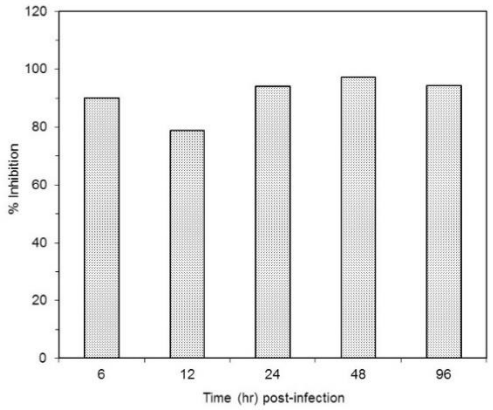

hMIP-1β (pg/ml) levels in supernatants
3000
2500
2000
1500
1000
500
0
4 hr
8 hr
24 hr
Donor 1 control
Donor 1 +hBD2
Donor 2 control
Donor 2 +hBD2
Donor 3 control
Donor 3 +hBD2

C

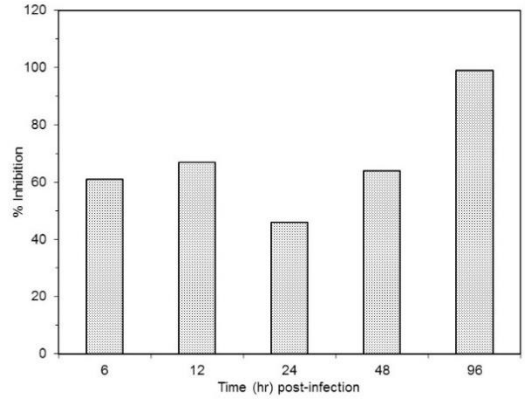

hRANTES (pg/ml) levels in supernatants
400
350
300
250
200
150
100
50
0
4 hr
8 hr
24 hr
Donor 1 control
Donor 1 +hBD2
Donor 2 control
Donor 2 +hBD2
Donor 3 control
Donor 3 +hBD2

Supplemental Figure S2. hBD2 does not induce expression of anti-viral cytokines or β-chemokines in macrophages. Culture supernatants from MDM treated with hBD2 were collected at times indicated and β-chemokines MIP-1α (A), MIP-1β (B), and RANTES (C) were quantified using commercial ELISA kits. Data are presented as average ± SEM of duplicates for independent experiments from 3 different donors.

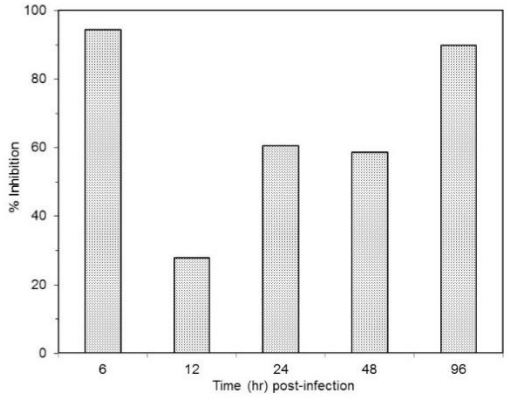
1


120
100
80
60
40
20
0
% Inhibition
6
12
24
48
96
Time (hr) post-infection

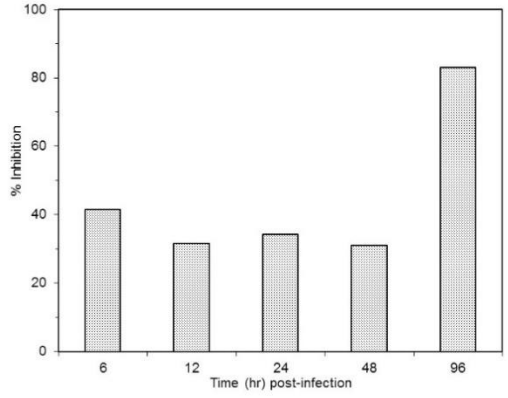
2


120
100
80
60
40
20
0
% Inhibition
6
12
24
48
96
Time (hr) post-infection

3


100
80
60
40
20
0
% Inhibition
6
12
24
48
96
Time (hr) post-infection

4


100
80
60
40
20
0
% Inhibition
6
12
24
48
96
Time (hr) post-infection

Supplemental Figure S3. hBD2 inhibits accumulation of early reverse transcription products of HIV-1. Inhibition for the four donors shown in Figure 3B was determined as the percentage of HIV-1 DNA copies in treated infected cells in reference to untreated infected cells.