LEGENDS TO SUPPLEMENTARY FIGURES

Figure 1S. **Fluorescence thresholding and gating strategy**

a. Threshold trigger setting on fluorescence height. Note the absence of events below the threshold.

b. Gating on single particles by excluding aggregates on height vs. width plot. Note the linearity of the lower fluorescence height resulting from the threshold defined in **a**. Note the exclusion (grey area) of out of range events on the fluorescence height parameter which are above the linear range of the detectors as defined by the Cytometer Setting and Tracking procedure (CST software, Becton Dickinson).

c. Dot plot of the threshold fluorescence parameter vs. an "empty" fluorescence channel. Note the linearity of the left border of the plotted events, resulting from the threshold defined in **a** (blue arrow), and the linear right limit of the plotted events, resulting from the exclusion of out of range events (grey zone) in **b** (black arrow).

d. Thresholding of fluorescence for DiO and DiD labeled HIV-1 virions. Thresholding was based on the lowest fluorescence channel value that gave no fluorescent events for filtered PBS (red arrows).

Note that this thresholding results in the linearity of the lower border of the plotted events in the corresponding channels (red and orange hatched zones in the left and center panels), and the combination of these two linear borders when the two thresholds are combined to detect the mixture of labeled viruses.

Note that all the linearities are the inherent properties of events triggered by fluorescence and not of subjective gating.

Figure 2S. **Flow virometry of Dengue virus**

Suspension of Dengue virus type 2 (strain Tongue/74) produced in BHK21 cells was stained with DiI and captured by MNPs carrying 3H5.1 antibodies against E-protein. Captured virions were separated by magnetic column. Both initial suspension and the flow-through fraction not bound to MNPs were subjected to flow analysis with volumetric control. 160 μL of viral suspensions were acquired.

a. Initial viral preparation. **b.** Virions that have not been captured by the MNPs. Note that more than 95% of virions have been captured by MNPs and retained by magnetic column.

Figure 3S: **Fluorescence minus one (FMO) control for staining virions for cellular antigens**

HIV-1_{SF162} produced by PBMCs were captured on MNPs via anti-gp120 antibody VRC01, visualized with labeled 2G12 antibodies and stained for either HLA-DR or LFA-1 or for both antigens with anti-HLA-DR APC-eFluor 780 and anti-LFA-1 PE-Cy7 antibodies. Fluorescence was acquired in all fluorescence channels irrespective of the presence of a labeled antibody. Left column: Staining with anti-LFA-1 but not with anti-HLA-DR antibodies. Center column: Staining with anti-HLA-DR but not with anti-LFA-1 antibodies. Right column: staining with both anti-HLA-DR and anti LFA-1 antibodies. Upper row: Acquisition in HLA-DR (APC-Fluor 780) channel. Bottom row: Acquisition in LFA-1 (PE-Cy7) channel.

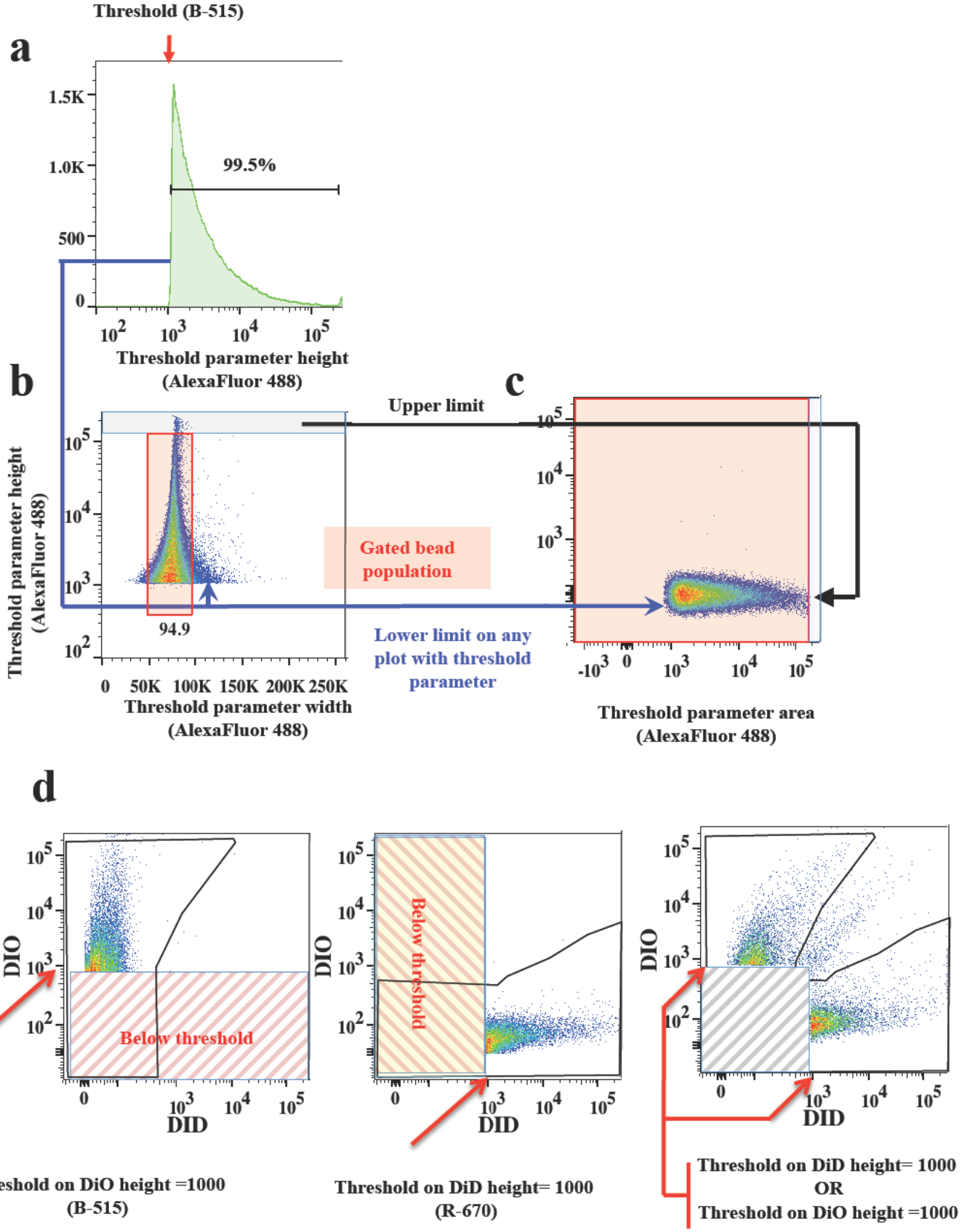
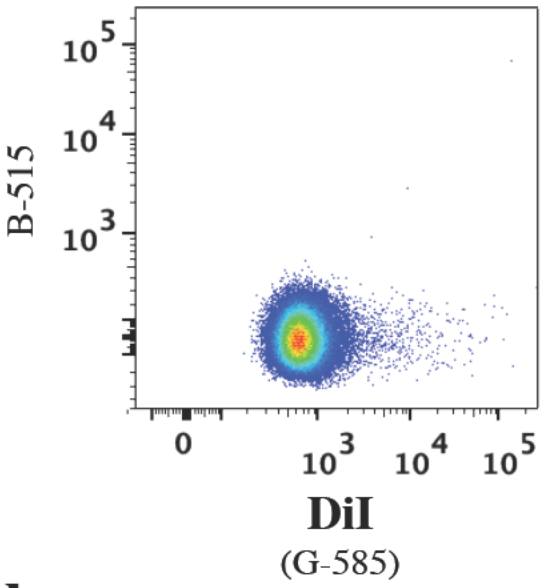

a
Threshold (B-515)
1.5K
1.0K
500
0
99.5%
10^2 10^3 10^4 10^5
Threshold parameter height
(AlexaFluor 488)
b
Threshold parameter height
(AlexaFluor 488)
10^5
10^4
10^3
10^2
94.9
0 50K 100K 150K 200K 250K
Threshold parameter width
(AlexaFluor 488)
Upper limit
Gated bead
population
Lower limit on any
plot with threshold
parameter
c
10^5
10^4
10^3
-10^3 0 10^3 10^4 10^5
Threshold parameter area
(AlexaFluor 488)
d
DIO
DID
Below threshold
Threshold on DiO height =1000
(B-515)
Below threshold
Threshold on DiD height= 1000
(R-670)
Threshold on DiD height= 1000
OR
Threshold on DiO height =1000

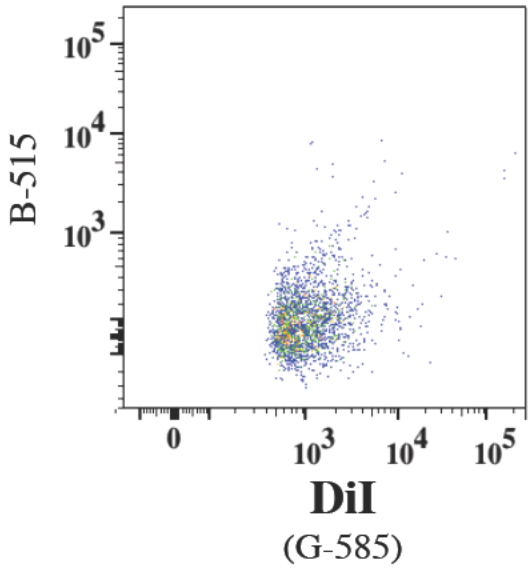
Figure 1S

a



Input virus:
97091 virions per 160μl

b



Flow through:
2246 virions per 160μl

Figure 2S

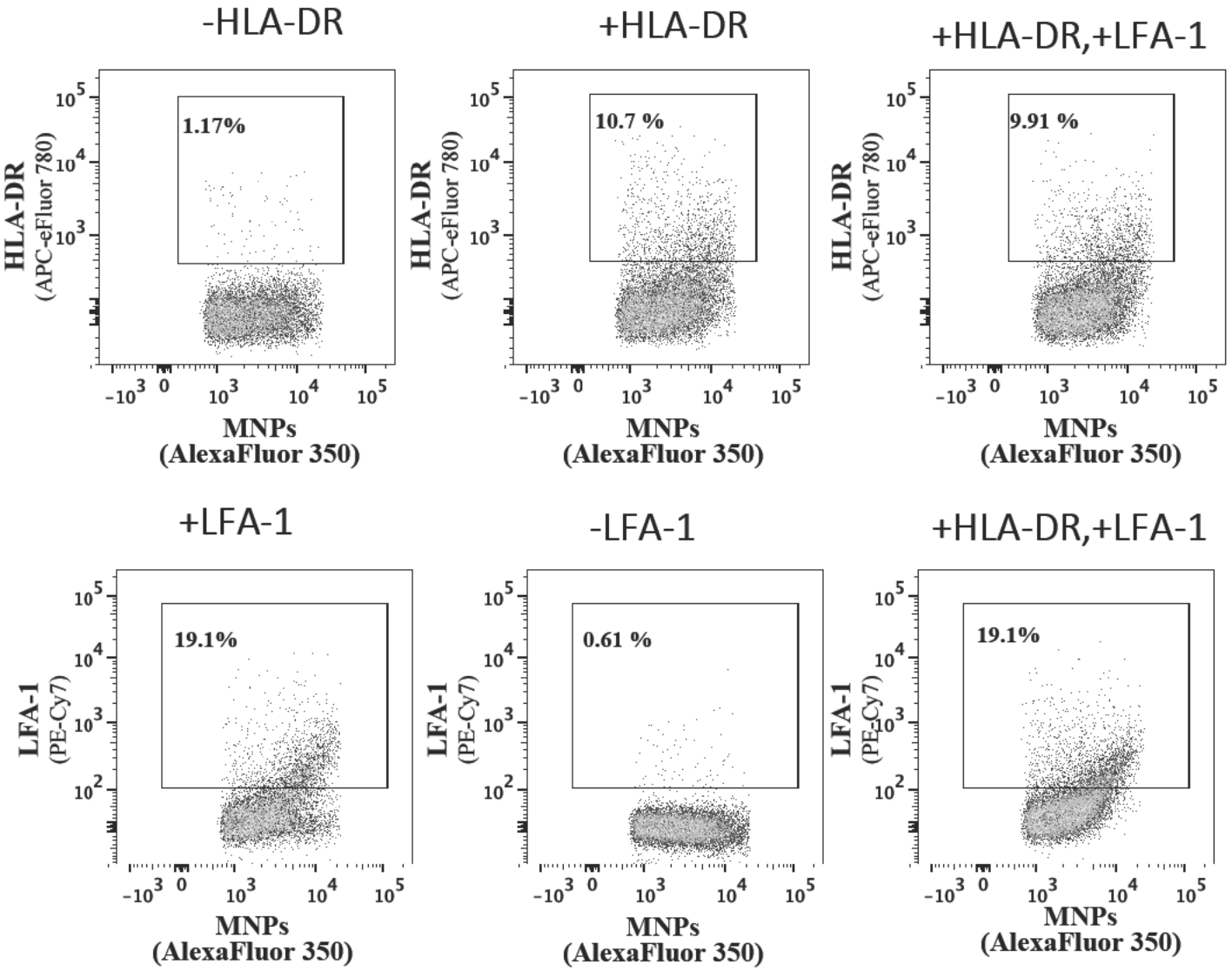

-HLA-DR
+HLA-DR
+HLA-DR,+LFA-1
1.17%
10.7 %
9.91 %
HLA-DR
(APC-eFluor 780)
MNPs
(AlexaFluor 350)
+LFA-1
-LFA-1
+HLA-DR,+LFA-1
19.1%
0.61 %
19.1%
LFA-1
(PE-Cy7)
MNPs
(AlexaFluor 350)

Figure 3S