SUPPLEMENTAL FIGURES LEGENDS

Figure S1. Depletion of T cells by CD25 beads

Depletion of CD25+ cells using magnetic beads conjugated to an anti-CD25 antibody efficiently removed FoxP3+ CD25+ Tregs but minimally changed bulk CD8 and CD4 T cell populations. These results are representative of 15 control experiments.

Figure S2. Flow cytometry gating scheme for isolation of mononuclear cells subsets

(A) Doublets were initially gated out using SSC-A vs SSC-H and monocytes were differentiated from T and B cells by scatter (FSC-A vs SSC-A). A dump channel was used containing viability dye and CD56. Monocytes were distinguished by expression of CD14. T cells were defined as CD3+ and then either CD4+ or CD8+. Tregs were defined as CD3+ CD4+ $CD25^{hi}$ $CD127^{lo}$ cells. B cells were defined as CD3- and CD19+. (B) DCs were distinguished using a separate staining panel using doublet discrimination and a dump channel containing viability dye, CD3, CD19, CD14, and CD56. Cells that were negative for staining by all of these reagents were then selected and the remaining population of cells were then distinguished based upon scatter and then pDCs defined as CD123+ and mDCs as CD11c+ HLA-DR+.

Figure S3. IL-10 secretion following stimulation of PMBCs with gag peptide pool

PBMCs from a chronic untreated subject were stimulated with gag peptide pool or media alone and ICS was then performed. Shown is a representative experiment of four.

Figure S4. Flow cytometry gating scheme for monocyte and T cell subset sorting

Doublet discrimination was performed by using SSC-A vs SSC-H. Monocytes and T cells were then differentiated based upon scatter (FSC-A vs SSC-A) and monocytes were identified as dump negative (viability dye, CD56, CD19) and CD14+. Tregs were identified as dump negative, CD8- and $CD25^{hi}$ $CD127^{lo}$.

Figure S5. Effect of PDL-1 and CTLA-4 blockade on IL-10 production

LPS stimulated PBMCs from 10 untreated, chronically HIV infected subjects were incubated with either an isotype control antibody or antibodies against PD-L1 (Panel A) or CTLA-4 (Panel B). Supernatants were then collected 3 days later and assessed for IL-10 by Luminex.

Figure S1

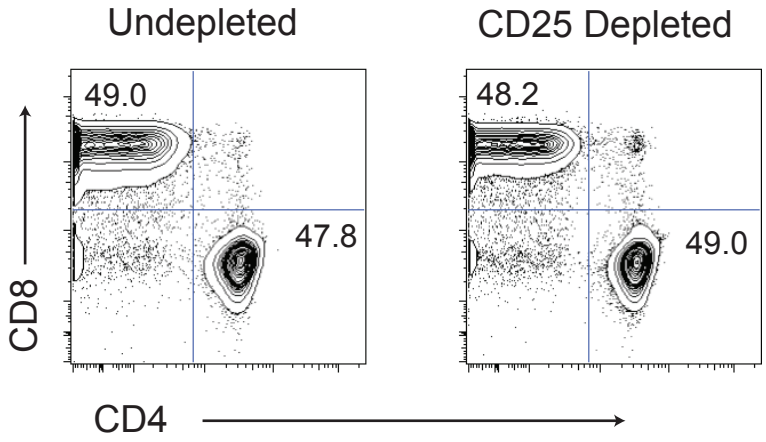


Figure S2

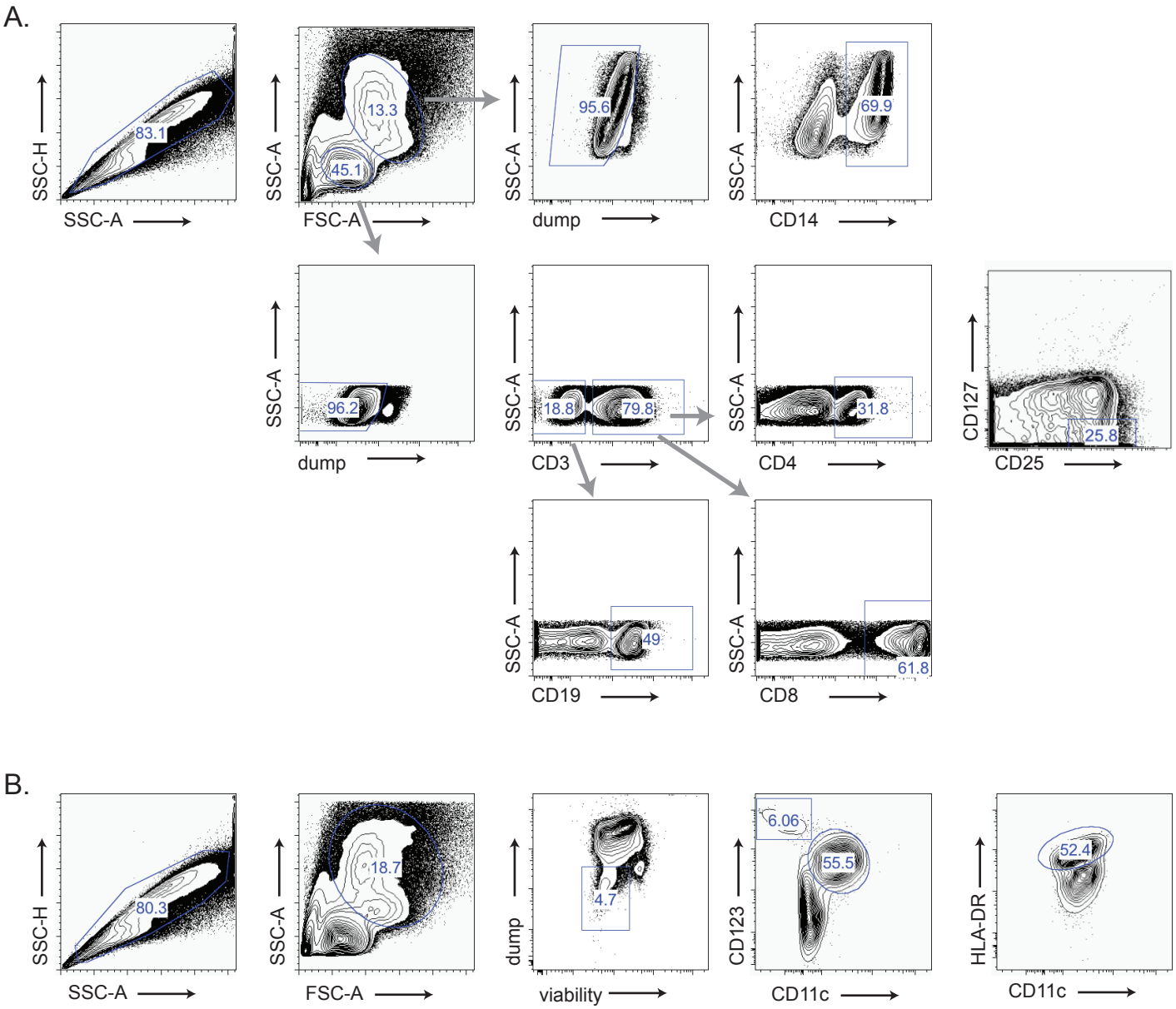

A.
SSC-H
SSC-A
83.1
SSC-A
FSC-A
13.3
45.1
SSC-A
dump
95.6
SSC-A
CD14
69.9
SSC-A
dump
96.2
SSC-A
CD3
18.8
79.8
SSC-A
CD4
31.8
CD127
CD25
25.8
SSC-A
CD19
49
SSC-A
CD8
61.8
B.
SSC-H
SSC-A
80.3
SSC-A
FSC-A
18.7
dump
viability
4.7
CD123
CD11c
6.06
55.5
HLA-DR
CD11c
52.4

Figure S3

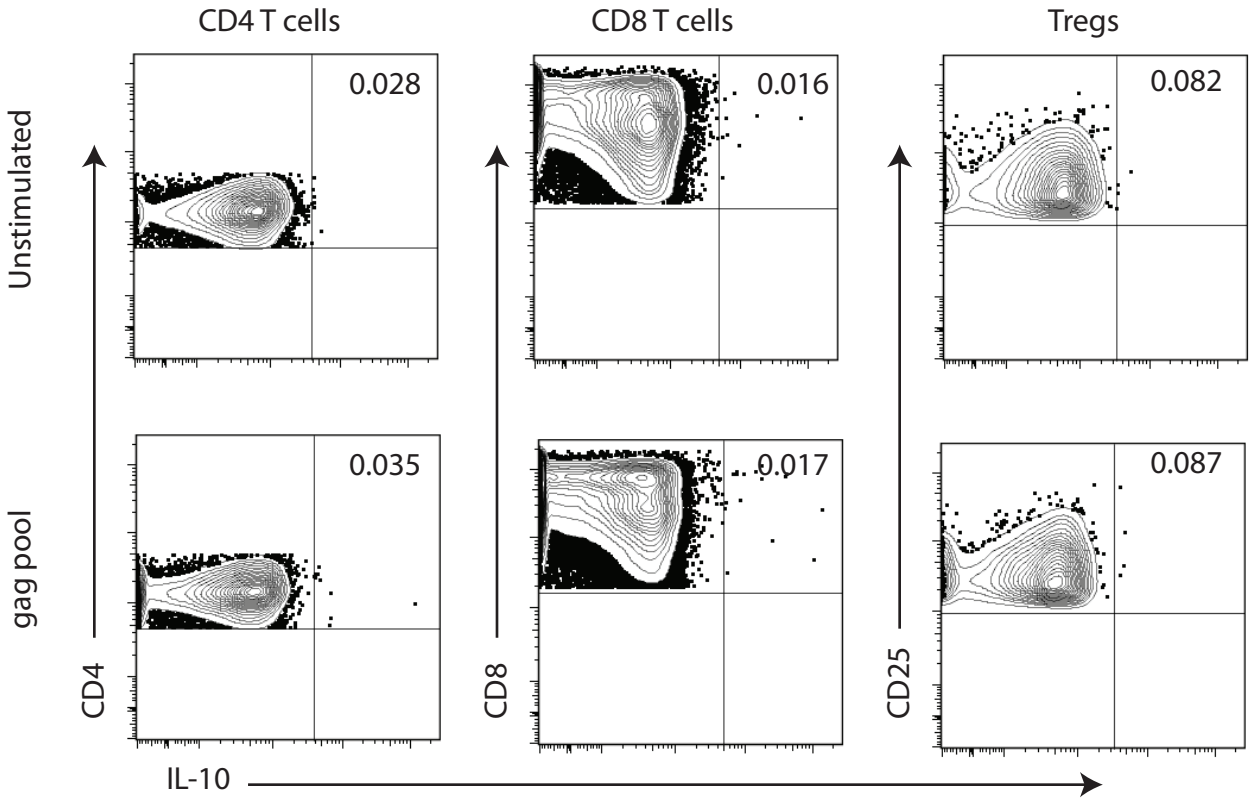

CD4 T cells
CD8 T cells
Tregs
Unstimulated
gag pool
0.028
0.016
0.082
0.035
0.017
0.087
CD4
CD8
CD25
IL-10

Figure S4

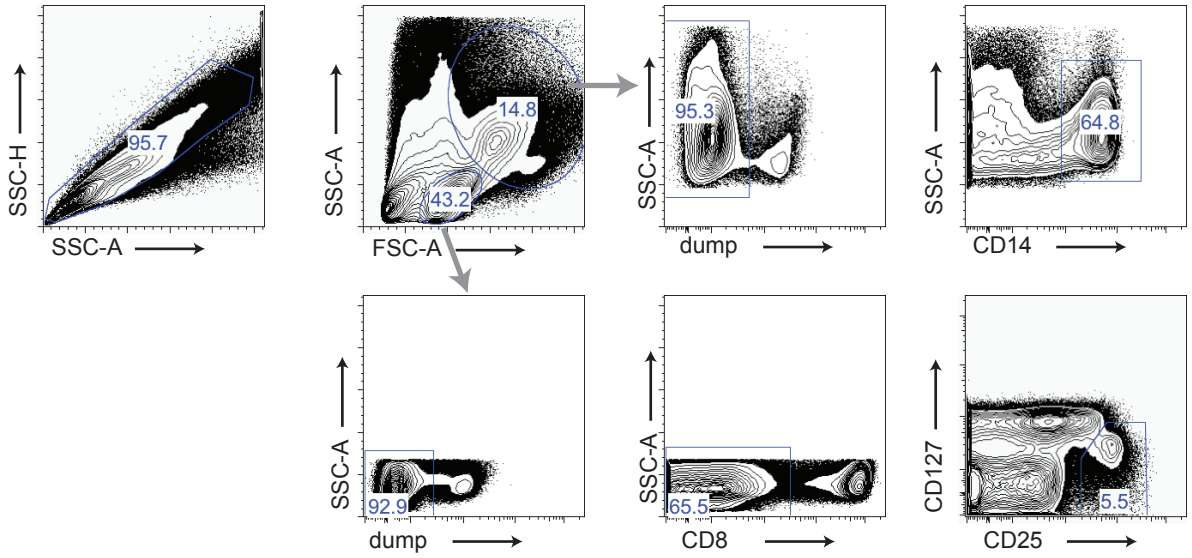
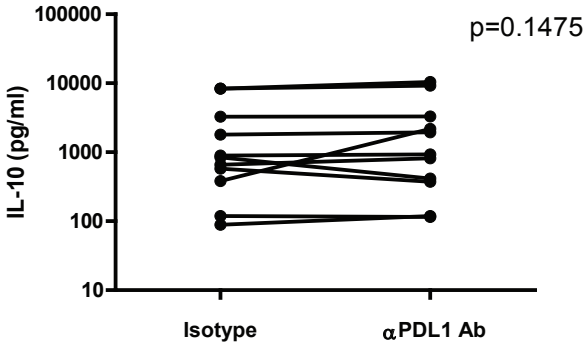
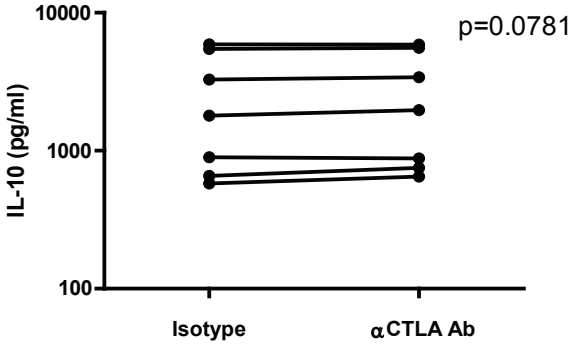

SSC-H
SSC-A
95.7
SSC-A
FSC-A
14.8
43.2
SSC-A
dump
95.3
SSC-A
CD14
64.8
SSC-A
dump
92.9
SSC-A
CD8
65.5
CD127
CD25
5.5

Figure S5

A.


100000
10000
1000
100
10
IL-10 (pg/ml)
p=0.1475
Isotype
αPDL1 Ab

B.


10000
1000
100
IL-10 (pg/ml)
p=0.0781
Isotype
αCTLA Ab