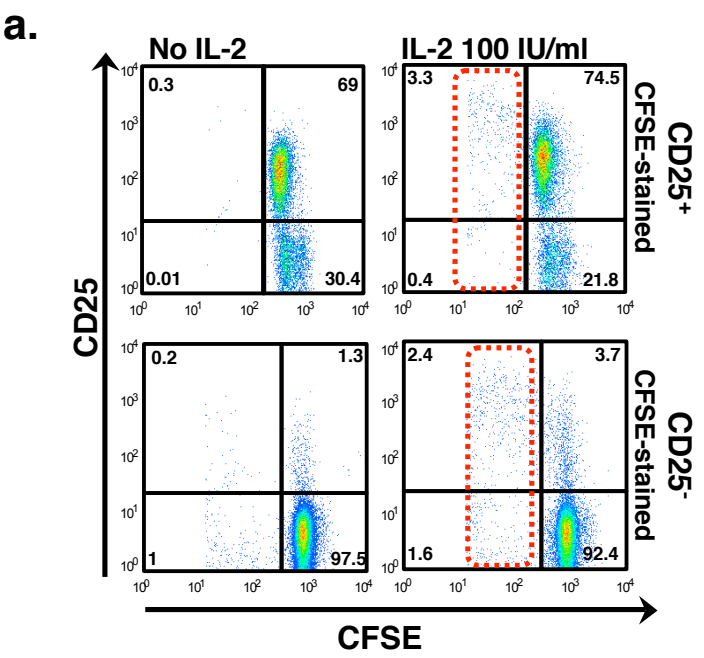


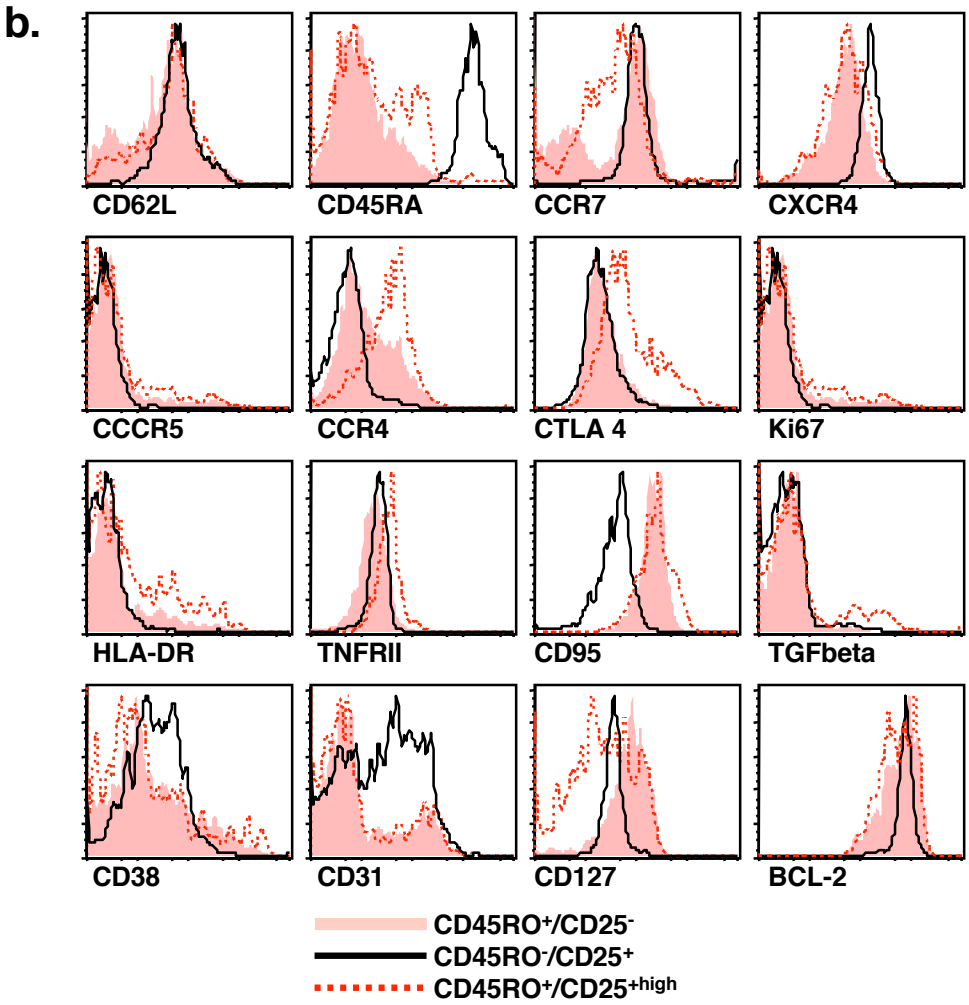


Figure 1 a and b

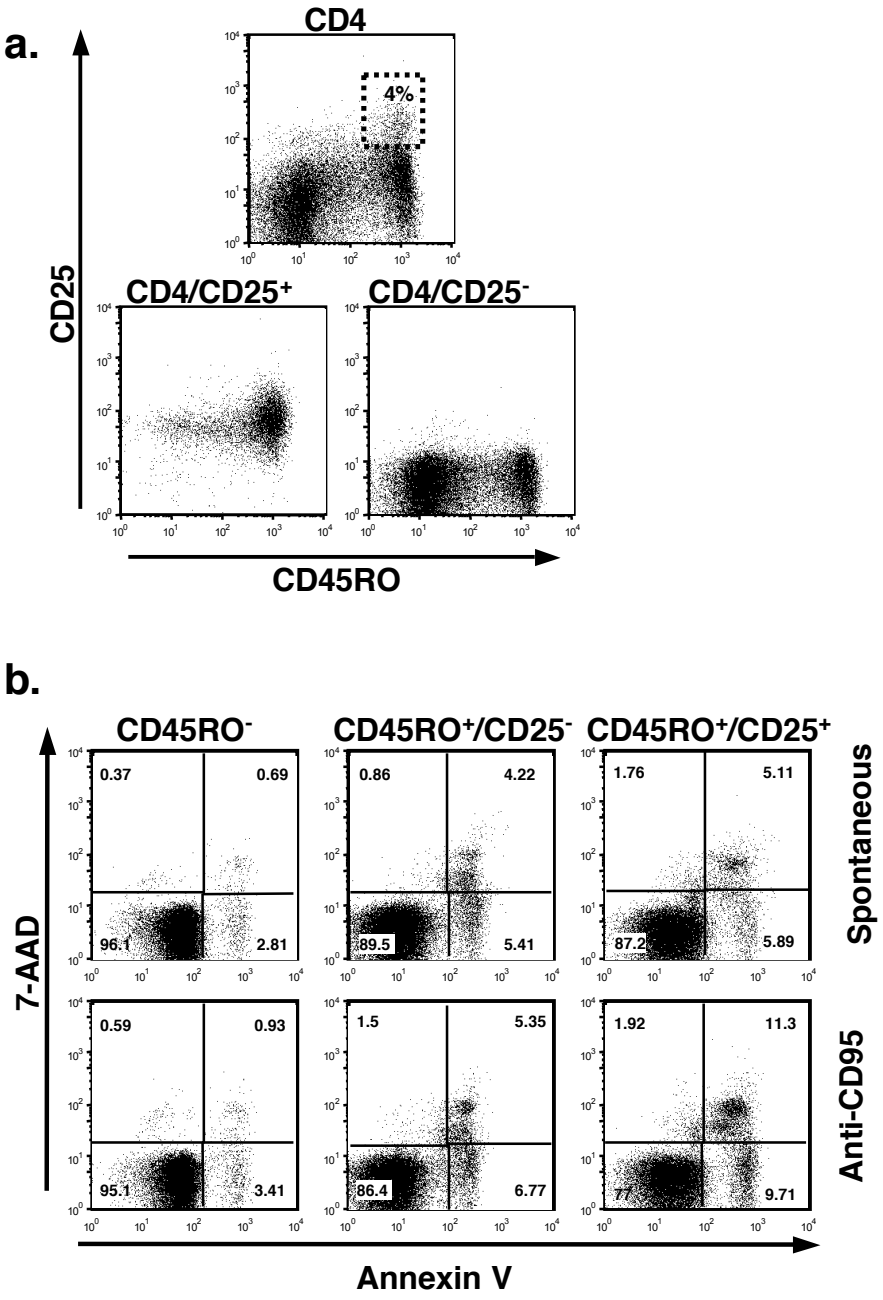
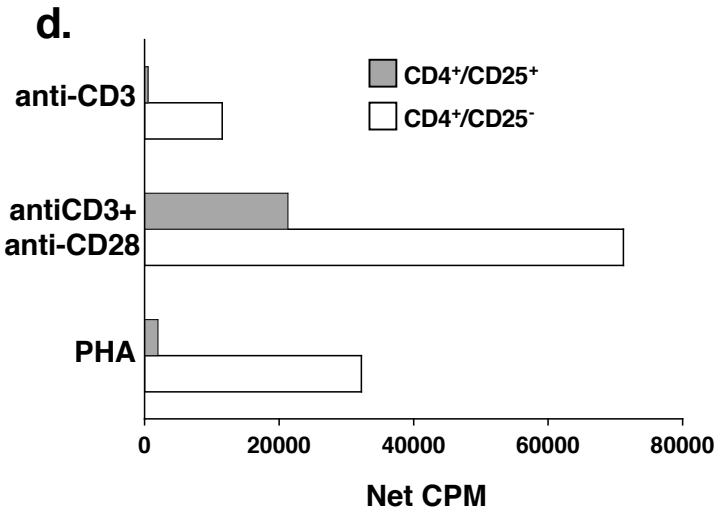

a.
CD4
4%
CD25
CD4/CD25+
CD4/CD25-
CD45RO
b.
CD45RO-
CD45RO+/CD25-
CD45RO+/CD25+
0.37
0.69
96.1
2.81
0.86
4.22
89.5
5.41
1.76
5.11
87.2
5.89
Spontaneous
0.59
0.93
95.1
3.41
1.5
5.35
86.4
6.77
1.92
11.3
77
9.71
Anti-CD95
7-AAD
Annexin V
c.
CD25+
CD25-
0.05
0.17
3.68
96.1
0.03
3.1
2.89
94
Interleukin 2
CD4

Figure 2 a, b & c


d.
anti-CD3
antiCD3+
anti-CD28
PHA
CD4+/CD25+
CD4+/CD25-
0
20000
40000
60000
80000
Net CPM

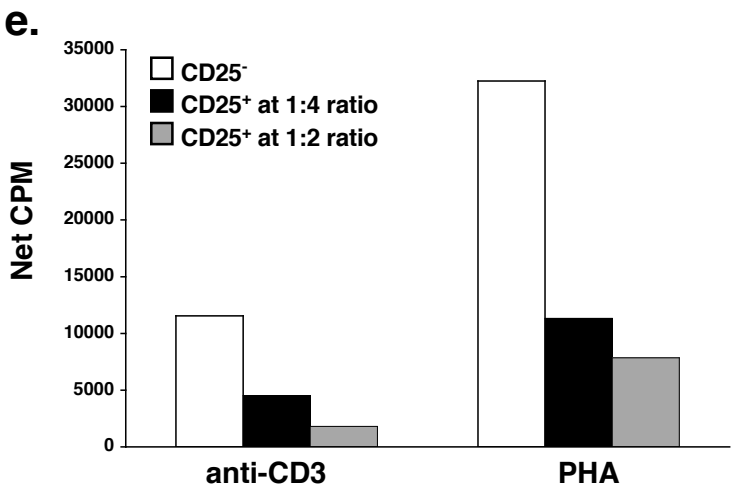

e.
Net CPM
35000
30000
25000
20000
15000
10000
5000
0
CD25-
CD25+ at 1:4 ratio
CD25+ at 1:2 ratio
anti-CD3
PHA

Figure 2 d & e

Legends of supplemental figures.

Supplemental Figure 1

a) $CD4^+$/CD25-depleted or $CD4^+/CD25^+$ cells from IL-2 patients were stained with CFSE and cultured in vitro for 5-7 days, in complete medium without or with IL-2 100 IU/ml. Cultures were done with CFSE stained $CD4^+$/CD25-depleted cells alone or mixed with CFSE unstained $CD4^+/CD25^+$ and vice versa, so that the baseline phenotype ($CD25^-$ or $CD25^+$) could be traced. The CD4-depleted fraction of PBMC (CFSE-unstained) was added in all culture conditions. $CD4^+$/CD25-depleted cells expressed CD25 and proliferated after stimulation with IL-2. A significant down-regulation of CD25 expression was seen after in vitro culture in the $CD25^+$ fraction in the absence of IL-2. Day 7 from one of four experiments (from independent donors) is shown. b) Overlay CD4 histograms of the same patient shown in figure 3a. Solid light red histograms represent $CD45RO^+/CD25^-$ cells. As in figure 3a, dashed red lines represent $CD45RO^+/CD25^{+high}$ cells and $CD45RO^-/CD25^+$ cells are shown as black lines.

Supplemental Figure 2

Phenotypic and functional characteristics of Treg cells isolated from healthy HIV-seronegative donors. a) A representative plot of CD45RO and CD25 expression on $CD4^+$ T cells as well as on the $CD25^+$ and $CD25^-$ bead separated subsets from a normal volunteer are shown. The majority of $CD4^+/CD25^{+high}$ (Treg) cells are expressing CD45RO. b) Treg cells are prone to spontaneous and fas-mediated apoptosis. A representative experiment from a normal volunteer is shown. c) Treg cells do not produce IL-2 after PMA/ionomycin stimulation. A representative experiment from a normal volunteer is shown. d) Lymphocyte proliferation assays of Treg and $CD4^+/CD25^-$ subsets. Treg cells are anergic and co-stimulation can only partially overcome their anergy (median values from four experiments from independent donors are shown). e) Treg cells are potent suppressors. Addition of Treg cells at increasing ratios in cultures of $CD4^+/CD25^-$ cells strongly suppressed proliferation of responder cells (median values from four experiments from independent donors are shown).