

**Figure 1: Analysis of FOXP3 expression in the compartment of nCD4+RTEs. An increased percentage was found in the group of non-treated individuals. The comparison between median values of the frequency of nCD4-RTE FOXP3+ in different study groups showed the highest values in the group of healthy control subjects, while in HIV-1 infected subjects under therapy and non-treated HIV-1 infected individuals it was significantly lower.**

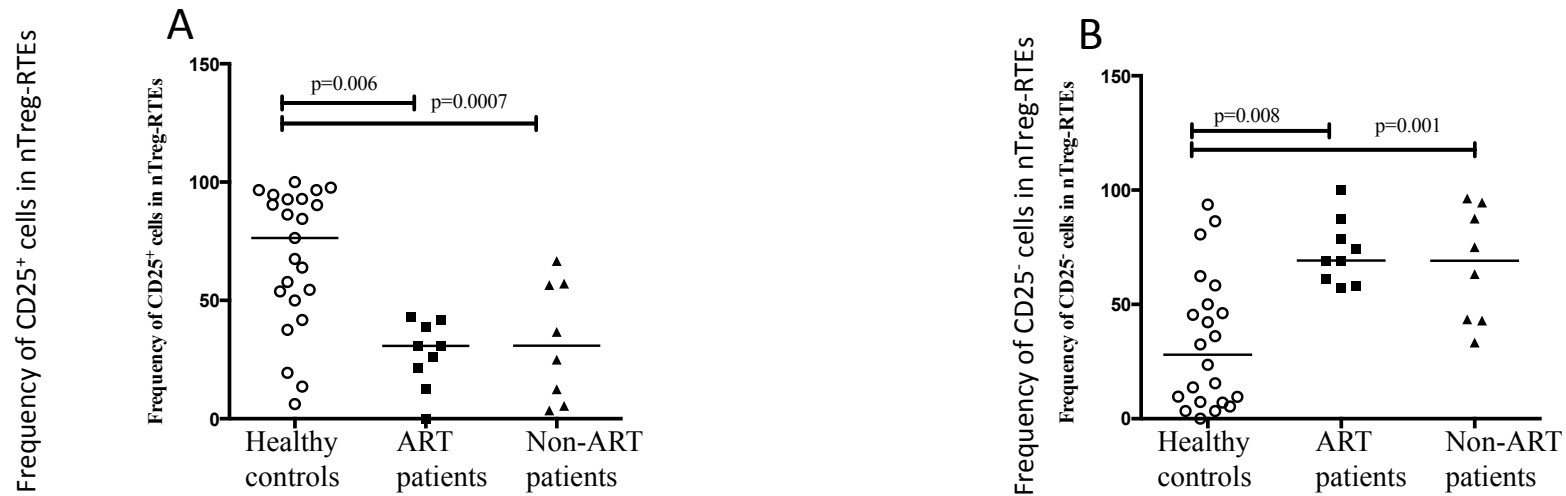


Figure 2. Expression of CD25 defines two subsets nTreg-RTEs (nCD4<sup>+</sup>CD31<sup>+</sup>FOXP3<sup>+</sup> T-cells). Both subsets are differently represented in healthy controls, ART-treated patients and non-treated individuals. In the compartment of nTreg-RTEs the frequency of CD25<sup>+</sup> (A) was higher than that of CD25<sup>low</sup> (B). That was not the case in HIV-1 infected participants, where an inverse ratio between the two fractions was found. The comparison between median values (non-parametric Mann-Whitney test) showed significant differences between patients groups and healthy controls in each fraction.

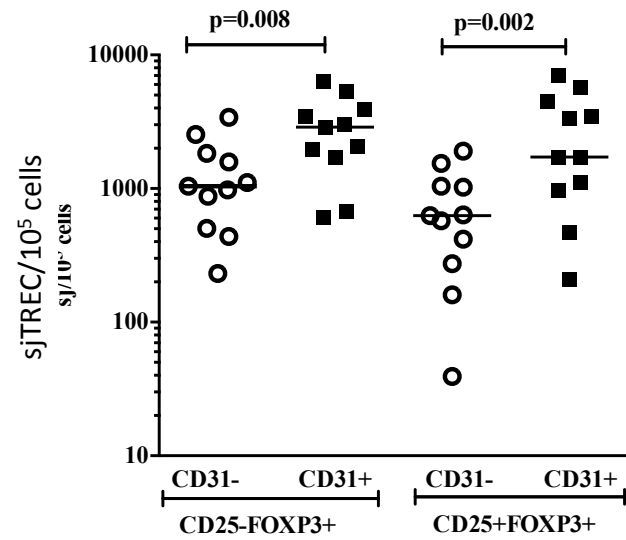
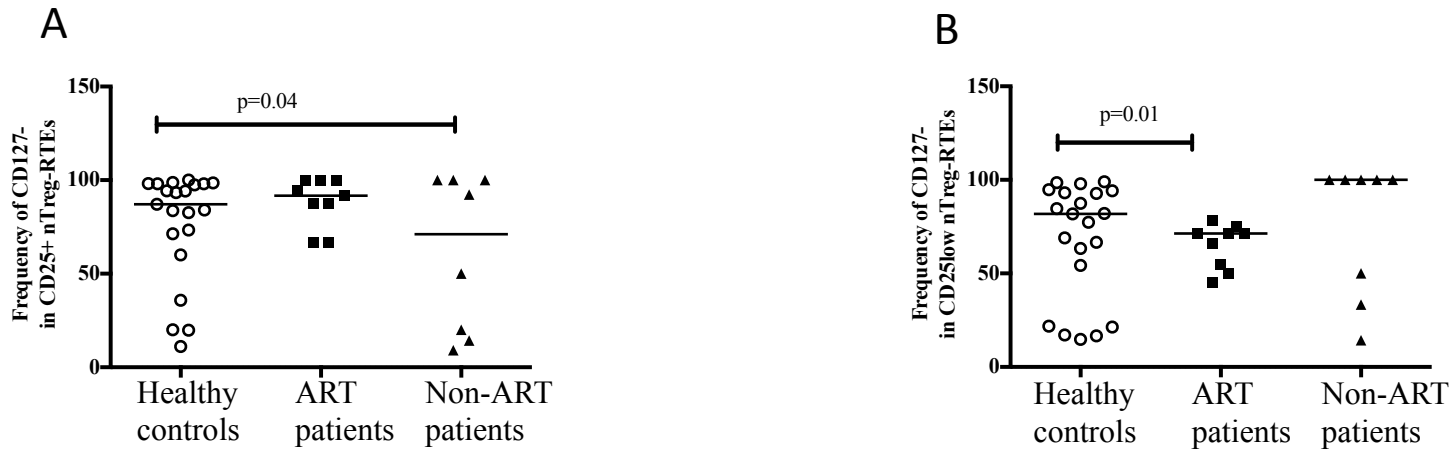


Figure 3. Comparison between sjTREC frequencies in CD25<sup>low</sup> and CD25<sup>+</sup> nTreg-RTEs defined as nCD4CD31<sup>+</sup>FOXP3<sup>+</sup> and in nCD4CD31<sup>-</sup>FOXP3<sup>+</sup> in healthy controls. In both fractions the CD31<sup>+</sup> nCD4 T cells contained more sjTRECs than CD31<sup>-</sup> nCD4 T cells.



**Figure 4. Expression of CD127 in CD25<sup>+</sup> (A) and CD25<sup>low</sup> (B) nTreg-RTEs.** The frequency of CD127<sup>low</sup> cells among CD25<sup>+</sup> and CD25<sup>-</sup> nTreg-RTEs for healthy controls, HIV-1 patients under therapy and non-treated HIV-1 infected individuals is presented. In healthy controls both fractions mostly contained CD127<sup>low</sup> cells. In HIV-1 infected individuals, CD127 was less expressed by CD25<sup>+</sup> cells or CD25<sup>-</sup> cells in treated or non treated patients respectively. The non-parametric Mann-Whitney test was applied to compare the median values between different groups.

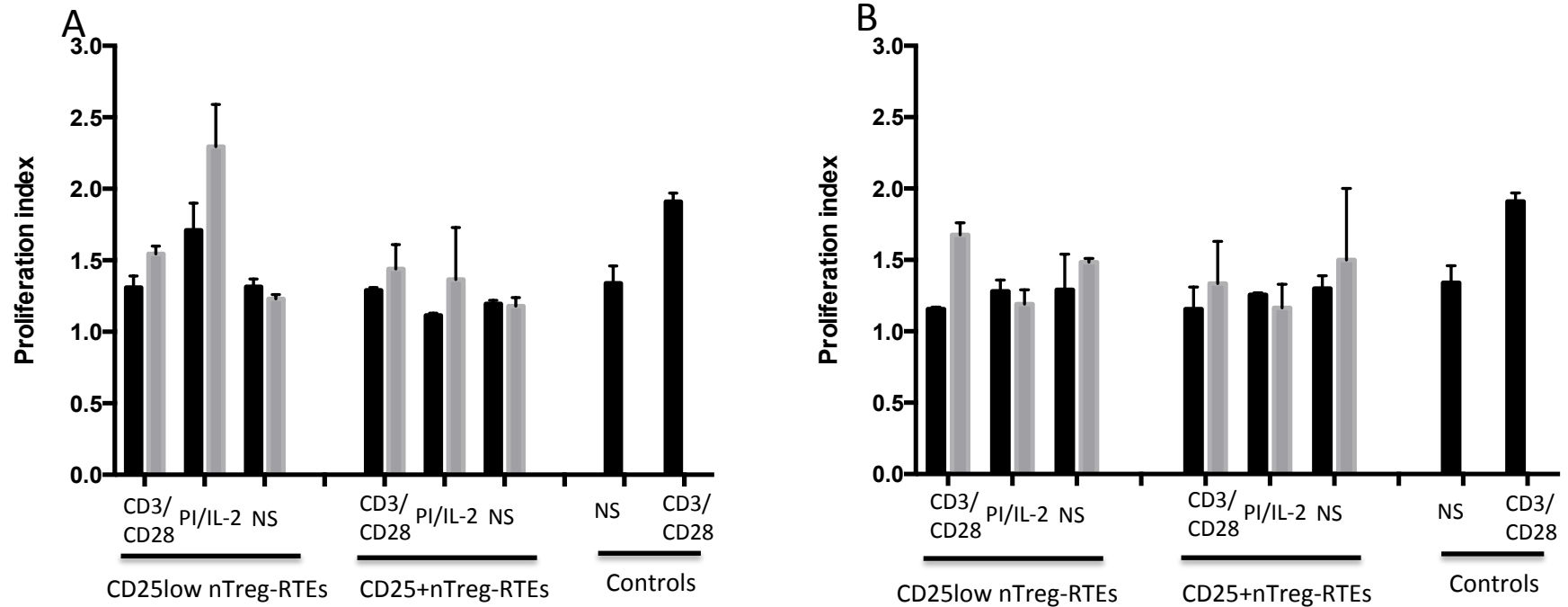


Figure 5. Dose-dependent changes in the proliferation index following an inhibition assay performed on naïve CD4 T cells, FOXP3<sup>-/-</sup> in the presence of different concentrations of CD25<sup>low</sup> and CD25<sup>+</sup> nTreg-RTEs – 1/6 (■) and 1/32 (◻). CD25<sup>low</sup>-nTreg-RTEs are less suppressive as compared to CD25<sup>+</sup>nTreg-RTEs: A) Conventional T cells were pre-stimulated with anti-CD3/CD28. nTreg-RTEs were pre-incubated with anti-CD3/CD28, Proinsulin+IL-2 or NS for 24h and added to the T conv. On day 4 the proliferation was measured by CFSE and proliferation index calculated by FlowJo software. B) Non-stimulated conventional T cells were incubated for 4 days in the presence of nTreg-RTEs pre-incubated with anti-CD3/CD28, Proinsulin+IL-2 or NS for 24h. As a controls non-stimulated and CD3/CD28 stimulated naïve CD4T conventional cells are used.