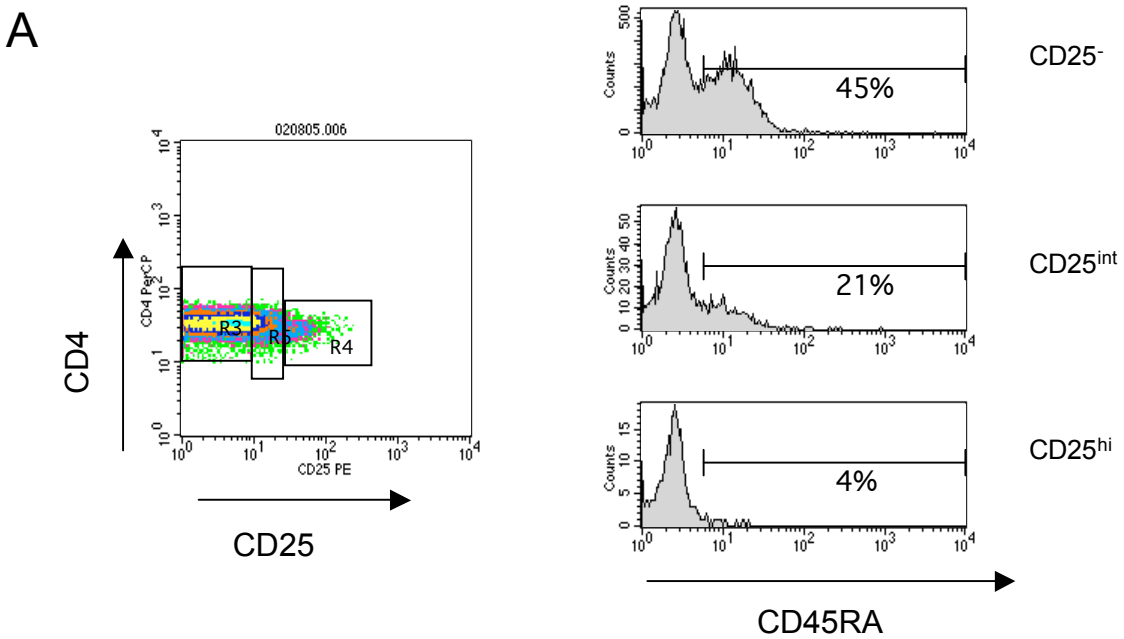
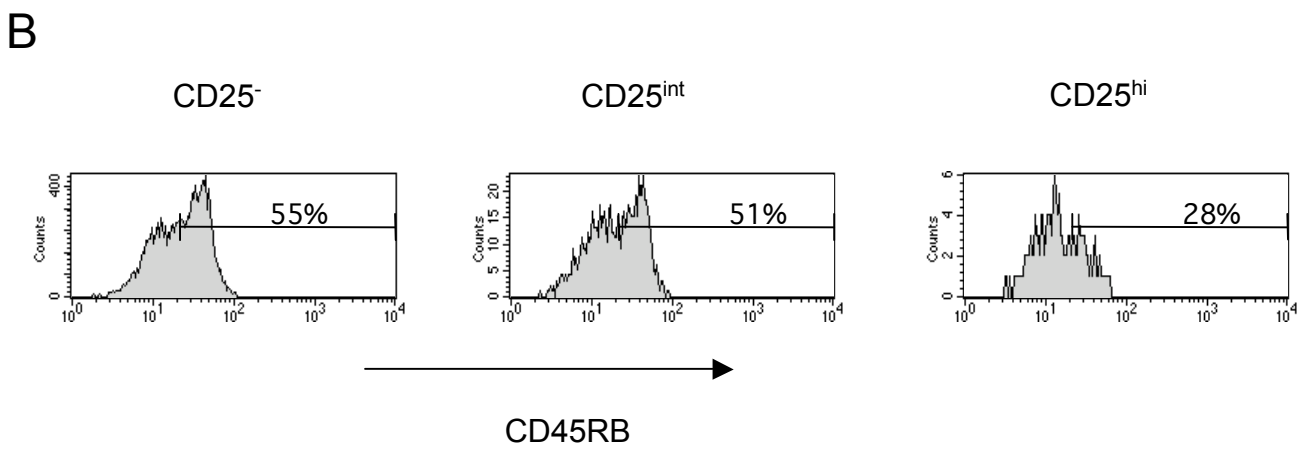
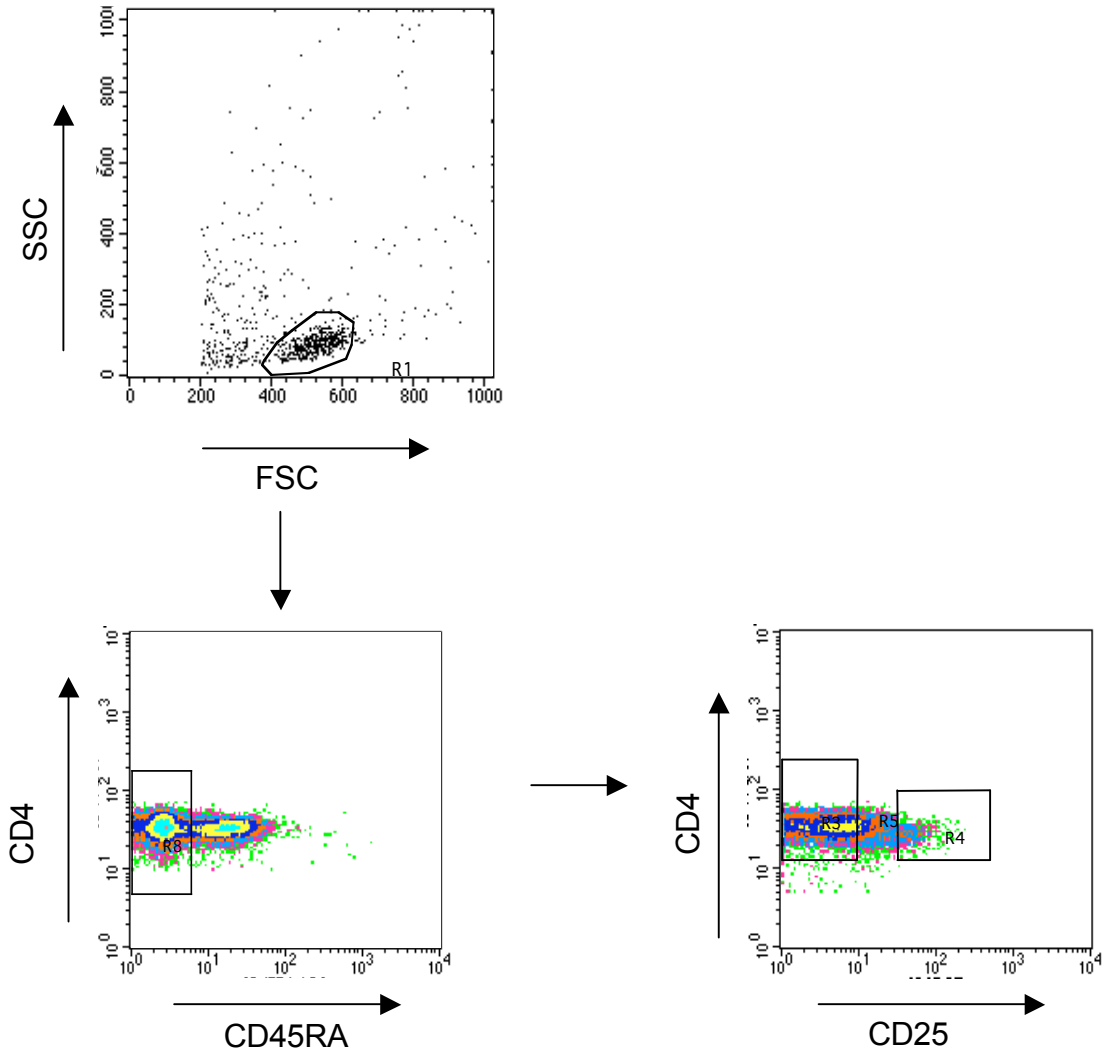
Supplemental figure 1

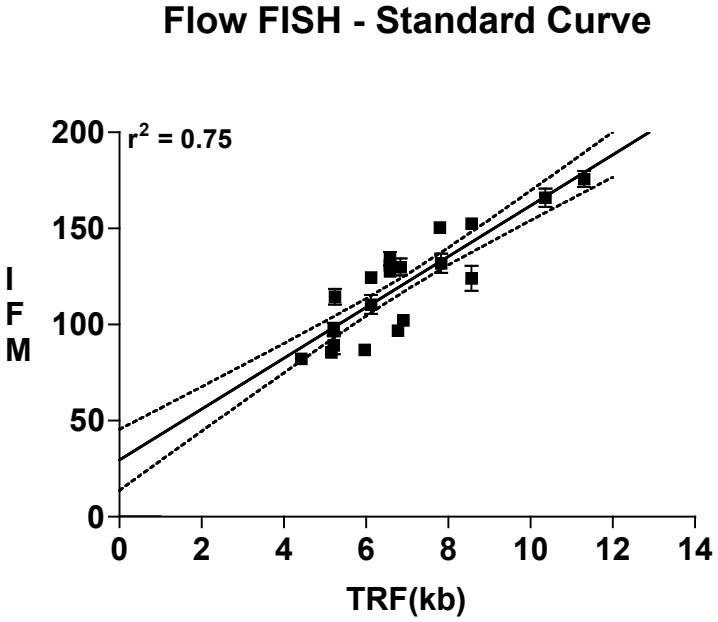




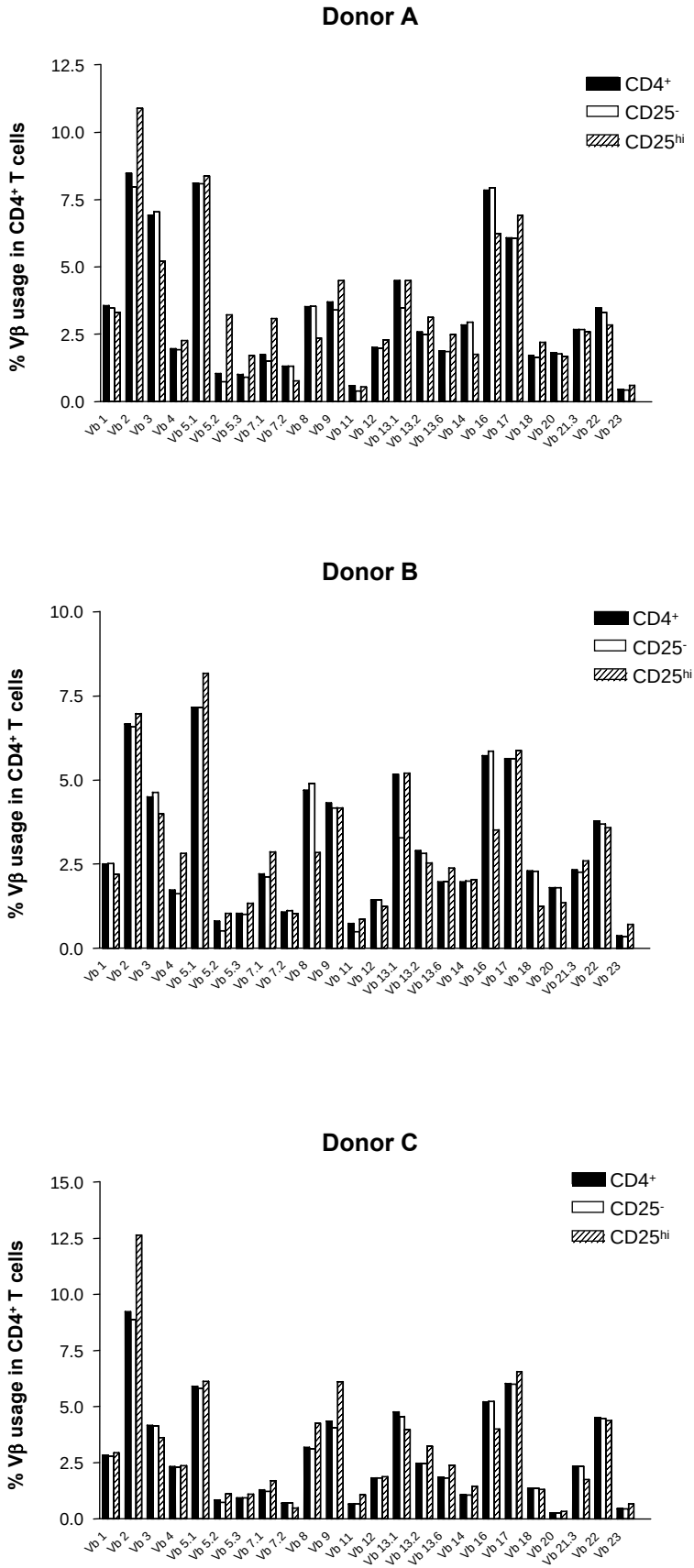
Supplemental figure 2



Supplemental figure 3



Supplemental figure 4



Supplementary Figure 1. CD4+CD25hi T cells have a highly differentiated memory phenotype. Freshly isolated PBMCs from young and old donors were stained with CD4-PerCP, CD25-PE and CD45RB-FITC and CD45RA-APC (**A**). Based on CD25 expression CD4 population is subdivided into $CD25^{-}$, $CD25^{int}$ and $CD25^{hi}$ (left panel) as described in Materials and Methods. Histograms illustrate the CD45RA expression in each population and the percentage of $CD45RA^{+}$ T cells is indicated. In (**B**), the histograms illustrate CD45RB expression in the 3 subsets. $CD4^{+}$ cells were first gated on the basis of CD45RA expression so that only $CD45RA^{-}$ ($CD45RO^{+}$) memory cells were analysed (as described in Materials and Methods). Percentage of $CD45RB^{hi}$ cells in each subset is indicated.

Supplementary Figure 2. Gating strategy used for FACS sorting of $CD25^{-}$, $CD25^{int}$ and $CD25^{hi}$ CD4 populations. Purified $CD4^{+}$ T cells were stained with anti-CD4 (PerCP), anti-CD45RA (FITC) and anti-CD25 (PE). Both $CD4^{+}CD25^{hi}$ and $CD4^{+}CD25^{-}$ populations were collected from the $CD45RA^{-}$ fraction and are therefore designated as $CD45RO^{+}$ for clarity. The $CD4^{+}CD25^{int}$ population was not collected to ensure stringency of the sorted $CD25^{-}$ and $CD25^{hi}$ populations. The same gating strategy was implemented for phenotypic analysis of freshly isolated PBMCs.

Supplementary Figure 3. Flow-Fish Standard curve. To construct the standard curve PBMC were collected from 20 donors of various ages. Half of each sample was used to isolate CD4+ T cells and telomere length was measured by Southern blot. The other half of the sample was used to measure telomeres by Flow Fish, gating on CD4+ T cells (in triplicate). All samples for the standard curve were run at the same time. TRF length (in kb) was plotted against mean fluorescence intensity (mean±SEM of triplicate samples) and a linear regression line was fitted using GraphPad Prism softwere. Dashed line indicates 95%confidence intervals.

Supplementary Figure 4. Comparison of TCR Vβ repertoires of $CD4^{+}CD25^{hi}$ and $CD4^{+}CD25^{-}$ cell populations. Freshly isolated PBMC from three young donors were stained with anti-CD4 and anti-CD25 and 24 different anti-TCR mAb

from the IOTest Beta Mark kit according to manufacturer's recommendations (Beckman Coulter). The TCR Vβ usage of the $CD4^+$ (black bars), $CD4+CD25^-$ (open bars) and $CD4^+CD25^{hi}$ (cross-hatched bars) T cell subsets was analysed on a FACSCalibur.