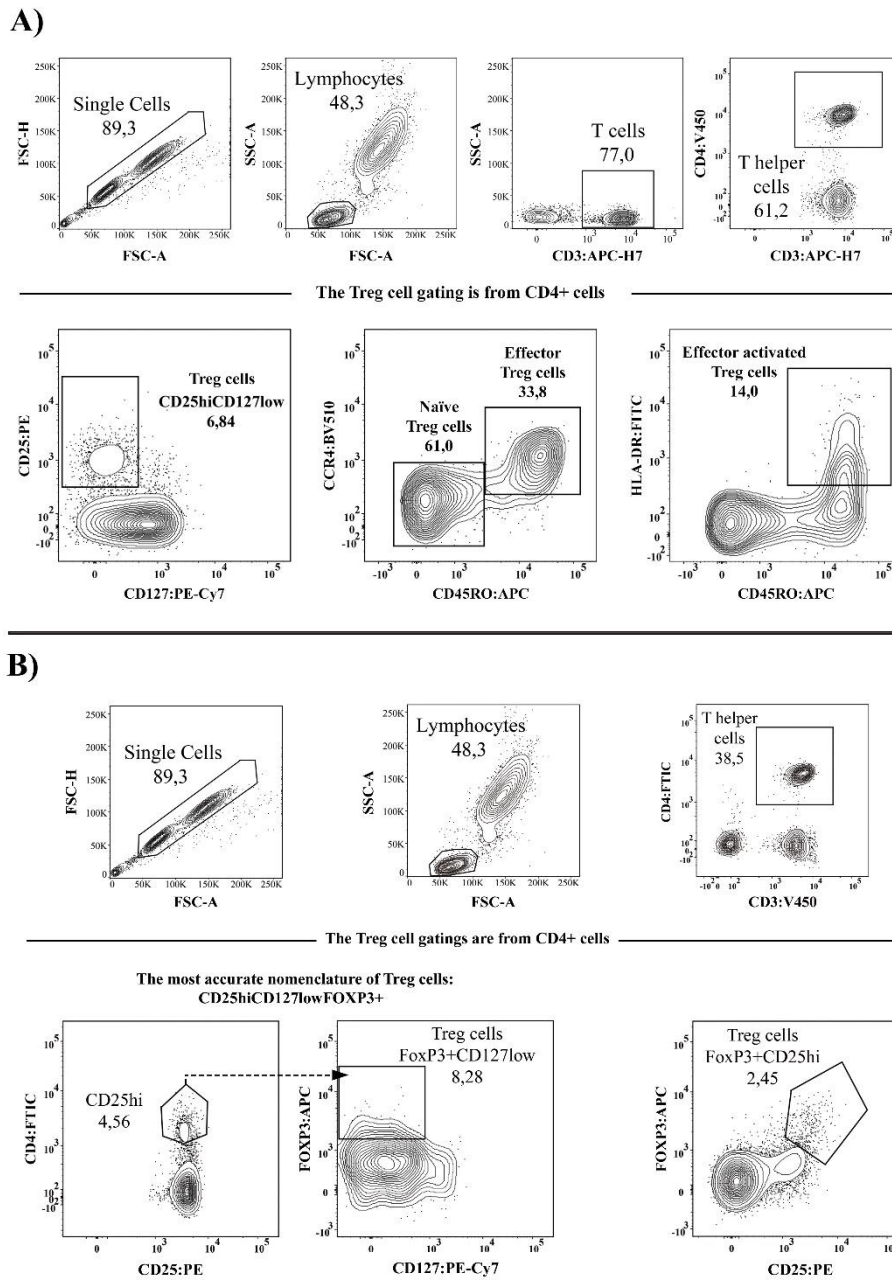


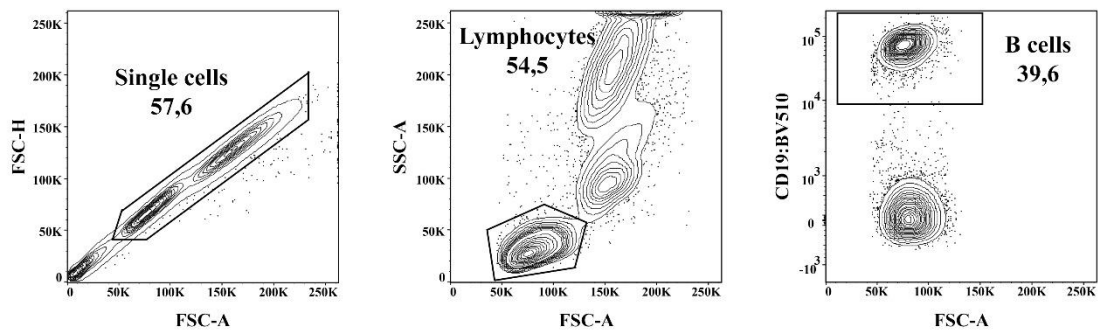
Title: Changes in Treg and Breg cells in a healthy pediatric population

Supplementary Material

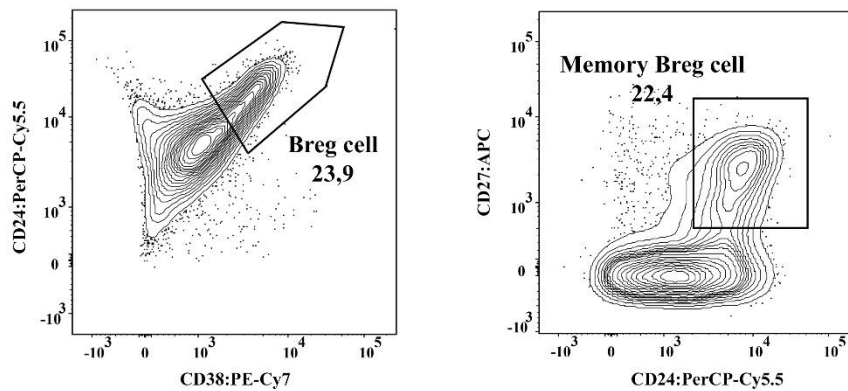
Supplementary Figure 1. Regulatory T cell (Treg) immunophenotyping gating strategy. A) Extracellular panel: $CD4^+CD25^{hi}CD127^{low}$, naïve Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^-CCR4^-$), memory Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^+CCR4^+$) and activated effector Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^+CCR4^+HLA-DR^+$). B) Intracellular panels: $CD4^+CD25^{hi}CD127^{low}Foxp3^+$, and $CD4^+CD25^{hi}FoxP3^+$.



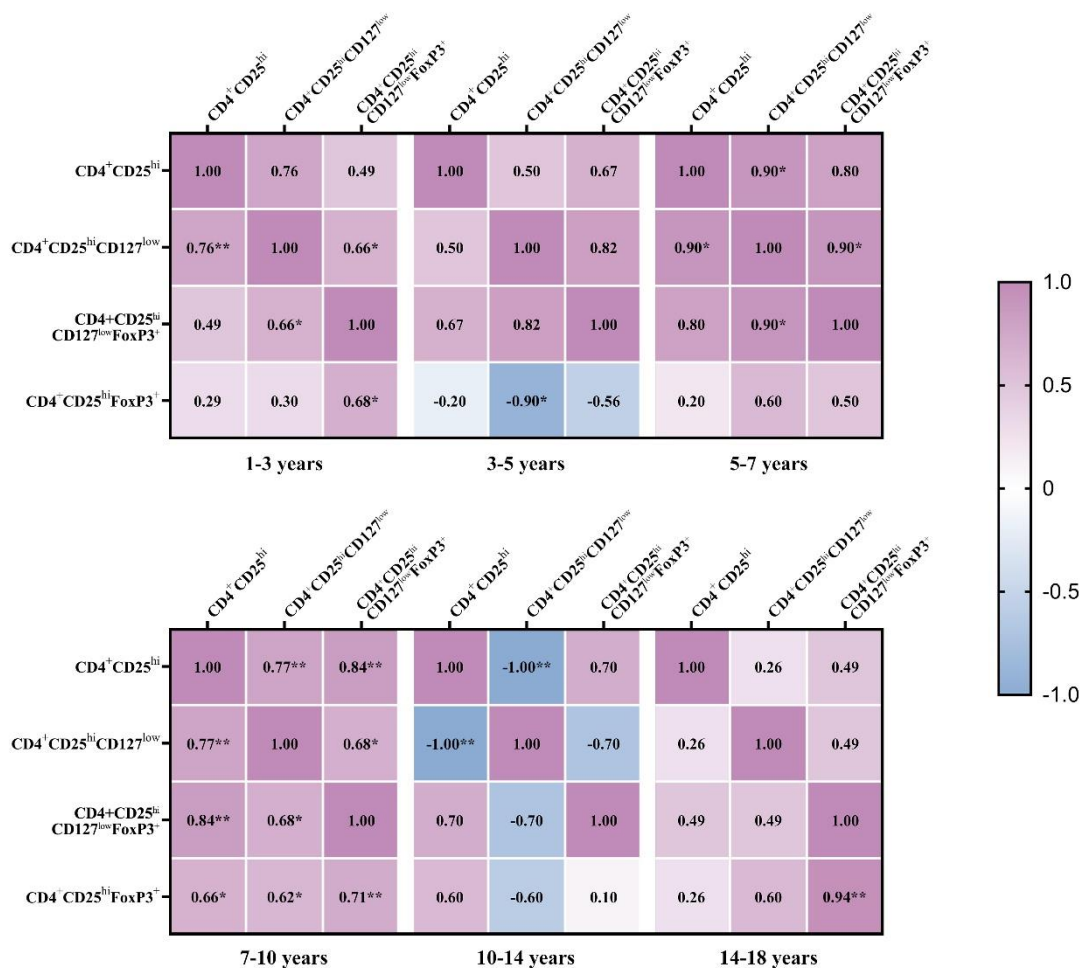
Supplementary Figure 2. Regulatory B cell (Breg) immunophenotyping gating strategy. Transitional Breg is defined as $CD19^+CD24^{hi}CD38^{hi}$ and memory Breg cell as $CD19^+CD24^{hi}CD27^+$.



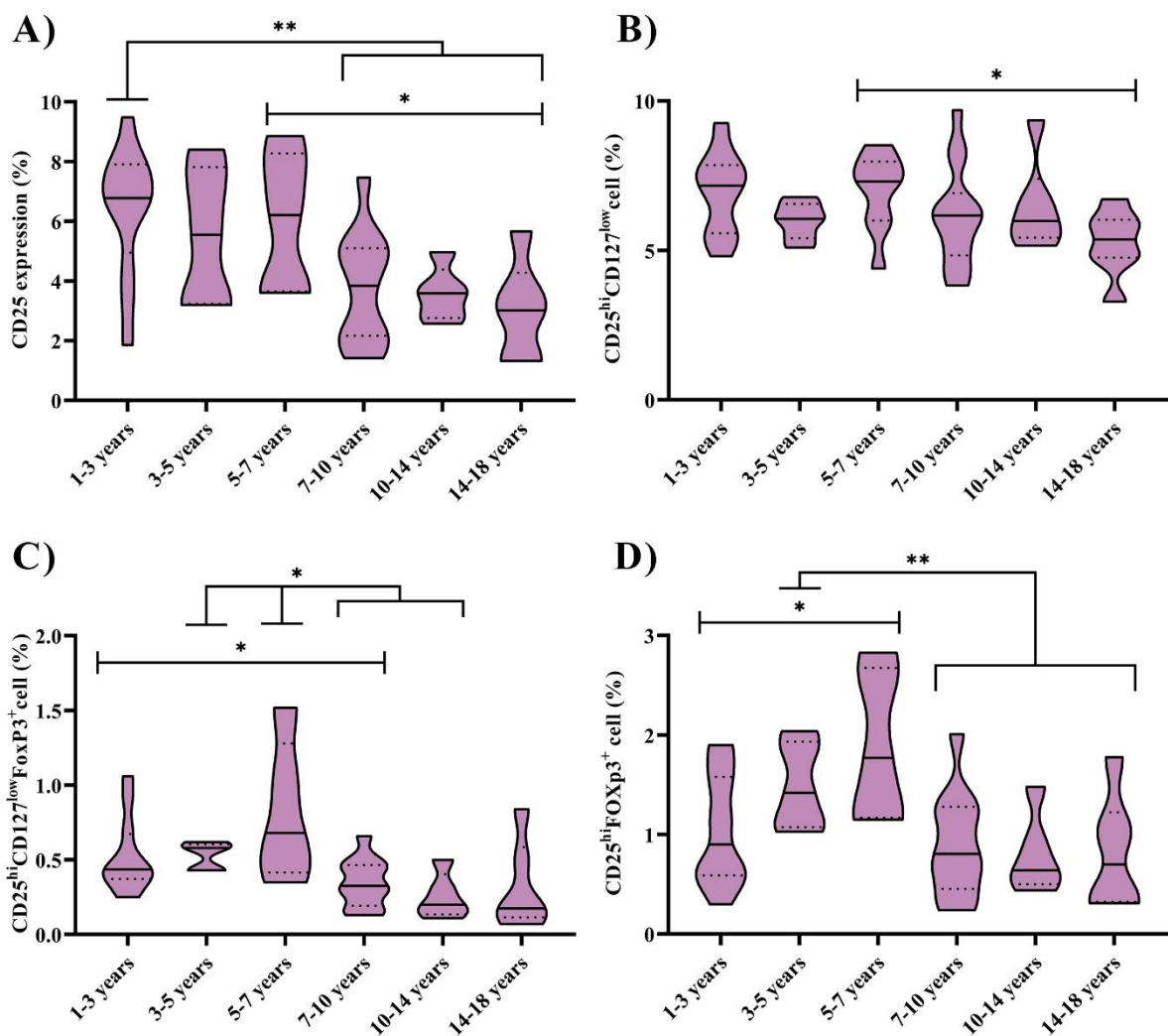
The Breg cell gating is from B cells



Supplementary Figure 3. Correlation between gating strategies of regulatory T cell (Treg) and CD4⁺CD25^{hi} by age. The three Treg definitions are CD4⁺CD25^{hi}CD127^{low}, CD4⁺CD25^{hi}CD127^{low}Foxp3⁺, and CD4⁺CD25^{hi}FoxP3⁺. The correlation between the three Treg definitions was moderate-strong for most of age ranges, being especially strong between the intracellular panels, and CD4⁺CD25^{hi} vs CD4⁺CD25^{hi}CD127^{low}. The values inside the boxes indicate the Spearman correlation strength. Low association 0.1-0.3; moderate positive association between 0.3-0.5; and strong positive association 0.5-1. (*) p value ≤ 0.05; (**) p value ≤ 0.01.

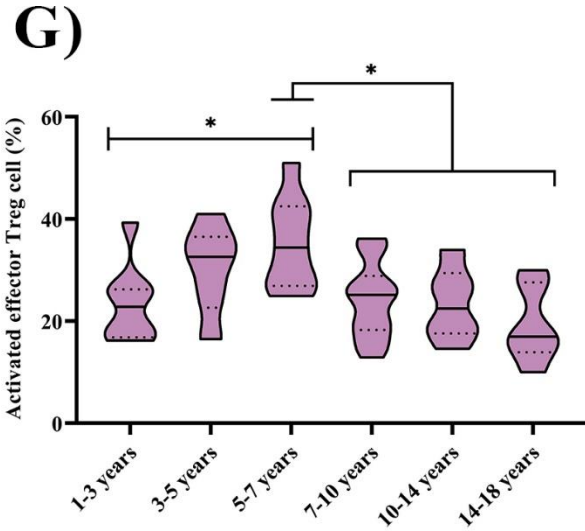
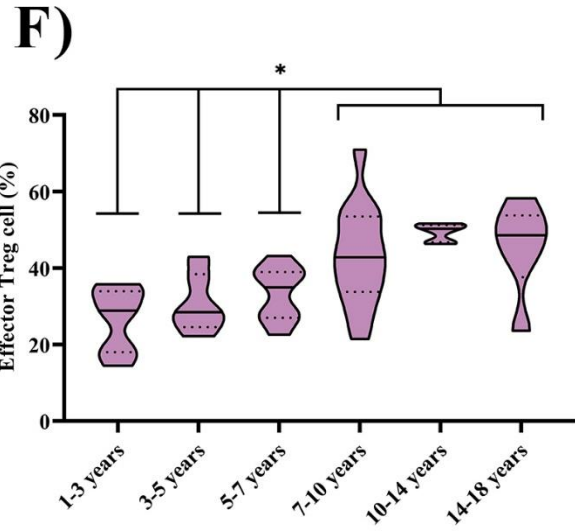
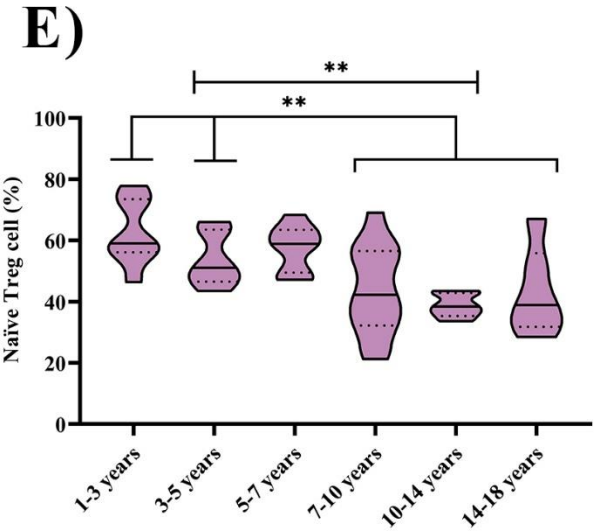


Supplementary Figure 4. Frequency of regulatory T cell (Treg) by age range. A), B), C) and D) Total Treg (median/%) decreases mainly after 7 years onwards. E) Decrease of naïve Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^-CCR4^-$) with age. F) Increase of effector Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^+CCR4^+$) with age. G) Increment of effector activated Treg ($CD4^+CD25^{hi}CD127^{low}CD45RO^+CCR4^+HLA-DR^+$) between 3-7 years. E), F) and G) suggest a process of maturation of Treg with age. The frequency of CD25, $CD25^{hi}CD127^{low}$, $CD25^{hi}CD127^{low}FoxP3^+$ and $CD25^{hi}FoxP3^+$ are calculated from the $CD4^+$ cells. The naïve and effector Treg are from $CD25^{hi}CD127^{low}$. (*) p value ≤ 0.05 ; () p value ≤ 0.01 . Discontinuous line: quartiles; Black solid line: median.**



(continued on next page)

(continued)



Supplementary Figure 5. Frequency of regulatory B cell (Breg) by age range. A) The total Breg (CD19⁺CD24^{hi}CD38^{hi}) (median/%) decrease mainly after 3 years onwards. **B)** Increment of memory Breg (CD19⁺CD24^{hi}CD27⁺) frequency with age. The frequency of both Breg and memory Breg was calculated from CD19⁺. (*) p value ≤ 0.05 ; (**) p value ≤ 0.01 . Discontinuous line: quartiles; Black solid line: median.

