

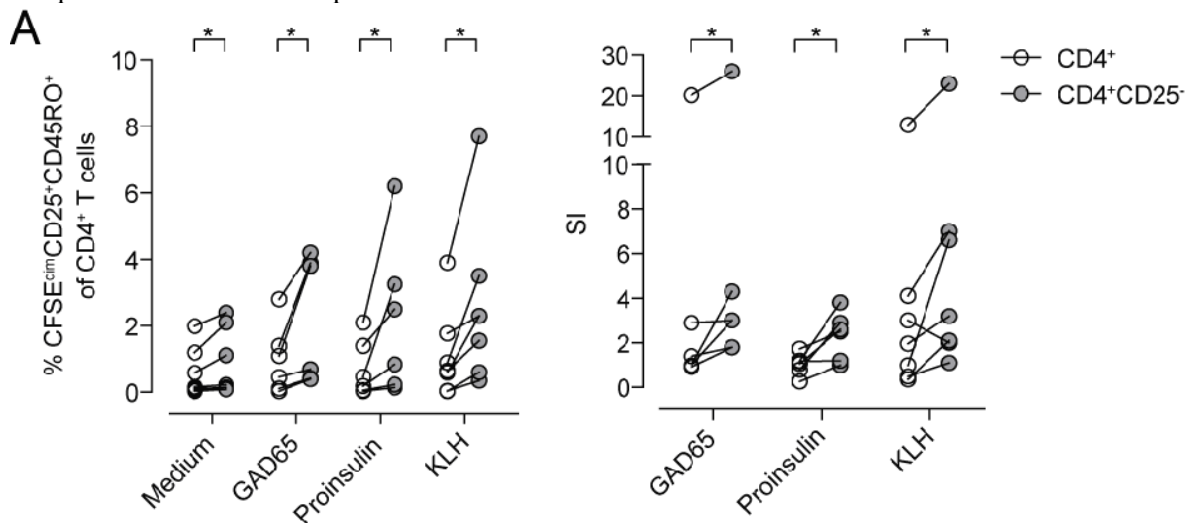
## SUPPLEMENTARY DATA

### Data justification:

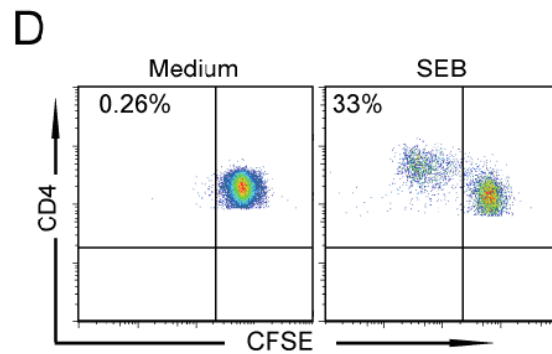
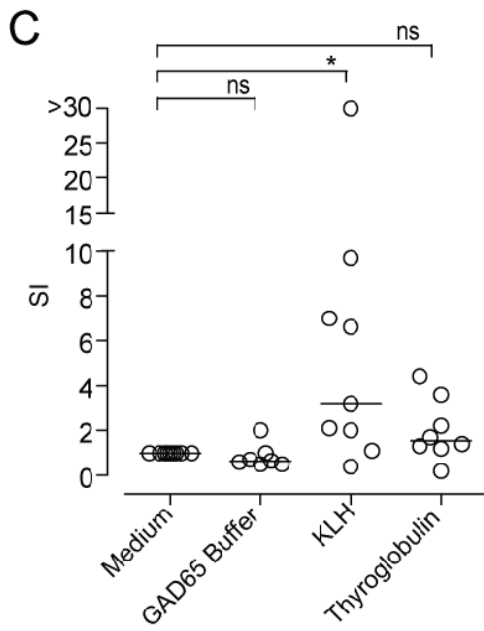
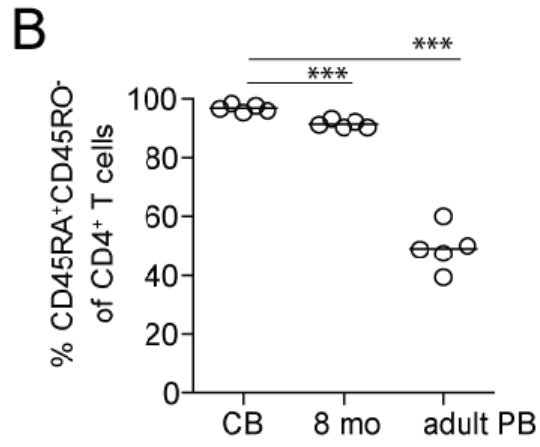
The additional figures are essential in supporting the understanding of the paper. They provide additional rationale and validation of methods (SFig. 1) and further control data (SFig. 2 and 3) and thus, give an additional support of the main conclusions made in the article. These results may also be utilized by others in their future research.

The 3 supplementary figures and their figure legends are below:

**Supplementary Figure 1.** Depletion of CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cells unmasks autoreactive proliferative responses. **A)** CB CD4<sup>+</sup> (open circles) or CD4<sup>+</sup>CD25<sup>-</sup> (filled circles) T cells from healthy newborns were stimulated for 6 days with autologous DC loaded with GAD65, proinsulin, or KLH. Responses were determined as %CFSE<sup>dim</sup>CD25<sup>+</sup>CD45RO<sup>+</sup> of CD4<sup>+</sup> T cells (A) and SI over medium (B) at the end of culture calculated from the mean of triplicate wells. (7 subjects for medium and KLH; 6 subjects for GAD65 and proinsulin); \* P<0.05, ns P>0.05. CB CD4<sup>+</sup> T cell responses to antigen were consistently higher when Treg had been removed (left panel). Background responses (medium) were only slightly higher in the absence of Treg (CFSE<sup>dim</sup>CD25<sup>+</sup>CD45RO<sup>+</sup> of CD4: median 0.16% [IQR 0.07-1.2%] with Treg vs. 0.22% [IQR 0.14-2.1] without Treg), and stimulation indices (right panel) were significantly increased in the absence of Treg cells when cells were stimulated with the autoantigens GAD65 (SI: median 1.2 [IQR 0.9-7.2] with Treg vs. 3.0 [IQR 1.8-9.7] without Treg, P=0.03) or proinsulin (SI: median 1.1 [IQR 0.7-1.3] with Treg vs. 2.6 [IQR 1.2-3.1] without Treg, P=0.03), or the exogenous antigen KLH (SI: median 1.9 [IQR 0.5-4.1] with Treg vs. 3.2 [IQR 2.0-7.0] without Treg, P=0.05). **B)** Percentage of CD4<sup>+</sup> T cells that express CD45RA and not CD45RO in CB (n=5) and PB from 8 month old infants (n=5), or adults (n=5). \*\*\* P<0.001. **C)** In a subset of the CB from healthy newborns (9 subjects), also responses of CD4<sup>+</sup>CD25<sup>-</sup> T cells against the GAD65 vehicle, KLH and thyroglobulin were tested. Shown here is the stimulation index (SI) over the control (medium) and represents for each condition the mean of triplicate wells; \* P<0.05, ns P>0.05. **D)** CBMC from a healthy newborn were labeled with CFSE and stimulated with medium or SEB (0.1 μg/ml) for 6 days. Proliferating CD4<sup>+</sup>CFSE<sup>dim</sup> of CD4<sup>+</sup> T cells are shown in % in the upper left quadrant of each FACS plot

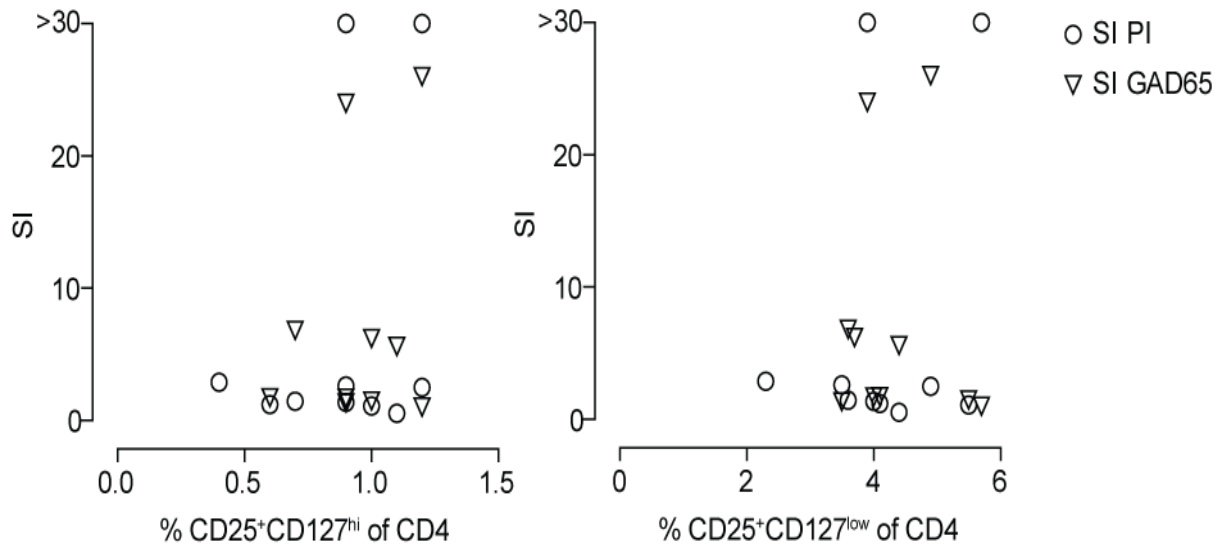


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**Supplementary Figure 2.** Correlation between the stimulation index (SI) for responses to autoantigen (GAD65, triangles; proinsulin, circles) and the percentage of CD25<sup>+</sup>CD127<sup>hi</sup> (left panel) and CD25<sup>+</sup>CD127<sup>low</sup> (right panel) of CD4<sup>+</sup> T cells present in the CB from 11 of the 20 healthy newborns reported in Figure 1.



**Supplementary Figure 3.** Treg in CB and Treg markers after culture of CB CD4<sup>+</sup>CD25<sup>-</sup> T cells with GAD65 and IL-7. A. Upper panel: FACS plots representing the gating strategy to identify CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> T cells in CB. The CD3<sup>+</sup>CD4<sup>+</sup> population (R1) was gated, analyzed to identify the CD25<sup>+</sup>CD127<sup>low</sup> population (R2), and the FOXP3 expression of R2 was determined. The graph on the right shows enumeration as the proportion of CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> amongst CD4<sup>+</sup> T cells in CB (n=16), 8 months old infants (n=5) or adult PB (n=3). Lower panel: FACS plots representing the expression of Helios in CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> T cells (R3) in CB (left) and adult PB (right). The graph on the right shows enumeration of Helios negative CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> Treg in CB (n=11) and adult PB (n=3). \*\*\* P<0.001. B. Upper panel: FACS plots representing the gating strategy to identify CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> T cells (R3) and Helios negative CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> T cells (R4) after 5 days stimulation of CB CD4<sup>+</sup>CD25<sup>low</sup> T cells with dendritic cells plus GAD65 in the presence of 0.1 ng/ml of IL-7. Lower panel: CD4<sup>+</sup>CD25<sup>-</sup> CB T cells from 4 of the healthy newborns (indicated as individual symbols) were stimulated with dendritic cells loaded with medium or GAD65 in the absence (0 ng/ml) or presence of IL-7 (0.1 and 1.0 ng/ml). After 5 days of culture the percentage of CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> of CD4<sup>+</sup> T cells (left) and CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup>Helios<sup>negative</sup> of CD4<sup>+</sup> T cells (right) was determined as shown in the upper panels.

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