

SUPPLEMENTARY ONLINE REPOSITORY

SUPPLEMENTARY FIGURE 1. Blood Tfh and Tfr cells in human T1D. (A) The non-parametric (Spearman) two-tailed test was used for correlation analyses between percentage of Tfh, Tfr, CXCR5⁻ Treg cells and years with T1D in living subjects with symptomatic T1D. (B) Percentage of Tfr (CXCR5⁺FOXP3⁺), CXCR5⁻Treg (CXCR5⁻FOXP3⁺), and Tfh (CXCR5⁺FOXP3⁻) cells in single AAb⁺ ($n = 7$), stage 1 ($n = 6$) and stage 2 ($n = 9$). (C) Graph shows Tfr (CXCR5⁺FOXP3⁺), cells in other (non-DR3 or DR4 HLA) ($n = 6$), DR3/DRX ($n = 9$), DR4/DRX ($n = 4$) and DR3/DR4 ($n = 4$). (D) The non-parametric (Spearman) two-tailed test was used for correlation analyses between percentage of Tfh, Tfr, CXCR5⁻ Treg cells and Tfh/Tfr ratio with plasma CXCL13 concentration.

SUPPLEMENTARY FIGURE 2. Distribution of Tfh subsets in patients with pre-symptomatic and symptomatic T1D. (A) Representative flow cytometry plots (B) and graph for Tfh2 (CXCR3⁻CCR6⁻), Tfh17 (CXCR3⁻CCR6⁺), Tfh1/17 (CXCR3⁺CCR6⁺) and Tfh1 (CXCR3⁺CCR6⁻), gated on singlets, lymphocytes, CD3⁺CD19⁻ and CD4⁺, CD4⁺CXCR5⁺ cells. (C) Representative flow cytometry plots for CXCR3⁻PD-1⁺ Tfh cells, gated on singlets, lymphocytes, CD3⁺CD4⁺, CD4⁺CXCR5⁺ cells. (D) Percentage of CXCR3⁻ PD-1⁺ Tfh cells from patients with presymptomatic and symptomatic T1D. Tfh cell subsets in AAb⁻ ($n = 14$), AAb⁺ ($n = 23$) and T1D ($n = 31$). In all graphs, each point represents an individual donor with the median indicated by a black line. Asterisks indicate statistical significance as calculated by Kruskal-Wallis. * $p < 0.05$ ** $p < 0.005$

SUPPLEMENTARY FIGURE 3. Expression of CD25 and functional properties of tissue-resident CXCR5⁺FOXP3⁺ Tfr cells. (A) gating strategy for sorting Tfh (CXCR5⁺CD25⁻), Tfr (CXCR5⁺CD25⁺), Tconv (CXCR5⁻CD25⁻) and Treg (CXCR5⁻CD25⁺) cells sorted from spleen of BDC2.5 mice. (B) Representative gating and (C) frequencies of Tfh (CXCR5⁺FOXP3⁻), Tfr (CXCR5⁺FOXP3⁺) and CXCR5⁻Treg cells (CXCR5⁻FOXP3⁺) from spleen and BDC2.5 mice ($n = 4$). (D) Representative gating and (E) frequencies of Tfh, Tfr and CXCR5⁻ Treg cells from peripheral blood of adoptively transferred mice at day 12 post injection. In all graphs, points

represent individual mice. Asterisks indicate statistical significance as calculated by paired-t test.
**p < 0.01.