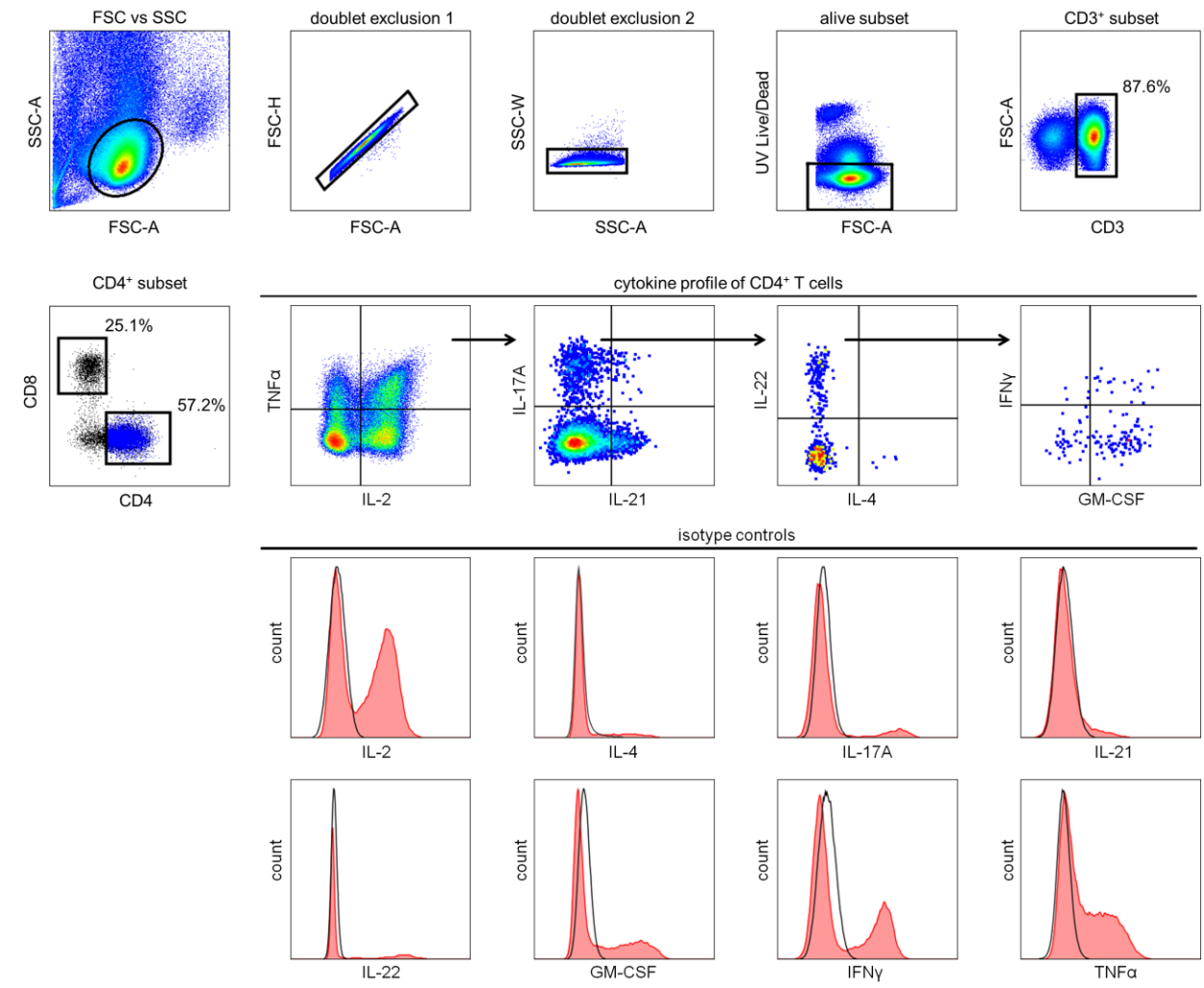
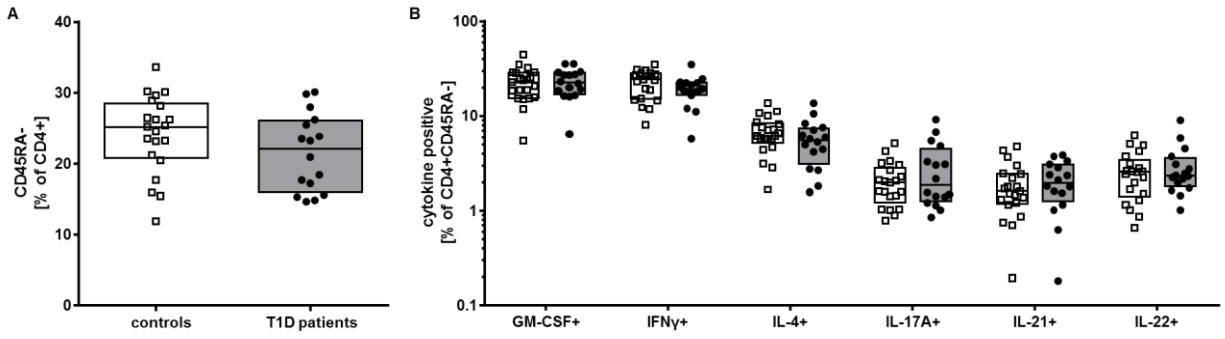


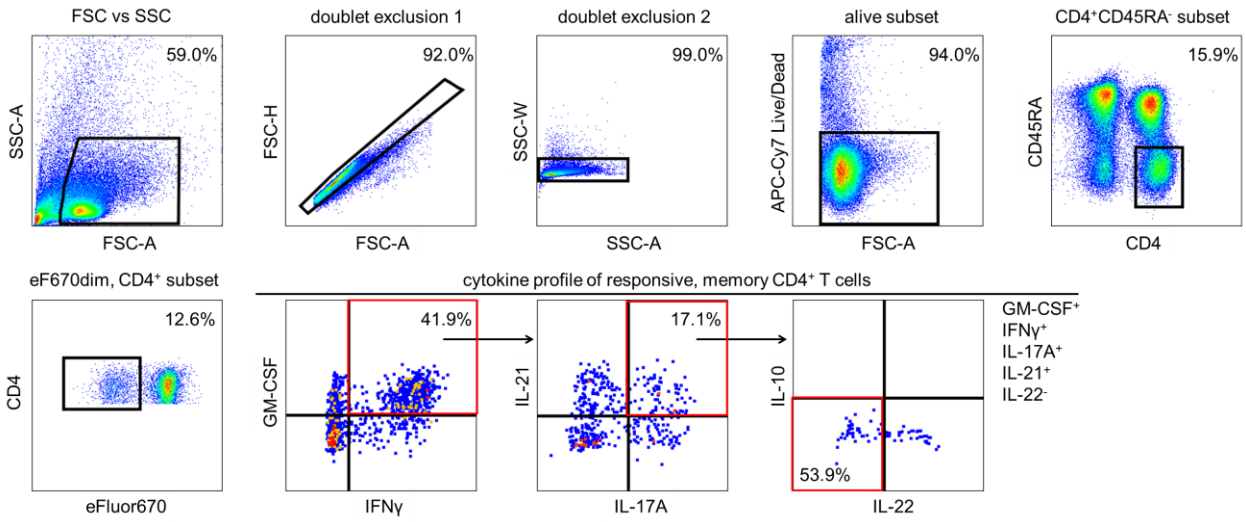
Supplementary Fig. S1: Representative FACS plot of established intracellular cytokine staining in cryo-conserved PBMCs.

Gating strategy used to analyze cytokine producing $CD4^+$ T cells. Single lymphocytes were identified by forward and side scatter, live cells gated as viability dye eFluor455UV negative, and $CD3^+$ and $CD4^+$ cells gated were analyzed for IL-2, TNFα, GM-CSF, IFNγ, IL-4, IL-17A, IL-21 and IL-22 according to respective isotype controls. Arrows in the cytokine staining plots indicate the double cytokine positive quadrant used in the analysis of the adjacent plot.



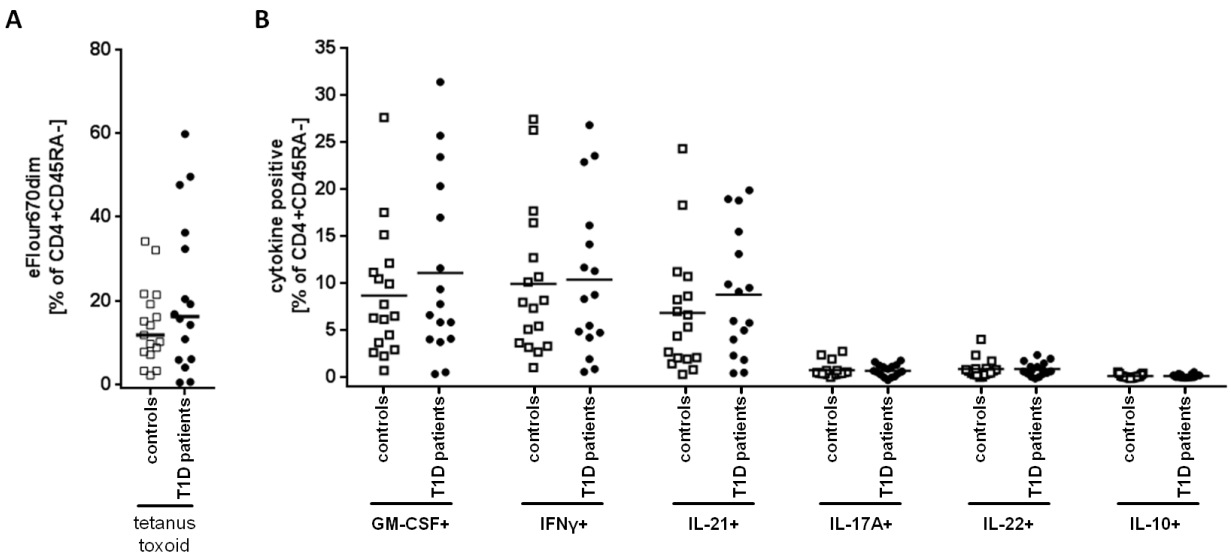
Supplementary Fig. S2: Distribution of memory CD4$^+$ T cells in children at onset of T1D.

A) The frequency of CD45RA$^-$ cells amongst CD4$^+$ T cells (y axis) is indicated for the control subjects (control, open squares, n=16) and patients with type 1 diabetes (T1D patients, circles, n=21). The median and interquartile range is indicated for each group (open for control, and shaded for T1D patients). No differences were observed between groups (Mann-Whitney U test). B) The frequency of cytokine-positive CD4$^+$CD45RA$^-$ T cells (y axis) for cytokines GM-CSF, IFNγ, IL-4, IL-17A, IL-21 and IL-22. The median and interquartile ranges are shown for the control subjects (open, n=16) and the T1D patients (shaded, n=21). The data were interpolated from the frequencies in total CD4$^+$ T cells shown in Figure 1 and the frequency of CD45RA$^-$ cells within the CD4$^+$ T cells shown in Supplementary Figure 2a. No differences were observed between groups (Mann-Whitney U test).



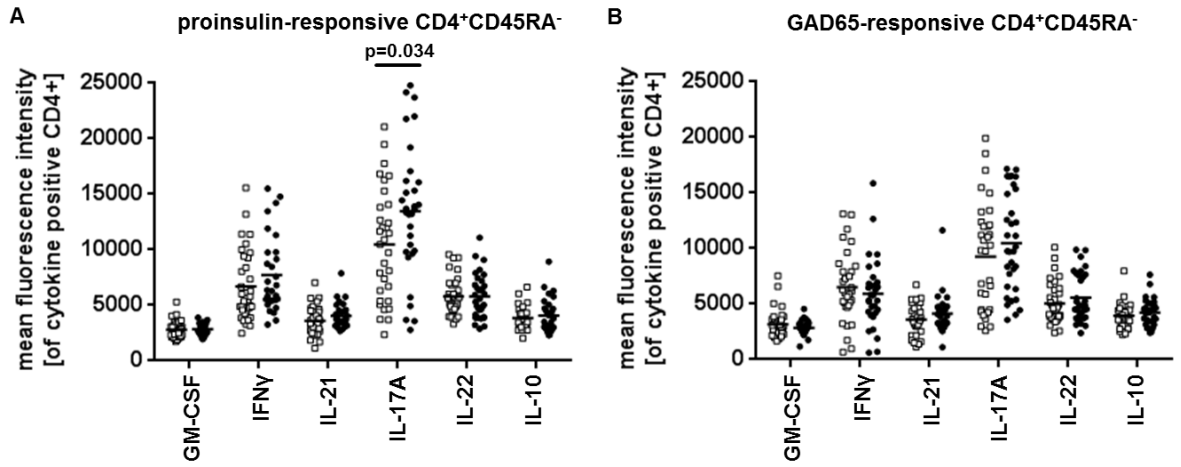
Supplementary Fig. S3: Gating strategy of the established cytokine staining of antigen-responsive CD4$^+$CD45RA$^-$ T cells.

Representative FACS plot of applied boolean gating approach to identify specific GM-CSF, IFNγ, IL-17A, IL-21, and IL-22 cytokine profiles in antigen-responsive CD4$^+$CD45RA$^-$ T cells following stimulation with proinsulin or GAD65. The example shows the identification of GM-CSF$^+$IFNγ$^+$IL-17A$^+$IL-21$^+$IL-22$^-$ proinsulin-responsive CD4$^+$CD45RA$^-$ T cells.



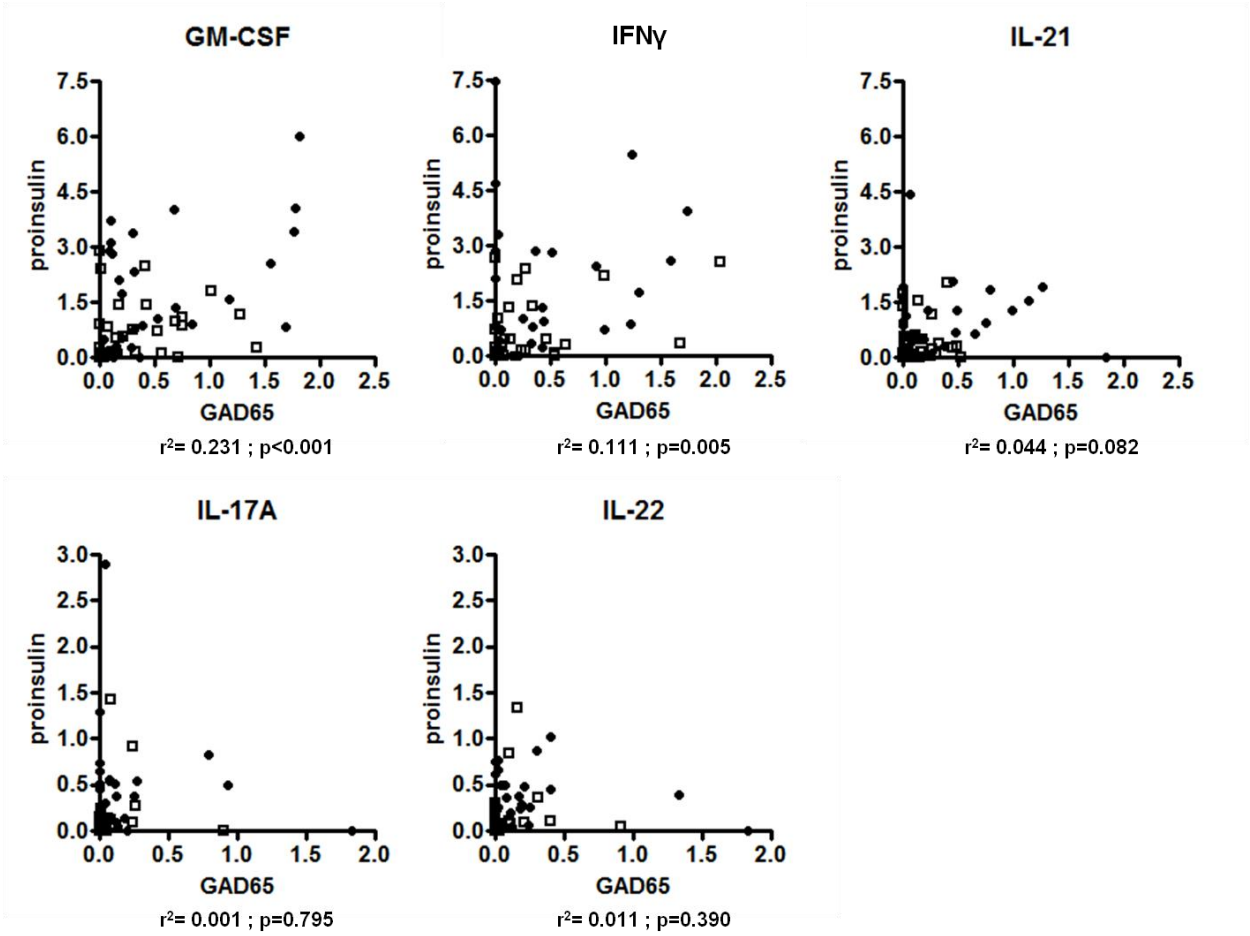
Supplementary Fig. S4: Proliferation and cytokine production of memory $CD4^+$ T cells in presence of tetanus toxoid.

A) Frequencies of tetanus toxoid-responsive $CD4^+CD45RA^-$ T cells and B) flow cytometry identification of cytokine production in $CD4^+CD45RA^-$ T cells responsive to tetanus toxoid in islet autoantibody-negative children (open squares, n=36) and patients with T1D (circles, n=33). Indicated in each scatter plot is the mean and no significant differences were obtained using the Students unpaired T test



Supplementary Fig. S5: Mean fluorescence intensity of antigen-specific, cytokine positive CD4$^+$CD45RA$^-$ T cells.

Cytokine expression of cytokine-positive CD4$^+$CD45RA$^-$ T cells in the presence of A) proinsulin, and B) GAD65 in islet autoantibody-negative children (open squares, n=36) and patients with T1D (circles, n=33). The mean fluorescence intensity (MFI) of cytokine positive, antigen-responsive CD4$^+$CD45RA$^-$ T cells is shown (y axis) for each sample. Indicated in each scatter plot is the mean and p-values were obtained using the Students unpaired T test. Significant p-values <0.05 (Students unpaired T test) are no longer significant after considering multiple testing (n = 6 cytokines).



Supplementary Fig. S6: Correlation between cytokine producing, GAD65- and proinsulin-responsive $CD4^+CD45RA^-$ T cells.

Linear regression of cytokine producing, proinsulin- and GAD65-responsive $CD4^+CD45RA^-$ T cell frequencies in islet autoantibody-negative children (open squares, n=36) and patients with T1D (circles, n=33). Linear regression of all data points for each cytokine tested is indicating by the coefficient of determination (r^2); p values are not adjusted for the number of cytokines analyzed (n = 6 cytokines). IL-10 producing cells were few and are not shown here.

Supplementary table S1: Subjects included in the study.

	Cytokine producing $CD4^+$ T cells after polyclonal stimulation		Cytokine production of antigen-responsive $CD4^+$ T cells	
sample group	controls	T1D patients	controls	T1D patients
sex (m/f)	(12/11)	(8/10)	(17/19)	(17/16)
median age, years (range)	10.8 (7.0 - 13.7)	10.1 (7.4 - 13.7)	13.2 (10.5 - 15.3)	14.1 (5.2 - 31.4)
median time after onset, days (range)	-	11 (3 - 31)	-	14 (4 - 343)
HLA DR3 (%, frequency of subjects)	32	33	31	50
HLA DR4 (%, frequency of subjects)	64	60	54	60