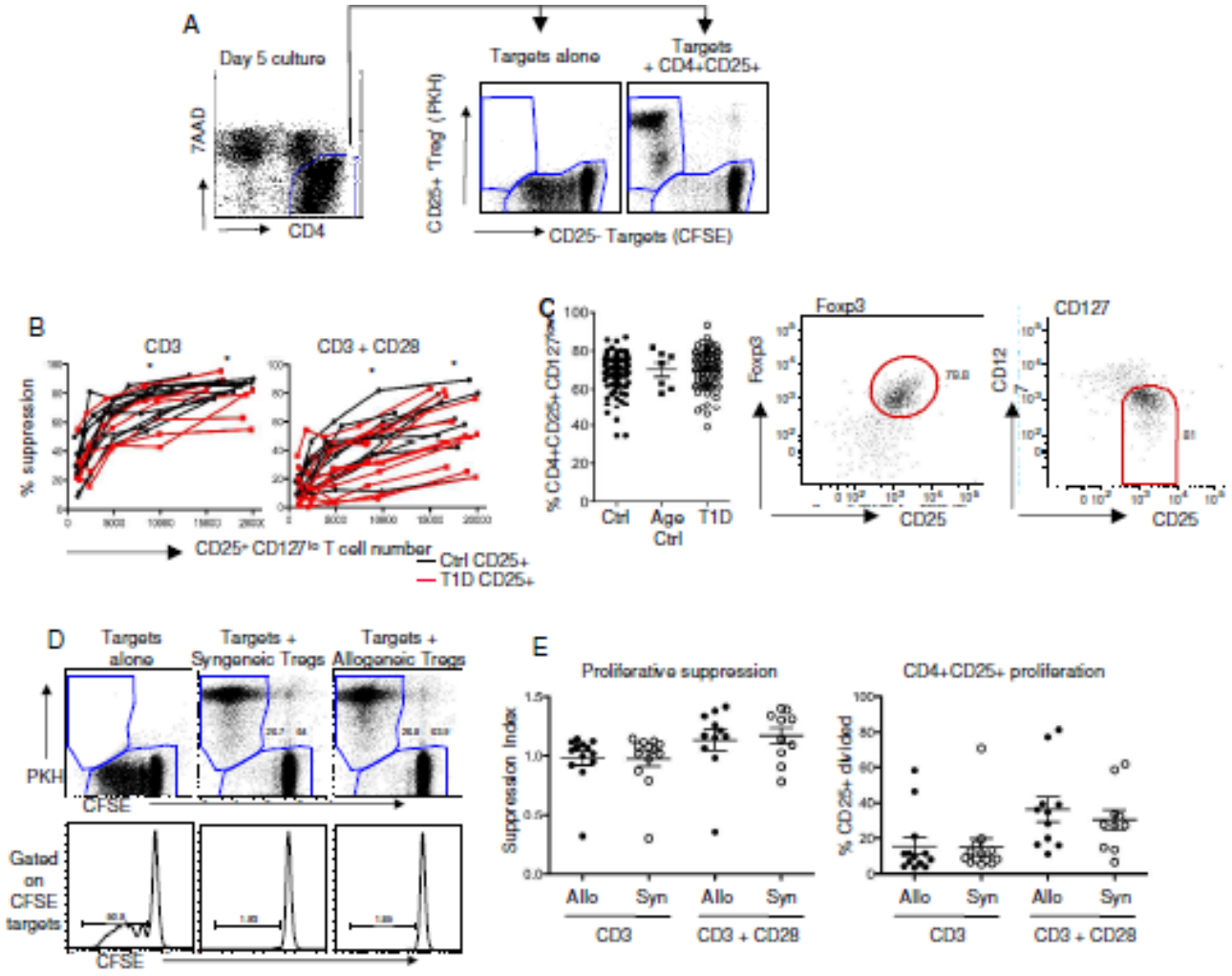
SUPPLEMENTARY DATA

Supplementary Figure 1. Single cell suppression assay using differentially labeled CD25- targets and CD25+ Tregs. A) Gating strategy for proliferation analyses on cultured cells Day 5. Representative dot-plots of cells in culture after anti-CD3 and anti-CD28 mAb stimulation. CFSE-labeled CD4+CD25- targets alone or in co-culture with PKH-labeled CD4+CD25+ Tregs. B) % suppression of CD4+CD25- target T cell proliferation by CD4+CD25+ T cells. Graphs are plotted by input number of CD25+CD127^{lo} T cells with a fixed number of targets (5 x 104). To directly test ability of T1D CD4+CD25+ T cells to suppress, T1D Tregs were co-cultured with control (non-diabetic) targets and stimulated with control APC and anti-CD3 or anti-CD3/CD28 as in A) and compared to co-cultures with control CD4+CD25+ T cells . * p=<0.05 by Wilcoxon rank between T1D and Control CD4+CD25+ suppression. C) Cumulative purity of CD4+CD25+ T cells after Miltenyi bead selection and representative dot plots of Foxp3 and CD127 staining. D-E) Effects of allo-stimulus on Treg suppression. D) Representative dot-plots of Targets cultured with syngeneic APC and anti-CD3 Ab alone or with syngeneic or allogeneic CD4+CD25+ Tregs. E) Collective analysis of the effect of allo-stimulation on Treg suppression (left panel) and CD4+CD25+ proliferation (right panel) under anti-CD3 and anti-CD3 + anti-CD28 stimulation conditions. Bars show mean and SEM. No statistical difference between allogeneic and syngeneic cultures using 2-tailed Mann-Whitney.



SUPPLEMENTARY DATA

Supplementary Figure 2. Clinical data for T1D study subjects. A) Summary table of demographic and clinical data for T1D subjects. B) Summary table of demographic data of control subjects. C) Individual HbA1c% over the study period. Onset = within 48 hours of diagnosis, months = time from diagnosis. Arrows indicate the three subjects that presented with diabetic ketoacidosis.

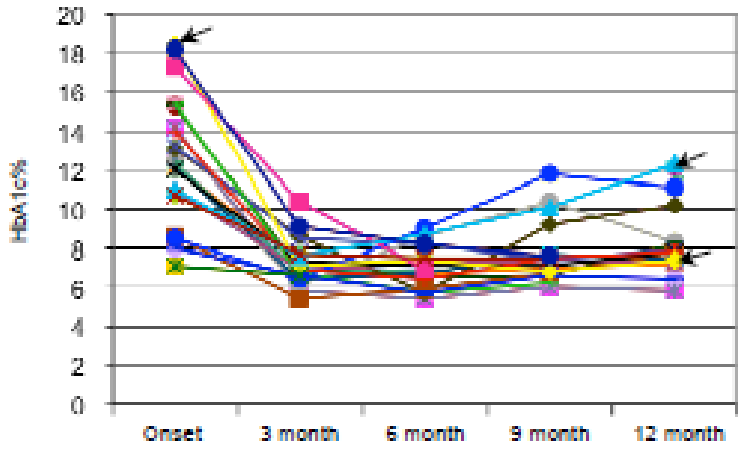
A

Demographic/clinical data for the patients in study with one or more autoAb positive DM1.					
n=21	Diagnosis	3 months	6 months	9 months	12 months
Age (years)	12.81 (SD 2.8)				
Body mass index	20.09 (SD 2.95)				
Glucose at Dx (mg/dl)	434.29 (SD 149.6)				
HbA1c % (normal <6%)	12.63 (SD 3.17)	7.20 (SD 1.16)	7.04 (SD 1.07)	7.79 (SD 1.54)	8.20 (SD 1.74)
Insulin dose at Dx (u/kg/d)	0.80 (SD 0.25)	0.69 (SD 0.30)	0.71 (SD 0.48)	0.70 (SD 0.47)	0.78 (SD 0.43)
15 males, 6 females. Mean (SD). Three subjects presented in diabetic ketoacidosis					

B

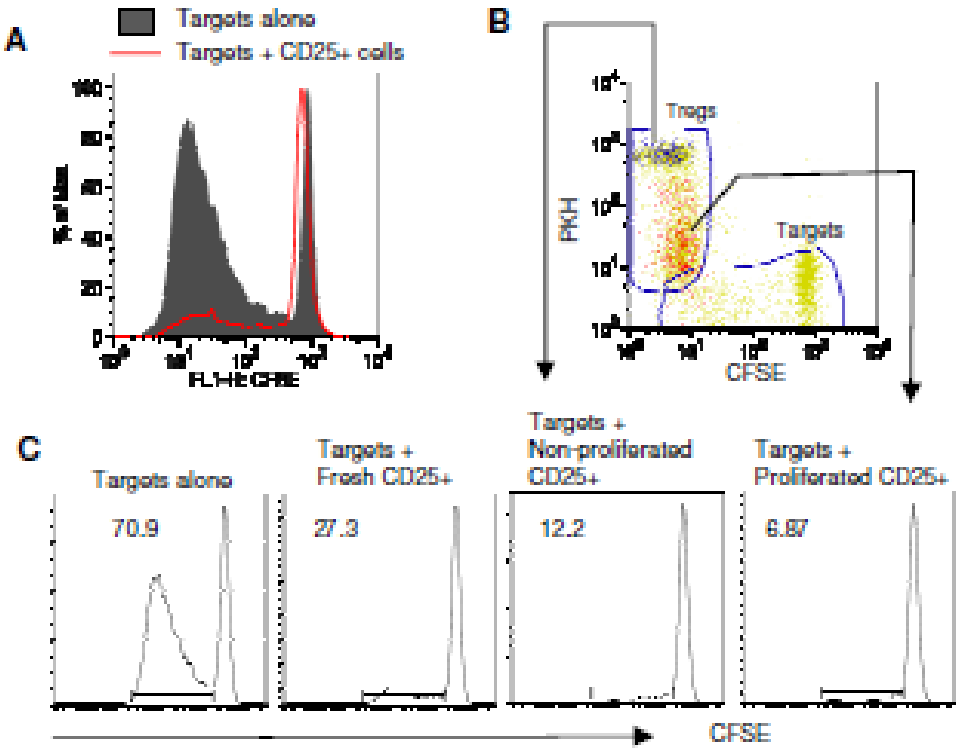
Demographic data for control subjects				
	Gender (M/F)	Mean Age	Median Age	Age Range
Age-matched n=22	13/9	13.23 (SD 3.023)	13.5	8-18
Controls n=70	33/37	35.14 (SD 10.53)	32	20-58

C



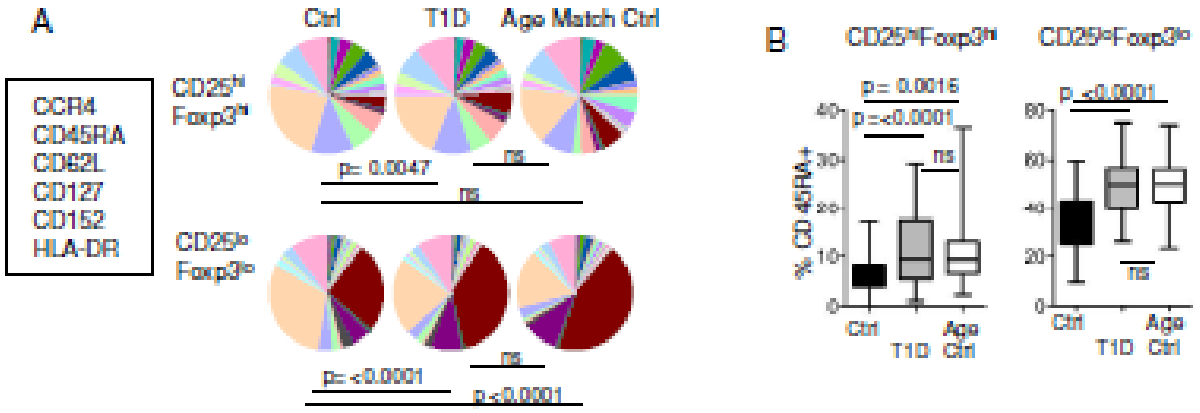
SUPPLEMENTARY DATA

Supplementary Figure 3. Proliferating CD4+CD25+ cells retain suppressive function CFSE-labeled CD4+CD25- targets were stimulated with anti-CD3/CD28 alone or in co-culture with PKH-labeled CD4+CD25+ Tregs. A) Histogram of CFSE gated on target T cells, day 5 of culture. B) Dot plot of co-culture on day 5. PKH-labeled CD4+CD25+ cells were sorted on the basis of PKH label into non-proliferated (PKH^{hi}, black events on post-sort analysis) and proliferated (PKH^{low}, red events on post-sort analysis). C) Sorted CD4+CD25+ populations were re-labeled with PKH and used as a source of potential Tregs in new co-cultures with fresh target cells. The suppressive capacity of the sorted cells was compared to freshly isolated bead-isolated CD4+CD25+ cells. Histograms are gated on the CFSE-labeled target T cells. Numbers are the percentage of target cells that had divided.



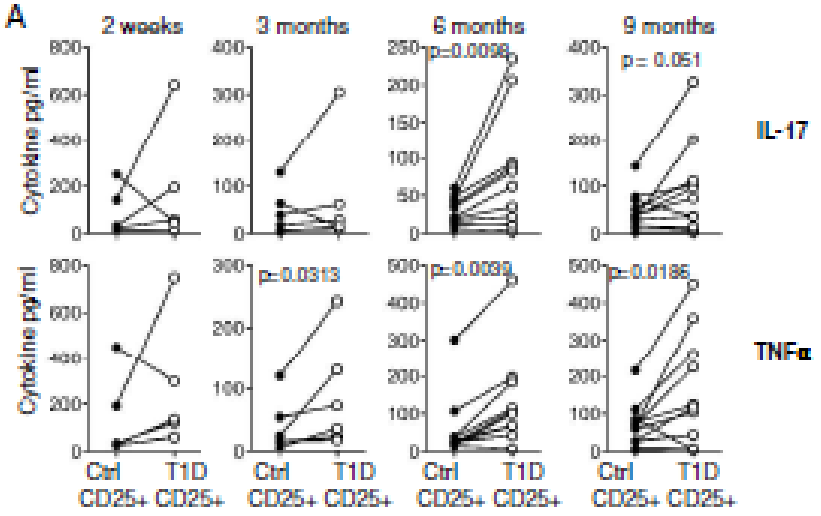
SUPPLEMENTARY DATA

Supplementary Figure 4. Multi-parameter phenotypic characterization of Foxp3^{hi} and Foxp3^{lo} CD4+ subsets. A) Multi-parameter FACS for the six markers shown in box at left. Collective subset analysis was performed using the SPICE analysis program. Cells were first gated on the CD25^{hi}Foxp3^{hi} and CD25^{lo}Foxp3^{lo} populations and then analyzed for the additional markers shown in the box at left. Frequencies of all possible combinations of the markers were obtained using boolean gating in FlowJo and then imported into the SPICE program. Pie charts show proportions of all 64 possible combinations of the six markers with each slice representing a single phenotype. Statistics of the overall differences between the pies was performed by Wilcoxon-Rank within the SPICE program. B) CD45RA expression within the CD4+ CD25^{hi}Foxp3^{hi} and CD25^{lo}Foxp3^{lo} populations. Boxed mean +/- SEM and min/max whiskers. Statistics by 2-tailed Mann Whitney.

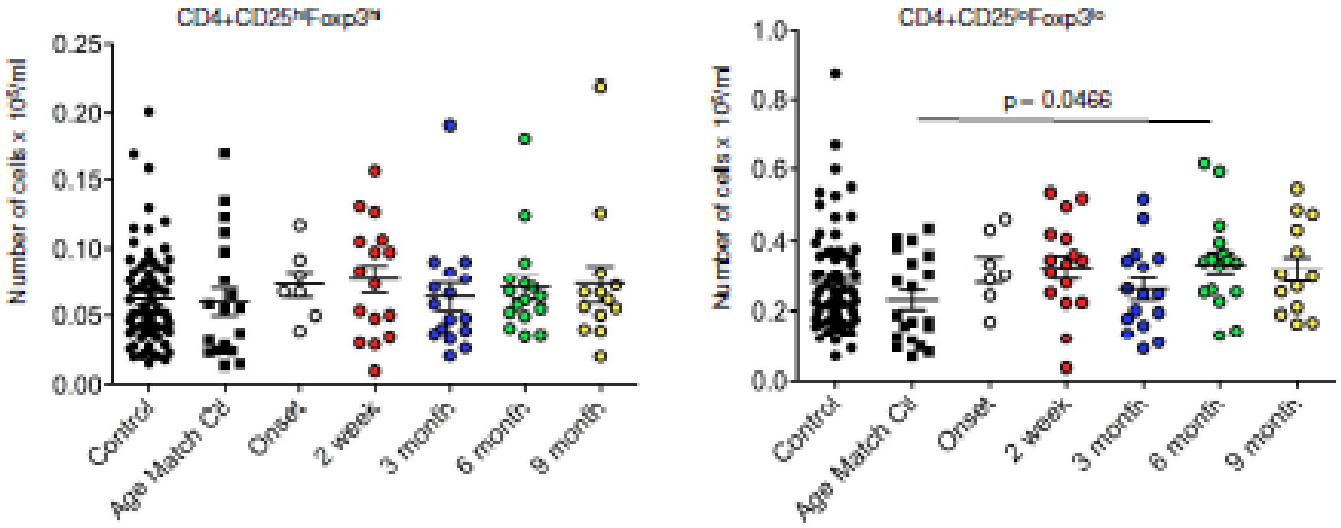


SUPPLEMENTARY DATA

Supplementary Figure 5. Cytokines in co-culture. A) Longitudinal analysis of IL-17 and TNFa production in co-cultures with Control or T1D CD4+CD25+ T cells. Time frame from diabetes diagnosis. Statistics by paired 2-tailed Wilcoxon. Pairs indicate cytokines from co-cultures with the same target T cells.

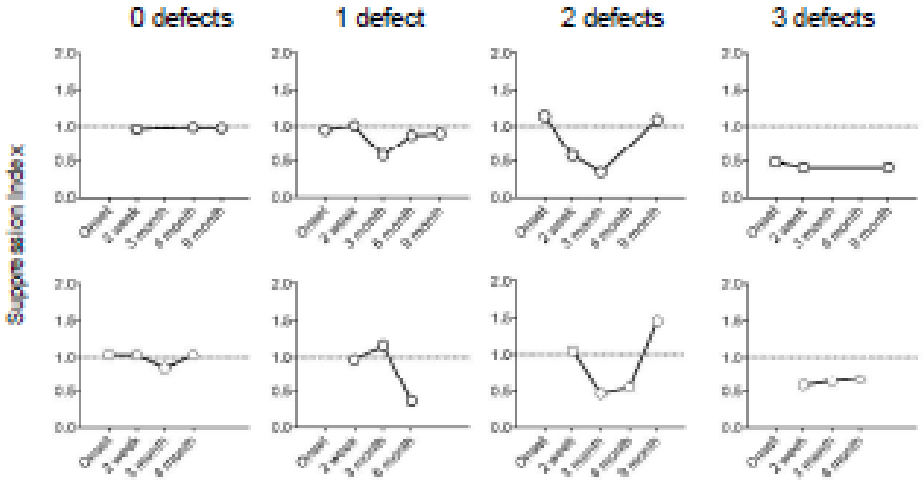


Supplementary Figure 6. Longitudinal analysis of numbers of CD25+ cells in PBMC from T1D. Data for CD25hiFoxp3hi cells not significant at any timepoints within T1D and between T1D and Controls or Age-matched controls, 2-tailed Mann Whitney. Only significant point for CD25loFoxp3lo, age-matched control and T1D at 6 months by 2-tailed Mann Whitney.



SUPPLEMENTARY DATA

Supplementary Figure 7. Longitudinal analysis of individual T1D subjects. Suppression index for T1D CD4+CD25+ T cells co-cultured with control CD4+CD25- targets and stimulated with anti-CD3/CD28 as described in Fig 3. Defects defined as suppression below the 99% confidence interval around the mean of all control CD4+CD25+ suppression.



SUPPLEMENTARY DATA

Supplementary Figure 8. T1D CD4+CD25- 'target' cells remain susceptible to CD4+CD25+ suppression. T1D CD4+CD25- were stimulated with anti-CD3 alone or anti-CD3 and anti-CD28 in co-culture with control CD4+CD25+ cells and control APC. A) Representative % suppression curves are plotted by input number of CD25+CD127lo Tregs with a fixed number of targets (5 x 10^4). To directly test ability of T1D non-regulatory T cell 'targets' to be suppressed, CD4+CD25-T1D targets were co-cultured with CD4+CD25+ control Tregs and compared to suppression of CD4+CD25- control targets. No significant difference in ability to be suppressed between control and T1D targets at any Treg:target ratio by Wilcoxon rank. B) collective analysis after anti-CD3 + anti-CD28 stimulation using Suppression index calculated as in Figure 4. C) collective analysis of target T cell proliferation in the absence of Tregs. D-E) longitudinal analysis as a group (D) and as individuals (E). SI from anti-CD3 + anti-CD28 cultures. Color of filled symbols in E) reflects time after T1D onset as depicted in D). All timepoints not statistically significant by Mann Whitney.