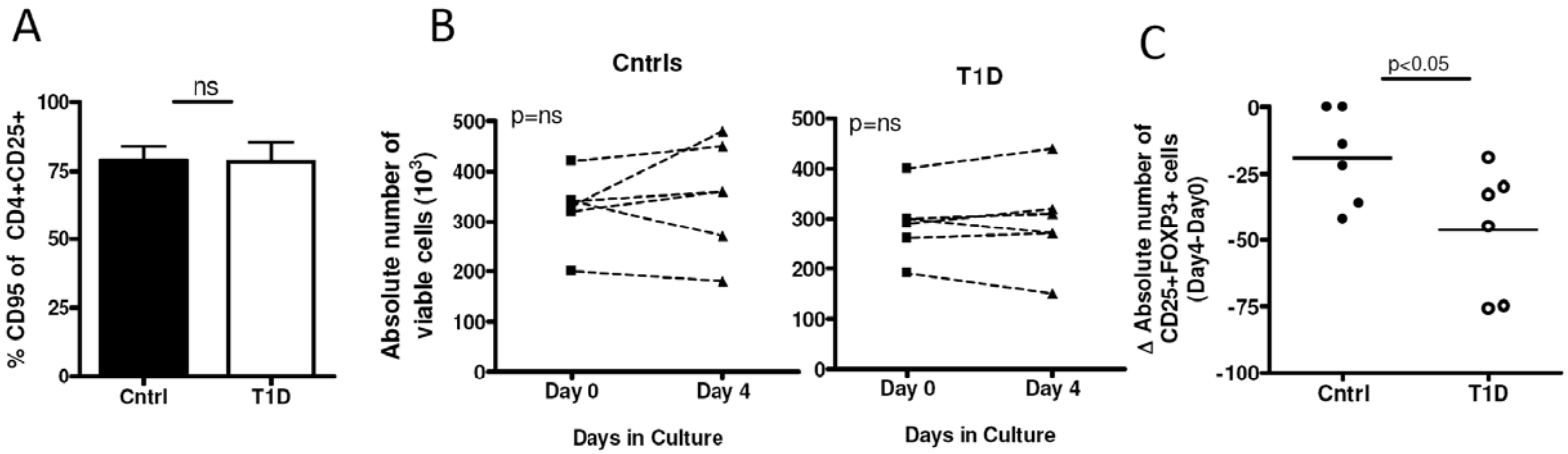
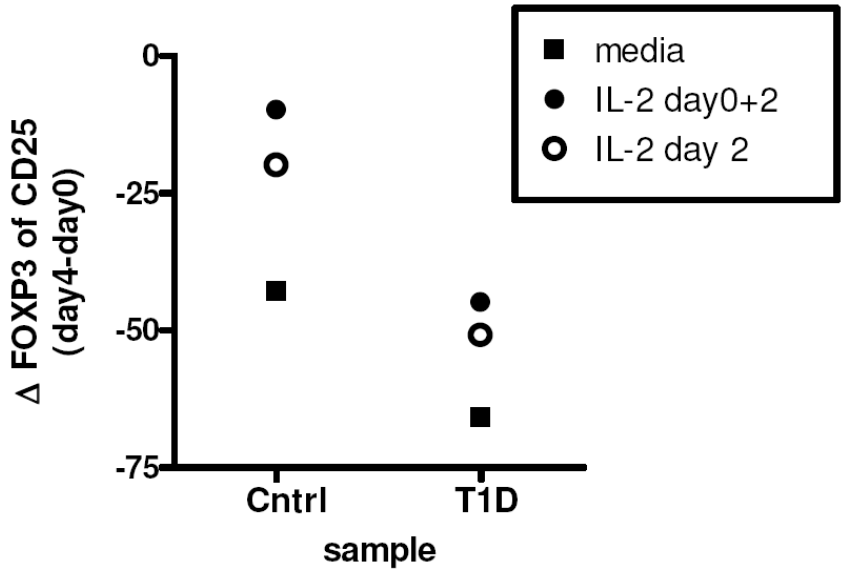
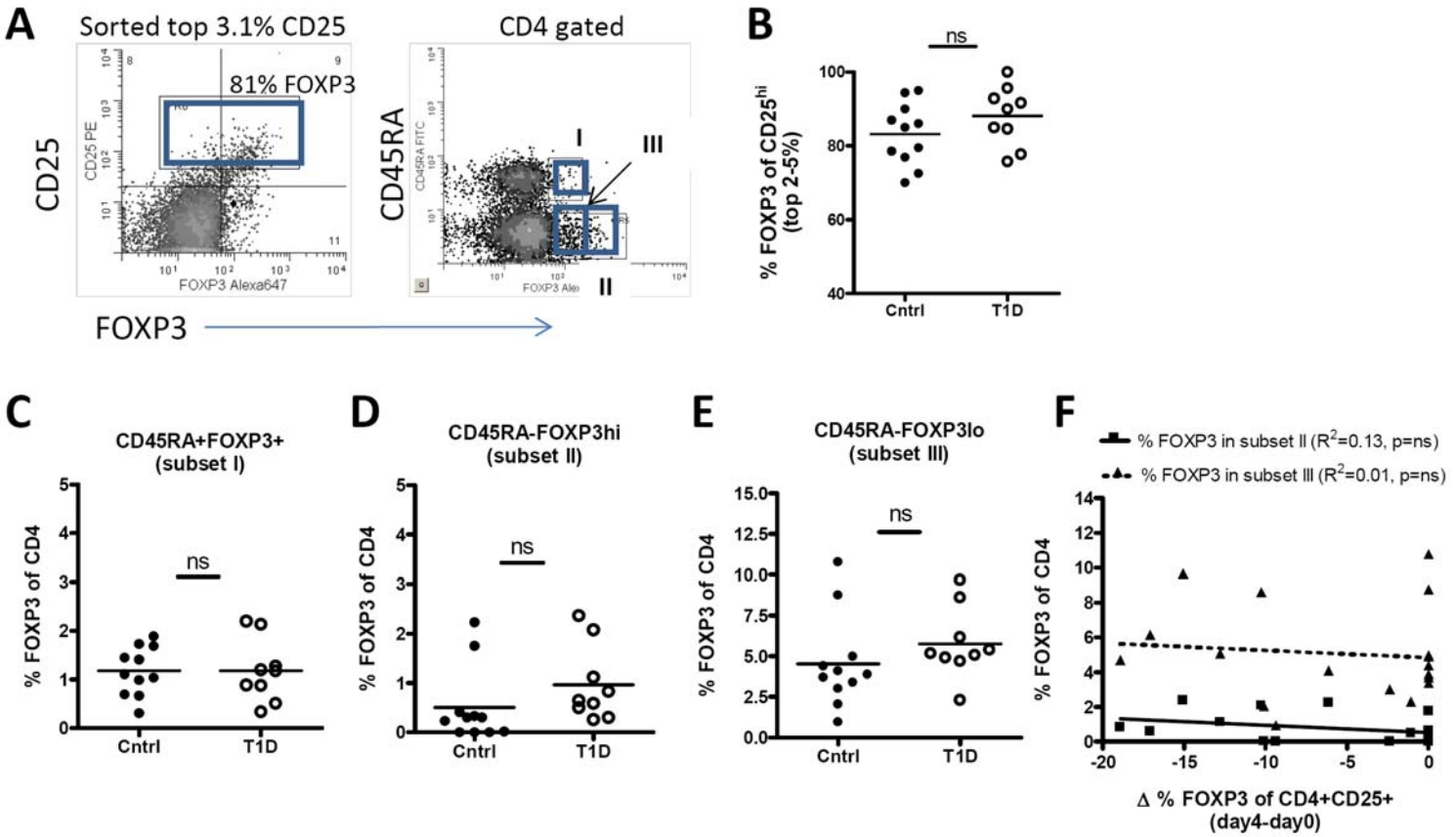
Online Appendix Supplemental Data:



Supplemental Data 1: Cell viability and expression of CD95 on $CD4^+CD25^{hi}$ sorted cells from control and T1D subjects. (A) CD4+CD25+ sorted cells from a subset of control (n=5) and T1D (n=5) subjects shown in Figure 1 were analyzed on Day0 by flow cytometry for expression of CD95. Statistical significance was determined using an independent student's t-test. (B) CD4+CD25+ sorted cells from a subset of control (n=6) and T1D (n=6) subjects shown in Figure 1 were cultured in IL-2 (200IU/ml) and counted on day0 and day4 . Data is shown for samples that were not analyzed mid-culture to avoid discrepancies in cell number due to sampling. Viability was determined by trypan blue staining and cell counts were determined using a hemacytometer. Statistical significance was determined using a paired student's t-test. (C) Change in the absolute number of FOXP3+ cells was determined for IL-2 cultures based on flow cytometry for FOXP3 and cell counts shown in (B). Statistical significance was determined using an independent student's t-test.



Supplemental Data 2: FOXP3 expression in cultured nTreg can be rescued by addition of IL-2 mid-culture. Sorted nTreg were placed in culture as described in Figure 1 with media alone, IL-2 (100 IU/ml) on day 0+2 or only on day2. One representative control of three and one representative T1D subject of two are shown.



Supplemental Data 3: nTreg phenotype in control and T1D subjects. (A) PBMC from samples shown in Figure 1 were thawed and stained for CD4, CD45RA, CD25 and FOXP3 or the corresponding isotype control. FOXP3 content in the CD25hi population is noted in the plot. FOXP3 subsets were determined based on FOXP3 and CD45RA staining as analyzed in (33) for CD4+ gated populations. Gating strategy is shown for one sample. (B) The percentage of FOXP3 was calculated for controls (n=11) and T1D (n=9) subjects for the CD4+CD25hi population and (C-E) nTreg subsets I, II and III. Closed circles denote control subjects and open circles denote T1D subjects. Statistical significance was determined using an independent student's t-test. (F) The correlation between nTreg persistence and % FOXP3 in subsets II and III was determined by linear regression.

Supplemental Data 4: Methylation of the FOXP3 gene in nTreg of T1D

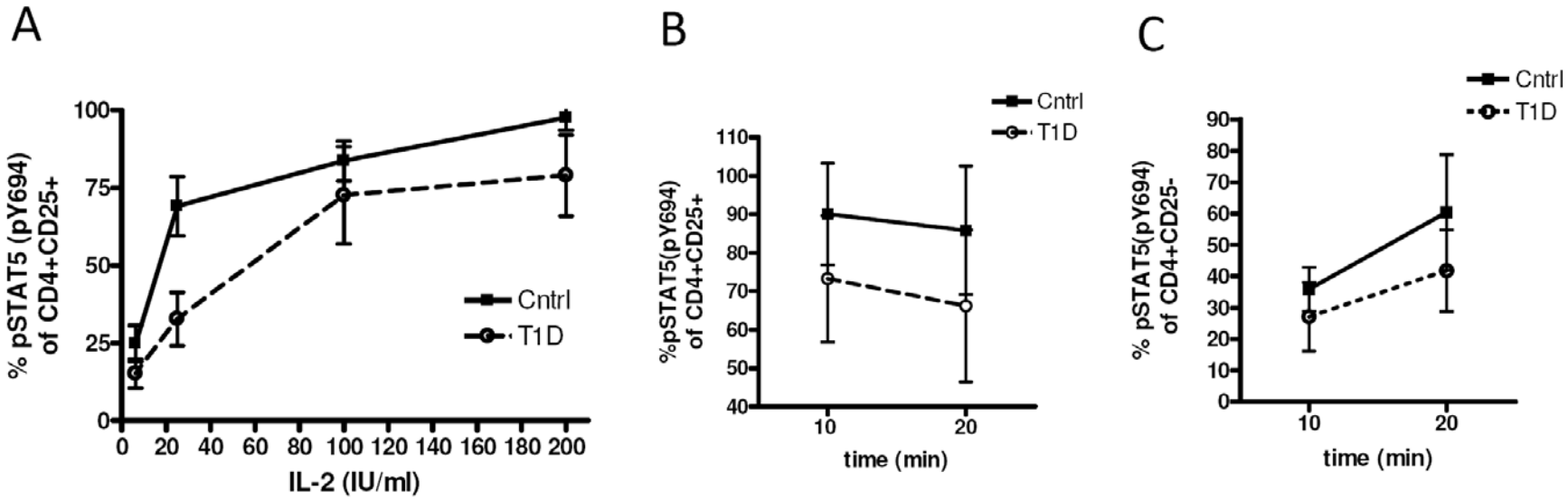
		% Methylation[2]	
Cells[1]	Subjects	Promoter[3]	TSDR[4]
CD4+CD25hi (nTreg)	Cntrl (n=11)	2±2	26±6
	T1D (n=6)	4±4	25±5
CD4+CD25-	Cntrl (n=11)	76±4	100±0
	T1D (n=6)	71±10	100±0

[1]$CD4^+CD25^+$ (top 2%) or $CD4^+CD25^-$ T cell were sorted from PBMC of male control (mean age 42.5, range of 27-59) and T1D (mean age 40.88, range 26-66) subjects. Purity was confirmed by FACS staining for CD25 and FOXP3. All populations were > 92% pure. Sorted cells from each individual were snap frozen and pooled for bisulfite analysis.

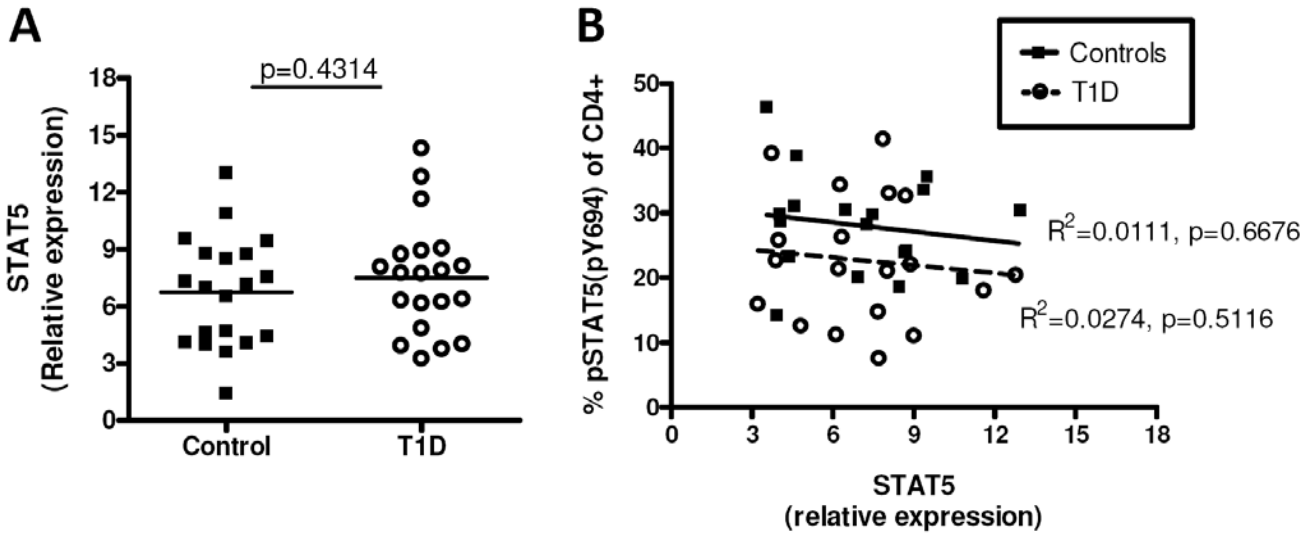
[2]Methylation analysis was performed by bisulfate sequencing. Genomic DNA was bisulfite converted using the EpiTect Bisulfite Kit (Qiagen).

[3]The FOXP3 promoter region was PCR-amplified using bisulfite forward and reverse primers described in Jansen et al. (31). PCR products were subcloned and the DNA from approximately 20 individual bacterial colonies were sequenced per sample. Values shown are mean percent methylation ±SD of 7 (out of 8 total) methylation sites within the promoter region PCR product, and represent variation in methylation across the amplified region for individual samples.

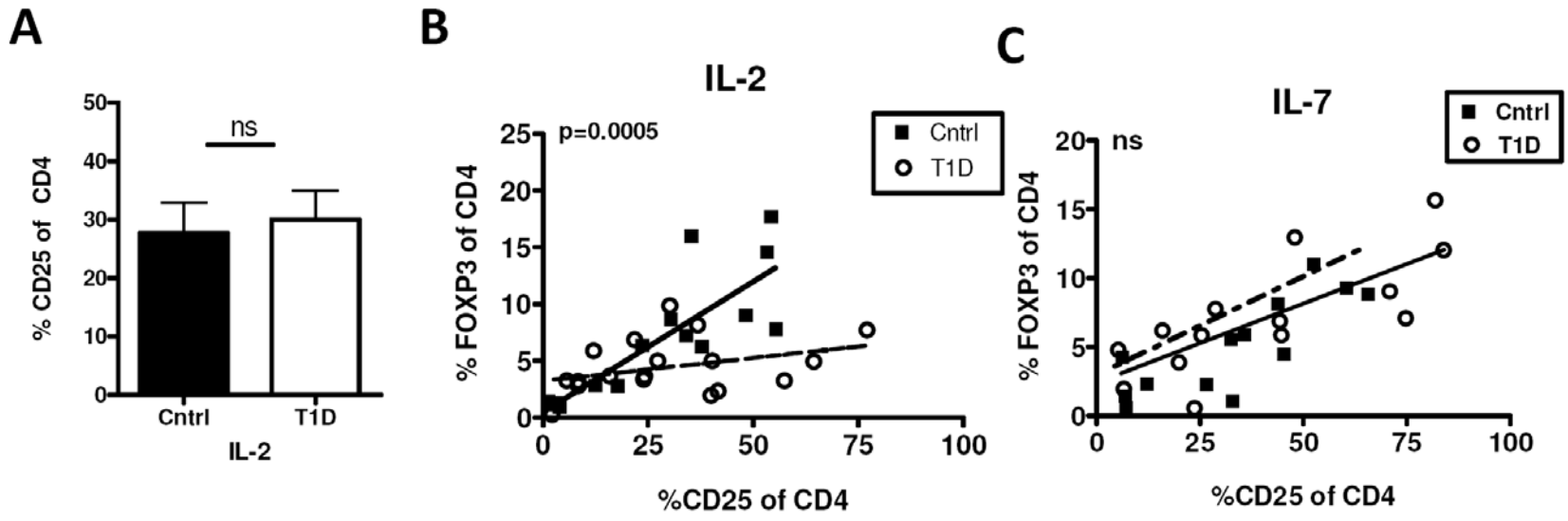
[4]The TSDR (Treg cell-specific demethylated region) was amplified using the primers: Amp5A1F-TTTGGGGGTAGAGGATTTAGAG and Amp5A1R-CCACCTAAACCAAACCTACTACAA (modified from Baron, et al. (30)). PCR products were directly sequenced with the same primers. Values shown are mean percent methylation ±SD of 11 (out of 15 total) methylation sites within the TSDR PCR product and represent variation in methylation across the amplified region for individual samples.



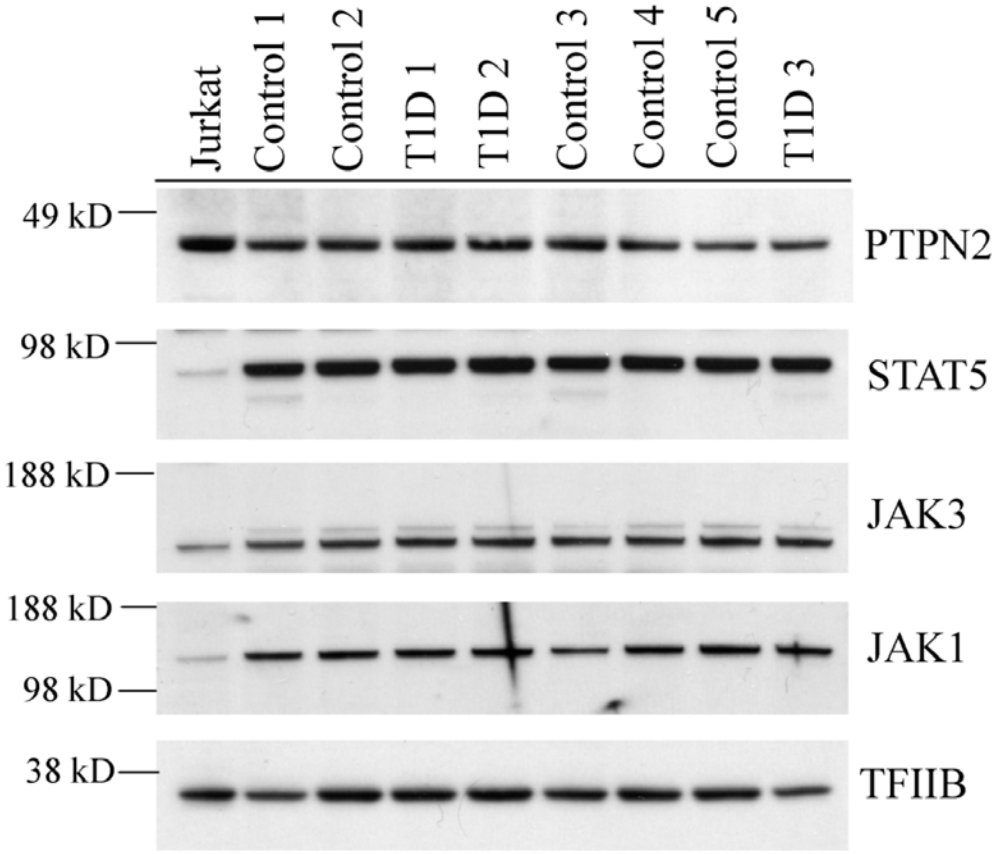
Supplemental Data 5: Kinetic and dose response to IL-2 in control and T1D subjects. (A) Freshly isolated PBMC from control (n=3) and T1D (n=3) subjects were stimulated with a range of doses of IL-2 for 10min. (B) PBMC from control (n=5) and T1D (n=5) subjects were stimulated with 100 IU/ml IL-2 for 10 or 20min. The frequency of pSTAT5+ cells, as compared to stimulation with media alone, was determined by flow cytometry. Analysis of the CD4+CD25+ population is shown. (C) The same population in (B) was analyzed for pSTAT5 in the CD4+CD25- population. For all graphs, the mean±SD for control (solid squares) and T1D (open circles) are shown.



Supplemental Data 6: Total STAT5 protein does not differ between control and T1D subjects and pSTAT5 is reduced in T1D subjects regardless of total STAT5 protein expression. (A) $CD4^+$ T cells were isolated from fresh PBMC of control and T1D subjects and whole cell protein lysates were analyzed by western blot. Immunoblots were probed with STAT5 specific antibodies and an anti-TFIIB antibody as a loading control. Protein expression was determined by densitometry, normalizing each sample to TFIIB and expressing total protein levels relative to a Jurkat control present on each blot. Total STAT5 protein expression was compared between control (n=20) and T1D (n=20) subjects. Significance was determined using an independent student's t-test . (B) PBMC from these same samples were assayed for pSTAT5 upon exposure with 100IU/ml IL-2 for 10min as in Figure 4. Using linear regression, protein expression was compared to pSTAT5 for control (n=19) and T1D (n=18) subjects. Solid trend line and squares denote controls and dashed trend line and open circles denote T1D subjects. The slopes of these trend lines do not differ.



Supplemental Figure 7: Diminished FOXP3 expression in iTreg of T1D activated in the presence of IL-2 is not due to decreased total activation. $CD4^{+}CD25^{-}$ T cells were isolated from previously frozen control (n= 15) and T1D (n=18) subjects and activated with 5μg/ml anti-CD3 antibody and irradiated accessory cells as described in Materials and Methods in the presence of 100 IU/ml IL-2 or 10ng/ml IL-7. FOXP3 and CD25 expression 48hrs following activation was determined by flow cytometry by gating on live, total $CD4^{+}$ T cells. CD25 expression is shown for each population in (A) as mean±SEM. The relationship between CD25 and FOXP3 expression, shown for IL-2 (B) and IL-7 (C) cultures of control and T1D cells, was determined by linear regression. p values in the plots represent the difference in the slopes of the lines determined using an ANCOVA.



Supplemental Data 8: Detection of PTPN2, JAK1, JAK3 and STAT5 protein by Western blot. $CD4^+$ T cells were isolated from fresh PBMC of control and T1D subjects and whole cell protein lysates were analyzed by western blot. Immunoblots were probed with STAT5, JAK1, JAK3 and PTPN2 specific antibodies and an anti-TFIIB antibody as a loading control. Protein expression was determined by densitometry, normalizing each sample to TFIIB and expressing total protein levels relative to a Jurkat control present on each blot.