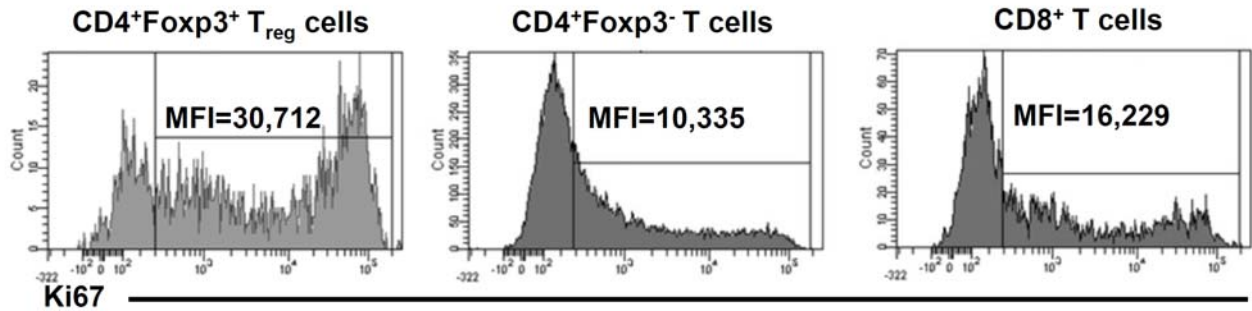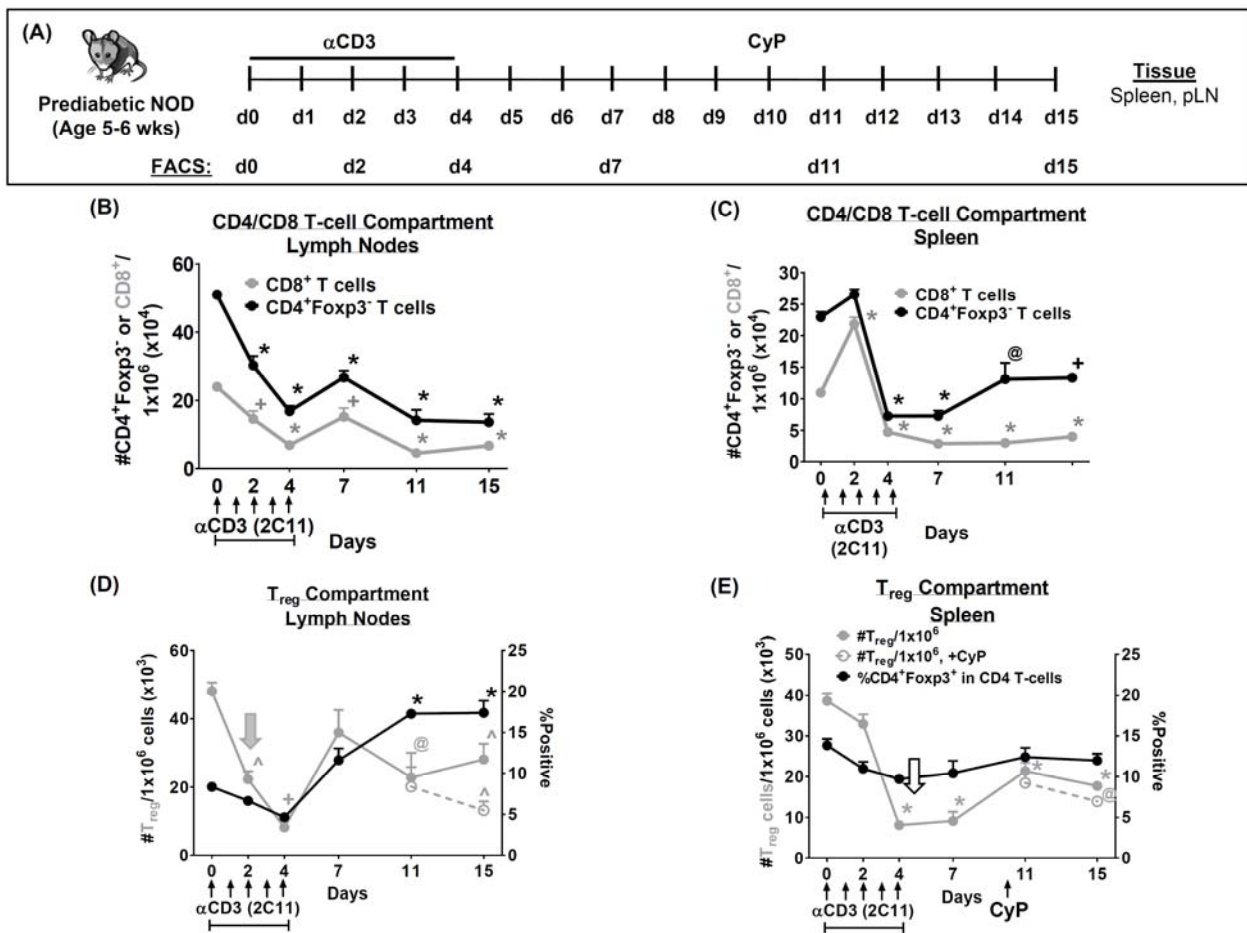SUPPLEMENTARY DATA

Supplementary Figure 1. T_{reg} proliferation is much higher compared to CD4+Foxp3- and CD8 T cells following aCD3 treatment. (A) Representative histograms showing Ki67 staining in the peripheral blood at d22 following the start of aCD3 treatment. Mean Fluorescent Intensity (MFI) is indicated in each histogram for gated CD4$^+$Foxp3$^+$ T_{reg} cells, and CD4$^+$Foxp3$^-$ and CD8$^+$ T cells.
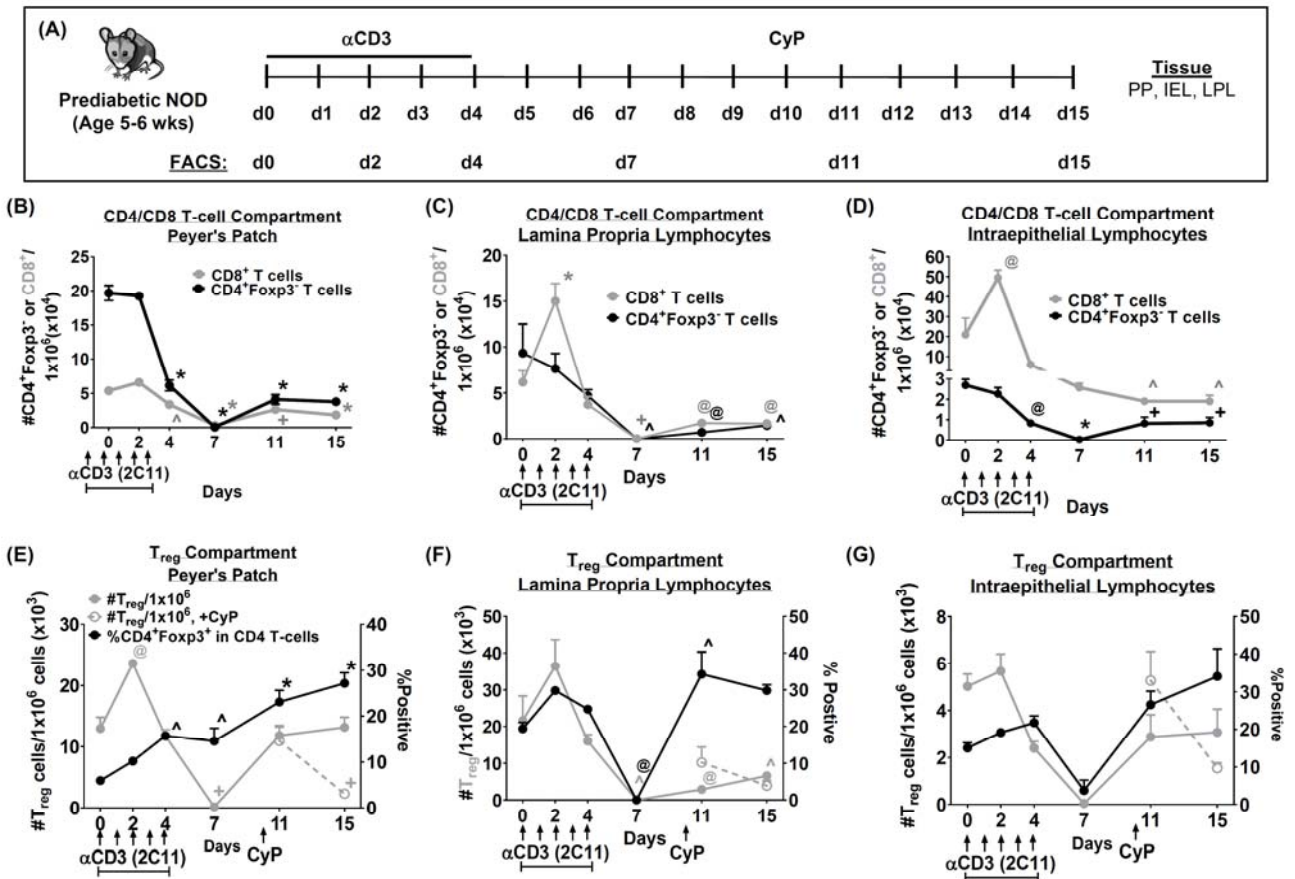
SUPPLEMENTARY DATA

Supplementary Figure 2. T_{reg} depletion and rebound in the pancreatic lymph nodes and spleen after αCD3 and CyP immunomodulation. (A) Experimental Scheme. Number of $CD8^+$ and $CD4^+Foxp3^-$ T-cells (per $1x10^6$ lymphocytes) in (B) lymph nodes and (C) spleen. Number of $CD4^+Foxp3^+$ cells (per $1x10^6$ lymphocytes) and percentage of $Foxp3^+$ CD4 T-cells in (D) lymph nodes and (E) spleen in the presence or absence of CyP of young, prediabetic NOD mice (aged 5-6 weeks). n=3-6 mice/group. *P<0.0001, +P<0.001, @P<0.01, and ^P<0.05 One-way ANOVA followed by Dunnett's Multiple Comparison Test compared to day 0. Unpaired T-Test, αCD3 compared to αCD3$^+$CYP at each time point. n=3-6 mice/group. In (D) grey-arrow indicates an early decrease in the number of $CD4^+Foxp3^+$ cells after the start of αCD3 LNs. In (E) white-arrow indicates a late decrease in the number of $CD4^+Foxp3^+$ cells after the start of αCD3.
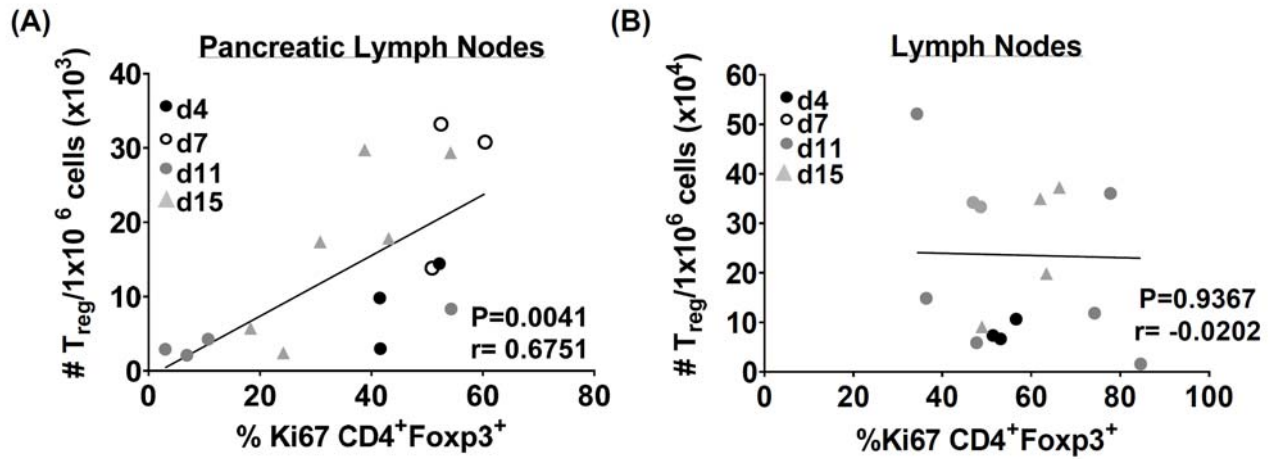
Supplementary Figure 3. T_{reg} depletion and rebound in the Peyer's Patches, Laminaia Propria and Intraepithelial of the small intestine after αCD3 and CyP immunomodulation. (A) Experimental Scheme. Number of $CD8^+$ and $CD4^+Foxp3^-$ T-cells (per 1×10^6 lymphocytes) in (B) Peyer's Patches, (C) LPL, and (D) IEL. Number of $CD4^+Foxp3^+$ cells (per 1×10^6 lymphocytes) and percentage of $Foxp3^+$ CD4 T-cells in (E) Peyer's Patches, (F) LPL, and (G) IEL in the presence or absence of CyP of young, prediabetic NOD mice (aged 5-6 weeks). n=3-6 mice/group. *P<0.0001, +P<0.001, @P<0.01, and ^P<0.05 One-way ANOVA followed by Dunnett's Multiple Comparison Test compared to day 0. Unpaired T Test, αCD3 compared to αCD3+CYP at each time point. n=3-6 mice/group.
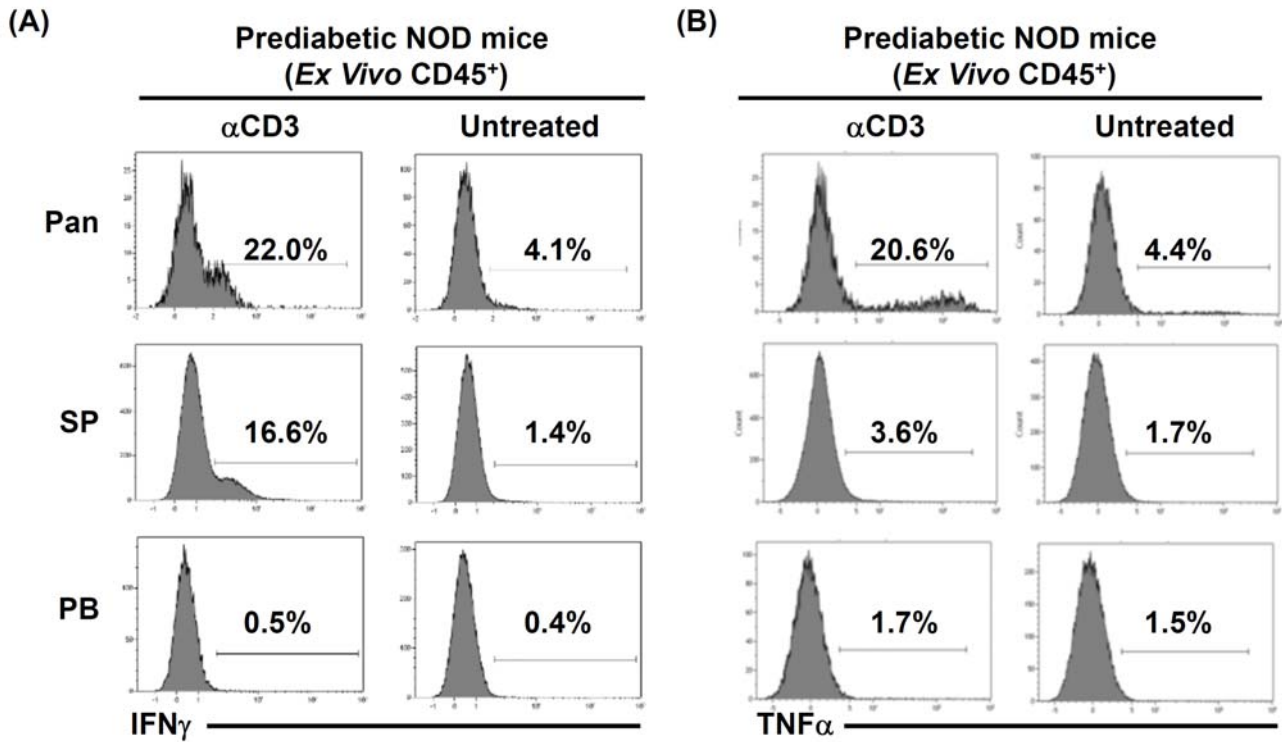
SUPPLEMENTARY DATA

Supplementary Figure 4. Link between the T_{reg} rebound and proliferation in some tissues but not all. Number of versus the percentage of Ki67$^+$ in gated CD4$^+$Foxp3$^+$ T cells in the (A) pLN or (B) LNs of young. prediabetic NOD mice (aged 5-6 weeks) receiving 5-day course of αCD3.
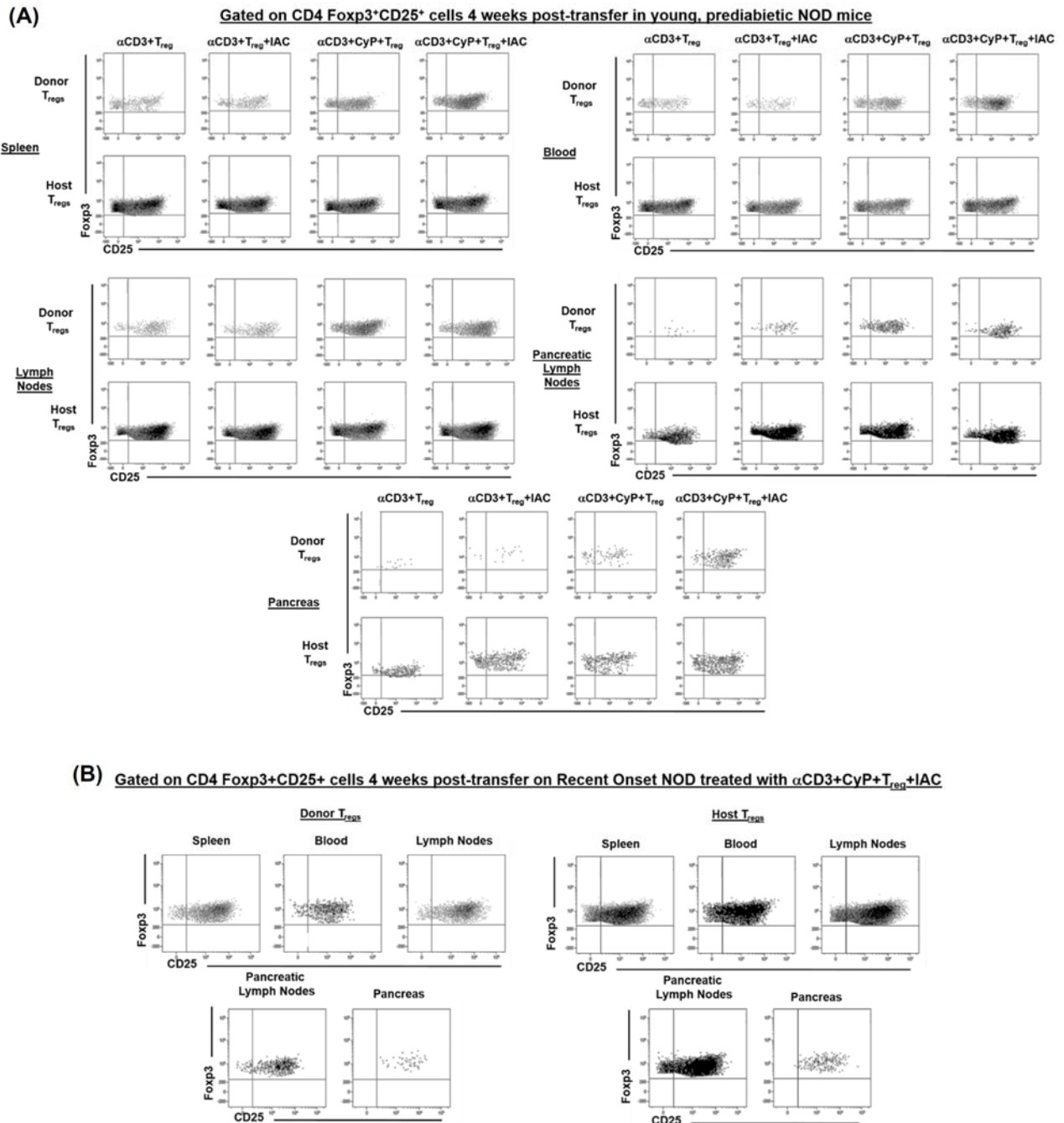
SUPPLEMENTARY DATA

Supplementary Figure 5. Depletion of T-cells with short-course αCD3 treatment in late-prediabetic NOD mice leads IFNγ-and TNFα-producing lymphocytes. Prediabetic NOD mice treated with 5-day course of intact αCD3 (50μg) were sacrificed 15 days after the start of treatment and compared to untreated, age- and sex-matched NOD mice. Histograms show the percentage of (A) IFNγ or (B) TNFα producing CD45^{+} cells isolated from pancreatic islets (Pan), spleen (SP), or peripheral blood after culturing cells directly ex-vivo for 4 hours in the presence of brefeldin A. n=3 mice/group.

Supplementary Figure 6. Representative staining of Foxp3 and CD25 expression on donor (Thy1.1+) or host (Thy1.2$^+$) T$_{regs}$ from the gated CD4$^+$Foxp3$^+$ population from the blood and SP, LN, pLN, and pancreas 4-weeks post-T$_{reg}$ infusion in (A) young, prediabetic NOD mice or (B) recent onset NOD mice treated with various immunomodulation followed by adoptive transfer of congenic, polyclonal Thy1.1+ T$_{regs}$.

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Supplementary Figure 7. (A) Percentage of Thy1.1$^+$ donor T$_{reg}$ engraftment among the total CD4$^+$Foxp3$^+$ T-cells in peripheral blood, (B) Number of donor T$_{regs}$/1x10^6 lymphocytes in peripheral blood, and (C) Percentage of CD4+Foxp3+ cells in the gated CD4 T cells in peripheral blood of late, prediabetic NOD mice. Percentage of Thy1.1$^+$ donor T$_{reg}$ engraftment among the total CD4$^+$Foxp3$^+$ T-cells 4wk post-T$_{reg}$ infusion in the SP, LN, pancreatic (p)LN, and pancreas (Pan) of (D) young, prediabetic NOD(E) late, female prediabetic NOD mice aged 16-22 weeks of age in mice treated with αCD3+T$_{reg}$, αCD3+T$_{reg}$+IAC, αCD3+CyP+T$_{reg}$, or αCD3+CyP+T$_{reg}$+IAC. IAC=anti-IL-2 (JES6)+rmIL-2 complex. (F) Percentage of Thy1.1$^+$ donor T$_{reg}$ engraftment among the total CD4$^+$Foxp3$^+$ T-cells 4wk post-T$_{reg}$ infusion in the SP, LN, pancreatic (p)LN, and pancreas (Pan) of recent onset NOD mice treated with αCD3+CyP+T$_{reg}$+IAC. n=3-4 mouse/treatment group
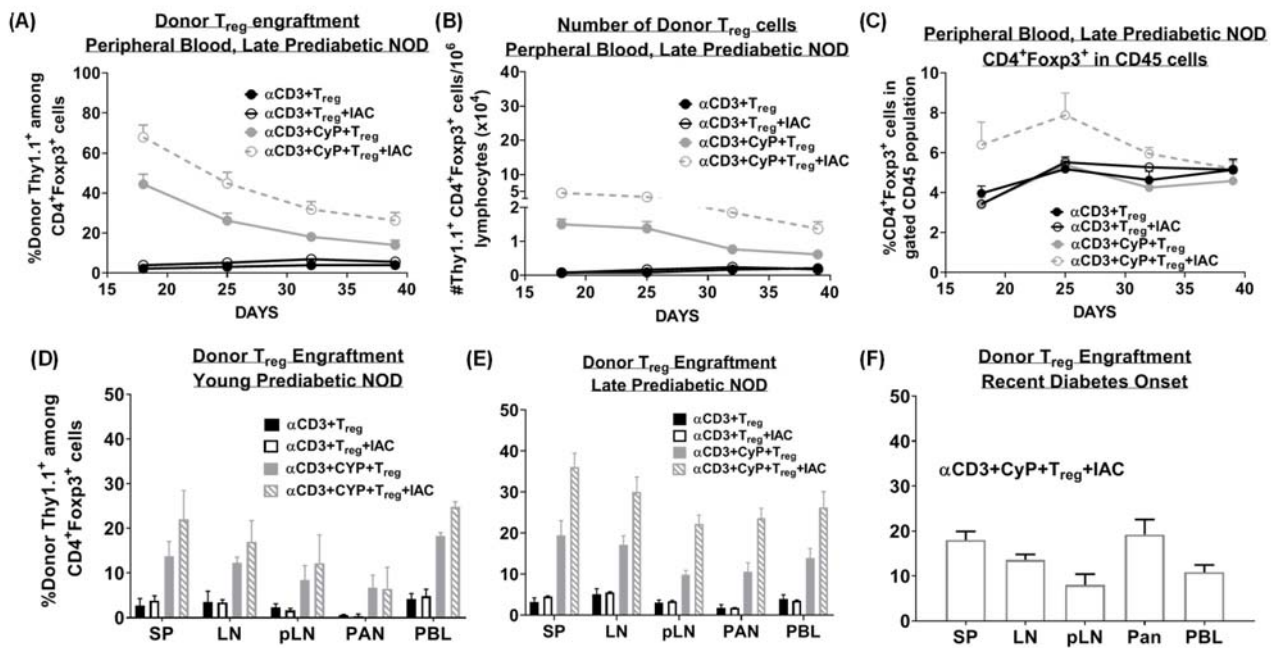
SUPPLEMENTARY DATA

Supplementary Table 1. Engraftment of Donor T$_{regs}$ following αCD3+CyP Immunomodulation.
Young, prediabetic female NOD mice were treated with combinational regimen of αCD3+T$_{reg}$, αCD3+T$_{reg}$+IAC, αCD3+CyP+T$_{reg}$, αCD3+CyP+T$_{reg}$+IAC, as described in Figure3A. Shown is number of donor T$_{reg}$/1x10^6 lymphocytes in the peripheral blood d15, 18, 22, 25, 32, and 39 after the start of αCD3 treatment.

	αCD3 + T$_{reg}$	αCD3 + T$_{reg}$ + IAC	αCD3 + CyP + T$_{reg}$	αCD3 + CyP + T$_{reg}$ + IAC
d15	2,242 ± 554	9,719 ± 4,421	23,999 ± 11,691	29,300 ± 12,512
d18	2,251 ± 468	15,108 ± 4,339	97,400 ± 6,249	256,480 ± 44,905
d22	4,003 ± 2,179	9,262 ± 1,964	20,724 ± 1,716	122,812 ± 12,262
d25	1,159 ± 169	2,164 ± 431	14,166 ± 1,062	58,178 ± 18,306
d32	1,683 ± 391	1,954 ± 330	12,325 ± 107	25,210 ± 3,612
d39	1,221 ± 244	1,431 ± 235	6,559 ± 167	10,754 ± 541

SUPPLEMENTARY DATA

Supplementary Table 2. Engraftment of Donor T_{regs} following αCD3+CyP Immunomodulation.
Recent onset diabetic female NOD mice were treated with combinational regimen of αCD3+CyP+T_{reg}+IAC as described in Figure3A. Shown is number of donor T_{reg}/1x10^6 lymphocytes in the peripheral blood, spleen, LN. pLN, and pancreas d22 and 39 after the start of αCD3 treatment.

	PBL	SP	LN	pLN	PAN
d22	22,207 ± 5,536	NA	NA	NA	NA
d39	5,277± 507	6,934± 2,172	6,582± 1,940	2,943± 1,235	4,500± 2,801