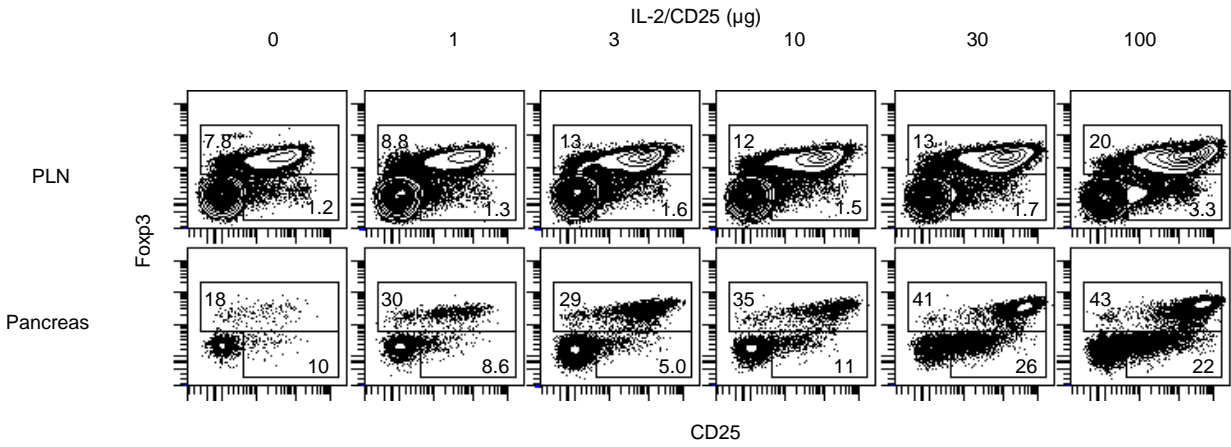
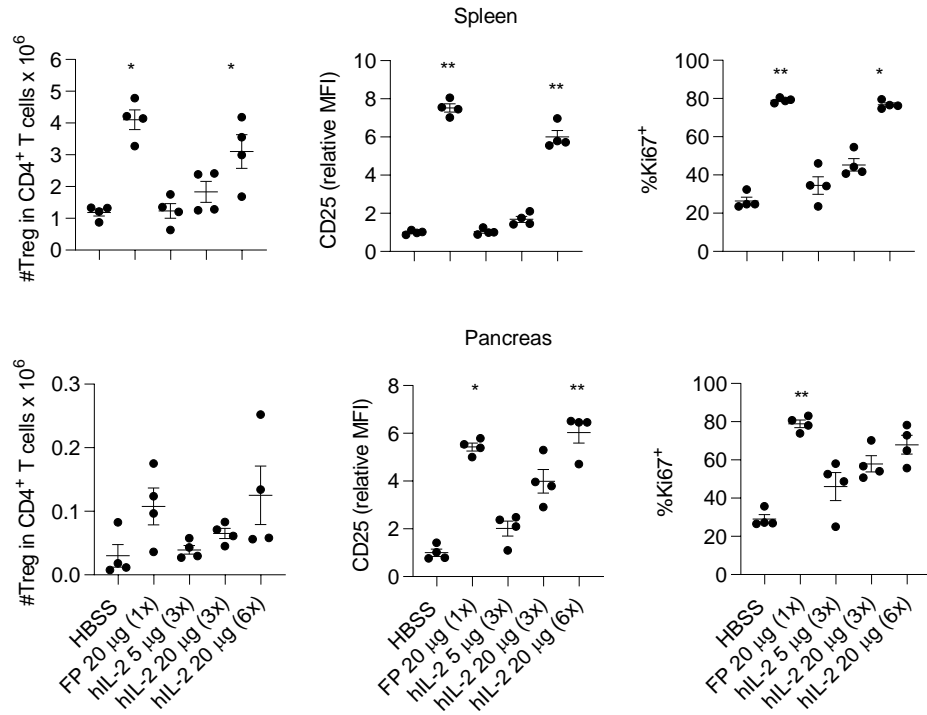


Gated on CD4⁺ T cells

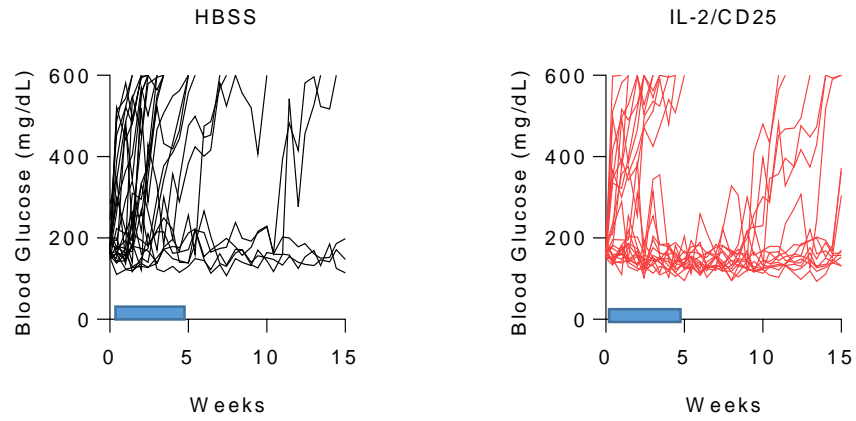


Supplemental Figure 1: Gating strategy to evaluate Treg:Teff ratio. 7-8 week-old female NOD mice received a single dose of IL-2/CD25 or HBSS. The gated CD4⁺ T cells from the PLN and pancreas were assessed 72 hours post-treatment for expression of Foxp3 and CD25. Tregs are shown in the upper gate of each histogram and were enumerated based on staining for Foxp3. Teff cells are shown in the lower gate of each histogram and were enumerated based on staining for CD25 but not Foxp3.

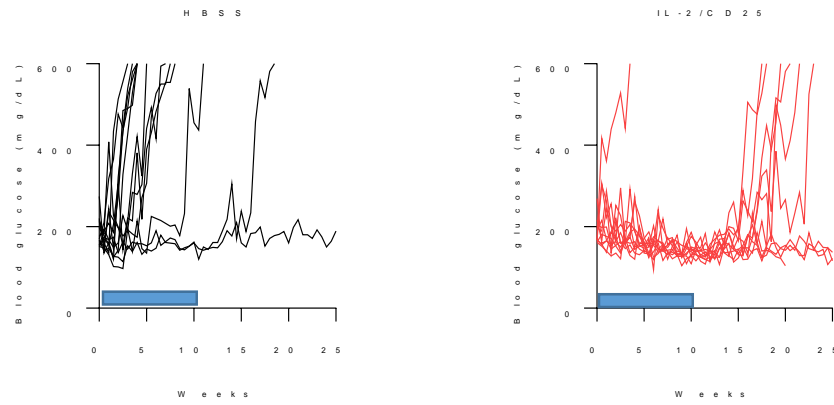


Supplemental Figure 2: The number of Tregs in the spleen and pancreas and their expression of CD25 and Ki67 3 days after administering a single injection of mIL2/CD25 or daily (3X) or every 12 hours (6x) injections of IL-2, as indicated (n=4/group). Data are shown as mean ± SEM and were analyzed by unpaired one-way ANOVA Kruskal-Wallis test.

5 weeks of therapy

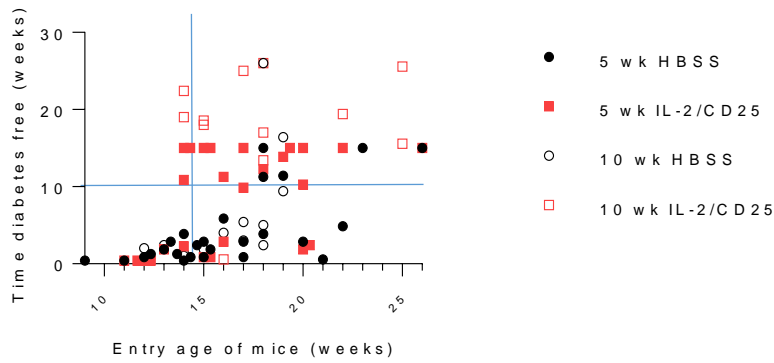


10 weeks of therapy

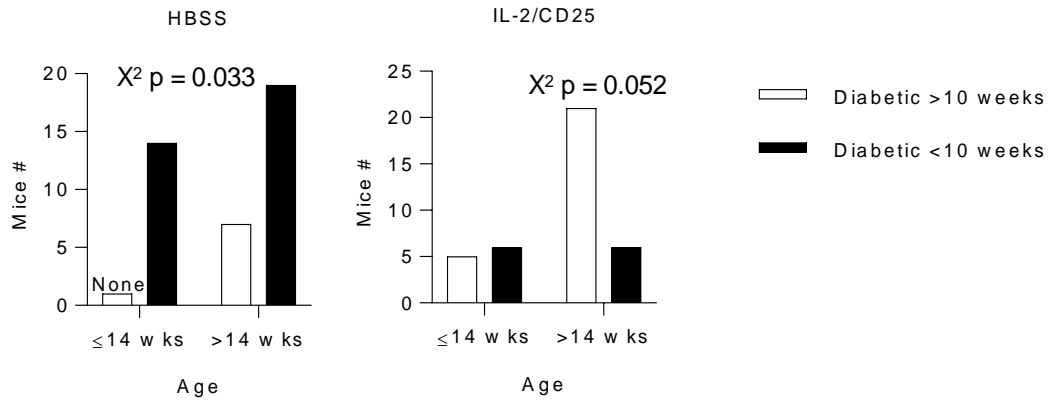


Supplemental Figure 3: Assessment of diabetes progression by individual mice shown in Fig. 2B, C, treated with HSSS or IL-2/CD25 for 5 or 10 weeks. The blue bar in each graph indicatedsthe treatment period.

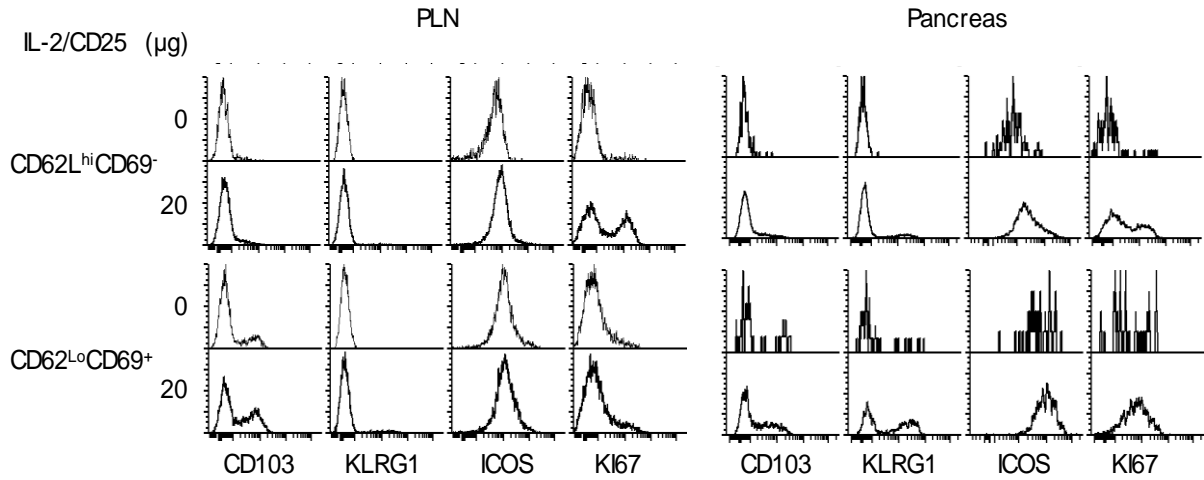
A



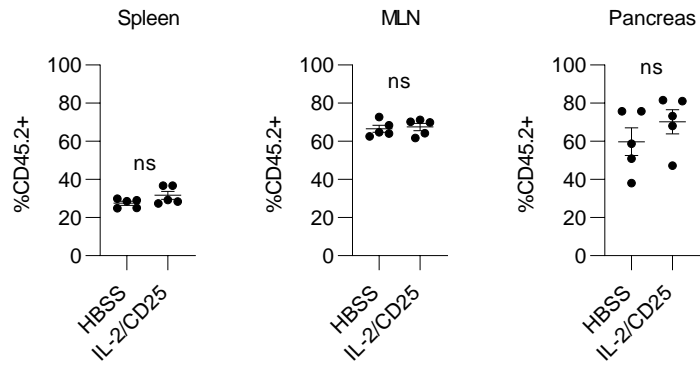
B



Supplemental Figure 4: Young (≤ 14 weeks old) pre-diabetic female NOD mice are less responsive to low-dose IL-2/CD25 therapy. (A) Data from Figure 2B-C were plotted based on the age when they were deemed pre-diabetic and the time from that point when they were determined to be diabetic (>400 mg/dl blood glucose levels). The vertical blue line demarcates data points at 14 weeks of age. The horizontal blue line marks 10 week time point use to show delayed diabetes progression. (B) Data from (A) for HBSS- (left) and IL-2/CD25-(right) treated mice were plotted based on their age, as indicated, when they were identified as pre-diabetic vs. whether they became diabetic in <10 weeks. All 14 HBSS-treated mice ≤ 14 weeks of age progressed to diabetes in <10 weeks. Data were analyzed by Chi-squared test to assess the change in diabetes instances between early (≤ 14 weeks) and a later (>14 weeks) age.



Supplemental Figure 5: 7-8 week-old female NOD mice received the indicated dose of IL-2/CD25 or HBSS twice a weeks for 10 weeks. Tregs from the PLN and pancreas were analyzed 72 hrs after the last injection. Representative histograms are shown for CD4⁺ Foxp3⁺ Tregs gated on the indicated subpopulation of Tregs for their expression of CD103, KlrG1, Ki67, or ICOS.



Supplemental Figure 6: The distribution of donor-derived lymphoid cells in the indicated tissues after NOD.SCID recipients received spleen cells from HBSS- or mIL-2/CD25-treated mice. Data (n=5/group) are combined from two independent experiments, are shown as mean \pm SEM, and were analyzed by an unpaired two-sided t-test.

Eotaxin/CCL11
G-CSF
GM-CSF
IFN-Gamma
IL-1 Alpha
IL-1 Beta
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-12 p40
IL-12 p70
IL-13
IL-15
IL-17A/CTLA8
IP-10/CXCL10
KC/GRO
alpha/CXCL1
LIF
LIX/CXCL6/GCP-2
M-CSF
MCP-1/CCL2
MIG/CXCL9
MIP-1 alpha/CCL3
MIP-1 beta/CCL4
MIP-2/CXCL2
Rantes/CCL5
TNF-alpha
VEGF-A

Supplemental Figure 7: Cytokines assayed in the Luminex mouse panel.