SUPPLEMENTARY METHODS AND MATERIALS

Mice and Reagents

NOD.BDC2.5 TCR transgenic mice were obtained from the JDRF Center for Immunological Tolerance at Harvard Medical School, and bred and distributed by Jackson Laboratories. NOD.IFN-$\gamma^{-/-}$ mice were from Jackson Laboratories and crossed with NOD.BDC25 TCR Tg. BDC2.5 mimetope, p79 (AVRPLWVRME) (1) was synthesized by SynBioSci. Anti-IL2 mAb (clone S4B6) was purchased from BD Biosciences.

Transfer of Diabetes

Naïve T cells were activated with BDC2.5 TCR mimetope under either Th1 (10 ng/ml IL-12 plus 10 μg/ml anti-IL-4 (clone 11B11)) or Th17 (3 ng/ml TGF-β and 30 ng/ml IL-6 with or without 20 μg/ml anti-IFN-γ (clone XMG1.2)) polarizing conditions in the presence of irradiated CD4-depleted splenocytes as APCs. Polarized Th1 and Th17 cells were expanded in IL-2- and IL-23-containing medium, respectively. When the cells stopped blasting, Th1 and Th17 cells were restimulated with 1 μg/ml each of plate-bound anti-CD3 and anti-CD28 in the presence of IL-12 plus anti-IL4 and IL-23 plus anti-IFN-γ, respectively, for 2 d before transfer. Cells were then washed and resuspended in PBS. Between $3 - 5 \times 10^6$ Th cells in 200 μl were transferred i.v. into 4 – 7 wk old recipient mice. Mice were monitored daily after adoptive transfer using diastix (Bayer) and urine glucose readings greater than 250 mg/dl, mice were considered diabetic. Blood glucose was measured using an Ascensia ELITE glucometer (Bayer) to confirm hyperglycemia. Statistical analysis using Logrank tests were performed using GraphPad Prism.

SUPPLEMENTARY FIGURES

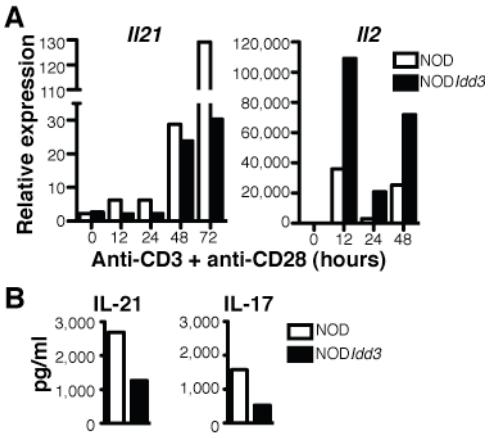

Figure S1. Naïve T cells from NOD mice express more IL-21 and less IL-2 than naïve T cells from NOD.*Idd3* mice. Naïve T cells were stimulated with plate-bound anti-CD3 and soluble anti-CD28 (**A)** or soluble anti-CD3 in the presence of autologous APCs (**B)**. IL-2 and IL-21 transcript expression was determined by real-time Taqman PCR and normalized to β-actin expression (**A)**. Supernatants were assessed for cytokine production after 48 h in culture (**B)**. Data are each representative of 3 experiments

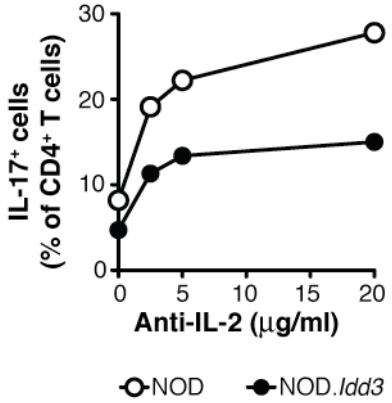

Figure S2. Neutralization of IL-2 in Th17 cell polarizations. Naïve T cells were cultured with plate-bound anti-CD3 and soluble anti-CD28 in the presence of IL-6, TGF-β and anti-IL-2 antibody. Th17 polarizations were assessed after 3 d by intracellular staining after restimulation with PMA and ionomycin. The graphs show frequency of IL-17^+ $CD4^+7AAD^-$ cells.

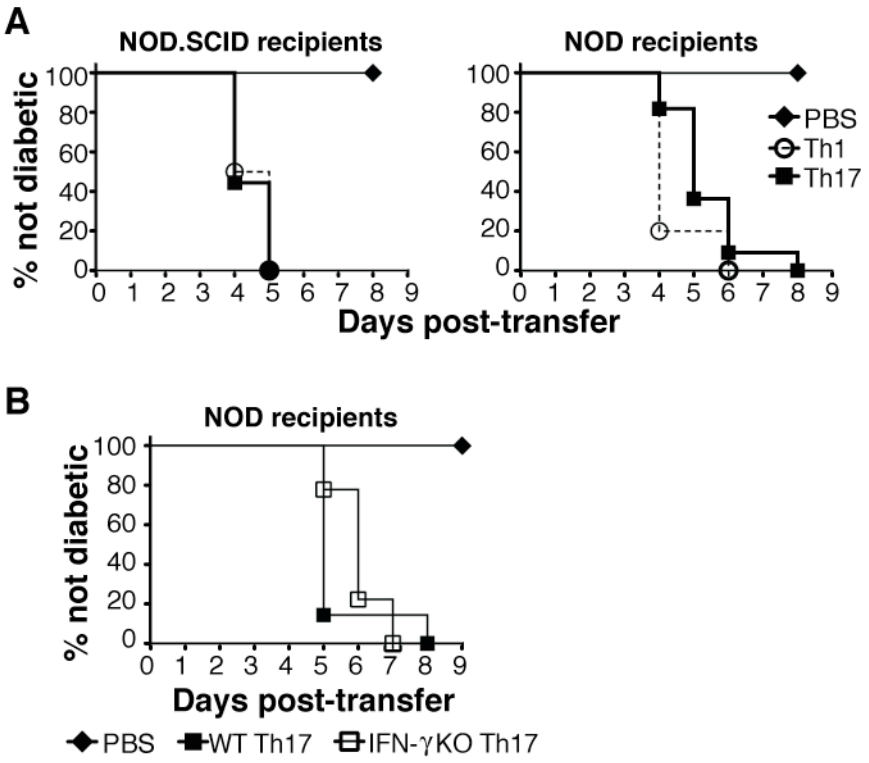

Figure S3. Islet-specific Th17 cells can transfer diabetes as well as Th1 cells. Th17 and Th1 cells polarized from naïve BDC2.5 TCR transgenic T cells and adoptively transferred into NOD.SCID or non-irradiated NOD mice and diabetes incidence was monitored daily (**A).** Data are pooled from 2 to 3 independent experiments. Islet-specific Th17 cells generated from T cells from IFN-$\gamma^{+/+}$ or IFN-$\gamma^{-/-}$ BCD2.5 TCR transgenic mice were transferred into NOD recipients and diabetes incidence was monitored daily (**B**). Data are pooled from 3 independent experiments. Logrank tests were performed using GraphPad Prism.

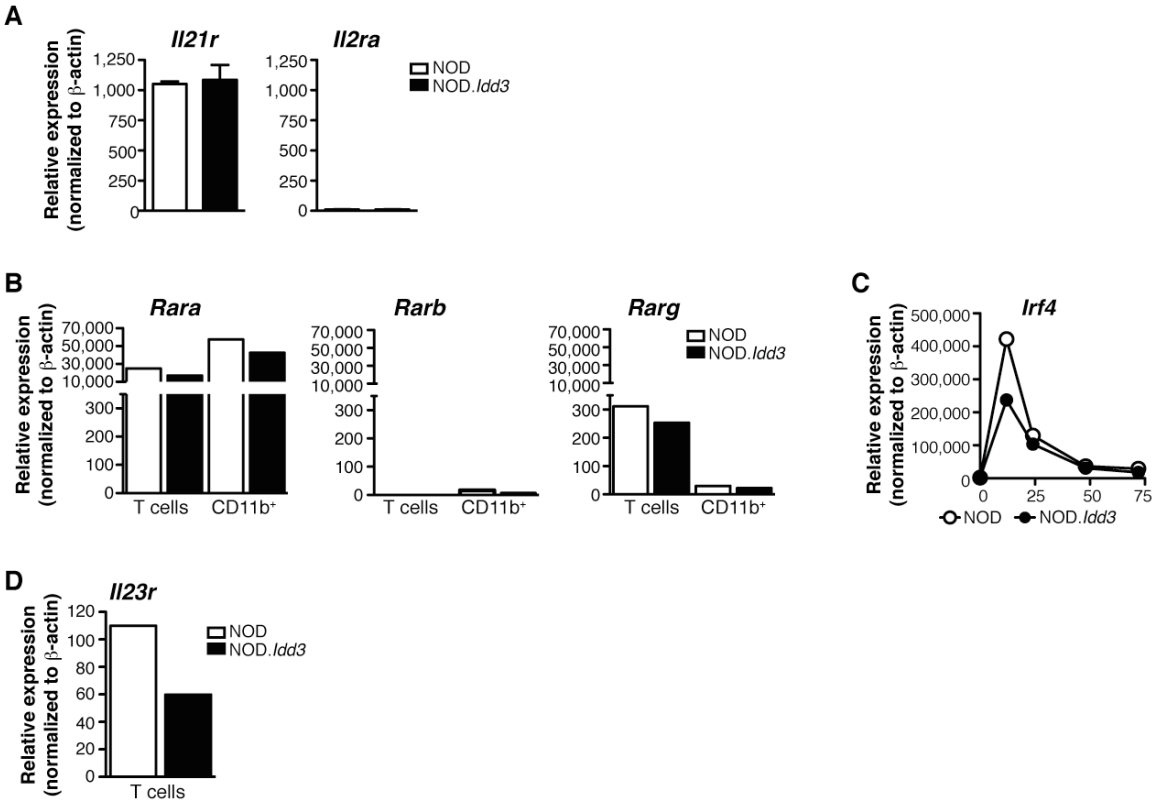

Figure S4. Real-time PCR analyses of cells from NOD and NOD.*Idd3* mice. Gene expression was analyzed by real-time PCR for $CD11c^{-}CD11b^{+}$ cells (**A** and **B**), and $CD4^{+}$Foxp3/GFP- T cells (**B** - **D**) from NOD and NOD.*Idd3* mice isolated *ex vivo* (**B** and **D**) or activated with plate-bound anti-CD3 and soluble anti-CD28 (**C**). Target gene expression was normalized to β-actin expression.

REFERENCES

1. Judkowski V, *et al*. (2001) Identification of MHC class II-restricted peptide ligands, including a glutamic acid decarboxylase 65 sequence, that stimulate diabetogenic T cells from transgenic BDC2.5 nonobese diabetic mice. J Immunol 166(2):908-917.