Supporting Information

Chen et al. 10.1073/pnas.1312911110

SI Materials and Methods

Insulin Autoantibodies, IgA, IgG, IgM, and IgE Detection. The serum levels of anti-insulin autoantibodies (IAA) were analyzed using the mouse insulin autoantibodies ELISA kit (TSZ ELISA). Serum levels of IgA, IgG, IgM, and IgE were detected using the mouse IgA, IgG, IgM, and IgE ELISA kits from Genway Biotech.

Histopathological Examination and Immunohistochemistry. Complete histopathology examinations were performed on mice with collections of all major organs. Tissues, including pancreas, were fixed in 10% buffered neutral formalin, paraffin embedded, and stained with hematoxylin and eosin (H&E). A portion of pancreas was embedded in optimal cutting temperature media and frozen for cryotomy. To detect transgene/s expression, pancreatic α/β/δ islet cells and infiltrating mononuclear cells, pancreatic tissues were stained with anti–IL-15, anti–IL-15Rα, anti-insulin, anti-glucagon, anti-somatostatin, anti-CD3, anti-B220, and anti-F4/80. Anti-CD4, anti-CD8, and anti- CD49b staining were done with frozen sections. Monoclonal anti–IL-15 (Abcam; ab55276) and anti-MxA antibody M143 were used for immunohistochemistry.

Mouse Islet Isolation, Flow Cytometry, and Islet Transplantation. Mouse pancreatic islets were isolated using standard techniques involving pancreatic inflation with collagenase type V (Sigma) and digestion. Isolated pancreatic islets from 10 mice were pooled together. Pooled islets were dissociated into a single cell suspension by washing in 2 mM EDTA/PBS and then incubation with 0.025% trypsin in Ca^{2+}-free PBS. Dissociated islet cells were washed and filtered with a 40-μm cell strainer and then blocked with anti-mouse CD16/32 (eBioscience) and control IgG mixture, and then stained with anti–IL-15Rα, anti-CD45, anti-CD80, anti-CD86, anti–ICAM-1, and anti-class I and anti-class II antibodies.

For transplantation, purified islets were hand-picked and pooled together. Approximately 1,500–2,000 islets were transplanted into the diabetic double transgenic mice under the kidney capsule following the methods described in ref. 1.

Nanostring, Real-Time RT-PCR, and RT^2 Profiler PCR Array. *IL-15* and *IL-15Rα* mRNA levels in microdissected islets were analyzed using NanoString Technologies.

For real-time RT-PCR analysis of *IL-15* and *IL-15Rα*, total RNAs from different tissues were extracted using RNeasy mini plus kits (Qiagen). Reverse transcription reactions were carried out for each sample (250 ng) using the SuperScript First-Strand synthesis system (Invitrogen). The Taqman Universal PCR Master Mix, the mouse *IL-15*, *IL-15Rα* primer/probe, and the *HPRT1* primer/probe sets were purchased from Applied Biosystems. The detection of mouse *IL-15*, *IL-15Rα*, and *HPRT1* was performed using an ABI Prism 7500 sequence detection system (Applied Biosystems) according to manufacturer instructions. The copy numbers of *IL-15* and *IL-15Rα* mRNA in the double transgenic mice were normalized to the copy numbers of the *HPRT1* gene and were expressed as fold increase versus normalized copy numbers of *IL-15* and *IL-15Rα* mRNA in age and sex matched wild-type C57BL/6 mice.

For RT^2 profiler PCR array, total RNA were obtained from isolated islets from 10 double transgenic mice (before the onset of diabetes) and 10 wild-type C57/BL6 mice using RNeasy mini plus kit (Qiagen). Three independent samples from each strain were collected. The RT^2 profiler PCR array for mouse inflammation and autoimmunity genes and data analysis were carried out by Invitrogen.

T-Cell Transfer. Total T cells or CD4 cells were purified from the spleen of diabetic RIP–IL-15/IL-15Rα double transgenic using magnetic beads (Miltenyi). Around 10–20 × 10^6 cells were transferred into irradiated C57BL/6 recipient mice or B6.Rag1$^{-/-}$ recipient mice.

1. Szot GL, Koudria P, Bluestone JA (2007) Transplantation of pancreatic islets into the kidney capsule of diabetic mice. *J Vis Exp* 9:e404.

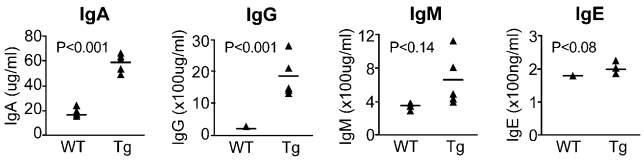

Fig. S1. Increased serum IgA and IgG levels in the IL-15/IL-15Rα double transgenic mice at the onset of diabetes.

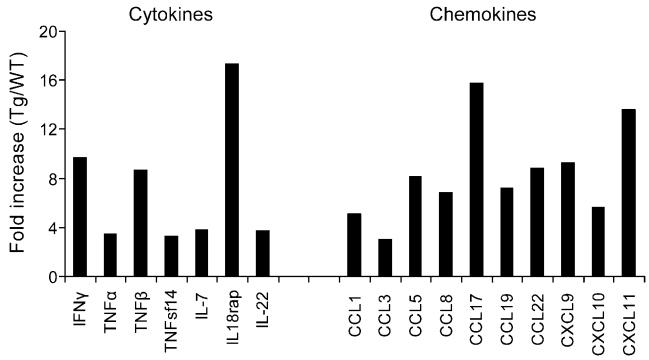

Fig. S2. Increased expression of proinflammatory cytokines/chemokines in the purified islet cells from IL-15/IL-15Rα double transgenic mice before the onset of diabetes.

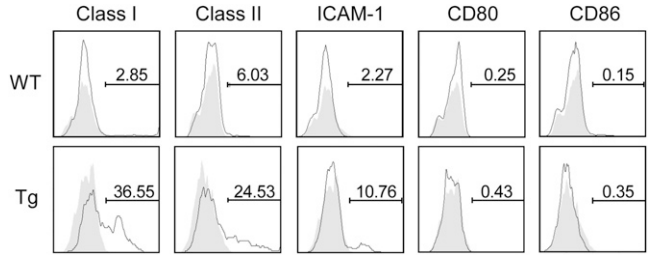

Fig. S3. Increased islet cell surface expression of MHC classes I/II and ICAM-1 in the purified islet cells from IL-15/IL-15Rα double transgenic mice before the onset of diabetes.

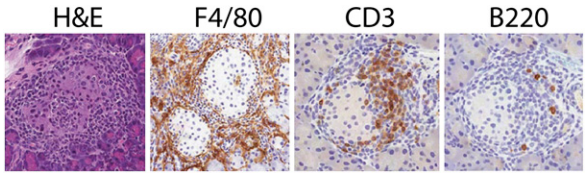

Fig. S4. Mononuclear cell infiltration of pancreas in the double transgenic mice depleted of CD4 cells.

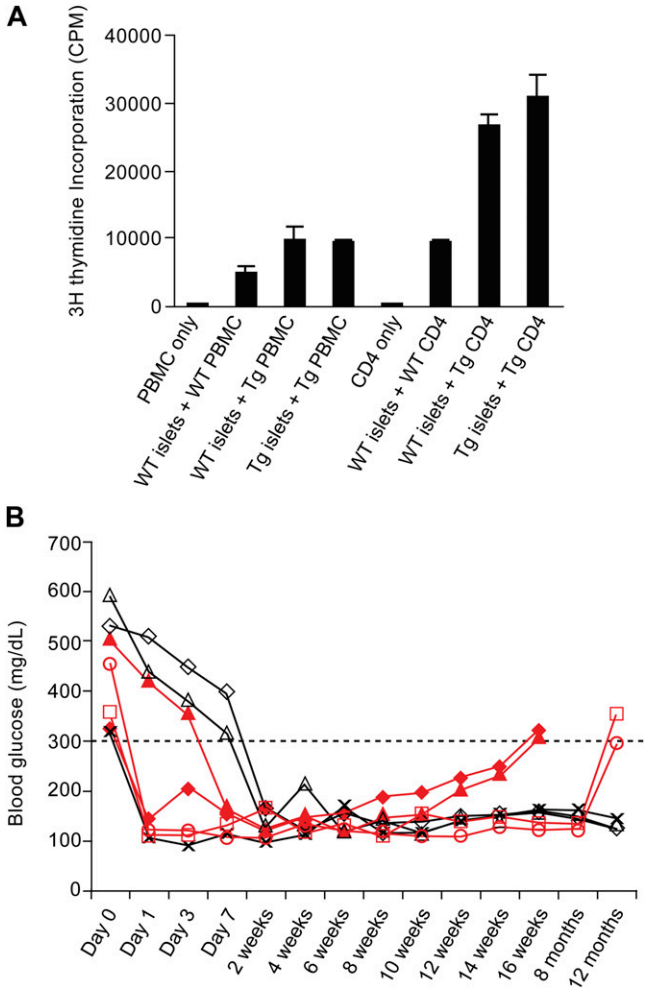

Fig. S5. CD4 cells (peripheral blood mononuclear cells) from the double transgenic mice responded to wild-type islets in vitro and wild-type islets were rejected when transplanted into diabetic double transgenic mice in vivo. (*A*) proliferation of islet CD4 cell (or PBMC) co-culture. (*B*) Blood glucose levels in diabetic transgenic mice received wild type islet transplants.

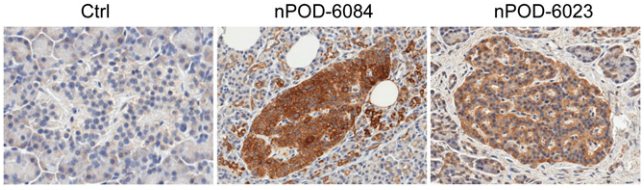

Fig. S6. Increased expression of MxA in the islets of a patient with type 1 diabetes [Network for Pancreatic Organ Donors (nPOD)-6084] and an autoantibody positive donor (nPOD-6023).

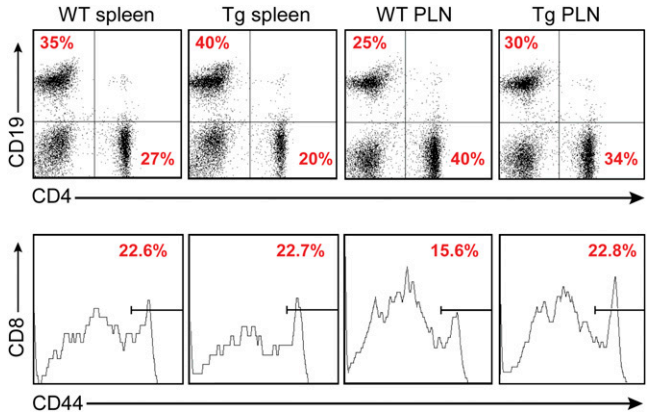

Fig. S7. Change of CD4, CD19, and CD8CD44^{high} cell percentages in the spleen and in the pancreatic lymph node (PLN) in the double transgenic mice at diabetes onset.

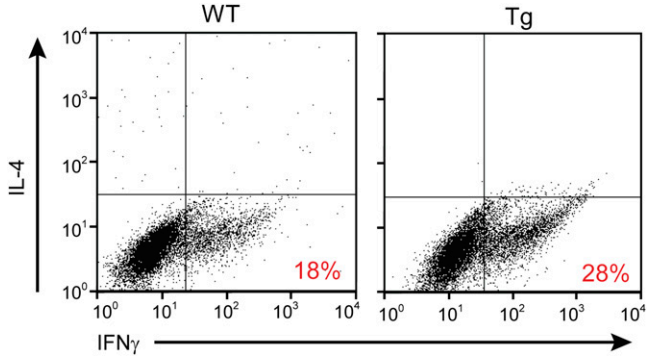

Fig. S8. CD4 cells from double transgenic mice are more prone to Th1 priming.