ONLINE APPENDIX-Supplemental Materials and Methods:

Bone marrow transplantation: NOD mice older than 13 weeks were routinely checked twice a week for urine and/or blood glucose. Mice having blood glucose levels higher than 300 mg/dl for 3 consecutive days were diagnosed as "diabetic". Diabetic NOD/LtJ mice were implanted with insulin pellets (Linshin, Toronto, ON, Canada) for control of hyperglycemia. The "late-stage" diabetic NOD mice were conditioned at least 21 days after diagnosis. Anti-CD3 (145-2C11) and anti-CD8 (116-13.1) antibodies were produced as described previously (26). Diabetic mice were injected i.v. with anti-CD3 and anti-CD8 mAb at a dose of 5 μg/g of body weight each, and the mice were given the second antibody injection 5 days after the first injection. HCT was usually performed 5 days after the second antibody injection. Each mouse was given one injection of donor $CD4^+$ T cell-depleted spleen cells and bone marrow cell (50×10^6 each) from FVB/N donor mice. Depletion of $CD4^+$ cell was performed with anti-CD4 micromagnetic beads and AutoMACS sorter (Miltenyi Biotec, Aubury CA). The purity was >95%.

Isolation of islets, islet transplantation, in vivo and ex vivo bioluminescent imaging (BLI), and glucose tolerance test: Pacreatic islets were isolated from WT FVB/N, luc^+ FVB/N mice or NOD mice after digestion with collagenase XI (Sigma) and isolated with Histopaque-1077 (Sigma). Islets sizing > 50 μm were hand-picked and evenly distributed to different groups. Islet transplantation into the liver or pancreas was always performed in pairs. For portal vein injection, islets were packed in a small tube connected to a $23^{\#}$ needle and then injected into the portal vein. For implantation of islets into pancreas, islets were packed in a small tubin with sharp ends and then the islets were directly injected into the pancreas tissue. In order to spread the islets and avoid clustering, the injecting small tubin was slowly retracted during injection. In vivo and ex vivo BLI was previously described (2). Glucose tolerance test and measurement of serum insulin was previously described (2; 26).

Histopathology of Pancreatic Islets, BrdU labeling of proliferating β cells, and immunofluoresent staining: Formalin-fixed tissue sections were stained with hematoxylin and eosin. Insulin staining of the tissue sections was performed using Tech-mate 1000 autostainer (Ventana, Tucson, AZ) as described previously (2; 24). BrdU labeling was performed as previously described (2). In brief, mice were injected i.p. daily with BrdU (Sigma, St. Louis, MO) in PBS at a dose of 50 μg/g of body weight for 2 weeks. Harvest of islet grafts in pancreas and liver tissues were performed under the guidance of in vivo and ex vivo BLI. Fomalin-fixed graft tissues were embedded in paraffin. Two sections of 5 μm thickness were cut with a distance of 100 μm between each section. Immunofluoresent staining of insulin, BrdU and nuclear were described in our previous publication (2). All $insulin^+$ and $insulin^+BrdU^+$ cells in a section were counted, and more than 500 islet β cells/organ in a mouse were evaluated. The proliferating insulin-secreting β cells were identified as $BrdU^+insulin^+$ after BudU-labeling. Photos were taken by using an Olympus BX51 fluorescent microscope equipped with a Pixera cooled CCD camera.

Sequential CldU and IdU labeling of proliferating β cells, and immunofluoresent staining: Sequential CldU and IdU labeling of graft islet β cells was performed as previously described (28) In brief, one day after islet transplantation, recipients were injected IP with CldU (50 μg/g) for 5 consecutive days. After 2 days rest, the recipients were injected IP with IdU (50μg/g) for 5 consecutive days. Two days after completion of IdU labeling, islet grafts were harvested. Harvest of islet grafts in the pancreas and liver

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tissues were performed under the guidance of in vivo and ex vivo BLI. Fomalin-fixed graft tissues were embedded in paraffin. Two sections of 5 μm thickness were cut with a distance of 100 μm between each section. Immunostaining of insulin, CldU and IdU were performed as described by Kushner and colleagues with minor changes on secondary antibodies (28). All insulin$^+$, CldU$^+$insulin$^+$, IdU$^+$insulin$^+$, and CldU$^+$IdU$^+$insulin$^+$ cells in a section were counted, and more than 500 islet β cells/organ in a mouse were evaluated. The replicating β cells were identified as CldU$^+$insulin$^+$ or IdU$^+$insulin$^+$ cells. The progenitor-derived β cells were identified as CldU$^+$IdU$^+$insulin$^+$ cells. Photos were taken by using an Olympus BX51 fluorescent microscope equipped with a Pixera cooled CCD camera.

Measurement of gene expression levels of the retrieved islet grafts: Retrieving islet grafts from the liver and pancreas was performed as described by Mattsson et al (30). The donor islets were identified with ex vivo BLI. The RNA was purifed with TRI Reagent (Sigma), and first-strand cDNA was synthesized by SuperScript III First Synthesis for RT-PCR (Invitrogen) following the manufacturer's protocol. Then mRNA was quantified by real-time quantitative polymerase chain reaction (PCR) using 7300 Fast Real-Time PCR System (Applied Biosystems, Forest City, CA). The following specific forward (F) and reverse (R) primers were used: insulin F TCTTCTACACACCCATGTCCC, insulin R GGTGCAGCACTGATCTAC; GLUT-2 F CATTCTTTGGTGGGTGGC, GLUT-2 R CCTGAGTGTGTTTGGAGCG; GCK F CATTGAATCAGAGGAGGGCAGC, GCK R TAGTGGACTGGGAGCATTTGTGGG; PDX-1 F CGGACATCTCCCCATACG, PDX-1 R AAAGGGAGCTGGACGCGG (31); glucagon F GATCATTCCCAGCTTCCCAG, glucagon R CTGGTAAAGGTCCCTTCAGC (32); luciferace F GCCTGAAGTCTCTGATTAAGT, luciferace R ACACCTGCGTCGAAGATGT. Relative expression levels of genes were normalized within each sample to the donor specific transgenic gene luciferace and were presented relative to the expression levels in islets before transplantation.

Evaluation of vascular density. Harvest of islet grafts in the pancreas and liver tissues were performed under the guidance of in vivo and ex vivo BLI. Fomalin-fixed graft tissues were embedded in paraffin. Five sections of 5 μm thickness were cut with a distance of 100 μm between each section. Staining of BS-1 was performed as previously described (34-37) and the secondary staining was Texas Red conjugated Streptavidin. Staining of insulin and DNA were described in our previous publication (2). Sections stained for DNA, insulin and BS-1 and were visualized with an Olympus IX50 fluorescent microscope and equipped with an Olympus DP7e CCD camera (Olympus America, Melville, NY). The images were analyzed by using Olympus Microsuite B35V image analysis software in the Measure-Area mode. Vascular density in the islet grafts was identified by BS-1 staining (red) inside of insulin staining (green). The ratio of total BS-1 stained areas versus total insulin stained areas of all five sections was calculated and compared.

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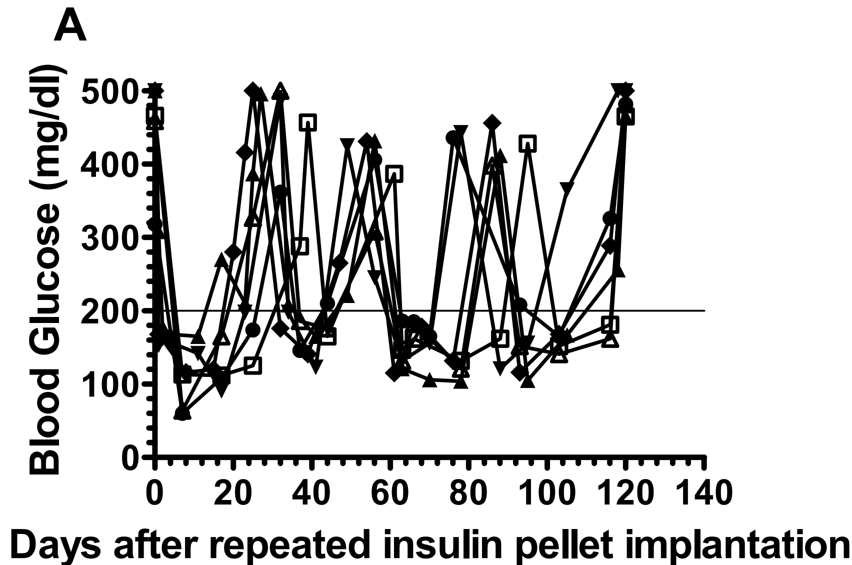
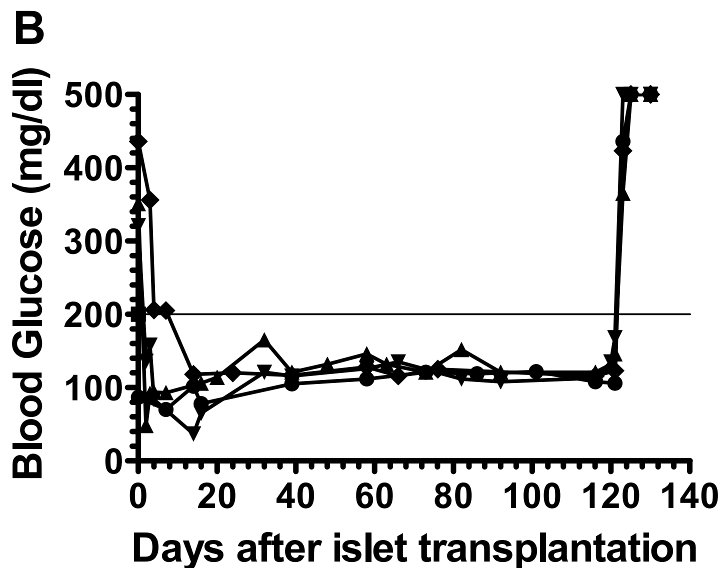
Sup. Fig. 1. Reversal of diabetes in chimeric late-stage diabetic NOD mice required islet transplantation. (A) Chimeric late-stage diabetic NOD recipients were repeatedly implanted with insulin pellets for the control of hyperglycemia for 120 days, but none (0/6) of the recipients showed reversal of diabetes. (B) Chimeric recipients were transplanted with 600 donor islets under kidney capsule. 4/4 of recipients showed normal glycemia for more 120 days after transplantation. However, all of the recipients showed hyperglycemia again after nephrectomy. These results indicate that islet transplantation is usually required for reversal of late-stage of diabetes.





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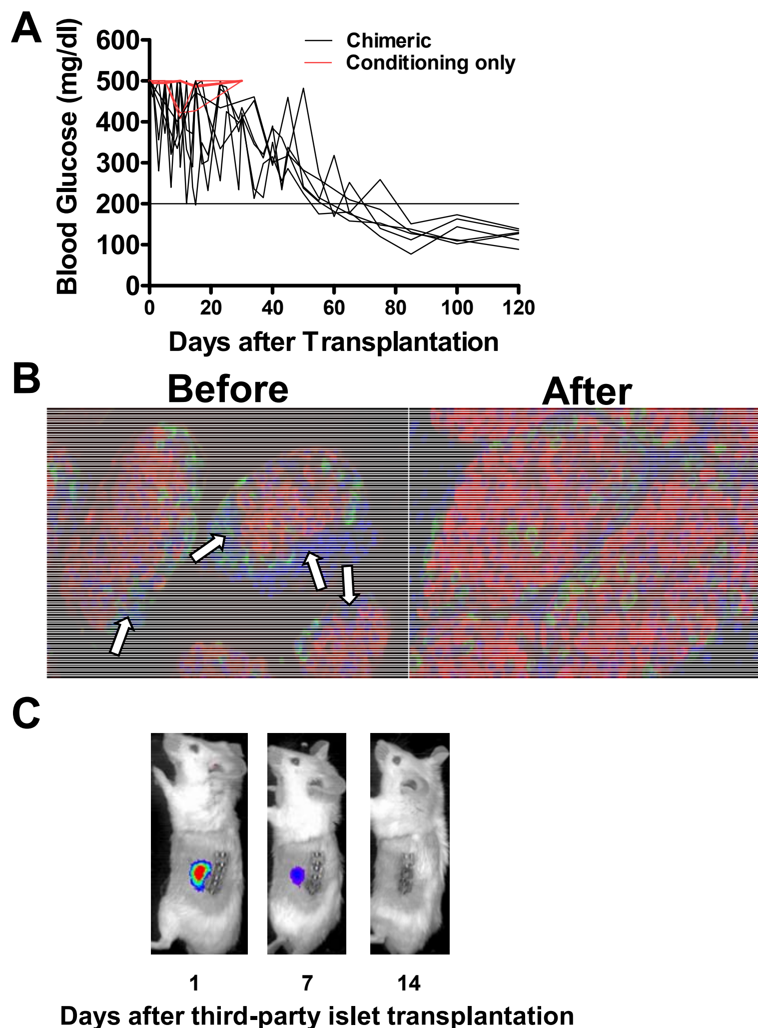
Sup. Fig. 2. Small amount of syngeneic NOD islet grafts implanted in the pancreas reversed late-stage diabetes in chimeric NOD mice. Late-stage diabetic NOD mice were conditioned with anti-CD3/CD8 and transplanted with bone marrow and $CD4^{+}$ T-depleted spleen cells. Next day, 100 islets from 10 weeks old female NOD mice were transplanted into the pancreas of chimeric recipients or control mice given anti-CD3/CD8 conditioning only. The blood glucose was checked up to 120 days after islet transplantation. 120 days after syngeneic islets transplantation, 100 islets from third-party donors (Luc^{+} C57BL/6) were transplanted into the pancreas of the chimeric recipients. (A) Blood glucose changes in chiemric (black line) recipients or in recipients given conditioning only (red line). (B) Histopathology of islet graft before and 120 days after transplantation. Islet tissues were stained for insulin (red), glucagon (green), and DNA (blue). Arrows indicated infiltration in islets before transplantation, and no infiltration was observed in islet grafts 120 days after transplantation. One representative of 3 examined grafts in each group is shown. (C) In vivo BLI of third-party donor islets, one representative of 3 recipients is shown.



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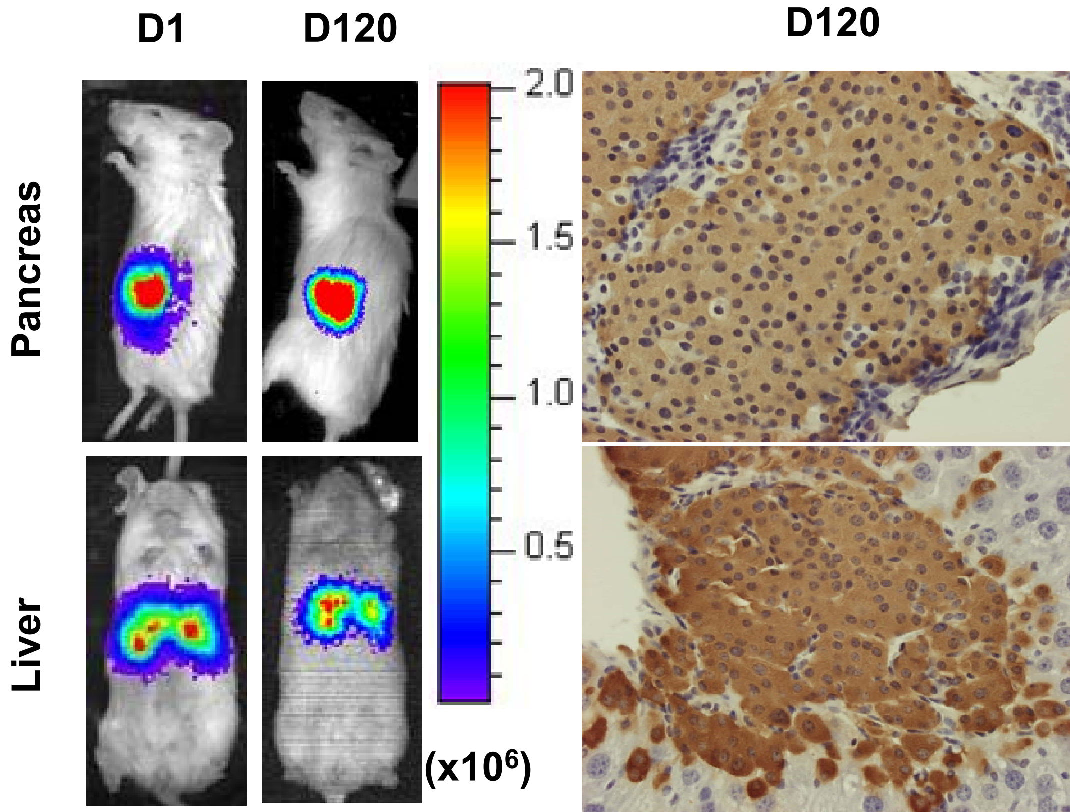
Sup. Fig. 3. No signs of rejection in islet grafts implanted in the liver of chimeric recipients. 50 islets from luc^+ FVB/N donors were transplanted into the pancreas or liver of chimeric late-stage diabetic NOD mice. The in vivo BLI and histopathology of islet grafts in pancreas of recipients with long-term normal glycemia and islet grafts in the liver of recipients with relapse of hyperglycemia were compared. In vivo BLI of islets on day 1 and day 120 and histopathology of islet grafts (40x) on day 120 are shown. One representative of 5 examined recipients is shown.



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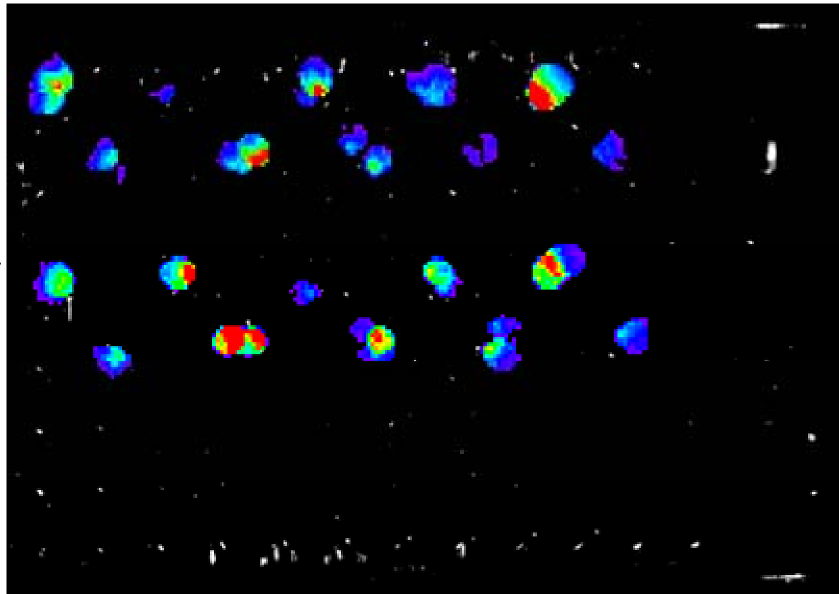
Sup. Fig. 4. Donor islets that were retrieved from pancreas and liver were identified with ex vivo BLI. After graft islets were retrieved from pancreas and liver, the islets were put into a black 96-well plate, respectively. Then luciferin was added into the wells and ex vivo BLI was performed to identify the donor-type islets. Islets in the wells of row A and B were retrieved from pancreas and the islets in wells of row D and E were retrieved from liver. One representative of experiments is shown.



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