Islet viability assay and Glucose Stimulated Insulin Secretion assay

Islet cell viability was determined by colorimetric (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay using CellTiter 96 non-radioactive cell proliferation assay kit (Promega, Madison, WI). After the treatment with experimental and control probes for 48 hrs, islets were washed with culture medium two times, and resuspended in 24 well non-treated plate followed by addition of 150 μl of a tetrazolium dye solution. After incubation at 37 °C for 4 hrs 1 ml of solubilization solution/stop mix was added to each well to dissolve the dark blue crystals. After overnight incubation, the solution was transferred to cuvette and was read on SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA; \( \lambda_{\text{test}} = 570 \text{nm} \) and \( \lambda_{\text{reference}} = 630 \text{nm} \)).

Insulin secretion was evaluated in glucose-stimulated insulin secretion test using static incubation of MN-siB2M-labeled or MN-siSCR-labeled islets at low (1.7 mmol/L) and high (20 mmol/L) glucose concentrations. Insulin concentrations in supernatants were measured using a human insulin ELISA kit (Mercodia, Uppsala, Sweden). A stimulation index was calculated as the ratio of stimulated to basal insulin secretion normalized by the insulin content.

RT-PCR and Western Blot

After incubation with MN-siB2M or MN-siSCR, total RNA was isolated from treated and control islets using an RNeasy Mini kit (Qiagen Inc., Valencia, CA). Relative levels of B2M mRNA were determined by real-time quantitative RT-PCR (TaqMan protocol). TaqMan analysis was performed using an ABI Prism 7700 sequence detection system (PE Applied Biosystems, Foster City, CA). The PCR primers and TaqMan probe specific for B2M were designed using Primer Express software 1.5. Primer and probe sequences were as follows:

- Forward primer, 5’-GTCTCGCTCCGTGGCCTTA-3’;
- Reverse primer, 5’-GAGTACGCTGGATAGCCTCCA-3’;
- TaqMan Probe 5’-CTGTGCTCGCGCTACTCTCTTTCTGG-3’.

Eukaryotic 18S rRNA TaqMan PDAR Endogenous Control reagent mix (PE Applied Biosystems) was used to amplify 18S rRNA as an internal control, according to the manufacturer’s protocol. All samples were run in duplicate.

For Western blot analysis of in vitro and ex vivo samples, islets or grafts were lysed in RIPA buffer containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, and a protease inhibitor cocktail (Pierce, Rockford, IL), and placed on ice for 30 minutes followed by sonication for 15 seconds. The samples were fractionated on a 10% SDS–PAGE gel, transferred to a nitrocellulose membrane (0.2μm; Bio-Rad Laboratories, Hercules, CA) for 1 hr and blocked for 1 hr at room temperature with TBST buffer in 50 mM Tris (pH 7.6), 150 mM NaCl and 0.05% Tween-20, and supplemented with 5% fat free milk. After a brief rinse, the membrane was incubated with a rabbit polyclonal antibody to B2M (1:500 dilution; Abcam, Cambridge, MA) and a mouse monoclonal antibody to β-actin (1:2000 dilution; Ambion, Austin, TX) at 4°C overnight. After washing, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2500 dilution; Cell Signaling Technology, Danvers, MA) or horseradish peroxidase-conjugated goat anti-mouse IgG (H+L; 1:2500 dilution; Invitrogen, Carlsbad, CA), which was followed by detection with SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). Western blot analysis of ex vivo islet grafts was performed two weeks after adoptive transfer. Two mice from each group (experimental and control) were sacrificed and their islet grafts collected for processing. As shown in Supplemental Figure 7B, the islet grafts could be easily identified on the surface of the recipient’s kidney. The islet grafts were dissected from the kidney and the total protein was purified from the collected tissue.
**Splenocyte isolation**

Splenocytes were isolated from 4-6 week-old NOD mice (The Jackson Laboratory, Bar Harbor, ME). Briefly, the spleen was put into the 70µm cell strainer (BD Biosciences, Bedford, MA) and mashed through the cell strainer into the petri dish. Then the suspended cells were washed with DMEM culture media. Cell pellet was resuspended in 5mL red blood cell lysing buffer (Sigma-Aldrich, St. Louis, MO) and incubated at RT for 5 minutes. Overall, $2.7 \pm 1.2 \times 10^7$ splenocytes were isolated per animal. After isolation of the splenocytes, the dead cells and debris were removed using MACS dead cell removal kit according to the manufacturer’s protocol (Miltenyi Biotec, Auburn CA). After separation, the lymphocyte compartment of the splenocytes was evaluated by FACS with PE-labeled rat anti-mouse CD8 monoclonal antibody (BD Biosciences, Bedford, MA) and FITC-labeled anti-mouse CD3 monoclonal antibody (Cedarlane labs, Hornby, Ontario, Canada). As shown in Supplemental Figure 8, there were $18.5 \pm 4\%$ of CD3+CD8+ cells, $36.5 \pm 2\%$ of CD3+CD8- cells (CD4+ T cells) and $44.5 \pm 1\%$ of CD3-CD8- cells (mainly B cells and macrophages).

**Supplementary Figure 1.** Schematic illustration of the conjugation of the probes.

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Supplementary Figure 2.
B: Quantitative analysis showed that conjugation resulted in 1.8pmole siRNA/µg Fe.

Supplementary Figure 3. Prussian Blue staining of the islets treated with MN-siB2M (A) or MN-siSCR (B) probe for 48 hr. Tiny blue dots in the cytoplasm of the islet cells represent clusters of nanoparticles (magnification bar = 10 μm).
**Supplementary Figure 4.** A: MTT assay revealed that MN-siB2M treatment did not induce significant toxicity to the islets (p > 0.05). B: Glucose stimulated insulin secretion assay of islets incubated with MN-siB2M or MN-siSCR probes for 48hrs. C: Glucose stimulation index was not significantly different in both treatment groups compared with sham islets (p > 0.05).
SUPPLEMENTARY DATA

**Supplementary Figure 5.** Purity of isolated CD8a+ cytotoxic T cells from NOD mouse splenocytes was confirmed to be 93.5% by FACS analysis.

**Supplementary Figure 6.** A: *In vivo* near infrared optical imaging of islet grafts (bright spot) 1 day before adoptive transfer. B: Post-mortem specimen showing islet grafts under the left kidney capsule two weeks after adoptive transfer (arrow).
Supplementary Figure 7. Blood glucose monitoring revealed restoration of normoglycemia after islet transplantation in STZ-induced diabetic animals from both groups followed by blood glucose increase after adoptive transfer.

![Graph showing blood glucose levels over time with a BG = 250 mg/dL marker.](image-url)
Supplementary Figure 8.
A: The content of isolated splenocytes was evaluated by FACS analysis.
B: The ratio of subpopulations in splenocytes used for adoptive transfer: CD3+CD8+ cells (18.5±4%), CD3+CD8- cells (36.5±2%) and CD3-CD8- cells (44.5±1%).

Supplementary Movie 1. 3D reconstruction of optical images indicating the location of the kidney with grafted islets.

Supplementary Figure 2. 3D reconstruction of optical images showing the location of the graft under the kidney capsule.