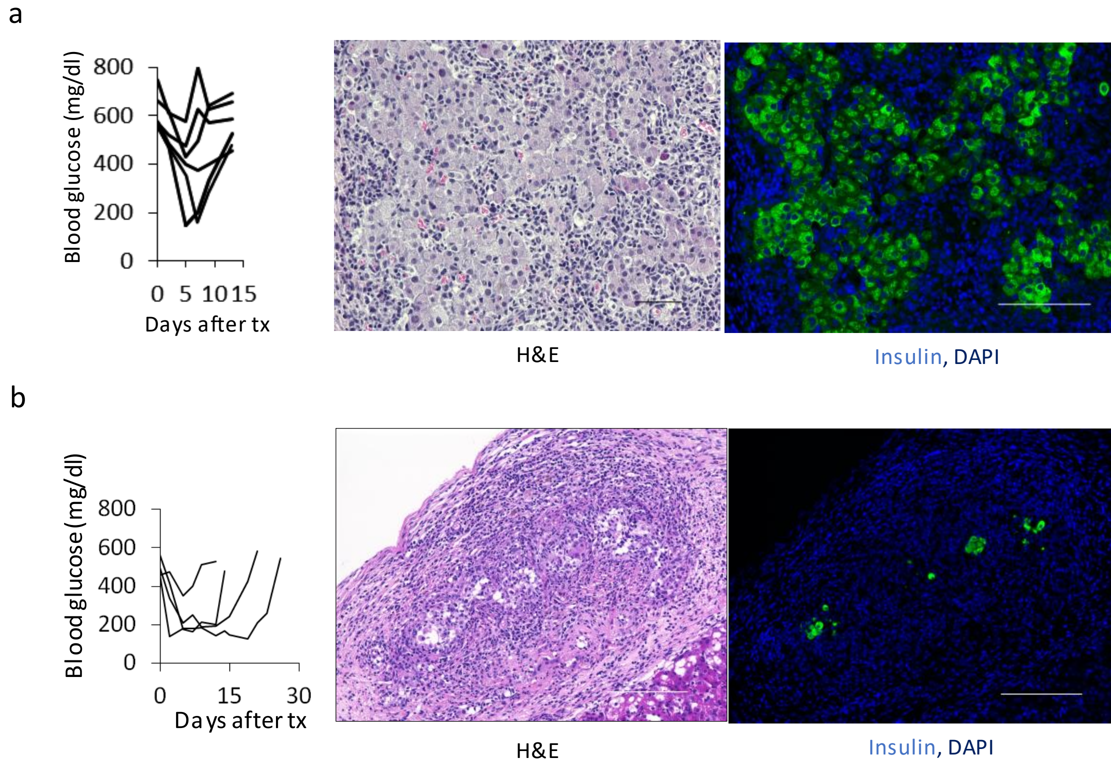
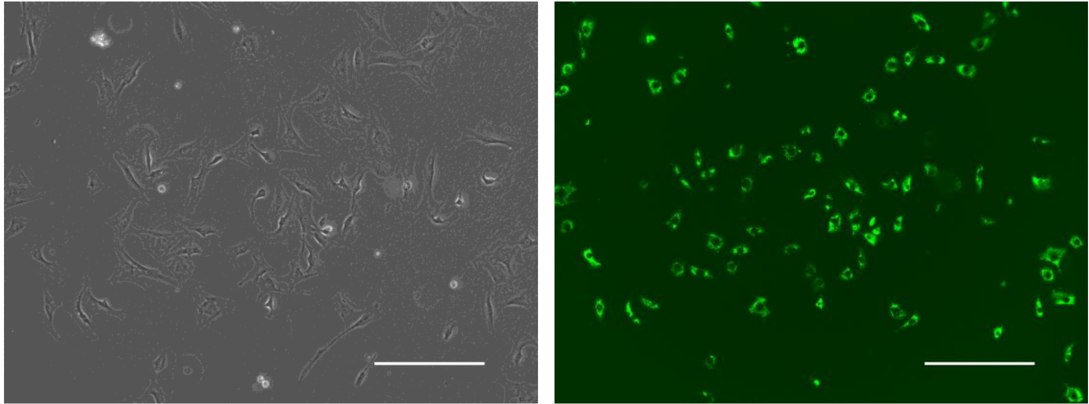
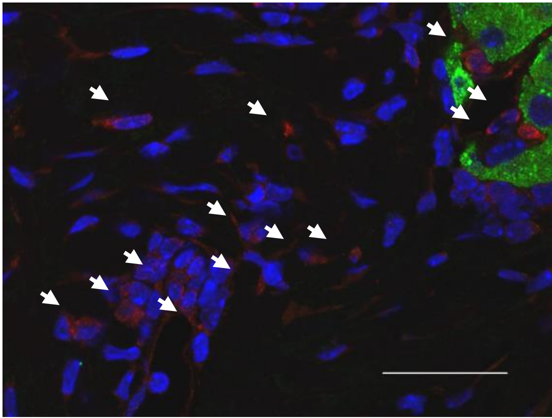
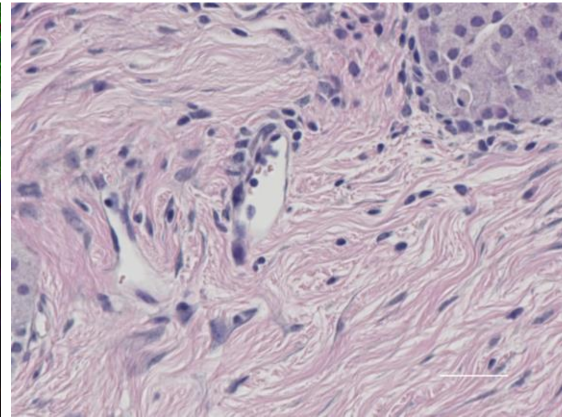


Supplemental figure 1. Rejection of islet allografts in the dorsal subcutaneous site and beneath the kidney capsule of recipient mice after the local pretreatment with bFGF.
An agarose rode containing bFGF was implanted in the dorsal subcutaneous site (a) or beneath the kidney capsule (b) of C57BL/6 mice. At 11 day after the implantation, mice were made diabetic with the i.v. injection of STZ (180 mg/kg) and then 3 days later, the agarose rod in the dorsal subcutaneous site or beneath the kidney capsule of recipient mice was extirpated and BALB/c islets were grafted in the space after the removal of a rod. Individual lines represent the blood glucose levels of each animal. The subcutaneous tissue or the kidney bearing allogeneic islets were removed by 24 day after transplantation and the sections were stained for H&E and for insulin (green) and DAPI (blue). Bars represent 100 and 150 μm in a and b, respectively.



Supplemental figure 2. MSCs expanded in the ISWAT after the treatment with bFGF were isolated as adherent cells in the tissue culture dish at 3 day after in vitro culture. Then, MSCs were labeled with fluorescent dye and cultured further. The photo was taken at 1 day after in vitro culture of labeled cells under fluorescent microscopy. Bars represent 300 μm.

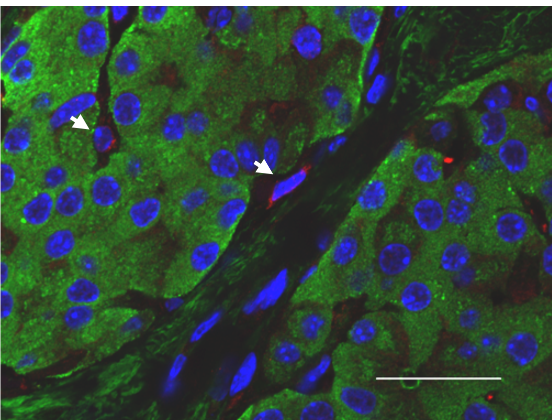
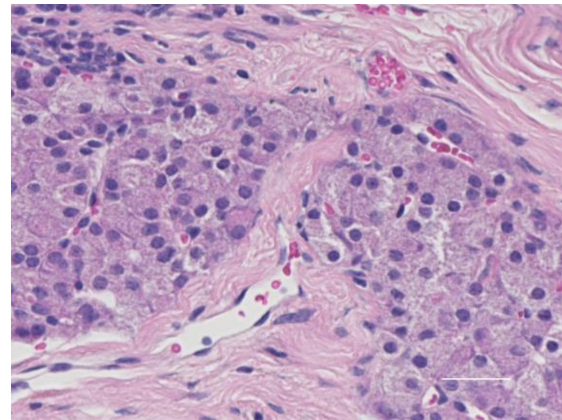
a

Insulin, TGFβ1, DAPI

H&E

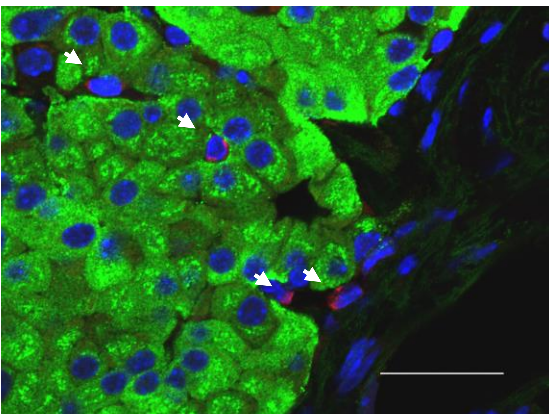
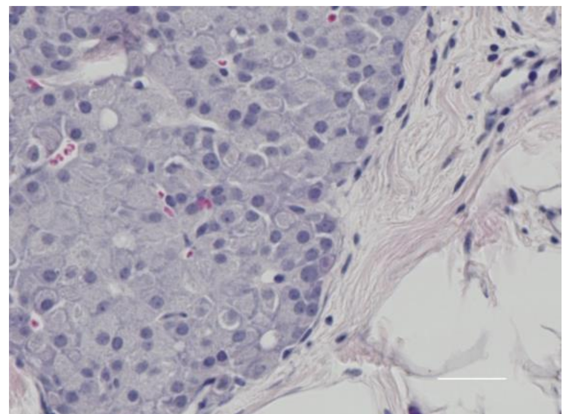
b

Insulin, TGFβ1, DAPI

H&E

c

Insulin, TGFβ1, DAPI

H&E

Supplemental figure 3. BALB/c islet allografts beneath the kidney capsule of normoglycemic C57BL/6 mice at 30 (a), 90 (b) and 240 day (c) after co-transplantation with syngeneic TGFβ-producing MSCs expanded in the inguinal subcutaneous white adipose tissue pretreated with bFGF. The sections were stained for insulin (green), TGFβ1 (red) and DAPI (blue). Bars represent 30 µm. Arrows indicate MNCs positive for TGFβ1.

Supplemental Materials

Islet isolation and transplantation

Islets were isolated from the mouse pancreas (1, 2) and those with a diameter of 150-250 μm were hand-picked, cultured in medium (DMEM, FUJIFILM-WAKO Pure Chemical Co., Osaka) supplanted with 10% fetal bovine serum (ThermoFisher Scientific, Waltham, MA) and Antibiotic-Antimycotic (ThermoFisher Scientific) at 24°C in a CO_2 incubator (95% air + 5% CO_2) for 1-3 days and then used as donors.

Blood sampling before and after transplantation

Blood samples were obtained from the tail vein of recipient mice. The non-fasting blood glucose levels of recipient mice were measured before and twice after the injection of STZ and 3 times a week until 30 days after transplantation and once a week thereafter.

Preparation and implantation of agarose rods as a bFGF delivery device. Freeze-dried agarose rods were prepared as described previously (7). The bFGF solution (Kaken Pharmaceutical Co., Tokyo) was uniformly dropped onto the freeze-dried agarose rod and placed in a 96 well plastic plate (BD Falcon, Bedford, MA) and allowed to absorb overnight at 4°C before use.

Primary and secondary antibodies for Immunohistochemistry of transplanted islets.

The primary and secondary antibodies used were the following: guinea pig anti-insulin (1:200, Agilent Cat# IR002, RRID:AB_2800361), mouse anti-glucagon (1:500, Sigma-Aldrich Cat# G2654, RRID:AB_259852) and 488-Alexa donkey anti-guinea pig IgG (Jackson ImmunoResearch Labs Cat# 706-545-148, RRID:AB_2340472), Cy3 anti-mouse IgG (Jackson ImmunoResearch Labs Cat# 115-165-072, RRID:AB_2338688), respectively. Nuclei of the cells were stained with DAPI (Sigma-Aldrich, 1mg/ml).

Antibodies for flow cytometry.

Antibodies used for FACS analysis on infiltrating cells in the ISWAT of mice before and after transplantation were as follows. Anti-mouse FcRgII/III (2.4G2, Cat# 101302, RRID：AB_312801), PE-conjugated anti-CD3e (145-2C11, Cat# 100308, AB_312673), peridinin–chlorophyll protein (PerCP)-conjugated anti-CD4 (RM4-5, Cat# 100538, RRID：AB_893325), PE-Cy7-conjugated anti-CD8 (53 6.7, Cat# 100722, RRID：AB_312761), allophycocyanin (APC)-conjugated anti-CD69 (H1.2F3,　Cat# 104513, RRID：AB_492844). All antibodies were purchased from BioLegend, San Diego.

Antibodies used for FACS analysis on MSCs expanded in the ISWAT after the treatment with bFGF were purchased by BioLegend as follows. Purified anti-mouse CD16/32 (Clone:93, Cat#101301, RRID: AB_312800), APC anti-mouse LAP(TGF-β1)

(Clone:TW7-16B4 (Mouse IgG1,κ) Cat#141405, RRID:AB_10898159), FITC anti-mouse CD106 (Clone:429 (MVCAM.A) (Rat IgG2a,κ) Cat#105705, RRID:AB_313206), FITC anti-mouse Ly-6A/E (Sca-1) (Clone:D7 (Rat IgG2a,κ) Cat#108105, RRID:AB_313342), FITC anti-mouse/human CD44 (Clone IM7 (Rat IgG2b,κ) Cat#103005, RRID:AB_312956), FITC anti-mouse/rat CD29 (Clone:HMβ1-1 (Armenian Hamster IgG) Cat#102205, RRID:AB_312882), FITC anti-mouse Lineage Cocktail with Isotype Ctrl (Clone:145-2C11; RB6-8C5; RA3-6B2; Ter-119; M1/70 Cat#133301, RRID:AB_10697030), FITC Armenian Hamster IgG Isotype Ctrl (Cat#4009051), FITC Rat IgG2b,κ Isotype Ctrl (Cat#400605, RRID:AB_326549), FITC Rat IgG2a,κ Isotype Ctrl (Cat#400505, RRID:AB_2736919), APC Mouse IgG1,κ Isotype Ctrl (Cat#400120, RRID:AB_2888687), PE Rat IgG2b,κ Isotype Ctrl (Cat#400636, RRID:AB_893669). PE anti-mouseMHC Class II (IA/IE) (Clone:M5/114.15.2 (Rat IgG2b,κ) Cat#12-5321-82, RRID:AB_465928) was purchased by Thermo Fisher Scientific.

Antibodies for immunohistochemical analysis of MSCs.

Primary and secondary antibodies were purchased from Abcam (Cambridge, UK) as follows. Anti-mouse CD44 (polyclonal, Abcam Cat# ab157107, RRID:AB_2847859),

anti-mouse Sca-1(ERP3355, Abcam Cat# ab109211, RRID:AB_10862573), anti-mouse CD29 (EPR16895, Abcam Cat# ab179471, RRID:AB_2773020), anti-mouse CD106 (EPR5047, Abcam Cat# ab134047, RRID:AB_2721053), anti-TGFβ1 antibody (EPR21143, Abcam Cat# ab215715, RRID: AB_2893156) conjugated with Cy3 (Jackson ImmunoResearch Labs Cat# 711-165-152, RRID:AB_2307443) was used as a secondary antibody.

Materials for co-transplantation of allogeneic islets with MSCs beneath the kidney capsule of streptozotocin-induced diabetic mice.

Individual batches of 400 BALB/c islets and MSCs in medium were separately transferred into 1.5 ml microfuge tubes (Trefflab, Degersheim, Switzerland). Islets and MSCs were placed in the bottom of the tube by 1 G and by centrifugation at 190 G for 1 min, respectively, and supernatants were removed from each tube, leaving behind approximately 50 μl of the medium. Then, islets and MSCs were aspirated into the same PE50 polyethylene tube (Beckton Dickinson, Sparks, MD), approximately 20 cm in length, with the aid of a Hamilton syringe (Hamilton Company, Reno, Nevada). The PE50 tubing was then bent and placed inside a 15 ml conical tube so that the center of the tubing was placed in the bottom of the conical tube, which was centrifuged at 190 G for 1 min, thus forming a pellet composed of islets with MSCs in the center of the PE50

tubing. After measuring the length of the pellet using an Electronic Digital Caliper (General, New York, NY) as an objective index of donor cell mass (Figure 6a), the PE50 tubing was cut with scissors approximately 1 mm distant from the edge of the pellet and then, a Hamilton syringe was connected to the other side of the PE50 tubing. Transplantation of islets beneath the kidney capsule was performed as reported previously (6) with minor modifications as follows. Briefly, after the exposure of the left kidney, a small incision was made on the kidney capsule and a subcapsular space was created with use of a glass spatula where islets and MSCs loaded in the PE50 tube were injected.

Administration of anti-TGFβ1 antibody.

Anti-TGFβ1 antibody (Bio X Cell Cat# BE0057, RRID:AB_1107757) or isotype control mouse IgG1 (Bio X Cell Cat# BE0083, RRID:AB_1107784) was administered intraperitoneally to mice receiving allogeneic islets in the ISWAT pretreated with bFGF 8 times (200 μg/injection/mouse, day 0, 2, 4, 6, 8, 10, 12, 14) after transplantation. In mice receiving co-transplantation of allogeneic islets with MSCs isolated from the ISWAT treated with bFGF beneath the kidney capsule, anti-TGFβ1 antibody or isotype control mouse IgG1 was administered 3 times (500 μg/injection/mouse, day 0, 2, 4) after transplantation.

Labeling of TGF-producing MSCs with the fluorescent dye and co-transplanted with allogeneic islets beneath the kidney capsule of diabetic recipient mice.

Isolated MSCs from the ISWAT after the treatment with bFGF were in vitro labeled with fluorescent dye (CellBriteTM Green Cytoplasmic Membrane Dye, Biotium, Inc.) according to the manufacture's instruction prior to transplantation and co-transplanted with allogenic islets beneath the kidney capsule of STZ-diabetic mice. At 7 days after transplantation, the kidney bearing islet grafts was removed, sliced and snap-frozen and prepared for observation by fluorescent microscopy. Nuclei were stained with DAPI. The frozen section was also stained for H&E

Materials for in vitro generation of inhibitory MSCs.

MNCs isolated as stromal cells from the ISWAT of mice as described were inoculated in tissue culture dish (Corning #3295) with 3ml medium containing 4 μg bFGF and cultured *in vitro* in a CO2 incubator (95% air + 5% CO2) at 37°C. After overnight *in vitro* culture, non-adherent cells in the culture dishes were removed by medium change and adherent cells were cultured further in the 3ml medium containing 0.4 μg bFGF. Three days later, another medium change was made by replacing the 3ml medium containing 0.4 μg bFGF. Then, at 7th day in vitro culture, adherent cells were detached, counted and used for co-transplantation with BALB/c donor islets beneath the kidney capsule of STZ-induced

diabetic C57BL/6 mice.

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2. Okeda T, Ono J, Takaki R, Todo S: Simple method for the collection of pancreatic islets by the use of Ficoll-Conray gradient. Endocrinol Jpn 1979;26:495-499.