Supplemental Materials

Expanded Materials and Methods

Intraperitoneal glucose tolerance test

For the intraperitoneal glucose tolerance test, mice were fasted for 16 h and then injected with 15% D-glucose (Wako Pure Chemical Industries Ltd., Osaka, Japan). The concentration of plasma insulin was measured using the Ultra Sensitive Mouse/Rat Insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan).

Histology and immunostaining

The pancreas was harvested and fixed overnight with 4% paraformaldehyde (Wako), embedded in paraffin, and sectioned. The sections were stained with anti-insulin (Abcam, Cambridge, UK), or anti-glucagon (Cell Signaling Technology, Danvers, MA, USA) antibodies, followed by incubation with Alexa Fluor 488-conjugated donkey anti-rat antibody or Alexa Fluor 594¬-conjugated donkey anti-rabbit antibody (Invitrogen, Carlsbad, CA, USA). ImageJ software version 1.6.0_24 (NIH, Bethesda, MD, USA) was used to measure the sensitivity of the immunoreaction.

Non-contact co-culture system

MIN6 cells were cultured in the lower chamber of a 24-well Transwell plate insert with a

74-μm mesh size polyester membrane. The MIN6 cells were washed and DMEM was replaced. Concurrently, peritoneal macrophages (2 × 105 cells/well) were cultured in the upper chamber, and then the two sets of cells were placed together by assembling both parts of the Transwell and incubated in DMEM at 37°C. After 14 h of incubation, the Transwell-attached macrophages were removed and incubated for 24 h, after which MIN6 cells were used for the assay.

In vitro GSIS assay

MIN6 cells or those cells incubated with ONO-AE1-329 were maintained in DMEM containing 5.5 mM glucose for 4 h, and then the medium was changed to DMEM containing 25 mM glucose. After 18 h of incubation, insulin release was measured using an enzyme-linked immunosorbent assay kit (Ultra Sensitive Mouse/Rat Insulin ELISA kit, Morinaga Institute of Biological Science).

Cytometric bead assay

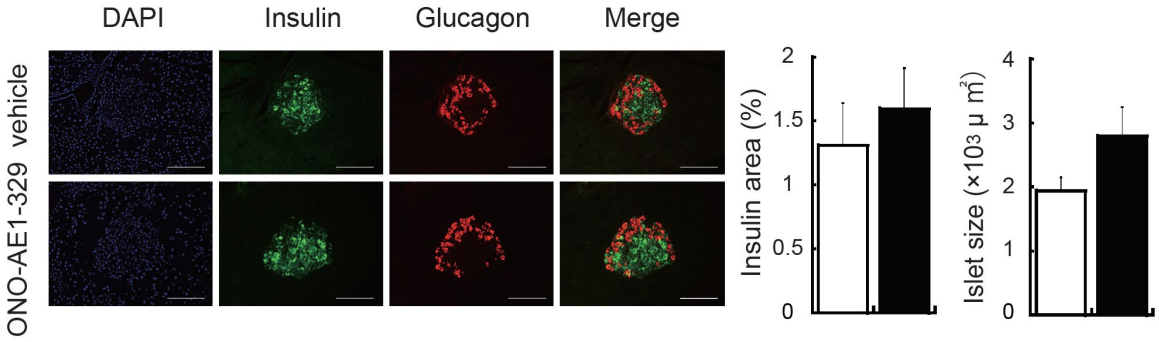
TNFα, IL-6 and IL-10 in the supernatant of macrophages after *in vitro* M1/M2 polarization was quantitatively estimated using the BD Cytometric Bead Array (CBA) kit (BD Biosciences, Franklin Lakes, NJ, USA).

Table S1

The PCR primers used for amplification were as follows:

Species	Gene	Primers
mouse	*β-actin*	5′-CCTGAGCGCAAGTACTCTGTGT-3′, 5′-GCTGATCCACATCTGCTGGAA-3′
	Tnfα	5′-CATCTTCTCAAAATTCGAGTGACAA-3′ 5′-TGGGAGTAGACAAGGTACAACCC-3′
	Tlr4	5′-ATGGCATGGCTTACACCACC-3′, 5′-GAGGCCAATTTTGTCTCCACA-3′
	Mcp-1	5′-GCTGGAGCATCCACGTGTT-3′, 5′-ATCTTGCTGGTGAATGTGTAGCA-3′
	Cd11c	5′-CTGGATAGCCTTTCTTCTGCTG-3′, 5′-GCACACTGTGTCCGAACTC-3′
	Cd68	5′-CTTCCCACAGGCAGCACAG-3′, 5′-AATGATGAGAGGCAGCAAGAGG-3′
	Cd163	5′-GGGTCATTCAGAGGCACACTG-3′, 5′-CTGGCTGTCCTGTCAAGGCT-3′
	Insulin	5′-GGAGCGTGGCTTCTTCTACA-3′, 5′-GGTGGGCCTTAGTTGCAGTA-3′
	Pdx1	5′-ACTTGA GCGTTCCAATACGC-3′, 5′-AGAGGGGGA ACGACTCTAGG-3′

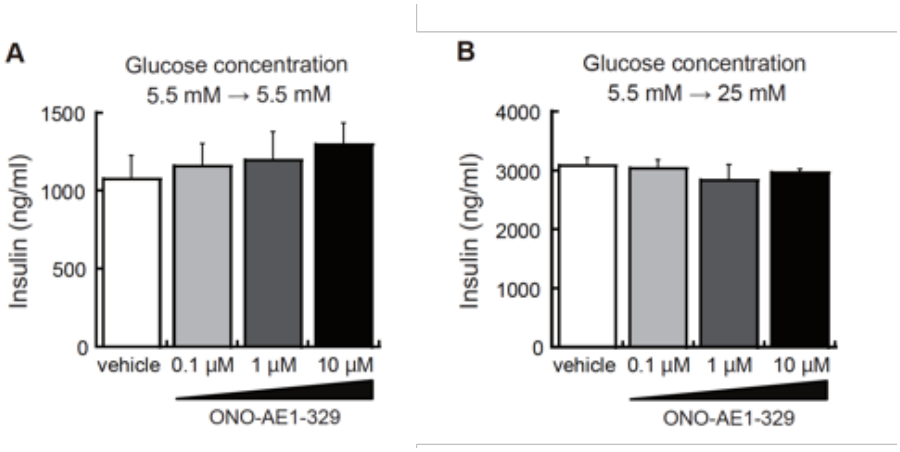
Figure S1



EP4 activation did not affect pancreatic morphology.

Islet morphology as analyzed by immunohistological examination. Sections of the pancreas from *db/db* mice were stained with anti-insulin antibody (green), anti-glucagon antibody (red), and DAPI (blue), and observed under a confocal fluorescence microscope. Scale bar, 100 μm. The islet area (%) or β-cell area of insulin-positive cells (%) is shown in the graph (right panel). EP4 agonist (ONO-AE1-329) -treated group, black square and black bar; vehicle-treated group, white square and white bar; n = 5–6 for each group. All values are the means ± SEM. *, $P < 0.05$; #, $P < 0.01$. versus control.

Figure S2



Effect of EP4 activation on GSIS in MIN6 cells.

MIN6 cells incubated with the EP4 agonist (ONO-AE1-329) did not respond in the insulin secretion assay; **A,** low-glucose medium (DMEM with 5.5 mM glucose) and **B,** high-glucose medium (DMEM with 25 mM glucose); n = 5–6 for each condition. All values are the means ± SEM.