

## SDC, Materials and Methods

**Islet transplantation** Recipient mice were made diabetic by a single intraperitoneal injection of streptozotocin (STZ; 200mg/kg; Sigma-Aldrich) 5-7 days prior to transplant. Diabetic mice are defined as having a nonfasting blood glucose >400 mg/dL for 2 consecutive days. Immediately after isolation, 150 Islets were transplanted intraportally via a 27 G winged infusion set into diabetic recipient mice under general anesthesia with isoflurane. After transplant, mice are considered cured on the first day of 2 consecutive blood glucose measurements <200 mg/dL. Blood glucose was measured with a Breeze 2 Blood Glucose Meter (Bayer HealthCare LLC, Mishawaka, IN, USA) by tail-vein prick. Mouse body weight was measured concurrently with blood glucose.

**Serum biomarker analysis** Immediately after isolation with or without TAK-242, 200 islets were transplanted into the kidney subcapsular space of STZ-induced diabetic mice. Serum was isolated from ~200 $\mu$ L of blood taken from mice via the tail vein prior to transplant, 4 hours posttransplant, then days 1, 2, 3, and 7. Serum samples were frozen at -80°C after collection until analysis.

miRNA was isolated from 50 $\mu$ L serum using the miRCURY RNA Isolation Kit – Biofluids (Exiqon Inc, Woburn, MA, USA) according to manufacturer instructions with optional DNase treatment, converted to cDNA with the miRCURY LNA Universal RT microRNA PCR kit with UniSp6 RNA for loading control according to manufacturer instructions (Exiqon). cDNA was diluted 1:40 in nuclease-free water and assayed by real-time PCR using ExiLent SYBR Green master mix with miRCURY LNA primers for hsa-miR-375 and UniSp6 (Exiqon) on a Bio-rad CFX Connect (Hercules, California, USA) with the following program: 95°C 10 min, then 40 cycles of 95°C, 10 s; 60°C, 1 min. Relative expression was calculated using the  $2^{-\Delta\Delta CT}$  method normalized to UniSp6.

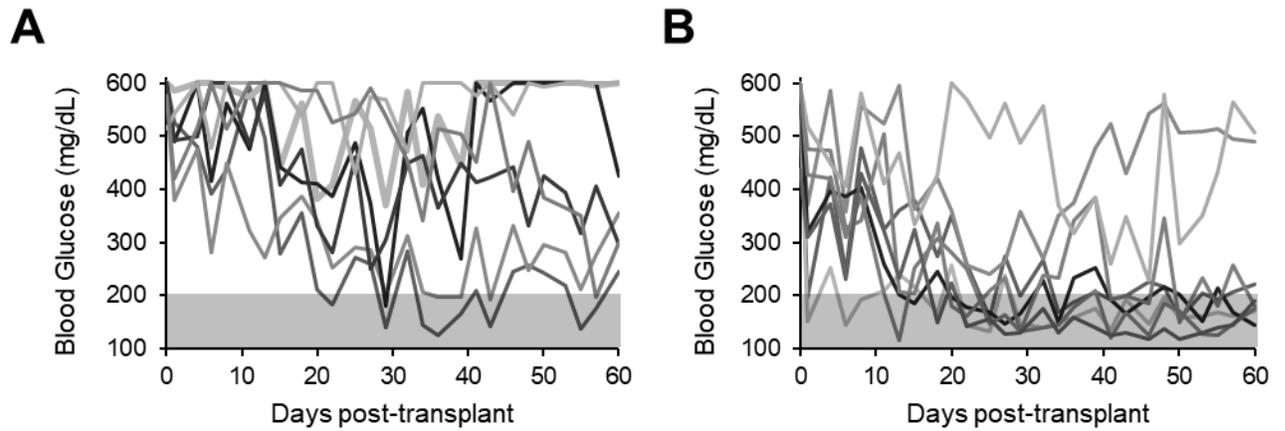
Serum cytokines for IL-6, CXCL1, and CXCL10 was measured by multiplex analysis with a Milliplex MCYTOMAG-70K kit (EMD Millipore Corporation, Billerica, MA, USA) according to manufacturer instructions with undiluted serum. Samples were incubated overnight at 4°C with shaking and analyzed on a Luminex 200 (Luminex Corporation, Austin, TX, USA).

**Western blotting** At different time points, islets from the different groups were rinsed 2x with ice-cold DPBS supplemented 1:100 with Halt Protease and Phosphatase Inhibitor (Halt) Cocktail (ThermoScientific, Rockford, IL, USA) lysed in RIPA buffer (ThermoScientific) supplemented 1:50 with Halt by sonication with a Bioruptor (Diagenode, Denville, NJ, USA) for 4 rounds of: 15 s ON, 1 min OFF, on low power at 4°C. After lysis, 4x Laemmli sample buffer (Bio-Rad) supplemented with 0.25% 2mercaptoethanol was added to the samples and immediately boiled at 95°C for 5 min or frozen at -20°C. Samples were then loaded into and resolved on 12% Tris-Glycine nUView gels (NuSep, Germantown, MD, USA) in a Mini-PROTEAN electrophoresis chamber (Bio-Rad) at 90V for 10 minutes, then 200V for 50 min. Proteins were transferred

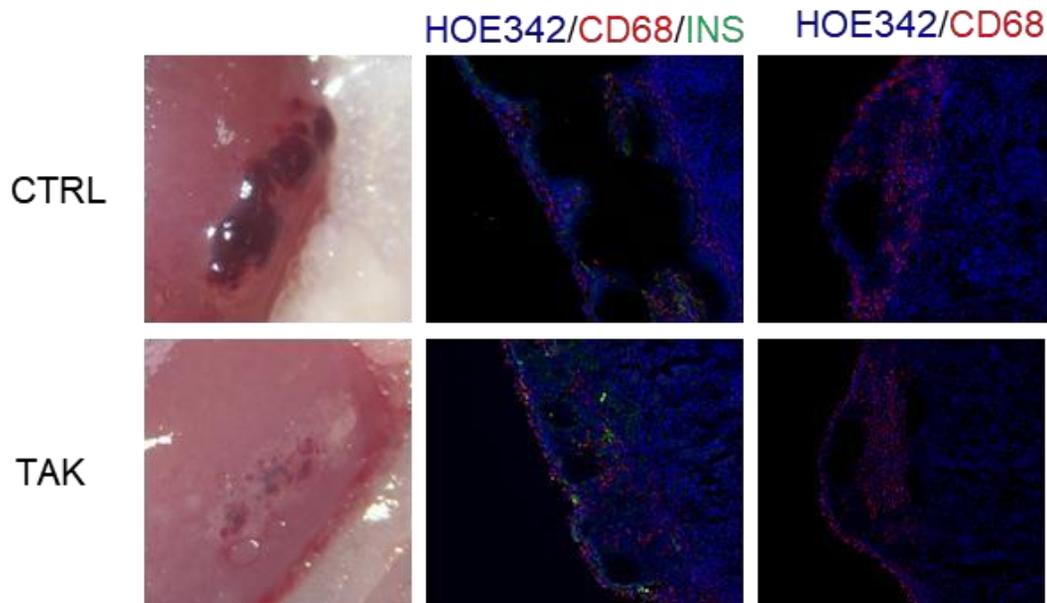
to 0.22µm PVDF membranes (azure biosystems, Dublin, CA, USA) via semidry transfer with a Trans-Blot SD (Bio-Rad) at 10V for 40 minutes using Schafer-Nielsen buffer with 10% methanol. After transfer, membranes are air-dried for at least 30 minutes prior to blocking with 5% BSA in TBST 0.1% for 1 hr at room temperature. Primary antibodies were incubated with the blot overnight at 4°C with gentle agitation, and secondary antibodies were incubated with the blot for 1 hr at room temperature before ECL development with SuperSignal West Dura Extended Duration Substrate (ThermoScientific) according to manufacturer instructions with 3 x 5 min TBST washes between each step. After development, blots were stripped with Restore Western Blot Stripping Buffer (ThermoScientific) for 20 min at 37°C with gentle agitation, reblocked with 5% BSA, then reprobbed. Stripping efficacy was confirmed by developing the stripped blots with SuperSignal West Femto Maximum Sensitivity Substrate and imaging for 3060s (ThermoScientific). All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA) and used at 1:1000 dilution unless otherwise stated. Antibodies used: β-actin (1:2000, #4970), phospho-ERK1/2 (1:2000, #4370), ERK1/2 (#9102), phospho-P38 (#4511), P38 (#9212), phospho-P65 (#3033), P65 (#8242), phospho-SAPK (#4668), SAPK (9252), α-Rabbit IgG HRP-linked (1:2000, #7074). Band density was measure using Fiji (<http://fiji.sc/>) [30]. Relative densities were calculated and compared using the  $([\text{phospho}]:[\text{actin}])/([\text{total}]:[\text{actin}])$  method to normalize relative activation across membranes.

**Islet viability assessment** Whole islets are stained with 1µg/mL Hoechst 33342 and propidium iodide (PI) for 20 minutes at room temperature, rinsed 3 x 5 min with DPBS, then mounted on slides with cover slips before imaging on an Olympus FSX100. Viability was determined by counting the total number of both Hoechst 33342- and PI-positive cells using Fiji (<http://fiji.sc/>) and calculating the percentage of PI-positive cells compared to Hoechst 33342-positive cells.

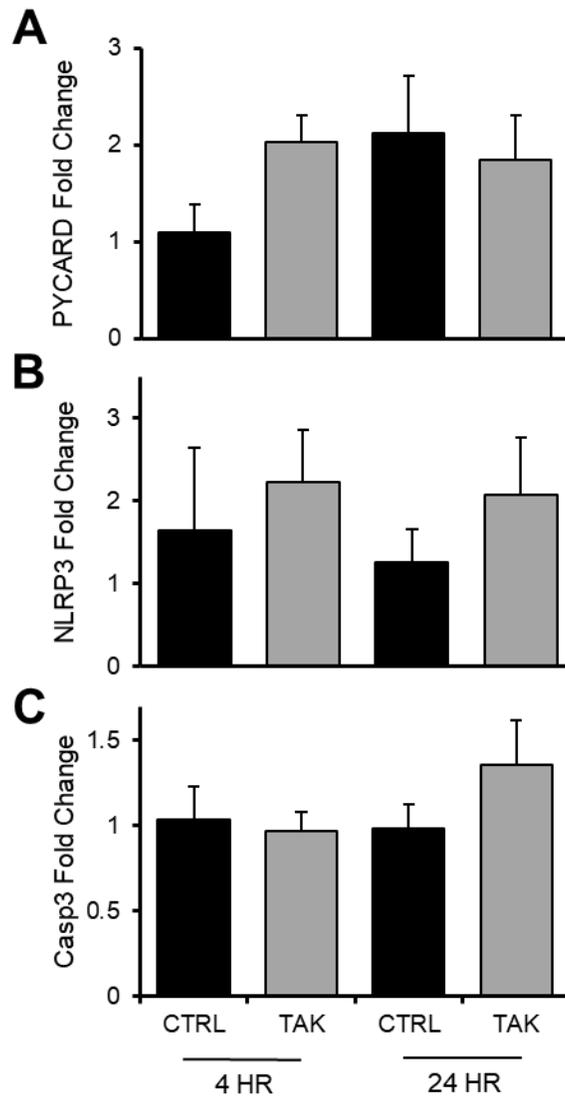
**Glucose-stimulated insulin secretion** Islets are incubated with low glucose (2mM) for 1 hour to equilibrate the islets, low glucose again for 1 hr, then 1 hr in high (20mM) glucose solution in KRBH + 0.2% BSA at 37°C. Media samples were collected immediately after islet incubation and frozen at -80°C or analyzed immediately for insulin content measured using a mouse insulin ELISA kit (ALPCO Diagnostics, Salem, NH, USA) according to manufacturer instructions. Stimulation index is defined by the concentration of insulin in high glucose solution divided by insulin concentration in low glucose solution after equilibration.



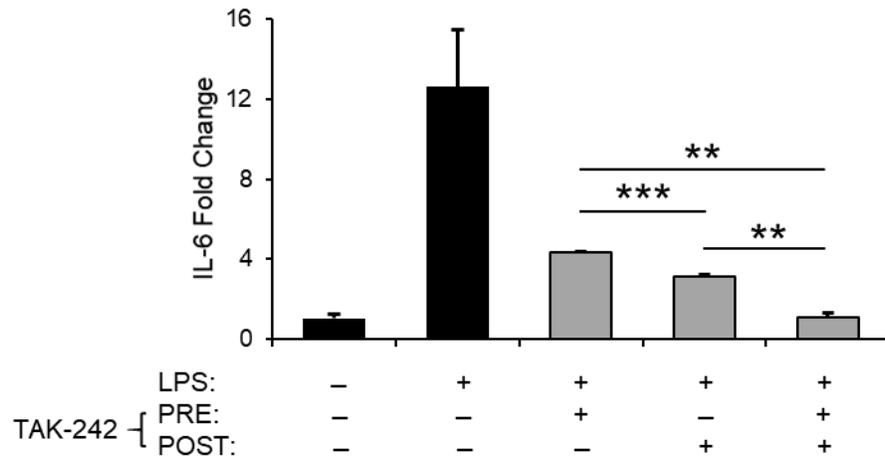
**Figure S1.** Blood glucose graphs of individual islet recipients. Nonfasting blood glucose from diabetic recipients receiving either islets isolated with untreated enzyme (CTRL; A) or early TLR4 blockade (TAK; B) was measured 3 times per week for 2 months. Grey box denotes the euglycemic threshold.



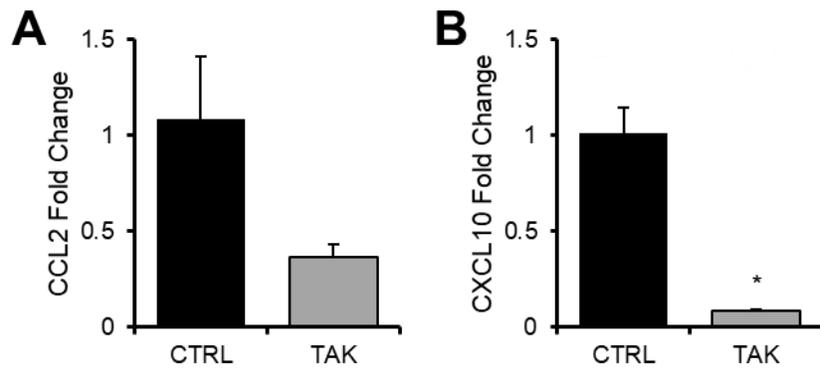
**Figure S2.** Islet kidney graft histological analysis. Islets isolated with untreated enzyme (CTRL) or TAK-242-treated enzyme (TAK) were transplanted into the subcapsular space of diabetic recipients and recovered on day 7 posttransplant. Grafts were frozen in OCT, sectioned into 8 $\mu$ m sections, and stained for insulin and/or CD68. Nuclei were stained with Hoechst 33342. The grafts from TAK mic displayed less edema than CTRL mice, but comparable levels of macrophage infiltration.



**Figure S3.** Gene expression levels of inflammasome proteins. 4- and 24-hours postisolation, mRNA from islets isolated with untreated enzyme (CTRL) or TAK-242-treated enzyme (TAK) was recovered for analysis of the major proteins of the inflammasome pathway, including PYCARD (A), NLRP3 (B), and caspase-3 (C). No statistically significant differences were detected among the groups as determined by unpaired *t* test. *n* = 3/group.



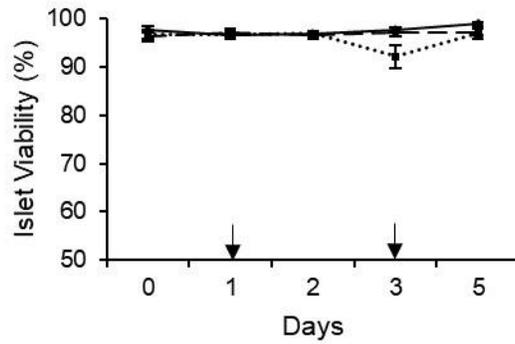
**Figure S4.** Proinflammatory gene expression in islets treated with TAK-242 before or after isolation. Islets were isolated with untreated enzyme or TAK-242-treated enzyme then further treated with or without TAK-242 before challenge with LPS.  $n = 3/\text{group}$ .  $**p < 0.01$ ,  $***P < 0.001$ , determined by unpaired  $t$  test.



**Figure S5.** Proinflammatory chemokine expression in islets isolated with Liberase TL.

Islets were isolated with Liberase TL with (TAK) or without (CTRL) TAK-242. mRNA was isolated 4 hours postisolation and analyzed for the expression of major chemokines CCL2 (A) and CXCL10 (B). CCLs was numerically, but not statistically lower by TAK treatment ( $p = 0.15$ ), however CXCL10 was significantly downregulated by TAK treatment (\* $p = 0.02$ ). Statistical significance was determined by unpaired  $t$  test.

$n = 3$ /group.



**Figure S6.** Islet viability after culture with TAK-242. One day after isolation, islets are cultured in normal media containing 0.01% DMSO (dotted line), 0.3µM TAK-242 (long dashes), and 3µM TAK-242 (solid line) for the indicated time. Black arrows indicate media changes. Viability was determined by Hoechst 33342/PI staining. No statistically significant differences were measured between the groups as determined by multiple t test. n = 10/group.