# Supporting Information

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### SI Materials and Methods

**Animals.** Characteristics of the GK rat model of type 2 diabetes maintained in our colony at the University Paris-Diderot, together with the Wistar control rats, have been previously described (1, 2). Animal experimentation was performed in accordance with accepted standards of animal care as established in the French National Center for Scientific Research guidelines.

Specific-pathogen-free laboratory mice were from the Institute of Labortierkunde of the veterinary facility of the University of Zurich (Zurich, Switzerland). Experiments were performed according to Swiss veterinary law and institutional guidelines. Male mice aged 8–10 weeks were used for all experiments and C57BL/6J mice (Harlan) were used as controls. Male MyD88 −/− mice backcrossed on C57BL/6J mice for 10 generations were kindly provided by S. Akira (Osaka University, Japan) (3). Male IL-1$\beta$ −/− mice on a C57BL/6J background were kindly provided by Y. Iwakura (University of Tokyo) as previously described (4, 5). MyD88 −/− mice were genotyped by PCR using the following primer sets: wtMyD88fw 5′-catggtggtggttgtttctg-3′, wtMyD88rv 5′-gggaaagtccttcttcatcgc-3′, MyD88neofw 5′-aaacgccggaacttttcg-3′, and MyD88neorv 5′-gcttgccgaatatcatg-gtg-3′.

**Pancreatic Islet Isolation.** Two-month-old male Wistar and GK rat islets were isolated by collagenase digestion, as previously described (6). Briefly, after animal euthanasia pancreata were digested with collagenase, and islets were handpicked under a stereomicroscope. Rat islets were cultured in RPMI medium 1640 containing 11 mM glucose, 100 units/mL penicillin, 100 $\mu$g/mL streptomycin, 40 $\mu$g/mL gentamycin, and 10% FCS. For RNA extraction, islets were cultured in suspension for 4 h before extraction, while islets used for protein cytokine/chemokine release were plated on extracellular matrix (ECM)-coated 3-cm dishes for 48 h (20 islets/2 mL of culture medium).

Mouse islets were isolated by collagenase digestion, followed by Histopaque gradient centrifugation (7). Mouse islets were cultured in the same medium as described above (hereafter referred to as islet media). Islets were initially cultured overnight in suspension and plated the following day. In all mouse islet experiments, islets were plated in 96-well plate ECM-coated dishes. Islet number and size were controlled for by plating 5 equally sized islets per well (as defined by J.A.E.). Islets were allowed to adhere and spread on the ECM dishes for 48 h before initiation of experiments.

**RNA Extraction and Real-Time PCR.** Total rat islet RNA was extracted as described and reverse transcribed using random hexamers (2). Liver, adipose, and muscle tissue RNA was extracted according to the manufacturer's instructions (Qiagen). Commercially available rat primers (Applied Biosystems) were used and real-time PCR was performed using the ABI 7000 system (Applied Biosystems). Changes in mRNA expression were calculated using difference of $C_T$ values as compared to a housekeeping gene (18S) and expressed relative to controls.

**Cytokines and Chemokines.** Rat and mouse islets were plated on ECM dishes and conditioned media were removed after 48 h without or with stimulatory and/or inhibitory conditions. Media were assayed for IL-6, chemokine KC, MCP-1, and MIP-1$\alpha$, using mouse and rat Luminex kits (Millipore). Released cytokines/chemokines were normalized to total islet protein content as previously described (7).

**Free Fatty Acid Preparation.** In some experiments islets were treated with 0.5 mM palmitate (Sigma) as previously described (7). The sodium salt of palmitic acid was dissolved at 10 mmol/L in RPMI-1640 medium containing 11% fatty acid-free BSA (Sigma) under an $N_2$ atmosphere. For control incubations, 11% BSA was prepared, as described above. Before use, the effective free fatty acid concentrations were controlled with a commercially available kit (Wako). Dissolved palmitic acid was tested for endotoxin, using the standard LAL test (Cambrex), and found to contain levels in the range of 50 pg/mL. Further, control BSA preparations had an equivalent amount of endotoxin, and islet media concentrations were below the limit of detection.

**In Vivo IL-1Ra Treatment.** IL-1Ra (kindly donated by Amgen) treatment of the GK rats was performed using miniosmotic pumps (Alzet 2004 model; 0.25 $\mu$L/h, 100 mg/mL) or by s.c. injections (10 and 50 mg/kg/injection twice daily). Treatment was initiated 2–3 days following weaning (4 weeks old), after onset of mild fed hyperglycemia (9). Thereafter, nonfasting glycemia was determined with a glucose analyzer (Beckman) 2–3 times per week at 9–10 a.m. Before rat euthanasia an i.p. insulin tolerance test (0.35 unit/kg) was performed as previously described (10). At euthanasia organs were harvested for immunohistochemistry, islet isolations, and total RNA isolation.

Miniosmotic pump experiments were terminated at 4 weeks and islets were isolated for determination of cytokine/chemokine release in vitro. s.c. injection experiments were also stopped at 4 weeks after initiation of treatment. Before rat euthanasia an i.p. insulin tolerance test (0.35 unit/kg) was performed as previously described (10). At euthanasia organs (livers, epididymal adipose tissues, quadriceps muscles, and pancreata) were harvested for immunohistochemistry, islet isolations, and total RNA isolation.

**Serum Parameters.** Serum insulin, proinsulin, and C peptide were assayed according to the manufacturer's instructions (Mercodia). Leptin and cytokines were assayed using Luminex technology (Millipore). FFA levels were quantified using an enzymatic colorimetric assay at 550 nm (NEFA C) from Wako Chemicals GmbH. Ketone levels were measured by the quantification of $\beta$-hydroxybutyrate, using the $\beta$-hydroxybutyrate LiquidColor kit from Stanbio Laboratory. Triglycerides and alkaline phosphatase activity were determined using enzymatic colorimetric assays according to the manufacturer's instructions (Pentra Triglycerid CP kit and Alp CP kit, all from ABX Diagnostics). Absorbance was measured on a Cobas Mira Chemstation (ABX Diagnostics). HOMA-IR was calculated as described (11).

**Immunohistochemistry.** Detection of apoptosis in isolated islets was determined using batches of 50 islets isolated from saline-treated or miniosmotic pump IL-1Ra-treated animals. Some islets from saline-treated animals were additionally incubated in the presence of IL-1Ra (500 ng/mL) for 24 h. Freshly or 24-h-cultured islets were fixed in aqueous Bouin's solution (71.4% picric acid, 23.8% formaldehyde, 4.8% acetic acid, all by volume; VWR International) and embedded in paraplast, according to standard procedures. Apoptotic cells in the islet sections were detected by TdT-dependent dUTP-biotin nick end labeling (TUNEL) assay, using the ApopTag peroxidase in situ apoptosis detection kit (Chemicon International) according to the manufacturer's instructions. Sections were then immunostained for insulin, using the indirect method with a guinea pig

anti-porcine insulin (1:300; MP Biomedicals) and an anti-guinea pig alkaline phosphatase-conjugated secondary antibody (MP Biomedicals). Immunoreactivity was localized using a peroxidase substrate kit (DAB; Vector Laboratories) or an alkaline phosphatase substrate kit (Vector Laboratories). Results were expressed as the percentage of TUNEL-positive total or $\beta$ cells. At least 300 $\beta$ cells and 500 total cells were counted per islet (2–9 islets per section), and 17–28 different sections were analyzed for each independent experiment.

GK rat pancreatic cryosections were incubated with anti-CD68, anti-MHC class II, anti-CD53, and anti-granulocyte antibodies as previously described (2). Staining was visualized using appropriate peroxidase-coupled secondary antibodies and subsequent incubation with 3-amino-9-ethylcarbazole (Sigma). Immune cells associated with islets were quantified blindly in 25–40 islets in 3 animals per treatment group. Images and islet morphometry were completed using a Sony color video camera (CDD-IRIS-/RGB) with biocom visiolab 1000 and Histolab software (Biocom). Percentage of $\beta$ cell area relative to pancreatic area was assessed by staining for insulin as previously published (12).

**Statistics.** Data are expressed as means ± SE with the number of individual experiments described. All data were analyzed using the nonlinear regression analysis program PRISM (GraphPad), and significance was tested using Student's *t* test and analysis of variance (ANOVA) with a Newman–Keuls posthoc test for multiple comparison analysis. Significance was set at $P < 0.05$.


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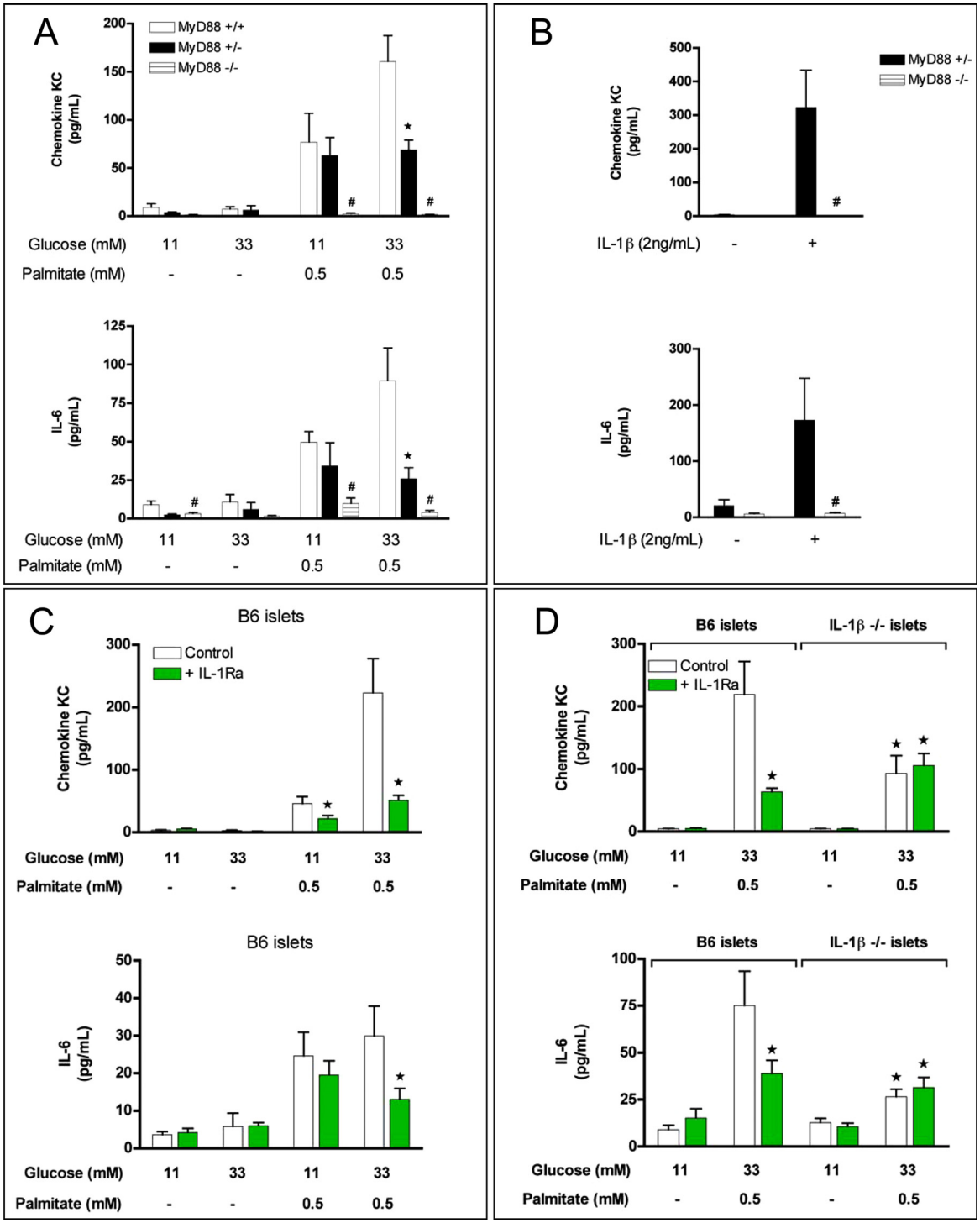


**Fig. S1.** Metabolic stress stimulates pancreatic islet chemokine KC and IL-6 release via MyD88 and IL-1$\beta$. (*A*) Pancreatic islets were isolated from MyD88 +/+, +/−, and −/− mice and stimulated in culture for 48 h with 11 mM glucose, 33 mM glucose, 0.5 mM palmitate, or both glucose and palmitate (33 mM + 0.5 mM; $n$ = 3–5). Conditioned media were removed and assayed for KC and IL-6. (*B*) MyD88 +/− and −/− islets were stimulated with 2 ng/mL IL-1$\beta$ in culture for 48 h ($n$ = 3–5) and conditioned media were assayed. (*C*) Mouse B6 wild-type islets were cultured in the absence (control) or the presence of 500 ng/mL IL-1Ra ($n$ = 3–10), and conditioned media were assayed. (*D*) B6 wild-type and IL-1$\beta$ −/− islets were cultured for 48 h in the presence of 11 mM glucose, or 33 mM glucose plus 0.5 mM palmitate, with and without 500 ng/mL IL-1Ra ($n$ = 5–8). Note that IL-1Ra was able to suppress KC and IL-6 release only in B6 wild-type islets. Each $n$ value represents separate islet isolations from $n$ individual animals, with experiments performed in triplicate. (A and B)* and $^{\#}$, $P < 0.05$ vs. +/+ and +/−, respectively as determined by ANOVA with Newman–Keuls posthoc analysis. (C) *, $P < 0.05$ vs. stimulated control as determined by Student's $t$ test. (D) *, $P < 0.05$ vs. B6 stimulated 33 mM glucose + 0.5 mM palmitate control as determined by ANOVA with Newman–Keuls posthoc analysis.

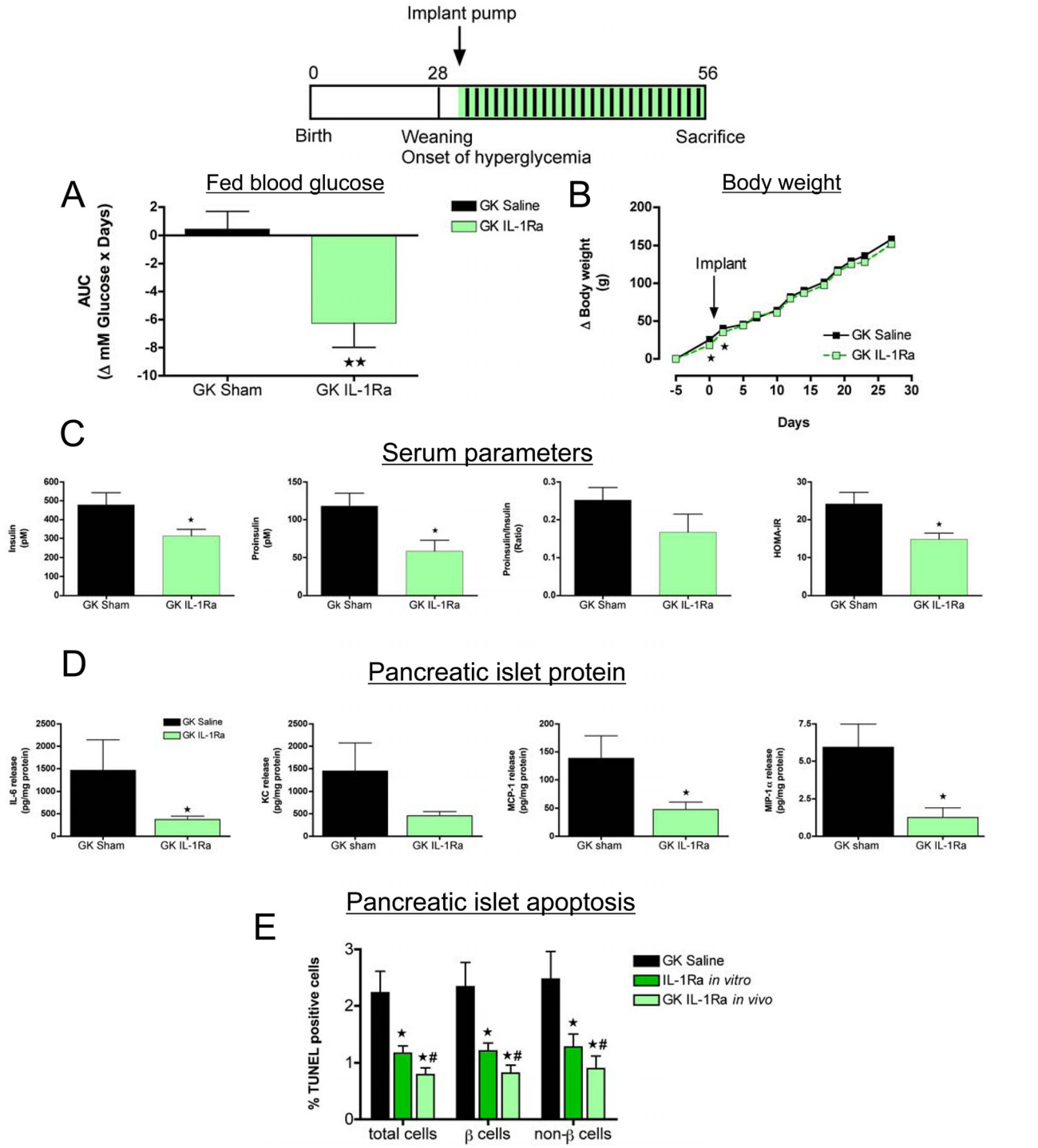


**Fig. S2.** IL-1Ra administered by miniosmotic pumps in the GK rat. Four-week-old male GK rats were implanted with Alzet 2004 miniosmotic pumps containing saline (GK saline, $n = 6$) or IL-1Ra (GK IL-1Ra, $n = 7$) for 4 weeks. This implantation resulted in an average dose of IL-1Ra of 6.75 mg/kg/day. (*A*) Area under the curve (AUC) for delta (Δ) fed blood glucose values and (*B*) Δ body weight over 4 weeks of treatment is shown. (*C*) At the end of treatment circulating fed insulin, proinsulin, proinsulin/insulin ratio, and HOMA-IR were determined. (*D*) After treatment, isolated islets were plated at 20 islets/well in quadruplicate, and conditioned media were removed after 48 h and assayed for the indicated cytokine/chemokine and normalized to total islet protein ($n = 4$ for GK saline and $n = 4$ for GK IL-1Ra). (*E*) After treatment, isolated islets were analyzed for apoptosis by immunohistochemistry. Additionally, islets from saline controls were also treated with IL-1Ra (500 ng/mL) for 24 h in vitro and analyzed for apoptosis ($n = 2$ for GK saline and IL-1Ra in vitro pooled from 6 animals, and $n = 4$ for GK IL1Ra in vivo pooled from 7 animals). (*A–D*) *n* represents the number of animals. *, $P < 0.05$; **, $P < 0.01$ as determined by Student's *t* test. (*E*) *, $P < 0.05$ vs. GK saline; $^{\#}$, $P < 0.05$ vs. GK IL-1Ra in vitro as determined by ANOVA with Newman–Keuls posthoc analysis.

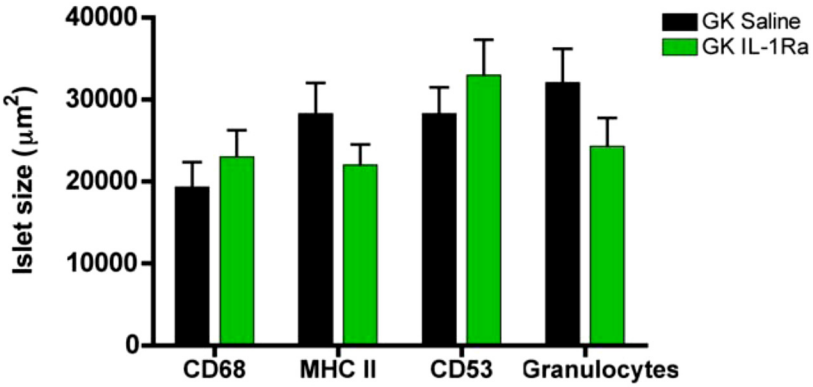


**Fig. S3.** Average islet area of islets analyzed for immune cell infiltration. Four-week-old male GK rats were injected s.c. twice daily with saline ($n = 3$; GK saline) or with 50 mg/kg/injection IL-1Ra ($n = 3$; GK IL-1Ra) for 4 weeks. Immune cells associated with islets were quantified blindly in 25–40 islets in 3 animals per treatment group. Average islet area is shown.

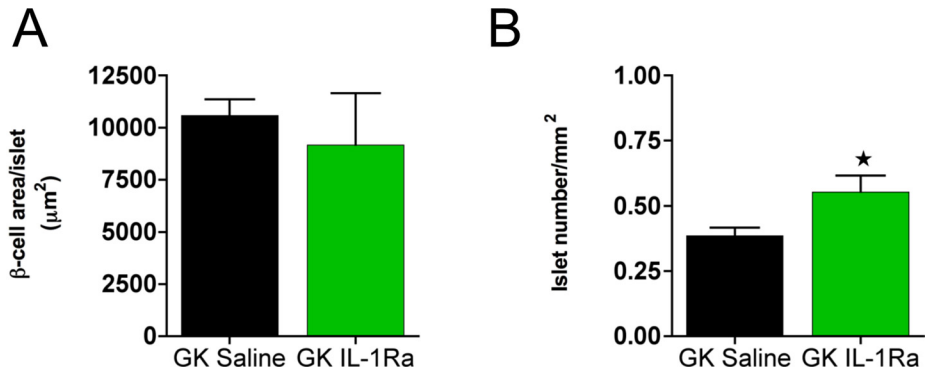


**Fig. S4.** IL-1Ra regulation of $\beta$ cell area per islet and islet number per square millimeter of pancreas area in the GK rat. Four-week-old male GK rats were injected s.c. twice daily with saline ($n = 3$; GK saline) or with 50 mg/kg/injection IL-1Ra ($n = 3$; GK IL-1Ra) for 4 weeks. (*A*) $\beta$ cell area per islet and (B) islet number per square millimeter of pancreas area were assessed by insulin immunohistochemistry. *n* represents the number of animals analyzed. *, $P < 0.05$ as determined by Student's *t* test.

**Table S1. Serum parameters in 2-month-old male Wistar and GK rats**

| Parameter | Wistar | GK/Par |
|---|---|---|
| Glucose (mM) | 5.9 ± 0.3 ($n$ = 4) | 8.3 ± 0.4* ($n$ = 4) |
| Insulin (pM) | 184 ± 43 ($n$ = 4) | 440 ± 115* ($n$ = 4) |
| Proinsulin (pM) | 32.4 ± 5.4 ($n$ = 4) | 115 ± 14* ($n$ = 4) |
| Proinsulin/insulin ratio | 0.18 ± 0.03 ($n$ = 4) | 0.26 ± 0.03* ($n$ = 4) |
| HOMA-IR | 4.6 ± 1.1 ($n$ = 4) | 15.6 ± 4.1* ($n$ = 4) |
| Leptin (pM) | 250 ± 33 ($n$ = 7) | 360 ± 17* ($n$ = 7) |
| Triglycerides (mM) | 1.5 ± 0.1 ($n$ = 9) | 2.1 ± 0.1* ($n$ = 9) |
| FFAs (mM) | 0.47 ± 0.04 ($n$ = 8) | 0.68 ± 0.05* ($n$ = 10) |
| Ketone $\beta$-HBA (mM) | 0.23 ± 0.02 ($n$ = 8) | 0.24 ± 0.03 ($n$ = 10) |
| Alkaline phosphatase (units/L) | 212 ± 13 ($n$ = 8) | 315 ± 21* ($n$ = 10) |
| IL-6 (pg/mL) | 78.9 ± 21.3 ($n$ = 7) | 224 ± 113 ($n$ = 7) |
| GRO/KC (pg/mL) | 312 ± 70 ($n$ = 7) | 337 ± 37 ($n$ = 7) |
| MCP-1 (pg/mL) | 153 ± 16 ($n$ = 7) | 219 ± 45 ($n$ = 7) |
| MIP-1$\alpha$ (pg/mL) | 6.4 ± 0.8 ($n$ = 7) | 7.6 ± 2.5 ($n$ = 7) |

*, $P < 0.05$ as analyzed by Student's $t$ test ($n$, number of animals used for analysis). All parameters were assayed under fed conditions. FFAs, free fatty acids; ketone $\beta$-HBA, ketone $\beta$-hydroxybutyric acid.