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FIGURE LEGENDS

Figure 1. *In vitro* cytotoxicity assay of neutrophil elastase against islets and optimum concentration of sivelestat in ET-Kyoto solution. (A) The cytotoxicity of neutrophil elastase against islets and the inhibitory effects of sivelestat were assessed by LDH assay. Data are mean±SD of three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$. (B) Islet yields under various concentrations of sivelestat. Data are mean±SD of three independent experiments. $^{*}p<0.05$.

Figure 2. Accumulation of activated neutrophils in the pancreas and neutrophil elastase activity during islet isolation. (A) Representative histology of the pancreas before and at the end of warm digestion. Activated neutrophils are stained red-brown (black arrow) by naphthol AS-D chloroacetate esterase. Calibration bars=100 μm. (B) Number of activated neutrophils per field (magnification, ×100) was counted under microscopy. Data are mean±SD of five sections. $^{**}p<0.01$, $^{***}p<0.001$. (C) Neutrophil elastase activity in the supernatant during islet isolation measured at various time points of islet isolation (before warm digestion, at the end of warm digestion, after purification). All experiments were done using each independent mouse. One mouse was used in each experiment (n=1). Data are mean±SD of five independent samples. $^{***}p<0.001$.

Figure 3. Assessment of apoptosis during islet isolation by TUNEL staining. (A) Representative histological sections of the islets at the end of warm digestion. Note the

brown TUNEL-stained apoptotic cells (arrowhead). Calibration bars = 100 μm. (B) The density of TUNEL-positive cells (cells/islet), was determined under × 400magnification. All experiments were done using each independent mouse. One mouse was used in each experiment (n=1). Data are mean±SD of five independent sections. $^{***}p<0.001$.

Figure 4. Results of islet isolation according to the type of isolation solution. (A) Size distribution for each diameter (50-150, 150-250, 250-350, 350- μm) of isolated islets assessed by using each isolation solution. All experiments were done using each independent mouse. One mouse was used in each experiment (n=1). Data are the mean percentage from five independent experiments. (B) Representative microscopic images of the islets immediately after isolation. The morphology of isolated islets was observed by scanning electron microscopy. Calibration bars= 100 μm.

Figure 5. *In vitro* viability assay of isolated islets by TMRE and 7-AAD. *Top panel:* Representative flow cytometry analysis by TMRE assay. *Bottom panel:* Representative flow cytometry analysis by 7-AAD. Percentage data represent percentages of cells with high fluorescence.

Figure 6. *In vitro* viability assay of isolated islets by MTS. (A) Viability of fresh islets (30 cells) was assessed using MTS assay. Data are mean±SD of five independent experiments. $^{*}p<0.05$. (B) Isolated islets (30 cells) were cultured for 0, 1, 2days and

their viabilities were assessed by MTS assay. Data are mean±SD of five independent experiments. $^{*}p<0.05$, $^{**}p<0.01$versus fresh islets

Figure 7. Results of *in vivo* transplant experiments. Islets were isolated using one of the isolation solutions (ET-Kyoto, S-Kyoto), then immediately transplanted (500 allogeneic islets) under the kidney capsule of diabetic mice. Sivelestat was injected intraperitoneally at 2 mg/day at 1 day before transplantation and every day until posttransplantation day 14. (A) Blood glucose levels of recipient mice transplanted with the islets. Data are mean±SD of five independent mice. (B) Serum neutrophil elastase activity of recipient mice was measured at day 1 before transplantation and days 4, 7, 14, 21, 28 post-transplantation. Data are mean±SD of three independent samples. $^{*}p<0.05$, versus S-Kyoto. (C) IPGTT of recipient mice was performed at posttransplantation day 7. Blood glucose was measured in each group (non-diabetic wild-type mice, untreated diabetic mice, recipient mice transplanted with islets isolated by the use of ET-Kyoto, S-Kyoto, and S-Kyoto with sivelestat) before injection and at 15, 60, 120 min after injection. Data are mean±SD of three independent mice. (D) Immunohistochemical analysis of insulin in a representative mouse kidney engrafted with islets was performed at posttransplantation day 7. Calibration bars = 100 μm.

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Figure 8. Assessment of proinflammatory cytokines in isolation solution during islet isolation. The levels of proinflammatory cytokines (IL-2, 4, 6, 10, 17A, IFN-γ, TNF-α) in the isolation solution were measured during islet isolation (before warm digestion,

at the end of warm digestion, after purification). Data are mean±SD of four independent experiments. $^{*}p<0.05$.

Figure 9. Assessment of proinflammatory cytokines in sera of islet mouse recipients after islet transplantation (ITx). The levels of proinflammatory cytokines (IL-2, 4, 6, 10, 17A, IFN-γ, TNF-α) in the sera of islet recipients were measured at pretransplantation day 1 and posttransplantation days 4, 7, 14, 21, and 28. Data are mean±SD of three independent samples. $^{**}p<0.01$, versus non-sivelestat ip group, $^{\#}p<0.05$ and $^{\#\#}p<0.01$, versus before transplantation (day -1).

Table 1. Results of islet isolation according to the isolation solution

	UW	S-UW	ET-Kyoto	S-Kyoto
Islet count after purification (cells/mouse)	248±92*	270±121	280±86	332±74
IEQ after purification	243±68[§]	276±126[¶]	367±70*	651±52
Recovery rate of purification	55.8±10.4[¶]	56.6±12.7*	56.6±4.6*	76.0±5.2
Isolation index	1.18±0.21[§]	1.20±0.38[¶]	1.56±0.21*	2.05±0.17
Islet purity (%)	82.7±3.1[§]	84.0±2.2[¶]	87.3±2.7	91.3±1.9

Data are expressed as mean±SD of five independent experiments.

*$p<0.05$, [¶]$p < 0.01$, [§]$p<0.001$,compared with S-Kyoto isolation solution.

Islet count and purity were measured by the VH-analyzer software. Isolation index was calculated as the ratio of IEQ to islet count.

The recovery rate of purification (%) = IEQ after purification / IEQ before purification ×100

IEQ, islet equivalents; UW, University of Wisconsin

Table 2. Insulin concentration under low (2.8mM) and high (20mM) glucose and stimulation index according to the isolation solution used in the present study

	UW	S-UW	ET-Kyoto	S-Kyoto
Insulin concentration (μg/l) under:				
low glucose (2.8 mM)	2.89±0.37	2.75±0.44	2.90±0.30	2.98±0.37
high glucose (20 mM)	3.68±0.52¶	3.64±0.54¶	3.78±0.44¶	4.46±0.79
Stimulation Index (SI)	1.30±0.06§	1.31±0.12§	1.38±0.12*	1.49±0.08

Data are expressed as mean±SD of five independent experiments.

*$p<0.05$, ¶$p<0.01$, §$p<0.001$, compared with the S-Kyoto solution.

SI was calculated as the ratio of insulin released during exposure to high glucose over the insulin released during low glucose incubation.

SI, Stimulation Index; UW, University of Wisconsin

Table 3. Survival of islet allografts in mice with streptozotocin-induced diabetes.

	without intraperitoneal injection of sivelestat		with intraperitoneal injection of sivelestat	
	ET-Kyoto	S-Kyoto	ET-Kyoto	S-Kyoto
Graft survival (days)	6, 7, 7, 8, 9	10, 10, 11, 12, 13	12, 13, 14, 17, 20	18, 19, 20, 22, 26
Mean Survival (days)	7.4±1.1	11.2±1.1[¶]	15.2±3.3[§]	21.0±3.2[†]

Data are mean±SD of five independent experiments.

[¶]$p<0.01$, [§]$p<0.001$, compared with the ET-Kyoto isolation solution.

[†]$p<0.001$, compared with the S-Kyoto isolation solution.

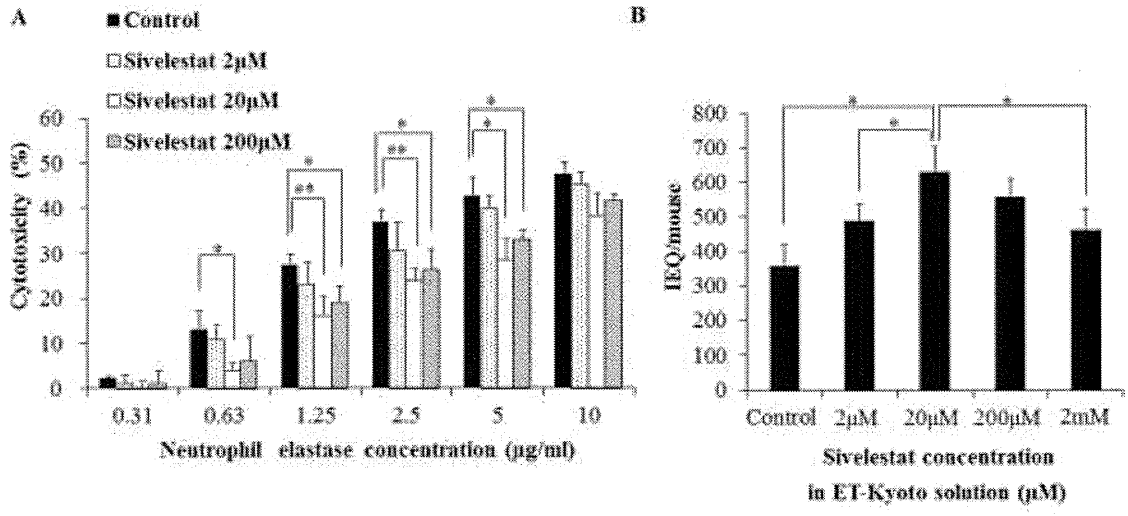

Figure 1

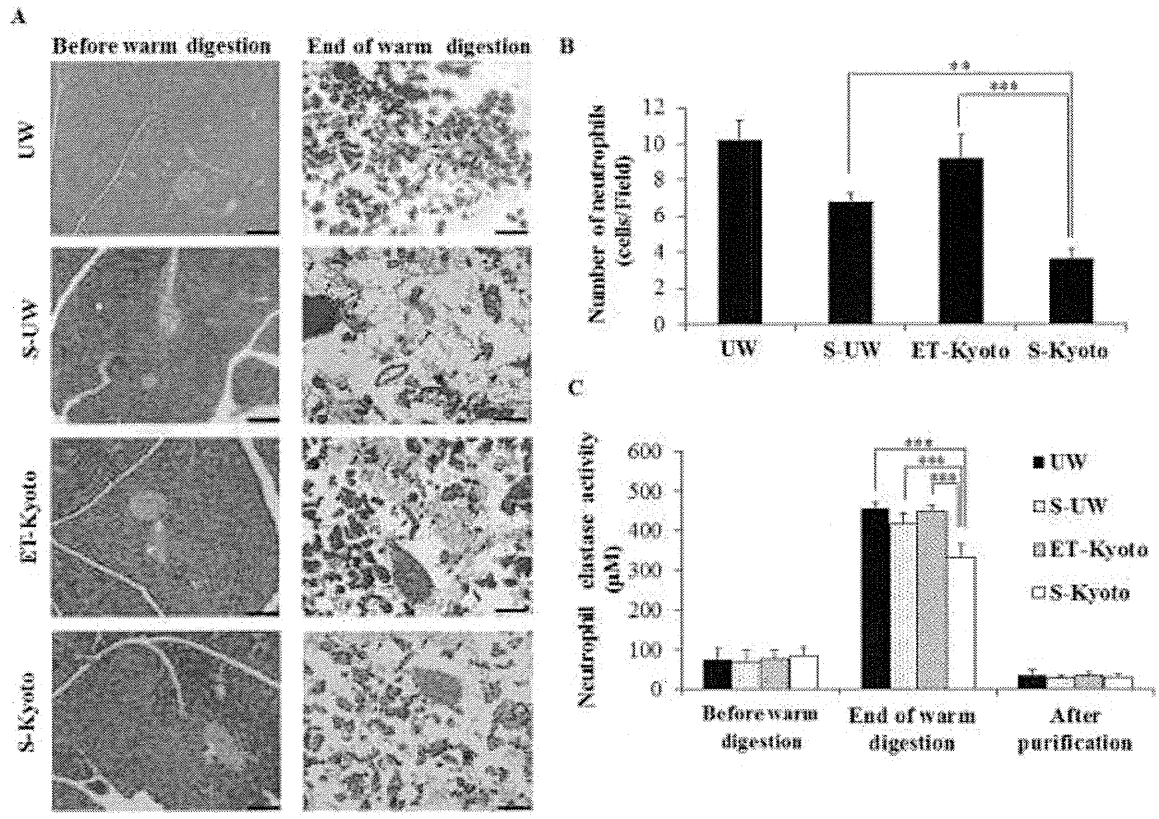

Figure 2

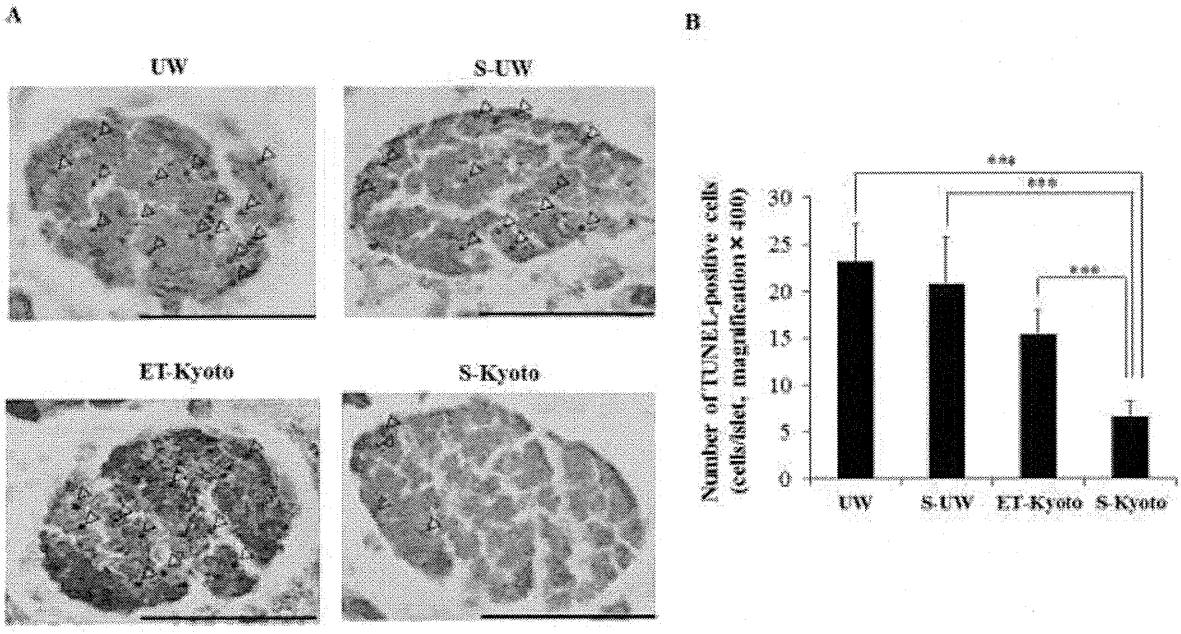

Figure 3

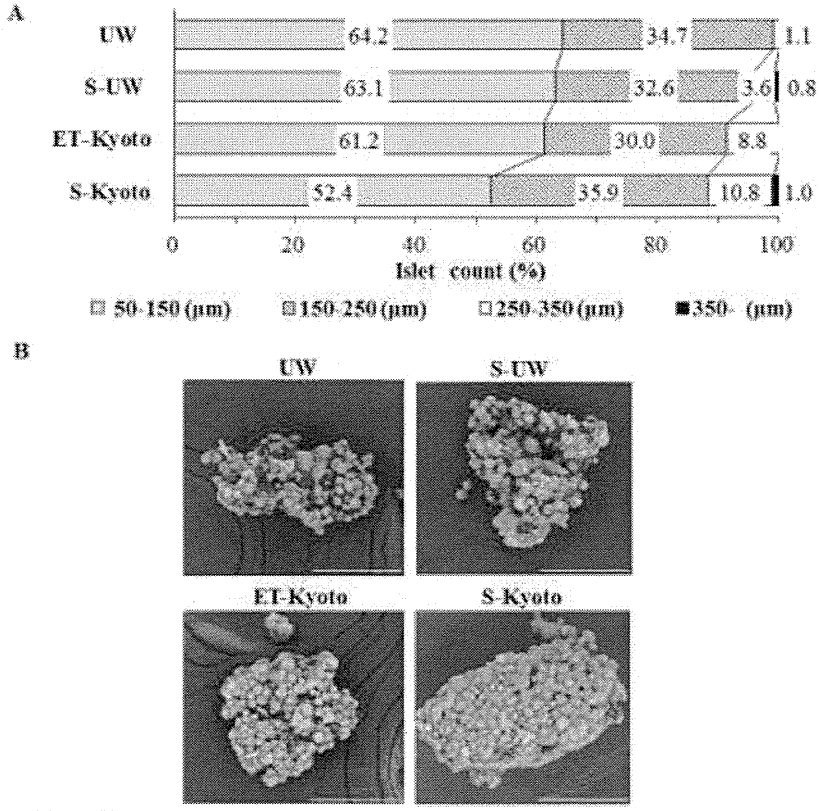

Figure 4