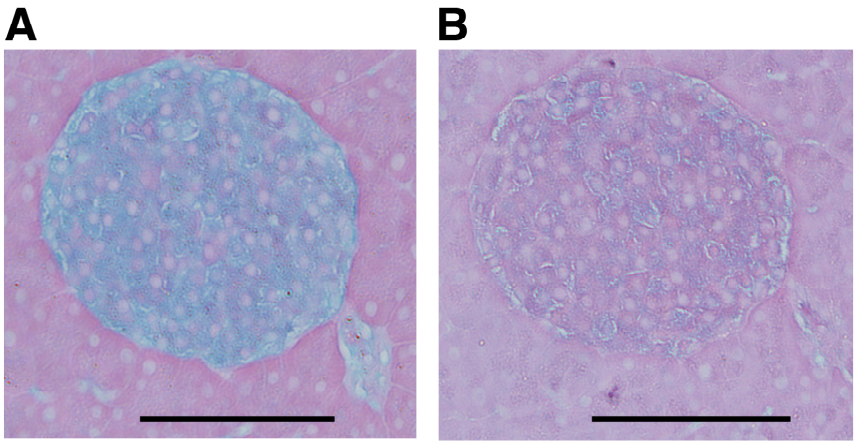
SUPPLEMENTARY TABLE 1

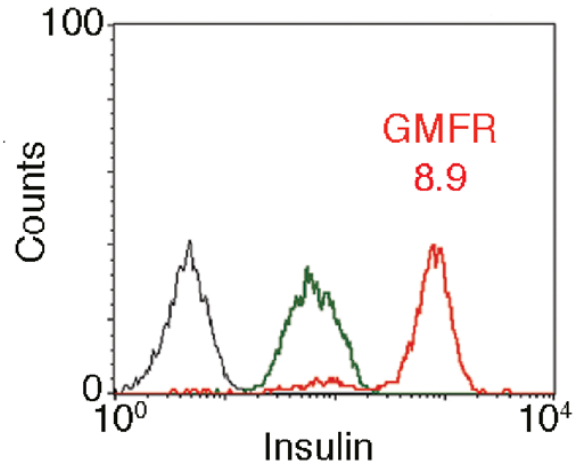
Heparin, highly sulfated heparan sulfate (HS^{hi}) and PI-88 protect mouse beta cells from culture-induced cell death

	% beta cells			
Treatment	Calcein+ PI- *	Calcein+PI+ *	Calcein-PI+ *	Calcein-PI-
Control	29.4±3.5	48.6±4.6	15.2±1.7	7.4±2.2
Heparin	82.8±2.0	6.3±0.6	1.1± 0.2	9.75±1.9
HS^{hi}	83.4±1.9	6.0±0.9	1.4±0.2	9.0±2.3
PI-88	88.3±1.3	4.3±0.7	0.9±0.3	6.3±0.9

Beta cells were cultured in the presence or absence of 50 μg/ml of heparin, HS^{hi} or PI-88 for 2 days and then Calcein/PI fluorescence staining was used to assess % viable cells (Calcein+PI-), % apoptotic cells (Calcein+PI+), % dead cells (Calcein-PI+) and cell debris (Calcein-PI-) by flow cytometry; n= 3-5/group. *For Calcein+PI-, Calcein+PI+ and Calcein-PI+: Control vs heparin, $P<0.05$; Control vs HS^{hi}, $P<0.05$; Control vs PI-88, $P<0.05$.

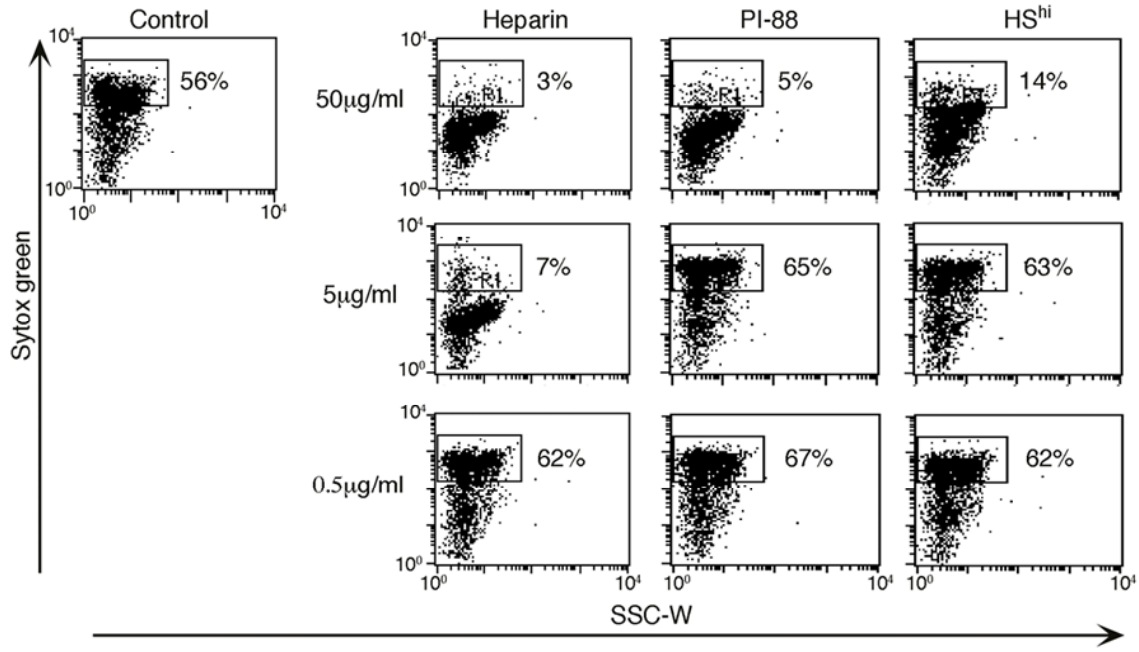


Supplemental Figure 1: Histochemical staining of pancreas sections with Alcian blue (0.65M $MgCl_2$, pH 5.8) is specific for HS. (A) Formalin-fixed NODscid pancreas section treated as a control with acetic acid (pH 4.0) shows intense intra-islet staining with Alcian blue. Scale bar = 50 μm. (B) Adjacent serial section in which HS is degraded by treatment with nitrous acid (adjusted to pH 4.0 using acetic acid) which specifically cleaves N-sulfated glucosamines found only in HS (or heparin) but not other glycosaminoglycans, shows essentially complete loss of staining of islet tissue with Alcian blue. Scale bar = 50 μm.



Supplemental Figure 2: Insulin expression by isolated islet beta cells. Representative intracellular staining with an anti-insulin serum (red histogram) confirms that the isolated islet cells used for the in vitro studies are approximately 90% insulin positive beta cells. Serum control (green histogram) and autofluorescence (black histogram). GMFR, geometric mean fluorescence ratio versus the isotype control.

A



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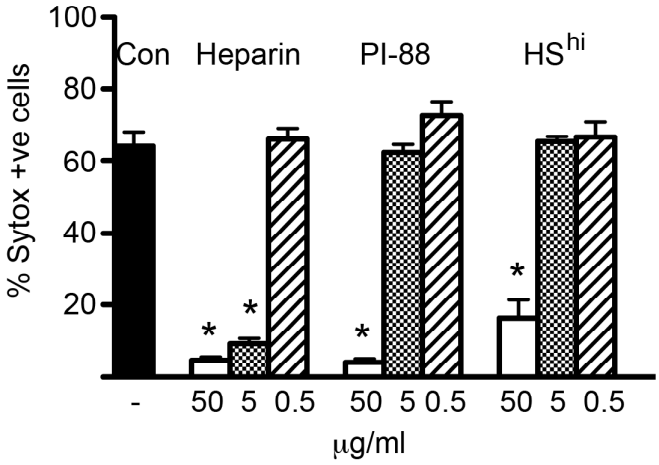
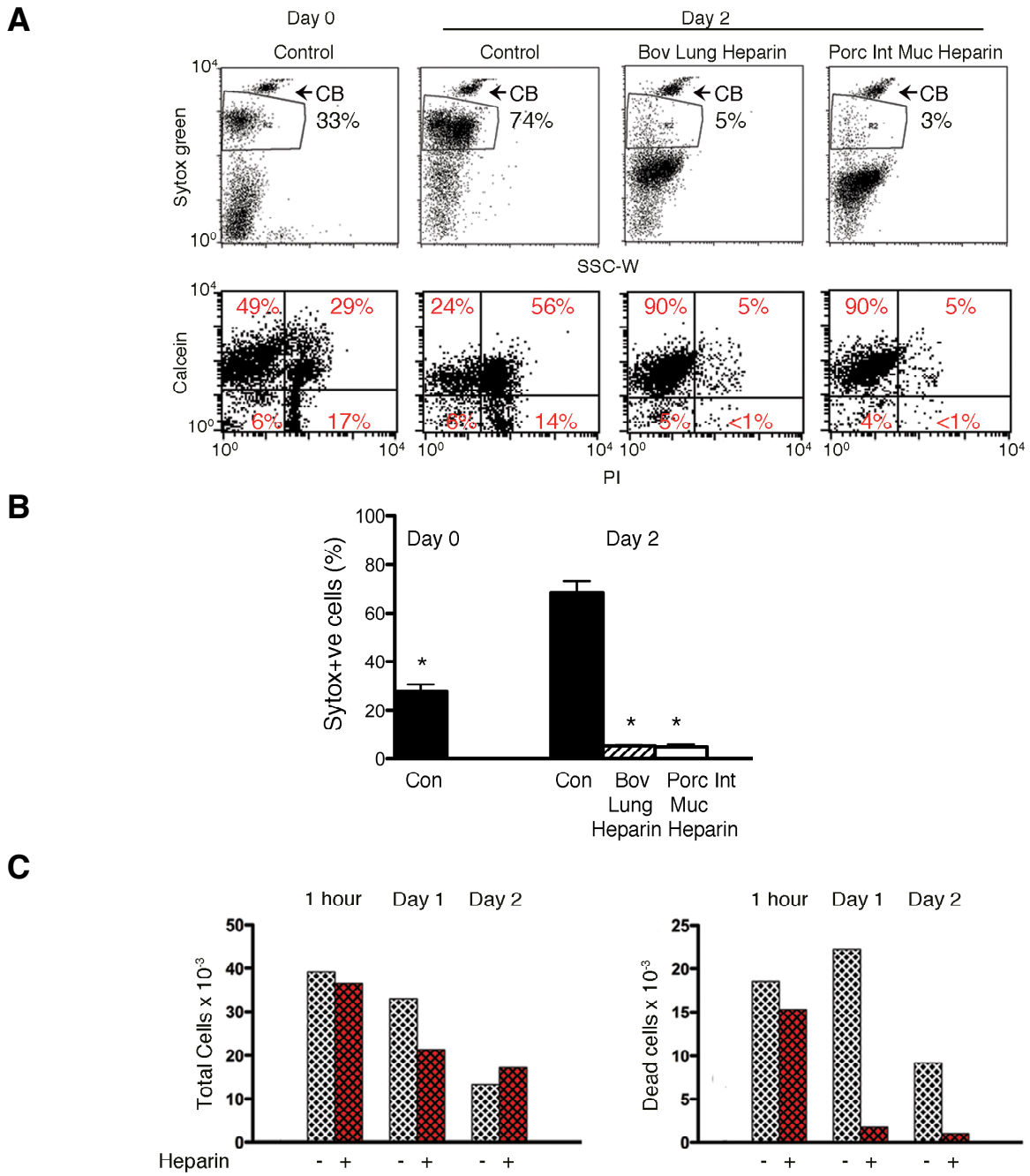
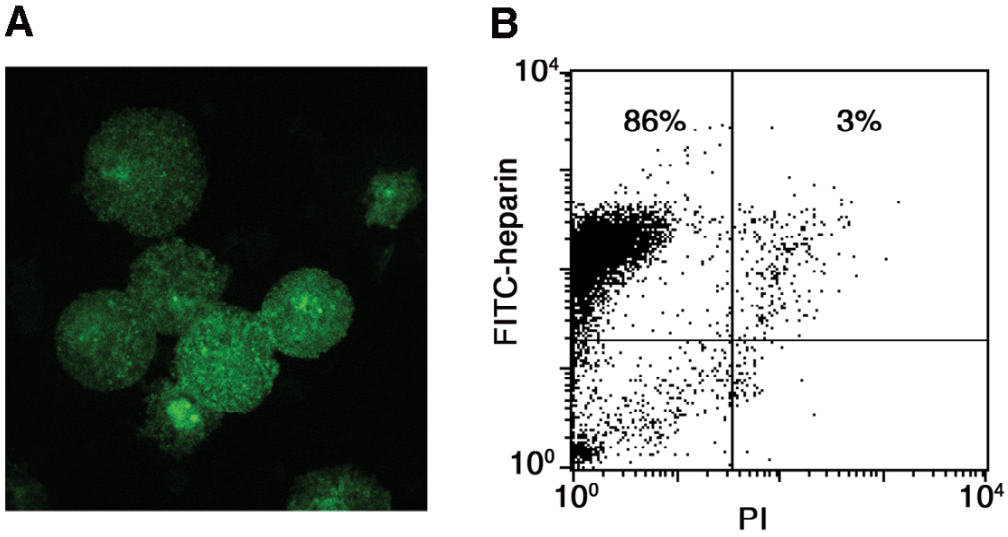


Supplemental Figure 3: Heparin, HS^{hi} and PI-88 can protect islet beta cells from culture-induced cell death. (A) Representative flow cytometry analysis using Sytox green staining shows that the protective effect of heparin, HS^{hi} and PI-88 on beta cell viability is dose dependent. In contrast to HS^{hi} and PI-88, heparin preserved beta cell survival at 5 μg/ml during 2 days of culture. (B) Statistical analyses of experimental groups from A, n=3/group. *Compared to control, $P<0.05$.

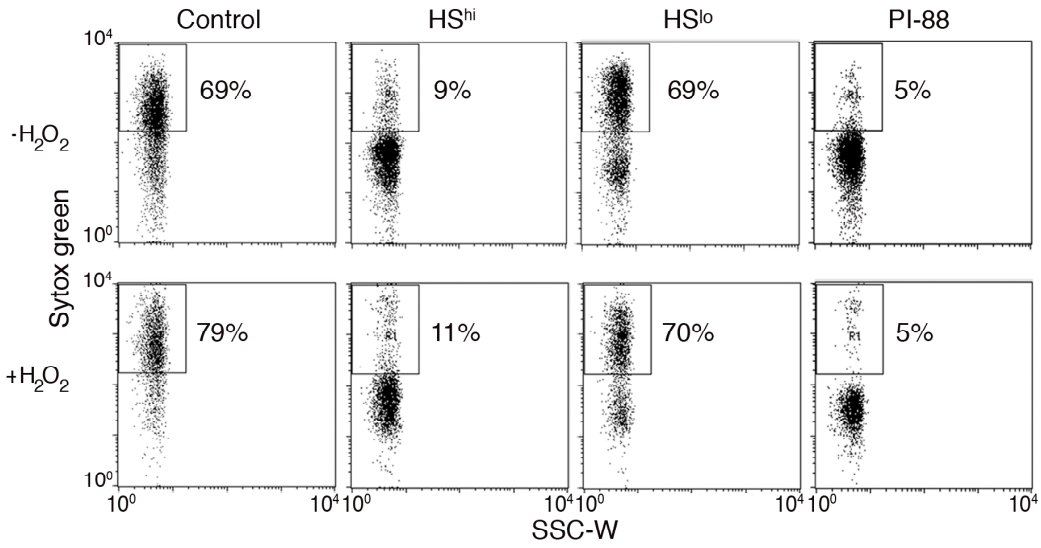


Supplemental Figure 4: Heparin from both bovine lung and porcine intestinal mucosa protects islet beta cells from culture-induced cell death. (A**)** Representative data showing that porcine intestinal mucosa (Porc Int Muc) heparin (50 μg/ml) is equally effective as bovine lung (Bov Lung) heparin (50 μg/ml) in protecting beta cells from culture-induced cell death after 2 days of culture. Viability was assessed by Sytox green uptake (upper panels) or by Calcein-AM (viable and apoptotic cells) and PI (dead and apoptotic cells) uptake (lower panels). The unbounded region at the top of the dot plots for Sytox green staining represents counting beads (CB) added to the cells prior to staining and flow cytometry analysis. (B) Statistical analyses of experimental groups from A (top panel), n=3/group. *Compared to control Day 2, P< 0.05. (C) Time course of absolute number of beta cells and number of sytox+ve (dead) beta cells after 1 hour, 1 day or 2 days of culture in the presence or absence of bovine lung heparin (50 μg/ml). Cell numbers were calculated using the counting beads shown in Figure 2D (top panels). See Table 1 for statistical analyses of experimental groups in C.



Supplemental Figure 5: Uptake of FITC-heparin by islet beta cells. (A) Confocal microscopy of mouse beta cells cultured for 2 days with FITC-labelled heparin (50 μg/ml) demonstrates substantial intracellular uptake of FITC-heparin. (B) Flow cytometry analysis of the beta cells from A revealed FITC-heparin uptake by 89% of the beta cells with 86% of the cultured beta cells being FITC-heparin$^+$PI$^-$, indicating that the FITC-heparin protected the beta cells from culture-induced cell death. See appendix for supplementary methods.

A



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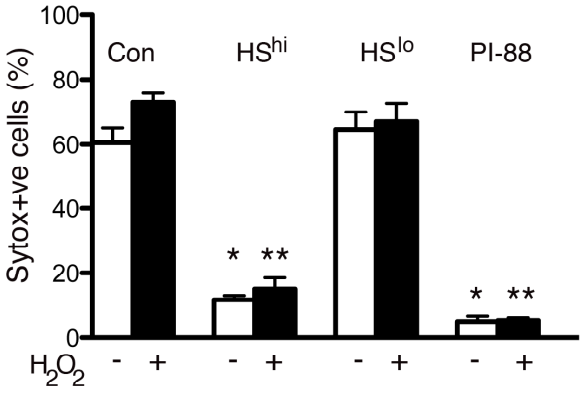
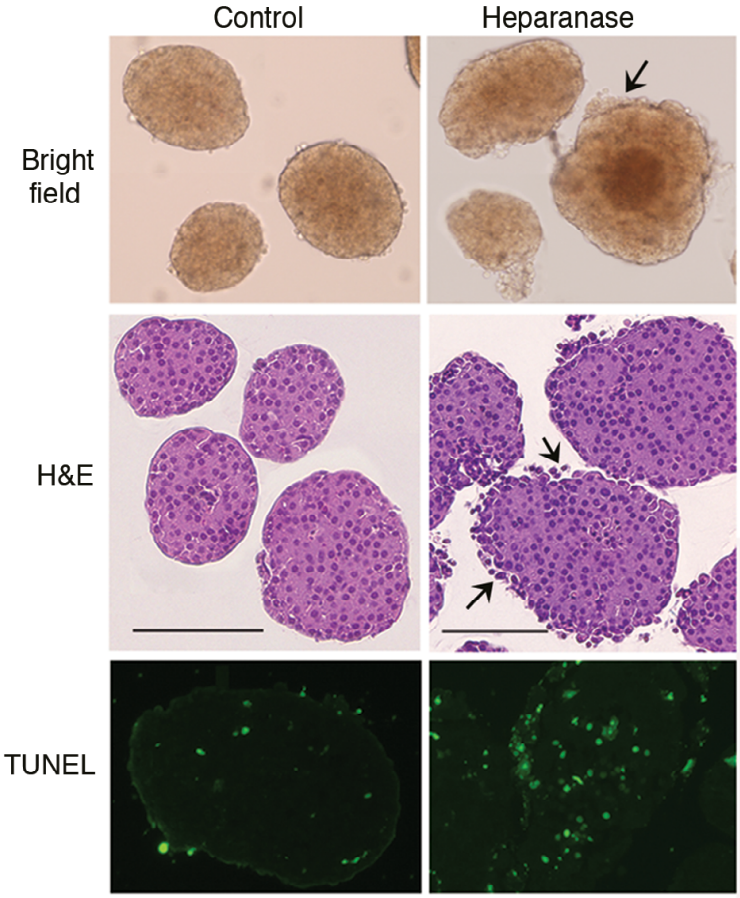


Supplemental Figure 6: Highly sulfated HS (HS^{hi}) and PI-88 protect islet beta cells from ROS-mediated cell death. (A) Mouse beta cells when cultured for 2 days with HS^{hi} (50 μg/ml) and PI-88 (50 μg/ml) but not when cultured with HS^{lo} (50 μg/ml), were protected from culture-induced and hydrogen peroxide (ROS)-mediated cell death, compared to control cultures. Note that 94% of beta cells, prior to culture, were killed by treatment with H_2O_2 (data not shown). (B) Statistical analyses of experimental groups from A, n=3-4/group. Compared to control $-H_2O_2$, *P<0.05. Compared to control $+H_2O_2$, **P< 0.05.



Supplemental Figure 7: Pancreatic islets are damaged by exogenous heparanase in vitro. Representative bright field images and histological appearance (H&E stain) of normal BALB/c mouse islets show that control islets cultured for 24 h remain intact but after 24 h of culture with purified human platelet-derived heparanase (20 μg/ml) the islets show peripheral damage and dissociation of islet cells (arrow). Note that no damage was observed after treatment of islets with 1 μg/ml heparanase. TUNEL immunofluorescence staining (lower panels) also shows increased apoptosis of islet cells following treatment of islets with 20 μg/ml human heparanase. Scale bar, 100 μm. See appendix for supplemental methods.

Appendix

Supplemental Methods

Treatment of islets *in vitro* with human heparanase. We isolated BALB/c mouse islets and cultured them in serum-free RPMI 1640 medium (50 islets/ml/well) in 6 well hydrophobic tissue culture plates (Greiner Labortechnik), in 5% CO_2, 95% air at 37°C. Human heparanase, purified from human platelets (56) and diluted in phosphate-buffered saline (PBS), was added to a final concentration of 20 μg/ml/well. We cultured control islets in culture medium with an equivalent volume of PBS. At 24 h post-culture, we photographed the islets using an inverted microscope (Olympus TH4-22) and then fixed the tissue in 10% neutral-buffered formalin for histological examination.

Localization of apoptotic cells by Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL). Apoptotic cells in paraffin sections of 10% neutral-buffered formalin-fixed islets cultured with or without heparanase, were detected using the *In Situ* Cell Death Detection Kit (Roche). Mouse pancreas sections treated with DNase (1500 U/ml; Sigma) for 10 min at room temperature were used as a positive control for TUNEL.

Detection of insulin in beta cells by flow cytometry. For intracellular staining, the beta cells were firstly fixed and permeabilised using BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Pharmingen/BD Biosciences). The cells were then stained with polyclonal guinea pig anti-swine insulin (Dako) and donkey anti-guinea pig IgG-FITC (Jackson ImmunoResearch). The geometric mean fluorescence ratio (GMFR) was calculated by dividing the GMFI (geometric mean fluorescence intensity) of cells stained with primary antibody by the GMFI of cells stained with the serum control. Cells were analysed using a BD LSRI flow cytometer and CellQuest™

software (version 6.0, BD Biosciences).

Preparation of FITC-heparin. Fluorescein isothiocyanate (FITC)-conjugated heparin was prepared by modification of the Watanabe method (S1). Briefly, 10 mg of porcine mucosal intestinal heparin (0.83 μmole; Sigma) in 2.5 ml of 0.5 M Na_2CO_3, pH 8.6 had fluorescein isothiocyanate isomer 1 (FITC, Sigma F7250; 50 mg, 125 μmol) added and the mixture was stirred overnight at 37ºC, before being applied to a PD-10 desalting column (GE Healthcare) equilibrated in water. The faster eluting FITC-heparin fraction was lyophilised and stored at -20ºC. Beta cells were cultured for 2 days with 50 μg/ml FITC-heparin in culture medium.

Confocal microscopy of beta cells cultured with FITC-heparin. Beta cells cultured for 2 days in FITC-heparin were cytospun for 5 min onto poly-L-lysine coated microscope slides using a Cytospin 2 (Shandon Southern Products). VECTOR Shield mounting medium was applied prior to cover-slip application and fluorescence was visualized using a confocal microscope (LEICA TCS SP5, Leica Microsystems CMS GmbH). No background staining was observed with control beta cells in the absence of FITC-heparin. Images were processed using Adobe Photoshop CS3, v10.0.1.

Degradation of HS in tissue sections with nitrous acid. For deaminative cleavage of HS in formalin-fixed sections of mouse pancreas, the sections were deparaffinised, rehydrated and incubated in 1M nitrous acid (adjusted to pH 4 using glacial acetic acid) at room temperature for 160 min (24,25). After 80 min, the nitrous acid was removed and fresh nitrous acid was re-applied for a further 80 min. The sections were washed and then stained with Alcian blue (0.65M $MgCl_2$, pH 5.8) for 40 min (23, 59).

Supplemental References

S1. Watanabe J, Muranishi H, Haba M, Yuasa H. Uptake of fluorescein isothiocyanate (FITC)-fractionated heparin by rat parenchymal hepatocytes in primary culture. *Biol Pharm Bull* 1993; 16: 939-941.