SUPPLEMENTARY INFORMATION

TITLE: A New Method for Targeted and Sustained Induction of Type 2 Diabetes in Rodents.

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Supplementary Methods:

Pancreas Histology

All animals were euthanized using carbon dioxide and the pancreas of each animal was immediately excised and fixed overnight using neutral buffered formalin (4% formaldehyde; Dako, Glustrup, Denmark). The following day, samples were rinsed in PBS for 60min, transferred to 70% ethanol and processed for paraffin wax embedding using a Leica Autoprocessor (Leica Biosystems, Nussloch, Germany). Pancreas samples were embedded in paraffin and 14µm sections collected, transferred to slides and left to dry overnight at 35°C. Sections were deparaffinised, rehydrated and heat-induced epitope retrieval was carried out in citrate buffer (pH 6.0) using a pressure cooker for 20 min. After cooling, sections were blocked for 60 min at room temperature with 5% goat serum (Sigma Aldrich) in 50 mM Tris-buffered saline (TBS; pH 7.6) containing 0.4% triton X-100 (Sigma Aldrich). Sections were incubated overnight at 4°C in primary antibody: rabbit anti-insulin/pro-insulin (1:1000; Catalogue number ab8304; Abcam, Cambridge, United Kingdom). The following day, endogenous peroxidase activity was blocked using 0.3% H_2O_2 (Sigma Aldrich) for 15 min at room temperature. Subsequently, sections were probed with horseradish peroxidase linked goat anti-rabbit secondary antibody (1:1000; Dako) for 60 min at room temperature. Finally, sections were incubated with 3,3'-diaminobenzidine (DAB; Dako) for 15 min at room temperature as per the manufacturer's instruction. Counterstaining was performed using haematoxylin (Meyers Haematoxylin; Australian Biostain, NSW, Australia) and coverslips were mounted using DPX neutral mounting media (Koch-Light

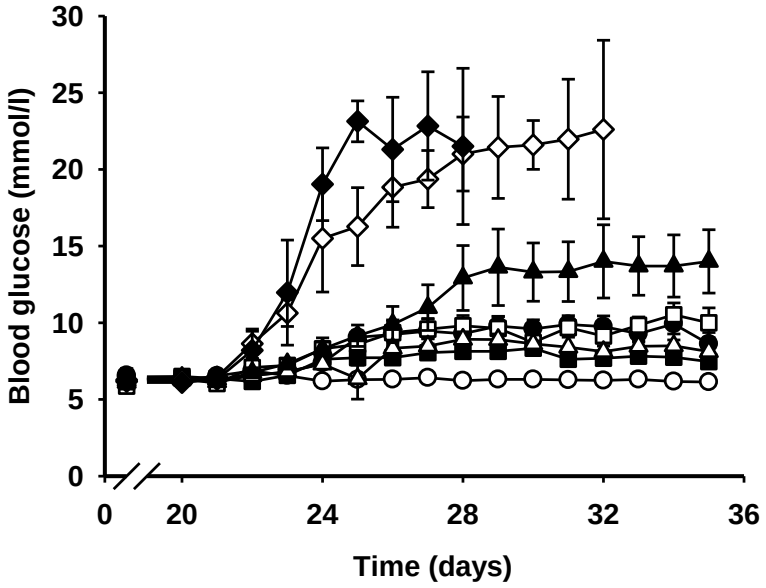
Laboratories, Suffolk, United Kingdom). Images were captured using a Leica DM2500 microscope and Leica DFC495 camera (Leica Biosystems).

Supplementary Table 1.

	Food intake (calories/day)			Water intake (ml/day)		
	Week 1	Week 3	Week 5	Week 1	Week 3	Week 5
CD	95±5	86±2	88±4	30±5	27±4	24±2
HFD	128±12#	106±17#	82±4*#	23±4	23±4	19±1
HFD + Vehicle	148±9#	94±3*	82±8*	24±4	20±4	21±6
HFD + 80 mg/kg STZ	128±15#	88±3*	78±4*	22±3	20±1	18±2
HFD + 90 mg/kg STZ	119±10#	90±5*	80±4*	25±4	20±1	23±2
HFD + 100 mg/kg STZ	146±8#	89±8*	81±7*	25±3	20±2	20±7
HFD + 110 mg/kg STZ	128±7#	97±6*	84±7*	21±1	20±1	21±1
HFD + 120 mg/kg STZ	130±6#	91±6*	79±4†	23±3	20±2	47±21†
HFD + 150 mg/kg STZ	103±15	94±1*	80±6†	27±5	25±3	69±14†
HFD + 200 mg/kg STZ	104±2*#	85±7*	40±4†	23±2	19±3	38±2†

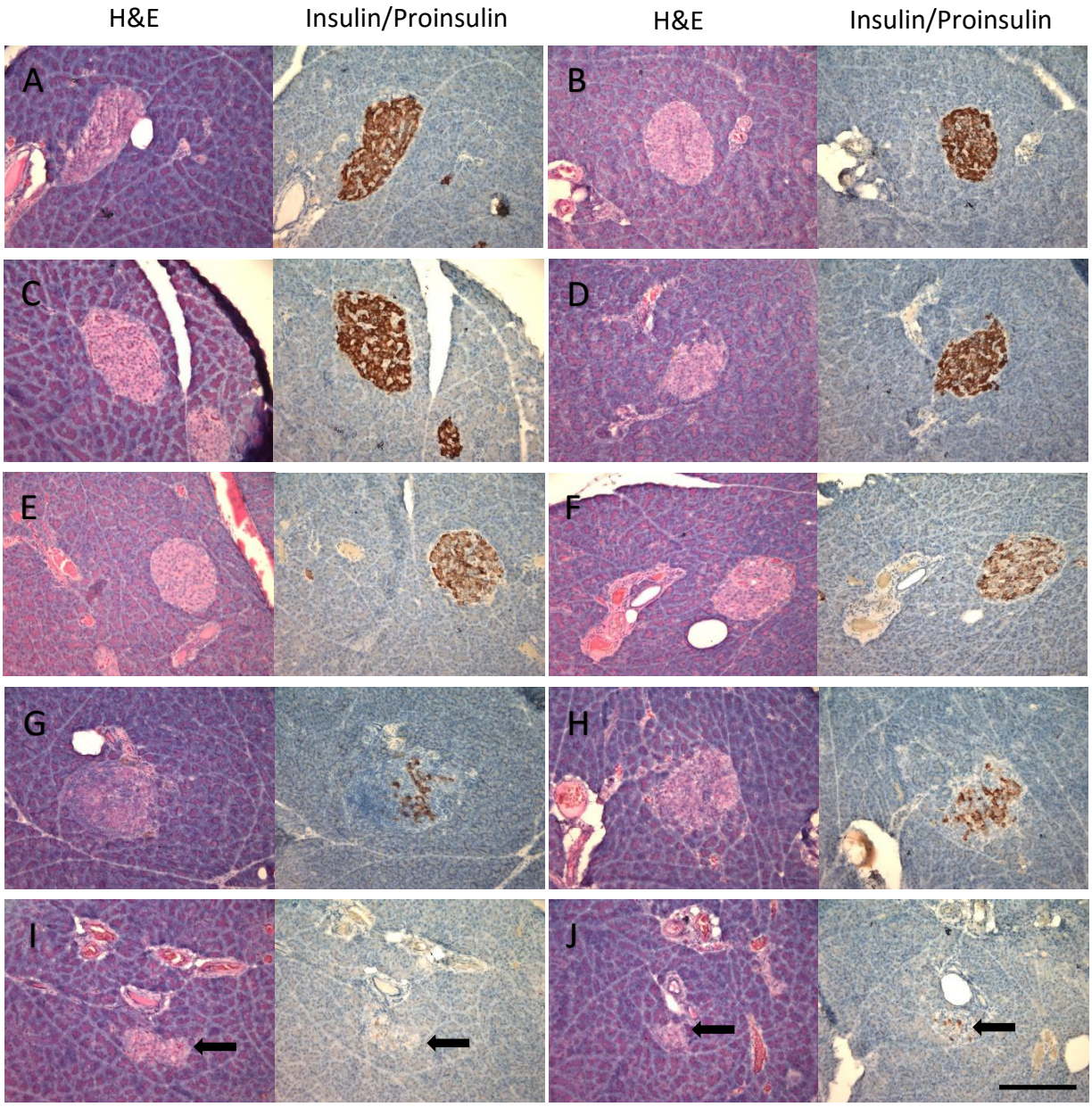
Effect of osmotic mini-pump delivered STZ on food and water intake. Food and water intake were assessed every 3-4 days from the start of the protocol. Data are means±SEM for n=6 in all groups except 150 mg/kg and 200 mg/kg STZ groups where n=3. * $p<0.05$ versus day 0 within the group; # $p<0.05$ versus CD at same time-point; † $p<0.05$ versus day 0 and day 20 within the group.

Supplementary Figure 1.



Osmotic mini-pump-infused STZ increased non-fasting blood glucose concentrations.
Daily non-fasting blood glucose concentrations are shown for vehicle (open circle), 80 mg/kg (closed circle), 90 mg/kg (open square), 100 mg/kg (closed square), 110 mg/kg (open triangle), 120 mg/kg (closed triangle), 150 mg/kg (open diamond) and 200 mg/kg STZ (closed diamond). Data are mean±SEM for n=6 in all groups except 150 mg/kg and 200 mg/kg STZ groups where n=3.

Supplementary Figure 2.



Osmotic mini-pump-infused STZ decreases insulin positive staining within the Islets of Langerhans. Sections were stained with either haematoxylin and eosin (H&E) to visualise islets or probed with a primary antibody for insulin/proinsulin, exposed to DAB and counterstained with haematoxylin. Representative images are shown for **A:** CD; **B:** HFD; **C:** Vehicle; **D:** 80 mg/kg; **E:** 90 mg/kg; **F:** 100 mg/kg: **G:** 110 mg/kg; **H:** 120 mg/kg; **I:** 150 mg/kg; and **J:** 200 mg/kg STZ. Arrows in panels I and J indicate Islets of Langerhans. Scale bar = 200µm.