

## Supplementary Data

### Deletion of the Mouse *Slc30a8* Gene Encoding Zinc Transporter-8 Results in Impaired Insulin Secretion.

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#### Generation of the *Slc30a8* targeting vector.

The ZnT-8 mutant mice were generated and analyzed in collaboration with Lexicon Pharmaceuticals, Inc. (The Woodlands, TX). An *Slc30a8* targeting vector was derived using the Lambda KOS system [1]. The Lambda KOS phage library, arrayed into 96 superpools, was screened by PCR using exon 3-specific primers (UTT047-1 [5'-GTGAGGATAGCCAGACTCC-3']) and (UTT047-2 [5'-CAGCTAGTAATTCAGCACAAC-3']). The PCR-positive phage superpools were plated and screened by filter hybridization using the 516 bp amplicon derived from primers UTT047-1 and UTT047-2 as a probe. A pKOS genomic clone, pKOS-36, was isolated from the library screen and the presence of the *Slc30a8* gene was confirmed by sequence and restriction analysis. Gene-specific arms (5'-GATATTGTGCATCTCACAGGTGGACACGTTG-3') and (5'-CTATATCATTATGCATTCACTATTGCGCAATCAG-3') were appended by PCR to a yeast selection cassette containing the URA3 marker. The yeast selection cassette and pKOS-36 were co-transformed into yeast, and clones that had undergone homologous recombination were isolated. DNA sequencing confirmed that recombination had replaced a 145 bp region, from bp 13 of exon 3 extending to the first 10 bp of the third intron, with the yeast selection cassette. To complete the *Slc30a8* targeting vector the yeast cassette was subsequently replaced with a selection cassette incorporating an internal ribosome entry site (IRES), the LacZ gene, the herpes simplex virus thymidine kinase (TK) promoter and a neomycin (Neo) selectable marker (Fig. 1A). The expression of LacZ mRNA is driven by the ZnT-8 promoter with translation dependent on the IRES whereas expression of Neo mRNA is driven by the TK promoter.

#### Generation of *Slc30a8* knockout mice.

The *Not I* linearized targeting vector was electroporated into 129/SvEv<sup>Brd</sup> (Lex-2) ES cells. G418/FIAU resistant ES cell clones were isolated, and correctly targeted clones were identified and confirmed by Southern blot analysis using a 354 bp 5' external probe (9/10), generated by PCR using wild type Lex-2 ES cell genomic DNA as the template with primers (UTT047-9 [5'-GCTGCAGACTCTTCTCATATGTAG-3']) and (UTT047-10 [5'-CATCTGTAGGCATATAAGTGCATGC-3']), and a 314 bp 3' internal probe (11/12), amplified by PCR using primers (UTT047-11 [5'-CACAGTCCTCTAAACCCACAGAGTG-3']) and (UTT047-12 [5'-GATGACTACACAAAGGTGAAGAGTG-3']). Southern blot analysis using probe 9/10 detected a 8.0 kbp wild type band and 11.1 kbp mutant band in *Pst I* digested genomic DNA while probe 11/12 detected a 7.6 kbp wild type band and 12.8 kbp mutant band in *Nhe I* digested genomic DNA. Correctly targeted clones were also confirmed by PCR using the following primers: The primers represented sequences in exon 3 (primer 1 [5'-GTGAGGATAGCCAGACTCC-3']), intron 2 (primer 2 [5'-CAGCTAGTAATTCAGCACAAC-3']), intron 3 (primer 4 [5'-CCCACAATAACTGCATTGACC-3']), and the Neo gene (Neo3a primer [5'-GCAGCGCATCGCCTTCTATC-3']). Cells from the correctly targeted ES cell clone, designated 2H8 (Fig. 1B), were microinjected into C57BL/6 (albino) blastocysts resulting in the generation of chimeric mice.

### **PCR genotyping of *Slc30a8* knockout mice.**

Mouse tail DNA was genotyped using PCR in conjunction with primers that distinguished between the wild type and targeted alleles. Primers A [5'-TGCGGCTCATCTCTTAATTG-3'] and B [5'-CCTCGATGACAACCACAAAG-3'] were used to amplify a 70 bp product from the wild type allele whereas primers C [5'-TTTCCATATGGGGATTGGTG -3'] and D [5'-CTGGAATTCCGCCGATACT-3'] were used to amplify a 61 bp product from the targeted allele. Tail DNA was isolated and purified by standard procedures [2]. The wild type and targeted allele fragments were amplified using 2.8 ng genomic DNA and the Bio-Rad iQ SYBR Green Supermix (Hercules, CA) under the following reaction conditions: 94°C, 30 sec; 60°C, 30 sec; 72°C, 30 sec for 40 cycles. Standard curve analyses were performed for each set of samples to determine the efficiencies of the two PCR reactions, which were both greater than 95%.

### **Analysis of islet number, size and cellular composition in *Slc30a8* knockout mice.**

At least 10 islets from a single or 20<sup>th</sup> consecutive pancreatic section were examined and scored from all groups of mice (n=6). Images of individual islets co-immunostained for insulin and glucagon were recorded with an Olympus BX51 microscope using a Pixera 600 digital color camera and analyzed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). Briefly, the islet perimeter was marked with a pen tracer tool and Cy3 stained glucagon-positive cells and Cy2 stained insulin-positive cells with associated nuclei (stained by Hoechst 33258) were counted manually in a double blind manner by two independent observers. Nuclei within the islet area that were not associated with either insulin or glucagon positive cells were designated non-alpha/beta cells.

Islet numbers and pancreatic area estimations were performed by scanning pancreatic sections immunostained for either insulin or glucagon using immunoperoxidase staining with diaminobenzidine as the pigment chromogen. The slides were counter-stained with hematoxylin and scanned into ScanScope GL (Aperio, Vista, CA). Using Imagescope viewing software (Aperio, Vista, CA), total pancreatic and all individual islet areas (endocrine) visualized within the sections were quantified using a pen tracer tool. Percent area was calculated as: 100 X (the sum of all individual islet areas)/ (total area of the pancreatic section) and averaged (n=6 each group).

## **Supplemental Figure Legend**

### **Supplemental Figure 1. Analysis of islet number, size and composition in *ZnT-8*<sup>-/-</sup> mice.**

Pancreas tissue was isolated from male wild type (WT) and *ZnT-8* knockout (KO) mice. Fixation, preparation of mouse pancreatic slices, immunohistochemical staining with antibodies raised to insulin and glucagon and quantitation of islet size, islet number and alpha and beta cell numbers were then performed as described in Materials and Methods. Results are presented as mean data ± S.E.M.

## **References**

- 1 Wattler, S., Kelly, M. and Nehls, M. (1999) Construction of gene targeting vectors from lambda KOS genomic libraries. *Biotechniques*. **26**, 1150-1156, 1158, 1160
- 2 Sambrook, J., Fritsch, EF and Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Plainview, NY

# Suppl. Fig. 1

