

SUPPLEMENTARY DATA

Generation of human *ZnT8* transgenic Mice, Breeding, and Genotyping. pIns -1 plasmid (26) containing the human insulin promoter (1.9 kB) fused to the rabbit β -globin intron (same construct used in 27), was used as the expression construct. Human *pZnT8WT-EGFP* and *pZnT8RW-EGFP* constructs (28) were used as templates for PCR reactions using the *ZnT8* cloning primers whose sequence is given in **Supplementary Table 1**. These primers generated new *EcoRI* sites to allow cloning, and removed the EGFP fusion protein. The PCR products were inserted into the *EcoRI* site in the pIns-1 plasmid and verified by sequencing, and expression was tested in MIN6 cells. Constructs were linearized, gel purified, and injected into CBA X B6 blastocysts by the Washington University Transgenic Core Facility. These mice were bred with C57BL/6J to syngeneity (Jackson Lab, Bar Harbor, ME). h*ZnT8* genotype of mouse lines was performed on tail DNA by PCR amplification using the T8hum RTF and RTR human specific primers (Supplemental Table 1). The h*ZnT8* and m*ZnT8* primers were chosen to cover regions of sequence divergence to differentiate the human and mouse genes.

***Zn*²⁺ Transporter Gene Expression.** Total RNA from harvested mouse pancreas was extracted with 0.5M guanidinium isothiocyanate. For details of the method see (29). The RNA concentration was measured, and RNA integrity was verified by electrophoresis in a 1% agarose gel. Reverse transcription (RT) was performed using the iScript cDNA synthesis kit (BioRad, Hercules, CA, USA) and 1 μ g total pancreatic RNA in 20 μ L. Real-time RT-PCR specific primers for human *ZnT8 R325W (RW)* and *ZnT8 wildtype (WT)* were the same as above (T8hum RTF and RTR). Mouse GAPDH, ZIP4, and *ZnT8* forward and reverse primers were also used (Supplemental Table 1)

The reactions used iQSYBR green Supermix (BioRad) and 1 μ L of the RT reaction with a final concentration of 100 nmol/L of the primer. The PCR cycle for *ZnT8* was as follows: 95°C 5 min, 40 cycles of 95°C/30 s, 62°C/30 s, and 72°C/ 30 s. The PCR cycle for mZIP4 was as described (30). The melt curve was 65°C to 95°C in increments of 0.5°C every 5 s to demonstrate a single PCR product, and the correct size product was verified by agarose gel electrophoresis. The melt-curve analyses were performed using an iCycler iQ Multicolor Real-Time PCR Detection System (BioRad). Samples were compared using the relative CT (The cycle number at the threshold level of log-based fluorescence) method. The fold increase or decrease (=Power) was determined relative to a transgenic negative control after normalizing to the GAPDH housekeeping gene using the $2^{-\Delta\Delta CT}$ (Livak) method, where ΔCT is (gene of interest CT) - (GAPDH CT) and $\Delta\Delta CT$ is (ΔCT *ZnT8RW*+ or *ZnT8WT*+) - (ΔCT *ZnT8RW* - or *ZnT8WT* -).

SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences used in this manuscript.

¹ hZnT8CloningF: 5'-CCCCGGATCCCGAATTCATGGAGTTTCTTGAA-3'

¹ hZnT8CloningR: 5'-CCCCGAATTCCGGATCCGTCACAGGGGTCTTC-3'

² hZnT8-RTF: 5'-CTCCTGGGCAACGTGCTGGTTGTTGTGCTG-3'

² hZnT8-RTR: 5'-TGGTCTCTCTCTGGGACACTGATCTTTATT-3' (190bp).

³ mZnT8-RTF: 5'-CAAGCCACCAAGATGTACGCCTTCCCTCTA-3'

³ mZnT8-RTR: 5'-TCGGTGCCCTGCTGTCTGTCCTTTGCATCT-3' (415bp)

³ mGAPDH-RTF: 5'-GGTGAAGGTCGGTGTGAACG-3'

³ mGAPDH-RTR: 5'-CTCGCTCCTGGAAGATGGTG-3' (230 bp).

³ mZIP4-long-RTF: 5'-AGAAGTCAGCACCTCTACAAGGAACGC-3'

³ mZIP4-long-RTR: 5'-AGTAGCTGGCTCAGACCCAGGGTC-3' (475bp).

¹ Cloning primers were used to generate restriction enzyme sites, and to remove the GFP portion of the fusion protein construct used as the template for the cDNA PCR reaction.

² hZnT8 primers were used both for DNA genotyping of the mouse transgenic lines, and for qRT-PCR of pancreatic RNA levels.

³ The other three pairs of primers were used for qRT-PCR of the specified pancreatic RNA. The number given in parentheses is the size of the PCR product in base pairs.

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Supplementary Table 2. Content of Harlan Teklad High Fat Diets

Ingredient	High Fat Diet (60% of calories) ¹ (Harlan Teklad TD.06414, g/kg)	High Fat Diet (60% of calories) ² (Harlan Teklad TD.10872, g/kg)
Casein	265.0	NA
Egg White Solids, spray-dried	NA	266.0
L-Cystine	4.0	NA
Dextrose, monohydrate	NA	151.5
Maltodextrin	160.0	160.0
Sucrose	90.0	NA
Lard	310.0	325.0
Soybean Oil	30.0	NA
Corn Oil	NA	20.0
Cellulose	65.5	30.0
Mineral Mix, AIN-93G-MX (3)	48.0	NA
Mineral Mix, Zn Deficient (81264) (31)	NA	34.2
Calcium Phosphate, dibasic	3.4	NA
Biotin	NA	0.005
Vitamin Mix, AIN-93G-VX (3)	21.0	NA
Vitamin Mix, Teklad (40060) (31)	NA	13.3
Choline Bitartrate	3.0	NA
Chromium Potassium Sulfate, dodecahydrate	NA	0.027
Blue Food Color	0.1	NA
Ethoxyquin, antioxidant	NA	0.004

¹ This diet contained 50-60 mg Zn²⁺/kg of diet.

² This diet contained 0.8-1.2 mg Zn²⁺/kg of diet.

NA specifies not added.

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Supplementary Table 3. Quantitation of RT-PCR of the *ZnT8 WT* and *ZnT8 RW* transgenic mouse lines ¹

Animal Line	Sample ID	hZnT8 Power	mZIP4 Power	mZnT8 Power
X-28-WT	8078+	1076 ± 137 ^a	NP	NP
X-40-WT	549+	1258 ± 94 ^a	0.5 ± 0.1 ^b	0.9 ± 0.2
T-12-RW	8038+	1643 ± 160 ^a	1.8 ± 0.2 ^b	1.1 ± 0.2
T-16-RW	852+	46 ± 15	NP	NP
T-8-RW	8024+	53 ± 12	NP	NP
Negative	615-	1 ± 3	1.0 ± 0.15	1.0 ± 0.17

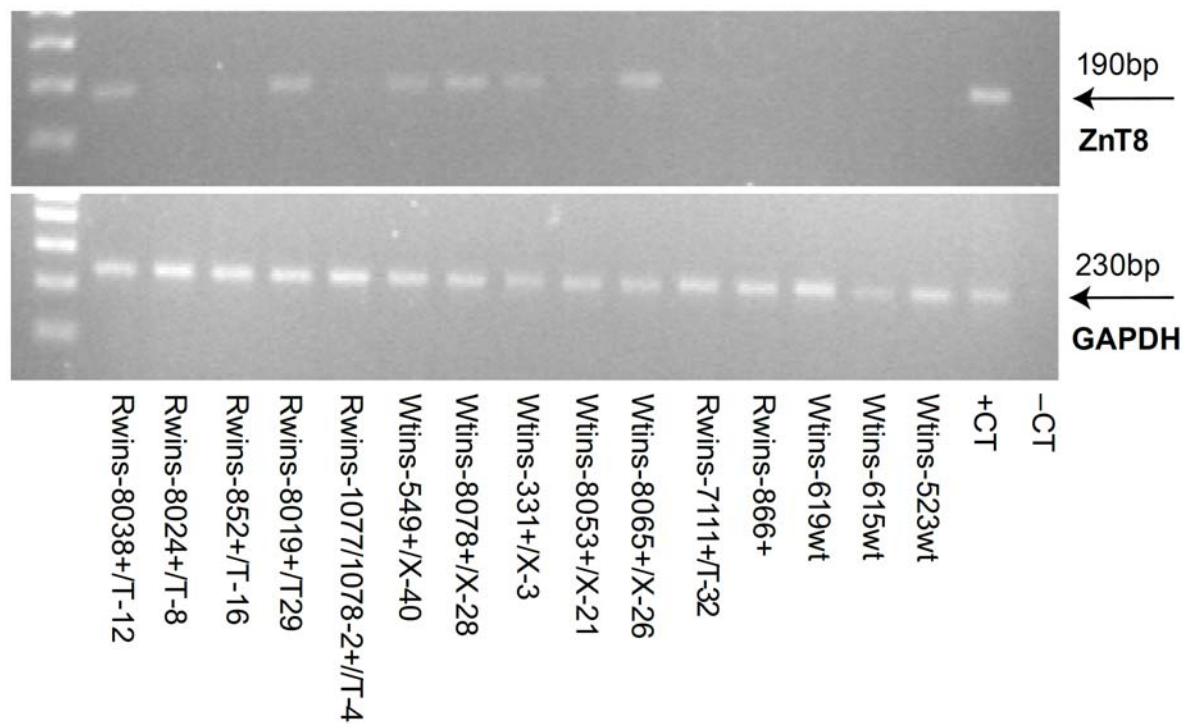
¹ Values are the average ± SEM, from 4 independent experiments, normalized to GAPDH, and compared to hZnT8 transgenic negative littermates (fold increase above negative control).

^a Indicates a significant difference of hZnT8 RNA expression from negative littermates at $P < 0.05$

^b Indicates a significant difference of mZIP4 RNA expression from negative littermates at $P < 0.05$

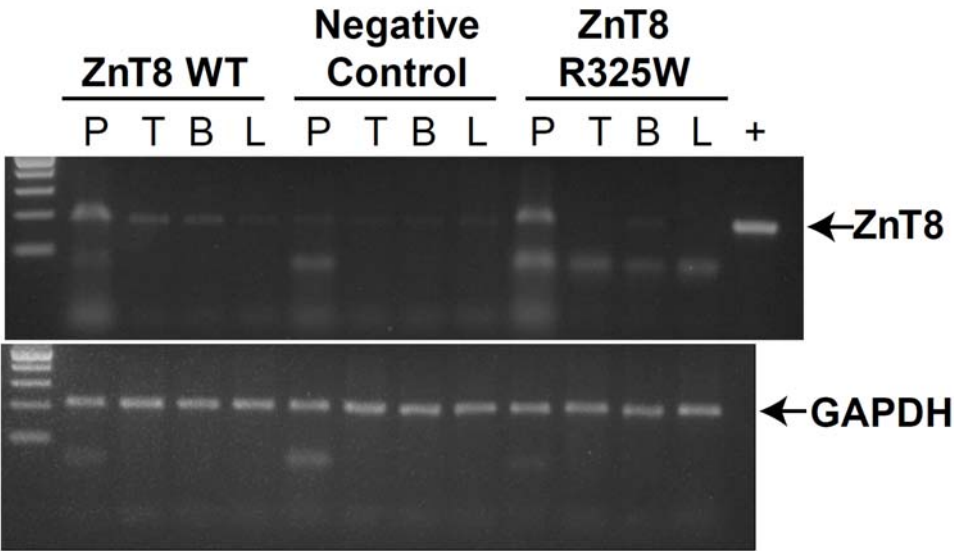
SUPPLEMENTARY DATA

Supplementary Figure 1. Expression levels of *hZnT8* WT and RW transgenic mouse lines. Human *ZnT8* mRNA expression for WT and R325W animal lines (upper panel) was assessed by RT-PCR performed on mouse pancreas RNA. Mouse GAPDH was used as an internal control (lower panel). The negative control (-CT) was performed without RNA, and the positive control (+CT) was performed using 1 ng of *ZnT8-GFP* plasmid DNA. The names of the different animal lines are given below.



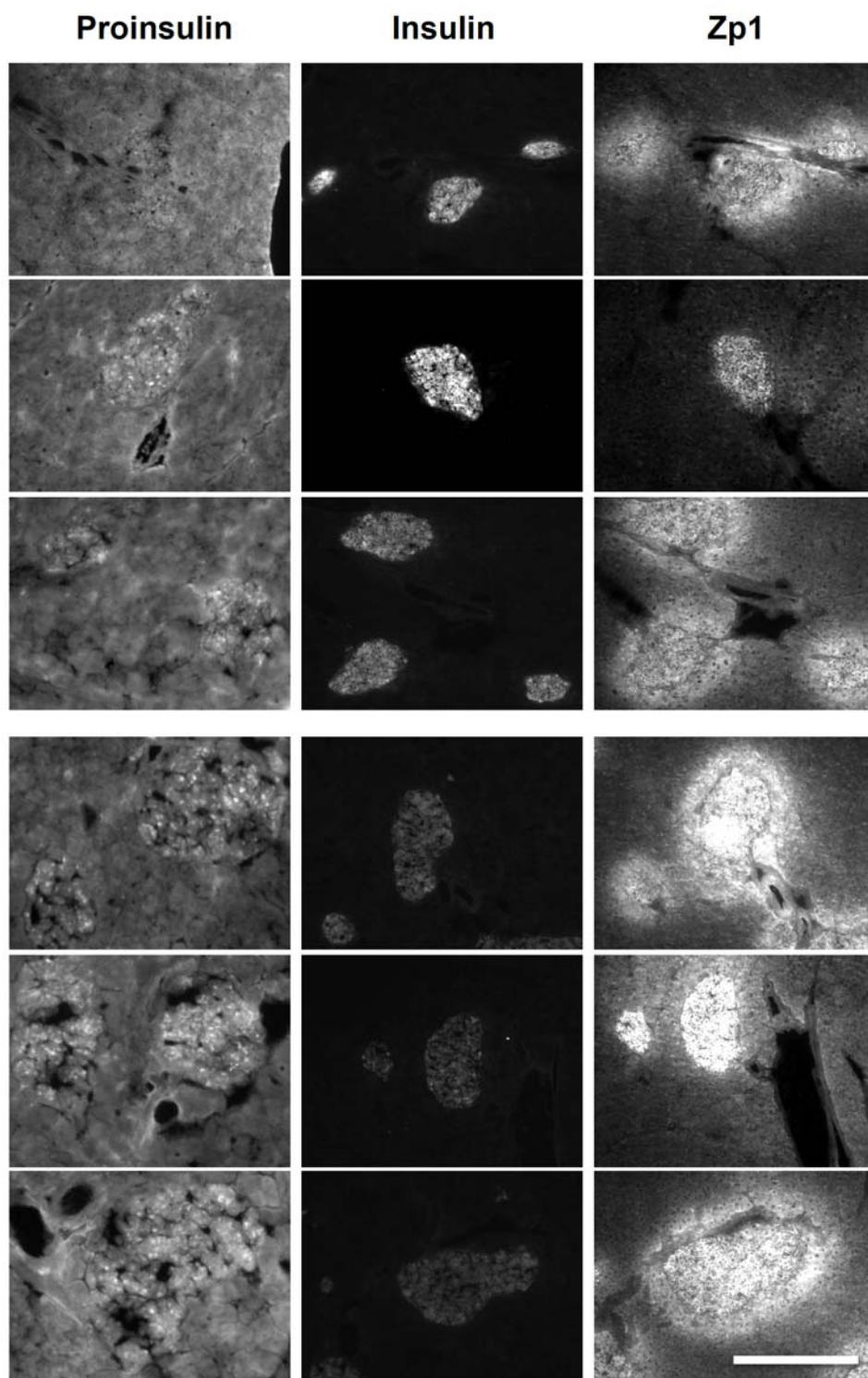
SUPPLEMENTARY DATA

Supplementary Figure 2. RT-PCR of tissues from *ZnT8* WT and RW transgenic mouse lines. To demonstrate the relative levels of expression of the transgene in different tissues, the pancreas (P), thymus (T), brain (B), and liver (L) were isolated, RNA extracted, and RT-PCR performed. The upper panel shows the *ZnT8* expression in each sample, whereas the lower panel shows the *GAPDH* loading control expression in each sample. The first lane is DNA size markers, and the last lane in the upper panel is the positive control for *ZnT8* (1 ng of *ZnT8-GFP* plasmid DNA).



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Supplementary Figure 3. Zn^{2+} , proinsulin, and insulin staining of *hZnT8* RW male mice (line T-12) after a normal diet (ND). Pancreatic sections from three different 16 week old *hZnT8* T-12 transgenic (+) mice fed ND (Top Panel), and three different transgenic (-) mice fed ND (Bottom Panel) were stained with anti-insulin (exposure time 500ms), anti-proinsulin (exposure time 100 ms), and Zp1 (exposure time 200ms) at magnification x 100. Scale bar represents 400 microns.



SUPPLEMENTARY DATA

Supplementary Figure 4. Zn^{2+} , proinsulin, and insulin staining of *hZnT8* WT male mice (line X-40) after a ND. Pancreatic sections from three different 16 week old *hZnT8* X-40 transgenic (+) mice fed ND (Top Panel) , and three different transgenic (-) mice fed ND (Bottom Panel) were stained with anti-insulin (exposure time 500ms), anti-proinsulin (exposure time 100 ms), and Zp1 (exposure time 200ms) at magnification x 100. Scale bar represents 400 microns.

