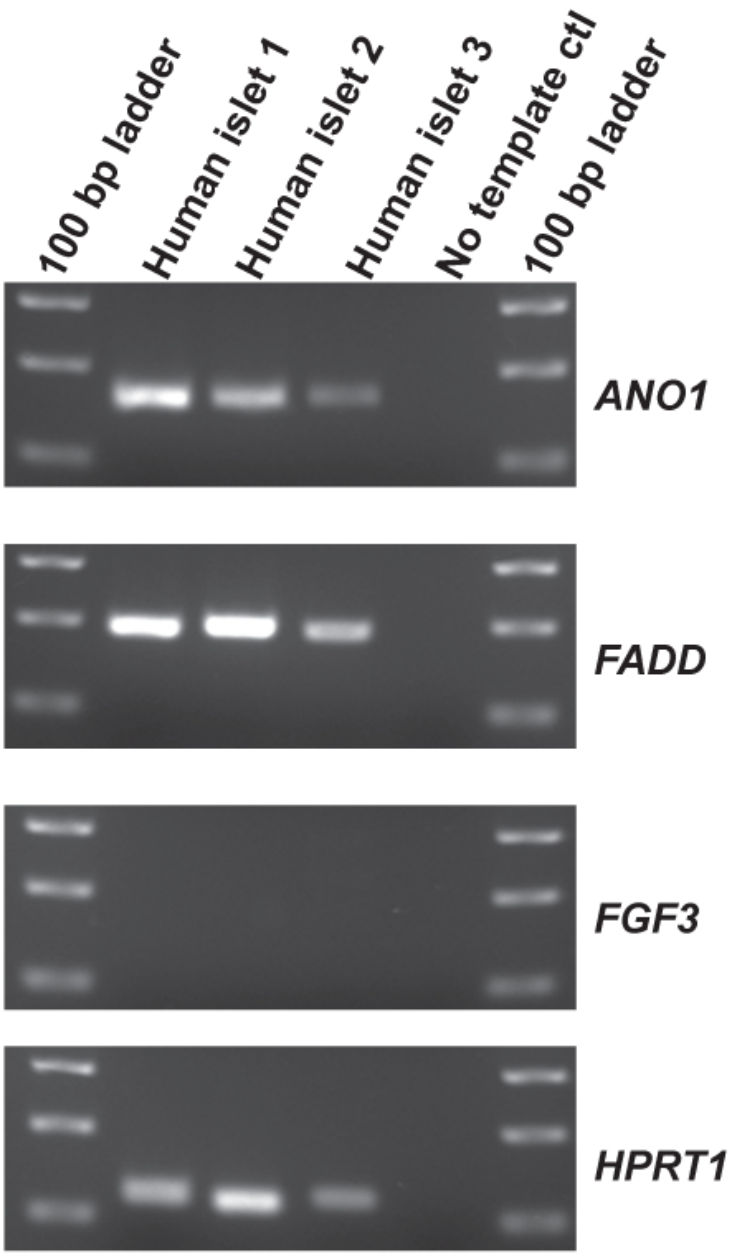


Figure S1 RT-PCR analysis of *ANO1*, *FADD*, *FGF3* and *HPRT1* gene expression in human islets from three different donors.

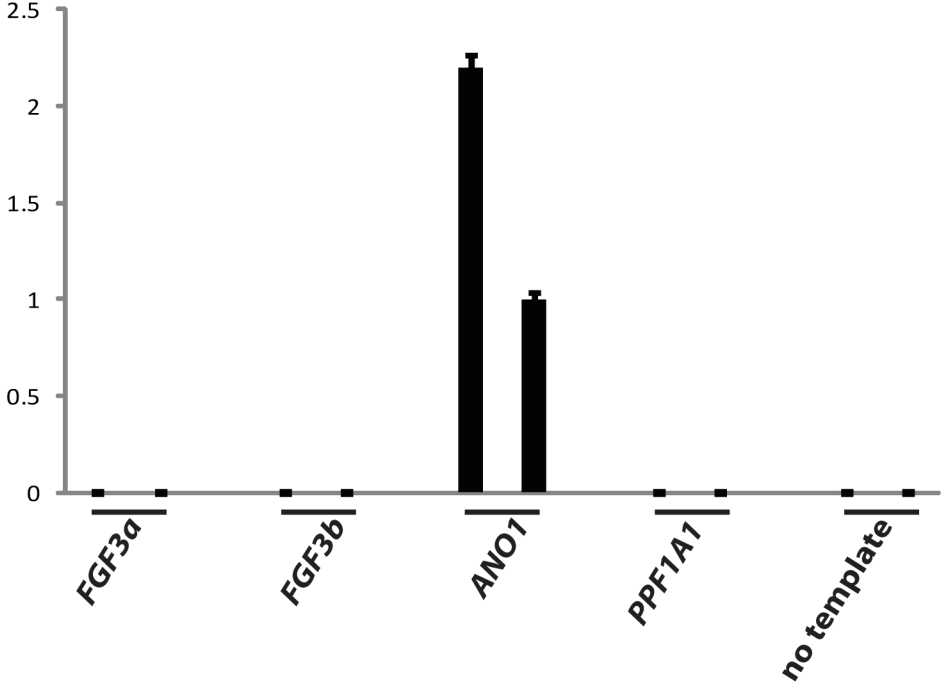
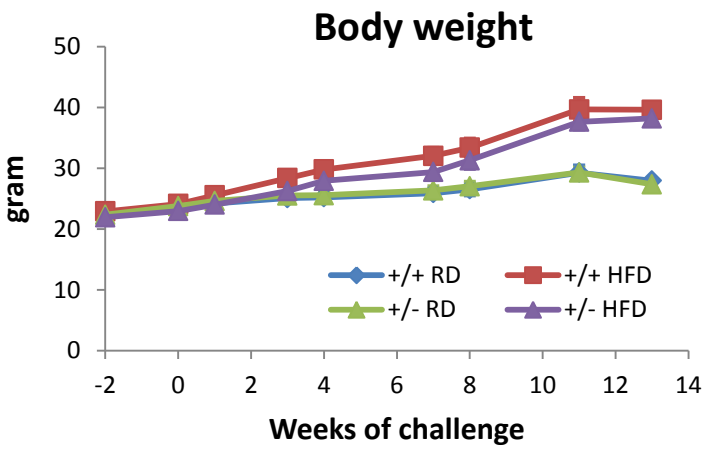


Figure S2 TaqMan 3C-PCR analysis of *INS* interactions with *FGF3*, *ANO1* and *PPF1A1* genes in human islets from two different donors. The interactions were normalized with ACTB internal ligation control.



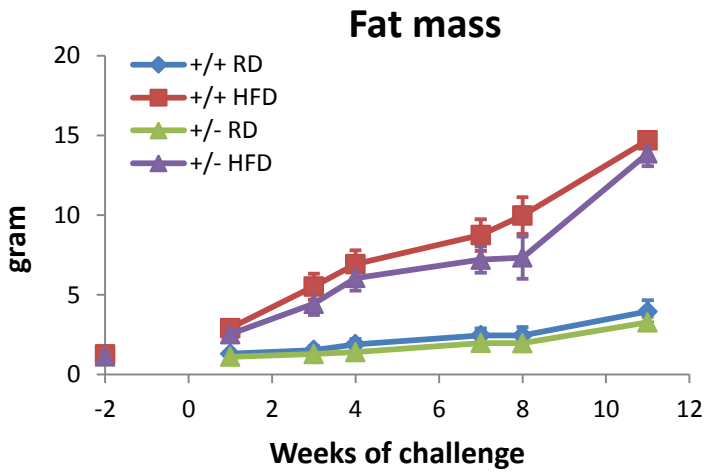
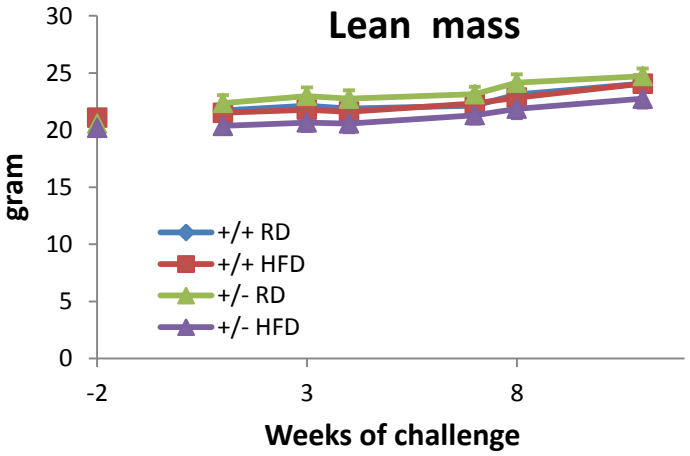


Figure S3. Analysis of *Ano1* mouse body composition (body weight, fat mass, and lean mass) over the 13 weeks of high fat feeding. Seven-weeks old *Ano1*$^{+/+}$ and *Ano1*$^{+/-}$ males were divided into 4 groups (8-9 mice per group). After 2 weeks of regular diet (RD), one group of each genotype was switched to a high-fat diet (HFD), whereas the others were left on a RD (week 0). Body weight, fat mass and lean mass were assessed weekly or bi-weekly.



Supplemental Table S1

The sequence of PCR primers for 3C analysis

Primer name	Sequence of primers (5′ to 3′)	Primer location
3C-INSR1	CACCGGGCCCCTGGTTAAGACTCTA	*INS*, anchored primer
3C-INS-q1R	GGCCCCTGGTTAAGACTCTAATGA	*INS*, nested PCR
3C-ANO1F	TAAAACAAACCCTGTGGCGGCAAGA	*ANO1* gene
3C-ANO1-q1F	CTGTGGCGGCAAGAAATAC	*ANO1*, nested PCR
3C-FGF3aF	GGGCATCATCATCAGGGTTCTGGTT	*FGF3* gene
3C-FGF3a-q1F	TCATCATCAGGGTTCTGGTT	*FGF3*, nested PCR
3C-FGF3bF	GTCTGTGGAGCAGAGACCAG	*FGF3* gene
3C-FGF3b_q1F	GTCTGTGGAGCAGAGACCAG	*FGF3*, nested PCR
3C-PPF1A1F	TGACCCCTTCCCTGGATTTTTGTCT	*PPF1A1* gene
3C-PPF1A1-q1F	CCCTGGATTTTTGTCTGTGT	*PPF1A1*, nested PCR

The sequence of PCR primers for TaqMan 3C analysis (

Primer name	Sequence of primers (5′ to 3′)	Primer location
3C-INS-q1R	GGCCCCTGGTTAAGACTCTAATGA	*INS*
3C-ANO1-q1F	CTGTGGCGGCAAGAAATAC	*ANO1*
TaqMan probe	[FAM]CAGATCTCCTTGGTCGTCAGCA CCTCTTC[TAMRA]	*INS* promoter
ACTB	F: TGGCACTGTTTTGAGAAATGAGA TaqMan probe: [FAM]AAGCACCCTGAAGAAAGCAAGCACAATTC[TAMRA] R: CTTGCCCACCTTGAAGATCTG	

The sequence of PCR primers for qRT-PCR analysis

Gene name	Sequence of primers (5′ to 3′)
ANO1	F: AGATGAGCTCAGCCTGCGCCACGG R: CAAAGCCCGTGAGGTCCCAGCGGT
FADD	F: CGTCGACGACTTCGAGGCGGGGG R: GCACACGCTCTGTCAGGTTGCGGG
FGF3	F: CGGCAGCCTGGAGAACAGCGCCTA R:TGGCCAGGTACCGCCCGGAGAAGA
INS	F: GCAGCCTTTGTGAACCAACACCTG R: GGCGGGTCTTGGGTGTGTAGAAGAAG
HPRT1	F: TGCTGAGGATTTGGAAAGGGTGTTT R: GCACACAGAGGGCTACAATGTGATG

Table S2 **Effects of genotype and diet on Ano1 mouse metabolic profiles**

		+/+		+/-	
	Weeks	**RD**	**HFD**	**RD**	**HFD**
Blood glucose	4	107.63±5.43	104.56±4.52	110.44±3.54	114.00±3.26
(mg/dL)	12	112.50±3.05	129.30±6.61	117.00±3.54	129.67±2.87
Insulin	4	0.63±012	1.42±015	0.68±010	**1.00±010**
(ng/mL)	12	0.76±0.15	2.98±0.76	0.90±0.11	1.77±0.21
Insulin/Glucose	4	6.00±1.13	13.30±1.50	6.25±0.95	**8.92±1.08**
(a.u.)	12	6.73±1.28	21.66±4.78	7.56±0.73	13.71±1.77
Free Fatty Acid	4	0.53±0.05	0.47±0.04	0.49±0.04	0.40±0.03
(mM)	12	0.67±0.05	0.54±0.04	0.64±0.06	0.52±0.02
Triglycerides	4	187.44±11.20	159.48±17.36	175.88±15.26	133.24±3.92
(mg/dL)	12	192.50±13.47	152.78±7.59	162.92±13.00	149.03±6.58
Cholesterol	4	126.64±3.82	216.47±8.75	124.00±4.15	207.58±6.85
(mg/dL)	12	120.03±5.59	225.38±8.21	116.25±2.84	234.89±9.89

t-test RD vs HFD per genotype, $p<0.05$

bold t-test +/+ vs +/- per diet, $p<0.05$

Table S2. Effects of genotype and diet on *Ano1* mouse metabolic profiles. The metabolic profiles of the 35 mice were established from blood drawn at 4 and 12 weeks of high fat feeding. The parameters that are statistically different based on genotype or diet are indicated in bold and grey, respectively.

Table S3 **3-way ANOVA analysis of GTT carried out after 5 and 13 weeks of HFD challenge**

	Blood glucose		Insulin		Insulin/Glucose	
Weeks of Challenge	5	13	5	13	5	13
Genot	0.13	0.77	0.078	0.11	0.13	0.17
Diet	3.09E-014	2.20E-016	3.79E-008	3.07E-007	1.80E-007	0.00095
Time	2.20E-016	2.20E-016	8.65E-007	8.28E-006	0.34	0.52
Genot:Diet	0.15	* 0.013	* 0.098	0.18	0.13	* 0.092
Genot:Time	0.83	0.43	0.24	0.27	0.48	0.35
Diet:Time	0.002	6.69E-007	0.14	0.3	0.13	0.47
Genot:Diet:Time	0.77	0.71	0.52	0.29	0.99	0.42

* Tukey HSD p <0.001 at 15 min

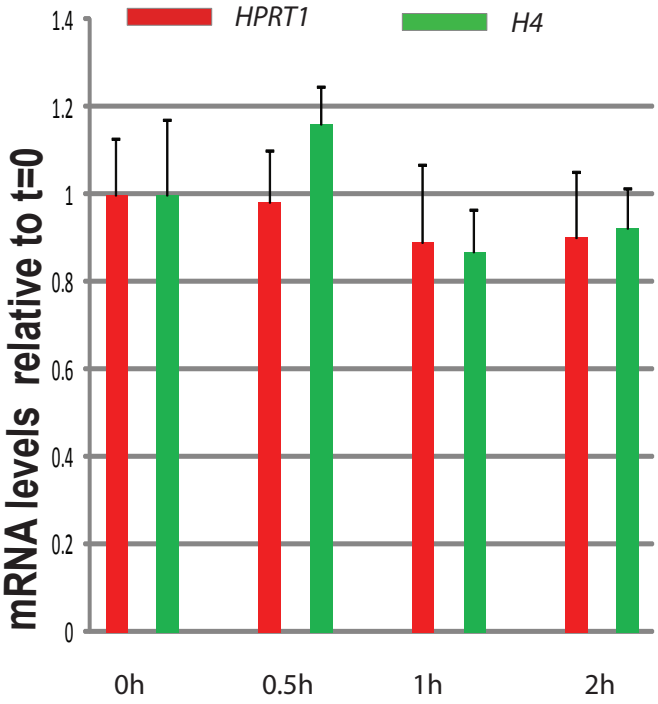


Fig. S4. Gene expression of *HPRT1* (red bar) and histone *H4* (green bar) in human islets before and after glucose treatment

Supplementary Materials and Methods

3C analysis and TaqMan quantitative 3C analysis. 3C ligated DNA libraries were made from human islets with and without glucose treatment, and 3C PCR and TaqMan quantitative 3C PCR were carried out as previously reported (1). Briefly, 60 ng of 3C ligated DNA was preamplified for 25 cycles with PicoMaxx High Fidelity PCR Master Mix using primers of *ANO1-INS* interactions or β actin (ACTB) internal controls. After passing through G25 column, the preamplified DNA products were diluted 100-fold with distilled water before TaqMan qPCR. The TaqMan PCR reactions were run in a 10-μl-volume using 2 μl of diluted preamplified 3C DNA, 0.5 μM of each of *ACTB* primers, or 0.5 μM of each primer for the *ANO1* and *INS* sites, 0.3 μM of TaqMan probe for *ACTB* or *INS* ligated DNA, and 1×TaqMan Universal PCR Master Mix (Applied Biosystems). The relative interactions between the *INS* promoter and the *ANO1* gene were determined by using a standard curve generated as described previously (1). The sequences of the primers used for 3C PCR and TaqMan qPCR analysis are provided in Supplementary Table S1.

Quantitative real-time RT-PCR analysis. SYBR Green qRT-PCR analyses were performed as previously (1). The steady-state levels of *ANO1*, *FADD* and *INS* mRNAs were determined by qRT-PCR and normalized to those of *HPRT1*. Each qRT-PCR assay was carried out in triplicate and independently repeated three times. The sequences of the primers used for qRT-PCR analyses are provided in Supplementary Table S1.

Luciferase reporter assays in mouse β cells. A human ANO1 promoter fragment whose nucleotide positions are from -998 to +16 was amplified by PCR and cloned into the XhoI and BglII sites of the pGL4 promoterless luciferase reporter vector (E665A, Promega) to generate *ANO1P*-Luc. A human *INS* promoter region with nucleotide positions from -6 to -346 was amplified by PCR and cloned in both orientations into the BamH1 site downstream of the Luciferase gene of the *ANO1P*-Luc reporter vector to generate *INSP-ANO1P*-Luc. The DNA sequences of the reporter vectors made were confirmed by DNA sequencing. The promoter luciferase reporter vectors were co-transfected with a Renilla luciferase reporter vector into mouse MIN6 β cells by Amaxa nucleofection using Solution V and T-020 program. Two days after transfection, cellular extracts were prepared and luciferase activities determined using Dual-luciferase reporter assay system (Promega).

Islet insulin secretion experiments. To determine the effects of *ANO1* knockdown on insulin secretion, siRNA-treated islets on cell strainers were washed twice with fresh islet media, resuspended in fresh media and cultured for 6 h in a 37ºC cell incubator. siRNA-treated islets were then resuspended in stimulating islet media and cultured for 1 hr. Finally, human islets were centrifuged down and the supernatants collected for measurement of the insulin levels while the pellets of human islets were used to prepare total cellular RNA. Human islets were also treated for 3h or overnight with mock (DMSO), 1.5 or 3 μM ANO1 channel inhibitor T16Ainh–A01, or 2 μM ANO1 channel activator Ecat. Islets were then washed once with fresh media and incubated in the islet media with or without 25 mM glucose for 30 min. The insulin levels in the islet media were determined using a human insulin ELISA assay (IS130D, Calbiotech) and were normalized to the amount of total RNA prepared from the islets. Each ELISA assay was done in duplicate and repeated twice.

Mice studies. *Ano1*$^{+/-}$ mice (2) were kindly provided by Jason Rock. *Ano1*$^{+/-}$ mice were selected to form 4 equal groups. At 9 weeks of age, two of these groups – one of each genotype - were switched to a high-fat diet, whereas the other two were maintained on a regular chow diet. Mice were housed three-four per cage with ad libitum access to water and chow diet (NIH-07, 11% kcal fat; Zeigler Brothers) or high fat diet (D12492, 60% kcal fat; Research Diets) and were maintained on a 12-h light/dark cycle (6 a.m./6 p.m.). Animal experiments were approved by the NIDDK Animal Care and Use Committee.

Body composition analysis. Body weight and body composition were measured every other week. Body composition was measured in conscious mice using an NMR analyzer (Echo MRI 3-in-1).

Biochemical assays. Blood was obtained from the tail vein in the non-fasted state after 4 and 12 weeks on HFD. Blood glucose levels were measured using Glucometer Contour (Bayer). Serum insulin was measured using radioimmunoassay (RIA) kits (Millipore). Free fatty acids (Roche Diagnostics Gmbh, Mannheim, Germany), triglycerides (Pointe Scientific), and cholesterol (Thermo Scientific) were measured by calorimetric assays.

Glucose tolerance test. Glucose tolerance test was performed at weeks 5 and 13 of high fat feeding. Mice were fasted overnight. Glucose (2 g/kg body weight) was injected intraperitoneally. Blood glucose levels were measured before and 15, 30, 60 and 120 min after the injection. Additional plasma samples were obtained from the tail vein at 0, 15 and 120 minute time point for measuring insulin concentration.

References

1. Xu, Z., Wei, G., Chepelev, I., Zhao, K. & Felsenfeld, G. (2011) Mapping of *INS* promoter interactions reveals its role in long-range regulation of *SYT8* transcription. ***Nat Struct Mol Biol*** **18, 372-8**

2. Rock, J. R., Futtner, C. R. & Harfe, B. D. (2008) The transmembrane protein TMEM16A is required for normal development of the murine trachea. ***Dev Biol*** **321, 141-9**