SUPPLEMENTARY DATA

The type-2 diabetes-associated K+ channel TALK-1 modulates beta-cell electrical excitability, 2nd-phase insulin secretion, and glucose homeostasis.

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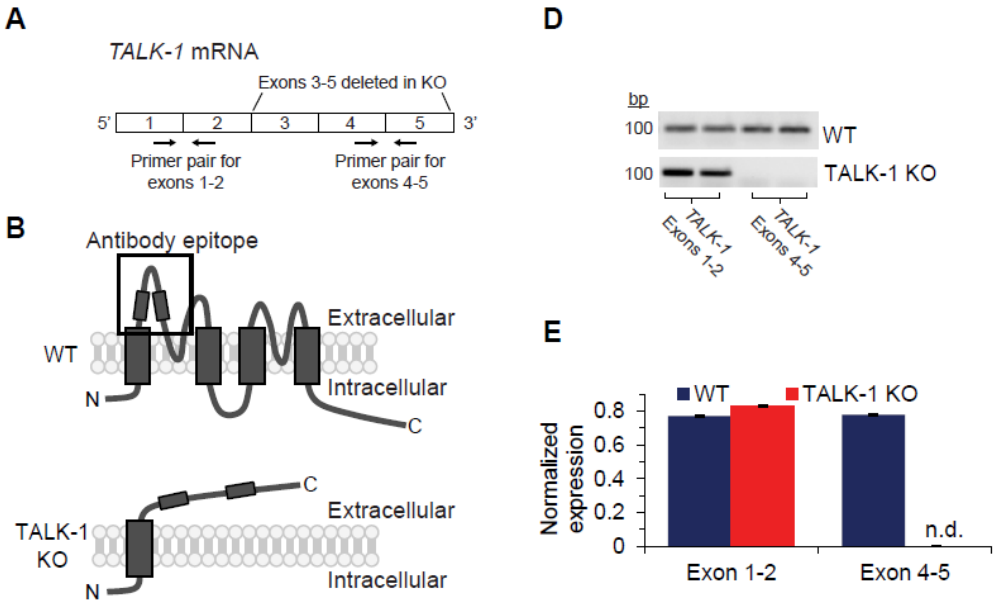
Supplementary Figure S1. Generation of TALK-1 KO mice. (*A*) Diagram illustrating predicted structure of *Kcnk16 (TALK-1)* mRNA. Exons 3-5 are removed in the targeted allele. (*B*) Illustration of TALK-1 protein membrane topology in WT (top) mice, and the structure of the predicted truncated TALK-1 protein in TALK-1 KO mice. Note that the antibody epitope is retained in the truncated TALK-1 protein. (*C*) Primer pairs used to validate expression of *Kcnk16* message in WT and TALK-1 KO islets, as illustrated in panel (*A*). (*D*) Gel images of RT-PCR products obtained using primers in (*C*) in WT (upper image) and TALK-1 KO (lower image); note that message corresponding to exons 1-2 is expressed in TALK-1 KO islets, predicting the expression of a truncated TALK-1 protein. (*E*) TALK-1 transcript abundance in WT and TALK-1 KO islets, as assessed by real-time qRT-PCR; primers as in (*D*), expression normalized to *GAPDH*.



C

Primer	Sequence (5′ 3′)	Product size (bp)
Kcnk16 Exons 1-2 (Forward)	GACCAGCAGGCCCTGGAGCA	100
Kcnk16 Exons 1-2 (Reverse)	AGTCCCAGTTGCTGGGATTG	100
Kcnk16 Exons 4-5 (Forward)	AGCACCATTGGCTTCGGGGACTA	100
Kcnk16 Exons 4-5 (Reverse)	GTCCTAGGAGGATCCATATAGCT	100

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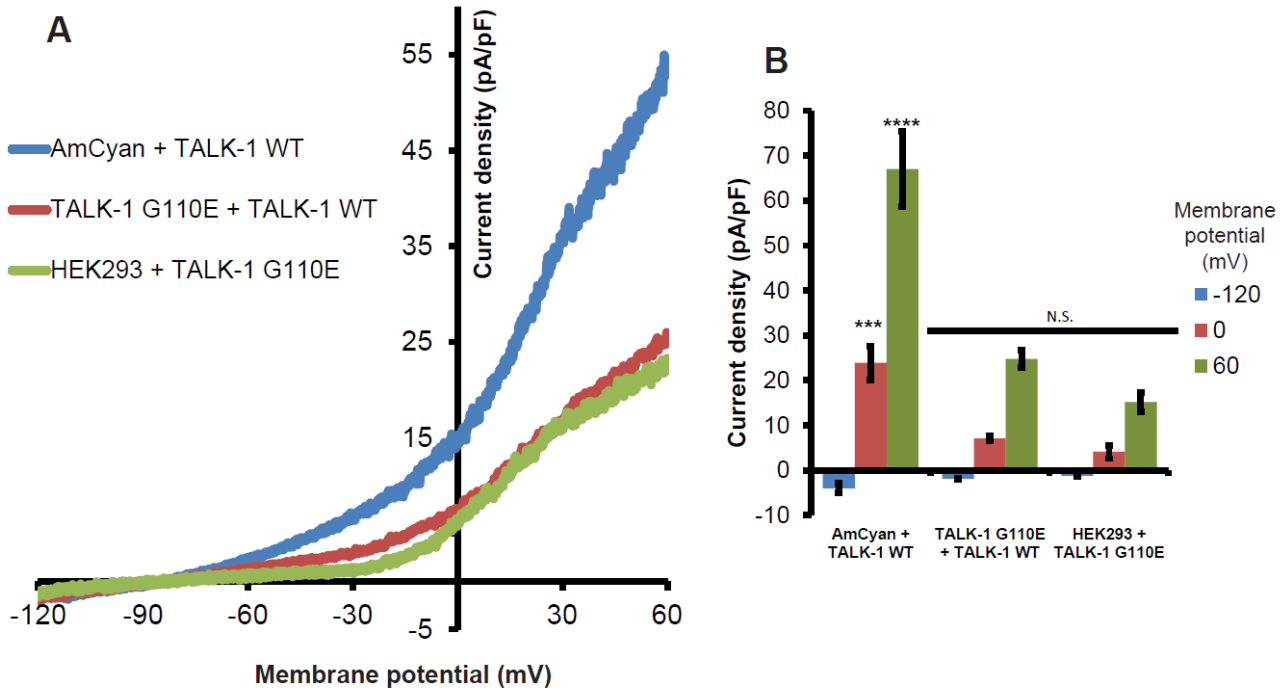
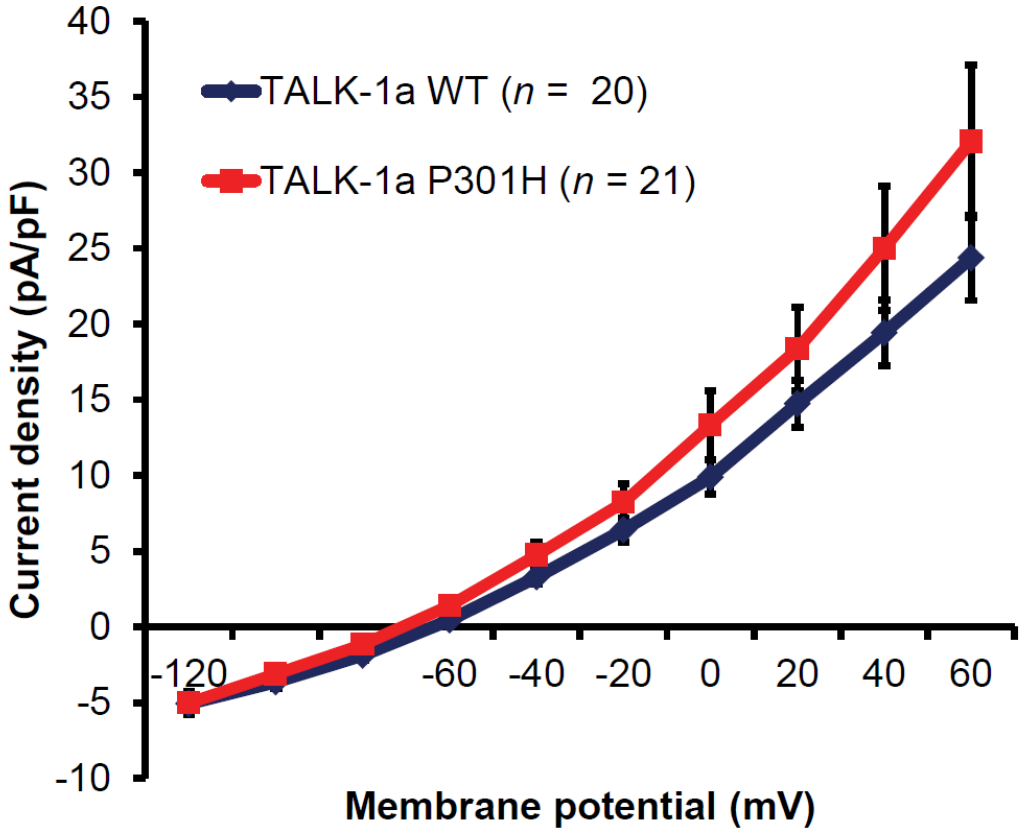
Supplementary Figure S2. The dominant negative TALK-1 G110E suppresses TALK-1 WT channel activity. (*A*) Whole-cell K2P currents in HEK293 cells expressing TALK-1 and TALK-1 G110E or a control plasmid (AmCyan) in response to a voltage ramp from -120 mV to +60 mV. Current densities are quantified in (*B*). Data are mean values ± SEM; ***P <0.001; ****P <0.0001, Student's *t*-test



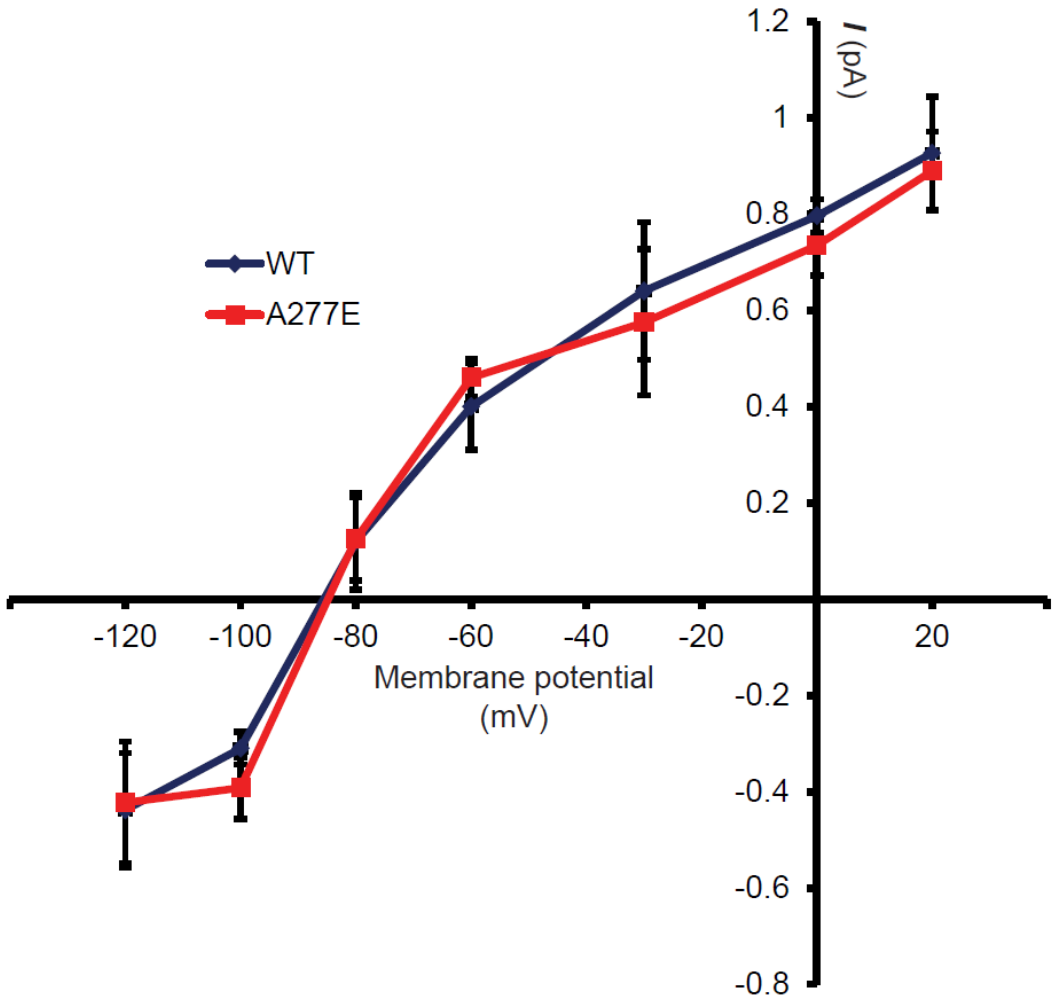
SUPPLEMENTARY DATA

Supplementary Figure S3. TALK-1a P301H does not exhibit changes in channel activity. Whole-cell current densities at selected membrane potentials for TALK-1a WT and TALK-1a P301H expressed in CHO cells. Data are mean values ± SEM.



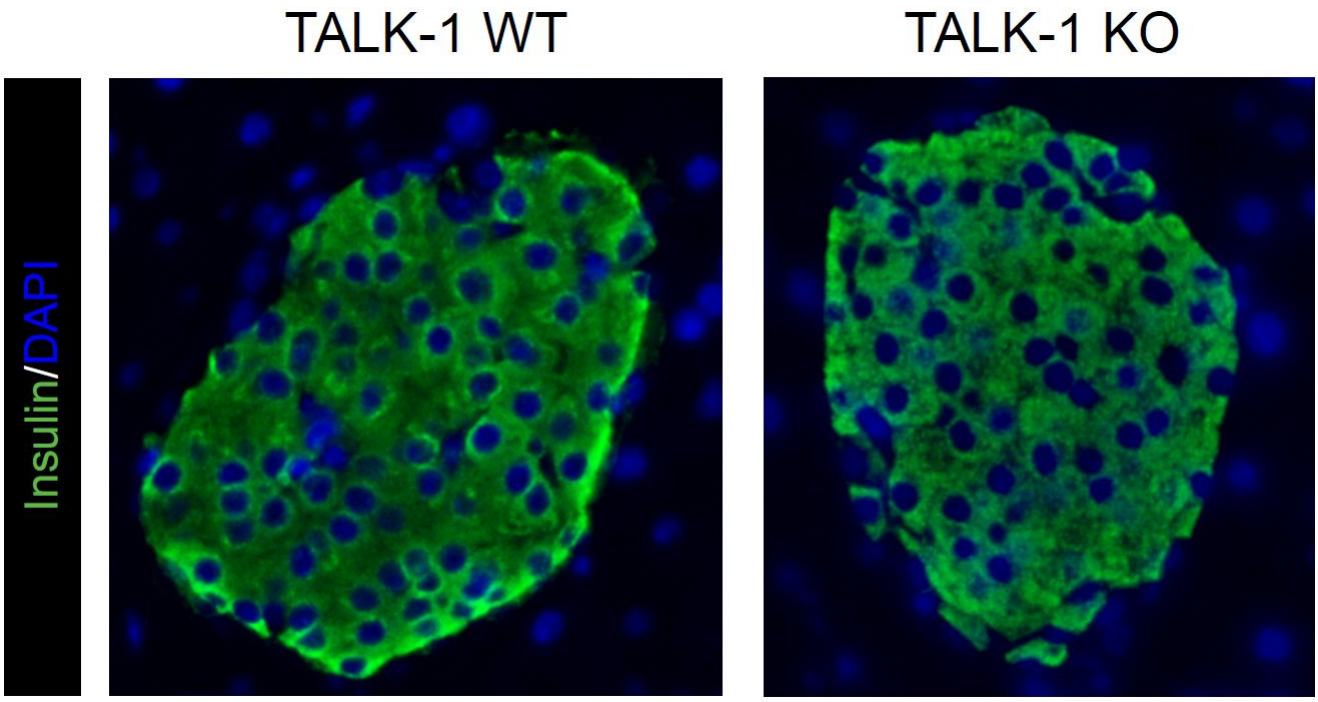
SUPPLEMENTARY DATA

Supplementary Figure S4. Unitary currents are not different between TALK-1 WT and TALK-1 A277E. Single channel currents measured in on-cell patches in HEK293 cells expressing TALK-1 WT or TALK-1 A277E at indicated membrane potentials. $n \geq 5$ for each point. Data are mean values ± SEM.



SUPPLEMENTARY DATA

Supplementary Figure S5. Pancreas sections from TALK-1 WT and TALK-1 KO mice. Sections were stained for insulin, as described in Research Designs and Methods.



SUPPLEMENTARY DATA

Supplementary Table S1. Action potential characteristics in WT and TALK-1 KO β-cells. Action potential parameters were determined over a period of 30 seconds in the second oscillation of electrical activity in islets stimulated with 14 mM glucose using the "Threshold search" event detection function in Clampfit 10 (pCLAMP 10; Molecular Devices). Data is presented as mean ± SD; Student's *t*-test.

Parameter	WT (n = 7)	TALK-1 KO (n = 7)	P value
Peak amplitude (mV)	37.92 ± 2.24	30.69 ± 2.24	0.04
Antipeak amplitude (mV)	22.74 ± 1.02	18.38 ± 1.01	0.009
Time to peak (ms)	6.28 ± 0.66	7.91 ± 1.00	0.21
Half-width (ms)	11.77 ± 1.28	14.71 ± 1.85	0.24
Area (mV·ms)	395.94 ± 47.65	393.38 ± 44.53	0.96
Instantaneous frequency (Hz)	3.92 ± 0.30	7.93 ± 2.17	0.09
Interevent interval (ms)	323.50 ± 33.59	351.22 ± 50.45	0.66
Event frequency (Hz)	3.27 ± 0.30	3.17 ± 0.33	0.82
Maximum rise slope (mV/ms)	4.41 ± 0.77	3.09 ± 0.42	0.14
Maximum decay slope (mV/ms)	-5.59 ± 0.90	-3.75 ± 0.49	0.08

Supplementary Table S2. Islet and pancreatic hormone content of WT and TALK-1 KO mice. Islet hormone content was determined after perifusion experiments by RIA. Pancreatic insulin was extracted using acid ethanol, and quantified using a rodent insulin ELISA (ALPCO). Data is presented as mean ± SEM; Student's *t*-test.

Parameter	WT	TALK-1 KO	P value
Islet insulin content (ng/IEQ)	37.24 ± 3.31 (n = 4)	38.87 ± 3.03 (n = 4)	0.73
Islet glucagon content (pg/IEQ)	912.10 ± 117.83 (n = 4)	718.42 ± 106.95 (n = 4)	0.27
Total pancreatic insulin (Chow diet) (ng/ml/mg tissue)	8.73 ± 3.43 (n = 3)	10.5 ± 4.89 (n = 3)	0.13
Total pancreatic insulin (HFD) (ng/ml/mg tissue)	15.56 ± 2.05 (n = 4)	11.49 ± 1.07 (n = 4)	0.16

SUPPLEMENTARY DATA

Supplementary Table S3. Islet donor characteristics for electrophysiology experiments using human islet cells. Characteristics of human donors for islets used to examine TALK-1 currents in _-cells expressing control mCherry or the TALK-1 dominant negative construct.

Donor	1	2
Sex	F	M
Age	32 yrs	52 yrs
BMI	39.4	22.5
Ethnicity	Caucasian	Caucasian
Type 2 Diabetes	No	No

Supplementary Table S4. Human pancreas donor characteristics for immunofluorescence

Donor	1	2
Sex	M	M
Age	31 yrs	58 yrs
Ethnicity	African American	Caucasian
Type 2 Diabetes	No	No