SUPPLEMENTARY INFORMATION

Cytokine-mediated changes in K^+ channel activity promotes an adaptive Ca^{2+} response that sustains β-cell insulin secretion during inflammation

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1. Average of all whole-cell K2P current recordings for WT and TALK-1 KO β-cells

Average K2P current densities were calculated for nontreated WT (N=39 cells) and TALK-1 KO (N=31 cells) β-cells by averaging all whole-cell K2P voltage-clamp recordings for voltages between -120 mV and 60 mV (Fig. S1a). WT and TALK-1 KO K2P currents were compared at selected voltages (-30 mV, 0 mV, 30 mV, and 60 mV; Fig. S1b) using an unpaired two-tailed t-test. TALK-1 KO K2P current density was lower than WT K2P current density at 0 mV (WT: 13.01±0.48 pA/pF and TALK-1 KO: 10.73±0.49 pA/pF, P<0.01), 30 mV (WT: 25.68±0.99 pA/pF and TALK-1 KO: 19.41±0.74 pA/pF, P<0.001), and 60 mV (WT: 40.52±1.34 pA/pF and TALK-1 KO: 29.75±1.19 pA/pF, P<0.001).

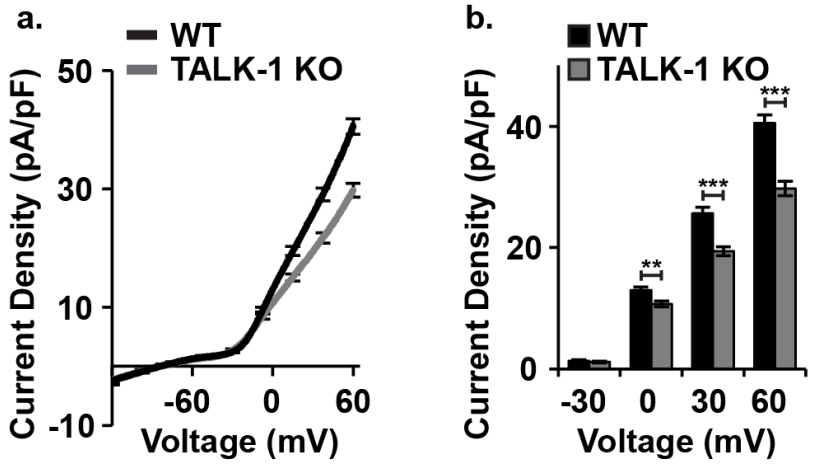

Fig. S1: Knockout of TALK-1 decreases β-cell whole-cell K2P currents. a. Average K2P current density of WT (N=39 cells) and TALK-1 KO (N=31 cells) β-cells and b. average K2P currents in WT (black) and TALK-1 KO (gray) mouse β-cells at selected voltages. Statistical analysis was conducted with unpaired two-tailed t-tests and uncertainty is expressed as SEM (**P<0.01, ***P<0.001).

2. Annexin-V-FITC apoptosis assay for WT and TALK-1 KO β-cells

WT and TALK-1 KO mouse islets were isolated and dispersed into single cells then cultured in islet media for 24 hrs at 37°C, 5% CO_2. The islet media was then replaced with fresh islet media or islet media with cytokines and the islets were cultured an additional 24 hrs at 37°C, 5% CO_2. Apoptotic cells were visualized using an ApoScreen® Annexin V Apoptosis Kit-FITC (SouthernBiotech) as per manufacturer's instructions. Cell nuclei were stained with Hoechst 33342 (Tocris Bioscience) and used to determine total cell number per field. Fluorescence was imaged using a Nikon Eclipse TE2000-U microscope equipped with an epifluorescence illuminator (Sutter Instrument Company), a CCD camera (HQ2; Photometrics, Inc), and Nikon Elements software (Nikon, Inc). Data analysis was performed using the ImageJ Fiji image processing pack.

Because $[Ca^{2+}]_{ER}$ handling is an important factor in apoptotic signaling we investigated the involvement of TALK-1 expression in cytokine-induced β-cell apoptosis (Fig. S2). In the absence of cytokines TALK-1 expression had no effect on β-cell apoptosis (WT nontreated: 22.1±1.1% apoptotic and TALK-1 KO nontreated: 23.8±1.3% apoptotic, Fig. S2). Cytokine exposure led to a significant increase in apoptosis for WT and TALK-1 KO β-cells (WT cytokines: 29.7±1.6% apoptotic, $P<0.05$, and 33.9±1.2% apoptotic, $P<0.001$). However, this increase in apoptosis was indistinguishable for WT and TALK-1 KO β-cells indicating that this rise is not due to changes in TALK-1 expression.

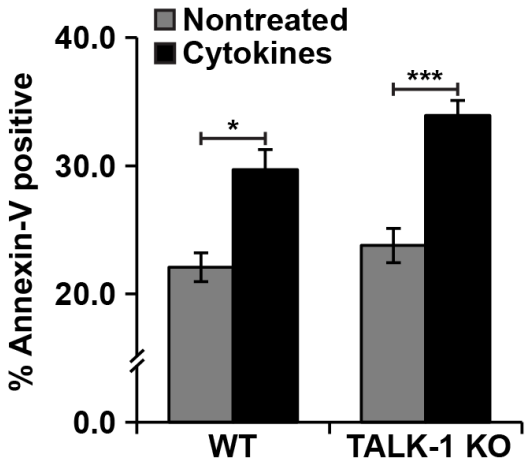

Fig. S2: Cytokine exposure triggers β-cells apoptosis in a TALK-1 independent manner. Percent apoptotic cells calculated as number of annexin-V-FITC positive cells divided by total number of cells (identified by Hoechst 33342 nuclear staining). Average % apoptotic cells for nontreated (gray) and cytokine treated (black) WT (N=3 animals) and TALK-1 KO (N≥3 animals) β-cells. Statistical analysis was conducted with a 1-way ANOVA and uncertainty is expressed as SEM (**P<0.01, ***P<0.001).

3. Quantification of $[Ca^{2+}]_i$ oscillation amplitudes in WT and TALK-1 KO β-cells

Because the amplitude of islet $[Ca^{2+}]_i$ oscillations during 2nd phase GSCI are greatly reduced after exposure to cytokines, the magnitude of this decrease was quantified. Whole islet Fura-2 AM $[Ca^{2+}]_i$ recordings (from Fig. 2) were examined and all islets exhibiting $[Ca^{2+}]_i$ oscillations for a period of at least 5 min were included in this analysis. A 5 min oscillatory period was selected for each recording and the amplitude of $[Ca^{2+}]_i$ oscillations was estimated as $F_{340/380, max} - F_{340/380, min}$ (Fig. S3a). The $[Ca^{2+}]_i$ oscillation amplitude of nontreated WT and TALK-1 KO islets were indistinguishable (WT: 0.285±0.022 $\Delta F_{340/380}$ and TALK-1 KO: 0.387±0.031 $\Delta F_{340/380}$) After cytokine exposure $[Ca^{2+}]_i$ oscillation amplitude decreased in magnitude ($P<0.001$) similarly in WT and TALK-1 KO islets (WT: 0.119±0.011 $\Delta F_{340/380}$ and TALK-1 KO: 0.130±0.013 $\Delta F_{340/380}$). These results suggest that the cytokine-mediated decrease in islet $[Ca^{2+}]_i$ oscillation amplitude in not related to TALK-1 expression.

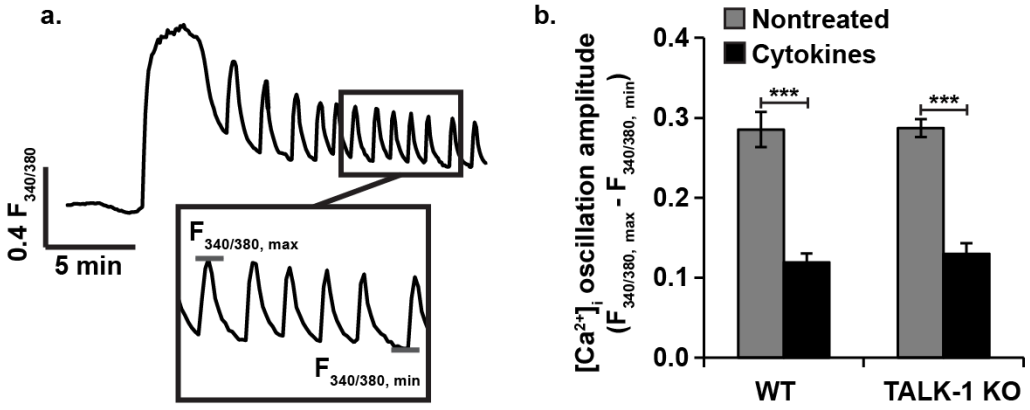

Fig. S3: Cytokine exposure decreased islet $[Ca^{2+}]_i$ oscillation amplitude independently of TALK-1 expression. a. Overview of approach used to quantify average islet $[Ca^{2+}]_i$ oscillation amplitude and b. average $[Ca^{2+}]_i$ oscillation amplitudes for nontreated (gray) and cytokine treated (black) WT (N≥41 islets) and TALK-1 KO (N≥23 islets). Statistical analysis was conducted with a 1-way ANOVA and uncertainty is expressed as SEM (***$P<0.001$).

4. Unmodified human islet TALK-1 western blots

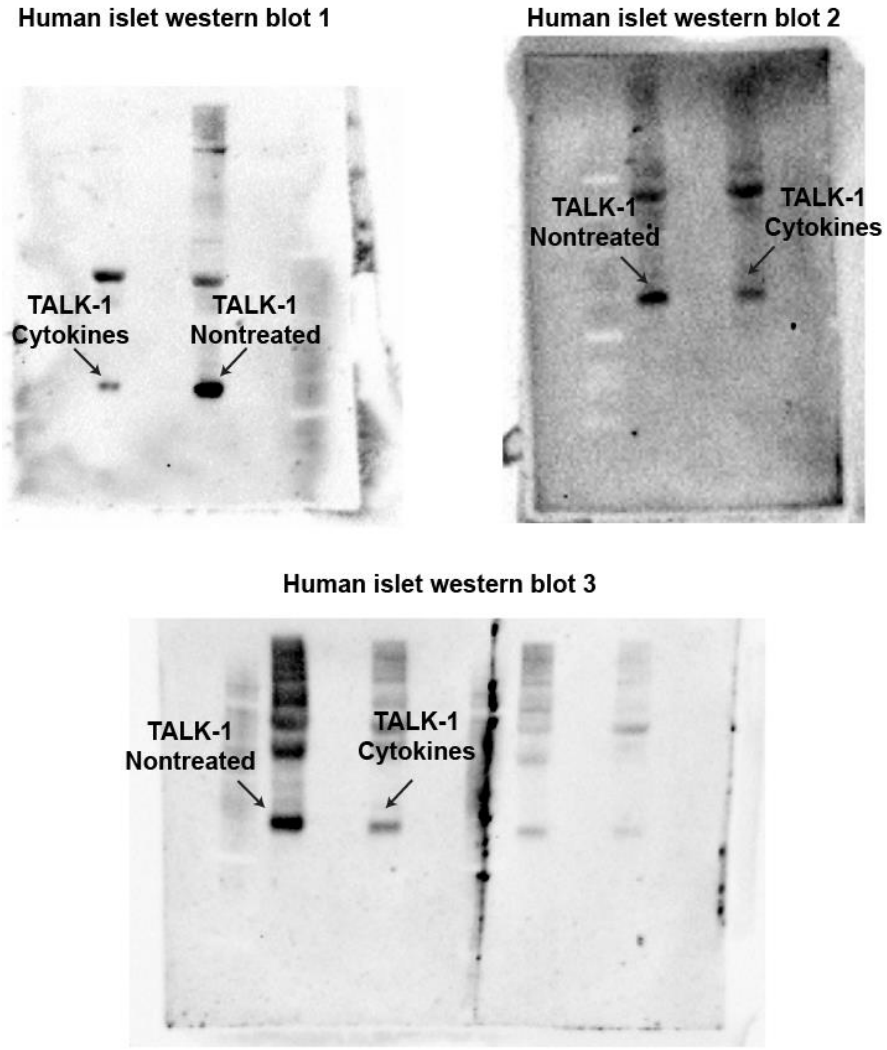

Fig S4: Cytokine exposure reduces TALK-1 protein expression in human islets. Unmodified western blots for TALK-1 protein from human islets quantified in Fig. 1c. For each western blot 40 mg total protein/lane loaded (as calculated by BCA assay).