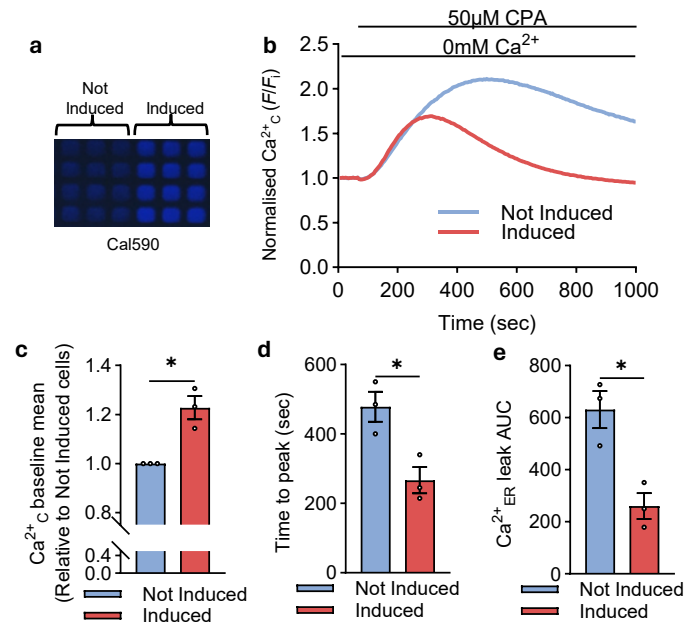


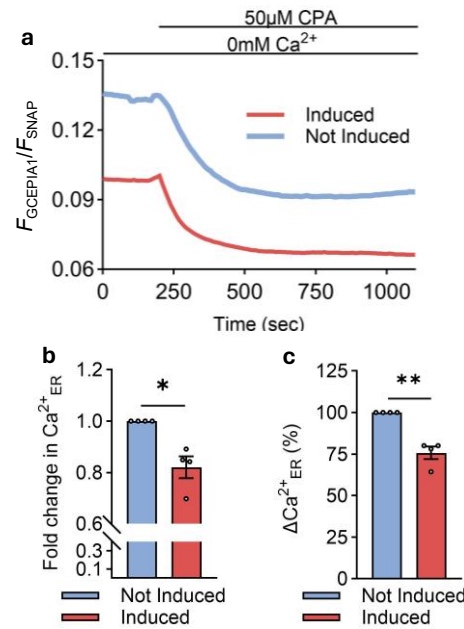
Electronic Supplementary Material

| Donor ID     | Sex | Age | Race | BMI  | Experiments                                 |
|--------------|-----|-----|------|------|---------------------------------------------|
| SAMN31697847 | M   | 30  | W    | 31.2 | qPCR                                        |
| SAMN32537360 | M   | 51  | W    | 24.8 | Ca <sup>2+</sup> imaging                    |
| SAMN32875450 | F   | 60  | W    | 25.2 | Ca <sup>2+</sup> imaging, qPCR              |
| SAMN33683883 | M   | 48  | W    | 28.5 | Ca <sup>2+</sup> imaging, qPCR              |
| SAMN33902447 | F   | 32  | W    | 19.1 | Ca <sup>2+</sup> imaging                    |
| SAMN34204018 | M   | 47  | W    | 27.5 | Ca <sup>2+</sup> imaging                    |
| SAMN34356133 | M   | 20  | H    | 38.5 | Ca <sup>2+</sup> imaging                    |
| SAMN35301006 | M   | 52  | W    | 31.6 | Ca <sup>2+</sup> imaging                    |
| SAMN35848421 | M   | 22  | B    | 31.8 | Ca <sup>2+</sup> imaging                    |
| SAMN36471214 | M   | 40  | W    | 24.2 | Ca <sup>2+</sup> imaging                    |
| SAMN36845032 | M   | 34  | H    | 33   | Ca <sup>2+</sup> imaging                    |
| SAMN36823227 | F   | 41  | W    | 38.4 | Ca <sup>2+</sup> imaging                    |
| SAMN37068473 | M   | 51  | W    | 30.4 | Ca <sup>2+</sup> imaging                    |
| SAMN37158106 | M   | 40  | W    | 32.3 | Ca <sup>2+</sup> imaging                    |
| SAMN37350251 | F   | 43  | W    | 29.9 | Ca <sup>2+</sup> imaging                    |
| SAMN37638596 | M   | 34  | NH   | 27.2 | Ca <sup>2+</sup> imaging                    |
| SAMN37973608 | F   | 55  | H    | 31.7 | Insulin secretion                           |
| SAMN38518088 | F   | 57  | H    | 30.1 | Insulin secretion                           |
| SAMN39447621 | M   | 49  | H    | 34.4 | Ca <sup>2+</sup> imaging, Insulin secretion |
| SAMN39639106 | M   | 49  | B    | 30.6 | Ca <sup>2+</sup> imaging                    |
| SAMN39912961 | M   | 39  | w    | 31   | Ca <sup>2+</sup> imaging                    |
| SAMN39980165 | F   | 60  | W    | 29.5 | Ca <sup>2+</sup> imaging                    |
| SAMN40100962 | M   | 58  | W    | 28.8 | Insulin secretion                           |
| SAMN40376066 | F   | 34  | W    | 25.7 | Ca <sup>2+</sup> imaging                    |
| SAMN40619409 | M   | 63  | B    | 34.1 | Ca <sup>2+</sup> imaging                    |
| SAMN41218531 | M   | 38  | W    | 29   | Electrophysiology                           |
| SAMN41475017 | F   | 63  | W    | 24.9 | Electrophysiology                           |
| SAMN43777471 | M   | 57  | W    | 28.8 | Electrophysiology                           |
| SAMN44562976 | F   | 62  | W    | 38.4 | Electrophysiology                           |
| SAMN44779828 | F   | 59  | W    | 26.3 | Electrophysiology                           |
| SAMN45232023 | F   | 55  | W    | 29.6 | Electrophysiology                           |

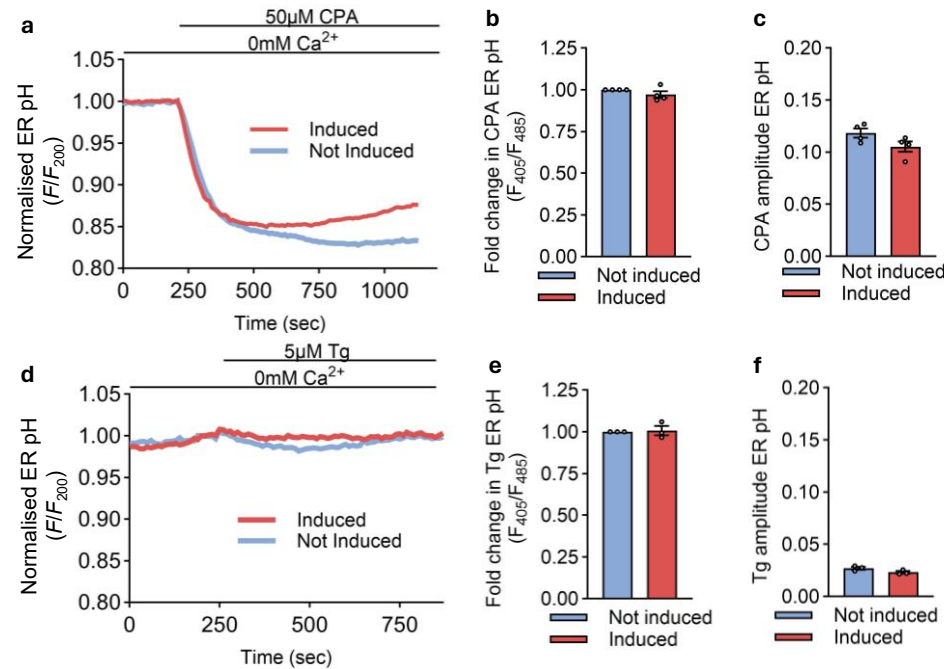
**ESM Table 1. Human islet donor descriptions used in this study.**



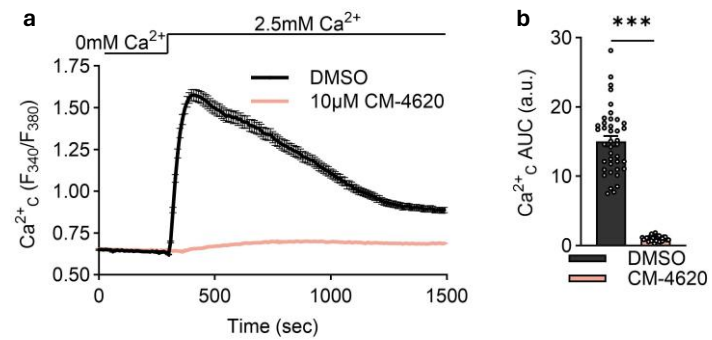
**ESM Fig 1. TALK-2 expressing cells have diminished  $\text{Ca}^{2+}_{\text{ER}}$  stores and higher baseline  $\text{Ca}^{2+}_c$ .** (a) Image of wells containing not induced and TALK-2 expressing cells (induced) loaded with Cal590 prior to  $\text{Ca}^{2+}$  imaging. (b) Representative  $\text{Ca}^{2+}_{\text{EC}}$  traces of CPA-mediated  $\text{Ca}^{2+}_{\text{ER}}$  release. (c) Analysis of relative baseline  $\text{Ca}^{2+}_c$  at 0mM  $\text{Ca}^{2+}_{\text{EC}}$  prior to CPA addition. (d) The time to CPA peak for not induced and TALK-2 expressing cells. (e) AUC of CPA-mediated  $\text{Ca}^{2+}_{\text{ER}}$  leak into the cytoplasm.  $n=3$  biological replicates; Statistical analysis conducted using one sample T-test (c) or unpaired students T-test (d-e). \* $p<0.05$



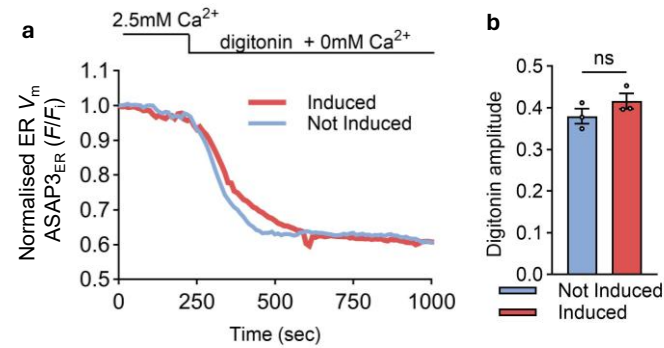
**ESM Fig 2. TALK-2 expressing cells have depleted  $\text{Ca}^{2+}_{\text{ER}}$  stores.** (a) Representative  $\text{Ca}^{2+}_{\text{ER}}$  traces in response to CPA-mediated  $\text{Ca}^{2+}_{\text{ER}}$  release. (b) Fold change analysis in  $\text{Ca}^{2+}_{\text{ER}}$  at 0mM  $\text{Ca}^{2+}_{\text{EC}}$  prior to CPA addition. (c) The percent change in  $\text{Ca}^{2+}_{\text{ER}}$  for not induced and TALK-2 expressing cells.  $n=4$  biological replicates; statistical analysis conducted using one sample T-test. \* $p<0.05$ , \*\* $p<0.01$



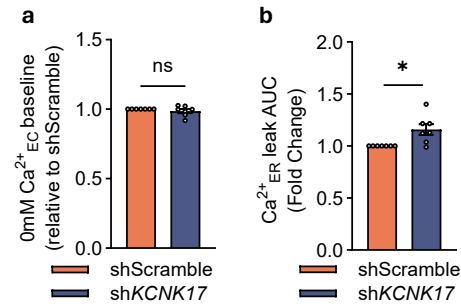
**ESM Fig 3. ER pH reduces upon CPA addition but not Tg addition.** (a) Representative ER pH changes in response to CPA from TALK-2 expressing and not induced cells. (b) Fold change in ER pH at 0mM  $Ca^{2+}$  relative to not induced cells. (c) Analysis of the ER pH amplitude in response to CPA. (d) Representative ER pH changes in response to thapsigargin (Tg) from TALK-2 expressing and not induced cells. (e) Fold change in ER pH at 0mM  $Ca^{2+}$  relative to not induced cells. (f) Analysis of the ER pH amplitude in response to Tg.  $n=4$  and 3 biological replicates for CPA and Tg respectively; statistical analysis conducted using one sample T-test (b,e) or unpaired Student's T-test (c,f).



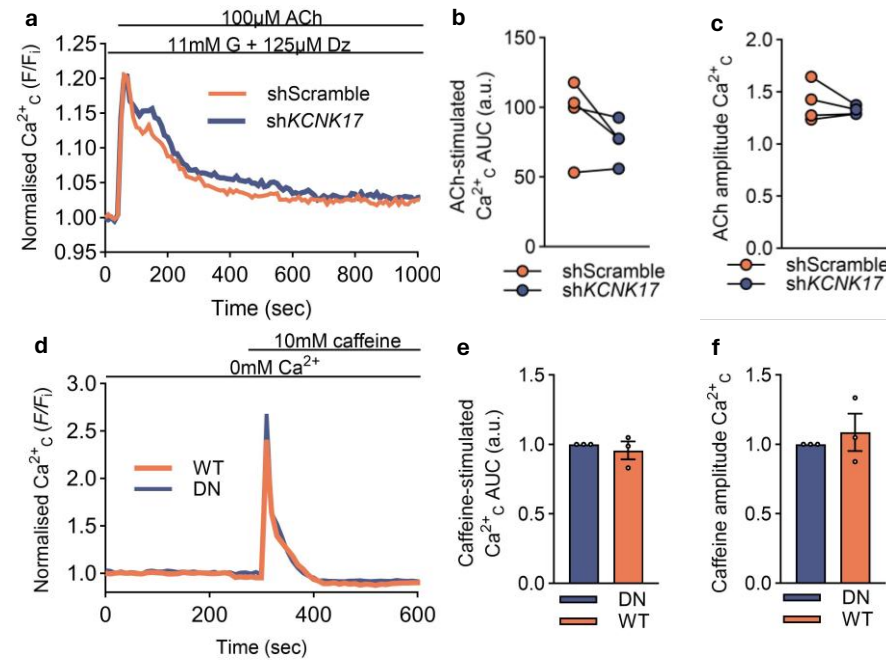
**ESM Fig 4. CM-4620 diminished SOCE.** (a) T-REx-293 cells preincubated with CPA and either CM-4620 or DMSO for 30 minutes and were then imaged in 0mM extracellular  $\text{Ca}^{2+}$  followed by the addition of 2.5mM  $\text{Ca}^{2+}$  to measure SOCE. (b) AUC analysis of the SOCE response in DMSO vs. CM-4620 treated cells.  $n =$  DMSO 40 cells, CM-4620 35 cells; statistical analysis conducted using Student's T-test. \*\*\* $p < .001$



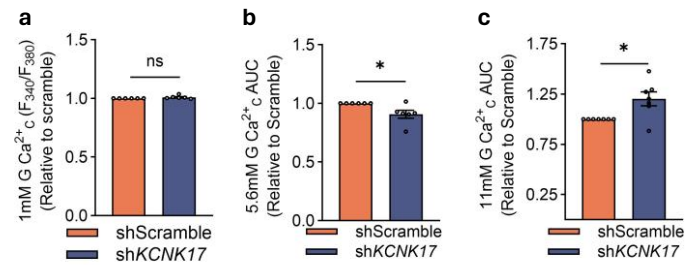
**ESM Fig 5. ER  $V_m$  hyperpolarisation reduces ASAP3<sub>ER</sub> fluorescence.** (a) Representative ER  $V_m$  traces from ER membrane hyperpolarisation with digitonin and 0mM  $Ca^{2+}$ . (b) Analysis of ASAP3<sub>ER</sub> amplitude in response to digitonin.  $n=3$  biological replicates; statistical analysis conducted using unpaired Student's T-test.



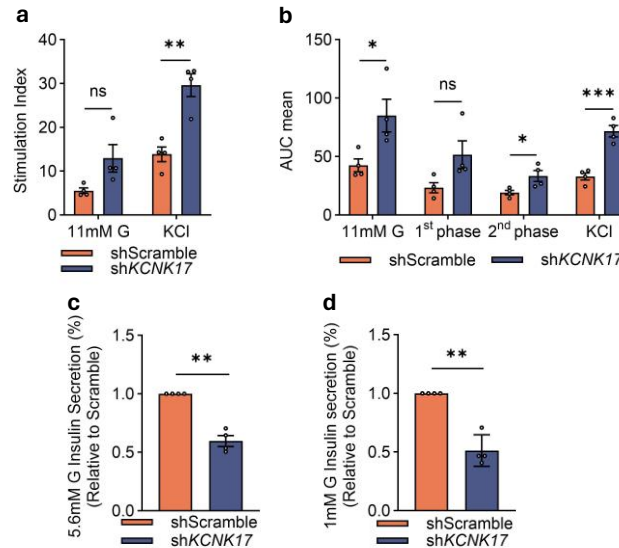
**ESM Fig 6. Human beta cell TALK-2 limits  $\text{Ca}^{2+}_{\text{ER}}$  stores.** (a)  $\text{Ca}^{2+}_{\text{C}}$  in shKCNK17 transduced beta cells at 0mM  $\text{Ca}^{2+}_{\text{EC}}$  relative to shScramble controls (one sample t-test from Fig. 6c). (b) Fold change in CPA-mediated  $\text{Ca}^{2+}_{\text{ER}}$  leak AUC relative to shScramble (one sample t-test from Fig. 6d).  $n=7$  donors;  $p<0.05$



**ESM Fig 7. TALK-2 expressing cells have diminished  $\text{Ca}^{2+}_{ER}$  stores and higher baseline  $\text{Ca}^{2+}_c$ .** (a) Representative  $\text{Ca}^{2+}$  traces from human beta cells of IP3R-mediated  $\text{Ca}^{2+}_{ER}$  release. (b) AUC analysis of  $\text{Ca}^{2+}_c$  from IP3R-mediated  $\text{Ca}^{2+}_{ER}$  release stimulated by acetylcholine (ACh). (c) Analysis of  $\text{Ca}^{2+}$  amplitude in response to IP3R activation with ACh. (d) Representative  $\text{Ca}^{2+}$  traces from INS-1 (832/13) of RyR-mediated  $\text{Ca}^{2+}_{ER}$  release transduced with either dominant negative (DN) or wild-type (WT) TALK-2. (e) AUC analysis of  $\text{Ca}^{2+}_c$  from RyR-mediated  $\text{Ca}^{2+}_{ER}$  release stimulated by caffeine. (f) Analysis of  $\text{Ca}^{2+}$  amplitude in response to RyR activation with caffeine.  $n=4$  donors (b-c) or 3 biological replicates (e-f); statistical analysis conducted using paired T-test (b-c) or one sample T-test (e-f).



**ESM Fig 8. TALK-2 promotes  $\text{Ca}^{2+}_c$  entry at basal glucose but limits  $\text{Ca}^{2+}_c$  entry at high glucose.** Beta cell  $\text{Ca}^{2+}_c$  at 1mM glucose (**a**), 5.6mM glucose (**b**), and 11mM glucose (**c**) relative to controls (one sample t-test from Fig. 6b, d, and f, respectively).  $n=6$  (**a,b**) or 7 donors (**c**);  $P<0.05$



**ESM Fig 9. TALK-2 limits insulin secretion under hyperglycemic conditions and promotes insulin secretion under euglycemic conditions.** (a) Stimulation index of beta cell TALK-2-KD pseudoislets vs Scramble in response to 11mM glucose and 25mM KCl (multiple unpaired t-tests of fig. 8c.). (b) Mean insulin secretion AUC of pseudoislets under the specified conditions (1<sup>st</sup> phase = 1<sup>st</sup> ten minutes in 11mM glucose; 2<sup>nd</sup> phase = time after 10 minutes in 11mM glucose) (multiple unpaired t-tests of fig. 8d.). Insulin secretion of TALK-2-KD pseudoislets at 5.6mM glucose (c) and 1mM glucose (d) relative to Scramble controls (one sample t-test of fig. 8f.).  $n = 4$  donors;  $P < 0.05$