**SUPPLEMENTAL INFORMATION**

**"Calcium release channel RyR2 regulates insulin release and glucose homeostasis"**

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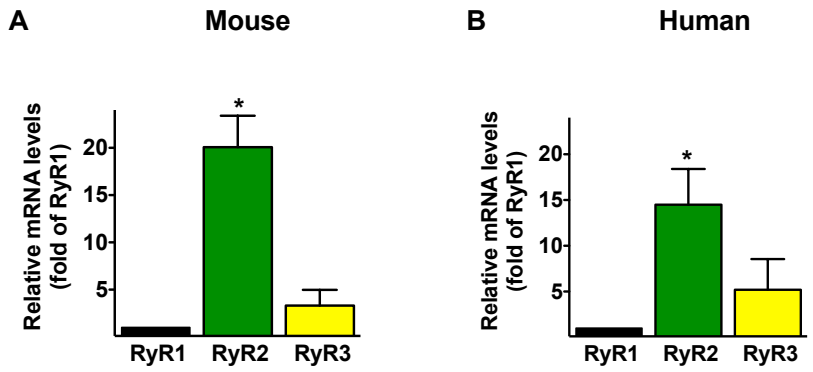


**Figure S1**. **RyR2 is the major form of RyR expressed in murine and human pancreatic islets.** The expression of RyR1, RyR2, and RyR3 in murine (**A**) and human (**B**) islets were determined by real-time RT-qPCR analysis of total RNA, using β actin as internal standard. Primer sequences are reported in Supplementary Table 3. Each bar represents mean±s.e.m. of five independent experiments in each of which reactions were performed in triplicate. **P*<0.05 vs RyR1; ANOVA.

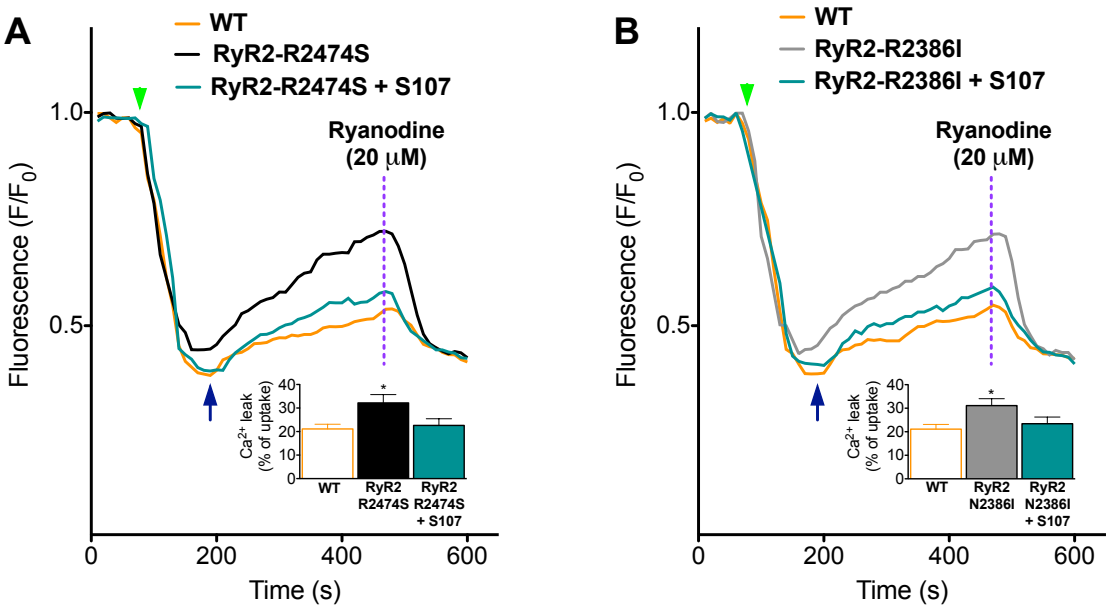


**Figure S2. Increased ER $Ca^{2+}$ leak in pancreatic islet microsomes from CPVT mice is reduced by oral treatment with S107 to fix the RyR leak.** Representative traces of ER $Ca^{2+}$ leak from pancreatic islet microsomes isolated from RyR2-R2474S (**A**) and RyR2-N2386I (**B**) mice treated with vehicle or S107 for four weeks (50 mg/kg/d in drinking water). The green arrowhead represents the addition of ATP; the blue arrow is thapsigargin (see Methods for details). The quantification of the $Ca^{2+}$ leak (from triplicate experiments) is shown in the bar graphs in the insets. *$P$<0.05 vs WT, ANOVA. Dashed line indicates the addition of ryanodine which identifies the source of the leak as the RyR channel.

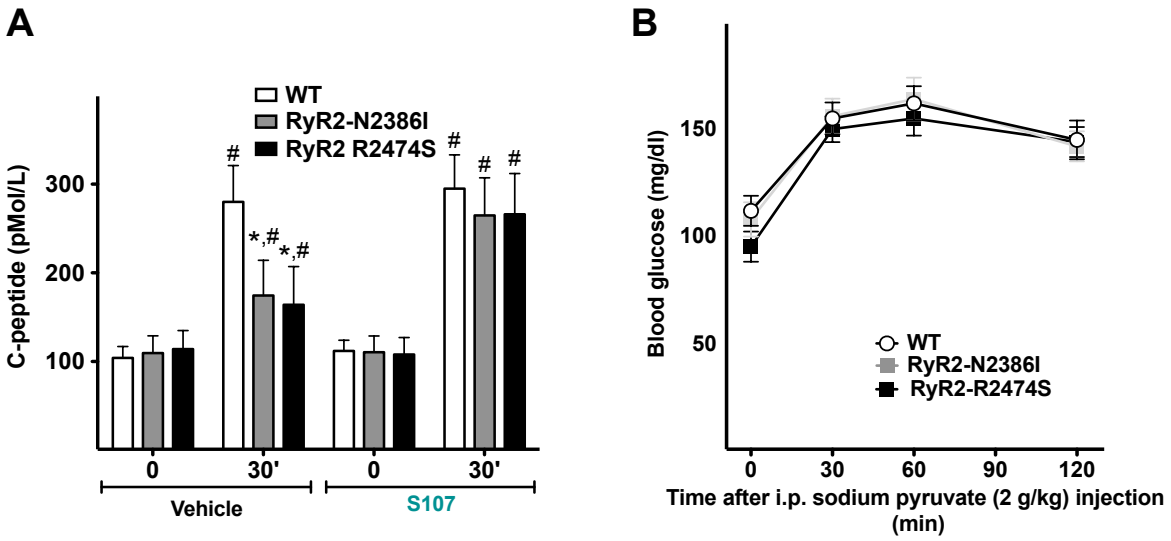


**Figure S3**. **Evaluation of C-peptide secretion and gluconeogenesis in CPVT mice.** *In vivo* determination of C-peptide release in basal conditions and 30' after an intraperitoneal (i.p.) injection (2 g/Kg) of glucose (**A**) and blood glucose levels following i.p. sodium pyruvate challenge in WT, RyR2-R2474S, and RyR2-N2386I mice (**B**). Data are expressed as mean±s.e.m. n=8-10/group. *:$P<0.05$ vs WT; #: $P<0.05$ vs baseline; ANOVA, Tukey-Kramer *post hoc* test.

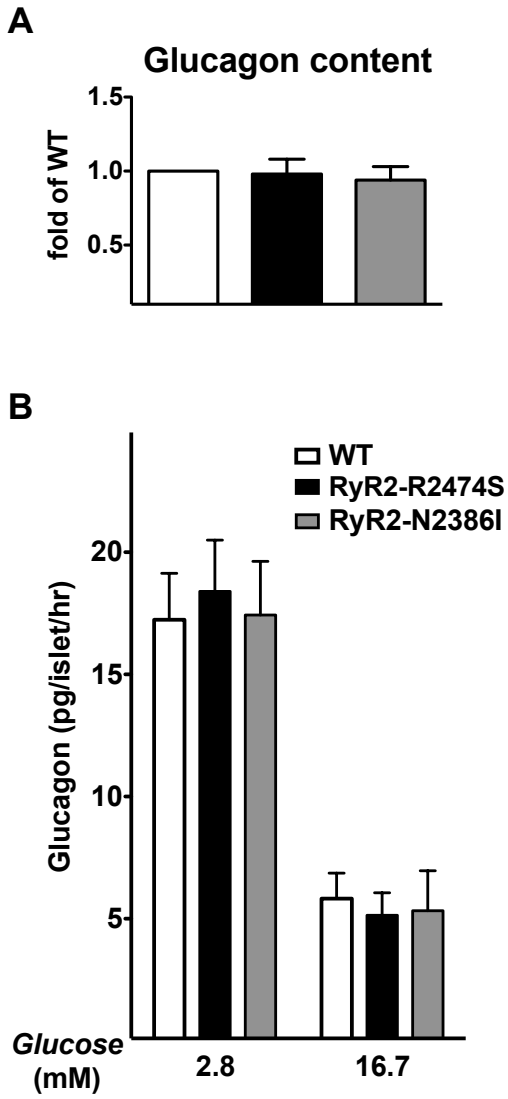


**Figure S4**. **Glucagon content and secretion in WT and CPVT islets.** Glucagon content (**A**) and secretion (**B**) in intact islets isolated from WT, RyR2-R2474S, and RyR2-N2386I mice. Data are shown as mean±s.e.m. from at least 6 different animals/group.

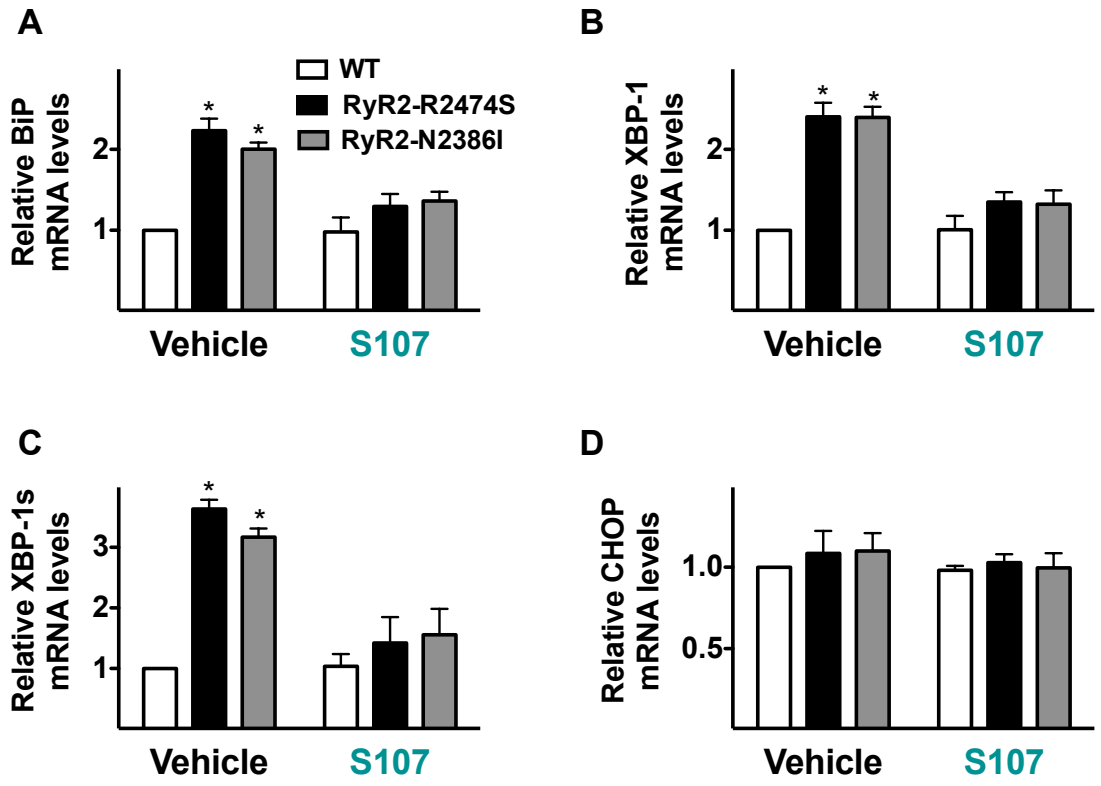


**Figure S5**. **Activation of ER stress response in CPVT islets.** The mRNA expression of BiP (**A**), total (unspliced) and spliced XBP-1 (**B**, **C**), and CHOP (**D**) was determined in murine WT and CPVT islets by real-time RT-qPCR analysis of total RNA, using β actin as internal standard. Primer sequences are reported in Supplementary Table 3. Each bar represents mean±s.e.m. of four independent experiments in each of which reactions were performed in triplicate. *:$P<0.05$ vs WT; ANOVA, Tukey-Kramer *post hoc* test.

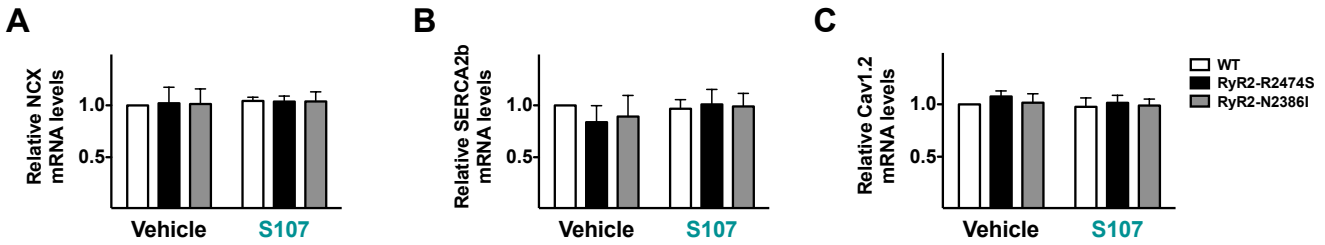


**Figure S6**. **Expression of $Ca^{2+}$ handling proteins in pancreatic islets of Langerhans isolated from CPVT mice.** The mRNA expression of NCX (**A**), SERCA2b (**B**), and L-type Cav1.2 $Ca^{2+}$ channel (**C**) was determined in murine WT and CPVT islets by real-time RT-qPCR analysis of total RNA, using β actin as internal standard. Primer sequences are reported in Supplementary Table 3. Each bar represents mean±s.e.m. of three independent experiments in each of which reactions were performed in triplicate.

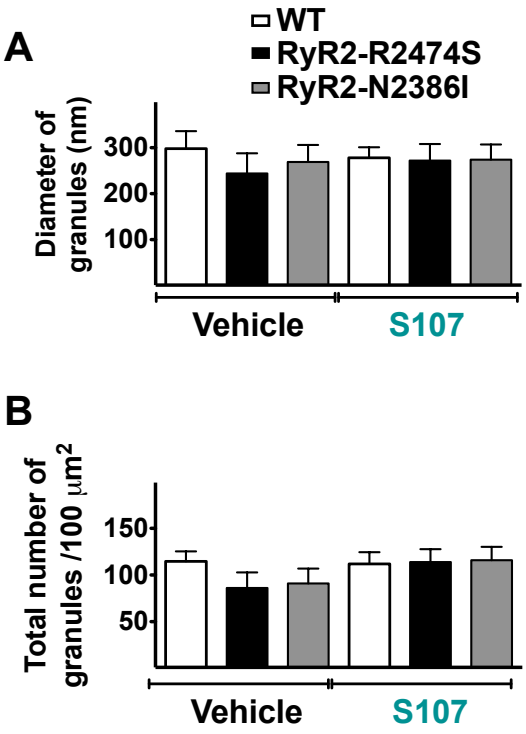


**Figure S7**. **Quantification of the ultrastructural analyses of insulin granules in pancreatic β-cells.** Diameter (**A**) and total number (**B**) of insulin secretory granules at the electron microscope analysis of murine WT and CPVT β-cells (representative pictures are shown in **Fig. 3A**). All data are shown as mean±s.e.m.

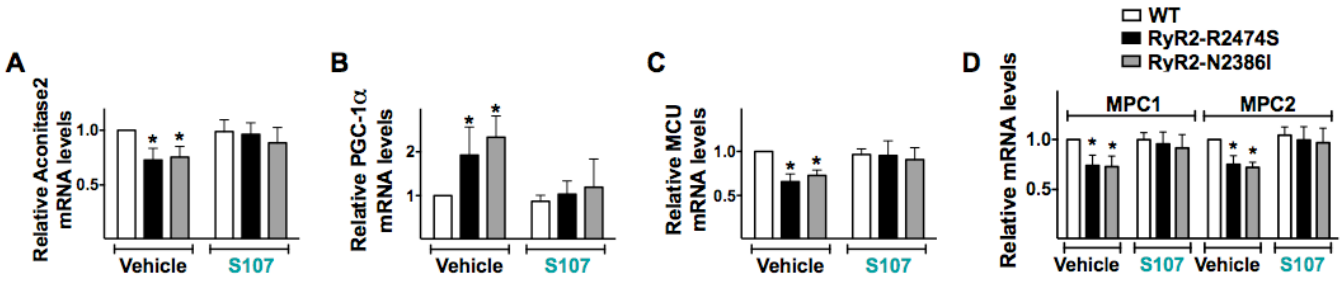


**Figure S8**. **Expression of markers of mitochondrial dysfunction in pancreatic islets of Langerhans isolated from CPVT mice.** The mRNA expression of aconitase2 (**A**), PGC-1α (**B**), MCU (**C**), and MPC1/2 (**D**) was determined in murine WT and CPVT islets by real-time RT-qPCR analysis of total RNA, using β actin as internal standard. Primer sequences are reported in Supplementary Table 3. Each bar represents mean±s.e.m. of three independent experiments in each of which reactions were performed in triplicate. *:$P<0.05$ vs WT; ANOVA, Tukey-Kramer *post hoc* test.

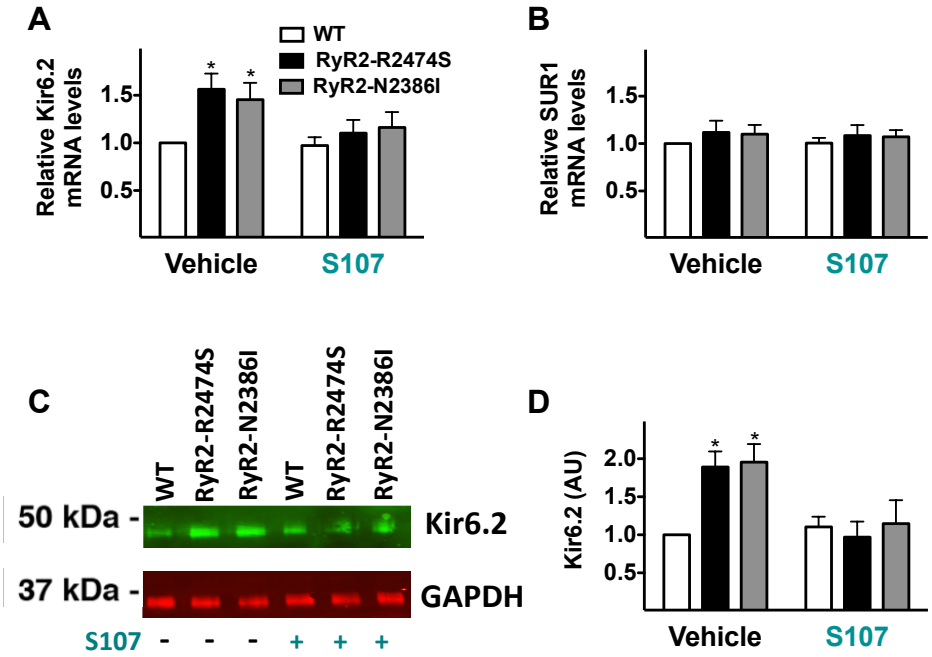


**Figure S9. Increased $K_{ATP}$ channel expression in pancreatic islets from CPVT mice.** (**A-B**) Kir6.2 (**A**) and SUR1 (**B**) mRNA levels determined in murine WT and CPVT islets by real-time RT-qPCR analysis of total RNA, using β actin as internal standard. Primer sequences are reported in Supplementary Table 3. Mice were treated with vehicle or S107. Each bar represents mean±s.e.m. of three independent experiments in each of which reactions were performed in triplicate. (**C**) Representative immunoblots of pancreatic islets from the indicated groups of mice treated or not with S107 (50 mg/kg/d, 4 weeks) and (**D**) relative quantification (triplicate experiments); AU: arbitrary units; *:$P<0.05$ vs WT vehicle; ANOVA, Tukey-Kramer *post hoc* test.

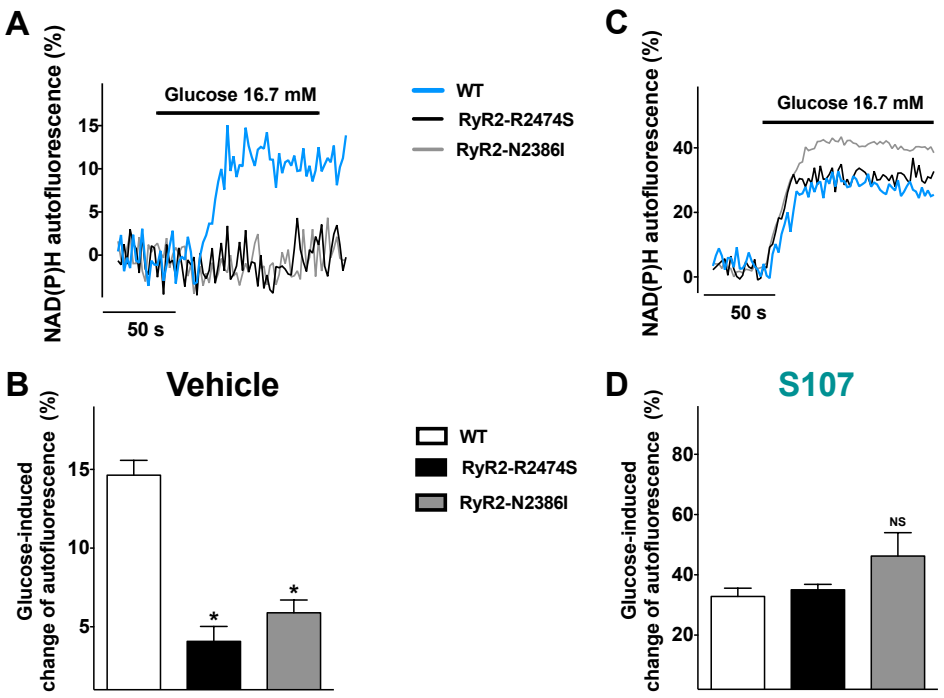


**Figure S10**. **Blunted glucose-induced NAD(P)H autofluorescence in CPVT mice.** (**A-D**) Evaluation of NAD(P)H autofluorescence in response to glucose dispersed cells from islets isolated from WT and CPVT mice treated with vehicle (**A**, **B**) or with S107 (50 mg/kg/d, 4 weeks, **C**, **D**). In **A** and **C** representative traces are depicted. 7-9 mice per group were used. Data are shown as mean±s.e.m. *:$P<0.05$ vs WT; ANOVA, Tukey-Kramer *post hoc* test. Additional details are given in Methods.

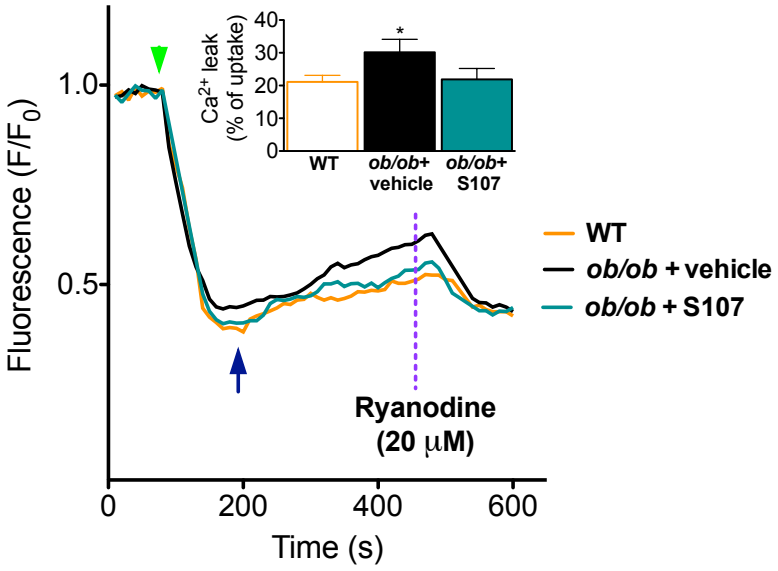


**Figure S11**. **Increased ER $Ca^{2+}$ leak in pancreatic islet microsomes from *ob/ob* mice is reduced by oral treatment with S107 to fix the RyR leak.** Representative traces of ER $Ca^{2+}$ leak from pancreatic islet microsomes isolated from *ob/ob* mice treated with vehicle or S107 for four weeks (50 mg/kg/d in drinking water). The green arrowhead represents the addition of ATP; the blue arrow is thapsigargin (see Methods for details). The bar graph in the inset is the quantification of $Ca^{2+}$ leak (from triplicate experiments). **P*<0.05 vs WT, ANOVA. Dashed line indicates the addition of ryanodine.

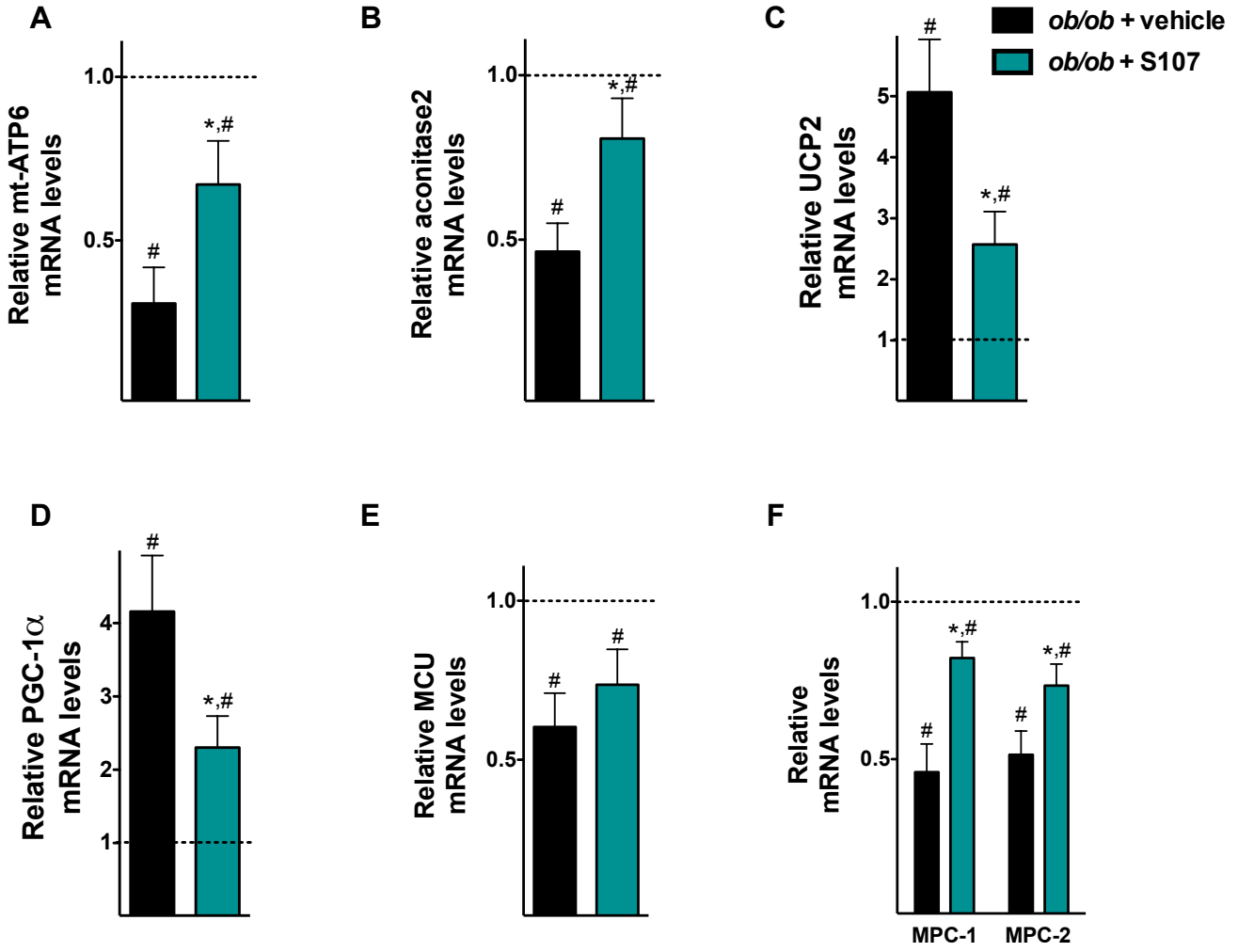


**Figure S12**. **Effects of chronic S107 treatment on markers of mitochondrial dysfunction in pancreatic islets of Langerhans isolated from *ob/ob* mice.** The mRNA expression of mt-ATP6 (**A**), aconitase 2 (**B**), UCP2 (**C**), PGC-1α (**D**), MCU (**E**), and MPC1/2 (**F**) in *ob/ob* mice treated or not with S107 (50 mg/kg/d, 4 weeks) was determined by real-time RT-qPCR analysis of total RNA, relative to untreated WT mice (horizontal dashed lines), using β actin as internal standard. 7-9 animals per group were used. Primer sequences are reported in Supplementary Table 3. Each bar represents mean±s.e.m. of three independent experiments, in each of which reactions were performed in triplicate. *:$P<0.05$ vs *ob/ob*+vehicle; #:$P<0.05$ vs WT; 2 tailed Student's *t* test.

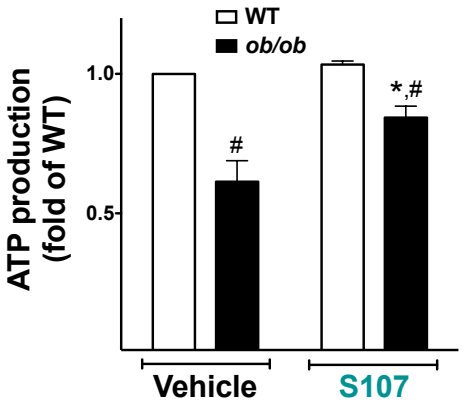


**Figure S13**. **Reduced ATP production in *ob/ob* islets is ameliorated by S107.** Chronic S107 treatment of *ob/ob* mice (50 mg/kg/d, 4 weeks) improved ATP production in response to methyl pyruvate (10 mM) in isolated islets of Langerhans. Each bars represents mean±s.e.m. from at least 6-8 animals/group. *:$P<0.05$ vs *ob/ob*+vehicle; #:$P<0.05$ vs WT; 2 tailed Student's *t* test.

**Supplementary Table 1. Baseline clinical characteristics of control and CPVT subjects.**

| | Control (n=27) | CPVT (n=27) |
|---|---|---|
| **Age (years)** | 27.2±4.1 | 26.9±3.7 |
| **Male (%)** | 59.25 | 62.9 |
| **BMI (kg/m$^2$)** | 23.6±2.4 | 21.7±2.9 |
| **Fasting blood glucose (mg/dl)** | 94.4±8.1 | 92.2±7.4 |
| **Fasting serum insulin (μU/ml)** | 7.76±2.11 | 7.93±2.29 |
| **Waist circumference (cm)** | 41.6±5.2 | 42.1±5.4 |
| **Waist-to-hip ratio** | 0.93±0.08 | 0.90±0.11 |
| **Systolic blood pressure (mmHg)** | 126.6±9.2 | 128.3±8.8 |
| **Diastolic blood pressure (mmHg)** | 75.8±3.6 | 72.4±3.8 |
| **Triglycerides (mg/dl)** | 121.8±32.5 | 118.1±29.8 |
| **Total cholesterol (mg/dl)** | 184.4±38.4 | 186.1±41.2 |
| **HDL cholesterol (mg/dl)** | 47.6±12.7 | 48.2±14.1 |
| **LDL cholesterol (mg/dl)** | 112.1±27.7 | 114.3±28.1 |
| **Creatinine (mg/dl)** | 0.86±0.18 | 0.91±0.24 |
| **Blood urea nitrogen (mg/dl)** | 11.4±3.1 | 10.8±3.2 |
| **Current cigarette smokers (%)** | 18.75 | 12.5 |
| **Familial sudden cardiac death (%)** | - | 40.7 |
| **Implanted cardioverter defibrillator (%)** | - | 81.48 |

Clinical characteristics of the subjects, including body mass index (BMI) high-density (HDL) and low-density (LDL) lipoprotein cholesterol. CPVT stand for Catecholaminergic Polymorphic Ventricular Tachycardia. Values of serum insulin were available in 19/27 individuals per group. Data are expressed as mean±s.e.m. or percentage.

**Supplementary Table 2. RyR2 mutations in CPVT patients.**

| # Patient | RyR2 Mutation | Blood glucose (mg/dl) 2h after OGTT |
|---|---|---|
| **#1** | P2404T | 187 |
| **#2** | K4392R | 147 |
| **#3** | L2534V | 192 |
| **#4** | T2510A | 188 |
| **#5** | R169Q | 166 |
| **#6** | R2401L | 188 |
| **#7** | R4959Q | 131 |
| **#8** | R2474S | 199 |
| **#9** | V2475F | 191 |
| **#10** | P2328S | 196 |
| **#11** | R169Q | 172 |
| **#12** | R2401H | 174 |
| **#13** | Q4201R | 133 |
| **#14** | H4108N | 122 |
| **#15** | I4587V | 135 |
| **#16** | E2296Q | 191 |
| **#17** | R1051P | 189 |
| **#18** | H4762P | 194 |
| **#19** | N2386I | 198 |
| **#20** | N1551S | 144 |
| **#21** | G4671R | 189 |
| **#22** | A2387P | 185 |
| **#23** | S4565R | 145 |
| **#24** | V2113M | 188 |
| **#25** | E1724K | 186 |
| **#26** | F2307L | 193 |
| **#27** | N1551S | 192 |

**Supplementary Table 3**. **Primer sequences.**

| Gene | Forward 5'-3' | Reverse 5'-3' | Product size (bp) |
|---|---|---|---|
| **Human RyR1** | TTCCCCAAGATGGTGACAAG | CTCCAGCAGGTAGCTCAGGT | 105 |
| **Human RyR2** | CCTTGCCTGAGTGCAGTTG | TTGAGGTATCAACAGGTTGTGG | 130 |
| **Human RyR3** | ATCAACACGCCATCTTTTCC | TTGTCCAAACCCAGGAGTTC | 119 |
| **Human β-Actin** | CTCTTCCAGCCTTCCTTCCT | AGCACTGTGTTGGCGTACAG | 116 |
| **Mouse RyR1** | CCTTGCCTGAGTGCAGTTG | TTGAGGTATCAACAGGTTGTGG | 130 |
| **Mouse RyR2** | TTCCCCAAGATGGTGACAAG | CTCCAGCAGGTAGCTCAGGT | 105 |
| **Mouse RyR3** | AGATCGAGCTGCTGAAGGAA | CCTTCCAGGAGGGACAGAAG | 73 |
| **Mouse Kir6.2** | GTAGGGGACCTCCGAAAGAG | CTGGTGGAGAGGCACAACTT | 102 |
| **Mouse SUR1** | GAACAGGCAAGACCAAGAGC | CAGAAGGCCATCTCTTGAGG | 106 |
| **Mouse CaV1.2** | CAGCCCAGAAAAGAAACAGG | GGATTCTCCATCGGCTGTAA | 100 |
| **Mouse NCX** | AGATCAAGCATCTGCGTGTG | CTCCACAACTCCAGGAGAGC | 104 |
| **Mouse SERCA2b** | AGGGACTGCAGTGGCTAAGA | GCCACAATGGTGGAGAAGTT | 69 |
| **Mouse UCP2** | AGCCTTCTGCACTCCTGTGT | TAGAAAATGGCTGGGAGACG | 82 |
| **Mouse Aconitase2** | CAGGTGCCAAGTATGGTGTG | GCCGATCAGAAGAACTCCAG | 101 |
| **Mouse PGC1-α** | CCGAGAATTCATGGAGCAAT | TTTCTGTGGGTTTGGTGTGA | 129 |
| **Mouse mt-ATP6** | CAACCGTCTCCATTCTTTCC | CGTCCTTTTGGTGTGTGGAT | 81 |
| **Mouse MPC1** | ACTTTCGCCCTCTGTTGCTA | ACATGGCATGCAAACAAAAG | 89 |
| **Mouse MPC2** | AATTGAGGCCGCTTTACAAC | GCTAGTCCAGCACACACCAA | 94 |
| **Mouse MCU** | TCATGGAATCCAAGTGCAAA | AGCAAACACCCAACATCCTC | 109 |
| **Mouse Bip** | TGCAGCAGGACATCAAGTTC | TTTCTTCTGGGGCAAATGTC | 111 |
| **Mouse unspliced XBP-1** | TCAAATGTCCTTCCCCAGAG | AAAGGGAGGCTGGTAAGGAA | 87 |
| **Mouse spliced XBP-1** | CTGAGTCCGCAGCAGGTG | GGCAACAGTGTCAGAGTCCA | 74 |
| **Mouse CHOP** | CTGCACCAAGCATGAACAGT | CTACCCTCAGTCCCCTCCTC | 131 |
| **Mouse β-Actin** | GCTCTTTTCCAGCCTTCCTT | GCCTCAGGAGTTTTGTCACC | 78 |
| **Mouse H19** | GTACCCACCTGTCGTCC | GTCCACGAGACCAATGACTG | 207 |
| **Mouse 18S** | CGCGGTTCTATTTTGTTGGT | AGTCGGCATCGTTTATGGTC | 219 |
| **Mouse mt12S** | ACCGCGGTCATACGATTAAC | CCCAGTTTGGGTCTTAGCTG | 178 |
| **Mouse mt16S** | CCTTGTTCCCAGAGGTTCAA | ATGCCGTATGGACCAACAAT | 169 |

RyR: Ryanodine Receptor/$Ca^{2+}$ release channel; Kir: Potassium inwardly-rectifying channel; SUR: sulfonylurea receptor; Cav1.2: $Ca^{2+}$ channel voltage-dependent, L-type, alpha1C subunit; NCX: $Na^{+}/Ca^{2+}$ exchanger; SERCA: Sarco/Endoplasmic Reticulum $Ca^{2+}$-ATPase; UCP2: Uncoupling Protein 2; PGC1-α: Peroxisome proliferator-activated receptor gamma (PPARγ)-coactivator 1-alpha; mt-ATPs: mitochondrially encoded ATP synthase 6; MPC: Mitochondrial Pyruvate Carrier; MCU: Mitochondrial $Ca^{2+}$ Uniporter; BiP: immunoglobin heavy-chain binding protein; XBP-1: X-box binding protein 1; CHOP: CCAAT/enhancer-binding protein homologous protein.

**Supplementary Table 4**. **Characteristics of human pancreatic islet donors.**

| | Age | BMI | T2DM |
|---|---|---|---|
| **#1** | 55 | 24 | No |
| **#2** | 41 | 35.5 | No |
| **#3** | 51 | 17.3 | No |
| **#4** | 32 | 28.2 | Yes |
| **#5** | 53 | 27.1 | Yes |
| **#6** | 42 | 30.2 | Yes |

BMI: Body-mass index; T2DM: Type 2 Diabetes Mellitus.

**Supplementary Table 5**. **Baseline characteristics of WT and CPVT mice (4-month-old).**

| | WT | RyR2-R2474S | RyR2-N2386I |
|---|---|---|---|
| **Body weight (g)** | 31.5±1.6 | 30.9±1.7 | 31.1±1.9 |
| **Food intake (g/day)** | 3.4±0.9 | 3.4±0.8 | 3.3±0.9 |
| **Water intake (ml/day)** | 5.9±0.8 | 6.3±1.1 | 6.2±1.1 |
| **Fasting blood glucose (mg/dl)** | 95.33±15.1 | 98.4±17.7 | 97.8±18.2 |
| **Fasting serum insulin (ng/dl)** | 0.46±0.06 | 0.41±0.07 | 0.43±0.07 |
| **Triglycerides (mM/l)** | 0.39±0.05 | 0.38±0.06 | 0.41±0.05 |
| **Free fatty acids (mM/l)** | 0.68±0.12 | 0.71±0.11 | 0.65±0.14 |
| **Mean arterial blood pressure (mmHg)** | 103.4±9.6 | 104.1±10.8 | 103.7±11.2 |

Data are expressed as mean±s.e.m from at least 6 mice per group.