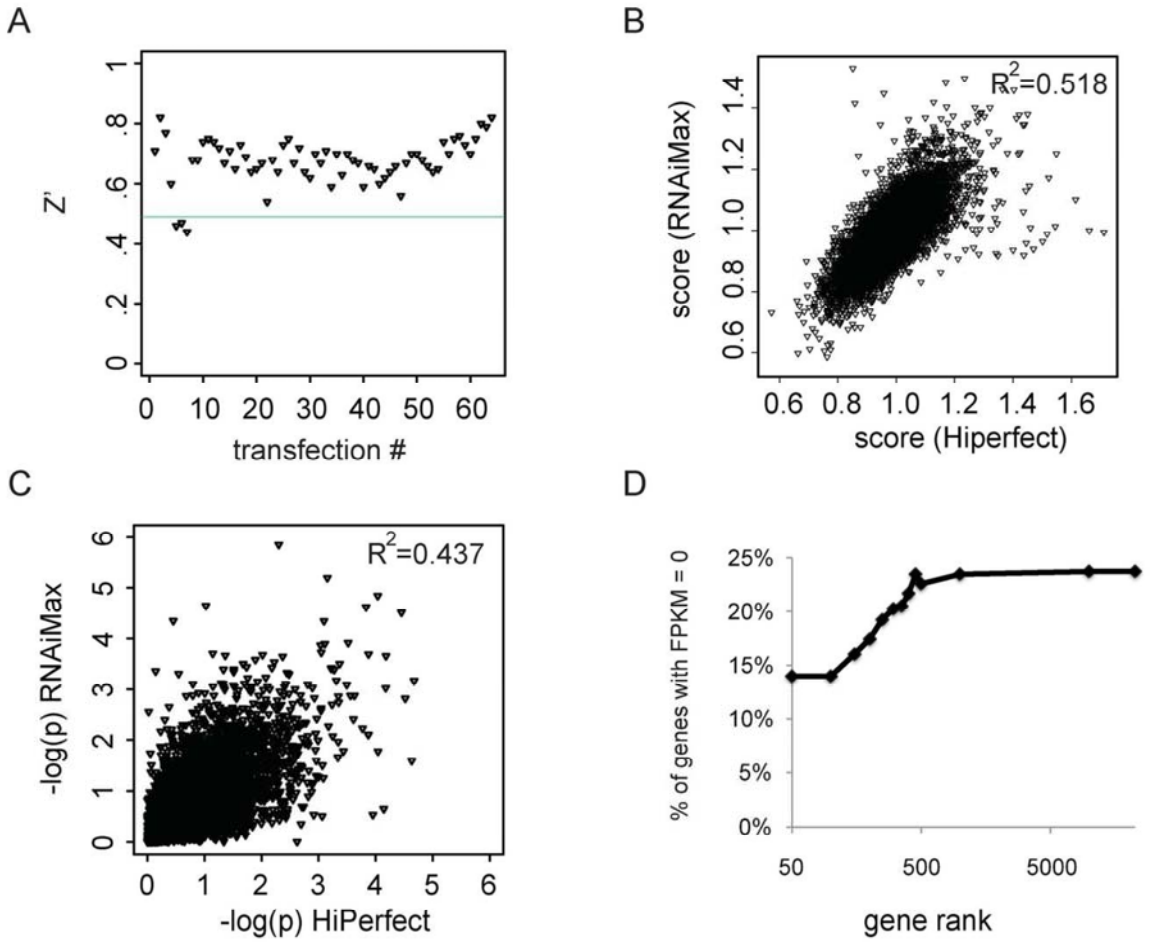
SUPPLEMENTARY DATA

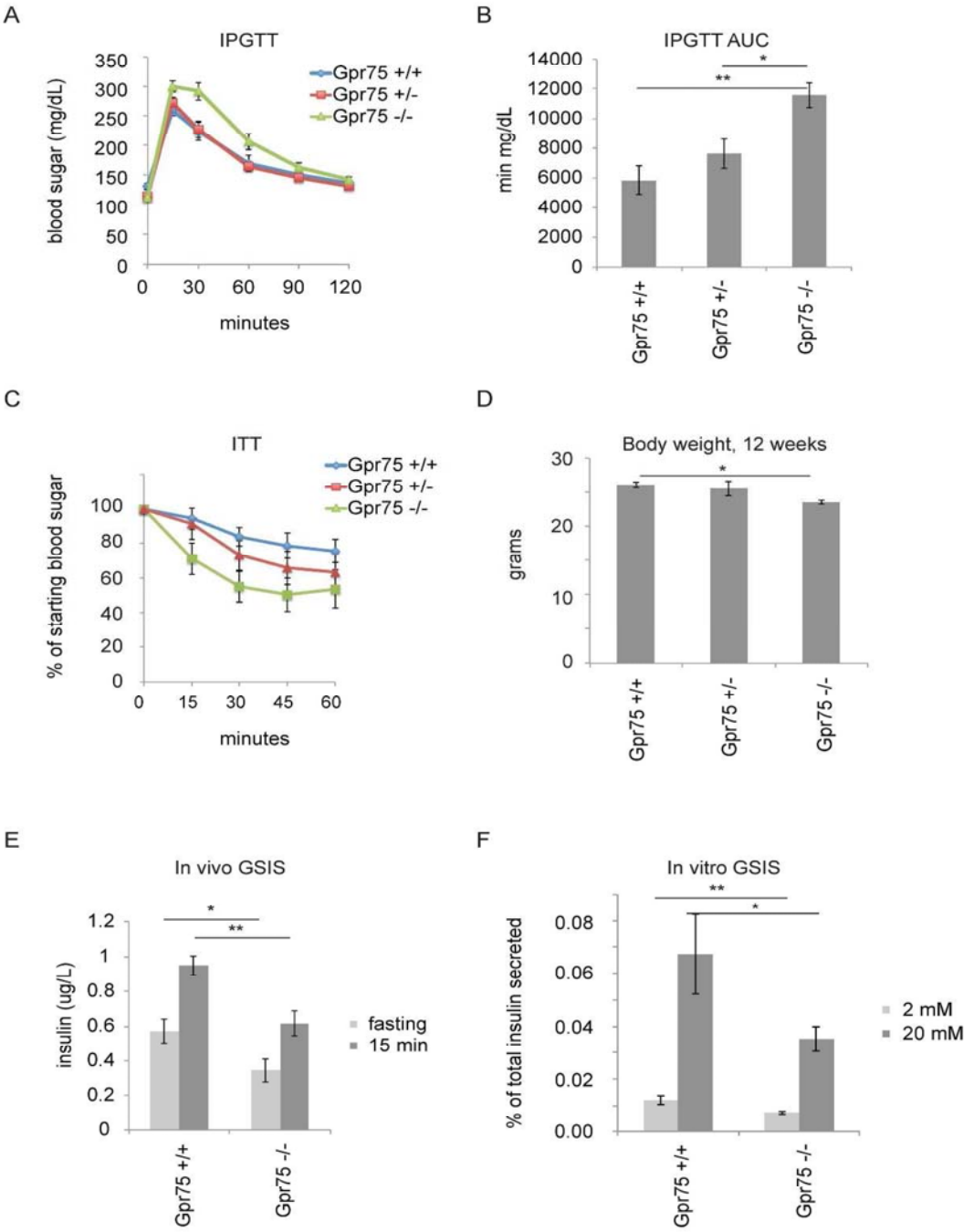
Supplementary Figure 1. Reproducibility of 384-well fluorescence screening assay. (A) Z' of each transfection performed in the primary screen. The green line indicates a Z' of 0.5, an acceptable value. Z' was calculated by comparing anti-Luciferase siRNA to anti-GFP siRNA. (B) Correlation plot of raw scores between siRNAs when transfected with two different transfection reagents, RNAiMax (Life Technologies) and Hiperfect (Qiagen). (C) Correlation plot of p values between screens performed with two different transfection reagents. (D) Fraction of genes that have FPKM = 0 in the screening cell line from rank 1 to the RSA ranked hit number indicated on the x-axis.



SUPPLEMENTARY DATA

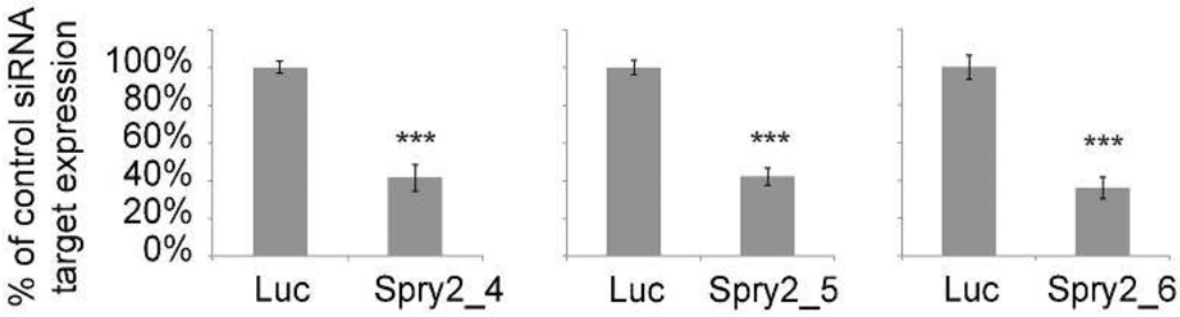
Supplementary Figure 2. *Gpr75* global knockout mice have reduced beta cell function. (A) Intraperitoneal glucose tolerance test of mice of the indicated genotype at 9 weeks of age. $p<0.05$ by ANOVA with repeated measures between wild type and knockout (n=9 wild type, n=12 heterozygous, n=7 knockout). (B) Area under the curve for data in A. (C) Intraperitoneal insulin tolerance testing (n=9 wild type, n=12 heterozygous, n=7 knockout). (D) Body weight at 12 weeks (n=9 wild type, n=12 heterozygous, n=7 knockout). (E) Plasma insulin level either fasting or 15 minutes after glucose injection. (n=6 wild type, n=7 knockout). (F) *In vitro* glucose stimulated insulin secretion of islets from mice of the indicated genotypes (n=3-5 mice per genotype with 1-3 technical replicates per mouse). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

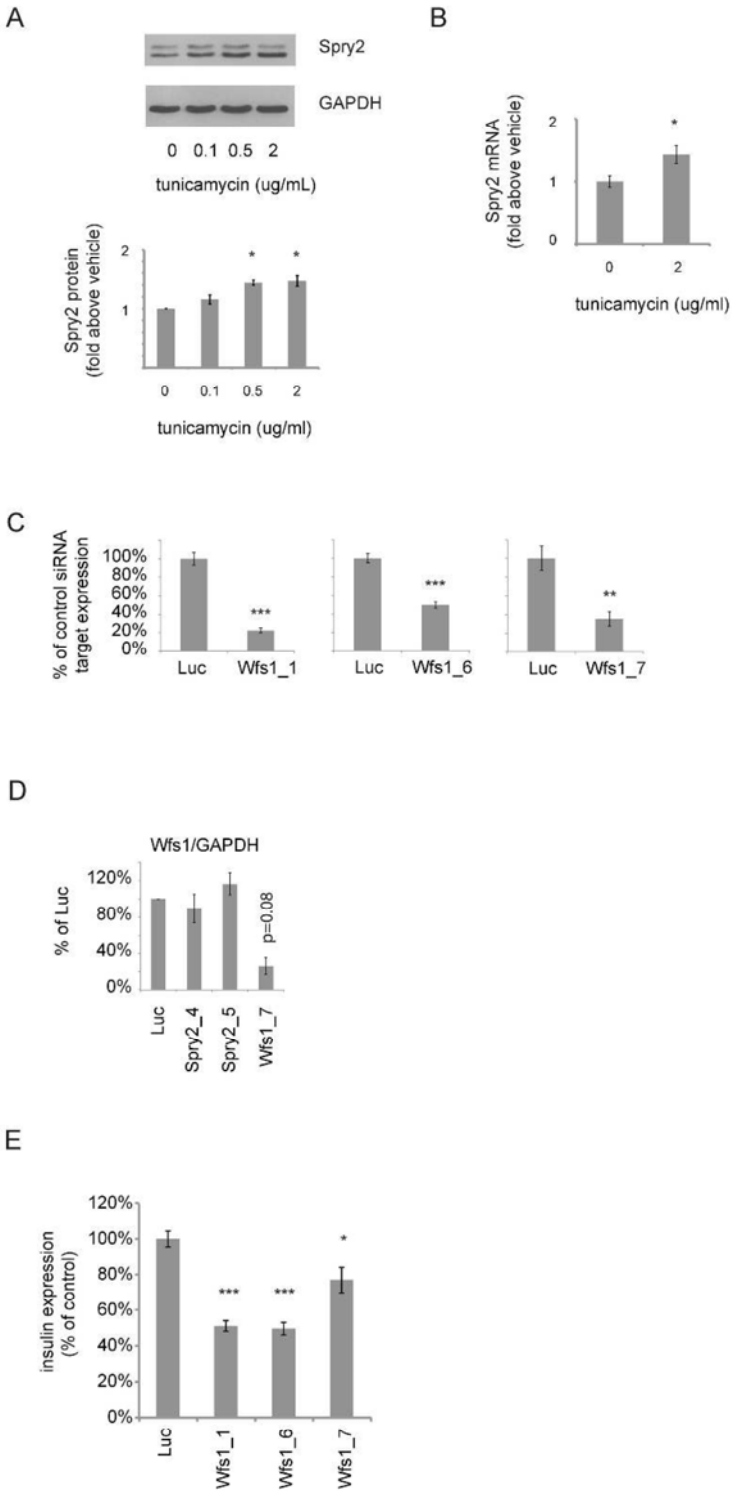
Supplementary Figure 3. Knockdown of *Spry2* mRNA by siRNAs. (A) qRT-PCR of *Spry2* mRNA after transfection with the indicated siRNAs (n=6). *** p<0.001.



SUPPLEMENTARY DATA

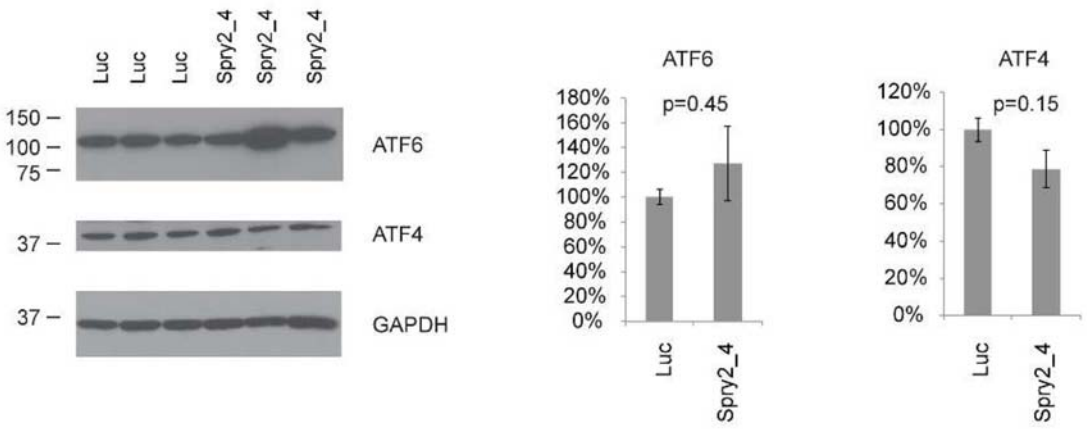
Supplementary Figure 4. *Spry2* is a UPR target gene. (A) MIN6 cells were treated with the indicated concentrations of tunicamycin for 12 hours and lysates were analyzed for Spry2 expression by western blot (n=4). (B) As in A, but quantitation of Spry2 mRNA is plotted. (C) qRT-PCR knockdown of *Wfs1* mRNA after transfection with the indicated siRNAs (n=6). (D) Quantitation of Wfs1 protein expression normalized to GAPDH from MIN6 cells transfected with the indicated siRNAs (n=3-6). (E) The indicated Wfs1 siRNAs were transfected into MIN6 cells and pre-ins1 was measured by RT-qPCR. *p<0.05, **p<0.01, ***p<0.001.

SUPPLEMENTARY DATA



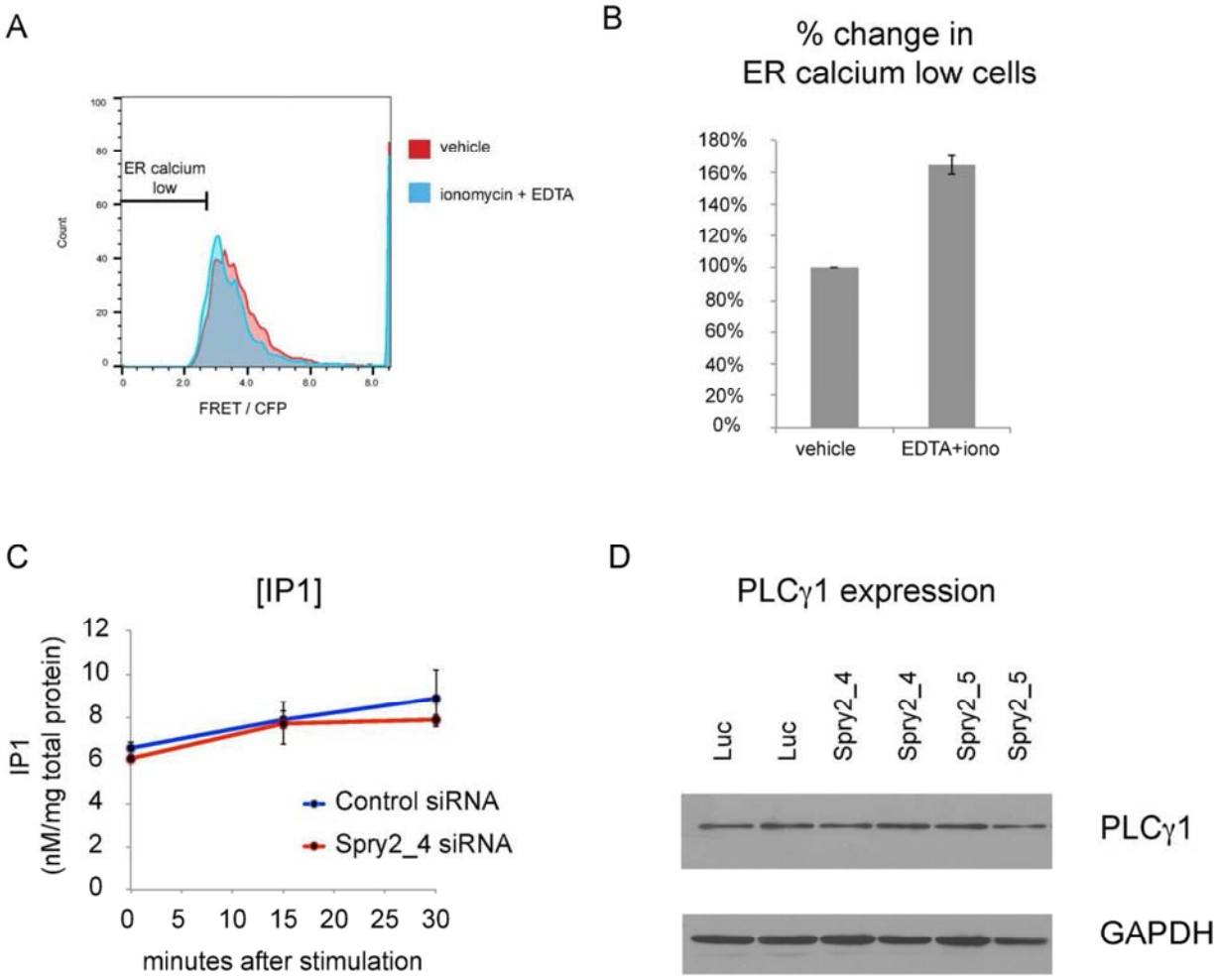
SUPPLEMENTARY DATA

Supplementary Figure 5. ATF4 and ATF6 are not affected by Spry2 knockdown. ATF4 and ATF6 protein levels in MIN6 cells transfected with the indicated siRNAs (n=3).



SUPPLEMENTARY DATA

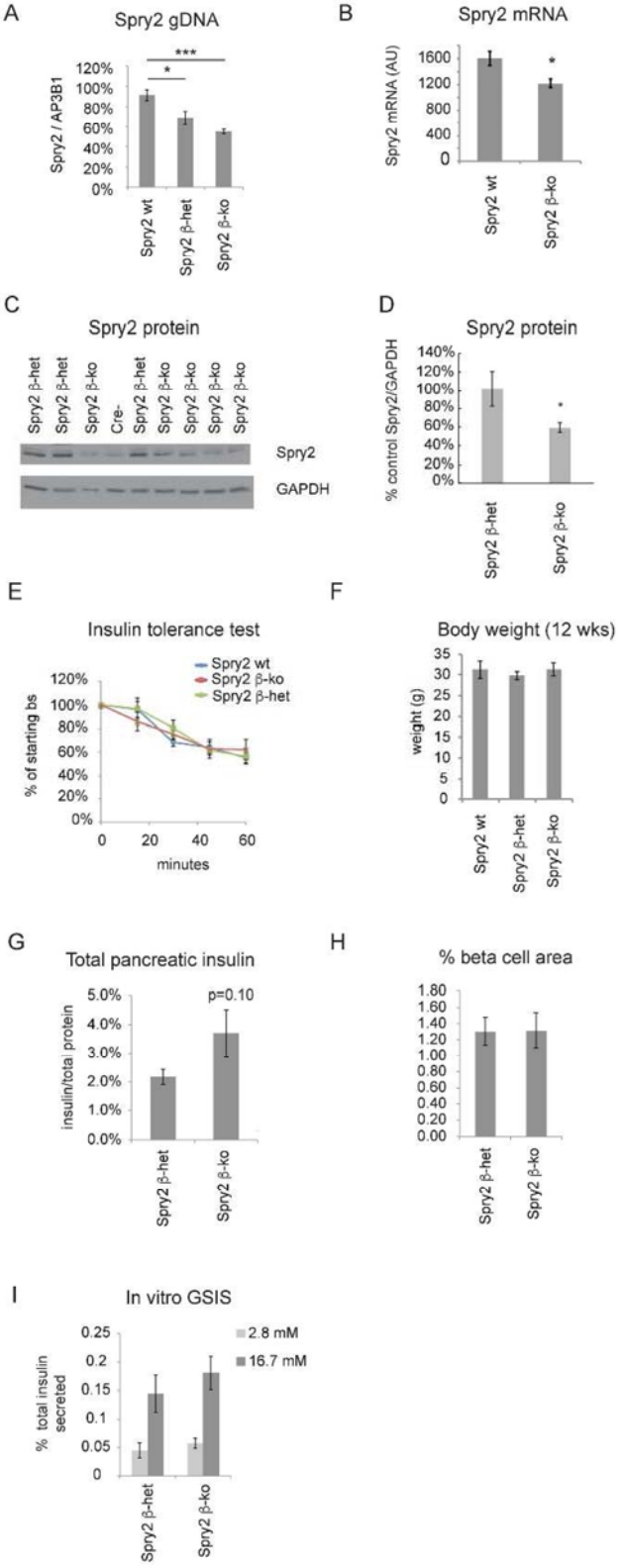
Supplementary Figure 6. Testing of D1ER MIN6 line and IP1 levels after *Spry2* knockdown. (A) MIN6 cells expressing the D1ER construct by lentivirus insertion were trypinized and resuspended in Hank's Buffered Saline Solution without calcium or magnesium. Cells were incubated with vehicle or 5 mM EDTA and 2 □M ionomycin at 37 degrees C for 15 minutes before FACS analysis. Plot of YFP emission with CFP excitation divided by CFP emission with CFP excitation. "ER calcium low" indicates the gating to identify low ER calcium cells. (B) Percent change in FRET low cells after ionomycin and EDTA treatment (n=3). (C) IP1 levels measured at indicated time points after shift from 2 mM to 20 mM glucose (n=3). (D) PLC□1 expression in MIN6 cells after transfection with the indicated siRNAs (n=3).



SUPPLEMENTARY DATA

Supplementary Figure 7. Beta cell specific deletion of Spry2 by MIP-Cre-ERT2. (A) The ratio of *Spry2* to *AP3B1* (a control locus) was measured from genomic DNA from isolated islets of mice of the indicated genotypes (n=7 wt, 4 □-het, 8 □-ko). The QPCR probe target sequence for *Spry2* is deleted by the Cre induced recombination at the *Spry2* locus. (B) Islets were isolated from the mice of the indicated genotype and RT-qPCR was performed for *Spry2 mRNA* (n=4-6 per genotype). (C) Islets were isolated from mice of the indicated genotypes and western blots were performed for the either Spry2 or GAPDH. (D) Quantitation by genotype of C. (E) Insulin tolerance testing of the indicated mice at 13 weeks of age (n=5 *Spry2* wt, 8 □-het, 5 □-ko). (F) Body weight at 12 weeks of mice of the indicated genotypes (n=7 wt, 11 □-het, 13 □-ko). (G) Total pancreatic insulin from mice of the indicated genotypes at 15-17 weeks of age (n=4 *Spry2* □-het, 3 Spry2 □-flox). (H) % beta cell area from mice of the indicated genotypes at 15-17 weeks of age (n=4 *Spry2* □-het, 4 Spry2 □-flox). (I) In vitro glucose stimulated insulin secretion from islets of the indicated genotypes at 15-17 weeks of age. Secretion shown as the percent of total insulin content. (n=2 *Spry2* □-het, 6 Spry2 □-flox, with 3 technical replicates per mouse)* $p<0.05$ by student's T-test.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 8. Additional UPR markers in Spry2 knockdown or knockout cells. (A) RT-qPCR for the indicated genes was performed on islets at 16-18 weeks of age. For CHOP, islets were harvested for RNA immediately after isolation. For the others, RNA was harvested 24 hours after isolation. (B) ATF4 protein levels from islets of the indicated genotypes at 16-18 weeks of age. (C) Fasting proinsulin levels from mice of the indicated genotypes at age 16-18 weeks of age (n=4 Spry2 wt and □-het, 7 Spry2 □-flox).