SUPPLEMENTARY DATA

Cell culture

Biopsy plating medium contained DMEM, FBS, GlutaMAX, Anti-Anti, NEAA, 2-Mercaptoethanol and nucleosides and culture medium was composed of DMEM, FBS, GlutaMAX and Pen-Strep (all from Invitrogen). For generation of induced pluripotent stem cells using Sendai viruses, 50,000 fibroblast cells were seeded in a well of six-well dish at passage three in fibroblast medium. Next day, Sendai viruses expressing human transcription factors Oct4, Sox2, Klf4 and C-Myc were mixed in fibroblast medium to infect fibroblast cells according to the manufacturer's instructions. 2 days later, the medium was exchanged to human ES medium supplemented by the MEK inhibitor PD0325901 (0.5 μM; Stemgent), ALK5 inhibitor SB431542 (2 μM; Stemgent), and thiazovivin (0.5 μM; Stemgent). Each iPS cell line was expanded from a single colony and cultured on MEF feeder cells with human ES medium. Human ES medium contained the following: KO-DMEM, KSR, GlutaMAX, NEAA, 2-Mercaptoethanol, PenStrep and bFGF (all from Invitrogen). Karyotyping of the cells was performed by Cell Line Genetics Inc. (Wisconsin).

Spontaneous differentiation of iPS cells

To generate embryoid bodies, 1-2 million iPS cells of each line were detached by TrypLE (Invitrogen) treatment and cultured in a low-attachment 6-well culture dish for 3 weeks. Cells assumed a spherical shape and were collected for immunostaining. For teratoma analysis, 1-2 million cells of each iPS cell line were collected and injected subcutaneously into dorsal flanks of a NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}*/SzJ* (NSG) mouse (Stock No. 005557, The Jackson Laboratory). 8-12 weeks after injection, teratomas were collected, fixed overnight with 4% paraformaldehyde and processed for paraffin embedding according to standard procedures. Then the samples were sectioned and HE (hematoxylin and eosin) stained.

Immunohistochemistry and microscopy

Cells and tissues were fixed by 4% paraformaldehyde. Tissues were embedded in OCT or paraffin and sectioned at 10 μm. Cells or sections were blocked in 5% normal donkey serum and stained with primary antibodies: mouse-anti-SSEA4 (MAB1435; R&D systems), rabbit-anti-SOX2 (09-0024; stemgent), mouse-anti-TRA1-60 (MAB4360; Millipore), goat-anti-NANOG (AF1997; R&D systems), mouse-anti-TRA1-81 (MAB4381; Millipore), mouse-anti-OCT4 (sc-5279; Santa Cruz Biotechnology), rabbit-anti-AFP (A000829; DAKO), mouse-anti-SMA (A7607; Sigma), rabbit-anti-TUJ1 (T3952; Sigma), goat-anti-SOX17 (AF1924; R&D systems), goat-anti-PDX1 (AF2419; R&D systems), mouse-anti-C-peptide (05-1109; Millipore), rabbit-anti-glucagon (A056501; DAKO), mouse-anti-ATF6α (sc-166659, Santa Cruz), rabbit-anti-Urocortin 3 (HPA038281, Sigma), Ddit3 (ab11419, Abcam). Anti-WFS1 antibody was generously provided by Dr. Urano, Fumihiko (Washington University School of Medicine). Secondary antibodies were obtained from Molecular Probes (Invitrogen). For fluorescence-activated cell sorting (FACS) analysis, cells were collected and fixed in 4% PFA for 15 min at room temperature and analyzed using a FACSAriaII. Images were acquired with an Olympus IX71 fluorescence microscope and confocal microscope (Zeiss LSM5 PASCAL).

SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences used in this study.

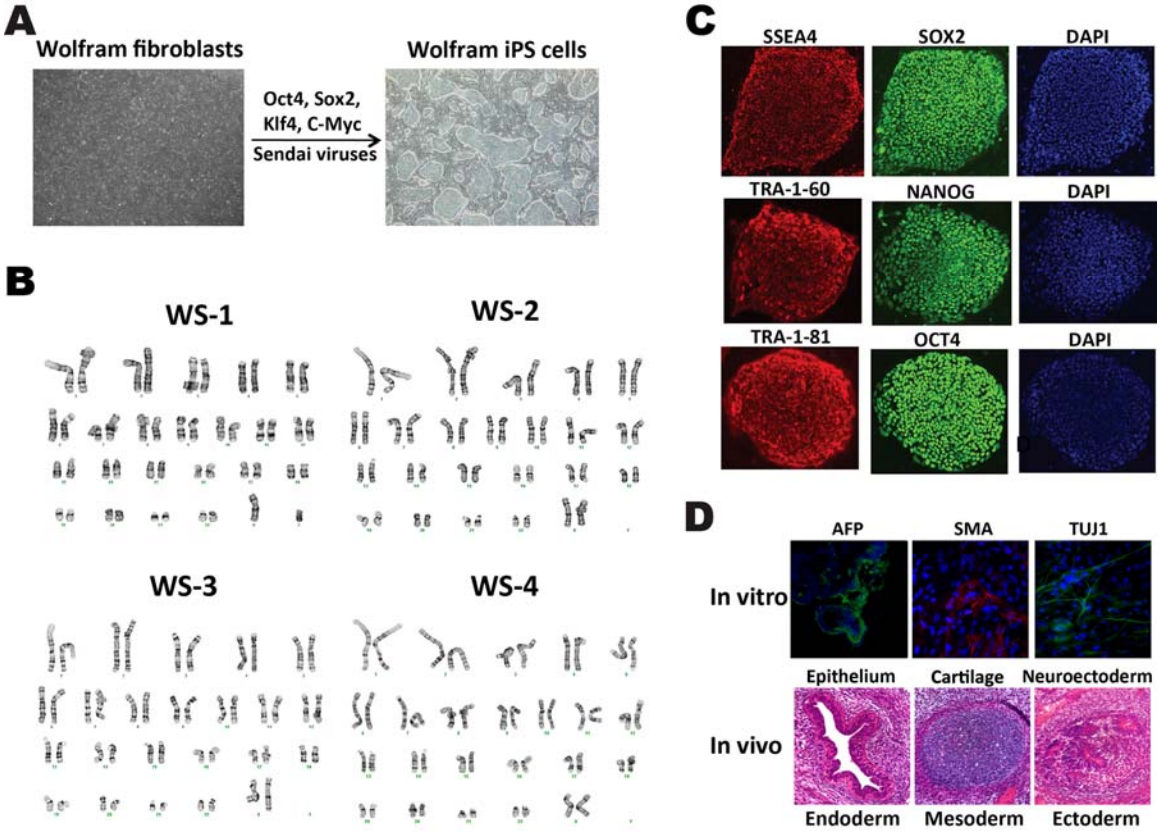
Gene	Primers
XBP-1 (for gel imaging)	forward 5' GAAGCCAAGGGGAATGAAGT 3'
	reverse 5' GGGAAGGGCATTTGAAGAAC 3'
Spliced-XBP-1 (for QPCR)	forward 5' CTGAGTCCGCAGCAGGTG 3'
	reverse 5' TGCCCAACAGGATATCAGACT 3'
ATF4	forward 5' GACCGAAATGAGCTTCCTGA 3'
	reverse 5' ACCCATGAGGTTTGAAGTGC 3'
GRP78	forward 5' CACAGTGGTGCCTACCAAGA 3'
	reverse 5' TGATTGTCTTTTGTCAGGGGT 3'
Insulin	forward 5' TTCTACACACCCAAGACCCG 3'
	reverse 5' CAATGCCACGCTTCTGC 3'
WFS1 mutation detection	Forward: 5'- TGACGAGCTGGCGGGGAAGA -3'
	Reverse: 5'- ACAGACCGCCACGGTGACTG -3'
	Forward: 5'- CGCCCGGTGGTTCACGTCTC -3'
	Reverse: 5'- CAGGCCGCCGACAGGAATGG -3'

Supplementary Table 2. Information on genotypes and phenotypes of the research subjects.

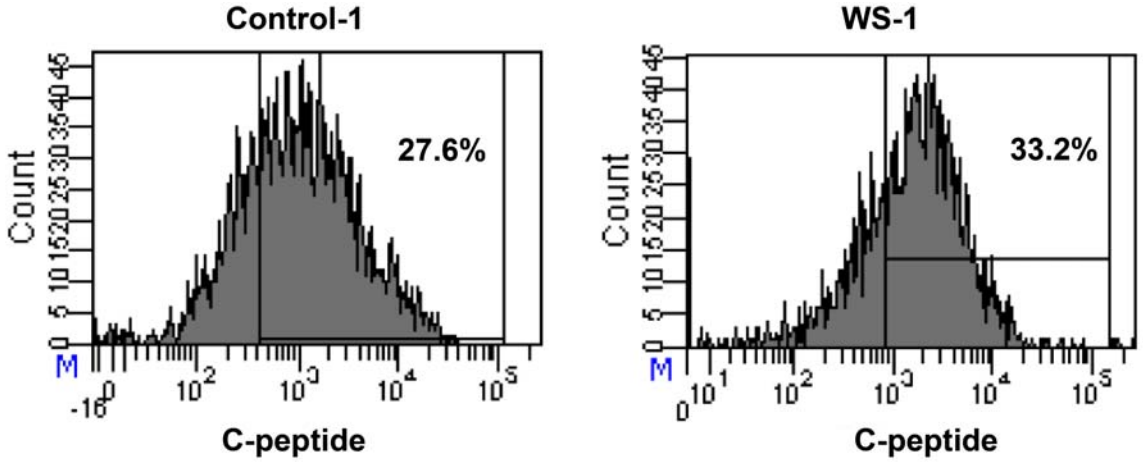
Cell Line	Source	Sex	Age of onset/ diagnosis	Mutations in *WFS1* gene	Remarks
WS-1	Naomi Berrie Diabetes Center	Male	12	1230-1233delCTCT (V412fsX440), P724L	Diabetes; Optic atrophy; Insulin dependent
WS-2	Naomi Berrie Diabetes Center	Female	2	1230-1233delCTCT (V412fsX440)	Diabetes; Optic atrophy; Insulin dependent
WS-3	Corriell Research Institute (GM01610)	Female	11	W648X, G695V	Diabetes; Optic atrophy; Insulin dependent
WS-4	Corriell Research Institute (GM01611)	Female	13	W648X, G695V	Diabetes; Optic atrophy; Insulin dependent
Carrier	Corriell Research Institute (GM01701)	Male	Not affected	G695V	Non-diabetic; Father of WS-3 and WS-4
Control-1 (HUES42)	Harvard University	Male	Not affected	None	Non-diabetic
Control-2 (iPSCs)	Naomi Berrie Diabetes Center	Male	Not affected	None	Non-diabetic

SUPPLEMENTARY DATA

Supplementary Figure 1. Induced pluripotent stem (iPS) cells generated from Wolfram fibroblasts using Sendai virus vectors. A. Wolfram subject fibroblasts and Wolfram subject iPS cells. B. Karyotypes of the iPS cells of four Wolfram research subjects. C. The Wolfram iPS cells expressed pluripotent marker genes, shown are SSEA4, SOX2, TRA-1-60, NANOG, TRA-1-81, OCT4, by immunocytochemistry. D. Immunohistochemistry of embryonic body cultures and histological analysis of teratomas derived from iPS cells.

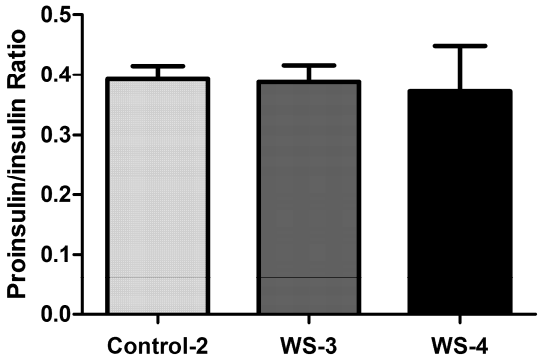


Supplementary Figure 2. FACS analysis for quantification of differentiated beta cells. At day 14 of differentiation, cells were stained with human C-peptide antibody and subjected to standard FACS preparation. Gating was set up based on fluorescent intensity of negative control cells.

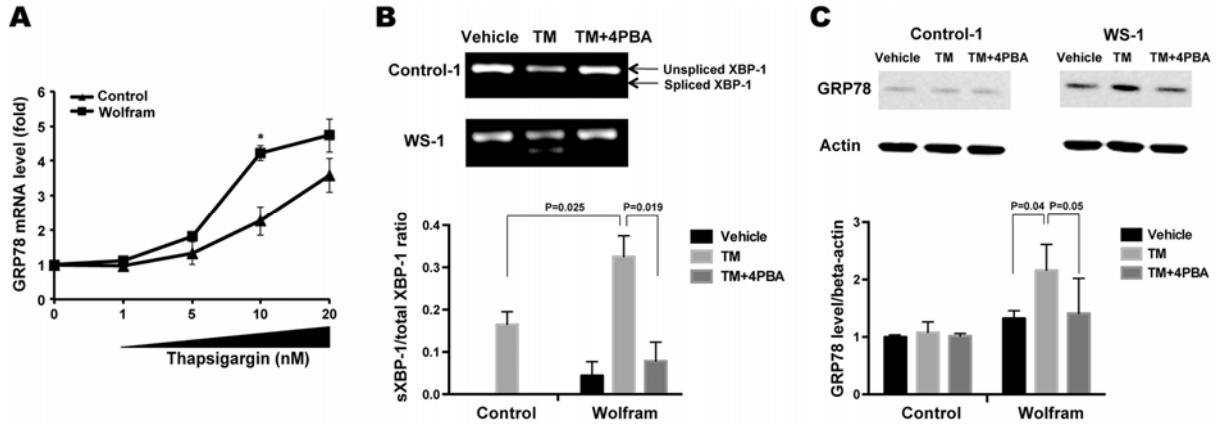


SUPPLEMENTARY DATA

Supplementary Figure 3. Proinsulin to insulin ratio of Control and Wolfram beta cells determined by ELISA. N=3 for each of two independent experiments.

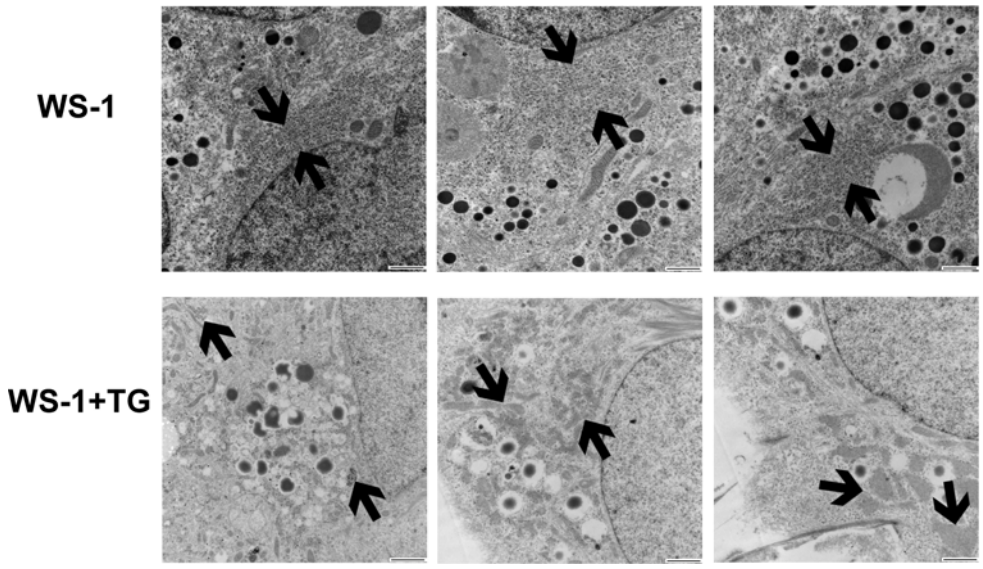


Supplementary Figure 4. Enhanced unfolded protein response in Wolfram cells. A. Fold change of GRP78 mRNA level in control and Wolfram iPS cells after 6 hours of thapsigargin (TG) treatment. * P< 0.05. B. Gel images showing spliced XBP-1 mRNA level in control and Wolfram iPS cells under indicated conditions and quantification represents the results from studies of 4 Wolfram subject lines of three independent experiments. Band intensities for vehicle and TM+4PBA conditions in control cells were below detection threshold. C. Western blot analysis showing fold change of GRP78 expression level in control and Wolfram fibroblasts under indicated conditions. Quantification represents the results from studies of 2 Wolfram subjects (WS-1 and WS-2) of three independent experiments. TM: tunicamycin; 4PBA: Sodium 4-phenylbutyrate. Cells were treated with TM or TG for 6 hours. 1 mM 4PBA was added one hour prior to and during TM or TG treatment.

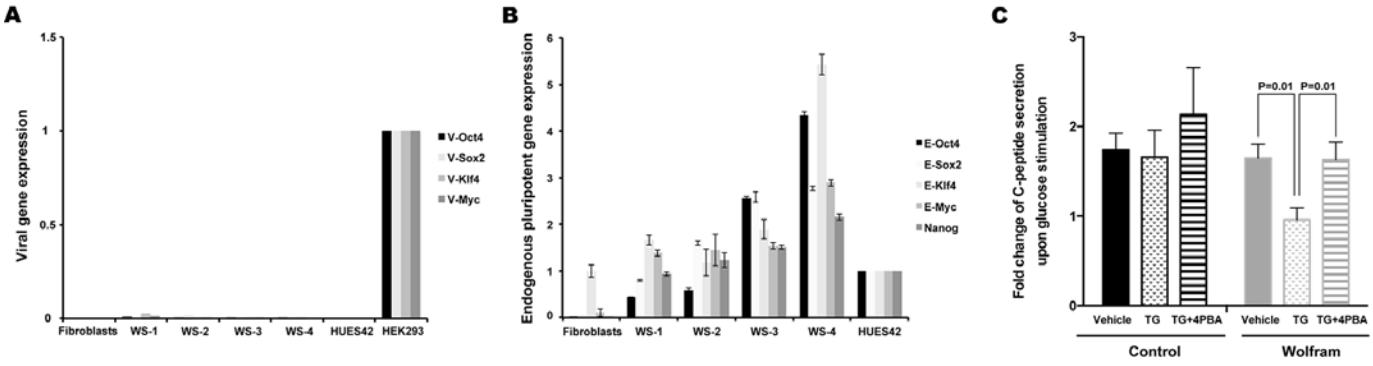


SUPPLEMENTARY DATA

Supplementary Figure 5. Transmission Electron Microscope showing endoplasmic reticulum morphology in Wolfram cells with or without treatment of 10 nM TG. Arrows point to the ER. Obvious ER dilation was observed in Wolfram cells after treatment with TG. Note the Scale bar: 500 nm.

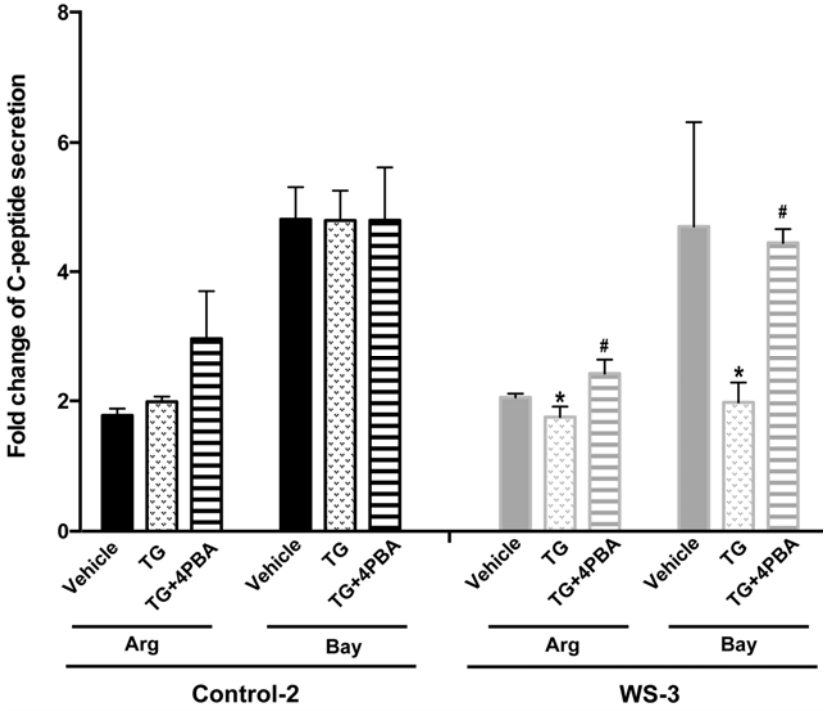


Supplementary Figure 6. Wolfram disease phenotypes can also be observed using iPS cells made by retroviral vectors, instead of Sendai virus. A. Expression from the retroviral transgenes in different cell lines as indicated. This shows that the viral vectors expression was silenced in the iPS cells. B. Expression of endogenous pluripotent genes in different cell lines as indicated. C. Fold change of human C-peptide secretion when the concentration of glucose in the media was changed from 5.6 mM to 16.9 mM glucose in control and Wolfram beta cells. N=3 for each experiment of three independent experiments.

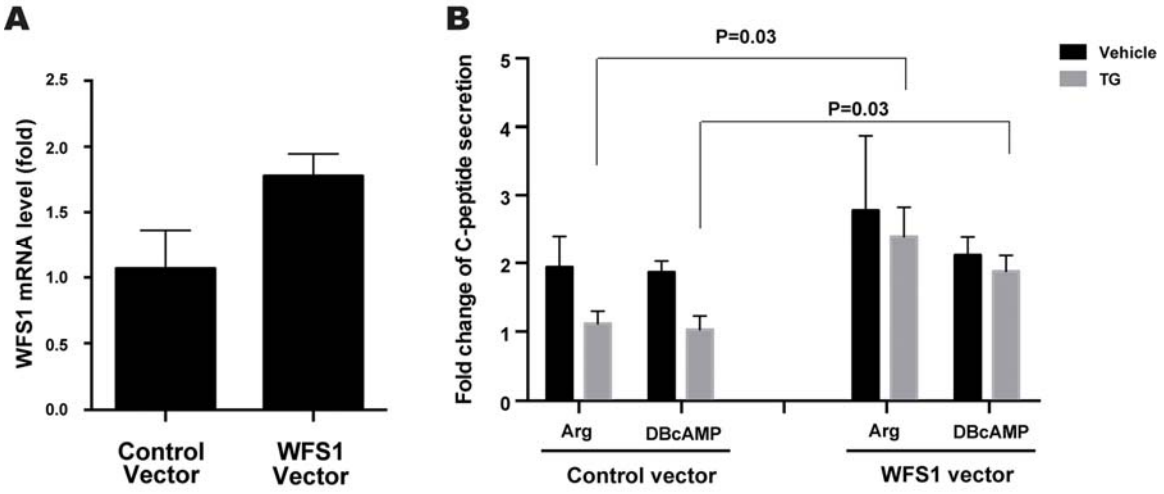


SUPPLEMENTARY DATA

Supplementary Figure 7. Fold change of human C-peptide secretion to 15mM arginine (Arg) or 1μM Bay K8644 stimulation in Control-2 and WS-3 beta cells upon thapsigargin (TG) treatment. N=3 for each experiment of three independent experiments. Bay: Bay K8644, a calcium channel agonist. 4PBA: Sodium 4-phenylbutyrate. * P<0.05 of TG vs. Vehicle; # P<0.05 of TG+4PBA vs. TG.

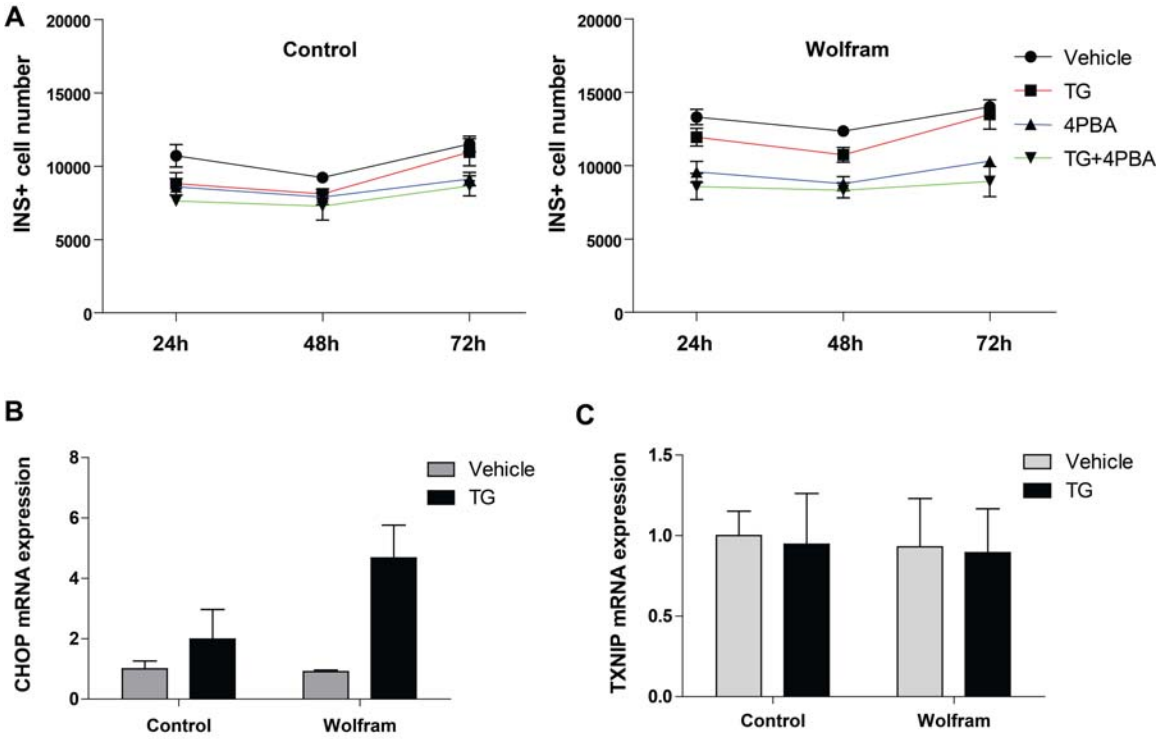


Supplementary Figure 8. WFS1 gene expression and insulin secretion of WS-1 cells carrying either control Lenti-virus vector without a cDNA, or with a wild-type *WFS1* cDNA driven by MSCV promoter. A. Quantitative PCR for WFS1 mRNA level (N=3). B. Fold change of human C-peptide secretion to 15 mM arginine or 1 mM DBcAMP+16.9mM glucose stimulation in WS-1 cells (N=3).



SUPPLEMENTARY DATA

Supplementary Figure 9. A. Control and Wolfram insulin positive cell number over 72 hours with or without treatment of 10nM thapsigargin (TG). B and C. CHOP (C/EBP-homologous protein, also known as DNA damage-inducible transcript 3 encoded by the DDIT3 gene) and TXNIP (thioredoxin-interacting protein) mRNA level in control and Wolfram cells with or without 10nM thapsigargin (TG) treatment for 12 hours.



SUPPLEMENTARY DATA

Supplementary Figure 10. A. Human C-peptide levels in the sera of mice transplanted with control or Wolfram cells. B. Human C-peptide levels in the sera of transplanted mice before and after nephrectomy. Negative control represents mice with sham surgery procedure. C. Fasting human C-peptide levels in mice transplanted with human islets and Wolfam cells before and after one dosage of 1mg/kg thapsigargin (TG) injection. D. Fold change of human C-peptide levels in transplanted mice before and after one dosage of 1mg/kg thapsigargin (TG) injection.