Supplementary Figure 1 *Fonseca et al.*

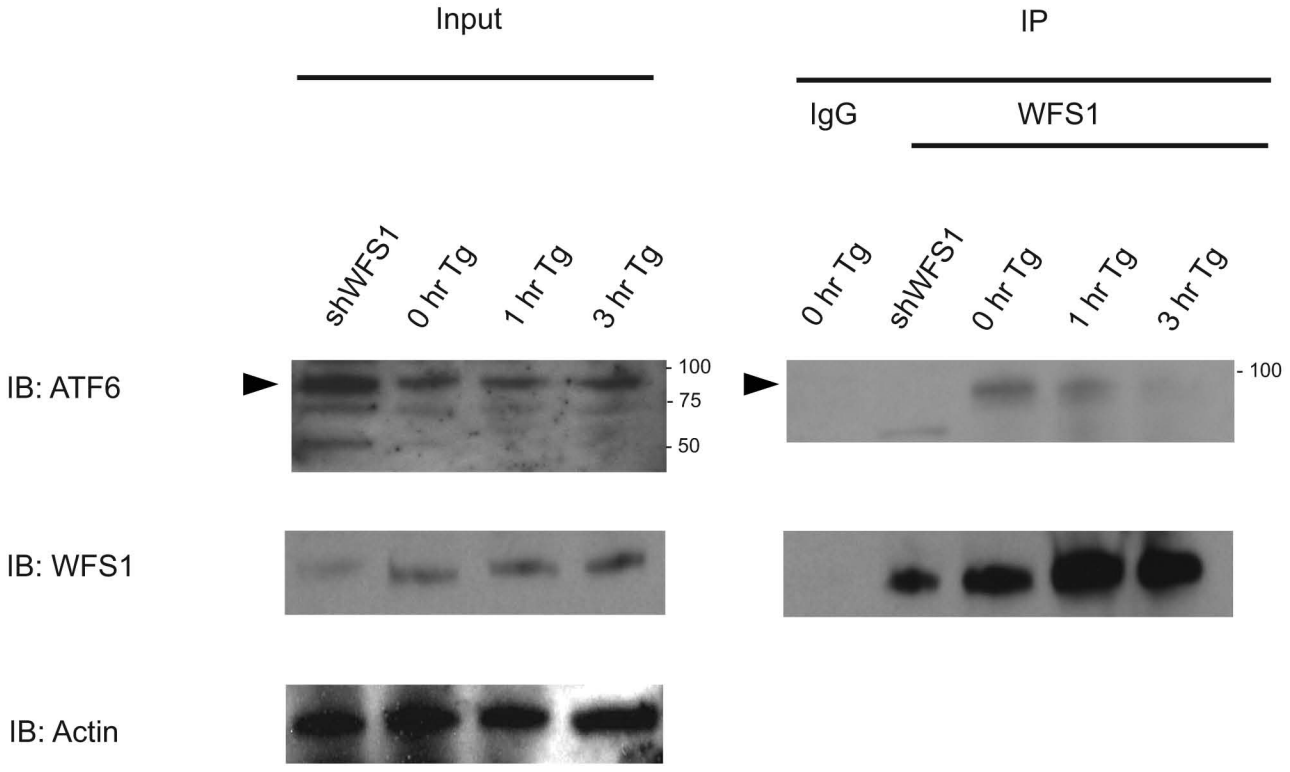

Figure S1. WFS1 interacts with ATF6 in an ER stress-dependent manner. An anti-WFS1 antibody was used to immunoprecipitate (IP) WFS1 protein from INS1 832/13 cells untreated (UT) or treated with 1 μM of the ER stress inducer thapsigargin (Tg) for 0, 1, or 3 hr. Immunoprecipitates were then subjected to immunoblot (IB) analysis using anti-ATF6, anti-WFS1, and anti-actin antibodies.

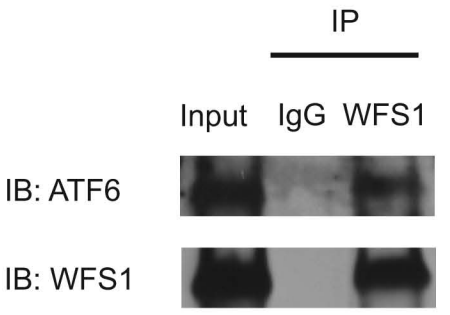

Figure S2. The WFS1-ATF6 complex in neuronal cells. An anti-WFS1 antibody was used to immunoprecipitate (IP) WFS1 protein from Neuro-2a cells. Immunoprecipitates were then analyzed using anti-ATF6 and anti-WFS1 antibodies (n=3).

Supplementary Figure 3 *Fonseca et al.*

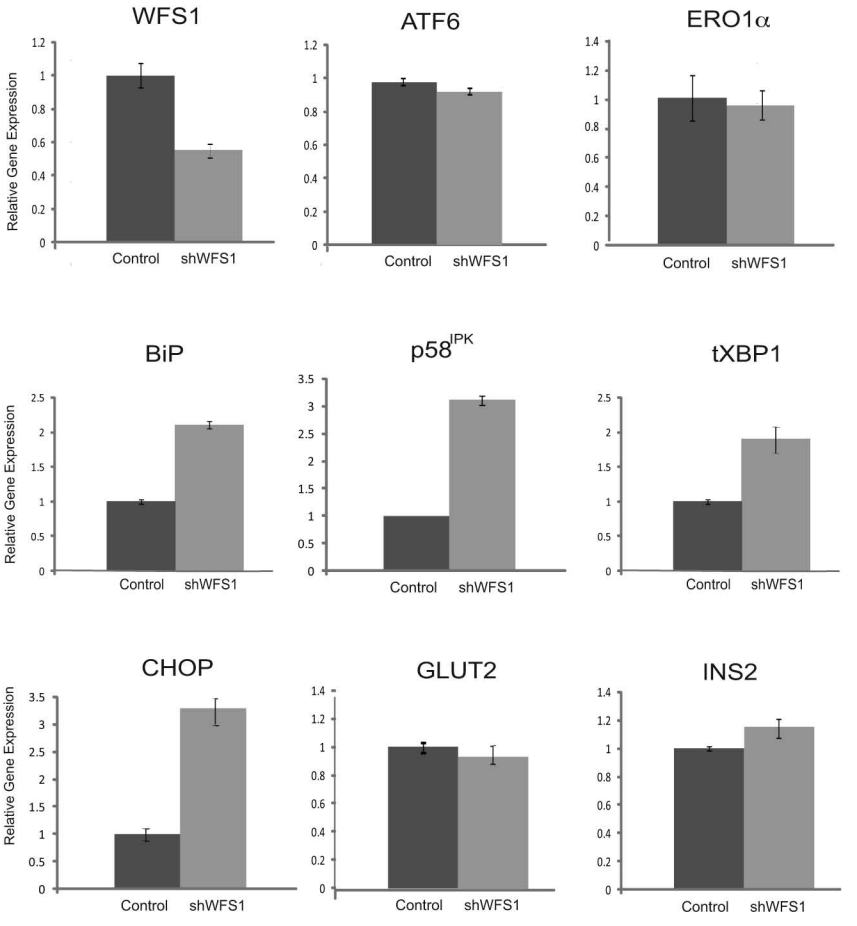

Figure S3. WFS1 inhibition enhances ATF6 target gene expression. Total mRNA was prepared from INS1 832/13 cells inducibly expressing shRNA against WFS1 (Control = UT, shWFS1 = 2 μM doxycycline treatment for 48 hrs). Expression levels of WFS1, ATF6, BiP, total Xbp-1, p58IPK, Chop, Ero1α, Glut2, and Ins2 were measured by quantitative real-time PCR (n=3; values are mean ± SD).

Supplementary Figure 4 Fonseca et al.

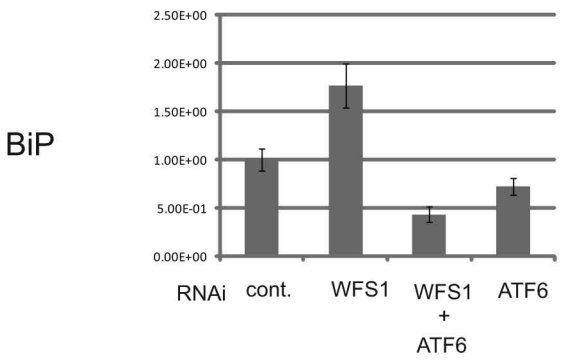

Figure S4. BiP upregulation by WFS1 inhibition is cancelled by ATF6α inhibition. Total mRNA was prepared from INS-1 832/13 cells transfected with WFS1 siRNA, ATF6α siRNA, or WFS1 and ATF6α siRNA. Expression levels of BiP were measured by quantitative real-time PCR (n=3). WFS1 expression was decreased by ~60% and ATF6α expression was decreased by 80%.

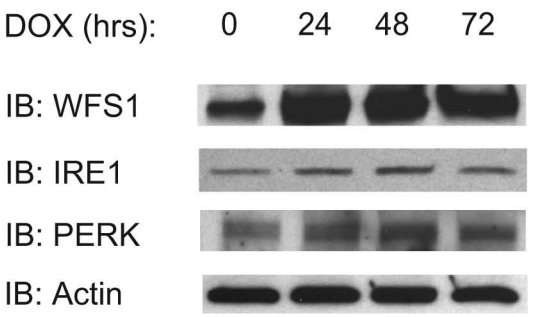

Figure S5. Varying levels of WFS1 protein expression does not affect IRE1 or PERK expression . pTetR 832/13 - WT WFS1 cells were treated with doxycycline for 0, 24, 48, or 72 hrs to induce varying WFS1 protein expression. Protein lysates were then subjected to immunoblot (IB) analysis using anti-WFS1, anti-IRE1, anti-PERK, and anti-Actin antibodies.

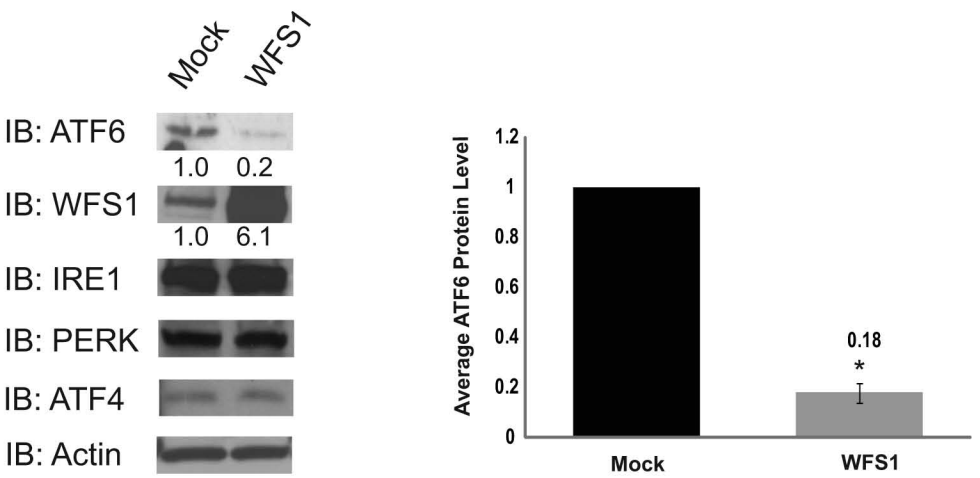

Figure S6. The WFS1-ATF6 relationship in neuronal cells. Neuro-2a cells were either mock transfected or transfected with a WFS1 expression plasmid. Lysates were analyzed by immunoblot (IB) using anti-ATF6, anti-WFS1, anti-IRE1, anti-PERK, anti-ATF4, and anti-actin antibodies (n=3; values are mean ± SD).

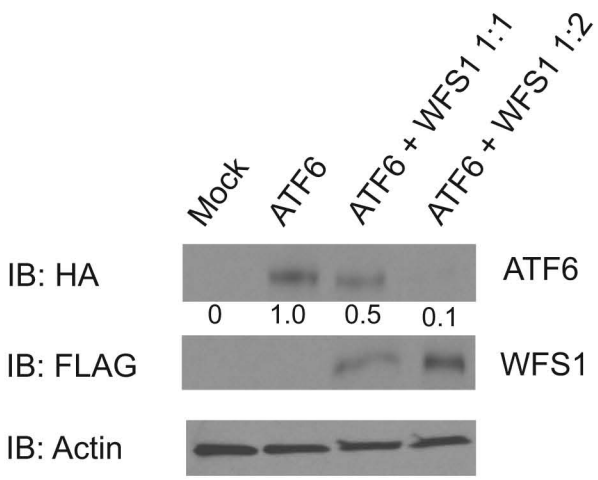

Figure S7. WFS1 controls ATF6 protein in a dose-dependent manner. COS7 cells were transfected with ATF6-HA or ATF6-HA and WFS1-FLAG at a 1:1 or 1:2 ratio of ATF6:WFS1. Whole cell extracts were then subjected to immunoblot (IB) using anti-HA, anti-FLAG, and anti-actin antibodies. Relative ATF6 protein level was measured using ImageJ software (n=3).

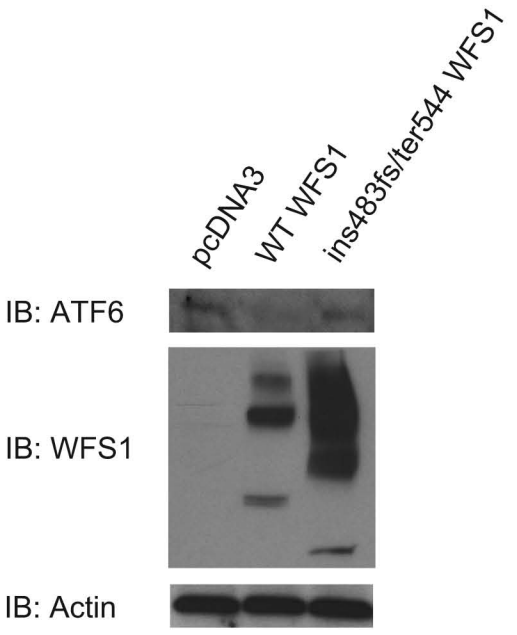

Figure S8. WFS1 inactivating mutants do not rescue ATF6 protein levels. shWFS1 MIN6 cells were transfected with WT or mutant (ins488fs/ter544) WFS1. Protein lysates were then subjected to immunoblot (IB) analysis using anti-WFS1, anti-ATF6, and anti-Actin antibodies.

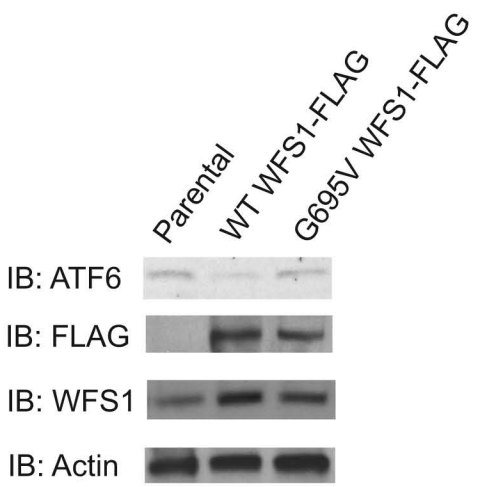

Figure S9. WFS1 degrades ATF6 protein in neuronal cells. Lysates from parental Neuro-2a cells and cells stably expressing WT WFS1 or the mutant variant G695V WFS1 were analyzed by immunoblot using anti-ATF6, anti-FLAG, anti-WFS1, and anti-Actin antibodies (n=3).

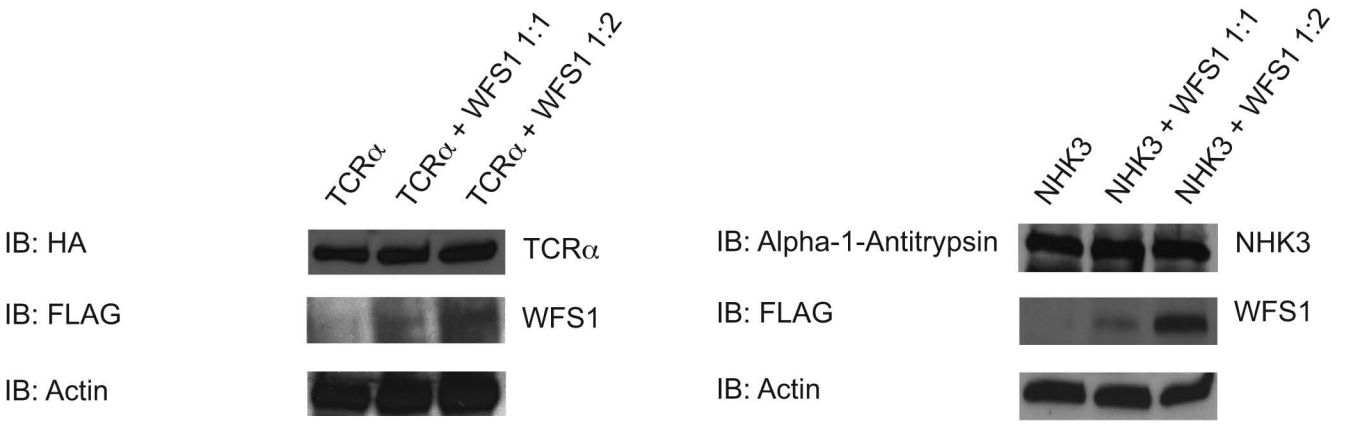

Figure S10. WFS1 does not degrade other ERAD proteins. COS-7 cells were transfected with TCRα-HA or TCRα-HA and WFS1-FLAG at a 1:1 or 1:2 ratio of TCRα:WFS1. Lysates were then immunoblotted with anti-HA, anti-FLAG, and anti-actin antibodies (left panel). COS-7 cells were transfected with mutant alpha-1-antitrypsin (NHK3) or NHK3 and WFS1-FLAG at a 1:1 or 1:2 ratio of NHK3:WFS1. Lysates were then immunoblotted with anti-alpha-1-antitrypsin, anti-FLAG, and anti-actin antibodies (right panel) (n=3).

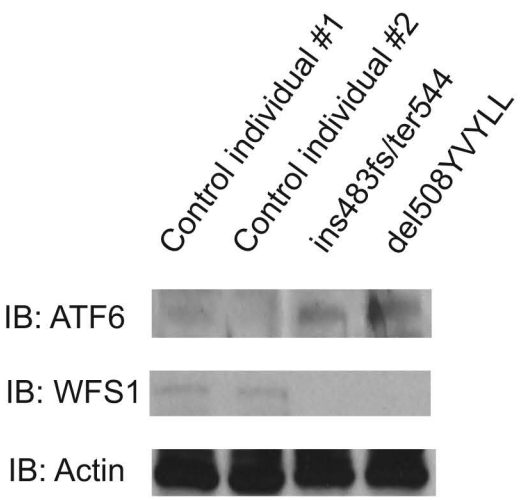

Figure S11. Patients with WFS1 mutations have a higher expression of ATF6. Lymphoblast lysates from Wolfram syndrome patients (ins483fs/ter544 and del508YVYLL) and control individuals were immunoblotted with anti-ATF6, anti-WFS1, and anti-actin antibodies.

Supplementary Figure 12 *Fonseca et al.*

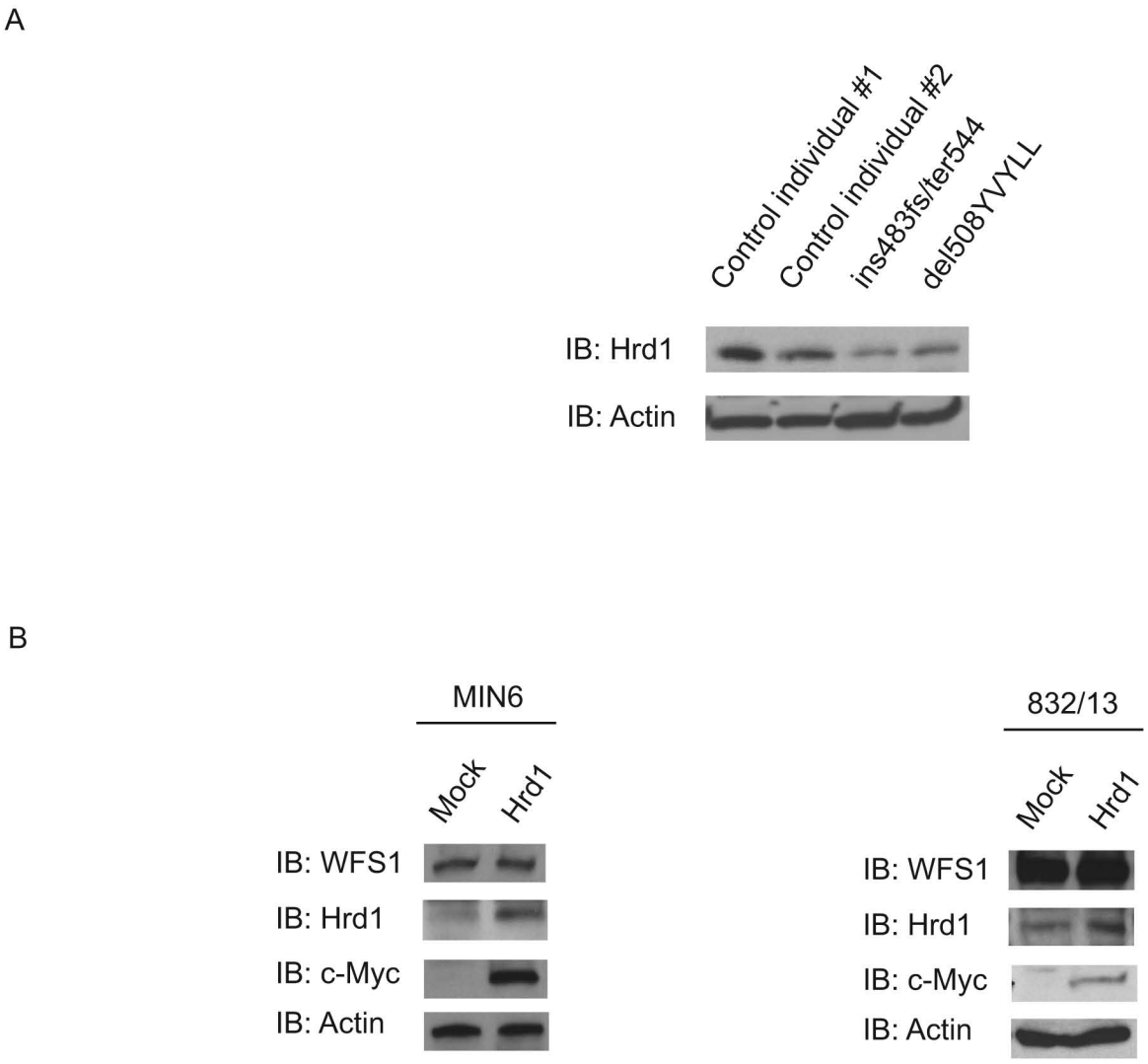

Figure S12. Patients with WFS1 mutations have a lower expression of HRD1. (A) Lymphoblast lysates from Wolfram syndrome patients (ins483fs/ter544 and del508YVYLL) and control individuals were immunoblotted (IB) with anti-Hrd1 and anti-actin antibodies (n=3). (B) MIN6 cells were mock transfected or transfected with a Hrd1-Myc expression plasmid and lysates were subjected to IB using anti-WFS1, anti-Hrd1, anti-c-Myc, and anti-actin antibodies (left panel). INS1 832/13 cells were mock transfected or transfected with a Hrd1-Myc expression plasmid and lysates were subjected to IB using anti-WFS1, anti-Hrd1, anti-c-Myc, and anti-actin antibodies (right panel) (n=3).

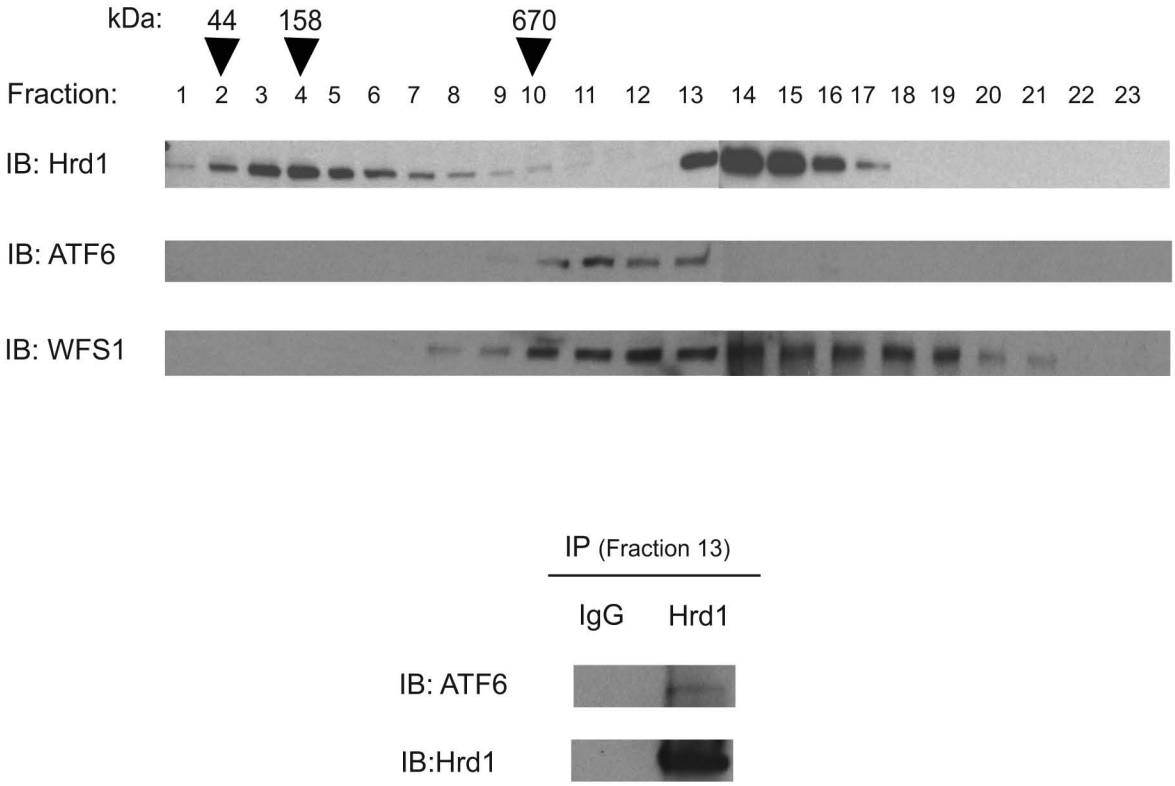

Figure S13. HRD1 is an E3 ligase for ATF6. ER-isolated lysates of INS1 832/13 cells were subject to fractionation using a 10-40% glycerol gradient. Fractions were analyzed by immunoblot using anti-Hrd1, anti-ATF6, and anti-WFS1 antibodies (upper panel). Hrd1 was immunoprecipitated from fraction 13, and IP products were analyzed by immunoblot with anti-Hrd1 and anti-ATF6 antibodies (lower panel) (n=3).

Supplementary Figure 14 Fonseca et al.

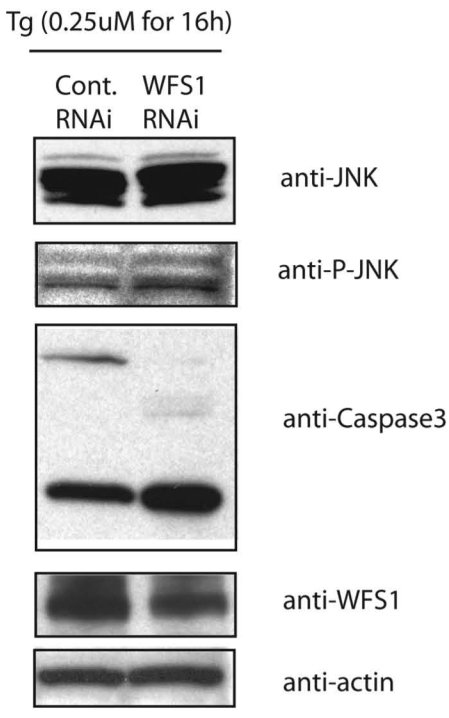

Figure S14. WFS1-deficient β cells are susceptible to ER stress-mediated apoptosis. INS1 832/13 cells were transfected with control scramble siRNA or siRNA directed against WFS1 and then treated with thapsigargin (Tg, 0.25 μM) for 16 hr. Expression levels of total JNK, phospho-JNK (P-JNK),caspase-3 (Casp3), WFS1, and actin were measured by immunoblot.

Supplementary Figure 15 Fonseca et al.

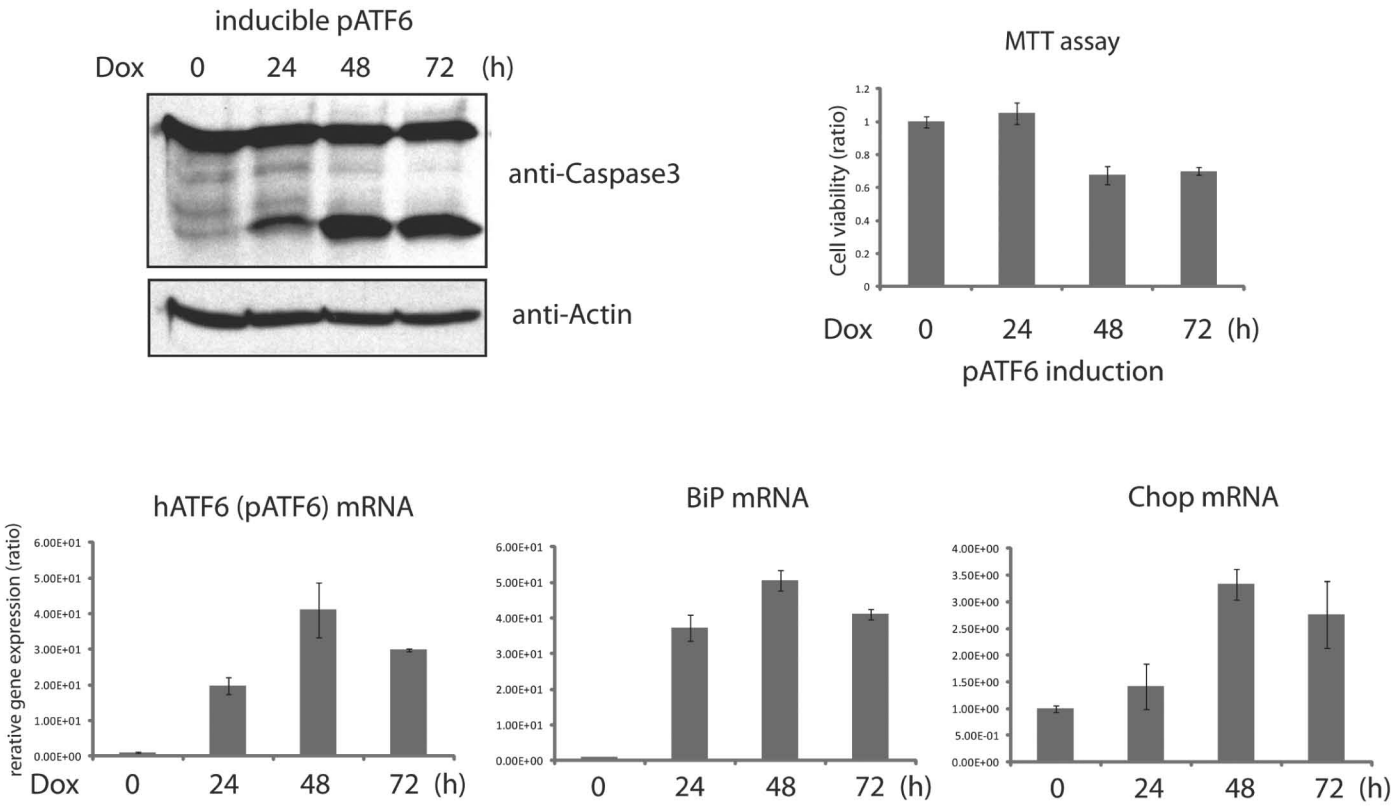

Figure S15. Chronic hyperactivation of ATF6α induces β-cell apoptosis. INS-1 832/13 cells were stably transduced with LV-TO/cleaved ATF6α, an inducible lentivirus expressing an active form of ATF6α. Cells were cultured with doxycycline (2 µg/ml) to induce AATF or without doxycycline for indicated times. Expression levels of caspase-3 (Casp3) and actin were measured by immunoblot. Expression levels of ATF6α, BiP, and Chop were measured by quantitative real-time PCR. Cell viabiliy was measure by MTT assay.

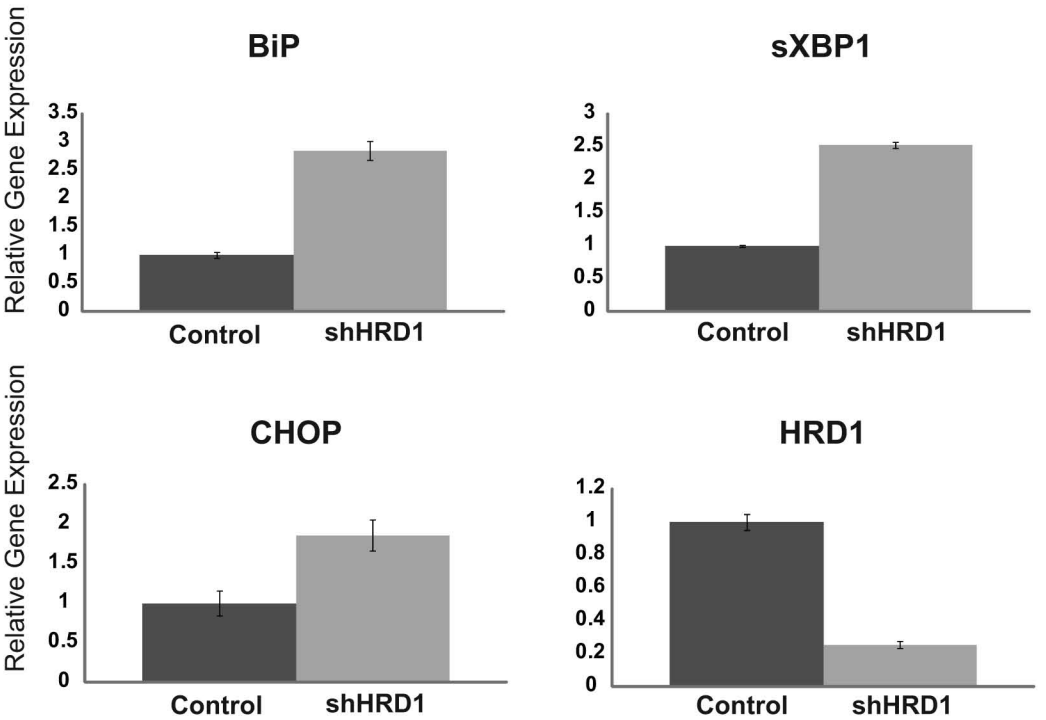

Figure S16. HRD1 suppression leads to mild ER stress. Total mRNA was prepared from MIN6 cells either expressing scramble shRNA (Control) or shRNA directed against HRD1. Expression levels of BiP, spliced XBP1 (sXBP1), CHOP, and HRD1 were measured by quantitative real-time PCR (n=3; values are mean ± SD).