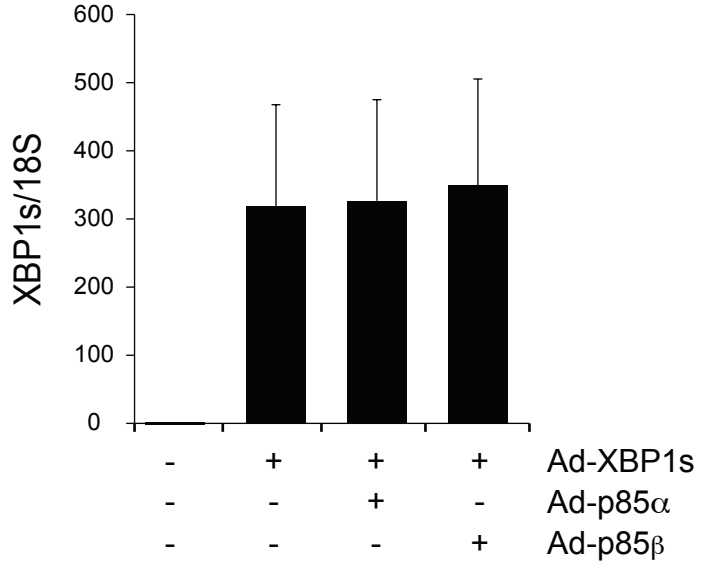
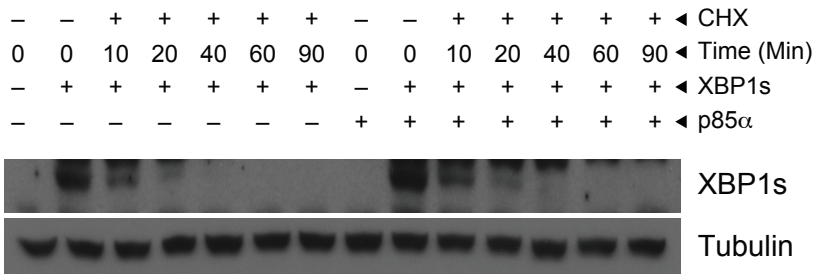
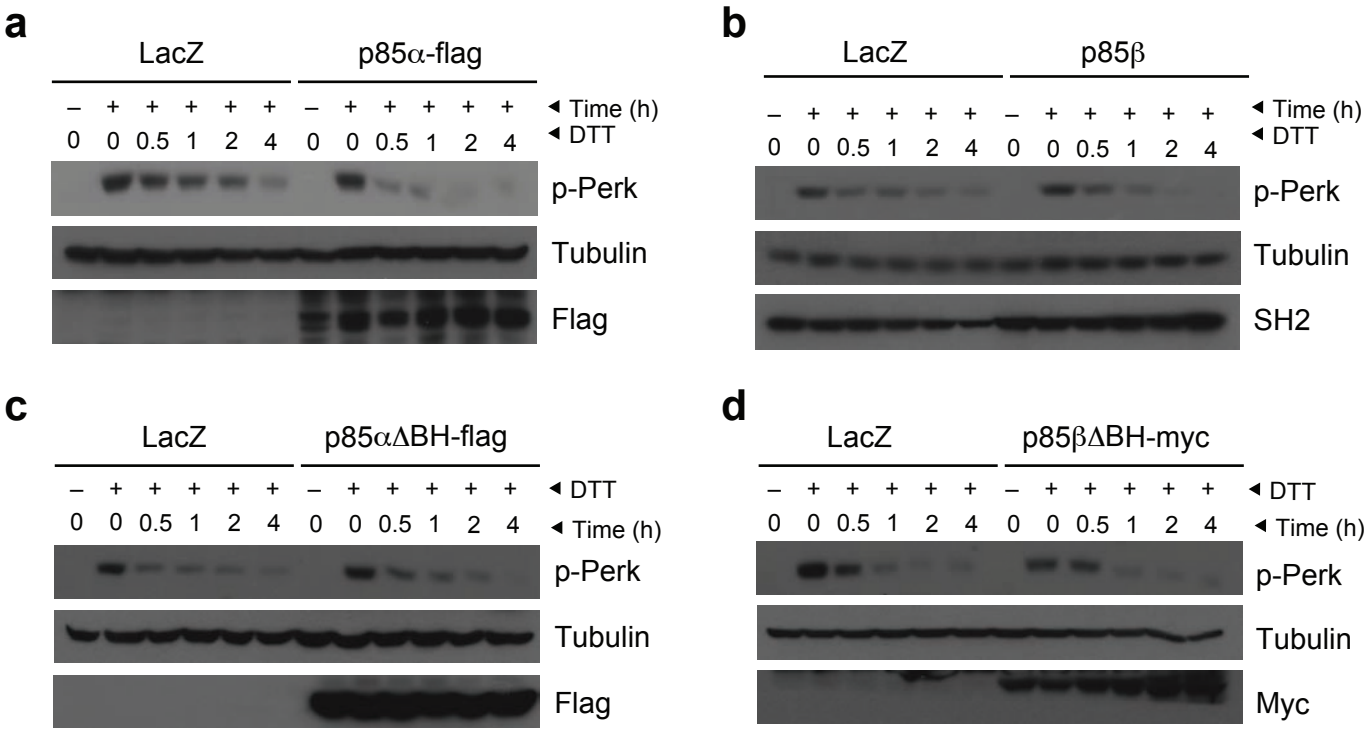
Supplementary Figures



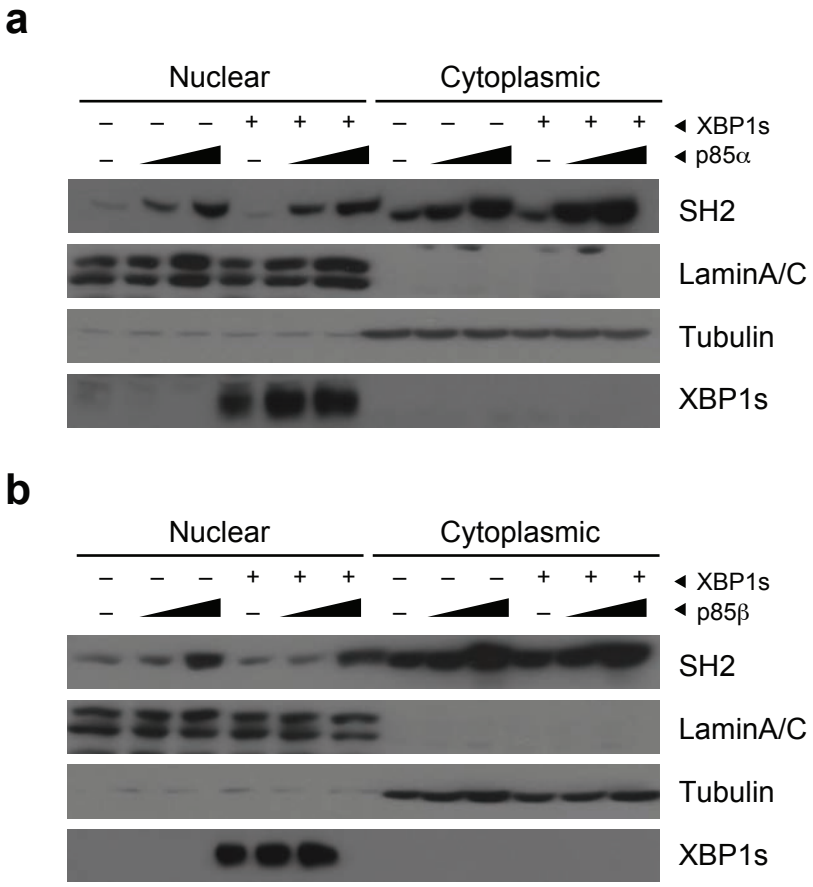
Supplementary Figure 1 p85α or p85β does not increase XBP-1s mRNA levels. The total mRNA levels of XBP-1s in MEF cells infected with Ad-XBP-1s alone or together with Ad-p85α or Ad-p85β. The expression levels were quantified with Q-PCR, and 18S was used as an internal control. Error bars are ± S.E.M.



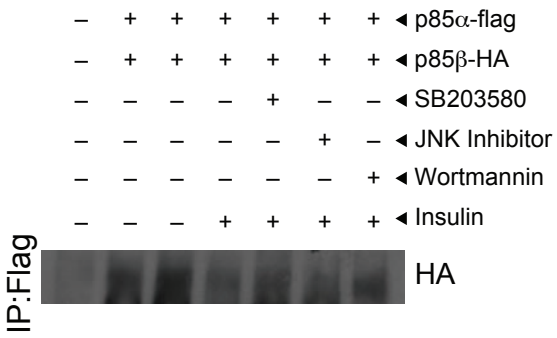
Supplementary Figure 2 Co-expression of XBP-1s with p85α does not alter the degradation rate of XBP-1s. Degradation rate of XBP-1s in MEFs that were infected with Ad-XBP-1s alone or together with Ad-p85α and treated with cycloheximide (100 g ml^{-1}).



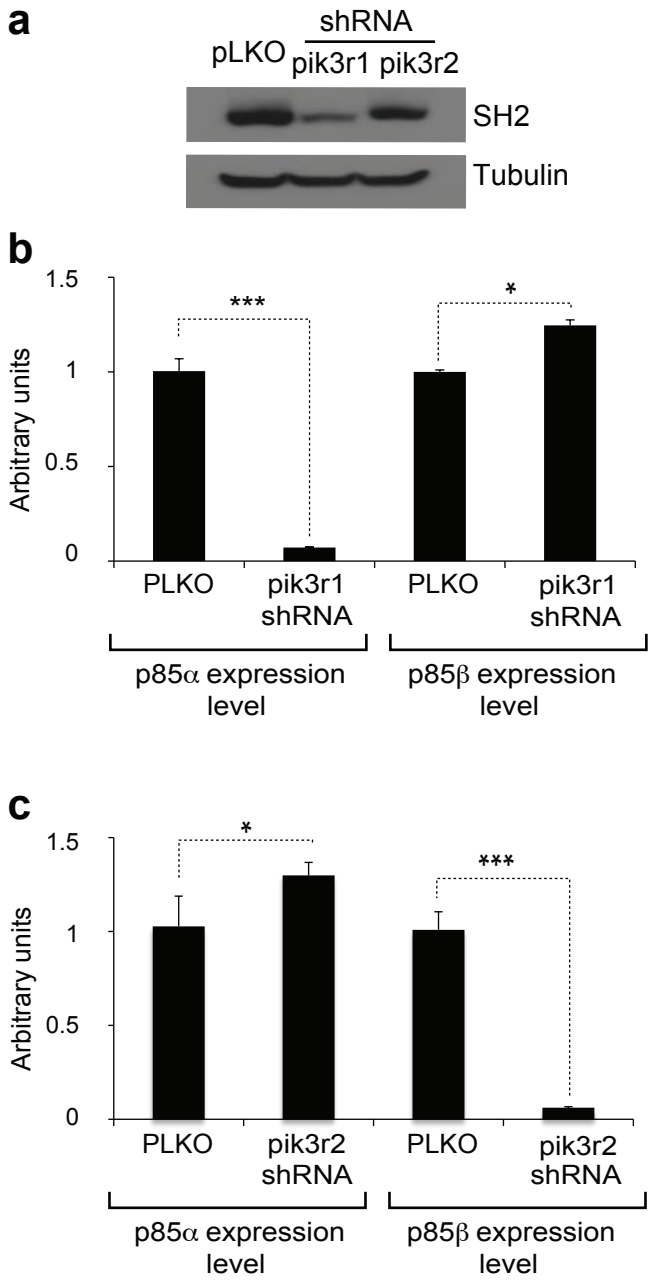
Supplementary Figure 3 p85α and p85β improve ability of cells to cope with endoplasmic reticulum (ER) stress. Western blot for phospho-PERK Thr980, tubulin and SH2, Flag, or Myc in MEFs infected with Ad-LacZ, or (**a**) Ad-p85α-flag, or (**b**) Ad-p85β or (**c**) Ad-p85αΔBH-flag or (**d**) Ad-p85βΔBH-myc. Infected MEFs were treated with 100 mM of DTT. After removing DTT from the media, the cells were incubated for 0, 0.5, 1, 2, and 4 h for recovery. Each experiment was independently repeated three times.



Supplementary Figure 4 Interaction of p85 and XBP-1 is unidirectional. (**a,b**) Nuclear and cytoplasmic proteins for SH2, lamin A/C, tubulin and XBP-1s in MEFs infected with increasing doses of p85α (**a**) or p85β (**b**) in the absence or presence of high expression level of XBP-1s.



Supplementary Figure 5 Inhibition of p38, JNK, or PI3K does not block association of p85α and p85β. Immunoblots of HA after flag immunoprecipitation from MEFs infected with Ad-p85α-Flag and Ad-p85β-HA and treated with DMSO, p38 inhibitor (20 nM) (SB203580), JNK inhibitor VIII (10 nM), or PI3K inhibitor (100 M) (wortmannin), followed by stimulation of insulin (500 nM).



Supplementary Figure 6 shRNA lentivirus system specific for p85α and p85β. (**a**) Protein amounts of p85 in the *pik3r1* or *pik3r2* silenced cells. (**b**) mRNA levels of p85α and p85β in the *pik3r1* silenced cells quantified by Q-PCR. PLKO cells were used as a control. (**c**) mRNA levels of p85α and p85β in the *pik3r2* silenced cells quantified by Q-PCR. Error bars are ± S.E.M., P values were determined by Student's t-test. (*p<0.05, **p<0.01, ***p<0.001).

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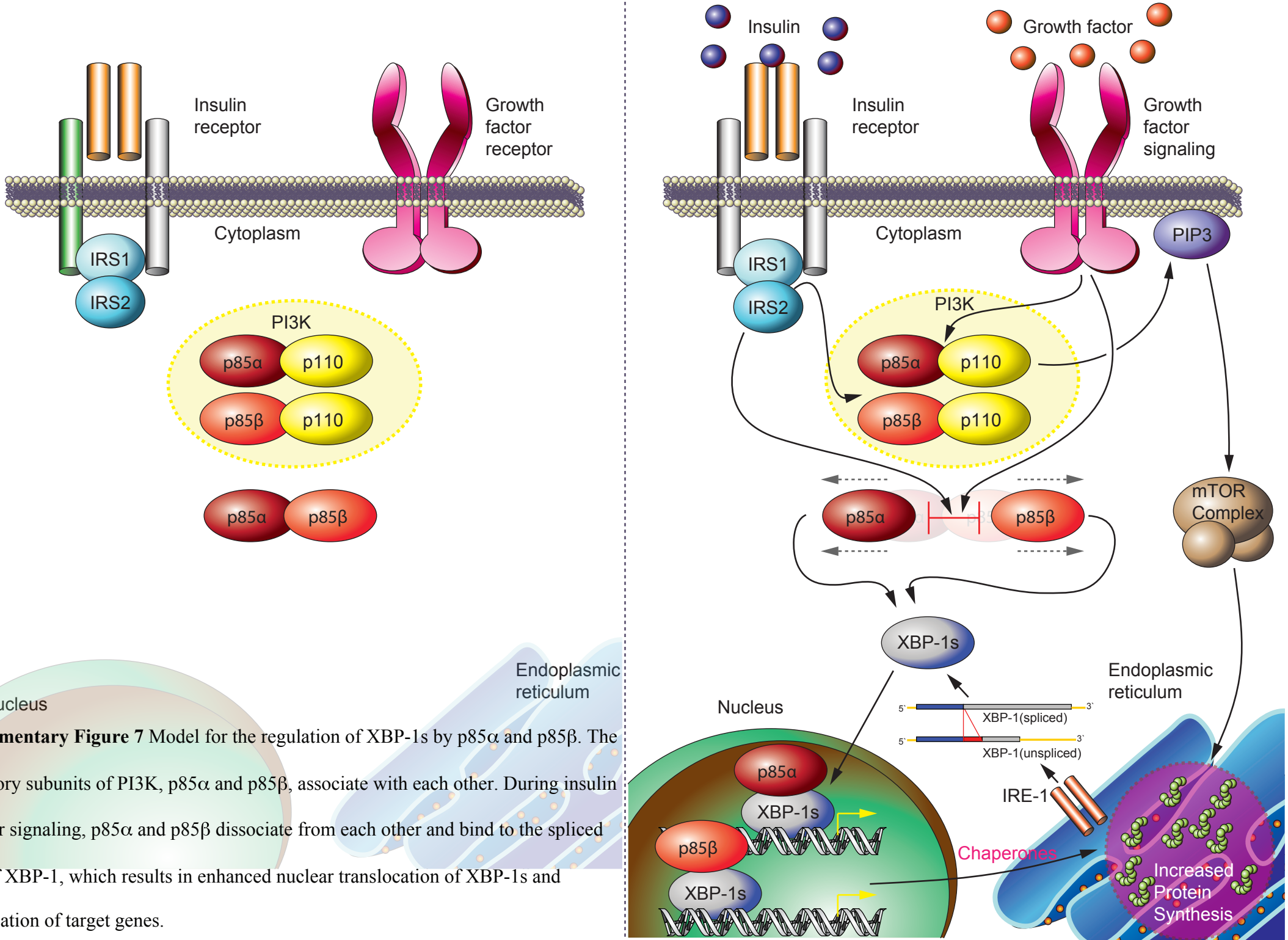

Supplementary Figure 7 Model for the regulation of XBP-1s by p85α and p85β. The regulatory subunits of PI3K, p85α and p85β, associate with each other. During insulin receptor signaling, p85α and p85β dissociate from each other and bind to the spliced form of XBP-1, which results in enhanced nuclear translocation of XBP-1s and upregulation of target genes.

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Regulatory subunits of PI3K, p85α and p85β, interact with XBP-1 and increase its nuclear translocation

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Supplementary Materials and Methods

Biochemical reagents. XBP-1-specific antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to p85 was from Upstate Biotechnologies (Billerica, MA). Flag-, HA-, Lamin A/C-, and Phospho-Perk (Thr980)-specific antibodies were from Cell Signaling Technology (Beverley, MA). FLAG M2 antibody was from Sigma Aldrich (St. Louis, MO). Silver staining kit was purchased from Bio-Rad (Hercules, CA). Dulbecco's Modified Eagle Medium, OptiMEM, fetal bovine serum, penicillin and streptomycin were purchased from Gibco (Grand Island, NY). All chemical reagents were purchased from Sigma Aldrich (St. Louis, MO). Okadaic acid was purchased from CalBiochem (Gibbstown, NJ). Leupeptin and aproptonin were from Sigma Aldrich (St. Louis, MO). Lipofectamine was from Invitrogen (Carlsbad, CA). Nuclear extraction kit for cell lines was purchased from Active Motif (Carlsbad, CA) and the kit for tissues was from Pierce Biotechnology (Rockford, IL). DSP was obtained from Thermo Scientific (Rockford, IL). Fast Start Taq DNA polymerase was from Roche Diagnostics (Indianapolis, IN). High Capacity cDNA kit and TaqMan were from Applied Biosystems (Foster City, CA). T4 ligase was from TaKaRa (Madison, WI). T4 Polynucleotide Kinase was purchased from New England BioLabs (Ipswich, MA).

Cell culture. Mouse Embryonic Fibroblast (MEF), 293A, and 293T cell were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 10 U ml^{-1} penicillin and 1 $\mu g\ ml^{-1}$ streptomycin. We split cells at a density of 3.5×10^5 in 10 cm dish. Cells were maintained at 37 ^oC in a 5% CO_2 humidified atmosphere.

Western blotting. We lysed cells in lysis buffer which contained 25 mM Tris (pH 7.4), 2 mM $NaVo_4$, 10 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM EGTA, 1 mM EDTA, 1% NP-40, 10 μg ml^{-1} Leupeptin, 10 μg ml^{-1} Aproptonin, 2 mM Phenylmethylsulfonyl fluoride (PMSF) and 20 nM Okadaic acid. Equivalent amounts of protein (100–400 μg) from each sample were placed in 1.5 ml tubes and the volume was brought to equal levels. We denatured the protein in 1× Laemmli buffer by boiling at 100 ^{o}C for 5 min. After boiling, the mixture was kept at 25 ^{o}C for 15 min and resolved by SDS-PAGE. Proteins were transferred onto polyvinylidene fluoride (PVDF) membrane and blocked in 10% blocking reagent with BM Chemiluminescence blotting substrate (POD) assay system for 1 h, followed by incubation with primary antibody in Tris-Buffered Saline-Tween (TBST) (pH:7.4)/10% blocking reagent overnight at 4 ^{o}C. Following three washes in TBST for 20 min, we incubated the membrane with secondary antibody for 1 h and washed again in TBST (three times for 20 min). We developed immunoblots using a chemiluminescence assay system, and proteins were visualized using Kodak exposure film. To strip membranes, we vigorously agitated membranes in stripping buffer (2% SDS, 100 mM 2-mercaptomethanol in TBS (pH 7.5)) at 50 ^{o}C for 20 min, followed by three washes with TBST.

DTT treatment. We starved MEFs in DMEM/1% FBS for 16 h and treated with 100 μl of 1 M DTT for 1 h. After the treatment, we removed the supernatant, and washed the cells three times with DMEM. We replaced media with 1% FBS for recovery. The cells were directly frozen in liquid nitrogen after 0.5, 1, 2 and 3 h of recovery.

RNA preparation and quantitative real-time qPCR. We extracted total RNA from cell lines or the liver of mice using Trizol reagent according to the manufacturer's instructions. We reverse transcribed 500 ng of RNA to cDNA using the High Capacity cDNA kit from Applied Biosystems. The reverse transcription conditions were as follows: 25 °C for 10 min, 37 °C for 2 h, and 85 °C for 5 sec. The transcripts were analyzed on ABI7900HT using TaqMan. We obtained the inventory probes from Applied Biosystems: *Gapdh* (assay ID: Mm99999915_g1), *Dnajb9* (Mm01622956_s1), *Pdia3* (Mm00433130_m1) and *Herpud1* (Mm00445600_m1). The primer sequences for *18S* and *Calr* were as follows:

18S (fwd) 5'-AGTCCCTGCCCTTTGTACACA-3'

18S (rev) 5'-CGATCCGAGGGCCTCACTA-3'

Calr (fwd) 5'-CCTGCCATCTATTTCAAAGAGCA-3'

Calr (rev) 5'-GCATCTTGGCTTGTCTGCAA-3'

XBP-1s splicing assay. We analyzed the splicing of XBP-1 from cDNA using Fast Start Taq DNA polymerase. The PCR conditions were as follows: 94 °C for 3 min; 29 cycles of 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 30 sec; and 72°C for 3 min. We used the following primers.

XBP-1 (fwd) 5'-ACACGCTTGGGAATGGACAC-3'

XBP-1 (rev) 5'-CCATGGGAAGATGTTCTGGG-3'

Production and transduction of shRNA lentivirus. We split 293T cells in 10 cm dish and transfected with pLKO, pLP1, pLP2, and pVSV-G using Lipofectamine. The shRNA

sequences carried in pLKO vector for p85α and p85β were as follows: p85α 5'-CCGGCAACCGAAACAAAGCGGAGAACTCGAGTTCTCCGCTTTGTTTCGGTTGTTTTTG-3' ; p85β 5'-CCGGCCTGTGTCCAAGTACCAACAACTCGAGTTGTTGGTACTTGGACACAGGTTTTT-3'. We replaced media with 10 ml of fresh media 16 h after the transfection. We incubated cells at 37 °C in a 5% CO_2 humidified atmosphere for 48 h. We harvested the viral particles by passing the supernatant through a 0.45 μm filter. To transduce cells with virus, we added viral supernatant to cells in the presence of polybrene at a final concentration of 2 μg ml^{-1}.

Glucose tolerance test (GTT). We fasted mice overnight and administrated D-glucose (0.5 g kg^{-1}) intraperitoneally. We measured blood glucose levels via tail clip using portable glucose meter (Contour, Bayer) at 0, 15, 30, 60, 90, 120 min following glucose administration.

PCR for testing recombination efficiency in pik3r1$^{f/f}$;pik3r2$^{-/-}$ mice. Recombination efficiency after expression of Cre-Recombinase in the liver of pik3r1$^{f/f}$;pik3r2$^{-/-}$ mice was performed using PCR with following conditions: 94 °C for 10 min; 32 cycles of 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 1 min; and 72 °C for 5 min. The following primer pair was used.

Loxp85 F (fwd) 5'-GGTTTCTTACTTTAGACGGACTG-3'

Loxp85 R (rev) 5'-CCAGTTACTTTCAAATCAGCACAG-3'

Construction of BH-mutant p85α and p85β adenoviruses. We deleted BH domains within p85α and p85β at 295–906 nucleotides and 385–762 nucleotides, respectively, from pENTR3C-p85α and pENTR3C-p85β using PCR. We used the following conditions for PCR: 94 °C for 10 min; 18 cycles of 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 5 min; and 72 °C for 15 min. We used the following primer pair to create pENTR3C-p85αΔBH.

(fwd) 5'-CCAGCACCAGCACTGCCC-3'

(rev) 5'-AGCAACAGGAAGCGGTCG-3'

We used the following primer pair to create pENTR3C-p85βΔBH.

(fwd) 5'-CTGCGCATACCTCCGTCA-3'

(rev) 5'-CAGAATCGGGGGAGCAGG-3'

After PCR, we cleaned the products with PCR cleanup kit (Qiagen) and phosphorylated the nucleotides with T4 Polynucleotide Kinase (New England BioLabs). We ligated the ends of the products with T4 ligase (TaKaRa) at 15 °C for 16 h. After screening with restriction enzymes and sequencing for the correct deletions, pENTR3C-p85αΔBH and pENTR3C-p85βΔBH were recombined to adenovirus backbone (pAd) to create pAd-p85αΔBH and pAd-p85βΔBH.

Transfection. We diluted DNA and Lipofectamine in 800 μl of OptiMEM and incubated at 25 °C for 5 min. We combined the diluted DNA and Lipofectamine and incubated at 25 °C for 30 min. After the incubation, we diluted the complexes in 1.6 ml of OptiMEM and added to cells with 3.2 ml of OptiMEM. We incubated the cells at 37 ^{o}C in a 5% CO_2 incubator for 2 h. Growth medium with 20% FBS were added to the cells to bring the

total volume to 12.8 ml.

Nuclear extraction. For nuclear extraction of cells, we used a commercially available Nuclear Extraction Kit (Active Motif) as directed by the manufacturer's instructions with minor modifications. We washed cells with 5 ml ice-cold PBS containing phosphatase inhibitors provided by the kit. We removed cells from dishes by scraping with 3 ml of PBS/phosphatase inhibitors. Subsequently, we centrifuged the cells, and the pellets were resuspended in 200 μl of 1× hypotonic buffer. Following 15 min of incubation on ice, we added 25 μl of detergent and vortexed for 10 sec. After this step, we centrifuged the cells for 30 sec at 14,000g, and we collected supernatants to obtain cytoplasmic fractions. The pellets were resuspended in 50 μl of lysis buffer containing 10 mM DTT and protease inhibitors provided by the kit. We incubated the suspension on ice for 30 min. During this incubation, we vortexed the lysates every 10 min for 30 sec. Finally, cells were centrifuged for 10 min at 14,000g to obtain nuclear proteins. For nuclear extraction of tissues, we cut the liver tissues in small pieces and washed once with ice-cold PBS. We isolated nuclear proteins by using a commercially available kit from Pierce following the manufacturer's instructions.

Adenovirus production. We constructed adenovirus expression clones that contain a gene of p85α, p85β, and XBP-1s using Gateway recombination system by Invitrogen. We digested adenovirus vectors with *Pac I*, then transfected the 293A producer cell line in a 6-well-plate. We replaced the media with DMEM containing 10% FBS and 1% penicillin/streptomycin the next day. We transferred the cells to 10 cm tissue culture

dishes 24 h after the transfection. We replaced the culture media with fresh media every 2–3 days until cytopathic effect (CPE) was observed. We collected the cells when 80% CPE were observed and harvested adenovirus by repeatedly freezing at –80 ^{o}C and thawing at 37 ^{o}C four times. We centrifuged cell lysates at 2,000g for 30 min at 25 ^{o}C and stored the supernatant containing adenovirus particles at –80 ^{o}C.

Adenovirus transduction. To transduce cells with adenovirus, we washed cells with culture medium containing 1% FBS and incubated with 2.5 ml of media containing 1% FBS and adenovirus. We gently rocked the dishes every 15 min for 1 h. We then added 7.5 ml of media containing 1% FBS and incubated for 16 h.

Adenovirus injection through tail vein. We thawed adenovirus immediately before injection at 25 ^{o}C and diluted adenovirus with saline to a final volume of 100 μL per mouse. We restrained mice in a restrainer and heated the tail mildly with a heating lamp to achieve vasodilatation. We injected adenovirus through tail vein slowly with a 30 gauge needle. After injection, we applied mild pressure at the spot of injection until no bleeding was achieved to prevent the backflow of virus solution.

Animal experiments. All animal experiments were approved by Children Hospital Boston, IACUC.