Product Datasheet

IRE1 alpha [p Ser724] Antibody - BSA Free
NB100-2323

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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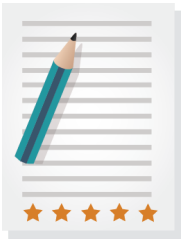


NB100-2323

IRE1 alpha [p Ser724] Antibody - BSA Free

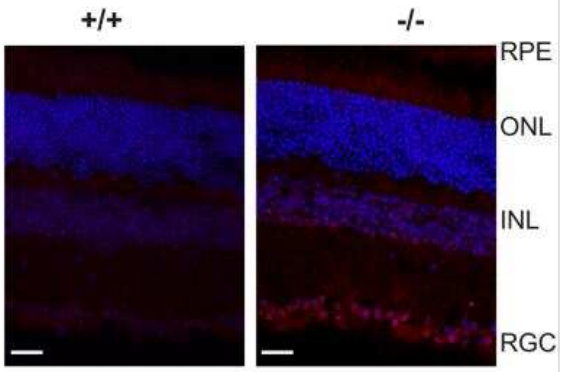
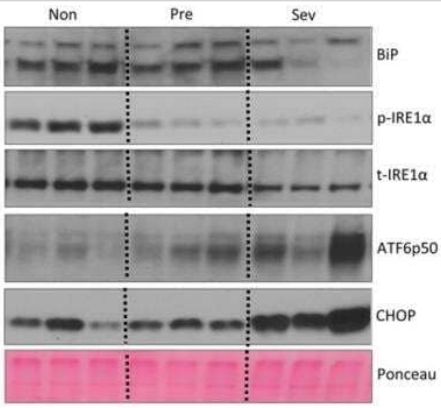
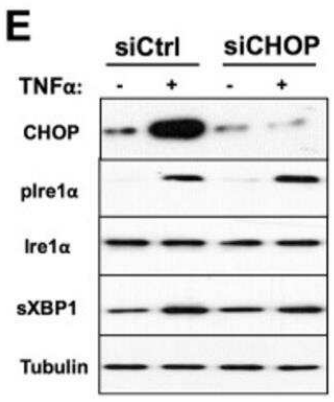
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	110 kDa

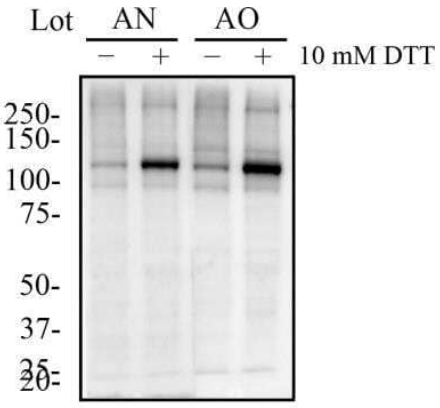
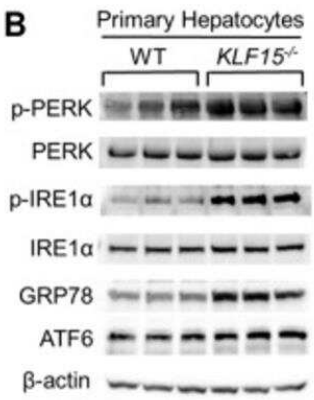
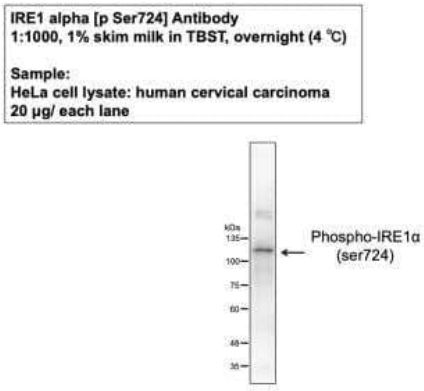
Product Description	
Description	When detecting phospho-IRE1 Alpha (Ser-724) using NB100-2323, it is recommended to normalize its band intensity/immunoreactivity with total-IRE1 alpha. NB100-2324 and NB110-59971 can be used for the detection of endogenous total IRE1 alpha.
Host	Rabbit
Gene ID	2081
Gene Symbol	ERN1
Species	Human, Mouse, Rat, Porcine, Drosophila, Goat, Mammal, Monkey, Primate, Rabbit, Golden Syrian Hamster
Reactivity Notes	Use in Porcine reported in scientific literature (PMID:35492579).Drosophila reactivity reported in scientific literature (PMID: 31641108). Goat reactivity reported in scientific literature (PMID: 29046053). Use in Golden Syrian Hamster reported in scientific literature (PMID:31167774).
Specificity/Sensitivity	NB100-2323 IRE1 alpha [p Ser724] Antibody detects IRE-1 alpha when phosphorylated at Ser724 residue.
Immunogen	This IRE1 alpha [p Ser724] antibody was raised against a synthetic peptide surrounding the phosphorylated serine 724 of the human IRE1 alpha protein. [Swiss-Prot #O75460]
Notes	Take a look at IRE1 alpha Antibody Sampler Pack [NBP2-50067] if you want to try 25ug aliquots of phospho-IRE1 alpha (Ser724) Antibody [NB100-2323SS] and total IRE1 alpha Antibody [NB100-2324SS] before purchasing 100ug full vials.

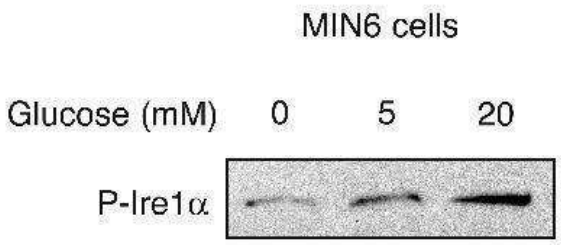
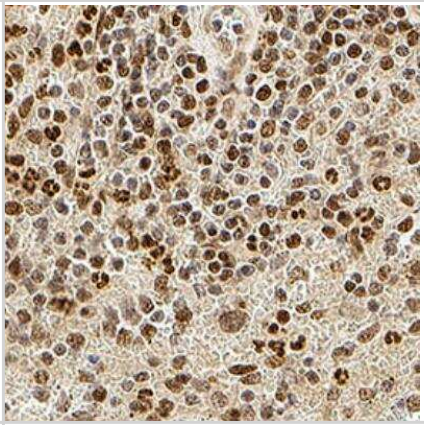
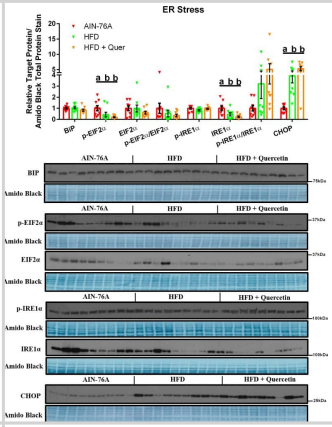
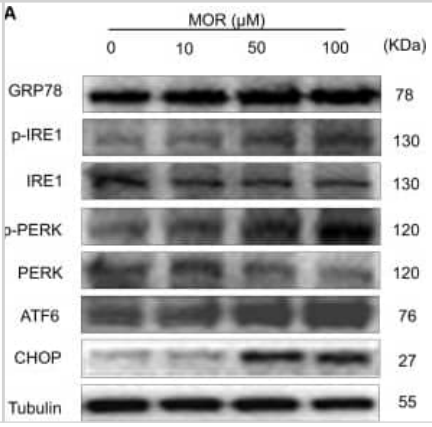
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, ELISA, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, In vitro assay, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:500 - 1:1000, Simple Western, ELISA 1:100 - 1:2000, Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence 1:10 - 1:500, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:10 - 1:500, Immunohistochemistry-Frozen 1:10 - 1:500, Immunoblotting, In vitro assay, Chromatin Immunoprecipitation (ChIP), Knockdown Validated

Application Notes	This IRE1 pS724 antibody is useful for WB, ELISA, and IHC-P sections (PMID: 19264902). Use in IHC-Frozen reported in scientific literature (PMID: 24823368). Use in ICC reported in scientific literature (PMID: 26762342). Use in immunoblotting reported in scientific literature (PMID: 24089213). Use In vitro assay reported in scientific literature (PMID: 24327956). Use in chromatin immunoprecipitation reported in scientific literature (PMID: 25225294). Knockdown Validated reported in scientific literature (PMID: 31159306). See Simple Western Antibody Database for Simple Western validation: Tested in lungs; separated by size; antibody dilution of 1:50.

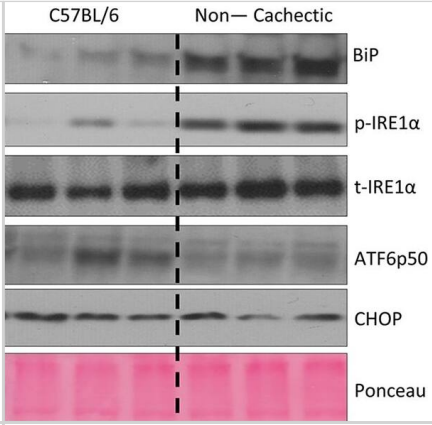
Images

P-Ire1a activation correlates with WFS1 cell expression. Cryosections of retina from 12 month old Wfs1+/+ and Wfs1-/- mouse were immunostained with anti-P-Ire1a antibody (red). DAPI was used for staining of cell nuclei (blue). RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer, RGC, retinal ganglion cells. Scale bars=50 um. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0097222) licensed under a CC-BY license.	
Hepatic ER stress markers with cachexia progression. ER stress markers Bip, IRE1a, ATF6p50 and CHOP were examined in the liver of non, pre and severely cachectic mice. (n = 6-8 per group, p < 0.05) Dotted line indicates levels of Non-cachectic mice. Non = Non-Cachectic Apc Min/+ Sev = severely cachectic Apc Min/+; * denotes significantly different from Non-cachectic Apc Min/+ Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0119888) licensed under a CC-BY license.	
IRF-1 plays a central role in ER stress-mediated modulation of VCAM-1 expression by TGRL. HAEC were conditioned for 4 hr with TNFa (0.3 ng/ml) E: CHOP knockdown decreased TNFa-induced VCAM-1 expression. n=4. **P<0.01 vs. siCtrl+TNFa. Shown are representative blots from 3 independent experiments with similar results. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0078322) licensed under a CC-BY license.	

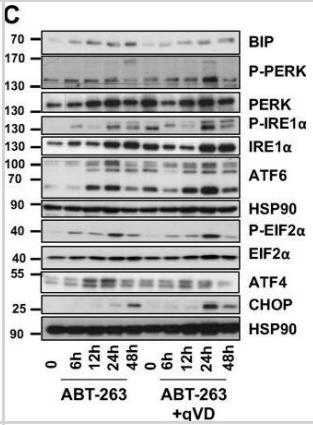
Analysis using HRP conjugate of NB100-2323. Detection of phosphorylated IRE-1 alpha using NB100-2323. Lane 1: COS-7 untransfected Lane 2: COS-7 expressing wild-type IRE1 alpha Lane 3: COS-7 expressing kinase-dead IRE1 alpha. Theoretical molecular weight: 110 kDa.	
Analysis of anti-IRE1 alpha (pSer724) using Lot AN and AO of NB100-2323. HeLa cells were treated (+) or untreated (-) with 10 mM DTT for 60 min to activate the UPR. Total protein was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% BSA in TBST. The membrane was probed with 2.0 ug/ml antibody in 5% BSA, and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Theoretical molecular weight: 110 kDa.	
Regulation of the unfolded protein response by KLF15. Western analysis of UPR activity in WT versus KLF15-/- primary hepatocytes. Hepatocytes were isolated from standard chow-fed 4-month-old male WT and KLF15 -/- mice. Two individual experiments were performed in triplicate; each lane indicates a technical replicate. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0077851) licensed under a CC-BY license.	
HeLa cell lysate, 20 ug. Antibody at 1:1000, 1% skim milk in TBST, overnight incubation at 4C. WB image submitted by a verified customer review.	

Detection in Min6 cells which were treated with different concentrations of glucose for 3 hours prior to lysates preparation.	
IRE1 (pS724) was detected in immersion fixed paraffin-embedded sections of human spleen using Rabbit Anti-Human IRE1 (pS724) polyclonal Antibody (Catalog # NB100-2323) at 1:300 for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the perinuclear cytoplasm in splenocytes.	
Hepatic ER Stress.Representative hepatic western blots of BiP, phosphorylated (Ser51), total EIF2α and phosphorylated:total EIF2α, phosphorylated (Ser724), total IRE1α and phosphorylated:total IRE1α, and CHOP (n = 9). Diets not sharing a common letter differ significantly from one another (P≤.05).	
Morphine suppressed 6-OHDA-induced ER stress through activation of UPR. (A,B) Morphine induced UPR in SH-SY5Y cells. Protein levels of GRP78, p-IRE1α, IRE1α, p-PERK, PERK, ATF6, CHOP and Tubulin in SH-SY5Y cells were analyzed (A) and quantified (B) by western blot. □P < 0.05, □□P < 0.01, □□□P < 0.001 vs. control.	

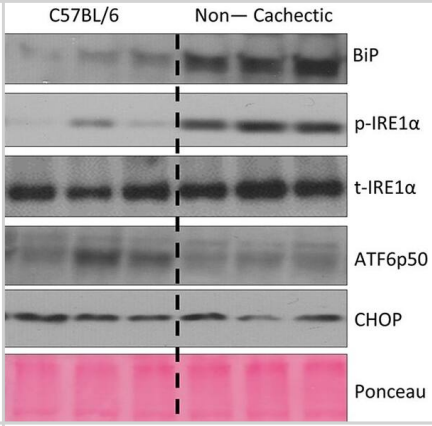
Effect of cancer on ER stress markers.Bip1, IRE-1, ATF-6 p50 and CHOP expression in the liver of non—cachectic ApcMin/+ mice (N = 6 per group), compared to healthy C57BL/6 mice. Dotted line on the western blot indicates two different sections of the same gel. Values are expressed as Mean ± SE. * denotes significantly different from the healthy C57BL/6 mice as analyzed by a pre—planned t—test. $p < 0.05$.



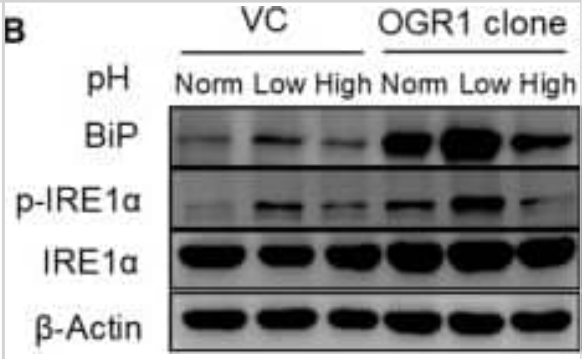
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ABT-263 induces ER stress & activation of the unfolded protein response.a Western blot of ER stress molecules in uveal melanoma cells treated with ABT-263 5 μM for 24 h & 48 h. HSP90 is used as a loading control. Representative western blots are shown. b XBP1 splicing in uveal melanoma cells exposed to ABT-263 5 μM for 15 h. *P-value < 0.05, **P-value < 0.01. c Kinetic analysis of ER stress proteins in Mel270 cells treated with ABT-263 5 μM in the absence or presence of qVD-OPh 20 μM. HSP90 is used as a loading control. Representative western blots are shown. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32337074), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



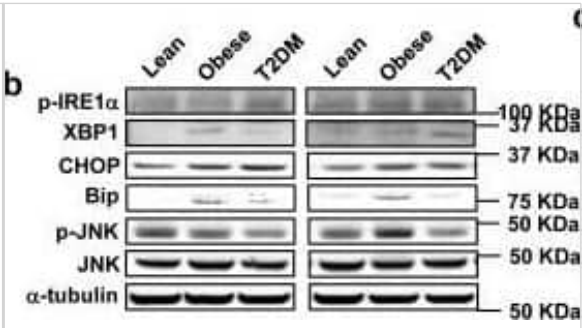
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Effect of cancer on ER stress markers.Bip1, IRE-1, ATF-6 p50 & CHOP expression in the liver of non—cachectic ApcMin/+ mice (N = 6 per group), compared to healthy C57BL/6 mice. Dotted line on the western blot indicates two different sections of the same gel. Values are expressed as Mean ± SE. * denotes significantly different from the healthy C57BL/6 mice as analyzed by a pre—planned t—test. $p < 0.05$. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0119888), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



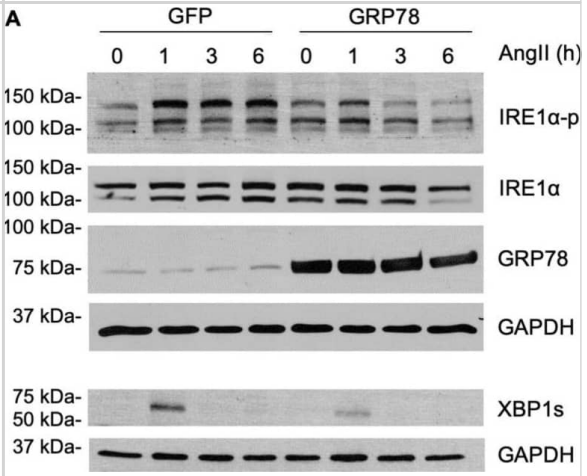
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ER stress is induced by acidosis activated OGR1-mediated signalling. Caco-2 cells subjected to different pH medium, following 4–6 h incubation in pH 7.6 serum free medium.(B) After 24 h pH shift, total protein was isolated & WB was performed. The results representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31996710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



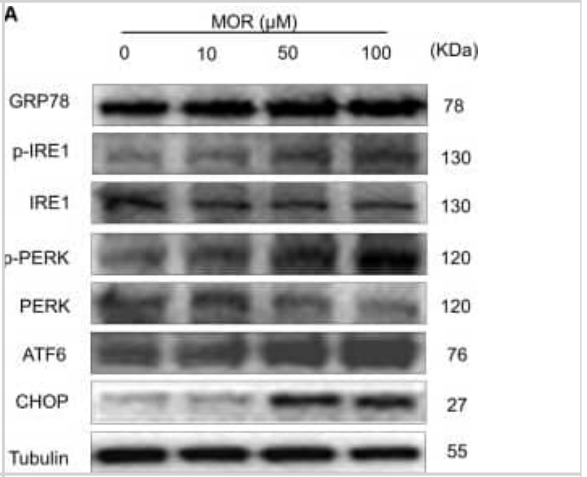
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Expression of ER stress markers in mouse & human skeletal muscle(a) TA muscle lysates from high fat fed mice were used to determine the expression of ER stress markers by Western blot analysis (n=6). (b-g) Western blot analysis was done to measure proteins involved in ER stress in vastus lateralis muscle from lean, obese, or diabetic subjects (n=6-13). Data are the means ± S.E.M. * indicates p<0.05 & ** indicates p<0.01 vs. chow or lean controls, using Student's t-test (a) & One way ANOVA. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms2851), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



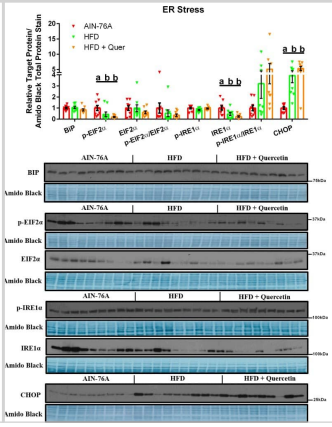
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - The inositol-requiring enzyme 1 α (IRE1α)/ X-box-binding-protein 1 spliced isoform (XBP1s) arm of unfolded protein response (UPR) is induced by angiotensin II in VSMCs. (A–C) The rat aortic VSMCs infected with adenovirus encoding GRP78 or control GFP (100 moi) for 48 h were stimulated with 100 nM AngII (AII) for 1–6 h & immunoblotting was performed as indicated. (A) Representative blots from 4 independent experiments. (B) Signal intensity was used to calculate the expression ratio of XBP1s to GAPDH. (C) Signal intensity was used to calculate the IRE1α Ser724 phosphorylation ratio to the total IRE1α. The bars in the graphs show the mean ± SD from 4 independent experiments. * indicates p < 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32679678), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



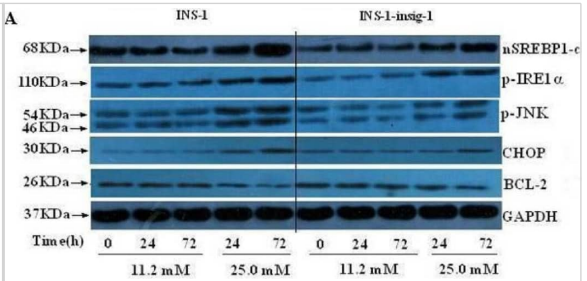
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Morphine suppressed 6-OHDA-induced ER stress through activation of UPR. (A,B) Morphine induced UPR in SH-SY5Y cells. Protein levels of GRP78, p-IRE1α, IRE1α, p-PERK, PERK, ATF6, CHOP & Tubulin in SH-SY5Y cells were analyzed (A) & quantified (B) by western blot. □P < 0.05, □□P < 0.01, □□□P < 0.001 vs. control. (C,D) Protein levels of GRP78, CHOP & Actin in SH-SY5Y cells were analyzed (C) & quantified (D) by western blot in the indicated groups. □P < 0.05, □□P < 0.01 vs. control & #P < 0.05, ##P < 0.01 vs. 6-OHDA-treated group. All data are expressed as mean ± SEM. Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/article/10.3389/fnmol.2018.00120/full), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



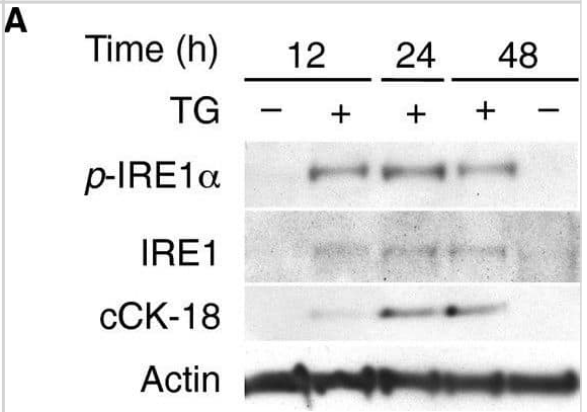
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Hepatic ER Stress.Representative hepatic western blots of BiP, phosphorylated (Ser51), total EIF2α & phosphorylated:total EIF2α, phosphorylated (Ser724), total IRE1α & phosphorylated:total IRE1α, & CHOP (n = 9). Diets not sharing a common letter differ significantly from one another (P≤.05). Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0167979), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



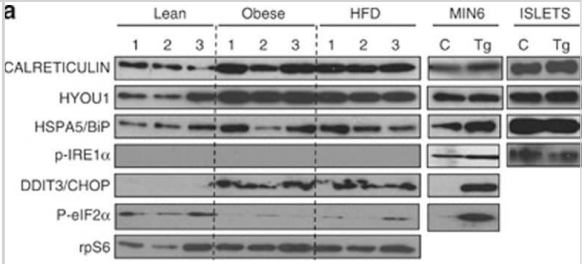
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Effect of Insig-1 overexpression on nSREBP-1c & proteins related to ER stress pathway in INS-1 cells. INS-1 & INS-1-Insig-1 cells were stimulated with 11.2 mM & 25.0 mM glucose for 0, 24 & 72 h. A) nSREBP-1c & p-IRE1α pathway of ER stress related proteins were detected by Western blot. GAPDH was used as control for protein loading. Equal amount of proteins (50 µg) were loaded. B) Relative protein expression compared to GAPDH, data are the means ± SE of three independent experiments; *, P < 0.05, **, P < 0.01 VS INS-1 cells at the same time point. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/21843373), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



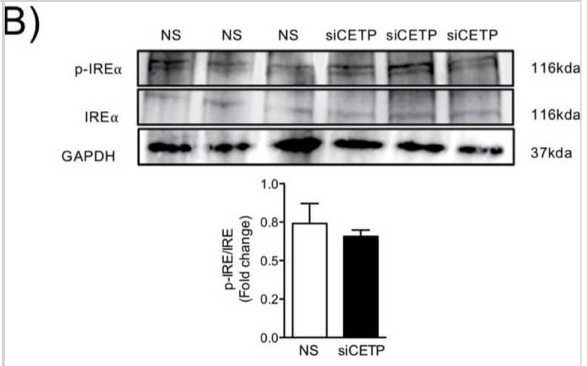
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - cCK-18 is formed following activation of the UPR. A. Immunoblots for phospho-IRE1 (p-IRE1), total IRE1 (IRE1), & cleaved cytokeratin 18 (cCK-18) showing time-dependent increase of cCK-18 in A549 cells exposed to 0.5 µM of thapsigargin (TG, +) for various lengths of time. Immunoblots showing an increase in total & phospho-IRE1 confirm thapsigargin activates the UPR in A549 cells compared to vehicle controls (−). B. Immunoblots for cCK-18 showing time-dependent appearance of cCK-18 in annexin V– or V+ A549 cells exposed to 0.5 µM of thapsigargin for various lengths of time. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23167970), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



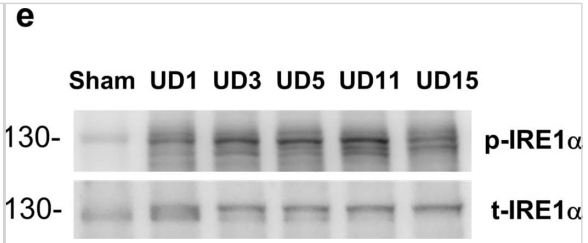
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Protein expression of downstream markers of ER stress in islets from lean, obese & high-fat-diet-fed fZDF rats. Protein lysates from islets isolated from three age-matched fZDF rats (obese), HF-fZDF (HFD) rats & their heterozygous lean littermates (lean) were separated by SDS-PAGE & analysed by western blotting. As controls, lysates from the islets isolated from Wistar rats or MIN6 cells treated with 1 µM thapsigargin for 2 h or sorbitol for 1 h were run alongside. Proteins were detected using antisera against (a) Calreticulin, HYOU1, GRP78, p-IRE1α, p-eIF2α & CHOP & as protein loading control rpS6 & (b) p-JNK & total JNK1/2 as loading control. Western blots were quantified using Image-J software. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/nutd201335), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



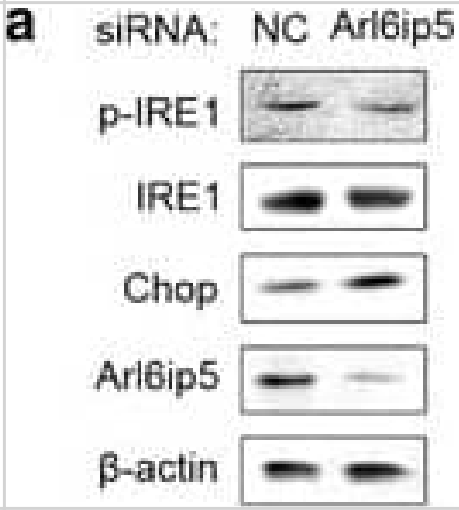
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - CETP inhibition reduces endoplasmic reticulum stress. HAECs transfected with CETP or control scrambled siRNA. (A) Western blot analysis of PERK (n = 3, * p < 0.01) & qPCR analysis of ATF6 mRNA. (B) Phosphorylated & total IRE (n = 3, ns). (C) Representative microscopic images of HAECs stained with GADD153, n = 5, & (D) Grp78/BIP, & DAPI. Scale bar, 10 µm. Mean ± SEM, n = 5, * p < 0.05. HAECs, human aortic endothelial cells. CETP, cholesteryl ester transfer protein. NS, non-silenced. siCETP, silenced CETP. PERK, protein kinase RNA-like ER kinase. ATF6, Activating transcription factor 6. IRE, inhibitor resistant esterase. BIP, binding immunoglobulin protein. GADD153, growth arrest- & DNA damage-inducible gene 153. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33430172), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



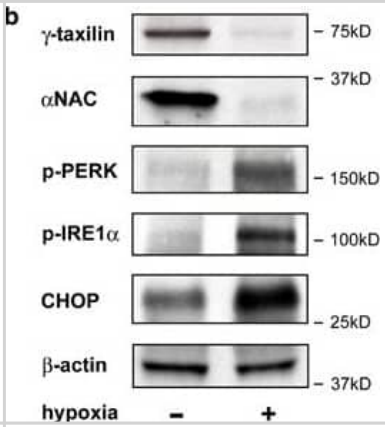
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Activation of UPR signaling pathways after UIRI. a & b The expression of p-PERK & t-PERK in kidneys of UIRI mice were evaluated with western blot analysis & quantified. c UIRI induces the activation of ATF6 as demonstrated by enhanced expression of cleaved ATF6 (cATF6). GAPDH was used as an internal control. d Fold-change expression of cATF6 in kidneys of UIRI mouse as compared with that of sham group mouse. e & f The expression of p-IRE1 & t-IRE1 in mice kidneys were evaluated with western blotting & quantified. Data are expressed as means ± SEM, n = 3 ~ 6 in each group. *P < 0.05, **P < 0.01, & ***P < 0.001, as compared with sham group Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35765067), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



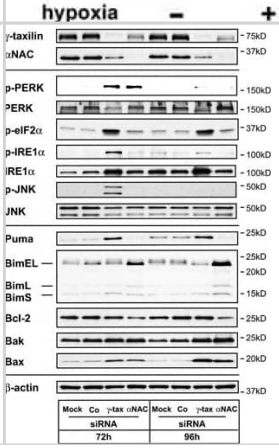
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Apoptosis mediated by Arl6ip5 interference is regulated by Chop. (a) Immunoblotting was used to detect the protein level for p-IRE1α, IRE1, Chop, Arl6ip5 & β-actin in the UAMS-32 cell with NC-siRNA or Arl6ip5-siRNA treatment for 72 h. Representative results were shown from three independent experiments. (b–e) UAMS-32 cells first received NC-siRNA or Chop-siRNA for 24 h, then the medium was changed & further received NC-siRNA or Arl6ip5-siRNA for 72 h. Q-PCR was used to analyze the mRNA levels of Chop (b), GADD34 (c) & Trib3 (d). The apoptotic cells were stained with Annexin-V & the proportion were calculated (e). n=4. In all panels, error bars represent the mean±S.E.M. **P<0.01, *P<0.05 based on ANOVA Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2014427), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



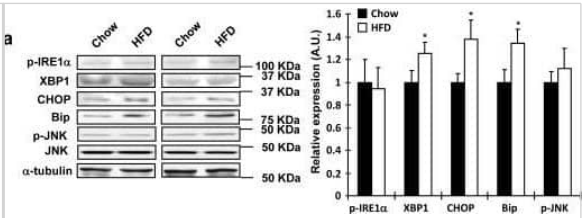
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Downregulation of γ-taxilin & αNAC, & ER stress responses in mouse brain slice cultured under hypoxic conditions. (a) Immunohistochemistry shows downregulation of γ-taxilin & αNAC expression in brain slices cultured for 48 h under hypoxic conditions. Scale bar, 20 μm. (b) Western blot shows downregulation of γ-taxilin & αNAC, phosphorylation of PERK & IRE1α & induction of CHOP in mouse brain slices cultured for 48 h under normoxic (−) or hypoxic conditions (+) Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25880086), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



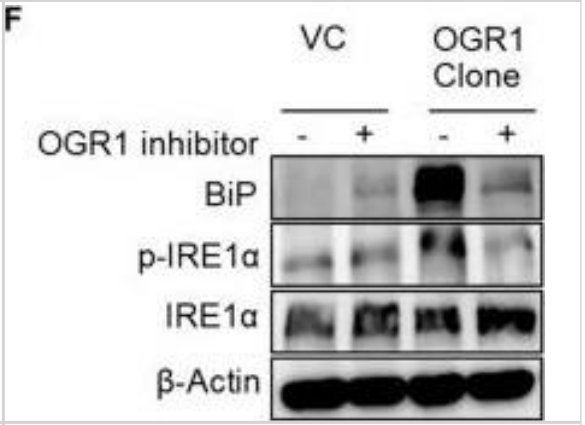
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Differential ER stress response pathways initiated by γ-taxilin or αNAC depletion. Proteins involved in UPR sensor (PERK, IRE1α), eIF2α, JNK, Bcl-2, & Bcl-2-related protein (Puma, Bim, Bak, & Bax) signaling pathways were analyzed by Western blotting of HeLa S3 cells that were depleted of γ-taxilin or αNAC by RNA intereference for 72 & 96 h. Upper panels, taxilin & NAC proteins; middle panels, UPR proteins; lower panels; apoptotic proteins; & bottom panel, β-actin Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25880086), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



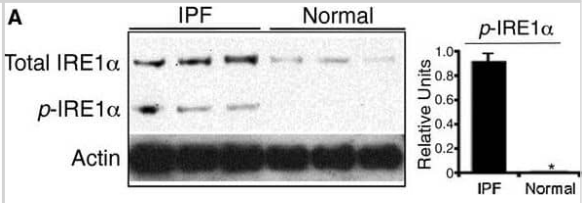
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Expression of ER stress markers in mouse & human skeletal muscle(a) TA muscle lysates from high fat fed mice were used to determine the expression of ER stress markers by Western blot analysis (n=6). (b-g) Western blot analysis was done to measure proteins involved in ER stress in vastus lateralis muscle from lean, obese, or diabetic subjects (n=6-13). Data are the means ± S.E.M. * indicates p<0.05 & ** indicates p<0.01 vs. chow or lean controls, using Student's t-test (a) & One way ANOVA. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms2851), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



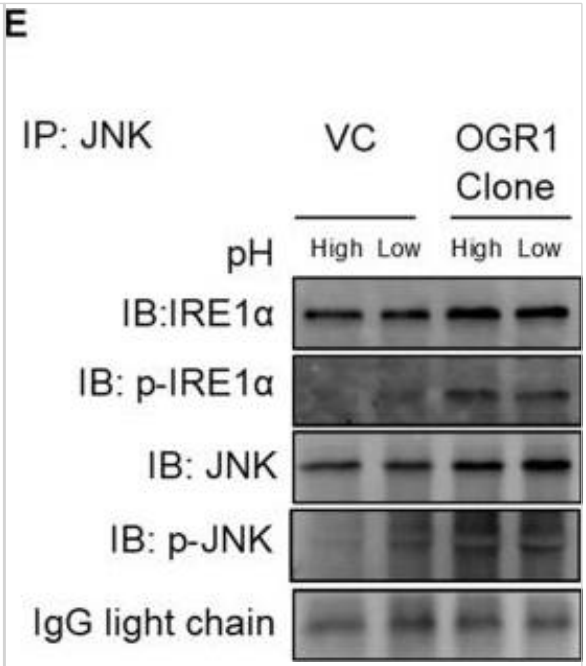
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ER stress is induced by acidosis activated OGR1-mediated signalling. Caco-2 cells subjected to different pH medium, following 4–6 h incubation in pH 7.6 serum free medium. (F) A specific small molecule OGR1 inhibitor (10 μM) was tested & the cells subjected to low pH for 24 h, following 4–6 h incubation in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & WB performed. Results representative of two independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31996710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



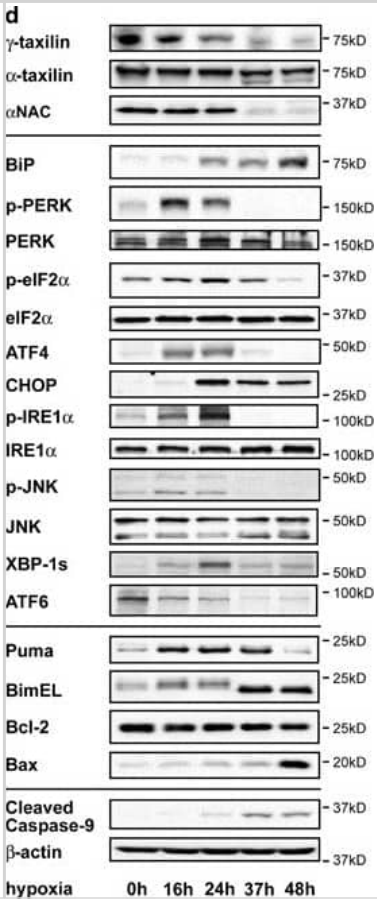
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - The UPR is activated in type II AECs isolated from IPF lungs. Phosphorylated IRE-1α (panel A) & spliced XBP-1 (panel B) were present in increased amounts in AECs from 3 different IPF lungs, but not in the 3 control subjects (* P < 0.05, **P = 0.0495 by Wilcoxon rank sum test). # indicates amplicon of unspliced XBP1 digested with Pst1. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23167970), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



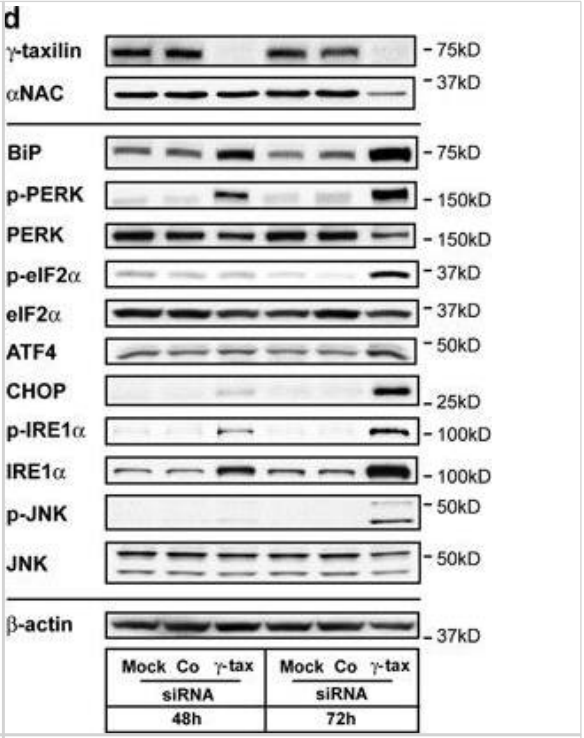
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ER stress is induced by OGR1 via IRE1α/JNK signalling. (A) Caco-2 cells were subjected to different pH medium, with or without an OGR1 inhibitor (10 μM), following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (B) Caco-2 cells were subjected to different pH medium After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (C) Caco-2 cells were subjected to different pH medium with or without a JNK inhibitor (10 μM) following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (D) Caco-2 cells were starved & subjected to an acidic pH with or without a JNK inhibitor as described in (C). After 24 h pH shift, total RNA was isolated & mRNA expression was investigated by qPCR. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Data are presented as means ± SE of three independent experiments (***$p < 0.001$). (E) Caco-2 cells were starved & subjected to different pH medium following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & co-IP using IRE1α antibody & JNK antibody was performed, followed by immunoblotting. Results are representative of two independent experiments. pH conditions: High pH 7.5–7.8; Normal pH 7.2–7.4; Low pH 6.6–6.8. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31996710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



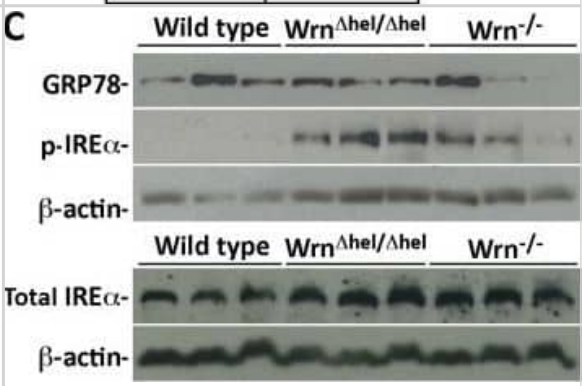
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Downregulation of γ-taxilin & αNAC & ER stress responses in hypoxic cells. (a) Phase-contrast micrographs of SK-N-SH cells cultures in hypoxic conditions for 0–48 h. Floating cells were evident by 37 h after the beginning of hypoxic treatment. (b) FACS (fluorescence-activated cell sorting) analysis of apoptosis in hypoxic SK-N-SH cells. Bars in the FACS profiles (left panel) indicate the fraction locations of annexin-positive cells. Bar graph indicates the relative numbers of annexin-positive cells (right panel). Bar graph data are shown as means±S.D. (n=3). *Significantly different from controls (0 h) ($P<0.001$, Tukey–Kramer test). (c) DAPI (4',6-diamidino-2-phenylindole) staining shows fragmented nuclei of apoptotic SK-N-SH cells after hypoxic treatment. Scale bar, 10 μm. (d) Downregulation of γ-taxilin & αNAC & induction of ER stress responses in hypoxic SK-N-SH cells. Western blot analysis was performed 0 to 48 h after hypoxic treatment of cells. Upper panels, taxilin & NAC proteins; middle panels, UPR proteins; lower panels, apoptotic proteins; & bottom panels, cleaved caspase-9 & β-actin proteins. (e) MG-132 treatment restore the γ-taxilin & αNAC protein level in hypoxic (16 h) HeLa S3 cells. MG-132 (−), DMSO; MG-132 (+), 20 μM. (f) Caspase inhibition does not affect the hypoxia-induced downregulation of γ-taxilin & αNAC in HeLa S3 cells. (g) Colocalization of γ-taxilin & αNAC in normoxic (0 h) & hypoxic (8 h) HeLa S3 cells. Note that the colocalization fraction increases with the appearance of apoptotic phenotypes. Scale bars, 10 μm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25880086), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



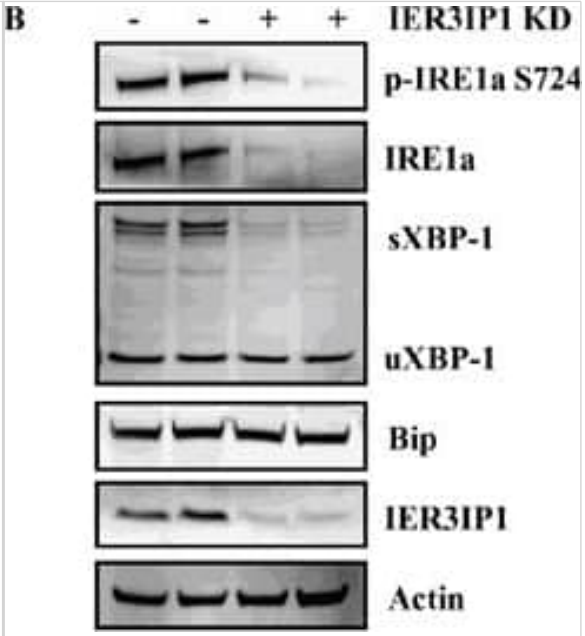
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - γ-Taxilin ablation causes ER stress responses & apoptosis in normoxic cells. (a) Knockdown of γ-taxilin expression by small interfering RNA (siRNA) in HeLa S3 cells. Two different siRNA constructs are equally effective. HeLa S3 cells were treated with solvent alone (Mock), control siRNA (Co), or γ-taxilin siRNA (γ-tax-1 or -2) for 96 h. (b) Induction of apoptosis by γ-taxilin ablation in HeLa S3 cells. γ-Taxilin ablation causes apoptosis in HeLa S3 cells, but Mock or Co treatment did not. Bar graph shows fractions of annexin-positive cells (means±S.D., n=3). *Significantly different from Mock- or Co treatment (P <0.001, Tukey–Kramer test). (c) Confocal microscopy demonstrates coincidence of γ-taxilin depletion & apoptotic nuclei in HeLa S3 cells treated with γ-taxilin siRNA. Scale bar, 10 μm. (d) γ-Taxilin depletion triggers ER stress responses in HeLa S3 cells. Upper panels, taxilin & NAC proteins; middle panels, UPR proteins; & lower panel, β-actin. (e) γ-Taxilin ablation induces accumulation of ubiquitinated proteins in HeLa S3 cells. Cell lysates were analyzed on 7.5% SDS-PAGE, followed by immunoblotting with antibodies specific for anti-mono- & polyubiquitinated conjugates (upper panel) & β-actin (lower panel) Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25880086), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



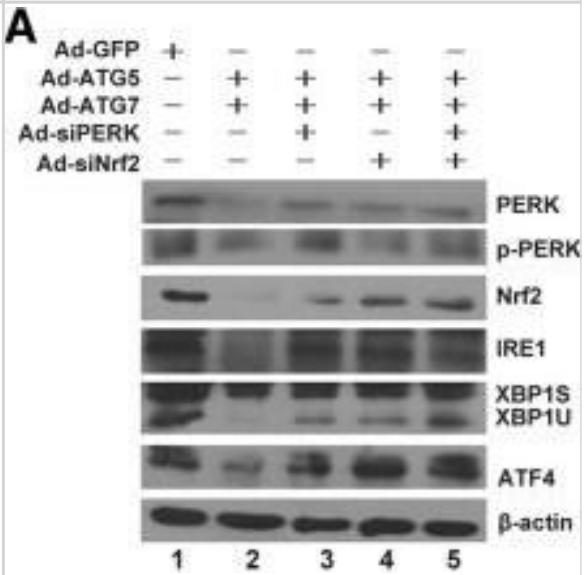
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Localization of the WT & mutant Wrn proteins in different subcellular fractions.(A) Example of western blots showing the presence of the Wrn WT protein in the nuclear fraction & the presence of the WrnΔhel mutant protein mainly in the cytosolic fraction. Fractionations were performed on WT, WrnΔhel/Δhel, & Wrn-/- MEFs. Topoisomerase I & β-tubulin are nuclear & cytoplasmic markers, respectively. As expected the molecular weight of the WrnΔhel mutant protein was lower than the Wrn WT protein. No band was detected in Wrn-/- MEFs fractions with the anti-Wrn polyclonal antibody. (B) Example of western blots showing only the presence of the WrnΔhel mutant protein in total ER & the peroxisomal fractions. Fractionations were performed on WT, WrnΔhel/Δhel, & Wrn-/- liver tissues. Catalase & calreticulin are used as peroxisomal & ER specific markers. This experiment was repeated three times. (C) Protein levels of GRP78, phosphorylated IREα, & β-actin (top panels) & of total IREα, & β-actin (bottom panels) in the spleen of three animals of each genotype. (D) Ratio of GRP78 signal over β-actin signal from western blots. (E) Ratio of IREα over β-actin signal from western blots. (F) Ratio of phosphorylated IREα signal over β-actin signal from western blots. Experiments were performed in triplicates. Bars in all histograms represent SEM. Tukey post ANOVA test P-values are shown (*P < 0.05 & **P < 0.01). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26447695), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



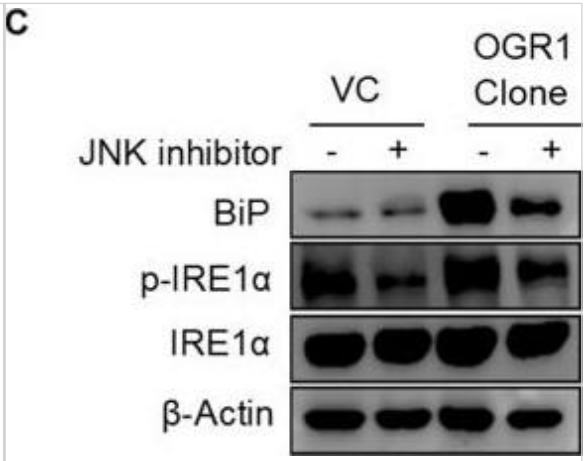
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - IER3IP1 suppression decreases the UPR activation(A) mRNA levels of IRE1α, Perk & Atf6 in IER3IP1 KD cells. 4 days after infection with IER3IP1 shRNA lentivirus, mRNA levels of IRE1α, Perk & Atf6 in IER3IP1 KD cells were determined by qRT-PCR. *P<0.05, **P<0.01 compared to control group. Values are mean ± SEM. (B) Effect of IER3IP1 suppression on the IRE1α arm of the UPR. 4 days after IER3IP1 KD in MIN6 cells, phosphorylation of IRE1α, Bip, sXBP-1 & uXBP-were determined by Western blot. The loaded proteins in the two "- or +" lines were the same but from the different plates. (C) mRNA levels of downstream targets of sXBP-1 in IER3IP1 KD cells. 4 days after IER3IP1 KD in MIN6 cells, mRNA levels of downstream targets of sXBP-1, Erdj4, p58IPK, EDEM & Sec61a, were determined by qRT-PCR. Values are mean ± SEM. (D) Effect of IER3IP1 suppression on the Perk arm of the UPR. 4 days after IER3IP1 KD in MIN6 cells, phosphorylation of Perk & eIF2a, & ATF4 were determined by Western blot. The loaded proteins in the two "- or +" lines were the same but from the different plates. (E) IER3IP1 KD decreases UPR activation induced by ER stress in MIN6. 4 days after IER3IP1 KD, MIN6 cells were treated with 10 μg/ml tunicamycin (TC) or 1 μM thapsigargin (TG) for 6 hours. Then the expression levels of Chop, sXBP-1, uXBP-1 & IER3IP1 were determined by Western blot. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28915629), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



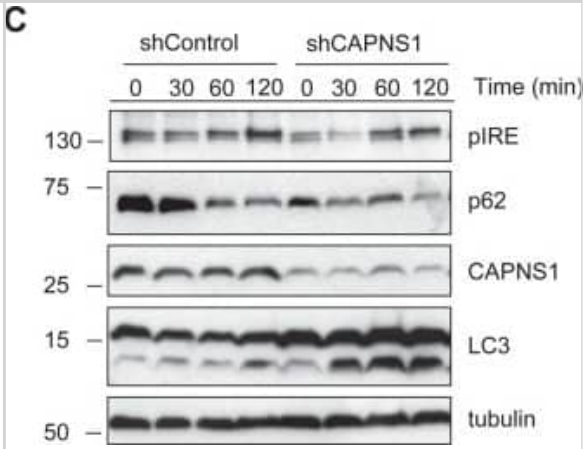
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ATG5 & ATG7 influenced on autophagy, apoptosis & cell cycle through PERK/Nrf2 signaling. a The ER stress proteins were analysed by western blotting in Ad-GFP, Ad-ATG5 + Ad-ATG7,Ad-ATG5 + Ad-ATG7 + siPERK,Ad-ATG5 + Ad-ATG7 + siNrf2 & Ad-ATG5+ Ad-ATG7 + siPERK +siNrf2 induced chondrocytes. b The levels of ER stress proteins were normalized to β-actin. c Determination of autophagy & apoptosis proteins expression by western blotting in Ad-GFP, Ad-ATG5 + Ad-ATG7, Ad-ATG5 + Ad-ATG7 + siPERK,Ad-ATG5 + Ad-ATG7 + siNrf2 & Ad-ATG5+ Ad-ATG7 + siPERK+ siNrf2 induced chondrocytes. dThe levels of related proteins were normalized to β-actin. e FCM analysis was used to calculate the percentage of apoptotic cells at the time point of 24 h. The apoptosis rate were increased when combined treatment with silencing of PERK or Nrf2. Experiments were repeated 3 times, Representative images are shown. f Analysis of cell apoptosis. Data come from 3 independent experiments. g FCM analysis indicated that the S phase percentage were decreased compared to that of the control groups when combine infected with silencing of PERK or Nrf2 in C28I2 cells. Experiments were repeated 3 times, Representative images are shown. h Percentage of cells at each phase in different groups. *P < 0.05, **P < 0.01 compared with the controls. Values are means ± SD n = 3). (1:Ad-GFP, 2:Ad-ATG5 + Ad-ATG7, 3:Ad-ATG5 + Ad-ATG7 + siPERK, 4:Ad-ATG5 + Ad-ATG7 + siNrf2, 5:Ad-ATG5 + Ad-ATG7 + siPERK+ siNrf2) Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31060556), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



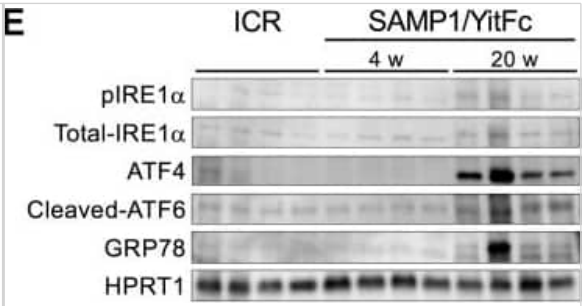
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - ER stress is induced by OGR1 via IRE1α/JNK signalling. (A) Caco-2 cells were subjected to different pH medium, with or without an OGR1 inhibitor (10 μM), following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (B) Caco-2 cells were subjected to different pH medium After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (C) Caco-2 cells were subjected to different pH medium with or without a JNK inhibitor (10 μM) following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & Western blot performed. Results are representative of two independent experiments. (D) Caco-2 cells were starved & subjected to an acidic pH with or without a JNK inhibitor as described in (C). After 24 h pH shift, total RNA was isolated & mRNA expression was investigated by qPCR. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Data are presented as means ± SE of three independent experiments (***$p < 0.001$). (E) Caco-2 cells were starved & subjected to different pH medium following 4–6 h in pH 7.6 serum free medium. After 24 h pH shift, total protein was isolated & co-IP using IRE1α antibody & JNK antibody was performed, followed by immunoblotting. Results are representative of two independent experiments. pH conditions: High pH 7.5–7.8; Normal pH 7.2–7.4; Low pH 6.6–6.8. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31996710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



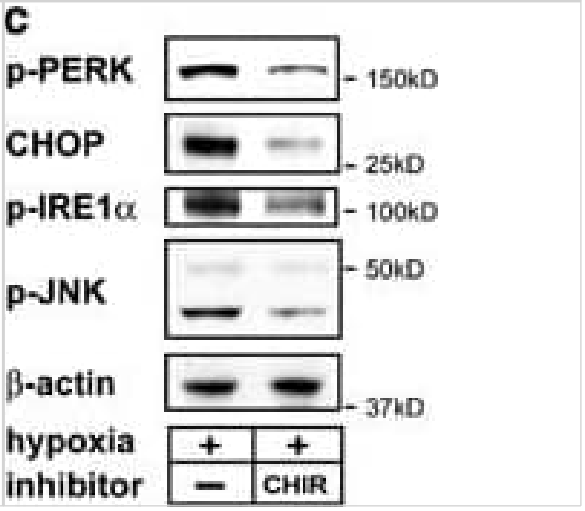
Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Thapsigargin treatment induces calpain activation & autophagy. (A) Control & shCAPNS1 cells were treated with 100 nM thapsigargin for 10, 20 & 30 min & the lysates subjected to western blot analysis to quantify the precursor & active form of CAPN1. The ratio between active CAPN1 & tubulin is reported below each lane. (B) Time course measurement of intracellular calcium concentration. The plot indicates the ratio of mean Indo Violet/Indo Blue emission values of each cell population at individual time points (minutes). The ratio corresponds to the relative calcium concentration of control & shCAPNS1 U2OS cells before & after addition of thapsigargin (100 nm final). Moving average was used as smoothing method. (C) shCAPNS1 cells were treated with 100 nM thapsigargin for 30, 60 & 120 min & the lysates subjected to western blot analysis to detect LC3, p62, pIRE, CAPNS1 & tubulin. (D) Levels of p62 & LC3 normalized to tubulin levels. Image collected & cropped by CiteAb from the following publication (https://journals.biologists.com/bio/article/doi/10.1242/bio.022806/256614/Calpain-mobilizes-Atg9-Bif-1-vesicles-from-Golgi), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Abnormal Paneth cells show ER stress.(A, B) Representative transmission electron microscopy images of Paneth cells at the base of ileal crypts in (A) ICR & (B) SAMP1/YitFc mice. Scale bars indicate 2 μm. (C, D) Quantitative analysis of (C) granule number & (D) ER lumen diameter in Paneth cells (n = 3/each week for SAMP1/YitFc mice). For the measurements, three Paneth cells were randomly selected from each mouse. (E) SDS–PAGE Western blot analysis of ER stress markers, pIRE1α, ATF4, cleaved-ATF6, & GRP78 in ileal crypts (n = 4/each group). Total-IRE1α & HPRT1 was used as loading control. (F) Relative expression level of ER stress markers calculated from the band intensity. Error bars represent mean ± SEM. (C, D, F) Statistical significance was evaluated by t test in (C, D), & one-way ANOVA followed by Tukey's post hoc test in (F). $P < 0.05$ was considered statistically significant. *$P < 0.05$, †$P < 0.01$, §$P < 0.001$. E, ER; G, granules; N, nucleus; n.s., not significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32345659), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: IRE1 alpha [p Ser724] Antibody - BSA Free [NB100-2323] - Hypoxia-induced downregulation of γ-taxilin & αNAC & the subsequent ER responses are GSK-3β-dependent. (a) GSK-3β activation in hypoxia. Western blot analysis shows downregulation of phosphorylated (Ser 9) GSK-3β (phospho-GSK-3β) in hypoxic (0–48 h) SK-N-SH & hypoxic (0–24 h) HeLa S3 cells. (b) Inhibition of GSK-3β activation by lithium chloride (LiCl, 100 mM) or CHIR-99021 (CHIR, 15 or 30 μM) maintained γ-taxilin & αNAC protein levels in hypoxic SK-N-SH & HeLa S3 cells. (c) GSK-3β inhibition suppresses the expression of ER stress response proteins that are induced in hypoxic SK-N-SH cells. (d) GSK-3β RNA interference almost completely blocked the protein expression & restored the expression of γ-taxilin & αNAC proteins in hypoxic HeLa S3 cells Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25880086), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Huber M, Widener A, Cuaycal A et al. Beta cell dysfunction occurs independently of insulitis in type 1 diabetes pathogenesis. Cell reports 2025-08-26 [PMID: 40875294]

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