On March 2024 Dr Laurie Glimcher decided to retract a paper published in Science in 2006 coauthored with Dr Hetz.

After finishing the institutional process at the University of Chile to investigate Pubpeer allegations two years ago, we as a laboratory started a process to perform formal actions and contacted the editorials of several journals, resulting in multiple corrections, in addition to improving work standards and making available responses to criticism received. In the case of this article, Dr Hetz sent the information that we were able to gather to Dr Glimcher in 2022 (see email correspondence), so that she could take actions on the matter as senior author. Unfortunately, Dr Glimcher never took formal actions to amend the article until recently. At that time, we posted the information we had available on our website for public review. We requested the original materials to amend the article (see email correspondence). However, since that work was performed 2 decades ago, there were no backups of original data, nor laboratory notebooks to trace the source of errors and/or manipulations. Importantly, practically all of the questioned figures involved supplementary data, showing negative results (no effects) or confirmations of previous published studies, and did not affect in any manner the central conclusions of the study.

In August of last year, Dr Glicmher contacted us to address the issue and we reached an agreement where we would repeat the experiments in question, and then take a decision. Dr Glimcher gave us a period of 6 months (end of February 2024) to carry out this task. In this process, in addition to repeat most of the requested experiments, **we generated additional confirmatory data with new methods of the field that is available as an ongoing confirmatory study in the *bioRxriv* repository (https://www.biorxiv.org/content/10.1101/2024.03.26.586784v1)**. In this report, we used up to date techniques to demonstrate that the biological phenomenon described is solid. On the other hand, it is important to mention that in 2012 we published a complete study that confirmed the proposed model in *Science 2006* and added new components to the pathway (Rodriguez et al EMBO 2012; PMID: 22510886).

In January of this year, an English blogger and *Pubpeer* questioned multiple scientific articles from Dana-Farber Cancer Institute (DFCI) as a center using artificial intelligence. Many researchers at the center, including Dr Glimcher, the CEO of the Institute, were questioned and our *Science* 2006 paper reappeared because unfortunately it remained uncorrected.

In January of this year, as a result of what happened to DFCI that was public, the editors of *Science* journal contacted us to make a formal correction and provide additional explanations, indicating in their email that **they themselves had reviewed the information posted by us years ago and considered the *PubPeer* comments solved and the issue closed** (see attached email extract). In February this year we submitted all the experiments that we were able to carry out, in addition to the additional studies that we posted in bioXriv. Unfortunately, Dr. Glimcher was not satisfied, considering the work incomplete and that essential controls were missing without providing any specific comments. I requested more time, and also proposed complementary solutions such as asking other laboratories to repeat key experiments or analyze the data generated by us by other labs working on the subject. Dr. Glimcher did not agree and proceed with the retraction of the study.

Dear Glimcher and Dr Hetz,

I hope that this email finds you well.

I'm writing to you with regard to your paper entitled "Proapoptotic BAX and BAK Modulate the Unfolded Protein Response by a Direct Interaction with IRE1α" which was published in *Science* in our 28 April 2006 issue (https://www.science.org/doi/10.1126/science.1123480).

As you are doubtless aware, a number of allegations have been made recently about the data in certain papers published by researchers at the Dana-Farber Cancer Institute.

Concerns have been raised previously about the western blot images in Fig. 1A and SM Figs. 2A, 2B, 2C, 3A, 5E, & 6A in this paper on PubPeer and we were provided in 2021 a copy of explanations from Claudio Hetz regarding evidence of splicing (this was not against journal policy at the time of publication), which were posted on his lab website in 2017. We were satisfied with the information provided and at the time marked these comments on PubPeer as resolved.

Preliminary study

BAX and BAK expression triggers multiple IRE1 signaling outputs under ER stress.

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Abstract

Adaptation to endoplasmic reticulum (ER) stress depends on the activation of the unfolded protein response (UPR) stress sensor inositol-requiring enzyme 1 alpha (IRE1). IRE1 is a central ER stress sensors, that signals through the activation of its RNase domain to catalyze the splicing the mRNA encoding the transcription factor X-box binding protein 1 (XBP1), resulting on the expression of a stable and active transcription factor termed XBP1s. The kinetics and amplitude of IRE1 signaling are regulated by different posttranslational modifications and the physical interaction of different factors. Early studies demonstrated that the expression of the proapoptotic proteins BAX and BAX enhance UPR signaling. However, the possible effects on the RNase activity were not defined. Here we provide preliminary evidence indicating that BAX and BAK deficiency increases the in 10 folds the threshold of ER stress to induce XBP1 mRNA splicing, and the upregulation of its target genes. In addition, the degradation of RIDD substrates was strongly reduced in BAX and BAK null cells. BAX and BAK double deficiency also attenuated the levels of IRE1 phosphorylation under mild ER stress. These results reinforce previous findings indicating that proapoptotic BAX and BAK have alternative functions at the ER regulating the UPR.

Key words: BAX, BAK, ER stress, UPR, IRE1

Introduction

Sustaining proteostasis is fundamental for organismal health, and its deregulation contributes to a series of chronic disease in addition to normal aging[1,2]. The endoplasmic reticulum (ER) is a central node of the proteostasis network involved in protein folding and secretion, in addition to operating as a central site for calcium storage and lipid synthesis. Multiple physiological and pathological conditions favor the accumulation of misfolded proteins in the ER lumen, resulting in a cellular state referred to as ER stress[3]. In fact, chronic ER stress is emerging as a relevant factor contributing to various diseases, including metabolic syndromes, cancer, diabetes, inflammatory diseases, and neurodegeneration[4]. The unfolded protein response (UPR) is the main adaptive mechanism to cope with ER stress and restore proteostasis[5]. Inositol-requiring enzyme 1 alpha (IRE1) is a type I ER transmembrane protein with a serine and threonine protein kinase and endoribonuclease activity, that upon activation, catalyzes the splicing of the mRNA encoding X-box binding protein 1 (XBP1), leading to the expression of a potent transcription factor termed XBP1s (for the spliced form)[5]. XBP1s regulates a cluster of genes involved in different aspects of the secretory pathway, including protein folding, ER-associated degradation (ERAD), protein quality control, among others[6,7]. The RNase activity of IRE1 also degrades selected mRNAs and microRNAs through a process known as regulated IRE1-dependent decay (RIDD), contributing to inflammation, DNA damage, apoptosis, and other processes[8].

There is increasing evidence that the signaling behavior of UPR transducers IRE1, PERK and ATF6 are modulated by the binding to specific factors[3]. Thus, the threshold of ER stress that triggers the UPR is determined by specific interactomes, which may influence the adaptive capacity of a cell and the susceptibility to undergo apoptosis under ER stress. Multiple laboratories have identified positive regulators of IRE1α signalling that function by controlling IRE1α dimerization, oligomerization, phosphorylation and dephosphorylation, impacting the amplitude and kinetics of the signalling response (reviewed in [3]). Our lab reported that several members of the BCL-2 family physically associate with IRE1 to enhance the amplitude of UPR signaling, including proapoptotic BAX and BAK and upstream regulators such as BIM and PUMA[9,10]. In contrast, the antiapoptotic protein BAX-inhibitor 1 BI-1 negatively regulates IRE1, impacting the UPR attenuation process[11]. More than 30 proteins have been identified to bind and regulate IRE1 function in different cellular systems, highlighting non-muscle myosin heavy chain IIB protein, the tyrosine-protein kinase ABL1, the collagen carrier HSP47, the core component of the translocon machinery Sec61PKA, among others (reviewed in [3]). Thus, IRE1 signaling is a

highly regulated process involving distinct checkpoints, defining the threshold to trigger an adaptive UPR or transit into a terminal cell death program.

Early studies showed that BAX and BAK double deficiency (DKO) reduces the expression of XBP1s protein in cells and animals exposed to experimental ER stress, associated with a physical interaction[9]. However, many assays were not available at that time to monitor the IRE1 activation process and its RNase activity. Here we investigated the possible impact of BAX and BAK expression on the endoribonuclease activity of IRE1 was not studied. Here we provide preliminary evidence indicating that the threshold to induce XBP1 mRNA slicing is reduced in BAX and BAK DKO cells. These effects were associated with a reduction in the upregulation of XBP1s target genes, in addition to the down regulation of classical RIDD targets. The phosphorylation of IRE1 was also attenuated as measured using PhosTag assays and non-denaturing electrophoresis. These preliminary results confirm the impact of BAX and BAK expression on IRE1 signaling.

Results

To define the possible impact of BAX and BAK expression on the activity of IRE1, we performed a dose response experiment in wild type (WT) and BAX and BAK DKO murine embryonic fibroblast (MEFs). We exposed cells to different concentrations of tunicamycin (Tm, 0.05-1 μg/ml) and measured XBP1 mRNA splicing after 4 h using conventional RT-PCR. Our results indicated that BAX and BAK DKO cells were 10-folds less sensitive to process of XBP1 mRNA splicing (Figure 1a). These results were confirmed using PCR primers that selectively amplify the processed XBP1s mRNA (Figure 1b). In agreement with these results, the expression of the XBP1s protein as reduced in BAX and BAK DKO cells (Figure 1c).

We also monitored the consequences of BAX and BAK double deficiency on IRE1-reppedent transcriptional responses under ER stress. The upregulation of XBP1s target genes *Erdj4*, *Edem1* and *Sec61* were reduced in BAX and BAK DKO cells treated with Tm (Figure 2a). In addition to catalyze XBP1 mRNA splicing, IRE1 degrades a subset of mRNAs through a process termed RIDD. We monitored the levels of *BlosC1*, a canonical RIDD target, under ER stress, and observed that BAX and BAK double deficiency reduced RIDD activity, suggesting the regulation of distinct signaling outputs (Figure 2b). Similar results were observed when the mRNA levels of the RIDD substrates *Spark* and *Col6a* were monitored by quantitative PCR (Figure 2c). Finally, we monitored other signaling outputs controlled by IRE1. Under ER stress, IRE1 binds TRAF2 to recruit JNK and induce its activation[12]. Phosphorylation of JNK was reduced under ER stress in BAX and BAK DKO cells. However, normal activation was observed in cells treated with

TNF alpha or UV exposure (Figure 3). BAX and BAK controls ER calcium content to regulate cell death, a phenomena that can ge genetically corrected by ovexpressing SERCA. WE used this system to assess the activation of the UPR in BAX and BAK DKO cells. Overexpression of SERCA did not increase the levels of XBP1 mRNA splicing and UPR signaling (suplementary figure 1).

Activation of IRE1 involves its oligomerization into large clusters. We used human HEK293 T cells expressing an inducible form of IRE1 fused to GFP. Then we knocked down BAX, BAK or both together (Figure 4A). After 24 h, IRE1-GFP expression was induced with doxicicline and then 24h later we stimulated cells with Tm. Analisis of IRE1-GFP foci formation indicated a reguction when BAR and BAK were knocked down (Figure 4B and C). IRE1 activation involves the auto-phosphorylation of the kinase domain, resulting on a conformational change that engages its RNase domain. We monitored the phosphorylation of IRE1 using a PhosTag assay. Dose response experiments indicated that BAX and BAK doble deficiency increased the threshold of ER stress required for IRE1 phosphorylation (Figure 5). Similar results were obtained using non-denaturing gels (Figure 5).

Conclusions

IRE1 initiates the most conserved signaling pathway of the UPR, determining the recovery of proteostasis under ER stress. IRE1 function has been implicated on a variety of diseases, and the use of small molecules to target the activity of IRE1 (RNase and kinase) have demonstrated important protective effects in various preclinical models of disease[13]. At the molecular level, a complex network of regulatory checkpoints tightly control IRE1 signaling behavior. The concept of the UPRosome (or IRE1 signalosome) was proposed to illustrate the idea that the activity of IRE1 is regulated by cofactors, in addition to crosstalk with other stress signaling pathways mediated by the assembly of adapter proteins and signaling mediators[14].

Our group have uncovered different IRE1 interactors using unbiased approaches (yeast two hybrids and IP-mass spectrometry analysis) identifying PUMA/BIM as novel regulators of IRE1 upstream of BAX and BAK[10], in addition to the chaperone and collagen carrier Hsp47[15], or the actin regulator Filamin A[16]. We also showed that cABL interacts with IRE1 to control RIDD[17], and or with the IP3R to regulate calcium transfer to the mitochondria and bioenergetics[18]. These examples illustrate the highly dynamic nature of the IRE1 interactome and the vast consequences to UPR regulation and the crosstalk with multiple biological processes. Here we have provided additional preliminary evidence confirming a regulatory role of BAX and BAK on the UPR. These

results will be part of a full study to determine the biochemical aspects involved in the interaction bteween BAX/BAK and IRE1.

Materials and methods

Cell lines

MEF cells used here were described in [9], and maintained in Dulbecco's modified Eagles medium supplemented with 5% fetal bovine serum, non-essential amino acids. The pMSCV-Hygro retrovirus vector expressing IRE1α-HA was previously described[10]. IRE1α contains two tandem HA sequences at the C-terminal domain and a precision enzyme site before the HA tag. COS-1 cells were maintained under standard tissue culture conditions using 10% fetal bovine serum in Dulbecco's modified Eagles medium (DMEM) (Sigma). HEK cells were maintained in DMEM supplemented with 5% fetal bovine serum.

RNA isolation, RT-PCR, and real-time PCR

Total RNA was prepared from cells and tissues using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized with SuperScript III (Invitrogen) using random primers p(dN)6 (Roche). Quantitative real-time PCR reactions employing SYBRgreen fluorescent reagent and/or EvaGreen™ were performed in the Stratagene Mx3000P system (Agilent Technologies, Santa Clara, CA 95051, United States). The relative amounts of mRNAs were calculated from the values of comparative threshold cycle by using Actin as a control and Rpl19 for RIDD. All methods for the *Xbp1* mRNA splicing assay, RIDD and the assessment of XBP1s-target genes used here were previously described[10,11,15]. Real time PCR primers are described in KEY RESOURSCES TABLE.

IRE1α oligomerization assay

TREX cells expressing IRE1α-3F6HGFP WT were obtained from Dr. Peter Walter at UCSF. TREX cells plated and treated with doxycycline (500 ng/mL for 24 h). Cells were treated with Tm and fixed with 4% paraformaldehyde for 30 min. Nuclei were stained with Hoechst dye. Coverslips were mounted with Fluoromount G onto slides and visualized by confocal microscopy (Fluoview FV1000). The number and size of IRE1α foci was quantified using segmentation and particle analysis of Image J software.

Immunoblot analysis and phostag gels

Immunoblot analysis was performed using standard conditions (Rojas-Rivera et al., 2012). The following antibodies and dilutions were used: Anti-β-actin (1:3000; 5125, Cell Signaling), anti-HA (1:2000; 901514, Biolegend) anti-IRE1alpha (1:1000; 3294, Cell Signaling), anti-Phospho-SAPK/JNK (1:1000; 4668, Cell Signaling) anti-SAPK/JNK (1:1000; 9252 Cell Signaling). Detection of the phosphorylated IRE1α form was performed using the Phostag™ assay loading 15 µg of total protein onto 4% SDS-PAGE minigels containing 80 µM of Phostag™ in the presence of 25 mM MnCl2.

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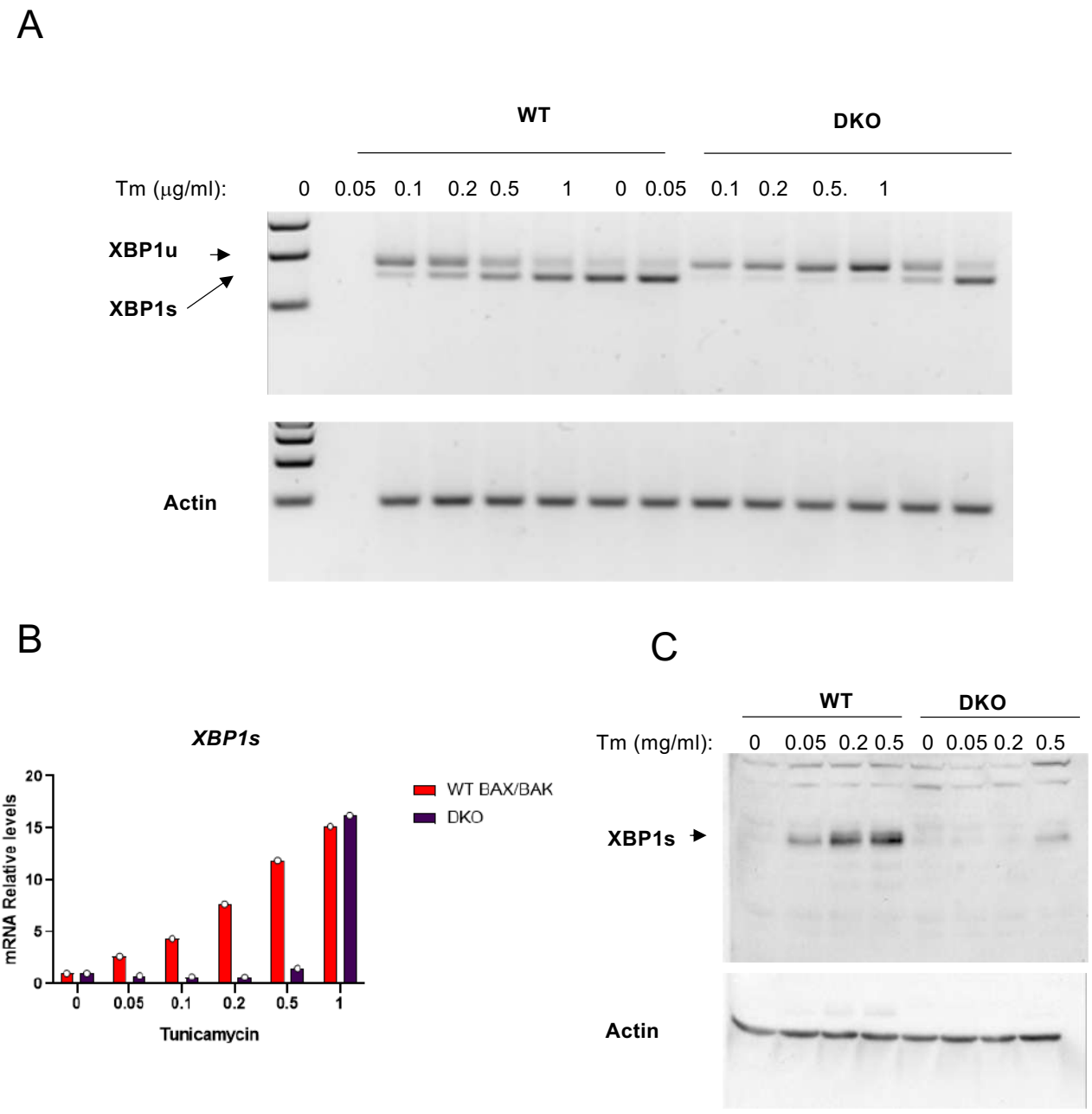


Figure 1. BAX and BAK double deficiency reduces XBP1 mRNA splicing under ER stress. (A) To monitor IRE1 RNase activity, BAX and BAK WT and double knockout (DKO) MEFs were treated with indicated concentrations of tunicamycin (Tm) for 4 h and then RNA was extracted and analyzed by RT-PCR to detect both the unspliced XBP1u and the XBP1s forms. Actin was amplified as control.

(B) We also confirmed the effects of BAX and BAK double deficiency on XBP1 mRNA splicing using real time PCR primers that specifically amplify the XBP1s form.

(C) BAX and BAK WT and DKO MEFs were treated with indicated concentrations of tunicamycin (Tm) for 6 h and then analyzed by Western blot to measure XBP1s expression.

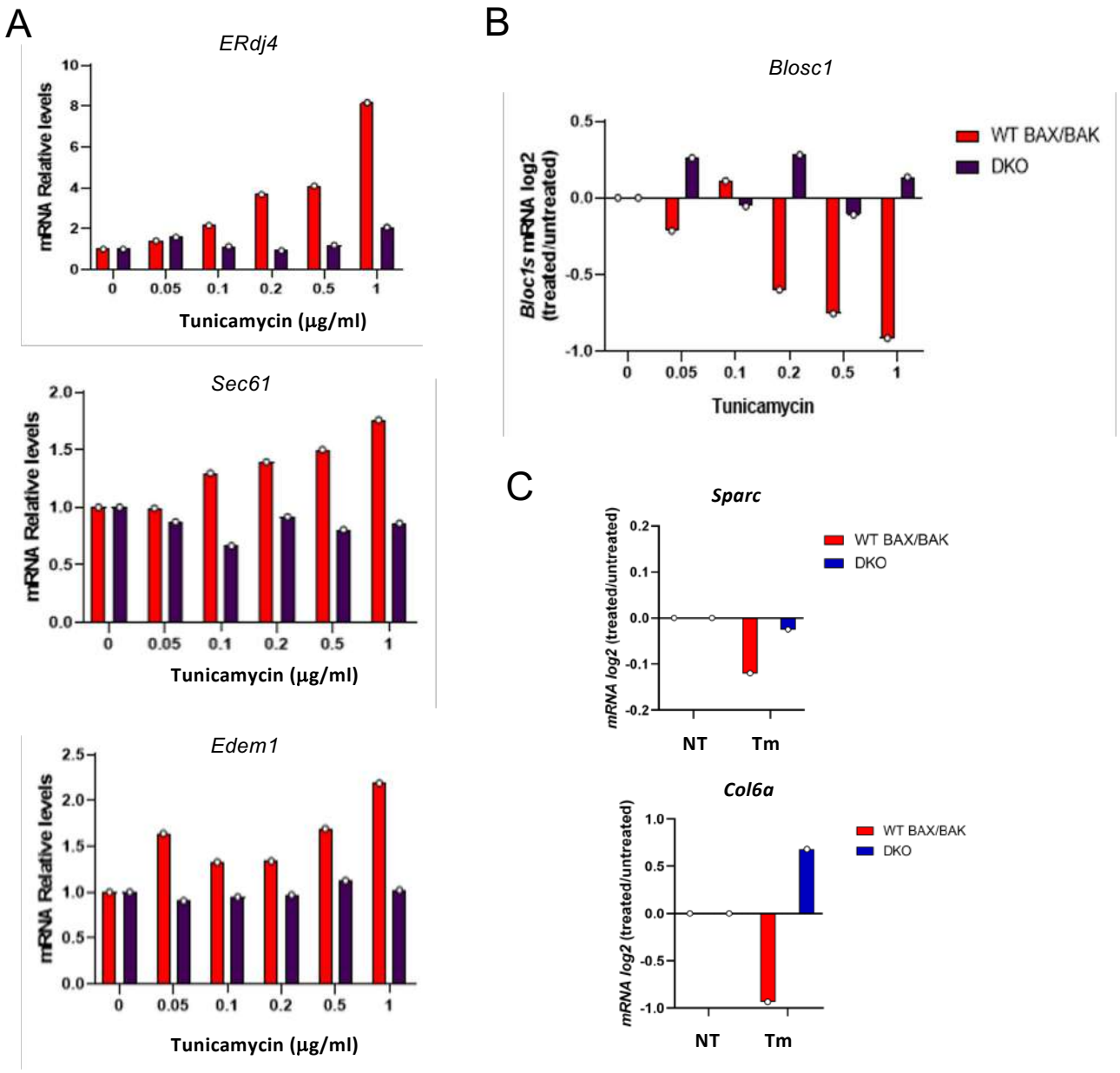


Figure 2. Reduced expression of XBP1s-target genes and RIDD substrate degradation in BAX and BAK double knockout cells.

(A) BAX and BAK WT and DKO MEFs were treated with indicated concentrations of tunicamycin for 4 h and then RNA was extracted and analyzed by real time PCR to measure the canonical XBP1s target genes *ERdj4*, *Sec61* and *Edem1*.

(B) In addition to process the XBP1 mRNA, IRE1 degrades certain mRNAs through RIDD. We measured the decay of the canonical RIDD substrate *Blosc1* under ER stress in the same samples.

(C) In addition, the mRNA levels of alternative RIDD substrates *Sparc* and *Col6a* were assessed in cells treated with 1 ug/ml of tunicamycin.

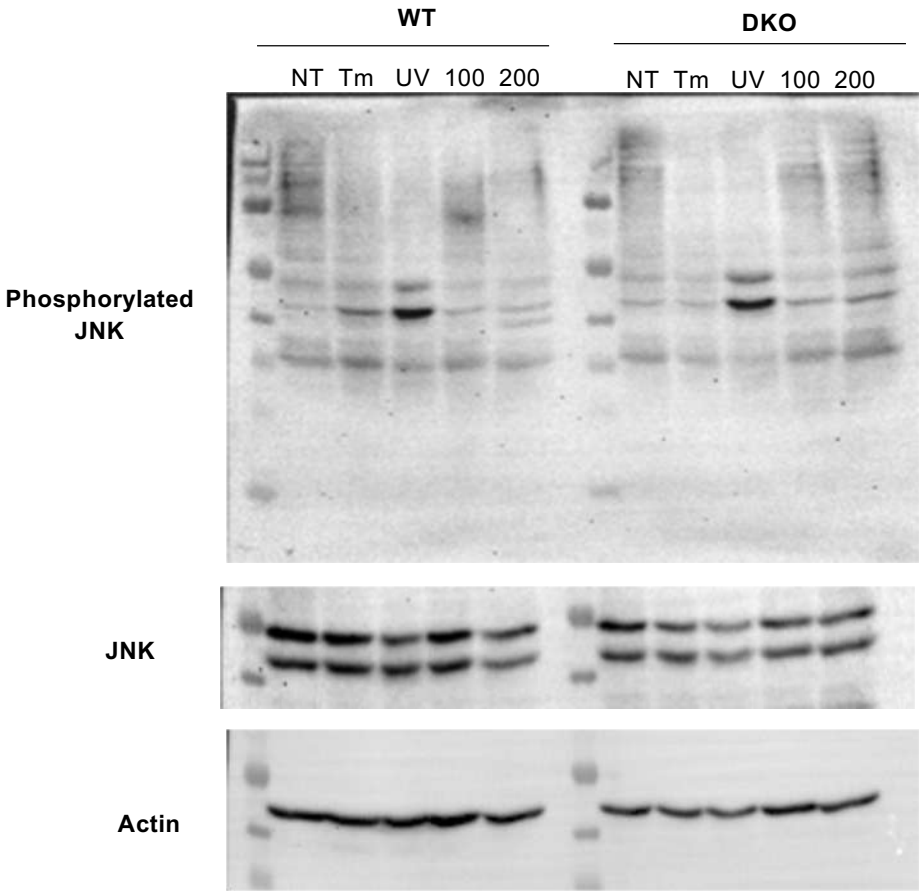
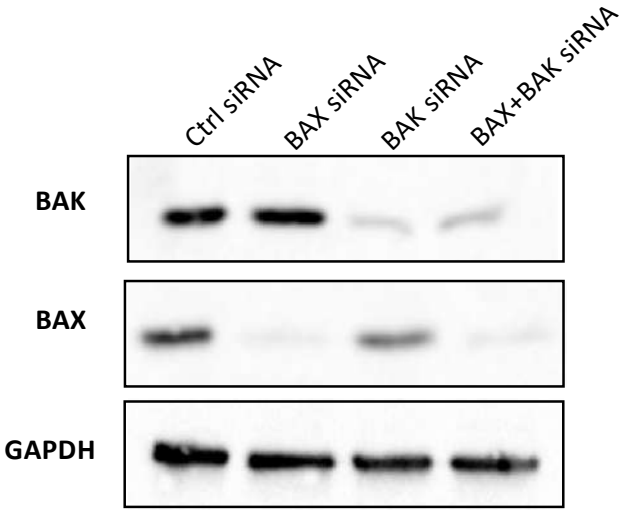


Figure 3. BAX and BAK DKO cell show reduced JNK phosphorylation under ER stress.
BAX and BAK WT and DKO cells were treated incubated in cell culture media with 1% FBS and then treated with 1 μg/mg tunicamycin (Tm), 100 or 200 ng/ml TNF (100, 200), or exposed to UV light (UV) or left untreated (NT) and phosphorylated JNK was monitored by western blot analysis.

A



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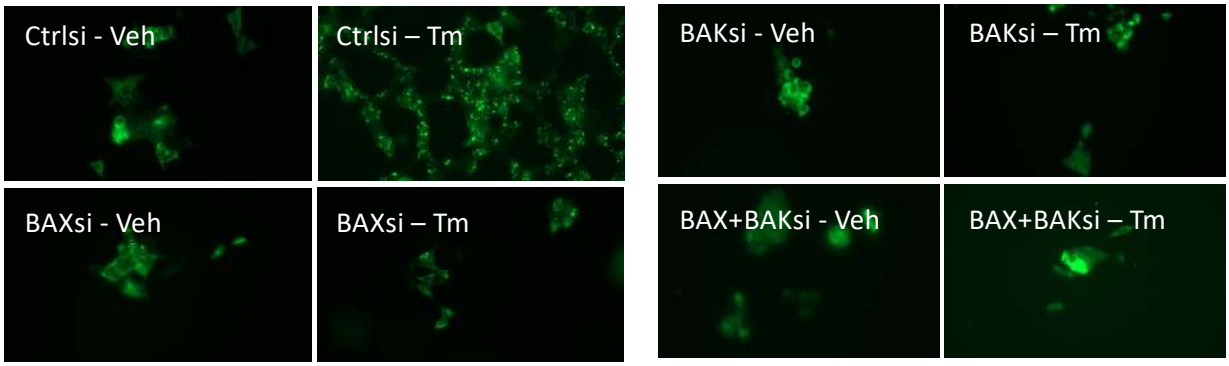

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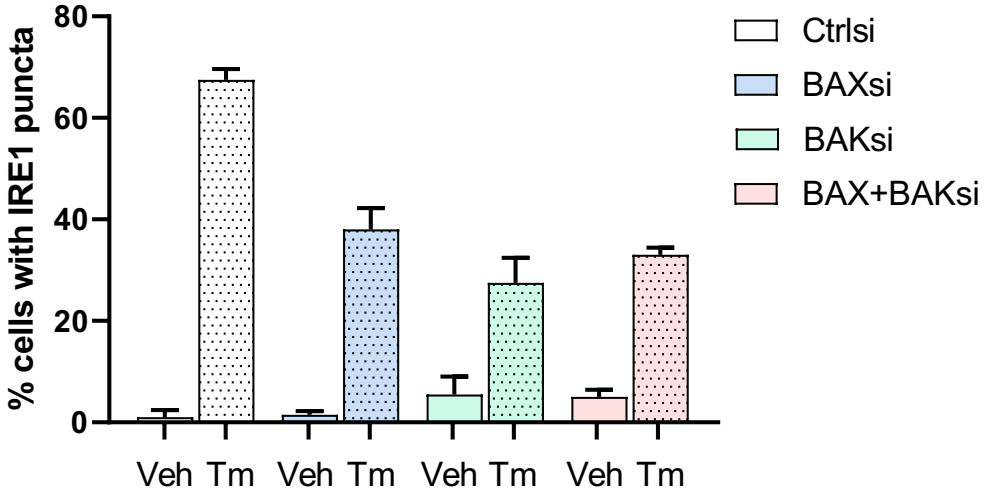


Figure 4. Analisis of IRE1 oligomerization (clustering) in Bax and BAK fedidcient cells. 293T cells expressing Ire1-GFP on an inducible manner were transfected witgh siRNAs for BAX, BAK, or both together or a control siRNA. Then, after 28 h cells were treated with 1 uM dox to induce IRE1 expression and 24 h later treated with 300 ng/ml of the ER stress agent tunicamycin (Tm). The percentage of cells containing IRE1-GFP foci was quantified by fluorescent microscopy at 4 h.

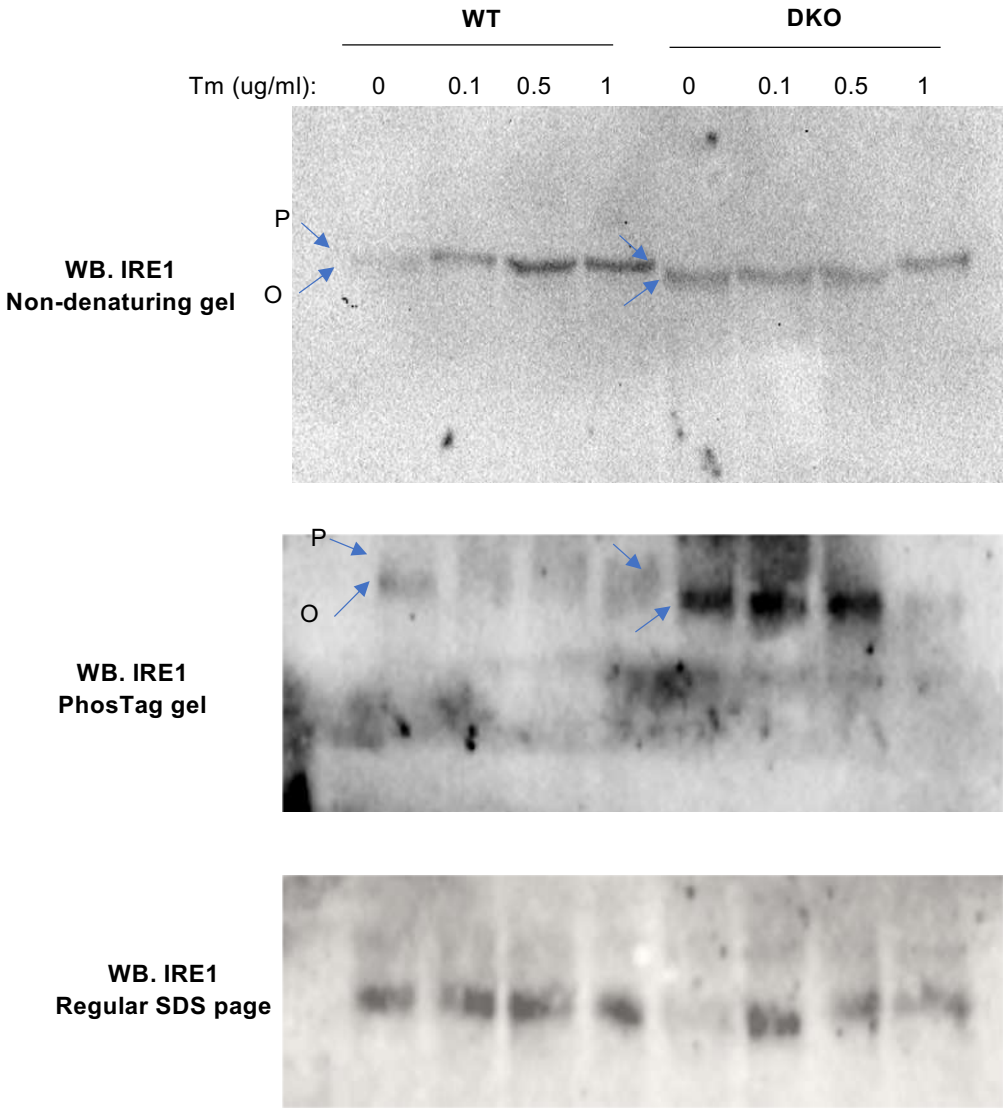
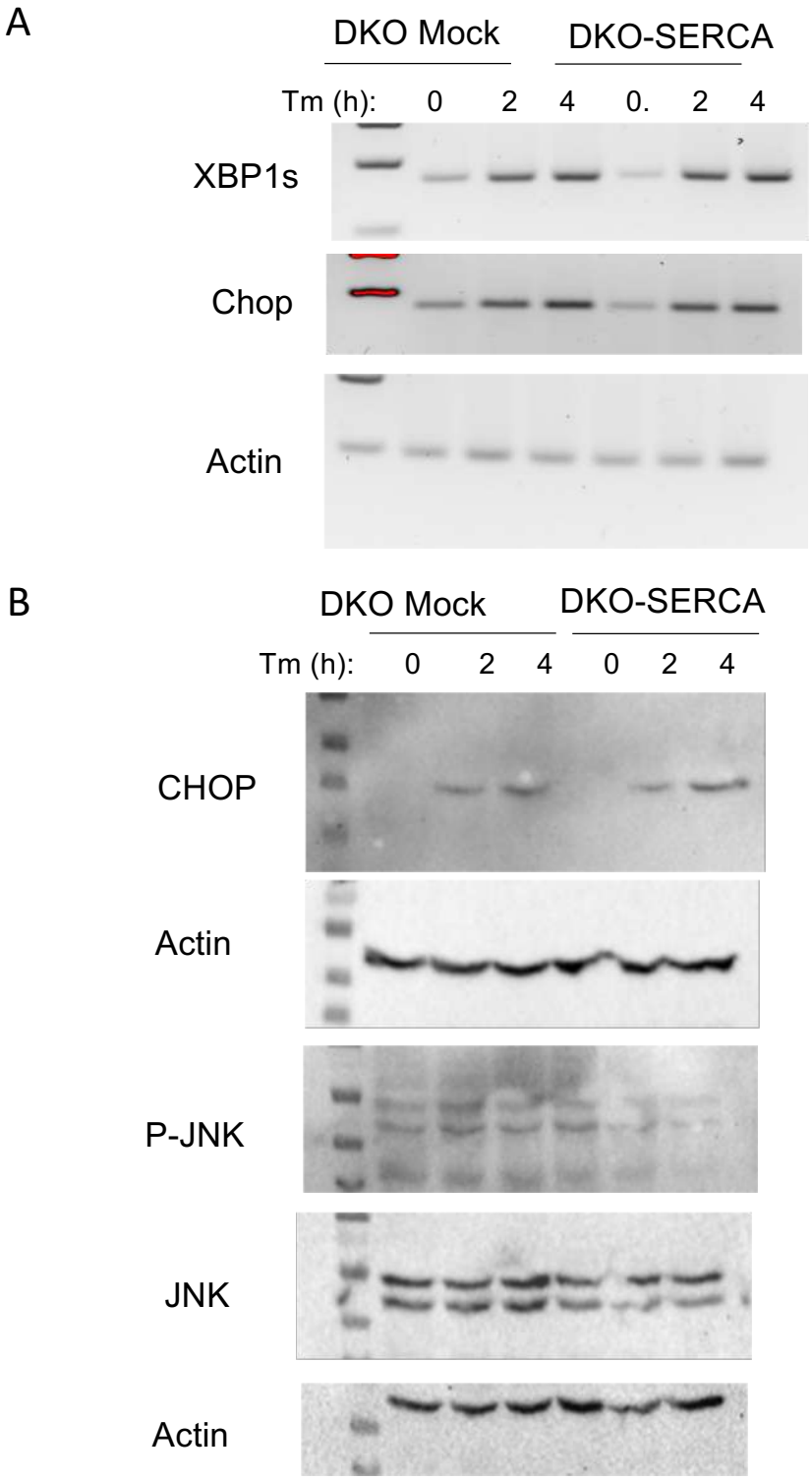


Figure 5. Effects of BAX and BAK deficiency on IRE1 phosphorylation.

BAX and BAK WT and DKO cells were treated with indicated concentrations of tunicamycin (Tm) for 6 h. Protein extracts were analyzed in 3 different types of western blot analysis: non denaturing gels, PhosTag gels and normal electrophoresis. A shift in the molecular weight of IRE1 was detected (P).



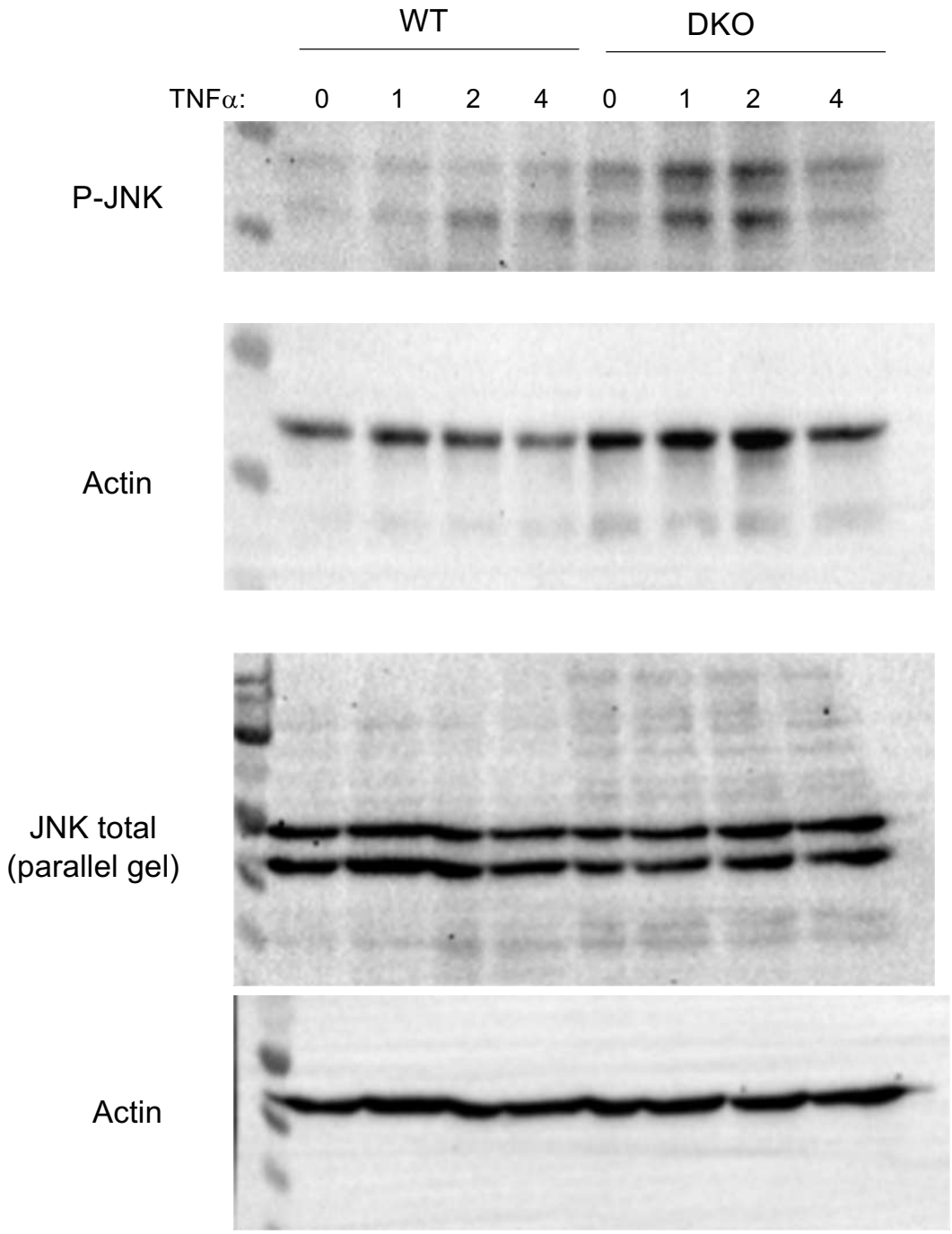
Supplementary Figure S1. Normal UPR activation in BAX and BAK DKO cells overexpressing SERCA. **(A)** BAX and BAK DKO cells were engineered to overexpress SERCA or empty vector (Mock) to restore the normal ER calcium phenotype (cells from Scorrano, Oakes et al., 2003 Science). Cells were treated with tunicamycin for 2 and 4 h or left untreated. XBP1s and Chop mRNA levels were monitored by semiquantitative RT-PCR. **(B)** In parallel CHOP and phospho-JNK were monitored by western blot analysis.

Repetition experiments Science 2006

Repetition Figure 1 A, Grp78 blot.

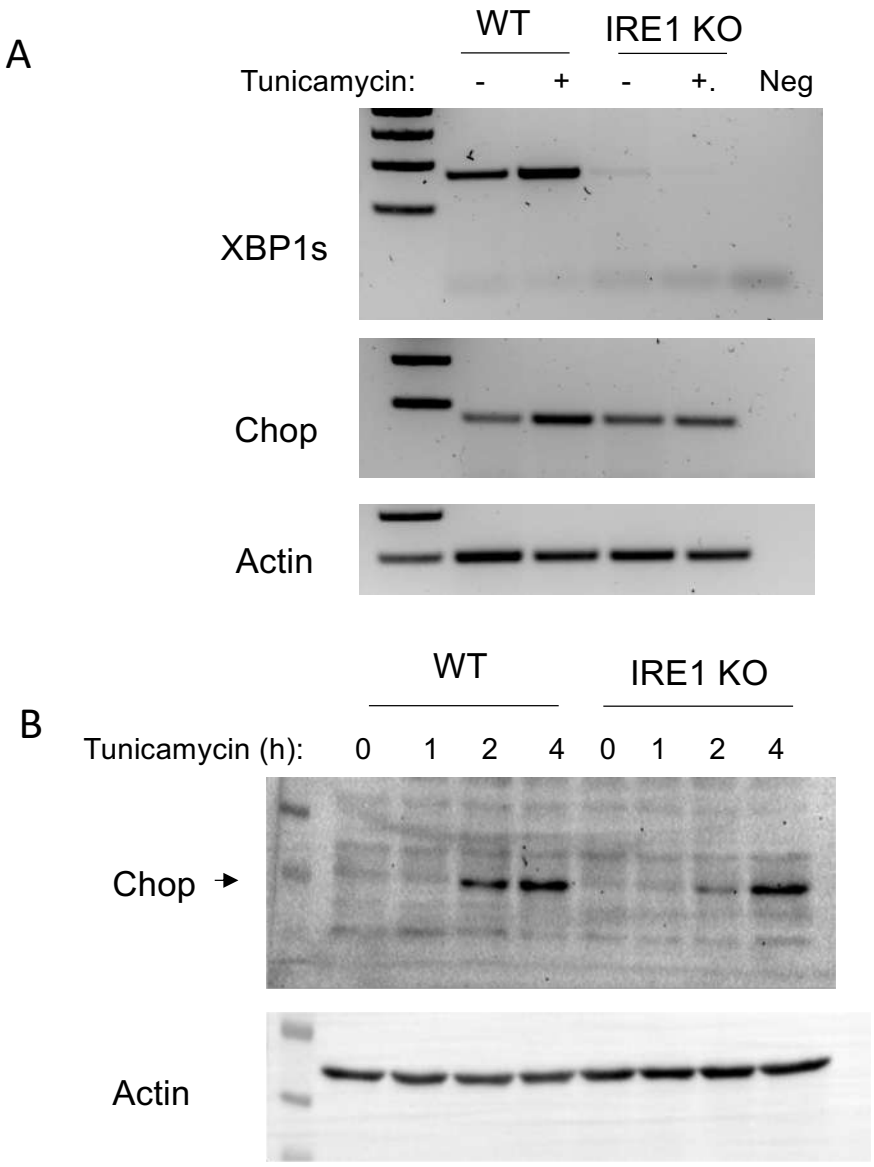
Upregulation of Grp78 in BAX and BAK double knockout liver after tunicamycin injection. BAX and BAK WT and DKO MEFs (BAK KO, Bax floxed animals, Mx Cre system) where treated with poly-IC to induce BAX deletion in the liver and then treated with a single IP injection of tunicamycin and tissue collected at indicated time points. Grp78/BiP expression was measured by Western blot analysis using total protein extracts. This experiments correspond to a repetition of the experiment to confirm main findings. Liver specific BAX and BAK DKO animals are no longer available.

These experiments were performed as repeat of the original experiments at the Kormeyer lab. This western blot should be replaced by the original one in the correction. DKO mice are no longer available.



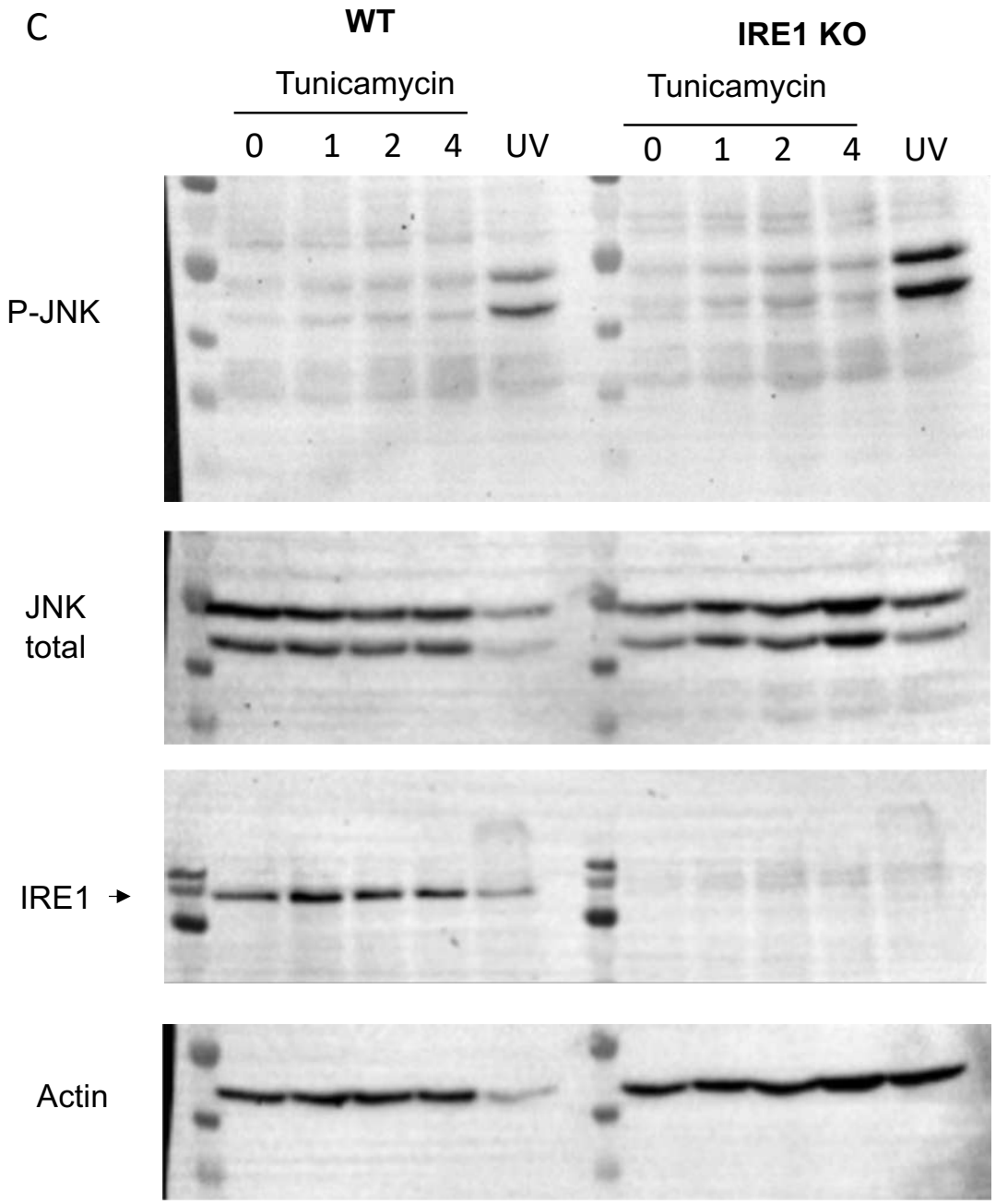
Repetition Supplementary Figure 2B, activation of JNK in BAX and BAK DKO cells treated with TNF alpha (ER stress-independent stimuli, control experiment).

BAX and BAK WT and DKO MEFs were treated with 100 ng/ml of TNFα for indicated time points and then phosphorylated JNK (Tyr183/185) levels were analyzed by Western blot analysis. Experiments performed by Claudia Sepulveda and Giovanni Tamburini.



Repetition Supplementary Figure 2B, confirmation of IRE1 KO phenotype.

(A) IRE1 WT and KO MEFs were treated with 1 mg/ml of tunicamycin and the XBP1s and Chop expression analyzed by semiquantitative RT-PCR after 4h of treatment. **(B)** IRE1 WT and KO MEFs were treated with 0.5 μg/ml of tunicamycin for indicated time points and CHOP expression analyzed by Western blot.



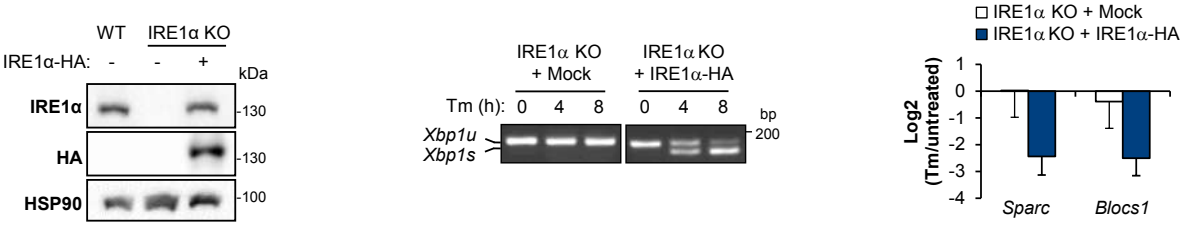
Repetition Supplementary Figure 2B (continuation).

(C) IRE1 WT and KO MEFs were treated with 1 ug/ml of tunicamycin for indicated time points and phosphorylated JNK analyzed by Western blot. As control, total JNK, actin and IRE1 were also analyzed. As positive control, cells were treated with UV to induce JNK activation.

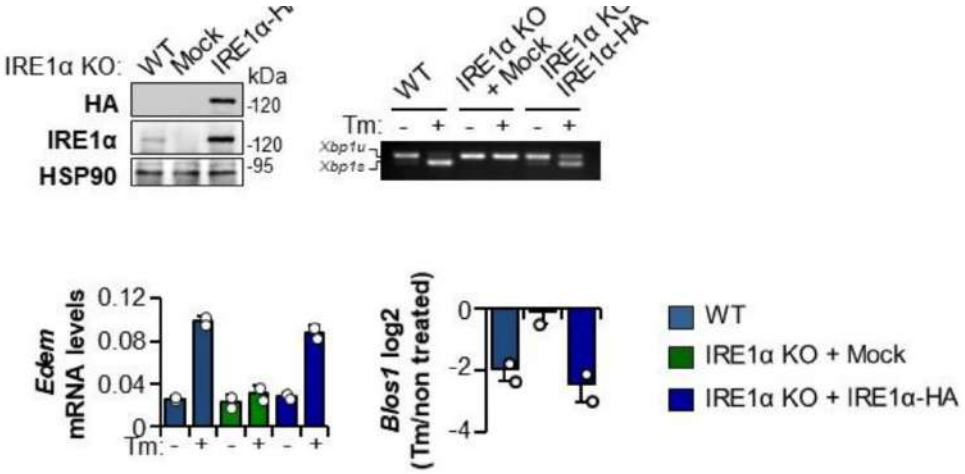
We were not able to get good JNK activation by ER stress in IRE1 and WT MEF pairs.

A Rodríguez et al. 2012, EMBO J. – Fig. 1A

B Sepulveda et al., 2018 Mol Cell – Fig S1A-C



C Urra et al., 2028 Nature Cell Biology - Fig S1



Other published confirmations from the lab using IRE1 KO MEFs.

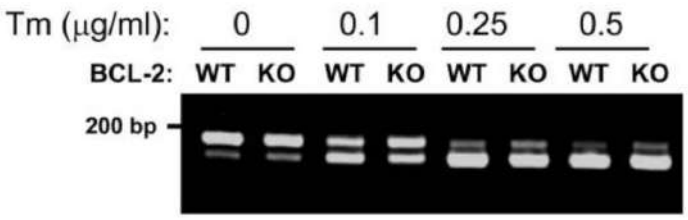
IRE1 KO MEFs were used in other studies from the lab and reconstituted with retroviruses to express IRE1-HA:

(A) Western blot to confirm HA-IRE1 expression (left panel). RT-PCR to monitor XBP1 mRNA splicing (mild panel) and real time PCR analysis to measure XBP1s target genes *edem* and *sec61* (right panel).

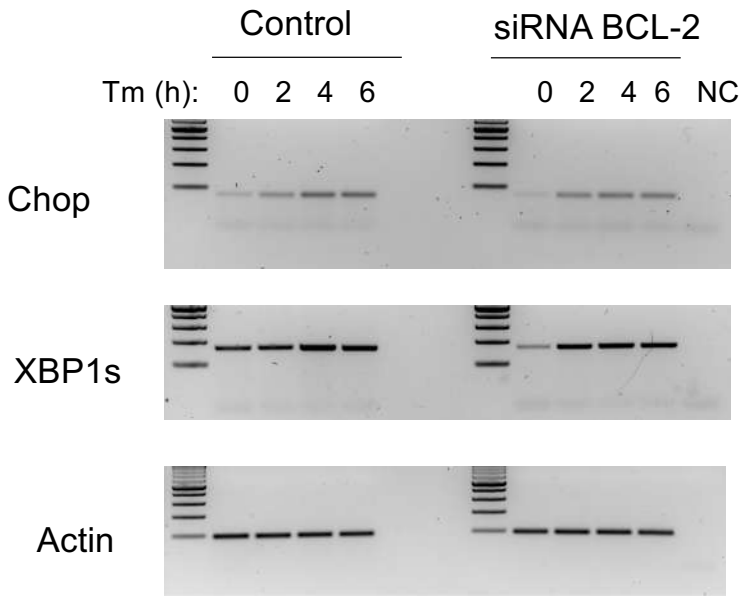
(B) Western blot to confirm IRE1 and HA-IRE1 expression (left panel). RT-PCR to monitor XBP1 mRNA splicing (by RT-PCR (right panel).

(C) Western blot to confirm IRE1 and HA-IRE1 expression. RT-PCR to monitor XBP1 mRNA splicing (by RT-PCR (upper panels). Real time PCR analysis to measure XBP1s target genes *edem* and the RIDD target *Blosc1*. WT cells were also included.

A



B

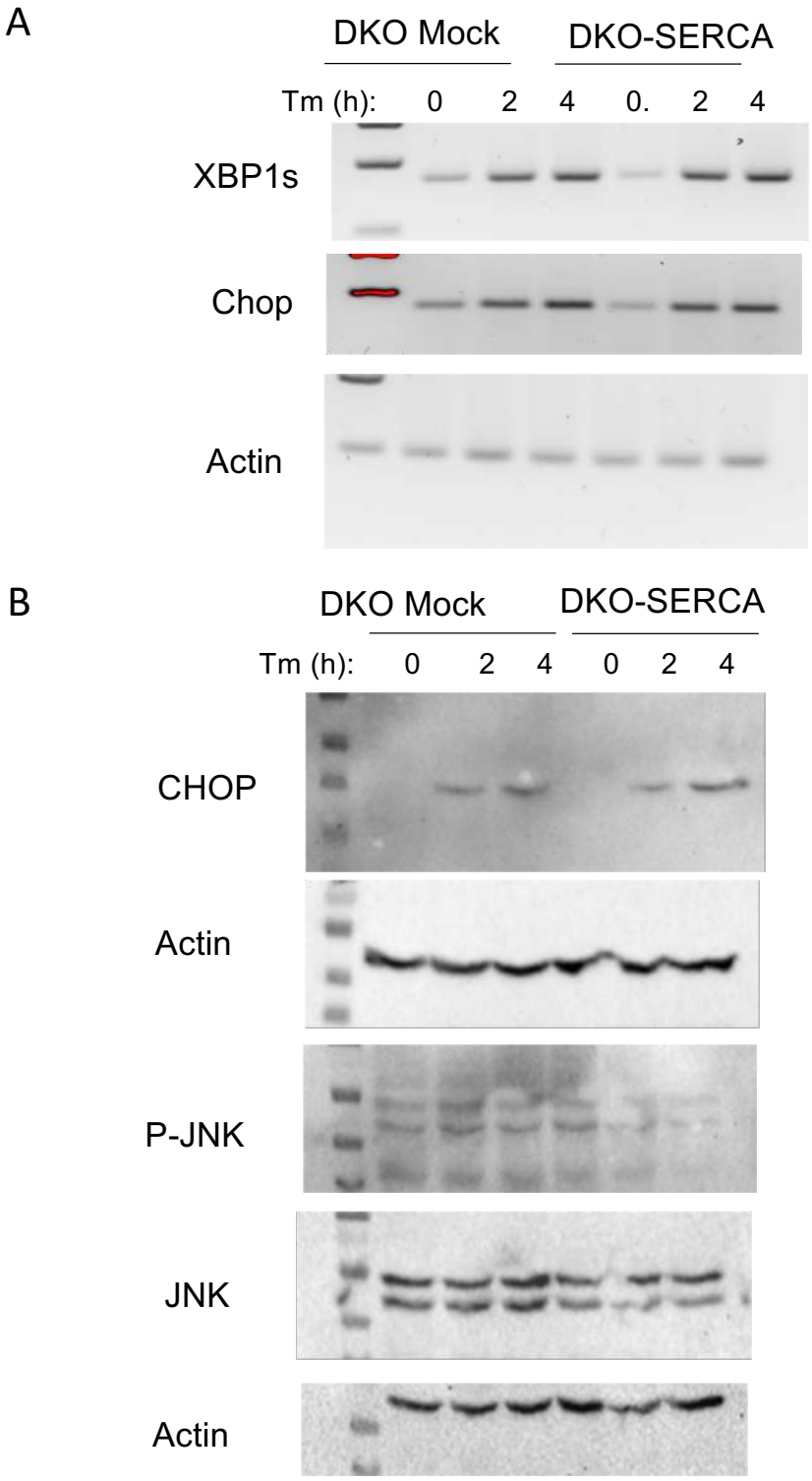


Repetition Supplementary Figure 2C, effects of BCL-2 deficiency

(A) The levels of XBP1 mRNA splicing was assessed in BCL-2 WT and KO MEFs, treated with indicated concentrations of Tm for 2.5 h. **Data from Lisboa et al Mol Cell 2009.**

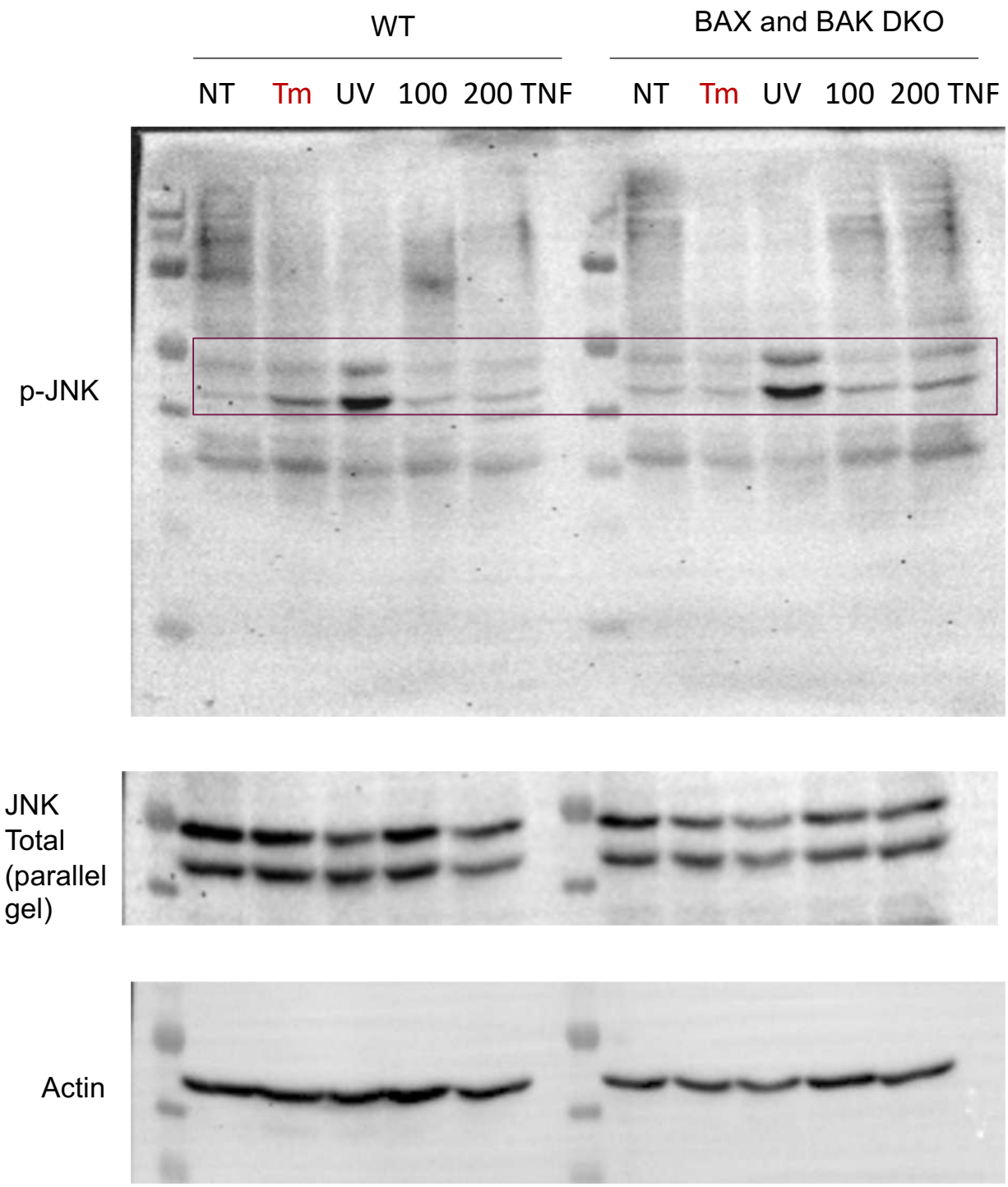
(B) BCL-2 was knockdown with siRNA in WT MEFs and after 72 h treated with 1 ug/ml tunicamycin for indicated time points. XBP1s and CHOP were analyzed by semi quantitative RT-PCR. NC: negative control without cDNA. Experiments performed by Hery Urra and Vania Morales.

Note: We defrosted two vials of cells from 2005 and 2012 of BCL-2 WT and KO cells in addition to human BCL-2 reconstituted MEFs. We were not able to recover them. We requested these cells to former Korsmeyer lab members, but nobody has them anymore.



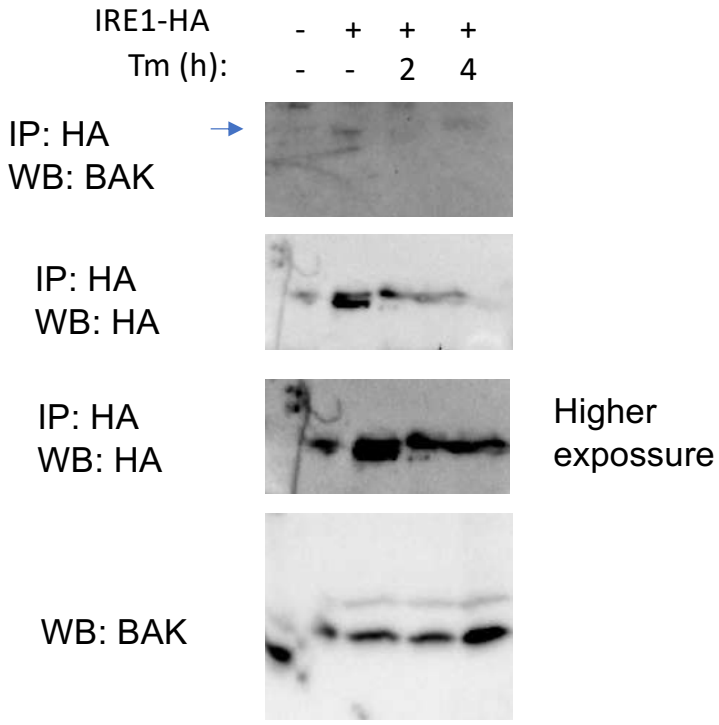
Repetition Supplementary Figure 3A, blots in BAX and BAK DKO cells overexpressing SERCA.

(A) BAX and BAK DKO cells were engineered to overexpress SERCA or empty vector (Mock) to restore the normal ER calcium phenotype (cells from Scorrano, Oakes et al., 2003 Science). Cells were treated with tunicamycin for 2 and 4 h or left untreated. XBP1s and Chop mRNA levels were monitored by semiquantitative RT-PCR. (B) In parallel CHOP and phospho-JNK were monitored by western blot analysis.



Additional data repetition Supplementary Figure 2A and 3A.
BAX and BAK WT and DKO cells were treated with 1 μg/mg tunicamycin (Tm), 100 or 200 ng/ml TNF (4 hours), or exposed to UV light and phospho-JNK were monitored by western blot analysis. Experiments performed by Nicolas Montes.

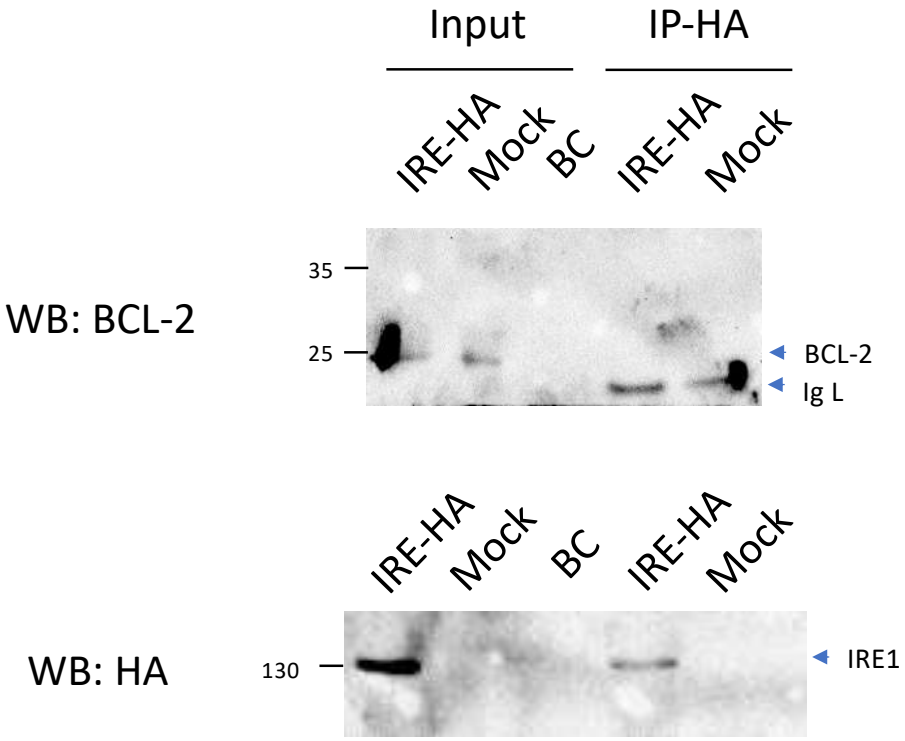
These experiments show that BAX and BAK DKO cells have lower phosphorylated JNK under ER stress. But not after TNF or UV exposure.



Repetition Supplementary Figure 5B, interaction between IRE1 and BAK.

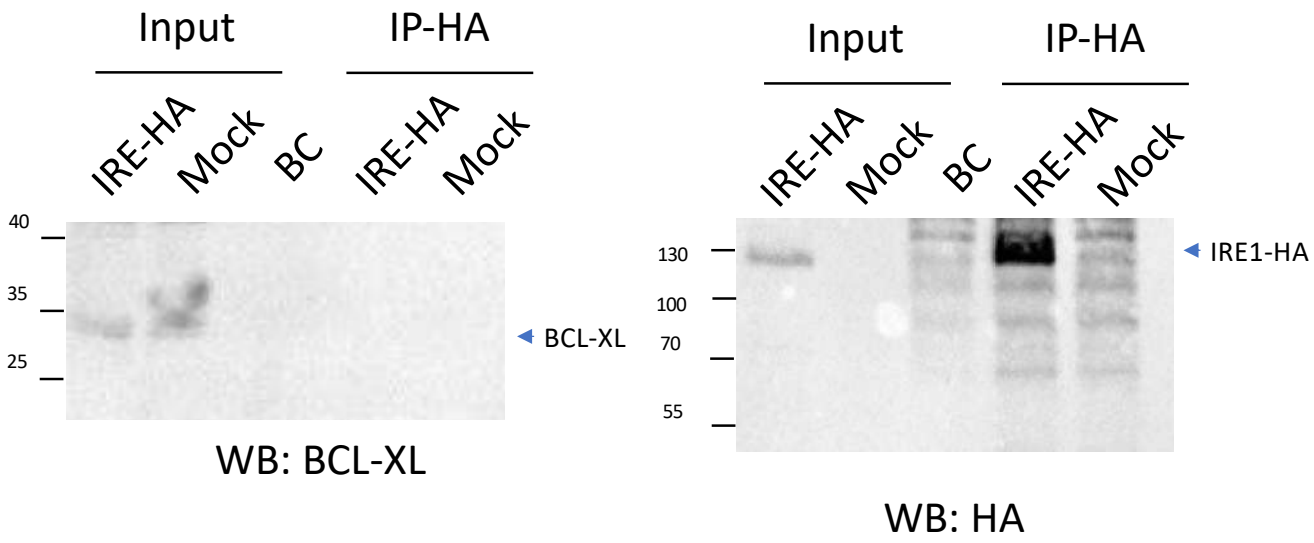
WT MEFs cells were stably transduced with retroviruses to express HA-tagged IRE1. Then protein extracts were immunoprecipitated (IP) using an anti-HA antibody conjugated with magnetics beads. Cells were also treated with tunicamycin for indicated time points. Then co-IP was assessed by western blot analysis of BAK. Total extracts are shown as control. Experiments performed by Hery Urra.

Note: The antibodies for BAK are giving low signals, the upstate antibody is discontinued (we used the last aliquot), got new ones with no luck. We are repeating the experiments to get better blots, but importantly the interaction is there, weak but detectable..



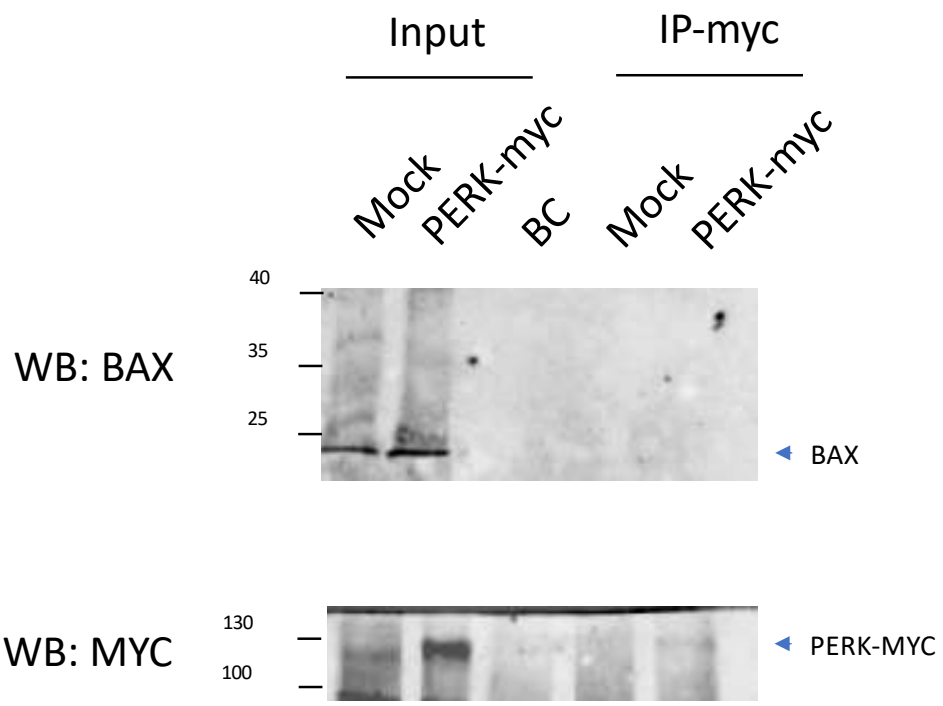
Repetition Supplementary Figure 5D, lack of interaction between IRE1 and BCL2.

WT MEFs cells were stably transduced with retroviruses to express HA-tagged IRE1. Then protein extracts were immunoprecipitated (IP) using an anti-HA antibody conjugated with magnetics beads. Then co-IP was eluted with HA peptides followed by western blot analysis of BCL-2. Total extracts are shown as control. Experiments performed by Mateus Milani.



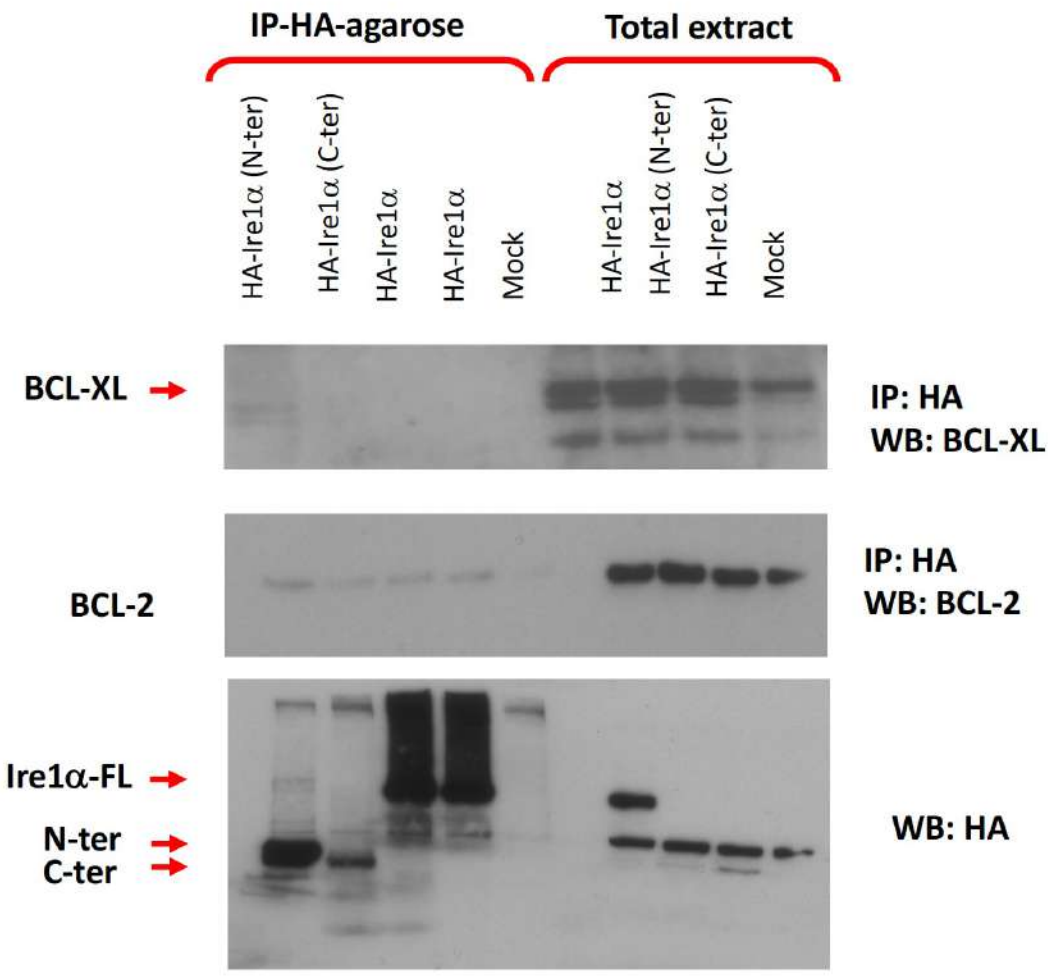
Repetition Supplementary Figure 5E, lack of interaction between IRE1 and BCL-XL.

WT MEFs cells were stably transduced with retroviruses to express HA-tagger IRE1. Then protein extracts were immunoprecipitated (IP) using an anti-HA antibody conjugated with magnetics beads. Then co-IP was assessed by western blot analysis of BCL-XL. Total extracts are shown as control. Experiments performed by Mateus Milani.



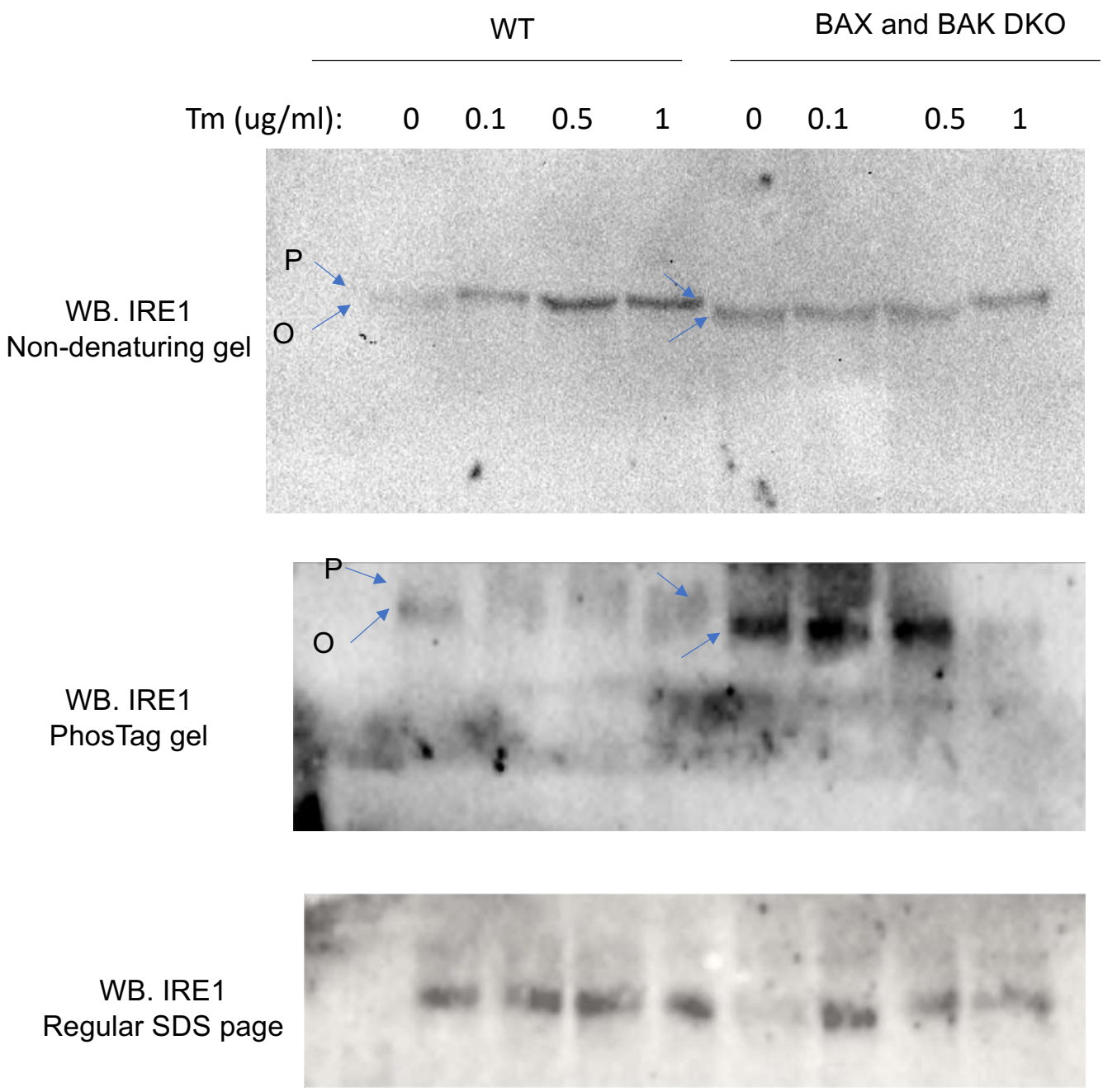
Repetition Supplementary Figure 5F, lack of interaction between PERK and BAX

293T HEK cells were transfected with a MYC-tagged PERK vector. Then protein extracts were immunoprecipitated (IP) using an anti-MYC antibody conjugated with magnetics beads. Then co-IP was assessed by western blot analysis of BAX. Total extracts are shown as control. Experiments performed by Mateus Milani.



Full scans Supplementary Figure 5E and F

In addition, to full length IRE1, the original experiments contained also mutants of IRE1-HA expressing either the C-terminal or N-terminal regions. In this particular experiments mutants didn't express well. Since data was negative (no interaction of IRE1 with BCL2 and BCL-XL) the experiments was simplified to generate the figure of the paper by cutting images obtained from the same film exposure.



Repetition Supplementary Figure 6A, reduced oligomerization/phosphorylation of IRE1 in BAX and BAK DKO cells.

BAX and BAK WT and DKO cells were treated with indicated concentrations of tunicamycin (Tm) for 6 h. The protein extracts were analyzed in 3 different types of western blot analysis: non denaturing gels, PhosTag gels and normal electrophoresis. A shift in the molecular weight of IRE1 was detected with lower doses of Tm in WT cells, however phenomena was only observed with high dose of Tm in DKO cells.

Gmail Claudio Hetz <claudio.hetz@gmail.com>

answers

6 messages

Claudio Hetz <claudio.hetz@gmail.com> Mon, Aug 3, 2020 at 3:38 PM
To: "Glimcher, Laurie,M.D." <Laurie_Glimcher@dfci.harvard.edu>

Hi Laurie

How are you?
I hope things are going well with all the COVID situation. Here it is really frustrating, 4 months already in mandatory quarantine and we started fase-1 to return working last week.

I wanted to update you about the comments you received about our papers from that website so you feed relief.

It is important to clarify that cutting gels from the same film were allowed at that time and for us it was normal. Editorial policies changed later and right now for all our papers we provide full scans, excels with data analysis and statistics; and we even generate an “open depository” with everything in our lab website:

1. I was able to track the data with Melissa and Soledad who perform the experiments from the *Genes and Development 2009*. **Everything is correct.** Basically, as you will see from the power point, the aggregation assays for SOD1 were done in different runs because to be able to detect SOD1 aggregation we need to eliminate DTT from the sampler buffer. That bot included additional samples and were eliminated to show just one mutant SOD1. **Importantly data comes from the same film.** But the controls are performed side by side: knockdowns were stable through puromycin selection and were confirmed for the selected sets of samples in a different set plus the loading control (with DTT).

2. Regarding the comment on ***the Hum Mol Gen 2012*** paper from Rene, the problem was very simple and was solved immediately years ago. Basically, the shRNAs for ATF4 and XBP1 were done in parallel and have the same shLuc control (3 sets), and were tested in the same western blot to confirm the knock down. Since the panels were performed on a period of 2 years we didn’t notice we were repeating an image. As you will see **the editor didn´t consider this a problem and give us the opportunity to change the supplementary information without any correction.** I am attaching what we sent to the editor and interchange of emails with the editor solving the problem.

   Laurie I really apologize I didn’t tell you at that time but I considered this a simple issue and there was no mistake behind.

3. They found a discrepancy between the N number of a survival curve of the *Cell Death Diff* 2007 paper. I contacted Peter to see if we can find the data set. I need to check my old computer backups. He made all the Kaplan Meyer curves since I didn’t have the software. But the important point is that the statistics and curve were generated simultaneously, so the conclusion and results are right, there was just a mistake in the figure legend. Nothing changes.

I hope this clarifies everything.

These people from this site are very aggressive and have bad intentions.

If we discover a mistake that is important we directly contact the editor, we never answer them directly, it is worst. I just saw you posted a comment on Rene ´s paper because you didn’t know we solved this issue before (they neither). If we find the data and the results are OK, we basically save it just in case the editor request it.

If the message of the paper doesn’t change I believe there is no need to file a correction. I discussed the strategy with various collogues (Guido Kroemer, Eric Chevet and Claudio Soto) that also have posted from this site and this is the way they approach it. We take this issue very seriously.

I am trying to generate a backup of our joint papers with what I have here to avoid any problem. There are experiments from 15 years ago!! I have the lab books but not all films. My boxes where sent to Cornel when the lab book. I ask Juan so I can re-scan everything for our records and he said that they were eliminated since the NIH polity request only 7 years of raw data storage.

I hope everything is well from your side. Here, been at home have been an amazing time to connect with my kids. Rethinking everything now...

All the best

Claudio

Data for Laurie.pptx

Claudio Hetz

Director
Biomedical Neuroscience Institute (BNI)
University of Chile

Adjunct Professor
Buck Institute for Research on Aging, Novato, USA.
and Visiting Professor Harvard School of Public Health, Boston USA.
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www.hetzlab.cl or www.bni.cl; www.neurounion.com; www.gerochile.org

2 attachments

Supplementary figure S4A Vidal et al HMG 2012 for laurie.pdf
2320K

email interchange with HMG editor Vidal et al 2012 .pdf
258K

Glimcher, Laurie,M.D. <Laurie_Glimcher@dfci.harvard.edu>
To: "<claudio. hetz@gmail. com>" <claudio.hetz@gmail.com>

Thank you, Claudio- this is very reassuring. I agree that the individuals running this site are extremely aggressive and best approach is to contact the editor if necessary. Sounds like you have all the
The pandemic is surreal and I don't think I have ever worked more hours each day than I have the last 4 months.
I'm on Zoom from early morning till evening and then spend another couple of hours catching up with email.
But I have gotten to spend more time with my grandsons and kids which has been very nice. Attaching pictures of my 3 grandsons, Ryan, 9; Aaron, 21/2 and Teddy 4 months.

Laurie H. Glimcher, M.D.
President and CEO
Dana-Farber Cancer Institute
Richard and Susan Smith
Professor of Medicine
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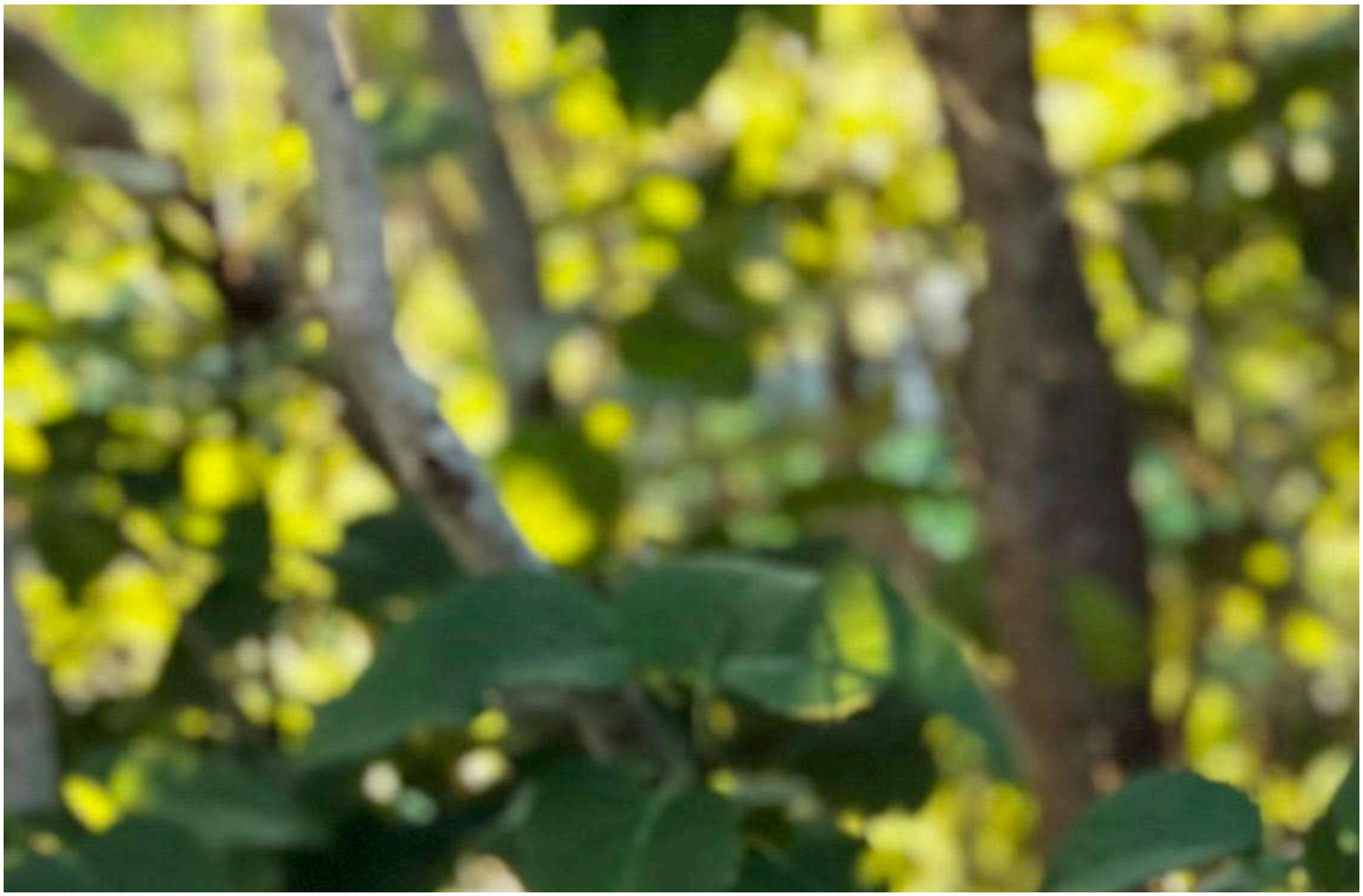

<Supplementary figure S4A Vidal et al HMG 2012 for laurie.pdf><email interchange with HMG editor Vidal et al 2012 .pdf>

Claudio Hetz <claudio.hetz@gmail.com> Tue, Aug 4, 2020 at 9:18 AM
To: "Glimcher, Laurie,M.D." <Laurie_Glimcher@dfci.harvard.edu>

Yes I feel the same, the amount of energy going from one zoom to another is exhausting. Yesterday I had 20 min free only to lunch!

I think we will post these answers and others actions with editorials to our website since we have a section on data resource. So this is freely available to everybody.

Thank you.

Did you see our DNA damage-RIDD paper? Very interesting thinking on cancer biology:

https://www.nature.com/articles/s41467-020-15694-y

All the best,

Claudio

Quick response sent by iPhone

El 03-08-2020, a la(s) 11:27 p. m., Glimcher, Laurie,M.D. <Laurie_Glimcher@dfci.harvard.edu> escribió:

Thank you, Claudio- this is very reassuring. I agree that the individuals running this site are extremely aggressive and best approach is to contact the editor if necessary. Sounds like you have all the data to back up what you explained below.
The pandemic is surreal and I don't think I have ever worked more hours each day than I have the last 4 months.
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<Aaron laughing great pic on beach 7-10-20.jpeg>
<Ryan Teddy.jpg>

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Glimcher, Laurie,M.D. <Laurie_Glimcher@dfci.harvard.edu> Wed, Aug 5, 2020 at 12:09 AM
To: "<claudio. hetz@gmail. com>" <claudio.hetz@gmail.com>

Goo idea to post these answers.
Laurie H. Glimcher, M.D.
President and CEO
Dana-Farber Cancer Institute
Richard and Susan Smith
Professor of Medicine
Harvard Medical School

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Claudio Hetz <claudio.hetz@gmail.com> Thu, Aug 6, 2020 at 2:46 PM
To: "Glimcher, Laurie,M.D." <Laurie_Glimcher@dfci.harvard.edu>

Gmail nih jrcubillos@gmail.com

34,418

claudio hetz <chetz@hsph.harvard.edu>
to Sarah, Sarah, Juan, Juan

Hi guys

how are tings going with the company? I saw the news about tall the money you raised with Laurie. a

We are doing also UPR and cancer research (3 projects) following new concepts. We could chat abc

Since we are patenting and also publishing a lot, as part of a lab effort we are organizyng data publis

I wanted to do the some with my papers.

I realized I only bring with me folders of papers I was finishing at that time. So the original data of the

Do you remember/known where are they? In the lab? Storage?
I know this is boring but if they are around it will be nice to get a picture of the folders, they are labell

THANK YOU!!!!!

Best

Claudio

--
Claudio Hetz

Professor

Gmail Claudio Hetz <claudio.hetz@gmail.com>

folders from HSPH - help

Juan R Cubillos-Ruiz <jur2016@med.cornell.edu> Thu, Jun 7, 2018 at 12:29 PM
To: "Hetz, Claudio" <chetz@hsph.harvard.edu>, Sarah Bettigole <sab2051@med.cornell.edu>, Sarah Bettigole <sarah.bettigole@gmail.com>, Juan Cubillos Ruiz <jrcubillos@gmail.com>

Claudio – I honestly do not know where these folders are. I think everything that was 7 years or older was discarded, according to NIH policies, and approved by Laurie back in the day. I do have the raw data for the EMBO J B cells paper that we published, but nothing else. Sorry I cannot be more helpful.

Juan

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