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Linking endoplasmic reticular stress and alternative splicing

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Abstract:

Endo-reticular stress induces the unfolded protein response including a highly conserved set of genes crucial for cell survival against a variety of onslaughts. Among the activated stress response genes is Ire1, which undergoes auto-phosphorylation and acquires a regulated Ire1-dependent mRNA decay activity. Ire1P non-canonically splices the mRNA for Xbp1 in the cytoplasm. This spliced Xbp1 serves as mRNA encoding a transcription factor for unfolded protein response genes. Meanwhile the mRNA decay function of Ire1P degrades other cellular mRNAs and can cause changes to the translation machinery by altering regulators in a cell specific manner. Naïve splenic B cells differentiate into Antibody Secreting Cells and activate Ire1 phosphorylation early on after LPS stimulation, within 18 hrs. When Ire1 is activated in B cells, in addition to Xbp1 splicing, there are large-scale changes in mRNA; inhibition of the mRNA degradation function of Ire1 both reduces the number and changes the type of genes involved in altered splicing patterns, including factors for snRNA transcription. Some of the splicing changes seen at 18 hrs after LPS persist into the late stages of antibody secretion, up to 72 hrs, while others are supplanted by new splicing changes introduced by the induction of ELL2, a transcription elongation factor. ELL2 changes mRNA processing patterns and is necessary for Immunoglobulin secretion. RNA splicing patterns in antibody secreting cells are thus shaped by endo-reticular stress, ELL2 induction, and are associated with changes in the levels of snRNAs.

Keywords:
B cells, RNA splicing, ER stress, unfolded protein response, RIDD

Abbreviations: ASC, antibody secreting cells; ER, endoplasmic reticulum, Ig, immunoglobulin, LPS, lipopolysaccharide a polyclonal B cell activator; RIDD, regulated Ire1-dependent mRNA decay; UPR, unfolded protein response.

ER stress in cell differentiation

In the transition from naïve B lymphocytes to antibody secreting cells a characteristic morphological change is the appearance of a large amount of endoplasmic reticulum (ER) along with the production of large quantities of the secreted form of the immunoglobulin (Ig) protein; these activated B cells can persist and secrete Ig for a long time (1). Similar large scale changes in the ER occur when pancreatic beta cells are induced to secrete insulin and when liver cells secrete bile or other soluble products (2). Understanding the enormous changes the cells go through in forming the ER has engrossed researchers for years.

The ER is composed of stacks of membranes separated by lumen where proteins synthesized for cellular export are made (rough ER) or where membrane lipids are produced (smooth ER). The lumen of the ER has a very high calcium (Ca++) ion concentration and is an oxidative environment in contrast to the cytoplasm. This facilitates proper protein folding, aided in large part by molecular chaperones, the so called heat shock proteins (3). Beyond facilitating proper folding, some heat shock proteins can stabilize their "client" proteins and prevent ubiquitination which might lead to client degradation (4). In cells that differentiate to produce large amounts of secreted proteins, the ER expands rapidly upon certain predetermined signals. Stress from disturbances in redox regulation, glucose deprivation, viral infection, or a back-up in the proteasome function can lead to too much protein as well; these all can contribute to the unfolded protein response (UPR), which is a highly conserved set of proteins seen throughout evolution (3). For example 24 hr after treating naïve B cells with lipopolysaccharide (LPS) there is minimal induction of Ig secretory specific protein but the cells are beginning to enter S phase from their previous quiescent state, their redox balance is altered, the amount of cytoplasmic and mitochondrial chaperones increases 5-fold, Ire1 auto-phosphorylates, Xbp1 is non-canonically spliced (5), and the highly conserved ER stress induced CHOP mRNA is induced 7-fold (6). CHOP is the DNA damage-inducible transcript 3, also known as C/EBP homologous protein, the induction of which causes calcium release from the endoplasmic reticulum into the cytoplasm, and if unchecked can result in apoptosis (7). Calcium release may serve as an energy deprivation signal to stimulate mitochondrial ATP production and thereby compensate for the large amount of ATP consumed by protein folding in the ER (2). Based on the severity of the ER stress, the UPR genes can re-establish normal ER function, sound an alarm and induce an adaptation to the higher levels of unfolded proteins, or result in cell death (3).

The UPR in many cells and across many phyla relies on three pathways: Ire1/ Xbp1; the ATF6 pathway; and a third involving PERK. All three pathways can be activated by factors that draw Grp78 (BiP) away from its steady state location to become involved in chaperoning the folding of nascent proteins. In mature antibody secreting cells the ER response is unique from that seen in other cells in that Ire1 phosphorylation and Xbp-1 activation have the largest effects on cell viability and antibody secretion, as the PERK pathway is suppressed (8, 9) and the ATF6 pathway has been shown to be dispensable (10).

ER stress activates RIDD

Ire1 auto-phosphorylation upon ER stress induces an Ire1 endogenous activity in numerous cell types called RIDD, regulated Ire1 dependent mRNA decay. The RIDD activity on a unique stem loop in the Xbp1 mRNA can lead to the splicing out of the loop to produce a shorter mRNA for a transcription factor that induces a series of UPR genes (11). However, the RIDD activity can also lead to the destruction of a number of other mRNAs including the immunoglobulin mRNA molecules under certain circumstances (12). The RNAs that are subject to RIDD share the stem loop structure of the Xbp1, a consensus CUGCAG, but not the ability to be non-canonically spliced like Xbp1 (13). It has been suggested that modulation of mRNA destruction by the Ire1 RNase and the overall ER stress response can lead to divergent cell fates of death or survival (14, 15). Cells may use a "multi-tiered mechanism" that results in distinct outputs for Ire1 mediated RIDD (14). Based on the crystal structure of the Ire1 dimer and its stabilization by ADP it has been suggested that its activation is transient and subordinate to ER-luminal stress signals (16).

RIDD can be regulated by eliminating the Perk function in some cells (17) or a ribonuclease inhibitor (RNH1) found associated with it in cells (18). A small molecule inhibitor of RIDD was found that blocks RNA substrate access to the active site of phosphorylated Ire1; this inhibitor is called 8-formyl-7-hydroxy-4-methylcoumarin / CB5305630 and is also known as 4u8C (4-methyl umbelliferon 8-carb-aldehyde) (19). The drug does not interfere with CHOP activation but does block amylase secretion after dexa-methasome induction of mouse embryo fibroblasts. As we discuss below 4u8C can influence mRNA splicing patterns.

Liver secretion and the effects of RIDD

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Activation of the Ire1 mediated RIDD pathway in liver cells can lead to degradation of certain microRNAs and prevent hepatic steatosis, aka fatty liver (20, 21). Micro-array studies on mRNA survival identified RIDD target genes for lipogenesis and lipoprotein metabolism with the characteristic stem loop and consensus sequence (22). In liver cells CReP/Ppp1r15b mRNA, encoding a regulatory subunit of eukaryotic translation initiation factor 2α (eIF2α) phosphatase, is a RIDD substrate. Decreased CReP expression results in more eIF2α phosphorylation and the attenuation of protein synthesis (23). This is at odds with the result of less phosphorylated eIF2 alpha obtained in ER stimulated B cells discussed below. This discrepancy should be explored.

Mice conditionally deficient in Xbp1 in the liver have profound hypo-lipidemia. The Xbp1 deficiency triggers abnormally high over-activation of Ire1 auto-phosphorylation and RIDD activity, in a feed-back mechanism. Loss of Ire1 phosphorylation was able to reverse the hypo-lipidemia resulting from Xbp1 deficiency in the whole animal (22).

B cell activation to antibody secreting cells

A schematic of the *in vitro* activation of naïve B cells, by bacterial lipopolysaccharide, to Antibody Secreting Cells (ASCs) is depicted in Figure 1. The time line recapitulates the principal features of the activation of B cells that do not enter germinal centers *in vivo*. Within 18 to 24 hrs of stimulation the Go resting cells leave their quiescent state and begin to proliferate and activate the ER stress response. This occurs prior to the massive up regulation of production of the secreted form of the immunoglobulin molecule (24, 25). After Ire1 auto-phosphorylation by ER stress, the typical regulated Ire1 dependent mRNA decay pathway (RIDD) is activated, producing a non-canonical spliced form of Xbp1 mRNA and degrading other cellular RNAs with a stem loop and consensus sequence (15). When mice are deficient for Xbp1, B cell differentiation to antibody secreting cells is blocked and the ER is distended (12, 26-28). *In vitro*, Ire1 cleaves the mRNA of secretory Ig μ chains (μs) in activated B cells. The IgM response is partially restored in Xbp1/Ire1 double knockout mice relative to the single Xbp1 knockout mice. RIDD can thus reduce Ig synthesis and secretion under certain circumstances (12).

mTOR signaling and the ER

Mammalian target of rapamycin or mTOR encodes a kinase that promotes anabolic activities along with phosphorylation of an eIF4E binding protein and ribosomal S6 protein

phosphorylation in many cells (29). ER stress attenuates the mTOR pathway, reducing protein synthesis, while induction of ER stress in B cells leads to a de-phosphorylation of eIF2 alpha (27). Treatment of B cells with rapamycin or by conditionally deleting RAPTOR, an essential signaling adaptor in the mTORC1 complex, blocked production of ASCs (30) in part by decreasing the expression of BiP, an unfolded protein chaperone. Artificial hyper-activation of the mTOR pathway occurs when the tuberous sclerosis complex (TSC1) is deleted in B cells; when B cells are engineered to over-activate the mTOR pathway there is a higher level of apoptosis following LPS stimulation (27). When mice are conditionally deleted for both Xbp1 and TSC1, the typical Xbp1 distended ER is alleviated and Ig secretion is enhanced (26) indicating that mTOR can work at cross purposes to Xbp1.

mTOR has also been shown to be upregulated in human marginal zone B cells, cells that contain more mitochondria, ER, lysosomes, and Golgi. Marginal zone B cells contrast with human follicular B cells that typically require T cell help for differentiation to secretion and have lower mTOR. The marginal zone B cells are more responsive to T cell independent stimuli and growth signals like TAC1 than the follicular B cells because mTOR links TAC1 signaling with metabolic events and differentiation (31).

Ire1 activation and ELL2 induction with alternative splicing

We examined the splicing patterns of genes, comparing B cells and cells stimulated for 18-24 hrs with LPS when Ire1 is phosphorylated. We saw that more than 11,144 genes had alternative splice forms between the two conditions, shown in (32) and illustrated here in Figure 2. A set of about 4,211 genes that changed splicing patterns early were seen to persist in cells at 72 hrs, the so called ELL2 independent genes. When we inhibited the Ire1 phosphorylation and the RIDD function of Ire1 with the drug 4u8C in LPS stimulated B cells, we saw the decrease in the short form of Xbp1 mRNA and a change in the alternative splicing pattern in the cells (32). The total number of spliced genes shown here in Figure 2 was reduced from 11,144 to 7,248 and the ELL2 independent genes were also reduced from 3,783 without drug to 2,659 with 4u8C. We conclude that altering Ire1 phosphorylation with 4u8C has major repercussions on the splicing pattern of a large number of genes.

After 24 hrs the activated B cell is induced to make the transcription factors Prdm1, Iref4, and ELL2, a transcription elongation factor; the cell begins a large scale shift to produce structural changes to become an antibody-producing and -secreting factory (6, 25, 32). By

comparing ELL2 sufficient and conditionally ELL2 deficient mice for the spliced products that result 72 hrs after LPS stimulation, we found that ELL2 alters global splicing patterns in ASCs, see Figure 2. Having ELL2 present accounts for the splicing changes seen in ~55% of the genes in a comparison of cells plus and minus ELL2 (32) and Figure 2.

In our previous work, to elucidate its mechanism of action we added ELL2 *in vitro* to B cells; ELL2 was able to modulate splicing and drive first poly(A) site use in test genes, facilitated the association of PAF and RNA processing factors with RNA polymerase II, and activated histone modifications on the IgH gene especially H3K79 tri-methylation (33-35). The role of ELL2 in the association of PAF and RNA processing factors to RNA polymerase was also seen in an HIV infection model (36, 37). So several factors are working to change RNA processing between B cells and their progeny, the cells at 18-24 hrs with Ire1 phosphorylation and RIDD, and cells at 72 hrs after LPS stimulation that have turned on ELL2.

We had previously shown that when ELL2 was conditionally knocked out in mouse B cells those cells had distended abnormal appearing ER, reduced amounts of the secretory-specific form of the Ig heavy chain, reduced BiP, ATF6, and cyclin B2 mRNAs and reduced Xbp1 mRNA, both spliced and unspliced, as well as almost no transcriptionally active 40 kDa Xbp1 protein. ELL2 also has the ability to efficiently drive transcription from a canonical UPR promoter as well as the cyclin B2 promoter. We concluded that ELL2 drives secretion and at least a part of the UPR at a point after it is induced (25).

But we saw that the remaining changes in RNA processing at 72 hrs that did not seem to require ELL2 had already occurred early after LPS stimulation, simultaneous with RIDD activation. Thus 3,783 of the 4,211 changes in splicing occur early after LPS stimulation (18 hrs) and persist through the 72 hrs of stimulation, a point at which maximal Ig secretion occurs (6), summarized in Figure 1. An additional 7,361 genes are alternatively spliced in this 0-18 hr interval. They do not persist until 72 hrs and seem to be replaced by the ELL2 dependent splicing events.

Changes in snRNA are correlated with the timing of RIDD

We wondered what might be causing the changes in RNA processing we had observed at 18-24 hrs and focused our thinking on the ER response and the UPR in the cells. We examined the levels of the small nuclear RNAs (snRNAs) in B cells after LPS stimulation and saw that they fell slightly at 18-24 hours post LPS induction and fell even further at 72 hrs post LPS,

reported in (32) and summarized here in Figure 3. Next we examined the levels of the mRNA encoding some of the transcription factors known to be important in snRNA synthesis. The transcription of snRNAs requires cooperative binding of five SnapC subunit proteins with Oct1 (and or Oct2) to the octamer sequence for each of the snRNA enhancers (38, 39). A little elongation complex including Ice1 and Zc3h8 with ELL1 forms and travels with RNAPII to synthesize the snRNAs (40). The Integrator (Ints) complex of at least five proteins is necessary for snRNA transcription termination.

We showed that the level of mRNA for Ints 10, SnapC1 and SnapC2 are decreased after LPS stimulation (32). This is also true for the SnapC1 (43 kDa) subunit protein. SnapC1 and 2 mRNA share the stem loop and consensus motif of other RIDD targets. When we treated B cells with 4u8C and LPS the snRNAs and SnapC1 levels paradoxically increase relative to both the time zero and the 18 hrs plus LPS levels, see Figure 3. Thus Ire1 phosphorylation under normal circumstances initiates a decrease of snRNA expression that might be expected to exert a number of the changes on mRNA splicing. We cannot ascertain if stopping the synthesis of the transcription factors for snRNAs is sufficient to explain their decreased levels by 72hrs or if there is an active process to degrade the snRNAs. Nuclear RNAs might not be expected to be subject to RIDD in the cytoplasm like the mRNAs encoding snRNA transcription factors might. The explanation for our observations thus far remain enigmatic.

snRNA levels in cell growth

We went on to ask if the changes in snRNA levels induced by ER stress in B cells is shared with other mouse cell types and other stressors. Splenic B cells are known to be in Go and enter S phase upon LPS stimulation within 24 hours. We had previously seen that CstF64 (Cstf2), a polyadenylation factor, increases as B cells leave Go and enter S phase just like it does in fibroblasts induced to enter the cell cycle (41). To investigate if snRNA levels change in the transition from Go to S, we cultured 3T6 mouse fibroblasts and arrested them in Go by serum starvation as previously described (41). Then we added serum, a treatment known to induce active mitosis within 8-12 hours (42). We harvested RNA samples from the Go and S phase cells and compared them as we did for the primary LPS treated cells. As shown in Figure 4, we saw a decrease in U4 levels in the growing, S phase 3T6 cells but no other significant decreases in the expression of either major or minor snRNAs between Go and S phase cells, in fact some of the snRNAs like U2 and U6atac increased significantly in growing cells relative to resting cells.

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It had previously been reported that when cells went from a state of rapid growth to stationary-phase there was a reduction in accumulation of all snRNAs except the 4.5S snRNAs. Those authors noted similar reductions in snRNAs when cells were induced to differentiate (43) not unlike what we saw following LPS stimulation. They offered no explanation for the decrease in snRNA amounts that they saw in differentiating Friend erythro-leukemia cells.

SnRNA levels in pancreatic beta cells undergoing ER stress

The mouse derived MIN6, a cultured pancreatic beta cell line, was used to recapitulate glucose regulated pro-insulin biosynthesis and protective effects of NR4AI induced resistance to ER stress-mediated apoptosis (44, 45). To further explore the effect of Ire1 phosphorylation on snRNA levels we treated MIN6 cells with thapsigargin (TG) a drug known to induce ER stress within 2 to 5 hours of treatment in the absence of any insulin synthesis (46). Ire1 and RIDD activation are confirmed in our experiments by the increased phosphorylation of Ire1 protein (Figure 4), a >10-fold increase in the Xbp1 short / spliced RNA form, and ~5-fold Chop (Ddit3) mRNA induction, all hallmarks of TG induced stress (47). The small RNA and SnapC1 mRNA levels are significantly decreased in 5 hrs with TG as compared with the time zero value. The levels of BiP, Chop, and Zc3h8 mRNAs are increased as would be expected with ER stress induction. Thus we conclude that Ire1 phosphorylation leads to reduced amounts of the snRNAs and some of the factors needed for their transcription like SnapC1 in the mouse pancreatic beta cells, just like in B cells (48). This alteration in snRNA levels could change splicing patterns.

Changes in snRNA levels can alter gene expression

Previous studies showing that altering snRNAs levels could change splicing patterns include during Drosophila development (49), in Alzheimer's disease where the changed levels of U1 snRNA lead to altered RNA processing of several mRNAs (50), and human diseases like hemato-lymphoid neoplasia, retinitis pigmentosa, and microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1) associated with a loss of U4atac snRNA (51). We and others have seen changes in U1A, a protein associated with U1, following B cell activation (52, 53); free U1A has been found associated with the Ig mu secretory poly(A) site where it can inhibit RNA processing (54). U1 snRNA has activities outside of it role in classic intron/ exon splicing; it has been shown to protect pre-mRNA from premature cleavage and polyadenylation (55, 56). Thus changes in U1 and U1A levels could have a plethora of effects. Meanwhile, the relative

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enrichment of minor snRNAs has been hypothesized to play a crucial role in retinal neuron development (57). Reducing the amount of the snRNAs following ER stress could therefore have a major effect on the splicing patterns in ASCs. The decline in snRNA expression and their transcription factors is visible by 18 hr post-LPS and can be perturbed by an inhibitor of the RIDD activity of Ire1 (32). The ER stress response overrides the natural tendency of the snRNAs to increase in response to cell growth, Figure 4. Some of these alterative splicing events induced early persist until the antibody secreting stage matures while other early splicing changes are supplanted by those introduced by ELL2, see Figure 1 for the summary.

Other changes associated with stress that could change snRNAs and splicing patterns

We have shown an association between falling snRNA levels and altered splicing but other explanations are possible for the changes in gene expression. A prime candidate for regulation is P-TEFb. CDK9 and Cyclin T1 associate to form P-TEFb which facilitates transcription elongation and phosphorylates the Ser2 in the Carboxyl-terminal domain of RNA Polymerase II to relieve pausing at viral and cellular heat shock genes (58, 59). P-TEFb can be sequestered by 7SK, LARP7 and HEXIM into an inactive state; alternatively P-TEFb can be brought into association with acetylated histones on chromatin by BRD4 where it activates transcription (60). P-TEFb was isolated in a complex with a number of other factors including ELL proteins in a “Super Elongation Complex” that can drive transcription elongation and alleviate pausing (40, 61). The distribution of P-TEFb between the active and inactive complexes can be altered by a variety of cellular stresses (62, 63) and perhaps by the available amount of ELL1. Since transcription elongation and RNA processing have been extensively linked (64) it is logical to predict that cellular stress and the increase in ELL2 in antibody secreting cells might change not only the transcription profile but also the splicing patterns of a number of genes.

Another possibility for changing splicing patterns in B cell development is the change-over in expression from predominantly ELL1 and ELL3 in a B cell to that of large amounts of ELL2 in ASCs. We hypothesize that this may also play a role in the splicing changes we have seen at the same time as the induction of ER stress and the UPR. ELL2 differs from the other two ELL family members (65) in the middle of the molecule where it can bind several different proteins in a yeast two hybrid screen (66). This could change the constellation of RNA

processing factors associated with RNA polymerase II and influence splicing patterns. This has not been explored further.

The reduction in ELL1 in ASCs may have other consequences. The snRNA genes are thought to be transcribed by a "little elongation complex" composed of ELL1 and KIAA0947 (ICE1), NARG2 (ICE2), and ZC3H8 (40). By having fewer ELL1 molecules available for the little elongation complex in ASCs, snRNA transcription could decrease and the amounts of snRNAs would be lower by 72 hr post LPS.

It has also been reported that p53 represses the transcription of snRNA genes (67) and does so by preventing the little elongation complex from forming (68). It has been suggested that p53 dissociates ELL1 from the little elongation complex. Mdm2 is a transcription target of ELL2 (32) and it attenuates p53 function by ubiquitination (69) which might counteract the dissociation of the little elongation complex. Thus finding snRNAs in such low abundance after LPS stimulation may involve the activation of several divergent pathways, some involving the ELL proteins and their pattern of expression.

Conclusions

Our findings of alternative splicing following ER induction may have major physiological and pathological implications. As we have described, the UPR activation is intrinsic in liver cells, pancreatic beta-cells and naïve B-cells. Beyond this, an induction of the UPR has been observed in clonal expansion of cell types such as smooth muscle cells, dysregulated calcium handling of cardiac myocytes, and protein misfolding in various brain regions. The linkage of ER stress and snRNA levels might be imagined to expand into disease states such as atherosclerosis, arrhythmogenesis, and neurodegenerative diseases such as Parkinson's and Alzheimer's disease (70, 71).

We have discussed the importance of defining the molecular mechanisms that contribute to the complex propagation and induction of the UPR. Further investigation should be conducted into the role of RIDD and the snRNAs as contributors to the induction of the UPR in other cell types. Our work has implicated the UPR as a regulator of splicing through RIDD, a powerful perturbation of the *status quo* in cells.

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1. Manz, R. A., and A. Radbruch. 2002. Plasma cells for a lifetime? *Eur. J. Immunol.* 32: 923-927.
2. Vincenz-Donnelly, L., and M. S. Hipp. 2017. The endoplasmic reticulum: A hub of protein quality control in health and disease. *Free Radical Biology and Medicine* 108: 383-393.
3. Xu, C., B. Bailly-Maitre, and J. C. Reed. 2005. Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Investigation* 115: 2656-2664.
4. Orthwein, A., A. Patenaude, E. B. Affar, A. Lamarre, J. C. Young, and J. M. Di Noia. 2010. Regulation of activation-induced deaminase stability and antibody gene diversification by Hsp90. *J Exp Med* 207: 2751-2765.
5. Back, S. H., M. Schröder, K. Lee, K. Zhang, and R. J. Kaufman. 2005. ER stress signaling by regulated splicing: IRE1/HAC1/XBP1. *Methods* 35: 395-416.
6. Bayles, I., and C. Milcarek. 2014. Plasma cell formation, secretion, and persistence: The short and the long of it. *Critical Reviews in Immunology* 34: 481-499.
7. Li, G., M. Mongillo, K.-T. Chin, H. Harding, D. Ron, A. R. Marks, and I. Tabas. 2009. Role of ERO1-α–mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress–induced apoptosis. *J. Cell Biol.* 186: 783-792.
8. Ma, Y., Y. Shimizu, M. J. Mann, Y. Jin, and L. M. Hendershot. 2010. Plasma cell differentiation initiates a limited ER stress response by specifically suppressing the PERK-dependent branch of the unfolded protein response. *Cell Stress and Chaperones* 15: 281-293.
9. Gass, J. N., H.-Y. Jiang, R. C. Wek, and J. W. Brewer. 2008. The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells. *Mol. Immunol.* 45: 1035-1043.
10. Aragon, I. V., R. A. Barrington, S. Jackowski, K. Mori, and J. W. Brewer. 2012. The Specialized Unfolded Protein Response of B Lymphocytes: ATF6α-Independent Development of Antibody-Secreting B cells. *Mol. Immunol.* 51: 347-355.
11. Reimold, A. M., N. N. Iwakoshi, J. Manis, P. Vallabhajosyula, E. Szomolanyi-Tsuda, E. M. Gravallese, D. Friend, M. J. Grusby, F. Alt, and L. H. Glimcher. 2001. Plasma cell differentiation requires the transcription factor XBP-1. *Nature* 412: 300-307.
12. Benhamron, S., R. Hadar, T. Iwawaky, J.-S. So, A.-H. Lee, and B. Tirosh. 2014. Regulated IRE-1 dependent decay participates in curtailing immunoglobulin secretion from plasma cells. *Eur. J. Immunol.* 44: 867-876.
13. Oikawa, D., M. Tokuda, A. Hosoda, and T. Iwawaki. 2010. Identification of a consensus element recognized and cleaved by IRE1α. *Nucleic Acids Research* 38: 6265-6273.
14. Hollien, J., J. H. Lin, H. Li, N. Stevens, P. Walter, and J. S. Weissman. 2009. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biology* 186: 323-331.

15. Han, D., A. G. Lerner, L. Vande Walle, J.-P. Upton, W. Xu, A. Hagen, B. J. Backes, S. A. Oakes, and F. R. Papa. 2009. IRE1α Kinase Activation Modes Control Alternate Endoribonuclease Outputs to Determine Divergent Cell Fates. *Cell* 138: 562-575.
16. Lee, K. P. K., M. Dey, D. Neculai, C. Cao, T. E. Dever, and F. Sicheri. 2008. Structure of the Dual Enzyme Ire1 Reveals the Basis for Catalysis and Regulation in Nonconventional RNA Splicing. *Cell* 132: 89-100.
17. Moore, K., and J. Hollien. 2015. Ire1-mediated decay in mammalian cells relies on mRNA sequence, structure, and translational status. *Mol Biol of the Cell* 26: 2873-2884.
18. Tavernier, Q., E. Bennana, V. Poindessous, C. Schaeffer, L. Rampoldi, N. Pietrancosta, and N. Pallet. 2018. Regulation of IRE1 RNase activity by the Ribonuclease inhibitor 1 (RNH1). *Cell Cycle* 17: 1901-1916.
19. Cross, B. C. S., P. J. Bond, P. G. Sadowski, B. K. Jha, J. Zak, J. M. Goodman, R. H. Silverman, T. A. Neubert, I. R. Baxendale, D. Ron, and H. P. Harding. 2012. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc. Nat. Acad. Sci. USA* 109: E869-E878.
20. Wang, J.-M., Y. Qiu, Z. Yang, H. Kim, Q. Qian, Q. Sun, C. Zhang, L. Yin, D. Fang, S. H. Back, R. J. Kaufman, L. Yang, and K. Zhang. 2018. IRE1α prevents hepatic steatosis by processing and promoting the degradation of select microRNAs. *Science Signaling* 11.
21. Herrema, H., Y. Zhou, D. Zhang, J. Lee, M. A. Salazar Hernandez, G. I. Shulman, and U. Ozcan. 2016. XBP1s Is an Anti-lipogenic Protein. *J Biol Chem* 291: 17394-17404.
22. So, J.-S., K. Y. Hur, M. Tarrio, V. Ruda, M. Frank-Kamenetsky, K. Fitzgerald, V. Koteliansky, A. H. Lichtman, T. Iwawaki, L. H. Glimcher, and A.-H. Lee. 2012. Silencing of lipid metabolism genes through IRE1α-mediated mRNA decay lowers plasma lipids in mice. *Cell Metabolism* 16: 487-499.
23. So, J.-S., S. Cho, S.-H. Min, S. R. Kimball, and A.-H. Lee. 2015. IRE1α-Dependent Decay of CReP/Ppp1r15b mRNA Increases Eukaryotic Initiation Factor 2α Phosphorylation and Suppresses Protein Synthesis. *Mol Cell Biol* 35: 2761-2770.
24. Kumazaki, K., B. Tirosh, R. Maehr, M. Boes, T. Honjo, and H. L. Ploegh. 2007. AID−/−μs−/− mice are agammaglobulinemic and fail to maintain B220−CD138+ plasma cells. *J. Immunol.* 178: 2192-2203.
25. Park, K. S., I. Bayles, A. Szlachta-McGinn, J. Paul, J. Boiko, P. Santos, J. Liu, Z. Wang, L. Borghesi, and C. Milcarek. 2014. Transcription elongation factor ELL2 drives immunoglobulin secretory specific mRNA production and the unfolded protein response. *J. Immunol.* 193: 4663-4674.
26. Benhamron, S., S. P. Pattanayak, M. Berger, and B. Tirosh. 2015. mTOR activation promotes plasma cell differentiation and bypasses XBP-1 for immunoglobulin secretion. *Mol Cell Biol* 35: 153-166.
27. Goldfinger, M., M. Shmuel, S. Benhamron, and B. Tirosh. 2011. Protein synthesis in plasma cells is regulated by crosstalk between endoplasmic reticulum stress and mTOR signaling. *Eur J Immunol* 41: 491-502.
28. Tirosh, B., N. N. Iwakoshi, L. H. Glimcher, and H. L. Ploegh. 2005. XBP-1 specifically promotes IgM synthesis and secretion, but is dispensable for degradation of glycoproteins in primary B cells. *J Exp Med* 202: 505-516.
29. Laplante, M., and David M. Sabatini. 2012. mTOR Signaling in Growth Control and Disease. *Cell* 149: 274-293.

30. Jones, D. D., B. T. Gaudette, J. R. Wilmore, I. Chernova, A. Bortnick, B. M. Weiss, and D. Allman. 2016. mTOR has distinct functions in generating versus sustaining humoral immunity. *J Clinical Invest* 126: 4250-4261.
31. Sintes, J., M. Gentile, S. Zhang, Y. Garcia-Carmona, G. Magri, L. Cassis, D. Segura-Garzón, A. Ciociola, E. K. Grasset, S. Bascones, L. Comerma, M. Pybus, D. Lligé, I. Puga, C. Gutzeit, B. He, W. DuBois, M. Crespo, J. Pascual, A. Mensa, J. I. Aróstegui, M. Juan, J. Yagüe, S. Serrano, J. Lloreta, E. Meffre, M. Hahne, C. Cunningham-Rundles, B. A. Mock, and A. Cerutti. 2017. mTOR intersects antibody-inducing signals from TACI in marginal zone B cells. *Nature Communications* 8: 1462.
32. Nelson, A. M., N. T. Carew, S. M. Smith, and C. Milcarek. 2018. RNA splicing in the transition from B cells to antibody secreting cells: the influences of ELL2, snRNA and ER stress. *J. Immunol.* 201.
33. Martincic, K., S. A. Alkan, A. Cheatle, L. Borghesi, and C. Milcarek. 2009. Transcription elongation factor ELL2 directs immunoglobulin secretion in plasma cells by stimulating altered RNA processing. *Nat Immunol* 10: 1102-1109.
34. Shell, S. A., K. Martincic, J. Tran, and C. Milcarek. 2007. Increased phosphorylation of the carboxyl-terminal domain of RNA polymerase II and loading of polyadenylation and cotranscriptional factors contribute to regulation of the Ig heavy chain mRNA in plasma cells. *J Immunol* 179: 7663-7673.
35. Milcarek, C., M. Albring, C. Langer, and K. S. Park. 2011. The Eleven-Nineteen Lysine-rich Leukemia gene (ELL2) influences the histone H3 modifications accompanying the shift to secretory Immunoglobulin heavy chain mRNA production. *J. Biol. Chem.* 286: 33795-33803.
36. He, N., M. Liu, J. Hsu, Y. Xue, S. Chou, A. Burlingame, N. J. Krogan, T. Alber, and Q. Zhou. 2010. HIV-1 Tat and Host AFF4 Recruit Two Transcription Elongation Factors into a Bifunctional Complex for Coordinated Activation of HIV-1 Transcription. *Mol Cell* 38: 428-438.
37. Qi, S., Z. Li, U. Schulze-Gahmen, G. Stjepanovic, Q. Zhou, and J. H. Hurley. 2017. Structural basis for ELL2 and AFF4 activation of HIV-1 proviral transcription. *Nat. Commun.* 8: 14076.
38. Murphy, S., J. B. Yoon, T. Gerster, and R. G. Roeder. 1992. Oct-1 and Oct-2 potentiate functional interactions of a transcription factor with the proximal sequence element of small nuclear RNA genes. *Molecular and Cellular Biology* 12: 3247-3261.
39. Henry, R. W., V. Mittal, B. Ma, R. Kobayashi, and N. Hernandez. 1998. SNAP19 mediates the assembly of a functional core promoter complex (SNAP(c)) shared by RNA polymerases II and III. *Genes Dev* 12: 2664-2672.
40. Smith, E. R., C. Lin, A. S. Garrett, J. Thornton, N. Mohaghegh, D. Hu, J. Jackson, A. Saraf, S. K. Swanson, C. Seidel, L. Florens, M. P. Washburn, J. C. Eissenberg, and A. Shilatifard. 2011. The little elongation complex regulates small nuclear RNA transcription. *Mol Cell* 44: 954-965.
41. Martincic, K., R. Campbell, G. Edwalds-Gilbert, L. Souan, M. T. Lotze, and C. Milcarek. 1998. Increase in the 64-kDa subunit of the polyadenylation/cleavage stimulatory factor during the G_0 to S phase transition. *Proc Natl Acad Sci USA* 95: 11095-11100.
42. Johnson, L. F., R. Levis, H. T. Abelson, H. Green, and S. Penman. 1976. Changes in RNA in relation to growth of the fibroblast. IV. Alterations in theproduction and processing of mRNA and rRNA in resting and growing cells. *J Cell Biol* 71: 933-938.

43. Kuncio, G. S., and L. Goldstein. 1981. Small nuclear rnas in cellular growth and differentiation. I: Metabolic alterations seen in friend erythroleukemic cells. *J Cell. Physiol.* 109: 235-241.
44. Skelly, R. H., G. T. Schuppin, H. Ishihara, Y. Oka, and C. J. Rhodes. 1996. Glucose-Regulated Translational Control of Proinsulin Biosynthesis With That of the Proinsulin Endopeptidases PG2 and PC3 in the Insulin-Producing MIN6 Cell Line. *Diabetes* 45: 37-43.
45. Yu, C., S. Cui, C. Zong, W. Gao, T. Xu, P. Gao, J. Chen, D. Qin, Q. Guan, Y. Liu, Y. Fu, X. Li, and X. Wang. 2015. The Orphan Nuclear Receptor NR4A1 Protects Pancreatic β-Cells from Endoplasmic Reticulum (ER) Stress-mediated Apoptosis. *J Biol Chem* 290: 20687-20699.
46. Gao, W., Y. Fu, C. Yu, S. Wang, Y. Zhang, C. Zong, T. Xu, Y. Liu, X. Li, and X. D. Wang. 2014. Elevation of NR4A3 expression and its possible role in modulating insulin expression in the pancreatic beta cell. *PLoS ONE* 9: e91462.
47. Ueda, K., J. Kawano, K. Takeda, T. Yujiri, K. Tanabe, T. Anno, M. Akiyama, J. Nozaki, T. Yoshinaga, A. Koizumi, K. Shinoda, Y. Oka, and Y. Tanizawa. 2005. Endoplasmic reticulum stress induces Wfs1 gene expression in pancreatic β-cells via transcriptional activation. *Eur. J. Endocrin.* 153: 167-176.
48. Aagaard-Tillery, K. M., and D. F. Jelinek. 1996. Phosphatidylinositol 3-kinase activation in normal human lymphocytes. *J Immunol* 156: 4543-4554.
49. Ray, R., K. Ray, and C. K. Panda. 1997. Differential alterations in metabolic pattern of the six major U snRNAs during development. *Mol. Cell Biochem* 177: 79-88.
50. Cheng, Z., Z. Du, Y. Shang, Y. Zhang, and T. Zhang. 2017. A preliminary study: PS1 increases U1 snRNA expression associated with AD. *J. Mol. Neuroscience*: 269-275.
51. Singh, R. K., and T. A. Cooper. 2012. Pre-mRNA splicing in disease and therapeutics. *Trends Mol Med* 18: 472-482.
52. Milcarek, C., K. Martincic, L.-H. Chung-Ganster, and C. S. Lutz. 2003. The snRNP-associated U1A levels change following IL-6 stimulation of human B-cells. *Mol Immunol* 39: 809-814.
53. Ma, J., S. I. Gunderson, and C. Phillips. 2006. Non-snRNP U1A levels decrease during mammalian B-cell differentiation and release the IgM secretory poly(A) site from repression. *RNA* 12: 122-132.
54. Phillips, C., N. Pachikara, and S. I. Gunderson. 2004. U1A Inhibits Cleavage at the Immunoglobulin M Heavy-Chain Secretory Poly(A) Site by Binding between the Two Downstream GU-Rich Regions. *Mol. Cell. Biol.* 24: 6162-6171.
55. Berg, M. G., L. N. Singh, I. Younis, Q. Liu, A. M. Pinto, D. Kaida, Z. Zhang, S. Cho, S. Sherrill-Mix, L. Wan, and G. Dreyfuss. 2012. U1 snRNP determines mRNA length and regulates isoform expression. *Cell* 150: 53-64.
56. Kaida, D., M. G. Berg, I. Younis, M. Kasim, L. N. Singh, L. Wan, and G. Dreyfuss. 2010. U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. *Nature* 468: 664-668.
57. Baumgartner, M., C. Lemoine, S. Al Seesi, D. K. P. Karunakaran, N. Sturrock, A. R. Banday, A. M. Kilcollins, I. Mandoiu, and R. N. Kanadia. 2014. Minor splicing snRNAs are enriched in the developing mouse CNS and are crucial for survival of differentiating retinal neurons. *Dev Neurobiology* 75: 895-907.

58. Barboric, M., T. Lenasi, H. Chen, E. B. Johansen, S. Guo, and B. M. Peterlin. 2009. 7SK snRNP/pTEFb couples transcription elongation with alternative splicing and is essential for vertebrate development. *Proc Natl Acad Sci, USA* 106: 7798-7803.
59. Lis, J. T., P. Mason, J. Peng, D. H. Price, and J. Werner. 2000. P-TEFb kinase recruitment and function at heat shock loci. *Genes Dev.* 14: 792-803.
60. Chen, R., J. H. N. Yik, Q. J. Lew, and S.-H. Chao. 2014. Brd4 and HEXIM1: Multiple Roles in P-TEFb Regulation and Cancer. *BioMed Research International* 2014: 11.
61. Smith, E., C. Lin, and A. Shilatifard. 2011. The super elongation complex (SEC) and MLL in development and disease. *Genes Dev* 25: 661-672.
62. Zhou, Q., and J. H. N. Yik. 2006. The Yin and Yang of P-TEFb Regulation: Implications for Human Immunodeficiency Virus Gene Expression and Global Control of Cell Growth and Differentiation. *Microbiol. Mol. Biol. Rev.* 70: 646-659.
63. Tan, J L., R D. Fogley, R A. Flynn, J. Ablain, S. Yang, V. Saint-André, Z P. Fan, B T. Do, A C. Laga, K. Fujinaga, C. Santoriello, C B. Greer, Y J. Kim, J G. Clohessy, A. Bothmer, N. Pandell, S. Avagyan, J E. Brogie, E. van Rooijen, E J. Hagedorn, N. Shyh-Chang, R M. White, D H. Price, P P. Pandolfi, B. Peterlin, Y. Zhou, T H. Kim, J M. Asara, H Y. Chang, R A. Young, and L I. Zon. 2016. Stress from Nucleotide Depletion Activates the Transcriptional Regulator HEXIM1 to Suppress Melanoma. *Mol Cell* 62: 34-46.
64. de la Mata, M., C. Lafaille, and A. R. Kornblihtt. 2010. First come, first served revisited: factors affecting the same alternative splicing event have different effects on the relative rates of intron removal. *RNA* 16: 904-912.
65. Shilatifard, A., D. R. Duan, D. Haque, C. Florence, W. H. Schubach, J. W. Conaway, and R. C. Conaway. 1997. ELL2, a new member of an ELL family of RNA polymerase II elongation factors. *Proc. Natl. Acad. Sci. USA* 94: 3639-3643.
66. Arumemi, F., I. Bayles, J. Paul, and C. Milcarek. 2013. Shared and discrete interacting partners of ELL1 and ELL2 by yeast two-hybrid assay. *Adv. Biosci. Biotech.* Paper ID: 7300601
67. Gridasova, A. A., and R. W. Henry. 2005. The p53 Tumor Suppressor Protein Represses Human snRNA Gene Transcription by RNA Polymerases II and III Independently of Sequence-Specific DNA Binding. *Mol Cell Biol* 25: 3247-3260.
68. Anwar, D., H. Takahashi, M. Watanabe, M. Suzuki, S. Fukuda, and S. Hatakeyama. 2016. p53 represses the transcription of snRNA genes by preventing the formation of little elongation complex. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* 1859: 975-982.
69. Hu, W., Z. Feng, and A. J. Levine. 2012. The Regulation of Multiple p53 Stress Responses is Mediated through MDM2. *Genes & Cancer* 3: 199-208.
70. Ortega, A., E. Roselló-Lletí, E. Tarazón, M. M. Molina-Navarro, L. Martínez-Dolz, J. R. González-Juanatey, F. Lago, J. D. Montoro-Mateos, A. Salvador, M. Rivera, and M. Portolés. 2014. Endoplasmic Reticulum Stress Induces Different Molecular Structural Alterations in Human Dilated and Ischemic Cardiomyopathy. *PLoS ONE* 9.
71. Santos, L. E., and S. T. Ferreira. 2018. Crosstalk between endoplasmic reticulum stress and brain inflammation in Alzheimer's disease. *Neuropharmacology* 136: 350-360.

Figure legends

Figure 1. Mouse splenic B cells cultured *ex vivo* with LPS differentiate into antibody secreting cells and experience changes in splicing and gene expression. The expression of the ELL family members changes from primarily ELL1 and ELL3 in B cells to induction of large amounts of ELL2 in ASCs (not shown). The exon splicing patterns are figuratively meant to show the increasing complexity seen in cells with time. We hypothesize that the decrease of RNA for SnapC and snRNA is linked to the RIDD activity induced by ER stress. About 4,211 genes spliced early (18 hrs) persist into the 72 hr samples and are seen as ELL2 independent. Meanwhile 7,361 genes alternatively spliced at 18 hrs are missing at 72 hrs and are replaced by the 5,115 genes that are ELL2 dependent.

Figure 2. Venn Diagrams of alternatively spliced genes. Panel A. Overlap between alternatively spliced genes in the ELL2+/+ and the ELL2-/- data sets at 72 hrs post LPS treatment. B. ELL2 independent genes (seen at 72 hrs) overlap with the alternatively spliced genes at 18hrs. C. Addition of 4u8C changes the splicing patterns at 18 hrs both reducing the number and omitting some of the ELL2 independent species. The data associated with these diagrams are deposited in NCBI Gene Expression Omnibus under accession numbers GSE113317, GSE113475 and GSE114435.

Figure 3. Expression of RNAs during LPS stimulation. Samples at 18 hr and 72 hrs post LPS were analyzed and compared with naïve B cells (To) set as 100%. RNA from treatment of cell with 18 hr LPS and 4u8C were similarly determined relative to the To samples.

Figure 4. snRNA levels uniformly respond to stress not growth. A. RT-QPCR was performed on RNA samples from growing or resting 3T6 mouse fibroblast cells. B. MIN6 mouse pancreatic beta cells were treated with thapsigargin at 5uM for 5 hours and the RNA subjected to RT-QPCR. C. Western blot showing protein expression in MIN6 cells stimulated with 5uM thapsigargin at t= 0, 2, and 5 hours. SnapC1 mRNA encodes a 43 kDa protein. Xbp1 long and short forms are indicated. Actin was used as a loading control. Mouse pancreatic beta cells (MIN6) were obtained from ATCC (CRL-11506), assayed as mycoplasma free and used at a low

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passage number. Cells were cultured in DMEM medium containing 50 μM 2-ME, 2 mM glutamine, 10% FBS, 1mM sodium pyruvate, nonessential amino acids, 1X Pen/Strep, and 1mM HEPES buffer. Semiconfluent cultures were treated with thapsigargin (TG, Tocris Bioscience #1138) in 100mM DMSO. Cells receiving only the DMSO were used as the control.

Figure 1

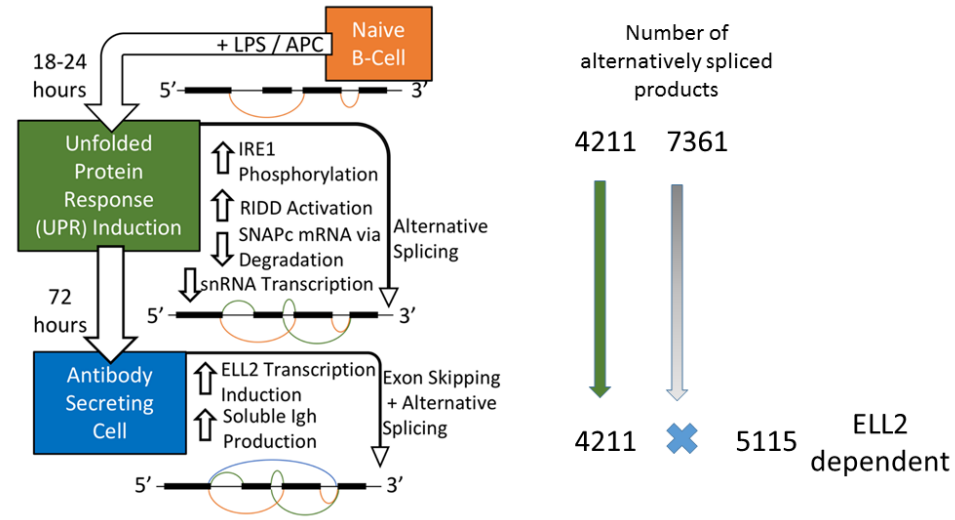

Figure 2

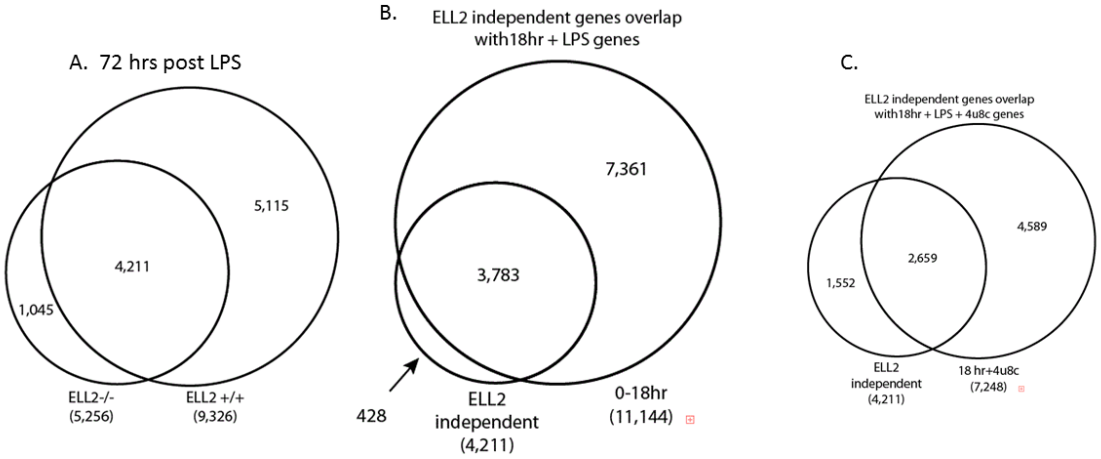

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Figure 3

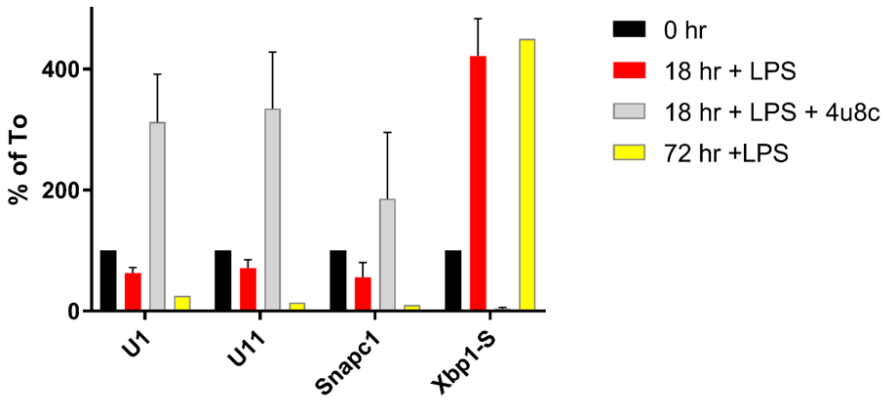

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Figure 4