Supporting Information

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SI Materials and Methods

Induction of GFP-b(5)tail Expression. GFP-b(5)tail–HeLa-TetOff cells were grown in DMEM supplemented with 100 μg/mL G418, streptomycin, penicillin, and 10% (vol/vol) Tet-approved FBS (Clontech) at 37 °C under a 5% CO_2 atmosphere. The culture medium normally also contained 0.1 μg/mL Dox. At the start of each experiment, cells were detached and replated at 15–20% confluence either in the presence or absence of Dox. After 8 h, the medium was replaced with Dox-free or Dox-containing medium. "Day 1" cells were collected after a further 16 h, and the medium was again replaced on the plates to be analyzed on the following days.

Immunoblotting. Blots were immunostained with the following primary antibodies obtained from the indicated sources: anti-GFP polyclonal antibody (MBL International Corporation); anti-eIF2α and anti–P-eIF2α polyclonal antibodies and anti-Ire1α monoclonal antibodies (Cell Signaling); anti-ATF6α monoclonal antibody (BioAcademia); anti-protein disulfide isomerase monoclonal antibodies (Stressgen); anti-Flag, anti-β-actin, and antitubulin monoclonal antibodies (Sigma); anti-GRP94 and anticalreticulin polyclonal antibodies (gifts from Marek Michalak, University of Alberta, Edmonton, AB, Canada); and ribophorin I polyclonal antibodies (gift from Gert Kreibich, New York University School of Medicine, New York, NY).

Incubation with antibodies was carried out with 5% (wt/vol) milk as a blocking agent or with BSA in some cases, as indicated by the supplier. Blots were developed with ECL or ECL plus reagent from GE Healthcare. The films were digitized, and band intensities were determined with ImageJ software (National Institutes of Health) after calibration with the optical density calibration step table (Stouffer Graphics Arts).

Total RNA Extraction and RT. Total RNA was extracted using the RNeasy Mini Kit and accompanying QIAshredder (Qiagen) according to the manufacturer's instructions. Contaminating DNA in the sample was degraded by on-column incubation with DNaseI (Qiagen) for 15 min. The amount of eluted total RNA was determined spectrophotometrically at 260 nm, and its purity was evaluated using the 260:280 ratio; subsequently, 1 μg per sample was reverse-transcribed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Ltd.) in accordance with the manufacturer's instructions.

Conventional RT-PCR of XBP1 mRNA and Cloning of the Exon 4-Exon 5 cDNA Fragment. Spliced and unspliced XBP1 cDNA was amplified in the same sample, using primers designed on GenBank RefSeqGene NG_012266.1, located in exon 4 (forward primer: 5′-AAG CCA AGG GGA ATG AAG TGA G-3′) and exon 5 (reverse primer: 5′-AAG AGT CAA TAC CGC CAG AAT CC-3′), respectively. Fifty nanograms of retrotranscribed RNA was used in a 50-μL reaction in the presence of GoTaq Flexi DNA polymerase (Promega) according to the manufacturer's instructions. The cycling conditions were as follows: one cycle of denaturation (95 °C for 2 min), 30 three-segment cycles of amplification (95 °C for 30 s, 60 °C for 45 s, and 72 °C for 45 s), and a final cycle of extension (72 °C for 5 min). The bands corresponding to unspliced and spliced mRNAs (from TM-treated cells; Fig. 3*A*) were size-separated on a 1.2% (wt/vol) agarose gel and eluted with the Nucleospin extract II Kit (Macherey–Nagel GmbH & Co. KG), according to the manufacturer's instructions. The cDNA fragments were inserted by means of TA cloning into the pCRII vector (Invitrogen, Ltd.), and plasmid purified from positive clones was used for the experiment in Fig. S1*B*.

RT-qPCR. Gene expression was quantitatively analyzed by SYBR green (BiP and RAMP4) or Taqman (spliced and unspliced XBP1) technology, using the ABI Prism 7000 Sequence Detection System and SDS software version 1.2.3 (Applied Biosystems). The $2^{-\Delta\Delta CT}$ method was used to calculate the results, thus allowing the normalization of each sample to the endogenous control and comparison with the calibrator for each experiment (set to a value of 1), as described in the figure legends.

BiP and RAMP4. The target sequences were amplified from 50 ng of cDNA in the presence of SYBR-Green PCR Master Mix (Applied Biosystems). Primers to detect BiP expression were designed on GenBank accession no. NM_005347.4. The primer sequences were as follows: forward primer, 5′-CCA ACG ACA AGC AAC CAA AGA TG-3′, and reverse primer, 5′-CCA CCC AGG TCA AAC ACC AGG ATG-3′. Primers to detect RAMP expression were designed on GenBank accession no. NM_014445.3 and were as follows: forward primer, 5′-CCA ACG AGA AGC ACA GCA AGA AC-3′, and reverse primer, 5′-AAT AAC CAG GGT CCT ACA GAC GCC-3′. In all analyses, GAPDH mRNA was used as an internal control for mRNA level normalization (forward primer, 5′-TCG GAG TCA ACG GAT TTG G-3′, reverse primer, 5′-TGG CAA CAA TAT CCA CTT TAC CA-3′; GenBank accession no. NM_002046.3).

Primers and amplicons were analyzed *in silico* with BLASTN for specificity, and the size of the PCR products was confirmed by gel electrophoresis. Each reaction consisted of 25 μL containing 2 μL of cDNA and 7.5 pmol of each primer (in the case of BiP) or 1.25 pmol of forward primer and 7.5 pmol of reverse primer (in the case of GAPDH) and 7.5 pmol of forward primer and 1.25 of pmol reverse primer for RAMP. The cycling conditions were one cycle of denaturation at 95 °C for 10 min, followed by 40 two-segment cycles of amplification (95 °C for 15 s and 60 °C for 1 min). At the end of the cycles, a dissociation protocol (95 °C for 15 s, 60 °C for 20 s, and 95 °C for 15 s) was run for each primer pair to verify the presence of one gene-specific peak and the absence of primer-dimer. All samples were amplified in triplicate, and the mean was used for further analysis.

Spliced and unspliced XBP1. The assays were designed to distinguish spliced and unspliced XBP1 using Assay-by-Design File Builder Software (Applied Biosystems). Assays for the spliced XBP1 (central FAM-labeled probe 5′-TGC TGA GTC CGC AGC AGG TGC A-3′ spanning the exon 4 and exon 5 splice junction) and unspliced XBP1 (central FAM-labeled probe 5′-CAG CAC TCA GAC TAC GTG-3′ spanning intron 4) were designed on GenBank RefSeqGene NG_012266.1 and used at a final concentration of 200 nM. The external primer sequences were as follows: forward primer, 5′-AAT GAA GTG AGG CCA GTG GC-3′, and reverse primer, 5′-TGA AGA GTC AAT ACC GCC AGA A-3′ (Fig. S1*A*), used at a final concentration of 300 nM. As an endogenous control, the TaqMan primer and probe assay *GADPH* (ID no. Hs99999905_m1) was used. Spliced and unspliced XBP1 was amplified from 50 ng of cDNA, usingTaqMan Universal PCR Master Mix Kit, No AmpEraseUNG (Applied Biosystems) according to the instructions of the manufacturer. The specificity of the two FAM-labeled probes for the two XBP1 forms was checked on cloned spliced and unspliced XBP1 cDNA fragments, as illustrated in Fig. S2.

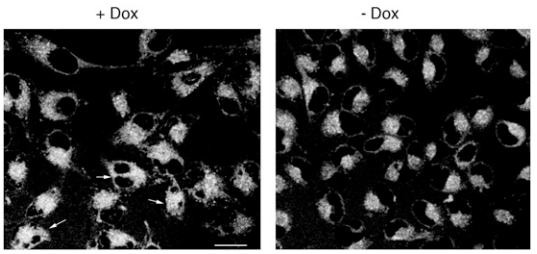

Fig. S1. Confocal analysis of cells grown in the presence or absence of Dox for 2 d and stained with ER-Tracker Blue-White (Invitrogen). No gross differences between the induced and noninduced cells are visible at this level of resolution. Some of the cells have indented or duplicated nuclei, whether grown in the presence (*Left*, arrows) or absence of Dox (Fig. 1*D*). (Scale bar: 20 μm.)

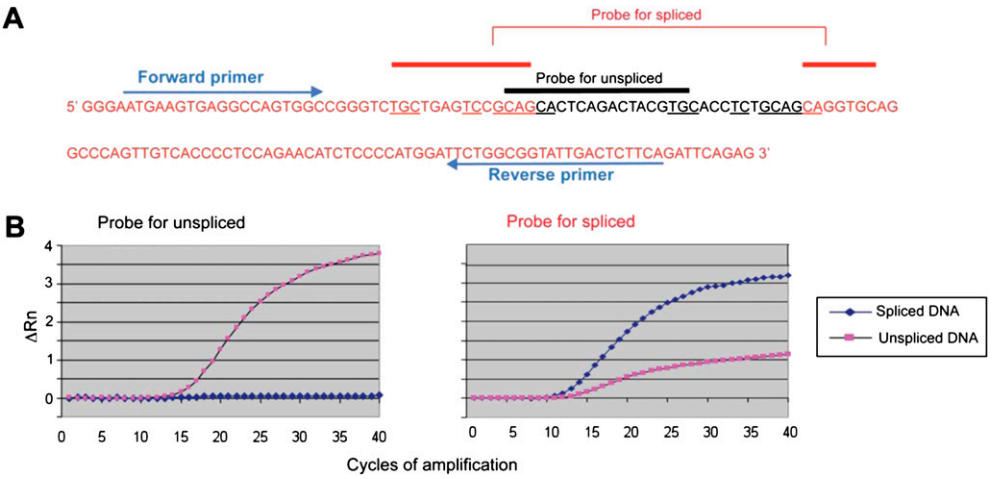

Fig. S2. Design of RT-qPCR experiments for the quantitative analysis of spliced and unspliced XBP1 mRNA. (*A*) Schematic representation of the region spanning XBP1 exons 4 and 5. The sequences common to the spliced and unspliced forms of XBP1 are shown in red, and the retained intron is shown in black. The common forward and reverse primers (blue) and the FAM-labeled probes for the spliced (red) and unspliced (black) forms are indicated. Sequences common to intron 4 and to the flanking regions of exons 4 and 5 are underlined. (*B*) Specificity of DNA amplification. The TaqMan probes designed to detect the expression level of the spliced and unspliced XBP1 isoform share a part of their sequence, as shown in *A*. To test whether the TaqMan assays were able to distinguish between the two XBP1 isoforms, equal amounts of XBP1 unspliced and spliced cDNA fragment, cloned in the plasmid vector pCRII, were amplified. (*Left*) Assay for detection of the unspliced form was specific for the corresponding DNA, because no amplification was measured in the presence of spliced XBP1 cDNA. (*Right*) Probe for the spliced form detected both forms, but unspliced cDNA was amplified with eightfold less efficiency (a difference of three cycles to reach the threshold cycle). Thus, the combined use of the two probes permits the discrimination between the two XBP1 forms. The amplification plots show the normalized reporter signals ΔRn vs. the cycle number. The data points are the means of two independent experiments, each performed in triplicate.

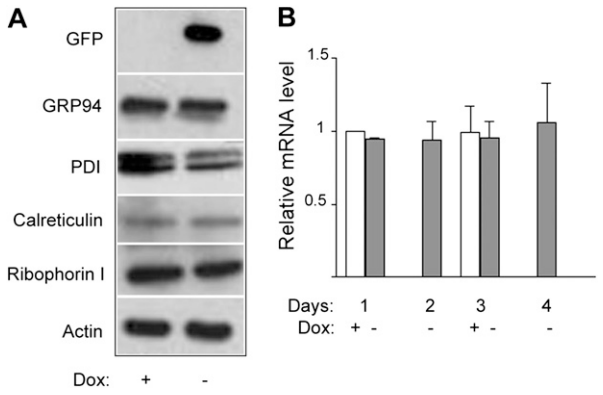

Fig. S3. GFP-b(5)tail induction does not alter the expression of stress-induced proteins. (*A*) GFP-b(5)tail–HeLa-TetOff cells grown in the presence or absence of Dox for 4 d were lysed and analyzed by immunoblotting with the indicated antibodies. PDI, protein disulfide isomerase. (*B*) RT-qPCR analysis of RAMP4 mRNA in cells induced or not induced to express GFP-b(5)tail. RNA was isolated from cells cultured for the indicated times with or without Dox, and cDNA was obtained by RT. RAMP expression was quantitatively analyzed by SYBR Green binding as described in *SI Materials and Methods*. The data were normalized as in Fig. 3. Data are from three experiments performed in triplicate, and the bars show the mean ± SEM.

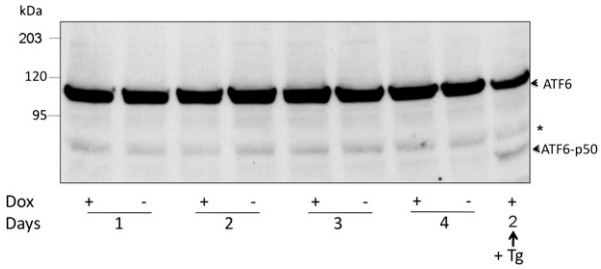

Fig. S4. Dox removal does not induce ATF6α cleavage in nontransfected HeLa-TetOff cells. ATF6α cleavage was analyzed as in Fig. 4*B*. The cleaved and uncleaved forms of ATF6α are indicated. The asterisk indicates a nonspecific band.