SUPPLEMENTAL FIGURE LEGENDS

Supplemental FIG. S1. RNase Protection Assay. (A) Cartoon of probe design for RNase protection assay (RPA) against murine *Xbp1* mRNA, pre-rRNA, and *7SL* (not to scale). The *Xbp1* probe contains sequences complimentary to nucleotides 207-505 of the *Xbp1* coding region. The *Xbp1* probe yields two distinct protected fragments (thin gray lines) corresponding to unspliced and spliced forms *Xbp1*. The pre-rRNA probe is complimentary to nucleotides 537-767 of the of the rRNA primary transcript yielding a protected fragment of 232 nt. If cleavage at site +650 has occurred, the protected fragments are half the size and migrate much faster on the polyacrylamide gel. *7SL* probe is complimentary to nucleotides 59-227 of the *7SL* RNA. All probes contain 16-18 nt of non-complementary sequence at the 5' end of the probe (black lines) in order to distinguish full length probe from full length protected fragments. (B) Autoradiograph of a representative RPA showing a single exposure of all three probes on the top panel, and lower panels show optimal exposures of *Xbp1* and *7SL* used in Figures 1-4; Figure 6; and supplemental Figures S2, S4, and S5. Lanes 1 and 2 are yeast tRNA control RNase protection reactions with (lane 2) or without (lane 1) addition of RNase. Lanes 3-8 are RNase protection reactions from the indicated concentration of wild type MEF RNA isolated from cells with (lanes 6-8) or without (lanes 3-5) treatment of 200 nM Tg for 2 hr. RNA was hybridized to all three probes overnight and the entire reaction was loaded onto a 6% denaturing acrylamide gel except 1/25th of the reaction was loaded in lane 1 to show undigested probes. Note the lack of protected fragments in lane 2 indicating probes do not hybridize

to one another, and in lanes 3-8 small protected fragments resulting from cleaved products are not shown.

Supplemental FIG. S2. Comparison of pre-rRNA analyzed by Northern or RPA. (A) Northern blot from wild type MEFs treated with Tg (200 nM) over a 12 hr time course. 10 µg of total RNA was run on 1% formaldehyde northern gel, transferred to zeta-probe membrane, and probed for full length pre-rRNA and RPA194 mRNA as loading control. (B) RPA probing for pre-rRNA and *7SL* using 2.5µg of the same RNA samples as in (A).

Supplemental FIG. S3. Nuclear Run On of Pol II and Pol III Transcripts. (A) Nuclear Run On analysis of Pol II transcripts *BiP*, inducible heat shock protein 70 (*Hsp70*), β-actin (*Actb*), α-tubulin (*Tuba2*), triosephosphate isomerase 1 (*Tpi1*), histone H1 (*H1.0*), and hexokinase II (*HKII*) as well as Pol III transcripts *7SK* and *7SL*. (B) Quantitation of nuclear run on as showed in (A). Each bar represents the mean and SD of three independent experiments.

Supplemental FIG. S4. (A) Western blots from *Perk*$^{+/+}$ and *Perk*$^{-/-}$ MEFs treated with Tg (200 nM) or DMSO (NT, 0.1%) over a 12 hr time course. Protein samples were run on denaturing polyacrylamide gels and probed with antibodies against phospho-eIF2α (top). Blots were stripped and then re-probed with antibodies against total eIF2α. (B) *eIF2*a$^{A/A}$ MEFs completely fail to downregulate pre-rRNA during 12 hrs of ER stress. RPA probing for pre-rRNA, *Xbp1*, and *7SL* in total

RNA isolated from *eIF2*$\alpha^{S/S}$ and *eIF2*$\alpha^{A/A}$ MEFs treated with Tg (200 nM) or DMSO (NT, 0.1%) over a 12 hr time course. (C) *eIF2*$\alpha^{S/S}$ and *eIF2*$\alpha^{A/A}$ MEFs were treated with Rap (20 nM). Total RNA was analyzed by RPA against pre-rRNA (top), and *7SL* (bottom).

Supplemental FIG. S5. PERK Regulation of rRNA is Specific to UPR. (A) Quantitation of rRNA promoter DNA amplified from ChIP samples using antibody against UBF. *Perk*$^{+/+}$ (black bars) and *Perk*$^{-/-}$ MEFs (white bars) were either untreated (NT) or treated with Tg (200nM) or H_2O_2 (30 µM) for 1 hr. Control ChIP was performed with samples from wild type MEFs using antibodies against GAPDH. Each bar represents the mean and SD of three independent experiments. Promoter DNA amplified from untreated cells were given the value of 1. (B) Ethidium staining of the first internal transcribed spacer (ITS-1) DNA amplified from ChIP samples using antibody against GAPDH, RRN3/TIF-IA, and Pol I (RPA194) upon treatment with Tg (200 nM). PCR was performed using two dilutions of ChIP template DNA for each sample representing a three-fold dilution of template DNA. Note that GAPDH and RRN3/TIF-IA ChIP do not pull down significant quantities of ITS-1 DNA. (C) Time course of *Perk*$^{+/+}$ and *Perk*$^{-/-}$ MEFs treated with ANS (10 µM). Cellular RNA was isolated at the indicated times and analyzed by RPA using probes against pre-rRNA, and *7SL*. (D) Quantitation of pre-rRNA relative to *7SL* during ANS treatment in *Perk*$^{+/+}$ (solid line) and *Perk*$^{-/-}$ cells (dashed line) as shown in (A). Each transcript at 0 hr was given the value of 1, and each point represents the mean ± SD of a minimum of three independent

experiments. (E) and (F) Quantitation of rRNA promoter DNA amplified from ChIP samples using antibody against Pol I (RPA194; C) or RRN3/TIF-IA (D) from *Perk*$^{+/+}$ (dark grey) and *Perk*$^{-/-}$ MEFs (light grey) treated with H_2O_2 (30 μM) for the indicated times. Control ChIP was performed with samples from wild type MEFs using antibodies against GAPDH. Each bar represents the mean and SD of three independent experiments. Promoter DNA amplified from untreated cells was given the value of 1.

Supplemental FIG. S6. Analysis of Purified RRN3/TIF-IA and UBF. (A) Western blot of indicated concentrations of purified FLAG-RRN3 protein (lanes 2-3) and 25μg of untreated nuclear extracts used for in vitro transcription (Ex; lane 1). Blots were probed with antibodies against RPA194 (Pol I), UBF, and FLAG (Sigma). Note the UBF antibody recognizes both isoforms of UBF protein, and that both UBF and Pol I are not detected in the FLAG-RRN3 purification (B) Coomassie stained gel of purified FLAG-RRN3 protein. Molecular weights of protein marker is indicated in KDa. (C) Western blot of partially purified UBF protein isolated from Novicoff hepatoma cells and 25μg of untreated nuclear extracts used for in vitro transcription (Ex). The amount of UBF protein used in transcription reactions in Figure 6D were estimated to be 2.5 and 5 fold the level of the endogenous UBF in the nuclear extract. Note the UBF antibody recognizes both isoforms of UBF protein.