The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress.

Brian F. Teske, Sheree A. Wek, Piyawan Bunpo, Judy K. Cundiff, Jeanette N. McClintick, Tracy G. Anthony, and Ronald C. Wek

SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1. Significant changes in gene transcript levels in livers from mice treated with tunicamycin or no ER stress condition.

p-value (ANOVA)	0.05	0.02	0.01	0.005	0.001
Number of probesets	9645	7421	6201	5187	3398
FDR (%)	15.0	7.0	4.2	2.5	0.8

Number of probe sets that changed significantly with respect to tunicamycin treatment.

Supplemental Table 2. Significant changes in transcript levels in WT and Ls*PERK-KO* livers.

p-value (ANOVA)	0.05	0.02	0.01	0.005	0.001
Number of probesets	4412	2738	1887	1263	504
FDR (%)	29.3	18.9	13.7	10.2	5.1

Number of probe sets that changed significantly with respect to genotype.

Supplemental Table 3. Interaction Term (condition*genotype) for microarray analysis.

p-value (ANOVA)	0.05	0.02	0.01	0.005	0.001
Number of probesets	7039	4806	3610	2660	1253
FDR (%)	18.4	10.8	7.2	4.9	2.1

Number of probe sets that changed significantly with respect to condition and genotype.

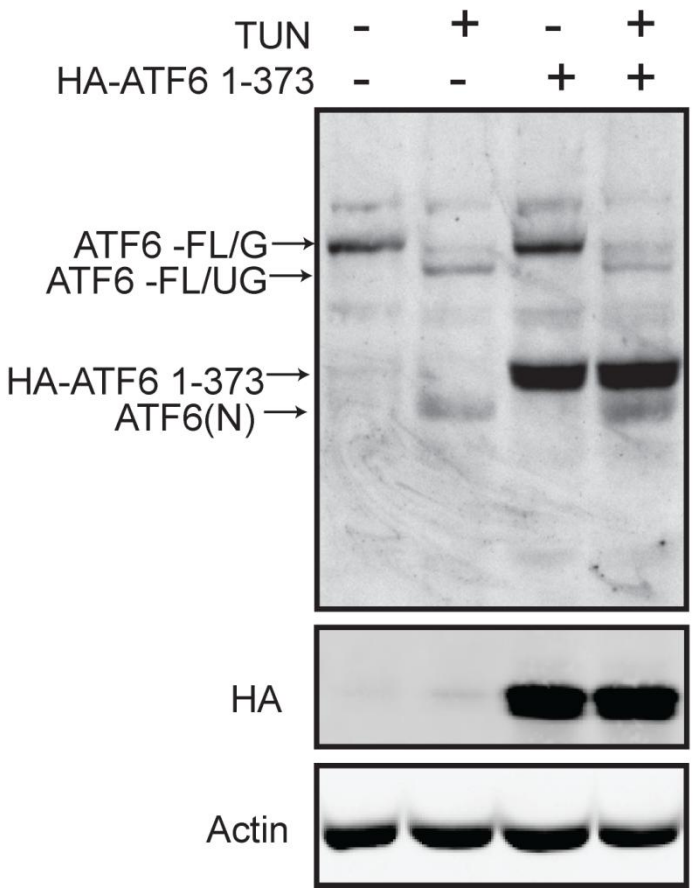
Supplemental Figure 1. ATF6 antibody detects endogenous and ATF6 over-expressed in MEF cells. WT MEF cells were transfected with an HA-tagged ATF6 expression plasmid encoding residues 1-373 (+), or no expression plasmid (-). Cells were then treated (+) for 3 hours with 2 μM tunicamycin, or left untreated (-). Cell lysates were prepared and analyzed by immunoblot using antibody that recognizes ATF6, the HA tag, or actin.

Supplemental Figure 2. PERK facilitates the expression of metabolic genes in response to ER stress. Graphical representation of the expression of genes involved in metabolism that are significantly reduced ($p<0.05$) in the LsPERK-KO. The mean fluorescent intensity (MFI) for each transcript is shown as a histogram, along with the S.D

Supplemental Figure 3. ATF4 induction is required for full activation of ATF6 in response to ER stress in MEF cells. WT and *ATF4*$^{-/-}$ MEF cells derived from an independent knockout (Hettmann *et al.*, 2000) were treated with thapsigargin for up to 6 hours as indicated, and the levels of ATF6, ATF4, and actin were measured by immunoblot analysis.

Supplemental Figure 4. PERK is required for full induction of *XBP1* mRNA in MEF cells treated with tunicamycin. WT and *PERK*$^{-/-}$ MEF cells were treated with 2 μM tunicamycin (TUN), as indicated, and mRNA levels for spliced *XBP1*, total *XBP1*, and *ATF4* were measured by qPCR. The "*" symbol indicates a significant difference ($p<0.05$) between tunicamycin treated and non-stressed samples, and the "#" symbol represents a significant difference between cell types.

Supplemental Figure 5. ATF4 is required for full induction of *XBP1* mRNA in response to ER stress. WT and *ATF4*$^{-/-}$ MEF cells were treated with 2 μM tunicamycin (TUN), as indicated. The mRNA levels for spliced *XBP1,* total *XBP1*, and *ATF4* were determined by qPCR. The "*" symbol highlights a significant difference (p<0.05) between tunicamycin treated and non-stressed samples, and the "#" symbol indicates a significant difference between WT and *ATF4*$^{-/-}$ cells.



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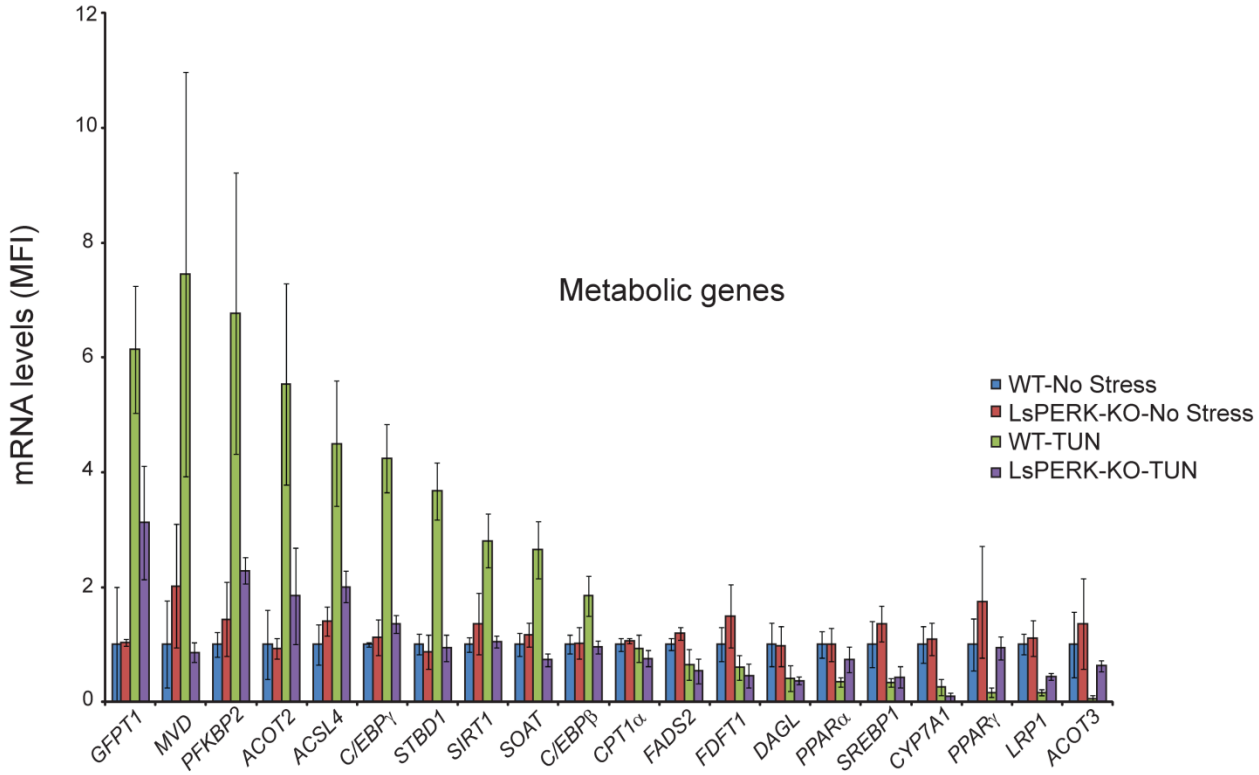

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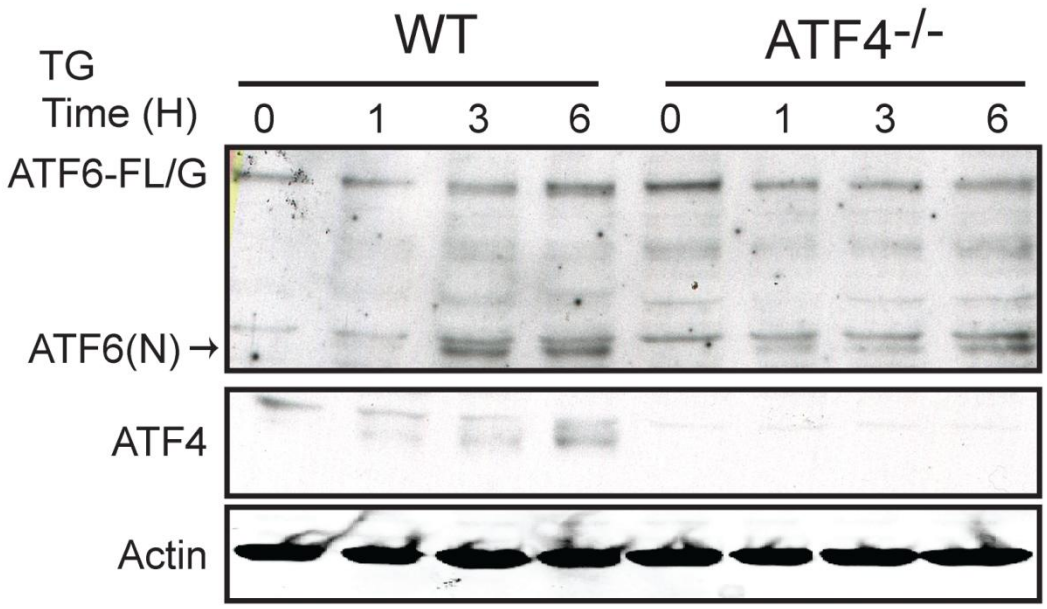

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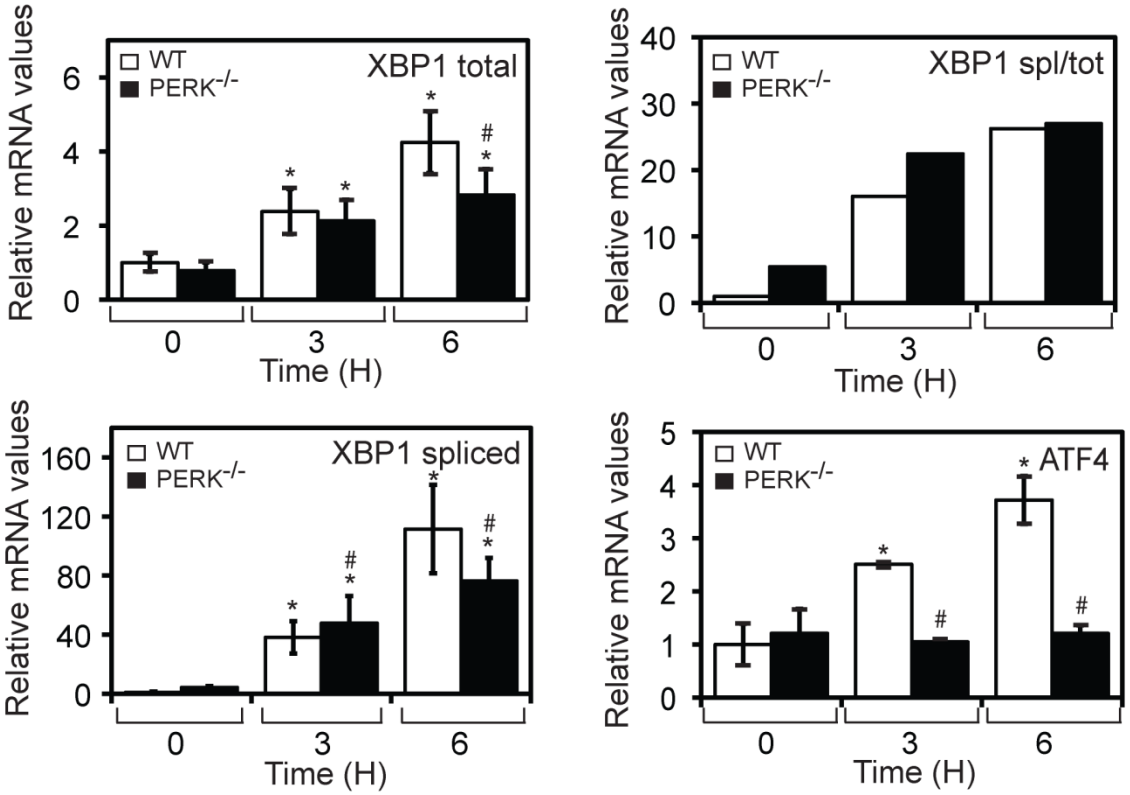
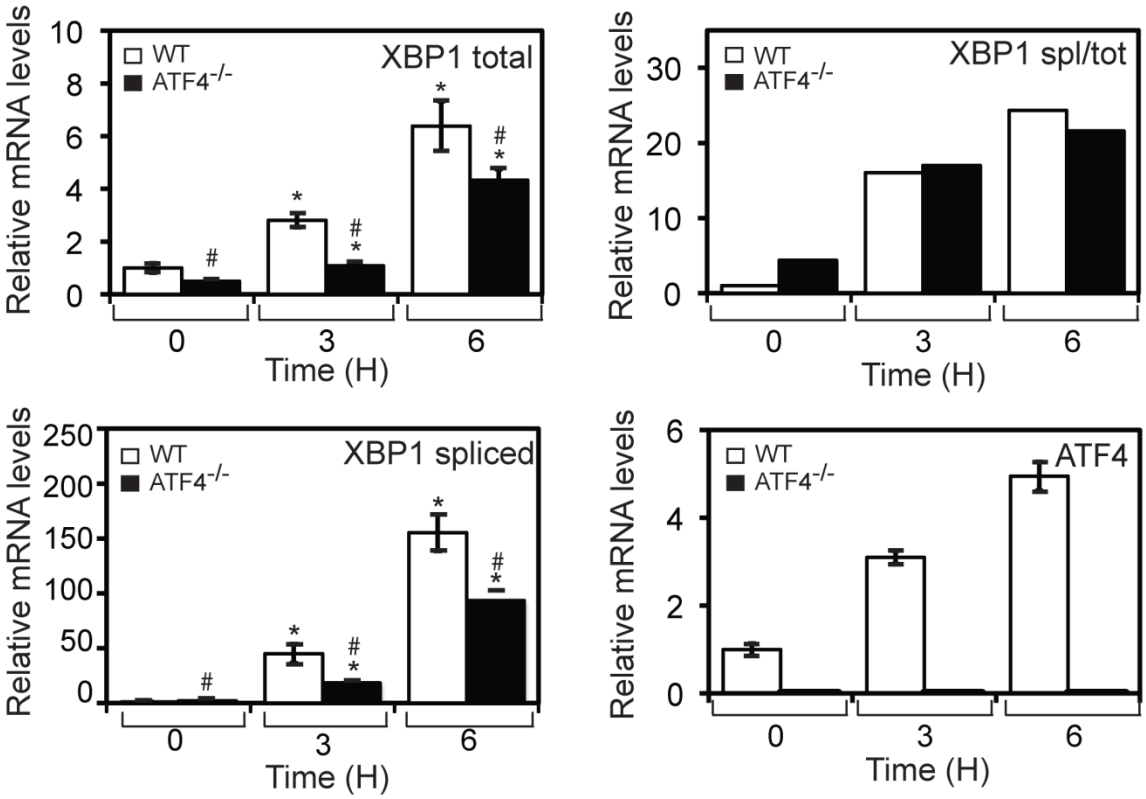


Supplemental Figure 4. PERK is required for full induction of *XBP1* mRNA in MEF cells treated with tunicamycin.



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