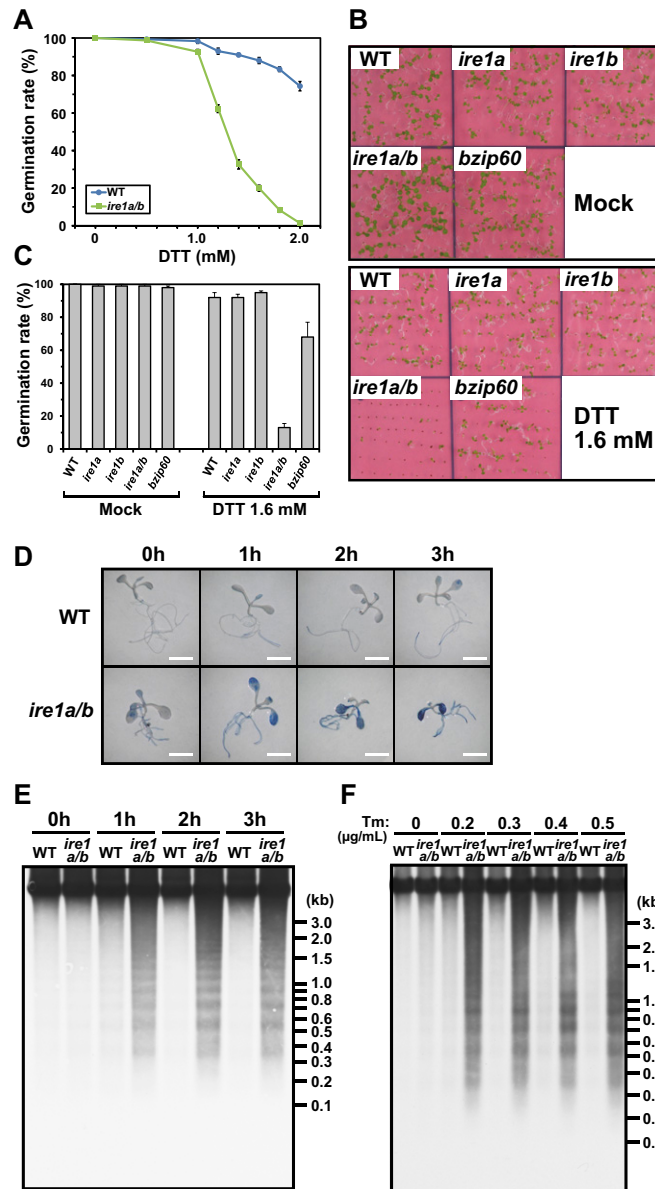# Supporting Information

Mishiba et al. 10.1073/pnas.1219047110

Fig. S1. Germination of mutants in the presence of DTT and observation of cell death in WT and *ire1a/b*. (*A*) Germination rates of WT and *ire1a/b* in different concentrations of DTT. The germination rate was determined as described (1) from three independent experiments (*n* = 100). (*B*) Germination of each mutant with (*Lower*) or without (*Upper*) 1.6 mM DTT in the medium. (*C*) Germination rates of the each mutant from three independent experiments (*n* = 100) with or without 1.6 mM DTT in the medium. (*D*) Evans blue staining of WT and *ire1a/b* seedlings treated with tunicamycin (Tm; 5 μg/mL) for 0–3 h. Seedlings were further cultured for 24 h and subjected to staining. (Bars: 5 mm.) (*E*) Detection of internucleosomal DNA fragmentation. DNA was extracted from whole tissues of WT and *ire1a/b* treated as in *D* and subjected to the assay. (*F*) Genomic DNAs extracted from aerial parts of WT and *ire1a/b* seedlings treated with Tm (0.2–0.5 μg/mL) for 72 h were subjected to the assay.

1. Nagashima Y, et al. (2011) Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor. *Sci Rep* 1:29.

Fig. S2. Relative transcript abundance of unfolded protein response (UPR)-related genes quantitated by qPCR. RNA was prepared from mock-treated (○) or Tm-treated (5 μg/mL; ●) seedlings of WT, *bzip60*, *ire1a*, *ire1b*, and *ire1a/b*. The relative abundance was presented according to the value of untreated (0 h) WT. *bZIP60u* and *bZIP60s* were detected by using specific primers for each mRNA. Data are means ± SD of four independent experiments.

Fig. S3. Relative transcript abundance of three selected genes and *BiP1* in WT, *bzip60*, *ire1a*, *ire1b*, and *ire1a/b* seedlings treated with DMSO (mock; ○) or Tm (5 μg/mL; ●). RNA samples used in Fig. S2 were used for qPCR, and the relative abundance is presented on a $\log_2$ scale. Data are means ± SD of four independent experiments. Data for WT, *bzip60*, and *ire1a/b* are also shown in Fig. 2*B*.

Fig. S4. Relative transcript abundance in WT and *ire1a/b* seedlings. (*A*) Seedlings were treated with various concentrations of Tm (black bars) or 2 mM DTT (gray bars) for 5 h. RNA samples used in Fig. 2*C* were used for qPCR to quantitate *bZIP60s* and *BiP3* transcripts. The abundance levels relative to those of untreated (0 h) WT were presented. (*B*) The abundance of *AT1G78850* transcripts is presented on a $\log_2$ scale as shown in Fig. 2*C*. (*C*) Seedlings were treated with DMSO (mock; ○), Tm (5 μg/mL; ●), DTT (2 mM; ▲), and heat (42 °C; ♦) for the indicated periods. RNA samples were subjected to qPCR, and the relative abundance is presented on a $\log_2$ scale. Data are means ± SD of four independent experiments.

Fig. S5. IRE1-dependent degradation of *PR-4* mRNA by DTT. (*A*) RNA gel blot analysis of *PR-4* mRNA in WT and *ire1a/b*. Total RNA (5 μg) extracted from seedlings treated with DTT (2 mM) for the indicated periods was used for hybridization. (*B*) CAP structure-independent 5′ RACE (cRACE) analysis of *PR-4* mRNA prepared from WT and *ire1a/b* treated with DTT (2 mM). Each lane corresponds to an independent reverse transcription. Arrow indicates the size of PCR products for intact *PR-4* mRNA.

Fig. S6. Bioinformatic analyses estimating regulated IRE1-dependent decay (RIDD) targets. (*A*) Gene Set Enrichment Analysis (GSEA) showing the correlation between RIDD targets and genes encoding secretory pathway proteins. Analyses similar to those performed in Fig. 3 were carried out using different combinations of microarray data. Microarray data from mock- and Tm-treated WT (*Upper*) and *ire1a/b* (*Lower*) in the presence of actinomycin D were subjected to GSEA. (*B*) Correlation between genes down-regulated by heat and genes encoding predicted signal sequences and/or transmembrane domains (SS/TM genes). In total, 22,746 genes retrieved from four independent heat-treatment experiments (GSE12619, GSE18666, GSE19603, and GSE26266) in the Gene Expression Omnibus database were serialized according to fold change. (*Upper*) Ratio of transcript levels after heat tress (HS/untreated) are presented on a log$_2$ scale. (*Lower*) The frequency of SS/TM gene appearance for every 20 genes was plotted as shown in *A Lower* and Fig. 3. (*C*) Enrichment of genes down-regulated by Tm in an IRE1-dependent manner in gene sets possibly encoding proteins synthesized in endoplasmic reticulum based on Gene Ontology (GO) terms with a false discovery rate of <0.01. The number of genes categorized in the leading-edge subset by GSEA in each gene set is shown in blue. The microarray data were the same as those used in Fig. 3.

A

Up-regulated genes in *ire1a/b*

- other cellular processes
- other metabolic processes
- protein metabolism
- unknown biological processes
- response to stress
- transport
- developmental processes
- response to abiotic or biotic stimulus
- cell organization and biogenesis
- other biological processes
- transcription,DNA-dependent
- signal transduction
- DNA or RNA metabolism
- electron transport or energy pathways

All genes

B

Up-regulated genes in *ire1a/b*

151

10   9

20

86   37   230

CPR   **Heat stress**

Fig. S7. Bioinformatic characterization of genes specifically up-regulated in *ire1a/b* by Tm. (*A*) Pie charts indicating the distributions of GO terms for the biological processes assigned to the 190 up-regulated genes in *ire1a/b* (*Upper*) and to all genes (*Lower*). (*B*) Venn diagram representing the relationships among the up-regulated genes in *ire1a/b* (listed in Dataset S2), the cytosolic protein response (CPR) genes in a previous report (1), and genes for response to heat (GO:0009408).

1. Sugio A, Dreos R, Aparicio F, Maule AJ (2009) The cytosolic protein response as a subcomponent of the wider heat shock response in *Arabidopsis. Plant Cell* 21(2):642–654.

Table S1. Primers used in this study

Primer	Sequence
qPCR	
Act8-F-rt for *ACT8*	TCAGCACTTTCCAGCAGATG
Act8-R-rt for *ACT8*	ATGCCTGGACCTGCTTCAT
AT1G78850-F-rt for AT1G78850	CTTTGATTCTCCCACCGACA
AT1G78850-R-rt for AT1G78850	CTTGGCTTCCATCACGAGAC
BiP1-F-rt for *BiP1*	TCAGTCCTGAGGAGATTAGTGCT
BiP1-R-rt for *BiP1*	TGCCTTTGAGCATCATTGAA
BiP3-F-rt for *BiP3*	CGAAACGTCTGATTGGAAGAA
BiP3-R-rt for *BiP3*	GGCTTCCCATCTTTGTTCAC
bZIP60s-F-rt for spliced form of *bZIP60*	AAGCAGGAGTCTGCTGTTGG
bZIP60s-R-rt for spliced form of *bZIP60*	TTTGTGTGGGACATATAAGGGAAT
bZIP60u-F-rt for unspliced form of *bZIP60*	AGTCTGCTGTGCTCTTGTTGG
bZIP60u-R-rt for unspliced form of *bZIP60*	GCAACACTTTGTGTGGGACATA
HsfA2-F-rt for *HSFA2*	AGCTTTGTGGTGTGGGATTCT
HsfA2-R-rt for *HSFA2*	TGCAAATTCCCATCTATCTGGA
HsfA2II-F-rt for *HSFA2-II*	CCACGTTACTTCAAGCATAGCA
HsfA2II-R-rt for *HSFA2-II*	TTCTGAATCCCTTTATAGCTGAAGAC
HsfA7a-F-rt for *HSFA7a*	TCTCCACGATTCTCCTTCCTC
HsfA7a-R-rt for *HSFA7a*	GCAAATTCCCATCTCTCTGCT
HSP90.1-F-rt for *HSP90.1*	AAAGAAATCTTCCTCCGTGAGC
HSP90.1-R-rt for *HSP90.1*	AATGAAGAGTTCAGGCTGTCCA
IRE1A-F-rt for *IRE1A*	GCGCTACAGGCGTTACAAATA
IRE1A-R-rt for *IRE1A*	TCGTCGAATCCTTCTGGAACT
IRE1B-F-rt for *IRE1B*	AGTGGGGAAAAACCAGTTCC
IRE1B-R-rt for *IRE1B*	AACCAAGTCTCGGAAACAGTG
PR-4-F-rt for *PR-4*	GTGGGATGCTGATAAGCCGTA
PR-4-R-rt for *PR-4*	TGCAGCATTTGTTCTTGTGTTCT
PRX34-F-rt for *PRX34*	ATGCGCAGATATGCTCACCA
PRX34-F-rt for *PRX34*	AATGGAGCTGGAAGATTTGC
cRACE	
PR-4-cRACE-RT(5′P) for RT	TTATGTAGACCG (5′ phosphorylated)
PR-4-cRACE-F1 for first PCR	TTCGGCCATTGATCGCGTTTG
PR-4-cRACE-R1 for first PCR	TTTCTGGAATCAGGCTGCCC
PR-4-cRACE-F2 for second PCR	ATTATGTAATGATTTTGAGGTCAATATCG
PR-4-cRACE-R2 for second PCR	ATGAGATGGCCTTGTTGATAGC
Preparation of probes	
PR-4-probe-F for *PR-4* probe	TCTGCTGCAGTCAGTACGGTTA
PR-4-probe-F for *PR-4* probe	GCTGCATTGGTCCACTATTCTC
ACT8-probe-F for *ACT8* probe	AACAGCAGAACGGGAAATTG
ACT8-probe-R for *ACT8* probe	CTGGAAAGTGCTGAGGGAAG

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)