Supporting Information

Deng et al. 10.1073/pnas.1314749110

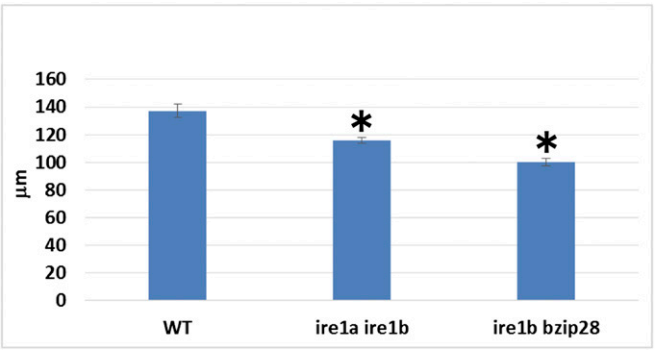

Fig. S1. The cellular phenotypes of the *inositol-requiring enzyme-1* (*ire1a*) (*ire1b*) and (*ire1b*) *basic leucine zipper transcription factor28* (*bzip28*) roots. Mean cell length for WT, *ire1a ire1b*, and *ire1b bzip28* in the cell maturation regions of the roots. Asterisks indicate significant differences between the mutants and WT (Student's test, $P < 0.001$). SE is indicated by bars.

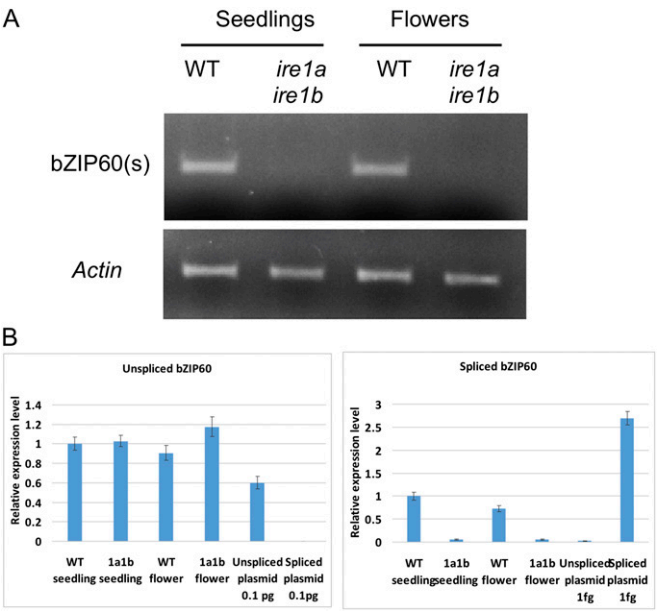

Fig. S2. bZIP60 spliced forms are found in unstressed seedlings and flowers. (*A*) RT-PCR assay shows the presence of bZIP60(s) in WT, but not *ire1a ire1b* seedlings or flowers under unstressed conditions. However, the levels are quite low because 35 cycles of PCR were used to detect the spliced transcripts. (*B*) Expression was quantified by quantitative RT-PCR assay. Relative expression level was normalized using ACTIN7 (At5g09810) as an internal control. SEs are indicated by bars.

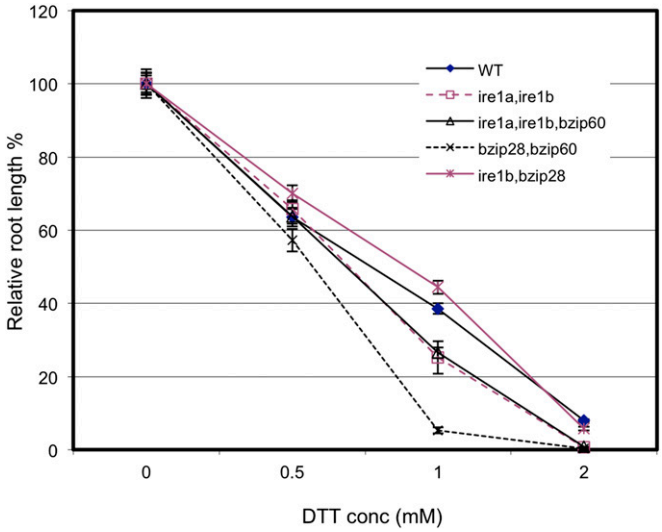

Fig. S3. DTT dose–response curve for the elongation of roots in multiple unfolded protein response (UPR) mutants. UPR mutants as indicated were grown on Linsmaier Skoog (LS) plates in the presence of various concentrations of DTT. Root lengths at 7 d relative to root lengths in the absence of DTT were plotted against DTT concentration. Error bars are SE.

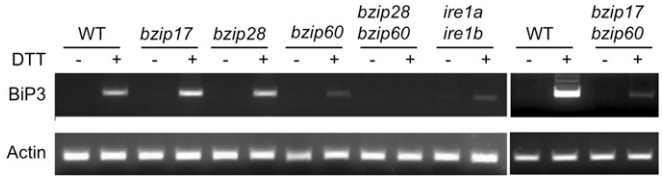

Fig. S4. Effect of UPR mutations on the induction of BiP3, a UPR biomarker. Seven-day-old seedlings were treated for 2 h with 2 mM DTT. BiP3 RNA accumulation was evaluated by semiquantitative RT-PCR using actin as a loading control. RT-PCR was carried using 24 thermocycles.

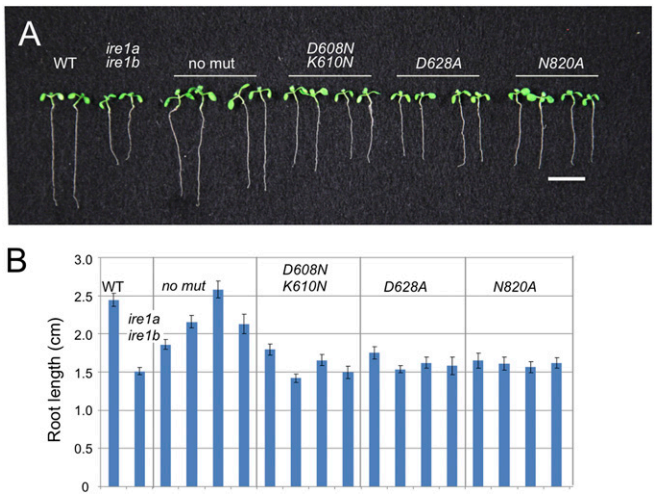

Fig. S5. Complementation by various *IRE1b* constructs of root elongation in *ire1a ire1b* seedlings. (*A*) Transgenic lines bearing the *IRE1b* constructs as indicated were grown under unstressed conditions. (*B*) Root lengths were measured in 7-d-old seedlings. Error bars are SE.

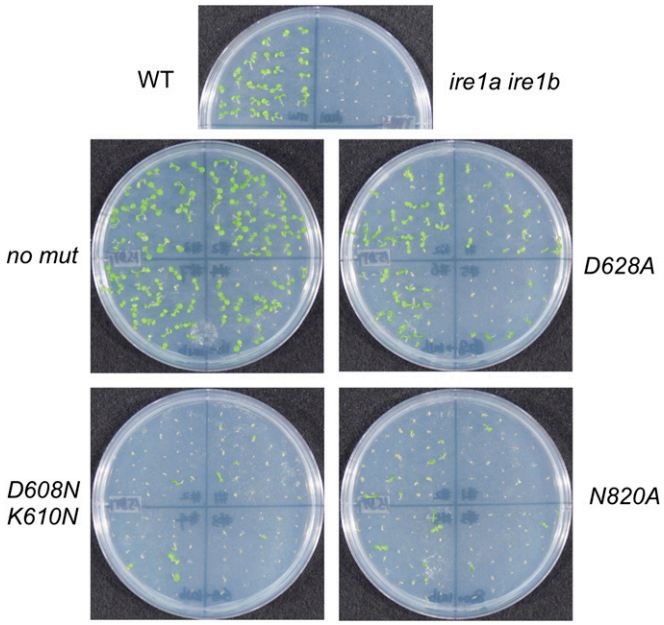

Fig. S6. Complementation by various *IRE1b* constructs of shoot growth in *ire1a ire1b* seedlings. Seedlings were grown under stress conditions (1.5 mM DTT) and photographed after 10 d of growth.

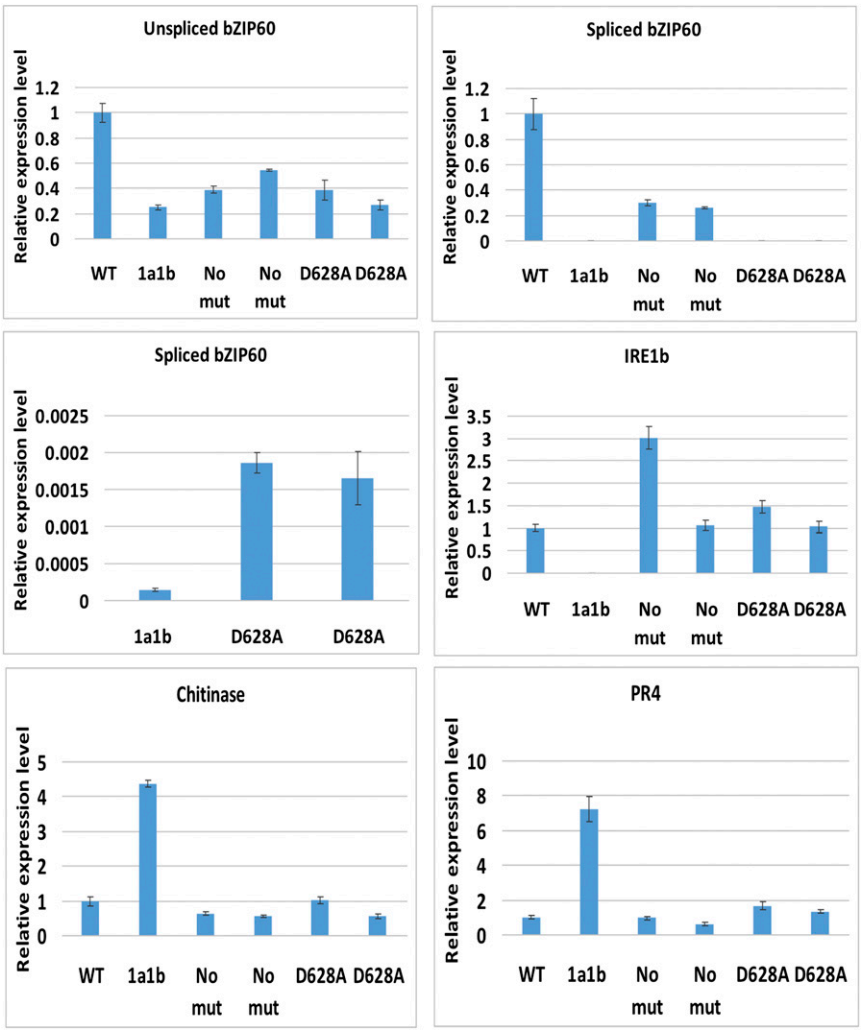

Fig. S7. bZIP60 RNA splicing and regulated IRE1-dependent decay target RNA degradation in *ire1a ire1b* transgenic seedlings expressing the IRE1b non mutant or D628A mutant construct and treated with 2 mM DTT for 5 h. Analyzed by quantitative RT-PCR. Relative expression level was normalized using *ACTIN7* (At5g09810) as an internal control. SEs are indicated by error bars.

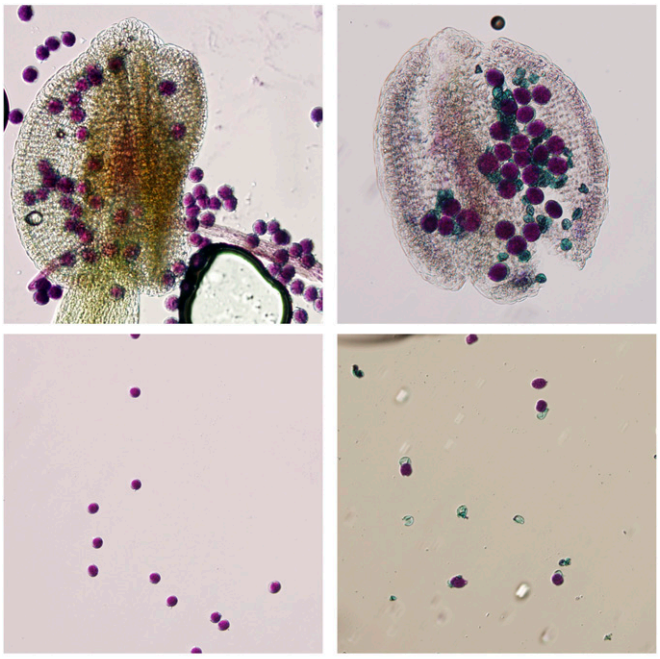

Fig. S8. Pollen viability is reduced in the triple *ire1a ire1b bzip28* mutant. Plants with the genotypes as indicated were selfed at room temperature conditions, and pollen grains in stamens were stained with Alexander's stain. Red-stained pollen is viable; blue- or green-stained pollen is not viable.

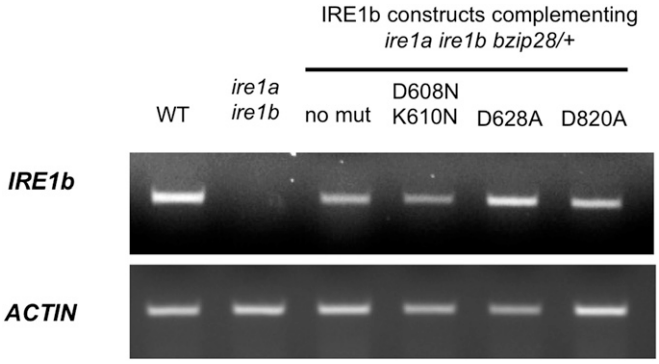

Fig. S9. Expression of IRE1b in transgenic lines used to test the ability of IRE1b mutant constructs to complement *ire1a ire1b bzip28*/+. Expression levels of *IRE1b* constructs were measured in untreated 7-d-old seedlings.

Table S1. Self-cross of *ire1a ire1b bzip28*/+ complemented with IRE1b mutations

	Genotype of progeny at *bZIP28* locus			
Genotype of complementing DNA	+/+	bzip28/+	bzip28/bzip28	Goodness of fit for expected complementation or noncomplementation ratio
No complementing DNA	53	41	0	Noncomplementation $\chi^2 = 1.53$, $P = 0.22$, df =1
+No mutation	26	44	12	Complementation $\chi^2 = 5.220$, $P = 0.77$, df = 2
+D608N K610N	49	58	0	Noncomplementation $\chi^2 = 0.757$, $P = 0.40$, df = 1
+D628A	49	53	0	Noncomplementation $\chi^2 = 0.156$, $P = 0.88$, df = 1
+N820A	65	46	0	Noncomplementation $\chi^2 = 3.25$, $P = 0.06$, df = 1
Expected ratio for complementation	2	3	1	
Expected ratio for noncomplementation	1	1	0	

Table S2. List of primers used in this study

Primers used in RT-PCR analysis	
IRE1bRP2	TGGCAAGGGAGTTGAGCTATGGAA
IRE1bLP2	TGTGCTTTCAAGTGCAGCCAAGAG
bZIP60F4	GAAGGAGACGATGATGCTGTGGCT
b60SB2	AGCAGGGAACCCAACAGCAGACT
ACTQF	GGAACTGGAATGGTGAAGGCTG
ACTQB	CGATTGGATACTTCAGAGTGAGGA
bip3F	TTCGACCCGAAACGTCTGATTGGA
bip3R	GCTTGCCTCTGCGCATCATTGAAA
ChitQF	CCAATCGTTCGACGCCTATAA
ChitQR	CATGTCTGGATCAGTCAAGAGAG
PR4QF	GAGAGCCGTGAGTGCTTATT
PR4QR	AAACCATCGGTGTCTATTTGATTG
Primers used in genotyping analysis	
LBa1	TGGTTCACGTAGTGGGCCATCG
b28LP2	CGAGAAGCGAAGCCAGAATAA
b28RP2	AGCGACATTCTCAGCCATAAC