# Figure S1

## A Wild-type and ΔIII Ire1

DTT 3mM

(hr) 0 0.25 0.5 1 1.5 2 3 4 5

u
i

(hr) 0 0.5 1 1.5 2 3 4 5

u
i

Non-stress
DTT 30min
1mM
3mM
10mM
–inositol 5hr

WT
u
i

ΔIII
u
i

–inositol

(hr) 1 2 3 4 5 6 7 8

WT
u
i

ΔIII
u
i

Non-stress
DTT 30min
1mM
3mM
10mM
–inositol 5hr

ΔIΔV
u
i

ΔIΔIIIΔV
u
i

## B bZip-Ire1

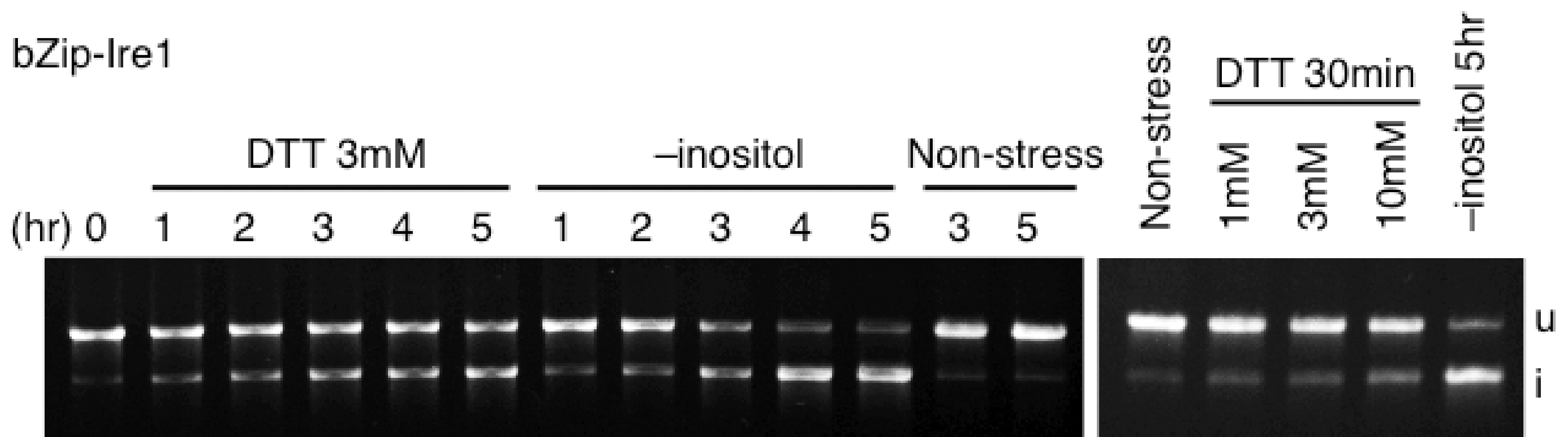



Figure S1 Raw-data examples for monitoring the *HAC1* mRNA splicing.
The *ire1Δ* cells KMY1516 carrying the *IRE1* gene (WT) or its mutants (on plasmid pRS313-IRE1 or its mutants) were stressed by the indicated stimuli, and total RNA samples were subjected to RT-PCR to amplify the *HAC1* products ($HAC1^u$ (u) and $HAC1^i$ (i)), which were then fractionated by 2% agarose-gel electrophoresis and visualized by EtBr staining.

Figure S2

*ire1Δ* cells

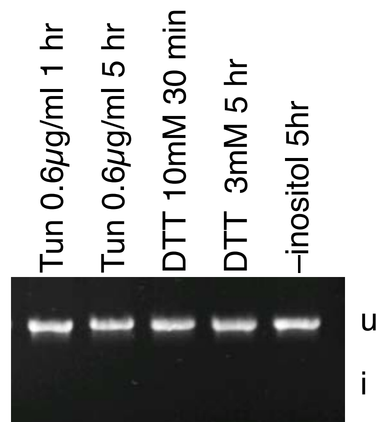
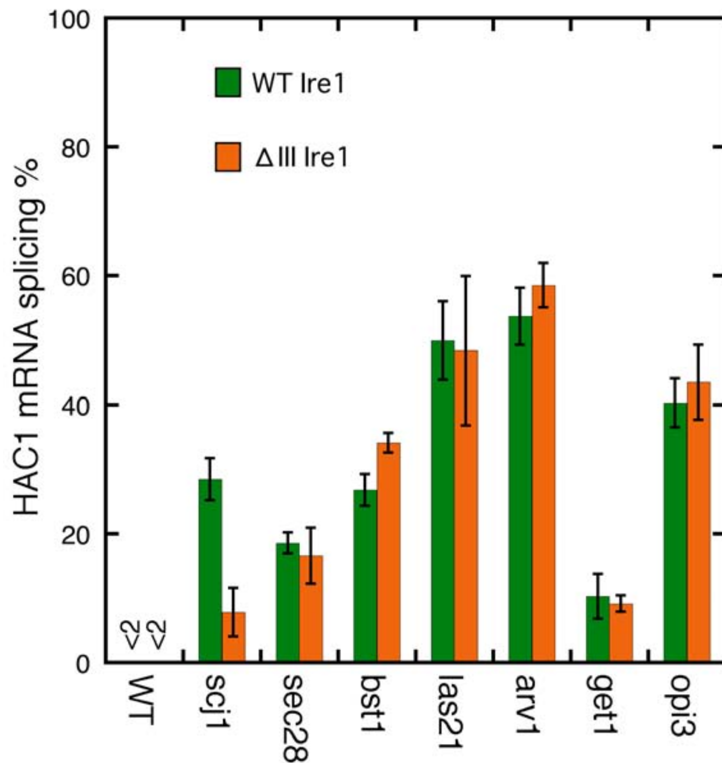



Figure S2 *ire1Δ* cells do not exhibit splicing of the *HAC1* mRNA.
The *ire1Δ* cells KMY1516 were stressed by the indicated stimuli, and total RNA samples were subjected to RT-PCR to amplify the *HAC1* products (*HAC1*$^{u}$ (u) and *HAC1*$^{i}$ (i)), which were then fractionated by 2% agarose-gel electrophoresis and visualized by EtBr staining.

Figure S3

A




B

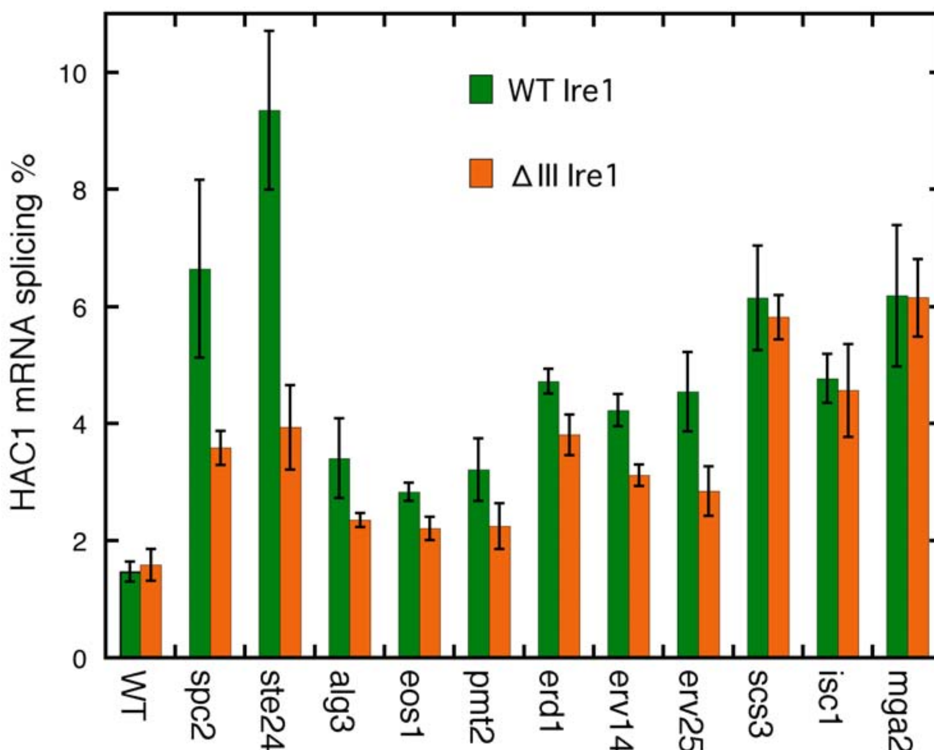



Figure S3 *HAC1* mRNA splicing induced by gene deletions.
Total RNA samples obtained from the strains employed in Figure 8 were analyzed by RT-PCR to evaluate splicing efficiency of the *HAC1* mRNA. In A, the RT-PCR products were conventionally fractionated by EtBr-containing gels, while in order to detect weak $HAC1^{i}$ signal, we employed a fluorescent dye-labeled PCR primer in B. Error bars represent the standard deviations from three independent transformants.

# Figure S4

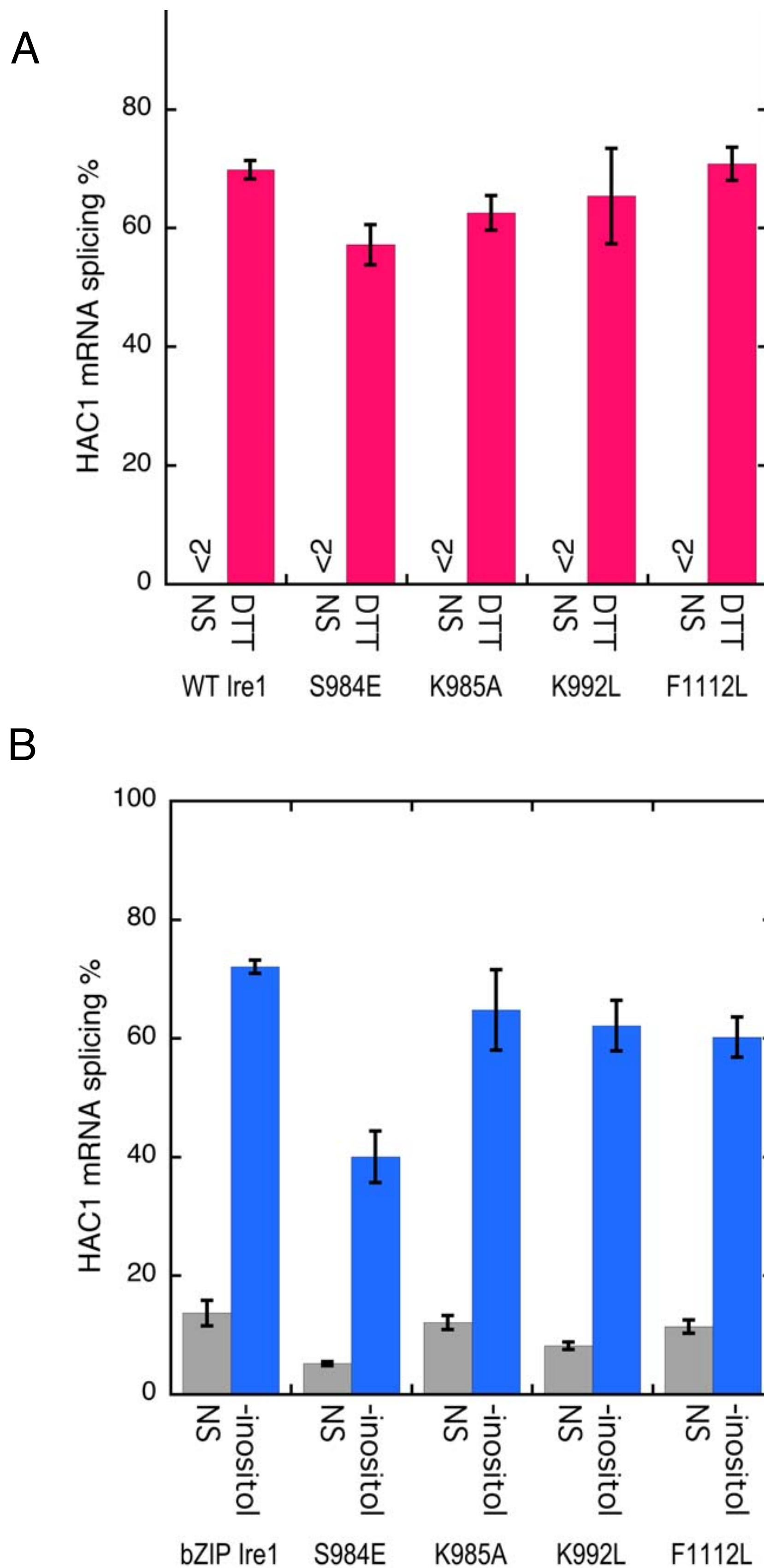



Figure S4 Activity of wild-type and bZIP-Ire1 carrying the quercetin-non-responding mutations.

The *ire1Δ* strain KMY1516 transformed with the wild-type IRE1 (A) or the bZIP-Ire1 (B) plasmid carrying the indicated mutations (pRS313-IRE1 or its derivatives) were cultured under non-stress conditions (NS) or stressed by DTT treatment (A; 3mM for 30 min) or inositol depletion (B; 5 hr) before RT-PCR amplification of the *HAC1* products from total RNA samples. Error bars represent the standard deviations from three independent transformants.

Table S1 **Oligomucleotide primers to generate CPY and CPY* plasmids.**

| Sequence | Target | Direction | Attached restriction site |
|---|---|---|---|
| atatatatgcggccgcATAGCTTCAAAATGTTTCTACTCC | TEF1 promoter | Forward | Not I |
| atatatactagtAAACTTAGATTAGATTGCTATGCT | TEF1 promoter | Reverse | Spe I |
| atatatgcggccgcACGGATTAGAAGCCGCCGAGC | GAL1 promoter | Forward | Not I |
| atatactagtGGTTTTTTCTCCTTGACGTTAAAGTATAGAGG | GAL1 promoter | Reverse | Spe I |
| gtttaactagtAAATGAAAGCATTCACCAGTTTACTATGTGG | CPY or CPY* (PRC1 or prc1-1) | Forward | Spe I |
| tttaagaattcTAAGGAGAAACCACCGTGGATCCAT | CPY or CPY* (PRC1 or prc1-1) | Reverse | Eco RI |
| ggtttagaattcAAAGGTGAAGAATTATTCACTGGTGTTG | GFP | Forward | Eco RI |
| ttaaactcgagTTATTTGTACAATTCATCCATACCATG | GFP | Reverse | Xho I |

The hybridizing sequences appear as capital letters.
The introduced restriction sites are highlighted by red letters.

Table S2 **Antibodies used in this study.**

| Description | Working concentration | Purpose | Source |
|---|---|---|---|
| 12CA5 mouse monoclonal IgG to the HA epitope | 1 $\mu$g/ml | Western blotting | Roche |
| | 2 $\mu$g/ml | Immunofluorescent staining | |
| | 4 $\mu$g/ml | Immunoprecipitation | |
| Rabbit anti-yeast BiP antiserum | 1: 1000 dilution | Western blotting | Higashio et al. (2000) (a) |
| | 1: 500 dilution | Immunofluorescent staining | |
| Chicken anti-GFP IgY | 1: 100 dilution | Immunofluorescent staining | Ave Labs |
| Rabbit anti-GFP IgG | 1: 1000 dilution | Western blotting | MBL International Corporation |
| | 1: 150 dilution | Immunoprecipitation | |
| HRP-conjugated goat antibody to mouse Igs | 1: 1000 dilution | Western blotting | Dako |
| HRP-conjugated donkey antibody to rabbit IgG | 1: 1000 dilution | Western blotting | GE Healthcare |
| FITC-conjugated goat antibody to mouse Igs | 1: 100 dilution | Immunofluorescent staining | Cappel |
| Cy5-conjugated donkey antibody to mouse IgG | 1: 100 dilution | Immunofluorescent staining | Jackson ImmunoResearch Laboratories |
| FITC-conjugated goat antibody to rabbit IgG | 1: 100 dilution | Immunofluorescent staining | Cappel |
| FITC-conjugated donkey antibody to chicken IgY | 1: 50 dilution | Immunofluorescent staining | Jackson ImmunoResearch Laboratories |
| Cy3-conjugated donkey antibody to rabbit IgG | 1: 200 dilution | Immunofluorescent staining | Jackson ImmunoResearch Laboratories |

(a) Higashio, H., Y. Kimata, T. Kiriyama, A. Hirata, and K. Kohno (2000) *J. Biol. Chem.* 275: 17900–17908.