Supplementary Information

# The unfolded protein response alongside the diauxic shift of yeast cells and its involvement in mitochondria enlargement

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# Figure S1

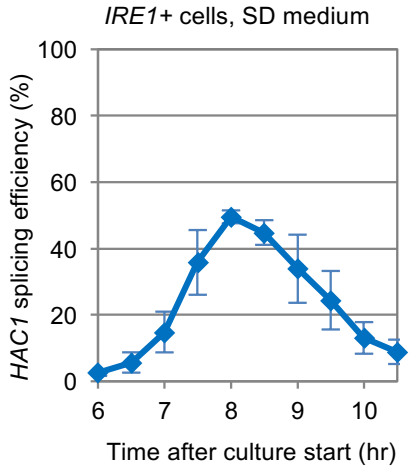


**Figure S1: Detailed time-course profiling of the transient *HAC1* mRNA splicing upon diauxic shift.** The same experiment as shown in Fig. 1b was performed with 30-min sampling intervals.

# Figure S2

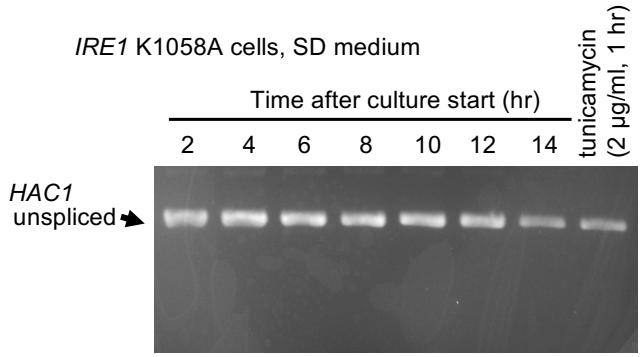


**Figure S2: The K1058A mutation of *IRE1* abolishes the *HAC1* mRNA splicing.** The same experiment as described in Fig. 1A was performed using cells carrying the K1058A mutation on the *IRE1* gene.

# Figure S3

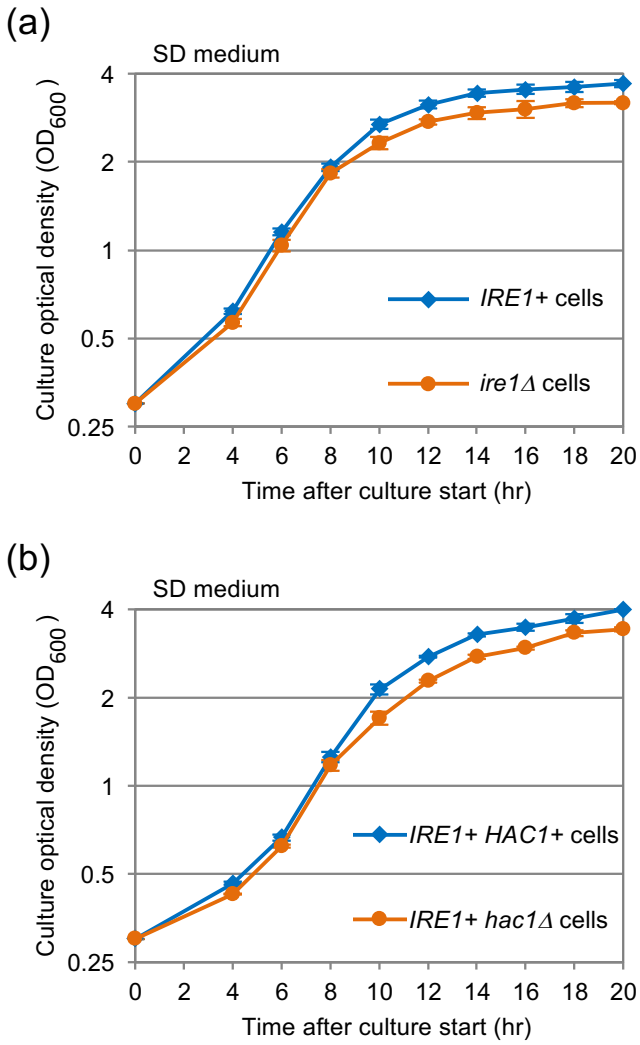


**Figure S3: The Ire1-*HAC1* pathway contributes to cell growth upon diauxic shift.** Cells were grown in SD medium, and optical density of cultures at the indicated time points was monitored.

# Figure S4

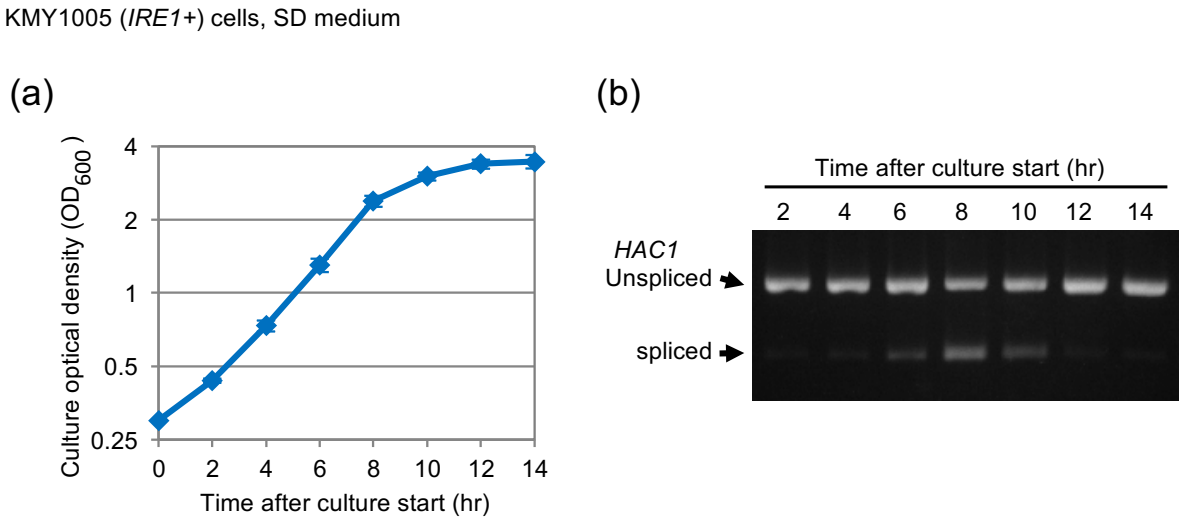


**Figure S4: Transient induction of the *HAC1*-mRNA splicing upon diauxic shift in yeast KMY1005 strain.** KMY1005 cells were cultured in SD medium and checked for cellular growth (a) and *HAC1*-mRNA splicing (b).

# Figure S5

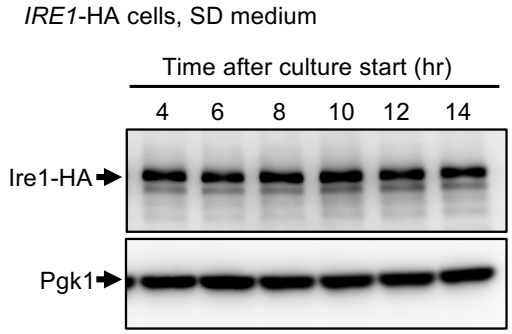


**Figure S5: Time-course change of the cellular Ire1 level.** Cells carrying Ire1-HA were cultured in SD medium for the indicated durations. Their lysates (equivalent to 0.1 $OD_{600}$ cells) were subjected to anti-HA Western-blot analysis. Anti-Pgk1 Western blot serves as a loading control.

# Figure S6

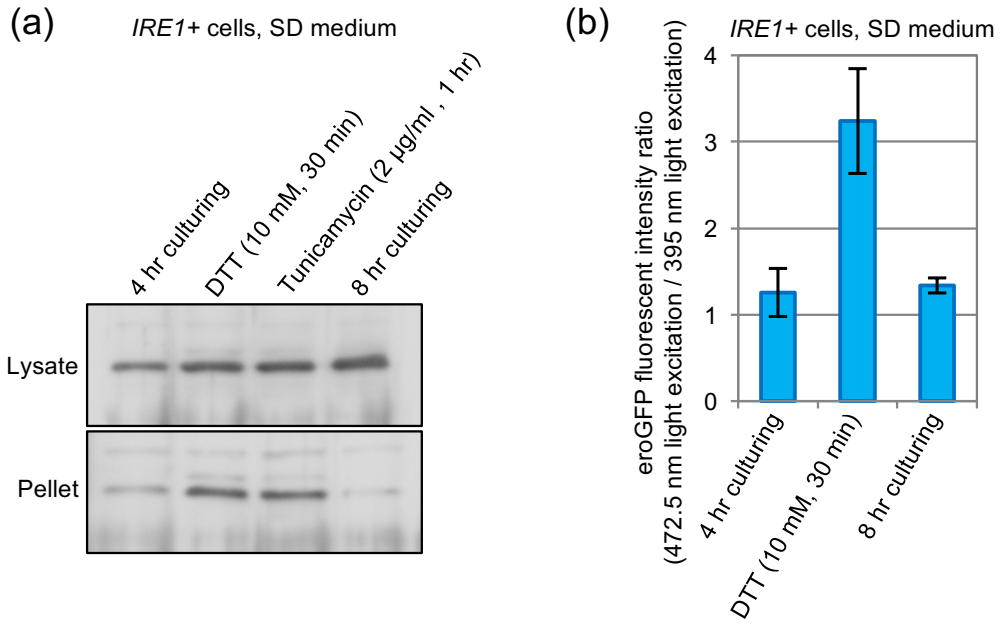


**Figure S6: ER protein-folding status is not impaired upon diauxic shift.** (a) After being cultured for the indicated durations or stressed by DTT or tunicamycin, *IRE1*+ cells were lysed and subjected to high-speed centrifugation as described in the Materials and Methods section. The total cell lysates (equivalent to 0.05 $OD_{600}$ cells) or the pellet fractions (equivalent to 1.0 $OD_{600}$ cells) were subjected to anti-BiP Western-blot analysis. (b) After being cultured for the indicated durations or stressed by DTT, *IRE1*+ cells producing eroGFP were illuminated by UV/violet light or blue light for detection of green fluorescent signals.

# Figure S7

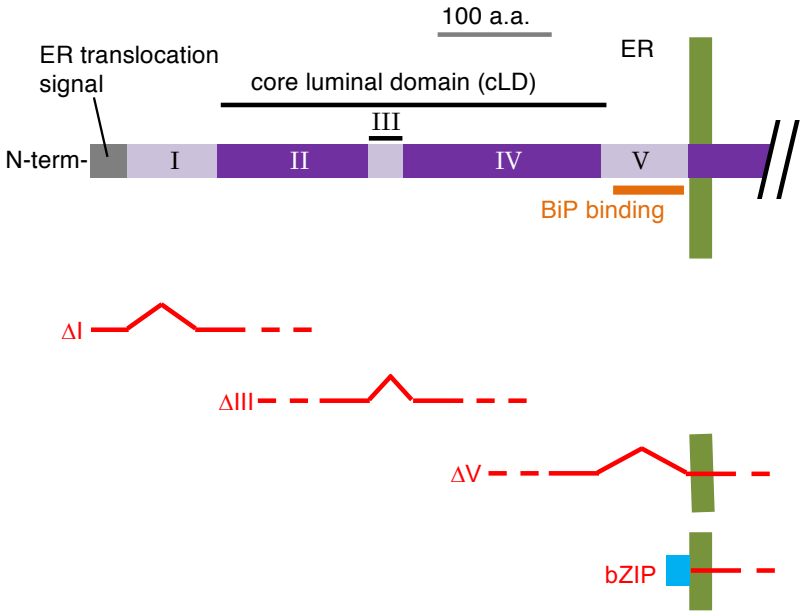


**Figure S7: Structure of the luminal domain of yeast Ire1 and its mutants.** Segmentation of the luminal domain of yeast Ire1 into Subregions I to V is described in Ref 5. Subregions II and IV form one tightly folded module, namely the core luminal domain, which is responsible for unfolded-protein sensing and self-association (12). Meanwhile, Subregions I and V are loosely folded and play suppressive roles in Ire1 regulation. The ΔI, and ΔIII and ΔV mutations are deletions of a.a 32-91, a.a. 253-272 and a.a. 463-524 of yeast Ire1, respectively (7).

# Figure S8

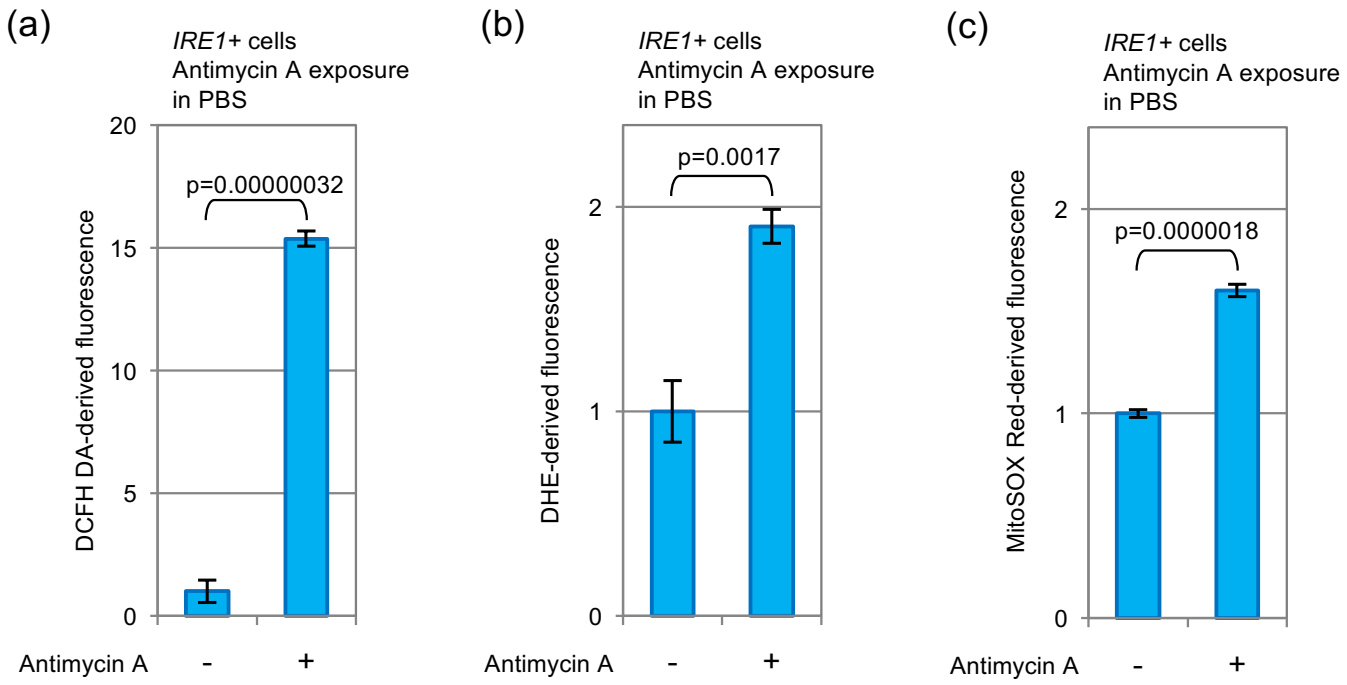


**Figure S8: Effect of antimycin A exposure on the ROS level in PBS-suspended cells.** After being cultured in SD medium and harvested, cells were suspended in PBS and incubated with or without 20 μM antimycin A in the presence of the indicated ROS-indicator dyes for 30 min, and were then analyzed by flowcytometry. The values are normalized against those of the left bars (antimycin A –), which are set at 1.00.

# Figure S9

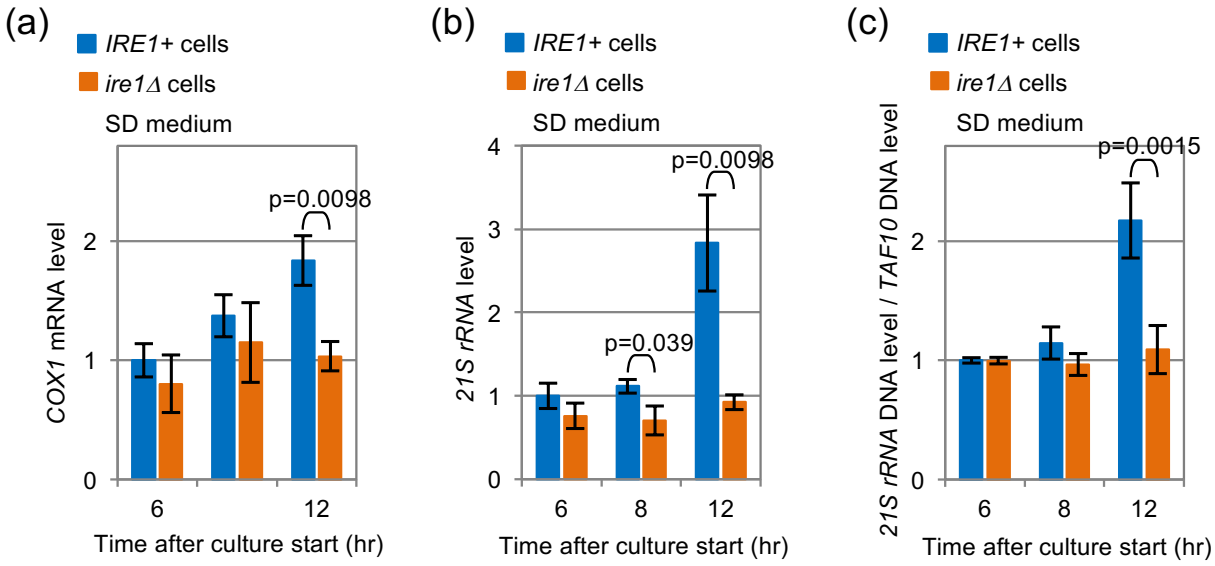


**Figure S9: Change of the mitochondrial RNA and DNA levels in *IRE1*+ cells and *ire1Δ* cells upon their long-time culturing.** RNA or DNA samples prepared in the experiments shown in Fig. 7 were subjected to RT-qPCR or genomic qPCR analyses using mitochondrial genome-specific oligonucleotide primers.

# Figure S10

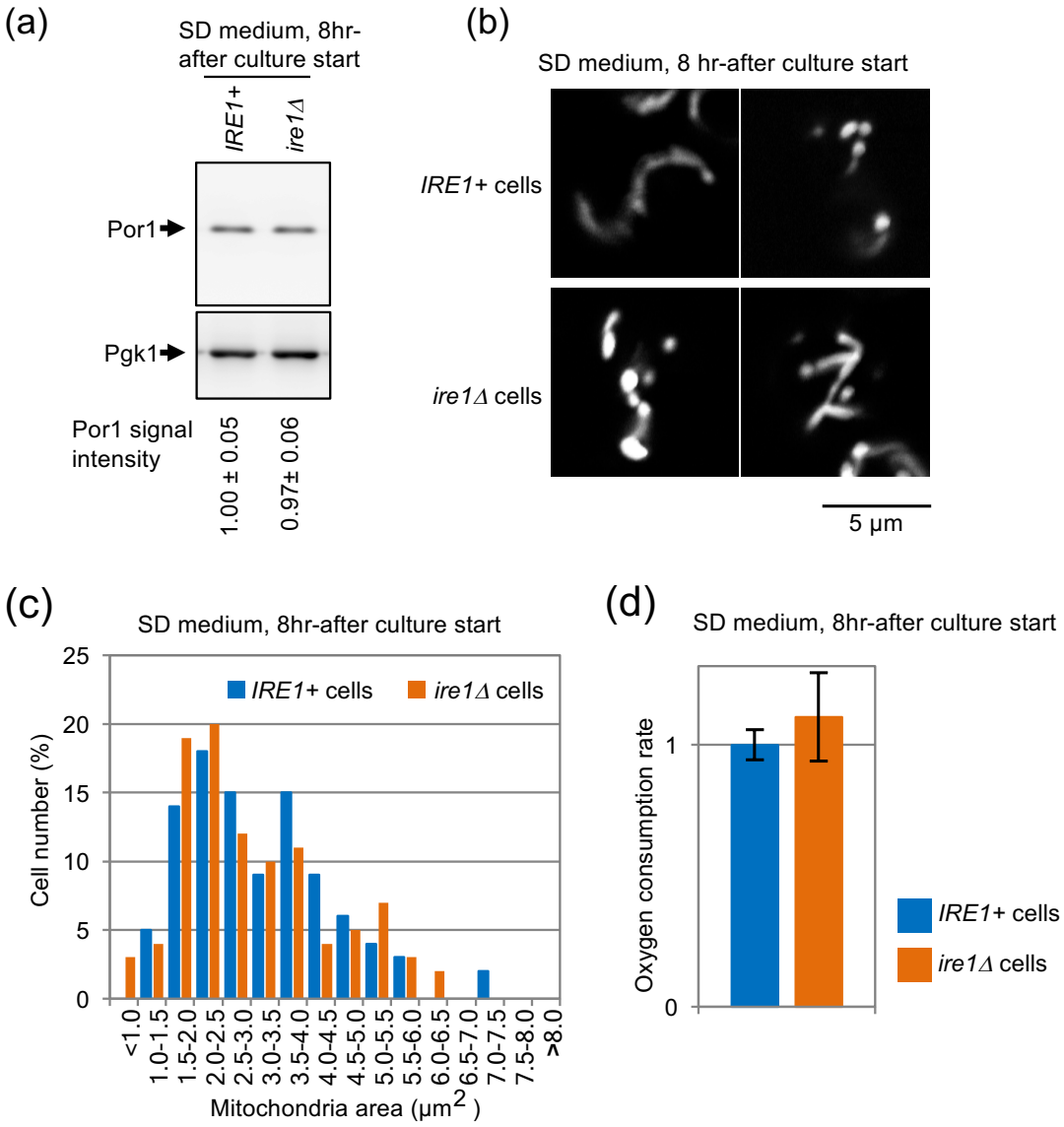


**Figure S10: Comparison of mitochondria-related phenotypes between 8hr-cultiured *IRE1*+ and *ire1Δ* cells.** After being cultured for 8 hr in SD medium, *IRE1*+ cells and *ire1Δ* cells were subjected to the same analyses as done in Fig. 8. In panel c, the mitochondria size distribution of *IRE1*+ cells was not statistically different from that of *ire1Δ* cells (p=0.29)

# Figure S11

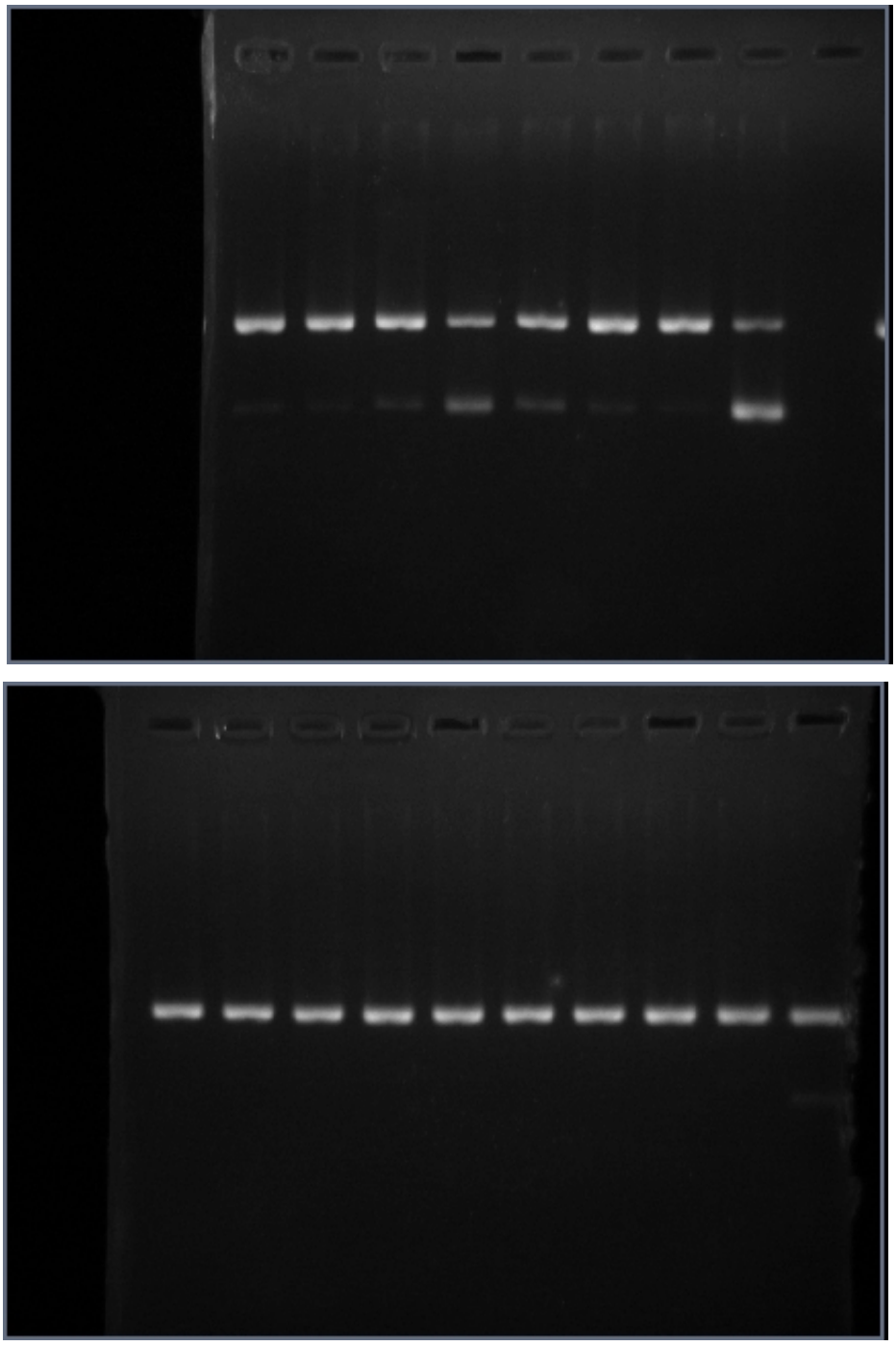

**Figure S11: Uncropped gel images for Figs 1a and d.**

# Figure S12

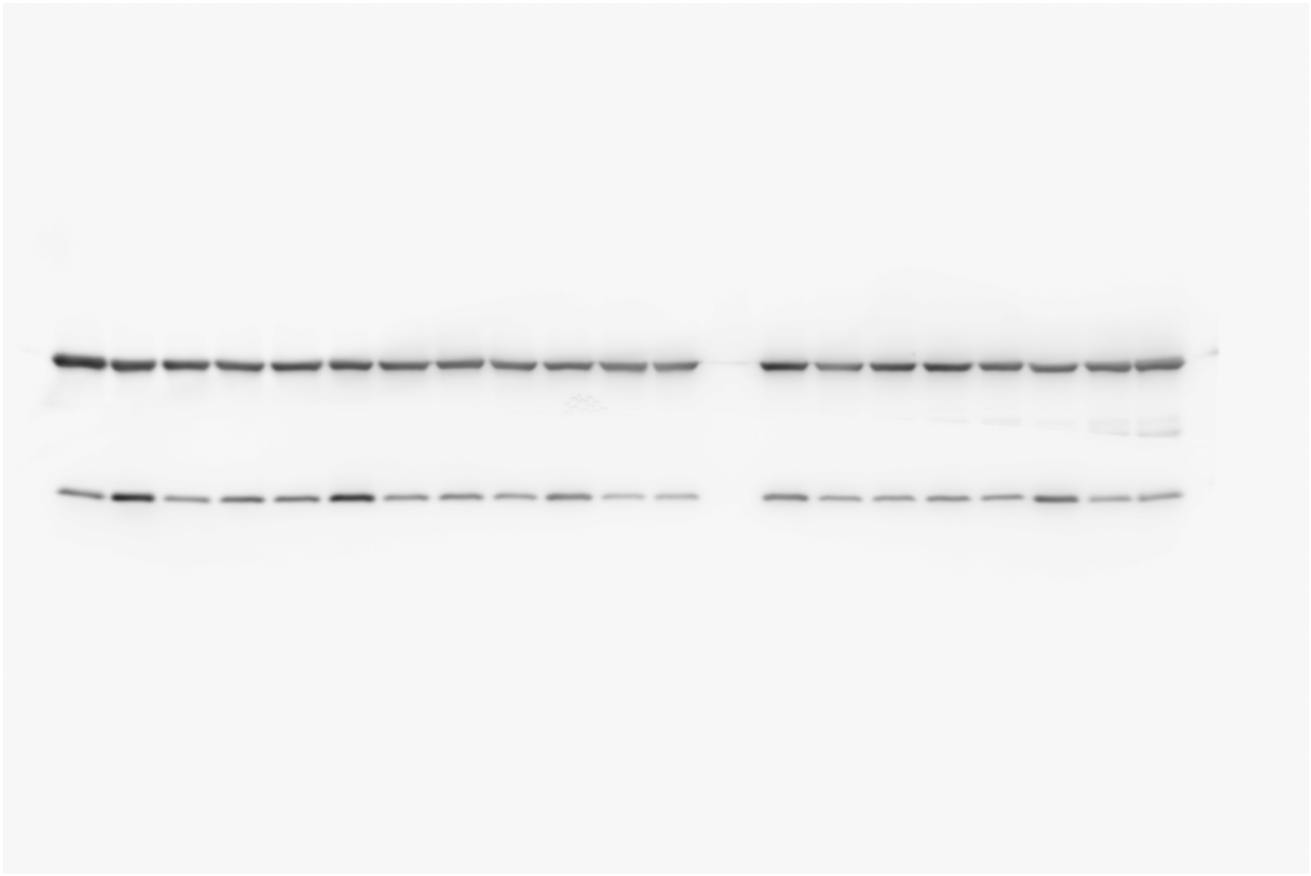

**Figure S12: Uncropped blot image for Fig. 8a.**

Table S1 Slopes of the growth charts shown in this paper

| Growth chart | Fig. 1C | Fig. 1F | Fig. 3A | Fig. 3A | Fig. 3C | Fig. 3E | Fig. 3E | Fig. 3E | Fig. 3E | Fig. 4A | Fig. 4C | Fig. 4C | Fig. 5A | Fig. 5A | Fig. 6A | Fig. 6A | Fig. S4A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IRE1 genotype | WT | WT | WT | ΔIII | bZIP | WT | ΔI | ΔV | ΔIΔV | ρ0 | WT | WT | WT | WT | WT | WT | WT KMY1005 |
| medium | SD | YPD | SD | SD | SD | SD | SD | SD | SD | SD | SD | SD+antimycinA | SD | SD+NAC | SCG | SCG | SD |
| HAC1 splicing chart | Fig. 1B | Fig. 1E | Fig. 3B | Fig. 3B | Fig. 3D | Fig. 3F | Fig. 3F | Fig. 3F | Fig. 3F | Fig. 4C | Fig. 4D | Fig. 4D | Fig. 5B | Fig. 5B | Fig. 6B | Fig. 6B | Fig. S5B |
| Fold change of the culture density during 2-hr culturing | | | | | | | | | | | | | | | | | |
| 0 hr to 2 hr | 1.47 | 1.42 | 1.49 | 1.64 | 1.38 | 1.07 | 1.20 | 1.15 | 1.11 | 1.25 | 1.45 | 1.52 | 1.43 | 1.28 | 2.27 | 2.22 | 1.46 |
| 2 hr to 4 hr | 1.62 | 2.21 | 1.68 | 1.68 | 1.50 | 1.76 | 1.85 | 1.80 | 1.72 | 1.31 | 1.70 | 1.57 | 1.63 | 1.32 | 1.14 | 0.93 | 1.68 |
| 4 hr to 6 hr | 1.80 | 2.28 | 1.95 | 1.92 | 1.73 | 1.83 | 1.79 | 1.72 | 1.63 | 1.18 | 2.02 | 1.91 | 1.88 | 1.54 | 1.45 | 1.22 | 1.77 |
| 6 hr to 8 hr | 1.72 | 1.26 | 1.72 | 1.69 | 1.49 | 1.77 | 1.67 | 1.97 | 1.65 | 1.76 | 1.78 | 1.89 | 1.47 | 1.37 | | | 1.83 |
| 8 hr to 10 hr | 1.26 | 1.10 | 1.27 | 1.22 | 1.48 | 1.36 | 1.28 | 1.15 | 1.50 | 1.38 | 1.26 | 1.30 | 1.46 | 1.53 | | | 1.26 |
| 10 hr to 12 hr | 1.16 | 1.06 | 1.09 | 1.06 | 1.09 | 1.14 | 1.08 | 1.14 | 1.17 | 1.49 | 1.06 | 1.02 | 1.16 | 1.20 | | | 1.12 |
| 12 hr to 14 hr | 1.08 | 1.11 | 1.06 | 1.06 | 1.08 | 1.05 | 1.09 | 1.05 | 1.03 | 1.13 | 1.04 | 1.08 | 1.06 | 1.15 | | | 1.02 |

The slopes of the lines in the indicated figures are indicated.

Table S2 Comparison of growth rate between two genotypes

| Growth chart | Fig. S2A | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | IRE1+ | | | | ire1Δ | | | | |
| Clone number | 1 | 2 | 3 | Average | 1 | 2 | 3 | Average | t.test |
| Fold change of the culture density during 2-hr culturing | | | | | | | | | |
| 4 hr to 6 hr | 1.81 | 1.88 | 1.91 | 1.87 | 1.92 | 1.76 | 1.82 | 1.83 | 0.56 |
| 6 hr to 8 hr | 1.62 | 1.75 | 1.61 | 1.66 | 1.76 | 1.92 | 1.63 | 1.77 | 0.32 |
| 8 hr to 10 hr | 1.39 | 1.36 | 1.45 | 1.40 | 1.24 | 1.32 | 1.25 | 1.27 | 0.02 |
| 10 hr to 12 hr | 1.18 | 1.21 | 1.12 | 1.17 | 1.21 | 1.11 | 1.23 | 1.18 | 0.78 |
| 12 hr to 14 hr | 1.10 | 1.06 | 1.13 | 1.09 | 1.11 | 1.07 | 1.04 | 1.07 | 0.47 |
| 14 hr to 16 hr | 1.02 | 1.05 | 1.03 | 1.03 | 1.06 | 1.02 | 1.01 | 1.03 | 0.95 |
| 16 hr to 18 hr | 1.08 | 0.95 | 1.03 | 1.02 | 0.96 | 1.11 | 1.09 | 1.05 | 0.62 |
| 18 hr to 20 hr | 0.98 | 1.10 | 1.00 | 1.03 | 1.02 | 0.97 | 1.02 | 1.00 | 0.58 |

| Growth chart | Fig. S2B | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | IRE1+HAC1+ | | | | IRE1+hac1Δ | | | | |
| Clone number | 1 | 2 | 3 | Average | 1 | 2 | 3 | Average | t.test |
| Fold change of the culture density during culturing for 2 hours | | | | | | | | | |
| 4 hr to 6 hr | 1.46 | 1.46 | 1.37 | 1.43 | 1.46 | 1.47 | 1.44 | 1.46 | 0.44 |
| 6 hr to 8 hr | 1.78 | 1.99 | 1.89 | 1.89 | 1.90 | 1.95 | 1.81 | 1.89 | 0.99 |
| 8 hr to 10 hr | 1.83 | 1.63 | 1.66 | 1.70 | 1.41 | 1.49 | 1.45 | 1.45 | 0.02 |
| 10 hr to 12 hr | 1.25 | 1.29 | 1.35 | 1.29 | 1.33 | 1.25 | 1.44 | 1.34 | 0.51 |
| 12 hr to 14 hr | 1.19 | 1.16 | 1.21 | 1.19 | 1.25 | 1.22 | 1.17 | 1.21 | 0.43 |
| 14 hr to 16 hr | 1.02 | 1.08 | 1.09 | 1.06 | 1.07 | 1.05 | 1.09 | 1.07 | 0.77 |
| 16 hr to 18 hr | 1.06 | 1.08 | 1.07 | 1.07 | 1.11 | 1.17 | 1.10 | 1.13 | 0.07 |
| 18 hr to 20 hr | 1.13 | 1.06 | 1.04 | 1.07 | 1.01 | 1.02 | 1.07 | 1.03 | 0.26 |

In Fig. S3A and S3B, three independent clones with the same genotype were monitored for culture optical density. We thus calculated its fold change during 2-hr culturing, and evaluated if the resulting values from two genotypes are statistically different as determined by Student's two-tailed t test. Yellow shaded rows: $p<0.05$.

## Table S4 IRE1 mutation primers used for overlap PCR

For luminal domain mutation

Internal mutation primers

| Mutation | Orientation | Sequence |
|---|---|---|
| ΔI | Forward | TGCTCAATCCCATTGTCGTCTCGCCGTGCTAACAAAAAAGGACGTAGG |
| | Reverse | CCTACGTCCTTTTTTGTTAGCACGGCGAGACGACAATGGGATTGAGCA |
| ΔIII | Forward | CAGCGTTCGGACCTGGTTCAAAAGAATCTGAAAATATGATTGTAATAGGC |
| | Reverse | GCCTATTACAATCATATTTTCAGATTCTTTTGAACCAGGTCCGAACGCTG |
| ΔV | Forward | CACCTTTATGAAAACTATGAAAAACAAAATTCTTTGCTACTGAAGTTTG |
| | Reverse | CAAACTTCAGTAGCAAAGAATTTTGTTTTTCATAGTTTTCATAAAGGTG |

External primers

| Orientation | Sequence |
|---|---|
| Forward | CCATTATCACTTTTCTCCATATCA |
| Reverse | CCTTGAAAACTTCCCTGAAAAACT |

For cytosolic domain mutation

Internal mutation primers

| Mutation | Orientation | Sequence |
|---|---|---|
| K1058A | Forward | GAGCACTTAGGAATGCATATCATCAT |
| | Reverse | ATGATGATATGCATTCCTAAGTGCTC |

External primers

| Orientation | Sequence |
|---|---|
| Forward | CGATGATGCTGATGAAGATGATGA |
| Reverse | ACGTTGTAAAACGACGGCCAGTGA |

**Table S5: Oligonucleotide primers for qPCR or RT-qPCR.**

| Treget gene | | Sequence |
|---|---|---|
| *HAC1* | Forward | TACAGGGATTTCCAGAGCACG |
| | Reverse | TGAAGTGATGAAGAAATCATTCAATTC |
| *KAR2* | Forward | TCTGAAGGTGTCTGCCACAG |
| | Reverse | TTAGTGATGGTGATAGATTCGGATT |
| *PDI1* | Forward | TACGAAGAAGCCCAGGAAAA |
| | Reverse | GTCAGCCAATTCAGCGTCA |
| *TSA1* | Forward | GTTCATCATCGACCCAAAGG |
| | Reverse | GTCAACGTTTCTACCGACTGG |
| *OLI1* | Forward | GCAGGTATTGGTATTGCTATCG |
| | Reverse | GCTTCTGATAAGGCGAAACC |
| *COX1* | Forward | TCCAACAGGAATTAAAATTTTCTCA |
| | Reverse | GCAATTGCATATAACATAGGTAGTGC |
| *21S rRNA* | Forward | AAATTGAAATCGTAGTGAAGATGCT |
| | Reverse | AAAGCTGCATAGGGTCTTTCC |
| *TAF10* | Forward | ATATTCCAGGATCAGGTCTTCCGTAGC |
| | Reverse | GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG |