Supplemental materials

Acquired Resistance to Severe Ethanol Stress on Protein Quality Control in *Saccharomyces cerevisiae*

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Legends for Supplemental Figures

Figure S1.

mRNA levels of various genes analyzed by qRT-PCR. Yeast cells were pre-treated with or without mild stress (6% ethanol stress for 180 min or thermal stress at 37˚C for 60 min) and then exposed to 10% (v/v) ethanol stress for 0, 60, 120, or 180 min. Yeast cells were also treated with 6% (v/v) ethanol for 30 min or at 37˚C for 30 min. *ACT1* was used as a reference gene for the normalization of qRT-PCR data, and $\log_2$ ratios between the stress treatment groups and control (w/o stress treatment) were calculated ($n = 3$).

Figure S2.

Confirmation of drug effects of MG132. Cells carrying a FLAG-tagged chromosomal copy of the *BTN2* gene were exposed to mild thermal stress at 37˚C for 60 min with or without 100 μM MG132. The levels of Btn2, which is rapidly turned over by the ubiquitin-proteasome system (1), were assessed by Western blotting. The inhibition of proteasomes by MG132 was confirmed by an elevated level of Btn2 in cells treated with MG132 at 37˚C. Ponceaus S staining was performed to confirm equal loading and the transfer of all proteins.

Figure S3.

Insoluble aggregated proteins were collected by the method of Roth *et al.* (2) using mortar-pestle lysis instead of glass bead lysis. Samples were separated using a 10% SDS-polyacrylamide gel and visualized by silver staining. Cells were treated under the same conditions as in Fig. 1B (*left*) and Fig. 3 (*right*).

Figure S4.

Other images used to generate graphs in Figs. 1B (A), 3A (B), 4 (C), 5 (D), 7A (E), and 8A (F).

Supplementary references

1. Malinovska L, Kroschwald S, Munder MC, Richiter D, Alberti S. 2012. Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. Mol Biol Cell 23:3041–3056.
2. Roth AF, Feng Y, Chen L, Davis NG. 2002. The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. J Cell Biol 159:23–28.

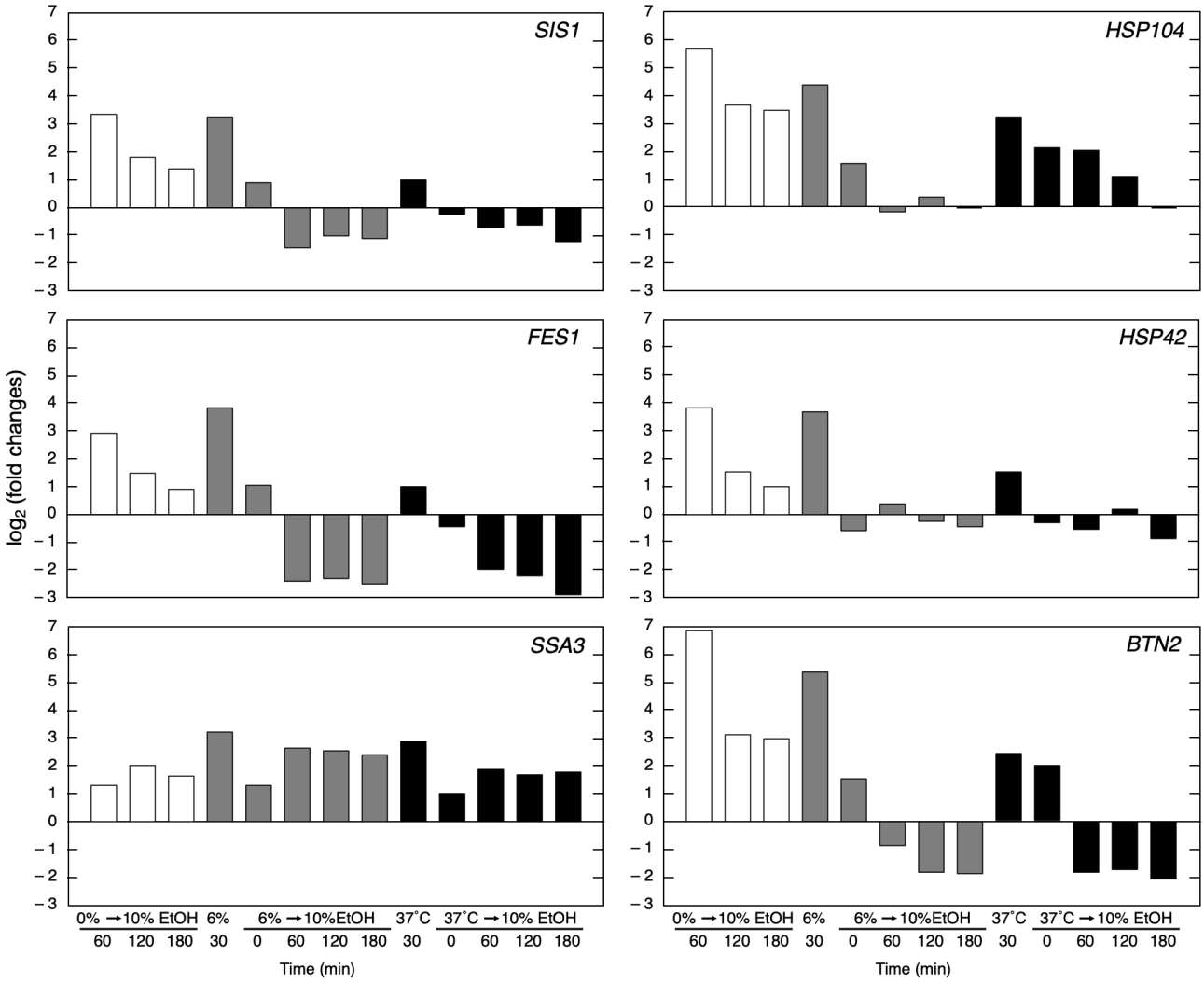

SIS1
HSP104
FES1
HSP42
SSA3
BTN2
log2 (fold changes)
0% → 10% EtOH
6%
6% → 10%EtOH
37˚C
37˚C → 10% EtOH
60 120 180 30 0 60 120 180 30 0 60 120 180
Time (min)

Fig. S1

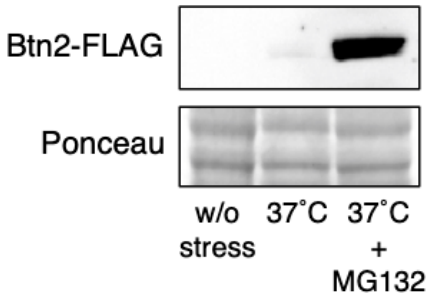


Fig. S2

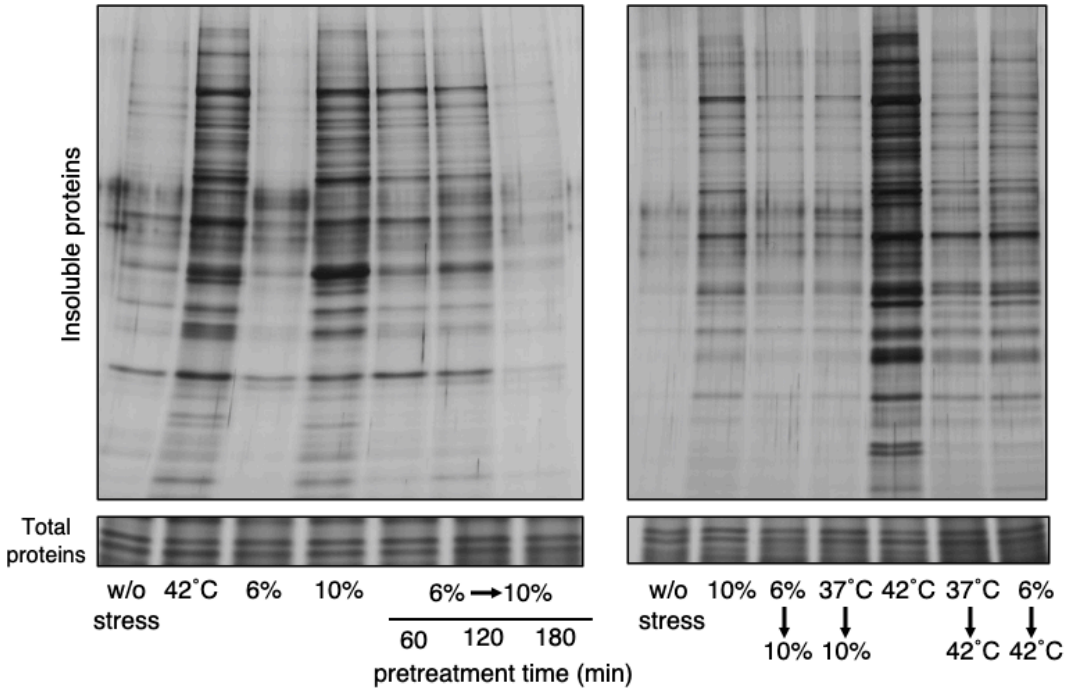
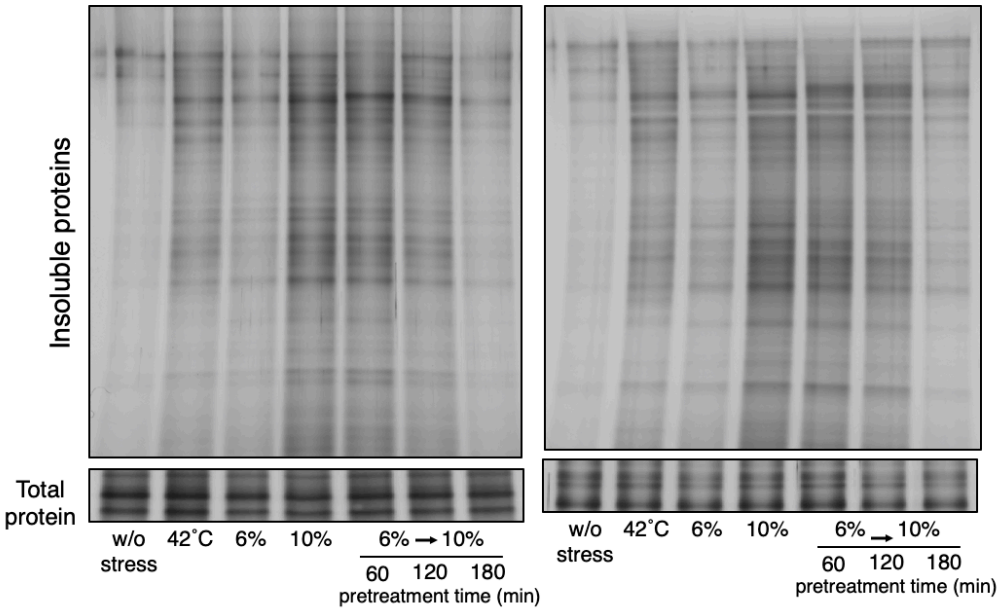

Insoluble proteins
Total proteins
w/o stress
42˚C
6%
10%
6% → 10%
60
120
180
pretreatment time (min)
w/o stress
10%
6%
37˚C
42˚C
37˚C
6%
10%
10%
42˚C
42˚C

Fig. S3

(A)


Insoluble proteins
Total protein
w/o stress
42˚C
6%
10%
6% → 10%
60 120 180
pretreatment time (min)
w/o stress
42˚C
6%
10%
6% → 10%
60 120 180
pretreatment time (min)

(B)

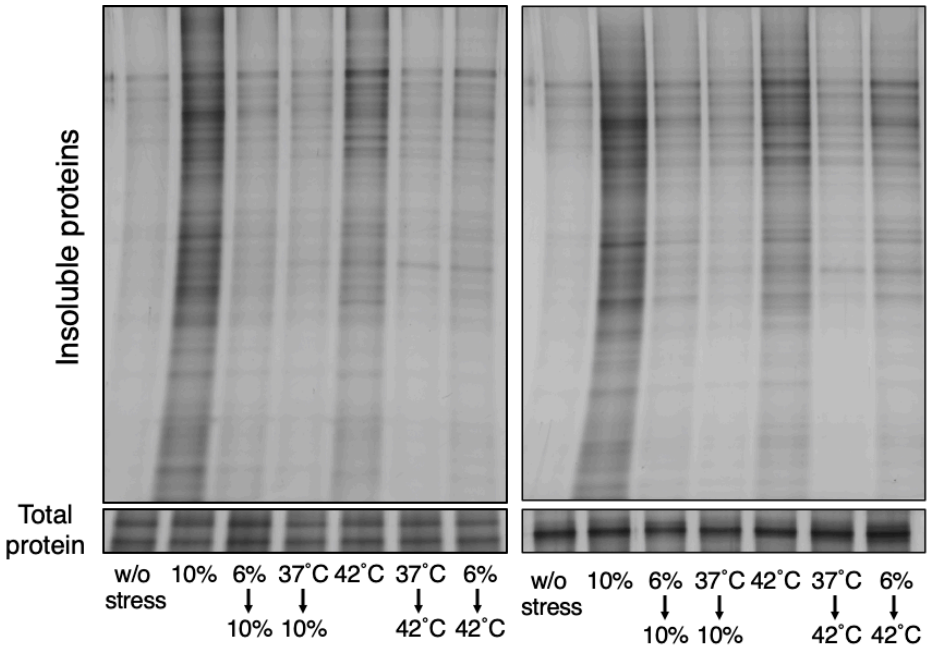

Insoluble proteins
Total protein
w/o stress
10%
6%
37˚C
42˚C
37˚C
6%
10% 10%
42˚C 42˚C
w/o stress
10%
6%
37˚C
42˚C
37˚C
6%
10% 10%
42˚C 42˚C

Fig. S4 (A)(B)

(C)

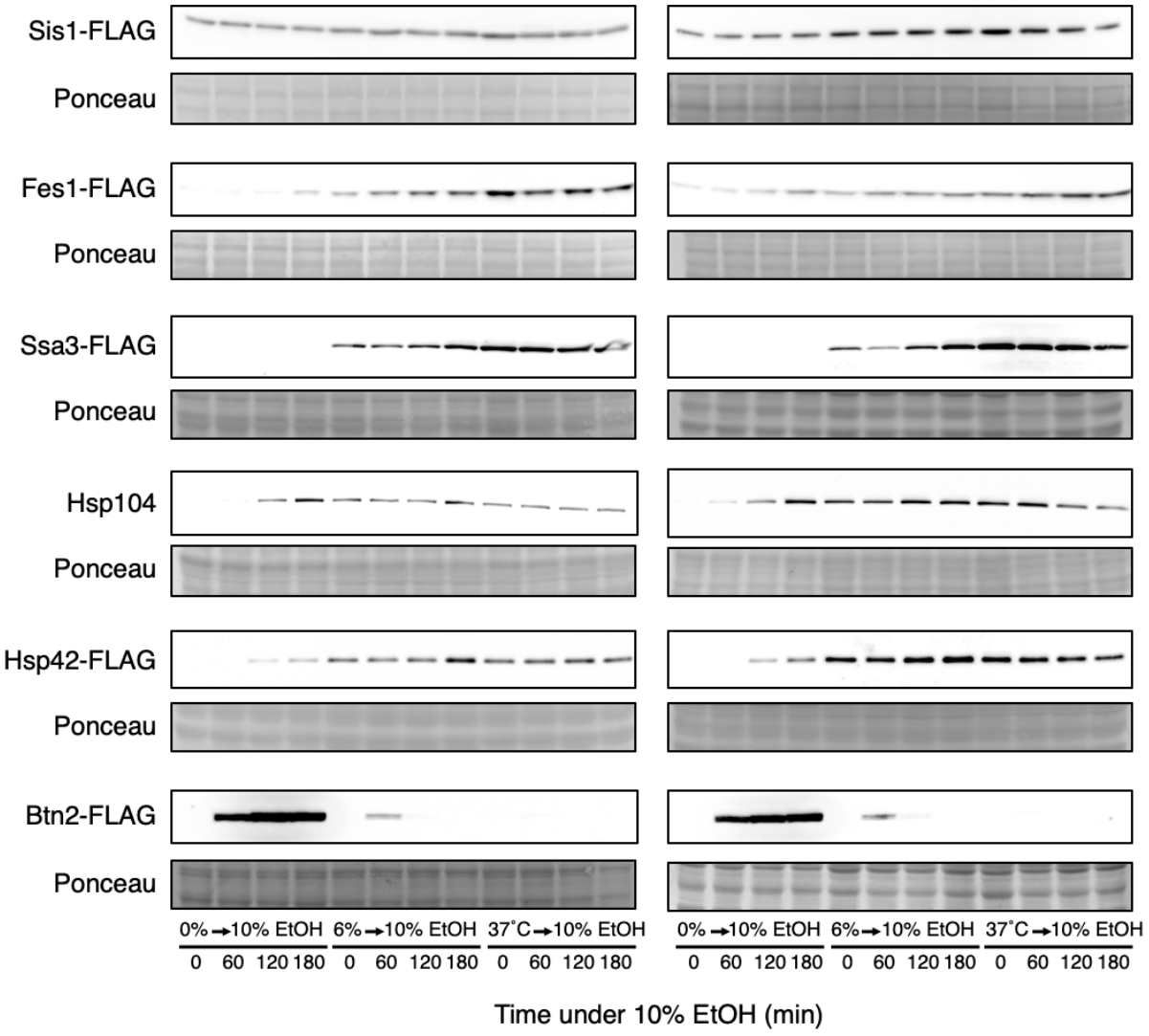

Sis1-FLAG
Ponceau
Fes1-FLAG
Ponceau
Ssa3-FLAG
Ponceau
Hsp104
Ponceau
Hsp42-FLAG
Ponceau
Btn2-FLAG
Ponceau
0%→10% EtOH
6%→10% EtOH
37°C→10% EtOH
0%→10% EtOH
6%→10% EtOH
37°C→10% EtOH
0 60 120 180 0 60 120 180 0 60 120 180 0 60 120 180 0 60 120 180 0 60 120 180
Time under 10% EtOH (min)

Fig. S4 (C)

(D)

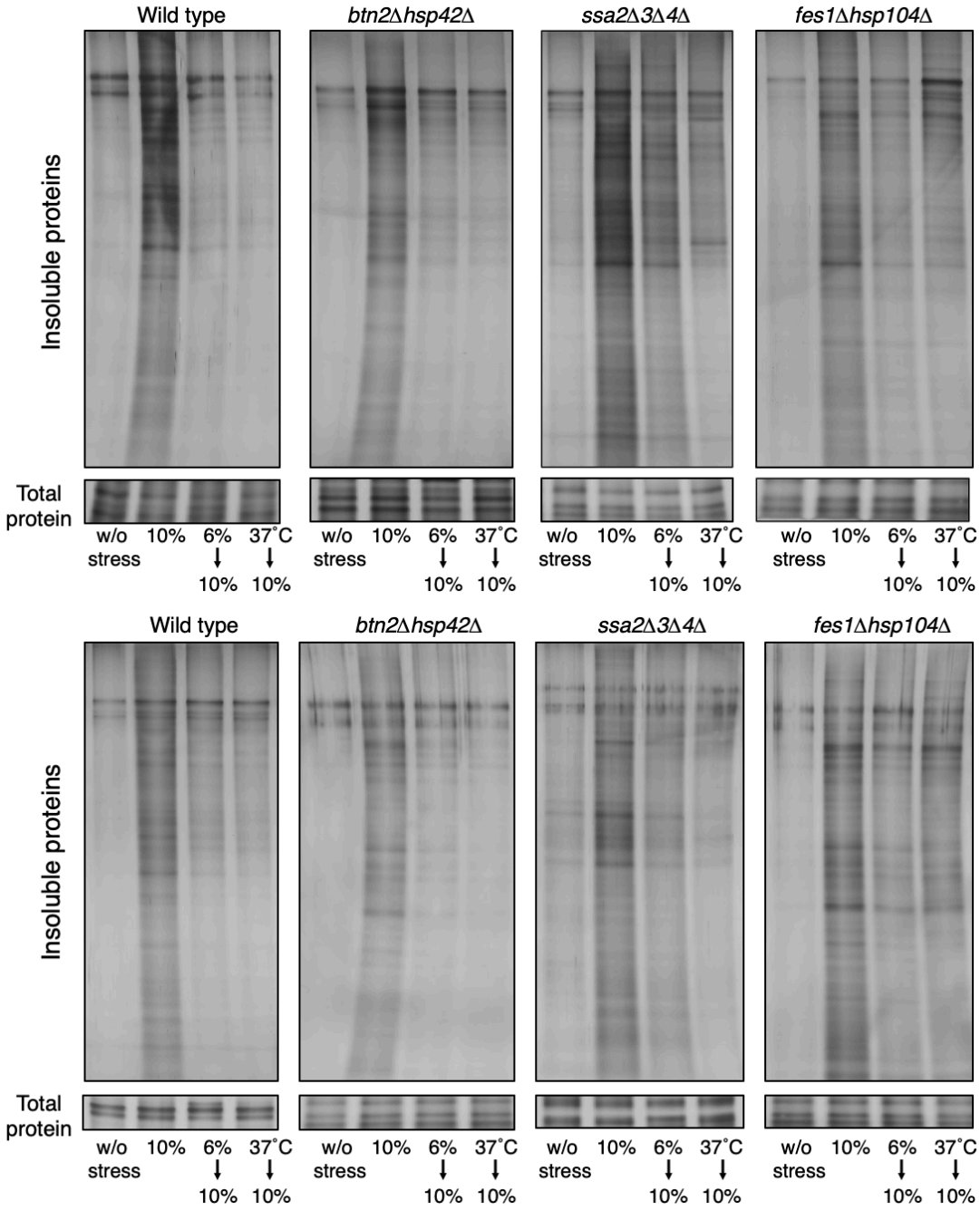

Wild type
btn2Δhsp42Δ
ssa2Δ3Δ4Δ
fes1Δhsp104Δ
Insoluble proteins
Total protein
w/o stress
10%
6% 10%
37˚C 10%
Wild type
btn2Δhsp42Δ
ssa2Δ3Δ4Δ
fes1Δhsp104Δ
Insoluble proteins
Total protein
w/o stress
10%
6% 10%
37˚C 10%

Fig. S4 (D)

(E)

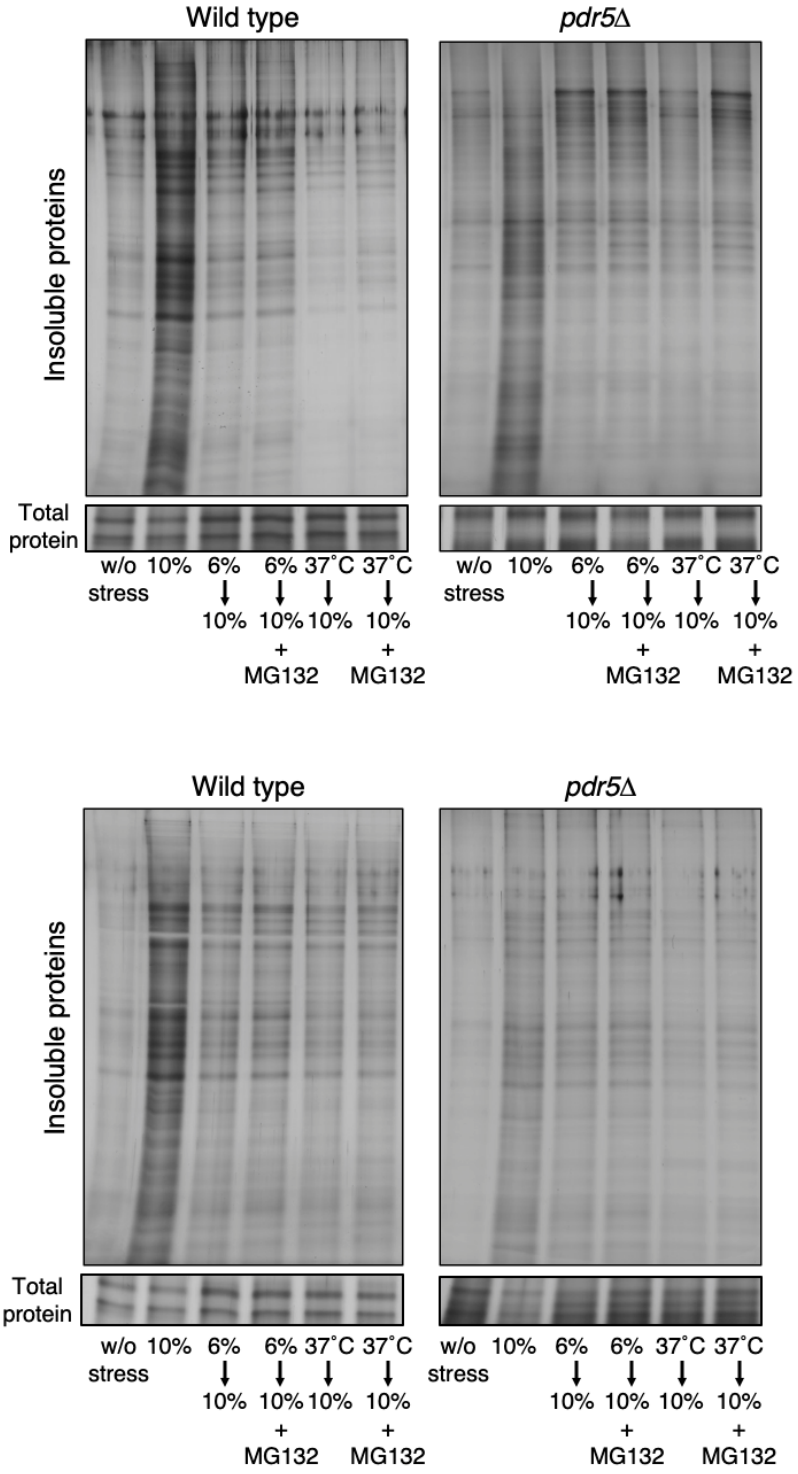
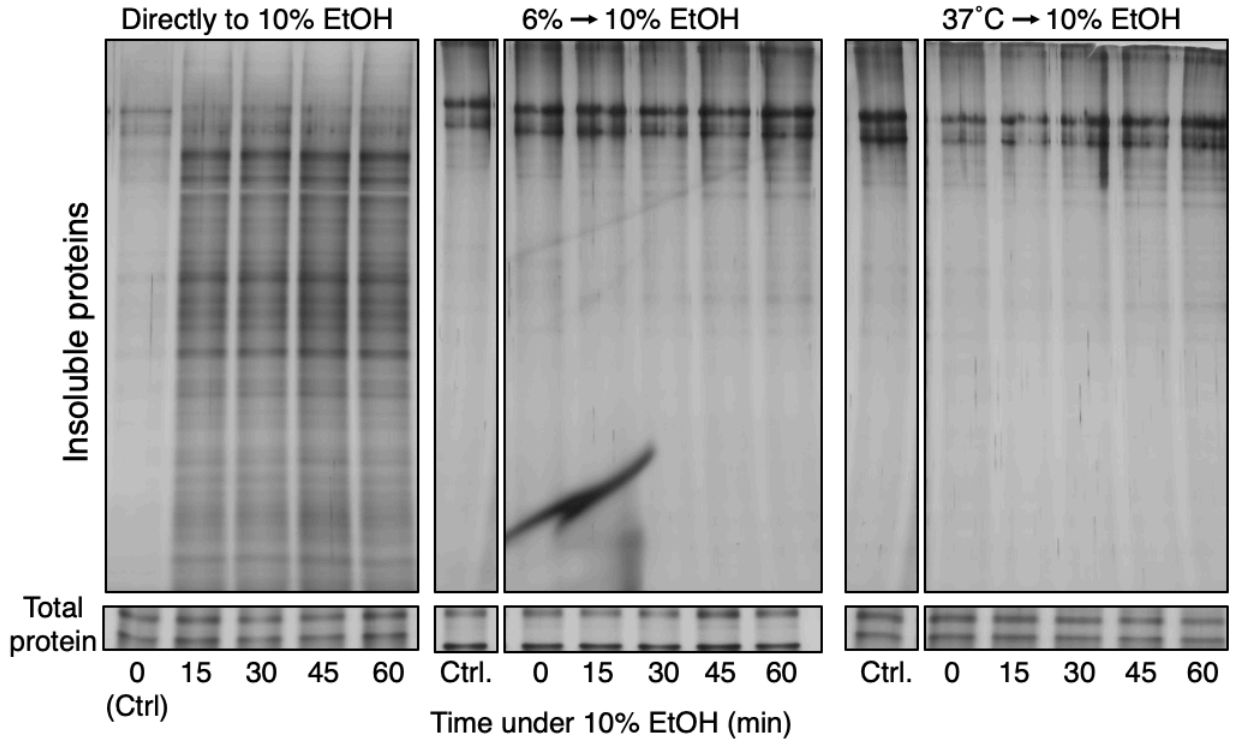

Wild type
pdr5Δ
Insoluble proteins
Total protein
w/o stress
10%
6% → 10%
6% → 10% + MG132
37°C → 10% + MG132
37°C → 10% + MG132

Fig. S4 (E)

(F)


Directly to 10% EtOH
6% → 10% EtOH
37˚C → 10% EtOH
Insoluble proteins
Total protein
0 15 30 45 60
(Ctrl)
Ctrl.
0 15 30 45 60
Ctrl.
0 15 30 45 60
Time under 10% EtOH (min)

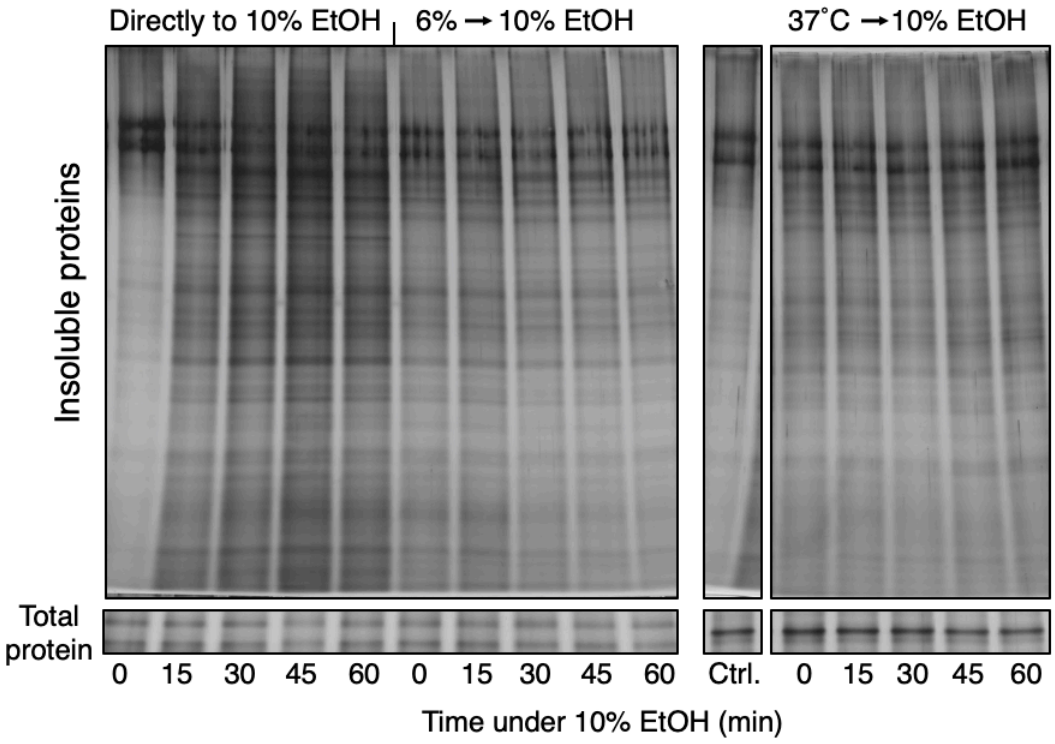

Directly to 10% EtOH
6% → 10% EtOH
37˚C → 10% EtOH
Insoluble proteins
Total protein
0 15 30 45 60 0 15 30 45 60
Ctrl.
0 15 30 45 60
Time under 10% EtOH (min)

Fig. S4 (F)