Supporting Information

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SI Materials and Methods

Strains and Cell Growth. All plasmids used in this article are listed in Table S1. All of these plasmids are single-integration plasmids and were transformed by the standard lithium acetate method into the *Saccharomyces cerevisiae* strain w303a to produce the strains listed in Table S2. The transcriptional reporters (P_{ERO1}–GFP, P_{HSP12}–GFP, and P_{RPL17A}–GFP) were built by amplifying the promoter of the genes from genomic DNA and cloned upstream of the GFP ORF. Cells were grown in complete medium [yeast extract, peptone, and 2% (wt/vol) dextrose; YPD] at 30 °C for all experiments.

RNA-Sequencing and Bioinformatic Analysis. RNA was purified from cells as described (1). Sequencing libraries were prepared, genomic alignment was performed, and fold changes were calculated as described (2). Fold-change values were then used to cluster the data via Cluster (Version 3.0) and plotted in Java Treeview. The correlation coefficient (r) was calculated for the fold-change values for the whole genome with the data from the latest time point (240 min) for both tunicamycin and estradiol + 1-naphthylmethyl-4-amino-1-*tert*-butyl-3-(*p*-methylphenyl) pyrazolo [3,4-*d*]pyrimidine (1NM-PP1).

Motif search analysis was conducted with the SCOPE software (http://genie.dartmouth.edu/scope/) (3). Genes induced or repressed under endoplasmic reticulum (ER) stress, Hac1 expression, and PKA inhibition were used to search for the motif sequences in their promoters (defined as 800 bp upstream of the start codon). The negative logarithm of the P value for each motif provided by the software was used to plot the results. Gene Ontology (GO) term analyses were conducted in YeastMine and in the Generic GO Term Finder (http://go.princeton.edu/cgi-bin/GOTermFinder).

PKA Activity Assay. PKA kinase activity was measured by using the ProFluor PKA assay (Promega). Cells were grown to $OD_{600} = 0.8$ in 200 mL of YPD, collected by splitting the culture into four 50-mL Falcon tubes, spinning for 2 min at full speed at 4 °C, pouring off the medium, and freezing in liquid N_2. Frozen pellets were lysed in a coffee grinder with crushed dry ice and no buffer. After 6 × 30-s pulses in the coffee grinder, the dry ice/yeast mixture was transferred to a glass beaker, and the dry ice was evaporated at room temperature. A total of 1 mL of kinase dilution/reaction buffer was added, and lysate was resuspended and immediately spun in a microfuge for 5 min at 4 °C. Cleared supernatant was used in the assay. Protein concentration was measured in a Nanodrop spectrometer. Samples were diluted to equalize protein concentrations. Six replicates of each lysate at two different concentrations (concentrated and diluted 1:2) were used in the ProFluor assay. We incubated the lysates with the fluorescent substrate and ATP, stopped the reaction with protease, and measured FITC fluorescence on a Tecan plate reader. We used recombinant PKA to generate a standard curve.

^{35}S Incorporation Measurements. Translation was measured by ^{35}S incorporation as described (4).

Transcriptional Activity Measurements. Cells bearing a transcriptional reporter (a given promoter fused to GFP) were grown in complete medium and treated with tumicamycin and/or estradiol. Samples of these cultures were measured in a flow cytometer (BD LSR-II) equipped with a high throughput sampler. Data were collected in FACS DIVA software and analyzed in Matlab. The mean of the green fluorescence (FITC) normalized by cell size (side-scatter; SSC) was calculated for each condition and time. Errors were calculated as the SD of the mean for three biological replicates. An automated high-throughput flow cytometry setup was used to calculate the expression rate of the transcriptional reporters P_{ERO1}-GFP, P_{HSP12}-GFP, and P_{RPL17A}-GFP. Cells bearing these reporters were grown in exponential phase for ∼24 h. Cells were taken to the automated setup, and measurements were taken as described (5). Tunicamycin was added after 1.6-h equilibration. The changes in fluorescence and growth rates were used to calculate the expression rates, following the equation:

$$\alpha_{cell,t} = \left(dlog\left(F_{total,t}\right) + dlog\left(N_t\right)\right) \times F_{total,t}$$

where $\alpha_{cell,t}$ is the expression rate at time t, $F_{total,t}$ is the mean fluorescence at time t, and N_t is the number of cells (calculated as the event rate in the cytometer).

Growth Measurements. Cells bearing the estradiol-inducible system driving the expression of Ras2(G19V) or with no ectopic targets were grown to saturation in complete medium overnight. The cells were diluted to $OD_{600} = 0.3$ and diluted 1:5 six times. A total of 5 μL of the dilutions were spotted in YPD plates containing 20 nM estradiol or 20 nM estradiol and 0.25 μg/mL tunicamycin. The plates were incubated at 30 °C, and pictures were taken 2 d after spotting.

To quantitatively capture the growth phenotypes of these strains under ER stress and PKA activation, we measured the ratio of cells over time. The Ras2(G19V) strain was cocultured with a control strain barcoded with a constitutive promoter driving the expression of mKate2 (P_{TDH3}–mKate2). Both strains were grown to saturation and then grown in exponential phase for ∼24 h. Cells were mixed 1:1 to a final $OD_{600} = 0.05$, and different concentrations of estradiol and tunicamycin were added to the cocultures. Next, 33-μL samples (diluted in 67 μL of Tris/EDTA buffer) were measured in the flow cytometer every 30 min. The relative change in ratio of cells expressing Ras2(G19V) to control was used to calculate the relative growth rate of the mutant strain. The steady state mean of the derivative of the logarithm of the ratio (dlogR) and the steady state mean of the growth rate of the control strain ($dlogN_0$) were used to calculate the relative growth rate (RGR):

$$RGR = dlogR/(dlogN\_0) + 1$$

for each combination of tunicamycin and estradiol. The RGR values for each concentration of estradiol were then normalized by the untreated values.

1. Pincus D, et al. (2010) BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLoS Biol* 8(7):e1000415.
2. Kimmig P, et al. (2012) The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *eLife* 1:e00048.
3. Carlson JM, Chakravarty A, DeZiel CE, Gross RH (2007) SCOPE: A web server for practical de novo motif discovery. *Nucleic Acids Res* 35(Web Server issue):W259–264.
4. Rubio C, et al. (2011) Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. *J Cell Biol* 193(1):171–184.
5. Zuleta IA, Aranda-Díaz A, Li H, El-Samad H (2014) Dynamic characterization of growth and gene expression using high-throughput automated flow cytometry. *Nat Methods* 11(4):443–448.

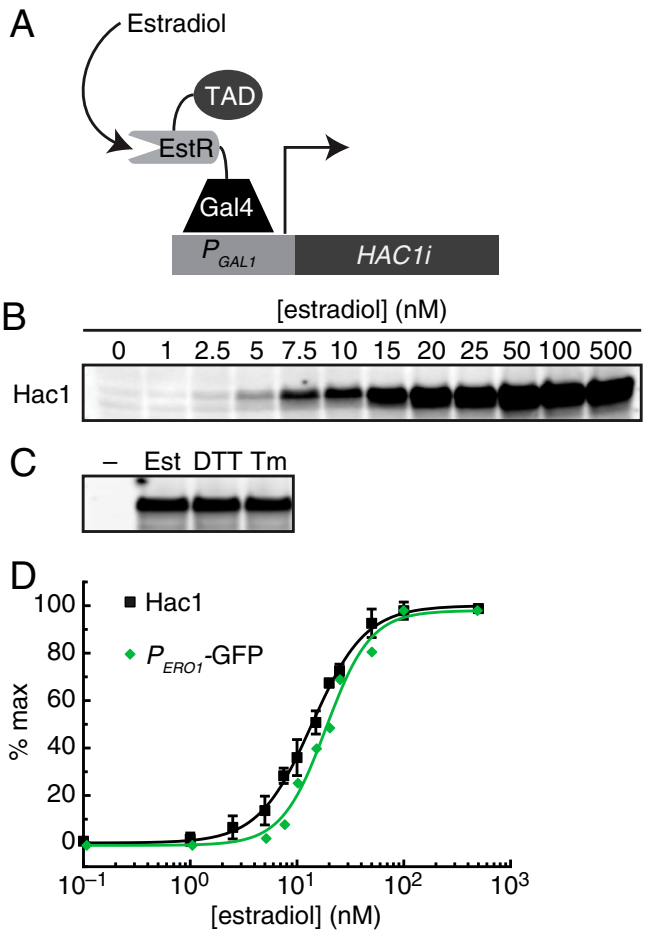

Fig. S1. Characterization of estradiol-inducible *HAC1*^{i} system. (*A*) Schematic of the estradiol-inducible *HAC1*^{i} system. A chimeric transcription factor, composed of the Gal4 DNA binding domain, the ligand binding domain of the human estrogen receptor (EstR), and a transcription activation domain (TAD), activates transcription of *HAC1*^{i}-driven by the *GAL1* promoter in the presence of estradiol. (*B*) Anti-HA Western blot showing expression of HA–Hac1 expressed as a function of estradiol concentration after 3 h of induction. (*C*) Western blot of HA-Hac1 showing comparable expression levels when induced by estradiol or ER stress [DTT, tunicamycin (Tm)]. (*D*) Quantification of HA-Hac1 levels measured by quantitative Western blot and the *ERO1* reporter measured by flow cytometry. Signals were normalized to their respective minimum and maximum values.

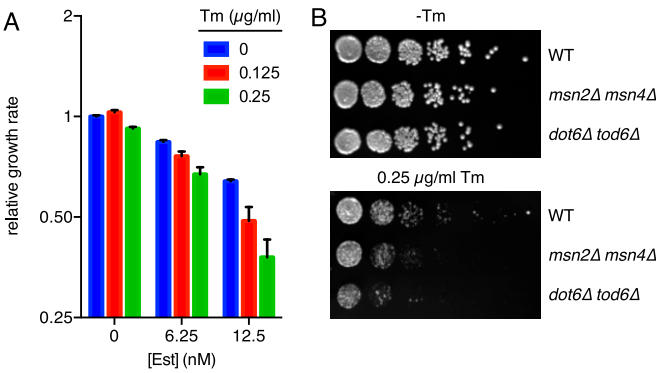

Fig. S2. Expression of Ras2(G19V) and deletion of PKA effectors *MSN2/4* and *DOT6/TOD6* decrease fitness in ER stress conditions. (*A*) Raw growth rates of cells expressing different levels of Ras2(G19V) in different concentrations of tunicamycin. (*B*) Dilution series spot assay of wild-type, *msn2*Δ*msn4*Δ, and *dot6*Δ*tod6*Δ cells in the absence and presence of 0.25 μg/mL tunicamycin.

Table S1. Plasmids used in this study

Plasmid	Construct	Marker
pDP001	pRS306-GEM(Gal4dbd-EstR-Msn2AD)	*URA3*
pDP008	pNH604-P_{GAL1}-*HA-HAC1*^{i}	*TRP1*
pDP010	pNH605-P_{ERO1}->GFP	*LEU2*
pDP012	pNH605-P_{HSP12}->GFP	*LEU2*
pAA001	pNH605-P_{RPL17A}->GFP	*LEU2*
pDP030	pRS303-GEM	*HIS3*
pAA002	pNH604-P_{GAL1}-*RAS2(G19V)*	*TRP1*
pAA003	pNH603*-P_{TDH3}->mKate2	*HIS3* (integrates at *can1*)

Table S2. Yeast strains used in this study

Strain	Genotype
DPY003	W303a; GEM(Gal4dbd-EstR-Msn2AD)::*URA3*; P_{GAL1}->*HA-HAC1*^{i}::*TRP1*
DPY007	W303a; GEM::*URA3*; P_{GAL1}-> *HA-HAC1*^{i}::*TRP1*; P_{ERO1}->GFP::*LEU2*
DPY015	W303a; GEM::URA3; P_{GAL1}-> *HA-HAC1*^{i}::*TRP1*; P_{HSP12}->GFP::*LEU2*
AAY001	W303a; GEM::URA3; P_{GAL1}-> *HA-HAC1*^{i}::*TRP1*; P_{RPL17A}->GFP::*LEU2*
DPY142	W303a; *tpk1/2/3-as*; *HSF1*-3xFLAG::*KANr*; P_{4xHSE}->emGFP::*URA3*
DPY268	W303a; *tpk1/2/3-as*; *HSF1*-3xFLAG::*KANr*; P_{4xHSE}-emGFP::*URA3*; *hac1*Δ::*HYGBr*; GEM::*HIS3*; P_{GAL1}-*HAC1*^{i}::*TRP1*
AAY002	W303a; GEM::*URA3*; P_{GAL1}->Ras2(G19V)::*TRP1*; P_{HSP12}->GFP::*LEU2*
AAY003	W303a; GEM::*URA3*; P_{GAL1}->Ras2(G19V)::*TRP1*; P_{ERO1}->GFP::*LEU2*
AAY004	W303a; GEM::*URA3*; P_{GAL1}->Ras2(G19V)::*TRP1*
AAY005	W303a; GEM::*URA3*; P_{TDH3}->mKate2:*HIS3*::*can1*

Dataset S1. RNA-sequencing (RNA-seq) data from Fig. 1

Dataset S1

Dataset S2. RNA-seq data from Fig. 4

Dataset S2