

SUPPLEMENTAL MATERIALS

Data availability

A website (<http://openwetware.org/wiki/Drummond/MisfoldingCost2010>) supporting this work includes fitness and mass-spectrometric data for download.

Yeast strains and construction

All strains used in this study (Table S3) are derived from BY4741 or BY4742 (1). All yeast transformations were performed using the lithium acetate method (2), were genomic PCR-confirmed, and, where noted, were genomic sequence-confirmed. The yeast galactose induction system was modified in BY4741 by replacing the P_{GAL3} promoter of *GAL3* with the P_{ACT1} promoter to produce MDY632, exactly as described in the Supplemental Data of ref. (3). The divergent *GAL1* and *GAL10* genes of MDY632 were deleted by replacement with the *LEU2* marker gene to produce MDY669. Briefly, oligos oMD01 and oMD02 (Table S4), each containing 60 flanking bases of homology to the downstream regions of both *GAL1* and *GAL10*, were used to amplify *LEU2* from plasmid pRS415 (4), and the PCR product was used to transform yeast to leucine prototrophy. The YFP*-containing fitness measurement strains KG001, KG006, KG039, KG041 and KG046 were isolated from G418 (Gibco)-resistant (5) colonies of MDY669 that was transformed with *NotI*-digested pKG001-pKG005 plasmids (Table S5), and the P_{GAL1} -YFP* insertions at the *leu2Δ0* locus were sequence-confirmed. YFP fluorescence, measured per cell by flow cytometry, increased with increasing galactose concentration exactly as predicted by the induction curve in the Supplemental Data of ref (3). The fitness reference strain KG007 was isolated from G418-resistant colonies isolated upon transformation of MDY669 with *NotI*-digested pKG006 (Table S5), and the P_{PGK1} -*mCherry* insertion at the *leu2Δ0* locus was sequence-confirmed. The mass spectrometry-analyzed strains KG071 and KG079 are progeny of BY4742 that was mated to KG001 and KG046, respectively. The resulting diploids were sporulated, and colony-purified haploid cells harboring the relevant genotype were obtained from random spores (6). The YFP-Ura3p*-containing fitness measurement strains SW001 and SW004 were isolated from G418 (Gibco)-resistant (5) colonies of MDY669 that was transformed with *NotI*-digested pSW001 and pSW003 plasmids (Table S5), and the P_{GAL1} -YFP-URA3* insertions at the *leu2Δ0* locus were sequence-confirmed.

Proteasome inhibition with bortezomib (ChemieTek) was performed on *rpn4Δ* and *pdr5Δ* strains (7, 8), constructed as follows. Oligos oMD03 and oMD04 (Table S4), containing ~50 flanking bases of homology to upstream and downstream regions of *RPN4*, were used to amplify *URA3* from pRS416 (4), and the PCR product was used to transform MDY669 to uracil prototrophy, producing MDY678. Similarly, *PDR5* was deleted from MDY678 upon transformation with *MET17* amplified from pRS411 (1) using oligos oMD05 and oMD06 (Table S4), producing the methionine prototroph MDY680. This strain was then genetically purified by backcrossing to BY4742. Diploids were sporulated, and colony-purified haploid cells harboring the relevant genotype were

obtained from random spores (6). Two additional rounds of backcrosses were performed with random spore isolates to BY4741, and the strain MDY711 was obtained. MDY727 was obtained upon transformation of MDY711 with a 3413 bp PCR product that was amplified from pMD47 (Table S5), using oligos oMD07 and oMD08 (Table S4). A hygromycin (Invitrogen)-resistant (5) isolate containing a $P_{GALI-3} \times Myc-YFP_{REF}$ insertion at the *leu2Δ0* locus was sequence-confirmed. The Western-blotted strains MDY729, MDY732, and KG030, KG032 and KG035 were obtained by transformation of MDY727 with *NotI*-digested pMD60-pMD64 plasmids (Table S5), and sequence confirmation of G418-resistant isolates containing a $P_{GALI-3} \times FLAG-YFP^*$ insertion at the *ura3Δ0* locus.

Misfolded yellow fluorescent proteins

Misfolded yellow fluorescent proteins YFPm1 – YFPm4 are named sequentially in order of least misfolded to most misfolded (Table S1). To find mutations that contribute to misfolding, we performed random PCR mutagenesis (9) on EmCitrine (yellow fluorescent protein, dubbed YFPwt) that had been codon-optimized for *S. cerevisiae* (10). We screened for temperature-sensitive YFPs that fluoresce at 25° C but not at 37° C; temperature sensitivity often results from protein misfolding at higher temperatures (11). Mutant YFPm1 was obtained from this screen; mutant YFPm3 is identical to YFPm1 except it lacks a mutation, V163A, previously reported to suppress temperature-sensitivity (12). Upon sequencing all temperature-sensitive YFPs we recovered a total of 10 mutations. We combined these mutations to synthesize (Integrated DNA Technologies) YFPm2 and YFPm4. The most severe misfolding mutant, YFPm4, was designed to contain all 10 mutations, while mutant YFPm2 contains only those mutations not present in YFPm1 or YFPm3. Fluorescence measurements in Table S1 are after 20 hours of exponential growth in induction media (SC-suc/raf/gal; see “Competitive growth-rate measurements” section below).

Misfolded Ura3p

To synthesize (Integrated DNA Technologies) misfolded *URA3m1* we combined 8 mutations previously known to cause temperature sensitivity to growth on media lacking uracil (13).

Competitive growth-rate measurements

We measured fitness using flow cytometry to monitor growth competitions of fluorescently tagged strains as in (14) but with controlled maximum culture densities and without the high throughput platform. Our procedure also differs in that some YFP mutants do not glow (YFPm2 and YFPm4) therefore separation often depends on only one fluorescent marker. We set a maximum flow rate of 800 threshold events to minimize the frequency that two cells are misidentified as a single cell, and included >95% of collected data in our calculations removing events that are extreme outliers in size (debris) compared to the main yeast population.

Each growth culture was a competition between one test strain (YFPwt, YFPm1–4, *URA3wt*, or *URA3m1*) and our reference strain: an isogenic yeast strain labeled with RFP (KG007). To begin a growth competition, two yeast strains (one test strain and the reference) were grown separately to saturation overnight. These cultures were then used

to inoculate the same 10 mL competition culture. These 10mL cultures were initially very dilute; approximately 2,500 -10,000 cells from each overnight culture were added to ensure that cultures never became saturated by the next time point (8-16 hours later).

After inoculation of the first 10mL competition culture from the overnight cultures, we grew cells at 30°C with shaking for 8 to 16 hours. Following this growth step, we placed an aliquot of each competition on ice and used flow cytometry to count the fraction of red versus yellow-labeled or non-fluorescent cells in a sample of 100,000 cells. (Note we wait one growth cycle to obtain our first data point to avoid including any lag phase growth in our measurements). At this time we also measured the total cell density of each competition using a Coulter counter (Beckman-Coulter) and diluted each competition into a fresh 10mL culture. Again, cultures were initially dilute. The amount of cells used to inoculate the 10mL competition cultures at each time point was adjusted such that cultures never exceeded final concentrations of 2×10^6 cells/mL. Cells were placed back at 30°C until the next time point. By controlling maximum culture density, we maintain exponential growth throughout the experiment. We repeated this procedure to obtain a total of four time points.

The rate at which the relative abundance of red-labeled and either yellow-labeled or unlabeled cells changed through time measures relative growth rate of the test and reference strains (see main text). All stages of these experiments were performed using synthetic complete media with 2% sucrose, 1% raffinose, either with 0.5% galactose (SC-suc/raf/gal, competition with induction) or without galactose (SC-suc/raf, competition without induction).

For each strain except YFPm2, induced and uninduced growth rate measurements were repeated on an isogenic independent isolate.

Correcting for the cost of induction to compare costs of expression and misfolding

Galactose, though rendered a gratuitous inducer in all strains due to the deletion of the metabolic genes *GAL1* and *GAL10*, also induces production of native yeast proteins (Fig. S3), slowing reference-strain growth by an average of 14.5% as determined by tracking the differences in generations between induced and uninduced measurements taken at the same time-points across all experiments (Fig. S3A). This implies that growth rates without induction must be corrected upward by a similar factor for direct comparison with induced cultures. Because growth rates are expressed as percentages of reference-strain growth, faster reference-strain growth in the absence of induction artificially depresses the uninduced growth defects, albeit by a small percentage. Correcting the uninduced relative growth rates for this effect does not alter the conclusions drawn in the main manuscript (Fig. S3B).

Cultures for protein isolations

Standard yeast growth media and conditions (15) were used, and relevant modifications are included as follows. Three mL yeast extract + peptone (YP) pre-cultures, containing 2% glucose, were inoculated with a single colony from fresh plates, and were grown overnight at 30°C with shaking. For yeast strains analyzed by Western blot, larger YP assay cultures, containing 2% sucrose and 1% raffinose, were equally inoculated with the overnight pre-cultures, and were grown for ~18 hours at 30° C with shaking. Cultures that achieved a mid-log density of ~ 0.3 to 0.5×10^7 cells/mL were

induced with 27.5 mM galactose, and were inhibited with 10 μ M bortezomib (ChemieTek) for an additional 2 hours at 30° C with shaking. For SILAC-labeled (16) yeast strains subjected to mass spectrometric analysis, larger SC (yeast nitrogen base, synthetic complete media) assay cultures, containing 2% sucrose, 1% raffinose, 27.5 mM galactose, and either ^{12}C - ^{14}N -lysine (Sigma-Aldrich) or ^{13}C - ^{15}N -lysine (Cambridge Isotope Laboratories, Inc.) were equally inoculated with overnight pre-cultures and were grown to a mid-log density for ~18 hours at 30° C with shaking.

Soluble, insoluble, and total protein isolation and relative quantification

Approximately 1.25×10^8 cells were harvested by centrifugation at $3000 \times g$ for 1 min from mid-log cultures. For the isolation of soluble and insoluble protein fractions, cell pellets were washed in 1.4 mL of ice-cold 50 mM Tris-HCl, pH 8.5, and were transferred to 1.5 mL snap-cap vials. The cells were repelleted at $20,000 \times g$ for 10 sec, the wash was discarded and the cell pellets were resuspended in 50 μ L of ice-cold *soluble* protein buffer (SPB; 500 mM NaCl, 50 mM Tris-HCl, pH 8.5, 1 mM PMSF, 1:100 dilution of Protease Inhibitor Cocktail Set IV (CalBiochem)). Cell suspensions were flash-frozen upon pipette-transfer to 2.0 mL snap-cap vials that were partially submerged in liquid nitrogen and that contained a 7 mm stainless steel ball (Retsch). The cells were disrupted using a Mixer Mill MM 400 (Retsch) set to 30 Hz for 2×90 sec, interspersed with a brief re-cooling of the tubes in liquid nitrogen. The lysed cells were thawed on ice for 5 min, and were transferred to 1.5 mL polyallomer tubes (Beckman Coulter, Inc.) upon further addition of 450 μ L of ice-cold SPB. The lysates were centrifuged at $100,000 \times g$ for 15 min at 4° C. The supernatant was decanted to a fresh tube, and the insoluble pellet was washed twice, each time with 500 μ L of ice-cold WB (SPB with 150 mM NaCl) and brief vortexing, followed by centrifugation at $100,000 \times g$ for 15 min at 4° C. The insoluble pellet was dissolved by vigorous vortexing in 500 μ L of *insoluble* protein buffer (IPBS; WB containing 2% SDS and 2 mM DTT). The resuspension was clarified by centrifugation at $20,000 \times g$ for 5 min, and the supernatant was decanted to a fresh tube.

For unfractionated, total protein extracts, cell pellets were washed in 1.8 mL of ice-cold 50 mM Tris-HCl, pH 8.5, and were transferred to 2 mL screw-cap vials (Biospec Products, Inc.). Cells were repelleted at $20,000 \times g$ for 10 sec, and were flash-frozen in liquid nitrogen after discarding the wash. The vials were filled two-thirds full with 0.5 mm glass beads, and 1 mL of IPBU (same as IPBS, but with 8 M urea instead of SDS). The cells were disrupted using a Mini-BeadBeater-8 (Biospec Products, Inc.) set to maximum speed for 1 min \times 5 in a 4° C cold room, interspersed with 45 sec rests. 500 μ L of the lysates were clarified by centrifugation at $20,000 \times g$ for 5 min, and the supernatant was decanted to a fresh tube.

Western blot analysis

Soluble protein fractions were mixed with 1 \times soluble protein loading buffer (6 \times SPLB; 50 mM Tris-HCl, pH 6.8, 0.05% bromophenol blue, 30% glycerol, 5% 2-mercaptoethanol, 10% SDS). Insoluble protein fractions, resuspended in IPB containing 2% SDS, were mixed with 1 \times insoluble protein loading buffer (6 \times IPLB; 6 \times SPLB lacking SDS). Proteins were resolved by SDS-PAGE using 12.5% Tris-HCl gels (Bio-Rad Laboratories), followed by electrotransfer to 0.2 μ m pore size nitrocellulose membranes

(GenScript). Hybridization against 3×FLAG-tagged proteins was performed using mouse monoclonal anti-FLAG M2 antibody (Sigma-Aldrich) and a ONE-HOUR Advanced Western Kit (GenScript), according to the manufacturer's instructions. Briefly, a 1:160 mixture of FLAG antibody and WB-1 Solution was pre-incubated for 1 hour. Membranes were blocked for 5 min in Pretreat Solution A-B, and were incubated overnight at 4° C in WB-2 Solution mixed 667:1 with the FLAG/WB-1 mixture. The membranes were washed 3 × 10 min in Wash Solution, and were reacted with LumiSensor HRP Substrate for 5 min. Chemiluminescent bands were digitally imaged with a ChemiImager 5500 CCD camera (Alpha Innotech Corp.). The membranes were then probed for 3×Myc-tagged proteins, as follows. A 3:1:30 mixture of rabbit polyclonal anti-c-Myc antibody (Sigma-Aldrich), alkalinephosphatase-conjugated goat anti-rabbit IgG (Fc) antibody (Thermo Scientific), and phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) was pre-incubated for 1 hour. The membranes were incubated overnight at 4° C in the antibody mix diluted 1:500 in PBS-T containing 3% nonfat milk. The membranes were washed 4 × 5 min in PBS-T, and were reacted with Immobilon AP Substrate (Millipore) for 5 min. Chemiluminescent bands were digitally imaged, and quantified using ImageQuant TL v2005 software (GE Healthcare).

All Western-analyzed strains contained two distinct epitope-tagged copies of YFP: 3×Myc-YFP_{pref} expressed from the *GALI* promoter at the *leu2Δ0* locus, and 3×FLAG-YFP_{wt/m1-4} expressed from the *GALI* promoter at the *ura3Δ0* locus. YFP_{REF} was tagged with 3×Myc, and the test YFPs were tagged with 3×FLAG. We used the reference protein YFP_{pref} as a control to ensure differences in signal intensities were not the result of differences in culture densities, expression resulting from galactose induction, or loading volume. The intensities of 3×Myc-YFP bands were roughly equal between strains, indicating differences in 3×FLAG-YFP intensities are likely due to specific properties of the 3×FLAG-tagged YFP variant. Still, soluble and insoluble 3×FLAG-YFP intensities were normalized by their respective 3×Myc-YFP_{pref} intensity measurements to control for differences in culture density or induction. For each test strain, we report the geometric mean over replicate experiments (soluble: $n = 5$, insoluble: $n = 4$, total: $n = 2$). For every Western-analyzed strain, cell fractionation and subsequent Western analysis were repeated using an isogenic independent isolate. A biological replicate was prepared from one of the independent transformant pairs, and the rest of the sample size was contributed from technical replicates on the other transformant.

Quantitative proteomics

Approximately 100 μg of 1:1 mixtures of ¹³C-¹⁵N-lysine-labeled (16) total yeast proteins and unlabeled total yeast proteins, in IPB containing 8M urea, were digested in solution by the endoproteinase LysC using a filter-assisted sample preparation protocol (17). The resulting peptide mixtures were cleaned by C18 cartridges (3M), and either separated on a PolyWAX LP column (PolyLC) and submitted in 40 fractions, or run directly, on an Orbitrap Velos instrument (Thermo Fisher Scientific) with 360-minute liquid chromatography and tandem mass spectrometry (LC-MS/MS) using a gas-phase fractionation procedure. Briefly: two m/z regions for small (350–750 m/z) and large (745–2000 m/z) ions were used to trigger MS/MS in the ion trap for the top 20 small ions from small region and top 10 large ions. Peptides were eluted with 200 nl flow rate using a

NanoAcquity pump (Waters). Samples were trapped for 15 minutes with flow rate of 2 μ l/min on a trapping column 100 micron ID packed for 5cm in-house with 5 μ m Magic C18 AQ beads (Waters) and eluted with a gradient to 20 cm 75 micron ID analytical column (New Objective) packed in-house with 3 μ m Magic C18 AQ beads (Waters).

Mass spectra were analyzed using MaxQuant 1.1.1.16 (18). The data were searched against the yeast ORF database downloaded from the *Saccharomyces Genome Database* (<http://www.yeastgenome.org>) supplemented with frequently observed contaminants using the MaxQuant search engine Andromeda; databases are provided in supplemental material. Carbamidomethylated cysteines were set as a fixed modification, with oxidation of methionine and N-terminal acetylation as variable modifications. A mass deviation of 0.5 Da was set as maximum allowed for MS/MS peaks and a maximum of two missed cleavages were allowed. Maximum false discovery rates (FDR) were set to 0.01 both on peptide and protein levels. Minimum required peptide length was six amino acids. For the mass spectrometry-analyzed strain expressing YFPm3, experiments were repeated using an isogenic independent isolate.

Plasmids for genomic integration of genes encoding fluorescent proteins

The pKG001-pKG005 series of plasmids are 5.9 kb yeast integration vectors that contain a 3129 bp insertion within the 2.8 kb cloning vector pJ204 (DNA 2.0 Inc.; Table S5). For yeast transformation, *NotI*-linearized plasmid is flanked with DNA homologous to 200 bp upstream and 200 bp downstream of the *leu2 Δ 0* locus of BY4741 and BY4742. The 494 bp *P_{GALI}* promoter was PCR-amplified from BY4741 genomic DNA. The 714 bp *YFP* sequence encodes a yeast-optimized yellow fluorescent protein variant EmCitrine (10), or destabilized variants of YFP (Table S1). *KanMX6* is a selectable marker that confers resistance to G418 in yeast(5).

leu2 Δ 0::P_{GALI}-YFP-KanMX6

NotI

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GCGGCCGctacatcaaaatccacgttcttttcatatggattcctaaatcctcgaggagaatTTtataatata
gtctgtatacataatattatagcttctaacgacaatggaactctaacaattatcaaattgtccgccagttc
ctcaaaaagatccatgtataatcttcattattacagccctcttgacctctaatacatgaatgttctcGCTAG
CAAGCTTCTCGAGTTTGGAACTTTCAGTAATACGCTTAACTGCTCATTGCTATATTGAAGTACGGATTAGA
AGCCGCCGAGCGGGGCGACAGCCCTCCGACGGAAGACTCTCCTCCGTGCGTCCCTCGTCTTCACCGGTGCGGT
TCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATG
GTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATTAACGAATCAAATTAACAACC
ATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTG
ATCTATTAACAGATATATAAATGGAAAAGTGCATAACCACCTTAACTAATACTTTCAACATTTTCAGTTT
GTATTACTTCTTATTCAAATGTCATAAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGT
CAAGGAGAAAACAATTTCATGTCTAAAGGTGAAGAATTATTCACTGGTGTGTCCTCCAAATTTTGTTGAATTAG
ATGGTGATGTTAATGGTACAAAATTTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACTTACGGTAAATTG
ACCTTAAATTTTATTTGTAATACTGTTAAATTGCCAGTTCATGGCCAACCTTAGTCACTACTTTAGGTTA
TGGTTTGATGTGTTTTGCTAGATACCCAGATCATATGAAACAACATGACTTTTTTCAAGTCTGCCATGCCAG
AAGGTTATGTTCAAGAAAGAACTATTTTTTTCAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAG
TTTGAAGGTGATACCTTAGTTAATAGAATCGAATTAAGGTTATTGATTTTTAAAGAAGATGGTAACATTTT
AGGTCACAAATTGGAATACAATACTATACTCTACAATGTTTACATCATGGCTGACAAACAAAAGAATGGTA
TCAAAGTTAACTTCAAATTTAGACACAACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAACAA
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AATACTCCAATTGGTGATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCTATCAATCTAGATTATC
CAAAGATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTTACTGCTGCTGGTATTACCCATG
GTATGGATGAATTGTACAAATAAGGATCCTGTACAAGTAA...

...[pUC ori, Amp^R of pJ204 (2.8 kb)]...

...GAGCTCGTTTTTCGACACTGGATGGCGGCGTTAGTATCGAATCGACAGCAGTATAGCGACCAGCATTCA
CATACGATTGACGCATGATATTACTTTCTGCGCACTTAACTTCGCATCTGGGCAGATGATGTGCGAGGCGAA
AAAAAATATAAATCACGCTAACATTTGATTAAAAATAGAACAACCTACAATATAAAAAAACTATACAAATGAC
AAGTTCTTGAAAACAAGAATCTTTTTATTGTCTGACTGATTAGAAAACTCATCGAGCATCAAATGAAAC
TGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAA
CTCACCGAGGCAGTTCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAA
TACAACCTATTAATTTCCCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAA
TCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTTCGTGATTGCGCCTGAGCGAGACGAAATACGCGAT
CGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACA
ATATTTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTGCGGGGATCGCAGTGGTGAG
TAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGCTCAGCCAGT
TTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGC
GCATCGGGCTTCCCATACAATCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCGAGCCCATTTATA
CCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAAACGTGAGTCTTTTCTTACCCATGG
TTGTTTATGTTCCGATGTGATGTGAGAAGTGTATCCTAGCAAGATTTTAAAAGGAAGTATATGAAAGAAGA
ACCTCAGTGGCAAATCCTAACCTTTTATATTTCTCTACAGGGGCGCGGCGTGGGGACAATTC AACCGCTCT
GTGAGGGGAGCGTTTTCCCTGCTCGCAGGTCTGCAGCGAGGAGCCGTAATTTTTGCTTCGCGCCGTGCGGCC
ATCAAAATGTATGGATGCAAATGATTATACATGGGGATGTATGGGCTAAATGTACGGGCGACAGTCACATC
ATGCCCCTGAGCTGCGCACGTCAAGACTGTCAAGGAGGGTATTCTGGGCCTCCATGTGCTGGCCGGGTGA
CCCGGCGGGGACGAGGCAAGCTAAACAGATCTGGCGCGCCAGATCTGTGACcgcacacgaaattacaaaat
ggaatatgttcatagggtagacgaaactatatacgcaatctacatacatttatcaagaaggagaaaaagga
ggatgtaaaggaatacaggttaagcaaatgataactaatggctcaacgtgataagggaaaaagaattgcactt
taacattaatattgacaaggaggaggccaccacacaaaa

pKG006 (Table S5) is identical to pKG001 except that P_{GAL1} was replaced with a 712 bp P_{PGK1} promoter region that was amplified from BY4741 genomic DNA, and YFP was replaced with a 708 bp sequence that encodes the red fluorescent protein mCherry (19), and is yeast codon-optimized.

P_{PGK1}-mCherry

CAAGAATTACTCGTGAGTAAGGAAAGAGTGAGGAACCTATCGCATACCTGCATTTAAAGATGCCGATTTGGG
CGCGAATCCTTTATTTTGGCTTACCCTCATACTATTATCAGGGCCAGAAAAAGGAAGTGTTCCTCCTT
CTTGAATTGATGTTACCCTCATAAAGCACGTGGCCTCTTATCGAGAAAAGAAATTACCGTCGCTCGTGATTT
GTTTGCAAAAAGAACAACAACTGAAAAAACCAGACACGCTCGACTTCTGTCTTCTTATTGATTGCAGCTT
CCAATTTCTGTCACACAACAAGGTCTAGCGACGGCTCACAGTTTTGTAAACAAGCAATCGAAGTTCTGGA
ATGGCGGGAAAGGGTTTTAGTACCACATGCTATGATGCCCACTGTGATCTCCAGAGCAAAGTTCTGTTCCGATC
GTACTGTTACTCTCTCTCTTTCAAACAGAATTGTCCGAATCGTGTGACAACAACAGCCTGTTCTCACACAC
TCTTTTCTTCTAACCAAGGGGGTGGTTTTAGTTTTAGTAGAACCTCGTGAAACTTACATTTACATATATATAA
ACTTGATAAATTTGGTCAATGCAAGAAATACATATTTGGTCTTTTCTAATTCGTAGTTTTTCAAGTTCTTA
GATGCTTTCTTTTTCTTTTTTACAGATCATCAAGGAAGTAATTATCTACTTTTTTACAACAAATATAAAA
CAgaattcATGGTTTTCTAAGGGTGAAGAAGACAACATGGCTATTATTAAGGAATTTATGAGATTCAAGGTT
CACATGGAAGGTTCTGTTAACGGTCACGAATTTGAAATTTGAAGGTGAAGGTGAAGGTAGACCATACGAAGG
TACTCAAACCGCTAAGTTGAAGGTTACCAAGGGTGGTCCATTGCCATTCGCTTGGGACATTTTGTCTCCAC
AATTCATGTACGGTTCTAAGGCTTACGTTAAGCACCCAGCTGACATTCAGACTACTTGAAGTTGTCTTTC
CCAGAAGGTTTTCAAGTGGGAAAGAGTTATGAACTTCGAAGACGGTGGTGTGTTACCCTTACCCAAGACTC

TTCTTTGCAAGACGGTGAATTTATTTACAAGGTTAAGTTGAGAGGTACTAACTTCCCATCTGACGGTCCAG
 TTATGCAAAAAGAAGACTATGGGTTGGGAAGCCTCTTCTGAAAGAATGTACCCAGAAGACGGTGCTTTGAAG
 GGTGAAATTAAGCAAAGATTGAAGTTGAAGGACGGTGGTCACTACGACGCTGAAGTTAAGACCACCTACAA
 GGCTAAGAAGCCAGTTCAATTACCAGGTGCTTACAACGTTAACATTAAGTTGGACATTACCTCTCACAACG
 AAGACTACACCATTGTTGAACAATACGAAAGAGCTGAAGGTAGACTCTACCGGTGGTATGGACGAATG
 TACAAG

pSW001 and pSW003 (Table S5) are identical to pKG001 except that an 806 bp sequence was inserted after *YFP* that specifies for yeast wild type Ura3p (13) or a destabilized Ura3p variant, Ura3m1, to create a contiguous YFP-Ura3p* protein. A short flexible linker sequence (10) was also included in between *YFP* and *URA3*.

YFP-linker-URA3

ATGTCTAAAGGTGAAGAATTATTCCTGCTGGTGTGTCCTCAATTTTGGTTGAATTAGATGGTGATGTTAATGG
 TCACAAATTTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACTTACGGTAAATTGACCTTAAAATTTATTT
 GTACTACTGGTAAATTGCCAGTTCATGGCCAACCTTAGTCACTACTTTAGGTTATGGTTTGTATGTGTTTT
 GCTAGATACCCAGATCATATGAAACAACATGACTTTTTTCAAGTCTGCCATGCCAGAAGGTTATGTTCAAGA
 AAGAACTATTTTTTTTCAAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAGTTTGAAGGTGATACCT
 TAGTTAATAGAATCGAATTTAAAGGTATTGATTTTTAAAGAAGATGGTAACATTTTAGGTCACAAATTGGAA
 TACAACATAACTCTCACAATGTTTACATCATGGCTGACAAACAAAAGAATGGTATCAAAGTTAACTTCAA
 AATTAGACACAACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAACAAAATACTCCAATTGGTG
 ATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCTATCAATCTAGATTATCCAAAGATCCAAACGAA
 AAGAGAGACCACATGGTCTTGTTAGAATTTGTTACTGCTGCTGGTATTACCCATGGTATGGATGAATTGTA
 CAAAggtgacggtgctggttaattaacATGTGCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTA
 GTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAACTTGTGTGCTTCATTGGATGTT
 CGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAATTTGTTTACTAAAAACACATGT
 GGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATCCGCCAAGTACAATT
 TTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTACTCTGCGGGTGTA
 TACAGAATAGCAGAATGGGCAGACATTACGAATGCACCGGTGTGGTGGGCCAGGTATTGTTAGCGGTTT
 GAAGCAGGCGGCAGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGG
 GCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATC
 GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGT
 GGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGAT
 CTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGAACGTTAC
 AGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTAA

The pMD60-pMD64 series of plasmids are 6.1 kb yeast integration vectors that contain a 3318 bp insertion within the 2.8 kb cloning vector pJ204 (DNA 2.0 Inc.; Table S5). The insertions are flanked with a pair of *NotI* restriction sites, and DNA homologous to 134 bp upstream and 134 bp downstream of the *ura3Δ0* locus of BY4741 and BY4742. *3×FLAG* encodes three tandem copies of the FLAG epitope DYKDDDDK (20) and is yeast codon-optimized. *NotI*-digested pMD60 yields two fragments, and only the larger 3.3 kb fragment targets the *ura3Δ0* locus, while the smaller 2.8 kb pJ204 cloning vector fragment has no homology to yeast DNA.

ura3A0::KanMX6-P_{GALI}-3xFLAG-YFP

NotI

ATTTAAATGTTTAAACGGCGCGCCGCGCCGCGCagatgctaagagatagtgatgatatttcataaataatgt
aattctatatatgttaattaccttttttgcgagggcatatttatggggaaggataagttttgaccatcaaag
aagggttaatgtggctgtggtttcaACTAGTGGATCTGATATCACCTAATAACTTCGTATAGCATAACATTAT
ACGAAGTTATATTAAGGGTTCTCGAGAGCTCGTTTTTCGACACTGGATGGCGGCGTTAGTATCGAATCGACA
GCAGTATAGCGACCAGCATTACATACGATTGACGCATGATATTACTTTCTGCGCACTTAACTTCGCATCT
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ATAAAAAAATATACAAATGACAAGTTCTTGAAAAACAAGAATCTTTTTATTGTCAGTACTGATTAGAAAAA
CTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCC
GTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCATAGGATGGCAAGATCCTGGTATCGGTCTGCG
ATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAA
ATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAA
CAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTTCGTGATTGCGCC
TGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAG
GAACACTGCCAGCGCATCAACAATATTTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTT
TGCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAAATGCTTGATGGTCGGAAGA
GGCATAAATTCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCC
ATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTGCGACCTGATTGCCCGA
CATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGAAACG
TGAGTCTTTTCCTTACCCATGGTTGTTTATGTTCCGGATGTGATGTGAGAAGTGTATCCTAGCAAGATTTTA
AAAGGAAGTATATAAAGAAGAACCTCAGTGGCAAAATCCTAACCTTTTATATTTCTCTACAGGGGCGCGGC
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TTTTGCTTCGCGCCGTGCGGCCATCAAAATGTATGGATGCAAAATGATTATACATGGGGATGTATGGGCTAA
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CTCCATGTGCTGGCCGGGTGACCCGGCGGGGACGAGGCAAGCTAAACAGATCTCTAGACCTAATAACTTC
GTATAGCATAACATTATACGAAGTTATATTAAGGGTTGTCGACGCTAGCAAGCTTCTCGAGTTTGGAACTTT
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CCGACGGAAGACTCTCCTCCGTGCGTCTCGTCTTACCAGGTCGCGTTTCTGAAACGCAGATGTGCCTCGC
GCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAG
TAACCTGGCCCCACAACCTTCAAATTAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTT
TTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAACAGATATATAAATGG
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TAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAACAATTGTTAATTAA
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CAAGGTTATGTCTAAAGGTGAAGAATTATTCAGTGGTGTGTCCTCAATTTTGGTTGAATTAGATGGTGATG
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TTCAAGAAAGAACTATTTTTTTCAAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAGTTTGAAGGT
GATACCTTAGTTAATAGAATCGAATTAAGGATTTGATTTTTAAAGAAGATGGTAACATTTTAGGTCACAA
ATTGGAATACAACATAACTCTCACAATGTTTACATCATGGCTGACAAACAAAAGAATGGTATCAAAGTTA
ACTTCAAATTAGACACAACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAACAAAATACTCCA
ATTGGTGATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCTATCAATCTAGATTATCCAAAGATCC
AAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTTACTGCTGCTGGTATTACCCATGGTATGGATG
AATTGTACAAAATAAGGATCCAAAACCTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGCT
TCAATTTAATTATATCAGTTATTACCCGggaatctcggtcgtaatgatttctataatgacgaaaaaaaaa
aattggaaagaaaaagcttcatggcctttataaaaaggaactatccaatacctcgccagaaccaagtaaca
gtattttacggggcacaatGCGGCCGCGCGCCGTTTTAAACATTTAAAT

NotI

pMD47 (Table S5) is identical to pMD60 except that *KanMX6* was replaced with *Hyg^R*, a selectable marker that confers resistance to hygromycin in yeast (5). *3×FLAG* was replaced with *3×Myc*, which encodes three tandem copies of the Myc epitope EQKLISEEDL (21), and is yeast codon-optimized. Finally, pMD47 contains a different YFP variant, called *YFPref*, with nucleotide substitutions that encode G40D and I123V mutations.

3×Myc

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GAACAAAAATTGATTTCTGAAGAAGATTTGGAACAAAAGTTAATCTCAGAAGAAGACTTAGAACAAAAGTT  
GATCTCTGAAGAAGATTTG
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