A

ZPR1 (E3 only)
DMSO 5-Ph-IAA *ssa2Δ*
120 min
60 min
30 min
0 min
Count/Max Count
Hsf1 reporter

B

ZPR1 (E3 only)
ZPR1-AID
OD_{600}
Treatment time (min)
DMSO
5-Ph-IAA

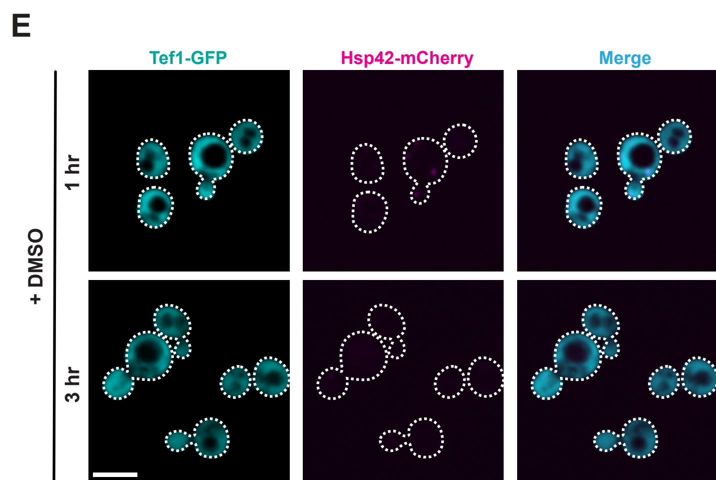
C

Replicate: 1 1 2 2
TEF1 *TEF1-GFP* *TEF1* *TEF1-GFP*
Tef1-GFP
eEF1A (Tef1/2 or Tef2)
IB: eEF1A
IB: Pgk1

D

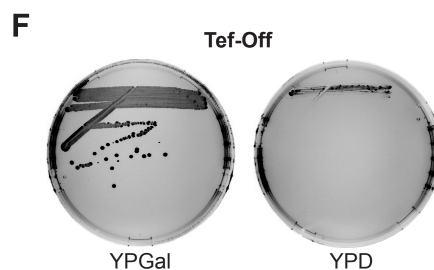
TEF1-GFP
log_2 Tef1-GFP
treatment time (hr): 0 3 6 0 3 6 0 3 6 0 3 6
DMSO 5-Ph-IAA DMSO 5-Ph-IAA
ZPR1 (E3 only) *ZPR1-AID*
treatment time (hr) 0 hr 3 hr 6 hr
+5-Ph-IAA +DMSO *ZPR1-AID*
+5-Ph-IAA +DMSO *ZPR1* (E3 only)
Count/Max Count
Tef1-GFP

E

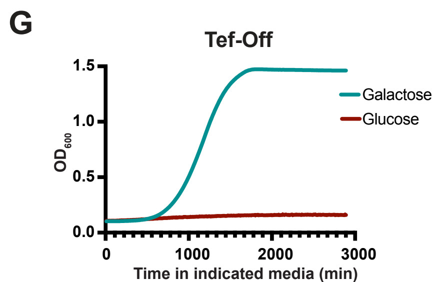


F

Tef-Off



G



H

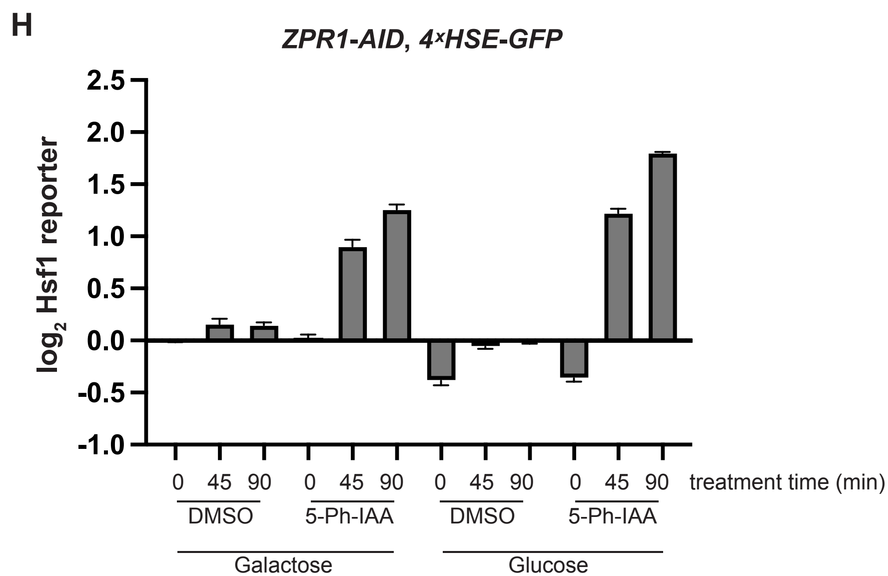
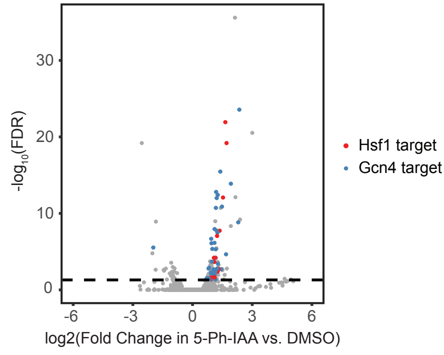
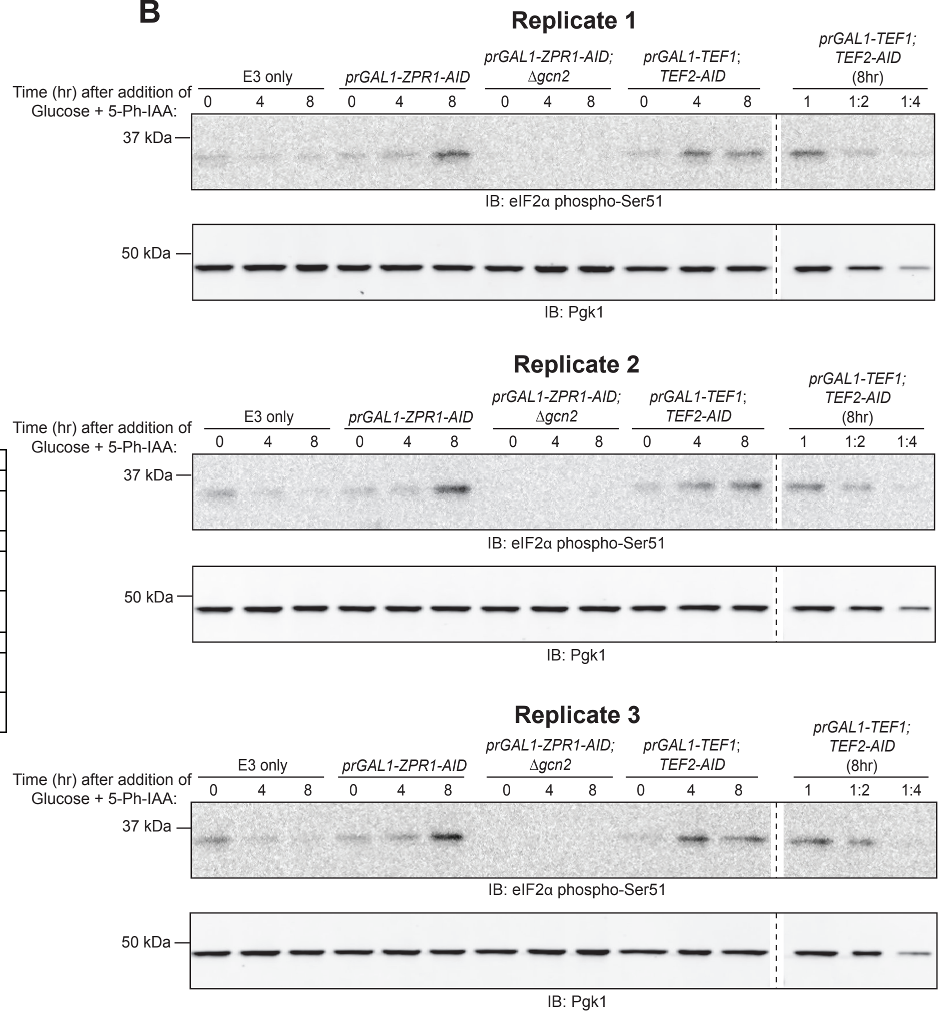


Figure S1. Further validation of Zpr1-AID, Tef1-GFP, and Tef-Off as tools for studying Zpr1 function in cells. Related to Figures 1 and 2. (A) Representative histogram of GFP intensity normalized to cell size from *ZPR1* (E3 only) or *ZPR1* (E3 only) *ssa2*Δ cells carrying the Hsf1 reporter (bar plot shown in Figure 1C). Data from *ZPR1* (E3 only) *ssa2*Δ cells is repeated from Figure 1C's histograms. (B) Growth curves of 96-well plate cultured *ZPR1* (E3 only) and *ZPR1-AID* cells. Cultures were inoculated at an OD600 of 0.01 and grown at 30˚C for two days with 5 μM of 5-Ph-IAA or mock treatment. (C) Immunoblots with the indicated antibodies of whole cell extracts derived from *TEF1, ZPR1-AID* or *TEF1-GFP, ZPR1-AID* strains after growth to mid-log phase in liquid YPD media. Two replicates are shown. (D) *TEF1-GFP, ZPR1* (E3 only) or *TEF1-GFP, ZPR1-AID* cells were grown to mid-log phase in liquid SD media and analyzed by flow cytometry at the indicated time points after treatment with 5 μM of 5-Ph-IAA or DMSO. Shown are bar graphs of the median values of GFP intensity normalized to cell size and to the average median GFP intensity of *ZPR1* (E3 only) DMSO cells at 0 min with standard deviation from three replicates along with representative histograms as indicated on the side. (E) *ZPR1-AID* cells expressing endogenously tagged Tef1-GFP and Hsp42-mCherry were grown to mid-log phase in liquid SD media and imaged at the indicated time points after mock treatment. Shown are confocal micrographs normalized to the same intensity with cells outlined. Scale bar: 2.5 μm. (F) Streak assay showing sensitivity of Tef-Off cells to glucose. Plates were incubated at 30 °C for two days prior to imaging. (G) Growth curves of 96-well plate cultured Tef-Off cells. Cultures were inoculated at 0.01 OD600 and grown at 30˚C for two days in the indicated liquid media. (H) *ZPR1-AID* cells carrying the Hsf1 reporter were grown in SGal before back-dilution into fresh SGal or SD for one hour and analyzed by flow cytometry at the indicated time points post-treatment with 5 μM 5-Ph-IAA or DMSO. Cells were analyzed as in Figure 1C. Bar graphs depict median GFP intensity values normalized to cell size and the average median GFP intensity of *ZPR1-AID* Galactose + DMSO cells at 0 min. Error bars represent standard deviation from three replicates. Representative histograms are shown. Compare figure to Figure 2E to see effects of carbon source shift vs. promoter shutoff of eEF1A.

A



B

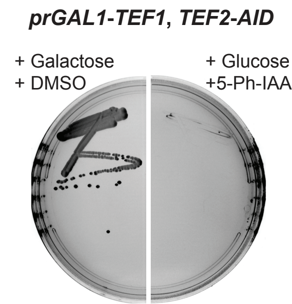


C

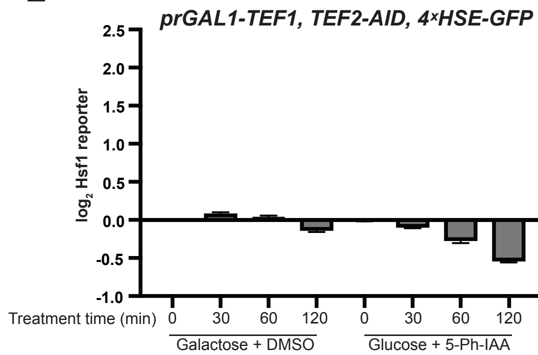
Sample	P/M Rep 1	P/M Rep 2
ZPR1; *TEF1*; *TEF2* (E3 only), YPG	3.77	3.87
ZPR1; *TEF1*; *TEF2* (E3 only), 8hr YPD + 5-Ph-IAA	3.49	3.55
prGAL1-ZPR1-AID, YPG	3.76	3.59
prGAL1-ZPR1-AID, 4hr YPD + 5-Ph-IAA	2.94	2.69
prGAL1-ZPR1-AID, 8hr YPD + 5-Ph-IAA	1.52	1.14
prGAL1-TEF1; TEF2-AID, YPG	3.51	3.20
prGAL1-TEF1; TEF2-AID, 4hr YPD + 5-Ph-IAA	1.91	1.76
prGAL1-TEF1; TEF2-AID, 8hr YPD + 5-Ph-IAA	1.38	1.52

D

prGAL1-TEF1, TEF2-AID



E



F

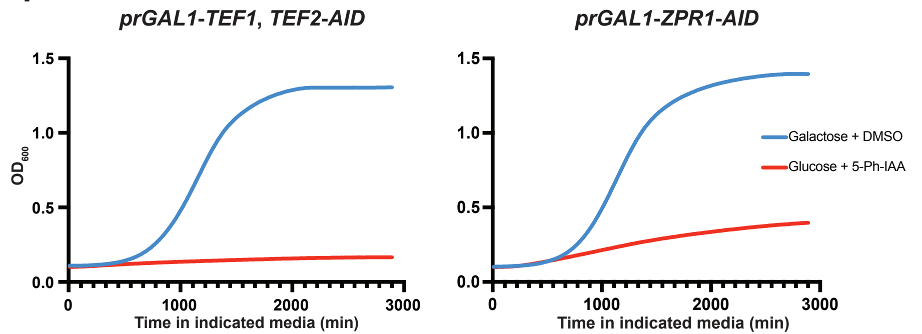
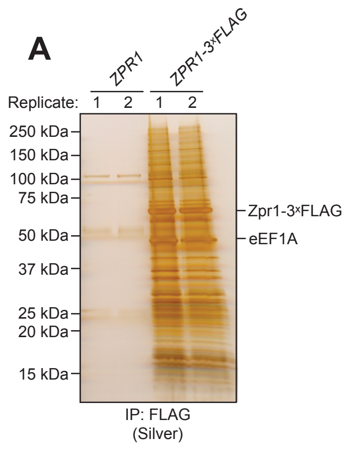
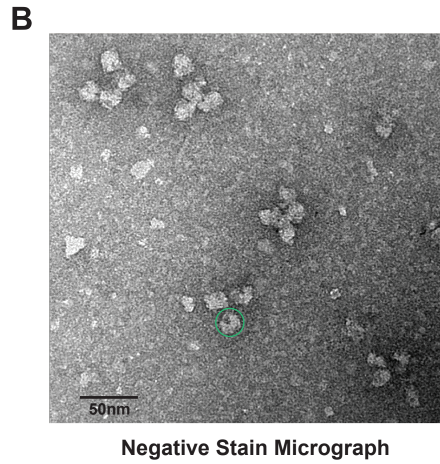
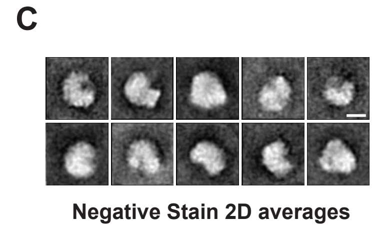
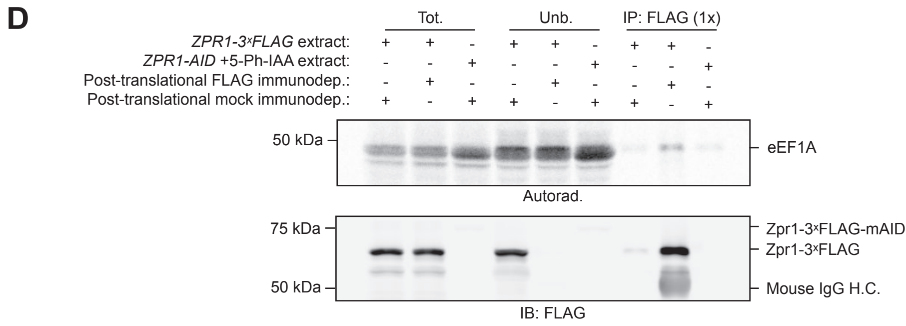


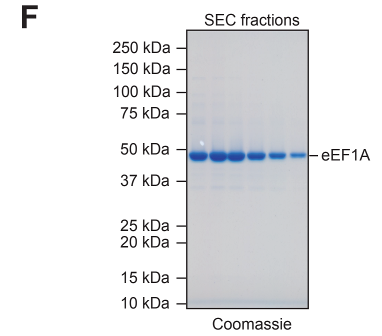
Figure S2. Additional evidence of integrated stress response activation in Zpr1-depleted and Tef1/2-depleted cells. Related to Figure 3. (A) *ZPR1-AID* cells grown to mid-log phase in liquid SD media were collected three hours after treatment with 5 μM 5-Ph-IAA or DMSO and subjected to isolation of total RNA, polyA enrichment, and NGS sequencing in triplicate. DESeq2 was used to determine genes differentially expressed in 5-Ph-IAA-treated cells relative to DMSO-treated cells. (B) Indicated strains were grown to mid-log phase in liquid media before adding glucose and 5 μM 5-Ph-IAA for the indicated treatment times. Shown are immunoblots of the corresponding whole cell extracts probed with the indicated antibodies. Band intensities were quantified using ImageJ for the scatter plot shown in Figure 3B. Dashed line indicates cropping from the same gel. (C) Table showing the polysome:monosome ratio of polysome profiles from two replicates of the experiment shown in Figure 3C. (D) Streak assay showing sensitivity of *prGAL1-TEF1, TEF2-AID* cells to glucose + 5 μM 5-Ph-IAA. Indicated plates were incubated at 30°C for two days prior to imaging. (E) *prGAL1-TEF1, TEF2-AID* cells carrying the Hsf1 reporter were grown to mid-log phase in liquid SD media with 5 μM 5-Ph-IAA or liquid SGal media with mock treatment. Cells were analyzed by flow cytometry after the indicated treatment times. Shown are bar graphs of the median values of GFP intensity normalized to cell size and to the average median GFP intensity of *prGAL1-TEF1, TEF2-AID* Galactose + DMSO cells at 0 min with standard deviation from three replicates. (F) Growth curves of 96-well plate cultured *prGAL1-TEF1, TEF2-AID* (left panel) or *prGAL1-ZPR1-AID* (right panel) cells. Cultures were inoculated at 0.01 OD600 and grown at 30˚C for two days in liquid SD media with 5 μM 5-Ph-IAA or liquid SGal media with mock treatment.

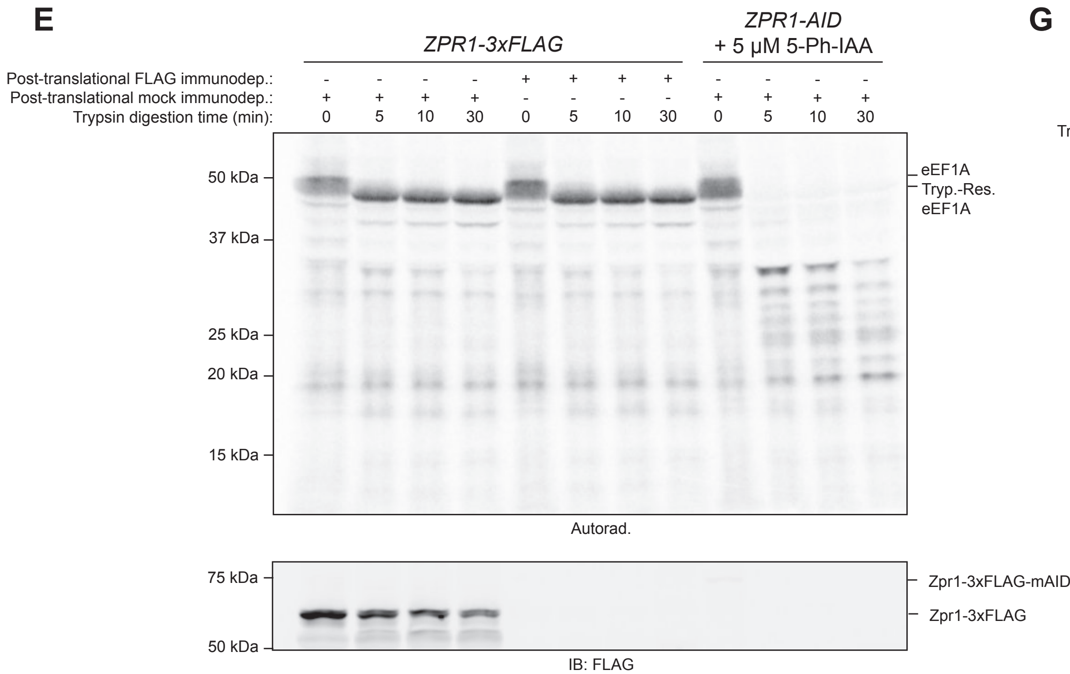

A
ZPR1
ZPR1-3xFLAG
Replicate: 1 2 1 2
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
Zpr1-3xFLAG
eEF1A
IP: FLAG
(Silver)


B
50nm
Negative Stain Micrograph


C
Negative Stain 2D averages


D
Tot.
Unb.
IP: FLAG (1x)
ZPR1-3xFLAG extract:
ZPR1-AID +5-Ph-IAA extract:
Post-translational FLAG immunodep.:
Post-translational mock immunodep.:
50 kDa
eEF1A
Autorad.
75 kDa
50 kDa
Zpr1-3xFLAG-mAID
Zpr1-3xFLAG
Mouse IgG H.C.
IB: FLAG


F
SEC fractions
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
eEF1A
Coomassie


E
ZPR1-3xFLAG
ZPR1-AID
+ 5 μM 5-Ph-IAA
Post-translational FLAG immunodep.:
Post-translational mock immunodep.:
Trypsin digestion time (min):
0 5 10 30 0 5 10 30 0 5 10 30
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
eEF1A
Tryp.-Res.
eEF1A
Autorad.
75 kDa
50 kDa
Zpr1-3xFLAG-mAID
Zpr1-3xFLAG
IB: FLAG

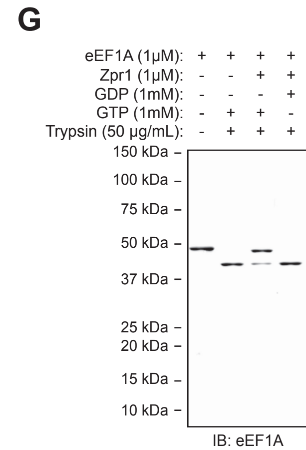
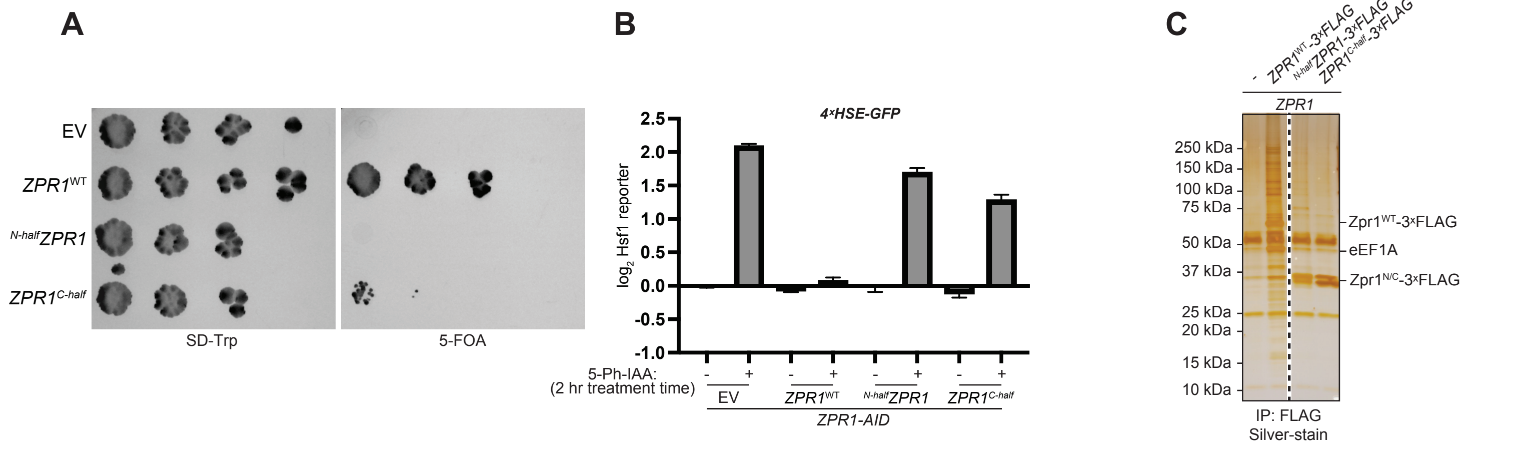
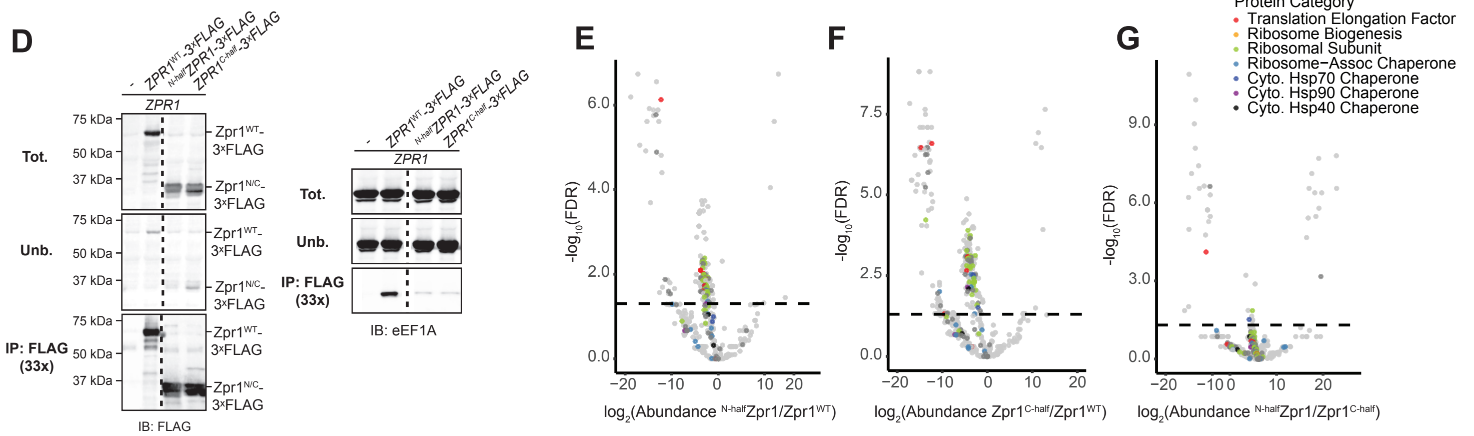
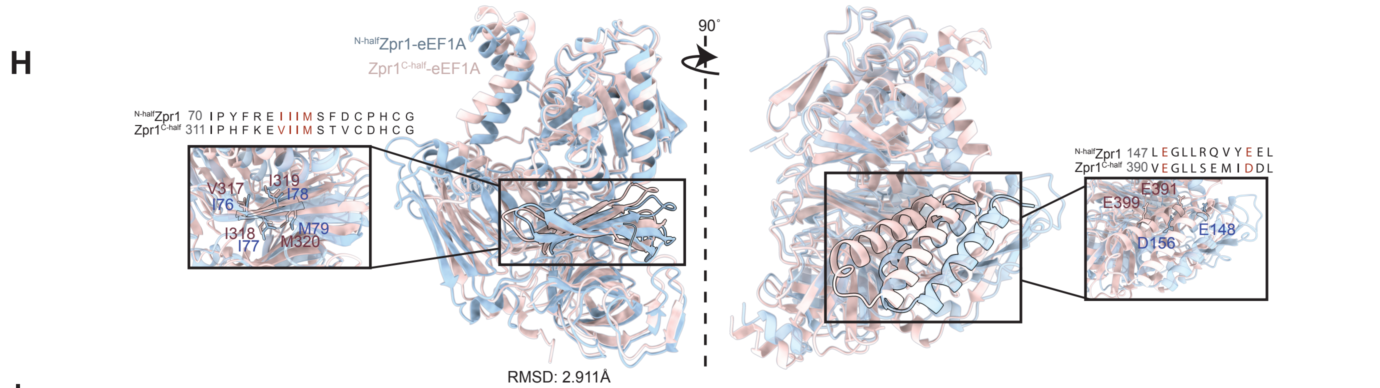

G
eEF1A (1μM):
Zpr1 (1μM):
GDP (1mM):
GTP (1mM):
Trypsin (50 μg/mL):
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
IB: eEF1A

Figure S3. Additional characterization of ribosomes associated with Zpr1 in the cell, Zpr1-mediated folding of eEF1A in yeast cell extracts, and eEF1A purity. Related to Figure 4. (A) The indicated strains were grown to mid-log phase in liquid YPD media and subjected to cryogenic lysis followed by immunoprecipitation (IP) with a FLAG-specific antibody. Shown is an image of a silver-stained SDS-PAGE gel containing two out of the four technical replicates that were analyzed by mass spectrometry as described in Figure 4C. (B) Zpr1-3xFLAG IP eluate from part A was analyzed by negative stain electron microscopy. Shown is a micrograph with an individual particle indicated (green circle). Scale bar: 50 nm. (C) Representative negative stain 2D averages of large particles from micrographs from part B. Scale bar: 10 nm. (D) Immunodepletion of Zpr1-3xFLAG from yeast translation extracts. *In vitro* transcribed eEF1A mRNA was translated in the presence of ^{35}S-Methionine in extracts derived from *ZPR1-3xFLAG* cells or *ZPR1-AID* cells treated with 5 μM 5-Ph-IAA for 1 hour. Translation was arrested using 100 μg/mL cycloheximide and extracts were depleted of Zpr1-3xFLAG via IP with anti-FLAG bound beads or mock depleted with empty beads. A total (Tot.) fraction of each translation was collected before IP. Equal amounts of Tot., unbound (Unb.), and immunoprecipitated (IP) fractions were resolved by SDS-PAGE followed by autoradiography. (E) Immuno-depleting Zpr1-3xFLAG after eEF1A translation does not render eEF1A sensitive to trypsin. The unbound ("Unb.") samples from panel B were treated with 50 μg/mL trypsin and aliquots were removed and quenched via SDS/Urea buffer at the indicated times and analyzed via SDS-PAGE, autoradiography, and immunoblotting. This experiment was repeated twice with similar results. (F) Shown is a coomassie-stained SDS-PAGE gel of SEC fractions from the native eEF1A purification as described in STAR Methods. Gel was cropped to remove irrelevant lanes. (G) Native eEF1A trypsin digest assay with WT Zpr1-3xFLAG protein and GDP as described in STAR Methods. Samples were analyzed by SDS-PAGE and immunoblotting.


A
EV
ZPR1WT
N-halfZPR1
ZPR1C-half
SD-Trp
5-FOA
B
4xHSE-GFP
log2 Hsf1 reporter
5-Ph-IAA:
(2 hr treatment time)
EV
ZPR1-AID
C
ZPR1
IP: FLAG
Silver-stain
Zpr1WT-3xFLAG
eEF1A
Zpr1N/C-3xFLAG


D
ZPR1
Tot.
Unb.
IP: FLAG (33x)
IB: FLAG
IB: eEF1A
E
F
G
Protein Category
Translation Elongation Factor
Ribosome Biogenesis
Ribosomal Subunit
Ribosome−Assoc Chaperone
Cyto. Hsp70 Chaperone
Cyto. Hsp90 Chaperone
Cyto. Hsp40 Chaperone
-log10(FDR)
log2(Abundance N-halfZpr1/Zpr1WT)
log2(Abundance Zpr1C-half/Zpr1WT)
log2(Abundance N-halfZpr1/Zpr1C-half)


H
N-halfZpr1-eEF1A
Zpr1C-half-eEF1A
N-halfZpr1 70 I P Y F R E I I I M S F D C P H C G
Zpr1C-half 311 I P H F K E V I I M S T V C D H C G
V317
I319
I76
I78
I318
M79
I77
M320
RMSD: 2.911Å
90°
N-halfZpr1 147 L E G L L R Q V Y E E L
Zpr1C-half 390 V E G L L S E M I D D L
E391
E399
E148
D156

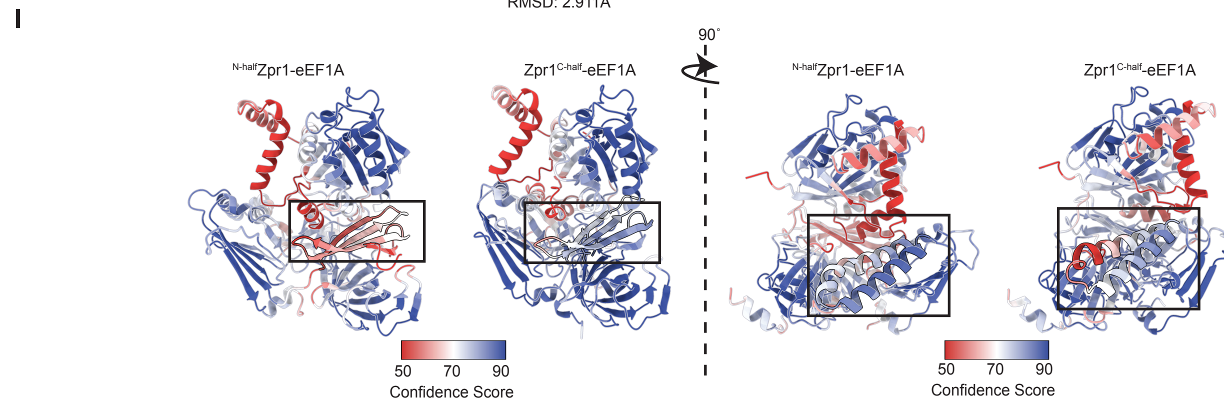

I
N-halfZpr1-eEF1A
Zpr1C-half-eEF1A
90°
50
70
90
Confidence Score

Figure S4: Phenotypic, interactor, and ColabFold analysis of individual Zpr1 halves to eEF1A biogenesis. Related to Figure 5. (A) *zpr1*Δ cells carrying a low-copy, URA3-based *ZPR1* expression plasmid were transformed with the indicated high-copy TRP1-based vectors. EV = empty vector. Following growth on plates with solid SD-Trp media, cells were spotted onto SD-Trp and SD + 5-FOA plates and incubated at 30 °C for two days prior to imaging. (B) *ZPR1-AID* cells carrying the Hsf1 reporter were transformed with high-copy vectors described in part A. Indicated transformants were grown to mid-log phase in liquid SD media and analyzed by flow cytometry two hours post-treatment with 5 μM of 5-Ph-IAA or mock treatment. Shown are bar graphs of the median values of GFP intensity normalized to cell size and to the average median GFP intensity of EV DMSO cells with standard deviation from three replicates. (C) The indicated strains with each Zpr1-3xFLAG construct (at an ectopic locus) were grown to mid-log phase in liquid YPD media and subjected to cryogenic lysis followed by immunoprecipitation with a FLAG-specific antibody. Shown is an image of a silver-stained SDS-PAGE gel of the pulldowns. (D) As in part C but analyzed via SDS-PAGE and immunoblotting with the indicated antibodies. Total (Tot.), unbound (Unb.), and IP were run on the same gel. Left and right panels are probed from the same membrane. (E-G) Mass spectrometry analysis of samples described in part A. The dashed line indicates the false discovery rate (FDR) at the adjusted p-value of 0.05. (H) Ribbon diagram of ColabFold-Fold Multimer model showing *S. cerevisiae* Zpr1$^{C\text{-}half}$ bound to eEF1A (tan) aligned to *S. cerevisiae* $^{N\text{-}half}$Zpr1 bound to eEF1A (blue) on eEF1A (RMSD: 2.911Å) in two orientations (rotated ~90˚). The left inset shows four hydrophobic residues of ZnF^{C} in stick representation (V317, I318, I319, M320) and the corresponding four hydrophobic residues of ZnF^{N} in stick representation (I76, I77, I78, M79). A sequence alignment of $^{N\text{-}half}$Zpr1 and Zpr1$^{C\text{-}half}$ highlighting the conserved region that contains the Zn finger is shown above the inset. The right inset shows the two negatively charged residues of aHH^{C} in stick representation (E391, E399) and the corresponding two negatively charged residues of aHH^{N} in stick representation (E148, D156). A sequence alignment of $^{N\text{-}half}$Zpr1 and Zpr1$^{C\text{-}half}$ highlighting the conserved region that contains the aHH is shown above the inset. (I) Ribbon diagrams of ColabFold-Fold Multimer model showing *S. cerevisiae* $^{N\text{-}half}$Zpr1 bound to eEF1A (left) *S. cerevisiae* Zpr1$^{C\text{-}half}$ bound to eEF1A (right) in two orientations (rotated ~90˚). Models are colored according to predicted confidence scores per position out of 100 with the color scale indicated.

A

Zpr1

aHH^{N}

eEF1A

aHH^{C}

Domain I

ZnF^{N}

Domain II

ZnF^{C}

Domain III

Zpr1 ZnF^{N} A ZnF^{C} B

B

isolate

EV 1 2

$ZPR1^{WT}$ 1 2

$ZPR1^{Zn\text{-}C}$ 1 2

$ZPR1^{Zn\text{-}NC}$ 1 2

SD -Trp

SD + 5-FOA

$ZPR1^{Zn\text{-}N}$ isolate 1 isolate 2

$ZPR1^{aH\text{-}C}$ isolate 1 isolate 2

$ZPR1^{aH\text{-}N}$ isolate 1 isolate 2

$ZPR1^{aH\text{-}NC}$ isolate 1 isolate 2

$ZPR1^{WT}$ isolate 1 isolate 2

EV isolate 1 isolate 2

C

isolate

EV 1 2

$ZPR1^{WT}$ 1 2

$ZPR1^{aH\text{-}N}$ 1 2

$ZPR1^{aH\text{-}C}$ 1 2

$ZPR1^{aH\text{-}NC}$ 1 2

$ZPR1^{Zn\text{-}N}$ 1 2

IB: FLAG

IB: eEF1A

D

isolate

$ZPR1^{WT}$ 1 2

$ZPR1^{Zn\text{-}C}$ 1 2

$ZPR1^{Zn\text{-}NC}$ 1 2

Zpr1-3xFLAG

IB: FLAG

eEF1A

IB: eEF1A

E

DMSO 5-Ph-IAA

Count/Max Count

$ZPR1^{aH\text{-}NC}$

$ZPR1^{aH\text{-}C}$

$ZPR1^{Zn\text{-}NC}$

$ZPR1^{Zn\text{-}C}$

$ZPR1^{Zn\text{-}N}$

$ZPR1^{WT}$

EV

2^{-8} 2^{-6} 2^{-4} 2^{-2}

Hsf1 reporter

F

1 2 3 4 5 6

250 kDa

150 kDa

100 kDa

75 kDa

50 kDa

37 kDa

25 kDa

20 kDa

15 kDa

10 kDa

Coomassie

1. 10xHis-3c-Zpr1-3xFLAG
2. 10xHis-3c-Zpr1$^{Zn\text{-}N}$-3xFLAG
3. 10xHis-3c-Zpr1$^{Zn\text{-}C}$-3xFLAG
4. 10xHis-3c-Zpr1$^{Zn\text{-}NC}$-3xFLAG
5. 10xHis-3c-Zpr1$^{aH\text{-}C}$-3xFLAG
6. 10xHis-3c-Zpr1$^{aH\text{-}NC}$-3xFLAG

G

CLUSTAL O (1.2.4) multiple sequence alignment

```
S. cerevisiae N           MSEQKEDLFKPVGEAAAEVEDESIAEQNKANDGVKLTGAQDAMG-HPVQ-EIESLCMNCG   58
S. cerevisiae C           -----------VGSAN--------------PQFLSDATDIENFN-NEVQ-TFRASCPSCT   299
Human N                   MAASGAVEPGPPGAAV---APSPAPAPPPAPDHLFRPISAEDEE-QQPT-EIESLCMNCY   55
Human C                   ---------------------------------PAEKPEEEDLR-NEVL-QFSTNCPECN   263
P. horikoshii (archaea)   MIYKACIRKRCGGVC-----------------------VGEELKPEKIQEIRLGDCPICG   37
A. pernix (archaea)       ----------MGGEAS-------------GGDILDRLLAESRKAPLRIAEVDNAVCPACG   37
S. islandicus (archaea)   ---------------------------------------MSEGP-KVIFEETLVCPVCK   19

                                      **** ZnF
S. cerevisiae N           KNGTTRL--LLTSIPYFREIIIMSFDCPHCGFKNCEIQPASQIQEKGSRYVLKVECREDF   116
S. cerevisiae C           QECETHM--KPVNIPHFKEVIIMSTVCDHCGYKSNEVKTGGAIPDKGRRITLYCDDAADL   357
Human N                   CNGMTRL--LLTKIPFFREIIVSSFSCEHCGWNNTEIQSAGRIQDQGVRYTLSVRALEDM   113
Human C                   APAQTNM--KLVQIPHFKEVIIMATNCENCGHRTNEVKSGGAVEPLGTRITLHITDASDM   321
P. horikoshii (archaea)   GKGTLKAIQFIHRIPYFGEVMESTVVCERCGYRNSDVIILEEREPR--LYEVKVEEEKDL   95
A. pernix (archaea)       AR-AMKLSEYLYSVPYFNNIVISVGECRNCGYRYRDVRLAEATEPK--KIVVAVRGEKQL   94
S. islandicus (archaea)   SK-TFKAIDYLYDTPHAGKLILSNWYCENCGYRFRDVKPYEAREPK--VIEMRIENENDL   76

                                                   *  aHH  *
S. cerevisiae N           NRQVIKSETATCKFVELDIEIPA---KRGQLTTVEGLLSEMIDDLSQDQE--MRKSIDEA   171
S. cerevisiae C           SRDILKSETCSMVIPELHLDIQEG-TLGGRFTTLEGLLRQVYEELESRIFTQTSDSMDEA   416
Human N                   NREVVKTDSAATRIPELDFEIPAF-SQKGALTTVEGLITRAISGLEQDQP--ARRANKDA   170
Human C                   TRDLLKSETCSVEIPELEFELGMA-VLGGKFTTLEGLLKDIRELVTKNPFTLG-DSSNPG   379
P. horikoshii (archaea)   FIRVVRSKSGTIELEELGIKIEPGPAAEGFVSNIEGVLERAKEVLLMARD-FKEQENDRE   154
A. pernix (archaea)       RYLLAKSPLSAVYIPEMDLEMVPGVASVGFITAVEGILHRFHEVAQVAC---KEGVVEAE   151
S. islandicus (archaea)   RTIIYRSAFAKITIPELGIEIEPAGMSQGYISTIEGVLEIILDK---VGD-FCDKECENR   132

S. cerevisiae N           LYKKIDDFIQKVKSYINCEPNTIPITFILDDPAGNSWIEYKPGEPQ-HKWSHTQYVRTDE   230
S. cerevisiae C           TKARWVEFFAKLKEAI---AGKVKFTVIMEDPLAGSYIQNVYAPDPDPNMTIEDYERTKE   473
Human N                   TAERIDEFIVKLKELK---QVASPFTLIIDDPSGNSFVENPHAPQKDDALVITHYNRTRQ   227
Human C                   QTERLQEFSQKMDQII---EGNMKAHFIMDDPAGNSYLQNVYAPEDDPEMKVERYKRTFD   436
P. horikoshii (archaea)   AVRRIDELLKYIEEVK---EGKKPLTVRIMDPFGNSALIGEKVKSRKLT--K----EEIR   205
A. pernix (archaea)       DLEKCRSVIRWLEEAI---EGRRAFTLIICDFDGLSRVIGEHPYVVEEA--I----DERC   202
S. islandicus (archaea)   I-----------RLAM---EGKIKFTLVLEDELGLSFIKSEKATVK--------------   164

S. cerevisiae N           QNVQVGIITRDQLEQRRQEQLKQLANRERNPSESVK   266
S. cerevisiae C           QNEDLGLSDIKVE-----------------------   486
Human N                   QEEMLGLQEEA-------------------------   238
Human C                   QNEELGLNDMKTEGYEAGL-----APQ-R-------   459
P. horikoshii (archaea)   KLSKGPYIVIEPDEVPQQ------------------   223
A. pernix (archaea)       QALKPGFME---------------------------   211
S. islandicus (archaea)   -----PLILTNSD-----------------------   172
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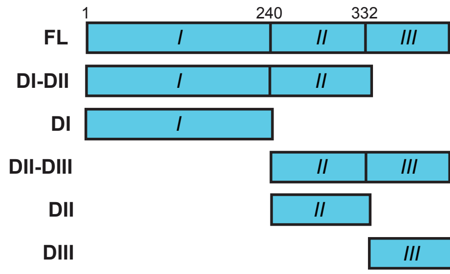
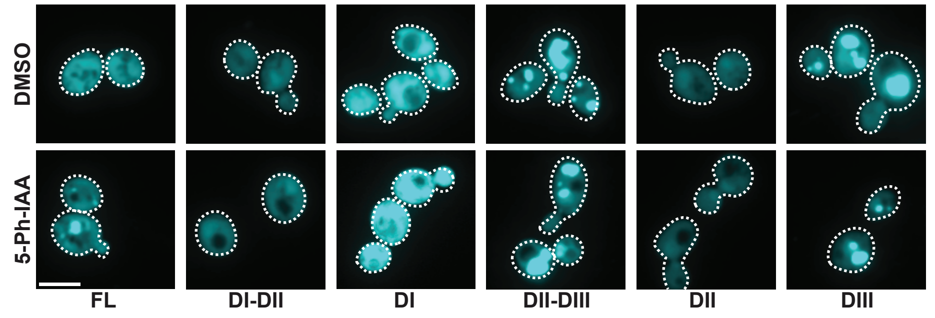
Figure S5. Supporting analysis of Zpr1 ZnF and aHH mutants. Related to Figures 4 and 5. (A) ColabFold-modeled Zpr1-eEF1A complex and cartoon schematic of Zpr1 domain architecture. (B) *zpr1* deletion cells containing a low-copy, URA3-based *ZPR1* expression plasmid were transformed with the indicated plasmid-borne Zpr1 mutants or empty (EV) (TRP1-based vectors) after selection on solid SD-Trp media, were spotted (left-panel) onto the indicated plates and incubated for at 30 °C for 2 days prior to imaging. The right-panel was treated similarly but streaked onto SD + 5-FOA plates. Images were cropped to remove irrelevant data. (C) Strains from part C were grown to mid-log phase in liquid SD-Trp media. Western immunoblots of the corresponding whole cell extracts were then probed with the indicated antibodies. Dashed lines indicate cropping from the same gel. (D) As in C but for ZnF mutants $Zpr1^{Zn-C}$ and $Zpr1^{Zn-NC}$. (E) Representative histograms of DMSO or 5-Ph-IAA treated cells of one replicate from Figure 5C. (F) Shown is a coomassie-stained SDS-PAGE gel of the recombinant Zpr1 proteins used in this study (after gel filtration). Proteins were purified as described in STAR Methods. Gel was cropped to remove irrelevant lanes. (G) CLUSTAL multiple sequence alignment of *S. cerevisiae* $^{N\text{-}half}$Zpr1, *S. cerevisiae* $Zpr1^{C\text{-}half}$, Human $^{N\text{-}half}$Zpr1, Human $Zpr1^{C\text{-}half}$, archaeal *P. horikoshii* Zpr1, archaeal *A. pernix* Zpr1, and archaeal *S. islandicus* Zpr1. The conserved region from part A is indicated with a black box. Residues are colored as follows: red (hydrophobic), blue (acidic), magenta (basic), green (hydroxyl, sulfhydryl, amine), gray (unusual). "*" indicates fully conserved, ":" indicates strongly similar, "." indicates weakly similar.

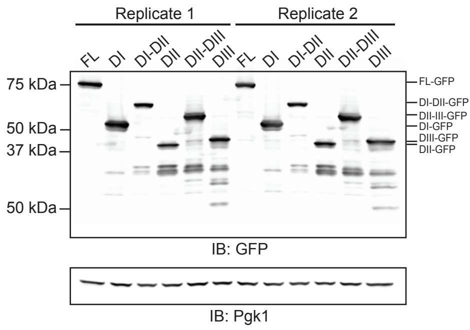
A



B



C



D

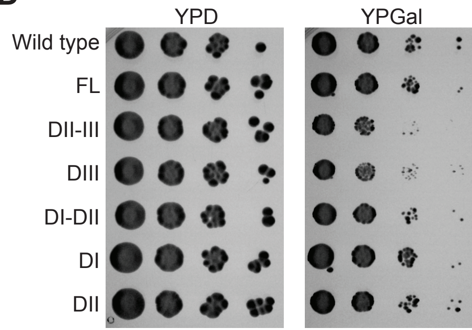


Figure S6. Characterization of eEF1A domain truncation constructs expressed in yeast. Related to Figure 5. (A) Cartoon schematic of full-length (FL) eEF1A and domain (D) truncation constructs. GFP is fused to the C-terminus of all constructs. (B) *ZPR1-AID* cells carrying *GAL1* promoter-driven GFP-tagged versions of eEF1A constructs (at an ectopic locus) were grown in synthetic media containing raffinose prior to addition of galactose and either 5 μM 5-Ph-IAA or DMSO for three hours. Confocal micrographs are normalized to the same intensity and cells are outlined. Scale bar: 2.5 μm. (C) *ZPR1-AID* cells carrying *GAL1* promoter-driven GFP-tagged versions of eEF1A constructs (at an ectopic locus) were grown to mid-log phase in liquid YPGal media for six hours. Western immunoblots of the corresponding whole cell extracts were then probed with antibodies specific to GFP and Pgk1 as the loading control. Two replicates of each are shown. (D) Spot assay showing fitness defect of DIII and DII-DIII cells grown on YPGal. Cells were serially diluted (1:10), spotted on the indicated plates, and incubated at 30 °C for two days prior to imaging.