Revised, Research Article, *Science*
December 4, 2016

# Supplementary Materials for

## An N-end rule pathway that recognizes proline and destroys gluconeogenic enzymes

Shun-Jia Chen[1], Xia Wu[1], Brandon Wadas[2], Jang-Hyun Oh[1], Alexander Varshavsky[1]*

[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

[2]Division of Cell Biology, Harvard Medical School, Boston, MA 02115

*Corresponding coauthor: Alexander Varshavsky (avarsh@caltech.edu)

**This PDF file includes:**

Materials and Methods.

Supplementary figures S1-S17 and their legends.

Tables S1-S3.

## Materials and Methods

### Antibodies and other reagents

"Complete Protease Inhibitor Cocktail" tablets and tetracycline (Tc) hydrochloride were from Roche and Sigma, respectively. 5'-Fluoroorotic acid monohydrate (5-FOA) was from Zymo Research. Antibodies to the following antigens were used for immunoblotting and/or immunoprecipitation: anti-flag M2 monoclonal antibody (Sigma, F1804), anti-c-Myc-9E10 monoclonal antibody (Sigma, M5546), anti-hemagglutinin (ha) tag monoclonal antibody (Sigma, H6908). Secondary antibodies for immunoblotting were Li-Cor IRDye-conjugated goat anti-mouse 800CW (Li-Cor, #C60405-05) or anti-rabbit 680RD (Li-Cor, C51104-08). Fluorescence patterns were detected and quantified using the Odyssey 9120 instrument (Li-Cor, Lincoln, NE).

### Yeast strains, media, and genetic techniques

The *S. cerevisiae* strains used in this study are cited in Table S1. Standard techniques (*1, 2*) were employed for strain construction and transformation. *S. cerevisiae* media included YPD medium (1% yeast extract, 2% peptone, 2% glucose; only most relevant components are cited); SD medium (0.17%

yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose); SE medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% ethanol); and synthetic complete (SC) medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose), plus a mixture of compounds required by a given auxotrophic strain. The alternative carbon sources, in either liquid or plate media, were 2% ethanol or 2% glucose. The *S. cerevisiae* strain AH109 was used for two-hybrid assays; the JD52 strain was used for split-ubiquitin (Ub) assays; and several BY4741-based strains (see Table S1) were used for tetracycline (Tc)-based chase-degradation assays.

**Construction of plasmids**

The *Escherichia coli* strains DH5α, SUREII (Stratagene), and STBL2 (Invitrogen) (Table S1) were used for cloning and maintaining plasmids. Phusion High-Fidelity DNA polymerase (New England Biolabs) was employed for PCR. The plasmids and PCR primers used in this study are described in Tables S2 and S3, respectively.

pJO629, a parental plasmid used to construct plasmids for the promoter reference technique (PRT), was a pRS313-based, low copy (*CEN*-based) plasmid that expressed a test protein and the $_{f}$DHFR$_{ha}$ reference protein from two identical (modified) $P_{TDH3}$ promoters (see the main Fig. 2A, B and the main text) (Table S2). Details of pJO629 construction are available upon request.

We describe here the construction of plasmids other than those used for 2-hybrid assays and split-ubiquitin (Ub) assays. Construction of the latter plasmids is described in sections below. To produce the low copy pCSJ95 and pCSJ98 plasmids, which expressed, respectively, **(M)P**-Fbp1$_{3f}$ and **(M)S**-Fbp1$_{3f}$ in *S. cerevisiae* from the $P_{TDH3}$-based promoter, the relevant *Fbp1* DNA fragments were amplified by PCR from *S. cerevisiae* genomic DNA using the primer pairs CSJ117/CSJ121 and CSJ120/CSJ121, respectively (Table S3). The resulting PCR products were digested with *Asc*I/*Bgl*II and ligated into *Asc*I/*Bam*HI-cut pJO629 (see above) downstream of its $P_{TDH3}$-based promoter, yielding pCSJ95 and pCSJ98 (Table S2).

To construct pCSJ125 and pCSJ126, which expressed **(M)P**-Mdh2$_{3f}$ and **(M)S**-Mdh2$_{3f}$ in *S. cerevisiae* from the $P_{TDH3}$-based promoter, the relevant *Mdh2* DNA fragments were amplified from *S. cerevisiae* genomic DNA using the primer pairs CSJ170/CSJ171 and CSJ172/CSJ171, respectively (Table S3). The resulting PCR products were digested with *Asc*I/*Bam*HI and ligated into *Asc*I/*Bam*HI-cut pJO629, yielding pCSJ125 and pCSJ126 (Table S2).

A two-step procedure was used to construct pCSJ121 and pCSJ122, which expressed **(M)SP**-Pck1$_{3f}$ and **(M)SS**-Pck1$_{3f}$ in *S. cerevisiae* from the $P_{TDH3}$-based promoter. First, a DNA fragment spanning a part of the *PCK1* open reading frame (ORF), from +382 to +1,647 bp, was amplified from *S. cerevisiae* genomic DNA using the primer pair CSJ162/CSJ163 (Table S3). The amplified fragment was digested with *Bam*HI/*Bgl*II and ligated into *Bam*HI-cut pJO629, yielding pCSJ120. Second, the relevant *PCK1* DNA fragments spanning the upstream region of the *PCK1* ORF (+1 to +381 bp) was amplified from *S. cerevisiae* genomic DNA using the primer pairs CSJ164/CSJ165 and CSJ166/CSJ165, respectively (Table S3). The resulting DNA fragments were digested with *Asc*I/*Bam*HI and ligated into *Asc*I/*Bam*HI-cut pCSJ120, yielding pCSJ121 and pCSJ122 (Table S2).

To construct pCSJ168, which expressed **(M)P**-Yhr020w$_{3f}$ in *S. cerevisiae* from the $P_{TDH3}$-based promoter, the *Yhr020w*$_{3f}$ DNA fragment was produced by a two-step PCR from *S. cerevisiae* genomic DNA, at first using the primers CSJ237 and CSJ238, and thereafter using the primers CSJ237 and CSJ182 (Table S3). The resulting PCR product was digested with *Asc*I/*Not*I and ligated into *Asc*I/*Not*I-

cut pJO629, yielding pCSJ168 (Table S2). Other plasmids, encoding many analogous constructs (Table S2), were constructed similarly to the plasmids described above. Additional details of plasmid construction are available upon request. Construction of plasmids for 2-hybrid assays and split-Ub assays is described below. All final constructs were verified by DNA sequencing.

**Tetracycline (Tc)-chase assays and immunoblotting**

Most protein degradation assays of this study employed a version of the promoter reference technique (PRT) described in the main Fig. 2A, B and in the main text. These PRT-based assays were carried out similarly to the previously described cycloheximide (CHX)-chase assays with *S. cerevisiae* (*3, 4*), but used low copy pJO629-based plasmids expressing a C-terminally $flag_3$-tagged test protein and the long-lived, also tagged ($_{f}DHFR_{ha}$) reference protein from a pair of identical (modified) $P_{TDH3}$ promoters, in a setting in which the synthesis of both proteins could be selectively extinguished by the addition of tetracycline (Tc) (see the main Fig. 2A, B and the main text). In sum, Tc was used to inhibit, in *cis*, the translation of two mRNAs, the one encoding a test protein and the one encoding the $_{f}DHFR_{ha}$ reference protein (the main Fig. 2A, B).

*S. cerevisiae* was grown to $A_{600}$ of 0.8-1.0 at 30°C in SC media whose exact composition was appropriate for a plasmid(s) carried by the yeast strain. Cells were centrifuged at 11,200*g* for 1 min, washed once in pre-warmed SE medium, then resuspended in SE to $A_{600}$ of 1.0 and grew in SE for 16 h at 30°C. The resulting cells were harvested by centrifugation at 11,200*g* for 1 min and resuspended in fresh pre-warmed SC to a final concentration of $A_{600}$ of 1.0, followed by the addition of Tc to the final concentration of 0.2 mM. Control cultures (ethanol-to-ethanol, as distinguished from ethanol-to-glucose) were processed identically, except that cells were resuspended in SE medium, instead of SC. At indicated times of a chase, a sample of cell suspension (adjusted to correspond to 1 ml of suspension with $A_{600}$ of 1.0) was centrifuged for 1 min at 11,200*g*. The pellet was resuspended in 0.8 ml of 0.2 M NaOH and incubated for 20 min on ice, followed by centrifugation at 11,200*g* for 1 min. The pellet was resuspended in 50 μl of HU buffer (8 M urea, 5% SDS, 1 mM EDTA, 0.1 M dithiothreitol (DTT), 0.005% bromophenol blue, 0.2 M Tris-HCl, pH 6.8) containing 1x-protease inhibitor cocktail (Roche), and heated for 10 min at 70°C. After centrifugation for 5 min at 11,200*g*, 15 μl of supernatant was subjected to SDS-4-10% PAGE, followed by immunoblotting as described previously (*4, 5*), using anti-ha (1:2,000) and anti-flag (1:2,000) antibodies as well as a secondary antibody (or antibodies) and quantification of resulting (green and/or red) fluorescence patterns using the Odyssey 9120 instrument (Li-Cor), its software, and manufacturer's manual. In some experiments, Tc-based, PRT-based chases were carried with cells that were grown solely in SC (glucose-containing) media, without an exposure to SE media. All Tc-chases in this study were performed at least twice, and yielded results that differed by less than 10%.

**Two-hybrid binding assays**

Two initial plasmids for 2-hybrid assays were pGADCg and pGBKCg (Table S2) (*6*) (Addgene plasmids # 20161 and # 20162). The pCSJ165 plasmid (Table S2), derived from pGBKCg, expressed **(M)P**-Fbp1-$Gal4^{DBD}$ fusion, i.e., the full-length, wild-type *S. cerevisiae* **(M)P**-Fbp1 that was fused, at its C-terminus, to $Gal4^{DBD}$, a 2-hybrid-specific DNA-binding protein domain (encoded by pGBKCg). pCSJ165 was constructed using the Gateway cloning technique (Invitrogen). In the first step, the BP recombination reaction was carried out, using Gateway BP clonase II enzyme mix (Invitrogen), the pDonor/Zeo vector (Invitrogen) and an *att*B-containing DNA fragment, produced by PCR from *S. cerevisiae* genomic DNA and the primer pair CSJ227/CSJ228 (Table S3). This step yielded

pCSJ161, which contained the attL-**(M)P**-Fbp1 DNA segment. In the second step, the LR reaction was carried out with the Gateway LR clonase II enzyme mix (Invitrogen), the pCSJ161 plasmid and the pGBKCg vector, yielding the pCSJ165 plasmid (Table S2).

Similar cloning steps were used to construct other, analogous plasmids, which encoded 2-hybrid-based fusions of test proteins such as **(M)X**-Fbp1, **(M)X**-Icl1, **(M)X**-Mdh2 or **(M)X**-Pck1, and other test proteins as well, whose intended (wild-type or modified) N-termini were unobstructed by either DBD or AD domains (Table S2). These polypeptides included those containing C-terminally truncated test proteins, which were fused, C-terminally, to the mouse DHFR moiety and the C-terminal (2-hybrid-specific) DBD domain (Table S2).

The pCSJ182 plasmid, derived from pGADCg (*6*) (Table S2), expressed NLS-Gid4-flag$_3$-Gal4$^{AD}$ fusion, which contained the full-length wild-type *S. cerevisiae* Gid4 protein bearing the C-terminal flag$_3$ tag and the 2-hybrid-specific AD domain. The N-terminus of Gid4 was fused to the SV40 nuclear localization signal (NLS), which was encoded by pGADCg (*6*). The pCSJ182 plasmid was also constructed by the two-step Gateway cloning technique. First, a BP reaction was carried out, using the pDonor/Zeo vector and an *att*B-containing DNA fragment encoding, in particular, the flag$_3$ tag. This fragment was produced by a two-step PCR from *S. cerevisiae* genomic DNA, at first with the primers CSJ248/CSJ249, and thereafter with the primers CSJ248 and CSJ245 (Table S3). This step yielded pCSJ177, containing the attL-GID4-flag$_3$ DNA segment. In the second step, the LR reaction was carried out, between pCSJ177 and the pGADCg vector, yielding the pCSJ182 plasmid (Table S2). Similar cloning steps were used to construct many other, analogous plasmids of the present study (Table S2).

To assay for possible interactions of specific GID subunits with the **(M)P**-Fbp1 protein, *S. cerevisiae* AH109 (Table S1) was cotransformed with pCSJ182 (expressing NLS-Gid4 flag$_3$-Gal4$^{AD}$) and either pCSJ165 (expressing **(M)P**-Fbp1-Gal4$^{DBD}$) or pCSJ179 (expressing **(M)S**-Fbp1-Gal4$^{DBD}$). Other tested 2-hybrid fusions encoding specific GID subunits were pCSJ164 (expressing NLS-Gid1-flag$_3$-Gal4$^{AD}$), pCSJ181 (expressing NLS-Gid2-flag$_3$-Gal4$^{AD}$), pCSJ234 (expressing NLS-Gid5-flag$_3$-Gal4$^{AD}$), pCSJ267(expressing NLS-Gid7-flag$_3$-Gal4$^{AD}$), pCSJ268 (expressing NLS-Gid8-flag$_3$-Gal4$^{AD}$), and pCSJ183 (expressing NLS-Gid9-flag$_3$-Gal4$^{AD}$) (Table S2). Analogous 2-hybrid assays, vis-à-vis specific GID subunits, involved other gluconeogenesis enzymes (as 2-hybrid fusions, specifically **(M)P**-Mdh2-Gal4$^{DBD}$ (pCSJ197) vs. **(M)S**-Mdh2-Gal4$^{DBD}$ (pCSJ198), **(M)P**-Icl1-Gal4$^{DBD}$ (pCSJ227) vs. **(M)S**-Icl1-Gal4$^{DBD}$ (pCSJ228), and **(M)SP**-Pck1-Gal4$^{DBD}$ (pCSJ199) vs. **(M)SS**-Pck1-Gal4$^{DBD}$ (pCSJ200) (Table S2).

Other 2-hybrid assays for mapping interactions between Gid4 and **(M)P**-Fbp1 included cotransformations of *S. cerevisiae* AH109 with pCSJ182 (expressing NLS-Gid4 flag$_3$-Gal4$^{AD}$) and plasmids that expressed N-terminal segments (e.g., the first 20 residues) of **(M)P**-Fbp1 fused to DHFR-Gal4$^{DBD}$ (pCSJ327, pCSJ328, and pCSJ348-pCSJ357) (Table S2). Additional 2-hybrid assays examined, in similar ways, other gluconeogenesis and non-gluconeogenesis proteins fused to DHFR-Gal4$^{DBD}$ (e.g., the plasmids pCSJ334, pCSJ335, pCSJ336 and pCSJ337) (Table S2). Positive 2-hybrid controls included plasmids that expressed the human WASP-Gal4$^{DBD}$ fusion (pCSJ167) and its known protein ligand Cdc42- flag$_3$-Gal4$^{AD}$ (pCSJ166) (Table S2), as described in the legends to specific figures.

Cotransformed cells were plated on SC plates lacking Trp and Leu. Single colonies of resulting cotransformants were grown in the otherwise identical liquid medium to a near-stationary phase, until $A_{600}$ of ~2.0. The cultures were thereafter serially diluted by 3-fold, and 20 µl samples of cell

suspensions were spotted onto triple-dropout plates (SC lacking Trp, Leu, and His) (*7*). The plates were incubated at 30°C for 2-3 days. As can be seen in Fig. S5B (the panel on the right, shown as an example), all examined *S. cerevisiae* strains could grow on double-dropout plates (lacking only Trp and Leu), but only some strains could grow on triple-dropout plates (lacking Trp, Leu and His). Expression of His3, the reporter of 2-hybrid assays (*8*), in otherwise $His^-$ cells, was a function of the binding affinity between test proteins.

**Split-ubiquitin (Ub) binding assays**

For the source plasmids and procedures of split-Ub assays see (*9-13*). The pCSJ408 and pCSJ409 plasmids were derived from the Ste14-$C_{Ub}$-**R**-Ura3-Met313 plasmid (Table S2) by replacing the Ste14-encoding DNA segment with PCR-produced, *Cla*I/*Sal*I-cut fragments encoding either **(M)P**-Fbp1 or **(M)S**-Fbp1. In addition, the $P_{MET17}$ promoter of the Ste14-$C_{UB}$-**R**-Ura3-Met313 plasmid (Table S2) was replaced by the $P_{CUP1}$ promoter, derived from pRS313, yielding the pCSJ473 and pCSJ474 plasmids (Table S2). They expressed, respectively, **(M)P**-Fbp1-$C_{Ub}$-**R**-Ura3 and **(M)S**-Fbp1-$C_{Ub}$-**R**-Ura3 from the $P_{CUP1}$ promoter.

The pCSJ418 plasmid was derived from the $N_{Ub}$-Ubc6-Cup314 plasmid (Table S2) by replacing the Ubc6-coding DNA segment with the PCR-produced, *Bam*HI/*Xho*I-cut, DNA fragment encoding Gid4-flag (*10*). A *Xho*I/*Kpn*I-cut DNA fragment containing the *CYC1* transcription terminator was inserted after the fragment encoding Gid4-flag, yielding the plasmid pCSJ418 (Table S2). It expressed $N_{Ub}$-Gid4-flag from the $P_{CUP1}$ promoter.

Split-Ub assays for mapping interactions between Gid4 and **(M)P**-Fbp1 vs. **(M)S**-Fbp1 involved cotransformations of *S. cerevisiae* JD52 (Table S1) with pCSJ418 (expressing $N_{Ub}$-Gid4-flag) and the plasmids pCSJ473 and pCSJ474 (Table S2), which expressed, respectively, **(M)P**-Fbp1 or **(M)S**-Fbp1 linked to the $C_{Ub}$-**R**-Ura3 moiety (see fig. S7 and the main text). Negative controls included otherwise identical split-Ub assays without either $N_{Ub}$-encoding or $C_{Ub}$-encoding plasmids.

Split-Ub assays were carried out by plating cotransformed cells on SC plates lacking Trp and His. Single colonies of resulting cotransformants were grown in the otherwise identical liquid medium to a near-stationary phase ($A_{600}$ of ~2.0). The resulting cultures were thereafter serially diluted by 3-fold, and 20 µl samples of cell suspensions were spotted onto triple-dropout plates (lacking Trp, His, and Ura) and containing 0.1 mM $CuSO_4$. The plates were incubated at 30°C for 1-2 days. All plated strains could grow on double-dropout plates (lacking only Trp and His), but only strains in which the $C_{Ub}$-**R**-Ura3 was <u>not</u> significantly cleaved (thereby releasing the short-lived **R**-Ura3) could grow on triple-dropout plates (lacking Trp, His and Ura), indicating little or no interaction between test protein moieties. The same dilutions of cell suspensions were also spotted onto plates lacking Trp and His but containing Ura as well as 5'-fluoroorotic acid (FOA; 2 mg/ml) and 0.1mM $CuSO_4$. The plates were incubated at 30°C for 2-3 days. Cell growth patterns on FOA plates were opposite to those on FOA-lacking, Ura-lacking plates. See fig. S7, its legend, and the main text for additional descriptions of the logic of split-Ub assays and their results.

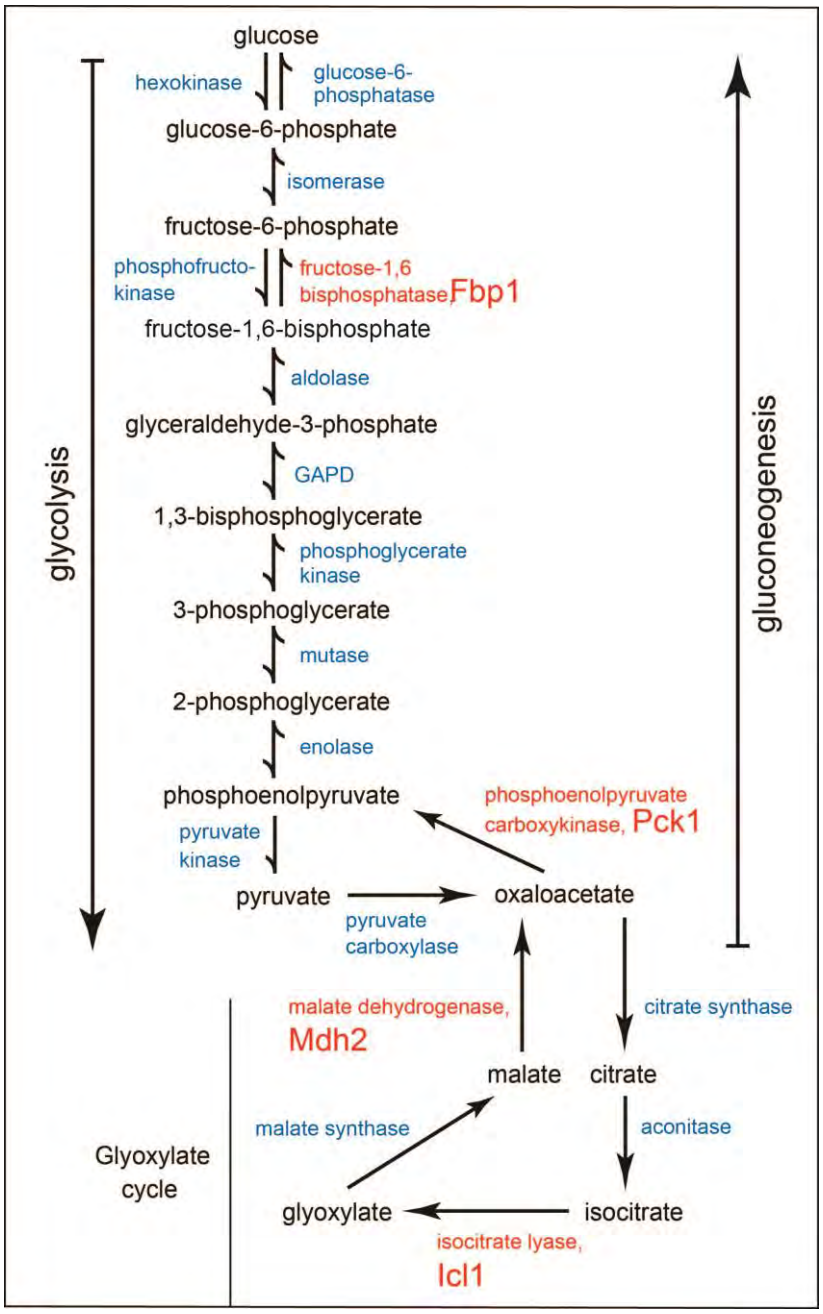


**Fig. S1. Glycolysis and gluconeogenesis.** Gluconeogenesis is, in effect, a reversal of glycolysis, in which glucose is converted to pyruvate, with production of ATP and NADH. Shown here are key enzymatic steps. The main gluconeogenesis-specific enzymes of *S. cerevisiae* are the Fbp1 fructose-1,6-bisphospatase, the Icl1 isocitrate lyase, the Mdh2 cytosolic malate dehydrogenase, and the Pck1 phosphoenolpyruvate carboxykinase. (There are also mitochondrial and peroxisomal counterparts of the cytosolic malate dehydrogenase.) The cited enzymes are highlighted in red in the diagram. The first three enzymes (**P**-Fbp1, **P**-Icl1, and **P**-Mdh2) bear N-terminal Pro, while **SP**-Pck1 contains Pro at position 2, in the N-terminal sequence Ser-Pro. Single two-headed arrows indicate substantially reversible steps, which gluconeogenesis and glycolysis have in common. Antiparallel double arrows, as well as the set of transitions between pyruvate, phosphoenolpyruvate, oxaloacetate and closely related compounds indicate steps whose net directions are determined by the relative activities of gluconeogenesis-specific and glycolysis-specific enzymes. **P**-Icl1 and **P**-Mdh2 are a part of the (also shown) glyoxalate cycle (*14-17*).

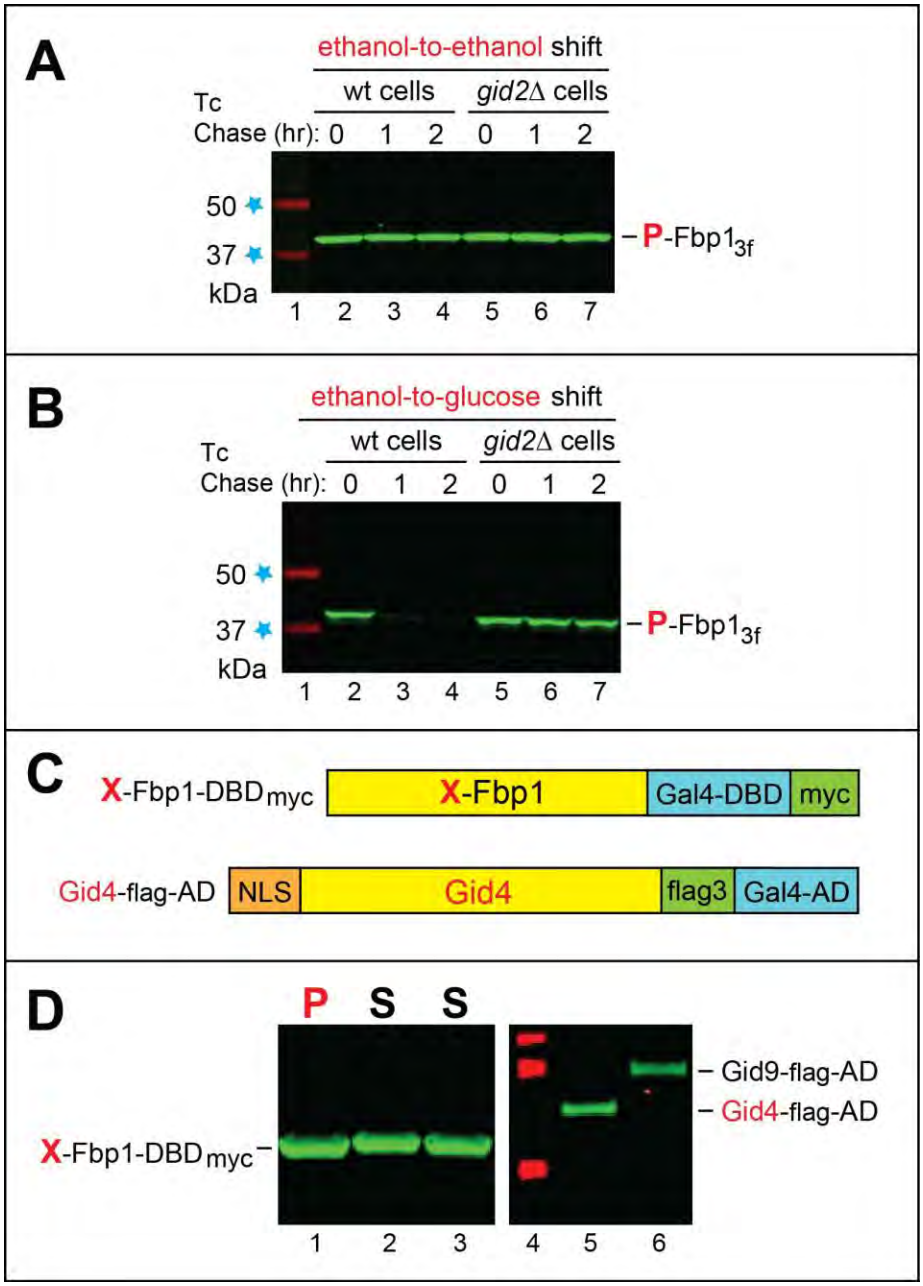


**Fig. S2. Conditional degradation of P-Fbp1 and designs of 2-hybrid fusions.**

(**A**) Lane 1, kDa markers. Tetracycline (Tc)-based chases were performed at 30°C with wild-type (lanes 2-4) and *gid2Δ S. cerevisiae* (lanes 5-7) expressing wild-type **P**-Fbp1$_{3f}$ in a glucose-lacking, ethanol-containing medium. At the indicated times of a chase, cell extracts were prepared, proteins in extracts were fractionated by SDS-PAGE, followed by immunoblotting with anti-flag antibody. Note stability of **P**-Fbp1$_{3f}$ in a medium containing ethanol as the sole carbon source (see Materials and methods).

(**B**) Same as in **A** but Tc-chases were initiated at the time of transfer of cells from ethanol to glucose. Note rapid degradation of **P**-Fbp1$_{3f}$ in wild-type cells (but not in *gid2Δ* cells) in the presence of glucose.

(**C**) Design of **X**-Fbp1 (**X**=Pro or other residues) and Gid4 2-hybrid fusions. These are examples of many 2-hybrid constructs produced and examined in the present study (Table S2). NLS, nuclear localization signal. Gal4-AD, activation domain of the *S. cerevisiae* Gal4 transcriptional activator. Gal4-DBD, DNA-binding domain of the Gal4 transcriptional activator. Epitope tags (myc and triple flag) are indicated as well (see Materials and methods).

(**D**) Expression of 2-hybrid test proteins in *S. cerevisiae* from the P$_{ADH1}$ promoter of 2-hybrid plasmids (*8*). Lanes 1-3, 2-hybrid fusions of **P**-Fbp1, **S**-Fbp1, and **S**-Fbp1 (the latter **S**-Fbp1 was from an independent yeast transformant). Lane 4, kDa markers (50, 75, and 100 kDa, respectively). Lanes 5 and 6, 2-hybrid fusions of Gid4 and Gid9, respectively. Proteins were detected by immunoblotting with anti-myc (lanes 1-3) and anti-flag antibodies (lanes 5, 6).

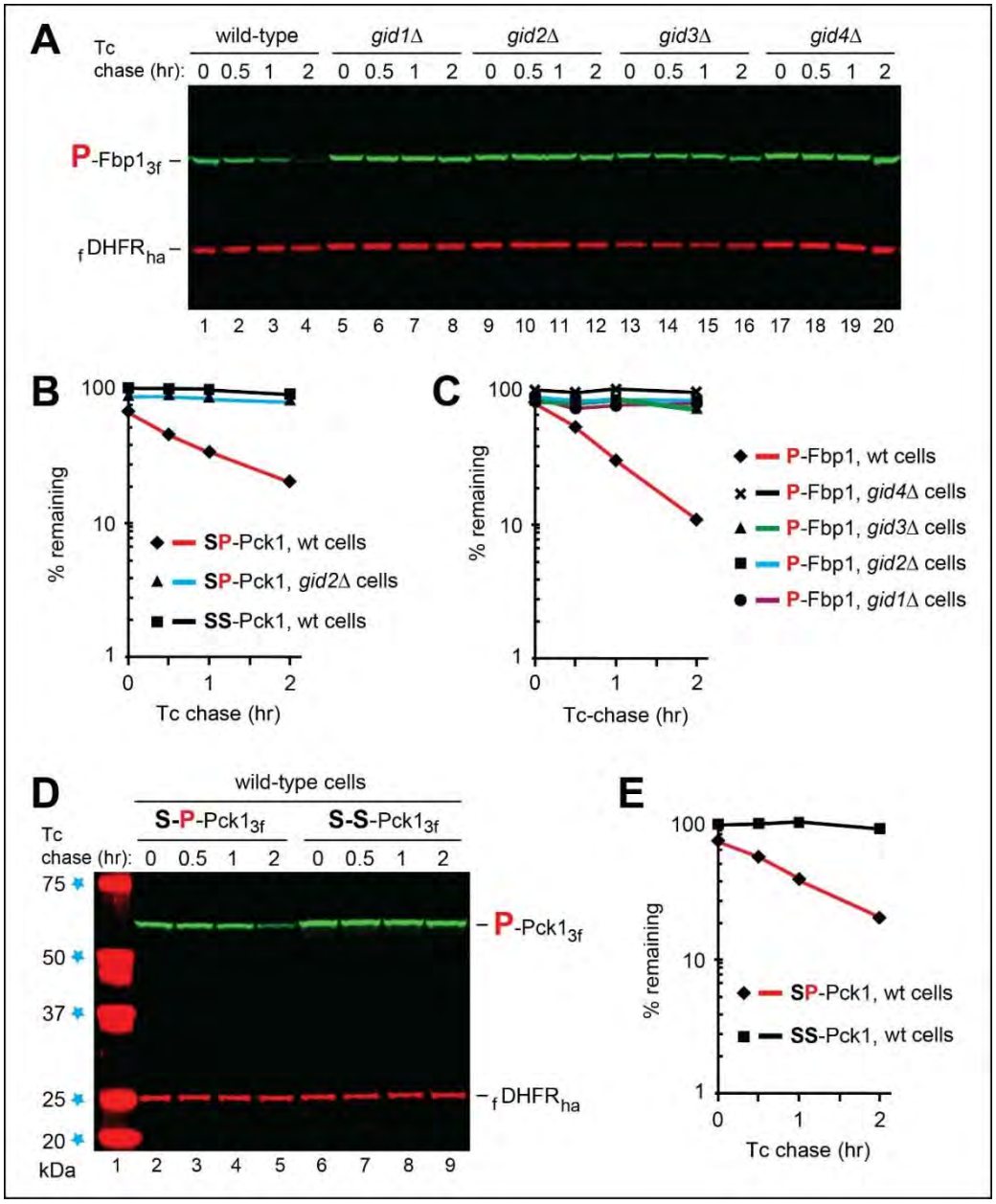


**Fig. S3. Degradation of P-Fbp1 and SP-Pck1 in wild-type and mutant *S. cerevisiae*.**
(**A**) Tc-chases, using PRT-based plasmids expressing **P**-Fbp1$_{3f}$ and the reference $_{f}$DHFR$_{ha}$ (see the main Fig. 2A, B), were performed at 30°C during transition from ethanol to glucose media with wild-type (lanes 1-4), *gid1Δ* (lanes 5-8), *gid2Δ* (lanes 5-8), *gid3Δ* (lanes 9-12), and *gid4Δ* (lanes 17-20) *S. cerevisiae* (see Materials and methods). The bands of **P**-Fbp1$_{3f}$ and $_{f}$DHFR$_{ha}$ are indicated on the left.
(**B**) Quantification of data shown in the main Fig. 2G (Tc-chases, in wild-type and *gid2Δ* *S. cerevisiae*, of wild-type **SP**-Pck1$_{3f}$ and its **SS**-Pck1$_{3f}$ mutant). All Tc-chases in this study were performed at least twice, and yielded results that differed by less than 10%.
(**C**) Quantification of data in **A**.
(**D**) Lane 1, kDa markers. Tc-chases of wild-type **SP**-Pck1$_{3f}$ (lanes 2-5) and its **SS**-Pck1$_{3f}$ mutant (lanes 6-9) in wild-type *S. cerevisiae* during transition from ethanol to glucose media (see Materials and Methods). This is an example of independently performed Tc-chases of Pck1 that were otherwise the same as some of the Tc-chases in the main Fig. 2G.
(**E**) Quantification of data in **D**. All Tc-chases in this study were performed at least twice, and yielded results that differed by less than 10%.

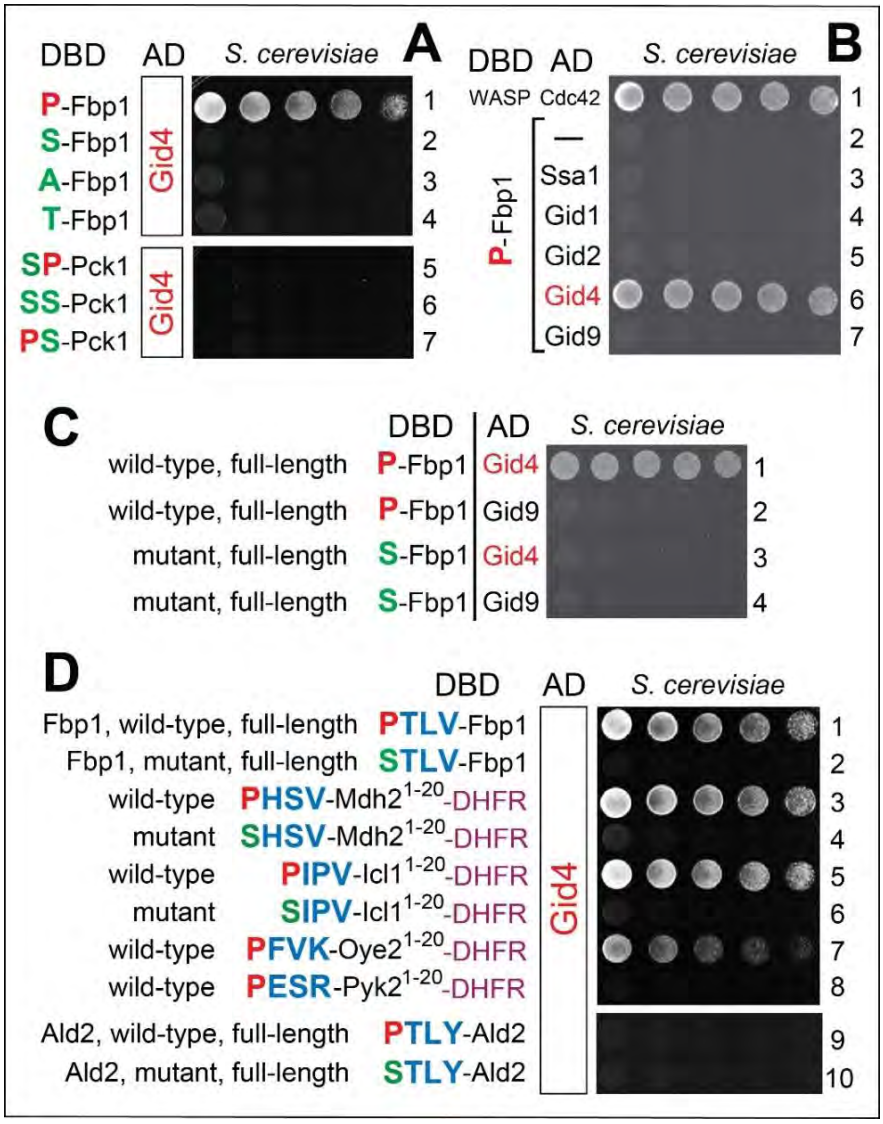


**Fig. S4. 2-hybrid assays of interactions among Gid4, P-Fbp1, derivatives of P-Fbp1, specific GID subunits, and other proteins.** In this and other figures describing 2-hybrid results, Pro residues are in red. Mutant (non-wild-type) residues are in green. Wild-type residues (other than Pro) are in blue. Expression of His3, the reporter of 2-hybrid assays (*8*) in otherwise $His^-$ cells, is a function of the binding affinity between test proteins. Histidine-lacking plates were incubated for 2 days at 30°C to detect the growth of $His^+$ cells. (**A**1-4) Gid4 binds to full-length **P**-Fbp1 but not to the otherwise identical **S**-Fbp1, **A**-Fbp1, or **T**-Fbp1. See also the main Fig. 2A1, 2 and Materials and Methods. (**A**5-7) Gid4 binds neither to full-length **SP**-Pck1, nor to **SS**-Pck1, nor to **PS**-Pck1. (**B**1) Human WASP vs. human Cdc42, a previously known protein interaction (*18*), used as a positive control. (**B**2-7) Assays with full-length **P**-Fbp1 vs. AD-containing vector alone (a negative control), Ssa1 (one of *S. cerevisiae* Hsp70 proteins), Gid1, Gid2, Gid4 (note the binding of **P**-Fbp1 to Gid4), and Gid9, respectively. (**C**1, 2) Full-length **P**-Fbp1 binds to Gid4 but not to Gid9. (**C**3, 4) The **S**-Fbp1 mutant binds neither to Gid4 nor to Gid9. (**D**1, 2) Gid4 binds to full-length **P**-Fbp1 but not to the otherwise identical **S**-Fbp1 (see also **A**1, 2, and, e.g., the main Fig. 2A1, 2 for the same but independently produced results). (**D**3, 4) Gid4 binds to **PHSV**-Mdh2$^{1\text{-}20}$-DHFR-DBD (derived from wild-type **PHSV**-Mdh2) but not to the otherwise identical **SHSV**-Mdh2$^{1\text{-}20}$-DHFR-DBD. (**D**5, 6) Gid4 binds to **PIPV**-Icl1$^{1\text{-}20}$-DHFR-DBD (derived from wild-type **PIPV**-Icl1) but not to the otherwise identical **SIPV**-Icl1$^{1\text{-}20}$-DHFR-DBD. (**D**7) Gid4 binds to **PFVK**-Oye2$^{1\text{-}20}$-DHFR-DBD (see also the main text). Note that the binding of Gid4 to **PFVK**-Oye2$^{1\text{-}20}$-DHFR-DBD is significantly weaker than the one to, e.g., **PHSV**-Mdh2$^{1\text{-}20}$-DHFR-DBD or **PIPV**-Icl1$^{1\text{-}20}$-DHFR-DBD. (**D**8) Gid4 does not bind to **PESR**-Pyk2$^{1\text{-}20}$-DHFR-DBD (see also the main text). (**D**9, 10) Gid4 binds neither to full-length **PTLY**-Ald2 nor to its **STLY**-Ald2 mutant (see also the main text).

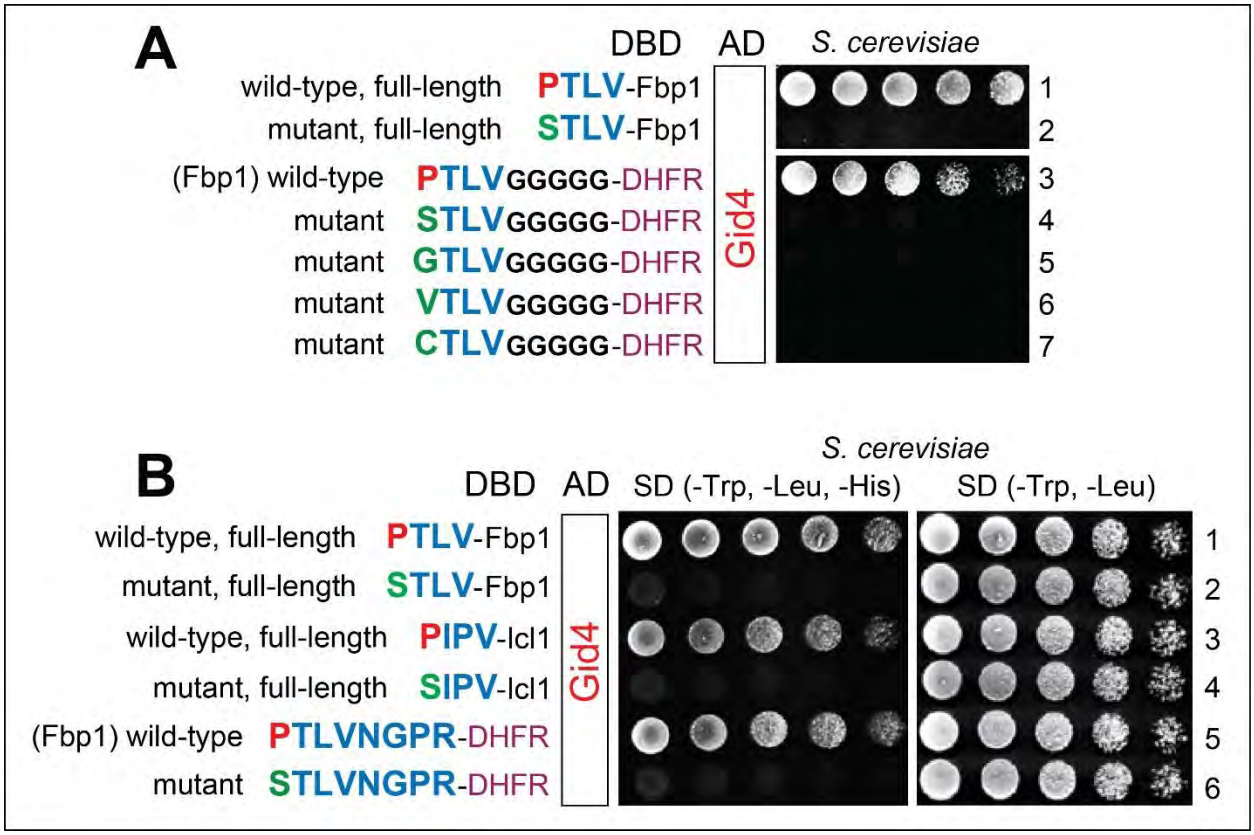


**Fig. S5. 2-hybrid assays of interactions among Gid4 and N-terminal sequences of P-Fbp1 and other proteins.** See the legend to fig. S4 for the residue color notations and related details.

(**A**1, 2) Gid4 binds to full-length **PTLV**-Fbp1 but not to **STLV**-Fbp1 (see, e.g., the main Fig. 2A1, 2 and fig. S4D1, 2 for the same but independently produced results).

(**A**3-7) Gid4 binds to **PTLV**-$G_5$-DHFR-DBD (bearing the first four N-terminal residues of wild-type **P**-Fbp1) but not to the otherwise identical 2-hybrid fusions bearing the indicated non-Pro N-terminal residues.

(**B**) The right panel (and the only control panel of this kind that is shown in the present study) displays the pattern of cellular growth on a histidine-containing plate, which supports the growth of all 2-hybrid-based strains, irrespective of expression levels of the His3 2-hybrid reporter. The left panel shows growth patterns of the identical set of strains on a histidine-lacking plate, used to detect the results of 2-hybrid assays (see Materials and Methods).

(**B**1, 2) Same as in **A**1, 2 but an independent 2-hybrid assay.

(**B**3, 4) Gid4 binds to full-length **PIPV**-Icl1 but not to the otherwise identical **SIPV**-Icl1 (see the main Fig. 2A3, 4 for the same but independently produced results).

(**B**5, 6) Gid4 binds to **PTLVNGPR**-DHFR-DBD (the first eight residues of wild-type **P**-Fbp1, in the context of a 2-hybrid fusion) but does not bind to the otherwise identical (N-terminal Ser-bearing) **STLVNGPR**-DHFR-DBD.

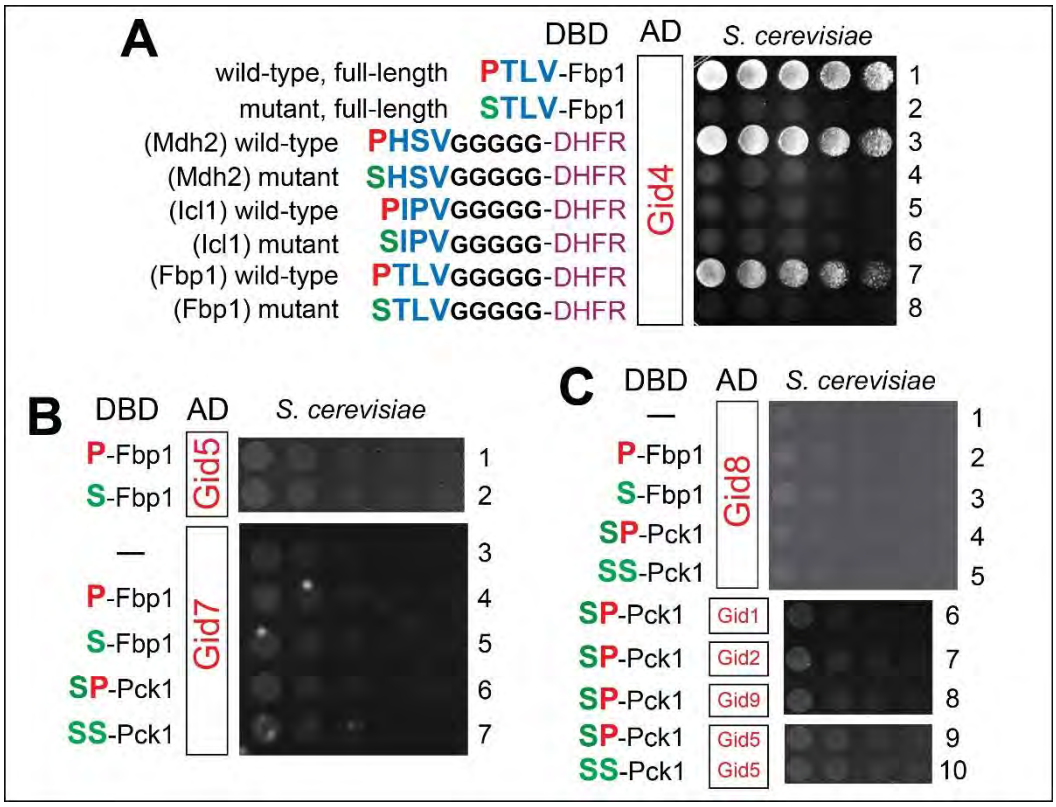


**Fig. S6. 2-hybrid assays of interactions among Fbp1, Pck1, and GID subunits.** See the legend to fig. S4 for the residue color notations and related details.

(**A**1, 2) Gid4 binds to full-length **PTLV**-Fbp1 but not to the **STLV**-Fbp1 mutant (see, e.g., the main Fig. 2A1, 2 and fig. S4D1, 2 for independently produced results).

(**A**3, 4) Gid4 binds to **PHSV**-$G_5$-DHFR-DBD (bearing the first four N-terminal residues of wild-type **PHSV**-Mdh2) but not to the otherwise identical **SHSV**-$G_5$-DHFR-DBD.

(**A**5) Gid4 does not bind to **PIPV**-$G_5$-DHFR-DBD (bearing the first four N-terminal residues of wild-type **PIPV**-Icl1), despite the presence of N-terminal Pro.

(**A**6) Same as in **A**5 but with **SIPV**-$G_5$-DHFR-DBD.

(**A**7, 8) **PTLV**-$G_5$-DHFR-DBD (but not **STLV**-$G_5$-DHFR-DBD) bind to Gid4 (see fig. 2D7, 8) for the same but independently produced results).

(**B**1, 2) Neither **PTLV**-Fbp1 nor its **STLV**-Fbp1 mutant bind to Gid5.

(**B**3) Gid7 vs. vector alone (a negative control).

(**B**4, 5) Neither **PTLV**-Fbp1 nor its **STLV**-Fbp1 mutant bind to Gid7.

(**B**6, 7) Neither **SPSK**-Pck1 nor its **SSSK**-Pck1 mutant bind to Gid7.

(**C**1) Gid8 vs. vector alone (a negative control).

(**C**2, 3) Neither **PTLV**-Fbp1 nor its **STLV**-Fbp1 mutant bind to Gid8.

(**C**4, 5) Neither **SPSK**-Pck1 nor its **SSSK**-Pck1 mutant bind to Gid8.

(**C**6) Wild-type **SPSK**-Pck1 does not bind to Gid1.

(**C**7) Wild-type **SPSK**-Pck1 does not bind to Gid2.

(**C**8) Wild-type **SPSK**-Pck1 does not bind to Gid9.

(**C**9, 10) Neither **SPSK**-Pck1 nor its **SSSK**-Pck1 mutant bind to Gid5.

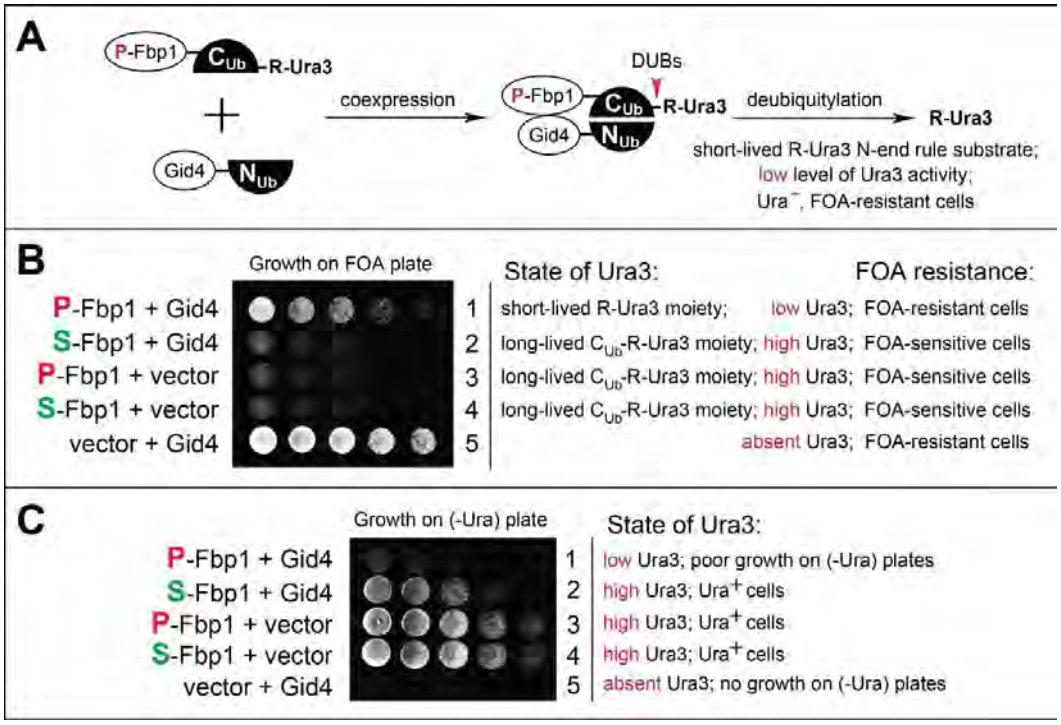


**Fig. S7. Verifying specificity of Gid4 binding to P-Fbp1 through the use of split-ubiquitin assay.** (**A**) Split-Ub assay. In this method, test proteins are expressed as fusions to a C-terminal half of Ub ($C_{ub}$) and to its mutant N-terminal half ($N_{ub}$), respectively (*9, 13*). An *in vivo* interaction between test protein moieties would reconstitute a quasi-native Ub moiety from $N_{ub}$ and $C_{ub}$. The readout of this technique is the *in vivo* cleavage, by *S. cerevisiae* deubiquitylases (DUBs), of a $C_{ub}$-containing fusion immediately after the reconstituted Ub moiety. In the version of the split-Ub assay employed in this study (see Materials and Methods), the C-terminal moiety of a split-Ub fusion that was released through the fusion's cleavage by DUBs was Arg-Ura3 (R-Ura3) (*9, 13*). This protein moiety, owing to its destabilizing N-terminal Arg residue, was a short-lived substrate of the Arg/N-end rule pathway (Fig. 5E). The Ura3 moiety was enzymatically active both in the initial (uncleaved) split-Ub fusion and in its cleavage-released R-Ura3 form, but the levels of DUB-released R-Ura3 were very low, owing to its degradation by the Arg/N-end rule pathway. Cells in which a Ura3-containing split-Ub fusion was largely uncleaved by DUBs (signifying little or no interaction between test protein moieties) contained relatively high levels of Ura3. Such cells were Ura+ (grew on Ura-lacking plates) and $FOA^-$ (did not grow on plates containing both uracil and fluoroorotic acid (FOA), since the activity of Ura3 converted FOA into a toxic compound). By contrast, in cells in which the bulk of the initial Ura3-containing split-Ub fusion was cleaved by DUBs (signifying an interaction between test protein moieties), the levels of the (short-lived) R-Ura3 were very low. Such cells, therefore, were $Ura^-$ and $FOA^+$ (since FOA was largely not converted to a toxic compound in these cells).

(**B**) Split-Ub assay was carried out with split-Ub-based fusions containing Gid4 and either **PTLV**-Fbp1 or **STLV**-Fbp1, as illustrated in **A**. The relative Ura3 levels were assayed, in experiments of this panel, by plating cells on FOA-containing, uracil-containing cells. Cell growth on these plates required (sufficiently) low levels of Ura3 and signified interaction between test protein moieties. **B**1: Gid4 binds to **PTLV**-Fbp1 (low levels of Ura3, growth on FOA plates). **B**2: Gid4 does not bind to **STLV**-Fbp1 (relatively high levels of Ura3, virtually no growth on FOA plates). These data were in agreement with the results obtained through the use of 2-hybrid binding assays (e.g., Fig. 2 and fig. S4). **B**3, 4: negative controls. Specifically, the **PTLV**-Fbp1 and **STLV**-Fbp1 split-Ub fusions (which contained the C-terminal Ura3 moiety) were expressed alone, without the split-Ub Gid4 fusion. This resulted in the absence of DUB- mediated cleavage of these fusions and therefore in high levels of Ura3 in both cases, leading to the absence of growth on FOA plates, as expected. **B**5: a converse negative control. The Gid4 split-Ub fusion (which lacked the Ura3 moiety) was expressed together with vector alone, a plasmid that lacked a split-Ub-based **X**-Fbp1 fusion. The complete absence of Ura3, in this case, led to robust cell growth on FOA plates, as expected.

(**C**) In this panel, the same *S. cerevisiae* strains as in panel **B** were assayed by growing cells on uracil-lacking plates, yielding, as expected, cell growth patterns opposite to those in **B** (see Materials and methods).

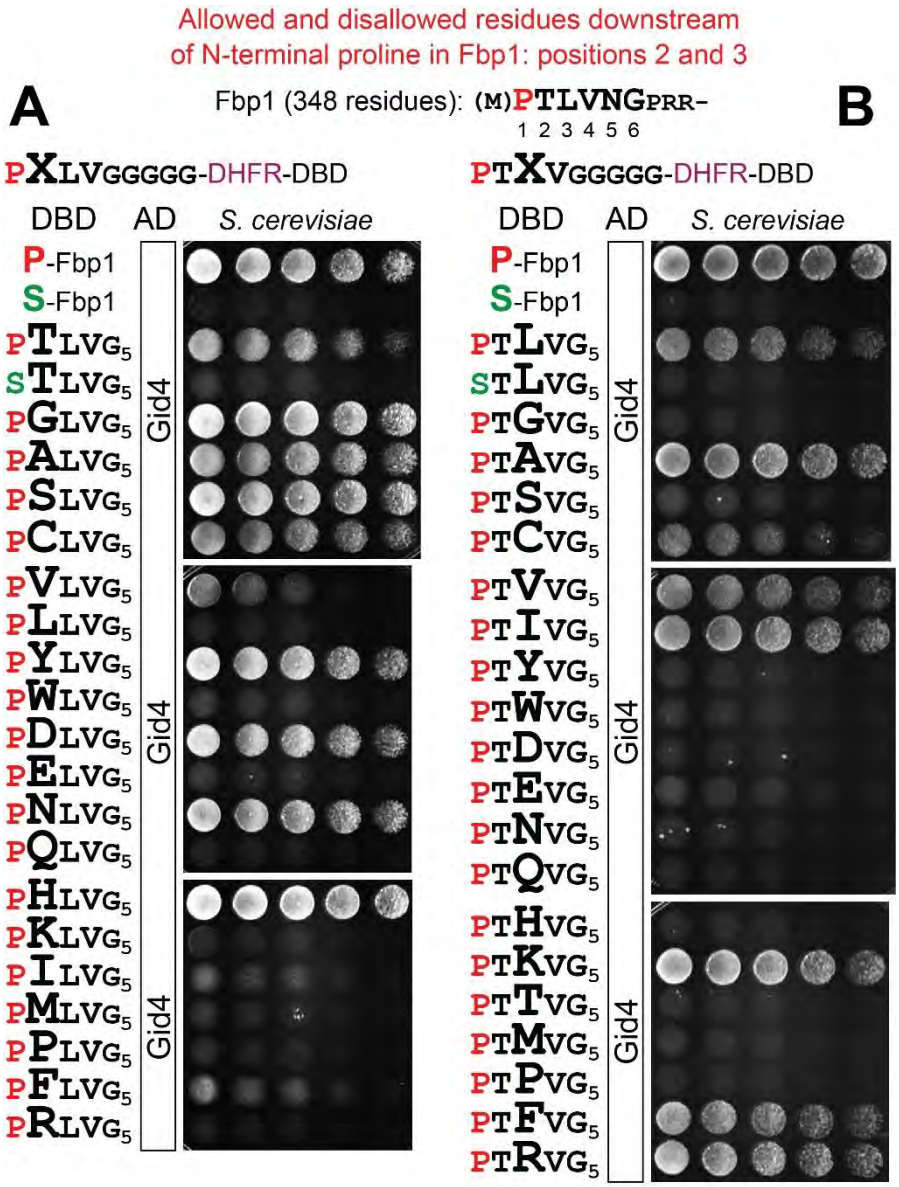


**Fig. S8. Allowed, suboptimal, and disallowed residues downstream of N-terminal proline in Fbp1: positions 2 and 3.**

(**A**) *Row 1, 2*: Gid4 binds to the wild-type full-length **PTLV**-Fbp1 but not to the mutant full-length **STLV**-Fbp1 (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). *Rows 3, 4*: the 2-hybrid-based Gid4 fusion and **X-T-L-V-G**$_5$-DHFR-DBD fusions (**X**=**P** or **S**). Gid4 binds to **P-T-L-V-G**$_5$-DHFR-DBD but not to **S-T-L-V-G**$_5$-DHFR-DBD. *Other rows*: otherwise identical 2-hybrid assays with the 2-hybrid-based Gid4 fusion vs. a set of **P-X-L-V-G**$_5$-DHFR-DBD fusions (**X**=**G**, **A**, **S**, **C**, **V**, **L**, **Y**, **W**, **D**, **E**, **N**, **Q**, **H**, **K**, **I**, **M**, **P**, **F**, **R**).

(**B**) *Row 1-4*: Same as *rows 1-4* in **A** but independent assays. *Other rows*: otherwise identical 2-hybrid assays with the 2-hybrid-based Gid4 fusion vs. a set of **P-T-X-V-G**$_5$-DHFR-DBD fusions (**X**=**G**, **A**, **S**, **C**, **V**, **I**, **Y**, **W**, **D**, **E**, **N**, **Q**, **H**, **K**, **T**, **M**, **P**, **F**, **R**). See the main Fig. 4 for a summary of the binding data in figs. S8-S10.

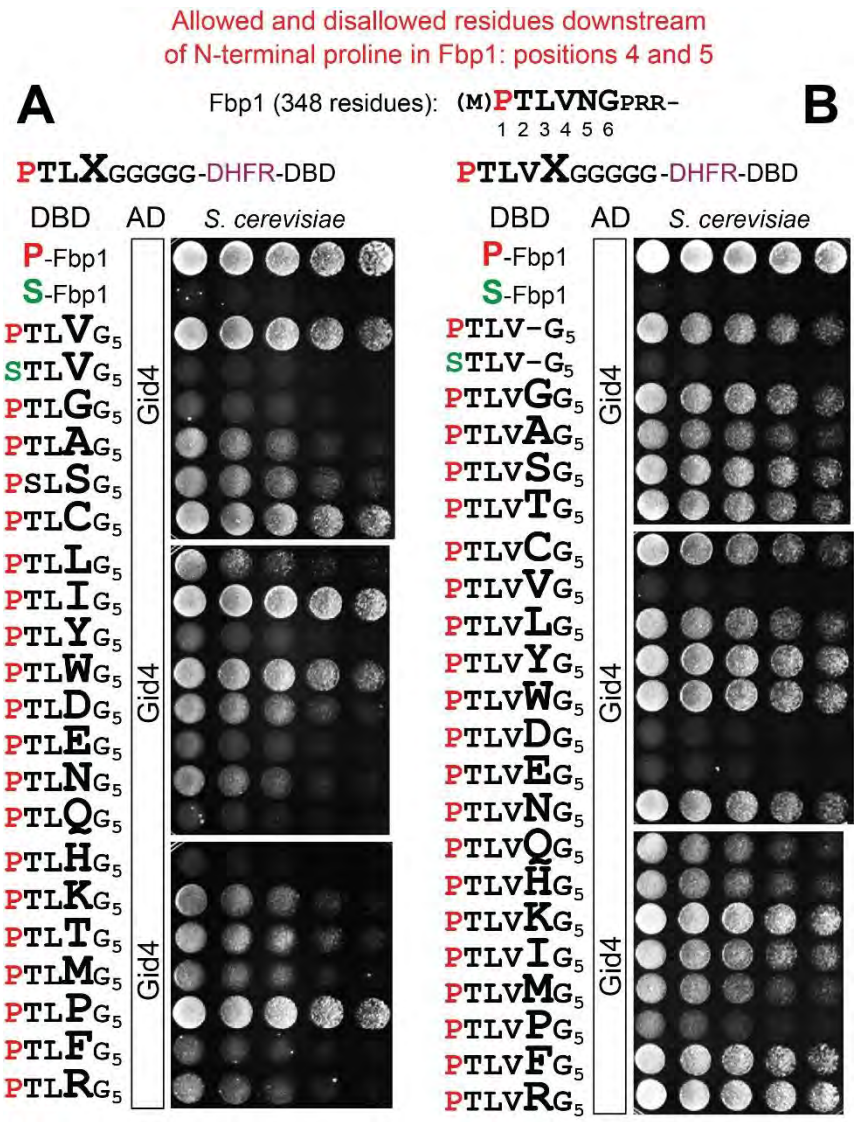


**Fig. S9. Allowed and disallowed residues downstream of N-terminal proline in Fbp1: positions 4 and 5.**

(**A**) *Row 1, 2*: Gid4 binds to the wild-type full-length **PTLV**-Fbp1 but not to the mutant full-length **STLV**-Fbp1 (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). *Rows 3, 4*: the 2-hybrid-based Gid4 fusion and **X-T-L-V-G**$_5$-DHFR-DBD fusions (**X**=**P**, **S**). Gid4 binds to **P-T-L-V-G**$_5$-DHFR-DBD but not to **S-T-L-V-G**$_5$-DHFR-DBD. *Other rows*: otherwise identical 2-hybrid binding assays with the 2-hybrid-based Gid4 fusion vs. a set of **P-T-L-X-G**$_5$-DHFR-DBD fusions (**X**=**G**, **A**, **S**, **C**, **L**, **I**, **Y**, **W**, **D**, **E**, **N**, **Q**, **H**, **K**, **T**, **M**, **P**, **F**, **R**).

(**B**) *Row 1-4*: Same as *rows 1-4* in **A** but independent assays. *Other rows*: otherwise identical 2-hybrid assays with the 2-hybrid-based Gid4 fusion vs. a set of **P-T-L-V-X-G**$_5$-DHFR-DBD fusions (**X**=**G**, **A**, **S**, **T**, **C**, **V**, **L**, **Y**, **W**, **D**, **E**, **N**, **Q**, **H**, **K**, **T**, **M**, **P**, **F**, **R**). In this set of fusions, the varying residue at position 5 (occupied by the Asn residue in wild-type **PTLVN**-Fbp1) was inserted between Val-4 and the first Gly residue of the Gly$_5$ repeat. See the main Fig. 4 for a summary of the binding data in figs. S8-S10.

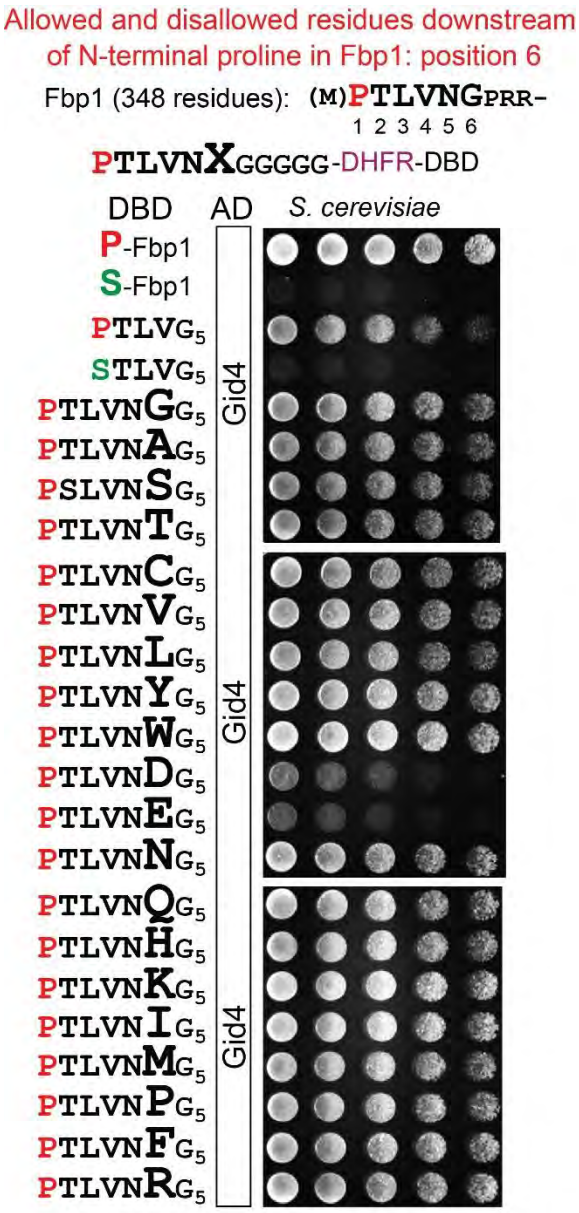


**Fig. S10. Allowed and disallowed residues downstream of N-terminal proline in Fbp1: position 6.**

*Row 1, 2*: Gid4 binds to the wild-type full-length **PTLV**-Fbp1 but not to the mutant full-length **STLV**-Fbp1 (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). *Rows 3, 4*: the 2-hybrid-based Gid4 fusion and **X-T-L-V-N-G**$_5$-DHFR-DBD fusions (**X**=**P**, **S**). Gid4 binds to **P-T-L-V-G**$_5$-DHFR-DBD but not to **S-T-L-V-G**$_5$-DHFR-DBD. *Other rows*: otherwise identical 2-hybrid binding assays with the 2-hybrid-based Gid4 fusion vs. a set of **P-T-L-V-N-X-G**$_5$-DHFR-DBD fusions (**X**=**G**, **A**, **S**, **C**, **L**, **I**, **Y**, **W**, **D**, **E**, **N**, **Q**, **H**, **K**, **T**, **M**, **P**, **F**, **R**). In this set of fusions, the varying residue at position 6 (occupied by the Gly residue in wild-type **PTLVNG**-Fbp1) was inserted between Asn-5 and the first Gly residue of the Gly$_5$ repeat. See the main Fig. 4 for a summary of the binding data in figs. S8-S10.

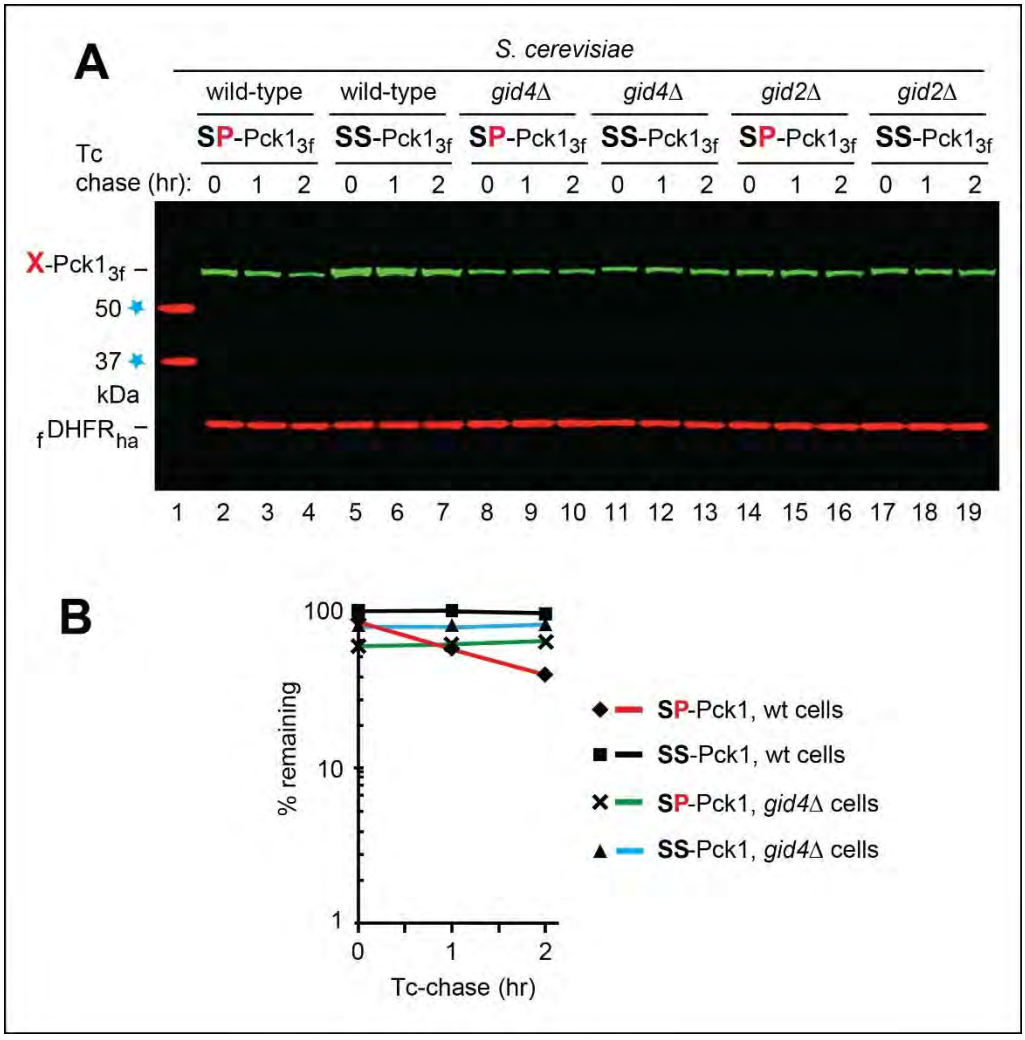


**Fig. S11. Degradation of wild-type SP-Pck1 and mutant SS-Pck1 in wild-type and mutant *S. cerevisiae*.**
(**A**) Lane 1, kDa markers. Lanes 2-4, tetracycline (Tc)-based chase, using a PRT-based plasmid expressing wild-type **SPSK**-Pck1$_{3f}$ and the reference $_{f}$DHFR$_{ha}$ (see the main Fig. 2A, B), was carried out at 30°C during transition from ethanol to glucose media with wild-type *S. cerevisiae*. Lanes 5-7, same as lanes 2-4 but with the mutant **SSSK**-Pck1$_{3f}$. Lanes 8-10, same as lanes 2-4 but in *gid4Δ* cells. Lanes 11-13, same lanes 8-10 but with the mutant **SSSK**-Pck1$_{3f}$. Lanes 14-16, same as lanes 8-10 but in *gid2Δ* cells. Lanes 17-19, same as lanes 14-16 but with the mutant **SSSK**-Pck1$_{3f}$ (see Materials and methods). The bands of **SXSK**-Pck1$_{3f}$ (**X**=**P** or **S**) and $_{f}$DHFR$_{ha}$ are indicated on the left.
(**B**) Quantification of data in **A**. All Tc-chases in this study were performed at least twice, and yielded results that differed by less than 10%.

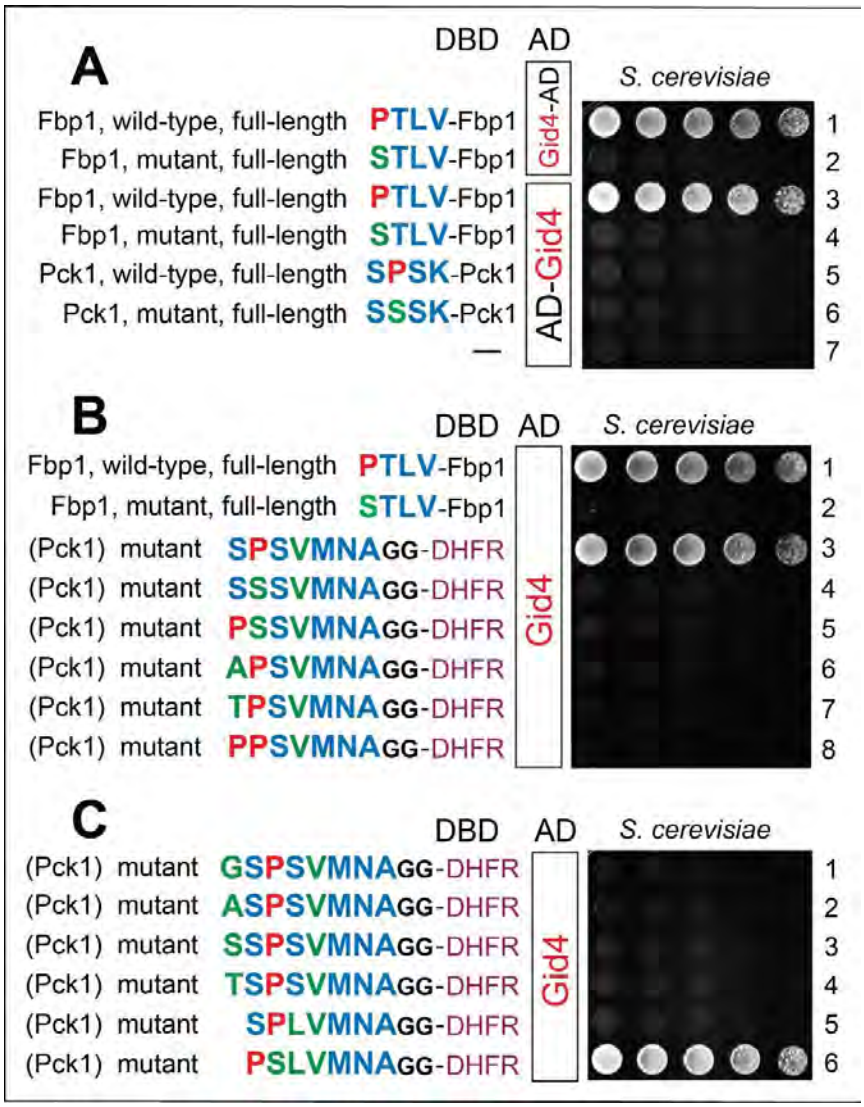


**Fig. S12. 2-hybrid assays that addressed analogies between the substrate-binding groove of Gid4 and peptide-binding grooves of MHC I proteins.** See the legend to fig. S4 for the residue color notations and related details. (**A**1, 2) Gid4 binds to full-length **PTLV**-Fbp1 but not to the otherwise identical **STLV**-Fbp1 mutant (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). (**A**3, 4) Same as in **A**1, 2, but with AD-Gid4, in which the 2-hybrid-specific AD domain was N-terminal, in contrast to C-terminal AD in other Gid4-based 2-hybrid assays of this study. (**A**5, 6) Same as in **A**3, 4, but with **SPSK**-Pck1 and its **SSSK**-Pck1 mutant, respectively. Note the absence of binding between Pck1 and either the AD-Gid4 fusion (this panel) or Gid4-AD (e.g., fig. S4A5-7). In contrast, **PTLV**-Fbp1 bound to Gid4 in either one of its configurations, Gid4-AD or AD-Gid4 (**A**1-4)
(**B**1, 2) Same as in **A**1, 2, but an independent 2-hybrid assay. (**B**3-8) 2-hybrid assays with Gid4 vs. **XZSVMNA-G**$_2$-DHFR-DBD (**X**=**S**, **P**, **A**, **T**; **Z**=**P**, **S**). The expressed protein constructs, here, and throughout this study, contained, initially, the N-terminal Met residue, which was cotranslationally cleaved off by Met-aminopeptidases (*19*). Note the binding of Gid4 to **SPSVMNA-G**$_2$-DHFR-DBD. Its N-terminal (bolded) sequence is identical to that of wild-type **SPSK**-Pck1, save for the replacement of wild-type Lys-4 with Val-4 (see the main text). Note, too, that Gid4 bound to **SPSVMNA-G**$_2$-DHFR-DBD (**B**3) but not to otherwise identical variants of this fusion that contained alterations at positions 1 or 2. (**C**1-4) 2-hybrid assays with Gid4 vs. **XSPSVMNA-G**$_2$-DHFR-DBD (**X**=**G**, **A**, **S**, **T**). The N-terminal sequences of these 2-hybrid fusions were one residue longer than that of **SPSVMNA-G**$_2$-DHFR-DBD, which interacted with Gid4 (see **B**3). See the main text for the logic of the assays in **C**1- **C**4. (**C**5) Same as in **C**1- **C**4 but with Gid4 vs. **SPLVMNA-G**$_2$-DHFR-DBD. Its N-terminal (bolded) sequence, derived from the **SPSKMNA** N-terminal sequence of wild-type **SPSK**-Pck1, contained the Lys-4 → Val-4 mutation (present in all constructs of **B** and **C**) and also the Ser-3 → Leu-3 mutation. (**C**6) Same as in **C**5 but with Gid4 vs. **PSLVMNA-G**$_2$-DHFR-DBD. Its (bolded) N-terminal sequence is identical to that of **SPLVMNA-G**$_2$-DHFR-DBD, save for inversion of N-terminal Ser-Pro, resulting in Pro-Ser. Gid4 interacted with both **SPSVMNA-G**$_2$-DHFR-DBD of **B**3 and **SPLVMNA-G**$_2$-DHFR-DBD of **C**6, but not with any other constructs in **B** and **C**. See the main text for the logic of assays in **C**5-**C**6.

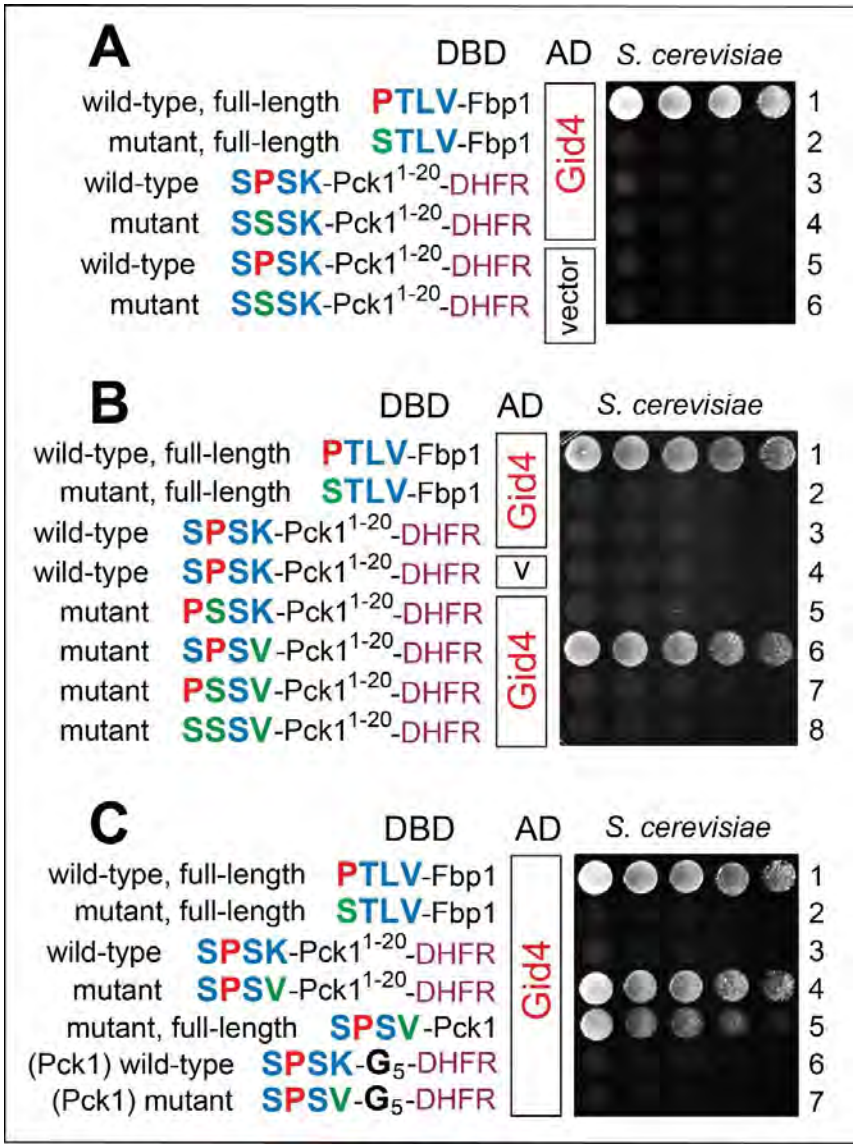


**Fig. S13. 2-hybrid assays of interactions among Gid4 and derivatives of SPSK-Pck1.** See the legend to fig. S4 for the residue color notations and related details. (**A**1, 2) Gid4 binds to full-length **PTLV**-Fbp1 but not to the otherwise identical **STLV**-Fbp1 mutant (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). (**A**3, 4) Gid4 binds neither to **SPSK**-Pck1$^{1-20}$-DHFR-DBD (derived from wild-type **SPSK**-Pck1; the first 20 residues of Pck1) nor the otherwise identical mutant **SSSK**-Pck1$^{1-20}$-DHFR-DBD. (**B**1, 2) Gid4 binds to the full-length **PTLV**-Fbp1 but not to the otherwise identical **STLV**-Fbp1 mutant (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). (**B**3) Same as in **A**3 (the absence of Gid4 binding to **SPSK**-Pck1$^{1-20}$-DHFR-DBD), but an independent assay. (**B**4) Same as in **A**5 (a vector-alone negative control with **SPSK**-Pck1$^{1-20}$-DHFR-DBD), but an independent assay. (**B**5) Gid4 does not bind to **PSSK**-Pck1$^{1-20}$-DHFR-DBD (a mutant of the wild-type N-terminal sequence **SPSK** of Pck1; its first 20 residues). (**B**6) Gid4 binds to **SPSV**-Pck1$^{1-20}$-DHFR-DBD (derived from wild-type **SPSK**-Pck1; its first 20 residues) which contains the Lys → Val mutation at position 4 (see the main text). (**B**7, 8) Gid4 binds neither to **PSSV**-Pck1$^{1-20}$-DHFR-DBD nor to **PSSV**-Pck1$^{1-20}$-DHFR-DBD fusions (derived from wild-type **SPSK**-Pck1; its first 20 residues), which contain the Lys → Val mutation at position 4. Compare with the result in **B**6. (**C**1, 2) Gid4 binds to full-length **PTLV**-Fbp1 but not to the otherwise identical **STLV**-Fbp1 mutant (see, e.g., the main Fig. 2A1, 2 and fig. S4A1, 2 for the same but independently produced results). (**C**3) Same as in **A**3 and **B**3 (the absence of Gid4 binding to "wild-type" **SPSK**-Pck1$^{1-20}$-DHFR-DBD (derived from wild-type Pck1; its first 20 residues). (**C**4) Gid4 binds to **SPSV**-Pck1$^{1-20}$-DHFR-DBD, which contains the Lys → Val mutation at position 4. The same result as in **B**6. (**C**5) Gid4 binds to the full-length **SPSV**-Pck1 protein that contains the same single Lys → Val mutation at position 4 as in the 2-hybrid fusion of **C**4. (**C**6, 7) Gid4 binds neither to **SPSK**-**G**$_5$-DHFR-DBD nor to **SPSV**-**G**$_5$-DHFR-DBD, although the latter fusion contains the Lys → Val mutation at position 4. These results show that the Lys → Val mutation at position 4 confers the binding of Gid4 in the context of the 20 N-terminal residues of Pck1 (see, e.g., **B**6) but not in the context of only four N-terminal residues.

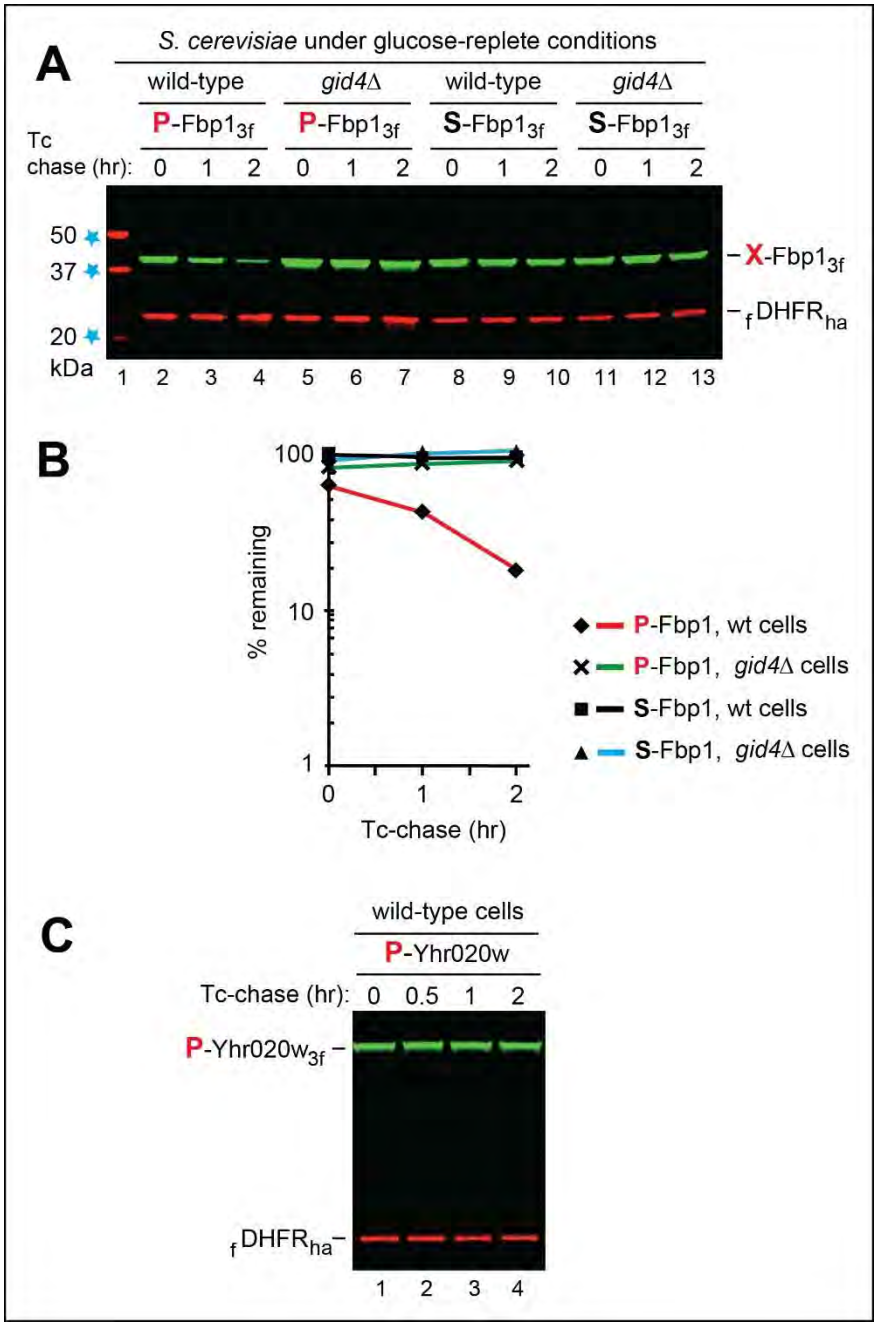


**Fig. S14. Degradation of P-Fbp1 in cells under glucose-replete conditions.**

(**A**) Tetracycline (Tc)-based, reference-based chases were performed as described in the legend to the main Fig. 2, at 30°C with indicated *S. cerevisiae* strains expressing either **PTLV**-Fbp1$_{3f}$ or **STLV**-Fbp1$_{3f}$, but with cells that did not undergo glucose deprivation. Lane 1, kDa markers. Lanes 2-4, wild-type cells expressing **PTLV**-Fbp1$_{3f}$. Lanes 5-7, same as lanes 2-4 but with *gid4Δ* cells. Lanes 8-10, same as in lanes 2-4 but with **STLV**-Fbp1$_{3f}$. Lanes 11-13, same as lanes 8-10 but with *gid4Δ* cells.

(**B**) Quantification of data in **A**. All chases in this study were performed at least twice, and yielded results that differed by less than 10%. The bands of **X**-Fbp1$_{3f}$ and $_{f}$DHFR$_{ha}$ are indicated.

(**C**) The *S. cerevisiae* **PVSE**-Yhr020w prolyl-tRNA synthetase bears N-terminal Pro but is long-lived during ethanol → glucose transitions. The 688-residue, C-terminally triple-flagged, **PVSE**-Yhr020w$_{3f}$ was assayed by a tetracycline (Tc)-based, PRT-based chase during transition from ethanol to glucose (see the main Fig. 2 and Materials and methods). The bands of **PVSE**-Yhr020w$_{3f}$ and $_{f}$DHFR$_{ha}$ are indicated. See also the main text.

N-terminal sequences of *S. cerevisiae* proteins bearing N-terminal Pro

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Fbp1:** | **PTLVN** | Cdc20: | **PESSR** | Fox2: | **PGNLS** | Ivy1: | **PDNNT** |
| **Icl1:** | **PIPVG** | Cef1: | **PPVPI** | Frs1: | **PTVSV** | Kin2: | **PNPNT** |
| **Mdh2:** | **PHSVT** | Cin8: | **PAENQ** | Ftr1: | **PNKVF** | Kog1: | **PEIYG** |
| Ade13: | **PDYDN** | Cka2: | **PLPPS** | Fui1: | **PVSDS** | Kpr3: | **PTNSI** |
| Adh5: | **PSQVI** | Cmk2: | **PKESE** | Fur4: | **PDNLS** | Kre6: | **PLRNL** |
| Aim21: | **PSEVT** | Cmr1: | **PELTE** | Fus3: | **PKRIV** | Kri1: | **PRKKS** |
| Alb1: | **PSKNS** | Crg1: | **PKTSY** | Gcy1: | **PATLH** | Kti12: | **PLVLF** |
| Ald2: | **PTLYT** | Crt10: | **PPQIP** | Gef1: | **PTTYV** | Ldb18: | **PGLKL** |
| Ald3: | **PTLYT** | Ctf8: | **PSVDI** | Gfd1: | **PLESI** | Lre1: | **PNTHT** |
| Aly2: | **PMDQS** | Ctf13: | **PSFNP** | Gga1: | **PQRIE** | Lsg1: | **PPKEA** |
| Ape2: | **PIVRW** | Ctk2: | **PSTFE** | Ggc1: | **PHTDK** | Mch1: | **PLSKV** |
| Apl2: | **PPLDK** | Ctr86: | **PMNNF** | Glt1: | **PVLKS** | Mdm1: | **PKFPQ** |
| Apn1: | **PSTPS** | Cwc23: | **PGHEL** | Glrx3: | **PVIEI** | Mds3: | **PLLQP** |
| Arb1: | **PPVSA** | Cyb5: | **PKVYS** | Gpb1: | **PQAST** | Met3: | **PAPHG** |
| Arc18: | **PAYHS** | Dal1: | **PINAI** | Gpi1: | **PNYIF** | Met10: | **PVEFA** |
| Arg5,6: | **PSASL** | Das1: | **PFQDY** | Gpi11: | **PAKKR** | Met17: | **PSHFD** |
| Asf2: | **PKNRG** | Dna2: | **PGTPQ** | Gpm1: | **PKLVL** | Mft1: | **PLSQK** |
| Asg1: | **PEQAQ** | Dog2: | **PQFSV** | Gpn2: | **PFAQI** | Mgr2: | **PPLPQ** |
| Atc7: | **PNPPS** | Dpb4: | **PPKGW** | Gpp1: | **PLTTK** | Mig2: | **PKKQT** |
| Atg26: | **PITQI** | Dse3: | **PRKFL** | Grs2: | **PLMSN** | Mip6: | **PNSHG** |
| Atp8: | **PQLVP** | Ecm15: | **PKIFC** | Gtt3: | **PTKST** | Mpc54: | **PEDTS** |
| Avt1: | **PEQEP** | Ecm21: | **PFITS** | Gus1: | **PSTLT** | Mrp20: | **PRLTV** |
| Avt5: | **PSNVR** | Egd1: | **PIDQE** | Gyp8: | **PLRSL** | Mrt4: | **PRSKR** |
| Azf1: | **PPPTA** | End3: | **PKLEQ** | Hem13: | **PAPQD** | Mrx7: | **PPRSI** |
| Bck1: | **PFLRK** | Ent4: | **PLLDT** | Her2: | **PLKRS** | Mtq1: | **PRIST** |
| Bck2: | **PKNSH** | Epl1: | **PTPSN** | Hip1: | **PRNPL** | Naa10: | **PINIR** |
| Bna6: | **PVYEH** | Esl2: | **PETSV** | His7: | **PVVHV** | Nam9: | **PRKAN** |
| Brf1: | **PVCKN** | Ess1: | **PSDVA** | Hmg1: | **PPLFK** | Ncp1: | **PFGID** |
| Bsd2: | **PEQEL** | Est3: | **PKVIL** | Hms1: | **PNFQK** | New1: | **PPKKF** |
| Btt1: | **PVDQE** | Exg2: | **PLKSF** | Hom3: | **PMDFQ** | Ngg1: | **PRHGR** |
| Bud16: | **PRLLA** | Exo70: | **PAEID** | Hri1: | **PALLK** | Nsa2: | **PQNDY** |
| Bud22: | **PSESS** | Fcy21: | **PQTHE** | Hrk1: | **PNLLS** | Nst1: | **PPNSK** |
| Bud31: | **PRIKT** | Fcy22: | **PEKLA** | Ies5: | **PSKDP** | Ntc20: | **PSLRD** |
| Cab1: | **PRITQ** | Flo10: | **PVAAR** | Iml3: | **PYTWK** | Nus1: | **PTMIK** |
| Cab2: | **PPLPV** | Flp1: | **PQFGI** | Isd11: | **PGFTA** | Ola1: | **PPKKQ** |

**Fig. S15. N-terminal sequences of *S. cerevisiae* DNA-encoded proteins that bear N-terminal Pro.**

This is one of three consecutive figures (S15-S17) that show the first five residues of 295 *S. cerevisiae* proteins that bear N-terminal Pro (after the cotranslational removal of their initial N-terminal Met by Met-aminopeptidases). This set of proteins was defined using the ScanProsite (http://prosite.expasy.org/scanprosite/) database, searching for the motif "<M-P" (N-terminal Met-Pro) and confining the search to *S. cerevisiae*, the strain S288C. An essentially identical set of proteins could also be identified through a search in the SGD (http://www.yeastgenome.org) database. **PTLVN**-Fbp1, **PIPVG**-Icl1, and **PHSVT**-Mdh2, the three (out of four) main gluconeogenic enzymes and the identified substrates of Gid4 and the rest of the Pro/N-end rule pathway (the main Fig. 1B), are highlighted in bold red at the beginning of this diagram. All other N-terminal Pro-bearing proteins in figs. S15-S17 are cited alphabetically.

N-terminal sequences of *S. cerevisiae* proteins bearing N-terminal Pro
(continuation of fig. S15)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ole1: | **PTSGT** | Rps25a: | **PPKQQ** | Tfc1: | **PVEEP** | Ydr340w: | **PNCFS** |
| Oye2: | **PFVKD** | Rps25b: | **PPKQQ** | Thr4: | **PNASQ** | Ydr444w: | **PYKIN** |
| Oye3: | **PFVKG** | Rps26a: | **PKKRA** | Tma16: | **PVTKS** | Ydr526c: | **PCLLP** |
| Pdr5: | **PEAKL** | Rps26b: | **PKKRA** | Tma46: | **PPKKG** | Ydr545ca: | **PAKLQ** |
| Pex4: | **PNFWI** | Rpt1: | **PPKED** | Tpo4: | **PSSLT** | Ydl196w: | **PSESR** |
| Pex21: | **PSVCH** | Rsp5: | **PSSIS** | Tpo5: | **PEYTL** | Ye090: | **PLEVL** |
| Pex22: | **PPPSR** | Rtt103: | **PFSSE** | Trk2: | **PTAKR** | Yel043w: | **PVSVI** |
| Pfa4: | **PVKLR** | Sac7: | **PNNTL** | Trl1: | **PSPYD** | Yer079w: | **PDSSH** |
| Phsg: | **PPAST** | Sct1: | **PAPKL** | Trs20: | **PQYFA** | Yfl012w: | **PKSRP** |
| Pif1: | **PKWIR** | Sdh6: | **PKRLS** | Tsc13: | **PITIK** | Ygl041c: | **PDFSN** |
| Pig1: | **PYSHG** | Sdo1: | **PINQP** | Tum1: | **PLFDL** | Ygl114w: | **PQSTP** |
| Pmt3: | **PYRVA** | Sds23: | **PQNTR** | Uba2: | **PRETS** | Ygl118c: | **PPEPV** |
| Pns1: | **PLNEK** | Sec63: | **PTNYE** | Ubp2: | **PNEDN** | Ygl177w: | **PIRIF** |
| Prp3: | **PPRNT** | Sec65: | **PRLEE** | Ubx2: | **PVVNH** | Ygr126w: | **PVPSV** |
| Prp39: | **PDETN** | Sen34: | **PPLVF** | Ubx4: | **PMVTV** | Ygr210c: | **PRDPL** |
| Psk1: | **PYIGA** | Sgo1: | **PKRKI** | Ura5: | **PIMLE** | Yhr180cb: | **PPARI** |
| Pst2: | **PRVAI** | Sgt1: | **PVEKD** | Utp8: | **PSLSQ** | Yhm2: | **PSTTN** |
| Pyk2 | **PESRL** | Shb17: | **PSLTP** | Wtm1: | **PKKVW** | Yhr020w: | **PVSEA** |
| Qcr7: | **PQSFT** | She4: | **PLCEK** | Yal067wa: | **PIIGV** | Yhr212wa: | **PYHYL** |
| Rad57: | **PRALS** | Shm2: | **PYTLS** | Yar068w: | **PQVQS** | Yhr214wa: | **PQVQS** |
| Raf1: | **PYKTA** | Sir4: | **PNDNK** | Yat1: | **PNLKR** | Yip5: | **PSNNS** |
| Ras2: | **PLNKS** | Snt2: | **PKEED** | Yb056: | **PPAQL** | Yil174w: | **PIIGV** |
| Reb1: | **PSGHN** | Snx3: | **PREFK** | Ybr056cb: | **PPIQL** | Yir007w: | **PAKIH** |
| Rfs1: | **PKVAI** | Sok2: | **PIGNP** | Ybl081w: | **PGQII** | Yir018ca: | **PSDYT** |
| Ria1: | **PRVES** | Sps2: | **PIWKT** | Ybl086c: | **PFNHN** | Yir020w: | **PHSEK** |
| Rim2: | **PKKSI** | Sps4: | **PSNLN** | Ybr113w: | **PLRPC** | Yj140: | **PKQTL** |
| Rir1: | **PKETP** | Srv2: | **PDSKY** | Ycr108c: | **PYSPS** | Yj77a: | **PGIAF** |
| Rpl12a: | **PPKFD** | Ssu72: | **PSHRN** | Ycr090c: | **PLFLV** | Yj77b: | **PILVW** |
| Rpl12b: | **PPKFD** | Stp1: | **PSTTL** | Yd269: | **PSTCL** | Yjr039w: | **PAGRI** |
| Rpl28: | **PSRFT** | Stp2: | **PILSL** | Ydl016c: | **PPIML** | Yjr140wa: | **PKQTS** |
| Rpn12: | **PSLAE** | Str3: | **PIKRL** | Ydr115w: | **PLFAR** | Yjl152w: | **PHLAA** |
| Rps9a: | **PRAPR** | Swc3: | **PAVLR** | Ydr149c: | **PFFVN** | Yjr107w: | **PVVHC** |
| Rps9b: | **PRAPR** | Swc5: | **PEVET** | Ydr179wa: | **PTILY** | Yk106: | **PFPSI** |
| Rps19a: | **PGVSV** | Swi4: | **PFDVL** | Ydl213wa: | **PVRSL** | Yl016: | **PITSS** |

**Fig. S16. N-terminal sequences of *S. cerevisiae* DNA-encoded proteins that bear N-terminal Pro.**

This is the second of three figures (S15-S17) that show the first five residues of 295 *S. cerevisiae* proteins that bear N-terminal Pro (after the cotranslational removal of their initial N-terminal Met). See the legend to fig. S15 for additional details.

N-terminal sequences of *S. cerevisiae* proteins bearing N-terminal Pro
(continuation of figs. S15 and S16)

| | | |
|---|---|---|
| Yl299: **PFSPD** | Ylr363wa: **PQKPL** | Ynr075c-a: **PIIVG** |
| Ylr031w: **PVLNT** | Yml089c: **PHAWQ** | Yor015w: **PHFKR** |
| Ylr101c: **PFLLH** | Yml122c: **PRNDS** | Yor292c: **PLQLF** |
| Ylr154we: **PPGIP** | Ymr099c: **PIKET** | Ypp1: **PNSNV** |
| Ylr202c: **PNFHL** | Ymr111c: **PAREY** | Ypr1: **PATLK** |
| Ylr345w: **PNVLS** | Ynl040w: **PTPMT** | |
| Ylr352w: **PDLKS** | Ynl181w: **PLNII** | |

**Fig. S17. N-terminal sequences of *S. cerevisiae* DNA-encoded proteins that bear N-terminal Pro.**

This is the third and last of three figures (S15-S17) that show the first five residues of 295 *S. cerevisiae* proteins that bear N-terminal Pro (after the cotranslational removal of their initial N-terminal Met). See the legend to fig. S15 for additional details.

**Table 1.** *E. coli* and *S. cerevisiae* strains used in this study.

| Strains | Relevant genotypes | Sources |
|---|---|---|
| *E. coli* strains: | | |
| DH5a | *F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1* | Invitrogen |
| SUREII | *endA1 glnV44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::Tn5 uvrC e14- Δ(mcrCB-hsdSMR-mrr)171 F'[ proAB$^+$ lacI$^q$ lacZΔM15 Tn10 Amy Cm$^R$]* | Stratagene |
| STBL2 | *F- endA1 glnV44 thi-1 recA1 gyrA96 relA1 Δ(lac-proAB) mcrA Δ(mcrBC-hsdRMS-mrr) λ-* | Invitrogen |
| *S. cerevisiae* strains: | | |
| BY4741 | *MAT**a** his3-1 leu2-0 Met15-0 ura3-0* | Open Biosystems |
| BY4594 | *GID1Δ::KanMX6* in BY4741 | Open Biosystems |
| BY3614 | *GID2Δ::KanMX6* in BY4741 | Open Biosystems |
| BY6577 | *GID3Δ::KanMX6* in BY4741 | Open Biosystems |
| BY3244 | *GID4Δ::KanMX6* in BY4741 | Open Biosystems |
| AH109 | *MAT**a**, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ* | Clontech |
| JD52 | *MAT**a** trp1-63 ura3-52 his3-200 leu2-3112. lys2-801* | Varshavsky lab collection. |

**Table 2.** Plasmids used in this study.

| Plasmid | Description | Source or Reference |
|---|---|---|
| pGADCg | Y2H expression vector. Contains the $P_{ADH1}$ promoter. Used to produce Gal4-AD$_{ha*}$ fusion by Gateway cloning. *contains a partial ha epitope sequence. | Addgene |
| pGBKCg | Y2H expression vector. Contains the $P_{ADH1}$ promoter. Produces Gal4–DBD$_{myc}$ fusion by Gateway cloning. | Addgene |
| pDONR/Zeo | Donor vector for Gateway cloning via BP reaction | Invitrogen |

| | | |
|---|---|---|
| pRS313Cup1 | pRS313 derivative containing the $P_{CUP1}$ promoter | Varshavsky lab collection |
| Ste14-$C_{Ub}$-R-Ura3-Met313 | Ste14-$C_{Ub}$-R-Ura3 in pRS313 with $P_{MET17}$ | Varshavsky lab collection |
| $N_{Ub}$-Ubc6-Cup314 | $N_{Ub}$-Ubc6 in pRS314 with $P_{CUP1}$ | Varshavsky lab collection |
| pJO629 | $Yap5_{3flag}$ and $_{flag}DHFR_{ha}$ in pRS313 with two identical (modified) $P_{TDH3}$ promoters. | This study |
| pCSJ95 | MP-$Fbp1_{3flag}$ in pJO629 | This study |
| pCSJ98 | MS-$Fbp1_{3flag}$ in pJO629 | This study |
| pCSJ120 | Yap5-Pck1 (+382~1647bp)$_{3flag}$ in pJO629 | This study |
| pCSJ121 | MSP-$Pck1_{3flag}$ in pJO629 | This study |
| pCSJ122 | MSS-$Pck1_{3flag}$ in pJO629 | This study |
| pCSJ125 | MP-$Mdh2_{3flag}$ in pJO629 | This study |
| pCSJ126 | MS-$Mdh2_{3flag}$ in pJO629 | This study |
| pCSJ160 | attL-$Gid1_{3flag}$ in pDONR/Zeo | This study |
| pCSJ161 | attL-MP-Fbp1 in pDONR/Zeo | This study |
| pCSJ162 | attL-$Cdc42_{3flag}$ in pDONR/Zeo | This study |
| pCSJ163 | attL-WASP in pDONR/Zeo | This study |
| pCSJ164 | SV40-NLS-$Gid1_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ165 | MP-Fbp1-Gal4-DBD in pGBKCg | This study |
| pCSJ166 | SV40-NLS-$Cdc42_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ167 | WASP-Gal4-DBD in pGADCg | This study |
| pCSJ168 | MP-$Yhr020w_{3flag}$ in pJO629 | This study |
| pCSJ174 | attL-MS-Fbp1 in pDONR/Zeo | This study |
| pCSJ175 | attL-$Ssa1_{3flag}$ in pDONR/Zeo | This study |
| pCSJ176 | attL-$Gid2_{3flag}$ in pDONR/Zeo | This study |
| pCSJ177 | attL-$Gid4_{3flag}$ in pDONR/Zeo | This study |
| pCSJ178 | attL-$Gid9_{3flag}$ in pDONR/Zeo | This study |
| pCSJ179 | MS-Fbp1-Gal4-DBD in pGBKCg | This study |
| pCSJ180 | SV40-NLS-$Ssa1_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ181 | SV40-NLS-$Gid2_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ182 | SV40-NLS-$Gid4_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ183 | SV40-NLS-$Gid9_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ193 | attL-MP-Mdh2 in pDONR/Zeo | This study |
| pCSJ194 | attL-MS-Mdh2 in pDONR/Zeo | This study |
| pCSJ195 | attL-MSP-Pck1 in pDONR/Zeo | This study |
| pCSJ196 | attL-MSS-Pck1 in pDONR/Zeo | This study |

| | | |
|---|---|---|
| pCSJ197 | MP-Mdh2-Gal4-DBD in pGBKCg | This study |
| pCSJ198 | MS-Mdh2-Gal4-DBD in pGBKCg | This study |
| pCSJ199 | MSP-Pck1-Gal4-DBD in pGBKCg | This study |
| pCSJ200 | MSS-Pck1-Gal4-DBD in pGBKCg | This study |
| pCSJ201 | attL-MP-Ald2 in pDONR/Zeo | This study |
| pCSJ202 | attL-MS-Ald2 in pDONR/Zeo | This study |
| pCSJ205 | MP-Ald2-Gal4-DBD in pGBKCg | This study |
| pCSJ206 | MS-Ald2-Gal4-DBD in pGBKCg | This study |
| pCSJ210 | attL-MP-Fbp1$^{1-175}$ in pDONR/Zeo | This study |
| pCSJ211 | attL-MS-Fbp1$^{1-175}$ in pDONR/Zeo | This study |
| pCSJ216 | MP-Fbp1$^{1-175}$-Gal4-DBD in pGBKCg | This study |
| pCSJ217 | MS-Fbp1$^{1-175}$-Gal4-DBD in pGBKCg | This study |
| pCSJ222 | attL-MP-Icl1 in pDONR/Zeo | This study |
| pCSJ223 | attL-MS-Icl1 in pDONR/Zeo | This study |
| pCSJ224 | attL-MPS-Pck1 in pDONR/Zeo | This study |
| pCSJ225 | attL-MP-Pyk2 in pDONR/Zeo | This study |
| pCSJ226 | attL-MS-Pyk2 in pDONR/Zeo | This study |
| pCSJ227 | MP-Icl1-Gal4-DBD in pGBKCg | This study |
| pCSJ228 | MS-Icl1-Gal4-DBD in pGBKCg | This study |
| pCSJ229 | MPS-Pck1-Gal4-DBD in pGBKCg | This study |
| pCSJ230 | MP-Pyk2-Gal4-DBD in pGBKCg | This study |
| pCSJ231 | MS-Pyk2-Gal4-DBD in pGBKCg | This study |
| pCSJ232 | attL-Gid5$_{3flag}$ in pDONR/Zeo | This study |
| pCSJ234 | SV40-NLS-Gid5$_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ261 | attL-Gid7$_{3flag}$ in pDONR/Zeo | This study |
| pCSJ263 | attL-Gid8$_{3flag}$ in pDONR/Zeo | This study |
| pCSJ267 | SV40-NLS-Gid7$_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ268 | SV40-NLS-Gid8$_{3flag}$-Gal4-AD in pGADCg | This study |
| pCSJ290 | attL-MP-Fbp1$^{1-62}$ in pDONR/Zeo | This study |
| pCSJ291 | attL-MS-Fbp1$^{1-62}$ in pDONR/Zeo | This study |
| pCSJ292 | attL-MP-Fbp1$^{1-87}$ in pDONR/Zeo | This study |
| pCSJ293 | attL-MS-Fbp1$^{1-87}$ in pDONR/Zeo | This study |
| pCSJ294 | MP-Fbp1$^{1-62}$-Gal4-DBD in pGBKCg | This study |

| | | |
|---|---|---|
| pCSJ295 | MS-Fbp1$^{1-62}$-Gal4-DBD in pGBKCg | This study |
| pCSJ296 | MP-Fbp1$^{1-87}$-Gal4-DBD in pGBKCg | This study |
| pCSJ297 | MS-Fbp1$^{1-87}$-Gal4-DBD in pGBKCg | This study |
| pCSJ323 | attL-MV-DHFR in pDONR/Zeo | This study |
| pCSJ324 | attL-MP-Fbp1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ325 | attL-MS-Fbp1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ326 | MV-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ327 | MP-Fbp1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ328 | MS-Fbp1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ330 | attL-MP-Mdh2$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ331 | attL-MS-Mdh2$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ332 | attL-MP-Icl1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ333 | attL-MS-Icl1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ334 | MP-Mdh2$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ335 | MS-Mdh2$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ336 | MP-Icl1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ337 | MS-Icl1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ358 | attL-MP-Oye2$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ359 | attL-MP-Pyk2$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ360 | MP-Oye$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ361 | MP-Pyk2$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ362 | attL-MA-Fbp1 in pDONR/Zeo | This study |
| pCSJ363 | attL-MT-Fbp1 in pDONR/Zeo | This study |
| pCSJ364 | MA-Fbp1-Gal4-DBD in pGBKCg | This study |
| pCSJ365 | MT-Fbp1-Gal4-DBD in pGBKCg | This study |
| pCSJ370 | attL-MP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ371 | attL-MS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ372 | attL-MP-Fbp1$^{1-3}$-(Gly)$_7$-DHFR in pDONR/Zeo | This study |
| pCSJ373 | attL-MS-Fbp1$^{1-3}$-(Gly)$_7$-DHFR in pDONR/Zeo | This study |
| pCSJ374 | attL-MP-(Gly)$_8$-DHFR in pDONR/Zeo | This study |
| pCSJ375 | attL-MS-(Gly)$_8$-DHFR in pDONR/Zeo | This study |
| pCSJ376 | MP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ377 | MS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |

| | | |
|---|---|---|
| pCSJ378 | MP-Fbp1$^{1-3}$-(Gly)$_7$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ379 | MS-Fbp1$^{1-3}$-(Gly)$_7$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ380 | MP-(Gly)$_8$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ381 | MS-(Gly)$_8$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ408 | MP-Fbp1-C$_{Ub}$-R-Ura3 in pRS313 with P$_{MET17}$ | This study |
| pCSJ409 | MS-Fbp1-C$_{Ub}$-R-Ura3 in pRS313 with P$_{MET17}$ | This study |
| pCSJ418 | N$_{Ub}$-Gid4$_{flag}$ in pRS314 with P$_{CUP1}$ | This study |
| pCSJ423 | attL-MSP-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ424 | attL-MSS-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ425 | MSP-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ426 | MSS-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ435 | attL-MPSSK-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ436 | attL-MSPSV-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ437 | attL-MPSSV-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ438 | MPSSK-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ439 | MSPSV-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ440 | MPSSV-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ445 | attL-MPG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ446 | attL-MPA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ447 | attL-MPS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ448 | attL-MPC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ449 | attL-MPV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ450 | attL-MPL-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ451 | attL-MPY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ452 | attL-MPW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ453 | attL-MPD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ454 | attL-MPE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ455 | attL-MPN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ456 | attL-MPQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ457 | attL-MPH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ458 | attL-MPK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ459 | MPG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ460 | MPA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |

| | | |
|---|---|---|
| pCSJ461 | MPS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ462 | MPC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ463 | MPV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ464 | MPL-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ465 | MPY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ466 | MPW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ467 | MPD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ468 | MPE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ469 | MPN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ470 | MPQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ471 | MPH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ472 | MPK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ473 | MP-Fbp1-C$_{Ub}$-R-Ura3 in pRS313 with P$_{CUP1}$ | This study |
| pCSJ474 | MS-Fbp1-C$_{Ub}$-R-Ura3 in pRS313 with P$_{CUP1}$ | This study |
| pCSJ475 | attL-MPTG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ476 | attL-MPTA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ477 | attL-MPTS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ478 | attL-MPTC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ479 | attL-MPTV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ480 | attL-MPTI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ481 | attL-MPTY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ482 | attL-MPTW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ483 | attL-MPTD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ484 | attL-MPTE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ485 | attL-MPTN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ486 | attL-MPTQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ487 | attL-MPTH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ488 | attL-MPTK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ489 | MPTG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ490 | MPTA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ491 | MPTS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ492 | MPTC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |

| | | |
|---|---|---|
| pCSJ493 | MPTV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ494 | MPTI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ495 | MPTY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ496 | MPTW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ497 | MPTD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ498 | MPTE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ499 | MPTN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ500 | MPTQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ501 | MPTH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ502 | MPTK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ503 | attL-MSSSV-Pck1$^{1-20}$-DHFR in pDONR/Zeo | This study |
| pCSJ504 | MSSSV-Pck1$^{1-20}$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ513 | attL-MPTLG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ514 | attL-MPTLA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ515 | attL-MPTLS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ516 | attL-MPTLC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ517 | attL-MPTLL-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ518 | attL-MPTLI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ519 | attL-MPTLY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ520 | attL-MPTLW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ521 | attL-MPTLD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ522 | attL-MPTLE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ523 | attL-MPTLN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ524 | attL-MPTLQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ525 | attL-MPTLH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ526 | attL-MPTLK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ527 | MPTLG-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ528 | MPTLA-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ529 | MPTLS-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ530 | MPTLC-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |

| | | |
|---|---|---|
| pCSJ531 | MPTLL-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ532 | MPTLI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ533 | MPTLY-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ534 | MPTLW-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ535 | MPTLD-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ536 | MPTLE-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ537 | MPTLN-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ538 | MPTLQ-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ539 | MPTLH-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ540 | MPTLK-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ541 | attL-MSPSV-Pck1 in pDONR/Zeo | This study |
| pCSJ542 | attL-MSPSK-Pck1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ543 | attL-MSPSV-Pck1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ545 | MSPSV-Pck1-Gal4-DBD in pGBKCg | This study |
| pCSJ546 | MSPSK-Pck1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ547 | MSPSV-Pck1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ549 | attL-MPI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ550 | attL-MPM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ551 | attL-MPP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ552 | attL-MPF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ553 | attL-MPR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ554 | MPI-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ555 | MPM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ556 | MPP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ557 | MPF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ558 | MPR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ559 | attL-MPTT-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ560 | attL-MPTM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ561 | attL-MPTP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ562 | attL-MPTF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |

| | | |
|---|---|---|
| pCSJ563 | attL-MPTR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ564 | MPTT-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ565 | MPTM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ566 | MPTP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ567 | MPTF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ568 | MPTR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ569 | attL-MPTLT-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ570 | attL-MPTLM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ571 | attL-MPTLP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ572 | attL-MPTLF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ573 | attL-MPTLR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ574 | MPTLT-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ575 | MPTLM-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ576 | MPTLP-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ577 | MPTLF-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ578 | MPTLR-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ579 | attL-MPTLV-Fbp1$^{1-5}$-G-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ580 | attL-MPTLV-Fbp1$^{1-5}$-A-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ581 | attL-MPTLV-Fbp1$^{1-5}$-S-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ582 | attL-MPTLV-Fbp1$^{1-5}$-T-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ583 | attL-MPTLV-Fbp1$^{1-5}$-C-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ584 | attL-MPTLV-Fbp1$^{1-5}$-V-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ585 | attL-MPTLV-Fbp1$^{1-5}$-L-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ586 | attL-MPTLV-Fbp1$^{1-5}$-Y-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ587 | attL-MPTLV-Fbp1$^{1-5}$-W-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ588 | attL-MPTLV-Fbp1$^{1-5}$-D-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ589 | attL-MPTLE-Fbp1$^{1-5}$-A-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ590 | attL-MPTLV-Fbp1$^{1-5}$-N-(Gly)$_5$-DHFR in pDONR/Zeo | This study |

| | | |
|---|---|---|
| pCSJ591 | attL-MPTLV-Fbp1$^{1-5}$-Q-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ592 | attL-MPTLV-Fbp1$^{1-5}$-H-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ593 | attL-MPTLV-Fbp1$^{1-5}$-K-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ594 | attL-MPTLV-Fbp1$^{1-5}$-I-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ595 | attL-MPTLV-Fbp1$^{1-5}$-M-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ596 | attL-MPTLV-Fbp1$^{1-5}$-P-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ597 | attL-MPTLV-Fbp1$^{1-5}$-F-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ598 | attL-MPTLV-Fbp1$^{1-5}$-R-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ599 | MPTLV-Fbp1$^{1-5}$-G-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ600 | MPTLV-Fbp1$^{1-5}$-A-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ601 | MPTLV-Fbp1$^{1-5}$-S-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ602 | MPTLV-Fbp1$^{1-5}$-T-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ603 | MPTLV-Fbp1$^{1-5}$-C-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ604 | MPTLV-Fbp1$^{1-5}$-V-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ605 | MPTLV-Fbp1$^{1-5}$-L-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ606 | MPTLV-Fbp1$^{1-5}$-Y-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ607 | MPTLV-Fbp1$^{1-5}$-W-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ608 | MPTLV-Fbp1$^{1-5}$-D-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ609 | MPTLV-Fbp1$^{1-5}$-E-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ610 | MPTLV-Fbp1$^{1-5}$-N-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ611 | MPTLV-Fbp1$^{1-5}$-Q-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ612 | MPTLV-Fbp1$^{1-5}$-H-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ613 | MPTLV-Fbp1$^{1-5}$-K-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ614 | MPTLV-Fbp1$^{1-5}$-I-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ615 | MPTLV-Fbp1$^{1-5}$-M-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ616 | MPTLV-Fbp1$^{1-5}$-P-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ617 | MPTLV-Fbp1$^{1-5}$-F-(Gly)$_5$-DHFR-Gal4-DBD | This study |

| | | |
|---|---|---|
| | in pGBKCg | |
| pCSJ618 | MPTLV-Fbp1$^{1-5}$-R-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ619 | attL-MP-Mdh2$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ620 | attL-MS-Mdh2$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ621 | attL-MP-Icl1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ622 | attL-MS-Icl1$^{1-5}$-(Gly)$_5$-DHFR in pDONR/Zeo | This study |
| pCSJ623 | MP-Mdh2$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ624 | MS-Mdh2$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ625 | MP-Icl1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ626 | MS-Icl1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ629 | attL-MSPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR in pDONR/Zeo | This study |
| pCSJ632 | MSPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ643 | MSSSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ644 | MPSSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ645 | MAPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ646 | MTPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ647 | MPPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ648 | MGSPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ649 | MASPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ650 | MSSPSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ651 | MTSPSSV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ652 | MSPLV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ653 | MPSLV-Pck1$^{1-8}$-(Gly)$_2$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ654 | MGTLV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ655 | MVTLV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ656 | MCTLV-Fbp1$^{1-5}$-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ681 | MPTLVN-Fbp1$^{1-6}$-G-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ682 | MPTLVN-Fbp1$^{1-6}$-A-(Gly)$_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ683 | MPTLVN-Fbp1$^{1-6}$-S-(Gly)$_5$-DHFR-Gal4-DBD | This study |

| | | |
|---|---|---|
| | in pGBKCg | |
| pCSJ684 | MPTLVN-Fbp1$^{1-6}$-T-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ685 | MPTLVN-Fbp1$^{1-6}$-C-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ686 | MPTLVN-Fbp1$^{1-6}$-V-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ687 | MPTLVN-Fbp1$^{1-6}$-L-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ688 | MPTLVN-Fbp1$^{1-6}$-Y-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ689 | MPTLVN-Fbp1$^{1-6}$-W-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ690 | MPTLVN-Fbp1$^{1-6}$-D-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ691 | MPTLVN-Fbp1$^{1-6}$-E-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ692 | MPTLVN-Fbp1$^{1-6}$-N-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ693 | MPTLVN-Fbp1$^{1-6}$-Q-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ694 | MPTLVN-Fbp1$^{1-6}$-H-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ695 | MPTLVN-Fbp1$^{1-6}$-K-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ696 | MPTLVN-Fbp1$^{1-6}$-I-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ697 | MPTLVN-Fbp1$^{1-6}$-M-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ698 | MPTLVN-Fbp1$^{1-6}$-P-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ699 | MPTLVN-Fbp1$^{1-6}$-F-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |
| pCSJ700 | MPTLVN-Fbp1$^{1-6}$-R-$(Gly)_5$-DHFR-Gal4-DBD in pGBKCg | This study |

**Table S3.** PCR primers used in this study.

| Primer | Sequence (5’ to 3’) |
|---|---|
| XW50 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGCCATTTGTTAAGGACTTTAAGCCAC |
| CSJ117 | ATATATGGCGCGCCATGCCAACTCTAGTAAATGGACC |
| CSJ120 | ATATATGGCGCGCCATGTCTACTCTAGTAAATGGACC |
| CSJ121 | ATATATAGATCTCTGTGACTTGCCAATATG |
| CSJ162 | GCAGGATGGGATCCAAAATACAG |
| CSJ163 | ATATATAGATCTCTCGAATTGAGGACCAGCGGC |
| CSJ164 | ATATATGGCGCGCCATGTCCCCTTCTAAAATGAATGC |
| CSJ165 | CTGTATTTTGGATCCCATCCTGC |
| CSJ166 | ATATATGGCGCGCCATGTCCTCTTCTAAAATGAATGCTACAGTAG |

| | |
|---|---|
| CSJ170 | ATATATGGCGCGCCATGCCTCACTCAGTTACACCATCCATAG |
| CSJ171 | ATATATGGATCCAGATGATGCAGATCTCGATGCAACGAATTC |
| CSJ172 | ATATATGGCGCGCCATGTCTCACTCAGTTACACCATCCATAG |
| CSJ225 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGAATATATGGATGACG |
| CSJ226 | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTGTCATCATCATCCTTATAG |
| CSJ227 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAAATGGACC |
| CSJ228 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGTGACTTGCCAATATGGTCTA |
| CSJ229 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCAGACAATTAAGTGTGTTG |
| CSJ230 | CTTGTCATCGTCATCCTTGTAATCGATATCATGATCTTTATAATCACCGTCATGGTCTTT ATAGTCCCCGGGTAGCAGCACACACCTGCGGCTCTTC |
| CSJ231 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTGTCATCGTCATCCTTGTAA |
| CSJ232 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAATAGTGGCCCTGGCCCTG |
| CSJ233 | GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCATCCCATTCATCATCCTCT |
| CSJ237 | ATATATGGCGCGCCATGCCTGTTTCGGAAGCGTTTGCC |
| CSJ238 | CATGATCTTTATAATCACCGTCATGGTCTTTATAGTCGGATCCATAAGAACGACCGAAC ATACAGTAATTGA |
| CSJ242 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTACTCTAGTAAATGGACC |
| CSJ243 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCAAAAGCTGTCGGTATTG |
| CSJ244 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGATCAACTTCTTCAACGGTTGGACC |
| CSJ245 | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTGTCATCATCATCCTTATAG |
| CSJ246 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGAATTACTAGATAGCT |
| CSJ247 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGAAGCATAACAAAACGAACCTTT |
| CSJ248 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATCAATAATCCTAAGGTAG |
| CSJ249 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGAGCAAACTCAAAAGAACAATCAC |
| CSJ250 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCAGAGAAATCAATATTTAATG |
| CSJ251 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGGGTTGGGTACATTTTGATAGAATC |
| CSJ261 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTCACTCAGTTACACCATC |
| CSJ262 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTCACTCAGTTACACCATC |
| CSJ263 | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGATGATGCAGATCTCGATGCAA |
| CSJ264 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTAAAATGAATGC |
| CSJ265 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCTCTTCTAAAATGAATGC |
| CSJ266 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCGAATTGAGGACCAGCGGCTA |
| CSJ267 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTACCTTGTATACTGATAT |
| CSJ268 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTACCTTGTATACTGATAT |
| CSJ269 | GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTGTCCAAAGAGAGATTTATGT |
| CSJ273 | GGGGACCACTTTGTACAAGAAAGCTGGGTCGGCATAGCAAGCGGCTACCATTT |
| CSJ277 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTATCCCCGTTGGAAATAC |
| CSJ278 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTATCCCCGTTGGAAATAC |
| CSJ279 | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTCTTTACGCCATTTTCTTTGA |
| CSJ280 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTTCCTCTAAAATGAATGC |
| CSJ281 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGAGTCCAGATTGCAGAG |
| CSJ282 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGAGTCCAGATTGCAGAGAC |

| | |
|---|---|
| CSJ283 | GGGGACCACTTTGTACAAGAAAGCTGGGTCGAATTCTTGACCAACAGTAGAAA |
| CSJ284 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACGGTGGCTTATTCCCTAGAG |
| CSJ285 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGTTTAACTTTCAAAAGCAGGTCCA |
| CSJ321 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCACACACTAATAAGATCGC |
| CSJ322 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGATTTCTTGAAATTTTCCAGATTT |
| CSJ323 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTATATCTACTCTTAGTAAC |
| CSJ324 | TTTGTCATCATCATCCTTATAGTCAATGTCATGATCCTTGTAATCACCATCATGATCCTT GTAATCCCCGGGGTTTTCGACCCTAGGAACCCCTA |
| CSJ347 | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGCACGTCTGATGGTGTGAGATAC |
| CSJ348 | GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGAACGTCCAACTTCTTTTGCTG |
| CSJ361 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTCGACCATTGAACTGCATC |
| CSJ362 | GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCTTTCTTCTCGTAGACTTC |
| CSJ363 | ATGCCAACTCTAGTAAATGGACCAAGAAGAGACTCTACCGAAGGGTTTGATACCGATA TCGTTCGACCATTGAACTGC |
| CSJ364 | ATGTCTACTCTAGTAAATGGACCAAGAAGAGACTCTACCGAAGGGTTTGATACCGATA TCGTTCGACCATTGAACTGC |
| CSJ375 | ATGCCTCACTCAGTTACACCATCCATAGAACAAGATTCGTTAAAAATTGCCATTTTAGG T GTTCGACCATTGAACTGC |
| CSJ376 | ATGTCTCACTCAGTTACACCATCCATAGAACAAGATTCGTTAAAAATTGCCATTTTAGG T GTTCGACCATTGAACTGC |
| CSJ377 | ATGCCTATCCCCGTTGGAAATACGAAGAACGATTTTGCAGCTTTACAAGCAAAACTAG AT GTTCGACCATTGAACTGC |
| CSJ378 | ATGTCTATCCCCGTTGGAAATACGAAGAACGATTTTGCAGCTTTACAAGCAAAACTAG AT GTTCGACCATTGAACTGC |
| CSJ379 | ATGCCATTTGTTAAGGACTTTAAGCCACAAGCTTTGGGTGACACCAACTTATTCAAACC A GTTCGACCATTGAACTGC |
| CSJ380 | ATGCCAGAGTCCAGATTGCAGAGACTAGCTAATTTGAAAATAGGAACTCCGCAGCAGC TC GTTCGACCATTGAACTGC |
| CSJ381 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTACTCTAGTAAATGGACC |
| CSJ382 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTACTCTAGTAAATGGACC |
| CSJ386 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGGAGGAGGGGG TGGAGTTCGACCATTGAACTGCATC |
| CSJ387 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTACTCTAGTAGGAGGAGGGGGT GGAGTTCGACCATTGAACTGCATC |
| CSJ388 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGGAGGAGGGGGTGGAGG CGGAGTTCGACCATTGAACTGCATC |
| CSJ389 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTACTGGAGGAGGGGGTGGAGG CGGAGTTCGACCATTGAACTGCATC |
| CSJ390 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGGAGGAGGGGGTGGAGGCGG AGGAGTTCGACCATTGAACTGCATC |
| CSJ391 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGGAGGAGGGGGTGGAGGCGG AGGAGTTCGACCATTGAACTGCATC |
| CSJ418 | ATATATATCGATATGCCAACTCTAGTAAATGG |
| CSJ419 | ATATATGTCGACCCACTCTGTGACTTGCCAATATGG |
| CSJ420 | ATATATATCGATATGTCTACTCTAGTAAATGG |
| CSJ440 | ATGTCCCCTTCTAAAATGAATGCTACAGTAGGATCTACTTCCGAAGTTGAACAAAAAA TCGTTCGACCATTGAACTGC |
| CSJ441 | ATGTCCTCTTCTAAAATGAATGCTACAGTAGGATCTACTTCCGAAGTTGAACAAAAAA TCGTTCGACCATTGAACTGC |
| CSJ453 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTTCCTCTAAAATGAATGCTACA GTAG |

| | |
|---|---|
| CSJ454 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTGTAATGAATGCTACA GTAG |
| CSJ455 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTTCCTCTGTAATGAATGCTACA GTAG |
| CSJ460 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGGTCTAGTAGGAGGAGGGGG T |
| CSJ461 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGCTCTAGTAGGAGGAGGGGG T |
| CSJ462 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATCTCTAGTAGGAGGAGGGGG T |
| CSJ463 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATGTCTAGTAGGAGGAGGGGG T |
| CSJ464 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGTTCTAGTAGGAGGAGGGGG T |
| CSJ465 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATTGCTAGTAGGAGGAGGGGG T |
| CSJ466 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATATCTAGTAGGAGGAGGGGG T |
| CSJ467 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATGGCTAGTAGGAGGAGGGGG T |
| CSJ468 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGATCTAGTAGGAGGAGGGGG T |
| CSJ469 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAGAACTAGTAGGAGGAGGGGG T |
| CSJ470 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAAATCTAGTAGGAGGAGGGGG T |
| CSJ471 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCACAACTAGTAGGAGGAGGGGG T |
| CSJ472 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCACATCTAGTAGGAGGAGGGGG T |
| CSJ473 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAAAACTAGTAGGAGGAGGGGG T |
| CSJ474 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGGTGTAGGAGGAGGGGG T |
| CSJ475 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGCTGTAGGAGGAGGGGG T |
| CSJ476 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTTCTGTAGGAGGAGGGGG T |
| CSJ477 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTTGTGTAGGAGGAGGGGG T |
| CSJ478 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGTTGTAGGAGGAGGGGG T |
| CSJ479 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTATTGTAGGAGGAGGGGG T |
| CSJ480 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTTATGTAGGAGGAGGGGG T |
| CSJ481 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTTGGGTAGGAGGAGGGGG T |
| CSJ482 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGATGTAGGAGGAGGGGG T |
| CSJ483 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTGAAGTAGGAGGAGGGGG T |
| CSJ484 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTAATGTAGGAGGAGGGGG T |
| CSJ485 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCAAGTAGGAGGAGGGGG T |

| | |
|---|---|
| CSJ486 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCATGTAGGAGGAGGGGGT |
| CSJ487 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTAAAGTAGGAGGAGGGGGT |
| CSJ488 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCTCCTCTGTAATGAATGCTACAGTAG |
| CSJ494 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGGTGGAGGAGGGGGTGG |
| CSJ495 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGCTGGAGGAGGGGGTGG |
| CSJ496 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATCTGGAGGAGGGGGTGG |
| CSJ497 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATGTGGAGGAGGGGGTGG |
| CSJ498 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATTGGGAGGAGGGGGTGG |
| CSJ499 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAATTGGAGGAGGGGGTGG |
| CSJ500 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATATGGAGGAGGGGGTGG |
| CSJ501 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATGGGGAGGAGGGGGTGG |
| CSJ502 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGATGGAGGAGGGGGTGG |
| CSJ503 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGAAGGAGGAGGGGGTGG |
| CSJ504 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAAATGGAGGAGGGGGTGG |
| CSJ505 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTACAAGGAGGAGGGGGTGG |
| CSJ506 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTACATGGAGGAGGGGGTGG |
| CSJ507 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAAAAGGAGGAGGGGGTGG |
| CSJ508 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTGTTATGAATGCTACAGT |
| CSJ509 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTAAAGGAGGAGGGGGTGG |
| CSJ510 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTGTTGGAGGAGGGGGTGG |
| CSJ512 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAATTCTAGTAGGAGGAGGGGGT |
| CSJ513 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAATGCTAGTAGGAGGAGGGGGT |
| CSJ514 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCACCACTAGTAGGAGGAGGGGGT |
| CSJ515 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCATTTCTAGTAGGAGGAGGGGGT |
| CSJ516 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAAGACTAGTAGGAGGAGGGGGT |
| CSJ517 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTACTGTAGGAGGAGGGGGT |
| CSJ518 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTATGGTAGGAGGAGGGGGT |
| CSJ519 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCCAGTAGGAGGAGGGGGT |
| CSJ520 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTTTTGTAGGAGGAGGGGGT |

| | |
|---|---|
| CSJ521 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTAGAGTAGGAGGAGGGGGT |
| CSJ522 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAACTGGAGGAGGGGGTGG |
| CSJ523 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAATGGGAGGAGGGGGTGG |
| CSJ524 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTACCAGGAGGAGGGGGTGG |
| CSJ525 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTATTTGGAGGAGGGGGTGG |
| CSJ526 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAAGAGGAGGAGGGGGTGG |
| CSJ527 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGGTGGAGGAGGGGGTGG |
| CSJ528 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGCTGGAGGAGGGGGTGG |
| CSJ529 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATCTGGAGGAGGGGGTGG |
| CSJ530 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAACTGGAGGAGGGGGTGG |
| CSJ531 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATGTGGAGGAGGGGGTGG |
| CSJ532 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGTTGGAGGAGGGGGTGG |
| CSJ533 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATTGGGAGGAGGGGGTGG |
| CSJ534 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATATGGAGGAGGGGGTGG |
| CSJ535 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATGGGGAGGAGGGGGTGG |
| CSJ536 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGATGGAGGAGGGGGTGG |
| CSJ537 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAGAAGGAGGAGGGGGTGG |
| CSJ538 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAAATGGAGGAGGGGGTGG |
| CSJ539 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTACAAGGAGGAGGGGGTGG |
| CSJ540 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTACATGGAGGAGGGGGTGG |
| CSJ541 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAAAAGGAGGAGGGGGTGG |
| CSJ542 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAATTGGAGGAGGGGGTGG |
| CSJ543 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAATGGGAGGAGGGGGTGG |
| CSJ544 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTACCAGGAGGAGGGGGTGG |
| CSJ545 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTATTTGGAGGAGGGGGTGG |
| CSJ546 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACTCTAGTAAGAGGAGGAGGGGGTGG |
| CSJ547 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTCACTCAGTTGGAGGAGGGGGT |
| CSJ548 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTCACTCAGTTGGAGGAGGGGGT |
| CSJ549 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCTATCCCCGTTGGAGGAGGGGGT |
| CSJ550 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTATCCCCGTTGGAGGAGGGGGT |

| | |
|---|---|
| CSJ553 | ATGTCCCCTTCTGTTATGAATGCTGGAGGAGTTCGACCATTGAACTGC |
| CSJ554 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCCCTTCTGTTATGAATG |
| CSJ564 | GGTCTTCTCGAGGAAAAATCAGTAGAAATAG |
| CSJ595 | ATATATACTAGTTTCATGTCCTCTTCTGTTATGAATGCTGGAGGA |
| CSJ596 | ATATATACTAGTTTCATGCCATCTTCTGTTATGAATGCTGGAGGA |
| CSJ597 | ATATATACTAGTTTCATGGCTCCTTCTGTTATGAATGCTGGAGGA |
| CSJ598 | ATATATACTAGTTTCATGACTCCTTCTGTTATGAATGCTGGAGGA |
| CSJ599 | ATATATACTAGTTTCATGCCACCTTCTGTTATGAATGCTGGAGGA |
| CSJ600 | ATATATACTAGTTTCATGGGTTCCCCTTCTGTTATGAATGCTGGAGGA |
| CSJ601 | ATATATACTAGTTTCATGGCTTCCCCTTCTGTTATGAATGCTGGAGGA |
| CSJ602 | ATATATACTAGTTTCATGTCTTCCCCTTCTGTTATGAATGCTGGAGGA |
| CSJ603 | ATATATACTAGTTTCATGACTTCCCCTTCTGTTATGAATGCTGGAGGA |
| CSJ604 | ATATATACTAGTTTCATGTCCCCTTTGGTTATGAATGCTGGAGGAGTTC |
| CSJ605 | ATATATACTAGTTTCATGCCATCTTTGGTTATGAATGCTGGAGGAGTTC |
| CSJ606 | ATATATACTAGTTTCATGGGTACTCTAGTAGGAGGAGGGGGTGGAG |
| CSJ607 | ATATATACTAGTTTCATGGTTACTCTAGTAGGAGGAGGGGGTGGAG |
| CSJ608 | ATATATACTAGTTTCATGTGTACTCTAGTAGGAGGAGGGGGTGGAG |
| CSJ616 | ATATATACTAGTTTCATGCCAACTCTAGTAAATGGTGGAGGAGGGGGTGGAGTTC |
| CSJ617 | ATATATACTAGTTTCATGCCAACTCTAGTAAATGCTGGAGGAGGGGGTGGAGTTC |
| CSJ618 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTCTGGAGGAGGGGGTGGAGTTC |
| CSJ619 | ATATATACTAGTTTCATGCCAACTCTAGTAAATACTGGAGGAGGGGGTGGAGTTC |
| CSJ620 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTGTGGAGGAGGGGGTGGAGTTC |
| CSJ621 | ATATATACTAGTTTCATGCCAACTCTAGTAAATGTTGGAGGAGGGGGTGGAGTTC |
| CSJ622 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTTGGGAGGAGGGGGTGGAGTTC |
| CSJ623 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTATGGAGGAGGGGGTGGAGTTC |
| CSJ624 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTGGGGAGGAGGGGGTGGAGTTC |
| CSJ625 | ATATATACTAGTTTCATGCCAACTCTAGTAAATGATGGAGGAGGGGGTGGAGTTC |
| CSJ626 | ATATATACTAGTTTCATGCCAACTCTAGTAAATGAAGGAGGAGGGGGTGGAGTTC |
| CSJ627 | ATATATACTAGTTTCATGCCAACTCTAGTAAATAATGGAGGAGGGGGTGGAGTTC |
| CSJ628 | ATATATACTAGTTTCATGCCAACTCTAGTAAATCAAGGAGGAGGGGGTGGAG |
| CSJ629 | ATATATACTAGTTTCATGCCAACTCTAGTAAATCATGGAGGAGGGGGTGGAGTTC |
| CSJ630 | ATATATACTAGTTTCATGCCAACTCTAGTAAATAAAGGAGGAGGGGGTGGAGTTC |
| CSJ631 | ATATATACTAGTTTCATGCCAACTCTAGTAAATATTGGAGGAGGGGGTGGAGTTC |
| CSJ632 | ATATATACTAGTTTCATGCCAACTCTAGTAAATATGGGAGGAGGGGGTGGAGTTC |
| CSJ633 | ATATATACTAGTTTCATGCCAACTCTAGTAAATCCAGGAGGAGGGGGTGGAGTTC |
| CSJ634 | ATATATACTAGTTTCATGCCAACTCTAGTAAATTTTGGAGGAGGGGGTGGAGTTC |
| CSJ635 | ATATATACTAGTTTCATGCCAACTCTAGTAAATAGAGGAGGAGGGGGTGGAGTTC |

## References


1. F. M. Ausubel *et al.*, *Current Protocols in Molecular Biology.*, (Wiley-Interscience, New York, 2010).
2. F. Sherman, Getting started with yeast. *Meth. Enzymol.* **194**, 3-21 (1991).
3. C. S. Hwang, A. Shemorry, A. Varshavsky, N-terminal acetylation of cellular proteins creates specific degradation signals. *Science* **327**, 973-977 (2010).

4. A. Shemorry, C. S. Hwang, A. Varshavsky, Control of protein quality and stoichiometries by N-terminal acetylation and the N-end rule pathway. *Mol. Cell* **50**, 540-551 (2013).
5. S. E. Park *et al.*, Control of mammalian G protein signaling by N-terminal acetylation and the N-end rule pathway. *Science* **347**, 1249-1252 (2015).
6. T. Stellberger *et al.*, Improving the yeast two-hybrid system with permutated fusions proteins: the Varicella Zoster Virus interactome. *Proteome Sci.* **8**, 8 (2010).
7. S. J. Chen, G. Lin, K. J. Chang, L. S. Yeh, C. C. Wang, Translational efficiency of a non-AUG initiation codon is significantly affected by its sequence context in yeast. . *J. Biol. Chem.* **283**, 3173-3180 (2008).
8. A. Bruckner, C. Polge, N. Lentze, D. Auerbach, U. Schlattner, Yeast two-hybrid, a powerful tool for systems biology. *Int. J. Mol. Sci.* **10**, 2763-2788 (2009).
9. N. Johnsson, A. Varshavsky, Split ubiquitin as a sensor of protein interactions in vivo. *Proc. Natl. Acad. Sci. USA* **91**, 10340-10344 (1994).
10. S. Wittke, N. Lewke, S. Müller, N. Johnsson, Probing the molecular environment of membrane proteins in vivo. *Mol. Biol. Cell* **10**, 2519-2530 (1999).
11. S. Wittke, M. Dunnwald, M. Albertsen, N. Johnsson, Recognition of a subset of signal sequences by Ssh1p, a Sec61p-related protein in the membrane of endoplasmic reticulum of yeast Saccharomyces cerevisiae. *Mol. Biol. Cell* **13**, 2223-2232 (2002).
12. J. Müller, N. Johnsson, Split-ubiquitin and the split-protein sensors: chessman for the endgame. *Chembiochem.* **9**, 2029-2038 (2008).
13. A. Dunkler, J. Muller, N. Johnsson, Detecting protein-protein interactions with the split-ubiquitin sensor. *Methods Mol. Biol.* **786**, 115-130 (2012).
14. D. G. Fraenkel, *Yeast Intermediary Metabolism*. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2011).
15. B. K. Han, S. D. Emr, The phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2)-dependent Tup1 conversion (PIPTC) regulates metabolic reprogramming from glycolysis to gluconeogenesis. *J. Biol. Chem.* **288**, 20633-20645 (2013).
16. L. Rui, Energy metabolism in the liver. *Compr. Physiol.* **4**, 177-197 (2014).
17. J. Wahren, K. Ekberg, Splanchnik regulation of glucose production. *Annu. Rev. Nutr.* **27**, 329-345 (2007).
18. L. Tian, D. L. Nelson, D. M. Stewart, Cdc42-interacting protein 4 mediates binding of the Wiskott-Aldrich syndrome protein to microtubules. *J. Biol. Chem.* **275**, 7854-7861 (2000).
19. Q. Xiao, F. Zhang, B. A. Nacev, J. O. Liu, D. Pei, Protein N-terminal processing: substrate specificity of Escherichia coli and human methionine aminopeptidases. *Biochemistry* **49**, 5588-5599 (2010).