Figure S1

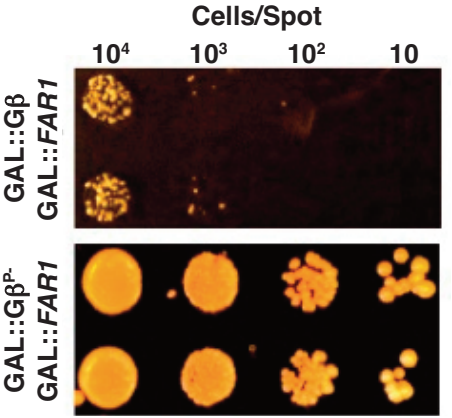
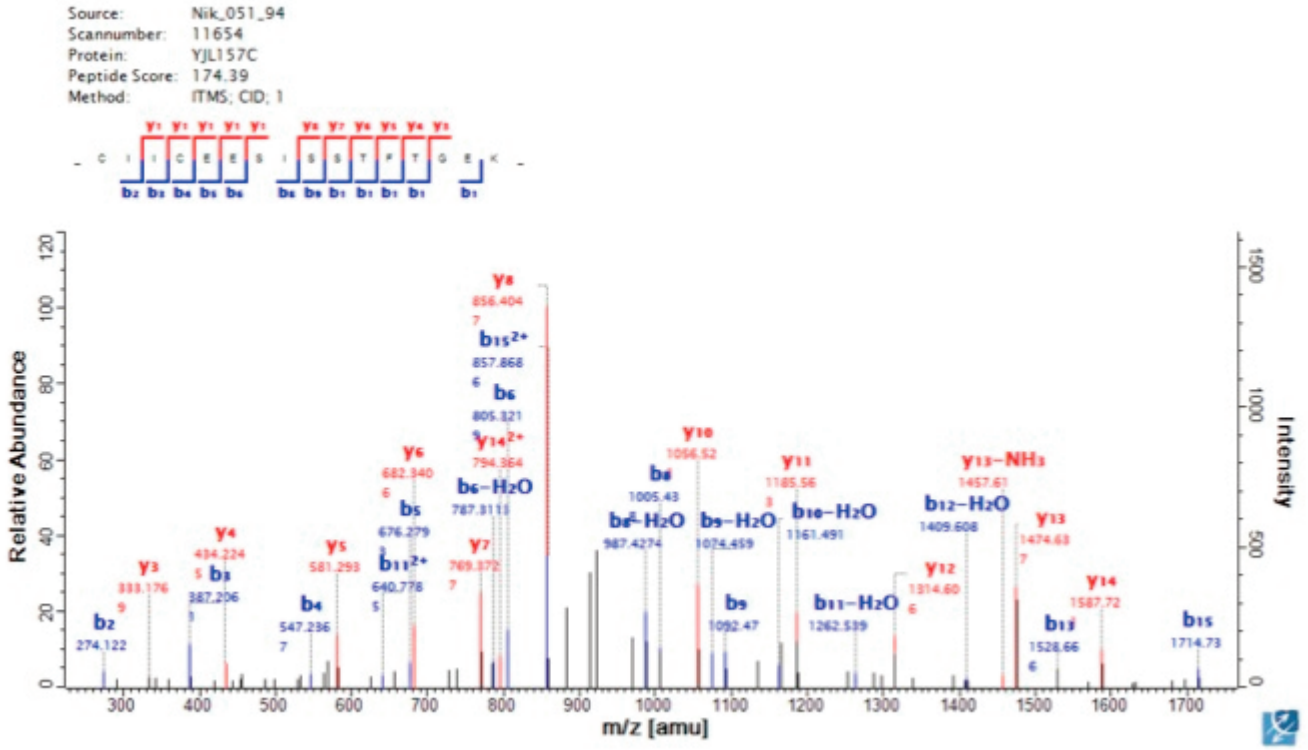


Figure S1. Gβ overexpression genetic assay. 15Dau *bar1*Δ cultures co-transformed with pESC/GAL1-FAR1 and either YCplac111/GAL1-STE4 or YCplac111/GAL1- STE4$^{T320A\ S335A}$ were grown to mid-log phase (~10^7 cells/ml) in selective sucrose medium. 10-fold serial dilutions from 10^5 to 1 were then spotted on selective galactose medium (to induce the overexpression of Far1, and Gβ or Gβ^{P-}) and selective glucose medium (to repress *GAL1*-driven gene expression), and incubated at 30˚C for 48 hr. Ten transformants of each strain were tested. Results for two representative strains of each type are shown. Far1 overexpression rescued overexpression of Gβ^{P-} but not of Gβ. All strains grew similarly on glucose-containing medium.

Figure S2

A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Scannumber: 11654
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Peptide Score: 174.39
Method: ITMS; CID; 1
Relative Abundance
Intensity
m/z [amu]

B

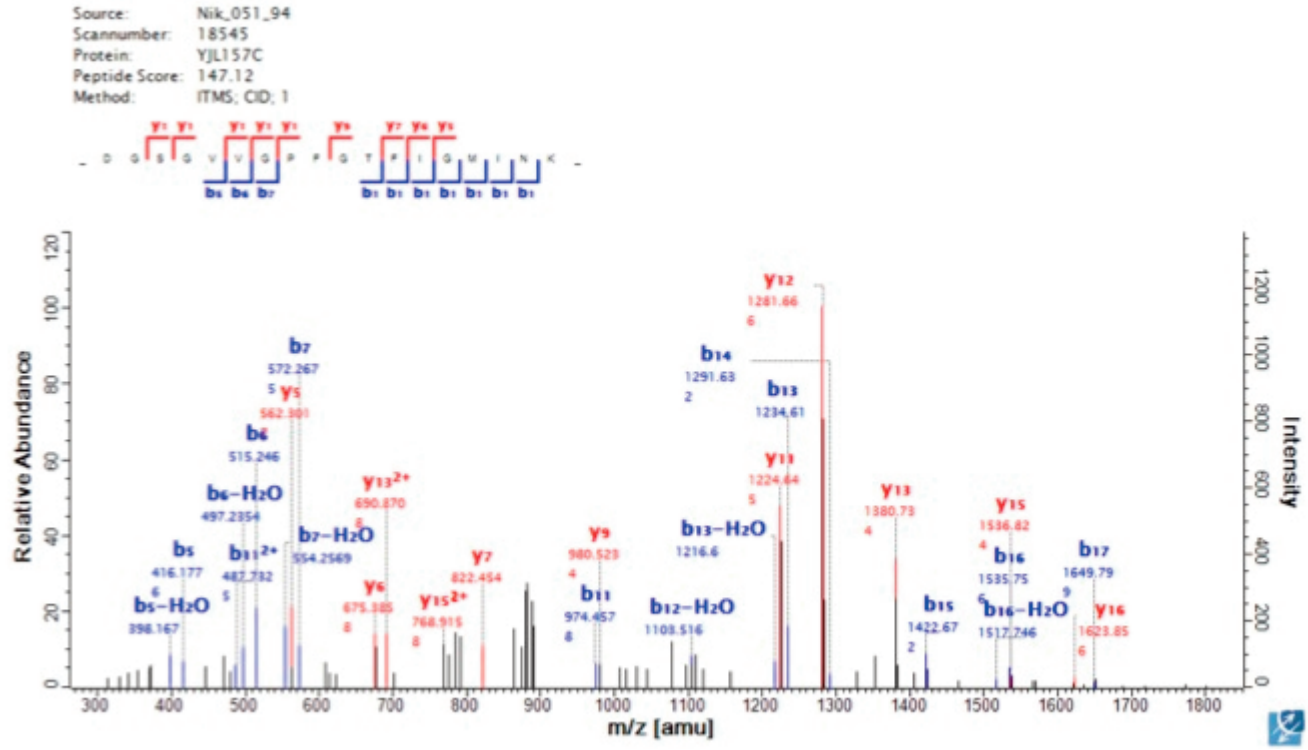
Source: Nik_051_94
Scannumber: 18545
Protein: YJL157C
Peptide Score: 147.12
Method: ITMS; CID; 1
Relative Abundance
Intensity
m/z [amu]

Figure S1. Detection of Far1 in the $G\beta^{P}\gamma$ pull-down. Pull-downs were performed as described in Cismowski et al. (2001). The proteins eluted from the Ni-NTA beads were separated by SDS-PAGE, digested by trypsin, and analyzed by nano-scale LC-MS/MS as previously described (Alldridge et al., 2008; Metodiev, 2011). The LTQ/Orbitrap Velos was operated in data-dependent mode where the high-resolution Orbitrap analyzer first executed two full scans at a resolution of 30,000 (at 400 m/z), followed by 20 MS/MS scans in the LTQ Velos analyzer to isolate and sequence the 20 most abundant peptide ions (Greenwood et al., 2012). Internal mass calibration was maintained using a lock mass ion of 445.12 m/z. Raw MS/MS data were analyzed by MaxQuant and X! Tandem as described previously (Greenwood et al., 2012; Cox and Mann, 2008; Cox et al., 2011). Each sample was analysed in triplicate by LC-MS/MS. Two high-scoring MS/MS spectra from LC-MS/MS runs analyzing the 94kDa fraction of the $G\beta^{P}\gamma$ pull-down sample are shown. (A) An MS/MS spectrum matching a peptide with sequence **CIICEESISSTFTGEK** from Far1. Andromeda, the search engine of MaxQuant, matched the spectrum to the peptide with a score of 174 and peptide expectation value (PEP) of 1.54E-06. (B) An MS/MS spectrum matching a peptide with sequence **DGSGVVGPFGTFIGMINK** from Far1. Andromeda matched the spectrum to the peptide with a score of 147 and PEP of 3.32E-08. The sequence-specific y and b fragment ion series are shown in red and blue as assigned by MaxQuant. For statistical analysis of spectral count data, the MS/MS data were filtered at 1% FDR at both the peptide and protein levels, and the filtered data was used to quantify the proteins using the spectral counting approach. The significance of the obtained differences in spectral counts reported by MaxQuant or X!Tandem were analyzed by the G-test (Greenwood et al. 2012), which does not require the data to be distributed normally (Heinecke, et al., 2010). Far1 was detected with a total of 19 spectral counts (7, 6, and 6 in the three separate replicates) and with 0 counts in the $G\beta^{P}\gamma$ and WT pull-downs, respectively. If the affinities of $G\beta^{P}\gamma$ and $G\beta\gamma$ for Far1 were similar, the total counts expected for Far1 in the WT pull-down would be 14 rather than 0 after normalizing for the amount of $G\beta\gamma$ on the beads ($p = 0.00013$).

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Supplementary Table 1. Yeast strains used in this study

Strain	Genotype	Source
DSY257	*MAT***a** *bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ*	Stone lab
ELY104	*MAT***a** *bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ ste4::URA3*	Stone lab
RDY114	*MAT***a** *ste4$^{T320A\ S335A}$ bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ*	This study
RDY103	*MAT***a** *bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ*	This study
RDY120	*MAT***a** *ste4$^{T320A\ S335A}$ bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ*	This study
MMY110	*MAT***a** *gpa1Δ::URA3 bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ* YCplac22/*gpa1$^{K21E\ R22E}$*	This study
MMY111	*MAT***a** *gpa1Δ::URA3 bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ* YCplac22/*GPA1*	This study
	*MAT***a** *gpa1Δ::URA3 bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ* YCplac22/*gpa1$^{K21E\ R22E}$*	This study
RDY130	*MAT***a** *ste4::URA3 GFP-STE4::ura3 bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1 ura3*	This study
RDY132	*MAT***a** *ste4::URA3 GFP- ste4$^{T320A\ S335A}$::ura3 bud1Δ::KAN bar1Δ ade1 his2 leu2-3, 112 trp1 ura3Δ*	This study
RDY126	*MAT***a** *ste4::URA3 GFP-STE4::ura3 bar1Δ ade1 his2 leu2-3, 112 trp1 ura3*	This study
RDY139	*MAT***a** *ste4::URA3 GFP- ste4$^{T320A\ S335A}$::ura3 bar1Δ ade1 his2 leu2-3, 112 trp1 ura3Δ*	This study
DSY246	*MATα bar1Δ ade1 his2 leu2-3, 112 trp1-1a ura3Δ*	Stone lab

RDY217	*MAT*α *ste4*$^{T320A\ S335A}$ *bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ	This study
RDY246	*MAT***a** *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS406/SPA2-GFP	This study
RDY247	*MAT***a** *ste4*$^{T320A\ S335A}$ *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS406/SPA2-GFP	This study
RDY259	*MAT***a** *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS424/GFP-CDC42	This study
RDY260	*MAT***a** *ste4*$^{T320A\ S335A}$ *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS424/GFP-CDC42	This study
EAY106	*MAT***a** *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS304/STE2$^{7XR/GPAAD}$	This study
EAY107	*MAT***a** *ste4*$^{T320A\ S335A}$ *bud1*Δ::*KAN bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ pRS304/STE2$^{7XR/GPAAD}$	This study
ODY111	*MAT***a** *bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ YCplac111/GAL1-Ste4 pESC /GAL10-FLAG-Far1	This study
ODY113	*MATa bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ YCplac111/GAL1- ste4$^{T320A\ S335A}$ pESC /GAL10-FLAG-Far1	This study
NWY051	*MAT***a** *bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ *ste18*Δ::*URA3 lys1*Δ::KanMX *arg5*/*arg6*Δ::G418 YCplac111/GAL1- ste4$^{T320A\ S335A}$	This study
NWY052	*MAT***a** *bar1*Δ *ade1 his2 leu2-3, 112 trp1-1a ura3*Δ *ste18*Δ::*URA3 lys1*Δ::KanMX *arg5*/*arg6*Δ::G418 YCplac111/GAL1-Ste4	This study

Strain construction: All strains used in this study were derived from strain 15Dau (*MAT***a** *bar1*D *ade1 his2 leu2-3*, *-112 trp1 ura3*D), which is congenic with strain BF264-15D (Reed et al. 1985). RDY114 was generated by *in situ* transplacement of *ste4*Δ::*URA3* in strain ELY104 (Li et al., 1998b) with $ste4^{T320A\ S335A}$, excised as an *Eco*RI-*Sph*I fragment from the plasmid YCplac33/ $ste4^{T320A\ S335A}$ (Li et al., 1998a). Recombinants were selected on 5′FOA and confirmed by sequencing. The *BUD1/RSR1* locus was deleted in strains 15Dau *bar1*Δ and RDY114 to create strains RDY103 and RDY120, respectively, using a *bud1*Δ*::KANMX4* cassette which was PCR-amplified from pFA6a-Kan (Wach, 1996) using the oligomers 5′- GCGCATTCATCCTCGACATTCTCAAACGCGAAATATCGTCGAACGTACGCTGCAG GTCGACGG - 3′ and 5′- GTTGTGAAGTAGCGCTAATTCCTGTCCTGTTGCTAGAACCAGATA TCGATGAATTCGAGCTCG - 3′. GFP-tagging was performed *in situ* by transplacement of an excised *Eco*RI-*Sma*I fragment from pRS316/*STE4*p-*GFP-STE4* (Kim et al., 2000) or pRS316/*STE4*p-$GFP\text{-}ste4^{T320A\ S335A}$ (RDB122, see construction below) into strain ELY104 to create strains RDY126 and RDY139, respectively. The *BUD1/RSR1* locus was deleted as described above in strains RDY126 and RDY139 to create strains RDY130 and RDY132, respectively. RDY114 was transformed with pGAL-HO and the mating type was switched to generate RDY217 as described (Guthrie and Fink, 2002). Strains EAY106 and EAY107 were created by transforming pRS304/$STE2^{7XR/GPAAD}$ cut with BsmI into strains RDY103 and RDY120, respectively, and confirmed by sequencing.

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Supplementary Table 2. Plasmids used in this study

Plasmid no	Plasmid name	Marker/Type	Source
DSB159	YCplac22/GPA1	TRP1/CEN	(Stratton et al., 1996)
MMB104	YCplac22/gpa1$^{K21E\ R22E}$	TRP1/CEN	(Metodiev et al., 2002)
RDB116	YCplac33/ste4$^{T320A\ S335A}$	URA3/CEN	(Li et al., 1998a)
BLT49	pRS316/GFP-Ste4	URA3/CEN	(Kim et al., 2000)
RDB122	pRS316/GFP- ste4$^{T320A\ S335A}$	URA3/CEN	This study
RDB151	pRS406/Spa2-GFP	URA3/INT	(Arkowitz and Lowe, 1997)
DLB2823	pRS305/Bem1-GFP-Snc2	LEU2/INT	(Howell et al., 2009)
	pRS424/GFP-Cdc42	URA3/2µm	(Barale et al., 2006)
DLB3217	pRS304/STE2$^{7XR/GPAAD}$	TRP1/INT	Lew lab
MCB26	YCplac111/GAL1-Ste4	LEU2/CEN	(Cismowski et al., 2001)
RDB131	YCplac111/GAL1- ste4$^{T320A\ S335A}$	LEU2/CEN	This study
pEB15.1	pESC /GAL10-FLAG-Far1	URA3/2µm	This study

Plasmid construction: The plasmids used in this study are listed in Table S2. RDB122 was created by sequential site-directed mutagenesis of pRS316/*STE4*p-*GFP-STE4*, and YCplac111/GAL1- STE4$^{T320A\ S335A}$ was created by sequential site-directed mutagenesis of YCplac111/GAL1- STE4, using QuikChange II XL kit (Qiagen). The oligomers used to create the T320A mutation were
5′- CGAGGTTATGAAGAACGTACCCCTGCCCCTACTTATATGGCAGC - 3′and
5′- GCTGCCATATAAGTAGGGGCAGGGGTACGTTCTTCATAACCTCG - 3′. The oligomers used to create the sequential S335A mutation were
5′ - GGAGTACAATACCGCGCAAGCGCCACAAACTTTAAAATCAAC -3′ and
5′ - GTTGATTTTAAAGTTTGTGGCGCTTGCGCGGTATTGTACTCC - 3′. To create

pEB15.1 (pESC/GAL10-FLAG-FAR1), *FAR1* was PCR-amplified from strain15Dau genomic DNA and the product was cloned into pESC-URA as a PacI-BglII fragment, thereby placing *FAR1* under *GAL10* promoter control. The priming oligonucleotides were:

5′ - CCTTAATTAAGCGTAGTATAGACGTGGAG - 3′ and

5′ - GAAGATCTTGAAGACACCAACAAGAGTTTCG - 3′.

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