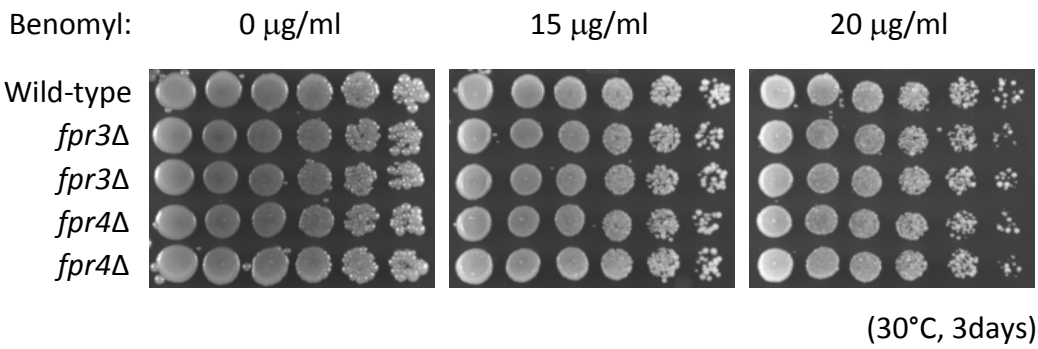

Figure S1 *fpr3*Δ or *fpr4*Δ cells don't show any benomyl sensitivity. Yeast cells were spotted in 5-fold dilutions from 5 x 10^4 cells per spot on YPD plates containing benomyl. The plates were incubated at 30°C for 3 days and photographed. Isogenic yeast strains were wild-type (YPH499), *fpr3*Δ (Y2243 and Y2244), and *fpr4*Δ (Y2245 and Y2246).

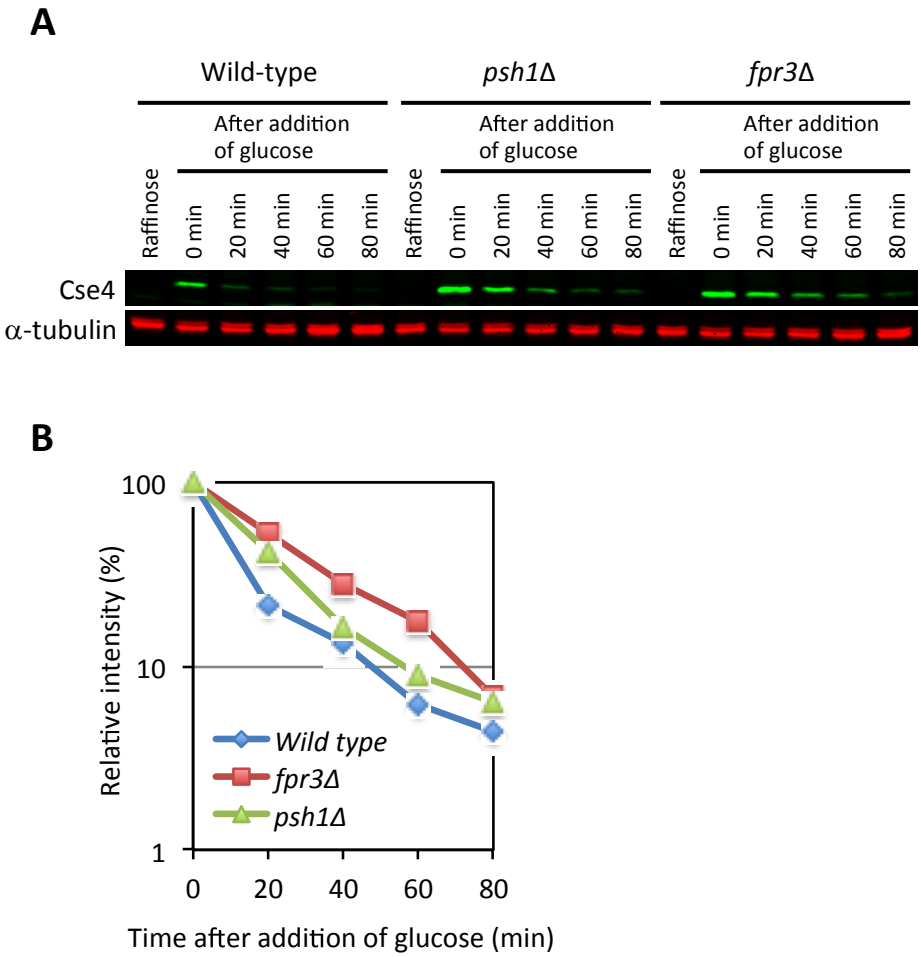

Figure S2 The stabilization level of Cse4 in *fpr3*Δ cells is higher than that in *psh1*Δ cells. The protein stability assay was performed as described in Figure 2. Isogenic yeast strains were wild-type (Y2255), *fpr3*Δ (Y2256), and *psh1*Δ (Y2258).

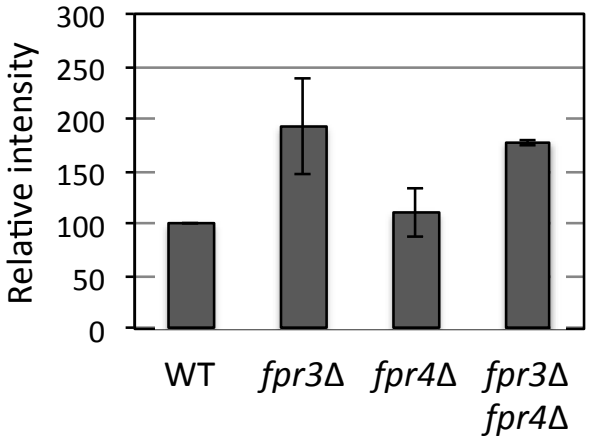

Figure S3 Enhanced *CEN* DNA interaction with Cse4 in *fpr3*Δ mutant. The indicated strains, Cse4-myc (Y2342), Cse4-myc *fpr3*Δ (Y2344), Cse4-myc *fpr4*Δ (Y2345), and Cse4-myc *fpr3*Δ *fpr4*Δ (Y2346), were grown to log phase, lysed, and anti-myc chromatin immunoprecipitation assay was performed as previously described (OHKUNI *et al.* 2008). We used ImageJ to quantify the signals.

File S1

Supplemental methods

Yeast strains and plasmids

Supplemental Table S1 and S2 present the genotype of yeast strains and plasmids used for this study, respectively.

Protein stability assay

Cells were grown in a 2% raffinose synthetic complete medium overnight. Cells were then diluted to an optical density at 600 nm of 0.3 and grown for an additional 3 h at 25°C. A sample was removed as a negative control before the addition of galactose (Raffinose). Galactose was added to the media to a final concentration of 2% to induce expression of Cse4 from the *GAL1* promoter for 2 h at 25°C. Glucose was then added to a final concentration of 5% to stop gene expression, and samples were taken at the indicated time points.

Antibodies

Anti-Cse4 antibody was generated as previously described (OHKUNI *et al.* 2008). Anti-Cdc28 was a gift from Dr. Deshaies's laboratory. Anti-myc (Roche, Indianapolis, IN), anti-HA (Roche, Indianapolis, IN), and anti-tubulin (Serotech, Oxford, UK) antibodies were purchased.

Western blotting and quantitation

Western blotting and quantitation were performed as described previously (ESCAMILLA-POWERS and SEARS 2007). In brief, protein from equal cell numbers were separated by SDS-PAGE gel and transferred to Immobilon-FL membrane (Millipore, Billerica, MA). Membrane was blocked with Odyssey Blocking buffer (LI-COR Biosciences, Lincoln, NE) after shaking the membrane in PBS for several minutes. Primary and secondary antibodies were diluted in Odyssey Blocking buffer with 0.1% Tween 20. Anti-Cse4 was used at a dilution of 1:1000, and anti-tubulin and anti-Cdc28 were used at 1:5000. Secondary antibodies were used at a dilution of 1:15000. Blots were scanned with a LI-COR Odyssey CLx Imager (Lincoln, NE). Protein levels at each time point were quantitated using Image Studio software version 2.0.

Table S1 ***S. cerevisiae*** **strains used in this study**

Strains	Genotype	Reference
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1*	*(SIKORSKI and HIETER 1989)*
YPH500	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1*	*(SIKORSKI and HIETER 1989)*
Y2243	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6*	This study
Y2244	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6*	This study
Y2245	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6*	This study
Y2246	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6*	This study
Y2247	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y2248	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y14	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11*	P. Hieter
Y2249	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2250	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2251	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2252	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::His3MX6*	This study
Y2253	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::kanMX6*	This study
Y2254	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::kanMX6*	This study
Y2255	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2256	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2257	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2258	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 psh1Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study

Y2340	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 psh1Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2259	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*FPR3*]	This study
Y2260	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416]	This study
Y2261	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*fpr3 W363L*]	This study
Y2262	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*fpr3 F402Y*]	This study
Y2263	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P53V*]	This study
Y2264	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P98V*]	This study
Y2265	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P100V*]	This study
Y2266	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P134V*]	This study
Y2267	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P157V*]	This study
Y2280	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1*	This study
Y2281	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr3Δ::kanMX6*	This study
Y2282	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr4Δ::kanMX6*	This study
Y2283	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y2284	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 cse4Δ::TRP1* [pRS415-*CSE4*]	This study
Y2285	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 cse4Δ::TRP1* [pRS415-*cse4 P134V*]	This study
Y2342	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6*	This study
Y2344	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr3Δ::kanMX6*	This study
Y2345	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr4Δ::kanMX6*	This study

Y2346	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study

Table S2 Plasmids used in this study

Plasmid	Relevant characteristics	Reference
B2883	pRS415-*PGAL1-3HA-CSE4*	This study
B2884	pRS416-*FPR3*	This study
B2885	pRS416-*fpr3 W363L*	This study
B2886	pRS416-*fpr3 F402Y*	This study
B2887	pRS415-*CSE4*	This study
B2891	pRS415-*cse4 P134V*	This study
B2893	pRS415-*PGAL1-3HA-cse4 P53V*	This study
B2894	pRS415-*PGAL1-3HA-cse4 P98V*	This study
B2895	pRS415-*PGAL1-3HA-cse4 P100V*	This study
B2896	pRS415-*PGAL1-3HA-cse4 P134V*	This study
B2897	pRS415-*PGAL1-3HA-cse4 P157V*	This study

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