Hovsepian et al., https://doi.org/10.1083/jcb.201610094

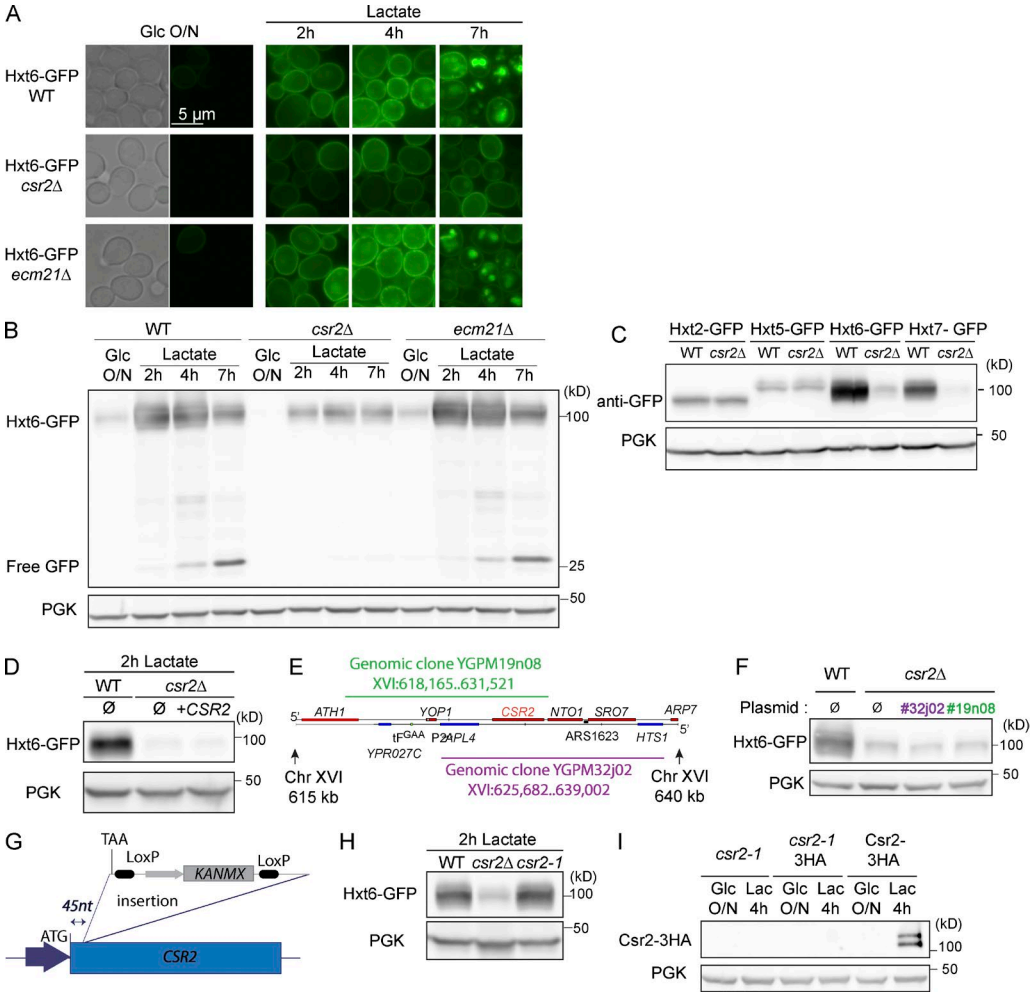


Figure S1. **Requirement of Csr2 for Hxt6 endocytosis after glucose removal, and rationale for the construction of the *csr2-1* mutant.** (A) Hxt6-GFP endocytosis in lactate-containing medium requires Csr2, but not its close paralogue Ecm21. Hxt6-GFP-expressing WT (ySL1184), *csr2*Δ (ySL1185), and *ecm21*Δ (ySL1650) cells were grown overnight in glucose medium (exponential phase) and were switched to lactate-containing medium. Cells were imaged for Hxt6 localization at the indicated times. Hxt6-GFP endocytosis occurs in an *ecm21*Δ mutant but not in *csr2*Δ cells. Note that Hxt6-GFP is expressed at a lower level in the *csr2*Δ strain as compared with WT cells. (B) From the experiment depicted in A, immunoblots were realized on total cell lysates with the indicated antibodies (PGK was used as a loading control). Note that Hxt6-GFP is expressed at a lower level in the *csr2*Δ strain compared with WT cells. (C) Hxt7-GFP is also expressed at a much lower level in the *csr2*Δ strain compared with WT cells. WT and *csr2*Δ cells expressing Hxt6-GFP (ySL1184 and ySL1185), Hxt7-GFP (ySL1551 and ySL1552), Hxt2-GFP (ySL1140 and ySL1654), or Hxt5-GFP (ySL1145 and ySL1653) were grown overnight in glucose medium (exponential phase) and switched to lactate-containing medium for 2 h. Total cell lysates were prepared at the indicated times and immunoblotted with anti-GFP and anti-PGK (loading control) antibodies. (D) The defect in Hxt6-GFP expression observed in the *csr2*Δ mutant is not complemented by a plasmid containing the *CSR2* gene. Hxt6-GFP-expressing WT (ySL1345), *csr2*Δ (ySL1316), or *csr2*Δ cells carrying a plasmid-encoded, unmodified *CSR2* gene (−500/+300; ySL1670) were grown overnight in glucose medium (exponential phase) and switched to lactate-containing medium for 2 h. Total cell lysates were immunoblotted with anti-GFP and anti-PGK (loading control) antibodies. (E) Schematic of the genomic locus around *CSR2*. The regions covered by two genomic clones, YGPM19n08 and YGPM32j02 (from the yeast genomic tiling collection; GE Healthcare) are represented in green and blue, respectively. (F) The defective expression of Hxt6-GFP observed in the *csr2*Δ mutant is not complemented by the genomic clones tested. Hxt6-GFP-expressing WT (ySL1345), *csr2*Δ (ySL1316), or *csr2*Δ cells carrying genomic clones indicated in E (YGPM32j02 or YGPM19n08: ySL1692 and ySL1693) were grown overnight in glucose medium (exponential phase) and switched to lactate-containing medium for 2 h. Total cell lysates were immunoblotted with anti-GFP and anti-PGK (loading control) antibodies. (G) Schematic of the strategy used to generate the *csr2-1* allele. A cassette containing a KanMx cassette, LoxP excision sites, and a stop codon in its 5′ was integrated 45 bp away from the start site of *CSR2*. Note that the cassette was not excised and thus is still present in the *csr2-1* mutant. (H) Hxt6-GFP expression is not altered in the *csr2-1* mutant. WT (ySL1184), *csr2*Δ (ySL1185), and *csr2-1* (ySL1706) cells expressing Hxt6-GFP were grown overnight in glucose medium (exponential phase) and were switched to lactate-containing medium for 2 h. Total cell lysates were prepared at this time and immunoblotted with anti-GFP and anti-PGK (loading control) antibodies. (I) Csr2 is not expressed in the disrupted mutant *csr2-1*. A 3HA-tagging cassette was introduced in 3′ of the *CSR2* ORF, in both the WT and the *csr2-1* genomic context. In both cases, the correct and in-frame insertion of the cassette with the *CSR2* ORF was verified by PCR and sequencing. The indicated strains (*csr2-1*: ySL1706; *csr2-1*-3HA: ySL1717; Csr2-3HA: ySL1037) were grown in lactate medium for 4 h, and total cell lysates were immunoblotted with anti-HA and anti-PGK (loading control) antibodies.

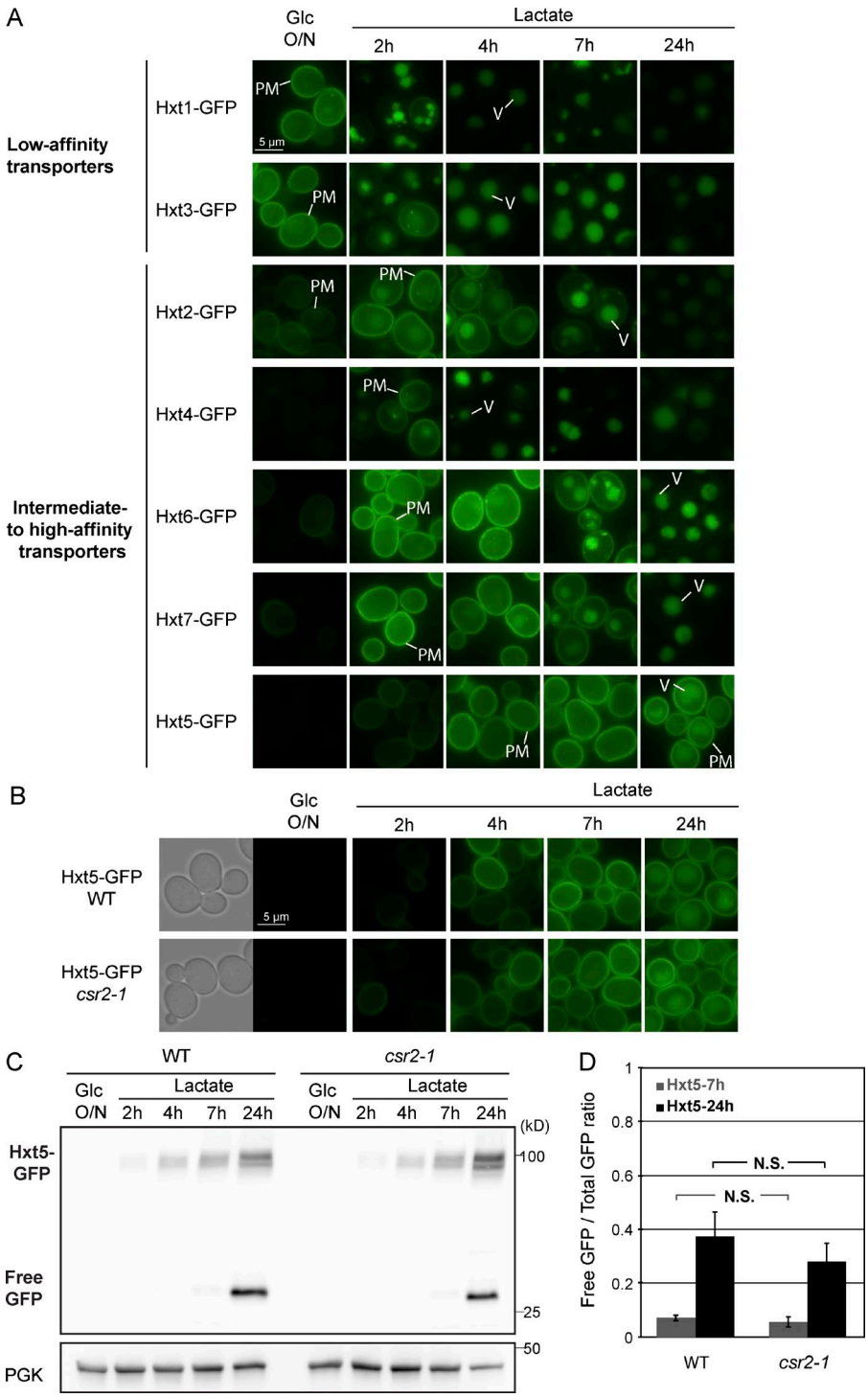


Figure S2. **Cargo selectivity of the ART Csr2 during endocytosis in the absence of glucose.** (A) Comparison of the expression and localization of seven hexose transporters (Hxt1 to Hxt7) fused to GFP. Cells were grown overnight in glucose medium (exponential phase) and then switched to lactate-containing medium and imaged at the indicated times. All hexose transporter genes were tagged at the chromosomal locus by homologous recombination, and their expression was driven by their endogenous promoter (Hxt1-GFP: ySL1186; Hxt2-GFP: ySL1140; Hxt3-GFP: ySL1027; Hxt4-GFP: ySL1852; Hxt5-GFP: ySL1145; Hxt6-GFP: ySL1184; Hxt7-GFP: ySL1551). (B) WT (ySL1145) and *csr2-1* cells (ySL2065) expressing Hxt5-GFP were grown overnight in glucose medium and switched to lactate medium for the indicated time and images by fluorescence microscopy. (C) From the experiment presented in B, immunoblots were realized from total cell lysates using anti-GFP and anti-PGK antibodies. (D) GFP signals were quantified (± SEM) at 7- and 24-h time points (n = 3 independent experiments).

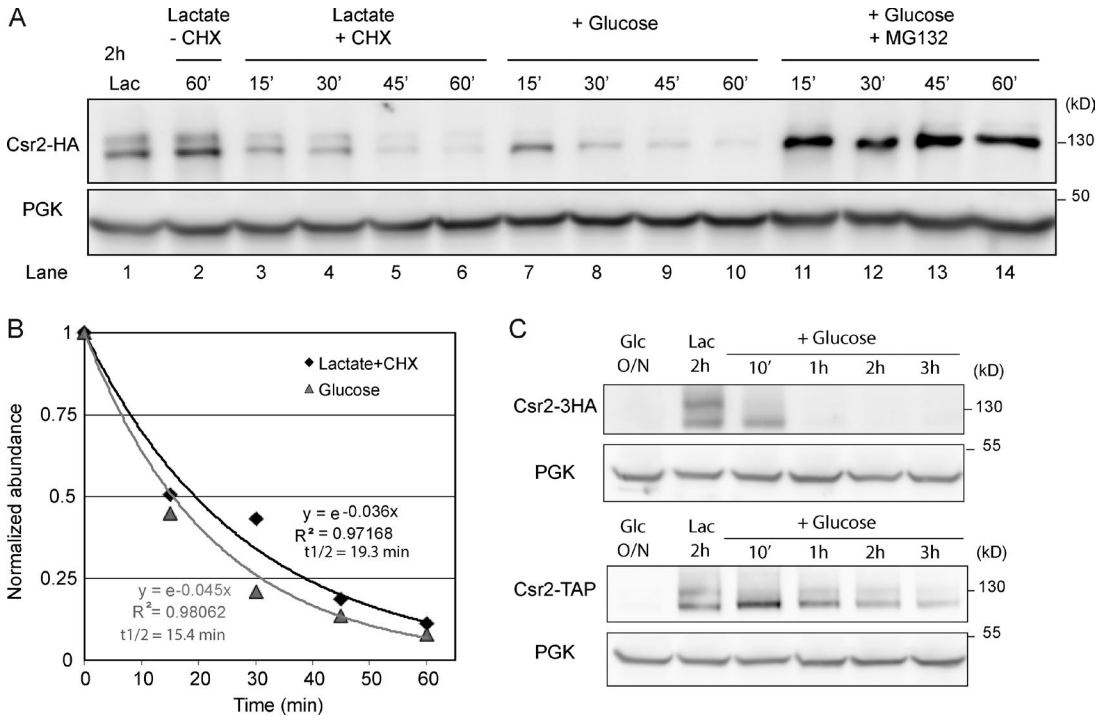
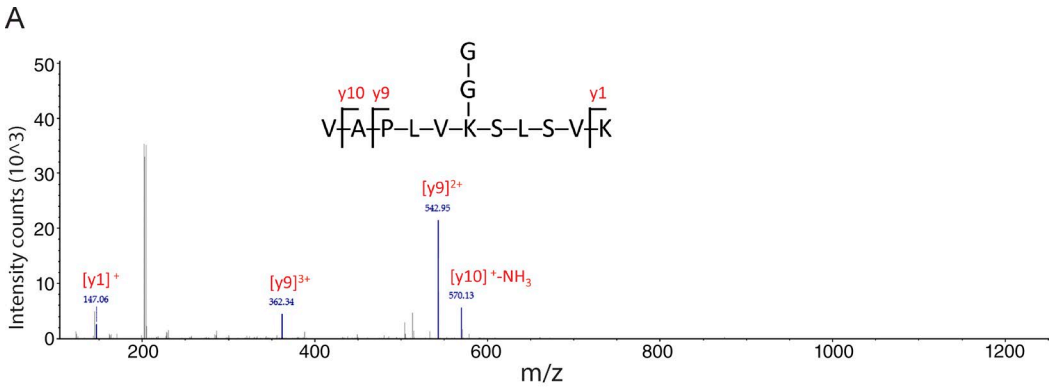


Figure S3. **Csr2-3HA is a short-lived protein.** (A) WT cells expressing Csr2-3HA (*pdr5*Δ background; ySL1826) were grown in glucose medium and were switched to lactate-containing medium for 2 h to induce Csr2-3HA synthesis (lane 1). They were then treated with cycloheximide (CHX) for various times (up to 1 h, lanes 3–6). As a control, no cycloheximide was added for 1 h (lane 2). Another batch of cells were treated with glucose for various times (up to 1 h, lanes 7–10) to repress Csr2-3HA expression. A similar experiment was performed in the presence of the proteasome inhibitor, MG-132 (lanes 11–14). (B) The blot displayed in A (nonsaturated blot acquired using a LAS-4000 imaging system) was used for quantification of band intensities and normalized to the initial Csr2-3HA signal (lane 1). The distribution of normalized abundance over time is shown, as well as exponential fits and its parameters, and the calculated half-life (t1/2). (C) WT cells expressing either Csr2-3HA (ySL1037, top) or Csr2-TAP (ySL1741, bottom) were grown in glucose medium and were switched to lactate-containing medium for 2 h. They were then treated with glucose for the indicated times. Total cell lysates were prepared at the indicated times and immunoblotted with anti-HA (top) or anti-peroxidase antibodies (PAP, bottom) and anti-PGK antibodies. Note that even though Csr2-TAP ubiquitylation vanishes at t = 10 min glucose treatment, it reappears at later time points, suggesting that the loss of Csr2 ubiquitylation is part of an early glucose response.



Sequence: VAPLVKSLSVK, K6-GlyGly (114.04293 Da)
Charge: +3, Monoisotopic m/z: 418.93048 Da (-0.39 mmu/-0.93 ppm), MH+: 1254.77689 Da, RT: 33.59 min,
Identified with: Mascot (v1.30); IonScore:13, Exp Value:4.3E-001, Ions matched by search engine: 11/94
Fragment match tolerance used for search: 0.5 Da
Q-value (FDR) = 0.037

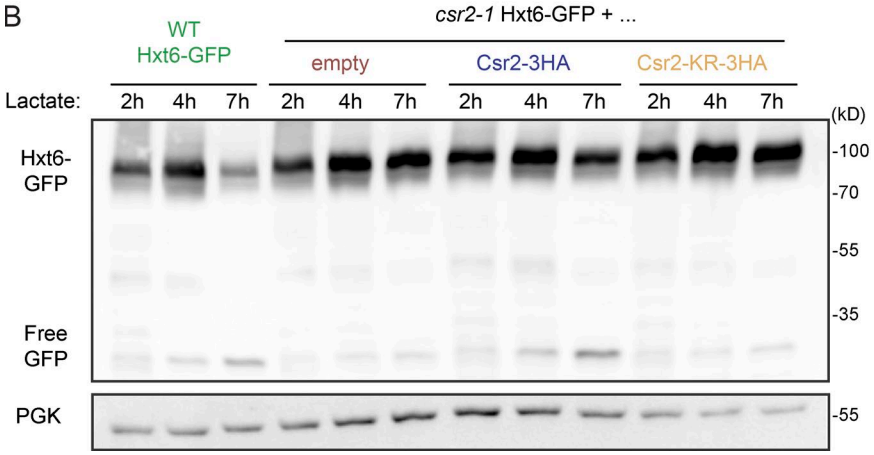


Figure S4. **Identification of the ubiquitylation site on Csr2.** (A) An immunoprecipitation was performed using lactate-grown Csr2-3HA cells (ySL1037) as starting material (see Materials and methods). The tandem mass spectrometry spectrum showing the intensities of the fragmented ions provides evidence of a diglycine remnant owing to Csr2 ubiquitylation at Lys670. Other features of this peptide are provided. FDR, false discovery rate. (B) From the experiment presented in Fig. 8 C, immunoblots were done on total cell lysates using anti-GFP and anti-PGK antibodies.

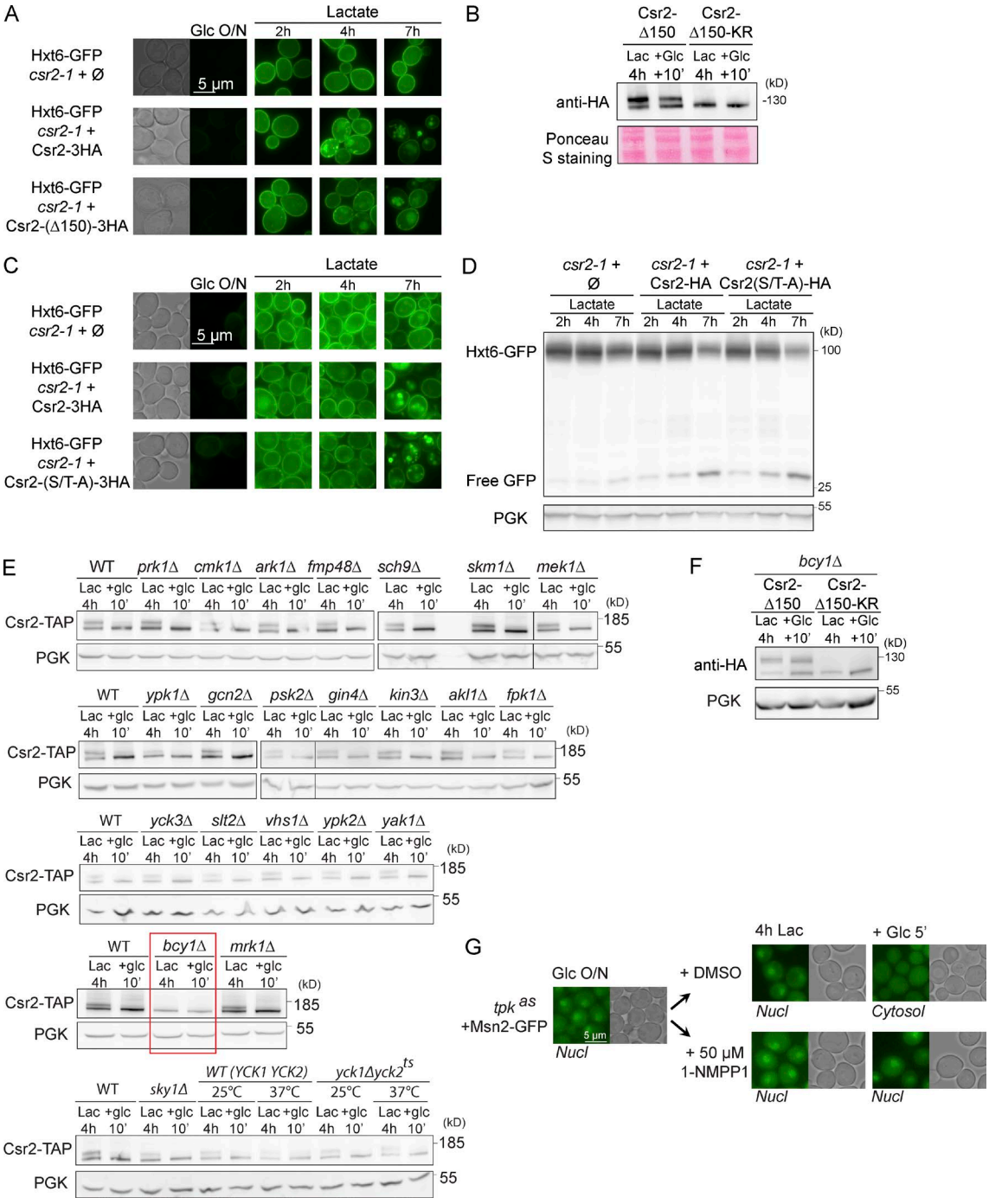


Figure S5. **Study of the N-terminal region of Csr2.** (A) *csr2-1* cells expressing Hxt6-GFP transformed with an empty plasmid (ySL1876) or a plasmid encoding either a full-length Csr2-3HA (ySL1714) or devoid of its first 150 residues in N-terminal (ySL1828) were grown in glucose medium and were switched to lactate-containing medium. They were imaged for Hxt6-GFP localization at the indicated times. (B) Glucose-grown WT cells expressing a truncated Csr2-3HA construct (Δ150), in which the conserved K670 residue is mutated or not (ySL1830 or ySL1818, respectively) were switched to lactate-containing medium for 4 h and then treated with glucose for 10 min. Total cell lysates were prepared at the indicated times and immunoblotted with anti-HA antibody. A scan of the Ponceau stain is provided as a loading control. (C) Glucose-grown *csr2-1* cells expressing Hxt6-GFP and either no Csr2, Csr2-3HA, or Csr2(S/T-A) (ySL1721, ySL1714, and ySL1849, respectively) were grown in glucose medium, switched to lactate-containing medium, and imaged at the indicated times. (D) From the experiment presented in C total cell lysates were prepared at the indicated times and immunoblotted using anti-GFP and anti-PGK antibodies. (E) Screening of kinase mutants potentially regulating Csr2 ubiquitylation. Mutant strains expressing a plasmid-encoded Csr2-TAP construct (see Table S2) were grown in glucose medium and switched to lactate-containing medium for 4 h. They were then treated with glucose for 10 min. Immunoblots were realized on total cell lysates using PAP and PGK (loading control) antibodies. (F) Evidence that the N-terminal region of Csr2 is required for the constitutive deubiquitylation of Csr2 observed in the *bcy1*Δ mutant: *bcy1*Δ cells expressing HA-tagged Csr2(Δ150) (ySL2070) or Csr2(Δ150,KR) (ySL2071) were grown in glucose medium and switched to lactate-containing medium for 4 h. They were then treated with glucose for 10 min. Immunoblots were realized on total cell lysates using anti-HA and anti-PGK (loading control) antibodies. (G) Evidence that PKA activity is induced during a lactate/glucose shift: WT (ySL2087) or *tpk*^{as} mutant cells(ySL2088) expressing Msn2 was grown overnight in glucose-containing medium (exponential phase), then switched to lactate-containing medium and treated with 50 µM 1-NMPP1 (or an equivalent volume of DMSO as a negative control) for 4 h. Glucose was added back (final concentration 2%), and cells were imaged at the indicated times.

Table S1. **List of yeast strains, per figure**

Figure	Yeast strains
Fig. 1	(A) ySL1184, ySL1551; (B) ySL1184; (C) ySL1184, ySL1643, ySL1539; (D) ySL1315, ySL1698, ySL1790, ySL1688; (E) ySL1184, ySL1706, ySL1551, ySL1718
Fig. 2	(A–D) ySL1184, ySL1706
Fig. 3	(A) ySL2027, ySL2028, ySL2023, ySL2025; (B) ySL2023; (C) ySL1643, ySL1809; (E) ySL1833, ySL1835
Fig. 4	(A) ySL1186, ySL1187, ySL1027, ySL1058; (C): ySL1140, ySL1654, ySL1852, ySL1853
Fig. 5	(A) ySL1037; (B): ySL1184, ySL1551; (C) ySL1188; (D) ySL1188, ySL1189 ySL1190; (E) ySL1188, ySL1779, ySL1816, ySL1780; (F) ySL1037, ySL1191, ySL1192; ySL1774
Fig. 6	(A) ySL1037, ySL1726; (B) ySL1184, ySL1734; (E) ySL1184; (F) ySL1953
Fig. 7	(A) ySL1212; (B) ySL1037, ySL1210; (C) ySL1655, ySL1680; (D) ySL820
Fig. 8	(B) ySL1655, ySL1677; (C) ySL1345, ySL1721, ySL1714, ySL1716; (E) ySL2023, ySL2026
Fig. 9	(A and B) ySL1037; (C) ySL1037, ySL1043; (D) ySL1043, ySL1896, ySL1897
Fig. 10	(B) ySL1826, ySL1827; (C) ySL1655, ySL1848; (D) ySL1655, ySL2021, ySL1848, ySL2043; (E) ySL2085, ySL2086; (F) ySL1876, ySL1877, ySL1881

For supplemental figures, please refer to their legends.

Table S2. **Description of the yeast strains used in this study**

Name/description	Genotype	Origin/reference
ySL820 WT + p_{CUP1}:6xHis-Ubi	Mat a, *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* (pSL206: p_{CUP1}:6xHis-Ubi, *URA3*)	This study
ySL1027 Hxt3-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, HXT3::GFP-HIS3MX6*	GFP collection (Huh et al., 2003)
ySL1037 Csr2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, CSR2*-3HA::*kanMX4*	This study
ySL1043 *ubp2Δ* Csr2-3HA	Mat α, *his3Δ1, leu2Δ0, lysΔ0, ura3Δ0, ubp2Δ::HISMX3; CSR2*-3HA::*kanMX*	This study
ySL1058 *csr2Δ* Hxt3-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX6, HXT3::GFP-HIS3MX6*	This study
ySL1140 Hxt2-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, HXT2::HIS3MX6*	GFP collection (Huh et al., 2003)
ySL1145 Hxt5-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, HXT5::GFP-HIS3MX6*	GFP collection (Huh et al., 2003)
ySL1184 Hxt6-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, HXT6::GFP-HIS3MX6*	This study
ySL1185 *csr2Δ* Hxt6-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX, HXT6::GFP-HIS3MX6*	This study
ySL1186 Hxt1-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, HXT1::GFP-HIS3MX6*	This study
ySL1187 *csr2Δ* Hxt1-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, HXT1::GFP-HIS3MX6 csr2Δ::kanMX*	This study
ySL1188 WT + p_{CSR2}-*lacZ*	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0* (pSL168, *URA3*)	This study
ySL1189 *snf1Δ* + p_{CSR2}-*lacZ*	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, snf1Δ::kanMX* (pSL168, *URA3*)	This study
ySL1190 *reg1Δ* + p_{CSR2}-*lacZ*	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, reg1Δ::HIS3MX6* (pSL168, *URA3*)	This study
ySL1191 *snf1Δ* Csr2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, CSR2*-3HA::*kanMX, snf1Δ:: hphNT1*	This study
ySL1192 *reg1Δ* Csr2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, CSR2*-3HA::*kanMX, reg1Δ::HIS3MX6*	This study
ySL1210 *npi1* Csr2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0,* p_{RSP5}*::HIS3MX6; CSR2*-3HA::*kanMX*	This study
ySL1212 Csr2-3HA + p_{CUP1}:6xHis-Ubi	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, CSR2*-3HA::*kanMX* (pSL206, *URA3*)	This study
ySL1315 Hxt6-GFP + pRS416	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, HXT6::GFP-HIS3MX* (pRHT95, *URA3*)	This study
ySL1316 *csr2Δ* Hxt6-GFP + pRS416	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX, HXT6::GFP-HIS3MX* (pRHT95, *URA3*)	This study
ySL1345 Hxt6-GFP + pRS315	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0,* Hxt6::*GFP-HIS3MX* (pRHT91, *LEU2*)	This study
ySL1539 *npi1* Hxt6-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0,* p_{RSP5}*::kanMX, HXT6::HIS3MX6*	This study
ySL1551 Hxt7-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, HXT7*-GFP:: *hphNT1*	This study
ySL1552 *csr2Δ* Hxt7-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ:: kanMX, HXT7*-GFP:: *hphNT1*	This study
ySL1643 *vrp1Δ* Hxt6-GFP	Mat α, *his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, vrp1Δ::kanMX; HXT6*-GFP:: *HIS3MX6*	This study
ySL1650 *ecm21Δ* Hxt6-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, ecm21Δ::hphNT1, HXT6::GFP-HIS3MX6*	This study
ySL1653 *csr2Δ* Hxt5-GFP	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::hphNT1, HXT5::GFP-HIS3MX6*	This study
ySL1654 *csr2Δ* Hxt2-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, HXT2::GFP-HIS3MX6, csr2Δ:: hphNT1*	This study
ySL1655 WT + p_{CSR2}-*CSR2*-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0* (pSL308, *LEU2*)	This study
ySL1670 *csr2Δ* Hxt6-GFP + p416-*pCSR2*	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX, HXT6::GFP-HIS3MX* (pSL316, *URA3*)	This study
ySL1677 WT + p_{CSR2}-*CSR2*(*KR*)-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0* (pSL318, *LEU2*)	This study
ySL1680 WT + p_{CSR2}-*CSR2*(*PYm*)-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0* (pSL319, *LEU2*)	This study
ySL1688 *9arrestinΔ* Hxt6-GFP + pRS416-*CSR2*	Mat a, *ura3, leu2, art1Δ, ecm21Δ::kanMX, aly1Δ, rod1Δ, art5Δ, aly2Δ, rog3::natMX; csr2Δ::kanMX, art10Δ::HIS, bsd2Δ HXT6*-GFP:: *hphNT1* (pSL316, *URA3*)	This study (*9arrestinΔ*: Nikko and Pelham, 2009)
ySL1692 *csr2Δ* Hxt6-GFP + YGPM32j02	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX, HXT6::GFP-HIS3MX6* + YGPM32j02 (pSL315, *LEU2*)	This study
ySL1693 *csr2Δ* Hxt6-GFP + YGPM19n08	Mat a, *his3Δ,1 leu2Δ0, met15Δ0, ura3Δ0, csr2Δ::kanMX, HXT6::GFP-HIS3MX6* + YGPM19n08 (pSL358, *LEU2*)	This study
ySL1698 *9arrestinΔ* Hxt6-GFP + pRS416	Mat a, *ura3, leu2, art1Δ, ecm21Δ::kanMX, aly1Δ, rod1Δ, art5Δ, aly2Δ, rog3::natMX; csr2Δ::kanMX, art10Δ::HIS, bsd2Δ HXT6*-GFP:: *hphNT1* (pRHT95, *URA3*)	This study
ySL1706 *csr2-1* Hxt6-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2-1::kanMX, HXT6::GFP-HIS3MX*	This study
ySL1714 *csr2-1* Hxt6-GFP + p_{CSR2}CSR2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2-1::kanMX, HXT6::GFP-HIS3MX* (pSL308, *LEU2*)	This study
ySL1716 *csr2-1* Hxt6-GFP + p_{CSR2}CSR2-KR-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2-1::kanMX, HXT6::GFP-HIS3MX* (pSL318, *LEU2*)	This study
ySL1717 *csr2-1*-3HA Hxt6-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2-1::kanMX, CSR2::3HA-hphNT1, HXT6::GFP-HIS3MX*	This study
ySL1721 *csr2-1* Hxt6-GFP + pRS315	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, csr2-1::kanMX, HXT6::GFP-HIS3MX* (pRHT91, *LEU2*)	This study
ySL1726 p_{TEF}-*CSR2*-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0,* p_{CSR2} :: *natNT2-pTEF, CSR2*:3HA ::*kanMX4*	This study
ySL1734 Hxt6-GFP p_{TEF}-*CSR2* Hxt6-GFP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0,* p_{CSR2} :: *natNT2-pTEF, HXT6::GFP-HIS3MX6*	This study
ySL1741 Csr2-TAP	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, CSR2*-TAP::*kanMX*	This study
ySL1774 *mig1Δmig2Δ* Csr2-3HA	Mat a, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, mig1Δ:: hphNT1, mig2Δ::HIS3MX6, CSR2*-3HA::*kanMX4*	This study

Table S2. **Description of the yeast strains used in this study** *(Continued)*

Name/description	Genotype	Origin/reference
ySL1779 *mig1*Δ + p_{CSR2}-*lacZ*	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mig1*Δ:: *hphNT1 CSR2*-3HA::*kanMX4* (pSL168, *URA3*)	This study
ySL1780 *mig1*Δ*mig2*Δ + p_{CSR2}-*lacZ*	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mig1*Δ:: *hphNT1*, *mig2*Δ::*HIS3MX6*, *CSR2*-3HA::*kanMX4* (pSL168, *URA3*)	This study
ySL1790 *9arrestin*Δ Hxt6-GFP + p_{ECM21} :*ECM21*-3HA	Mat a, *ura3, leu2, art1*Δ, *ecm21*Δ::*kanMX, aly1*Δ, *rod1*Δ, *art5*Δ, *aly2*Δ, *rog3*::*natMX; csr2*Δ::*kanMX, art10*Δ::*HIS, bsd2*Δ *HXT6*-GFP:: *hphNT1* (pSL324, *LEU2*)	This study
ySL1809 *vrp1*Δ *csr2-1* Hxt6-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0, vrp1*Δ::*kanMX; HXT6*-GFP:: *HIS3MX6 csr2-1*::*kanMX*	This study
ySL1810 *mig1*Δ *mig2*Δ Hxt6-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mig1*Δ::*hphNT1; mig2*Δ::*KanMX; HXT6*::*GFP-HIS3MX6*	This study
ySL1811 *mig1*Δ *mig2*Δ *csr2-1* Hxt6-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mig1*Δ::*hphNT1; mig2*Δ::*KanMX; csr2-1*::*NAT; HXT6*::*GFP-HIS3MX6*	This study
ySL1816 *mig2*Δ + p_{CSR2}-*lacZ*	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mig2*Δ::*HIS3MX6*, *CSR2*-3HA::*kanMX4*	This study
ySL1818 WT + p_{CSR2}-*CSR2*(Δ*150*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0* (pSL325, *LEU2*)	This study
ySL1826 *pdr5*Δ + p_{CSR2}-*CSR2*-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, pdr5*Δ::*HIS3MX6* (pSL325, *LEU2*)	This study
ySL1827 *pdr5*Δ + p_{CSR2}-*CSR2*(Δ*150*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, pdr5*Δ::*HIS3MX6* (pSL308, *LEU2*)	This study
ySL1828 Hxt6-GFP *csr2-1* + p_{CSR2}-*CSR2*(Δ*150*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*::*kanMX, HXT6*::*GFP-HIS3MX6*, (pSL325, *LEU2*)	This study
ySL1830 WT + p_{CSR2}-*CSR2*(Δ*150,KR*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0* (pSL345, *LEU2*)	This study
ySL1833 *vrp1*Δ Hxt6-GFP + p_{CUP1}:6xHis -Ubi	*vrp1*Δ::*kanMX; HXT6*::*GFP-HIS3MX6*, Matα, lys-, met? (pSL206: p_{CUP1}:6xHis-Ubi, *URA3*)	This study
ySL1835 *vrp1*Δ *csr2-1* Hxt6-GFP + p_{CUP1} :6xHis-Ubi	Mat a, *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0, vrp1*Δ::*kanMX, csr2-1*::*kan*MX, *HXT6*::*GFP-HIS3MX6* (pSL206, *URA3*)	This study
ySL1848 WT + p_{CSR2}:*CSR2*(*S/T-A*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0* (pSL351, *LEU2*)	This study
ySL1849 *csr2-1* Hxt6-GFP + p_{CSR2}-*CSR2*(*S/T-A*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*::*kanMX, HXT6*::*GFP-HIS3MX* (pSL351, *LEU2*)	This study
ySL1852 Hxt4-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT4*::*GFP-HIS3MX6*	This study
ySL1853 *csr2-1* Hxt4-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT4*::*GFP-HIS3MX6, csr2-1*::*kanMX*	This study
ySL1876 *csr2-1* Hxt6-GFP + pRS315	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*::*kanMX, HXT6*::*GFP-HIS3MX* (pRHT91, *LEU2*)	This study
ySL1877 *csr2-1* Hxt6-GFP + p_{CSR2}:*CSR2* -TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*::*kanMX, HXT6*::*GFP-HIS3MX* (pSL386, *LEU2*)	This study
ySL1881 *csr2-1* Hxt6-GFP + p_{CSR2}:*CSR2*(*S /T-A*)-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*::*kanMX, HXT6*::*GFP-HIS3MX* (p_{CSR2}-*CSR2*(S/T-A)-TAP, *CEN*, *LEU2*)	This study
ySL1896 *ubp2*Δ Csr2-3HA + p_{CUP1}:Ub-WT	Mat α, *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0, ubp2*Δ::*HISMX3*, *CSR2*-3HA::*kanMX* (pRHT86, *LYS2*)	This study
ySL1897 *ubp2*Δ Csr2-3HA + p_{CUP1}:Ub -K63R	Mat α, *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0, ubp2*Δ::*HISMX3*, *CSR2*-3HA::*kanMX* (pRHT88, *LYS2*)	This study
ySL1953 Hxt6-GFP + p_{CSR2}:*CSR2*-3HA	Mat a, *his3*Δ*,1 leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT6*::*GFP-HIS3MX6*	This study
ySL1982 *gin4*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, gin4*Δ::*KanMX* (pSL386, *LEU2*) [*gin4*Δ*/ySL1957* = deletion collection, checked by PCR]	This study
ySL1983 *ypk2*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, ypk2*Δ::*KanMX* (pSL386, *LEU2*) [*ypk2*Δ*/ySL1958* = deletion collection, checked by PCR]	This study
ySL1985 *yak1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, yak1*Δ::*KanMX* (pSL386, *LEU2*) [*yak1*Δ*/ySL1960* = deletion collection, checked by PCR]	This study
ySL1986 *slt2*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, slt2*Δ::*KanMX* (pSL386, *LEU2*) [*slt2*Δ*/ySL1961* = deletion collection, checked by PCR]	This study
ySL1987 *ark1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, ark1*Δ::*KanMX* (pSL386, *LEU2*) [*ark1*Δ*/ySL1962* = deletion collection, checked by PCR]	This study
ySL1988 *akl1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, akl1*Δ::*KanMX* (pSL386, *LEU2*) [*akl1*Δ*/ySL1963* = deletion collection, checked by PCR]	This study
ySL1989 *fpk1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, fpk1*Δ::*KanMX* (pSL386, *LEU2*) [*fpk1*Δ*/ySL1964* = deletion collection, checked by PCR]	This study
ySL1990 *prk1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, prk1*Δ::*KanMX* (pSL386, *LEU2*) [*prk1*Δ*/ySL1965* = deletion collection, checked by PCR]	This study
ySL1991 *fmp48*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, fmp48*Δ::*KanMX* (pSL386, *LEU2*) [*fmp48*Δ*/ySL1966* = deletion collection, checked by PCR]	This study
ySL1992 *cmk1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, cmk1*Δ::*KanMX* (pSL386, *LEU2*) [*cmk1*Δ*/ySL1967* = deletion collection, checked by PCR]	This study
ySL1993 *yck3*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, yck3*Δ::*KanMX* (pSL386, *LEU2*) [*yck3*Δ*/ySL1968* = deletion collection, checked by PCR]	This study
ySL1994 *vhs1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, vhs1*Δ::*KanMX* (pSL386, *LEU2*) [*vhs1*Δ*/ySL1969* = deletion collection, checked by PCR]	This study

Table S2. **Description of the yeast strains used in this study** *(Continued)*

Name/description	Genotype	Origin/reference
ySL1995 *sch9*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, sch9*Δ::*KanMX* (pSL386, *LEU2*) [*sch9*Δ*/ySL1970* = deletion collection, checked by PCR]	This study
ySL1996 *psk2*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, psk2*Δ::*KanMX* (pSL386, *LEU2*) [*psk2*Δ*/ySL1971* = deletion collection, checked by PCR]	This study
ySL1997 *mek1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mek1*Δ::*KanMX* (pSL386, *LEU2*) [*mek1*Δ*/ySL1972* = deletion collection, checked by PCR]	This study
ySL1998 *skm1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, skm1*Δ::*KanMX* (pSL386, *LEU2*) [*skm1*Δ*/ySL1973* = deletion collection, checked by PCR]	This study
ySL1999 *kin3*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, kin3*Δ::*KanMX* (pSL386, *LEU2*) [*kin3*Δ*/ySL1974* = deletion collection, checked by PCR]	This study
ySL2000 WT *YCK* + p_{CSR2}:*CSR2*-TAP	Mat a *his3 leu2 ura3-52* (LRB341) (pSL386, *LEU2*)	This study (LRB341: Panek et al., 1997)
ySL2001 *yck1*Δ *yck2*^{ts} + p_{CSR2}:*CSR2*-TAP	Mat a *his3 leu2 ura3-52 yck1-1*::*ura3 yck2-2ts* (LRB362) (pSL386, *LEU2*)	This study (LRB362: Panek et al., 1997)
ySL2021 *bcy1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, bcy1*Δ::*KanMX* (pSL386, *LEU2*) [*bcy1*Δ*/ySL2019* = deletion collection, checked by PCR]	This study
ySL2022 *mrk1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, mrk1*Δ::*KanMX* (pSL386, *LEU2*) [*mrk1*Δ*/ySL2020* = deletion collection, checked by PCR]	This study
ySL2023 Hxt6-VC + p_{TEF}:Csr2-VN	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT6-VC*::*KanMX* (pSL391, *LEU2*)	This study
ySL2025 *vrp1*Δ Hxt6-VC + p_{TEF}:Csr2-VN	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, vrp1*Δ::*hphNT1 HXT6-VC*::*KanMX* (pSL391, *LEU2*)	This study
ySL2026 Hxt6-VC + p_{TEF}:Csr2(KR)-VN	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT6-VC*::*KanMX* (pSL393, *LEU2*)	This study
ySL2027 Hxt6-VC + p_{TEF}:VN	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, HXT6-VC*::*KanMX* (pSL394, *LEU2*)	This study
ySL2028 *vrp1*Δ Hxt6-VC + p_{TEF}:VN	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, vrp1*Δ::*hphNT1 HXT6-VC*::*KanMX* (pSL394, *LEU2*)	This study
ySL2031 *ypk1*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, ypk1*Δ::*KanMX* (pSL386, *LEU2*) [*ypk1*Δ*/ySL2029* = this study]	This study
ySL2032 *gcn2*Δ + p_{CSR2}:*CSR2*-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, gcn2*Δ::*KanMX* (pSL386, *LEU2*) [*gcn2*Δ*/ySL2030* = this study]	This study
ySL2043 *bcy1*Δ + p_{CSR2}:*CSR2*(*S/T-A*)-TAP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, bcy1*Δ::*KanMX* (pSL387, *LEU2*)	This study
ySL2067 *csr2-1* Hxt5-GFP	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, csr2-1*:: *hphNT1*, *HXT5*::*GFP-HIS3MX*	This study
ySL2070 *bcy1*Δ + p_{CSR2}:*CSR2*(Δ*150*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, bcy1*Δ::*KanMX* (pSL325, *LEU2*)	This study
ySL2071 *bcy1*Δ + p_{CSR2}:*CSR2*(Δ*150, KR*)-3HA	Mat a, *his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0, bcy1*Δ::*KanMX* (pSL345, *LEU2*)	This study
ySL2085 WT (PKA) + p_{CSR2}:*CSR2*-TAP	Mat α *gal1*::*HIS3 ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 GAL* (= Y2864) (pSL386, *LEU2*) trp1-1, GAL; mat α	This study (Y2864: Zaman et al., 2009)
ySL2086 *pka*^{as} + p_{CSR2}:*CSR2*-TAP	Mat α *ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 tpk1*^{M164G} *tpk2*^{M147G} *tpk3*^{M165G} (=Y3561) (pSL386, *LEU2*)	This study (Y3561: Zaman et al., 2009)
ySL2085 WT (PKA) + p_{CSR2}:*CSR2*-TAP	Mat α *gal1*::*HIS3 ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 GAL* (= Y2864) (pSL386, *LEU2*)	This study (Y2864: Zaman et al., 2009)
ySL2086 *pka*^{as} + p_{CSR2}:*CSR2*-TAP	Mat α *ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 tpk1*^{M164G} *tpk2*^{M147G} *tpk3*^{M165G} (=Y3561) (pSL386, *LEU2*)	This study (Y3561: Zaman et al., 2009)

Table S3. **Plasmids used in this study**

Name	Description	Origin/reference
pSL168	p_{CSR2}:*lacZ*, 2µ, *URA3* (Yep358-based)	This study
pSL206 (Yep352-6xHis-Ub)	p_{CUP1}:6xHis-Ubi, 2µ, *URA3* (Yep352-based)	Gwizdek et al., 2006
pSL308	p_{CSR2}:*CSR2*-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL315	Genomic clone YGPM32j02 in pGP564-based vector (genomic tiling collection; chr.XVI:625682-639002)	Jones et al., 2008
pSL316	p_{CSR2}:*CSR2*, CEN, URA3 (pRS416-based; −500/+300)	This study
pSL318	p_{CSR2}:*CSR2-KR*-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL319	p_{CSR2}:*CSR2-PYm*-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL324	p_{ECM21}:*ECM21*-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL325	p_{CSR2}:*CSR2*(Δ*150*)-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL345	p_{CSR2}:*CSR2*(Δ*150,KR*)-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL351	p_{CSR2}:*CSR2*(*S/T-A*)-3HA, CEN, *LEU2* (pRS415-based)	This study
pSL358	Genomic clone YGPM19n08 in pGP564-based vector (genomic tiling collection; chr.XVI:618165-631521)	Jones et al., 2008
pSL386	p_{CSR2}:*CSR2*-TAP, CEN, *LEU2*	This study
pSL387	p_{CSR2}:*CSR2*(*S/T-A*)-TAP, CEN, *LEU2*	This study
pSL391	pTEF:CSR2-VN, CEN, *LEU2* (pRS415-based)	This study
pSL393	pTEF:CSR2-KR-VN, CEN, *LEU2* (pRS415-based)	This study
pSL392	pTEF:VN, CEN, *LEU2* (pRS415-based)	This study
pSL404	P_{MSN2}:*MSN2*-GFP, CEN, *LEU2* (pRS415-based)	Durchschlag et al., 2004
pRHT91	pRS315, CEN, *LEU2*	Sikorski and Hieter, 1989
pRHT95	pRS416, CEN, *URA3*	Christianson et al., 1992

Table S4. **Primers used in this study**

Plasmid number	Primer sequence (cloning)
pSL168	oSL437: 5′-TCCGGAATTCTATGGTGTTCAGGTCTTTCTCG-3′
	oSL438: 5′-TCCGGGATCCCATGATTGACTTTGCTTACTAGTCTG-3′
pSL316	oSL713: 5′-GCCTCTCGAGACTTGTCCCAACCTCATTCC-3′
	oSL714: 5′-GCCTTCTAGATAGGCCCATCATCTAATGAT-3′
pSL318	oSL715: 5′-CCTCTAGTAAGATCTCTTAGTGTCAAGAGAATTC-3′
	oSL716: 5′-GACACTAAGAGATCTTACTAGAGGTGCCACTTTA-3′
pSL319	oSL448: 5′-ATACACAGCGAACCAGCCC-3′
	oSL656: 5′-GCCTGTCGACTTATGATGAAATCTCGTCAGCTCGAGGTGGTTCAG-3′
	oSL662: 5′-TCTACCACCAGCTGGTATCGATCTTTTCGACC-3′
	oSL663: 5′-ATCGATACCAGCTGGTGGTAGACTTGCAGCTT-3′
pSL324	oSL751: 5′-TCCGCTCGAGTTCTTCATCACTCATCAAAGGCAC-3′
	oSL770: 5′-TCCGACTAGTAAATTTATTATTTCGACAGTC-3′
pSL325	oSL768: 5′-CCCGTCTAGAATGGTCGCGGCAAAACAAATATCTAG-3′
	oSL440: 5′-GCCTCTCGAGTGATGAAATCTCGTCATATCTTGG-3′
pSL345	oSL768: see above
	oSL440: see above
pSL386	oSL806: 5′-TCCAATAATTCATCCGGGTCCTGAACCACCAAGATATGACGAGATTTCATCACGTACGCTGCAGGTCGAC-3′
	oSL807: 5′-GGGGGGAGGGCGTGAATGTAAGCGTGACATAACTAATTACATGACTCGACCTAAAGAGCCGCGGAATTCG-3′
pSL387	oSL806: see above
	oSL807: see above
pSL391	oSL1007: 5′-TTGTTCTAGAATGCAATCTACTGTCCCA-3′
	oSL1008: 5′-CGCCTCGAGTTACTCGATGTTGTGGCG-3′
pSL392	oSL1046: 5′-TTGTGGATCCATGAGATCCATCGCCACC-3′
	oSL1047: 5′-CGCCTCGAGTTAACTTATAATACAACA-3′
	Primer sequence (quantitative RT-PCR)
oSL685	5′-CCAGCCCCTCTAATACAGCA-3′
oSL686	5′-ACTCGCAACAAAGGAATCCG-3′
oSL704	5′-ACGTTACCCAATTGAACACG-3′
oSL705	5′-AGAACAGGGTGTTCTTCTGG-3′

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