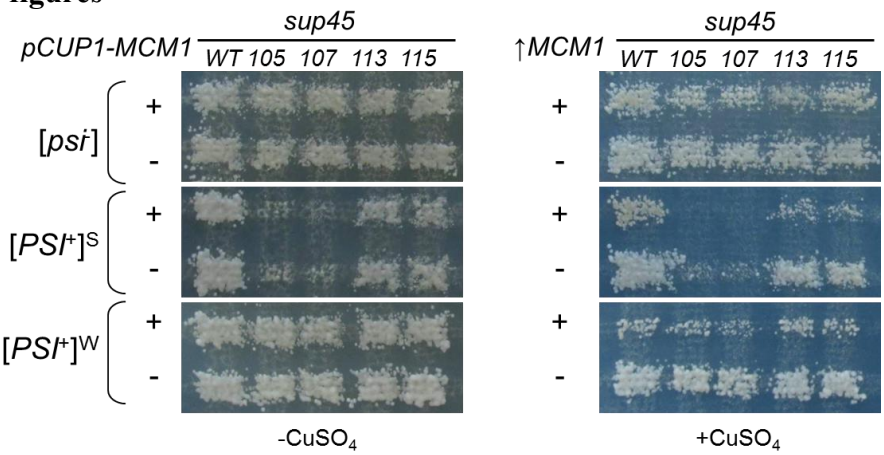
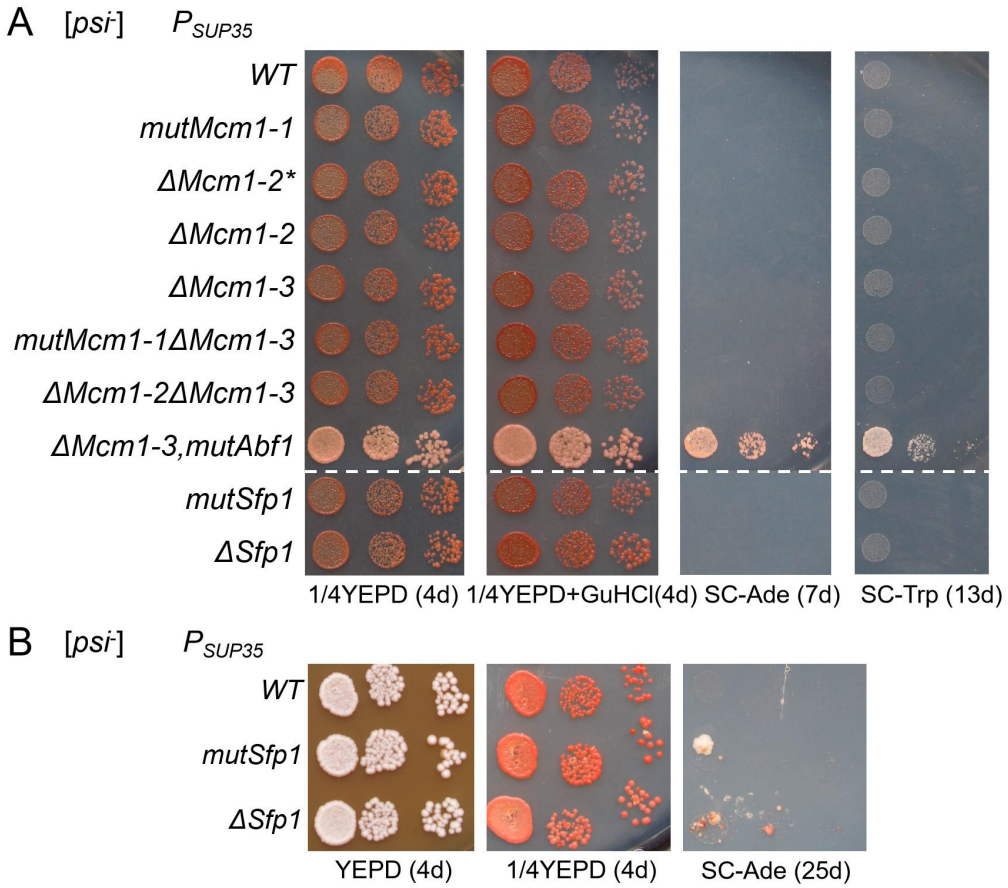
Transcription factors Mcm1 and Sfp1 may affect [*PSI*$^+$] prion phenotype by altering the expression of the *SUP35* gene.

Andrew G. Matveenko, Anastasiia S. Mikhailichenko, Polina B. Drozdova, Galina A. Zhouravleva

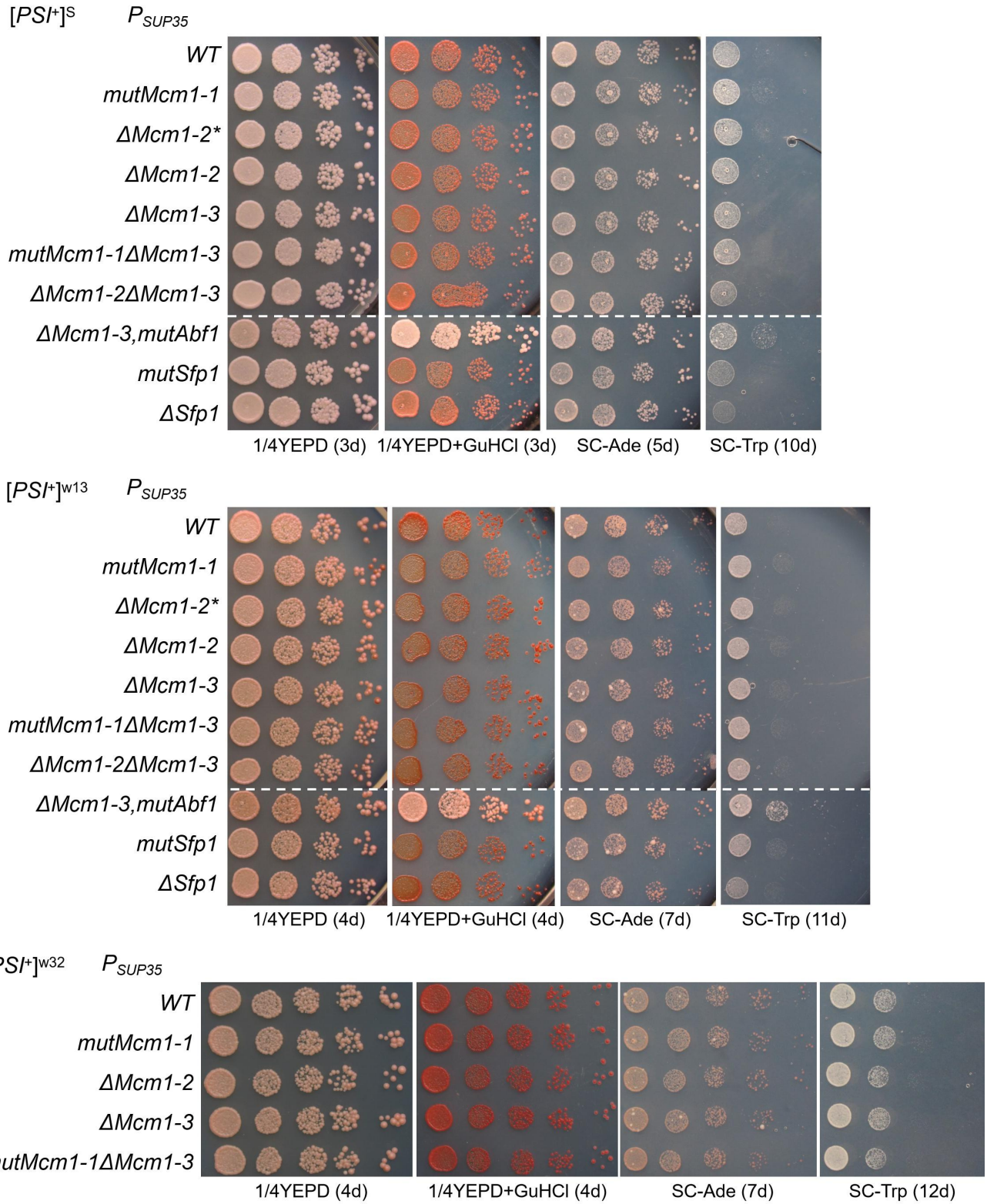
Supplementary figures



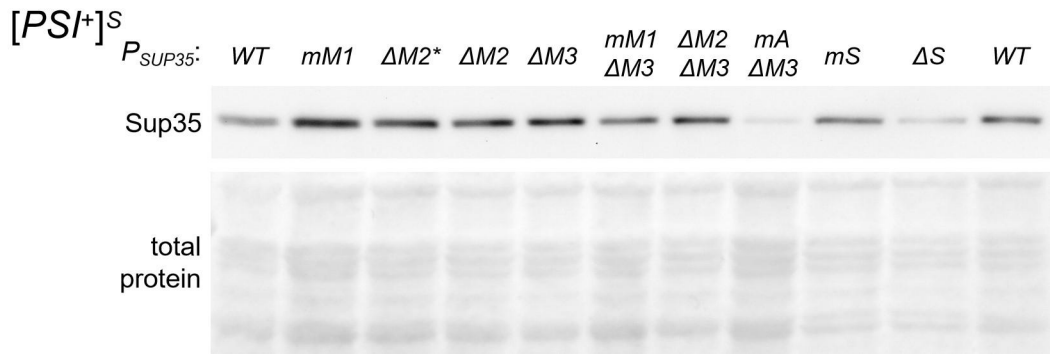
Supplementary Figure S1. The synthetic lethality of [*PSI*$^+$] with *sup45* mutations is increased upon *MCM1* transient overexpression. U-1A-D1628 derivatives containing *SUP45* (*WT*), *sup45-105, sup45-107, sup45-113*, or *sup45-115* allele (designated with the respective number) on *LEU2* vectors were mated to OT56 ([*PSI*$^+$]^S), OT55 ([*PSI*$^+$]^W), or 2-OT56 ([*psi*$^-$]), each transformed with either pRS316CG (-) or pU-MCM1 (+). The selective medium for hybrid selection is SD supplemented only with adenine and tryptophan. 150 μM $CuSO_4$ was added to the media for the *CUP1* promoter induction. Representative crosses are shown. S – strong and W – weak variants of [*PSI*$^+$], respectively.



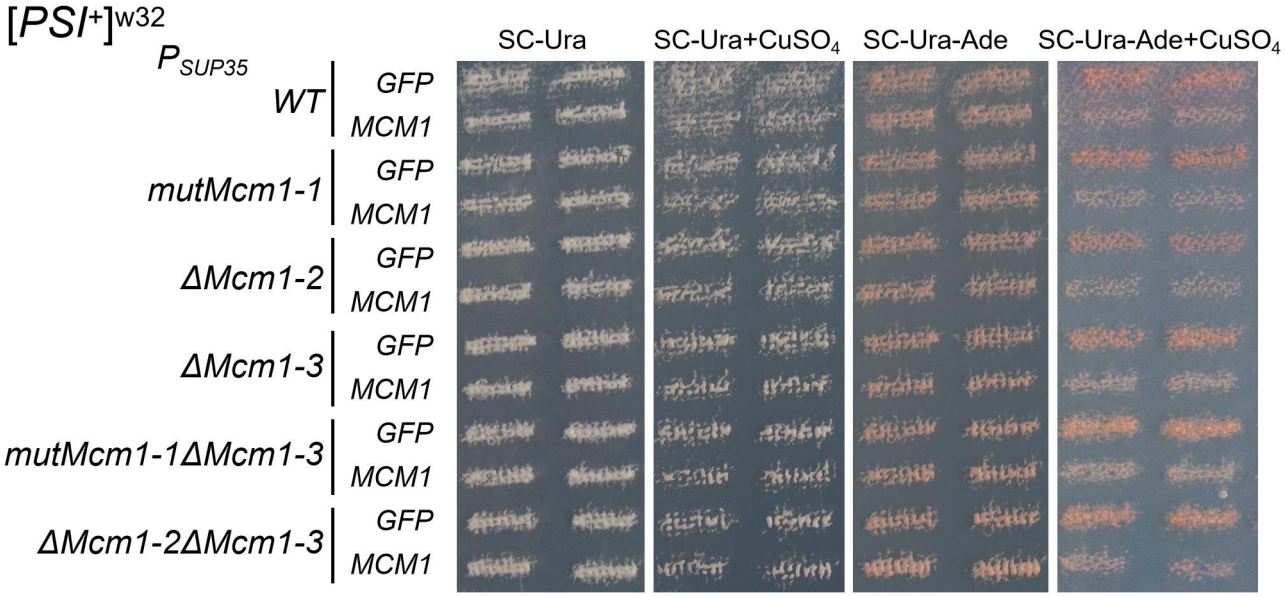
Supplementary Figure S2. Alterations in the potential Mcm1 TFBSs do not affect nonsense suppression in [*psi*$^-$] strains, while deletion of the potential Sfp1 binding site in the *SUP35* promoter leads to an extremely small increase in the nonsense suppression. Shown are representative tenfold serial dilutions of the derivatives of U-12-D1682 (A) or U-GT671 (B) with indicated *SUP35* promoter alleles.



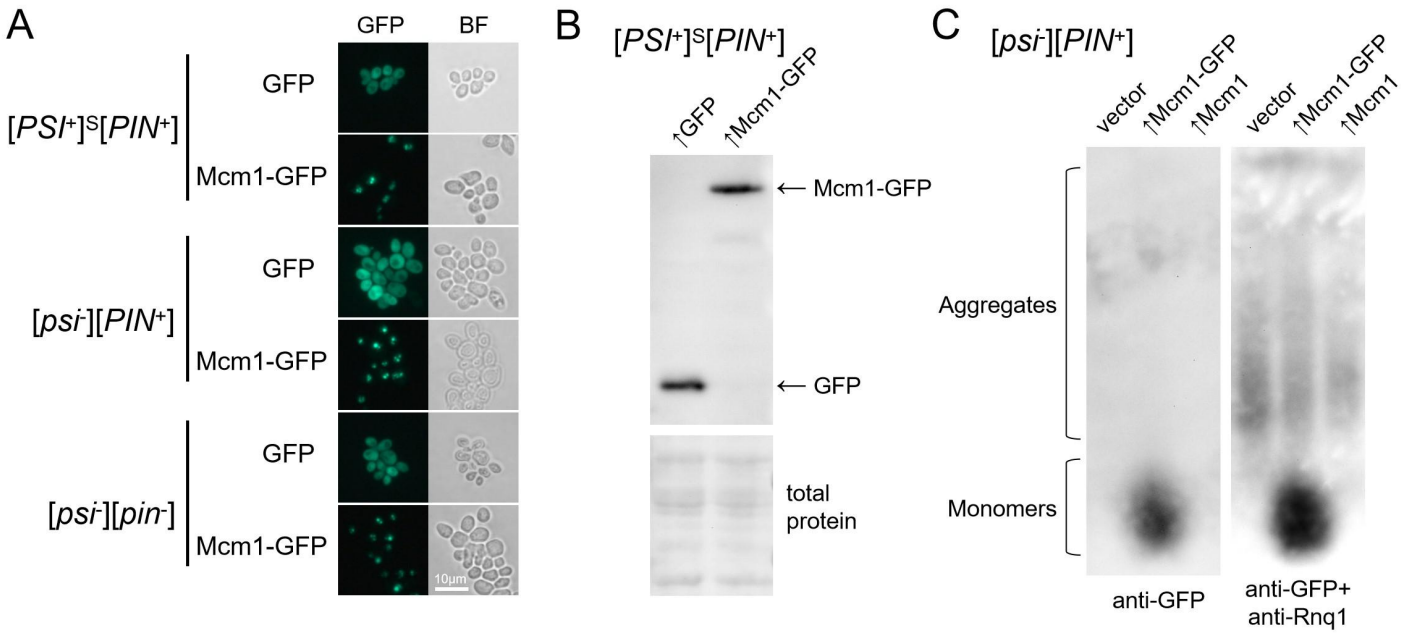
Supplementary Figure S3. Mutations and deletions of the potential TFBSs of Mcm1 and Sfp1 do not affect nonsense suppression in various [*PSI*$^{+}$] strains. Shown are tenfold serial dilutions of the strains derived from U-P^{S}-A-GT671 ([*PSI*$^{+}$]^{S}), w13-U-12-D1682 ([*PSI*$^{+}$]^{w13}), and w32-U-12-D1682 ([*PSI*$^{+}$]^{w32}) bearing indicated *SUP35* promoter variants.



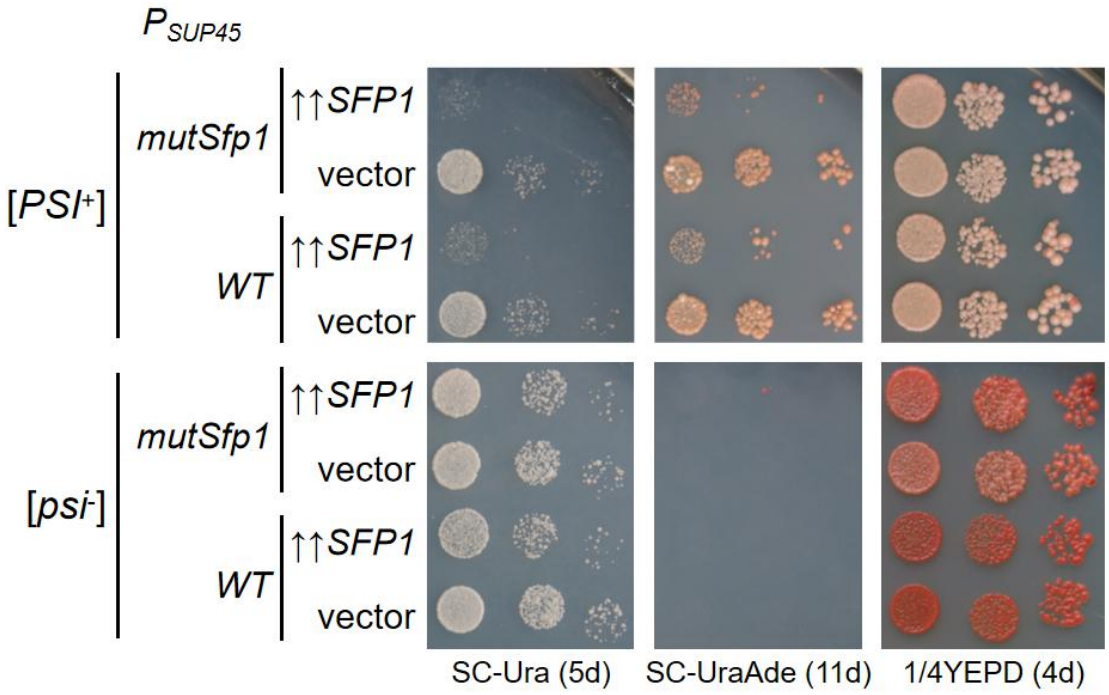
Supplementary Figure S4. Analysis of the Sup35 protein levels in U-P^S-A-GT671 derivatives using SDS-PAGE, followed by Western blotting with anti-Sup35 antibodies. Coomassie R-250 staining (total protein) was used as a loading control. Promoter variants are denoted as on the Fig. 3B.



Supplementary Figure S5. Deletions and mutations of potential Mcm1 binding sites in the *SUP35* promoter do not affect the suppressor effect of Mcm1 overproduction. Derivatives of the w32-U-12-D1682 strain with *SUP35* under control of the mutant promoters were transformed with pRS316CG (*GFP*) or pU-MCM1 (*MCM1*). Obtained clones were replica plated onto the selective media, supplemented with $CuSO_4$ at a final concentration of 50 μM where indicated. Shown is growth of two independent transformants per each combination of promoter and plasmid.



Supplementary Figure S6. The [*PIN*$^+$] prion does not affect Mcm1 aggregation, and Mcm1 overproduction does not affect the [*PIN*$^+$] prion. A. Fluorescence microscopy of the OT56 ([*PSI*$^+$]^S[*PIN*$^+$]), 74-D694 ([*psi*$^-$][*PIN*$^+$]), and 2-74-D694 ([*psi*$^-$][*pin*$^-$]) cells transformed with either pRS316CG (GFP), or pUGC-MCM1-GFP (Mcm1-GFP) plasmid. BF, bright field. B. Analysis of the Mcm1-GFP protein levels in the OT56 strain transformed with plasmids from A using SDS-PAGE and Western blotting with anti-GFP antibodies. Coomassie R-250 staining was used to visualize total protein for a loading control. C. SDD-AGE analysis of the protein samples extracted from 74-D694 transformants, performed as in Fig. 5C. Western blotting was performed, first, with anti-GFP primary antibodies (anti-GFP), and then the same membrane was blotted using anti-Rnq1 antibodies (anti-GFP + anti-Rnq1).



Supplementary Figure S7. Mutation of the potential Sfp1 binding site in the *SUP45* promoter does not affect [*PSI*$^+$] toxicity. The derivatives of P1-U-1A-D1628 or U-1A-D1628 with indicated P_{SUP45} variants were transformed with either pRS426-SFP1 (↑↑*SFP1*) or pRS426 (vector). Shown are tenfold serial dilutions of representative clones.

Supplementary tables

Supplementary Table S1. Plasmids used in this work.

Name	Description	Reference
pRS316	*CEN URA3*	Sikorski & Hieter, 1989
pRS316CG	*CEN URA3* P_{CUP1}*-GFP*	Serio et al., 1999
pU-MCM1	*CEN URA3* P_{CUP1}*-MCM1*	Nizhnikov et al., 2013
pUGC-MCM1-GFP	*CEN URA3* P_{CUP1}*-MCM1-GFP*	This work
pRS315CNMG	*CEN URA3* P_{CUP1*}*-SUP35NM-GFP*	Danilov et al., 2019
pYX242-Nab2NLS-2mCherry	2μ *LEU2* P_{TPI}*-Nab2NLS-2mCherry*	Malinovska et al., 2012
pRS426	2μ *URA3*	Christianson et al., 1992
pRS426-SFP1	2μ *URA3* P_{SFP1}*-SFP1*	Rogoza et al., 2010
pRSU1	*CEN LEU2* P_{SUP35}*-SUP35*	Volkov et al., 2002
pRSU1-mutMcm1-1	*CEN LEU2* $P_{SUP35\text{-}mutMcm1\text{-}1}$*-SUP35*	This work
pRSU1-ΔMcm1-2	*CEN LEU2* $P_{SUP35\text{-}\Delta Mcm1\text{-}2}$*-SUP35*	This work
pRSU1-ΔMcm1-2*	*CEN LEU2* $P_{SUP35\text{-}\Delta Mcm1\text{-}2*}$*-SUP35*	This work
pRSU1-ΔMcm1-3	*CEN LEU2* $P_{SUP35\text{-}\Delta Mcm1\text{-}3}$*-SUP35*	This work
pRSU1-ΔMcm1-3mutAbf1	*CEN LEU2* $P_{SUP35\text{-}mutAbf1\Delta Mcm1\text{-}3}$*-SUP35*	This work
pRSU1-ΔMcm1-3mutMcm1-1	*CEN LEU2* $P_{SUP35\text{-}mutMcm1\text{-}1\Delta Mcm1\text{-}3}$*-SUP35*	This work
pRSU1-ΔMcm1-3ΔMcm1-2	*CEN LEU2* $P_{SUP35\text{-}\Delta Mcm1\text{-}2\Delta Mcm1\text{-}3}$*-SUP35*	This work
pRSU1-flipSfp1	*CEN LEU2* $P_{SUP35\text{-}mutSfp1}$*-SUP35*	This work
pRSU1-ΔSfp1	*CEN LEU2* $P_{SUP35\text{-}\Delta Sfp1}$*-SUP35*	This work
pRS315-SUP45	*CEN LEU2* P_{SUP45}*-SUP45*	Moskalenko et al., 2003
pRS315-SUP45-flipSfp1	*CEN LEU2* $P_{SUP45\text{-}flipSfp1}$*-SUP45*	This work

Supplementary Table S2. Oligonucleotides used in this work.

Name	Sequence (5'-3')	Constructed plasmids or qPCR target genes
SUP35-mutMcm1-1-F	agttcatagcaaaattcttacgcaaatcatgaatcttagttctcagcc	pRSU1-mutMcm1-1, pRSU1-ΔMcm1-3 mutMcm1-1
SUP35-mutMcm1-1-R	gctgagaactaagattcatgatttgcgtaagaattttgctatgaacttc	pRSU1-mutMcm1-1, pRSU1-ΔMcm1-3 mutMcm1-1
SUP35-delMcm1-2-F	atcttagttctcagcccaccggtacatgctaagatcatac	pRSU1-ΔMcm1-2, pRSU1-ΔMcm1-2*, pRSU1-ΔMcm1-3 ΔMcm1-2
SUP35-delMcm1-2-R	gtatgatcttagcatgtaccggtgggctgagaactaagat	pRSU1-ΔMcm1-2, pRSU1-ΔMcm1-2*, pRSU1-ΔMcm1-3 ΔMcm1-2
SUP35-delMcm1-3-F	catcgtataatatgatctttctagaaaatttttttttcactcga	pRSU1-ΔMcm1-3, pRSU1-ΔMcm1-3mutAbf1
SUP35-delMcm1-3-R	tcgagtgaaaaaaaaattttctagaaagatcatattatacgatg	pRSU1-ΔMcm1-3, pRSU1-ΔMcm1-3 mutAbf1
SUP35_flipSfp1-F	atgatctttctttatggagtttttttaaaattcactcgaccaaagctccc	pRSU1-flipSfp1
SUP35_flipSfp1-R	gggagctttggtcgagtgaattttaaaaaaactccataaagaaagatcat	pRSU1-flipSfp1

SUP35_delSfp1-F	tttctttatggagaattcactcgaccaaagctcccattgc	pRSU1-ΔSfp1
SUP35_delSfp1-R	ggagctttggtcgagtgaattctccataaagaaagatcat	pRSU1-ΔSfp1
SUP45_flipSfp1_F	attattccgttgaccctgaatgattttaaaattcagaaatccagtgctaa	pRS315-SUP45-flipSfp1
SUP45_flipSfp1_R	ttagcactggatttctgaattttaaaatcattcagggtcaacggaataat	pRS315-SUP45-flipSfp1
SUP35_F	ACAACAAGGTAACAACAGATACC	*SUP35* (qPCR)
SUP35_R	GGATTGAATTGCTGCTGATAAC	*SUP35* (qPCR)
SUP45_F	CGATCCAAGACTAGCATGTAAG	*SUP45* (qPCR)
SUP45_R	CTTGAACATACTTGACATTGGC	*SUP45* (qPCR)
ACT1_F	TAACGGTTCTGGTATGTGTAAAGC	*ACT1* (qPCR)
ACT1_R	GCTTCATCACCAACGTAGGAGTC	*ACT1* (qPCR)

Supplementary Table S3. Yeast strains used in this work.

Name	Description	Reference
74-D694	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*psi*$^-$] [*PIN*$^+$]	Chernoff et al., 1995
P-74-D694	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*PSI*$^+$] [*PIN*$^+$]	Drozdova et al., 2016
2-74-D694	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*psi*$^-$] [*pin*$^-$]	Matveenko et al., 2022
OT56	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*PSI*$^+$]^S [*PIN*$^+$]	Derkatch et al., 1997; Newnam et al., 1999
OT55	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*PSI*$^+$]^W [*PIN*$^+$]	Derkatch et al., 1997; Newnam et al., 1999
2-OT56	*MAT*a *ade1-14 his3-Δ200 ura3-52 leu2-3,112 trp1-289* [*psi*$^-$] [*pin*$^-$]	Matveenko et al., 2016
U-12-D1682	*MAT*a *ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup35::HIS3MX* [*psi*$^-$] [*PIN*$^+$] [pRSU2]	Danilov et al., 2019
L-12-D1682	U-12-D1682 [pRSU1] (instead of [pRSU2])	This work
mutMcm1-1L-12-D1682	U-12-D1682 [pRSU1-mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-2L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-2] (instead of [pRSU2])	This work
ΔMcm1-2*L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-2*] (instead of [pRSU2])	This work
ΔMcm1-3L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-3] (instead of [pRSU2])	This work
ΔMcm1-3mutAbf1L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-3mutAbf1] (instead of [pRSU2])	This work
ΔMcm1-3mutMcm1-1L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-3mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-3ΔMcm1-2L-12-D1682	U-12-D1682 [pRSU1-ΔMcm1-3ΔMcm1-2] (instead of [pRSU2])	This work
flipSfp1L-12-D1682	U-12-D1682 [pRSU1-flipSfp1] (instead of [pRSU2])	This work
ΔSfp1L-12-D1682	U-12-D1682 [pRSU1-ΔSfp1] (instead of [pRSU2])	This work
U-P^S-A-GT671	*MAT*a *ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup35::HIS3MX* [*PSI*$^+$]^S [*PIN*$^+$] [pRSU2]	Matveenko et al., 2019
L-P^S-A-GT671	U-P^S-A-GT671 [pRSU1] (instead of [pRSU2])	This work
mutMcm1-1L-P^S-A-GT671	U-P^S-A-GT671 [pRSU1-mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-2L-P^S-A-GT671	U-P^S-A-GT671 [pRSU1-ΔMcm1-2] (instead of [pRSU2])	This work

ΔMcm1-2*L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔMcm1-2*] (instead of [pRSU2])	This work
ΔMcm1-3L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔMcm1-3] (instead of [pRSU2])	This work
ΔMcm1-3mutAbf1L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔMcm1-3mutAbf1] (instead of [pRSU2])	This work
ΔMcm1-3mutMcm1-1L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔMcm1-3mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-3ΔMcm1-2L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔMcm1-3ΔMcm1-2] (instead of [pRSU2])	This work
flipSfp1L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-flipSfp1] (instead of [pRSU2])	This work
ΔSfp1L-P^{S}-A-GT671	U-P^{S}-A-GT671 [pRSU1-ΔSfp1] (instead of [pRSU2])	This work
w13-U-12-D1682	*MAT*a *ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup35::HIS3MX* [*PSI*$^{+}$]^{w13} [*PIN*$^{+}$] [pRSU2]	Danilov et al., 2019
L-w13-12-D1682	w13-U-12-D1682 [pRSU1] (instead of [pRSU2])	This work
mutMcm1-1L-w13-12-D1682	w13-U-12-D1682 [pRSU1-mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-2L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-2] (instead of [pRSU2])	This work
ΔMcm1-2*L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-2*] (instead of [pRSU2])	This work
ΔMcm1-3L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-3] (instead of [pRSU2])	This work
ΔMcm1-3mutAbf1L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-3mutAbf1] (instead of [pRSU2])	This work
ΔMcm1-3mutMcm1-1L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-3mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-3ΔMcm1-2L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔMcm1-3ΔMcm1-2] (instead of [pRSU2])	This work
flipSfp1L-w13-12-D1682	w13-U-12-D1682 [pRSU1-flipSfp1] (instead of [pRSU2])	This work
ΔSfp1L-w13-12-D1682	w13-U-12-D1682 [pRSU1-ΔSfp1] (instead of [pRSU2])	This work
w32-U-12-D1682	*MAT*a *ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup35::HIS3MX* [*PSI*$^{+}$]^{w32} [*PIN*$^{+}$] [pRSU2]	Danilov et al., 2019
L-w32-12-D1682	w32-U-12-D1682 [pRSU1] (instead of [pRSU2])	This work
mutMcm1-1L-w32-12-D1682	w32-U-12-D1682 [pRSU1-mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-2L-w32-12-D1682	w32-U-12-D1682 [pRSU1-ΔMcm1-2] (instead of [pRSU2])	This work
ΔMcm1-3L-w32-12-D1682	w32-U-12-D1682 [pRSU1-ΔMcm1-3] (instead of [pRSU2])	This work
ΔMcm1-3mutMcm1-1L-w32-12-D1682	w32-U-12-D1682 [pRSU1-ΔMcm1-3mutMcm1-1] (instead of [pRSU2])	This work
ΔMcm1-3ΔMcm1-2L-w32-12-D1682	w32-U-12-D1682 [pRSU1-ΔMcm1-3ΔMcm1-2] (instead of [pRSU2])	This work
U-GT671	*MATα ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup35::HIS3MX* [*psi*$^{-}$] [*pin*$^{-}$] [pRSU2]	Danilov et al., 2019
L-GT671	U-GT671 [pRSU1] (instead of [pRSU2])	This work
flipSfp1L-GT671	U-GT671 [pRSU1-flipSfp1] (instead of [pRSU2])	This work
ΔSfp1L-GT671	U-GT671 [pRSU1-ΔSfp1] (instead of [pRSU2])	This work
U-1A-D1628	*MATα ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup45::HIS3MX* [*psi*$^{-}$] [*PIN*$^{+}$] [pRS316-SUP45]	Moskalenko et al., 2003; Barbitoff et al., 2021

L-1A-D1628	U-1A-D1628 [pRS315-SUP45] (instead of [pRS316-SUP45])	Moskalenko et al., 2003; This work
flipSfp1L-1A-D1628	U-1A-D1628 [pRS315-SUP45-flipSfp1] (instead of [pRS316-SUP45])	This work
105L-1A-D1628	U-1A-D1628 [pRS315-sup45-105] (instead of [pRS316-SUP45])	Moskalenko et al., 2003
107L-1A-D1628	U-1A-D1628 [pRS315-sup45-105] (instead of [pRS316-SUP45])	Moskalenko et al., 2003
113L-1A-D1628	U-1A-D1628 [pRS315-sup45-105] (instead of [pRS316-SUP45])	Matveenko et al., 2016
115L-1A-D1628	U-1A-D1628 [pRS315-sup45-105] (instead of [pRS316-SUP45])	Matveenko et al., 2016
P1-U-1A-D1628	*MATα ade1-14 his3-Δ200 lys2 ura3-52 leu2-3,112 trp1-289 sup45::HIS3MX* [*PSI*$^{+}$] [*PIN*$^{+}$] [pRS316-SUP45]	Matveenko et al., 2022
L-P1-1A-D1628	P1-U-1A-D1628 [pRS315-SUP45] (instead of [pRS316-SUP45])	This work
flipSfp1L-P1-1A-D1628	P1-U-1A-D1628 [pRS315-SUP45-flipSfp1] (instead of [pRS316-SUP45])	This work