Supplementary Information

Cdc48$^{Ufd1/Npl4}$ segregase removes mislocalized centromeric histone H3 variant CENP-A from non-centromeric chromatin

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Supplementary Tables S1-S3

Supplementary Figures S1-S20

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

Strains	Parent/Source	Genotype	Reference
PK1/YMB8853		*MAT***a** *bar1Δ ura3Δns ade1 his2 leu2-3,112 trp1-1 ARG4*	P. Kaiser
PK1658/YMB9055		*MAT***a** *cdc48-3*	P. Kaiser
PK1743/YMB9056		*MAT***a** *ufd1-2*	P. Kaiser
PK1670/YMB9057		*MAT***a** *npl4-1*	P. Kaiser
YMB10546	PK1	*MAT***a** [pYES2]	This study
YMB10547	PK1	*MAT***a** [pMB1345]	This study
YMB10549	PK1658	*MAT***a** *cdc48-3* [pYES2]	This study
YMB10550	PK1658	*MAT***a** *cdc48-3* [pMB1345]	This study
YMB10552	PK1743	*MAT***a** *ufd1-2* [pYES2]	This study
YMB10553	PK1743	*MAT***a** *ufd1-2* [pMB1345]	This study
YMB10555	PK1670	*MAT***a** *npl4-1* [pYES2]	This study
YMB10556	PK1670	*MAT***a** *npl4-1* [pMB1345]	This study
YMB10649	YMB10550	*MAT***a** *cdc48-3* [pMB1345] [pRS425]	This study
YMB10650	YMB10550	*MAT***a** *cdc48-3* [pMB1345] [pMB1897]	This study
YMB10651	PK1	*MAT***a** *cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB10652	PK1658	*MAT***a** *cdc48-3 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB10653	PK1743	*MAT***a** *ufd1-2 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB10654	PK1670	*MAT***a** *npl4-1 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11092	YMB10652	*MAT***a** *cdc48-3 psh1Δ::kanMX6 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11148	YMB10652	*MAT***a** *cdc48-3 slx5Δ::kanMX6 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11448	PK1	*MAT***a** *NPL4-Myc::kanMX6*	This study
YMB11449	YMB10651	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11450	YMB10652	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR cdc48-3*	This study
YMB11522	YMB10651	*MAT***a** *psh1Δ::kanMX6 cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11457	PK1658	*MAT***a** *cdc48-3* [pYES2]	This study
YMB11458	PK1658	*MAT***a** *cdc48-3* [pMB1345]	This study
YMB11459	PK1658	*MAT***a** *cdc48-3* [pMB1768]	This study
YMB11460	PK1658	*MAT***a** *cdc48-3* [pMB1766]	This study
YMB11510	YMB10654	*MAT***a** *npl4-1 cse4Δ::6His-3HA-CSE4::NatR* [pMB1345] [pRS425]	This study
YMB11511	YMB10654	*MAT***a** *npl4-1 cse4Δ::6His-3HA-CSE4::NatR* [pMB1345] [pMB1996]	This study
YMB11601	PK1658	*MAT***a** *cdc48-3 hhf1Δ::kanMX*	This study

YMB11602	PK1658	*MAT***a** *cdc48-3 hhf2Δ::kanMX*	This study
YMB11605	YMB11601	*MAT***a** *cdc48-3 hhf1Δ::kanMX* [pYES2]	This study
YMB11606	YMB11601	*MAT***a** *cdc48-3 hhf1Δ::kanMX* [pMB1345]	This study
YMB11607	YMB11602	*MAT***a** *cdc48-3 hhf2Δ::kanMX* [pYES2]	This study
YMB11608	YMB11602	*MAT***a** *cdc48-3 hhf2Δ::kanMX* [pMB1345]	This study
YMB11653	PK1658	*MAT***a** *cdc48-3* [pMB1787]	This study
YMB11619	PK1658	*MAT***a** *cdc48-3 cse4Δ::6His-3HA-cse4 Y193A::NatR*	This study
YMB11620	PK1658	*MAT***a** *cdc48-3 cse4Δ::6His-3HA-cse4 Y193F::NatR*	This study
YMB11628	YMB11450	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR cdc48-3* [pMB433]	This study
YMB11629	YMB11450	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR cdc48-3* [pMB1458]	This study
YMB11648	YMB11450	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR cdc48-3 psh1Δ::hphMX*	This study
YMB11665	YMB11648	*MAT***a** *NPL4-Myc::kanMX6 cse4Δ::6His-3HA-CSE4::NatR cdc48-3 psh1Δ::hphMX* [pMB1458]	This study
YMB11677	PK1743	*MAT***a** *ufd1-2* [pMB1345] [pRS425]	This study
YMB11678	PK1743	*MAT***a** *ufd1-2* [pMB1345] [pMB2011]	This study
YJW15		*MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 ura3-1::ADH1-OsTIR1-9Myc (URA3)*	(1)
YMM203		*MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 cdc48-aid (hphNT1) ura3-1::ADH1-OsTIR1-9Myc (URA3 & klTRP1)*	(1)
YMB11428	YJW15	*MAT***a** *cse4Δ::6His-3HA-CSE4::NatR*	This study
YMB11429	YMM203	*MAT***a** *cse4Δ::6His-3HA-CSE4::NatR*	This study
BY4741		*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
BY4742		*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
YMB8734	BY4742	*MATα cdc48-1::NatR* (9 mutations)	Charles Boone
YMB8735	BY4741	*MAT***a** *cdc48-1::kanMX6* (3 mutations)	Charles Boone
YMB8736	BY4741	*MAT***a** *cdc48-4601::kanMX6*	Charles Boone
YMB9673	BY4741/BY4742	*MAT***a** *cse4Δ::6His-3HA-CSE4::NatR*	(2)
YMB9470	BY4741/BY4742	*MATα cse4Δ::kanMX6* [pMB1725]	(3)
YMB9887	BY4741/BY4742	*MATα cse4Δ::kanMX6* [pMB1781]	This study
YMB9888	BY4741/BY4742	*MATα cse4Δ::kanMX6* [pMB1782]	This study
YMB10904	BY4741	*MAT***a** *cdc48-3::kanMX6*	Charles Boone
YMB11204	YMB10904	*MAT***a** *cdc48-3::kanMX6 cse4Δ::6His-3HA-CSE4::NatR*	This study

Table S2. Plasmids used in this study.

Plasmid	Relevant characteristics	Reference
pYES2	Vector (*pGAL*, *2μ*, *URA3*)	(4)
pMB433	Vector (p426-*pGAL1*, *2μ*, *URA3*)	(5)
pRS415	Vector (*CEN*, *LEU2*)	(6)
pRS416	Vector (*CEN*, *URA3*)	(6)
pRS425	Vector (*2μ*, *LEU2*)	(7)
pMB1345	pYES2-*pGAL-8His-HA-CSE4* (*2μ*, *URA3*)	(8)
pMB1458	pMB433-*pGAL-6His-3HA-CSE4* (*2μ*, *URA3*)	(9)
pMB1551	MoBY Vector (*2μ*, *LEU2*)	(10)
pMB1604	MoBY *UBI4* (*2μ*, *LEU2*)	(10)
pMB1725	pRS415-*6His-3HA-CSE4* (*CEN, LEU2*)	(3)
pMB1766	pYES2-*pGAL-8His-HA-cse4 Y193A* (*2μ*, *URA3*)	(11)
pMB1768	pYES2-*pGAL-8His-HA-cse4 K215/216R* (*2μ*, *URA3*)	(12)
pMB1787	pYES2-*pGAL-8His-HA-cse4 Y193F* (*2μ*, *URA3*)	(11)
pMB1781	pRS415-*6His-3HA-cse4 Y193A* (*CEN, LEU2*)	This study
pMB1782	pRS415-*6His-3HA-cse4 Y193F* (*CEN, LEU2*)	This study
pMB1897	MoBY *CDC48* (*2μ*, *LEU2*)	This study
pMB1996	MoBY *NPL4* (*2μ*, *LEU2*)	This study
pMB2011	MoBY *UFD1* (*2μ*, *LEU2*)	This study
pLG41	*pGAL-H3* (*GAL1/10-HHT1*, *2μ*, *URA3*)	M. M. Smith
pLG39	*pGAL-H4* (*GAL1/10-HHF1*, *2μ*, *URA3*)	M. M. Smith

Table S3. Primers used in this study.

Primers	Description	Sequence	Reference
OMB244/OMB1690	*CEN3* forward	GATCAGCGCCAAACAATATGG	(13)
OMB245/OMB1691	*CEN3* reverse	AACTTCCACCAGTAAACGTTTC	(13)
OMB1692	*periCEN3* R1 forward	TTTACTGGTGGAAGTTTTGCTCA	(13)
OMB1693	*periCEN3* R1 reverse	GTCAACGAGTCCTCTCTGGCTA	(13)
OMB2824/SB3735	*SAP4* promoter forward	ACAGCACAACACGCTTACCA	(14)
OMB2825/SB3736	*SAP4* promoter reverse	CCAGCCCTAAATCCCCTAAA	(14)
OMB2826/SB4768	*RDS1* promoter forward	GACCCGTGCAGATCACTATTACA	(14)
OMB2827/SB4769	*RDS1* promoter reverse	GCAGTTTATCACATTTCCGTTTG	(14)

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Variant CENP-A/Cse4 into Chromatin Is Facilitated by Its C-Terminal Sumoylation. *Genetics*, **214**, 839-854.
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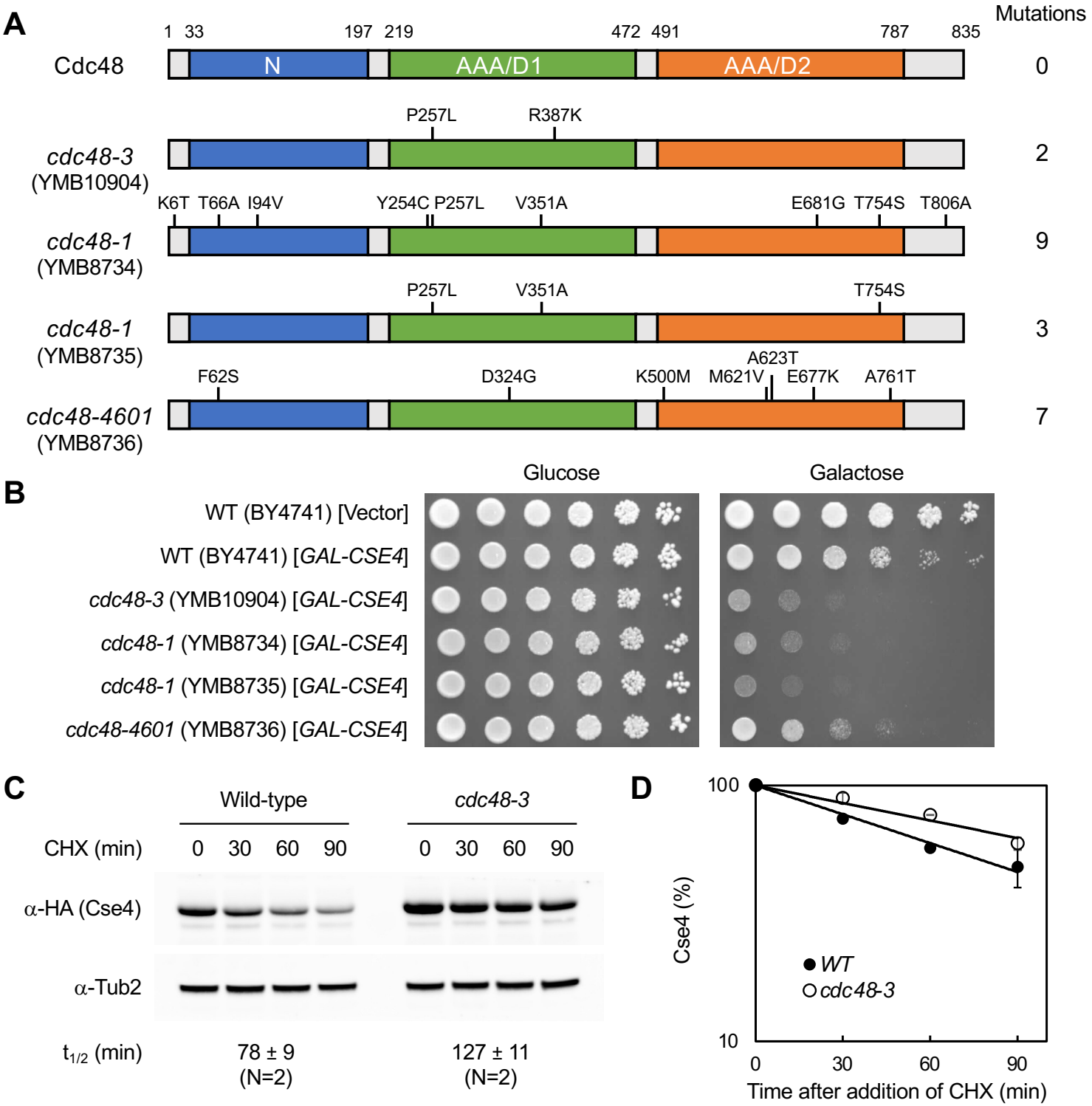
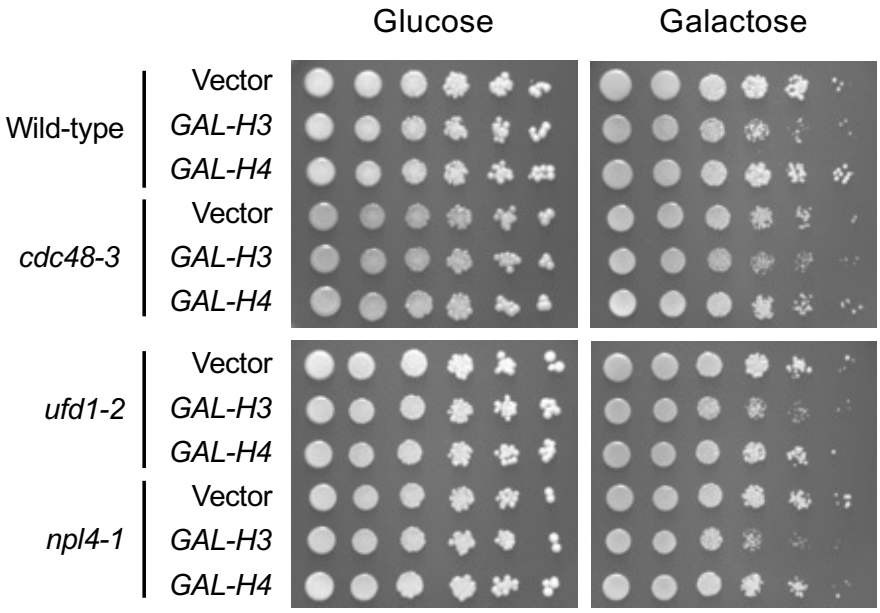
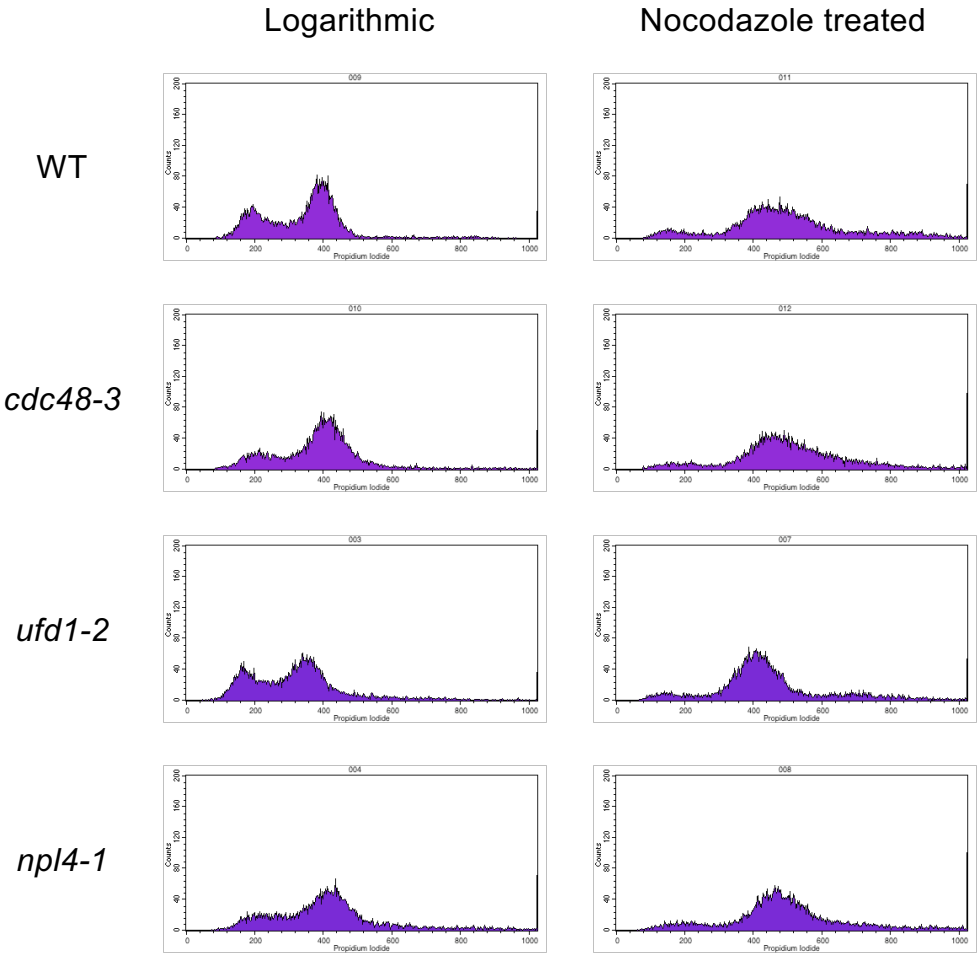


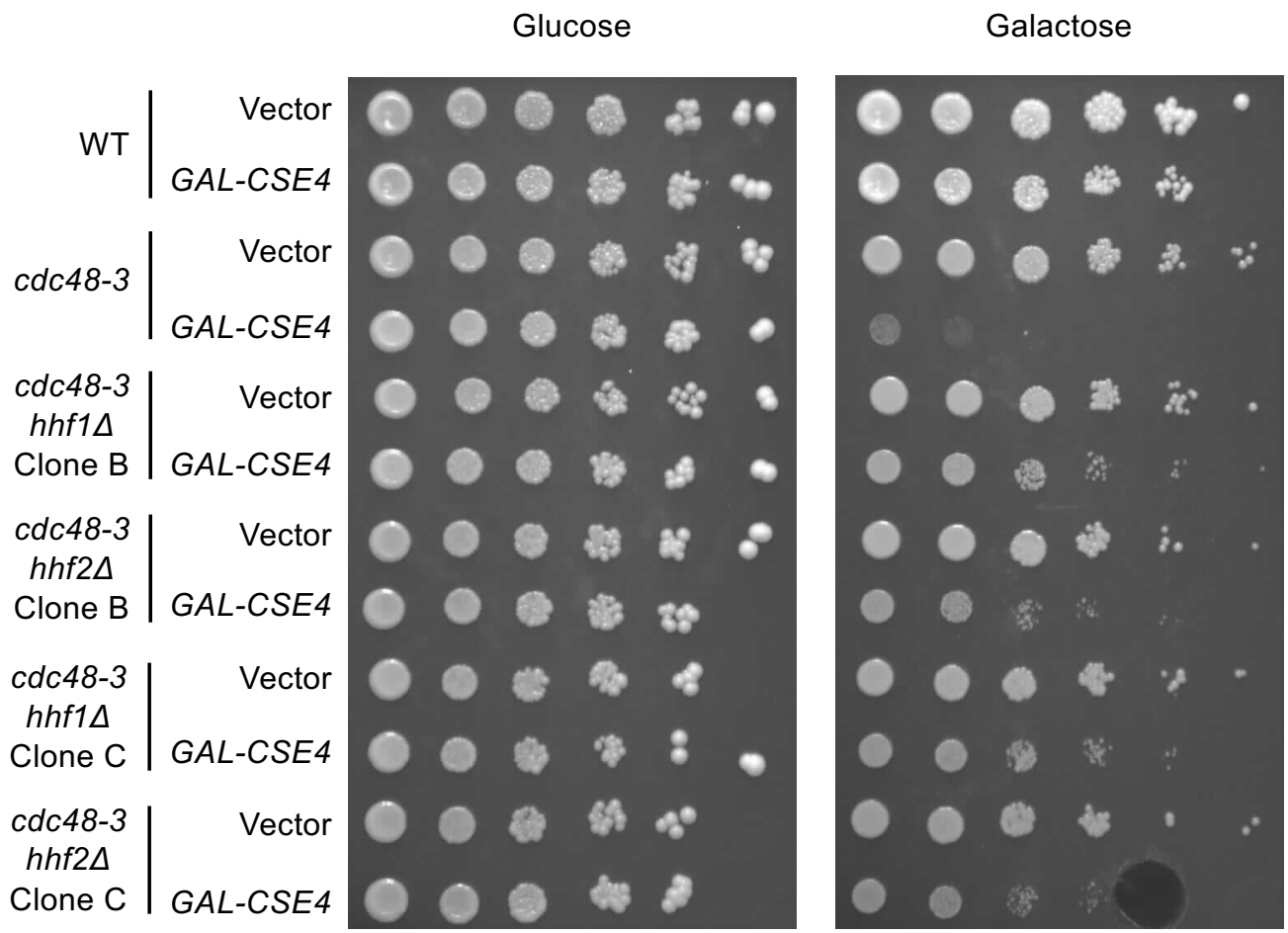
Supplementary Figure S1. The SGA screen identified *GAL-CSE4* SDL in *cdc48-3* mutant. (A) Sequence analysis of various *cdc48* mutants. The N, D1, and D2 domains of Cdc48 are blue, green, and orange, respectively. (B) Overexpression of *CSE4* results in SDL in *cdc48* mutants. The indicated strains transformed with vector (pYES2) or *GAL-CSE4* (pMB1345) were spotted in five-fold serial dilutions on glucose (2%)- or galactose (2%)-containing synthetic medium selective for the plasmids. The plates were incubated at 25°C for 3 days. (C) Endogenous Cse4 is stabilized in *cdc48-3* mutant. Protein extracts from wild-type (YMB9673) or *cdc48-3* (YMB11204) strain were prepared using logarithmically growing cells in YPD, treated with cycloheximide (CHX, 50 μg/ml) for various time points. Blots were probed with anti-HA (Cse4) or anti-Tub2 (loading control) antibody. Cse4 protein half-life ($t_{1/2}$) represents the mean of two biological repeats with average deviation. (D) Kinetics of turnover from C. Error bars indicate average deviation from the mean.



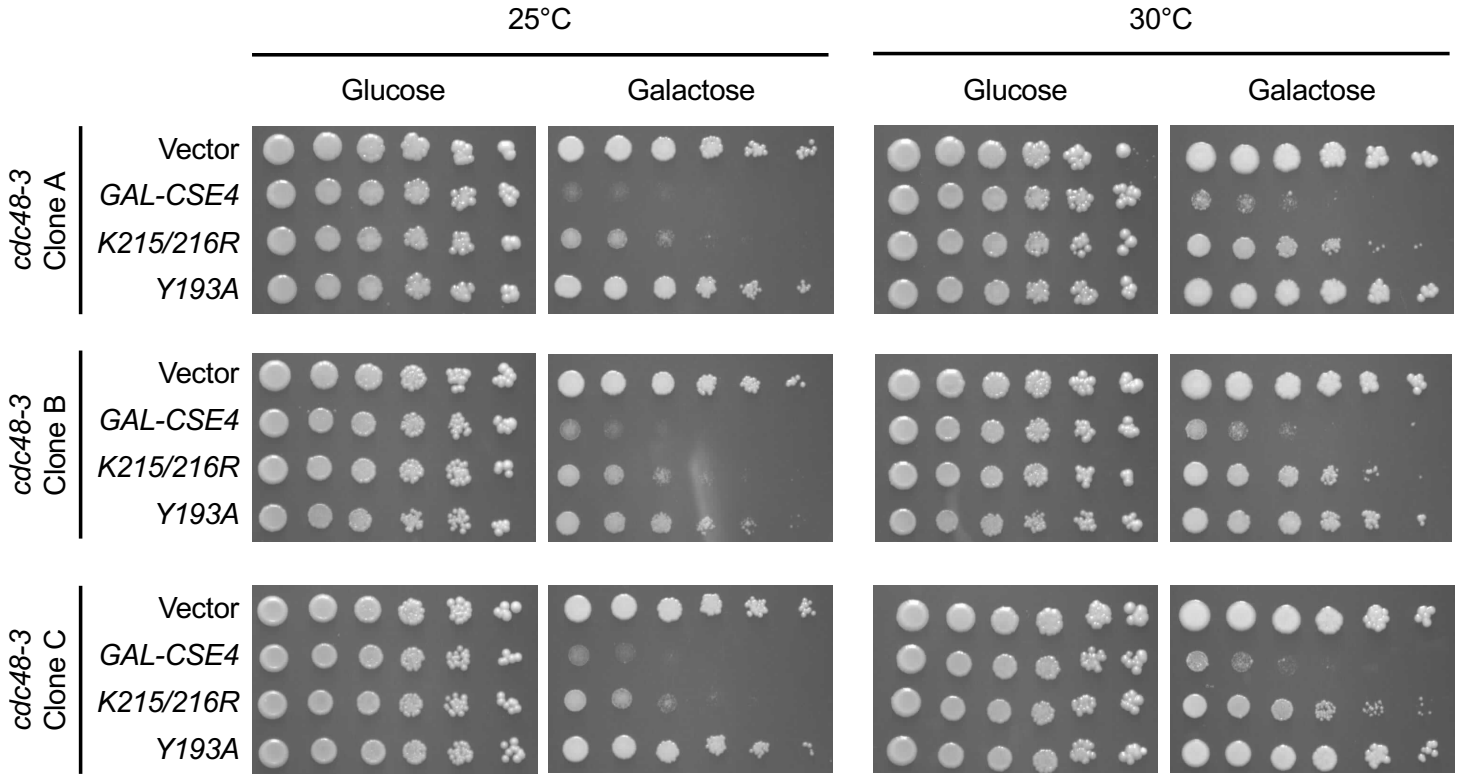
Supplementary Figure S2. Overexpression of histone H3 or H4 does not cause a growth defect in *cdc48-3*, *ufd1-2*, and *npl4-1* strains. Wild-type (PK1)**,** *cdc48-3* (PK1658), *ufd1-2* (PK1743), and *npl4-1* (PK1670) cells containing either vector (pMB433), *GAL-H3* (pLG41), or *GAL-H4* (pLG39) were spotted in five-fold serial dilutions on glucose (2%)- or galactose (2%)-containing synthetic medium selective for the plasmid. The plates were incubated at 25°C for 4 days.



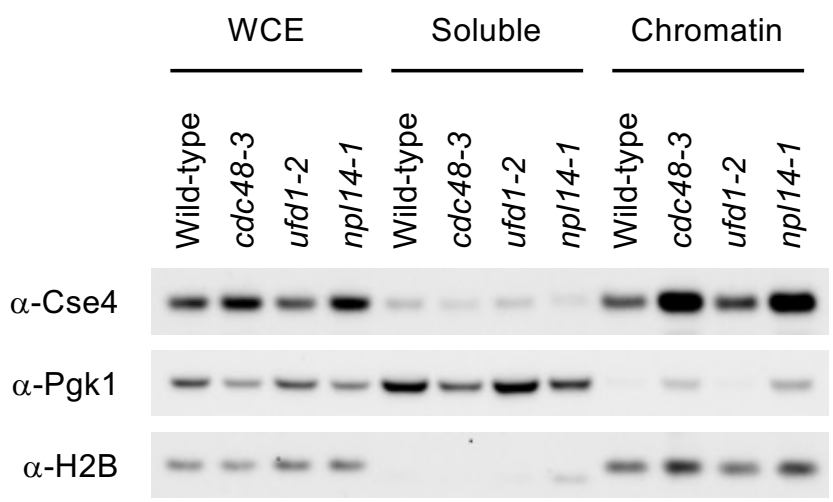
Supplementary Figure S3. FACS profiles used in Figure 1C for chromosome spreads with nocodazole arrested cells. Cells grown to logarithmic phase or arrested with nocodazole were processed by flow cytometry.



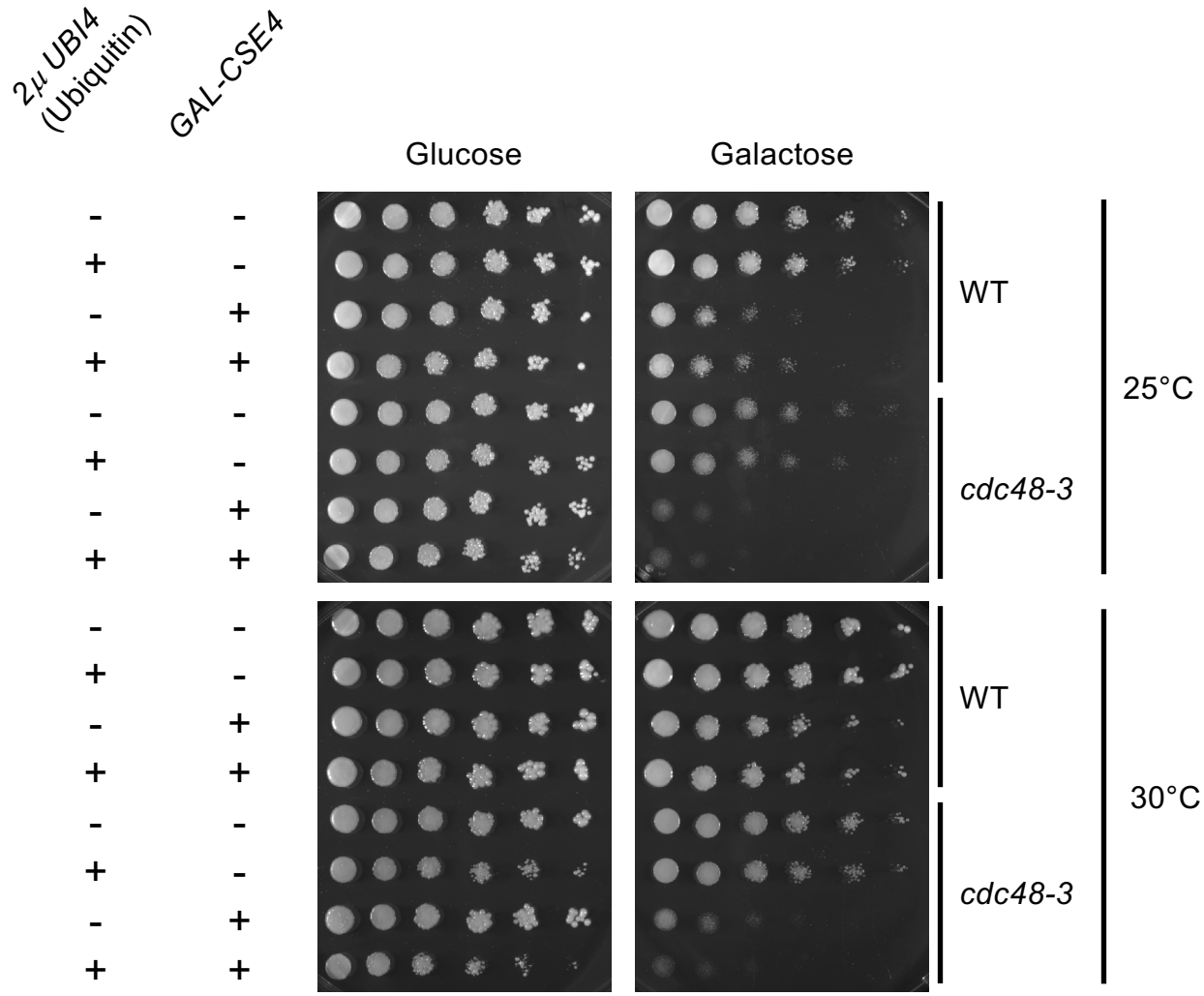
Supplementary Figure S4. Reduced dosage of histone H4 (*hhf1*Δ or *hhf2*Δ) suppresses the *cdc48-3 GAL-CSE4* SDL. Biological repeats for the growth assay shown in Figure 2A. The plates were incubated at 25°C for 5 days.



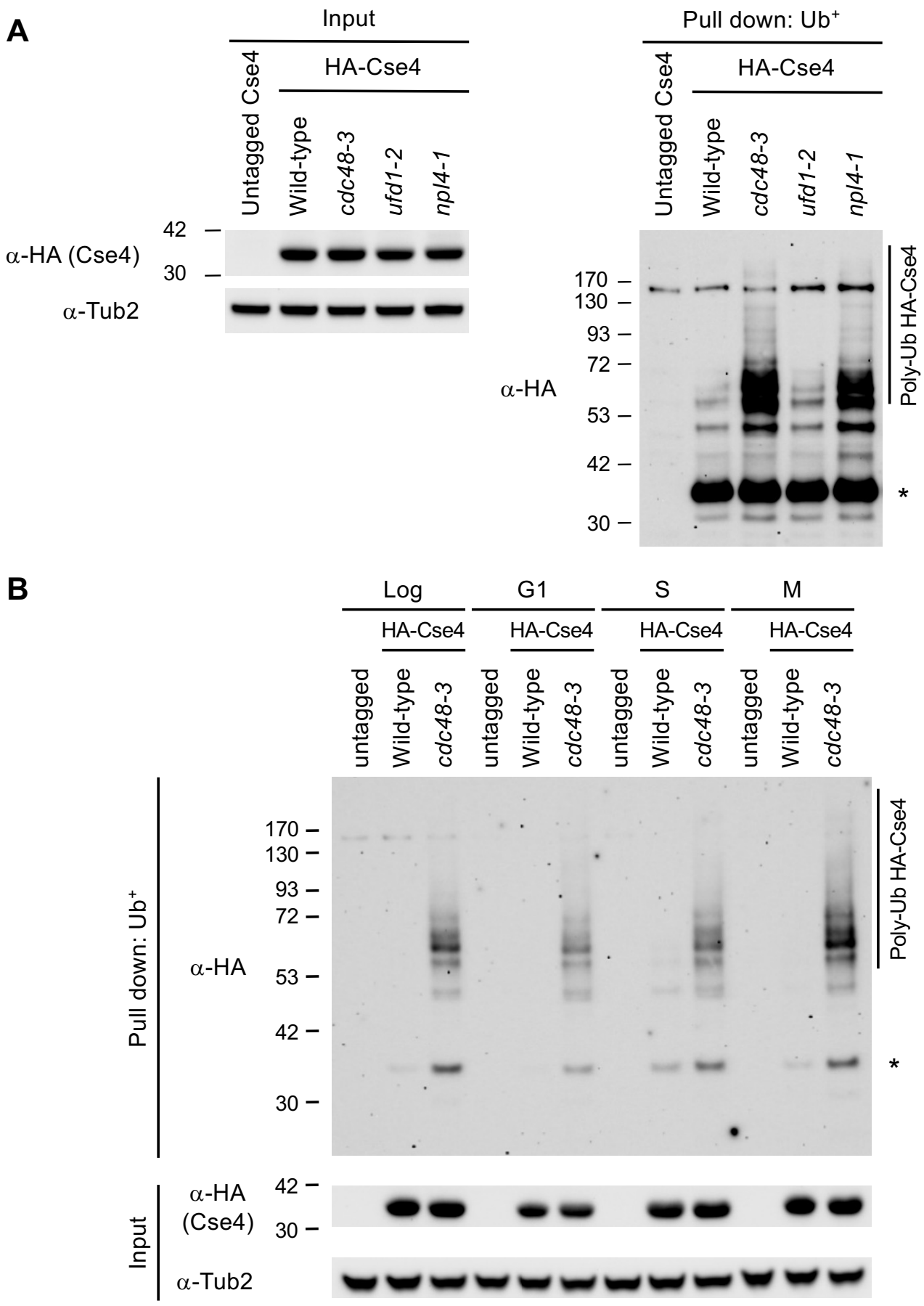
Supplementary Figure S5. *cse4 K215/216R* and *cse4 Y193A* mutations suppress *cdc48-3 GAL-CSE4* SDL. Biological repeats for the growth assay shown in Figures 2B and 4B. The plates were incubated at 25°C or 30°C for 5 days.



Supplementary Figure S6. Chromatin-bound endogenous Cse4 is enriched in *cdc48-3* and *npl4-1* strains. Biological repeats shown in Figure 2C. Whole cell extracts (WCEs) prepared from equal numbers of logarithmically growing cells in YPD were fractionated into soluble and chromatin fractions. Cse4 levels in each fraction were monitored by Western blot analysis with anti-Cse4 antibody. Pgk1 and histone H2B were used as markers for soluble and chromatin fractions, respectively.

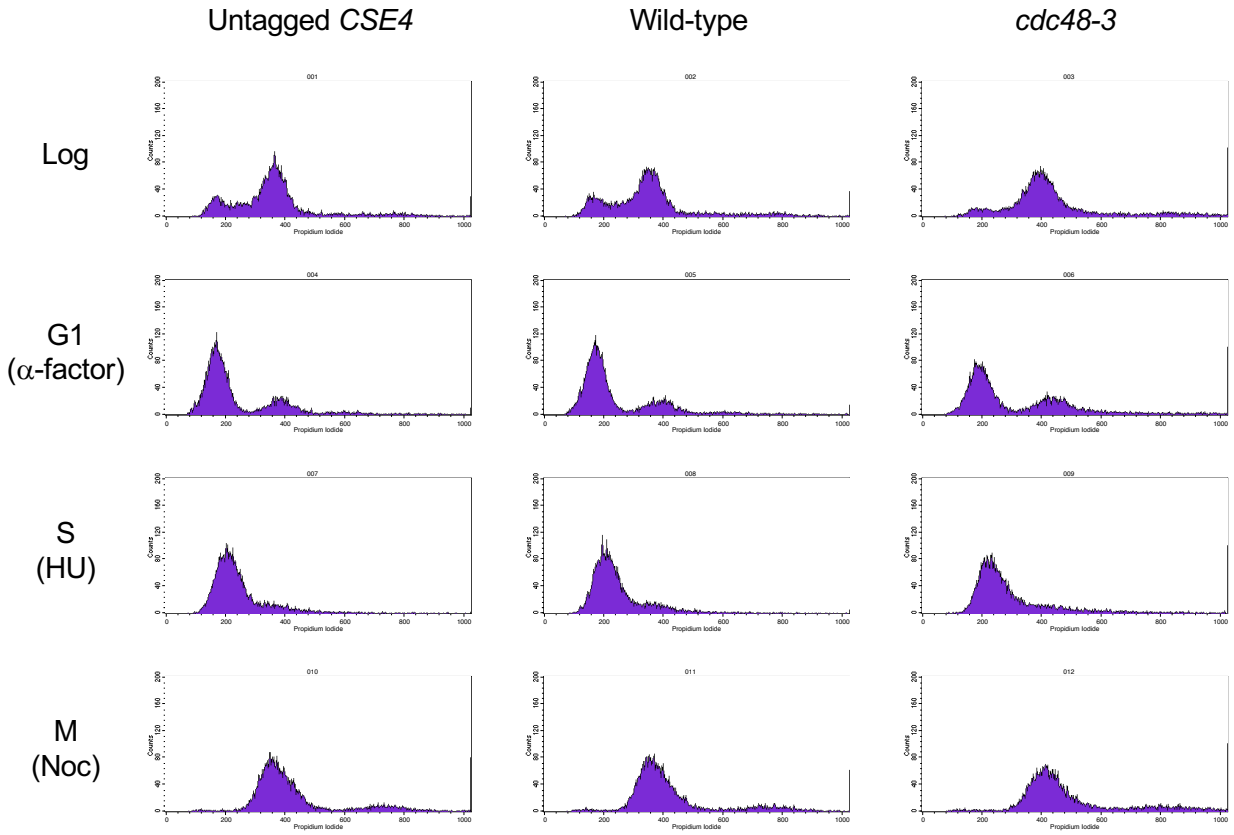


Supplementary Figure S7. Overexpression of *UBI4* does not suppress the SDL of a *cdc48-3 GAL-CSE4* strain. Wild-type (PK1) and *cdc48-3* (PK1658) strains transformed with vector (pYES2) or *GAL-CSE4* (pMB1345) and subsequently transformed with empty vector (pMB1551) or *2μ UBI4* (pMB1604) were spotted in five-fold serial dilutions on glucose (2%)- or galactose (2%)-containing synthetic medium selective for the plasmids. The plates were incubated at 25°C or 30°C for 3 days.



Supplementary Figure S8. Polyubiquitinated Cse4 is enriched in *cdc48-3, ufd1-2,* and *npl4-1* mutants under normal physiological conditions. Biological repeats for the Ub pull down assay shown in Figure 3A. Asterisk shows nonmodified Cse4. (A) Levels of Cse4 polyubiquitination are enhanced in *cdc48-3*, *ufd1-2*, and *npl4-1* strains. (B) Higher levels of Cse4 polyubiquitination are not specific to any cell cycle stage in *cdc48-3* mutants.

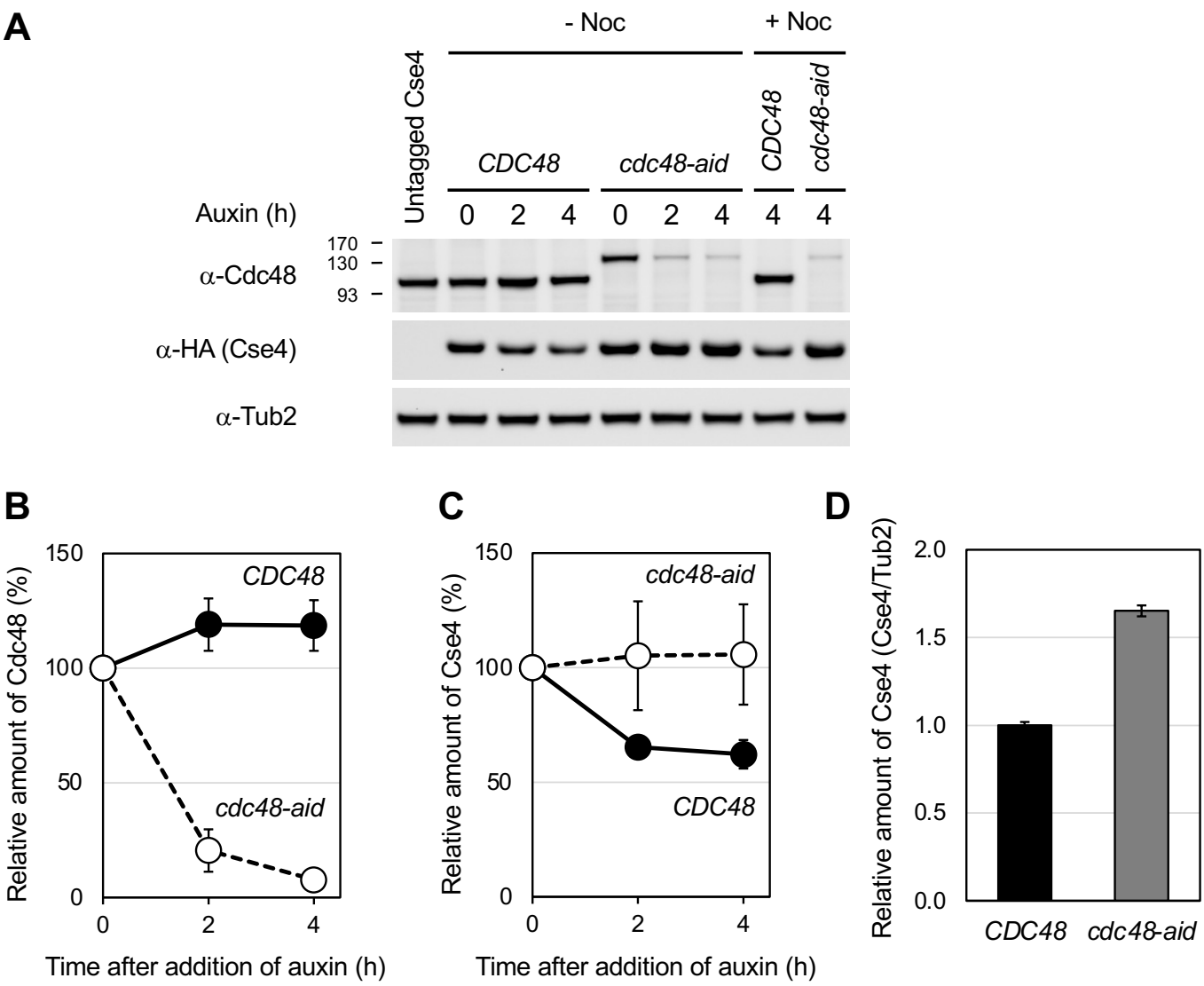
A



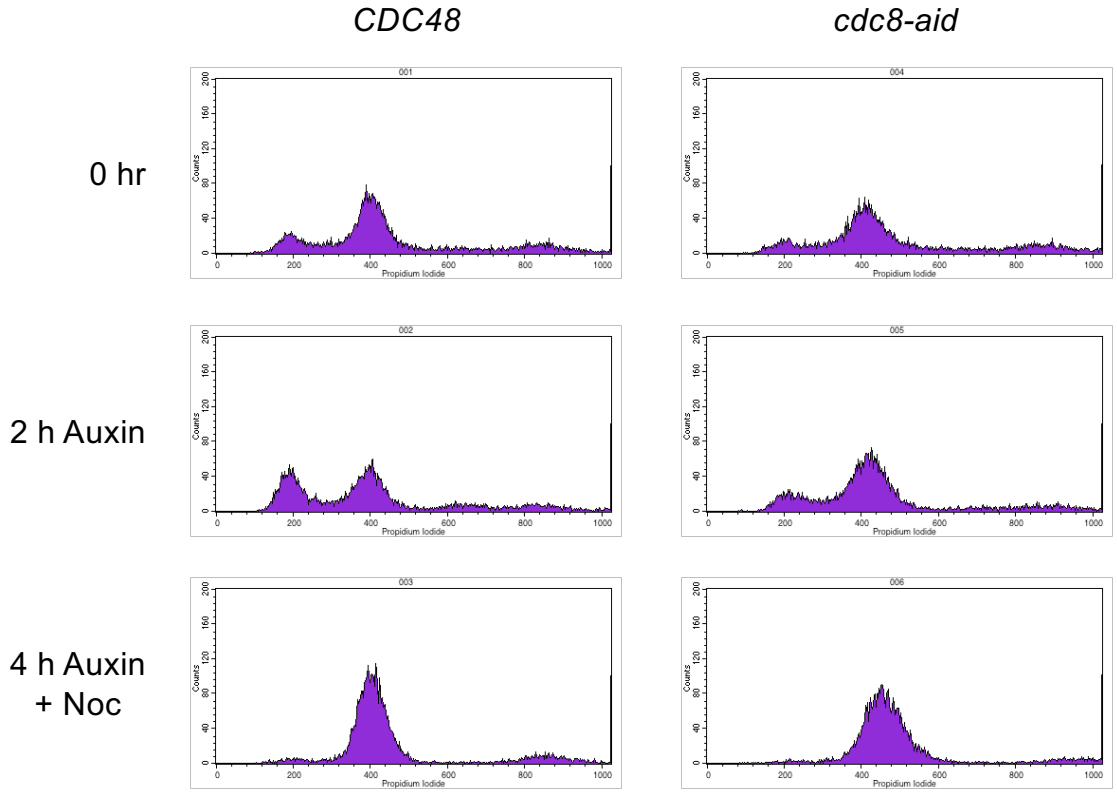
B

Strain	Cell cycle	G1	S	M	A	T
Untagged *CSE4*	LOG	19	28	13	2	38
Wild-type	LOG	20.5 ± 0.5	35.0 ± 2.0	5.0 ± 2.0	5.0 ± 2.0	34.5 ± 1.5
cdc48-3	LOG	14.0 ± 2.0	39.0 ± 4.0	5.5 ± 2.5	7.5 ± 0.5	34.0 ± 0.0
Untagged *CSE4*	G1 (α-factor)	90	2	0	0	8
Wild-type	G1 (α-factor)	90.5 ± 1.5	0	0.5 ± 0.5	1.0 ± 1.0	8.0 ± 0.0
cdc48-3	G1 (α-factor)	84.0 ± 1.0	5.5 ± 4.5	0	1.5 ± 0.5	9.0 ± 3.0
untagged *CSE4*	S (HU)	2	72	10	4	12
Wild-type	S (HU)	2.5 ± 0.5	77.0 ± 1.0	6.0 ± 6.0	7.5 ± 1.5	7.0 ± 3.0
cdc48-3	S (HU)	2.0 ± 0.0	80.5 ± 2.5	5.5 ± 2.5	5.5 ± 2.5	6.5 ± 2.5
Untagged *CSE4*	G2/M (Nocodazole)	0	11	88	1	0
Wild-type	G2/M (Nocodazole)	1.5 ± 0.5	18.0 ± 2.0	73.5 ± 2.5	5.0 ± 2.0	2.0 ± 2.0
cdc48-3	G2/M (Nocodazole)	1.5 ± 0.5	23.5 ± 2.5	66.0 ± 1.0	6.0 ± 2.0	3.0 ± 0.0

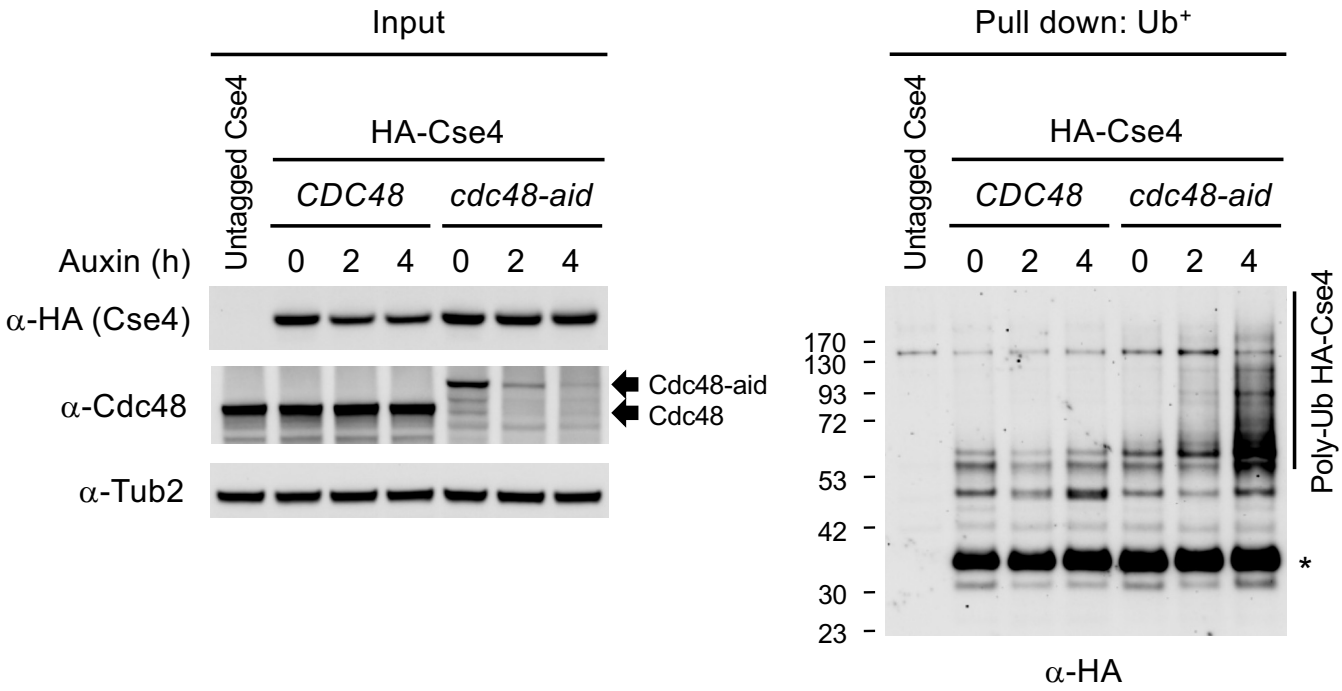
Supplementary Figure S9. Cell cycle profiles of untagged *CSE4*, wild-type, and *cdc48-3* strains grown to logarithmic phase or arrested with α-factor, HU, or nocodazole (Noc). (A) FACS analysis used in Supplementary Figure S8B. (B) Nuclear morphology was used to determine the percentage of cells that show unbudded (G1), small budded (S), and large budded (M) arrest phenotype of cells used in Figure 3A and Supplementary Figure S8B. 100 cells were counted for each strain for each arrest.



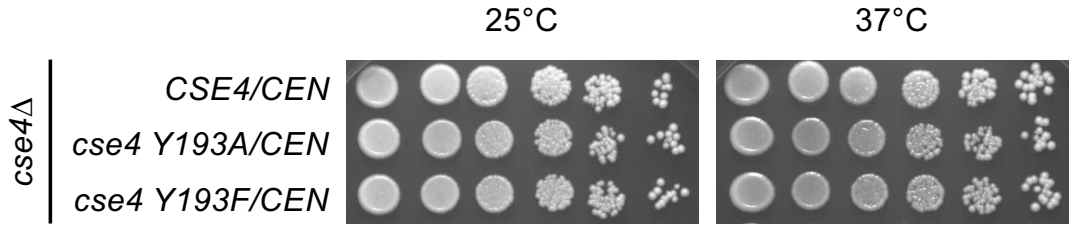
Supplementary Figure S10. Levels of Cse4 are increased by inactivation of Cdc48. (A) Auxin treatment leads to efficient depletion of Cdc48 in the *cdc48-aid* strain. *CDC48* (control, YMB11428) and *cdc48-aid* (YMB11429) cells were grown in YPD to logarithmic phase at 25°C, then treated with auxin at the indicated time point. For M phase arrested cells, nocodazole (Noc) was added to the media after 2 hours auxin treatment. The levels of Cdc48, Cse4, and Tub2 were monitored by immunoblotting with anti-Cdc48, anti-HA, and anti-Tub2 antibodies, respectively. (B) The level of Cdc48 was greatly reduced in the *cdc48-aid* strain after auxin treatment (-Noc). (C) The level of Cse4 was maintained in the *cdc48-aid* strain after auxin treatment (-Noc). (D) The level of Cse4 was increased in the *cdc48-aid* strain after auxin and nocodazole treatments when compared to control *CDC48*.



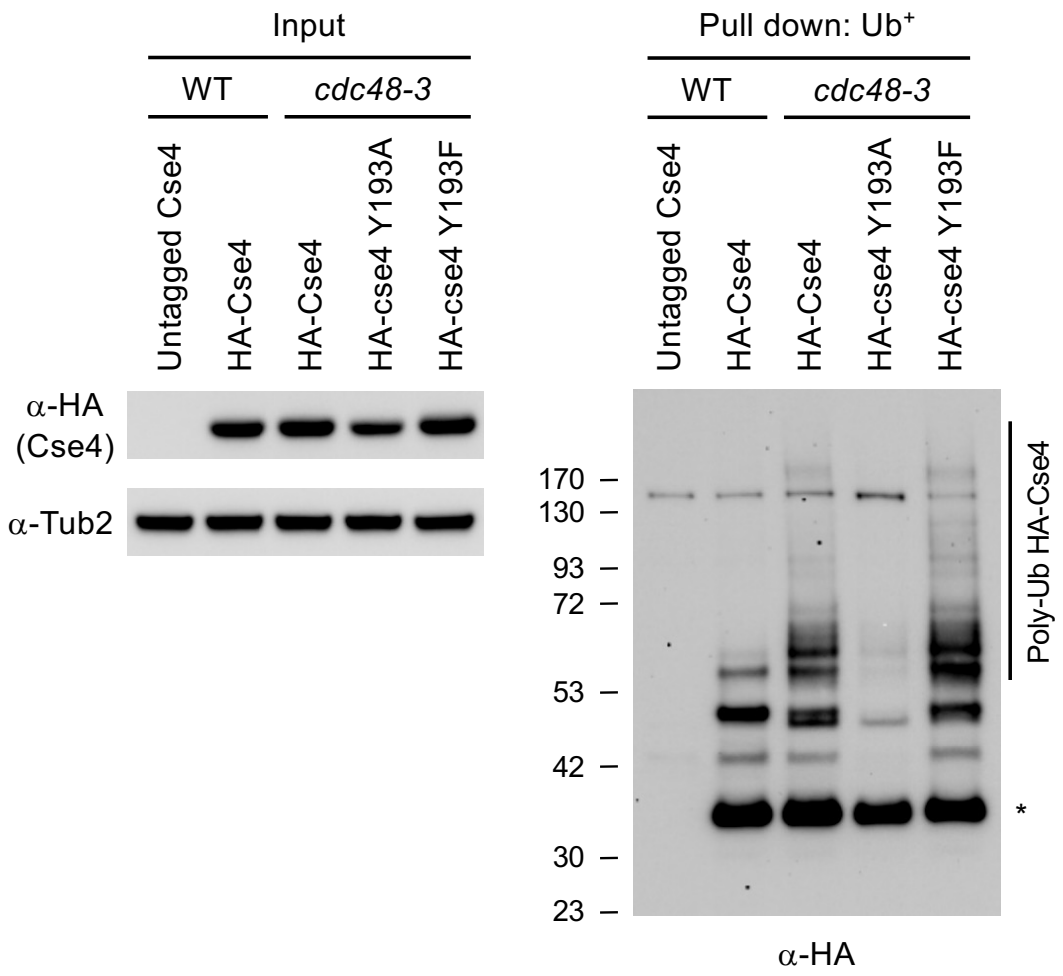
Supplementary Figure S11. FACS profiles for strains used in Figure 3B and Supplemental Figure S10. Untreated or nocodazole-treated cells were processed by flow cytometry.



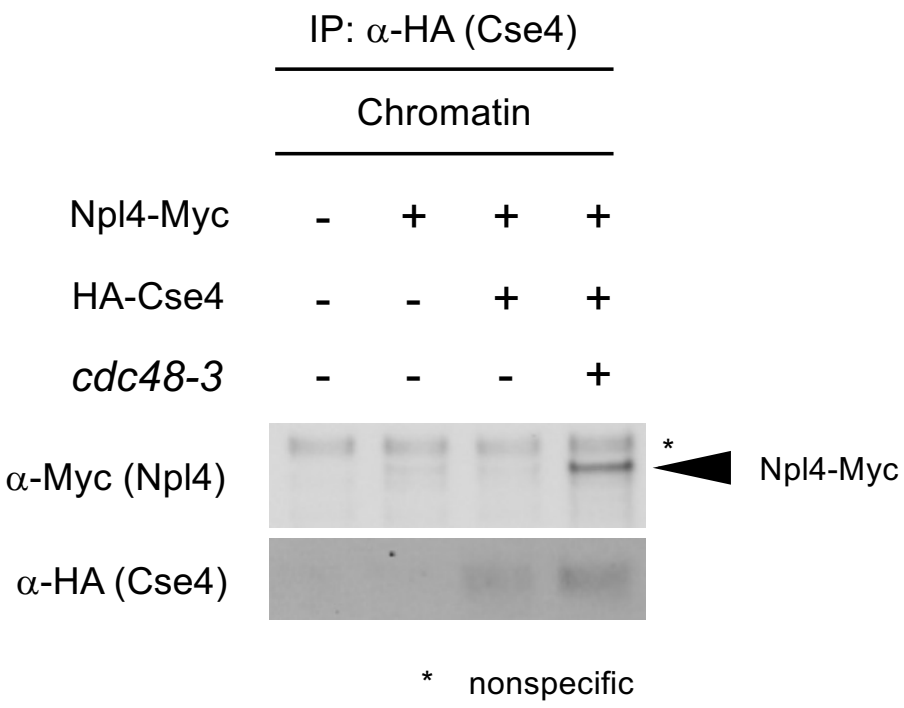
Supplementary Figure S12. Auxin-mediated depletion of Cdc48 contributes to accumulation of polyubiquitinated Cse4. Biological repeats for the Ub pull-down assay shown in Figure 3B. Ub pull-down assay was performed using protein extracts from *CDC48* (control, YMB11428) and *cdc48-aid* (YMB11429) strains, grown in YPD to logarithmic phase at 25°C and treated with auxin at the indicated time point. Input and ubiquitin pull down samples were analyzed using anti-HA (Cse4), anti-Cdc48, and anti-Tub2 antibodies. Untagged Cse4 was used as a negative control. Asterisk shows nonmodified Cse4.



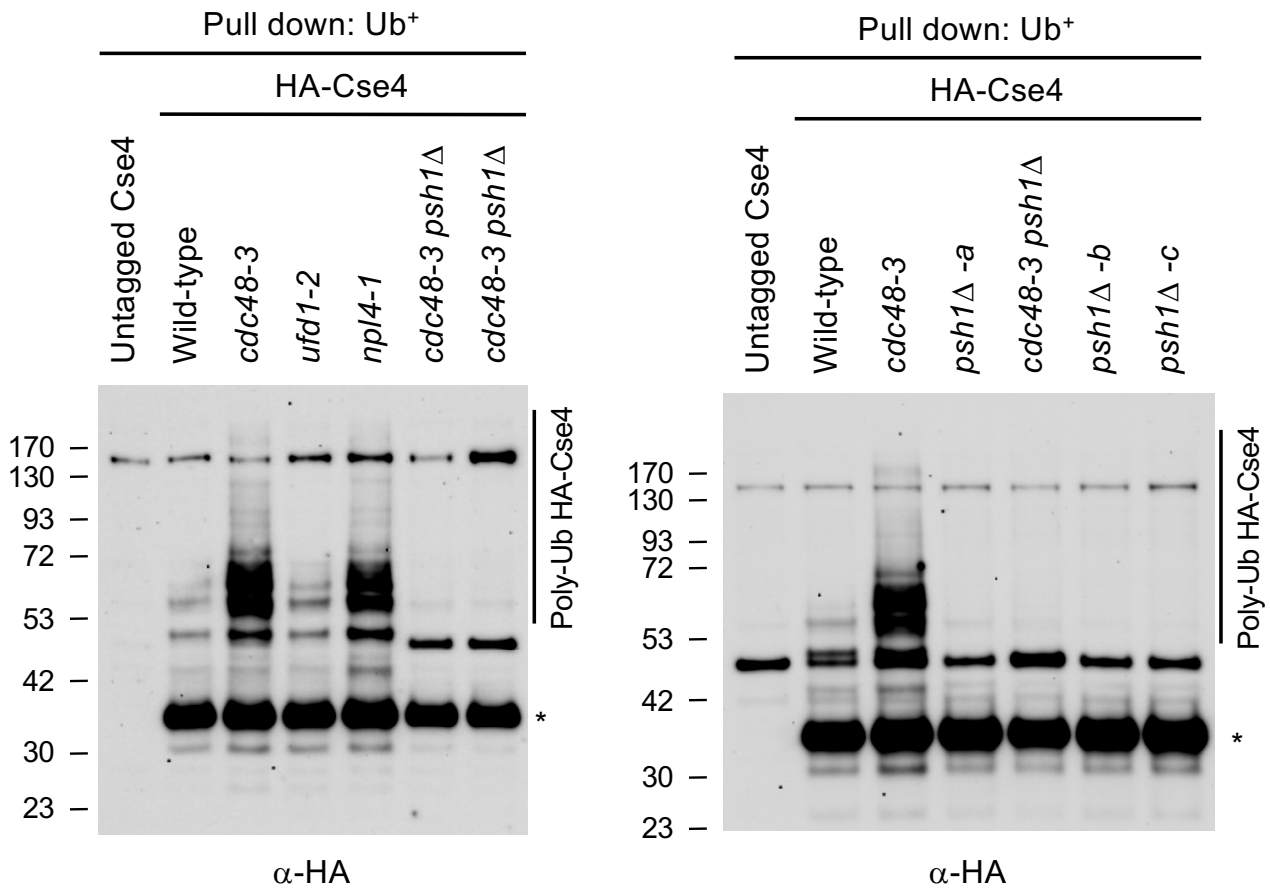
Supplementary Figure S13. Both *cse4 Y193A/F* alleles complement *cse4*Δ strain and do not show temperature sensitivity. Cells were spotted in five-fold serial dilutions on YPD plates and incubated at 25°C or 37°C for 3 days. Isogenic yeast strains are *CSE4* (YMB9470), *cse4 Y193A* (YMB9887), and *cse4 Y193F* (YMB9888).



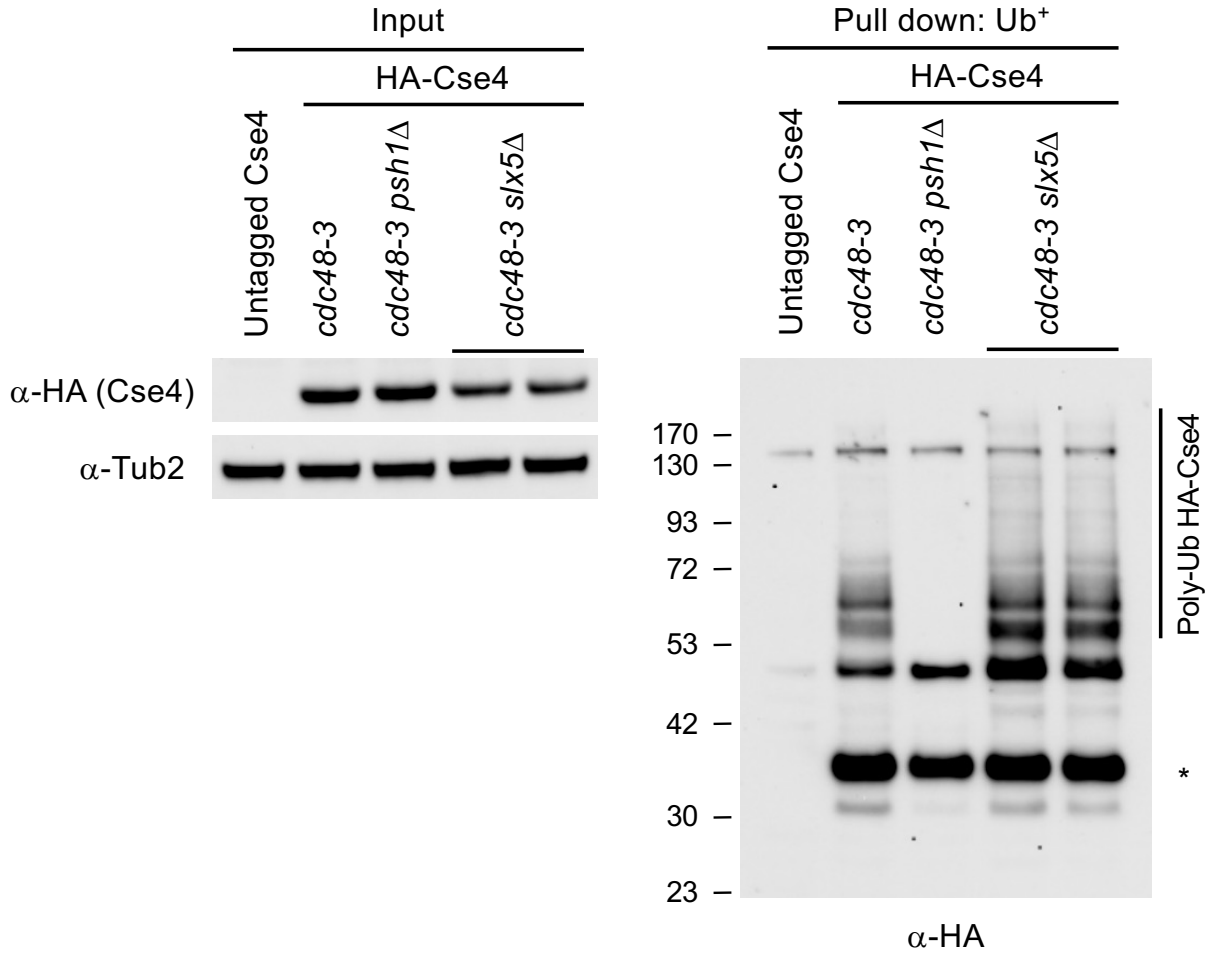
Supplementary Figure S14. Endogenous Cse4 Y193A exhibits reduced Cse4 polyubiquitination. Biological repeats shown in Figure 4E. Asterisk shows nonmodified Cse4.



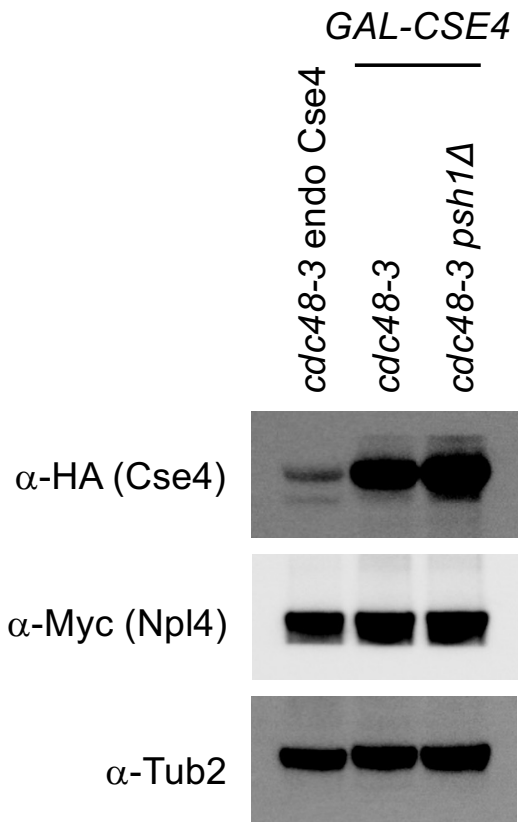
Supplementary Figure S15. Npl4 interacts with chromatin-bound Cse4. Biological repeats shown in Figure 5C.



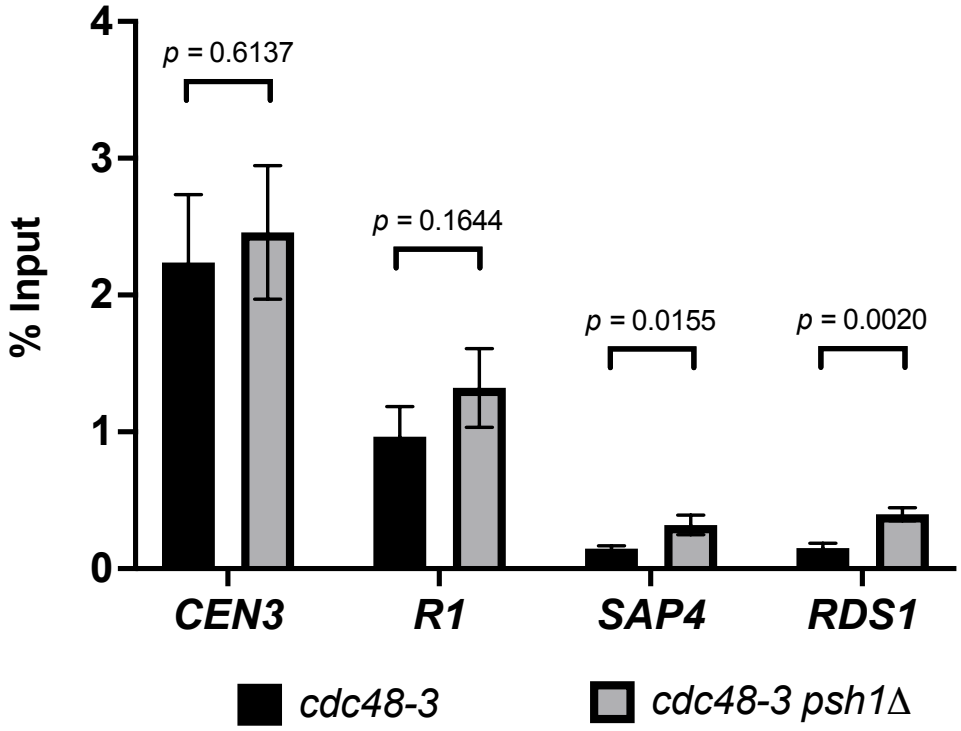
Supplementary Figure S16. Psh1 contributes to polyubiquitination of endogenous Cse4 in *cdc48-3* strain. Biological repeats for the Ub pull-down assay shown in Figure 6. Asterisk shows nonmodified Cse4.



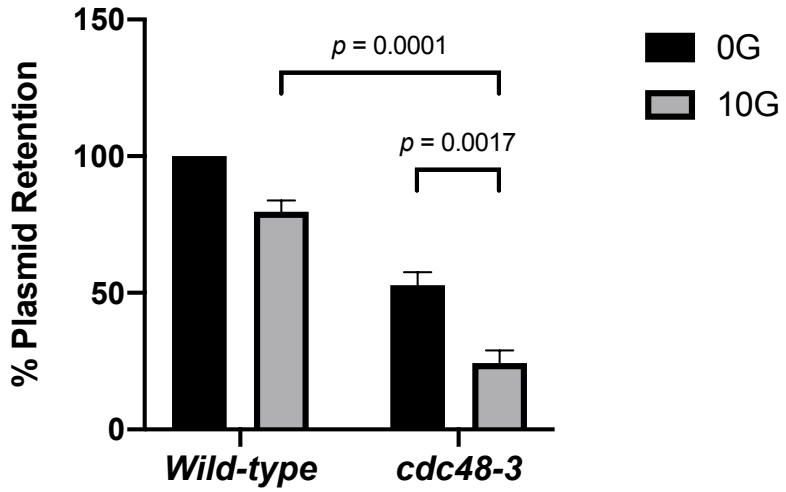
Supplementary Figure S17. Higher levels of endogenous Cse4 polyubiquitination in *cdc48-3* are not reduced by deletion of *SLX5*. Ub pull-down assay was performed using protein extracts from logarithmically growing cells in YPD at 25°C. Input and ubiquitin pull down samples were analyzed using anti-HA (Cse4) and anti-Tub2 antibodies. Untagged Cse4 was used as a negative control. Asterisk shows nonmodified Cse4.



Supplementary Figure S18. Deletion of *PSH1* does not affect levels of Npl4-Myc. Cells were grown in sucrose/galactose (2% final concentration each) media for 3-3.5 hours to induce expression of *HA-CSE4* under *GAL* promoter. The levels of Cse4, Npl4, and Tub2 were monitored by Western blot analysis with anti-HA, anti-Myc, and anti-Tub2 antibodies, respectively.



Supplementary Figure S19. Increased mislocalization of Cse4 at the promoters of *SAP4* and *RDS1* in a *cdc48-3 psh1*Δ strain, compared to a *cdc48-3* strain. ChIP-qPCR was performed as described in Figure 7D. Statistical significance was assessed by unpaired *t*-test. Error bars represent standard deviation of the mean of three independent experiments.



Supplementary Figure S20. *cdc48-3* strain exhibits CIN. Wild-type and *cdc48-3* strains containing *CEN* plasmid (pRS416 *URA3*) were grown selectively in SC-Ura plus glucose (2%) medium (SD-Ura) as 0 generation (0G). Equal OD_{600} of each strain were inoculated in SD complete medium and allowed to grow for 10 generations without selection (10G). Equal OD_{600} of cells from 0G and 10G were plated on SD-Ura and SD complete medium. Colony number of SD-Ura/SD complete is calculated as the rate of plasmid retention. The percentage of plasmid retention in wild-type cells at 0G is set to 100%. Error bars represent the standard deviation of three replicates. Statistical significance was assessed by unpaired *t*-test.