Deposition of centromeric histone H3 variant CENP-A/Cse4 into chromatin is facilitated by its C-terminal sumoylation

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Supplemental Tables S1-S3

Supplemental Figures S1-S4

Supplemental Information

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

Strains	Parent	Genotype	Reference
BY4741		*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
BY4742		*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
YMB7277	BY4741/BY4742	*MAT***a** *siz1Δ::kanMX siz2Δ::kanMX*	(OHKUNI *et al*. 2016)
YMB8785	BY4742	*MAT*α *hir2Δ::clonNAT*	This study
YMB9034	BY4741	*MAT***a** *psh1Δ::kanMX*	Open Biosystems
YMB9035	BY4741	*MAT***a** *slx5Δ::kanMX*	Open Biosystems
YMB10975	BY4741	*MAT***a** *CAC2-3FLAG:kanMX*	This study
YMB10976	BY4741	*MAT***a** *SCM3-3FLAG:kanMX*	This study
W303a		*MAT***a** *ura3-1 leu2, 3-112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ*	(HEWAWASAM *et al.* 2018)
JG1689	W303a	*MAT***a** *pGAL1-10-3HA-SCM3::TRP1* [pSB17 (vector) *URA3*]	(HEWAWASAM *et al.* 2018)
JG1690	W303a	*MAT***a** *pGAL1-10-3HA-SCM3::TRP1* [pSB873 (*pCUP1-CSE4*) *URA3*]	(HEWAWASAM *et al.* 2018)
YMB11165	W303a	*MAT***a** *pGAL1-10-3HA-SCM3::TRP1* [pMB1969 (*pCUP1-cse4 K215/216R*) *URA3*]	This study
YPH1018		*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CFIII (CEN3L.YPH278) HIS3 SUP11*	P. Hieter
YMB10298	YPH1018	*MAT*α *cse4Δ::6His-3HA-CSE4::mx4NAT*	This study
YMB10299	YPH1018	*MAT*α *cse4Δ::6His-3HA-cse4 K215/216A::mx4NAT*	This study
YMB10300	YPH1018	*MAT*α *cse4Δ::6His-3HA-cse4 K215/216R::mx4NAT*	This study

Table S2. Plasmids used in this study.

Plasmid	Relevant characteristics	Reference
pYES2	Vector (*pGAL*, *2μ*, *URA3*)	(BOECKMANN *et al.* 2013)
pMB433	Vector (p426-*pGAL1*, *2μ*, *URA3*)	(MUMBERG *et al.* 1994)
pMB1344	pYES2-*pGAL-8His-HA-cse4 16KR* (*2μ*, *URA3*)	(OHKUNI *et al.* 2016)
pMB1345	pYES2-*pGAL-8His-HA-CSE4* (*2μ*, *URA3*)	(OHKUNI *et al.* 2016)
pMB1458	pMB433-*pGAL-6His-3HA-CSE4* (*2μ*, *URA3*)	(AU *et al.* 2013)
pMB1597	pMB433-*pGAL-3HA-CSE4* (*2μ*, *URA3*)	(OHKUNI *et al.* 2016)
pMB1675	pMB433-*pGAL-6His-3HA-cse4 K215/216A* (*2μ*, *URA3*)	This study
pMB1677	pMB433-*pGAL-3HA-cse4 K215/216A* (*2μ*, *URA3*)	This study
pMB1762	pYES2-*pGAL-8His-HA-cse4 K65R* (*2μ*, *URA3*)	(OHKUNI *et al.* 2018)
pMB1768	pYES2-*pGAL-8His-HA-cse4 K215/216R* (*2μ*, *URA3*)	This study
pMB1769	pYES2-*pGAL-8His-HA-cse4 K65/215/216R* (*2μ*, *URA3*)	This study
pMB1789	pMB433-*pGAL-6His-3HA-cse4 K215/216R* (*2μ*, *URA3*)	This study
pMB1791	pMB433-*pGAL-6His-3HA-cse4 K65R* (*2μ*, *URA3*)	(OHKUNI *et al.* 2018)
pMB1792	pMB433-*pGAL-6His-3HA-cse4 K65/215/216R* (*2μ*, *URA3*)	This study
pMB1815	pMB433-*pGAL-3HA-cse4 K215/216R* (*2μ*, *URA3*)	This study
pMB1969	*pCUP1-cse4 K215/216R* (*URA3*)	This study

Table S3. Primers used in this study.

Primers	Description	Sequence	Reference
OMB244/OMB1690	*CEN3* forward	GATCAGCGCCAAACAATATGG	(CIFTCI-YILMAZ *et al.* 2018)
OMB245/OMB1691	*CEN3* reverse	AACTTCCACCAGTAAACGTTTC	(CIFTCI-YILMAZ *et al.* 2018)
OMB426	*CEN1* forward	CTCGATTTGCATAAGTGTGCC	(CIFTCI-YILMAZ *et al.* 2018)
OMB427	*CEN1* reverse	GTGCTTAAGAGTTCTGTACCAC	(CIFTCI-YILMAZ *et al.* 2018)
OMB2824/SB3735	*SAP4* promoter forward	ACAGCACAACACGCTTACCA	(HILDEBRAND AND BIGGINS 2016)
OMB2825/SB3736	*SAP4* promoter reverse	CCAGCCCTAAATCCCCTAAA	(HILDEBRAND AND BIGGINS 2016)
OMB2826/SB4768	*RDS1* promoter forward	GACCCGTGCAGATCACTATTACA	(HILDEBRAND AND BIGGINS 2016)
OMB2827/SB4769	*RDS1* promoter reverse	GCAGTTTATCACATTTCCGTTTG	(HILDEBRAND AND BIGGINS 2016)
OMB3274	*FIG4* and *LEM3* divergent forward	ACCAGAACGGCAGACAAAGT	This study
OMB3275	*FIG4* and *LEM3* divergent reverse	TGAACGTGCTGCAATAAACC	This study
OMB3276	*COQ3* tandem forward	CCTTTCATAATGTATTATCACCCTTT	This study
OMB3277	*COQ3* tandem reverse	AGACTTCGCTGTACCTGTTTCC	This study
OMB3278	*GUP2* gene forward	CGATGGTATTGATGCACCTG	This study
OMB3279	*GUP2* gene reverse	GACTGTTAACCACCCCCAAA	This study
OMB3280/SB3781	*SLP*1 promoter forward	TCCTAGGTTATCTCATCGGTACT	(HILDEBRAND AND BIGGINS 2016)
OMB3281/SB3782	*SLP1* promoter reverse	ACTATATCCATTGCGTCCTTTCT	(HILDEBRAND AND BIGGINS 2016)
OMB3282	*PHO5* promoter forward	CCCATTTGGGATAAGGGTAAAC	(HEWAWASAM *et al.* 2018)
OMB3283	*PHO5* promoter reverse	GATGAAGCCATACTAACCTCGA	(HEWAWASAM *et al.* 2018)
OMB3298	*UGA3* and *UGX2* convergent forward	CCCCCTGGGTGTCTTAAATTAT	This study
OMB3299	*UGA3* and *UGX2* convergent reverse	TTCTATGTCTCAACGTTAGCATTTC	This study

References

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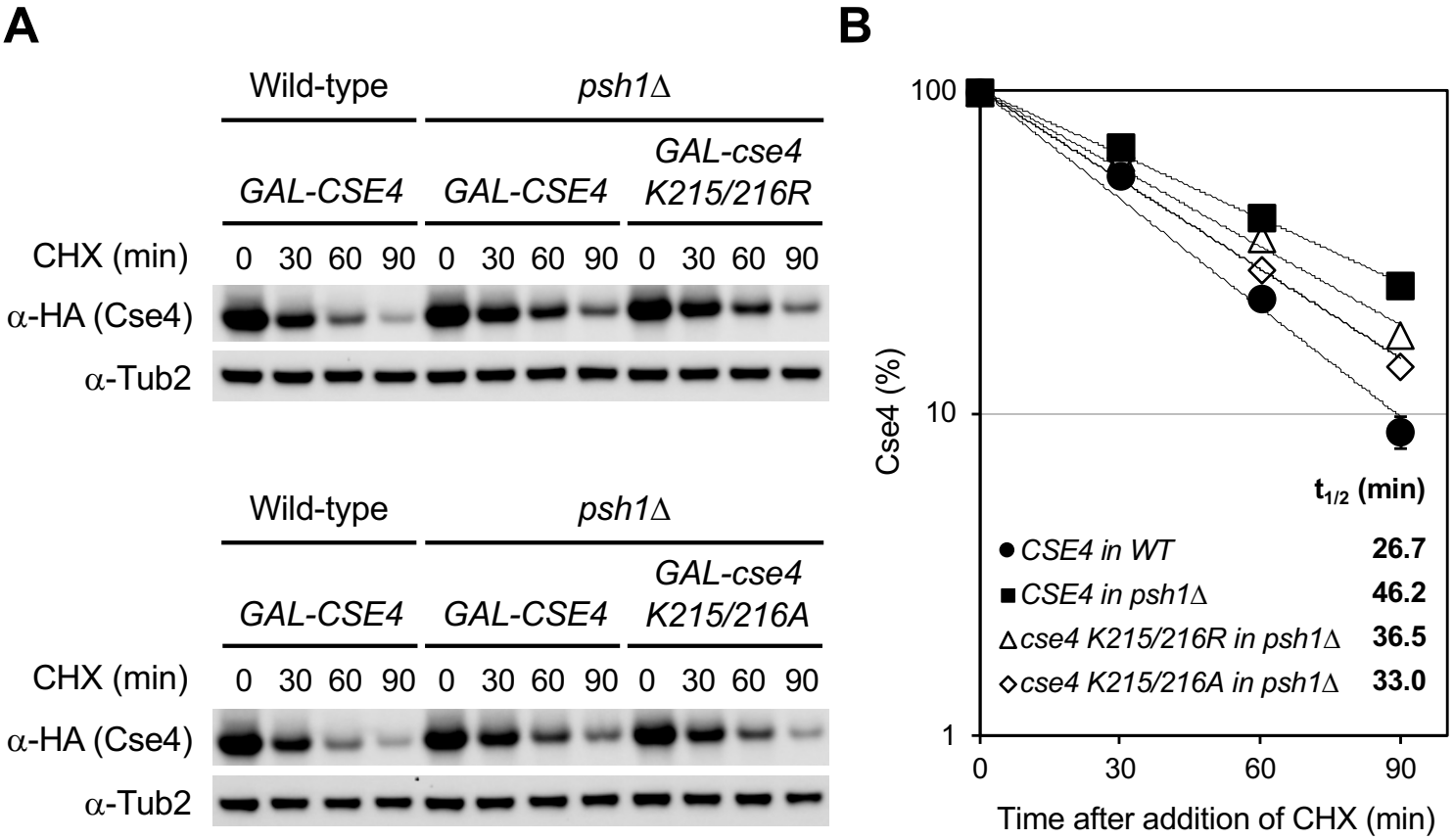

Figure S1. The stability of HA-Cse4 K215/216R/A is not increased when compared to HA-Cse4 in a *psh1*Δ strain. Biological repeat of Figure 2C-2D is shown. Protein stability assay was done as described in Figure 2C-2D.

Figure S1

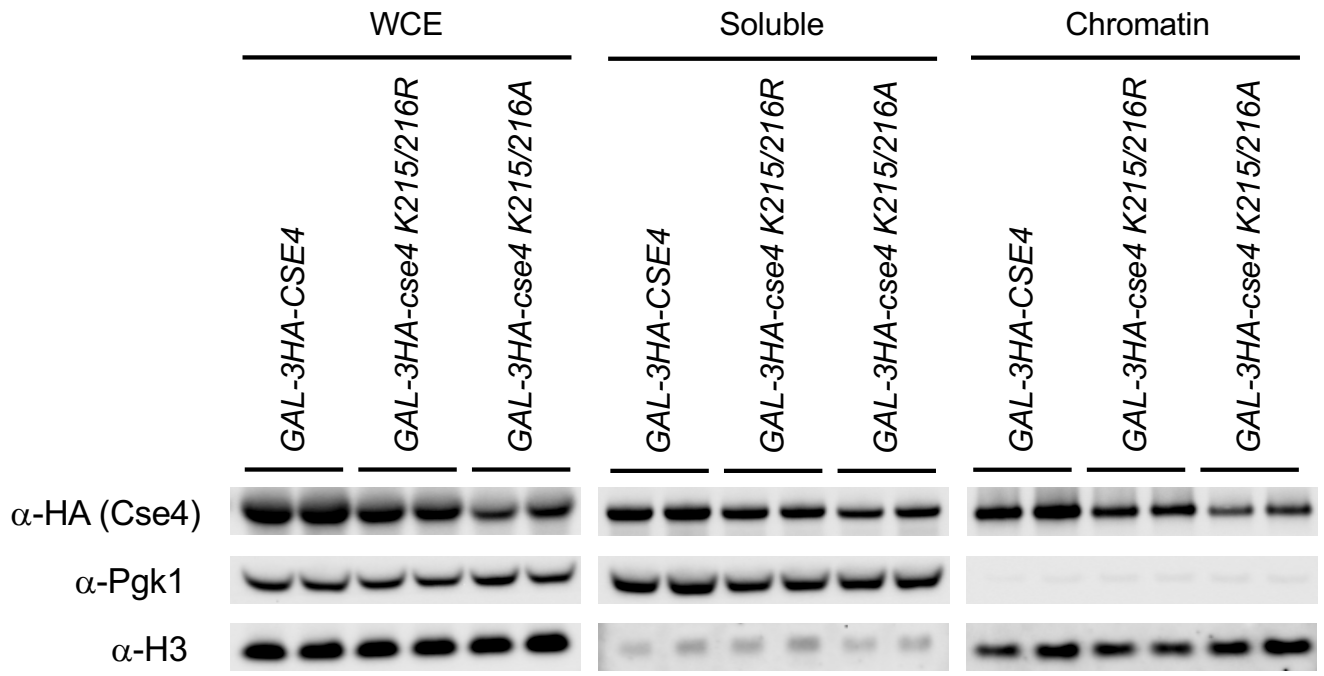

Figure S2. Levels of chromatin associated Cse4 K215/216R/A are reduced when compared to wild-type Cse4. Biological repeat of Figure 4A is shown. Subcellular fractionation experiments were done as described in Figure 4A.

Figure S2

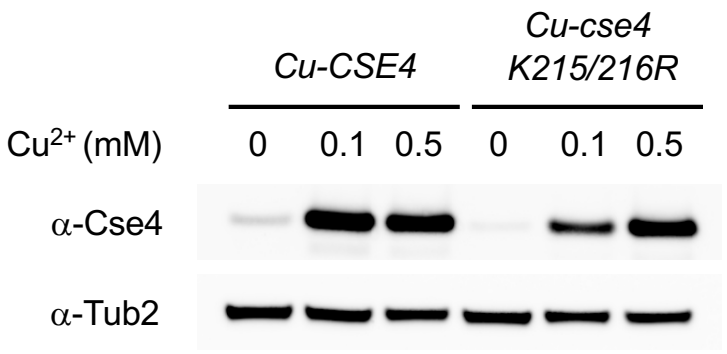

Figure S3. Protein levels of Cse4 and Cse4 K215/216R under the copper-inducible promoter. Isogenic yeast strains, *GAL-SCM3* transformed with vector (JG1689), *Cu-CSE4* (JG1690), or *Cu-cse4 K215/216R* (YMB11165), were grown to logarithmic phase of growth in a galactose/raffinose (2%) synthetic complete medium at 30°C. *CSE4* or *cse4 K215/216R* was induced by the addition of copper sulfate (0, 0.1, 0.5 mM Cu^{2+}) for 4 hours. Blots were probed with anti-Cse4 and anti-Tub2 (loading control) antibodies.

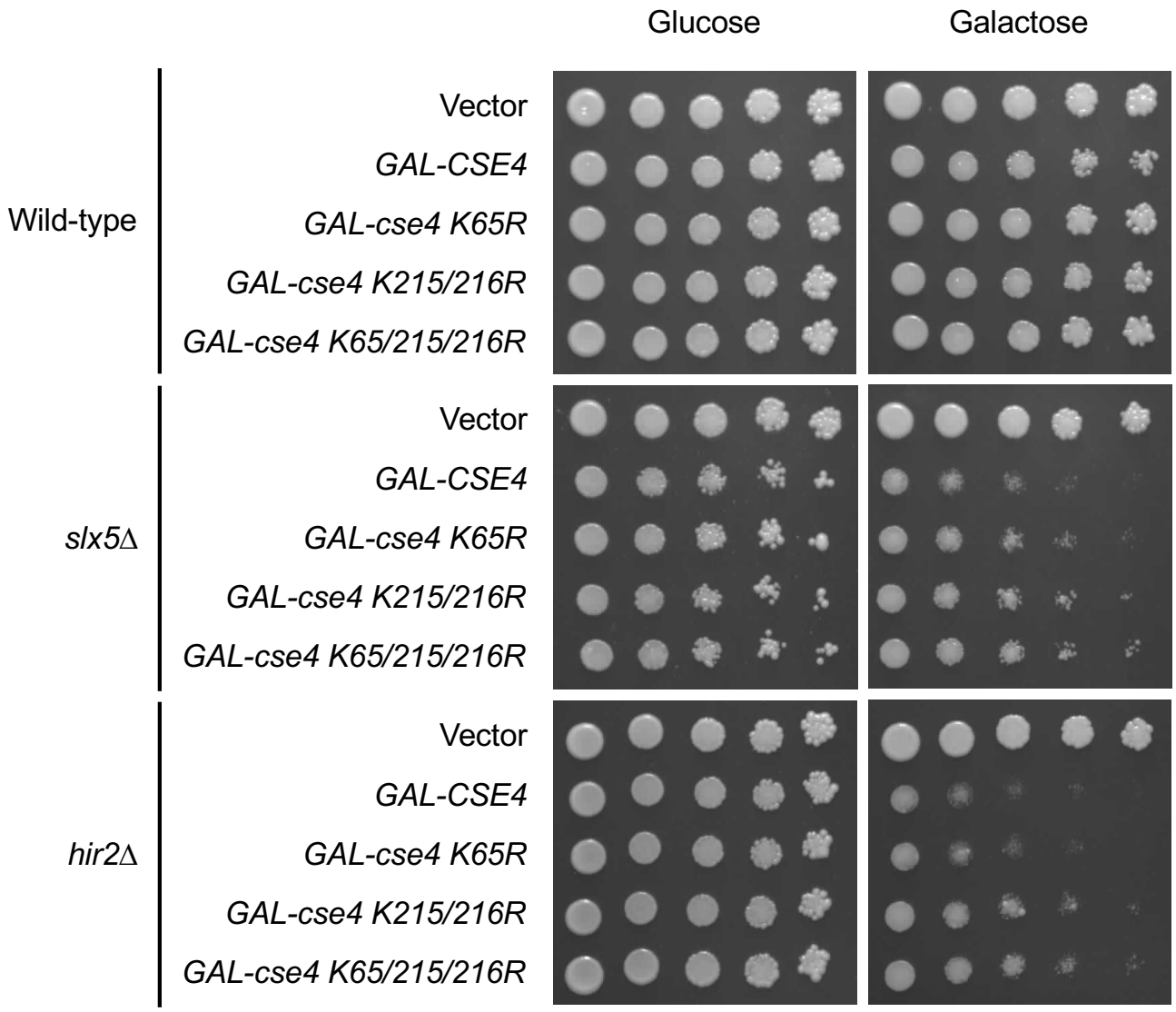

Figure S4. *GAL-cse4 K65R* exhibits SDL in *slx5*Δ and *hir2*Δ strains and those are suppressed by *cse4 K215/216R*. Yeast strains, wild-type (BY4741), *slx5*Δ (YMB9035), or *hir2*Δ (YMB8785), transformed with vector (pYES2), *pGAL-8His-HA-CSE4* (pMB1345), *pGAL-8His-HA-cse4 K65R* (pMB1762), *pGAL-8His-HA-cse4 K215/216R* (pMB1768), or *pGAL-8His-HA-cse4 K65/215/216R* (pMB1769) 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 4 days and photographed.

Figure S4