Table S2: Yeast strains used in this study

|  |  |  |
| --- | --- | --- |
| **Strain** | **Genotype** | **Parent Strain/Source** |
| BY4741 | MAT**a** *his3∆1 leu2∆O met15∆O ura3∆O* | Open Biosystems |
| BY4742 | MAT**α** *his3∆1 leu2∆O met15∆O ura3∆O* | Open Biosystems |
| Y7092 | MAT**α** *his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1∆::STE2pr-SpHIS5* | Charlie Boone |
| YMB7290 | MAT**α** *cse4Δ::KanMX6 (pRB199=pRS316-6His-3HA-Cse4)* | (Ohkuni *et al.* 2016) |
| YMB8995 | MAT**α** *psh1*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2* | Charlie Boone |
| YMB9034 | MAT**a** *psh1Δ::G418R his3∆1 leu2∆O met15∆O ura3∆O* | BY4741 |
| YMB9802 | MAT**α** *his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1∆::STE2pr-SpHIS5 pMB433* | Y7092 |
| YMB9803 | MAT**α** *his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1∆::STE2pr-SpHIS5 pMB1458* | Y7092 |
| YMB10478 | MAT**α** *psh1*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB433* | YMB8995 |
| YMB10479 | MAT**α** *psh1*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB1458* | YMB8995 |
| YMB10766 | MAT**a** *hhf1Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB10767 | MAT**a** *hhf2Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB10825 | MAT**a** *hhf1Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pMB433* | YMB10766 x YMB9802 |
| YMB11166 | MAT**a***hhf2Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* *pMB433* | YMB10767 x YMB9802 |
| YMB10937 | MAT**a** *hhf2∆::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pMB1458* | YMB10767 x YMB9803 |
| YMB10938 | MAT**a** *hhf1∆::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pMB1458* | YMB10766 x YMB9803 |
| YMB10821 | MAT**a** *psh1Δ::NATR hhf2Δ::G418R pMB433* | YMB10767 x YMB10478 |
| YMB10822 | MAT**a** *psh1Δ::NATR hhf2Δ::G418R pMB1458* | YMB10767 x YMB10479 |
| YMB10823 | MAT**a** *psh1Δ::NATR hhf1Δ::G418R GAL-pMB433* | YMB10766 x YMB10478 |
| YMB10824 | MAT**a** *psh1Δ::NATR hhf1Δ::G418R pMB1458* | YMB10766 x YMB10479 |
| YMB8994 | MAT**α** *slx5*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2* | Charlie Boone |
| YMB10963 | MAT**α** *slx5*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB1458* | YMB8994 |
| YMB11046 | MAT**a** *slx5*∆*::NATR hhf*∆*1::G418R pMB1458* | YMB10963 x YMB10766 |
| YMB11047 | MAT**a** *slx5*∆*::NATR hhf2*∆*::G418R pMB1458* | YMB10963 x YMB10767 |
| SN2176 | MAT**α** doa*1*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2* | Charlie Boone |
| YMB11032 | MAT**α** doa*1*∆*::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB1458* | SN2176 |
| YMB11050 | MAT**a** *doa1Δ::NATR hhf1Δ::G418R pMB1458* | YMB11032 x YMB10766 |
| YMB11053 | MAT**a** *doa1Δ::NATR hhf2Δ::G418R pMB1458* | YMB11032 x YMB10767 |
| YMB8785 | MAT**α** *hir2*∆*::NATR his3∆1 leu2∆O lys2∆0 ura3∆O* | BY4742 |
| YMB8332 | MAT**a** *hir2∆::NATR his3∆1 leu2∆O met15∆O ura3∆O pMB1458* | BY4741 |
| YMB11105 | MAT**α** *hir2*∆*::NATR hhf2*∆*::G418R pMB1458* | YMB8785 x YMB10937 |
| YMB11107 | MAT**a** *hir2∆::NATR hhf1∆::G418R pMB1458* | YMB8785 x YMB10938 |
| YMB8933 | MAT**α** *cdc4-1::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2* | Charlie Boone |
| YMB9756 | MAT**α** *cdc4-1::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB1458* | YMB8933 |
| YMB11051 | MAT**a** *cdc4-1::NATR hhf1Δ::G418R pMB1458* | YMB9756 x YMB10766 |
| YMB11054 | MAT**a** *cdc4-1::NATR hhf2Δ::G418R pMB1458* | YMB9756 x YMB10767 |
| tsa131 | MAT**α** *cdc7-4::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2* | Charlie Boone |
| YMB9760 | MAT**α** *cdc7-4::NATR his3∆1 leu2∆0 ura3∆0 met15∆0 lyp1∆ can1^::STE2pr-Sp\_his5 lyp1^::STE3pr-LEU2 pMB1458* | tsa131 |
| YMB11052 | MAT**a** *cdc7-4::NATR hhf1Δ::G418R pMB1458* | YMB9760 x YMB10766 |
| YMB11055 | MAT**a** *cdc7-4::NATR hhf2Δ::G418R pMB1458* | YMB9760 x YMB10767 |
| YMB11178 | MAT**a** *hta1Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB11258 | MAT**a** *hta1Δ::G418R pMB433* | YMB11178 x YMB10478 |
| YMB11262 | MAT**a** *hta1Δ::G418R pMB1458* | YMB11178 x YMB10479 |
| YMB11260 | MAT**a** *psh1Δ::NATR hta1Δ::G418R pMB433* | YMB11178 x YMB10478 |
| YMB11264 | MAT**a** *psh1Δ::NATR hta1Δ::G418R pMB1458* | YMB11178 x YMB10479 |
| YMB11179 | MAT**a** *hta2Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB11266 | MAT**a** *hta2Δ::G418R pMB433* | YMB11179 x YMB10478 |
| YMB11270 | MAT**a** *hta2Δ::G418R pMB1458* | YMB11179 x YMB10479 |
| YMB11268 | MAT**a** *psh1Δ::NATR hta2Δ::G418R pMB433* | YMB11179 x YMB10478 |
| YMB11272 | MAT**a** *psh1Δ::NATR hta2Δ::G418R pMB1458* | YMB11179 x YMB10479 |
| YMB11180 | MAT**a** *hht1Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB11274 | MAT**a** *hht1Δ::G418R pMB433* | YMB11180 x YMB10478 |
| YMB11278 | MAT**a** *hht1Δ::G418R pMB1458* | YMB11180 x YMB10479 |
| YMB11276 | MAT**a** *psh1**Δ::NATR hht1Δ::G418R pMB433* | YMB11180 x YMB10478 |
| YMB11280 | MAT**a** *psh1Δ::NATR hht1Δ::G418R pMB1458* | YMB11180 x YMB10479 |
| YMB11181 | MAT**a** *hht2Δ::G418R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Open Biosystems |
| YMB11282 | MAT**a** *hht2Δ::G418R pMB433* | YMB11181 x YMB10478 |
| YMB11286 | MAT**a** *hht2Δ::G418R pMB1458* | YMB11181 x YMB10479 |
| YMB11284 | MAT**a** *psh1Δ::NATR hht2Δ::G418R pMB433* | YMB11181 x YMB10478 |
| YMB11288 | MAT**a** *psh1Δ::NATR hht2Δ::G418R pMB1458* | YMB11181 x YMB10479 |
| JG1689 | MAT**a** *pGAL1-10-3HA-SCM3::TRP1 <pSB17 (empty vector) URA3>* | (Hewawasam *et al.* 2018) |
| JG1690 | MAT**a** *pGAL1-10-3HA-SCM3::TRP1 <pSB873 (Cu-CSE4) URA3>* | (Hewawasam *et al.* 2018) |
| YMB11252 | MAT**a** *hhf2Δ::G418R pGAL1-10-3HA-SCM3::TRP1 <pSB17 (empty vector) URA3>* | JG1689 |
| YMB11253 | MAT**a** *hhf2Δ::G418R pGAL1-10-3HA-SCM3::TRP1 <pSB873 (Cu-CSE4) URA3>* | JG1690 |
| MSY559 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1HHF1* Δ(*HHT2-HHF2*) | (Glowczewski *et al.* 2000) |
| MSY535 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1 hhf1-10* Δ(*HHT1-HHF1*) Δ(*HHT2-HHF2*) | (Glowczewski *et al.* 2000) |
| MSY534 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1 hhf1-20* Δ(*HHT1-HHF1*) Δ(*HHT2-HHF2*) | (Glowczewski *et al.* 2000) |
| YMB11346 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1HHF1* Δ(*HHT2-HHF2*) | MSY559 |
| YMB11347 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1 hhf1-10* Δ(*HHT1-HHF1*) Δ(*HHT2-HHF2*) *psh1Δ* | MSY535 |
| YMB11348 | *MAT***a** *leu2,3-112 ura3-52 lys2*Δ*200 HHT1 hhf1-20* Δ(*HHT1-HHF1*) Δ(*HHT2-HHF2*) *psh1Δ* | MSY534 |
| YPH1800 | *MAT****a*** *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 trp1Δ63 NDC80-13Myc::His3MX6* | (Montpetit *et al.* 2006) |
| YMB7862 | *MAT****a*** *his3 leu2 ura3 ade2 trp1 6His-Flag-SMT3::LEU2 NDC80-13Myc::His3MX6* | (Ohkuni *et al.* 2015) |
| YMB11599 | *MAT****a*** *his3 leu2 ura3 ade2 trp1 6His-Flag -SMT3::LEU2 NDC80-13Myc::His3MX6 hhf1∆::kanMX6* | YMB7862 |
| YMB11600 | *MAT****a*** *his3 leu2 ura3 ade2 trp1 6His-Flag -SMT3::LEU2 NDC80-13Myc::His3MX6 hhf2∆::kanMX6* | YMB7862 |
| SBY8904 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3* | (Ranjitkar *et al.* 2010) |
| YMB11609 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3 pMM230* | SBY8904 |
| YMB11603 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3 hhf1∆::kanMX6* | SBY8904 |
| YMB11611 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3 hhf1∆::kanMX6 pMM230* | YMB11603 |
| YMB11604 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3 hhf2∆::kanMX6* | SBY8904 |
| YMB11612 | *MAT****a*** *ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 ade2-1 bar1-1 pGAL-FLAG-CSE4::URA3 hhf2∆::kanMX6 pMM230* | YMB11609 |

Table S3: Plasmids used in this study.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Promoter, Gene, and Marker** | **Source** |
| pRS415 | *CEN LEU2* | (Sikorski and Hieter 1989) |
| pMB1725 | *CEN 6His-3HA-CSE4 LEU2* | This Study |
| pMB1935 | *CEN 6His-3HA-cseD217A LEU2* | This Study |
| pMB1936 | *CEN 6His-3HA-cseD217E LEU2* | This Study |
| pRS425 | *2µ LEU2* | (Christianson *et al.* 1992) |
| pMB433 | *2µ pGAL1 URA3* | (Mumberg *et al.* 1994) |
| pMB1458 | *2µ pGAL1-6His-3HA-CSE4 URA3* | (Au *et al.* 2013) |
| pMB1892 | *2µ pGAL1-3HA-cse416KR URA3* | (Au *et al.* 2020) |
| pMB1928 | *MoBy 2µ HHF1 LEU2* | This Study |
| pMB1929 | *MoBy 2µ HHF2 LEU2* | This Study |
| pMM230 | *CEN pFZO1-FZO1HA LEU2* | (Metzger *et al.* 2017) |
| pYES2 | *2µ pGAL URA3* | (Boeckmann *et al.* 2013) |
| pMB1344 | *2µ* pYES2-*pGAL-8His-HA-cse416KR* *URA3* | (Ohkuni *et al.* 2016) |
| pMB1345 | *2µ* pYES2-*pGAL-8His-HA-CSE4* *URA3* | (Ohkuni *et al.* 2016) |
| pMB1766 | *2µ* pYES2-*pGAL-8His-HA-cse4 Y193A* *URA3* | This Study |
| pMB1787 | *2µ* pYES2-*pGAL-8His-HA-cse4 Y193F* *URA3* | This Study |
| pMB1910 | *2µ* pYES2-*pGAL-8His-HA-cse4 D217A* *URA3* | This Study |
| pMB1920 | *2µ* pYES2-*pGAL-8His-HA-cse4 D217E* *URA3* | This Study |
| pMB1984 | *2µ* pYES2-*pGAL-8His-HA-cse4-102* *URA3* | This Study |
| pMB1985 | *2µ* pYES2-*pGAL-8His-HA-cse4-107MB URA3* | This Study |
| pMB1986 | *2µ* pYES2-*pGAL-8His-HA-cse4-108* *URA3* | This Study |
| pMB1987 | *2µ* pYES2-*pGAL-8His-HA-cse4-110* *URA3* | This Study |
| pMB1988 | *2µ* pYES2-*pGAL-8His-HA-cse4-111* *URA3* | This Study |

Table S4: Primers used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Region** | **Primer** | **Sequence (5’ to 3’)** | **Source** |
| **ChIP-qPCR** |  |  |  |
| *ACT1* For | OMB444 | ACAACGAATTGAGAGTTGCCCCAG | (Dimova *et al.* 1999) |
| *ACT1* Rev | OMB445 | AATGGCGTGAGGTAGAGAGAAACC | (Dimova *et al.* 1999) |
| *SAP4* For | SB3735 | ACAGCACAACACGCTTACCA | (Hildebrand and Biggins 2016) |
| *SAP4* Rev | SB3736 | CCAGCCCTAAATCCCCTAAA | (Hildebrand and Biggins 2016) |
| *RDS1* For | SB4768 | GACCCGTGCAGATCACTATTACA | (Hildebrand and Biggins 2016) |
| *RDS1* Rev | SB4769 | GCAGTTTATCACATTTCCGTTTG | (Hildebrand and Biggins 2016) |
| *SLP1* For | SB3781 | TCCTAGGTTATCTCATCGGTACT | (Hildebrand and Biggins 2016) |
| *SLP1* Rev | SB3782 | ACTATATCCATTGCGTCCTTTCT | (Hildebrand and Biggins 2016) |
| *PHO5* For | OMB3282 | CCCATTTGGGATAAGGGTAAAC | (Hewawasam *et al.* 2018) |
| *PHO5* Rev | OMB3283 | GATGAAGCCATACTAACCTCGA | (Hewawasam *et al.* 2018) |
| *FIG4*/*LEM3* For | OMB3274 | ACCAGAACGGCAGACAAAGT | (Ohkuni *et al.* 2020) |
| *FIG3*/*LEM3* Rev | OMB3275 | TGAACGTGCTGCAATAAACC | (Ohkuni *et al.* 2020) |
| *UGA3*/*UGX2* For | OMB3298 | CCCCCTGGGTGTCTTAAATTAT | (Ohkuni *et al.* 2020) |
| *UGA3*/*UGX2* Rev | OMB3298 | TTCTATGTCTCAACGTTAGCATTTC | (Ohkuni *et al.* 2020) |
| *COQ3* For | OMB3276 | CCTTTCATAATGTATTATCACCCTTT | (Ohkuni *et al.* 2020) |
| *COQ3* Rev | OMB3277 | AGACTTCGCTGTACCTGTTTCC | (Ohkuni *et al.* 2020) |
| *GUP2* For | OMB3278 | CGATGGTATTGATGCACCTG | (Ohkuni *et al.* 2020) |
| *GUP2* Rev | OMB3279 | GACTGTTAACCACCCCCAAA | (Ohkuni *et al.* 2020) |
| peri*CEN3* L3 For | OMB1684 | GCCATACCATGCTTTGTTATCGTC | (Choy *et al.* 2011) |
| peri*CEN3* L3 Rev | OMB1685 | TATTATGCTCCCCTGGATTTTATGCG | (Choy *et al.* 2011) |
| peri*CEN3* L2 For | OMB1686 | TCATCTTTGAAAAGTTCATCAAGG | (Choy *et al.* 2011) |
| peri*CEN3* L2 Rev | OMB1687 | GATAACAAAGCATGGTATGGCG | (Choy *et al.* 2011) |
| peri*CEN3* L1 For | OMB1688 | ATATTGTTTGGCGCTGATCGCC | (Choy *et al.* 2011) |
| peri*CEN3* L1 Rev | OMB1689 | TTGATGAACTTTTCAAAGATGAC | (Choy *et al.* 2011) |
| *CEN3* For | OMB244 | GATCAGCGCCAAACAATATGG | (Choy *et al.* 2011) |
| *CEN3* Rev | OMB245 | AACTTCCACCAGTAAACGTTTC | (Choy *et al.* 2011) |
| peri*CEN3* R1 For | OMB1692 | TTTACTGGTGGAAGTTTTGCTCA | (Choy *et al.* 2011) |
| peri*CEN3* R1 Rev | OMB1693 | GTCAACGAGTCCTCTCTGGCTA | (Choy *et al.* 2011) |
| peri*CEN3* R2 For | OMB1694 | GAGAGGACTCGTTGACGTAGAA | (Choy *et al.* 2011) |
| peri*CEN3* R2 Rev | OMB1695 | GAATATGATAATGGTTACACCAGTAGG | (Choy *et al.* 2011) |
| peri*CEN3* R3 For | OMB1696 | TGTAACCATTATCATATTCATGAC | (Choy *et al.* 2011) |
| peri*CEN3* R3 Rev | OMB1697 | GATTTAATGCACGTTATGTTTCG | (Choy *et al.* 2011) |
| **RT-qPCR** |  |  |  |
| *SIZ1* transcript For | OMB3465 | GGAAACCATCACTCTGATGG | This Study |
| *SIZ1* transcript Rev | OMB3466 | GGATCAGACTTGCCTACTAC | This Study |
| *SIZ2* transcript For | OMB3467 | AACCCTTTGTCCAACACAGG | This Study |
| *SIZ2* transcript Rev | OMB3468 | AGCCTTTTTGCCCGTTATGG | This Study |
| *UBI4* transcript For | OMB3471 | TTCGTCAAGACTTTGACCGG | This Study |
| *UBI4 transcript* Rev | OMB3472 | GTTCTACCGTCTTCTAGCTG | This Study |
| *SMT3* transcript For | OMB3475 | TGTCCGATGGATCTTCAGAG | This Study |
| *SMT3* transcript Rev | OMB3476 | ATCTGTTCTCTGTGAGCCTC | This Study |

CITATIONS

Au, W. C., A. R. Dawson, D. W. Rawson, S. B. Taylor, R. E. Baker *et al.*, 2013 A Novel Role of the N-Terminus of Budding Yeast Histone H3 Variant Cse4 in Ubiquitin-Mediated Proteolysis. Genetics 194**:** 513-518.

Au, W. C., T. Zhang, P. K. Mishra, J. R. Eisenstatt, R. L. Walker *et al.*, 2020 Skp, Cullin, F-box (SCF)-Met30 and SCF-Cdc4-Mediated Proteolysis of CENP-A Prevents Mislocalization of CENP-A for Chromosomal Stability in Budding Yeast. PLoS Genet 16**:** e1008597.

Boeckmann, L., Y. Takahashi, W. C. Au, P. K. Mishra, J. S. Choy *et al.*, 2013 Phosphorylation of centromeric histone H3 variant regulates chromosome segregation in Saccharomyces cerevisiae. Mol Biol Cell 24**:** 2034-2044.

Choy, J. S., R. Acuna, W. C. Au and M. A. Basrai, 2011 A role for histone H4K16 hypoacetylation in Saccharomyces cerevisiae kinetochore function. Genetics 189**:** 11-21.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene 110**:** 119-122.

Dimova, D., Z. Nackerdien, S. Furgeson, S. Eguchi and M. A. Osley, 1999 A role for transcriptional repressors in targeting the yeast Swi/Snf complex. Mol Cell 4**:** 75-83.

Glowczewski, L., P. Yang, T. Kalashnikova, M. S. Santisteban and M. M. Smith, 2000 Histone-histone interactions and centromere function. Mol Cell Biol 20**:** 5700-5711.

Hewawasam, G. S., K. Dhatchinamoorthy, M. Mattingly, C. Seidel and J. L. Gerton, 2018 Chromatin assembly factor-1 (CAF-1) chaperone regulates Cse4 deposition into chromatin in budding yeast. Nucleic Acids Res 46**:** 4440-4455.

Hildebrand, E. M., and S. Biggins, 2016 Regulation of Budding Yeast CENP-A levels Prevents Misincorporation at Promoter Nucleosomes and Transcriptional Defects. PLoS Genet 12**:** e1005930.

Metzger, M. B., J. L. Scales, M. F. Dunklebarger and A. M. Weissman, 2017 The Ubiquitin Ligase (E3) Psh1p Is Required for Proper Segregation of both Centromeric and Two-Micron Plasmids in Saccharomyces cerevisiae. G3 (Bethesda) 7**:** 3731-3743.

Montpetit, B., T. R. Hazbun, S. Fields and P. Hieter, 2006 Sumoylation of the budding yeast kinetochore protein Ndc10 is required for Ndc10 spindle localization and regulation of anaphase spindle elongation. J Cell Biol 174**:** 653-663.

Mumberg, D., R. Muller and M. Funk, 1994 Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res 22**:** 5767-5768.

Ohkuni, K., E. Suva, W. C. Au, R. L. Walker, R. Levy-Myers *et al.*, 2020 Deposition of Centromeric Histone H3 Variant CENP-A/Cse4 into Chromatin Is Facilitated by Its C-Terminal Sumoylation. Genetics 214**:** 839-854.

Ohkuni, K., Y. Takahashi and M. A. Basrai, 2015 Protein purification technique that allows detection of sumoylation and ubiquitination of budding yeast kinetochore proteins Ndc10 and Ndc80. J Vis Exp**:** e52482.

Ohkuni, K., Y. Takahashi, A. Fulp, J. Lawrimore, W. C. Au *et al.*, 2016 SUMO-Targeted Ubiquitin Ligase (STUbL) Slx5 regulates proteolysis of centromeric histone H3 variant Cse4 and prevents its mislocalization to euchromatin. Mol Biol Cell.

Ranjitkar, P., M. O. Press, X. Yi, R. Baker, M. J. MacCoss *et al.*, 2010 An E3 ubiquitin ligase prevents ectopic localization of the centromeric histone H3 variant via the centromere targeting domain. Mol Cell 40**:** 455-464.

Sikorski, R. S., and P. Hieter, 1989 A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122**:** 19-27.