Supplemental Materials

Molecular Biology of the Cell

Howard and Tansey

Supplemental Material for "Interaction of Gcn4 with target gene chromatin is modulated by proteasome function" by Howard and Tansey

Contents:

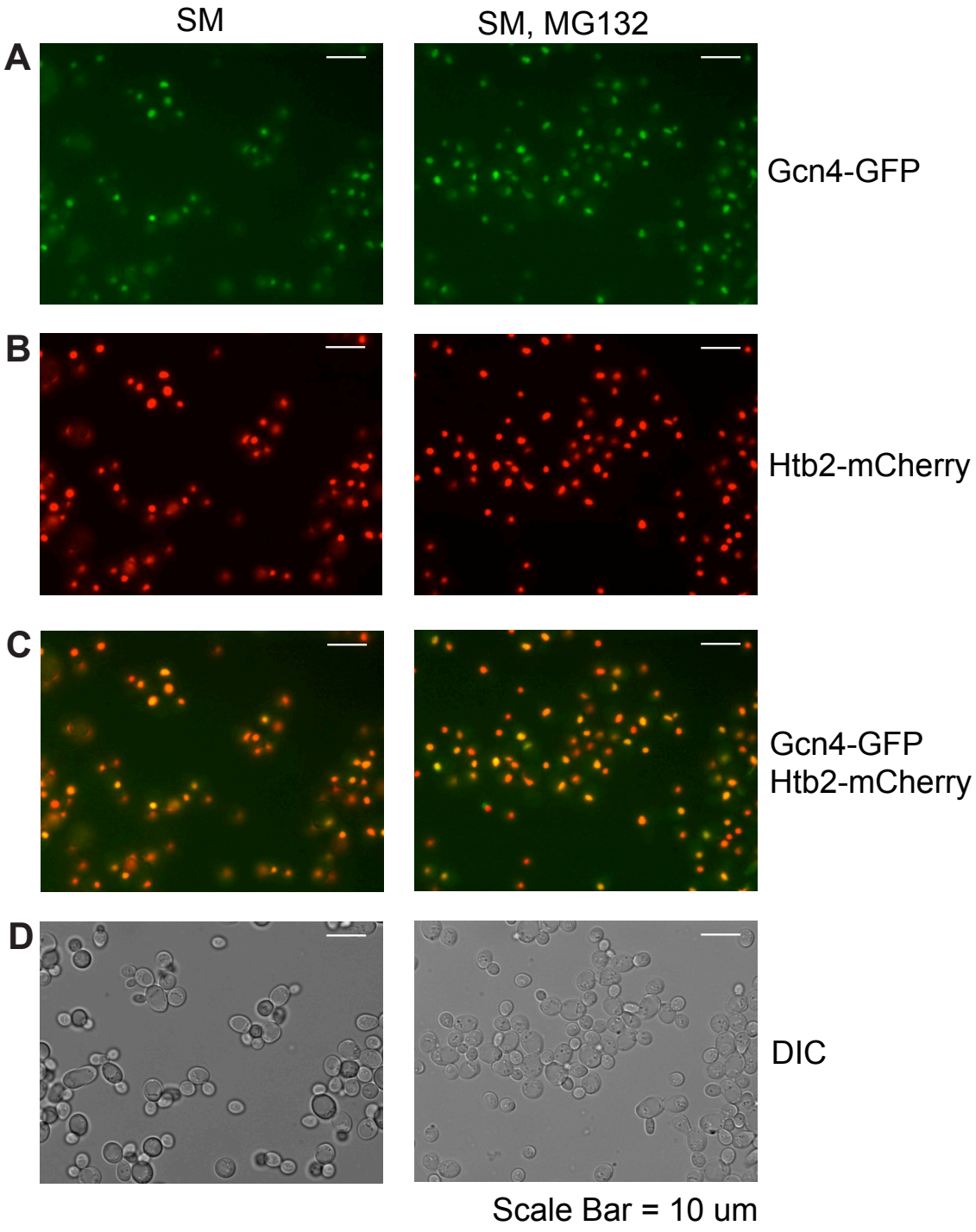
Legend to Supplemental Figure S1

Supplemental Figure S1. Nuclear localization of Gcn4 is not impacted by proteasome inhibition. *GCN4-GFP HTB2-mCherry* (GHY339) yeast were grown to log phase at 30°C in minimal media, treated with either DMSO or MG132 for one hour, and induced with SM for 1.5 hours. Samples were imaged using fluorescent microscopy to visualize (A) Gcn4-GFP and (B) Htb2-mCherry. (C) Overlay of Gcn4-GFP and Htb2-mCherry images. (D) Differential interference contrast (DIC) microscopy of corresponding fields in (A-C). Scale bars represent 10 μm. This image is a wider field view of that presented in Figure 2D.

Supplemental Figure S2. Proteasome inhibition reduces the ability of native, untagged, Gcn4 to bind the ARG1 UAS. (A) *gcn4* (GHY004) and *GCN4* (GHY010) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with a polyclonal antibody against Gcn4. Co-precipitating *ARG1* promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. *n=3.* (B) *GCN4* (GHY010) and *GCN4-Myc* (GHY021) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with an antibody against the Myc epitope. Co-precipitating *ARG1* promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. *n=4.* Error bars represent SEM.

Supplemental Figure S3. Mutations in Gcn4 that modulate its ubiquitylation status. (A) Graphical representation of Gcn4 showing the functional domains of the protein (TAD, transcriptional activation domain, blue; DBD, DNA-binding domain, gray). The wild-type (WT) Gcn4 protein is represented on top, the 3T2S mutant (showing the location of five alanine substitution mutations) in the middle, and the lysine free, K0, mutant at the bottom. (B) *GCN4-HA* (GHY356) and *3T2S-GCN4-HA* (GHY360) yeast carrying a copper-inducible His-Myc-Ubiquitin expression plasmid (pUB221) were grown to log phase at 30°C in minimal media and treated with 0.5 mM $CuSO_4$ and either DMSO or 50 μM MG132 for one hour. Yeast were induced with 0.5 μg/ml SM, or a DMSO control, for an additional 1.5 hours, at which time protein lysates were collected under denaturing conditions. Ubiquitin-conjugates were captured by nickel-resin (Ni-NTA) chromatography, resolved by SDS-PAGE, and probed for HA-tagged Gcn4 protein by western blotting. A sample of the input material to the nickel resin was also probed for HA-tagged Gcn4. IB, immunoblot. A single Ub-conjugate of Gcn4 (arrow) persists in the 3T2S Gcn4 mutant. (C–D) *GCN4 (*GHY010) and *3T2S-GCN4* (GHY008) yeast were grown to log phase in minimal media and treated with either DMSO or 50 μM MG132 for one hour. Strains were then treated with 0.5 μg/ml SM, or DMSO control, for 1.5 hours, at which time RNA was collected and *ARG1* (C) and *HIS4* (D) mRNA levels quantified by RT-qPCR, relative to an *ACT1* control. Relative mRNA levels were then normalized to the SM-induced, DMSO-treated, sample for each gene. Error bars represent SEM. *n=3*. (E) *pup1–T30A pre3–T20A GCN4-HA* (GHY356) and *pup1–T30A pre3–T20A K0 GCN4-HA* (GHY052) yeast carrying either empty vector or a copper-inducible His-Myc-Ubiquitin expression plasmid (pUB221) were grown to log phase at 30°C in minimal media and treated with 0.5 mM $CuSO_4$ and 50 μM MG132 for one hour. Yeast were induced with 0.5 μg/ml SM for an additional 1.5 hours, at which time protein lysates were collected under denaturing conditions. Ubiquitin-conjugates were captured by nickel-resin (Ni-NTA) chromatography, resolved by SDS-PAGE, and probed for HA-tagged Gcn4 protein by western blotting. A sample of the input material to the nickel resin was also probed for HA-tagged Gcn4. IB, immunoblot. Ni-NTA pull-down material was also probed for total His-Myc-Ubiquitin. (F) *GCN4-HA* (GHY025) and *K0 GCN4-HA* (GHY052) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with either IgG or antibody against the HA epitope. Co-precipitating *ARG1* promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. *n=3*. Error bars represent SEM.

Supplemental Figure S4. Model. In this model, unmodified Gcn4 binds its cognate UAS element but the resulting complex is inactive for gene activation (OFF). Ubiquitylation of Gcn4 by the SCF^{Cdc4} complex converts Gcn4 into a state that is competent for gene activation (ON) but at the same time renders it a substrate for a Cdc48-containing complex. Cdc48 mediates stripping of Gcn4–Ub from DNA, allowing Gcn4 to be destroyed by the 26S proteasome. Although not shown in the figure, it is possible that Gcn4 could be deubiquitylated after extraction (recycled) and not destroyed. It is also possible that Gcn4 could be ubiquitylated before it encounters DNA, in which case the model still predicts that it would be stripped from promoters in a Cdc48-dependent manner.



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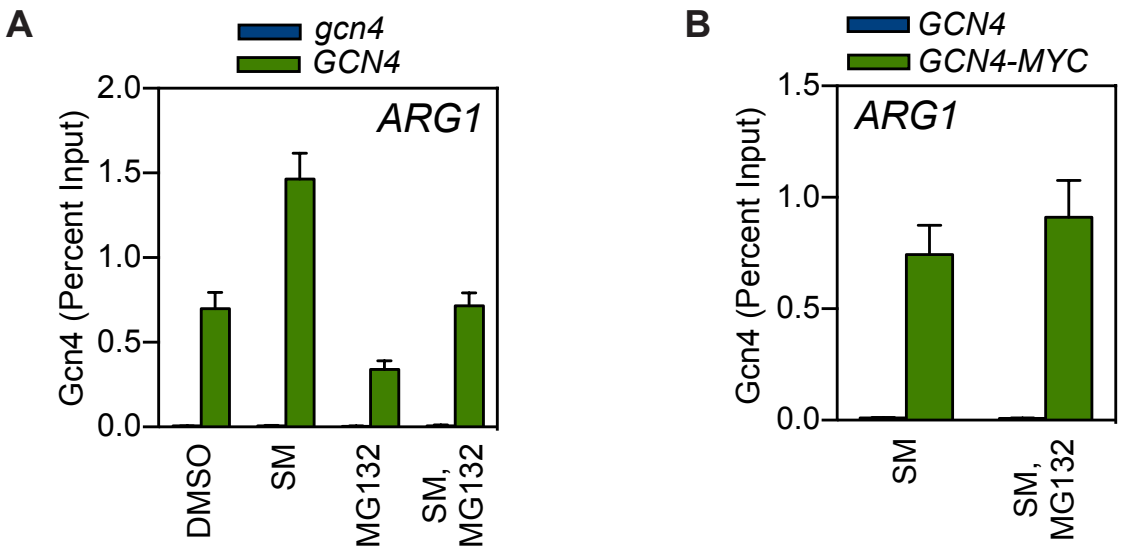

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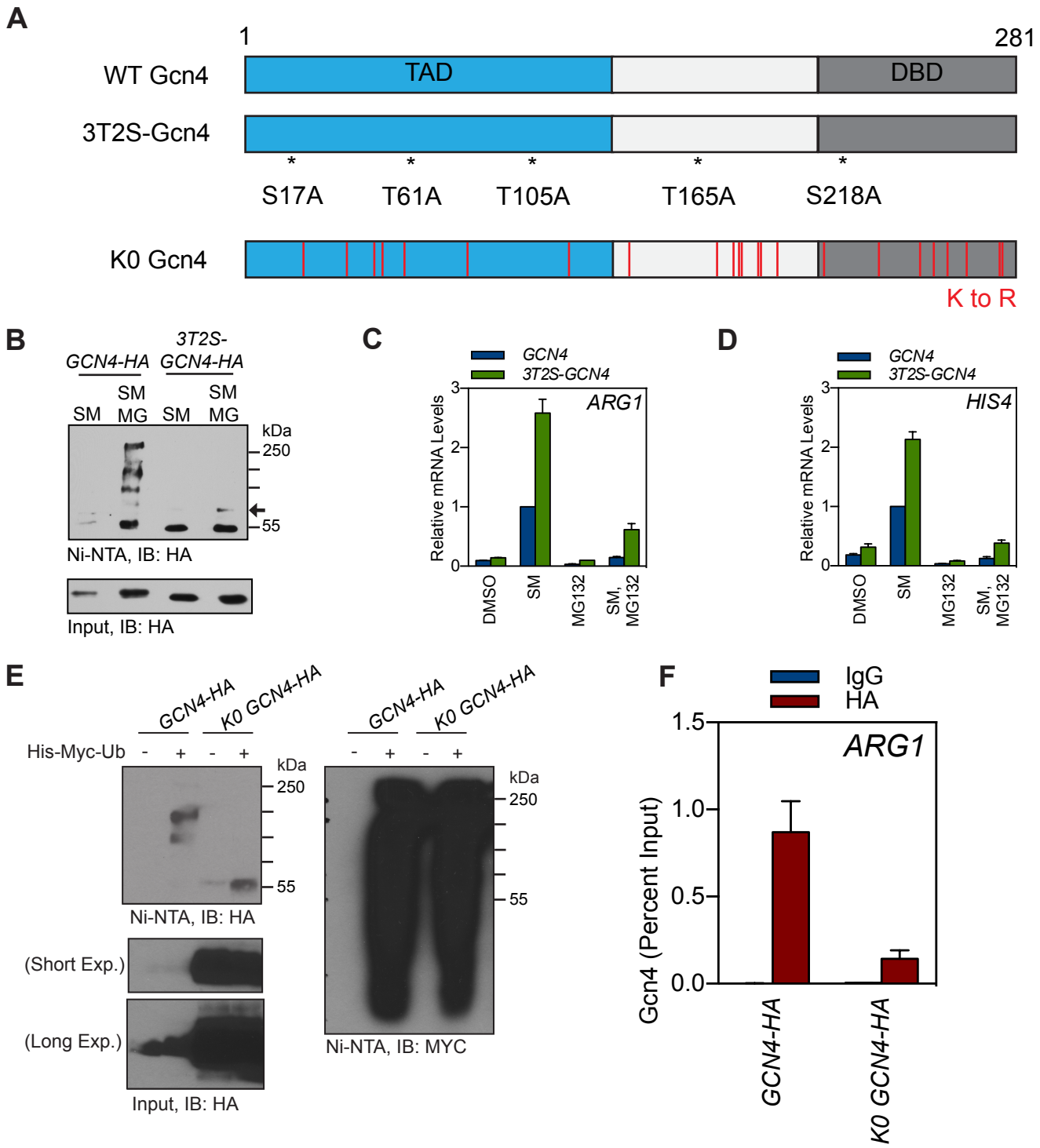

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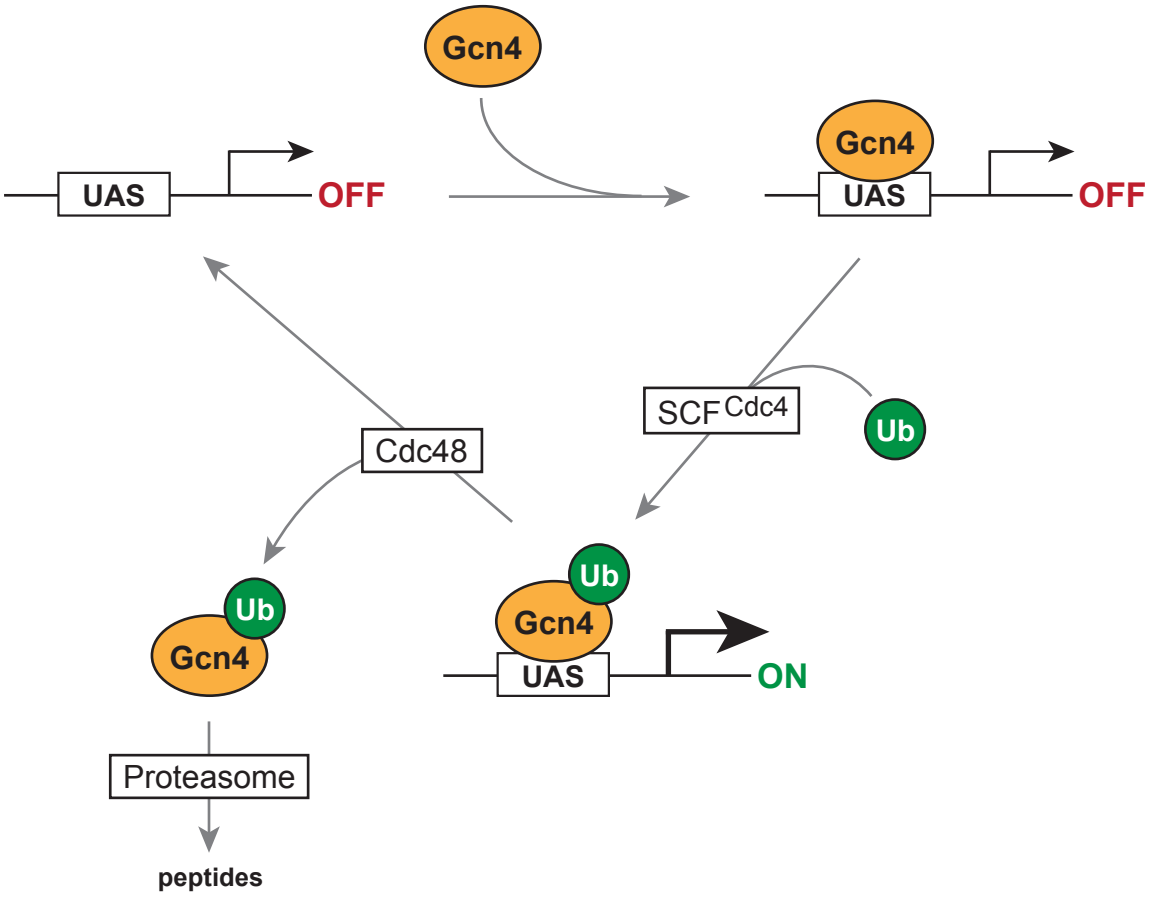

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Supplemental Table S1. Yeast strains used in this study		
Strain	**Genotype**	**Origin**
W303-1a	*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	Patton et al., 1998
MT670	W303-1a *cdc34-2*	Patton et al., 1998
MT668	W303-1a *cdc4-1*	Patton et al., 1998
GHY107	MT668 *GCN4-HA::KAN*	This study
HHY168	MATα *tor1-1 can1-100 leu2-3,112 ura3-1 ade2-1 his3-11,15*	Haruki et al., 2008
GHY139	HHY168 *pdr5::LEU2 GCN4-HA::KAN*	This study
GHY149	HHY168 *pdr5::LEU2 GCN4-HA::KAN CDC34-FRB::HIS*	This study
GHY145	HHY168 *pdr5::LEU2 GCN4-FRB::HIS*	This study
YUS5	WCG4a *pup1-T30A pre3-T20A*	Heinemeyer et al., 1997
GHY010	YUS5 *GCN4*	This study
GHY025	YUS5 *GCN4-3xHA::KAN*	This study
GHY021	YUS5 *GCN4-9xMYC::HIS3*	This study
GHY339	YUS5 *GCN4-yEGFP::KAN HTB2-mCherry::HIS3*	This study
GHY004	YUS5 *gcn4::URA3*	This study
GHY081	YUS5 *GCN4 arg80::NAT*	This study
GHY079	YUS5 *gcn4::URA3 arg80::NAT*	This study
GHY356	YUS5 *GCN4-3xHA::KAN* [pUB221]	This study
RHY2455	MATα *ura3–52 leu2–3,112*	Sato and Hampton, 2006
RHY2457	MATα *ura3–52 leu2–3,112 cdc48–3*	Sato and Hampton, 2006
GHY116	RHY2455 *GCN4-3xHA::KAN*	This study
GHY118	RHY2457 *GCN4-3xHA::KAN*	This study
GHY279	RHY2457 *cdc48-3::CDC48*	This study
GHY304	RHY2455 *gal80::NAT*	This study
GHY305	RHY2457 *gal80::NAT*	This study
GHY285	YUS5 *GCN4 CDC48-3xMYC::HIS3*	This study
GHY287	YUS5 *GCN4-3xHA::KAN CDC48-3xMYC::HIS3*	This study
GHY124	YUS5 *K0 GCN4-3xHA::KAN CDC48*	This study
GHY293	YUS5 *K0 GCN4-3xHA::KAN CDC48-3xMYC::HIS3*	This study
GHY008	YUS5 *3T2S-GCN4*	This study
GHY027	YUS5 *3T2S-GCN4-HA::KAN*	This study
GHY360	YUS5 *3T2S-GCN4-HA::KAN* [pUB221]	This study
GHY052	YUS5 *K0 GCN4-HA::KAN* [pUB221]	This study

Supplemental Table S2. Primers used in this study		
Purpose	**Name**	**Sequence**
RT-qPCR	ACT1 F	AGCCGTTTTGTCCTTGTACTCTTCC
RT-qPCR	ACT1 R	AGCGTAAATTGGAACGACGTGAGTA
RT-qPCR	ARG1 F	GCCAACGGTGTTGGTAGAAT
RT-qPCR	ARG1 R	AGTCAATGGAGCCTGTTCGT
RT-qPCR	ARG4 F	GTCATCCAAACGACGAGGAT
RT-qPCR	ARG4 R	ACCGGTGTGGACTTTACCAG
RT-qPCR	HIS4 F	ACAACTGCCACGTGTGGATA
RT-qPCR	HIS4 R	TTGGACATTTCAAGGGCTTC
ChIP	ARG1 Gcn4 BS2 F	GCTGTCGCAACCTATTTCCA
ChIP	ARG1 Gcn4 BS2 R	TCAATCTGATCCAATGAAGATGA
ChIP	ARG1 TATA F	ATCTGAGCAGTTGCGAGACC
ChIP	ARG1 TATA R	AACTGTGGGCAAGAACAAGG
ChIP	ARG1 ORF3 F	CAAGCCCACATTTCTTACGAG
ChIP	ARG1 ORF3 R	ATCGACGATCAATTTCCACA
ChIP	ARG4 Gcn4 BS F	GGTTACTCATTGGCAGAATCC
ChIP	ARG4 Gcn4 BS R	TTCAATTTGCGCCAGCTTAT
ChIP	ARG5 Gcn4 BS F	TCCGAATGACTCAGTCTACATCA
ChIP	ARG5 Gcn4 BS R	GCGCGCAAGCTCTTTATATG
ChIP	ARO4 Gcn4 BS F	CACCCTGTGCATTTTGTACG
ChIP	ARO4 Gcn4 BS R	CGTCCCGCACATCTTTTT
ChIP	CPA2 Gcn4 BS F	GAGATAGGAACCTCCATGTCG
ChIP	CPA2 Gcn4 BS R	TGGCAGAAATGCTTATGACG
ChIP	HIS4 Gcn4 BS F	TGCACAGTGACTCACGTTTTT
ChIP	HIS4 Gcn4 BS R	TCGGAGGTGAATATAACGTTCC
ChIP	HIS7 Gcn4 BS F	GGCTAATTAGGTGATCATGAAAAA
ChIP	HIS7 Gcn4 BS R	AACCTGATTGAGTAGTCGTCGAT
ChIP	LEU3 Gcn4 BS F	TCTAGCTATTCTAAATCATCTGCATGT
ChIP	LEU3 Gcn4 BS R	CCTCCGATCGAAGAGAGGTT
ChIP	LYS1 Gcn4 BS F	TTTGGAATTCCGCTCTCAAC
ChIP	LYS1 Gcn4 BS R	ATCGTGGTTTCTCGAGGATG
ChIP	SNZ1 Gcn4 BS F	AGCCGGGCTTTTTCACTACT
ChIP	SNZ1 Gcn4 BS R	GTAACTAACGGTGCGGCAGA
ChIP	THR4 Gcn4 BS F	CAACGAGGAAATAGAAGAAAATGAA
ChIP	THR4 Gcn4 BS R	CCAAATGGAAAAATATAAGATACACAA
MNase	GAL1 NB F	CCCCACAAACCTTCAAATTAACG
MNase	GAL1 NB R	CGCTTCGCTGATTAATTACCC
MNase	GAL1 NUB F	CGGATTAGAAGCCGCCGA
MNase	GAL1 NUB R	ATCTTTATTGTTCGGAGCAGTG
MNase	ARG1 -820 F1	ACGTCCGCATGGAAGACCTA
MNase	ARG1 -716 R1	AAAGAGGCAACAGGAAAGATCAGA

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Purpose	**Name**	**Sequence**
MNase	ARG1 -740 F2	CTCTGATCTTTCCTGTTGCCTCTT
MNase	ARG1 -653 R2	CTGTAGTAATGTTACTAGTAGTAGTTGTAGAACTTGTG
MNase	ARG1 -690 F3	CACAAGTTCTACAACTACTACTAGTAACATTACTACAGTT
MNase	ARG1 -541 R3	CGGTGATGTGATATGTAAACGATAATAG
MNase	ARG1 -580 F4	CCATTATACACGCTATTATCGTTTACATATC
MNase	ARG1 -471 R4	ATAGATAACAGAAAAGGTTATGGCGATTA
MNase	ARG1 -557 F5	TACATATCACATCACCGTTAATGAAAGA
MNase	ARG1 -448 R5	TCGAAGAAACAGCTTTAAGGGCTAT
MNase	ARG1 -510 F6	ACACAATTAAATAATCGCCATAACCTT
MNase	ARG1 -415 R6	GGCCCATGTGGAGAATTACTG
MNase	ARG1 -492 F7	CATAACCTTTTCTGTTATCTATAGCCCTTA
MNase	ARG1 -382 R7	GTGACTAACATAGCGCTCTTATCTCAGT
MNase	ARG1 -469 F8	GCCCTTAAAGCTGTTTCTTCGAG
MNase	ARG1 -361 R8	ATGACTGGAGAGCCGTCAGTAGT
MNase	ARG1 -444 F9	TTTTCACTGCAGTAATTCTCCACAT
MNase	ARG1 -326 R9	CCAAATGCGACATGAGTCACTAA
MNase	ARG1 -410 F10	CACTGAGATAAGAGCGCTATGTTAGTC
MNase	ARG1 -297 R10	AATAGGTTGCGACAGCGGAA
MNase	ARG1 -383 F11	ACTACTGACGGCTCTCCAGTCAT
MNase	ARG1 -281 R11	CGGCACCGTTAATGGAAATAG
MNase	ARG1 -348 F12	TTAGTGACTCATGTCGCATTTGG
MNase	ARG1 -256 R12	CCTGCCTTTAAATGACTCTTCCATAC
MNase	ARG1 -312 F13	GCTGTCGCAACCTATTTCCATTA
MNase	ARG1 -211 R13	ACGCAGTCATCAATCTGATCCA
MNase	ARG1 -290 F14	AACGGTGCCGTATGGAAGAG
MNase	ARG1 -179 R14	TCGCAACTGCTCAGATTACACTATCT
MNase	ARG1 -231 F15	GGATCAGATTGATGACTGCGTA
MNase	ARG1 -141 R15	CCCATTAATATACTATTGAGACAGTGC
MNase	ARG1 -207 F16	GGCAGATAGTGTAATCTGAGCAGTTG
MNase	ARG1 -114 R16	GCAAGAACAAGGGAGTACGAATGT
MNase	ARG1 -170 F17	CTGGCACTGTCTCAATAGTATATTAATGG
MNase	ARG1 -76 R17	AGACAAGATACAAGAAGTAAAGAGAGAGAGAA
MNase	ARG1 -142 F18	GGCATACATTCGTACTCCCTTGT
MNase	ARG1 -51 R18	TGTTCCTTATCGCTGCACAATG
MNase	ARG1 -109 F19	AGTTCTCTCTCTCTTTACTTCTTGTATCTTGTC
MNase	ARG1 -15 R19	TGTGTATTTCTTTTGTATCCGTGTATATTAGA
MNase	ARG1 -43 F20	GCAGCGATAAGGAACATTGTTCTA
MNase	ARG1 +20 R20	CAAACTTTTCCCTTAGACATTATTTTATGC
MNase	ARG1 -35 F21	CGGATACAAAAGAAATACACATAATTGC
MNase	ARG1 +60 R21	CAAAATGACGGAGGTATCTAAACCA

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MNase	ARG1 -10 F22	TGCATAAAATAATGTCTAAGGGAAAAGTT
MNase	ARG1 +79 R22	CTTGGTCTAGTAGCCAAGCCAAA
MNase	ARG1 +21 F23	TTTGGCTTATTCTGGTGGTTTAGAT
MNase	ARG1 +111 R23	TACATTAGCCATGAAAGCTACAACTTC
MNase	ARG1 +33 F24	TGGTGGTTTAGATACCTCCGTCAT
MNase	ARG1 +137 R24	GCGGCATCGAAATCTTCTTCT
MNase	ARG1 +84 F25	CGAAGTTGTAGCTTTCATGGCTAAT
MNase	ARG1 +170 R25	TTGCAGGCACCGATCTTCA
MNase	ARG1 +102 F26	GGCTAATGTAGGGCAAGAAGAAGAT
MNase	ARG1 +197 R26	TCTTCACGACAATCCACACAAAC
MNase	ARG1 +153 F27	GAAGATCGGTGCCTGCAAGT
MNase	ARG1 +235 R27	TGACCTGTACAGCTGGGAATAGAAT
MNase	ARG1 +175 F28	GTTTGTGTGGATTGTCGTGAAGA
MNase	ARG1 +269 R28	GTACCCAACAGATAAACGTCTTCGT
MNase	AGR1 +190 F29	CGTGAAGATTTTGTCAAGGATATTCTATT
MNase	ARG1 +291 R29	AATAACAGGTCTTGCCAAAGAGGTA
MNase	ARG1 +213 F30	TCTATTCCCAGCTGTACAGGTCAA
MNase	ARG1 +312 R30	GACGTCAATTTGGGCTTTGG
MNase	ARG1 +234 F31	GTACGAAGACGTTTATCTGTTGGGTA
MNase	ARG1 +349 R31	AACCATGAGAGACCGCGAAA
MNase	ARG1 +279 F32	AAGACCTGTTATTGCCAAAGCC
MNase	ARG1 +383 R32	TCGAATCTGATTTGATCATTACCTTT
MNase	ARG1 +325 F33	GGCTGTTTCGCGGTCTCTC
MNase	ARG1 +426 R33	TGTAATACACTTAACGTCTGGCTTCA
MNase	ARG1 +341 F34	CTCATGGTTGTACCGGTAAAGGTAA
MNase	ARG1 +444 R34	TTCAGGCATTCTCCATGGTGTA
MNase	ARG1 +381 F35	CGAATTGTCATTTTACGCTCTGAA
MNase	ARG1 +471 R35	CTTTCTGCCAGCAAATCTTTCG
MNase	ARG1 +411 F36	CGTTAAGTGTATTACACCATGGAGAATG
MNase	ARG1 +506 R36	GGAATACCCTTTTGTGCAGCATAG
MNase	HIS4 -757 F1	TGTCGTAAGCCAACTACACGA
MNase	HIS4 -679 R1	TCAGGAGTTCGACATCTTCG
MNase	HIS4 -707 F2	TTTCTCATAATCAACCCACTGGT
MNase	HIS4 -618 R2	CAAATTGGTCTTCTATGTTGCGTA
MNase	HIS4 -679 F3	CGAAGATGTCGAACTCCTGA
MNase	HIS4 -586 R3	GCGTTCTTTAGCCCACTTTG
MNase	HIS4 -640 F4	ACGCAACATAGAAGACCAATTT
MNase	HIS4 -550 R4	TTTACTGAGCGAATCGTTATGC
MNase	HIS4 -598 F5	GGCTAAAGAACGCGAACAAT
MNase	HIS4 -493 R5	CGATGAGGAATCTTGTGGTTT

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MNase	HIS4 -557 F6	TCAGTAAAGAATACCAAAATTTGAGC
MNase	HIS4 -470 R6	TCAGTAAAGAATACCAAAATTTGAGC
MNase	HIS4 -509 F7	CACAAGATTCCTCATCGGAAG
MNase	HIS4 -412 R7	TGTTTGTGCTTGAGCCTGTT
MNase	HIS4 -469 F8	AAAACTTGAAGAGGCTAATGAAAAA
MNase	HIS4 -385 R8	GTCGAAAATTGGCAACGATT
MNase	HIS4 -419 F9	CACAAACAGCCGTGGAATC
MNase	HIS4 -328 R9	ATCGCAATGCTCACACCACT
MNase	HIS4 -381 F10	CCTGCACCAGTCGATACCAC
MNase	HIS4 -297 R10	GGGGGCATTCTGCTGTATTA
MNase	HIS4 -342 F11	TGTGAGCATTGCGATACGAT
MNase	HIS4 -257 R11	TCGACTGCCTAGAAGAACTGC
MNase	HIS4 -299 F12	CCCATCACAATCCTGACAAC
MNase	HIS4 -196 R12	TCACTGTGCATGGGTTTAGC
MNase	HIS4 -257 F13	AACTGACTCTAATAGTGACTCCGGTAA
MNase	HIS4 -161 R13	CCTTCTATATCGAATGACTGATAAAA
MNase	HIS4 -205 F14	TGCACAGTGACTCACGTTTTT
MNase	HIS4 -88 R14	CGGAGGTGAATATAACGTTCC
MNase	HIS4 -176 F15	CATTCGATATAGAAGGTAAGAAAAGGA
MNase	HIS4 -78 R15	CAACACACATCGGAGGTGAA
MNase	HIS4 -96 F16	TCACCTCCGATGTGTGTTGT
MNase	HIS4+23 R16	GGTAGAATCGGCAAAACCATT
MNase	HIS4 -56 F17	GCACAACTGCGCTGTGTAAT
MNase	HIS4 +44 R17	CATGAGGCCAGATCATCAAT
MNase	HIS4 -6 F18	CTGAATAATGGTTTTGCCGATT
MNase	HIS4 +83 R18	ACCTGACCAACAAGTGAAACG
MNase	HIS4 +31 F19	GATCTGGCCTCATGGAATAG
MNase	HIS4 +140 R19	TCCTCTTTGGAGAACTGGAGA
MNase	HIS4 +71 F20	TTGTTGGTCAGGTACTTTTGGA
MNase	HIS4 +162 R20	CAAAGCCACCAATGGAACTT
MNase	HIS4 +120 F21	TCTCCAGTTCTCCAAAGAGGA
MNase	HIS4 +218 R21	CCGTTGTTCAAGAAGGCAAT
MNase	HIS4 +158 F22	CTTTGTCCTTGCCAAGTGGT
MNase	HIS4 +266 R22	TGTTCGGCTGTTTTAGCATC
MNase	HIS4 +202 F23	GCCTTCTTGAACAACGGAGT
MNase	HIS4 +299 R23	CGCTCCTTTGGTACATTCAA
MNase	HIS4 +246 F24	AGATGCTAAAACAGCCGAACA
MNase	HIS4 +335 R24	TGATTGGAGAAAACACCGTTC
MNase	HIS4 +285 F25	TGTACCAAAGGAGCGTGTTG
MNase	HIS4 +375 R25	CACAATTTTATCTTGCGAGAATTT

Supplemental Table S2. Primers used in this study		
Purpose	**Name**	**Sequence**
MNase	HIS4 +328 F26	TCCAATCAATTCATGGTAAAACA
MNase	HIS4 +422 R26	CCAAGCACTTCTTTGGTCAAC
MNase	HIS4 +390 F27	AAGCAAGGATATGTTGACCAAAG
MNase	HIS4 +476 R27	TGGTCGACAACTAGGGTGGT
MNase	HIS4 +444 F28	TGACGGTTTATATACCACCCTAGTT
MNase	HIS4 +536 R28	TCGATGGCCTTTGCTATAGATT