SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Immunoprecipitation of Swi6p for glutathionylation. Cells were grown in SC-leu to OD_{600} of 0.4 and exposed to 30 μM LoaOOH for 45 min at 30°C. Yeast protein extract was prepared and Swi6p was immunoprecipitated as described in Experimental Procedures. Protein eluates were subjected toSDS-PAGE and glutathionylated proteins were detected by immunoblotting using anti-GSH antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Detection of dimersiation of Swi6p -To analyse whether dimer could form through the Cys404 residue in Swi6p, *swi6*Δ strains expressing pSWI6 or pC404A were used. Cultures of 100 ml were grown to OD_{600} of 0.4 and 20 ml of cells collected in 2 ml of trichloroacetic acid (100% w/v) to a final concentration of 10% (w/v) as zero time point. The rest of the cultures were treated with 30 μM LoaOOH treatment at 30°C and samples (20 ml) were collected at 5, 10, and 20 min intervals into tubes containing 2 ml of 100% (w/v) TCA. Cells were collected by centrifugation and washed once in 20% (w/v) TCA. Cell pellets were stored at –80°C until lysis. Cell lysis and alkylation of cysteine residues using iodoacetamide were performed according to Vivancos et al. (2005) (1). Protein samples were separated by non-reducing SDS-PAGE (8% w/v). Swi6p was detected by immunoblotting using anti-Swi6p (yN-19) antibody.

SUPPLEMENTARY TABLES

TABLE S1

Yeast strains and plasmids used in this study

Strains/Plasmids	Description	Source or Reference
BY4741	*MAT***a**, *his3, leu2, met15, ura3*	(2)
swi6Δ	*MAT***a**, *his3, leu2, met15, ura3, swi6ΔkanMX*	(2)
pRS415	*CEN LEU2*	Lab collection
pSWI6	*SWI6, CEN, LEU2, Amp*^{R}	(3)
PC354A	*SWI6 C354A, CEN, LEU2, Amp*^{R}	This study
PC380A	*SWI6 C380A, CEN, LEU2, Amp*^{R}	This study
pC404A	*SWI6 C404A, CEN, LEU2, Amp*^{R}	This study
pC473A	*SWI6 C473A, CEN, LEU2, Amp*^{R}	This study
pGPD-9HisSWI6	*GPDp::9His-SWI6, CEN, URA3, Amp*^{R}	This study
pGPD-9HisC404A	*GPDp::9His-C404A, CEN, URA3, Amp*^{R}	This study

TABLE S2

Primers used in PCR for cloning and sited-directed mutagenesis by SLIM

Primers	Sequence*	Applications
Swi6-C354A F	GGGGAGTCA**GC**CCTAGTGAAAGCTGTCAAATC	Mutagenesis of Cys354 to alanine
Swi61050bp R	CATATTATCACCATATAATCTGTTT	
Swi61069bp F	AAAGCTGTCAAATCAGTAAATAATT	
Swi6-C354A R	CACTAGG**GC**TGACTCCCCCATATTATCACC	
Swi6-C380A F	TTATATCCA**GC**TTTAATTTTGGAAGACTCAATGAA	Mutagenesis of Cys380 to alanine
Swi61128bp R	ATAATCTAGAAGTGCCTCAAAAG	
Swi61147bp F	TTGGAAGACTCAATGAATAGAAC	
Swi6-C380A R	AATTAAA**GC**TGGATATAAATAATCTAGAAGTGC	
Swi6-C404A F	ACTGGC**GC**TTCAGCAGCTGCGAAATATTATTTA	Mutagenesis of Cys404 to alanine
Swi61203bp R	CATACCAGACGTAATAATGATAT	
Swi61222bp F	GCGAAATATTATTTAGATATTTTAAT	
Swi6-C404A R	AGCTGCTGAA**GC**GCCAGTCATACCAGACGTAAT	
Swi6-C473A F	GGCGATACT**GC**CTTGAACATTGCAGCAAGATTGG	Mutagenesis of Cys473 to alanine
Swi61407bp R	ATTAGAGTCCTGCGCATTGAGC	
Swi61426bp F	ATTGCAGCAAGATTGGGAAACATT	
Swi6-C473A R	GTTCAAG**GC**AGTATCGCCATTAGAGTCCTGC	
attB1-N9HisSwi6 F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCACCATCACCATCACCAT CACCATCACGCG	Gateway cloning of Swi6p into pAG416GPD-ccdB
attB2-Swi6Stop R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATGAAGCATGCTTTTTTAAAAA	

*Restriction enzyme sites *Bam*HI, *Pst*I and *Not*I are shown in italics. The adapter sequences used in SLIM, recombination sequences in attB1 and attB2 sites used in gateway cloning are underlined. Point mutations introduced in primers are in bold.

TABLE S3

Primers used in real time PCR for target gene transcript

Target gene	Primer sequence*	T_m (°C)	Product size (bp)
SIR3	F – TCTCAGCGAGGAACGTCAAC	61.55	102
	R – AGGTGATGTGAGCGCAGAAG	61.57	
CLN1	F – GATCAGCAACCGGAGATGAA	61.16	114
	R – CCTGACAGCGTGGAAGAAGA	61.53	
CLN2	F – TGGGACTCGTCATCAATGCT	61.64	190
	R – GTTTCCACGGGGTTCATTTC	61.48	
PCL1	F – CAAGTCGCCAGTAACGGATG	61.62	190
	R – GGTGAGCAGAGTGGGTGTGT	61.22	
PCL2	F – ATACGGACGGCCTTCTCATC	61.38	174
	R –ACGTGCGACAGTCTCTTTGC	61.60	

*Primers were designed using Primer 3 (4) according to the following parameters: 19-23 bases in length with greater than 50% GC content and melting temperatures (T_m) in the range of 60 – 63°C.

TABLE S4

Functional groupings enriched[*] in gene clusters (1-14) from hierarchical clustering of differentially expressed genes from the six test conditions

Cluster	Category	*p-value*[†]	Genes[‡]	Source[§]
1	Regulation of translation	2.53×10^{-10}	*NUP60 SKT5 MNN2 SHM1 NAT1 COP1 DHH1 SSB1 LCB2 SEC7 CCT6 YDR266C NPL3 GCN20 PUF4 STT3 AFT1 NUP145 NAB2 GCN1 HXK2 ADH4 TIF4631 MTM1 BIO2 TIR3 RHR2 URA6 YLL032C HCR1 KAP95 SKI2 SPT5 ERG13 ORC5 YOL098C ABP140 DIG1 KAP120 FAS2 SGV1*	(2)
	Translation initiation	6.22×10^{-7}	*FUN12 RPG1 RLI1 TIF4631 SUI2 HCR1 NIP1 PRT1 TIF5 TIF3*	(1)
	Biopolymer biosynthetic process	3.63×10^{-6}	*SKT5 CHS3 SSB1 BAP3 LCB2 NPL3 STT3 GCN1 HXK2 YHB1 TNA1 TIR3 KAP95 ERG13 FET3 NIS1 FAS2*	(2)
	Aminoacyl-tRNA-synthetases	4.09×10^{-5}	*ILS1 KRS1 VAS1 YNL247W GLN4 ALA1 CDC60 HTS1*	(1)
	rRNA metabolic process	3.21×10^{-4}	*FUN30 YBL036C FUI1 SKT5 YBL081W SMY2 YBR238C SIR2 RBA50 NUP145 STE20 RRN7 TRL1 PHO86 TRK1 FRE1 FPR3 UBP8 ALR1 NUP1 RPO31 KIN4 MOT1*	(2)
2	Transcriptional Control	1.48×10^{-4}	B*DF2 ADR1 YAP6 SWR1 TOS8 INO80 SPT16 ASK10 SIP4 SWI3 RIC1 YAP1 SUB1 GAT2 TAF7 FKH2 NRD1 THI20 HRT1 HMS1 HAL1 HOS1 NOT5*	(1)
	Homeostasis of metal ions (Na, K, Ca, etc)	2.11×10^{-3}	*CCC2 TOS8 ARN2 MNR2 OCT1 HMX1 ATX1 MMT2*	(1)
3	No functional groupings with *p-value*<0.005			

4	Spindle pole body/centrosome and microtubule cycle	9.50×10^{-4}	*DAD1 SPC42 DAD2*	(1)
	Mitotic cell cycle	1.61×10^{-3}	*YOX1 CLN2*	(1)
	Cell division	3.03×10^{-3}	*DAD1 AXL2 DAD2 PCL1 CLN2*	(2)
	Regulation of microtubule polymerisation or depolymerisation	3.85×10^{-3}	*DAD1 DAD2*	(2)
5	Anti-apoptosis	3.78×10^{-3}	*SNO1*	(2)
6	Aminoacyl-tRNA-synthetases	5.02×10^{-4}	*MSW1 MSK1*	(1)
7	Cytoplasmic and nuclear protein degradation	9.25×10^{-4}	*BAR1 UBP7 APE2 UBP15*	(1)
	Modification by ubiquitination, deubiquitination	2.70×10^{-4}	*CDC53 UBP7 UBA1 UBP15*	(1)
8	Iron-sulfur cluster assembly	6.60×10^{-5}	*NBP35 DRE2 NAR1*	(2)
	Transposition, RNA-mediated	2.72×10^{-3}	*DBR1*	(2)
	snoRNA metabolic process	4.65×10^{-3}	*DBR1 NAR1*	(2)
9	Protein polymerisation	8.67×10^{-5}	*TUB4 TUB1*	(2)
	Microtubule-based movement	3.87×10^{-4}	*TUB1 KIP2*	(2)
	Nuclear migration along microtubule	1.45×10^{-3}	*TUB1 KIP2*	(2)
	Cell division	3.57×10^{-3}	*BUD14 SKG6 CLB4 KIP2*	(2)
10	Cell cycle	1.36×10^{-5}	*LTE1 CLN3 ALK2 SCC2 ECO1 BUD9 BUB1 IRR1 SLD2 CDC45 MEC3 MCM4 DPB2*	(2)
	Regulation of gene expression, epigenetic	5.15×10^{-5}	*NSE4 CDC45 DPB2*	(2)
	DNA replication	7.16×10^{-5}	*NSE4 PLM2 ECO1 SLD2 CDC45 SPH1 MCM4 DPB2*	(2)
	Cell growth	2.93×10^{-4}	*BEM1 CLA4*	(2)
	S phase of mitotic cell cycle	1.11×10^{-3}	*ECO1 CDC45 MCM4*	(2)
11	Meiotic mismatch repair	7.11×10^{-5}	*RAD10 PMS1 MSH2*	(2)
	Cell cycle checkpoints (checkpoints of morphogenesis, DNA-damage,-replication, mitotic phase and spindle)	1.02×10^{-4}	*RFA1 KCC4 PDS1 DMA1 MSH2*	(1)
	Mitotic recombination	1.44×10^{-4}	*RFA1 RAD10 MSH2*	(2)
	Somatic/mitotic recombination	4.11×10^{-4}	*RAD10 TOP2 MSH2*	(1)

12	Biopolymer biosynthetic process	1.51×10^{-4}	*PMT1 KSS1 SFB3 SEC24*	(2)
	ER to Golgi transport	3.82×10^{-4}	*ERP2 SFB3 SEC24*	(1)
	Phosphate metabolism	1.02×10^{-3}	*PHO3 APA 1 VTC2 KSS1 SEC59*	(1)
	Thiamine and derivative metabolism process	2.12×10^{-3}	*PHO3*	(2)
13	Peptidyl-amino acid modification	1.05×10^{-5}	*ATS1 FUN26 ALG14 GIC2 SEC20 HLR1 IES6 MAK10 AVT2 GLE2 YER186C POR2 FLX1 YKL047W YLL054C ADY4 CPR8 TPT1 HNT3*	(2)
	Regulation of translation	1.67×10^{-3}	*FES1 RNQ1 CDC50 GCS1 GIC2 SAC7 GCN4 MCM3 HYP2 GLY1 VMA8 PMI40 VTC1 PTC2 TRP2 GLE2 SCS2 PMD1 PET122 OXA1 PAB1 BRR2 FLX1 VMA5 YLL054C CNA1 ERG12 YNL035C GPM3 RKM1*	(2)
	Modification with fatty acids (e.g. myristylation, palmitylation, farnesylation)	2.12×10^{-3}	*RAM1 GWT1 RAM2 ERF2 GPI12 BET2*	(1)
	Homeostasis of protons	3.03×10^{-3}	*PMP2 CUP5 VMA8 PMA1 YHC3 VMA5 VPH2*	(1)
	Regulation of nitrogen metabolism	3.42×10^{-3}	*GLN3 GZF3 URE2*	(1)
14	Negative regulation of protein metabolic process	1.78×10^{-4}	*CHO1 GAL83 RAD4 RAD54 TWF1 YGR207c ARC15*	(2)
	Protein binding	2.29×10^{-4}	*APC11 ARP2 DLD2 PEX7 HSP78 VAB2 UBC8 VAC8 TIM9 SEC3 TSC11 SSA4 RSP5 DDI1 BMH1 MST27 VPS45 TWF1 PDX1 CAP2 ARC15 CDC6 ENT3 DID4 CAP1 VPS51 APC9 UBC12 SLG1 HSP10*	(1)
	Catabolism of nitrogenous compound	5.44×10^{-4}	*DAL2 DAL7 DAL3 ADE2*	(1)
	Purine base metabolic process	5.87×10^{-4}	*DAL2 DAL7 DAL3 ADE2*	(2)
	Biological process	8.51×10^{-4}	*SAW1 YBR096W AIM5 FMP21 YCL049C YCR023C YDR061W TMS1 YDR182W-A YDR307W SNA2 IRC22 VAB2 YEL020C YEL023C PAU2 RMD6 FMP52 YER010C ZRG8 PHM8 HVG1 YER039C-A*	(2)

		YEN1 SAP1 CAJ1 HMF1 FCY22 YER067W YER071C YER078W-A YER079W AIM9 AIM10 IES5 AIM11 AST2 YER130C YER134C YER152C YER158C YER163C FMP10 AIM13 YGL108C MTC3 YGR207C CPR2 YIR018C-A PRY1 NIT2 YJL133C-A YJL185C IML1 YJR142W ALY1 YLR001C YLR049C YLR050C YLR156W YLR253W YLR281C YLR408C CUE4 YMR155W YMR178W YMR253C YMR262W YOL114C AIM43 AIM45	
Heteroduplex formation	4.07×10^{-4}	*RAD51 DMC1 RAD54*	(2)

*Enrichment was determined using FunSpec (5). Complete list of genes in each clusters and their functional groupings can be found in excel spreadsheet in supplementary information.

†The *p-value* is the probability of accidental enrichment and top five catagories with $p<0.005$ are shown.

‡Paralogs were not included in the analysis of functional groupings since microarray data could not differentiate the signals from paralogs.

§Source refers to the classification source: 1 – MIPS Functional Classification, 2- GO Biological Process.

TABLE S5

Transcription factor binding sites in genes up-regulated in C404A identified using YEASTRACT (6,7)

Transcripton Factor	**Percentage (%)**	**Genes***
Swi4p	27.5	*YBL109w HTA1 YDR544c YHR217c AXL2 YOX1 HHF2 PCL1 YNL338w CSI2 CLN2*
Mbp1p	27.5	*YBL109w YDR544c YHR217c AXL2 APN1 YLR104w YOX1 HHF2 PCL1 YNL338w CLN2*
Ste12p	25.0	*HHF1 YHR214w YHR214w-a YEH1 YLL066w-b NUP116 ERB1 PCL1 CSI2 YOR192c-c*
Yap6p	17.5	*YBL109w YDR544c YJL218w YEH1 PCL1 YNL338w YPL034w*
Rap1p	15.0	*YDR544c YJL218w YLR104w YMR244w PCL1 YNL338w*

*Paralogs were not included in the analysis.

SUPPLEMENTARY FIGURE LEGENDS

FIGURE S1. **Identification of a potentially reactive cysteine in the Swi6p ankyrin repeat.** (A) The crystal structure of the ANK motif in Swi6p (PDB: 1SW6). All cysteine residues (Cys354, Cys380, Cys404 and Cys473) in the structure are highlighted in orange. The Cys404 residue projects outward and is not involved in structural conformation. Cys404 was mutated to Ala (green) in the C404A mutant. Structures were generated using PyMol Graphics System (B) Alignment of the ankyrin repeats from Swi6p, Swi4p and Mbp1p. Swi6p Cys404 is a non-conserved residue, indicated by an asterisk (*). Sequence alignment was performed by ClustalW (8).

FIGURE S2. **Characterisation of cell shape and morphology of various strains.** Cells were grown to mid-exponential phase on YEPD and viewed under a phase contrast microscope. Enlarged cells with irregular cell shape observed in *swi6Δ* and C404A mutants are indicated by while arrowheads.

FIGURE S3. **Cell cycle response of the wild type, C404A and *swi6Δ* mutants to diamide.** Cultures were synchronised with alpha-factor and were tyreated with 1 mM diamide for 30 min. Aliquots were taken every 15 min and cells were fixed for FACS analysis of DNA content as described in Experimental Procedures. For FACS analysis, 25000 cells were analysd per sample: 1N represents single copy of genome per cell and 2 N represents two copies.

FIGURE S4. **Detection of Swi6p modification upon LoaOOH treatment.** (A) No detection of protein dimerisation in cells upon LoaOOH treatment. Cells were grown to OD_{600}=0.4 and subjected to 30 μM LoaOOH. Samples were taken at indicated time intervals and cells were lysed with 12.5% (w/v) TCA as in Supplementary Experimental Procedures. Total lysates were subjectd to non-reducing SDS-PAGE and Swi6p was detected by immunoblotting. (B) Lack of detection of glutathionylation in Swi6p. Cells were treated with 30 μM LoaOOH for 45 min and protein extracts were prepared for immunoprecipitation using anti-Swi6p antibody as described in Experimental Procedures and immunoblotting by anti-GSH antibody.

FIGURE S5. **Hierarchical clustering of differentially expressed genes from microarray analysis observed in wild type, C404A and *swi6Δ* mutants subjected to LoaOOH treatment.** Cultures of each strains were grown to OD600=0.4, treated with 30 30 μM LoaOOH for 45 min and cell extracts prepared for RNA extraction. Three biological replicates of each strain and treatment were performed. Gene expression from other genotypes and conditions was normalised as the intensity ratio relative to the untreated wild type control. Genes enriched in functional groups are listed in Table S4.

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A

B

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Swi6   288  QHVSFDSLLQEVN----------DAFPNTQLNLNIPVDEHGNTPLHWLTSIANLELVKHL
Swi4   481  KQTILTILSSERSSDVDQALLATLYPAPKNFNINFEIDDQGHTPLHWATAMANIPLIKML
Mbp1   355  SKLVDYFISNEMKSNKSLPQVLLHPPPHSAPYIDAPIDPELHTAFHWACSMGNLPIAEAL

Swi6   338  VKHGSNRLYGDNMGESCLVKAVKSVNNYDSGTFEALLDYLYPCLILEDSMNRTILHHIII
Swi4   541  ITLNANALQCNKLGFNCITKSIFYNNCYKENAFDEIISILKICLITPDVNGRLPFHYLIE
Mbp1   415  YEAGTSIRSTNSQGQTPLMRSSLFHNSYTRRTFPRIFQLLHETVFDIDSQSQTVIHHIVK

                     *
Swi6   398  TSGMTGCSAAAKYYLDILMGWIVKKQNRPIQSGTNEKESKPNDKNGERKDSILENLDLKW
Swi4   601  LSVNKSKNPMIIKSYMDSIILSLGQQD-------------------------------YN
Mbp1   475  R---KSTTPSAVYYLDVVLSKIKDFSP-------------------------------QY

Swi6   458  IIANMLNAQDSNGDTCLNIAARLGNISIVDALLDYGADPFIANKSGLRPVDFGAGTSKLQ
Swi4   630  LLKICLNYQDNIGNTPLHLSALNLNFEVYNRLVYLGASTDILNLDNESPASIMNKFNTPA
Mbp1   501  RIELLLNTQDKNGDTALHIASKNGDVVFFNTLVKMGALTTISNKEGLTANEIMNQQYEQM

Swi6   518  NTNGGDENSKMVSKGDYDGQKNGKAKKIR--------------------------------
Swi4   690  GGSNSRNNNTKADRKLARNLPQKNYYQQQQQQQQPQNNVKIPKIIKTQHPDKEDSTADVN
Mbp1   561  MIQNGTNQHVNSSNTDLNIHVNTNNIETKN---------------------------DVN
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Fig. S1

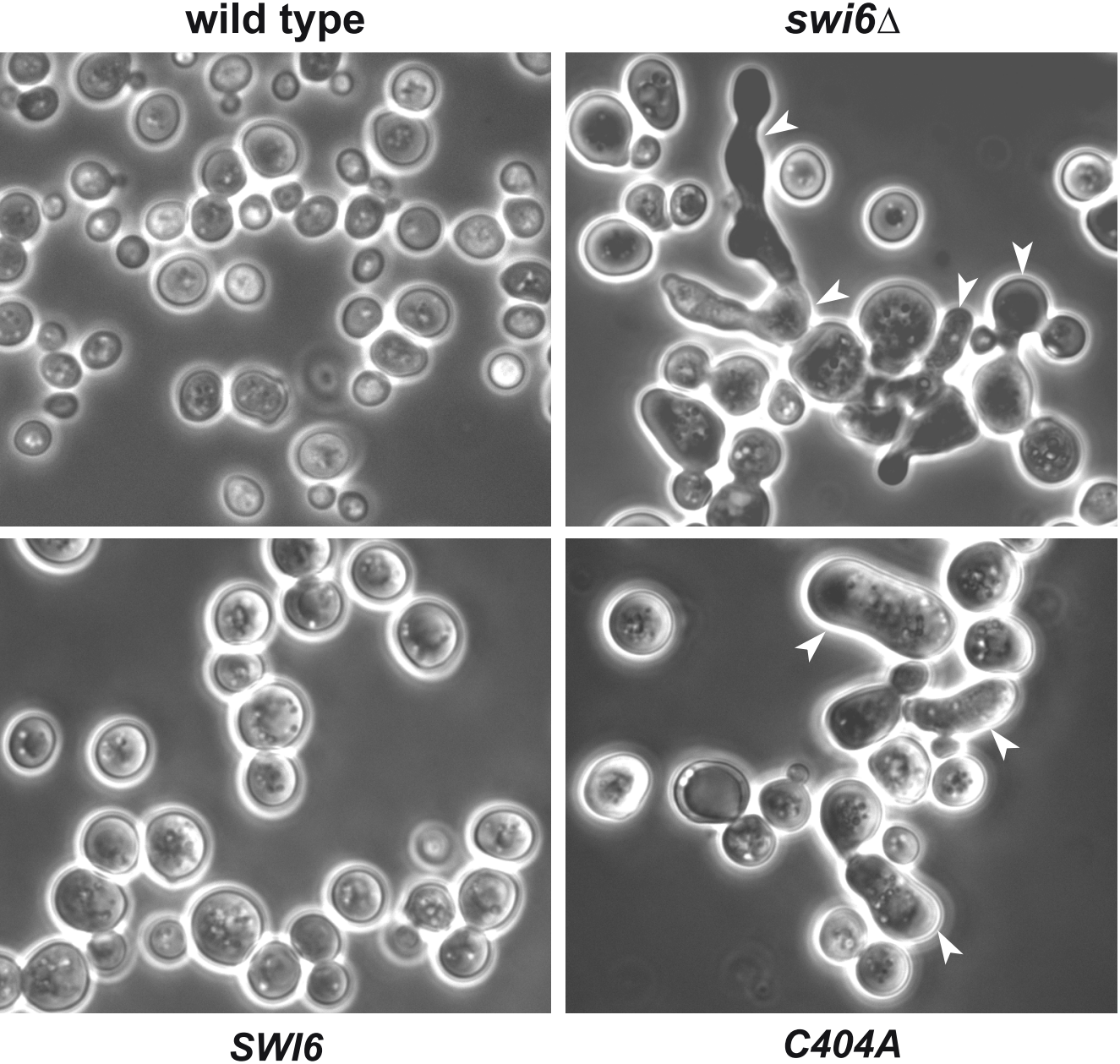
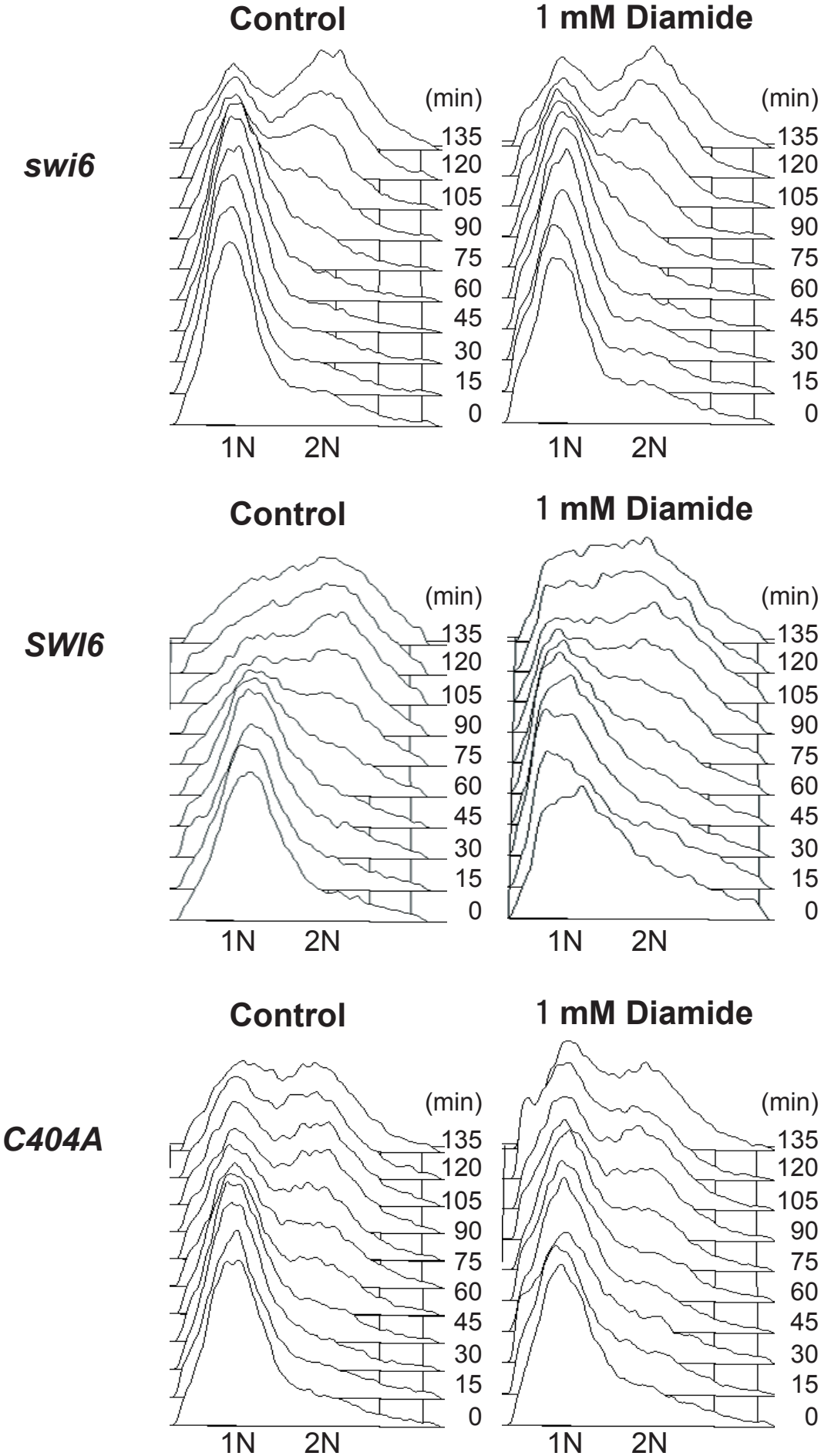


Fig. S2


Control
1 mM Diamide
swi6
SWI6
C404A
(min)
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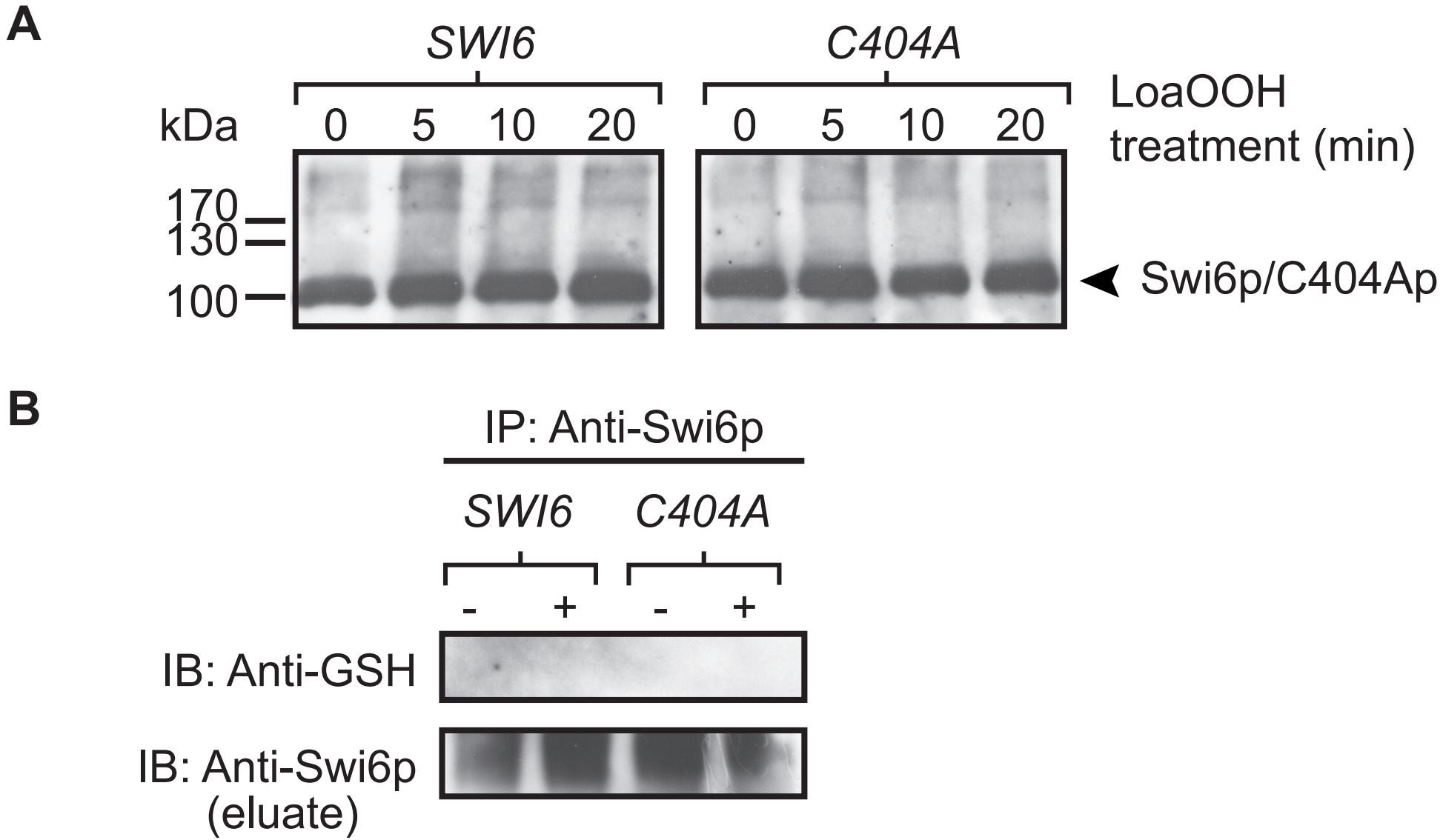
120
105
90
75
60
45
30
15
0
1N
2N

Fig. S3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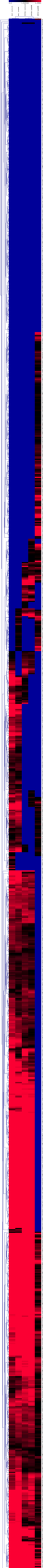
SWI6
C404A
kDa
0
5
10
20
0
5
10
20
LoaOOH
treatment (min)
170
130
100
Swi6p/C404Ap
B
IP: Anti-Swi6p
SWI6
C404A
-
+
-
+
IB: Anti-GSH
IB: Anti-Swi6p
(eluate)

Fig. S4


Cluster 1
Cluster 2
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Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9
Cluster10
Clluster 11
Cluster 12
Cluster 13
Cluster 14