Unexpected Function of the Glucanosyltransferase Gas1 in the DNA Damage Response Linked to Histone H3 Acetyltransferases in *Saccharomyces cerevisiae*

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ABSTRACT Chromatin organization and structure are crucial for transcriptional regulation, DNA replication, and damage repair. Although initially characterized in remodeling cell wall glucans, the β-1,3-glucanosyltransferase Gas1 was recently discovered to regulate transcriptional silencing in a manner separable from its activity at the cell wall. However, the function of Gas1 in modulating chromatin remains largely unexplored. Our genetic characterization revealed that *GAS1* had critical interactions with genes encoding the histone H3 lysine acetyltransferases Gcn5 and Sas3. Specifically, whereas the *gas1 gcn5* double mutant was synthetically lethal, deletion of both *GAS1* and *SAS3* restored silencing in *Saccharomyces cerevisiae*. The loss of *GAS1* also led to broad DNA damage sensitivity with reduced Rad53 phosphorylation and defective cell cycle checkpoint activation following exposure to select genotoxins. Deletion of *SAS3* in the *gas1* background restored both Rad53 phosphorylation and checkpoint activation following exposure to genotoxins that trigger the DNA replication checkpoint. Our analysis thus uncovers previously unsuspected functions for both Gas1 and Sas3 in DNA damage response and cell cycle regulation.

CHROMATIN packages DNA in the nucleus and regulates accessibility to the underlying genome. Tightly compacted chromatin, or heterochromatin, impedes nuclear processes including transcription, DNA replication, and DNA damage repair (reviewed in Li and Reinberg 2011; Papamichos-Chronakis and Peterson 2013). Thus, genes found within heterochromatic regions are repressed or silenced (reviewed in Rusche *et al.* 2003). However, the degree of chromatin compaction is highly dynamic, as cells must continuously alter transcriptional programs in response to environmental or metabolic demands while promoting replication and repair processes.

The basic unit of chromatin is the nucleosome, consisting of DNA wrapped around an octamer of conserved core histone proteins (Kornberg and Lorch 1999). Post-translational modification (PTM) of histones is a prime means for altering chromatin structure. These modifications are dynamic and tightly controlled as they regulate higher order chromatin structure and DNA accessibility by altering the interaction between DNA and histones in addition to recruiting chromatin-modifying enzymes (reviewed in Kouzarides 2007; Campos and Reinberg 2009). The localization of chromatin within the nucleus also plays a fundamental role in chromatin dynamics, such that localization to the nuclear periphery regulates processes including silencing and the DNA damage response (DDR) (reviewed in Bermejo *et al.* 2012; Taddei and Gasser 2012).

The β-1,3-glucanosyltransferase Gas1, a member of the Gas family of proteins, was initially characterized at the cell wall where it remodels chains of β-1,3-glucan (Ragni *et al.* 2007). However, a pool of Gas1 also localizes to the nuclear periphery (Huh *et al.* 2003) and genome-wide studies have identified genetic and physical interactions between Gas1 and diverse components of the chromatin modifying machinery (www.thebiogrid.org). Reflecting these findings, deletion of *GAS1* was recently discovered to lead to a unique constellation of silencing defects in yeast. Specifically, loss of

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Gas1 catalytic activity increases rDNA silencing and decreases telomeric silencing, yet has no observable change at the *HM* cryptic mating-type loci. These alterations in silencing are not remediated by the osmoregulator sorbitol (Koch and Pillus 2009), which rescues the cell wall-associated defects of *gas1* and other cell wall mutants (Turchini *et al.* 2000; Levin 2005). Combined, these data support a function for Gas1 in chromatin-mediated processes that is separable from its role at the cell wall.

A genome-wide screen reported that *GAS1* has a negative genetic interaction with *GCN5* (Costanzo *et al.* 2010), which encodes a prominent lysine acetyltransferase (KAT). Gcn5-catalyzed acetylation of histone and nonhistone substrates affects numerous chromatin-dependent processes (reviewed in Lee and Workman 2007; Koutelou *et al.* 2010). Gcn5 functions in several important complexes including SAGA, ADA, and SLIK/SALSA (Grant *et al.* 1997; Pray-Grant *et al.* 2002) to acetylate nucleosomal substrates on histone H3, with lysine 14 (K14) as a predominant target (Kuo and Andrews 2013). Gcn5 acts as a coactivator, with H3K14 acetylation correlating with active transcription (Pokholok *et al.* 2005) and Gcn5 is enriched at the promoters of active genes (Robert *et al.* 2004).

Gcn5 functionally overlaps with another KAT, Sas3. Gcn5 and Sas3 share nucleosomal H3 targets (reviewed in Lafon *et al.* 2007) and deletion of both *GCN5* and *SAS3* is synthetically lethal (Howe *et al.* 2001). Further, both Gcn5 and Sas3 are recruited to similar genomic regions (Rosaleny *et al.* 2007). Whereas Gcn5 has been studied extensively, less is known about Sas3, due in part to the functional overlaps with Gcn5 as well as the limited independent phenotypes defined for *SAS3* mutants. Deletion of *SAS3* leads to a modest increase in silencing at the *HM* loci (Reifsnyder *et al.* 1996) and Sas3 localizes at the boundary of the *HM* loci, blocking the spread of silent chromatin (Tackett *et al.* 2005). Sas3 physically associates with the N terminus of Spt16, a subunit of the FACT elongation complex (John *et al.* 2000), which is essential for recovery from replication stress (O'Donnell *et al.* 2004) and boundary formation (Tackett *et al.* 2005).

In addition to functions in transcriptional regulation and silencing, Gcn5 and other histone modifying enzymes also have crucial roles in the DDR. One of the earliest marks associated with DDR activation in yeast is the phosphorylation of H2A at serine 129 (S129), which serves as a scaffold that amplifies the DNA damage signal in part by recruiting the repair machinery (reviewed in Rossetto *et al.* 2010). Subsequently, phosphorylation of other mediators, prominently including the Rad53 kinase, triggers a cascade that leads to changes in transcription and activation of cell cycle checkpoints, which foster the repair of damaged DNA (reviewed in Branzei and Foiani 2006; Sirbu and Cortez 2013).

Deletion of *GCN5* renders cells sensitive to DNA damaging agents such as the topoisomerase I inhibitor camptothecin (CPT), the radiomimietic drug methyl methanesulfonate (MMS) and the replication inhibitor hydroxyurea (HU) (Choy and Kron 2002; Burgess *et al.* 2010). Indeed, Gcn5-catalzyed acetylation of both histone and nonhistone substrates features prominently at numerous stages of the DNA damage response (Burgess *et al.* 2010; Lee *et al.* 2010; Charles *et al.* 2011).

There is also some evidence that Sas3 may play a role in the DDR. For example, Sas3 has a reported physical interaction with the DNA damage checkpoint effector kinase Chk1 (Liu *et al.* 2000), although the functional significance of this interaction has not been established. Further, mutants of H3K14 and H3K23, nucleosomal substrates of Gcn5 and Sas3, are sensitive to DNA-damaging agents (Qin and Parthun 2002; Tamburini and Tyler 2005). However, what role, if any, Sas3 may play in DNA damage has not been defined.

Here we report that *GAS1* has strong genetic interactions with the histone H3 lysine acetyltransferases encoded by both *GCN5* and *SAS3*. The *gas1 gcn5* combination was synthetically lethal. In contrast, the *gas1 sas3* double mutant was viable and, moreover, displayed selective mutual suppression of each individual mutant's phenotypes. We also discovered that *gas1* has broad DNA damage sensitivity following exposure to the genotoxins MMS, HU, and CPT. Sensing and initial activation of the DNA damage response was intact in *gas1* strains, as evidenced by phosphorylation of histone H2A. However, the MMS and HU sensitivity of *gas1* reflects failure to trigger the DNA damage cell cycle checkpoint as demonstrated by loss of both the cell cycle delay and Rad53 phosphorylation. The deletion of *SAS3* in the *gas1* background specifically suppressed both MMS and HU sensitivity, leading to restoration of cell cycle delay and Rad53 phosphorylation. These findings define a role for Gas1 in the DNA damage response that is separable from its cell wall function. We have also identified a specific role for Sas3 in antagonizing the replication checkpoint, which is unique and opposite to the role previously identified for Gcn5.

Materials and Methods

Yeast strains and plasmids

Strains are listed in Supporting Information, Table S1, plasmids in Table S2, and oligonucleotides in Table S3. All mutations are deletions, unless otherwise noted, and were constructed using standard techniques (Amberg *et al.* 2005).

Growth, silencing, and DNA damage assays

Plate assays are fivefold serial dilutions adjusted to an A_{600} of 1.0 after growth to saturation in synthetic complete (SC) medium. Dilution assays were incubated at 30°, except where noted. Telomeric silencing assays were performed with the TELVR::*URA3* reporter strain grown in SC medium and plated on SC as growth control or SC supplemented with 0.1% 5-fluoroorotic acid (5-FOA) to assay silencing (Renauld *et al.* 1993; Van Leeuwen and Gottschling

2002). Silent mating-type analysis was performed with the *hml*::*TRP1* reporter (Le *et al.* 1997). Silencing of the rDNA was assayed using the *RDN*::*Ty-1-mURA3* construct (Smith and Boeke 1997). Strains were plated on SC as a growth control and SC −Ura for rDNA silencing. HU sensitivity was analyzed with 0.2 M HU. MMS sensitivity was analyzed with 0.015% MMS. CPT sensitivity was analyzed using 20 μg/ml CPT dissolved in DMSO added to plates buffered with 100 mM potassium phosphate (pH 7.5) to maintain CPT activity (Nitiss and Wang 1988) with growth control plates at the same concentration of DMSO. DMSO is shown as a control with all CPT images as *gas1* is mildly sensitive to DMSO. For ultraviolet light (UV) sensitivity, strains were plated at A_{600} of 1.0 and exposed to 60 J/m^2. Where indicated, plates were supplemented with 1 M sorbitol.

Protein immunoblots

Strains for analysis of H2AS129 and Rad53 phosphorylation following genotoxin exposure were incubated at 30° to an A_{600} of 0.4. Cultures were then treated with either indicated genotoxin or untreated as a control. The concentrations of HU, MMS, and CPT were the same as in dilution assays. Cells were incubated with genotoxin for 2 hr at 30° with shaking. Cell extracts were prepared by bead beating (Clarke *et al.* 1999). Proteins were separated on 18% (H2A) or 8% (Rad53) SDS-polyacrylamide gels and transferred to nitrocellulose. H2AS129 phosphorylation levels were analyzed with the primary antiserum anti-H2A phospho S129 (1:5000, Abcam) and blots were imaged using ECL Plus (GE Healthcare Amersham) with anti-H2A (1:5000, Abcam) used as a probe for protein loading. For analysis of Rad53 phosphorylation, the primary antiserum was anti-Rad53 (1:5000 dilution; Pike *et al.* 2003, a gift from J. Heierhorst). Antitubulin (1:10000; Bond *et al.* 1986) used as a probe for protein loading.

Flow cytometry

Cells were grown in SC with genotoxin conditions as used for immunoblots, fixed with ethanol overnight, and then treated with RNase A (Clarke *et al.* 1999). Cells were stained with propidium iodide for 2 days at 4°, sonicated, and analyzed with Accuri (BD).

Results

The synthetic lethality of GAS1 with GCN5 is separable from cell wall functions

The function of Gas1 at the cell wall has been studied extensively (reviewed in Popolo and Vai 1999; Orlean 2012), but less is known about the pool of Gas1 that is contiguous with the nuclear periphery (Huh *et al.* 2003). Genome-wide studies report >50 interactions of *GAS1* with genes encoding nuclear proteins, many of which are active in chromatin dynamics and/or the DDR (www.thebiogrid.org). However, few of these interactions have been independently validated. Based on the silencing defects of *gas1* and its reported interactions, we chose to further define the chromatin-based functions of Gas1 by analyzing interactions with genes encoding nuclear factors. We selected these based on previous genome-wide analysis of synthetic interactions, such as the synthetic lethality for *gas1* and *orc2-1* (Suter *et al.* 2004) or based on independent observations from our laboratory. The initial analysis included genes encoding the Orc2 subunit of the DNA replication origin recognition complex, the histone lysine deacetylase Rpd3, and the ATPase Swr1. The double mutants *gas1 rpd3* and *gas1 orc2-1* were synthetically lethal; however, these interactions were at least partially rescued by the osmoregulator sorbitol (Figure S1, A and B), which rescues phenotypes of cell wall-defective mutants, including *gas1* (Turchini *et al.* 2000; Levin 2005). Conversely, deletion of *SWR1* rescued both *gas1* temperature and calcofluor white (CFW) sensitivity (Figure S1C), which disrupts the cell wall by inhibiting chitin synthesis (Roncero and Duran 1985). Although these results do not eliminate the possibility that the proteins encoded by these genes may also be significant for Gas1-related chromatin functions, we directed our focus to other chromatin modifying enzymes as a means to define the roles of Gas1 in chromatin dynamics that are separable from its cell wall function.

A recent genome-wide study indicated that *GAS1* and *GCN5* have a negative genetic interaction (Costanzo *et al.* 2010). We found that the *gas1 gcn5* heterozygous double mutant failed to sporulate unless covered by a plasmid encoding either *GAS1* or *GCN5*. When dissected, the resulting haploid double mutants were not viable without the covering plasmid as demonstrated in two ways: first by the inferred genotype of dead spores and second by inability to grow on 5-FOA, which selects against the *URA3*-marked covering plasmids. The catalytic activity of both Gas1 and Gcn5 is required for viability, as neither of the previously defined catalytically inactive mutants, *gcn5-KQL* (*gcn5**; Wang *et al.* 1998) or *gas1-E161Q, E262Q* (*gas1***; Carotti *et al.* 2004), rescued the lethality of the double mutant in plasmid-shuffle tests. Additionally, the osomoregulator sorbitol did not rescue the synthetic lethality of *gas1 gcn5* (Figure 1A). Thus, the synthetic lethality of *gas1 gcn5* is due to loss of the catalytic activities of Gas1 and Gcn5 and is separable from cell wall-associated functions.

The substrate specificity of Gcn5 is largely defined by the macromolecular complexes in which it is found, including SAGA, ADA, and SLIK/SALSA (Grant *et al.* 1999; Lee *et al.* 2011; Figure 1B). To determine whether the synthetic lethality observed for *gas1 gcn5* was specifically mediated through one complex or functional module, double mutants were generated with *gas1* to include genes encoding components of the SAGA modules and unique subunits for both SLIK/SALSA and ADA. These included genes encoding a central component of the HAT module (*ADA2*), key structural or functional components of other SAGA modules including DUB (*SGF73*) and SPT (*SPT20*), in addition to genes encoding unique components of SLIK/SALSA (*RTG2*) and ADA

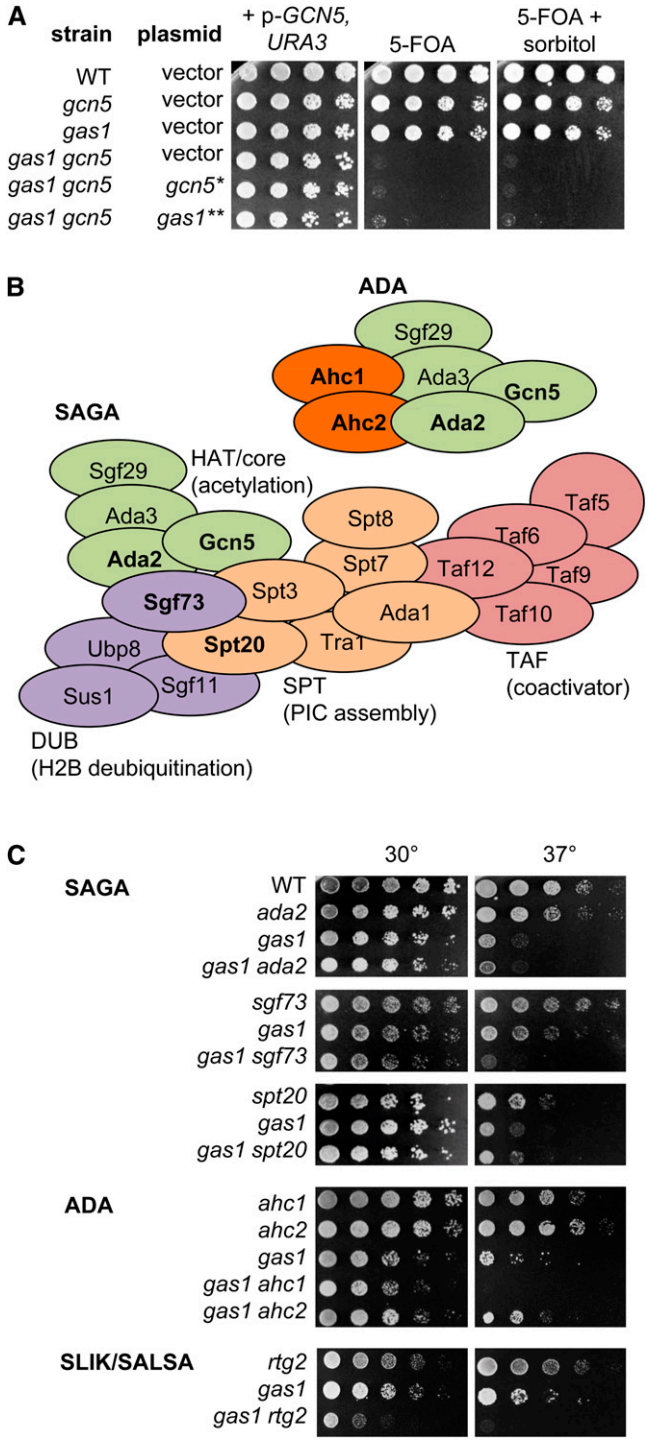

Figure 1 The *gas1 gcn5* double mutant is synthetically lethal. (A) Synthetic lethality of *gas1 gcn5* is due to loss of catalytic activity of both Gas1 and Gcn5 and is not rescued by the osomoregulator sorbitol. Serial dilutions of wild type (LPY18050), *gcn5* (LPY12264), *gas1* (LPY18081), *gas1 gcn5* (LPY16798), and *gas1 gcn5* covered by plasmid-born p-*gcn5-KQL* (*gcn5**; LPY16800) or p-*gas1-E161Q*, *E262Q* (*gas1***; LPY16801) were plated on selective media with 5-FOA, to counterselect the p-*GCN5*, *URA3* plasmid, with or without 1 M sorbitol at 30°. (B) Primary Gcn5-containing complexes are shown with color coding to highlight defined subunits in each functional module. Boldface type indicates subunits analyzed in this study (adapted from Lee *et al.* 2011). (C) *gas1* has modest synthetic interactions with components of all three complexes tested, including increased temperature sensitivity with *gas1 sgf73* and *gas1 ahc1* at 37°. A more severe effect is observed in which *gas1 rtg2* is synthetic sick at 30° and dead at 37°. Serial dilutions of wild type (LPY5), *ada2* (LPY6439), *gas1* (LPY10129), *gas1 ada2* (LPY19197), *sgf73* (LPY19816), *gas1 sgf73* (LPY19771), *spt20* (LPY16914), *gas1 spt20* (LPY19630), *ahc1* (LPY17370), *ahc2* (LPY18518), *gas1 ahc1* (LPY19467), *gas1 ahc2* (LPY19414), *rtg2* (LPY18206), and *gas1 rtg2* (LPY18372) were plated on SC at either 30° or 37°. Here, and in other figures, *gas1* and *gcn5* refer to the null alleles, whereas the *gas1* catalytic mutant (Carotti *et al.* 2004) is denoted as *gas1*** and the *gcn5* catalytic mutant (Wang *et al.* 1998) as *gcn5**.

(*AHC1* and *AHC2*). The TAF module subunits are essential and shared with TFIID (Grant *et al.* 1998) and thus were not analyzed.

Deletion of *ADA2*, which is required for Gcn5 association with all complexes and nucleosome acetylation (Candau *et al.* 1997; Balasubramanian *et al.* 2002), did not have a synthetic interaction with *gas1*; however, modest interactions were observed with distinct subunits of each Gcn5 complex (Figure 1C). As deletion of no single subunit defining modules or complexes recapitulated the synthetic lethality of *gas1 gcn5* at 30°, it is likely that Gcn5 catalytic activity itself is the critical factor in the interaction with Gas1, as is observed for the *gcn5 sas3* synthetic lethality (Howe *et al.* 2001).

The gas1 sas3 double mutant mutually suppresses select phenotypes

In addition to the synthetic lethality, Gcn5 and Sas3 have overlapping sites of genomic localization (Rosaleny *et al.* 2007) and share nucleosomal substrates (Howe *et al.* 2001). Based on the similarities between Gcn5 and Sas3, we chose to analyze the *gas1 sas3* double mutant to determine if the synthetic lethality observed with *gas1 gcn5* was gene specific.

In sharp contrast to *gas1 gcn5*, not only was *gas1 sas3* viable but the double mutant also displayed mutual suppression of select phenotypes (Figure 2A). Deletion of *SAS3* suppressed phenotypes of *gas1*, including temperature sensitivity and telomeric and rDNA silencing defects. In turn, deletion of *GAS1* restored normal levels of cryptic mating-type silencing in *sas3*. Deletion of *SAS3* did not suppress the sensitivity of *gas1* to CFW. This suggests that, like the *gas1 gcn5* mutant, the interaction between *GAS1* and *SAS3* is separable from cell wall functions of Gas1.

Sas3 is targeted to specific chromatin regions by the NuA3 complex (Howe *et al.* 2002; Figure 2B), which includes the subunit Yng1, a PHD-finger protein that recognizes methylated H3K4 (Martin *et al.* 2006). To determine whether the NuA3 complex plays a role in suppression of *gas1* phenotypes, we generated the double mutant *gas1 yng1*. This mutant did not display synthetic interactions and phenocopied *gas1* (Figure 2C). Thus the interaction observed between *GAS1* and *SAS3* depends on Sas3 activity but is independent of specific substrate targeting properties of NuA3.

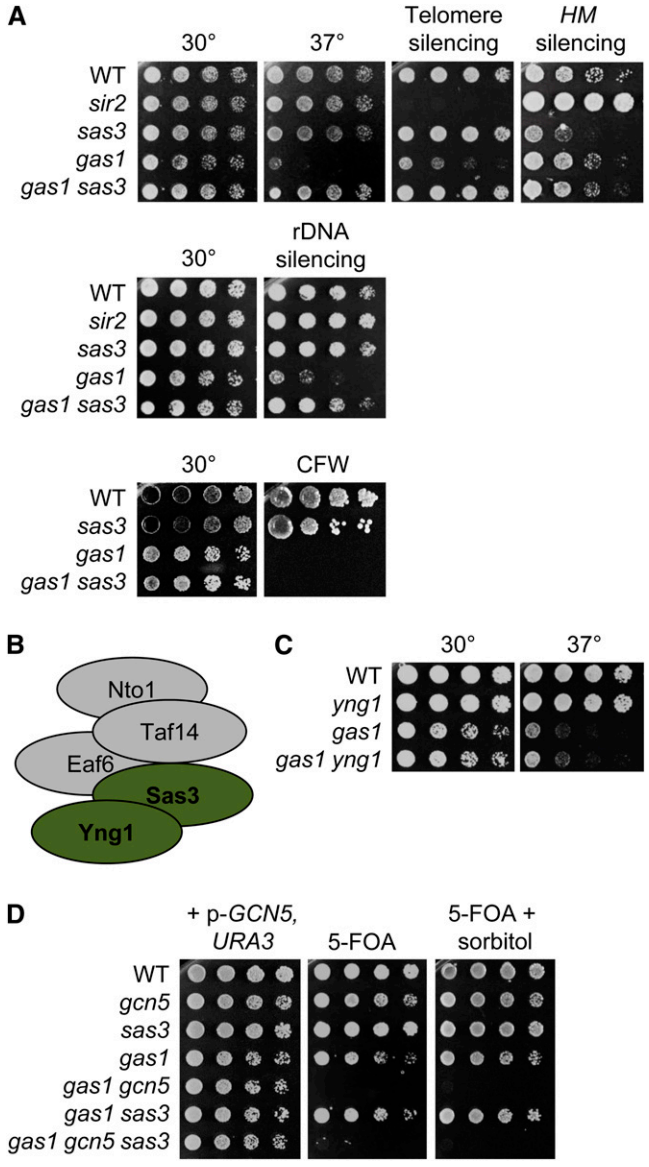

Figure 2 Mutual suppression of phenotypes in the *gas1 sas3* double mutant. (A) Deletion of *SAS3* rescues *gas1* temperature sensitivity and silencing defects at the telomere and rDNA array but not CFW sensitivity. In turn, deletion of *GAS1* restores *HM* silencing in *sas3* to wild-type levels. The *sir2* mutant is included as a positive control for disruption of silencing. Top panel: Serial dilutions of wild type (LPY4924), *sir2* (LPY5035), *sas3* (LPY19731), *gas1* (LPY19773), and *gas1 sas3* (LPY16444) were plated on SC at 30° and 37°, SC with 5-FOA (TELVR::*URA3*) or SC −Trp (*hml*::*TRP1*). Middle panel: Serial dilutions of wild type (LPY2444), *sir2* (LPY2447), *sas3* (LPY17686), *gas1* (LPY10074), and *gas1 sas3* (LPY17685) were plated on SC or SC −Ura (*RDN*::*Ty-1-mURA3*) at 30°. Bottom panel: wild type (LPY5), *sas3* (LPY8256), *gas1* (LPY10129), and *gas1 sas3* (LPY17520) were plated on either SC or SC with 10 μg/ml CFW. (B) NuA3 complex with subunits analyzed herein shaded green (adapted from Lafon *et al.* 2007). (C) Deletion of *YNG1* does not have synthetic interactions with *gas1*. Serial dilutions of wild type (LPY6285), *yng1* (LPY5526), *gas1* (LPY9820), and *gas1 yng1* (LPY16997) were plated on SC at either 30° or 37°. (D) Analysis of *GAS1*, *GCN5*, and *SAS3* reveals distinct and opposing outcomes for synthetic interactions. Serial dilutions of wild type (LPY5), *gcn5* (LPY8242), *sas3* (LPY16039), *gas1* (LPY10129), *gas1 gcn5* + p-*GCN5*, *URA3* (LPY16736), *gas1 sas3* (LPY19823), and *gas1 gcn5 sas3* + p-*GCN5*, *URA3* (LPY19101) were plated on SC or SC with 5-FOA, to select against p-*GCN5*, *URA3*, at 30° with and without 1 M sorbitol.

Based on the mutual suppression observed in the *gas1 sas3* double mutant, we next tested whether deletion of *SAS3* suppressed the *gas1 gcn5* synthetic lethality. The triple mutant *gas1 gcn5 sas3* was not viable (Figure 2D). These results suggest that the interactions between *GAS1* and *GCN5* or *SAS3* are of distinct and opposite outcomes.

Due to the strength of the genetic interactions with H3 KATs, we analyzed H3 acetylation (H3Ac) levels under suppressing conditions. As previously reported, deletion of *GAS1* did not alter levels of H3K9Ac, H3K14Ac at 30° (Koch and Pillus 2009), which are targets of both Gcn5 and Sas3 (reviewed in Lafon *et al.* 2007). At 37°, a condition under which deletion of *SAS3* suppresses *gas1* temperature sensitivity, neither the *gas1* strain nor *gas1 sas3* had altered global levels of H3K9Ac, H3K14Ac (Figure S2). This suggests that the suppression phenotypes of *gas1 sas3* are not mediated through changes in global H3K9Ac, H3K14Ac levels, which are largely intact in *sas3* strains due to Gcn5.

Deletion of GAS1 leads to broad DNA damage sensitivity with specific suppression in the absence of SAS3

Several studies have demonstrated a role for Gcn5-based acetylation of histone and nonhistone substrates in the DDR (Choy and Kron 2002; Qin and Parthun 2002; Tamburini and Tyler 2005; Liang *et al.* 2007; Burgess *et al.* 2010; Wang *et al.* 2012). *GAS1*, *SAS3*, and *GCN5* also all have numerous genetic and physical interactions with key components of the DDR, as defined from previous genome-wide screens (www.thebiogrid.org). Based on these connections, we asked whether the chromatin functions of *GAS1* may also influence DDR.

We evaluated the sensitivity of *gas1* to a spectrum of DNA damaging agents including MMS, HU, CPT, and UV irradiation, which generates bulky DNA adducts (Sertic *et al.* 2012). Deletion of *GAS1* led to sensitivity to all chemical agents tested, but not to UV. The genotoxin sensitivity was due to loss of the β-1,3-glucanosyltransferase activity of Gas1 and was not rescued by sorbitol (Figure 3A). DNA damage sensitivity was not shared with other members of the *GAS* family, nor other components of the cell wall machinery tested (Figure S3), demonstrating that the sensitivity was not a general phenotype of mutants with cell wall defects.

As deletion of *SAS3* suppressed specific phenotypes of *gas1*, we analyzed the *gas1 sas3* double mutant upon DNA damage. Deletion of *SAS3* suppressed both the MMS and HU sensitivity of *gas1* but did not rescue the CPT sensitivity (Figure 3B). These results indicated that, whereas Gas1 has a broad role in the DDR, Sas3 has a more specific, and antagonistic, function.

Based on the DNA damage phenotypes, we performed genetic analysis of nucleosomal targets of Sas3 that have been implicated in DDR. The residues, H3K14 and H3K23, have increased sensitivity to DNA damaging agents when

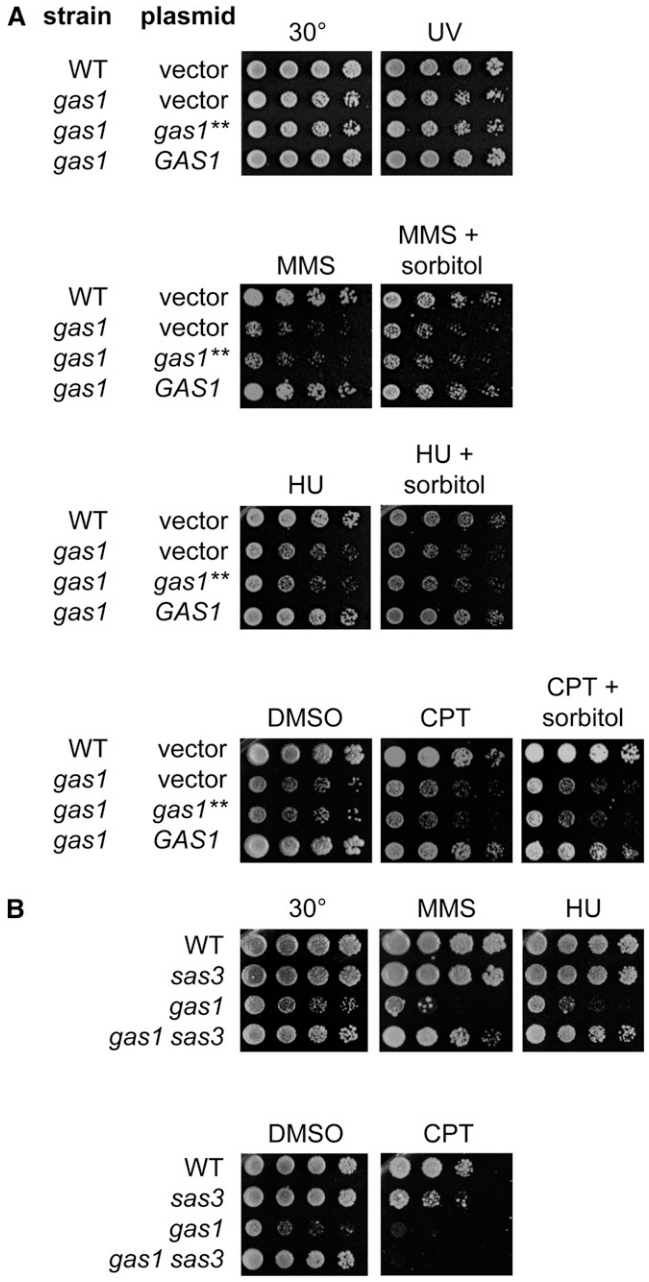

Figure 3 Loss of *GAS1* leads to broad DNA damage sensitivity with phenotype-specific suppression by deletion of *SAS3*. (A) *gas1* mutants are sensitive to MMS, HU, and CPT but not exposure to UV. Sensitivity is due to loss of Gas1 catalytic activity and separable from cell wall function as demonstrated by failure of sorbitol to rescue these phenotypes. Serial dilutions of wild type (LPY18050), *gas1* (LPY12247), *gas1* + p-*gas1-E161Q*, *E262Q* (*gas1***; LPY12251), and *gas1* + p-*GAS1* (LPY12326) were plated on selective media with 0.015% MMS, 0.2 M HU, or 20 μg/ml CPT in DMSO with or without 1 M sorbitol or on SC buffered with phosphate and supplemented with DMSO as a control. UV exposure was 60 J/m^2. (B) Deletion of *SAS3* specifically suppressed the MMS and HU sensitivity of *gas1*, but not CPT sensitivity. Serial dilutions of wild type (LPY5), *sas3* (LPY8256), *gas1* (LPY10129), and *gas1 sas3* (LPY17520) were plated on SC plates using the same concentration of genotoxins and plate conditions as in A.

mutated to arginine (Qin and Parthun 2002; Tamburini and Tyler 2005). Lysine-to-arginine mutations both block acetylation and maintain a positive charge, thus mimicking the nonacetylated form. Conversely, mutation of lysine to alanine, which neutralizes lysine's positive charge, leads to disruption of DNA-nucleosome contacts, as occurs with acetylation.

The H3K23A mutant suppressed all *gas1* phenotypes, including temperature- and genotoxin sensitivity, whereas the H3K14A, H3K23A double mutant suppressed temperature sensitivity alone (Figure S4A). Conversely, the H3K23R mutant had no obvious phenotype compared to the single *gas1* mutant and the double H3K14R, H3K23R was synthetically sick at elevated temperature and with DNA damage (Figure S4B). The H3K14A mutant was also synthetically sick with *gas1* (Figure S4A) as were wild type and *sas3* (Figure S4C). The increased sensitivity of the H3K14R, H3K23R mutant is consistent with reports that this double mutant has increased sensitivity to genotoxic stress (Tamburini and Tyler 2005) and was also observed in both the wild-type and *sas3* histone mutant background (Figure S4D).

These findings indicate that changes in the acetylation status of Sas3 histone substrates can influence *gas1* phenotypes. However, analysis of whether this is mediated by Sas3 is complicated in the histone mutant background. Here, *gas1* growth is improved and suppression by deletion of *SAS3* is less apparent (compare growth in Figure 2A and Figure 3B to Figure S4, A and B). It is possible that *HHT1-HHF1* may modulate suppression of *gas1* phenotypes, as this histone locus is deleted in the histone mutant background. Indeed, we found that adding a centromeric plasmid containing wild-type *HHT1-HHF1* restored suppression of *gas1* phenotypes by deletion of *SAS3* (Figure S5A), although global histone levels remained comparable to the histone mutant strain (Figure S5B).

Deletion of SAS3 selectively restores DNA damage cell cycle arrest control in gas1

Genotoxin exposure triggers activation of cell cycle checkpoints via a kinase cascade that allow cells time to repair damaged DNA (reviewed in Sirbu and Cortez 2013). To determine how the DNA damage sensitivity of *gas1* and its suppression by deletion of *SAS3* may be linked to events in the DDR pathway, we analyzed *gas1*, *sas3*, and *gas1 sas3* cell cycle profiles by flow cytometry. Whereas the wild-type and *sas3* strains had the expected cell cycle delay blocking replication following MMS and HU treatment, the *gas1* strain did not have a delayed cycle, with cells remaining distributed throughout the cell cycle. The genotoxin-associated delay was restored with deletion of *SAS3*, although to a lesser extent with MMS treatment (Figure 4A). Conversely, upon treatment with CPT, the *gas1* strain displayed a clear cell cycle arrest at G_2/M similar to wild type, and this response was not altered in the *gas1 sas3* double mutant (Figure 4B). Thus, whereas there are distinct functions for Gas1 under a spectrum of DNA damage conditions, Sas3 may act

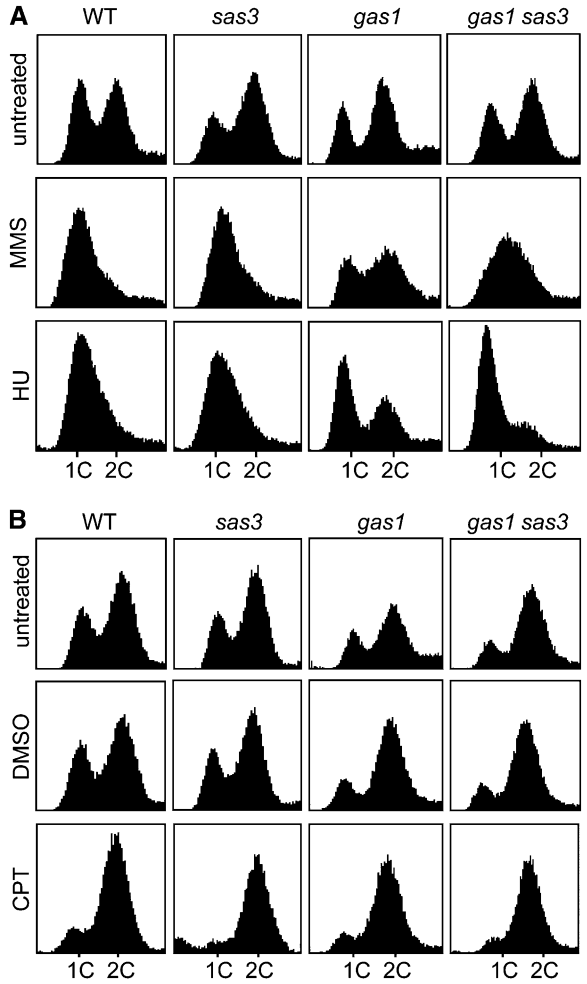

Figure 4 Deletion of *SAS3* rescues *gas1* defects in cell cycle arrest. (A) Treatment of *gas1* with HU fails to trigger the cell cycle delay observed in wild type, whereas the cell cycle delay following treatment with MMS is severely impaired in *gas1*. Cycle delay is significantly restored in the double mutant *gas1 sas3*. (B) CPT treatment triggers cell cycle arrest in all strains tested. Strains and genotoxin concentrations are as in Figure 3A.

specifically to antagonize the DNA replication checkpoint (DRC), as both MMS and HU trigger the DRC, but CPT does not (Redon *et al.* 2003).

Cell cycle defects in gas1 correspond to loss of Rad53 phosphorylation and are restored by deletion of SAS3

Analysis of cell cycle profiles following DNA damage suggested that the rescue of *gas1* by *SAS3* deletion may specifically occur by restoring activation of the cell cycle checkpoint. One of the initial events following DNA damage in yeast is the phosphorylation of histone H2A at serine 129, which is indicative of sensing of DNA damage (reviewed in Rossetto *et al.* 2010). Downstream of H2AS129 phosphorylation, the effector kinase Rad53 is hyperphosphorylated, which is largely responsible for triggering cell cycle delay or arrest (Branzei and Foiani 2006; Sirbu and Cortez 2013).

To determine whether *gas1* is defective in sensing DNA damage we analyzed H2A phosphorylation in *gas1* and *gas1 sas3* by immunoblotting. In all mutants, H2AS129 phosphorylation levels are comparable to WT levels following exposure to MMS, HU, and CPT (Figure 5), consistent with accurate sensing of damage.

To monitor subsequent activation of the downstream effectors, we evaluated Rad53 phosphorylation. In the *gas1* strain, Rad53 phosphorylation was severely impaired upon treatment with MMS and HU. However, moderate phosphorylation of Rad53 was evident following MMS exposure (Figure 6), consistent with the partial activation of the cell cycle checkpoint observed in Figure 4A. The reduced level of Rad53 phosphorylation was due to loss of Gas1 activity, as the catalytically inactive *gas1-E161Q, E262Q* mutant was also defective for Rad53 phosphorylation (Figure S6). As with the rescue of *gas1* MMS and HU sensitivity by deletion of *SAS3*, in the double mutant, Rad53 phosphorylation was restored to near wild-type levels (Figure 6). Rad53 phosphorylation following CPT treatment is negligible (Figure S7), consistent with previous reports that CPT only minimally triggers Rad53 phosphorylation (Redon *et al.* 2003). Together, these data demonstrate that *sas3* suppression of *gas1* MMS and HU sensitivity is linked to reactivation of the cell cycle delay via restoration of Rad53 phosphorylation.

Discussion

Our findings demonstrate that *GAS1* has striking yet distinct genetic interactions with genes encoding the lysine acetyltransferases Gcn5 and Sas3, which themselves are synthetically lethal and have overlapping nucleosomal substrates (Howe *et al.* 2001) and genome-wide localization patterns (Rosaleny *et al.* 2007). Whereas the *gas1 gcn5* double mutant is dead, there is mutual suppression of specific phenotypes in the *gas1 sas3* strain. The suppression phenotypes include both silencing defects and specific relief of the newly identified *gas1* sensitivity to genotoxins. The strong genetic interactions with the acetyltransferases and the DNA damage sensitivity of the *gas1* mutant demonstrate that Gas1 plays an important role in chromatin dynamics, which is separable from its cell wall function. Further, whereas Gcn5 and Sas3 have often been considered to be largely functionally overlapping, our results distinguish the biological roles of Sas3 and Gcn5 in the important process of DNA repair.

Gas1 and Sas3 counterbalance silencing at all three silenced regions

Previous research indicates that Gas1 and Sas3 contribute to transcriptional silencing at distinct loci. Whereas loss of *SAS3* leads to an increase in silencing at the *HM* loci (Reifsnyder *et al.* 1996), *gas1* mutants have impaired silencing at telomeric loci and improved silencing within rDNA (Koch and Pillus 2009). We demonstrate that deletion of both enzymes leads to restoration of silencing to wild-type levels at all loci analyzed (Figure 2A). Locus-specific silencing

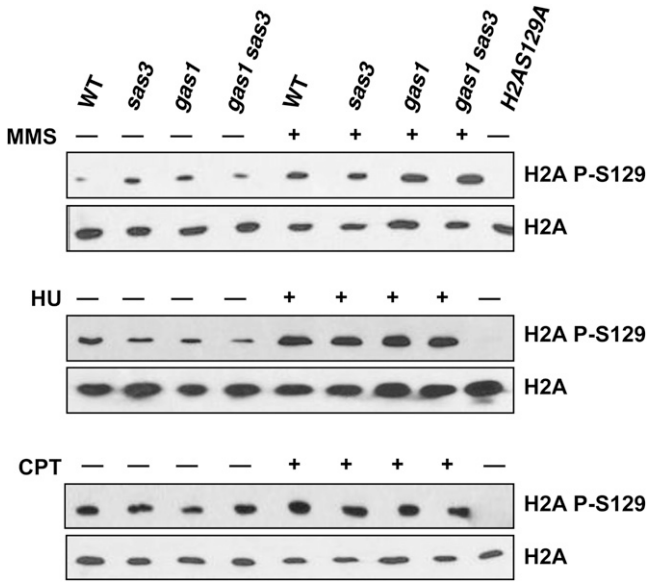

Figure 5 H2AS129 is phosphorylated following genotoxin exposure in all strains. Levels of H2AS129 phosphorylation following exposure to MMS (top), HU (middle), and CPT (bottom) are comparable to wild type in all strains analyzed. Strains and genotoxin concentrations are as in Figure 3A.

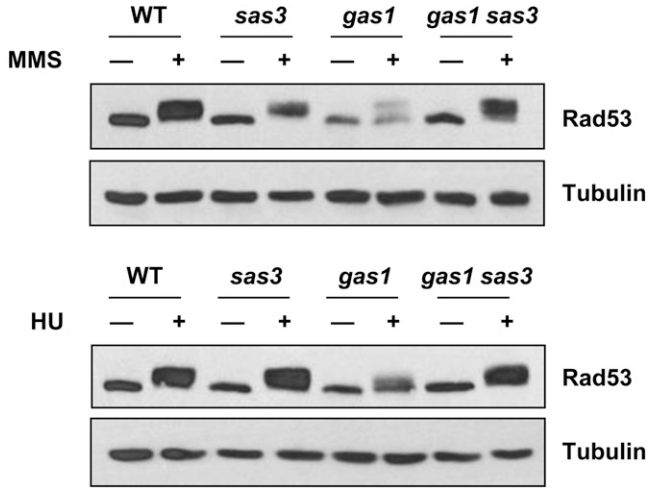

Figure 6 Rad53 phosphorylation is significantly reduced in *gas1* and restored in *gas1 sas3* following exposure to MMS (top) and HU (bottom). Note that overall levels of Rad53 are diminished in *gas1*. Strains and genotoxin concentrations are as in Figure 3A.

relies on a balance of silencing proteins and other chromatin factors, some of which are limiting (Smith *et al.* 1998; Benbow and Dubois 2008). Altering the distribution of these factors can lead to changes in the strength of silencing between loci (Lustig *et al.* 1996). As silencing is both strengthened and/or disrupted at specific loci in the mutants under study, one potential explanation for the mutual suppression observed in the *gas1 sas3* strain is that localization of limiting silencing factors is normalized. In this case, Sas3 and Gas1 counteract the influence of each other, such that in the absence of both enzymes balance is restored. This idea is in agreement with our previous observation of a physical interaction between Gas1 and the deacetyltransferase Sir2 (Koch and Pillus 2009), a limiting factor essential for establishment and maintenance of silencing (Rusche *et al.* 2003).

Analysis of DNA damage sensitivity in gas1 cells reveals that Sas3 antagonizes the DNA replication checkpoint

In addition to previously defined silencing defects (Koch and Pillus 2009) we found that deletion of *GAS1* led to DNA damage sensitivity. Strains lacking Gas1, or with defective catalytic activity, were sensitive to the genotoxins MMS, HU, and CPT but not UV (Figure 3A). Thus, although Gas1 plays a broad role in DNA damage, there are distinctions for particular types of damage or repair pathways.

Whereas H2AS129 phosphorylation, indicating sensing and initial DDR activation, was intact in all strains analyzed, the levels of Rad53 phosphorylation were significantly reduced in *gas1* and restored by deletion of *SAS3*. Impairment of the MMS or HU DNA damage-associated cell cycle delay and Rad53 phosphorylation levels in *gas1* strains (Figure 4 and Figure 5) indicates that Gas1 may function in triggering hyperphosphorylation of Rad53 and the subsequent cell cycle checkpoint. Although *GAS1* mutants failed to arrest in response to MMS and HU they did undergo CPT-induced G_2/M arrest. These observations strengthen the idea that Gas1 is broadly relevant to DDR, yet its contributions appear to depend on the type of lesion.

Distinct mechanistic roles for Gas1 in DNA damage are further supported by the suppression seen with deletion of *SAS3*, which rescued MMS and HU sensitivity but not CPT sensitivity (Figure 3B). MMS and HU elicit a largely overlapping transcriptional response, which is primarily dependent on Rad53 phosphorylation of substrates. By contrast, CPT leads to induction of a markedly different set of genes (Travesa *et al.* 2012; Travesa and Wittenberg 2012). Both MMS and HU trigger the replication checkpoint via fork arrest or by slowing fork progression by reducing dNTP pools, respectively (reviewed in Branzei and Foiani 2007). Conversely, CPT is considered to be "checkpoint blind" as exposure leads to only modest induction of Rad53 phosphorylation and does not trigger the replication checkpoint (Redon *et al.* 2003; Tourriere and Pasero 2007).

The primary checkpoints activated by DNA damage include delay of the G_1/S transition and block of the G_2/M transition and the S-phase checkpoints. Although there are overlaps in the proteins mediating these checkpoints there are also distinctions that depend on the phase of the cell cycle, type of DNA damage, and repair pathway choice (reviewed in Warmerdam and Kanaar 2010; Symington and Gautier 2011; Gobbini *et al.* 2013). Cell cycle checkpoints and DNA damage repair require both positive and negative regulation to ensure proper spatiotemporal dynamics and maintenance of genomic integrity (reviewed in Panier and Durocher 2013). Thus, Sas3 may be particularly relevant in antagonizing activation of the replication

checkpoint pathway, specific to the repression of the cell cycle delay prior to DNA replication mediated by Rad53 phosphorylation.

DNA damage occurs within the context of chromatin, yet the functions of the chromatin-modifying enzymes and histone post-translational modifications in the DNA damage response remain incompletely defined (reviewed in Papamichos-Chronakis and Peterson 2013). Multiple chromatin factors, including key silencing enzymes, are known to dynamically redistribute from telomeres to sites of double-strand breaks (Martin *et al.* 1999; Mills *et al.* 1999). Further, silencing at the *HM* loci was recently found to involve key factors of the homologous recombination pathway (Kirkland and Kamakaka 2013). If Sas3 and Gas1 act to balance chromatin-modifying enzymes, as proposed above, the suppression of *gas1* genotoxin sensitivity could relate to redistribution of the same or similar factors that alter silencing phenotypes in the double mutant. Indeed, localization of chromatin to the nuclear periphery is linked to both maintenance of silencing (reviewed in Zimmer and Fabre 2011; Taddei and Gasser 2012) and regulation of the DNA damage response (reviewed in Bermejo *et al.* 2012). Thus the pool of Gas1 at the nuclear periphery may be optimally localized at the interface of both silencing and DDR.

We found that the *HHT1-HHF1* locus may, at least in part, mediate the suppression observed by deletion of *SAS3* in the *gas1* background (Figure S5A). The role of histones in the DNA damage response is complex, such that even modest imbalances in histone levels can alter DNA damage sensitivity (see for example Gunjan and Verreault 2003; Sanders *et al.* 2004; Du *et al.* 2006). Whereas the duplicate histone loci are believed to be largely redundant, there are distinctions both at the level of dosage (Cross and Smith 1988; Libuda and Winston 2010) and in regulation of their expression (Zunder and Rine 2012). Our findings here and previous work of others (Sanders *et al.* 2004; Du *et al.* 2006) suggest that the *HHT1-HHF1* locus may indeed have a unique function in DNA damage. Histones are highly regulated at multiple levels including expression, localization, PTM, and degradation (reviewed in Kurat *et al.* 2013). Whether the restoration of suppression by *HHT1-HHF1* is relevant to precise histone levels or some other aspect of this locus's biology has yet to be determined.

Distinct functions for Gcn5 and Sas3

Although the function of Gcn5 in both transcription and DNA damage has been analyzed extensively (Robert *et al.* 2004; Burgess *et al.* 2010; Lee *et al.* 2010), less is known about functions of Sas3. Several lines of research indicate that Sas3 may have a role in cell cycle regulation and DDR. Using a *sas3* allele with diminished function, it was found that both Gcn5 and Sas3 play a role in cell cycle regulation, with decreased Sas3 activity coupled with deletion of *GCN5* leading to G_2/M arrest (Howe *et al.* 2001). Loss of *SAS3* leads to a decrease of H3K14Ac, primarily at genes involved in cell cycle regulation and cell division (Rosaleny *et al.* 2007). Sas3 physically interacts with Chk1 (Liu *et al.* 2000), a Mec1 DNA damage pathway effector kinase and Dpb4, which regulates DNA replication and telomere silencing (Tackett *et al.* 2005). As noted above, Sas3 physically associates with the FACT remodeling complex via interaction with the N terminus of Spt16 (John *et al.* 2000), which is necessary for the DNA replication stress response (O'Donnell *et al.* 2004). Several chromatin-remodeling complexes have been linked to the synthetic lethality observed between *SAS3* and *GCN5*, including RSC (Choi *et al.* 2008) and ISWI (Lafon *et al.* 2012). Chromatin remodeling complexes have well-established roles in the DDR, with Gcn5-based acetylation of Rsc4 identified as a key factor in replication stress resistance (Charles *et al.* 2011).

Although Sas3 has often been considered to be largely functionally redundant with Gcn5, previous research indicated that Sas3 can disrupt Gcn5-based acetylation of H3K14 at distinct genomic loci (Rosaleny *et al.* 2007). They may also compete during other dynamic processes. Whereas Gcn5 has primarily been implicated as a broad positive regulator of the DNA damage response, our finding that Sas3 may function antagonistically in DDR further demonstrates a unique, and opposing, function for Sas3. This possibility is consistent with the strong yet opposing genetic interactions observed between *GAS1* and *GCN5* and *SAS3*. Future studies should reveal how the protein modifications controlled by these three enzymes are balanced to respond to distinct forms of cellular and genotoxic stresses.

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Unexpected Function of the Glucanosyltransferase Gas1 in the DNA Damage Response Linked to Histone H3 Acetyltransferases in *Saccharomyces cerevisiae*

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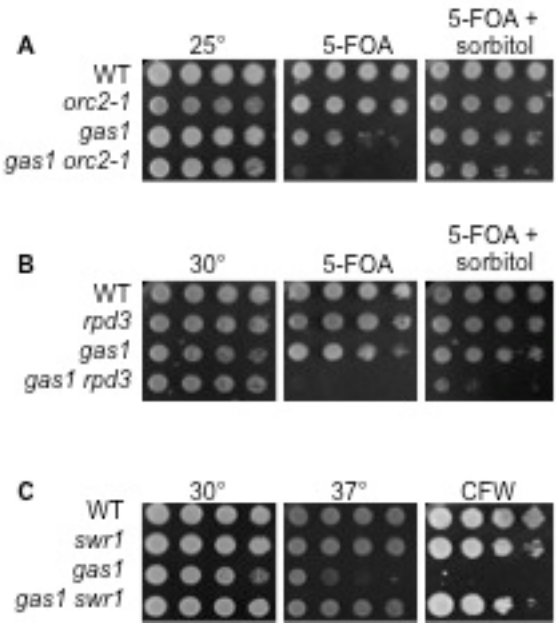

Figure S1 The synthetic lethality of *gas1* with *orc2-1* or *rpd3* is at least partially rescued by sorbitol, whereas deletion of *SWR1* rescued both *gas1* temperature and CFW sensitivities. (A) Wild type (LPY10266), *orc2-1* (LPY10267), *gas1* (LPY10271) and *gas1 orc2-1* covered by p-*GAS1* (LPY10270) were plated on SC or SC with 5-FOA, to counterselect p-*GAS1, URA3*, with or without 1M sorbitol at 25°. (B) Wild type (LPY4196), *rpd3* (LPY14355), *gas1* (LPY19200), *gas1 rpd3* covered by p-*GAS1, URA3* (LPY15695) were plated at 30° on SC and SC with 5-FOA, to counterselect p-*GAS1, URA3*, with or without 1M sorbitol. (C) Wild type (LPY5), *swr1* (LPY16104), *gas1* (LPY10129) and *gas1 swr1* (LPY17161) were plated on SC at 30°, 37°, and SC with CFW at 30°.

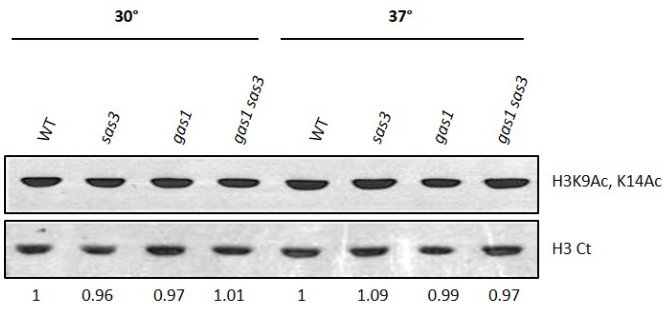

Figure S2 Neither *gas1* nor *gas1 sas3* have significantly reduced global levels of H3K9Ac, K14Ac. Whole cell lysates from wild type (LPY5), *sas3* (LPY8256), *gas1* (LPY10129) and *gas1 sas3* (LPY17520) were separated on 18% SDS-PAGE after growth at either 30° or 37° and probed with anti-H3K9Ac, K14Ac (1:10000; Millipore). Blots were reprobed with anti-H3 C-terminal (Ct) (1:10000; Millipore) as a loading control.

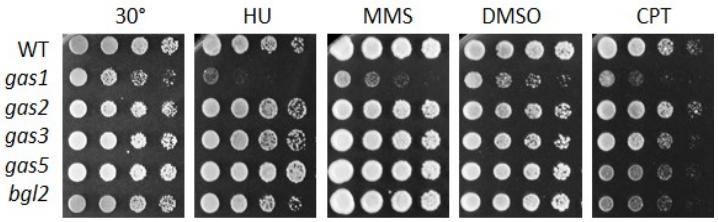

Figure S3 Genotoxin sensitivity is not a common feature of the *GAS* family or cell wall disruption. Wild type (LPY5), *gas1* (LPY10129), *gas2* (LPY10047), *gas3* (LPY10051), *gas5* (LPY11544) and *bgl2* (LPY13102) were plated on SC or SC with HU, MMS or CPT, with DMSO as a control, and incubated at 30°. Among the five-membered *GAS* family, *GAS2*, like *GAS4* (not shown) is expressed meiotically, whereas *GAS1*, *GAS3*, and *GAS5* are vegetatively expressed (Ragni *et al.* 2007). *BGL2* encodes a cell wall endo-β-1,3-glucanase (Mrsa *et al.* 1993).

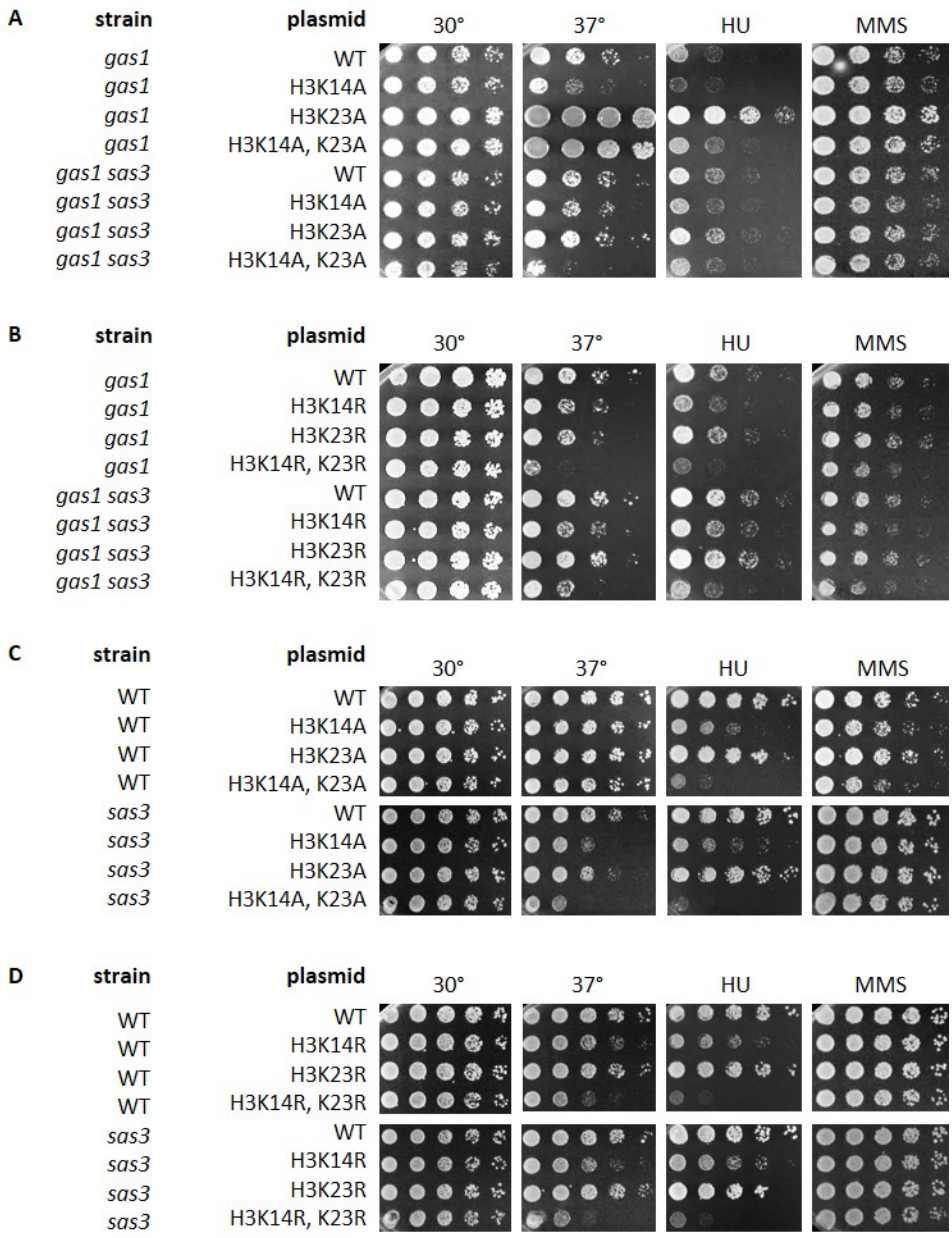

Figure S4 H3K23A mutants suppress *gas1* temperature and DNA damage sensitivity phenotypes. (A) H3K23A mutant in *gas1* rescues temperature, HU and MMS sensitivity. This suppression is decreased in the absence of *SAS3* as well as in the double mutant H3K14A, K23A. (B) Mutation of the same single residues to arginine does not alter phenotypes of either *gas1* or *gas1 sas3* yet, as in A, the double mutant exacerbates the phenotypes. (C/D) Wild type and *sas3* controls analyzed as in A and B. Although phenotypes are similar to wild type, *sas3* decreased growth at elevated temperature. For these experiments *gas1* (LPY18343), *gas1 sas3* (LPY19878), wild type (LPY12242) and *sas3* (LPY16432) were freshly transformed with indicated histone mutants and struck out on 5-FOA to select against the covering wild type plasmid (pJH33; Ahn *et al.* 2005). Transformations were performed with plasmids containing wild type H3-H4 (*HHT2-HHF2*; pLP1775), H3K14A (pLP1777), H3K23A (pLP3086), H3K14A, K23A (pLP3078), H3K14R (pLP3018), H3K23R (pLP3050) and H3K14R, K23R (pLP3064). Mutants were generated with site-directed mutagenesis with oligonucleotides listed in Table S3.

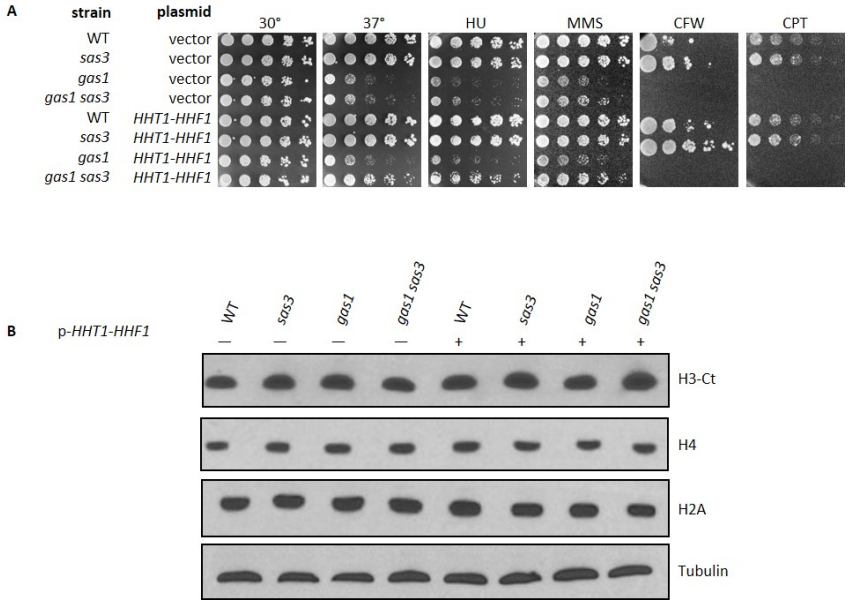

Figure S5 Suppression of *gas1* phenotypes by deletion of *SAS3* is at least partially dependent on the presence of *HHT1-HHF1.* (A) Diminished suppression by deletion of *SAS3* is observed in the histone mutant background deleted for *HHT1-HHF1*. Suppression is restored when the *HHT1-HHF1* locus is provided on a CEN plasmid in the *gas1 sas3* double mutant. (B) However, this is not due to global changes in histone levels. Genotoxin and growth conditions are the same as in Figure S4. Strains are as in Figure S4, except those carrying the p-*HHT1-HHF1* (pLP3145), which also have *HHT2-HHF2* (pLP1775). Strains plated in (A) were subsequently used for analysis in (B). The immunoblot was probed with anti-H3-Ct (1:10000; Millipore), anti-H4 (1:10000; Millipore), anti-H2A (1:5000; Abcam) and anti-tubulin (1:10000; Bond *et al.* 1986) as a loading control.

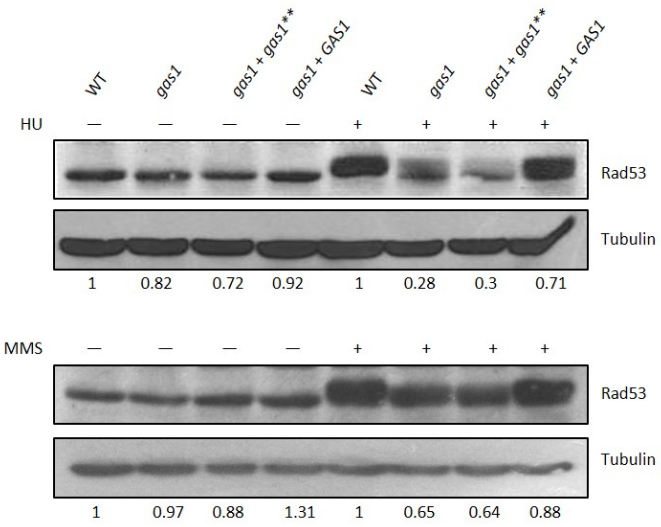

Figure S6 Reduction of Rad53 protein levels and phosphorylation isoforms is dependent on the β-1,3-glucanosyltransferase activity of Gas1. Wild type (LPY5), *gas1* (LPY10129), *gas1* + p-*gas1*** (LPY12251) and *gas1* + p-*GAS1* (LPY122326) were treated with HU or MMS. Whole cell lysates were separated on 8% SDS-PAGE and probed with anti-Rad53 followed by anti-tubulin as loading control, as done for Figure 5.

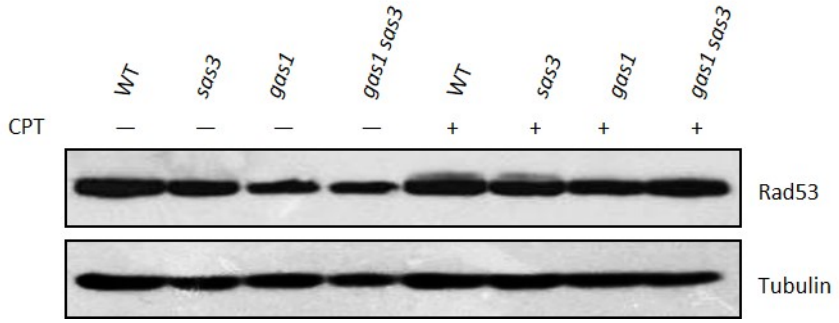

Figure S7 Rad53 is only minimally, if at all, phosphorylated following exposure to CPT as previously reported (Redon *et al.* 2003). Strains and treatment are the same as in Figure 5.

Table S1 Yeast strains used in this study

Strain	Genotype	Source
LPY5 (W303-1a)	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein
LPY1597	W303 *MATa sas2Δ::TRP1*	
LPY2444	*MATα his3Δ200 leu2Δ1 ura3-52* with rDNA Ty mURA insert	J.S. Smith
LPY2447	*MATα his3Δ200 leu2Δ1 ura3-52* with rDNA Ty mURA insert *sir2Δ2::HIS3*	J.S. Smith
LPY4196	LPY5 + pLP60	
LPY4924	W303 *MATa hmr::TRP1* TELVR::*URA3*	
LPY5035	W303 *MATa sir2Δ::HIS3 hmr::TRP1* TELVR::*URA3*	
LPY5526	W303 *MATa yng1Δ::HIS3 rDNA::ADE2-CAN1 TELVR::URA3*	
LPY6285	W303 *MATa rDNA::ADE2-CAN1 TELVR::URA3*	K. Runge
LPY6439	W303 *MATa ada2Δ::kanMX*	R. Rothstein
LPY8242	W303 *MATa gcn5Δ::HIS3*	
LPY8256	W303 *MATa sas3Δ::HIS3*	
LPY9820	W303 *MATa gas1Δ::kanMX rDNA::ADE2-CAN1 TELVR::URA3*	
LPY10047	W303 *MATa rDNA::ADE2-CAN1 hmr::TRP1 gas2Δ::kanMX*	
LPY10051	W303 *MATa rDNA::ADE2-CAN1 hmr::TRP1 gas3Δ::kanMX*	
LPY10074	*MATα his3Δ200 leu2Δ1 ura3-52* with rDNA Ty mURA insert *gas1Δ::kanMX*	
LPY10129	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 gas1Δ::kanMX*	
LPY10266	W303 *MATα rDNA::CAN:ADE2 +* pLP1823	
LPY10267	W303 *MATα orc2-1 rDNA::CAN:ADE2 +* pLP1823	
LPY10270	W303 *MATα gas1Δ::kanMX orc2-1 rDNA::CAN:ADE2 +* pLP1823	
LPY10271	W303 *MATα gas1Δ::kanMX orc2-1 rDNA::CAN:ADE2 +* pLP1823	
LPY11544	W303 *MATa gas3Δ::kanMX rDNA::CAN:ADE2 hmr::TRP1*	
LPY12232	W303 *MATa hht1-hhf1Δ::kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH* + pJH33	M.M. Smith
LPY12247	LPY10129 *+* pLP135	
LPY12251	LPY10129 *+* pLP2114	
LPY12264	W303 *MATa rDNA::CAN:ADE2 hmr::TRP1 gcn5Δ::NatMX*	
LPY12326	LPY10129 + pLP1951	
LPY13102	W303 *MATa rDNA::CAN:ADE2 hmr::TRP1 bgl2Δ::kanMX*	
LPY14355	W303 *MATa rpd3Δ::kanMX +* pLP60	
LPY15695	W303 *MATa gas1Δ::kanMX rpd3Δ::kanMX +* pLP60 + pLP1823	
LPY16039	W303 *MATa sas3Δ::HIS3*	
LPY16104	W303 *MATa swr1Δ::kanMX*	
LPY16432	W303 *MATa hht1-hhf1Δ::kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH sas3Δ::HIS3* + pJH33	
LPY16444	W303 *MATa sas3Δ::HIS3 hmr::TRP1* TELVR::*URA3*	
LPY16736	W303 *MATa gas1Δ::kanMX gcn5Δ::HIS3 +* pLP1640	
LPY16798	W303 *MATa gas1Δ::kanMX gcn5Δ::HIS3* + pLP 1640 + pLP 135	
LPY16800	W303 *MATa gas1Δ::kanMX gcn5Δ::HIS3* + pLP 1640 + pLP 1950	

LPY16801	W303 *MATa gas1Δ::kanMX gcn5Δ::HIS3* + pLP 1640 + pLP 2114	
LPY16914	W303 *MATa spt20Δ::HIS3*	D. Stillman
LPY16997	W303 *MATa gas1Δ::kanMX yng1Δ::HIS3 rDNA::ADE2-CAN1 TELVR::URA3*	
LPY17161	*W303 MATa swr1Δ::kanMX gas1Δ::kanMX* TELVR*::URA3*	
LPY17370	W303 *MATa ahc1Δ::kanMX*	
LPY17685	*MATa his3Δ200 leu2Δ1 ura3-52* with rDNA Ty mURA insert *gas1Δ::kanMX sas3Δ::HIS3*	
LPY18050	LPY5 + pLP 135	
LPY18081	LPY10129 + pLP 135	
LPY18206	W303 *MATa rtg2Δ::kanMX*	
LPY18343	W303 *MATa hht1-hhf1Δ::kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH gas1Δ::kanMX* + pJH33	
LPY18372	W303 *MATa gas1Δ::kanMX rtg2Δ::kanMX*	
LPY18518	W303 *MATa ahc2Δ::kanMX*	
LPY19101	W303 *MATa gas1Δ::kanMX gcn5Δ::HIS3 sas3Δ::HIS3* + pLP 1640	
LPY19200	LPY10129 *+* plp60	
LPY19272	W303 *MATa gas1Δ::kanMX ada2Δ::kanMX*	
LPY19414	W303 *MATa gas1Δ::kanMX ahc2Δ::kanMX*	
LPY19467	W303 *MATa gas1Δ::kanMX ahc1Δ::kanMX*	
LPY19630	W303 *MATa gas1Δ::kanMX spt20Δ::HIS3*	
LPY19670	W303 *MATa gas1Δ::kanMX sas2Δ::HIS3*	
LPY19731	W303 *MATa sas3Δ::HIS3 hmr::TRP1* TELVR::*URA3*	
LPY19771	W303 *MATa gas1Δ::kanMX sgf73Δ::URA3*	
LPY19773	W303 *MATa gas1Δ::kanMX hmr::TRP1* TELVR::*URA3*	
LPY19816	W303 *MATa sgf73Δ::URA3*	
LPY19823	W303 *MATa gas1Δ::kanMX sas3Δ::HIS3*	
LPY19878	W303 *MATa hht1-hhf1Δ::kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH sas3Δ::HIS3 gas1Δ::kanMX* + pJH33	

All strains were constructed during the course of this study or are part of our standard lab collection unless otherwise indicated.
All strains are W303 unless otherwise indicated.

Table S2 Plasmids used in this study

Plasmid	Description	Alias	Source
pJH33	*HTA1 HTB1 HHF2 HHT2 URA3* CEN		Ahn *et al.* 2005
pLP60	vector HIS3 CEN	pRS313	
pLP135	vector *LEU2* 2μ	YEP351	
pLP1640	*GCN5 URA3* CEN		S. Lo
pLP1775	*HHT2-HHF2 TRP1* CEN		S.L. Berger
pLP1777	*hht2-K14A HHF2 TRP1* CEN		S. Lo
pLP1823	vector *TRP1* 2μ	pRS424	C. Nislow
pLP1950	*gcn5-KQL LEU2* 2μ		
pLP1951	*GAS1 LEU2* 2μ		
pLP2114	*gas1-E161Q, E262Q LEU2* 2μ		
pLP3018	*hht2-K14R HHF2 TRP1* CEN		
pLP3050	*hht2-K23R HHF2 TRP1* CEN		
pLP3064	*hht2-K14R, K23R HHF2 TRP1* CEN		
pLP3078	*hht2-K14A, K23A HHF2 TRP1* CEN		
pLP3086	*hht2-K23A HHF2 TRP1* CEN		
pLP3145	*HHT1-HHF1 URA3* CEN		

All plasmids were constructed during the course of this study or are part of our standard lab collection unless otherwise indicated.

Table S3 Oligonucleotides used in this study

Oligo #	Sequence	Name
oLP1965	CCA CTG GTG GTA GAG CCC CAA G	H3K14R sense
oLP1966	CTT GGG GCT CTA CCA CCA GTG G	H3K14R antisense
oLP1969	CAA TTA GCC TCC AGG GCT GCC AG	H3K23R sense
oLP1970	CTG GCA GCC CTG GAG GCT AAT TG	H3K23R antisense
oLP1985	CAA TTA GCC TCC GCG GCT GCC AG	H3K23A sense
oLP1986	CTG GCA GCC GCG GAG GCT AAT TG	H3K23A antisense

Supplemental Literature Cited

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