Inventory of Supplemental Information

Supplementary Data

Table S1: A table showing the genetic interactions that led to the study of Gcn5 in the maintenance of genome integrity. The genetic interactions support the studies in Figure 1.

Figure S1: A complete representation of the genetic data presented in Figure 1A.

Figure S2: A complete representation of the genetic data and interactions presented in Figure 2A-C.

Figure S3: A complete representation of the genetic data and interactions presented in Figure 5C-F.

Figure S4: Control data and experiments for the ChIP assays shown in Figure 6. Figure S4B is a plasmid supercoiling assay that supports data and conclusions presented in Figure 6.

Supplementary Experimental Procedures

The Supplementary Experimental Procedures elaborate on the shorter explanations given in the main text (Experimental Procedures) and provide further detail for others to replicate the studies.

Supplemental Yeast Strain Table: A complete list of the yeast strains used in the study.

Supplementary References

This section includes references used in the supplemental information that were not previously listed in association with the main text.

Supplemental Information

A role for Gcn5 in replication-coupled nucleosome assembly

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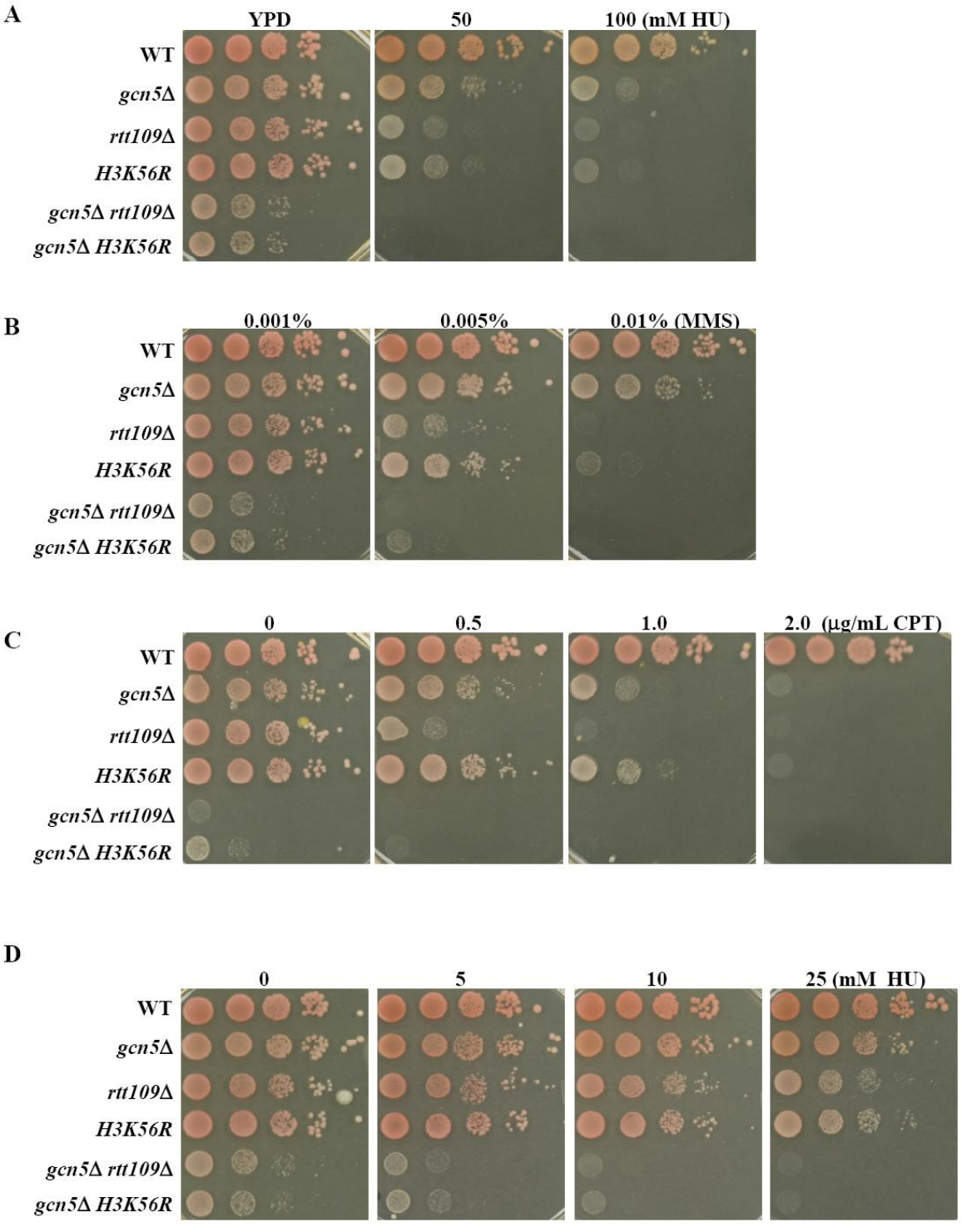
Supplemental Data

Gene Name	Synthetic slow growth with *asf1*Δ	Sensitivity towards DNA damaging agents with *asf1*Δ
ELP3	++	++
GCN5	++++	++
HAT1	-	-
HPA2	-	-
HPA3	-	-
SAS2	-	-
SAS3	-	-
SPT21	-	-

Table S1, related to Figure 1. *ASF1* genetically interacts with *GCN5* and *ELP3*. The *asf1*Δ, single deletion mutant in each gene listed above, and double mutant that contains *asf1*Δ and each of the deletion mutants were assayed for cell growth and sensitivity towards the DNA damaging agents methyl methane sulfonate (MMS), camptothecin (CPT) and hydroxyurea (HU). ++ represents synthetic effect on growth on YDP or media containing at least one DNA damaging agent. ++++ indicates that *gcn5*Δ *asf1*Δ double mutant cells grew much more slowly than *elp3*Δ *asf1*Δ double mutant cells.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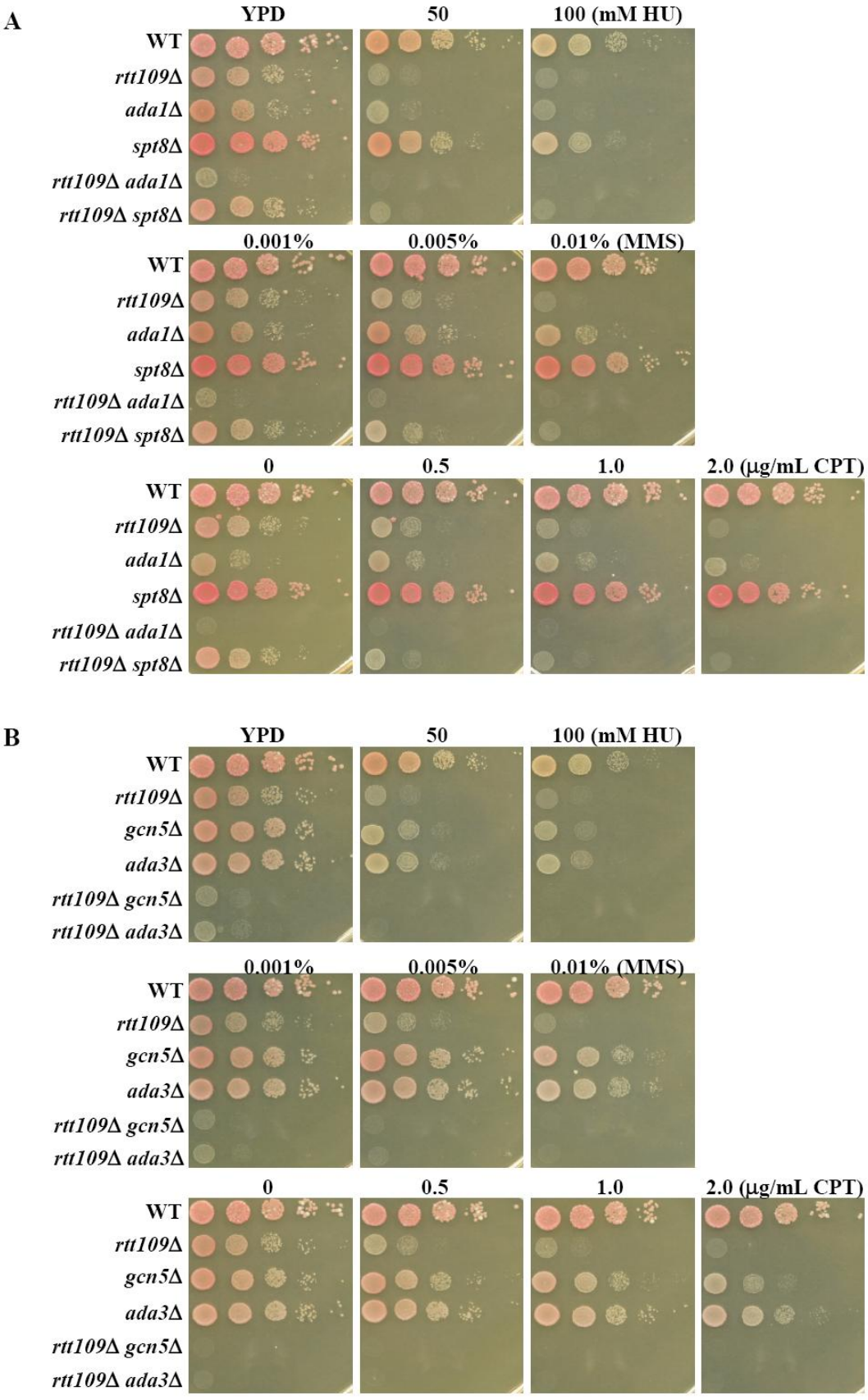
YPD
50
100 (mM HU)
WT
gcn5Δ
rtt109Δ
H3K56R
gcn5Δ rtt109Δ
gcn5Δ H3K56R
B
0.001%
0.005%
0.01% (MMS)
WT
gcn5Δ
rtt109Δ
H3K56R
gcn5Δ rtt109Δ
gcn5Δ H3K56R
C
0
0.5
1.0
2.0 (μg/mL CPT)
WT
gcn5Δ
rtt109Δ
H3K56R
gcn5Δ rtt109Δ
gcn5Δ H3K56R
D
0
5
10
25 (mM HU)
WT
gcn5Δ
rtt109Δ
H3K56R
gcn5Δ rtt109Δ
gcn5Δ H3K56R

Figure S1

Figure S1, related to Figure 1. Gcn5 and H3 lysine 56 acetylation function in parallel in growth and response to DNA damaging agents. (A) The *gcn5*Δ mutant exhibits a synthetic phenotype with the *rtt109*Δ and *H3K56R* mutants. Ten fold serial dilutions of yeast cells with relevant genotype indicated at the left were spotted onto normal growth media, YPD, or YPD media containing the indicated concentration of the DNA damaging agents HU (B) MMS and (C) CPT. (D) DNA damage sensitivity at lower concentrations of HU. This figure is a complete representation of data presented in Figure 1A.


A
YPD
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100 (mM HU)
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rtt109Δ
ada1Δ
spt8Δ
rtt109Δ ada1Δ
rtt109Δ spt8Δ
0.001%
0.005%
0.01% (MMS)
WT
rtt109Δ
ada1Δ
spt8Δ
rtt109Δ ada1Δ
rtt109Δ spt8Δ
0
0.5
1.0
2.0 (μg/mL CPT)
WT
rtt109Δ
ada1Δ
spt8Δ
rtt109Δ ada1Δ
rtt109Δ spt8Δ
B
YPD
50
100 (mM HU)
WT
rtt109Δ
gcn5Δ
ada3Δ
rtt109Δ gcn5Δ
rtt109Δ ada3Δ
0.001%
0.005%
0.01% (MMS)
WT
rtt109Δ
gcn5Δ
ada3Δ
rtt109Δ gcn5Δ
rtt109Δ ada3Δ
0
0.5
1.0
2.0 (μg/mL CPT)
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gcn5Δ
ada3Δ
rtt109Δ gcn5Δ
rtt109Δ ada3Δ

Figure S2

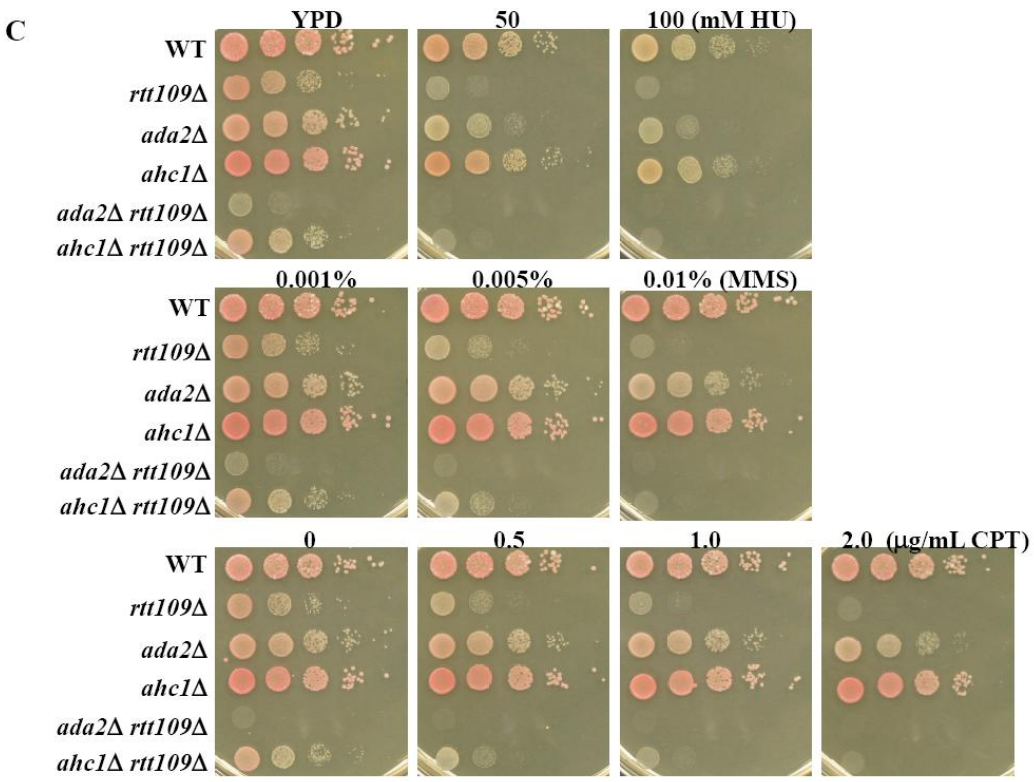


Figure S2 continued

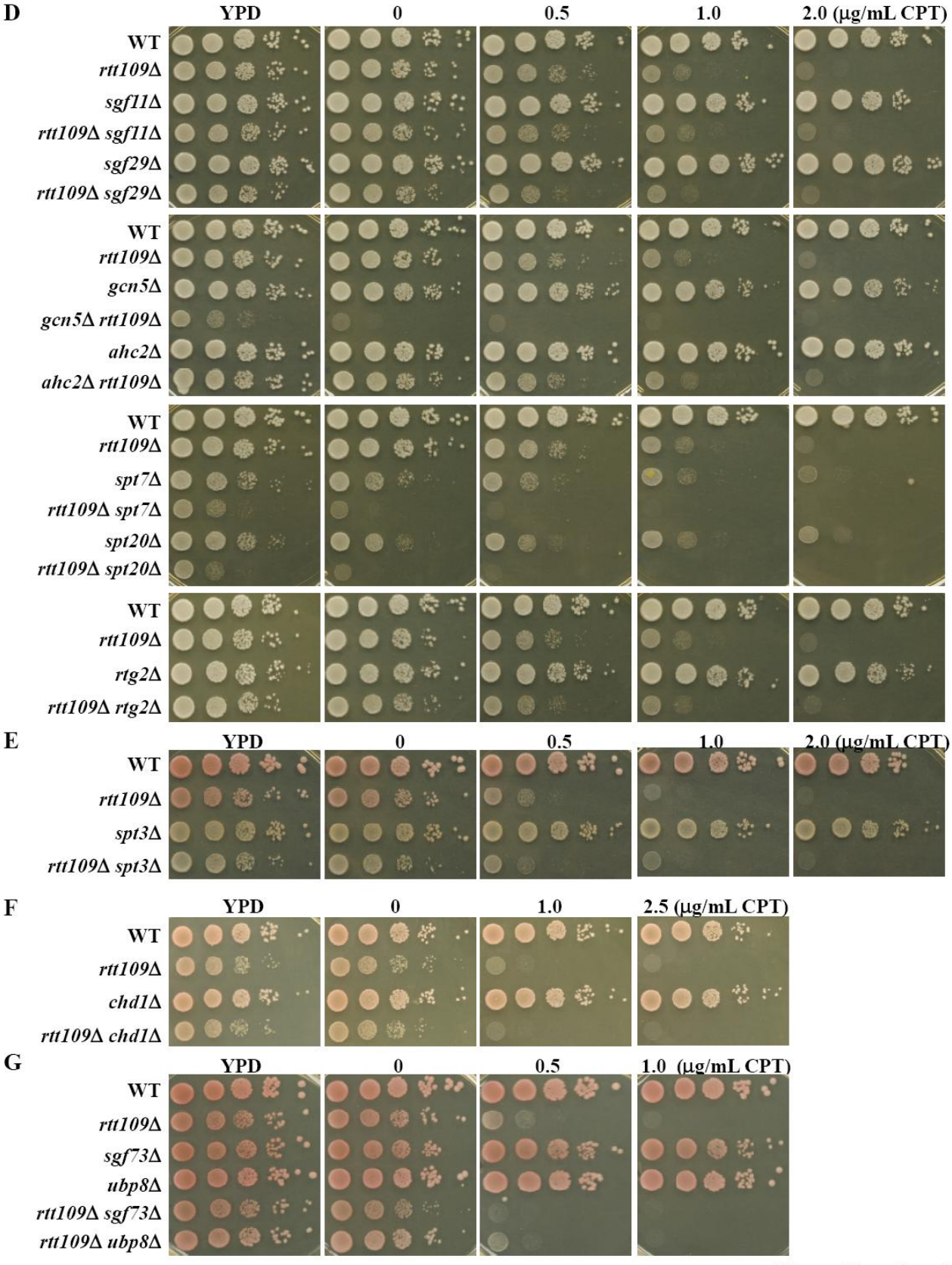
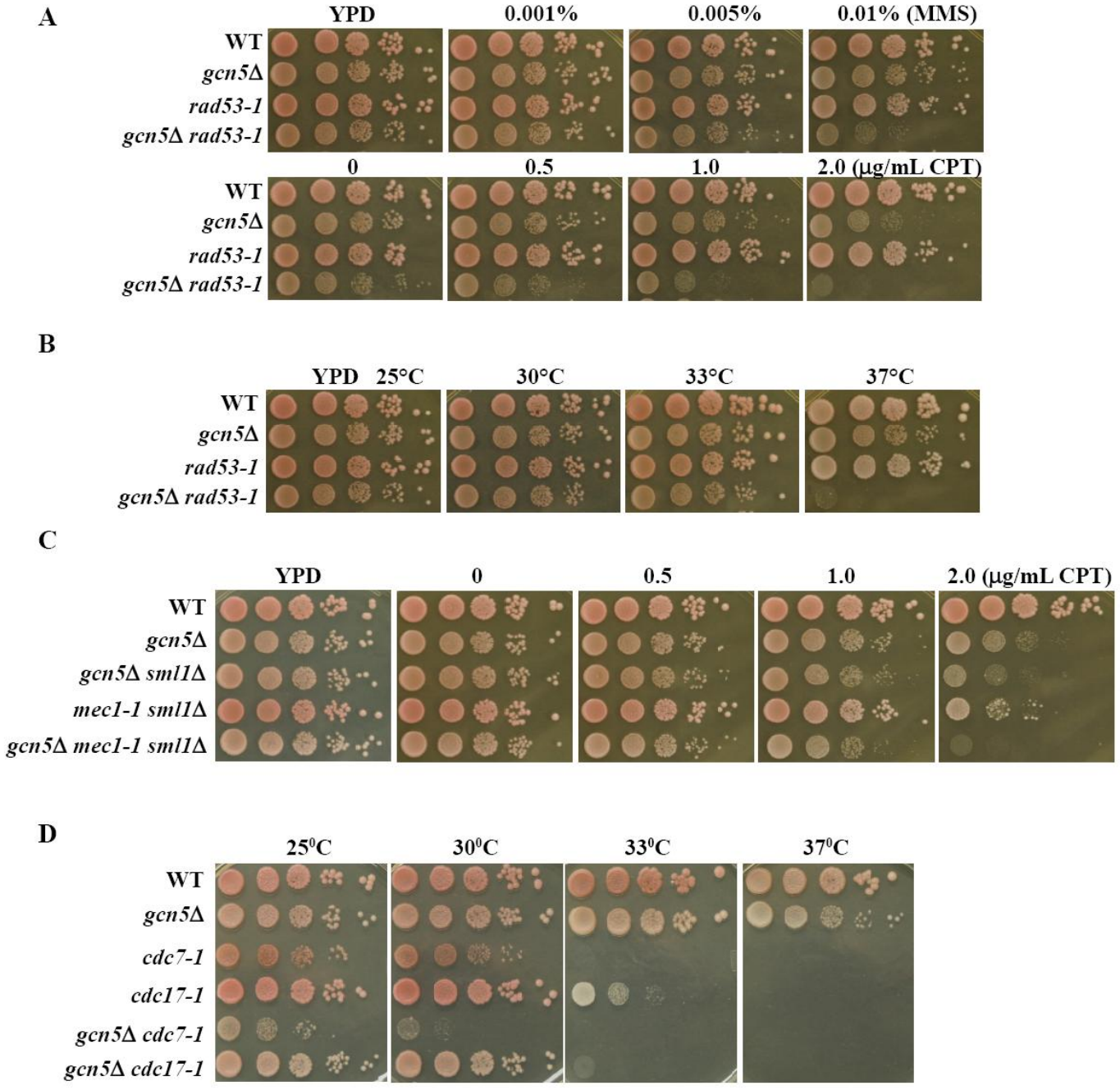


Figure S2 continued

Figure S2, related to Figure 3. Mutations in *ADA1, ADA2, ADA3, SPT7,* and *SPT20,* but not other Gcn5-containing complex components in *rtt109*Δ cells phenocopy *gcn5*Δ *rtt109*Δ mutant cells (A) Mutations in *ADA1*, but not *SPT8*, in *rtt109*Δ mutant cells phenocopy loss of *GCN5* in the absence of *RTT109*. (B) Mutations in *ADA3* in *rtt109*Δ mutant cells phenocopy the *gcn5*Δ *rtt109*Δ mutant. (C) Mutations in *ADA2*, but not *AHC1*, in *rtt109*Δ mutant cells phenocopy loss of *GCN5* in the absence of *RTT109.* (A-C) Ten fold serial dilutions of yeast cells (in W303 background) with indicated genotype were plated on YPD or YPD media containing the indicated concentrations of HU, MMS and CPT. (D) Deletion of *SPT7* and *SPT20* in *rtt109*Δ mutant cells phenocopied that of *GCN5* in the absence of *RTT109.* Ten fold serial dilutions of yeast cells of the BY4741 background were plated on YPD or YPD containing CPT. (E-G) Deletion of *SPT3, CHD1, SGF73*, or *UBP8* with *RTT109* shows no significant phenotype over the corresponding single mutant. Cells (in W303 background) were prepared as in A (HU and MMS data not shown).


A
YPD
0.001%
0.005%
0.01% (MMS)
WT
gcn5Δ
rad53-1
gcn5Δ rad53-1
0
0.5
1.0
2.0 (μg/mL CPT)
WT
gcn5Δ
rad53-1
gcn5Δ rad53-1
B
YPD 25°C
30°C
33°C
37°C
WT
gcn5Δ
rad53-1
gcn5Δ rad53-1
C
YPD
0
0.5
1.0
2.0 (μg/mL CPT)
WT
gcn5Δ
gcn5Δ sml1Δ
mec1-1 sml1Δ
gcn5Δ mec1-1 sml1Δ
D
25⁰C
30⁰C
33⁰C
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gcn5Δ cdc17-1

Figure S3

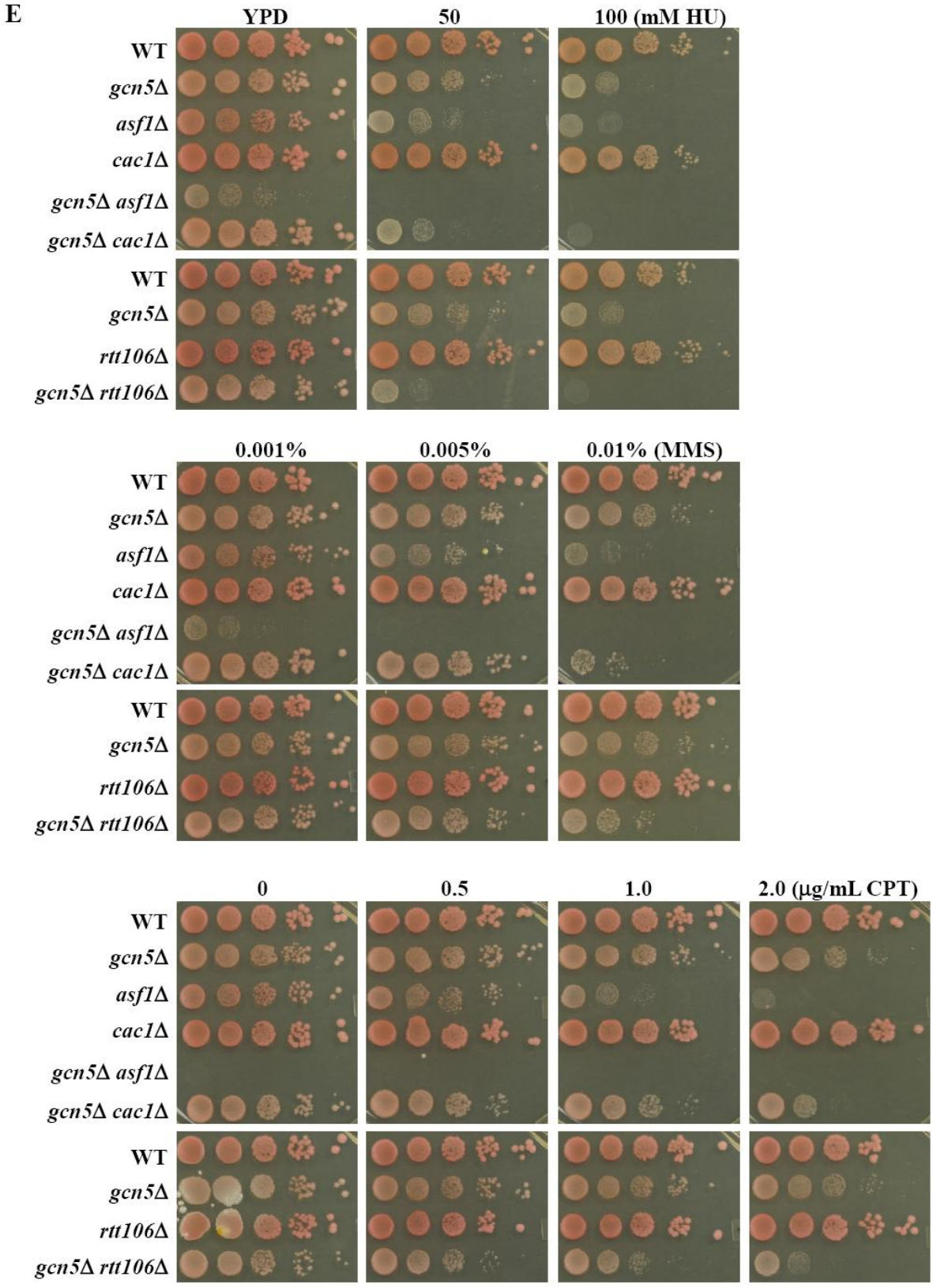
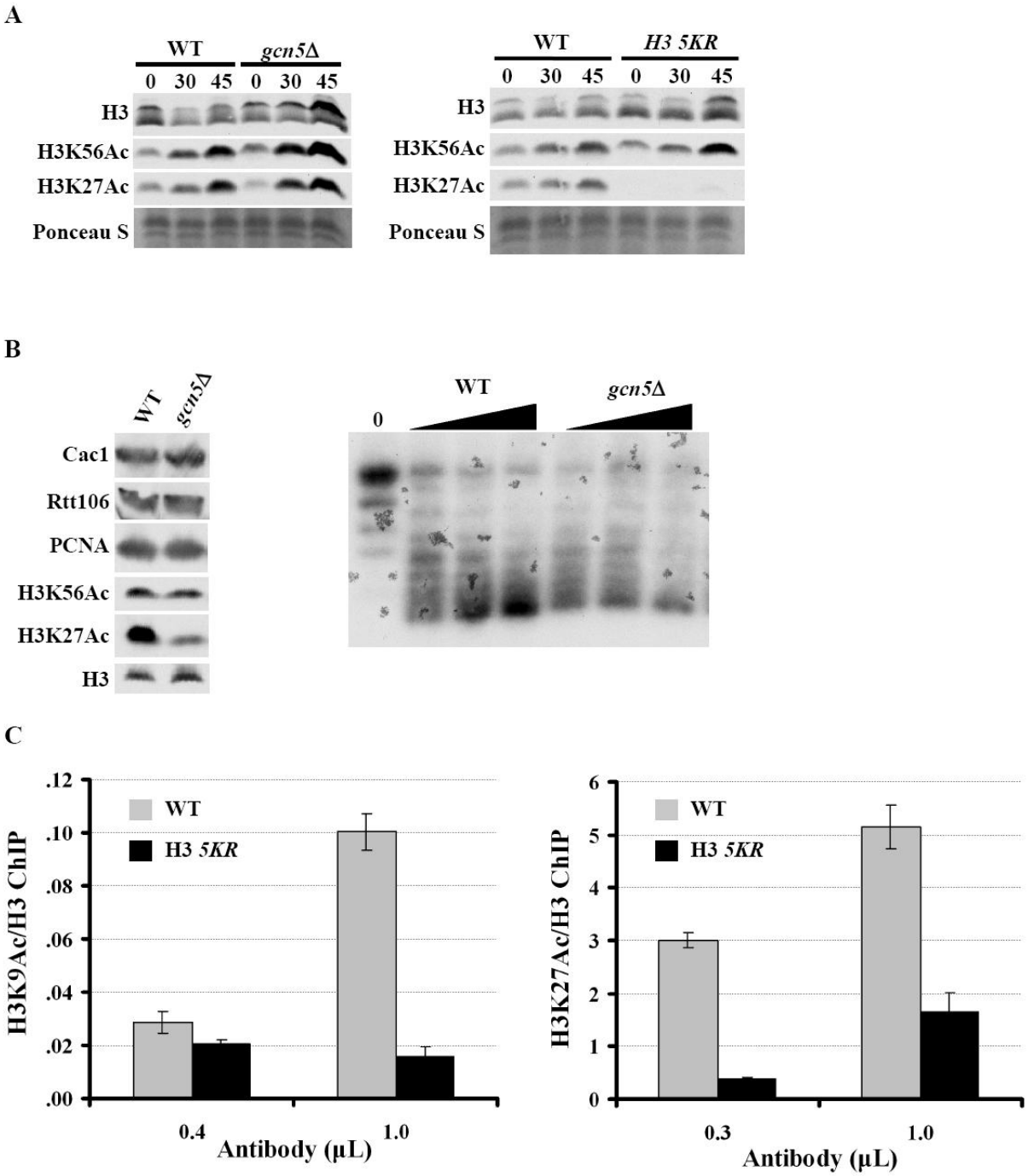


Figure S3 continued

Figure S3, related to Figure 5. Genetic interactions with checkpoint kinases, DNA replication proteins, and histone chaperones suggest that Gcn5 has a role in replication-coupled nucleosome assembly. (A-B) *GCN5* interacts genetically with the checkpoint kinases *RAD53* and *MEC1*. *GCN5* shows a synthetic interaction with *rad53-1* in DNA damage sensitivity (A) and temperature sensitivity (B). (C) *GCN5* shows a synthetic interaction with *mec1-1* in response to the DNA damaging agent CPT. Figure S3A-C is a complete representation of Figure 5C and 5D. (D) *GCN5* shows a synthetic interaction with the temperature sensitive mutants, *cdc7-1* and *cdc17-1*. (E) *GCN5* genetically interacts with nucleosome assembly factors. The *gcn5*Δ mutation exhibited slow growth and increased DNA damage sensitivity with the nucleosome assembly factors *ASF1*, *CAC1*, and *RTT106*. Yeast cells were spotted onto YPD and YPD containing the indicated cytotoxic agent. Plates were incubated at 30˚C unless otherwise indicated. Figure S3E is a complete representation of Figure 5E.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WT
gcn5Δ
0 30 45 0 30 45
H3
H3K56Ac
H3K27Ac
Ponceau S
WT
H3 5KR
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H3K56Ac
H3K27Ac
Ponceau S
B
WT
gcn5Δ
Cac1
Rtt106
PCNA
H3K56Ac
H3K27Ac
H3
0
WT
gcn5Δ
C
H3K9Ac/H3 ChIP
.12
.10
.08
.06
.04
.02
.00
WT
H3 5KR
0.4
1.0
Antibody (µL)
H3K27Ac/H3 ChIP
6
5
4
3
2
1
0
WT
H3 5KR
0.3
1.0
Antibody (µL)

Figure S4

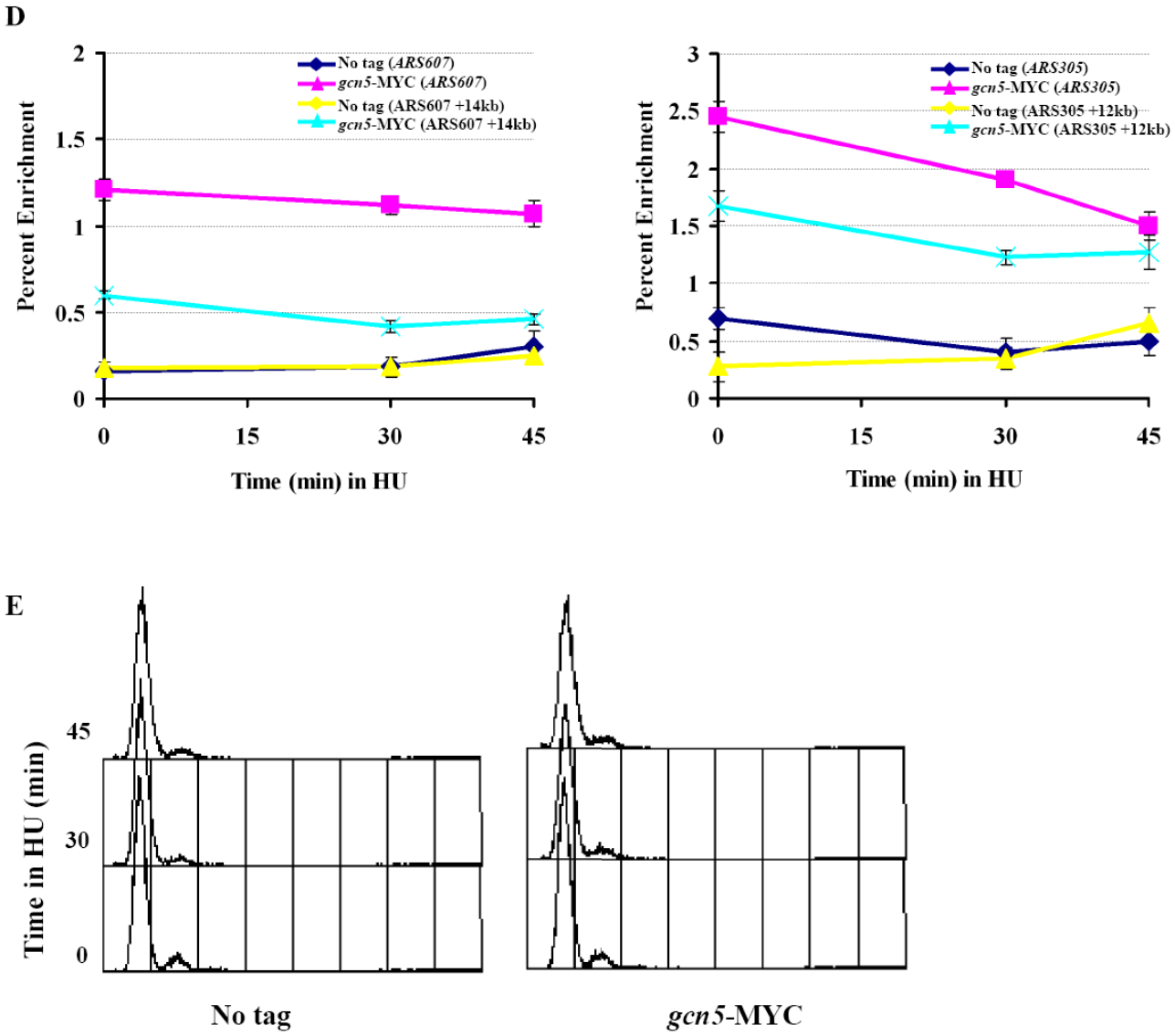

Figure S4 continued

Figure S4, related to Figure 6. Mutant *gcn5*Δ cells show defects in nucleosome assembly. (A) (Left) Levels of H3K56Ac in *gcn5*Δ and H3 *5KR* mutant cells are similar to that of wild-type cells. Western blot was used to analyze H3, H3K56Ac, and H3K27Ac in wild type and *gcn5*Δ mutant cells when these cells were released into media containing 200 mM HU and collected at

different times following release from G1. (Right) Similar experiments were performed to analyze H3, H3K56Ac, H3K27Ac in wild-type and H3 *5KR* cells. Note that the same membrane was used for probing H3 and modified forms of H3. (B) Cell extracts prepared from *gcn5*Δ cells exhibit defects in nucleosome assembly via an *in vitro* plasmid supercoiling assay. (Left) Western blot analysis of cell extracts prepared from wild-type (WT) and *gcn5*Δ mutant cells for the proteins or H3 modifications indicated at the left. (Right) Compared to wild-type cells, cell extracts prepared from *gcn5*Δ mutant cells show defects in the ability to assemble plasmid DNA into nucleosomes. Briefly, ^{32}P-labeled plasmid DNA was relaxed by Topoisomerase I and incubated with increasing amounts of cell extracts as indicated. After purification, DNA was resolved on an agarose gel and detected by autoradiography. (C) H3 antibodies against H3K9Ac and H3K27Ac are specific for ChIP assay. (Left) The antibody against H3K9Ac was specific for ChIP. Cell extracts were prepared from wild-type cells and cells with five lysine residues of the N-terminus of H3 mutated (H3 *5KR* : H3K9R, K14R, K18R, K23R, and K27R) and used for ChIP assays using increasing amounts of H3 and H3K9Ac antibody. Precipitated DNA was analyzed using real-time PCR as described in Experimental Procedures. (Right) The H3K27Ac antibody is specific for ChIP assay. The experiments were performed as just described except with an antibody against H3K27Ac. (D-E) Gcn5 is present at replication origins in G1 and early S phase. (D) Gcn5 was present at *ARS305* and *ARS607* at G1 and early S phase. Gcn5, tagged with the Myc epitope at its C-terminus (*gcn5*-MYC) was precipitated using antibody against the MYC epitope, and the precipitated DNA was analyzed via real-time PCR using primers amplifying *ARS607* and a fragment 14 kb downstream of the *ARS607* (ARS607 + 14kb) (Left), as well as *ARS305* and a fragment downstream of this origin, ARS305 + 12kb (Right). As a

negative control for the ChIP, the same ChIP assay procedures were also performed in a yeast strain without the MYC epitope (No tag). (E) FACS analysis of the cells analyzed in A and B.

Supplemental Experimental Procedures

Supplemental Yeast Strain Table: A list of the yeast strains used in this study.

Antibodies

Antibodies against Ada2, Ada3, Spt8, and Spt7 were purchased from Santa-Cruz. Antibodies against H3, H3K56Ac and H3K27Ac were produced in the lab. The antibody against H3K9Ac was a gift from Dr. Alain Verreault.

CPT cytotoxicity assay

Cells were grown to OD_{600} of 0.4 and treated with the indicated concentration of CPT for two hours. Cells were then diluted, plated onto YPD plates and incubated at 30 ˚C for 2-4 days before counting colonies. The percentage of cells that survived was calculated for each strain based on untreated cells exhibiting 100% survival.

Whole cell extraction and Western blot

To prepare whole cell extracts, cells grown to 0.8 OD_{600} were harvested and re-suspended with 1X TBS buffer containing 1mM PMSF, 1mM benzamidine and 1mM DTT. After beads-beating, cells were mixed with 2X SDS buffer and boiled for 3 minutes. To analyze histones by Western

blot, proteins prepared by acid extracts or whole cell extraction were resolved on 15% SDS PAGE gels, transferred to a nitrocellulose membrane and detected by Western blot using indicated antibodies.

Histone acetyltransferase (HAT) assays

Briefly, samples were incubated at 30 °C for 1-2 hours in 15 µl reactions containing 50 mM Tris-HCl, pH 8.0, 5% (w/v) glycerol, 0.1 mM EDTA, 1 mM DTT, 5 mM PMSF, 6 pmol acetyl-CoA (4.3mCi/mmol, Amersham Life Science), 1 µg of recombinant *Drosophila* H3/H4 tetramers, Asf1-H3/H4, core histones, or mono-nucleosomes purified from 293 cells, and the indicated histone acetyltransferase (or complex). Negative samples contain no HAT. After incubation, 7.5 µl was placed on P81 filter paper (Upstate Biotechnology Co.). The paper was washed 5 times, 5 minutes each, with 50 mM $NaHCO_3$, pH 9.0 and counts were recorded using a liquid scintillation counter. After HAT assays, the remaining sample was resolved by 15% SDS-PAGE, stained with Coomassie and dried with a gel drier. Acetylated proteins were detected using autoradiography. Samples used to detect site-specific acetylation were treated with recombinant Rtt109-Vps75 complex as the HAT and REGα as a negative control. Following HAT assays, the samples were resolved by 15% SDS-PAGE and detected using Western blot with specific antibodies.

Chromatin Immunopreciptation (ChIP) assays

To determine whether modified forms of histones were deposited onto replicating DNA, cells were first synchronized at G1 using α-factor for 3 hours and then released into fresh media containing 0.2 M hydroxyurea (HU). At different times after release into HU, cells were

collected for analysis of DNA content by FACS or for ChIP assays. A standard protocol was followed to perform ChIP assays as described (Huang et al., 2007) using antibodies against H3, H3 acetylated at lysine 9 (H3K9Ac), H3 acetylated at lysine 27 (H3K27Ac), and H3 acetylated at lysine 56 (H3K56Ac). After obtaining ChIP DNA, the ChIP DNA was quantified via real-time PCR using indicated PCR primers. ChIP signals were reported as the ratio of ChIP signal from one antibody over H3 ChIP signal. Alternatively, the percentage of immunoprecipitated DNA by antibodies to modified forms of H3 over whole cell DNA was calculated. Data normalized in these two ways agreed with each other.

Tandem Affinity Purification (TAP) to detect binding of H3 with histone chaperones

One liter of cells expressing C-terminally TAP-tagged proteins were grown to OD_{600} 2.0 and harvested. The volume of cells was estimated and an equal volume of buffer A (25 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.01% NP-40, 1 mM DTT, 1 mM PMST, 1 mM Benzamidine, 1 mM Pefabloc, and 15kU/mL DNase I) was added. The suspension was frozen into pellets using liquid nitrogen. Proteins were extracted by grinding cells using the 6870 Freezer Mill. After centrifugation, the supernatant was treated with 75μg/mL ethidium bromide for 30 minutes followed by 30 minutes of centrifugation at 14,000 rpm. Next, the supernatant was incubated with IgG-Sepharose beads (Pharmacia) for 2 hours. After washing, proteins were eluted using TEV protease and incubated with calmodulin beads for 1 hour. After washing, proteins were eluted with SDS sample buffer, resolved in SDS-PAGE and detected by Western blot using antibodies against H3, H3K56Ac, H3K27Ac or CBP. To serve as controls, proteins in soluble cell extracts (SCE) were also analyzed by Western blot.

Gcn5 complex purification

Gcn5 containing complexes were purified using two different procedures. First, to purify complexes containing Gcn5, we followed the standard TAP purification procedure described above to purify Gcn5-TAP (Gcn5-all) except that co-purified proteins were eluted using buffers containing EGTA. The proteins were then dialyzed against EGTA free buffer before being used for HAT assays. Second, to purify the Ada1-Gcn5 complex, we constructed a yeast strain to allow for purification of a complex containing both Ada1 and Gcn5 (*ada1-TAP, gcn5*Δ *Gcn5-Flag*). After collecting cells, we followed the standard procedure described to purify Ada1-TAP using IgG sepharose. Following elution of proteins from IgG beads via TEV protease, the eluted proteins were mixed with buffer A (20mM Tris, pH8, 300mM potassium acetate, 1mM EDTA, 10% glycerol), loaded onto to the M2 beads from Sigma and incubated at 4˚C overnight. After washing the M2 beads extensively, the protein complexes were eluded using 2 mg/mL Flag peptide in Buffer A. Associated proteins were analyzed via silver staining and western blot using specific antibodies. Activity was assessed using HAT assays as described above.

Cell extract preparation for plasmid supercoiling assay

Exponentially growing cells were collected and harvested. The pellet was washed in cold H_2O and then weighed. Next, the pellet was successively washed with YEB extraction buffer (100 mM Hepes-KOH, pH 7.9, 245 mM KCl, 5 mM EGTA, 1 mM EDTA, and 2.5 mM dithiothreitol) (1.3mL per gram of cells) and YEB extraction buffer containing protease inhibitors. The pellet was then re-weighed and resuspended in YEB extraction buffer containing protease inhibitors (250 μL per gram of cells). The suspension was frozen into liquid nitrogen drop by drop and stored at -80˚C. To make the cell extracts, cells were broken up using the 6870 Freezer Mill and

resuspended in 1.3 volume (by pellet mass) YEB with protease inhibitors. The lysate was then subjected to centrifugation at 30,000 rpm for 2 hours, and the supernatant was collected and dialyzed for 4 hours against YDBI buffer (20 mM Hepes-KOH, pH 7.9, 50 mM KCl, 5 mM EGTA, 0.05 mM EDTA, 2.5 mM dithiothreitol, 20% vol/vol glycerol, 0.2 mM PMSF, 0.5 μg/mL leupeptin). Following dialysis, extracts were frozen and stored at -80˚C.

In vitro Plasmid Supercoiling Assay

Template pSV011^{+} preparation and labeling, as well as the supercoiling assay, has been previously described in detail (Li et al., 2008). Briefly, the negatively supercoiled plasmid pSV011 was labeled with ^{32}P and a mixture of 1 part labeled to 4 parts unlabeled relaxed plasmid was relaxed using DNA topoisomerase I (Promega). The plasmids were then treated with increasing amounts of whole cell extracts at 30˚C for 1.5 hours. Following digestion with RNAase A and Pronase, purified DNA products were resolved on a 1.25% native agarose gel. Signal was detected via a phosphorimager or autoradiography.

Supplemental References

Huang, S., Zhou, H., Katzmann, D., Hochstrasser, M., Atanasova, E., and Zhang, Z. (2005). Rtt106p is a histone chaperone involved in heterochromatin-mediated silencing. Proceedings of the National Academy of Sciences of the United States of America *102*, 13410-13415.

Thomas, B.J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. Cell *56*, 619-630.

Weinreich, M., and Stillman, B. (1999). Cdc7p-Dbf4p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway. Embo J *18*, 5334-5346.

Zhou, H., Madden, B.J., Muddiman, D.C., and Zhang, Z. (2006). Chromatin assembly factor 1 interacts with histone h3 methylated at lysine 79 in the processes of epigenetic silencing and DNA repair. Biochemistry *45*, 2852-2861.