SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Loss of *SET1* Does Not Create Cell Cycle Abnormalities, Related to Figure 1.

(A) Serial five fold dilution assay of wild-type (WT) and mutants with the indicated genotype grown on either control plates (YPD) or plates containing the indicated amounts of benomyl. Cells were incubated at 30^0C for 2 days. (B) Whole cell extracts were prepared from strains with the indicated genotype. The amount of H3K4 dimethylation (αH3K4me2) and H3K4 trimethylation (αH3K4me3) was assessed using immunoblot analysis. Total H3 (αH3) protein levels were used as a loading control. (C) Cell cycle analysis was assessed in both wild-type (blue) and *set1Δ* (red) asynchronous cultures. Cells were subjected to DNA labeling, flow cytometry analysis, and corresponding histograms were generated. Peaks associated with cells in G1 and G2 are labeled accordingly. (D) The budding index of wild-type (WT) and *set1Δ* cells were assessed by dividing fixed cells into three categories; cells with no bud, cells with a small bud, and cells with a large bud. These categories correspond to G1, S, and G2/M, respectively. (E) Overnight cultures were diluted into fresh media and cell growth was assessed by Optic Density (O.D) over time in both wild-type (WT) and *set1* cells. (F) Equal amounts of wild-type (WT) and *set1Δ* cell cultures were taken from growth assays at the indicated time points and assessed for cell viability by counting colony forming units on normal YPD plates. (G) Yeast strains were fixed and Differential Interface Contrast (DIC) images were taken to assess overall cell shape, size, and morphology.

Figure S2: H3K4 Mutants Do Not Alter Set1 Protein Levels or Incorporation Into the COMPASS Complex, Related to Figure 3.

(A and B) Serial five fold dilution assay of wild-type (WT) and mutant yeast with the indicated genotypes. Cells were placed onto YPD plates (YPD) with or without 30μg/mL of benomyl (BENOMYL) and incubated at 30^0C for 2 days. (C) Whole cell extracts were isolated from strains with the indicated genotype. The amount of total Set1 protein was assessed using western blot analysis with an antibody against Set1. Pgk1 protein levels were used as a loading control. (D) Set1 proteins in wild-type (WT), *set1G951S,* and *H3K4R* strains similarly associate with the COMPASS component Bre2. Immunoprecipitation (IP) assays were used to pull down Bre2 and the associated levels of Set1 were assessed. Bre2-TAP (α-Protein A) and Pgk1 (α-Pgk1) were used as loading controls. (E) Immunofluorescence images of wild-type (WT) and mutant yeast with the indicated genotype. Images of mitotic cells were taken on a confocal microscope. Tubulin (green) and DAPI (blue) were used to identify cells undergoing mitosis from an asynchronous culture. Mitotic cells are outlined in white. (F) RT-PCR was performed with isolated RNA from the indicated strains with primers specific to the gene indicated. RNA levels were compared to the *ACT1* gene transcript, which was used as a control. (G) Heat maps of H3K4me2 and H3K4me3 enrichment at the centromeric and pericentromeric regions of the indicated chromosomes.

Figure S3: Two Histone H3 regions result in benomyl resistance when mutated, Related to Figure 5.

(A) Serial five fold dilution assay of histone H3 mutant yeast with the indicated genotypes. Benomyl resistance is observed both for mutants that disrupt methylation at

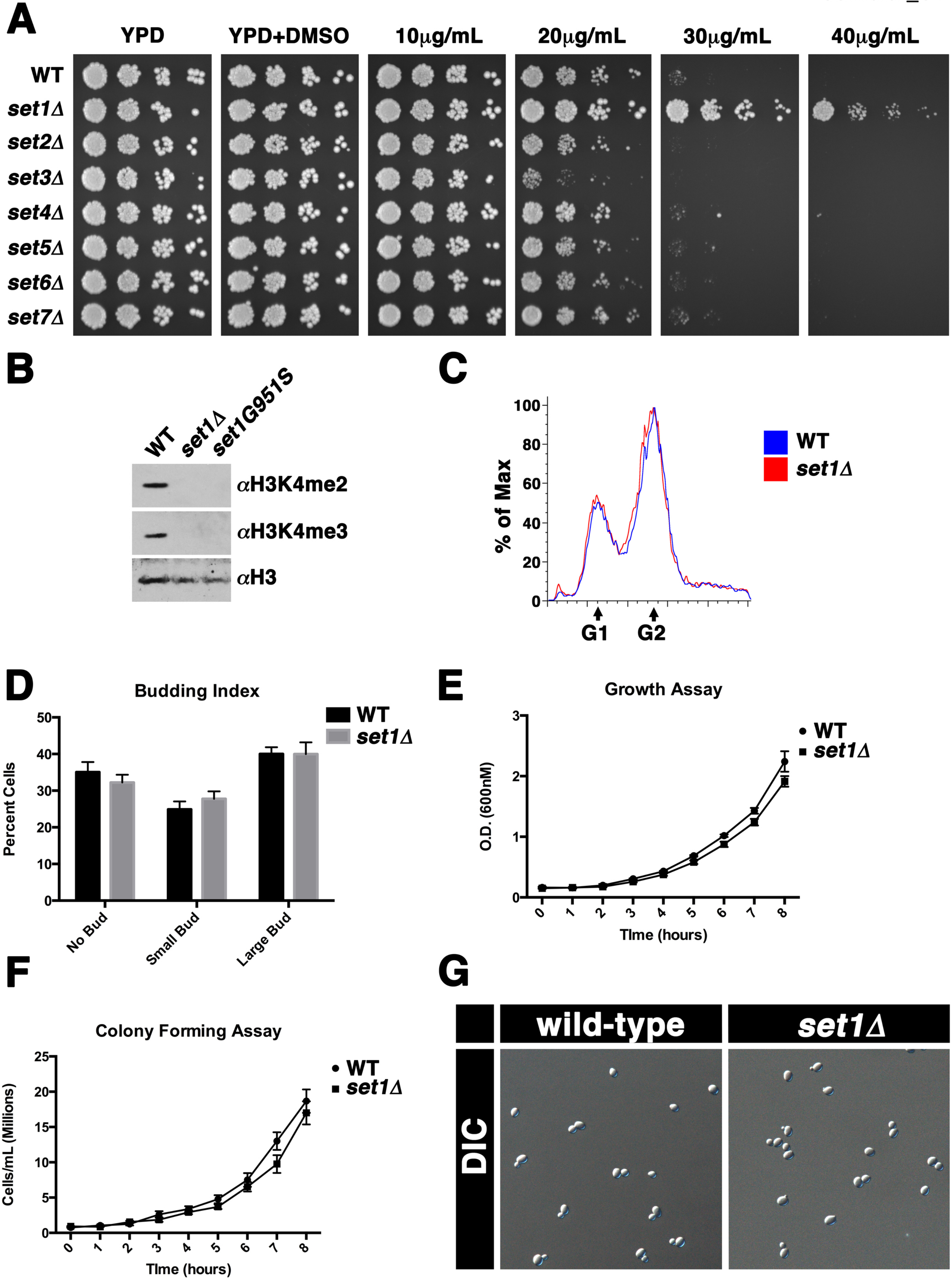
K4 (*H3R2A* (Kirmizis et al. 2007) and *H3K4A*) and mutations that disrupt a consensus Mad2 binding motif (amino acids 23-30; marked with a black bar). (B) C-terminal epitope tags in Mad2 suppress the benomyl resistance of *set1* mutants, indicating disruption of the Mad2 C-terminus affects *in vivo* function of Mad2. Cells were grown onto YPD plates (YPD) with or without 30μg/mL of benomyl (BENOMYL) and incubated at 30^0C for 2 days.

Figure S4: GST-Mad2 localizes to the nucleus, cytoplasm, and the bud neck.

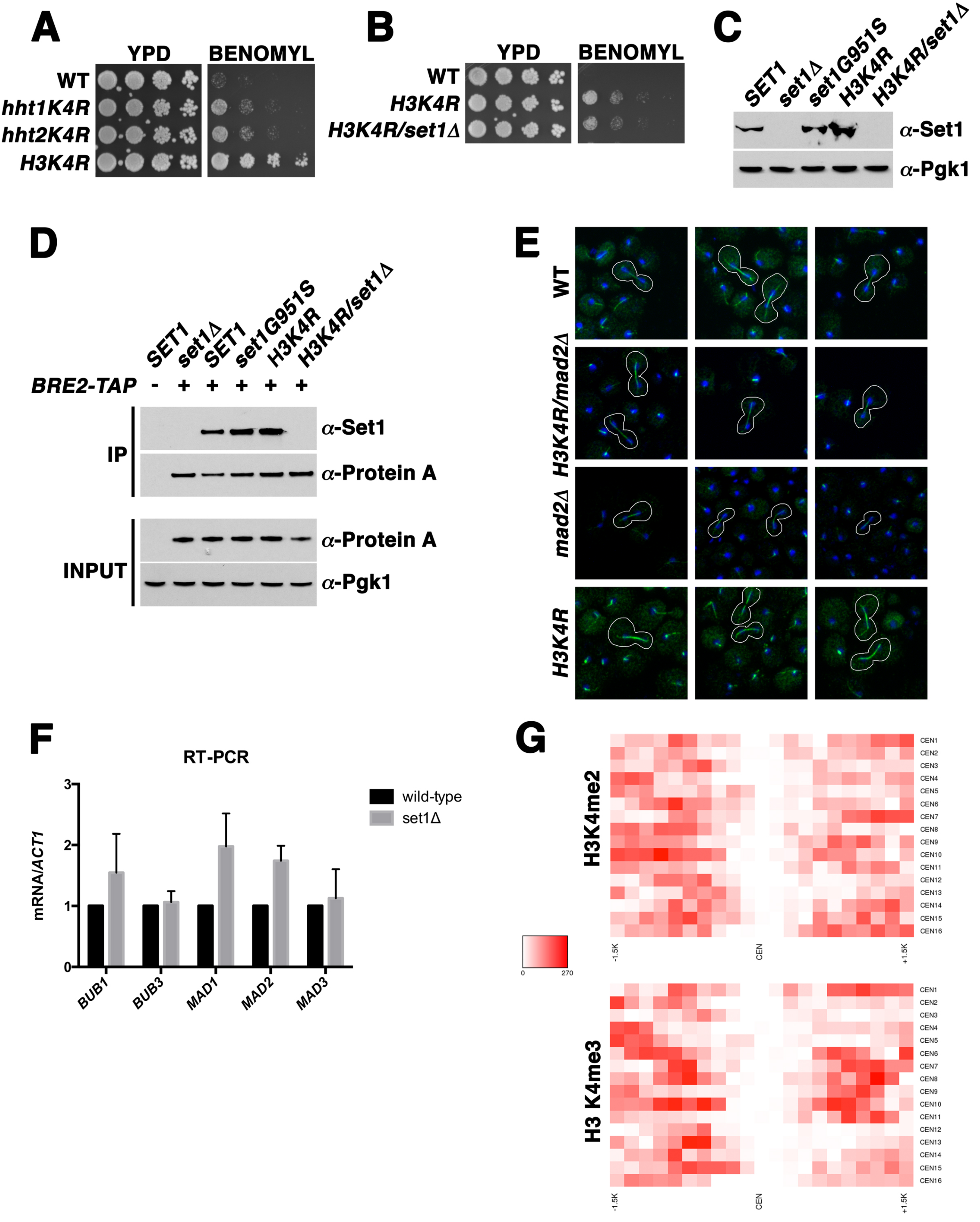
(A) Expression of GST-Mad2 was induced in wild-type, *set1G951S*, and *H3K4R* cells by addition of galactose 30 minutes prior to harvest and Mad2 localization was analyzed with immunofluorescence . DAPI staining (blue) was used to visualize DNA and an α-GST antibody was used to monitor expression of the GST-Mad2 fusion protein. (B) Mad2 localization was assessed in wild-type, *set1G951S*, and *H3K4R* mutants by counting the percentage of GST-Mad2 positive cells with staining localized to the bud neck.

Kirmizis A, Santos-Rosa H, Penkett CJ, Singer MA, Vermeulen M, Mann M, Bahler J, Green RD, Kouzarides T. 2007. Arginine methylation at histone H3R2 controls deposition of H3K4 trimethylation. *Nature* **449**: 928-932.

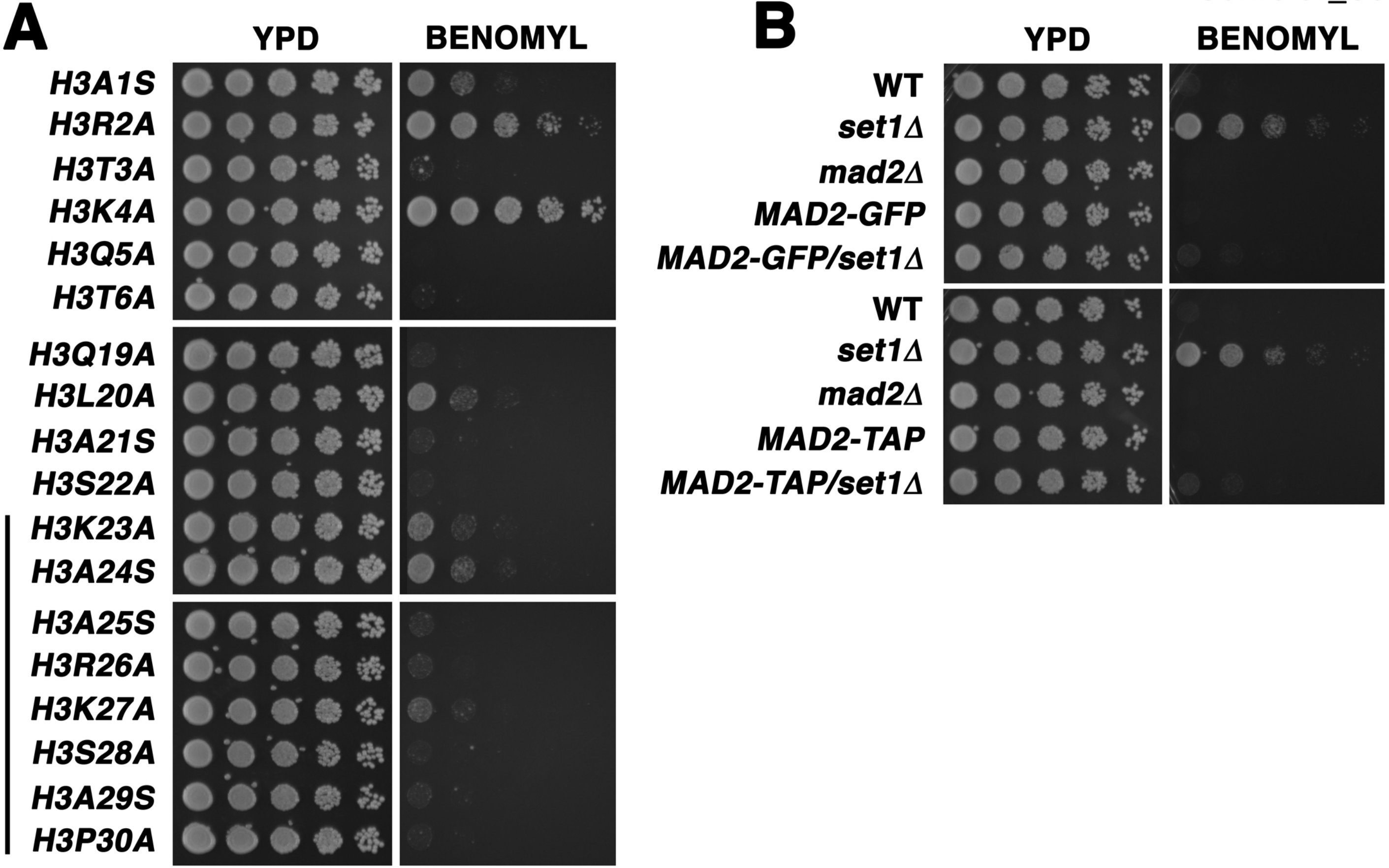
Schibler_S1


A
YPD
YPD+DMSO
10μg/mL
20μg/mL
30μg/mL
40μg/mL
WT
set1Δ
set2Δ
set3Δ
set4Δ
set5Δ
set6Δ
set7Δ
B
WT
set1Δ
set1G951S
αH3K4me2
αH3K4me3
αH3
C
% of Max
100
80
60
40
20
0
G1
G2
WT
set1Δ
D
Budding Index
Percent Cells
50
40
30
20
10
0
No Bud
Small Bud
Large Bud
WT
set1Δ
E
Growth Assay
O.D. (600nM)
3
2
1
0
0
1
2
3
4
5
6
7
8
TIme (hours)
WT
set1Δ
F
Colony Forming Assay
Cells/mL (Millions)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
TIme (hours)
WT
set1Δ
G
wild-type
set1Δ
DIC

Schibler_S2

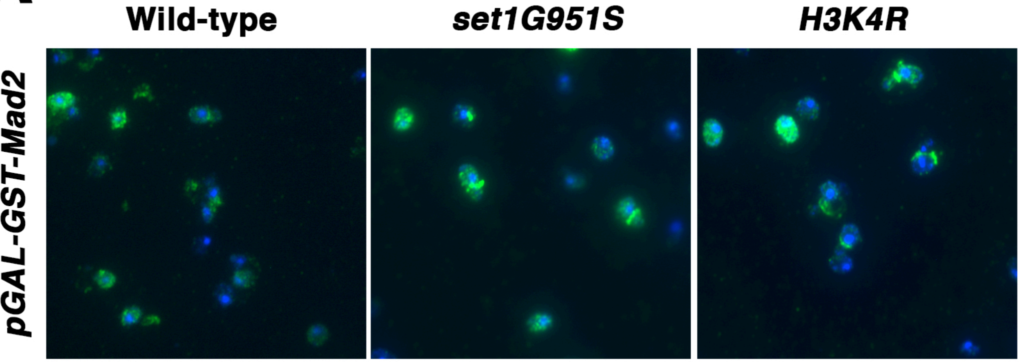

A
YPD
BENOMYL
WT
hht1K4R
hht2K4R
H3K4R
B
YPD
BENOMYL
WT
H3K4R
H3K4R/set1Δ
C
SET1
set1Δ
set1G951S
H3K4R
H3K4R/set1Δ
α-Set1
α-Pgk1
D
SET1
set1Δ
SET1
set1G951S
H3K4R
H3K4R/set1Δ
BRE2-TAP
-
+
+
+
+
+
IP
α-Set1
α-Protein A
INPUT
α-Protein A
α-Pgk1
E
WT
H3K4R/mad2Δ
mad2Δ
H3K4R
F
RT-PCR
mRNA/ACT1
0
1
2
3
wild-type
set1Δ
BUB1
BUB3
MAD1
MAD2
MAD3
G
H3K4me2
CEN1
CEN2
CEN3
CEN4
CEN5
CEN6
CEN7
CEN8
CEN9
CEN10
CEN11
CEN12
CEN13
CEN14
CEN15
CEN16
-1.5K
CEN
+1.5K
0
270
H3 K4me3
CEN1
CEN2
CEN3
CEN4
CEN5
CEN6
CEN7
CEN8
CEN9
CEN10
CEN11
CEN12
CEN13
CEN14
CEN15
CEN16
-1.5K
CEN
+1.5K

Schibler_S3


A
YPD
BENOMYL
H3A1S
H3R2A
H3T3A
H3K4A
H3Q5A
H3T6A
H3Q19A
H3L20A
H3A21S
H3S22A
H3K23A
H3A24S
H3A25S
H3R26A
H3K27A
H3S28A
H3A29S
H3P30A
B
YPD
BENOMYL
WT
set1Δ
mad2Δ
MAD2-GFP
MAD2-GFP/set1Δ
WT
set1Δ
mad2Δ
MAD2-TAP
MAD2-TAP/set1Δ

Schibler_S4

A



B

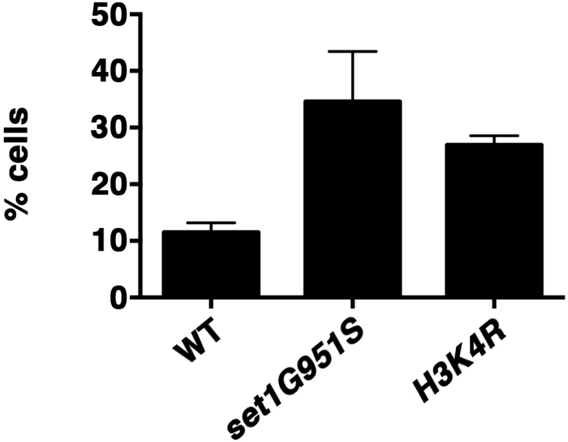


Table S1: Yeast Strains Used in this Study

Strain	Genotype	Source
yAS1	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	EUROSCARF
yAS14	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1Δ::KANMX	This Study
yAS15	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set2Δ::kan^r	This Study
yAS21	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set3Δ::kan^r	This Study
yAS17	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set4Δ::kan^r	This Study
yAS25	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set5Δ::kan^r	This Study
yAS26	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set6Δ::kan^r	This Study
yAS19	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set7Δ::kan^r	This Study
yAS203	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre1Δ::kan^r	This Study
yAS28	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S	This Study
yAS225	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2-TAP::HIS	This Study
yAS226	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2-TAP::HIS set1Δ::KANMX	This Study
yAS227	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2-TAP::HIS set1G951S	This Study
yAS93	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 swd1Δ::kan^r	This Study
yAS75	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 swd3Δ::kan^r	This Study
yAS90	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sdc1Δ::kan^r	This Study
yAS10	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2Δ::kan^r	This Study
yAS12	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 spp1Δ::kan^r	This Study
yAS92	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 shg1Δ::kan^r	This Study
yAS42	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bub1Δ::kan^r	This Study
yAS44	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bub3Δ::kan^r	This Study
yAS30	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S bub1Δ::kan^r	This Study
yAS32	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S bub3Δ::kan^r	This Study
yAS45	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mad1Δ::kan^r	This Study
yAS46	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mad2Δ::kan^r	This Study
yAS47	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mad3Δ::kan^r	This Study
yAS33	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S mad1Δ::kan^r	This Study
yAS34	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S mad2Δ::kan^r	This Study
yAS35	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1G951S mad3Δ::kan^r	This Study
yAS129	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dam1K233R	This Study
yAS153	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R	This Study
yAS36	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R bub1Δ::kan^r	This Study
yAS38	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R bub3Δ::kan^r	This Study
yAS39	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R mad1Δ::kan^r	This Study
yAS40	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R mad2Δ::kan^r	This Study
yAS41	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1K4R hht2K4R mad3Δ::kan^r	This Study
yAS229	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2-TAP::HIS hht1K4R hht2K4R	This Study
yAS228	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bre2-TAP::HIS hht1K4R hht2K4R set1Δ::KANMX	This Study
yAS232	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cdc20-127	This Study
yAS233	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cdc20-127 set1G951S	This Study
yAS237	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cdc20-127 hht1K4R hht2K4R	This Study
yAS234	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PDS1-18MYC	This Study
yAS235	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PDS1-18MYC set1G951S	This Study
yAS147	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MAD2-GFP::HIS set1Δ::KANMX	This Study
yAS162	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MAD2-GFP::HIS	This Study
yAS187	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pGAL-GST-MAD2::URA3	This Study
yAS188	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pGAL-GST-MAD2::URA3 set1G951S	This Study
yAS189	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pGAL-GST-MAD2::URA3 hht1K4R hht2K4R	This Study
yAS230	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MAD2-TAP::HIS	This Study
yAS231	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MAD2-TAP::HIS set1Δ::KANMX	This Study

Table S2: Plasmids Used in this Study

Construct	**Content**	**Content (Amino Acids) Mutations**	**Source:**
pGEX4T1	GST	GST	GE Healthcare
pAS48	GST-yMad2-full length	GST-yMad2 (1-197)	This study
pAS119	GST-yMad2-Open	GST-yMad2 (1-191)	This study
pAS120	GST-yMad2-RQEA	GST-yMad2 (1-197) R126E/Q127A	This study
pAS43	GST-hMad2	GST-hMad2 (1-206)	Dr. Toshiyuki Habu
pAS133	GST-hMad2-RQEA	GST-hMad2 (1-206) R133E/Q134A	This study
pAS134	GST-hMad2-Open	GST-hMad2 (1-196)	This study
pAS56	H3.1-6xHIS	H3.1-6xHIS	This study
pAS146	H3.1(K23A)-6xHIS	H3.1(K23A)-6xHIS	This study
pAS148	H3.1(R26A/K27A)-6xHIS	H3.1(R26A/K27A)-6xHIS	This study