Histone H2B ubiquitylation regulates histone gene expression by suppressing antisense transcription in fission yeast

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Supplemental Figures S1-S6

Supplemental Figure Legends

Figure S1. H2Bub1 promotes expression of histone genes in *S. pombe* cells grown asynchronously. Steady-state RNA levels of the indicated genes were quantified by RT-qPCR and normalized to *act1*$^{+}$ mRNA levels for each of the indicated strains (JTB204, JTB86-3, JTB429, JTB335). For each gene the wild-type expression level was set to 1. Error bars denote standard deviations; asterisks indicate significant differences from wild-type (n=3, unpaired t test, *p<0.05).

Figure S2. Strand-specific RNA-seq data from wild-type and *htb1-K119R* strains in the region surrounding *ams2*$^{+}$. Genome browser view generated in IGV (https://software.broadinstitute.org/software/igv/). The red arrow denotes the position of the *ams2*$^{+}$ transcription start site (TSS). The red bar denotes position of the *ams2*$^{+}$ qPCR amplicon analyzed in this study.

Figure S3. Primer specificity of *ams2*$^{+}$ antisense qPCR signals. Steady-state *ams2*$^{+}$ antisense RNA levels were quantified by qPCR after reverse transcriptase reactions with or without a strand-specific primer. The indicated strains (JTB425, JTB508, respectively) were treated with DMSO. Values for the *cdk9*^{as} strain (with primer) were set to 1. Error bars denote standard deviations (n=3).

Figure S4. H2Bub1 and Cdk9 activity do not affect Ams2 protein stability. **(A)** Immunoblotting of whole-cell extracts from the indicated strains (JTB914 and JTB916) after treatment with DMSO (-) or 3-MB-PP1 (+) for 2 hours and subsequent treatment with cycloheximide for the indicated time. Antibodies are indicated on the right. **(B)** Quantification of the experiment in (A) using Image J software. Intensities for the myc immunoblot were normalized to tubulin and the t=0 time point was set to one for each condition. Error bars indicate standard deviations (n=4). No significant differences were found between conditions (multiple t-tests corrected using Holm-Sidak method, $p<0.05$).

Figure S5. Loss of H2Bub1 and reduced Cdk9 activity both increase levels of histone H3 lysine 9 methylation in the central core of the centromere. Levels of methylated histone H3 lysine 9 (H3K9me2) detected by chromatin immunoprecipitation (ChIP) in the indicated strains (JTB362, JTB98-1, JTB321, JTB429) at the central core of centromere on chromosome 1 (detected by cnt2 primers) or at pericentric heterochromatin (detected by otr2 primers)(see schematic at the top). Signals in immunoprecipitated samples are expressed as % of input. Error bars denote standard deviations; asterisks denote significant differences between immunoprecipitated samples and controls lacking antibody (n=3; *$p<0.05$).

Figure S6. Ams2 overexpression does not rescue septation defects associated with H2Bub1 loss. **(A)** Immunoblotting of whole-cell extracts from the indicated strains (JTB98-1, JTB362, respectively), expressing either empty vector (pREP41-HA; -) or HA-tagged *ams2*$^+$ (pREP41-HA-*ams2*$^+$; +) under the control of the *nmt1*$^+$ promoter. Two transformants

for each strain are shown. Antibodies are indicated on the right. **(B)** Steady-state mRNA levels for the indicated histone genes were quantified by RT-qPCR in the indicated strains (JTB362 and JTB98-1) transformed with either empty vector or vector expressing HA-tagged *ams2*$^{+}$ as in (A). Values were normalized to *act1*$^{+}$ and those for wild-type, empty vector were set to 1. Error bars denote standard deviations; asterisks indicate significant differences from wild-type, empty vector (n=3, unpaired t test, *p<0.05). **(C)** Fraction of cells harboring division septa in the indicated strains as assessed by fluorescence microscopy. “septa” denotes total with division septa; “multisep” denotes cells with multiple septa between two nuclei. Error bars denote standard deviation from two independent experiments. At least 100 cells were counted for each measurement.

Figure S1

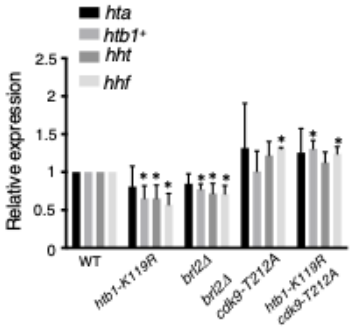


Figure S2

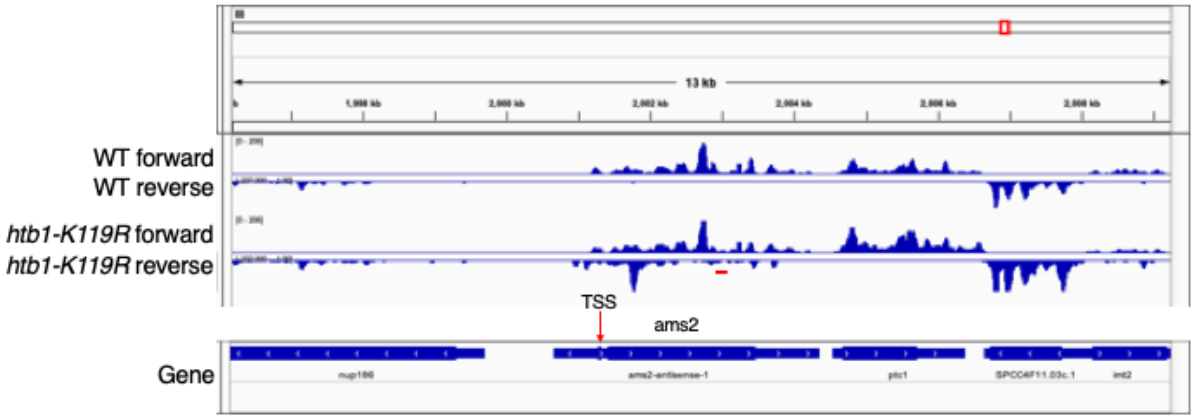


Figure S3

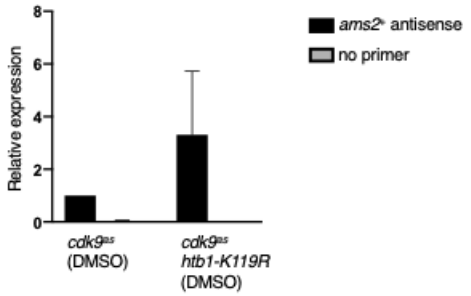

8
6
4
2
0
Relative expression
ams2 antisense
no primer
cdk9as
(DMSO)
cdk9as
htb1-K119R
(DMSO)

Figure S4

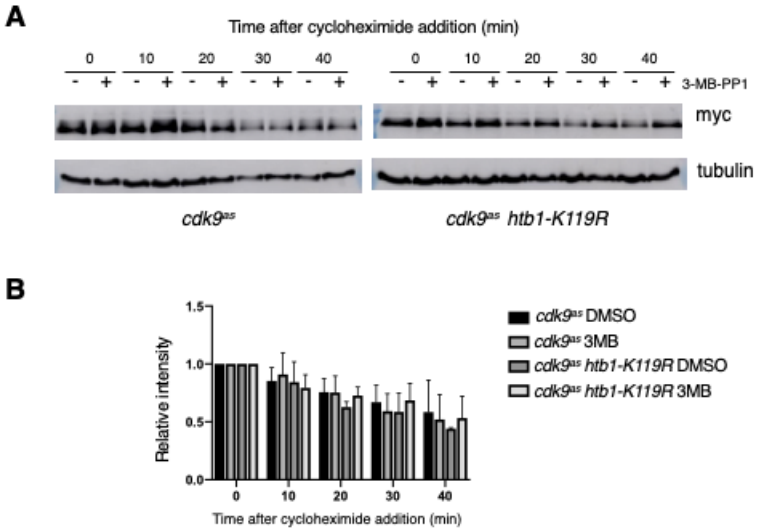

A
Time after cycloheximide addition (min)
0 10 20 30 40 0 10 20 30 40
- + - + - + - + - + - + - + - + - + - +
3-MB-PP1
myc
tubulin
cdk9as
cdk9as htb1-K119R
B
Relative intensity
1.5
1.0
0.5
0.0
0 10 20 30 40
Time after cycloheximide addition (min)
cdk9as DMSO
cdk9as 3MB
cdk9as htb1-K119R DMSO
cdk9as htb1-K119R 3MB

Figure S5

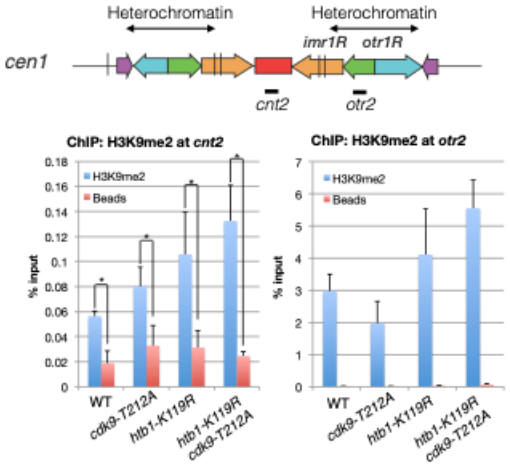
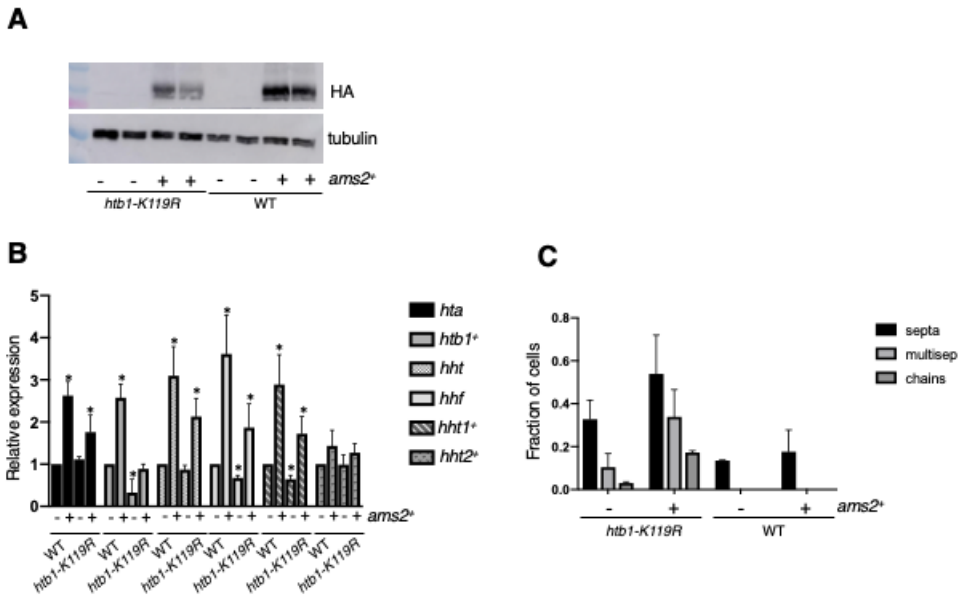

Heterochromatin
Heterochromatin
imr1R
otr1R
cen1
cnt2
otr2
ChIP: H3K9me2 at cnt2
ChIP: H3K9me2 at otr2
H3K9me2
Beads
% input
WT
cdk9-T212A
htb1-K119R
htb1-K119R cdk9-T212A

Figure S6


A
HA
tubulin
- - + + - - + + ams2+
htb1-K119R
WT
B
Relative expression
hta
htb1+
hht
hhf
hht1+
hht2+
ams2+
WT
htb1-K119R
C
Fraction of cells
septa
multisep
chains
ams2+
htb1-K119R
WT