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Supplemental Information

The Histone Acetyltransferase Mst2 Protects Active Chromatin from Epigenetic Silencing by Acetylating the Ubiquitin Ligase Brl1

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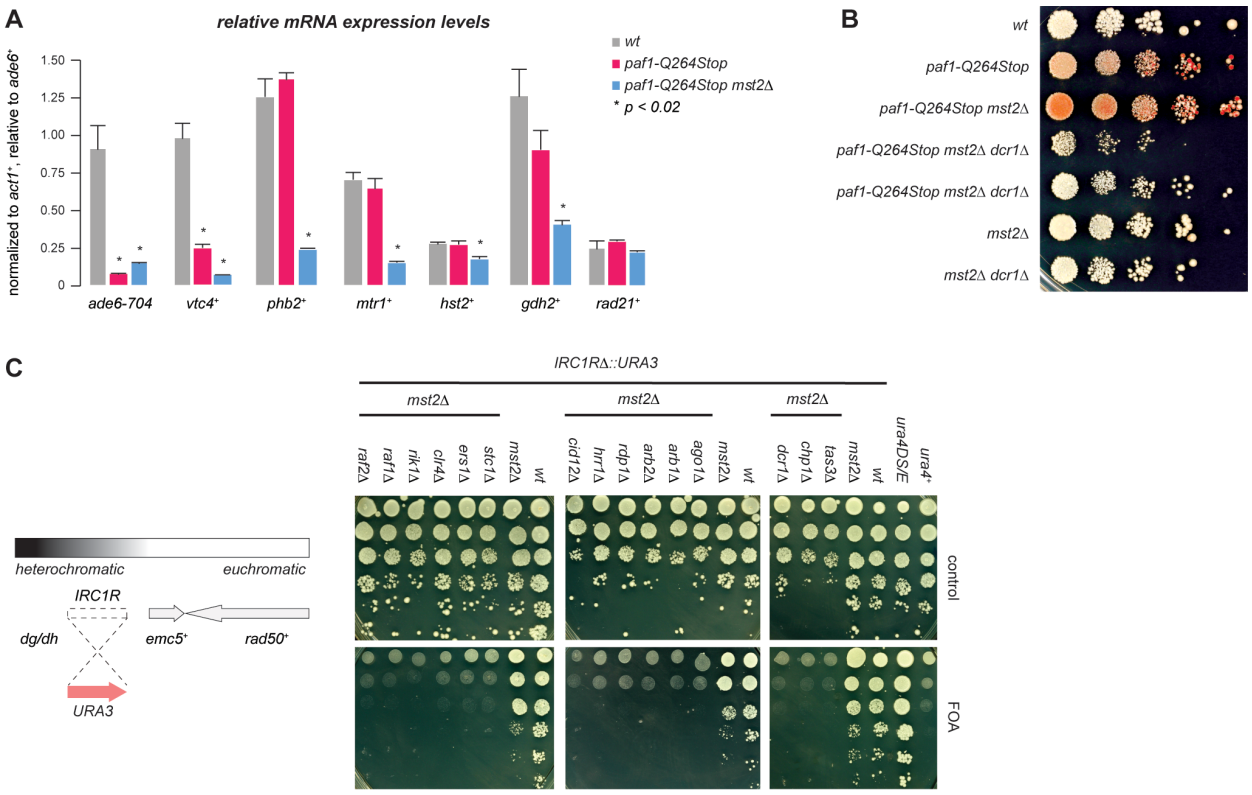

Figure S1 (related to Figure 2)

(A) Relative mRNA expression levels determined by RT-qPCR analysis in indicated mutants. Shown are transcript levels relative to *ade6-704* in *wt* (grey) after normalization to *act1*$^{+}$. *paf1-Q264Stop* and *paf1-Q264Stop mst2Δ* are shown in red and blue. Error bars indicate SD. n=3 independent biological replicates.

(B) Silencing assay with *ade6*$^{+}$ reporter in indicated strains to monitor siRNA-directed de novo heterochromatin assembly (see text for details). Cells were plated in a 10-fold dilution series onto YE-Nat (100 ug/mL nourseothricin).

(C) Silencing assay with *IRC1RΔ::URA3* in indicated strains to monitor siRNA-dependent heterochromatin spreading (see text for details). Cells were plated in a 10-fold dilution series on PMGc plates (control) or PMGc plates with 2g/L FOA.

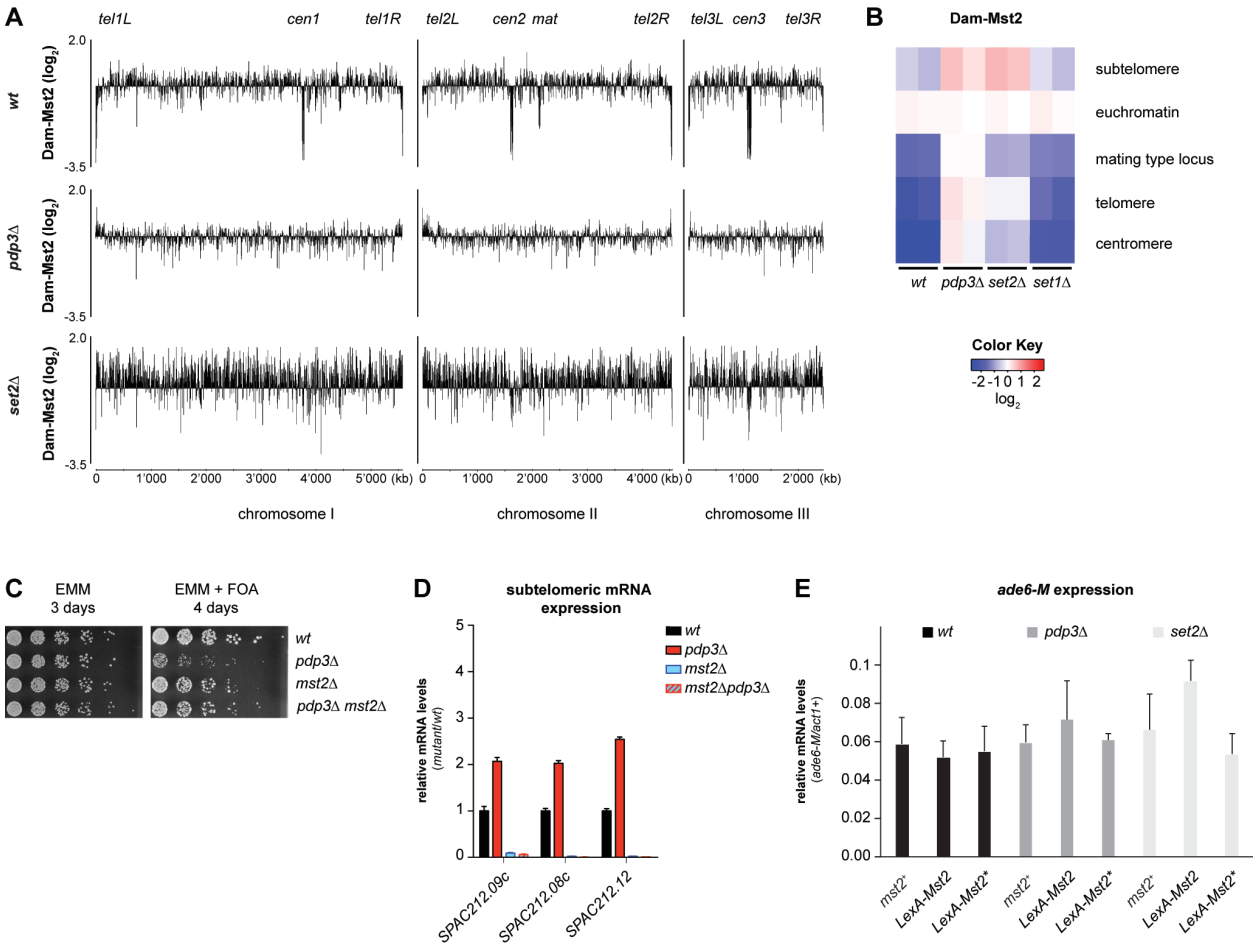

Figure S2 (related to Figure 5)

(A) Mst2 DamID maps of all three chromosomes in *wt*, *pdp3*Δ, and *set2*Δ cells. The signal of DamMst2 (normalized to Dam-only) was averaged over 500 probes and is shown in $\log_2$ scale. X-axis shows position on chromosomes.

(B) Enrichment of Dam-Mst2 at different genomic regions in *wt*, *pdp3*Δ, *set1*Δ, and *set2*Δ cells. Two independent replicates are shown (scale in $\log_2$).

(C) Silencing assay with *imr1L::ura4*$^+$ reporter in indicated strains to monitor heterochromatin maintenance (see text for details). Cells were plated in five-fold serial dilutions on EMM plates (control) or EMM plates containing 1g/L FOA and incubated for the indicated time.

(D) Relative RNA expression levels of subtelomeric genes at telomere 1 in *mst2*Δ, *mst2*Δ*pdp3*Δ, and *pdp3*Δ relative to WT. Transcript levels relative to wild type after normalization to *act1*$^+$ are shown. Data are represented as mean ± SEM from 4 independent biological experiments.

(E) Relative RNA expression levels at the endogenous *ade6-M210* locus in wild type (black), *pdp3*Δ (dark grey), and *set*Δ (light grey) cells with Mst2-tethering variants. Error bars indicate SD. n≥3 independent biological replicates.

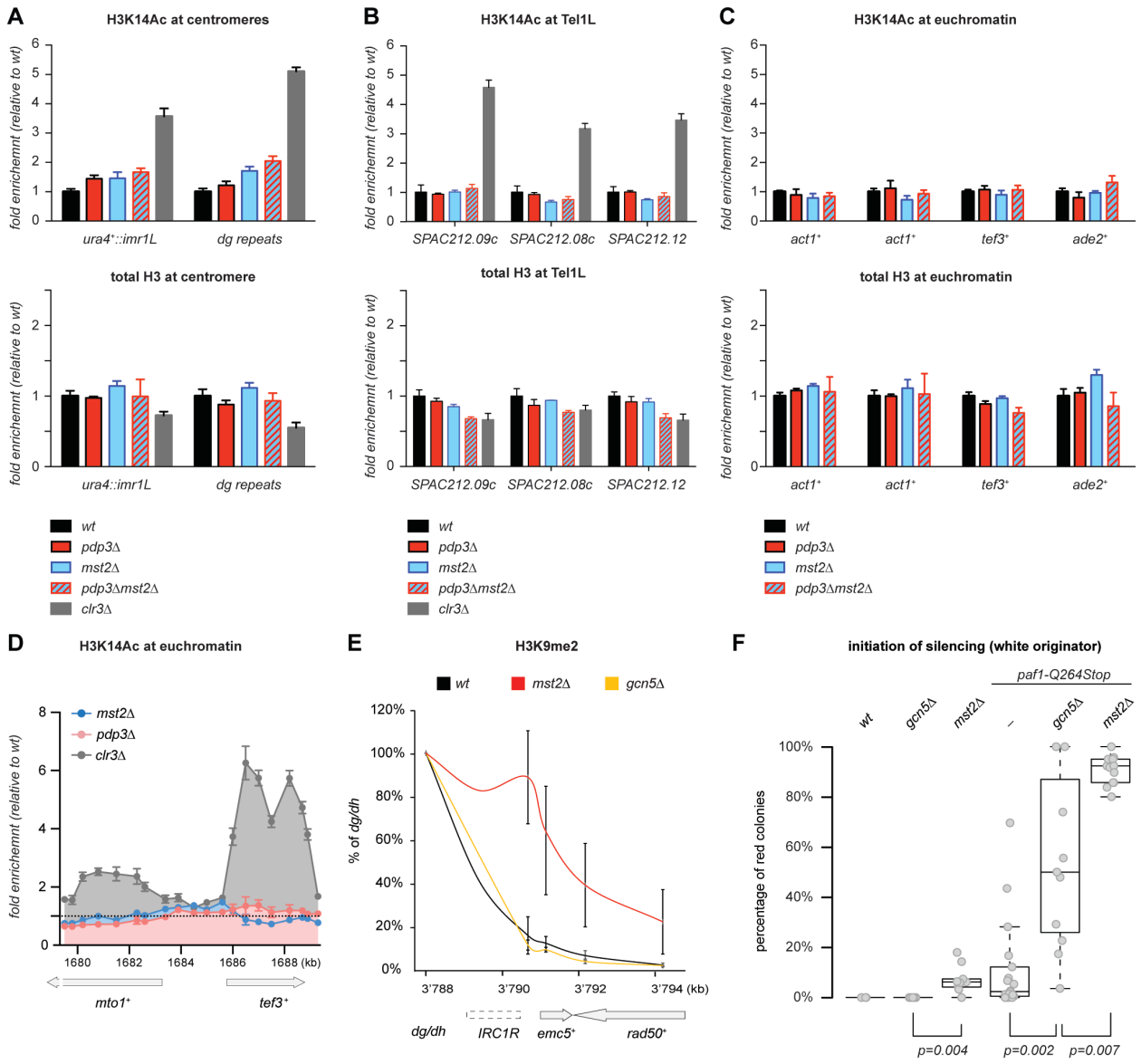

Figure S3 (related to Figure 6)

(A-C) ChIP enrichment of H3K14ac and H3 at centromere 1 (A), telomere 1L (B), and euchromatic loci (C) in indicated strains. Cells lacking the H3K14ac HDAC Clr3 served as a positive control. ChIP data at the indicated loci have been normalized to mitochondrial DNA and to input, and are shown relative to wild type. n=3 ± SEM from independent biological experiments.

(D) ChIP enrichment of H3K14ac at the *mto1*$^{+}$*/tef3*$^{+}$ locus. ChIP data have been normalized to mitochondrial DNA and to input, and are shown relative to wild type for each target, respectively. n=3 ± SEM from independent biological experiments.

(E) ChIP enrichment of H3K9me2 at the boundary of IRC1R in *wt*, *mst2*Δ, and *gcn5*Δ cells. Error bars indicate SD. n≥2 independent biological replicates.

(F) Initiation frequencies of siRNA-directed de novo heterochromatin assembly in different strains. Frequency was calculated as in Figure 1D. P-value was calculated using the two-sided, two sample Student t-test. n≥3 different white colonies. Exact numbers are listed in the STAR methods.

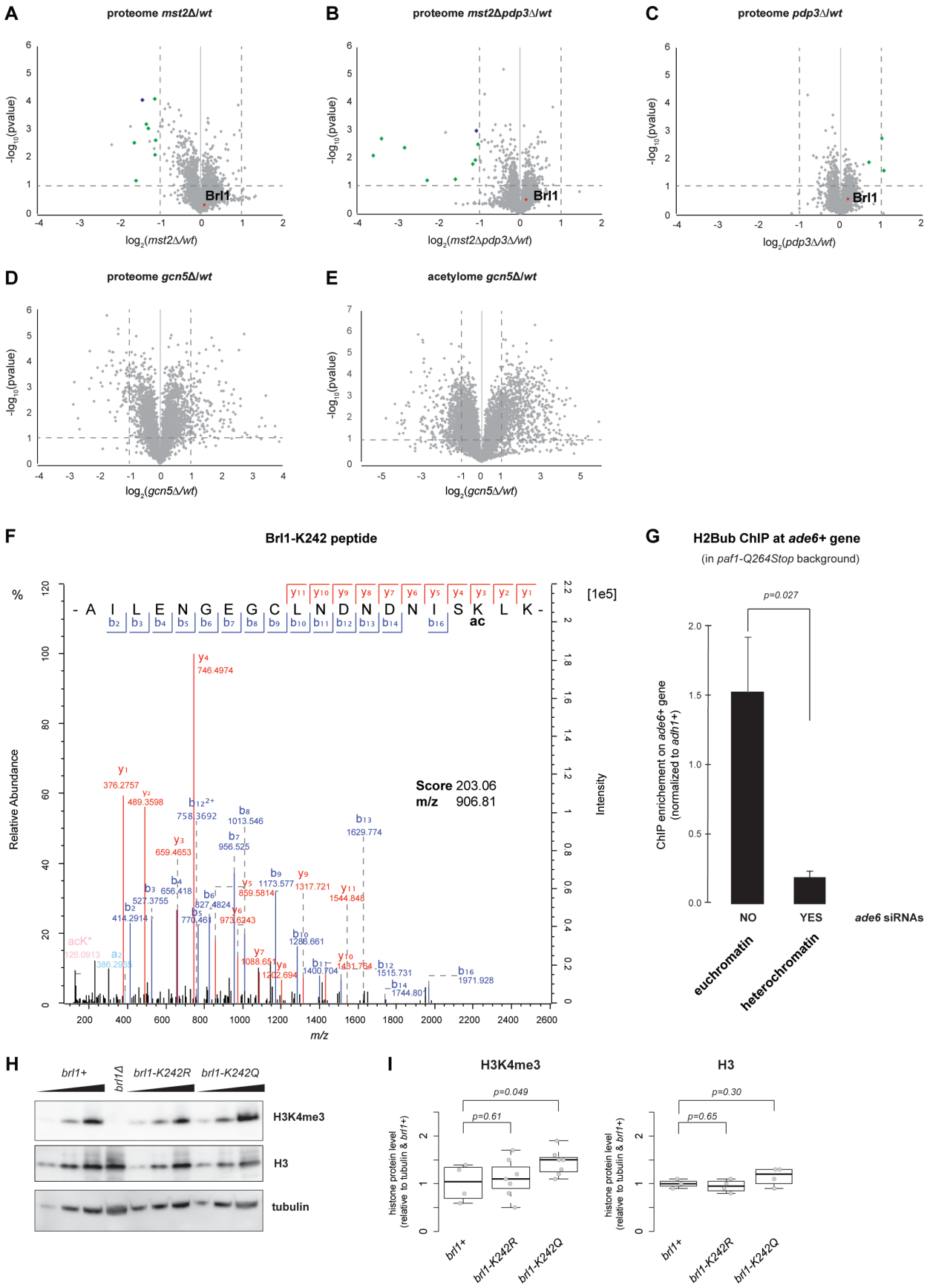
A
proteome mst2Δ/wt
-log10(pvalue)
log2(mst2Δ/wt)
Brl1
B
proteome mst2Δpdp3Δ/wt
-log10(pvalue)
log2(mst2Δpdp3Δ/wt)
Brl1
C
proteome pdp3Δ/wt
-log10(pvalue)
log2(pdp3Δ/wt)
Brl1
D
proteome gcn5Δ/wt
-log10(pvalue)
log2(gcn5Δ/wt)
E
acetylome gcn5Δ/wt
-log10(pvalue)
log2(gcn5Δ/wt)
F
Brl1-K242 peptide
- A I L E N G E G C L N D N D N I S K L K -
ac
Score 203.06
m/z 906.81
Relative Abundance
Intensity
[1e5]
m/z
G
H2Bub ChIP at ade6+ gene
(in paf1-Q264Stop background)
p=0.027
ChIP enrichement on ade6+ gene
(normalized to adh1+)
NO
YES
ade6 siRNAs
euchromatin
heterochromatin
H
brl1+
brl1Δ
brl1-K242R
brl1-K242Q
H3K4me3
H3
tubulin
I
H3K4me3
p=0.049
p=0.61
histone protein level
(relative to tubulin & brl1+)
brl1+
brl1-K242R
brl1-K242Q
H3
p=0.30
p=0.65
histone protein level
(relative to tubulin & brl1+)
brl1+
brl1-K242R
brl1-K242Q

Figure S4 (related to Figure 6)

(A-E) Volcano plots showing the relative changes in different strains. X-axis is in log_2 scale, y-axis depicts the inverted p-value. All experiments were performed in three independent biological replicates. A-C, relative proteome changes in *mst2*Δ (A), *mst2*Δ *pdp3*Δ (B), and *pdp3*Δ cells (C) compared to wild type. Proteins encoded by subtelomeric genes are highlighted in green, whereas Per1 (encoded by a locus adjacent to the cen1L boundary) is shown in blue. Brl1 is highlighted in red. (D) proteome changes in *gcn5*Δ cells compared to wild type. E, changes in the acetylome in *gcn5*Δ compared to *wt* cells.

(F) Annotated high resolution MS/MS spectrum of acetylated peptide fragmented with higher-energy collisional dissociation (HCD). The triply charged precursor ion located at m/z of 906.801 was isolated using quadrupole filter, fragmented with HCD and analyzed in the orbitrap detector. The acetylated peptide AILENGEGcamCLNDNDNISacKLK was identified and annotated by the Andromeda search engine assigning b- and y-ions with an Andromeda score of 203.

(G) ChIP enrichment of H2BK119ub at the *ade6+* locus relative to *adh1+*. n=3 ± SD from independent biological experiments. P-value was calculated using the two-sided, two sample Student t-test.

(H) Immunodetection of H3K4me3 and total H3 in different strains. Dilution series of 1/9, 1/3 and 1/1 of the respective protein extracts. Tubulin served as a loading control. A representative experiment is shown.

(I) Quantification of H3K4me3 (top) and H3 (bottom panel) levels normalized to tubulin and relative to *brl1+*. Multiple independent biological replicates for H3K4me3 (WT: n=4; brl1-KR/KQ: n=7) and H3 (WT: n=3; brl1-KR/KQ: n=4). P-value was calculated using the two-sided, two-sample Student *t*-test with equal/unequal variance according to prior evaluation with the F-test.