Supplemental Material for

Loss of HP1 causes depletion of H3K27me3 from facultative heterochromatin and gain of H3K27me2 at constitutive heterochromatin

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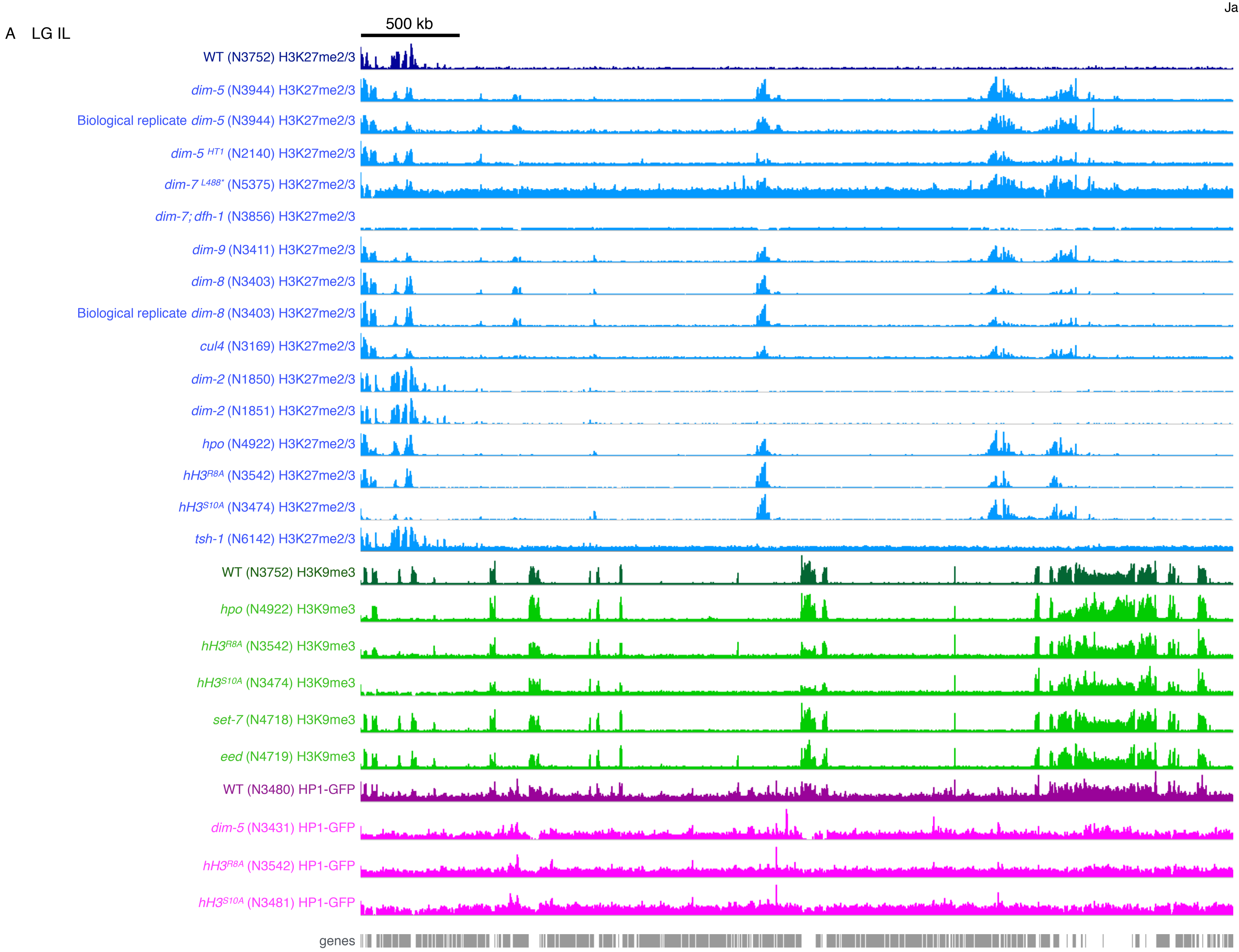
Supplemental Figure S1. ChIP-seq tracks for all seven linkage groups.

ChIP-seq data for the each of the seven linkage groups. (A) LG I has been split into two parts (LG IL and IR), while all other linkage groups are displayed intact. Enrichment of H3K27me2/3 in indicated strains is grouped (blue tracks). H3K27me2/3 is shown in a wild-type (WT) strain (dark blue track) and in mutant strains (light blue tracks). Biological replicates for some strains are shown. Enrichment of H3K9me3 in wild-type (dark green track) and mutant strains (light green tracks) are displayed for *hpo*, *hH3*^{R8A}, *hH3*^{S10A}, *set-7* and *eed* strains followed by enrichment of HP1-GFP in a wild-type strain (purple track) and HP1-GFP in *dim-5*, *hH3*^{R8A} and *hH3*^{S10A} strains (pink tracks). For reference, annotated genes are displayed below (gray rectangles). A scale bar (500 kb) is shown at the top left. While the majority of domains are virtually identical, representative minor differences between the distribution of H3K27me2/3 in a *dim-5* deletion strain and a *dim-5*^{HT1} strain are indicated by two red bars on LG VI below the track for *dim-5*^{HT1}. (B) Three novel peaks of H3K27me2/3 are visible in both *dim-2* deletion strains that we tested (N1850 and N1851) that were not detected in our wild-type strain and are overlapping or located on either side of an H3K27me2/3 peak that is visible in our *dim-5* deletion strain on LG VI. The area indicated by the black bar in the upper panel is expanded in the lower panel to show the novel H3K27me2/3 domains in greater detail. Scale bars (500 and 50 kb, respectively) are shown for the upper and lower panels.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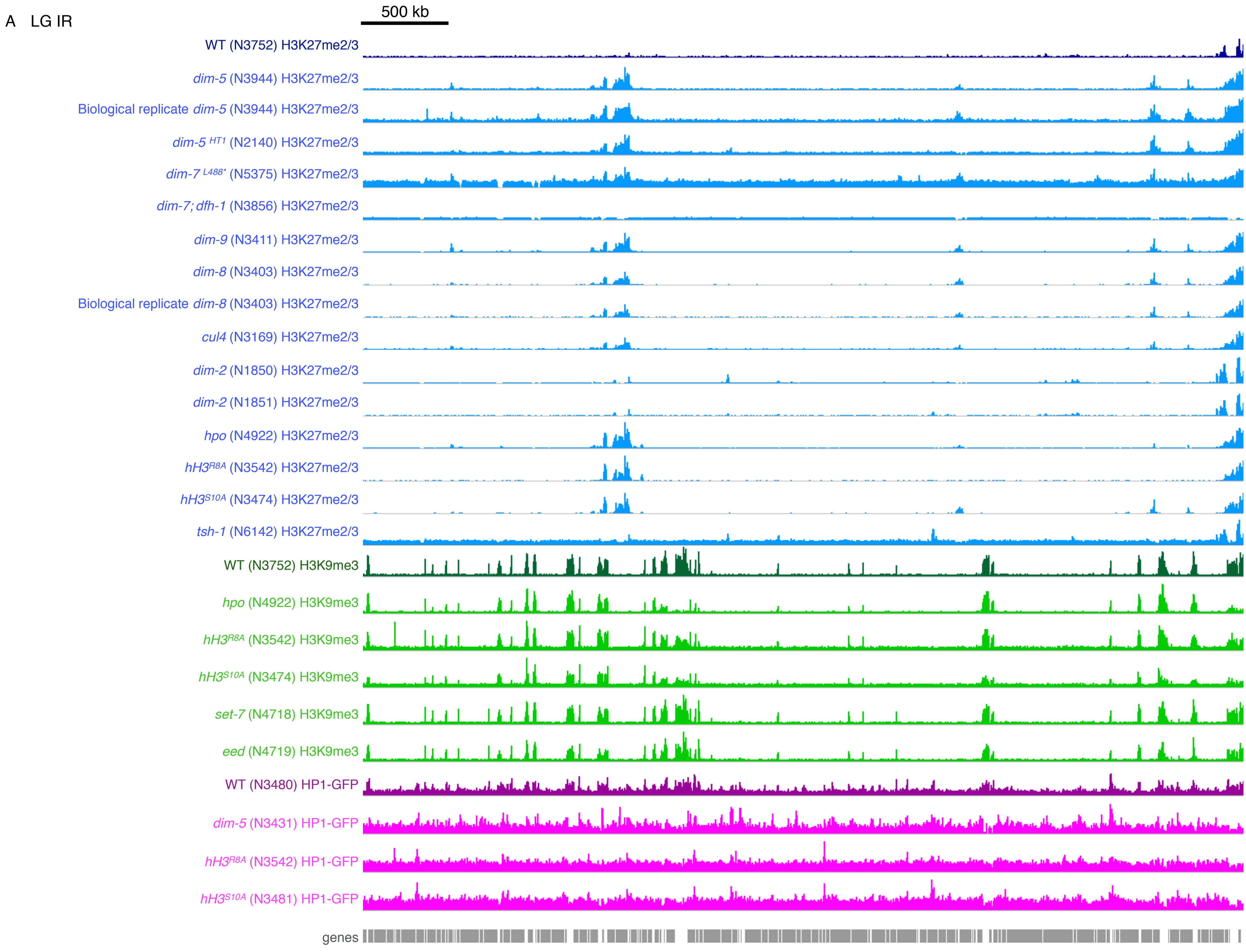
genes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dim-8 (N3403) H3K27me2/3
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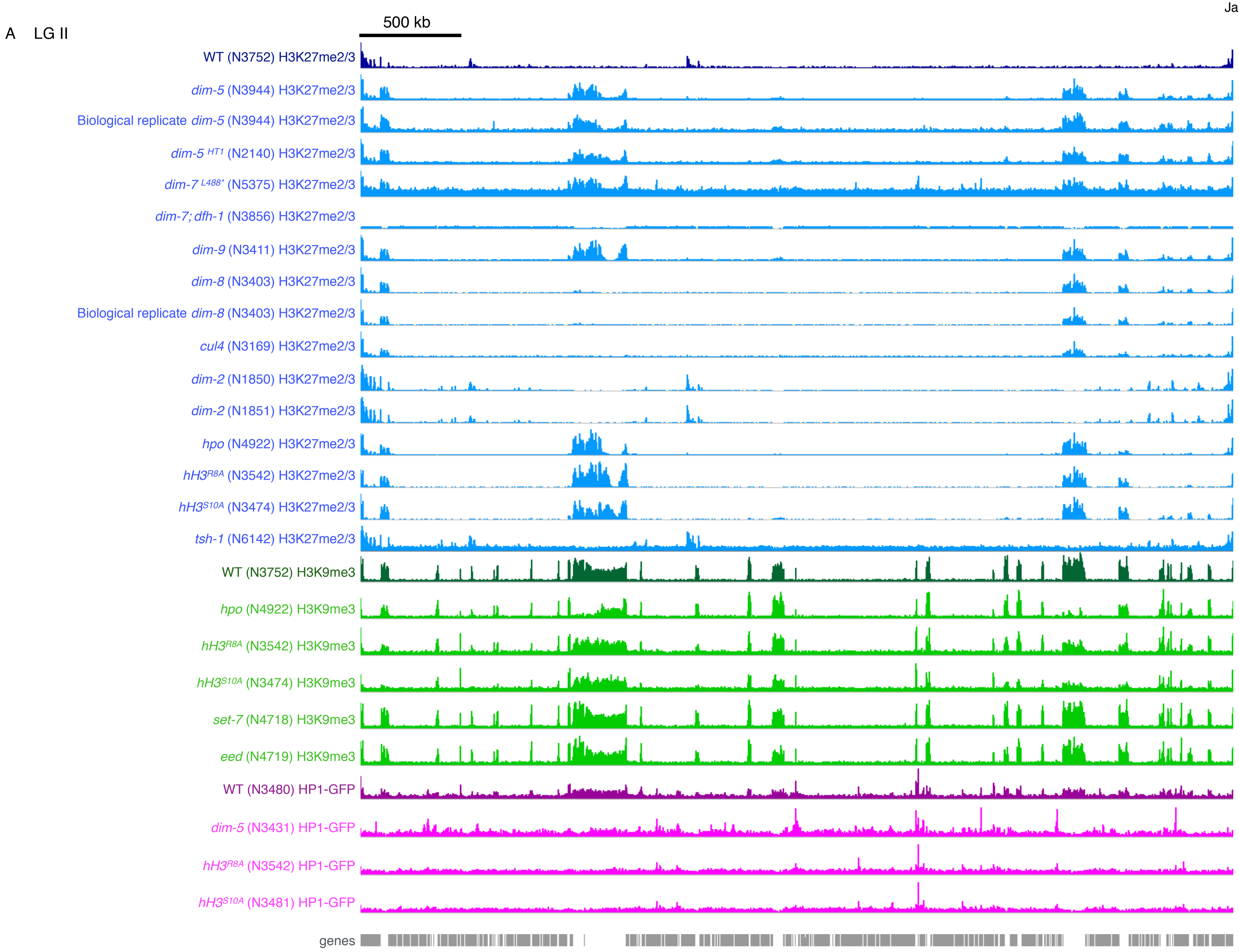
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dim-7; dfh-1 (N3856) H3K27me2/3
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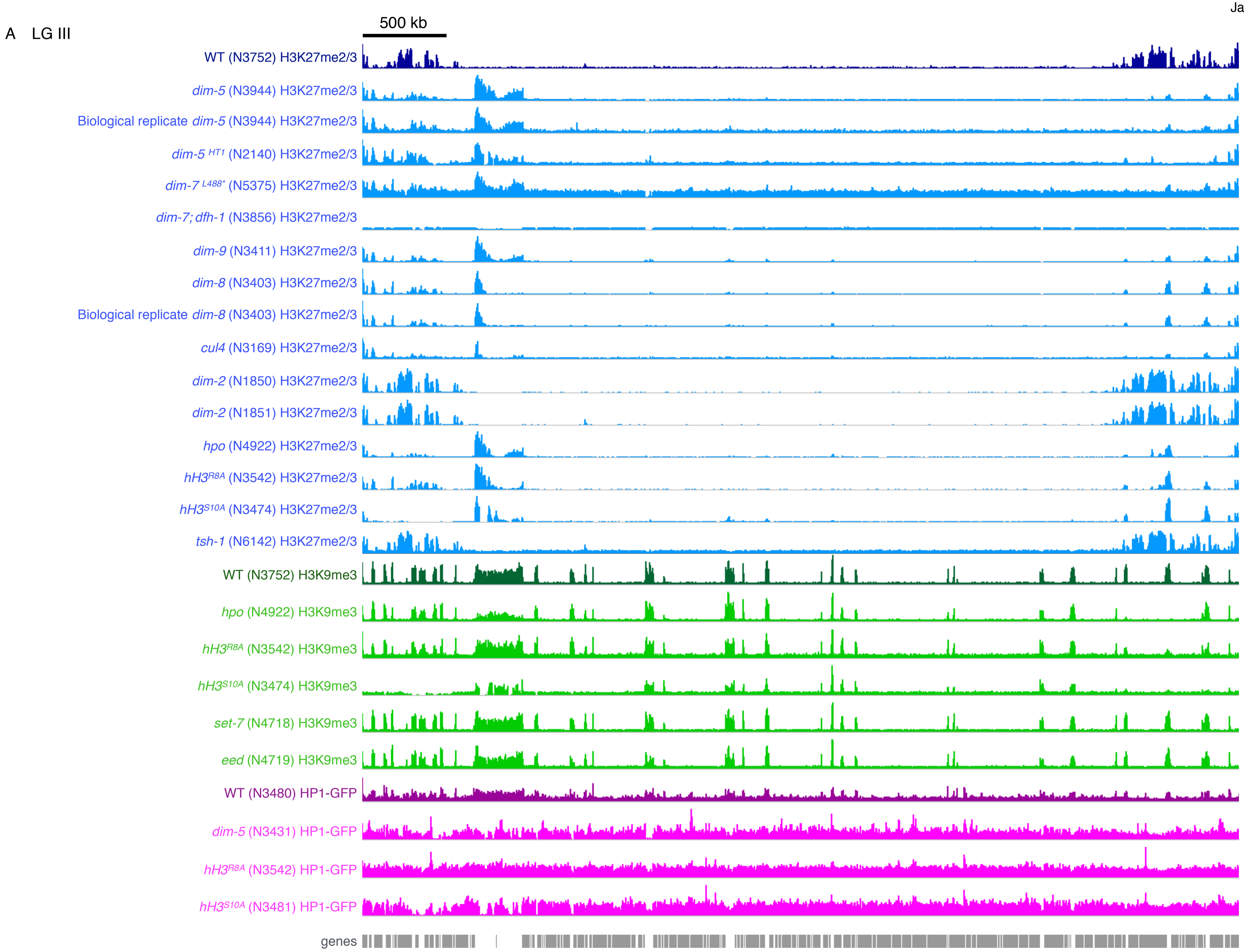
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dim-7 L488* (N5375) H3K27me2/3
dim-7; dfh-1 (N3856) H3K27me2/3
dim-9 (N3411) H3K27me2/3
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Biological replicate dim-8 (N3403) H3K27me2/3
cul4 (N3169) H3K27me2/3
dim-2 (N1850) H3K27me2/3
dim-2 (N1851) H3K27me2/3
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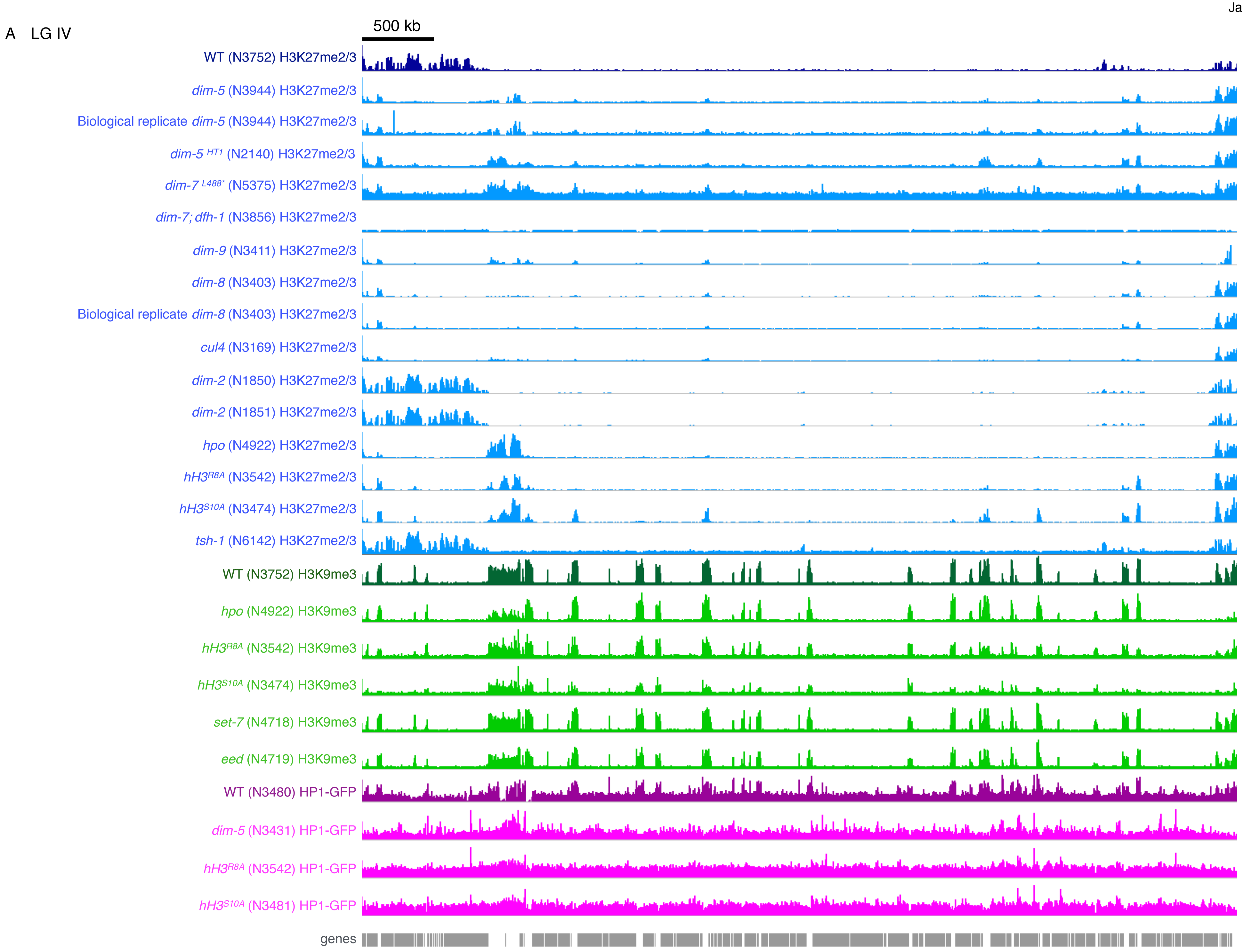
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dim-7; dfh-1 (N3856) H3K27me2/3
dim-9 (N3411) H3K27me2/3
dim-8 (N3403) H3K27me2/3
Biological replicate *dim-8* (N3403) H3K27me2/3
cul4 (N3169) H3K27me2/3
dim-2 (N1850) H3K27me2/3
dim-2 (N1851) H3K27me2/3
hpo (N4922) H3K27me2/3
hH3 ^{R8A} (N3542) H3K27me2/3
hH3 ^{S10A} (N3474) H3K27me2/3
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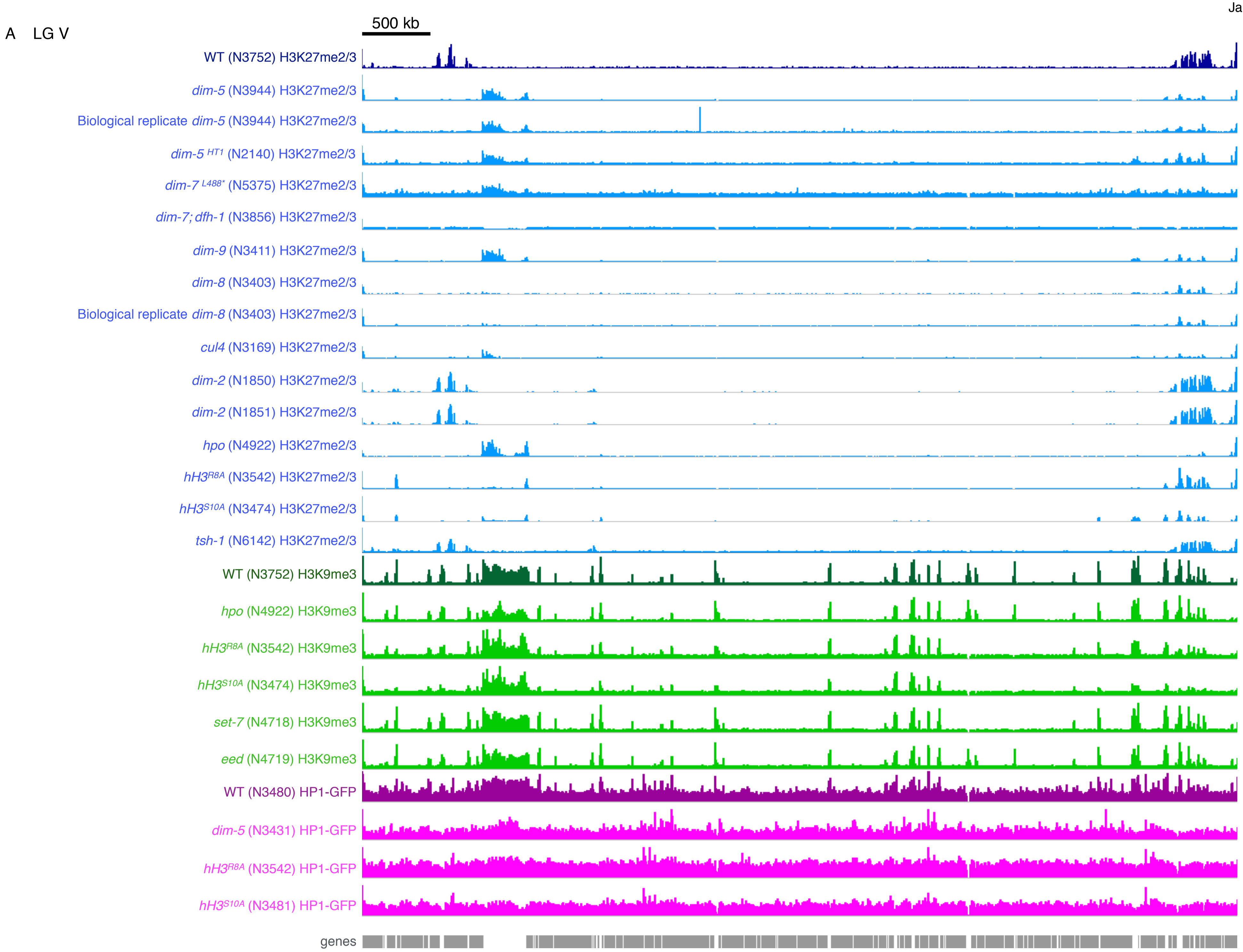
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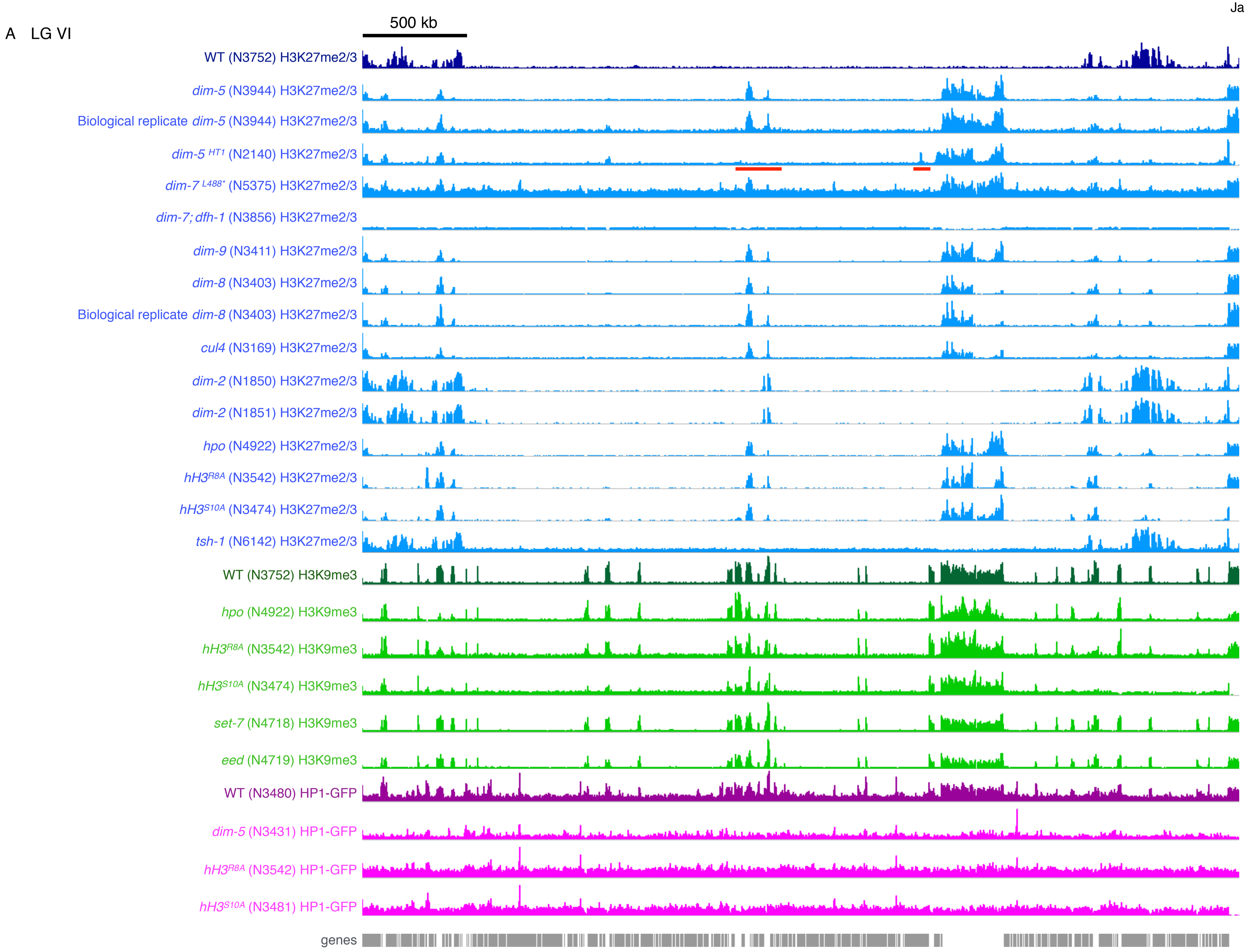
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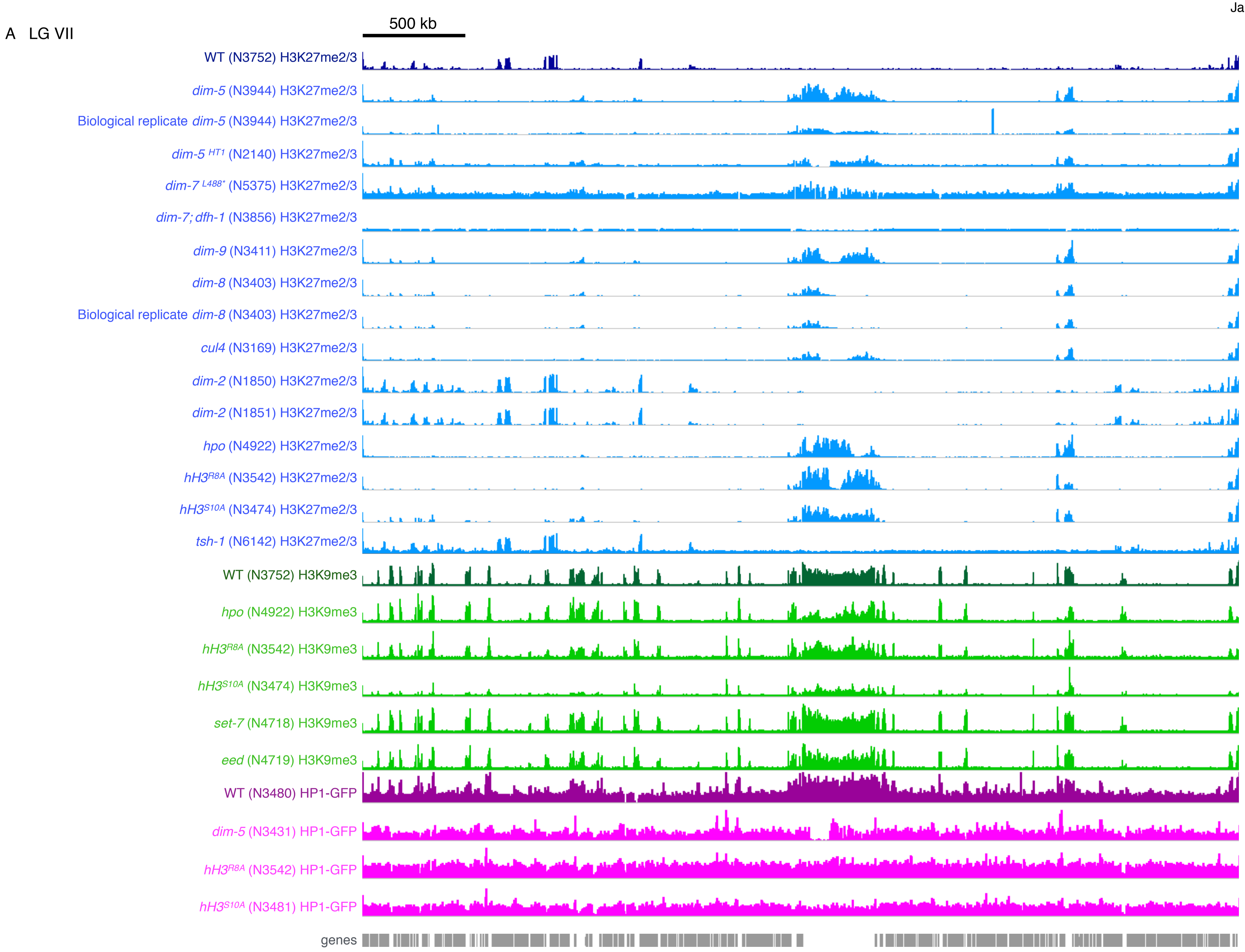
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dim-7; dfh-1 (N3856) H3K27me2/3
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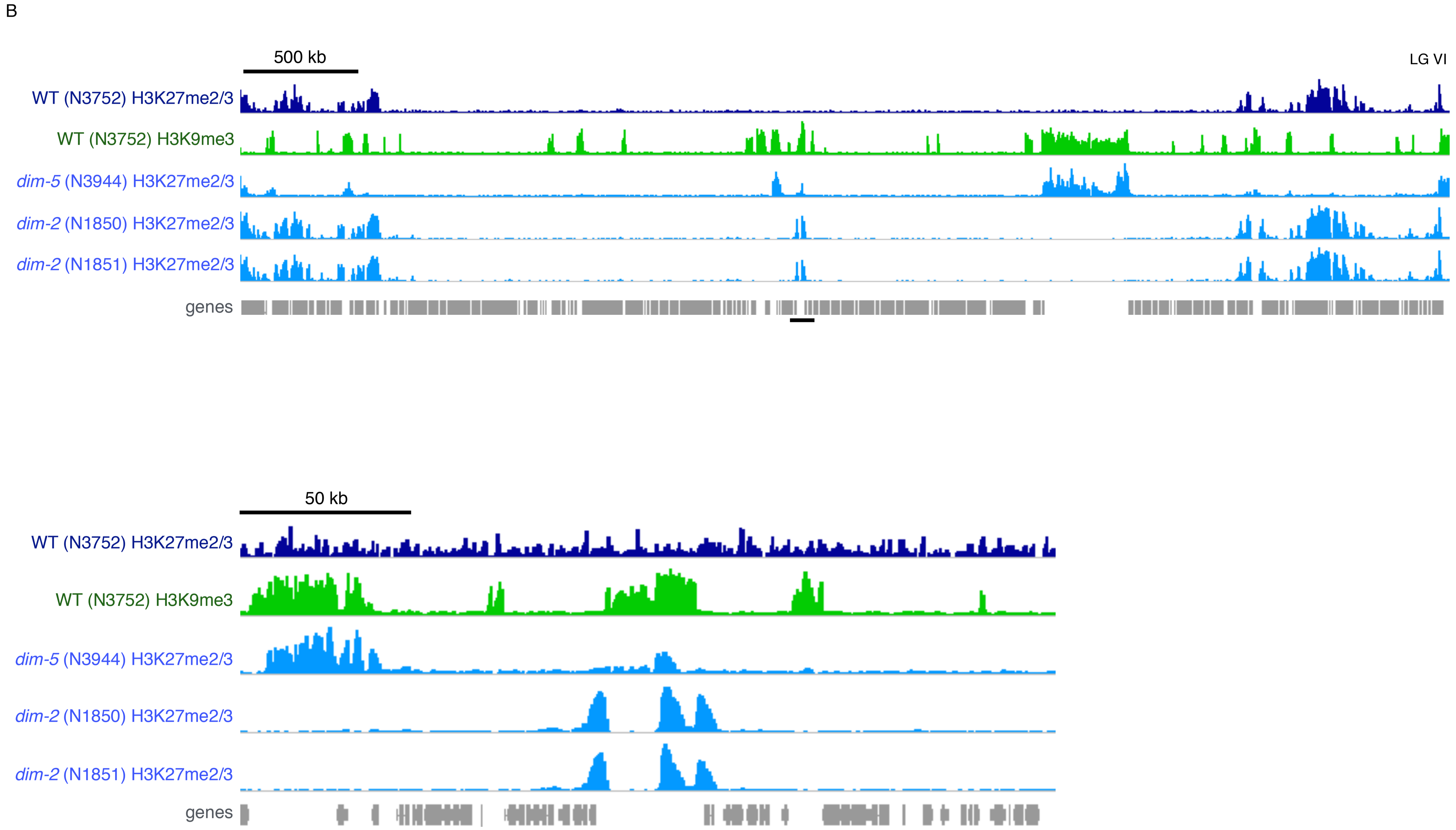
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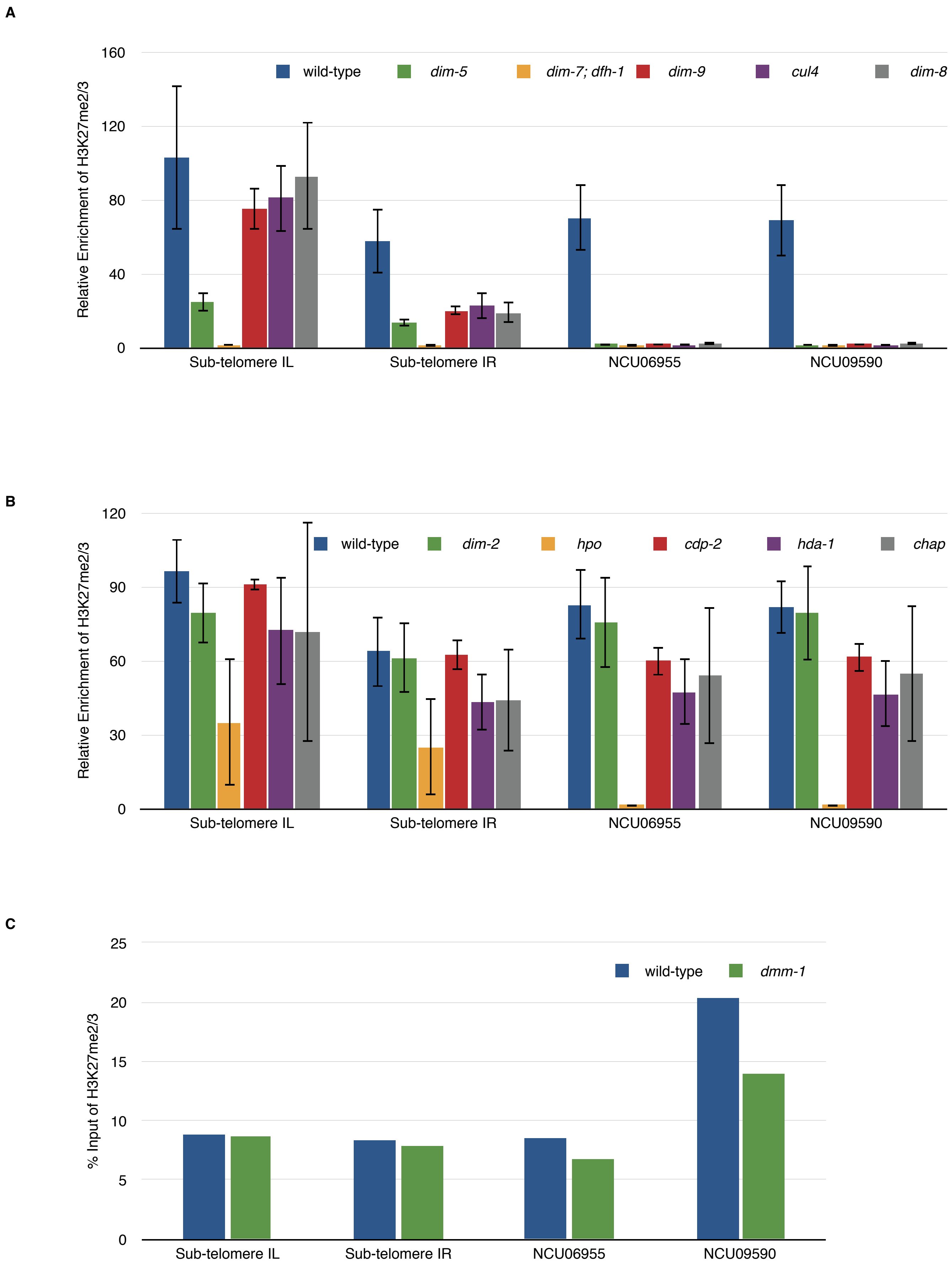
dim-2 (N1851) H3K27me2/3
genes

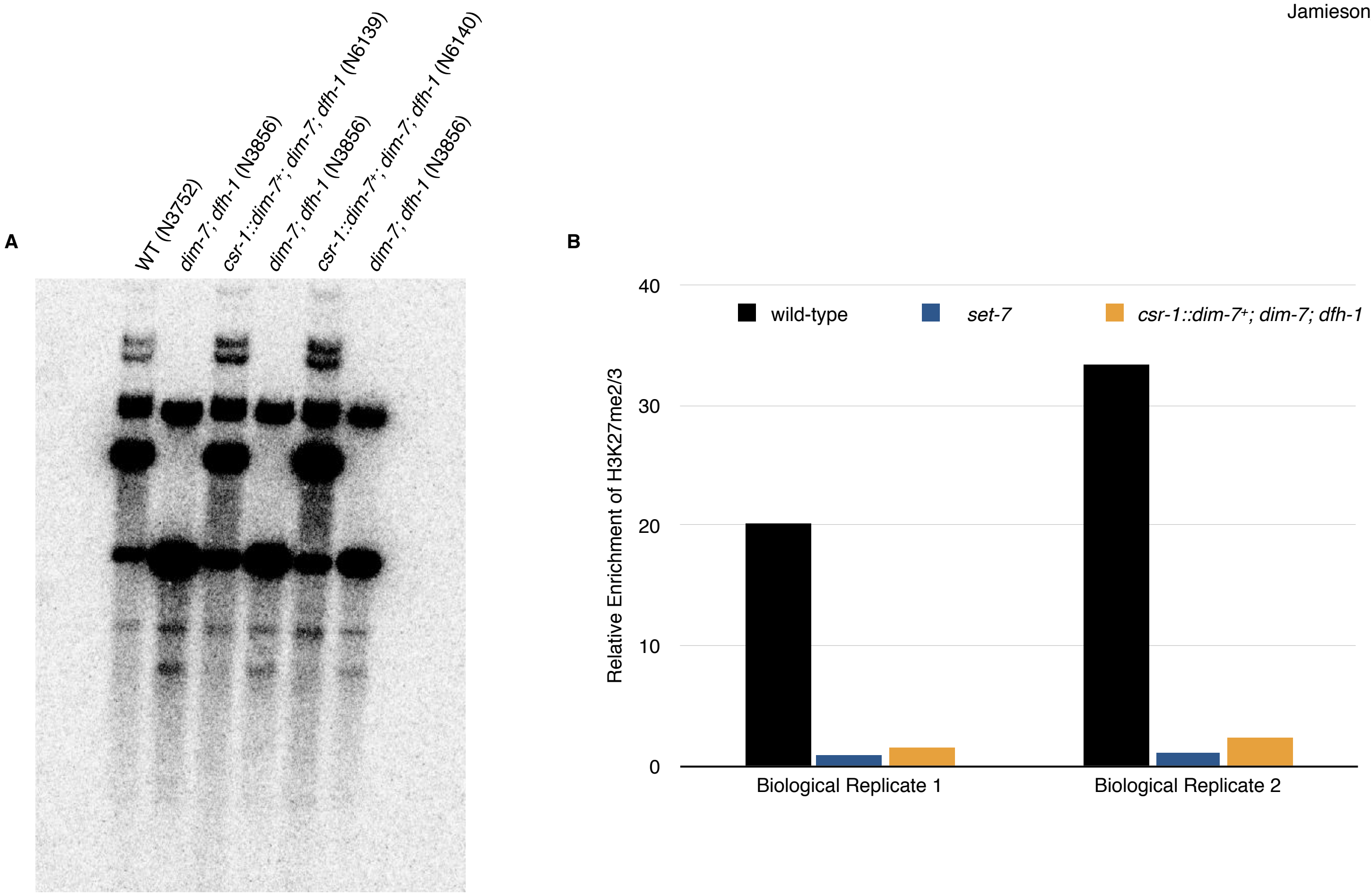
Supplemental Figure S2. Deletions of DCDC members and HP1 perturb the distribution of H3K27me2/3. (A) ChIP-qPCR to evaluate relative enrichment of H3K27me2/3 in strains with deletions of DCDC members (*dim-5*, *dim-7; dfh-1*, *dim-8*, *cul4* or *dim-9*), compared to a wild-type strain. The relative enrichment of H3K27me2/3 was evaluated at four loci normally associated with H3K27me2/3, two in sub-telomere domains on LG I (IL and IR) and two in genes distal to the telomeres on LG VII (NCU06955 and NCU09590). Enrichment at each of the four facultative heterochromatin loci was normalized to background enrichment at histone H4. (B) ChIP-qPCR to measure relative enrichment of H3K27me2/3 in *dim-2*, *hpo*, *cdp-2* and *hda-1* strains as in (A). (C) ChIP-qPCR to measure the enrichment of H3K27me2/3 in a *dmm-1* strain. The percent enrichment (% input) of H3K27me2/3 was evaluated at the four loci as described in (A).

Jamieson_FigS2



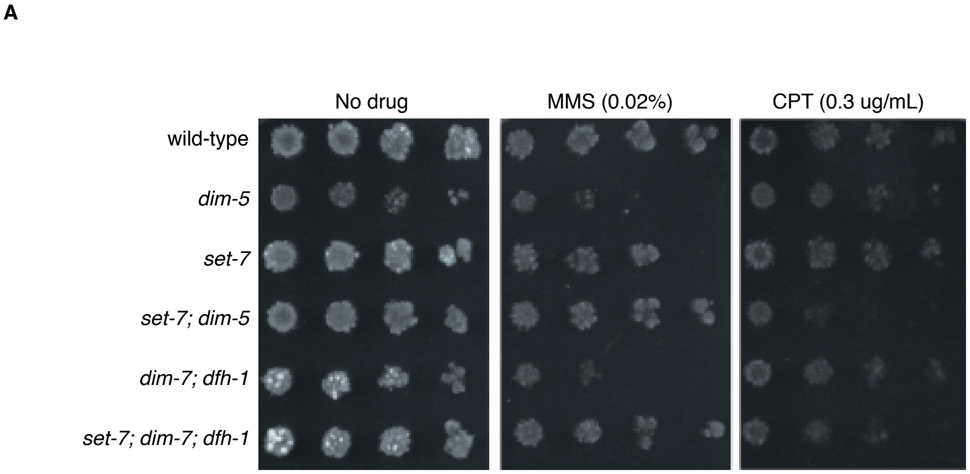
Supplemental Figure S3. Ectopic *dim-7* restores the DNA methylation defect but not the H3K27me2/3 defect in a *dim-7; dfh-1* strain. (A) DNA methylation levels were tested in wild-type, *dim-7; dfh-1* and *dim-7; dfh-1* complemented strains by Southern blot with DNA digested with the AvaII methylation-sensitive restriction enzyme and probed with the 8:A6 region, which has strong levels of DNA methylation (Selker et al. 2003). (B) H3K27me2/3 is not restored when *dim-7* is reintroduced into a *dim-7; dfh-1* strain. ChIP-qPCR results for two biological replicates are shown. Enrichment of H3K27me2/3 is measured at sub-telomere IL (Tel IL).

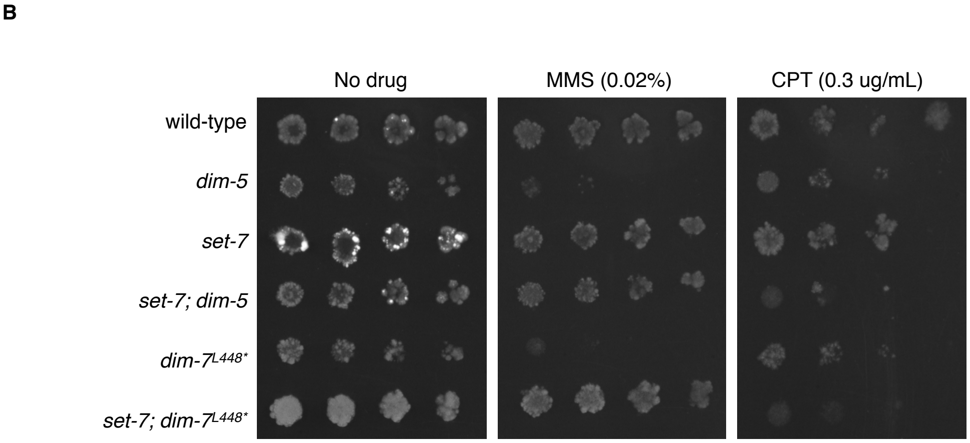
Jamieson_FigS3



Supplemental Figure S4. Effects of a *set-7* mutation on sensitivity of DCDC mutants to MMS and CPT. (A) The *set-7* strain shows nearly equivalent growth compared to wild-type on MMS. The poor growth of the *dim-5* deletion strain and the *dim-7; dfh-1* strain on MMS was largely alleviated in a *set-7* mutant background. *set-7*; *dim-5*, and *set-7*; *dim-7*; *dfh-1* mutants show greater sensitivity to CPT compared to their counterparts lacking the *set-7* mutant background. Serial dilutions of conidia (from left to right: 10^4, 10^3, 10^2, 10^1) were tested on both minimal (no drug), MMS and CPT-containing media, growth at 72 h is shown. (B) The poor growth of the *dim-7*^{L448*} strain is largely alleviated in a *set-7; dim-7*^{L448*} strain. CPT sensitivity is greater in the *set-7; dim-7*^{L448*} strain compared to its single mutant counterparts. Growth at 72 h is shown for minimal (no drug) and MMS-containing media, growth at 96 h is shown for CPT-containing medium.

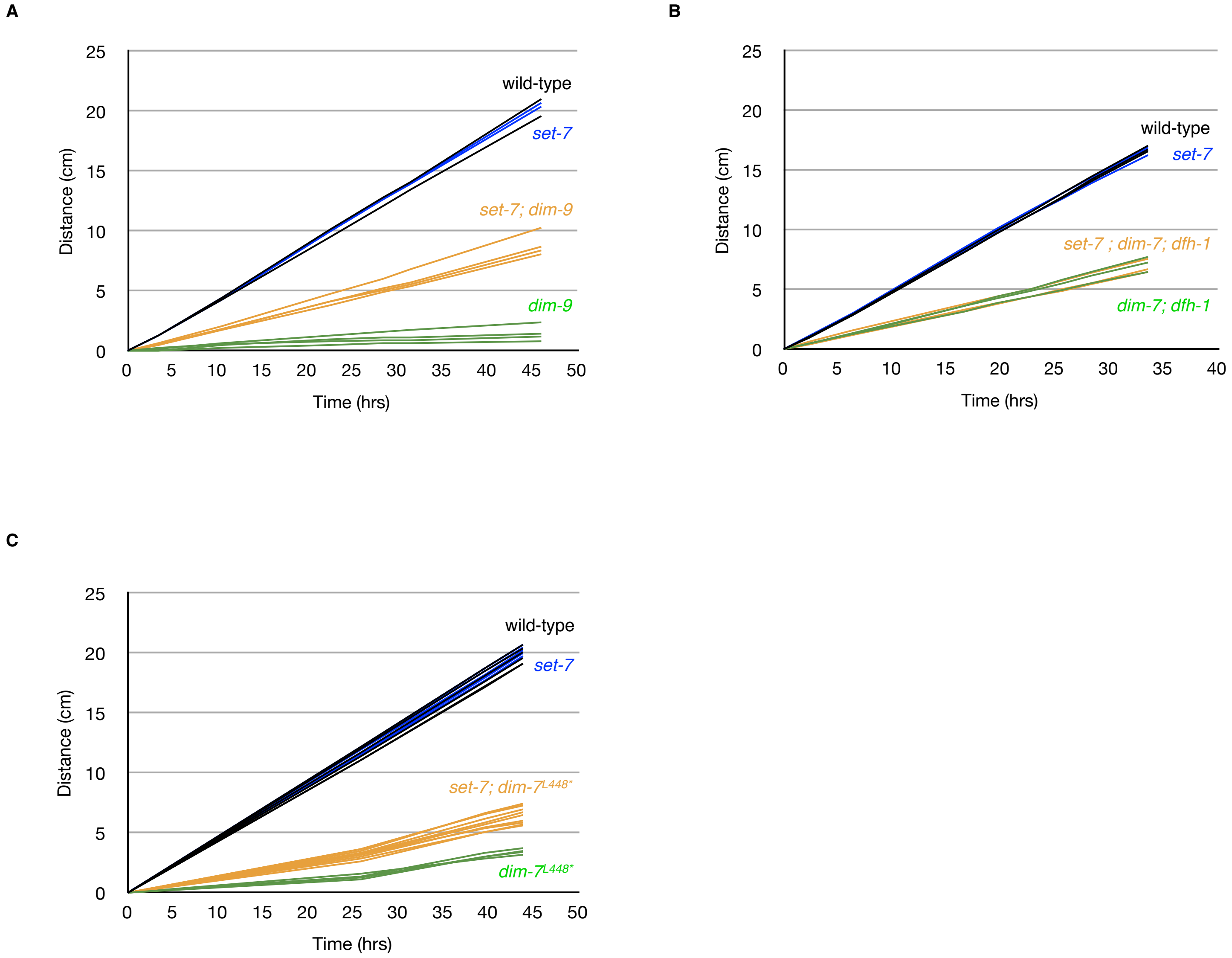
Jamieson_FigS4



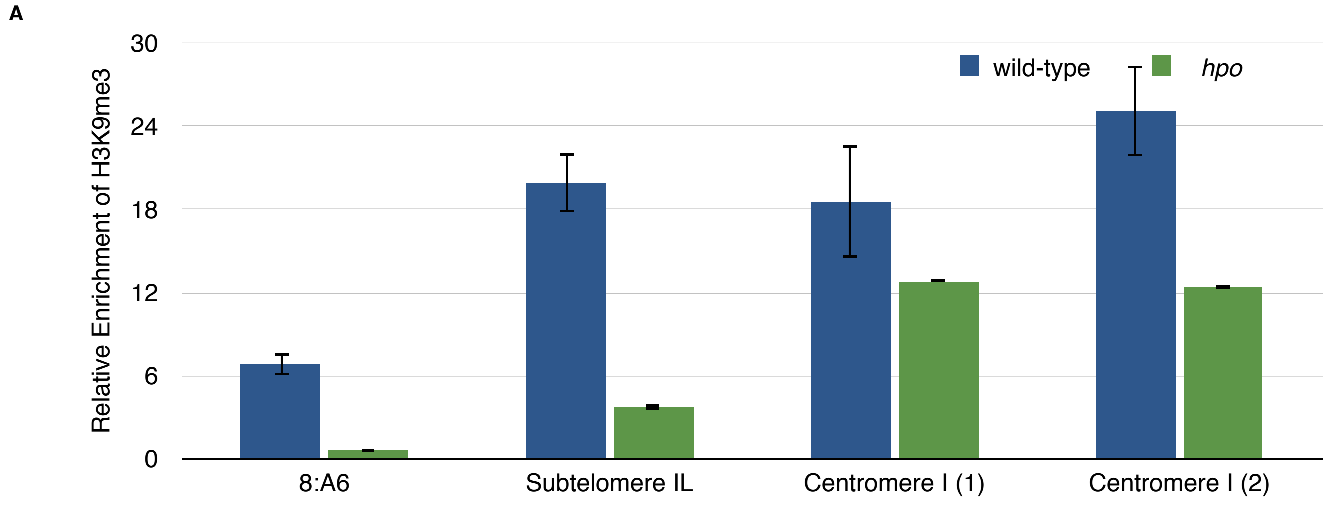


Supplemental Figure S5. Deletion of set-7 partially alleviates the slow growth of *dim-9* and *hpo* strains. (A) Comparison of growth rates for wild-type, *set-7*, *dim-9* and *set-7*; *dim-9* double mutant strains. The slow growth phenotype of the *dim-9* strain (green lines) is partially relieved in the *set-7; dim-9* double mutant strains (yellow lines). (B) The linear rate of growth was measured for siblings obtained from a cross between *dim-7; dfh-1* and *set-7*. Mutation of *set-7* does not rescue the poor growth of *dim-7; dfh-1* strains. The slow growth rate of the *dim-7; dfh-1* double mutants (green lines) is approximately equivalent to that of *set-7; dim-7* double mutants (yellow lines). (C) The slow growth phenotype of the *dim-7*^{L448*} strains (green lines) is rescued by the *set-7* deletion (yellow lines). The growth rates of wild-type (black lines) and *set-7* strains (blue lines) are shown in all panels for comparison.

Jamieson_FigS5



Supplemental Figure S6. Deletion of HP1 reduces H3K9me3 at some constitutive heterochromatin regions. H3K9me3 was evaluated in wild-type and *hpo* strains by ChIP-qPCR. The relative enrichment of H3K9me3 in these strains was measured at 8:A6, a region of constitutive heterochromatin at sub-telomere IL and at two loci within the centromere located on LG I. Relative enrichment was calculated as described in Supplemental Fig. S2.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18
12
6
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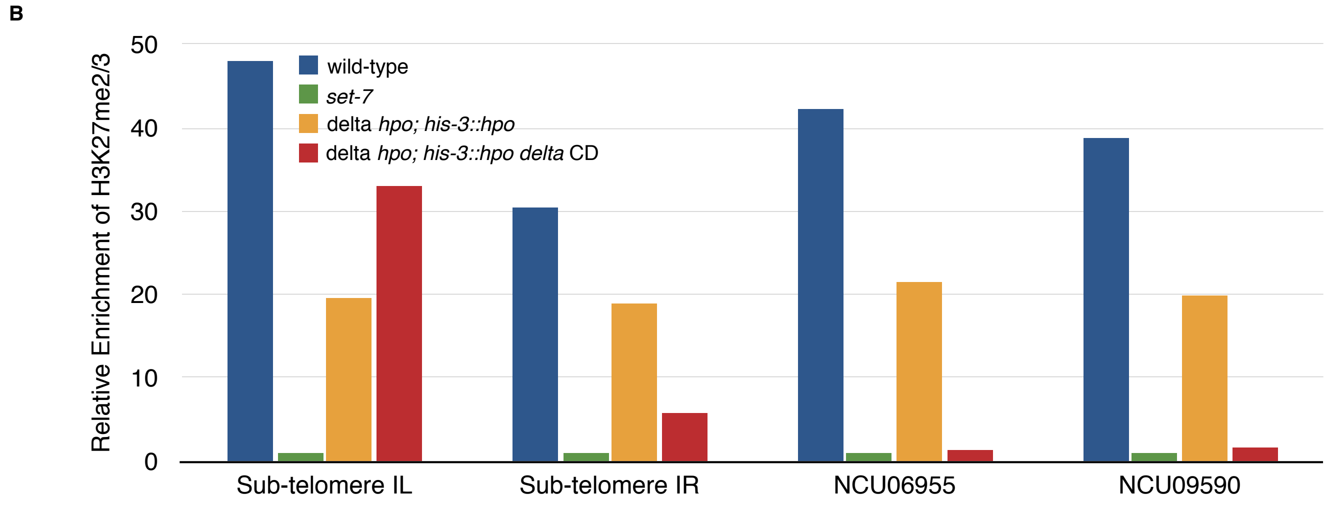
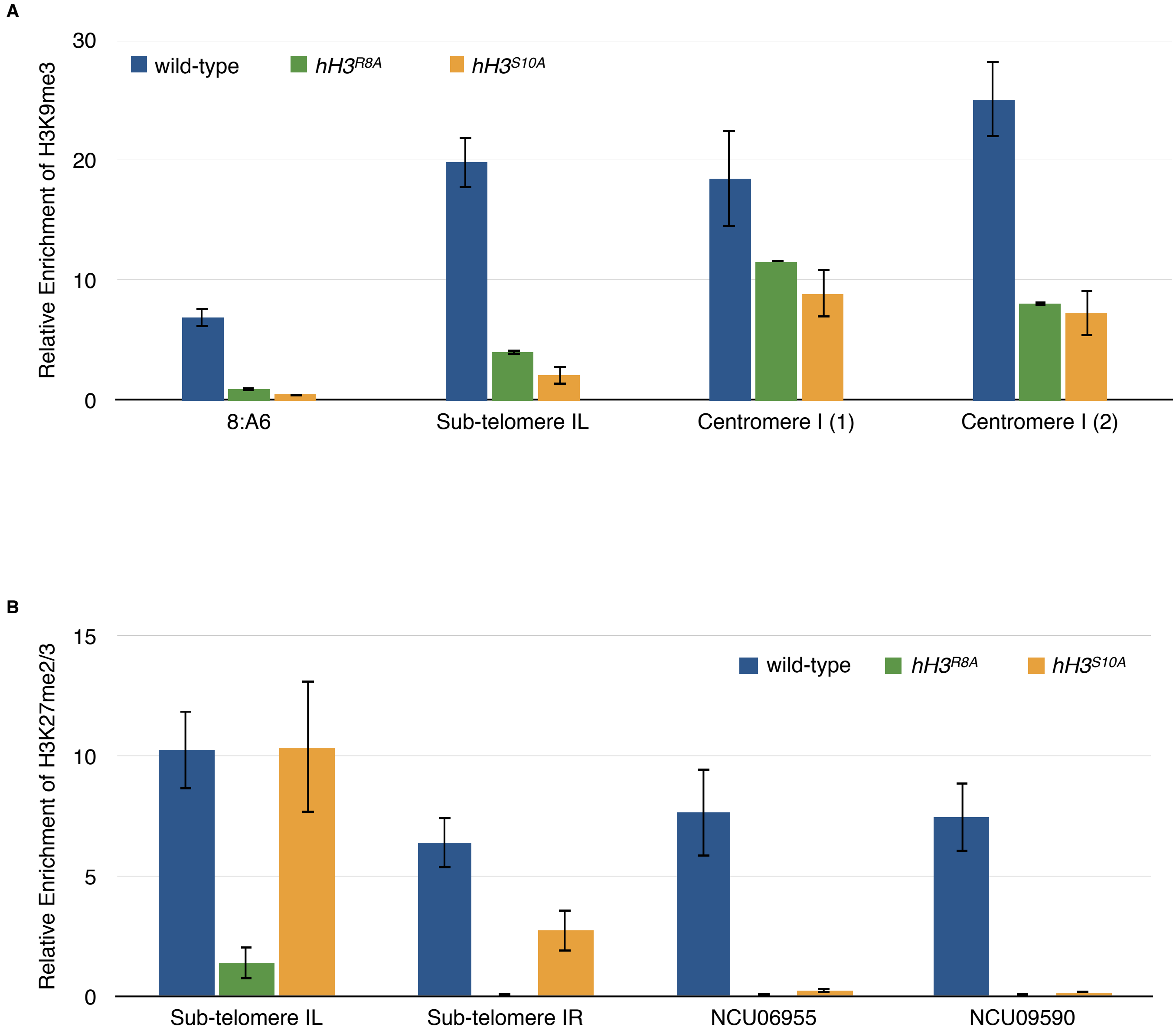
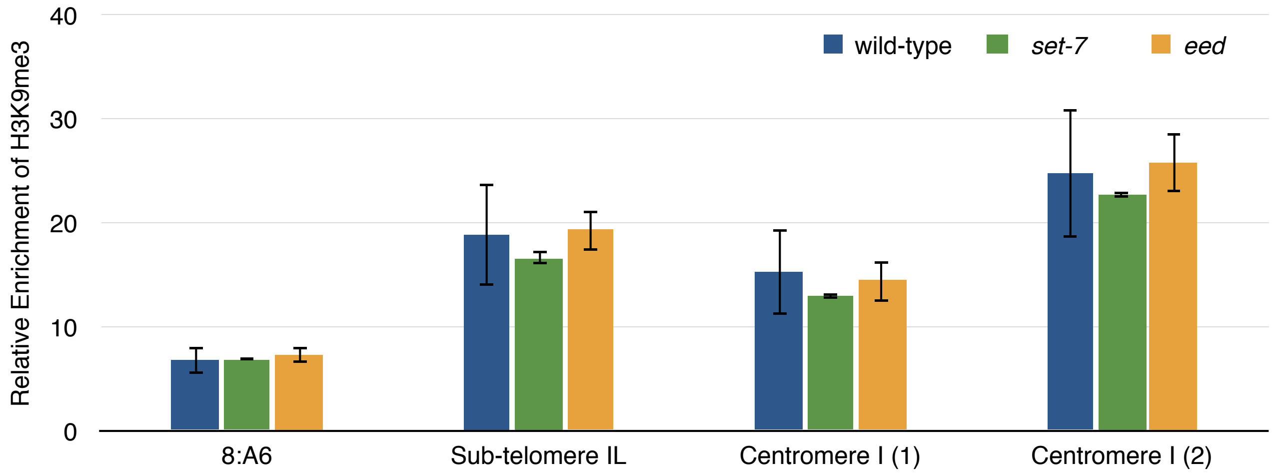
wild-type
hpo
8:A6
Subtelomere IL
Centromere I (1)
Centromere I (2)


B
Relative Enrichment of H3K27me2/3
50
40
30
20
10
0
wild-type
set-7
delta *hpo; his-3::hpo*
delta *hpo; his-3::hpo delta* CD
Sub-telomere IL
Sub-telomere IR
NCU06955
NCU09590

Supplemental Figure S7. (A) Analysis of H3K9me3 by ChIP-qPCR in histone H3 mutants, *hH3*^{R8A} and *hH3*^{S10A}, and wild-type at four loci: 8:A6, a region of constitutive heterochromatin on sub-telomere IL and two loci located within centromere I. Relative enrichment of H3K9me3 was calculated as described in Supplemental Fig. S2. (B) ChIP-qPCR measurements of H3K27me2/3 in two histone H3 mutants, *hH3*^{R8A} and *hH3*^{S10A}, and in a wild-type strain at regions of facultative heterochromatin on sub-telomere IL and IR as well as two genes typically enriched for H3K27me2/3, NCU06955 and NCU09590.


A
Relative Enrichment of H3K9me3
30
20
10
0
wild-type
$hH3^{R8A}$
$hH3^{S10A}$
8:A6
Sub-telomere IL
Centromere I (1)
Centromere I (2)
B
Relative Enrichment of H3K27me2/3
15
10
5
0
wild-type
$hH3^{R8A}$
$hH3^{S10A}$
Sub-telomere IL
Sub-telomere IR
NCU06955
NCU09590

Supplemental Figure S8. H3K9me3 is not significantly influenced by absence of H3K27me2/3. ChIP-qPCR to measure the relative enrichment of H3K9me3 in *set-7*, *eed* and wild-type strains. The relative enrichment of H3K9me3 was analyzed at 8:A6, a region of constitutive heterochromatin on sub-telomere IL and at two loci located within centromere I.


Relative Enrichment of H3K9me3
40
30
20
10
0
wild-type
set-7
eed
8:A6
Sub-telomere IL
Centromere I (1)
Centromere I (2)

Supplemental Table 1. List of strains

Strain	Genotype	Experiment	Reference
N1850	*mat a; delta dim-2::hph*$^{+}$	ChIP	(Kouzminova and Selker 2001)
N1851	*mat A; delta dim-2::hph*$^{+}$	ChIP	(Kouzminova and Selker 2001)
N2140	*mat A; pan-1; dim-5* ^{HT1}*, leu-2*	ChIP	(Tamaru and Selker 2001)
N3064	*mat A; delta dmm-1::hph*$^{+}$	ChIP	(Honda et al. 2010)
N3135	*mat a; delta cdp-2::hph*$^{+}$	ChIP	(Honda et al. 2012)
N3169	*mat a; delta cul4::hph*$^{+}$	ChIP	(Lewis et al. 2010a)
N3403	*mat a; delta dim-8::hph*$^{+}$	ChIP	(Lewis et al. 2010a)
N3411	*mat a; delta dim-9::hph*$^{+}$	ChIP	(Lewis et al. 2010a)
N3474	*mat a; sad-1 his-3*$^{+}$*::hH3*^{S10A} *; hH3*^{RIP1}	ChIP	(Adhvaryu et al. 2011)
N3480	*mat A*; *pan-2::hpo-sgfp*	ChIP	(Adhvaryu et al. 2011)
N3481	*mat a*; *sad-1 his-3*$^{+}$*::hH3*^{S10A} *; hH3*^{RIP1}*; pan-2*$^{+}$*::hpo*$^{+}$*-sgfp*$^{+}$	ChIP	(Adhvaryu et al. 2011)
N3552	*mat A*; *rid*^{RIP4}*; his-3*$^{+}$*::hH3*^{R8A}*; hH3*^{RIP1}*; pan-2*$^{+}$*::hpo*$^{+}$*-sgfp*$^{+}$	ChIP	(Adhvaryu et al. 2011)
N3542	*mat A; rid*^{RIP4} *his-3*$^{+}$*::hH3*^{R8A} *; hH3*^{RIP1} *; pan-2*$^{+}$*::hpo*$^{+}$*-sgfp*$^{+}$	ChIP	(Adhvaryu et al. 2011)
N3610	*mat a; delta hda-1::hph*$^{+}$	ChIP	(Honda et al. 2012)
N3612	*mat a; delta chap::hph*$^{+}$	ChIP	(Honda et al. 2012)
N3752	*mat A*	ChIP, Southern blot	(Jamieson et al. 2013)
N3856	*mat a; delta dim-7::hph*$^{+}$*; dfh-1*	ChIP	(Lewis et al. 2010b)
N3944	*mat A; delta dim-5::bar*$^{+}$	ChIP	(Lewis et al. 2010a)
N4718	*mat a*; *delta set-7::hph*$^{+}$	ChIP	(Jamieson et al. 2013)
N4719	*mat A*; *delta eed::hph*$^{+}$	ChIP	(Jamieson et al. 2013)
N4729	*mat a*; *delta set-7::bar*$^{+}$	ChIP	This study
N4922	*mat a*; *delta hpo::hph*$^{+}$	ChIP	This study
N5375	*mat A*; *dim-7*^{L448*}*; am-132; inl*	Race tubes	This study
N5542	*mat a*; *delta set-7::hph*$^{+}$*; delta dim-5::bar*$^{+}$	Race tubes	This study
N5543	*mat a*; *delta set-7::hph*$^{+}$*; delta dim-5::bar*$^{+}$	Race tubes	This study
N5544	*mat a*; *delta set-7::hph*$^{+}$*; delta dim-5::bar*$^{+}$	Race tubes	This study
N5546	*mat a*; *delta set-7::hph*$^{+}$*; delta dim-5::bar*$^{+}$	Spot tests	This study

N5867	*mat A*; *CenVIR::bar, trp-2, delta hpo::hph, his-3::Phpo::hpo* WT-10xGly-HAT-3xFLAG	ChIP	This study
N5869	*mat A; CenVIR::bar, trp-2, delta hpo::hph, his-3::Phpo::hpo* delta CD-10xGly-HAT-3xFLAG	ChIP	This study
N5904	*mat a*	Race tubes	This study
N5905	*mat A*	Race tubes	This study
N5906	*mat A*	Race tubes	This study
N5907	*mat A*	Race tubes	This study
N5908	*mat a*; *delta set-7::hph*$^{+}$	Race tubes	This study
N5909	*mat a*; *delta set-7::hph*$^{+}$	Race tubes	This study
N5910	*mat a*; *delta set-7::hph*$^{+}$	Race tubes	This study
N5911	*mat a*; *delta set-7::hph*$^{+}$	Race tubes	This study
N5912	*mat a*; *delta dim-5::bar*$^{+}$	Race tubes	This study
N5913	*mat a*; *delta dim-5::bar*$^{+}$	Race tubes	This study
N5914	*mat A*; *delta dim-5::bar*$^{+}$	Race tubes	This study
N5915	*mat A*; *delta dim-5::bar*$^{+}$	Race tubes	This study
N5916	*mat A*; *delta set-7::hph*$^{+}$; *delta dim-5::bar*$^{+}$	Race tubes	This study
N5936	*mat a*	Race tubes	This study
N5937	*mat a*	Race tubes	This study
N5938	*mat A*	Race tubes	This study
N5939	*mat A*; *delta set-7::bar*$^{+}$	Race tubes	This study
N5940	*mat A*; *delta set-7::bar*$^{+}$	Race tubes	This study
N5941	*mat A*; *delta set-7::bar*$^{+}$	Race tubes	This study
N5942	*mat A*; *delta set-7::bar*$^{+}$; *delta dim-7::hph*$^{+}$	Race tubes	This study
N5943	*mat A*; *delta set-7::bar*$^{+}$; *delta dim-7::hph*$^{+}$	Race tubes	This study
N5944	*mat A*; *delta set-7::bar*$^{+}$; *delta dim-7::hph*$^{+}$	Race tubes	This study
N5945	*mat a*; *delta dim-7::hph*$^{+}$*; dfh-1*	Race tubes	This study
N5946	*mat A*; *delta dim-7::hph*$^{+}$*; dfh-1*	Race tubes	This study
N5947	*mat a*; *delta dim-7::hph*$^{+}$*; dfh-1*	Race tubes	This study
N5948	*mat A; delta set-7::bar*$^{+}$; *delta hpo::hph*$^{+}$	Race tubes	This study
N5949	*mat A; delta set-7::bar*$^{+}$; *delta hpo::hph*$^{+}$	Race tubes	This study
N5950	*mat A; delta set-7::bar*$^{+}$; *delta hpo::hph*$^{+}$	Race tubes	This study
N5951	*mat A; delta set-7::bar*$^{+}$; *delta hpo::hph*$^{+}$	Race tubes	This study
N5953	*mat a*; *delta hpo::hph*$^{+}$	Race tubes	This study

N5954	*mat A; delta set-7::bar*$^{+}$; *delta dim-9::hph*$^{+}$	Race tubes	This study
N5955	*mat A; delta set-7::bar*$^{+}$; *delta dim-9::hph*$^{+}$	Race tubes	This study
N5956	*mat A; delta set-7::bar*$^{+}$; *delta dim-9::hph*$^{+}$	Race tubes	This study
N5957	*mat A; delta set-7::bar*$^{+}$; *delta dim-9::hph*$^{+}$	Race tubes	This study
N5958	*mat a*; *delta dim-9::hph*$^{+}$	Race tubes	This study
N5959	*mat a; delta dim-9::hph*$^{+}$	Race tubes	This study
N6023	*mat A; delta hpo::hph*$^{+}$	Race tubes	This study
N6033	*mat a; delta dim-9::hph*$^{+}$	Race tubes	This study
N6034	*mat a*; *delta dim-9::hph*$^{+}$	Race tubes	This study
N6139	*mat a*; *csr-1::dim-7*$^{+}$; *delta dim-7::hph*$^{+}$*; dfh-1*	ChIP, Southern blot	This study
N6140	*mat a*; *csr-1::dim-7*$^{+}$; *delta dim-7::hph*$^{+}$*; dfh-1*	ChIP, Southern blot	This study
N6149	*mat a*; *delta set-7::bar*$^{+}$; *dim-7*^{L448*}*; am::hph::am; inl*	Race tubes	This study
N6150	*mat a*; *delta set-7::bar*$^{+}$; *dim-7*^{L448*}*; am::hph::am; inl*	Race tubes	This study
N6151	*mat a*; *delta set-7::bar*$^{+}$; *dim-7*^{L448*}*; am::hph::am; inl*	Race tubes	This study
N6152	*mat a*; *delta set-7::bar*$^{+}$; *dim-7*^{L448*}*; am::hph::am; inl*	Race tubes	This study
N6153	*mat a*; *dim-7*^{L448*}*; am::hph::am; am-132; inl*	Race tubes	This study
N6154	*mat a*; *delta set-7::bar*$^{+}$; *dim-7*^{L448*}*; am::hph::am; inl*	Race tubes	This study

Supplemental Table 2. List of primers

Primer	Description	Sequence (5' to 3')	Reference
1877	8:A6 FP	TGGTTGGTCGATTGTGGTGG	(Honda and Selker 2008)
1878	8:A6 RP	TTTTGAGGATCCGCCATCCG	(Honda and Selker 2008)
2972	Gene 1 NCU06955 FP	GTCTTCGGGCATGGGTATAA	(Jamieson et al. 2013)
3004	Gene 1 NCU06955 RP	GATCAATCCTCTCGACTGGG	(Jamieson et al. 2013)
2977	Gene 2 NCU09590 FP	AGCATCCTCCACTGAGCACT	(Jamieson et al. 2013)
3006	Gene 2 NCU09590 RP	TCGAGTTTGGTAAGTGCTGTT	(Jamieson et al. 2013)
3565	Sub-tel IL NCU10129 FP (Facult. Het.)	AGCGTTCAAATGCCGTGACCTGT	(Jamieson et al. 2013)
3566	Sub-tel IL NCU10129 RP (Facult. Het.)	AGTCCAATGGTGCTAACGGCGA	(Jamieson et al. 2013)
3908	Sub-tel IR NCU10130 FP (Facult. Het.)	GACGGACCTCTTCCGCTCGC	(Jamieson et al. 2013)
3909	Sub-tel IR NCU10130 RP (Facult. Het.)	CCCTGCACGAGACGGTTCGA	(Jamieson et al. 2013)
3998	*hh4* NCU01634 FP	CATCAAGGGGTCATTCAC	(Jamieson et al. 2013)
3999	*hh4* NCU01634 RP	TTTGGAATCACCCTCCAG	(Jamieson et al. 2013)
4020	8:A6 FP	AGCGCGCGTTGACCCTAACC	This study
4021	8:A6 RP	GTGCATCCACAGCGACCCCT	This study
4038	Sub-tel IL FP (Constit. Het.)	TCCCCAACGCCTTAAATCGCCT	This study
4039	Sub-tel IL RP (Constit. Het.)	TGGGATGCCGTTTGGGTTGCT	This study
4082	*hh4* NCU01634 FP	CATCAAGGGGTCATTCAC	This study, see also 3998
4083	*hh4* NCU01634 RP	TTTGGAATCACCCTCCAG	This study, see also 3999
4218	Centromere I (1) FP	CCGGGTTGGTTTAGTTAATTATTGC	This study
4219	Centromere I (1) RP	ACCGAACTTTATACCCACTTACA	This study
4220	Centromere I (2) FP	GGAGGCCGTAGTAACCAGTG	This study
4221	Centromere I (2) FP	CTTGACTGGGGCGTCGTTAT	This study
4947	Sub-tel IL NCU10129 FP (Facult. Het.)	AGCGTTCAAATGCCGTGACCTGT	This study, see also 3565
4948	Sub-tel IL NCU10129 RP (Facult. Het.)	AGTCCAATGGTGCTAACGGCGA	This study, see also 3566
5250	Centromere III (1) FP	CGAGTATCCGAACCGGAAGGAG	This study
5251	Centromere III (1) RP	CCGAATCCGGCTCAGTACAGC	This study
5252	Centromere III (2) FP	CGATATTACCCTCCTAGCAGTTCCC	This study

5253	Centromere III (2) RP	GGGAGTAACCGGACTATAGATCG	This study

Supplemental Methods

Isolation of genomic DNA and Southern blotting

The isolation of genomic DNA was modified from (Oakley et al. 1987). Southern blotting were performed as described in (Miao et al. 2000). Primers for the 8:A6 probe are listed in Supplemental Table 2.

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