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Supplemental Information

Timing of Transcriptional Quiescence during Gametogenesis Is Controlled by Global Histone H3K4 Demethylation

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Inventory of Supplemental Information

- Figure S1 supports main Figure 1
  - Expression microarray of WT and *jhd2*Δ vegetative cells
  - Additional quantitative western blots showing H3K4me dynamics during sporulation in WT and *jhd2*Δ cells
- Figure S2 supports main Figure 2
  - Additional western blots that support Figure 2B-C
  - Global microarray data related to Figure 2D
  - Additional RT-qPCR data from loci not included in Figure 2F
- Figure S3 supports main Figure 3
  - The Histone H3 K4A mutant is epistatic to *JHD2* in meiotic timing
- Figure S4 supports main Figure 4
  - TTS H3K4me3 signal for genes that have converging 3' ends
  - A biological replicate of Figure 4D, with RNA signals normalized to rRNA
- Figure S5 supports main Figure 5
  - Statistical analyses that show that the depth of the average NDR changes significantly during sporulation
- Figure S6 supports main Figure 6
  - Analysis of RP genes from Figure 6 is extended to all Rap1-bound genes

- Table S1: Expression microarray data for Figure S1A. Supports Figure 1D

- Table S2: Expression microarray data for Figures 2D-E and S2C-D

- Table S3: Chromosome III ChIP chip data for Fig 4A-B and S4A-B

- Supplemental Experimental Procedures: Yeast Strains

- Supplemental Experimental Procedures: Oligos

Supplementary Figures

Xu *et al*. Fig S1

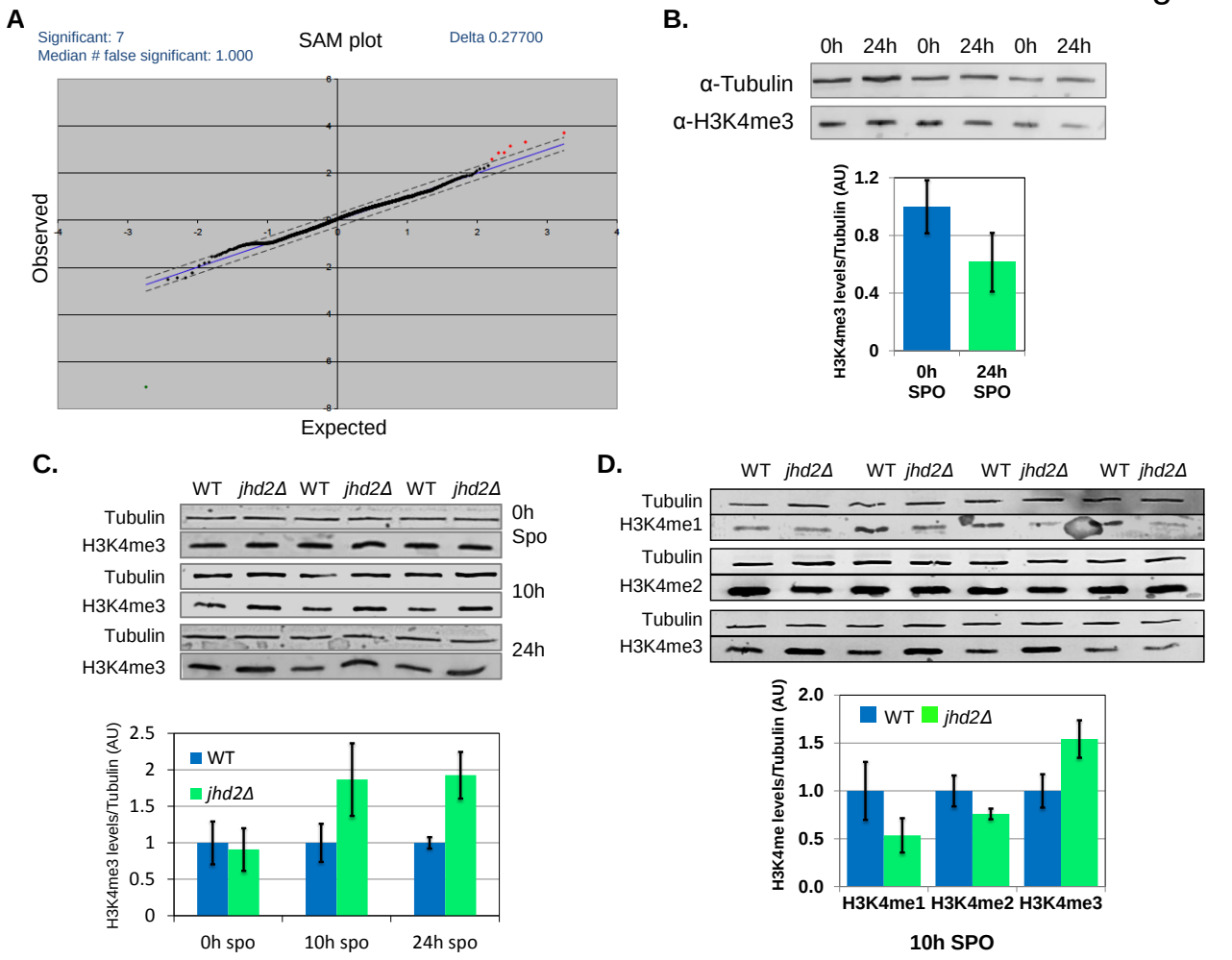

Figure S1. *JHD2*-mediated control of H3K4me in vegetative and sporulating cells.

(A) Significance Analysis of Microarrays (SAM) was used to identify misregulated genes in *jhd2Δ* compared with a WT isogenic strain. Red and green dots represent significantly UP and DOWN regulated genes, respectively. The only green dot is the *JHD2* transcript, which is absent from the *jhd2Δ* strain. The false discovery rate cutoff was set to 10%. See also Table S1. **(B)** Western blot quantification of H3K4 me3 levels in WT cultures at 0h and 24h of sporulation from three independent sporulation cultures. In the graph, H3K4 me3 signals were normalized to tubulin, and then to the average

signal at 0h of sporulation. Error bars represent one standard deviation from three biological replicates. **(C)** Western blot quantifications of bulk H3K4 me3 levels in WT and *jhd2Δ* cultures at 0h, 10h, and 24h of sporulation. In the graph, H3K4 me3 signals were normalized to tubulin and *jhd2Δ* signals are normalized to WT signal at each timepoint. Error bars represent one standard deviation from three biological replicates. **(D)** Western blot quantifications of bulk H3K4 me1, me2, and me3 levels in WT and *jhd2Δ* cultures at 10h of sporulation. Data was graphed as in **(C)**. Error bars represent one standard deviation from four biological replicates.

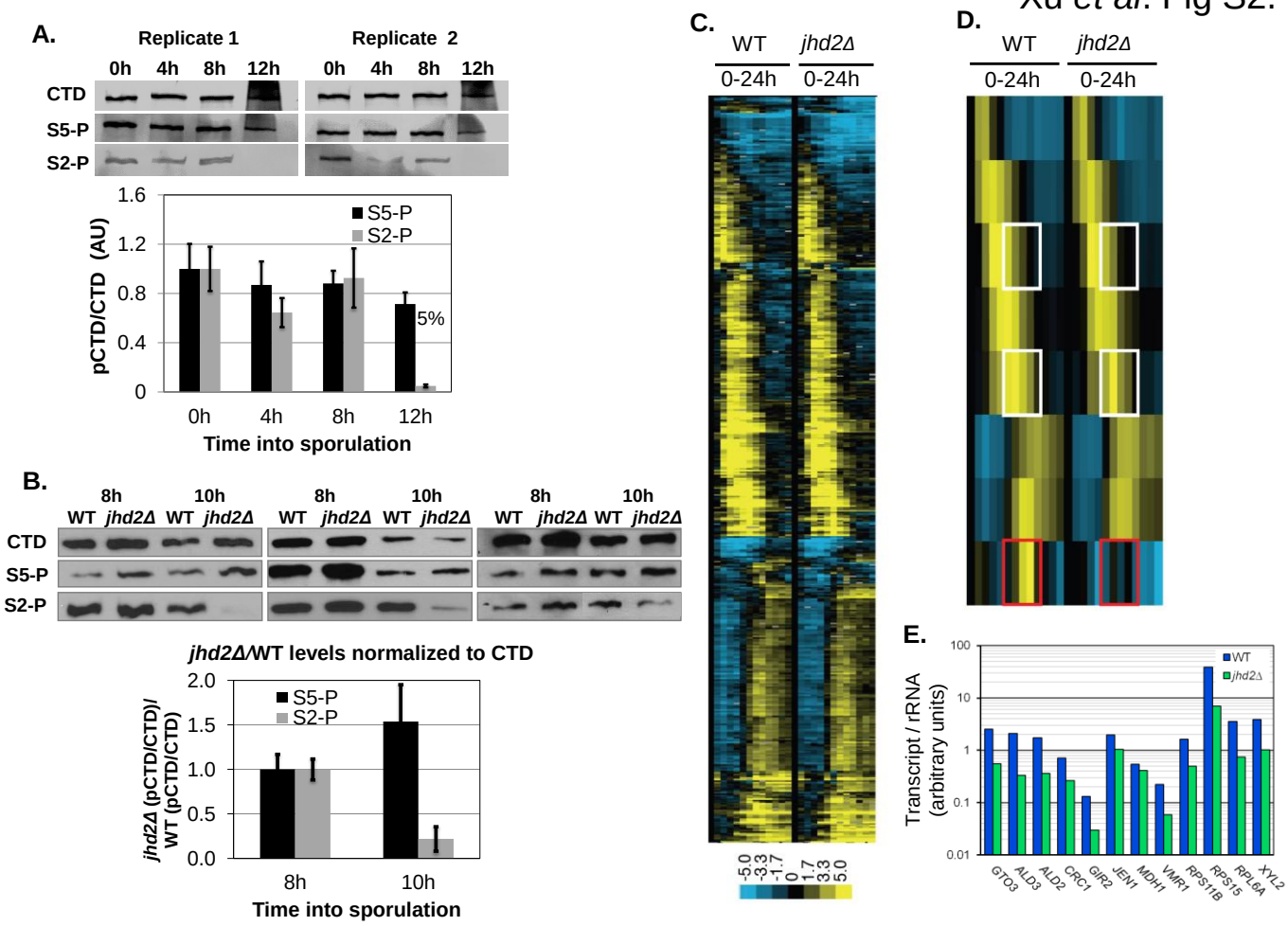

Figure S2. *JHD2* promotes global transcription in opposition to programmed quiescence during sporulation. (A) Quantitative western blots of bulk RNA pol II CTD (CTD), CTD heptad repeat Serine 2 phosphorylated Pol II (S2-P), and Serine 5 phosphorylated Pol II (S5-P) levels in WT cultures collected at 0h, 8h, 10h, and 12h of sporulation. Two biological replicates are shown. The bar graph is plotted as described in **Figure 2(C).** Error bars represent one standard deviation. **(B)** Quantitative western blots as described in **(A)**, performed on WT and *jhd2Δ* cultures at 8h and 10h of sporulation, in three biological replicates. The bar graph is plotted as described before.

(C) Sporulation microarray data were filtered and hierarchically clustered. Data are depicted as a heat map. **(D)** The same data shown in **(C)** was analyzed using Self Organizing Maps. The red boxes highlight a gene cluster that is specifically induced in WT, but not in *jhd2Δ*, at 10-18 hours. White boxes highlight classes of genes that are induced normally in *jhd2Δ* during mid-late meiosis but show reduced transcript abundance during the 10-18 hour window of spore differentiation. **(E)** RT-qPCR was used to measure transcript abundance of the indicated genes at 10h of sporulation.

Xu *et al*. Fig S3.

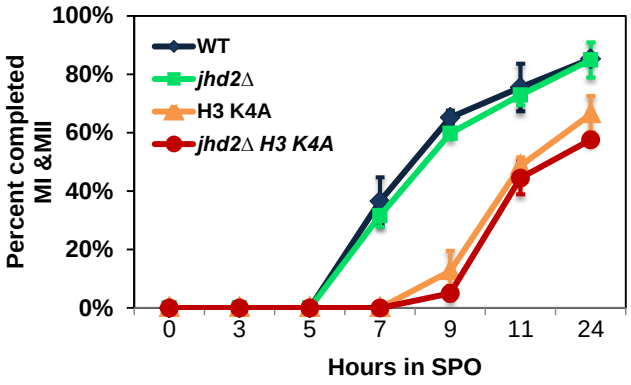

Figure S3. Meiotic progression of cultures of the genotypes indicated was quantified as percent of cells that have completed meiosis I or meiosis I and II. Error bars indicate one standard deviation from three biological replicates.

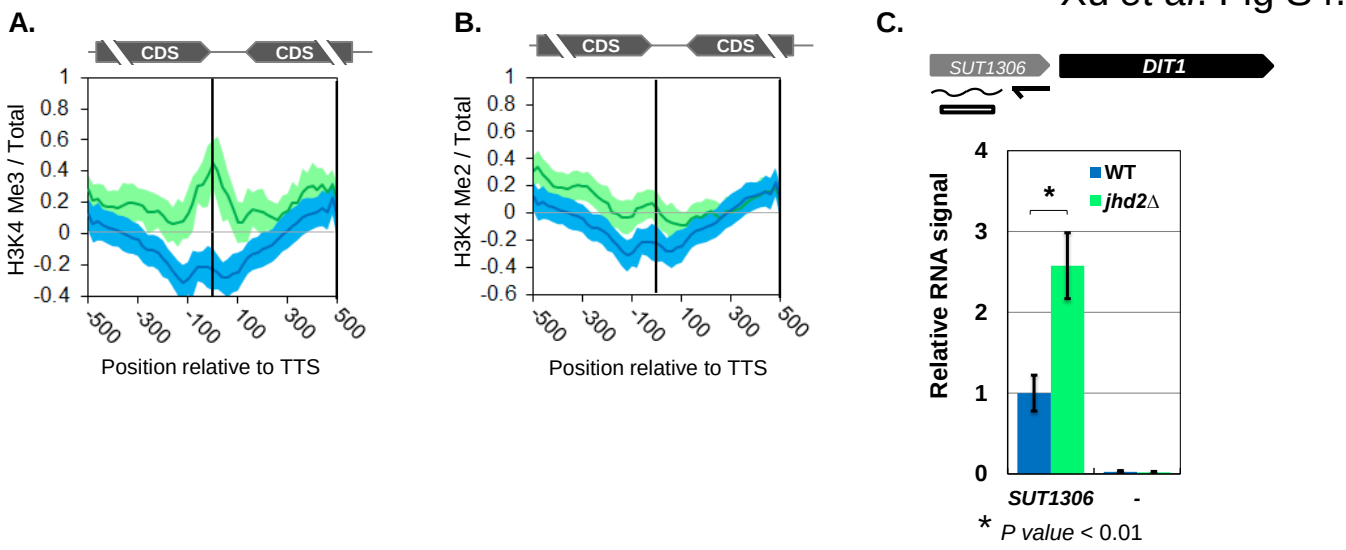

Figure S4. Accumulation of H3K4me signal at 3' ends of genes in *jhd2Δ* cells is not due to H3K4me deposition from downstream coding gene transcription. ChIP chip was performed as described in **Figure 4 (A)** & **(B)**. Shown are TTS-centered average profiles of H3K4 me3 **(A)** and me2 **(B)** ChIP signal from genes that have converging 3' ends (54 genes). Error-"clouds" represents one standard error. **(C)** RT q-PCR was performed with transcript-specific primers to measure levels of *SUT1306* RNA and *DIT1 m*RNA in WT and *jhd2Δ* cells at 10h of sporulation. RNA signals were normalized to *RDN25-1* rRNA, and shown as relative abundance compared to WT *SUT1306* transcript levels. Negative control reaction "no RT" was identical to RT, except no reverse transcriptase enzyme was used. Error bars represent one standard deviation of technical replicates. * $P < 0.01$.

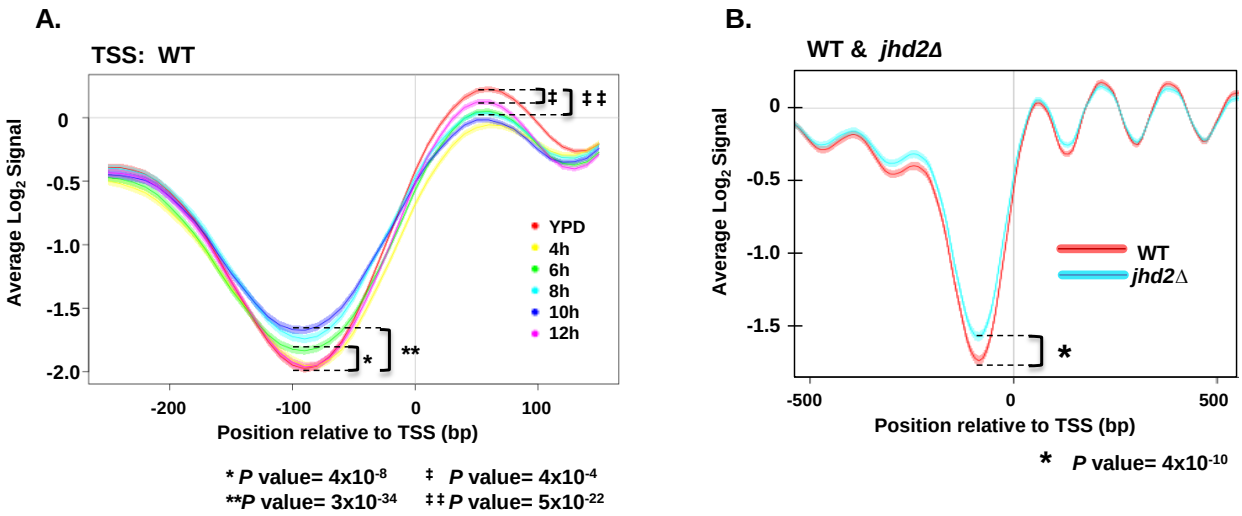

Figure S5. Global mean NDR and +1 nucleosome occupancy signals change significantly over the course of sporulation. Global average signals from 5147 genes are shown with 95% confidence intervals (±97.5%) (represented by colored clouds). *P* values from two-component t-tests are shown. **(A)** During sporulation in WT cells, global mean nucleosome occupancy at the 5' NDR and +1 nucleosome becomes significantly different from the basal state observed in YPD. **(B**) TSS-proximal NDR occupancy is significantly increased in *jhd2*Δ cells at 8h into sporulation.

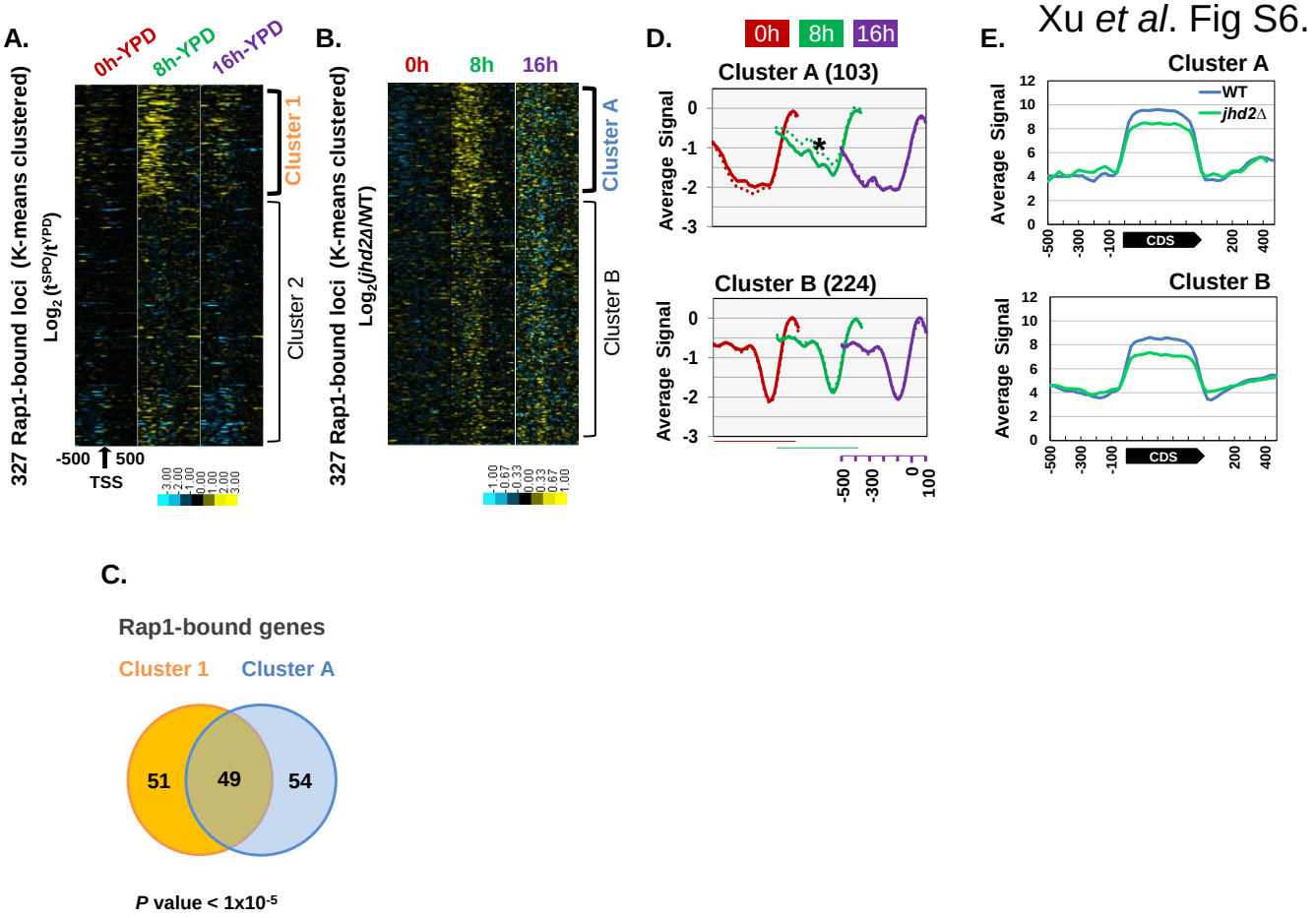

Figure S6. *JHD2* opposes Transcriptional Interference that Targets Rap1-bound genes and TSS-associated nucleosome accumulation in post-meiotic cells. (A) Log_2 (t^{SPO}/t^{YPD}) signal of 326 Rap1-bound genes (Rhee & Pugh 2011) (RP genes were excluded) was K-means clustered into two groups (Cluster 1 and Cluster2) using a Euclidian distance matrix. **(B)** Log_2(*jhd2*Δ/WT) signal of the same genes as in **(A)** were independently K-means clustered into two groups (Cluster A and Cluster B). **(C)** There is significant overlap of genes in Cluster 1 and Cluster A. *P*-value from the hypergeometric distribution test is shown. **(D)** Shown are the average nucleosome occupancy signals of the genes in Cluster A and Cluster B, respectively. Timepoints in

sporulation are represented by color as shown in the color-key. For clarity, data from different sporulation timepoints are shown offset from each other, with the x-axes for each timepoint shown underneath. * *P value* <0.05. **(E)** The average normalized Affymetrix RNA transcript signals at 20h of sporulation for clusters in **(D)** are shown. Signal associated with coding region are scaled into 20 bins. An additional 500bp of flanking signals are shown on each side of the CDS.

Table S1. Expression microarray data for vegetative WT and *jhd2*Δ cells

See accompanying Excel file

Contains the $\log_2$ normalized *jhd2*Δ/WT ratio transcript signal for each gene.

Table S2. Sporulation timecourse expression microarray data for WT and *jhd2*Δ

See accompanying Excel file

Contains the $\log_2$ normalized transcript signal for WT and *jhd2*Δ cells at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24h into the sporulation program.

Table S3. Tiling resolution H3K4me ChIP chip data

See accompanying Excel file

Contains tiling resolution H3K4 3Me, and 2Me ChIP signal from Chromosome III, for WT and *jhd2*Δ cells at 10h of the sporulation program.

Supplemental Experimental Procedures: Yeast Strains

Strain	Background	Genotype
MMY718	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1*
MMY1879	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 jhd2Δ::Nat/jhd2Δ::Nat*
MMY1622	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 JHD2(H427A)/JHD2(H427A)*
MMY1657	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 JHD2(H427A)/JHD2(H427A) SPS2-GFP::Hyg/SPS2-GFP::Hyg*
MMY1662	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 SPS2-GFP::Kan/SPS2-GFP::Kan*
MMY1898	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 HTA2-GFP::Kan/ HTA2*
MMY1996	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 jhd2Δ::Nat/jhd2Δ::Nat HTA2-mCherry::Hyg/ HTA2*
MMY1995	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 HTA2-mCherry::Hyg/HTA2*
MMY3032	SK1	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 jhd2Δ::Nat/jhd2Δ::Nat HTA2-GFP::Kan/ HTA2*
MMY2773	SK1	*MATa/α ura3/ura3 trp1/trp1 hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN Plasmid HHT2-HHF2 CEN ARS TRP1 HTA2-GFP::KAN/HTA2*
MMY2777	SK1	*MATa/α ura3/ura3 trp1/trp1 jhd2Δ::NAT/jhd2Δ::NAT hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN Plasmid HHT2-HHF2 CEN ARS TRP1 HTA2-GFP::KAN/HTA2*
MMY2781	SK1	*MATa/α ura3/ura3 trp1/trp1 jhd2Δ::NAT/jhd2Δ::NAT hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN Plasmid HHT2-HHF2 CEN ARS TRP1 HTA2-mCherry::KAN/HTA2*
MMY2826	SK1	*MATa/α ura3/ura3 trp1/trp1 hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN Plasmid HHT2(K4A)-HHF2 CEN ARS TRP1 HTA2-GFP::KAN/HTA2*
MMY2828	SK1	*MATa/α ura3/ura3 trp1/trp1 hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN*

		Plasmid HHT2(K4A)-HHF2 CEN ARS TRP1
		HTA2-mCherry::KAN/HTA2
MMY2829	SK1	*MATa/α ura3/ura3 trp1/trp1 jhd2Δ::NAT/jhd2Δ::NAT*
		hht1-hhf1::KAN/hht1-hhf1::KAN hht2-hhf2::KAN/hht2-hhf2::KAN
		Plasmid HHT2(K4A)-HHF2 CEN ARS TRP1
		HTA2-mCherry::KAN/HTA2

Supplemental Experimental Procedures: **Oligos**

Primer set	Oligo sequences
Q-PCR primers	
CIT2	GCGAGGTTCCAACTCAAG / GCAAGTCTAGTGAATCTTCAAA
ICL1	GGACACCGTTCCAAACAAAG / GGGTATAACACATCTTCCTGCCA
ACO1	GCCTCTGCCACTGCGAAAT / TGACAGTTGTGATACCAGCCA
DIT1	TTCCTGACAAAGGCGAAGAA / ATCGTTCAATCCACAAAAGC
PEX18	AACAACGGGCTACGGACATC / ATTTTCCTCTTCTGTATGGACATCTTC
UGO1	CGATTCTTTCAATGCTGGGTA / TTTCGCCATTACTTCCCTCC
RDN25	TGCGATGGTCAGAAAGTGATG / ATTCTGCCAAGCCCGTTC
ALD3	CAAACCTGCTGAAAATACCTCT / TTCAAATACAAGAGCAGGAGACT
MRPL32	CCATTTGAATAAGTGCCCATC / TCCTTGGTATATTTGGTATCTCTTC
GFA1	GCTGGTCCTGAAATTGGTGT / TCAACTCACCTGCCAAAACA
HXT3	CGGCCATGTTTAAGCGTACT / CATACAGTTACCAGCACCCTTG
GTO3	ATTCAAAAGGCAGTCATCGC / TCAAGGAATCGCCAGCC
ALD2	CATCAAACCTGCTGAGAATACCT / CAGGAGACTTACCACCGCATT
CRC1	GGTGTTATCCCGCCCTTATT / CGCCTGAATGAAAGAGCCC
VMR1	GCCGAGTGCGATTTAGTGAAC / CGTTAGTCCACCATGATTTTTAG
RPS11B	GGATTCAAGACCCCAAAGACC / ACGTTGAATCTAACAGTCTTGGAG
RPS15	CTGAAGATTTCGTCAAGTTGGC / ACTGGGGTGTAAGTAATGGAGAA
RPL6A	CTAAAGAAGACCAGAAAGGCTGC / TGGACCAGAAATCAACAAAGTG
XYL2	TGCCACCCACATCGTCA / ACAGTATCGGAAACAGCCTTG
GIR2	AAGGAAGAACAGAAGCAGGAACT / GTTGTCATTGAGGGCTACTTCTTG
JEN1	GCACATCCACGAGTTTTCTTG / AACACCAATCCCAACCCC
MDH1	TGTTGCCACCGATTTGTCTC / AGCATTGGGAGCGGATTC
RT-primers	for ncRNAs and associated CDSs
RPS6A ncRNA	TTG TCA CTT CTC TTA AGC AA
RPS6A CDS	GAG CTT GAG CGT TTC TGA CC
RPL10 ncRNA	GTCAGCATTATTGTGCTAAC
RPL10 CDS	TCCAAAGAACCCTTCTTGGACA

RPL11B ncRNA	CCTCTCTTAACAGTATACTTC
RPL11B CDS	ACCAAGAGACGGTGTCTTCC
RPS1B ncRNA	TCACACCGGCACCACCTTGATT
RPS1B CDS	CCGGAACCTTCACCATGCAA
Q-PCR primers	for ncRNAs and associated CDSs
RPS6A ncRNA	GAAAGTTATCTCTCCTGGTAAGCG/CAATGACGAATAAGCATTAACCAC
RPS6A CDS	CGGGTCTCAAAAGACCT/ GGC AAC AAA ACA CCT TGC TTC
RPL10 ncRNA	GTCAGCATTATTGTGCTAAC/ TATAGTAGCGGTTATTTCCG
RPL10 CDS	AGAAGGCTACCGTCGATGAA/ ACCGGCACAAGACAACATCT
RPL11B ncRNA	TCTTTTGGAACCCGCTCTGC/ CTTTAATGATGAGTTATGTCC
RPL11B CDS	ACATCTCCGTTGGTGAATCTGG/ TTGGACCTCTGACGGTAACGT
RPS1B ncRNA	AAGAGCAGCATGGATGTCCG/ ACCACCTTGATTCCACACTGGA
RPS1B CDS	AGAAGAAGGTCGTTGACCCA/ ACTCTACCCTTCAAAGCATCGG