Supporting Information

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SI Materials and Methods

Yeast Strains, Growth Conditions, and Tagging. All yeast strains used in this study are listed in Table S1. Strains described in Table S1 are isogenic to S288C, with the exception of Lea1-HA and Msl1-HA strains used in ChIP studies as described (1). All strains were propagated according to standard procedures in rich media (YPD).

Strains harboring histone H3 point mutations or truncation were obtained from Open Biosystems, as described by Dai et al. (2). The *hos3*Δ deletion strain was created by first replacing the KanMX4 marker in *HOS3::KanMx4* with the CLONAT resistance gene using the "marker swap" method (3), and then crossing the *hos3Δ* strain with *hos2Δ* to generate the *hos3Δ hos2Δ* double mutant. CLONAT- and Kan-resistant colonies were examined by genomic PCR to confirm the genotype.

Gcn5-13XMyc was constructed using a PCR-based method for tagging chromosomal genes by yeast transformation (4). The pFA6a-13Myc-His3MX6 plasmid was used as a template, and transformants were selected on synthetic complete medium lacking histidine (SC-His medium). His^+ colonies were analyzed by colony PCR to verify the presence of the tag in the gene of interest and by Western analysis to verify expression of the tagged protein.

Standard methods for mating, sporulation, transformations, and tetrad analysis were used as described in *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual* (5). The genotype of each viable spore was confirmed by PCR.

Quantitative RT-PCR. Quantitative PCR was performed using an ABI Prism 7700 Sequence Detector as described previously (1). Complementary DNA was diluted 1:20 and 1 μL of this was used in a 25-μL reaction volume. Reactions consist of 12.5 μL SYBR GREEN Master Mix (Applied Biosystems), 1 μM primers. All samples were run in triplicate for each independent experiment. Primers were designed to amplify unspliced (precursor) message using intron-specific primer and total message amplification using primers specific to exon 2. Primer sequences were previously described (1).

For quantification, standard curves using wild-type genomic DNA were generated for each primer set. To calculate ratio of precursor to total RNA, the amount of unspliced (precursor) transcript was divided by the total amount of transcript (both spliced and unspliced). Data were then normalized to wild-type. Data are represented as a fold-increase in the ratio of precursor/total relative to wild-type. Error bars represent the SEM.

1. Gunderson FQ, Johnson TL (2009) Acetylation by the transcriptional coactivator Gcn5 plays a novel role in co-transcriptional spliceosome assembly. *PLoS Genet* 5:e1000682.
2. Dai J, et al. (2008) Probing nucleosome function: A highly versatile library of synthetic histone H3 and H4 mutants. *Cell* 134:1066–1078.
3. Voth WP, Jiang YW, Stillman DJ (2003) New 'marker swap' plasmids for converting selectable markers on budding yeast gene disruptions and plasmids. *Yeast* 20:985–993.
4. Longtine MS, et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* 14:953–961.
5. Amberg D, et al. (2005) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

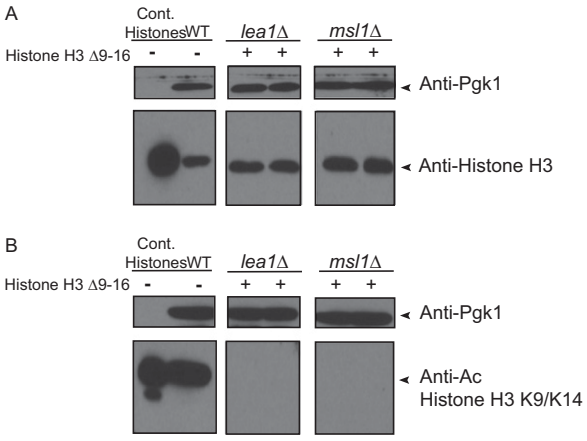

Fig. S1. Protein expression analysis of histone H3 truncation mutant used in this study. (*A*) Protein immunoblot of either wild-type cells (second from the left lane) or strains harboring histone H3 N-terminal tail truncation (Δ9–16) and *MSL1* or *LEA1* deletion (used in Fig. 1). Each strain was analyzed in duplicate. Cells were grown in YPD media to the same OD_{600} (between 0.6 and 0.8), and whole-cell extracts were prepared from equivalent cell numbers (*SI Materials and Methods*). As a control, acid-treated histones purified from HeLa cells were loaded (Millipore). Proteins were resolved on a 15% SDS/PAGE and probed with Anti-Histone H3 CT PAN (Upstate/Millipore) or Anti-Pgk1 (Invitrogen) as a loading control. (*B*) Protein immunoblot of acetylated histone H3 in truncation mutant in the absence of *MSL1* or *LEA1*. Lysates were treated as in *A*, except using the following antibodies: anti-acetylated Histone H3 K9/14 (Upstate/Millipore) and anti-Pgk1 (Invitrogen) as a loading control.

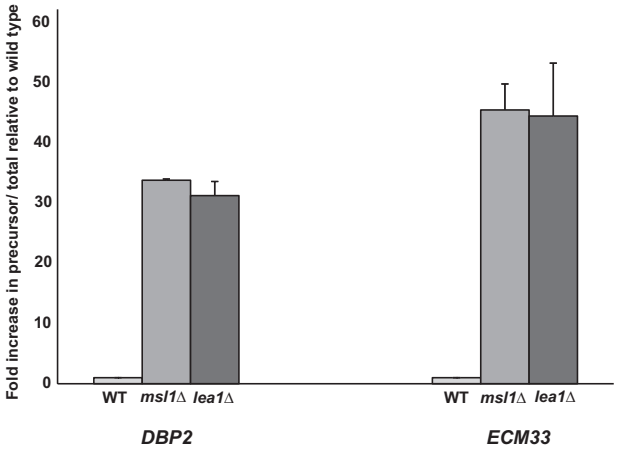

Fig. S2. Deletion of *MSL1* or *LEA1* alters splicing of the *DBP2* and *ECM33* transcripts. Quantitative RT-PCR of *DBP2* and *ECM33* in the absence of the genes encoding Msl1 or Lea1. Data are represented as a fold-increase in the ratio of precursor (unspliced) RNA to total message relative to wild-type. Error bars represent the SEM for at least three independent experiments.

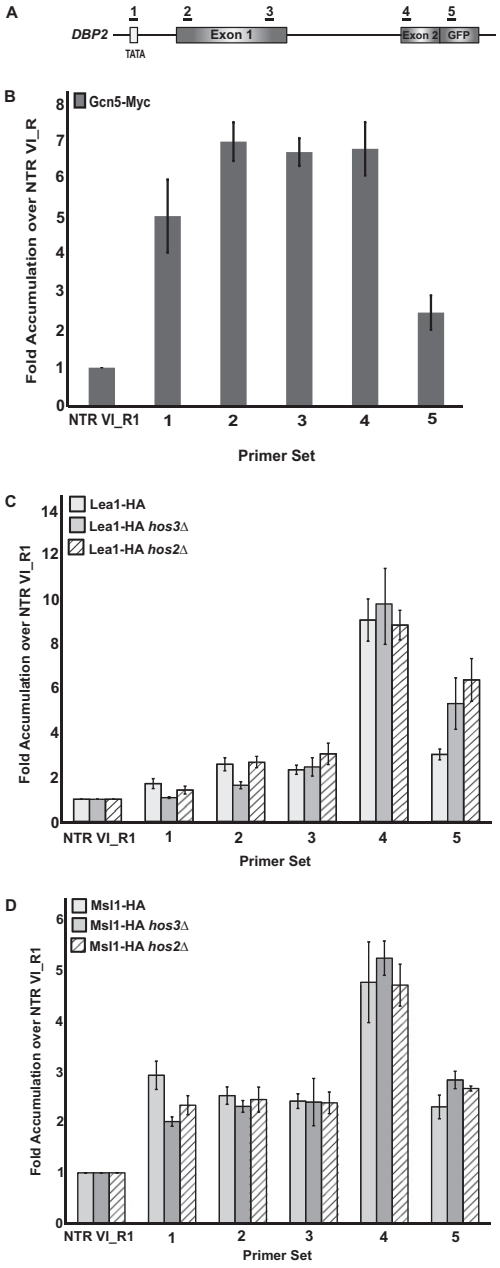

Fig. S3. Gcn5 is associated with intron-containing gene *DBP2*, but single deletion of *HOS3* or *HOS2* does not affect cotranscriptional recruitment of Lea1 or Msl1. (*A*) Schematic of the intron-containing gene *DBP2*. Underlined numbers represent the amplicons for each primer set used in the present study. (*B*) Graph depicting the recruitment of Gcn5-13xMyc to *DBP2* relative to the nontranscribed region (average of three independent experiments, ±SD). (*C*) Graph depicting the occupancy of Lea1-HA at each region of *DBP2* in the presence or absence of Hos3 or Hos2 relative to the nontranscribed control. Light gray bars depict the occupancy of Lea1 in wild-type. Dark gray bars represent Lea1-HA occupancy in the absence of Hos3, and dash marked bars represent Lea1-HA occupancy in cells lacking Hos2. (*D*) Graphs showing the occupancy of Msl1-HA at each region of *DBP2* in the presence and absence of Hos3 or Hos2 relative to the nontranscribed control. Light gray bars depic the occupancy of Msl1 in wild-type. Dark gray bars represent Msl1-HA occupancy in the absence of Hos3 and dash marked bars represent Msl1-HA occupancy in the absence of Hos2. Graphs represent the average of three independent experiments, ±SD.

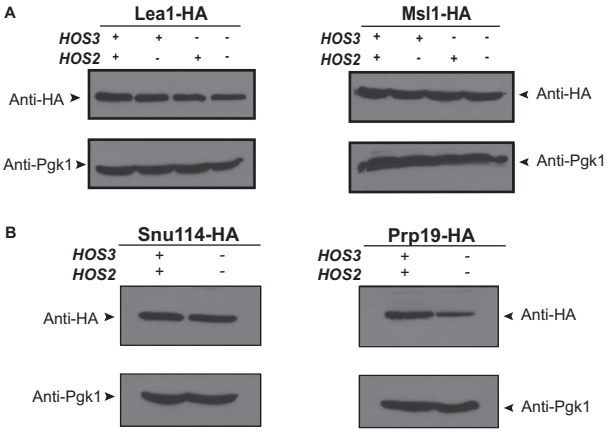

Fig. S4. Deletion of *HOS3* and *HOS2* does not alter protein levels of spliceosomal proteins used in ChIP assays. Protein Immunoblot of strains used for ChIP assays. (*A*) U2 snRNP proteins, Lea1-HA and Msl1-HA, in either wild-type or deletion of *HOS3* and *HOS2* were grown in YPD liquid medium and whole-cell extracts were prepared (*Materials and Methods*) and probed with anti-HA 12CA5 (Roche) (*Upper*). Extracts were also probed with anti-PGK1 (Invitrogen) as a loading control (*Lower*). (*B*) Protein immunoblot of U5 snRNP component, Snu114-HA, and Nineteen Complex component, Prp19-HA cells from deleted of *HOS3* and *HOS2*. Cells were treated as described in *A*.

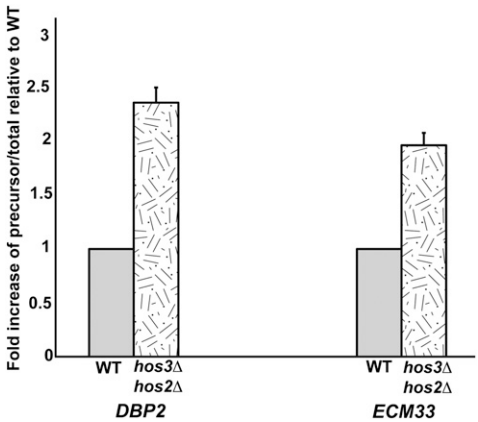

Fig. S5. Deletion of the genes encoding Hos3 and Hos2 alters the splicing of *DBP2* and *ECM33*. Quantitative RT-PCR of *DBP2* and *ECM33* in the absence of Hos3 and Hos2. Data are represented as fold-increase of the ratio of precursor (unspliced) RNA to total message relative to wild-type. Error bars represent the SEM from three independent experiments.

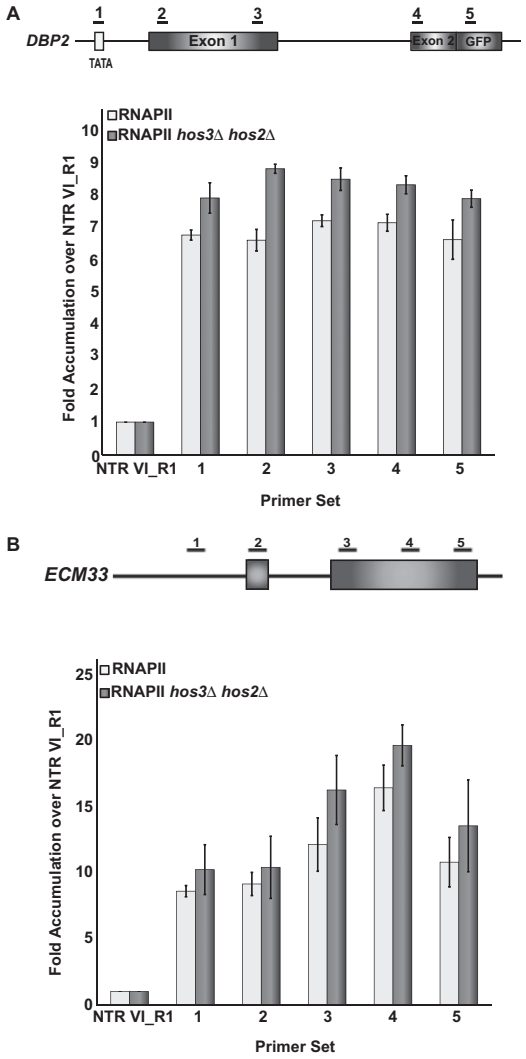

Fig. S6. RNA Pol II occupancy is only mildly altered throughout intron-containing genes in the absence of histone deacetylases (HDACs). (*A*) Graph represents recruitment of RNAPII (using 8WG16 antibody) across the intron-containing gene *DBP2* in the presence and absence of genes encoding Hos3 and Hos2. Light gray bars represent wild-type and dark gray bars represent *HOS3* and *HOS2* double deletion. Data are normalized to a nontranscribed control. (*B*) Recruitment of RNAPII to the intron-containing gene *ECM33* in the presence and absence of HDACs Hos3 and Hos2. Light gray bars represent RNAPII recruitment for wild-type and dark gray bars represent recruitment in the *HOS3* and *HOS2* double deletion. Data are normalized to a nontranscribed control. Graphs represent the average of three independent experiments, ±SD.

Table S1. List of strains used in this study

Name	Parent	Relevant phenotype	Reference
TJY4318	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K9A]*-URA3*	2
TJY4319	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K14A]*-URA3*	2
TJY4321	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3Δ9–16]*-URA3*	2
TJY4339	S288C	*Isogenic to TJY4318 except msl1Δ::KanMX4*	Present study
TJY4341	S288C	*Isogenic to TJY4319 except msl1Δ::KanMX4*	Present study
TJY4343	S288C	*Isogenic to TJY4321 except msl1Δ::KanMX4*	Present study
TJY4334	S288C	*Isogenic to TJY4318 except lea1Δ::KanMX4*	Present study
TJY4335	S288C	*Isogenic to TJY4319 except lea1Δ::KanMX4*	Present study
TJY4337	S288C	*Isogenic to TJY4321 except lea1Δ::KanMX4*	Present study
TJY3522	BY4743	*GCN5-13XMyc:HIS3*	Present study
TJY3401	BY4742	*hos2Δ::KanMX4*	Present study
TJY4315	BY4742	*hos3Δ::NatMX4*	Present study
TJY4312	BY4742	*hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4325	LG1	*LEA1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4327	LG1	*MSL1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4430	BY4743	*msl1Δ::KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4431	BY4743	*lea1Δ::KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY0474	LG1	*PRP19-HA$_3$:TRP1 DBP2-GFP:KanMX4*	Present study
TJY4434	LG1	*SNU114-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4435	LG1	*PRP19-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4 hos3Δ::NatMX4*	Present study
TJY4307	LG1	*LEA1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4*	Present study
TJY3753	LG1	*LEA1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos3Δ::NatMX4*	Present study
TJY4309	LG1	*MSL1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos2Δ::KanMX4*	Present study
TJY3755	LG1	*MSL1-HA$_3$:TRP1 DBP2-GFP:KanMX4 hos3Δ::NatMX4*	Present study