Sorenson et al. Supplemental Information contains the following:

1. **Supplemental Methods**
2. **Supplemental Tables S4, S5, and S6**
3. **Supplemental Data Set Information**
4. **Supplemental Figures with legends**

Supplemental Methods:

Yeast strain construction:

To construct the *splicing factorXΔ::NAT set2D::G418* and *splicing splicing factorXΔ::NAT jhd1Δ::G418* double mutant strains (X=mud1, mud1, isy1, snu66, or ist3), we first created *Mat a splicing factorXΔ::NAT SGA* strains by transforming *MATa splicing factorXD::G418* strains (see Table S1 for details) with a PCR product encoding the clonNAT[R] (NAT) cassette sequence that included regions of homology with the G418 cassette for integration (see Table S2 for primer sequences and TableS3 for plasmid used for PCR). *Splicing factorXΔ::NAT* strains were selected on YEPD containing 200mg/mL clonNAT-Nourseothricin (Werner Biological) and their genotype was confirmed by PCR. Next, we mated the *MATa splicing factorXΔ::NAT* strains with the *MATα* SGA strain (YTK609), which is a derivative of the original SGA strains (Tong and Boone ref) and selected for diploids on YEPD containing 200μg/mL clonNAT. We used SM-LEU+NAT media (Min-LEU with monosodium glutamate, 100μg/mL cancavanine, 100μg/mL s-AEC, 100μg/mL clonNAT) to isolate the *MATα splicing factorΔ::NAT SGA* strains confirmed genotypes by PCR (see YTK649, 660, 663,664, and 679). Next we mated the *MATα splicing factorΔ::NAT SGA* strain with either the *MA a set2Δ::G418* or *jhd1 Δ::G418* (see Table S1), isolated diploids on YEPD+NAT+G418 media, sporulated, and subsequently isolated haploid *MATa* double mutants on DM-His media (Min-HIS with monosodium glutamate, 100μg/mL cancavanine, 100μg/mL S-(2-Aminoethyl)-L-cysteine hydrochloride, 150μg/mL G418, 100μg/mL clonNAT). The genotypes of the double mutant strain were strain confirmed by PCR. See Table S1 for a full list of strains used in this study.

To generate the *splicing factorXΔ::G418 H3K36A/R/Q* double mutants (X=mud1, mud1, isy1, snu66, or ist3) we mated the *MATa splicing factorΔ::G418* strains (OpenBiosystems) with either the *MATα H3K36A*, *H3K36R* or *H3K36Q* point mutant strains from a histone point mutant collection (GE Healthcare, Ref. 1 below). Diploids were isolated on Min-URA containing 100μg/mL of G418 and 150 μg/mL clonNAT, sporulated, and haploid double mutants were isolated by tetrad dissection and subsequent selection on Min-URA containing 100ug/mL of G418 and 150 ug/mL clonNAT. The genotypes of the double mutants were confirmed by PCR. See Table S1 for a full list of strains.

To generate the yeast strains for chromatin immunoprecipitation analysis, we transformed Prp42-HA, Lea1-HA, and Snu114-HA with a PCR product encoding the hygromycin^R cassette flanked by 50 nts of homology to the sites of integration (SET2 5' and 3' UTR; see Table S2 for primer sequences and TableS3 for plasmid used for PCR). Transformants were isolated on YEPD containing 150μg/mL clonNAT. We utilized PCR to confirm the genotype of each strain. See Table S1 for the full list of strains.

1. Dai J, Hyland EM, Yuan DS, Huang H, Bader JS, et al. (2008) Probing nucleosome function: A highly versatile library of synthetic histone H3 and H4 mutants. Cell 134(6): 1066-1078.

Whole cell extract preparation and immunoblotting

To monitor H3K36 methylation status in *set2* mutants, overnight cultures were grown for *set2* mutants in selective medium and it was used to start fresh cultures (starting A_{600} 0.1). After growing the cultures to an A_{600} 0.6-0.8, cells were centrifuged (13,000 rpm) for 5 min and the cell pellet was lysed by vortexing (for 5-7 min.) using 200 µL of SUMEB buffer (1% SDS, 8M Urea, 10mM MOPS, pH 6.8, 10mM EDTA, 0.01% bromophenol blue) and 200 µL equivalent of glass beads. The protein extract were centrifuged before loading onto the SDS- PAGE gels.

For immunoblotting, 15% SDS-PAGE gels were run at 200V for about 30 minutes and the gels were transferred using Hoefer semi-dry apparatus (45mA/gels for 1.5hr) onto PVDF membranes. Primary antibodies were incubated overnight in 5% milk and secondary antibody incubation was done at room temperature for an hour. Three washes with 1XTBST were performed after primary and secondary incubations and the blots were developed by ECL-prime (Amersham; RPN 2232). The primary antibodies and their dilutions were: H3K36me1 (Abcam ab9048; 1:1000); H3K36me2 (Active Motif, 39255; 1:1000); H3K36me3 (Abcam ab9050; 1:10,000) and C-terminus H3 (1:5000; Epicypher 13-0001).

To monitor the levels of HA-tagged splicing factors when *SET2* is deleted, the indicated yeast strain cultures were grown at 31°C to OD_{585} between 0.25-0.4 and shifted to 37°C for 30 mins. Yeast pellets equivalent to 10 mL of OD_{585}= 0.5 were harvested by centrifugation at 3000g for 5 min. Pellets were resuspended in 200μl of protein extraction buffer (10mM Tris pH7.5, 125 mM NaCl, 0.1% Igepal/NP-40, 100 ug/ml of aprotonin, leupeptin, and antipain, and 200 μM PMSF) plus ~50μL of 0.5mM glass beads, and were vortexed 5 x 1 min. intervals with a 30 sec. rest on ice between intervals. Supernatant was isolated by centrifugation at 10,000g for 10 min. The protein extracts (supernatants) were quantified using standard Bradford Assay. Protein extracts (6ug of total protein for Lea1-HA and Snu114-HA strains and 12μg total protein for Prp42-HA strains) was separated on 10% Biorad TGA gel, transferred to nitrocellulose and probed with α-HA (Roche 12CA5, Cat #11583816001) or α-Glucose-6-phosphate dehydrogenase (G-6-PDH; Sigma A9521) using traditional Western Blot technique using ECL detection (Pierce 32132).

Table S1. Yeast strains used in this study.

Name	Genotype	Ref.
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	
BY4742	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	
YTK609	*MATα SGA leuΔ0 his3Δ1 LYS2+ met15Δ0 ura3Δ0 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU3*	1
YTK846	*MATα SGA leuΔ::NAT his3Δ1 LYS2+ met15Δ0 ura3Δ0 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU3 cyh-s*	1
YDC135	*MATα SGA his3Δ::G418 leuΔ0 LYS2+ met15Δ0 ura3Δ0 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU3 cyh-s*	1
YDC161	*MATa SGA his3Δ::G418 leuΔ::NAT LYS2+ met15Δ0 ura3Δ0 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU3 cyh-s*	1
YTK660	*MATα mud1Δ::NAT can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK663	*MATα mud2Δ::NAT can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK664	*MATα isy1Δ::NAT can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK649	*MATα snu66Δ::NAT can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK769	*MATα ist3Δ::NAT can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
	MATa jhd1Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATa set2Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
YTK799	*MATa mud1Δ::NAT set2 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK759	*MATa mud1Δ::NAT set2 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK705	*MATa isy1Δ::NAT set2 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK800	*MATa snu66Δ::NAT set2 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK841	*MATa ist3Δ::NAT set2 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK743	*MATa mud1Δ::NAT jhd1 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK780	*MATa mud2Δ::NAT jhd1 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK752	*MATa isy1Δ::NAT jhd1 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK754	*MATa snu66Δ::NAT jhd1 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
YTK757	*MATa ist3Δ::NAT jhd1 Δ::G418 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 uraΔ0*	this study
	MATα mud1Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2

	MATα mud2Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATα isy1Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATα snu66Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATα ist3Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATα lea1Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATα bud13Δ::G418 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	2
	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]-URA3*	3
	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]-URA3*	3
	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]-URA3*	3
YTK795	*mud1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK796	*mud2Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK798	*isy1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTL801	*snu66Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK803	*ist3Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK802	*lea1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK797	*bud13Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36A-HHFS]*-URA3*	this study
YTK804	*mud1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK805	*mud2Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK850	*isy1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK807	*snu66Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK808	*ist3Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK806	*lea1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK809	*bud13Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36R-HHFS]*-URA3*	this study
YTK815	*mud1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YTK816	*mud2Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YTK817	*isy1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YTK843	*snu66Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-*	this study

	hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]-URA3*	
YTK810	*ist3Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YTK828	*lea1Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YTK818	*bud13Δ::G418 his3Δ leu2Δ0 lys2Δ0 ura3Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[H3K36Q-HHFS]*-URA3*	this study
YGMW187	*Prp42-HA (HIS3); MATα; ade2-1 can1-100 his3-11 leu2-3 112 trp1-1 ura3-1 (W303)*	4
#268	*LG1/DBP2-GFP (G418) (JG12)/Lea1-HA (TRP); MATa ura3 his3-11 leu-2 112 trp1-1 can1-100 flo8 psi ADE+ GAL+ SSD1+ BUD4 (W303)*	5
#302	*LG1/DBP2-GFP (G418) (JG12)/Snu114-HA (TRP); MATa ura3 his3-11 leu-2 112 trp1-1 can1-100 flo8 psi ADE+ GAL+ SSD1+ BUD4 (W303)*	5
YTK847	*Prp42-HA (HIS3) set2Δ::HYG MATα ade2-1 can1-100 his3-11 leu2-3 112 trp1-1 ura3-1 (W303)*	this study
YTK880	*LG1/DBP2-GFP (G418) (JG12)/Lea1-HA (TRP) set Δ::HYG MATa ura3 his3-11 leu-2 112 trp1-1 can1-100 flo8 psi ADE+ GAL+ SSD1+ BUD4 (W303)*	this study
YTK881	*LG1/DBP2-GFP (G418) (JG12)/Snu114-HA (TRP) set2Δ::HYG MATa ura3 his3-11 leu-2 112 trp1-1 can1-100 flo8 psi ADE+ GAL+ SSD1+ BUD4 (W303)*	this study
KLY78	*MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 kanMX-GAL1pr-FLO8-HIS3*	gift from Michael Keogh
YSM138	*MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 kanMX-GAL1pr-FLO8-HIS3set2Δ::NATMX*	Strahl Lab
set2Δ	*MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 set2Δ::NATMX*	6
spt16-11 (DY11848)	*MATa spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3 (DY8107)*	gift from David Stillman
spt16-11set2Δ	*MATa spt16-11 set2::HpHMX ade2 can1 his3 leu2 ura3*	Strahl Lab
References		
1	strains derived from strains described in Tong A.H.Y., Boone, C. (2006) Synthetic genetic array analysis in *Saccharomyces cerevisiae*. Methods Mol Biol 313: 171–192. YDC strains a gift from Dale Cameron.	
2	yeast deletion collection from OpenBiosystems (www.openbiosystems.com formerly, Research Genetics, Huntsville AL)	
3	histone point mutant collection from GE Healthcare #YSC5106; as described in Dai, J., Hyland, E.M., Yuan, D.S., Huang, H., Bader, J.S., Boeke, J.D.(2008) Probing nucleosome function: a highly versatile library of synthetic histone H3 and H4 mutants. Cell 2008 Sep 19;134(6):1066-78.	

4	a gift from G. Wilmes and C. Guthrie	
5	Gornemann, J., Kotovic, K. M., Hujer, K., and Neugebauer, K. M. (2005). Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex. Mol Cell 19, 53-63	
6	Deepak K. Jha and Brian D. Strahl; Nature Communications 5, Article number: 3965 doi:10.1038/ncomms4965	

Table S2. Plasmids used in this study.

Plasmid	Description	Assay	Reference
pAG25 *(natMX4)*	resistance to clonNAT-Nourseothricin	to replace G418 with NAT	Goldstein et al Yeast. 1999 Oct;15(14):1541-53.
pAG32 *(hphMX4)*	resistance to hygromycin B	to KO *SET2*	Goldstein et al Yeast. 1999 Oct;15(14):1541-53.
SET2 WT	WT *SET2* cloned into pRS416, driven by endogenous promoter	transcriptional assays and rescue of splicing experiments	Du *et.al* Genes Dev. Oct 15, 2008; 22(20): 2786–2798.
set2 H199L	QuikChange Mutagenesis of WT *SET2*	transcriptional assays and rescue of splicing experiments	Jha and Strahl, Nature Communications 5, Article number: 3965
set2 R195C	QuikChange Mutagenesis of WT *SET2*	transcriptional assays and rescue of splicing experiments	this study
set2 K663L	QuikChange Mutagenesis of WT *SET2*	transcriptional assays and rescue of splicing experiments	this study
set2 H199L K663L	QuikChange Mutagenesis of WT *SET2*	transcriptional assays and rescue of splicing experiments	this study
*set2 SRI*Δ	*set2 1-618* in pRS416	transcriptional assays and rescue of splicing experiments	Du *et.al* Genes Dev. Oct 15, 2008; 22(20): 2786–2798.
pRS316 Reporter	*GPD*-Reporter *CEN URA3*	Gene expression reporter	Sorenson and Stevens, RNA. 2014; 20(5):732-45
pRS415 Reporter	*GPD*-Reporter *CEN LEU2*	Gene expression reporter	Sorenson and Stevens, RNA. 2014; 20(5):732-45

Table S3. Primers used in this study.

Primer name	Primer Sequence	Purpose	Reference
NatCassetteF	ACATGGAGGCCCAGAATACCC	to replace G418 with NAT	this study
NatCassetteR	CAGTATAGCGACCAGCATTCAC	to replace G418 with NAT	this study
Set2KOF	CTGCATAGTCGTGCTGTCAAACCTTTCTCCTT TCCTGGTTGTTGTTTTACGTGATCACATGGAG GCCCAGAATACCC	to KO Set2	this study
Set2KOR	GACTTCCTTTGGGACAGAAAACGTGAAACAAG CCCCAAATATGCATGTCTGGTTAACAGTATAC GCGACCAGCATTCA	to KO Set2	this study
TEF5intFWDjp qPCR	GATAGCACAGAGCAGAGTATCATTA	qPCR	this study
TEF5intREVjp qPCR	CTGGAGAATTCTGGGTAAGCAGATT	qPCR	this study
TEF5 total FWD qPCR	CCATTGTCACTCTAGATGTCAAGCC	qPCR	this study
TEF5 total REV qPCR	GATACCGAAACCAATTGGGATAAAT	qPCR	this study
DBP2intFWDms qPCR	ACAGAATAACGAACCAAATTACTAACAGT	qPCR	this study
DBP2intREVms qPCR	CCTGGCATATCGTAGTTGATAACG	qPCR	this study
DBP2exonFWDms qPCR	CTTCACCGAACAAAACAAAGGTT	qPCR	this study
DBP2exonREVms qPCR	TCGGGAGGAATATTTTGATTAGCT	qPCR	this study
SRB2 pre-mRNA FWD qPCR	GGGAAAATCAGCGTATGTAAAGC	qPCR	this study
SRB2 pre-mRNA REV qPCR	GAGTGGCTCTTTCCACGAATATAA	qPCR	this study
SRB2 total FWD qPCR	CATCGAAGGACACCTAGCTGAA	qPCR	this study
SRB2 total REV qPCR	TCCGGCCCCAACGAG	qPCR	this study
RPS21B pre-mRNA FWD qPCR	CAAATAGGGTGGGACCAACA	qPCR	this study
RPS21B pre-mRNA REV qPCR	TCAGGTAACACTTGTGCCAAT	qPCR	this study
RPS21B total FWD qPCR	ATCCAGAGGTGAATCCGATG	qPCR	this study
RPS21B total REV qPCR	AACGACTTCCCCTCTTCTTTTT	qPCR	this study
Rps21BIntronF3 (Region 1)	TCCGAATTAGTGGGTTTCCA	ChIP	1
Rps21BIntronR3 (Region 1)	TCAATGTTAGTACATTTGCGCTTA	ChIP	1

Rps21BintronF1 (Region 2)	AATCATCAAAGCCGATGACC	ChIP	1
Rps21BIntronR1 (Region 2)	CATCGGATTCACCTCTGGAT	ChIP	1
Rps21bExon2-2 (Region 3)	ATCCAGAGGTGAATCCGATG	ChIP	1
Rps12b3'UTR-2 (Region 3)	AACGACTTCCCCTCTTCTTTTT	ChIP	2
IR V-1(intergenic region for Rps21B ChIPs)	GGCTGTCAGAATATGGGGCCGTAGTA	ChIP	2
IR V-2 (intergenic region for Rps21B ChIPs)	CACCCCGAAGCTGCTTTCACAATAC	ChIP	2
DBP2 1409 F (Region 1)	TGA CAA CCA TGA TAG TAC AGA AGA GAG	ChIP	3
DBP2 1558 R (Region 1)	TTT CCG ATA CTC CCC ATC G	ChIP	3
DBP2 1877 F (Region 2)	ATG CCG TCA TCC TTC TTG AC	ChIP	3
DBP2 1970 R (Region 2)	TCG AAC TTG GGA TGC AAC AG	ChIP	3
DBP2 2392 F (Region 3)	TTC ACC GAA CAA AAC AAA GG	ChIP	3
DBP2 2612 R (Region 3)	CCA CCA TCT CTC TGC CTG TT	ChIP	3
NTR VI_R F (intergenic region for Dbp2 ChIPs)	CAG GCA GTC CTT TCT ATT TC	ChIP	3
NTR VI_R R (intergenic region for Dbp2 ChIPs)	GCT TGT TAA CTC TCC GAC AG	ChIP	3
R195C F	CTGCAAGAGTGATTGCAAAAGCAGGCCAACG AACCCTTTATTG	QuikChange	this study
R195C R	CAATAAAGGGTTCGTTGGCCTGCTTTTGCAAT CACTCTTGCAG	QuikChange	this study
K663L F	GTAGTTAGGATTTTCACGATGTCTAAAGCACA TTGTTTAATGTTTTCATGATC	QuikChange	this study
K663L R	GATCATGAAAACATTAAACAATGTGCTTTAGA CATCGTGAAAATCCTAACTAC	QuikChange	this study
References			
1	Tracy L. Kress, Nevan J. Krogan, and Christine Guthrie (2008). A single SR-like protein, Npl3, promotes pre-mRNA		
2	Komarnitsky, P, Cho, E.J. and Buratowski, S. (2000). Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes. Dev.		

	14(19):2452-60.		
3	Gunderson FQ, Johnson TL. (2009). Acetylation by the transcriptional coactivator Gcn5 plays a novel role in co-		

Table S4. Tukey method p-values for Figure 3.

Gene analyzed by RT-qPCR	Treatment Pair	p-value
DBP2	WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053
	WT vs jhd1Δ	0.0010053
	WT vs H3K36A	0.001384
	WT vs H3K36Q	0.0217177
	WT vs H3 K36R	0.2046892
	snu66Δ vs set2Δ	0.0010053
	snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs H3K36A	0.0010053
	snu66Δ vs H3K36Q	0.0010053
	snu66Δ vs H3K36R	0.0010053
	set2Δ vs H3K36A	0.0010053
	set2Δ vs H3K36Q	0.0010053
	set2Δ vs H3K36R	0.0010053
	jhd1Δ vs H3K36A	0.8999947
	jhd1Δ vs H3K36Q	0.3693971
	jhd1Δ vs H3K36R	0.0454708
	H3K36A vs H3K36Q	0.7020738
	H3K36A vs H3K36R	0.1364809
	H3K36Q vs H3K36R	0.8233492
SRB2	WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053
	WT vs jhd1Δ	0.8999947
	WT vs H3K36A	0.0010053
	WT vs H3K36Q	0.0010053
	WT vs H3 K36R	0.0010053
	snu66Δ vs set2Δ	0.0010053
	snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs H3K36A	0.0277639
	snu66Δ vs H3K36Q	0.0158005
	snu66Δ vs H3K36R	0.0422296
	set2Δ vs H3K36A	0.0010053
	set2Δ vs H3K36Q	0.0010053
	set2Δ vs H3K36R	0.0010053
	jhd1Δ vs H3K36A	0.0010053
	jhd1Δ vs H3K36Q	0.0010053
	jhd1Δ vs H3K36R	0.0010053

	H3K36A vs H3K36Q	0.8999947
	H3K36A vs H3K36R	0.8999947
	H3K36Q vs H3K36R	0.8999947
RPS21B	WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053
	WT vs jhd1Δ	0.8999947
	WT vs H3K36A	0.5746997
	WT vs H3K36Q	0.0010053
	WT vs H3 K36R	0.0869973
	snu66Δ vs set2Δ	0.0379223
	snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs H3K36A	0.0010053
	snu66Δ vs H3K36Q	0.0049439
	snu66Δ vs H3K36R	0.0010053
	set2Δ vs H3K36A	0.0010053
	set2Δ vs H3K36Q	0.8999947
	set2Δ vs H3K36R	0.0037169
	jhd1Δ vs H3K36A	0.2877402
	jhd1Δ vs H3K36Q	0.0010053
	jhd1Δ vs H3K36R	0.0314379
	H3K36A vs H3K36Q	0.0025908
	H3K36A vs H3K36R	0.8103717
	H3K36Q vs H3K36R	0.0284683
TEF5	WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.8999947
	WT vs jhd1Δ	0.8999947
	WT vs H3K36A	0.8141013
	WT vs H3K36Q	0.2261035
	WT vs H3 K36R	0.0989161
	snu66Δ vs set2Δ	0.0010053
	snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs H3K36A	0.0010053
	snu66Δ vs H3K36Q	0.0010053
	snu66Δ vs H3K36R	0.0010053
	set2Δ vs HK36A	0.8999947
	set2Δ vs H3K36Q	0.363122
	set2Δ vs H3K36R	0.1711983
	jhd1Δ vs H3K36A	0.8999947
	jhd1Δ vs H3K36Q	0.349774
	jhd1Δ vs H3K36R	0.1638143
	H3K36A vs H3K36Q	0.8774383
	H3K36A vs H3K36R	0.6105911
	H3K36Q vs H3K36R	0.8999947

Table S5. Tukey method p-values for Figure 4.

Mud1 Double Mutants					
Gene Analyzed by PCR	**treatments pair**	**Tukey HSD inferfence**		**treatments pair**	**Tukey HSD inferfence**
SRB2	WT vs mud1Δ	0.0016635		WT vs mud1Δ	0.0010053
	WT vs set2Δ	0.0241081		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud1Δ	4.43E-05		WT vs jhd1Δ mud1Δ	0.0290273
	mud1Δ vs set2Δ	0.2250149		mud1Δ vs jhd1Δ	0.0010053
	mud1Δ vs set2Δ mud1Δ	0.0179447		mud1Δ vs jhd1Δ mud1Δ	0.0707268
	set2Δ vs set2Δ mud1Δ	0.0013105		jhd1Δ vs jhd1Δ mud1Δ	0.03731
DBP2	WT vs mud1Δ	0.7834077		WT vs mud1Δ	0.836575
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0010053
	WT vs set2Δ mud1Δ	0.4940669		WT vs jhd1Δ mud1Δ	0.5366688
	mud1Δ vs set2Δ	0.0010053		mud1Δ vs jhd1Δ	0.0010053
	mud1Δ vs set2Δ mud1Δ	0.1585598		mud1Δ vs jhd1Δ mud1Δ	0.2045859
	set2Δ vs set2Δ mud1Δ	0.0010053		jhd1Δ vs jhd1Δ mud1Δ	0.0019992
RPS21B	WT vs mud1Δ	0.1997401		WT vs mud1Δ	0.5283174
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud1Δ	0.209323		WT vs jhd1Δ mud1Δ	0.326054
	mud1Δ vs set2Δ	0.0010053		mud1Δ vs jhd1Δ	0.7110421
	mud1Δ vs set2Δ mud1Δ	0.0099871		mud1Δ vs jhd1Δ mud1Δ	0.0474535
	set2Δ vs set2Δ mud1Δ	0.0022707		jhd1Δ vs jhd1Δ mud1Δ	0.2054051
TEF5	WT vs mud1Δ	0.8067788		WT vs mud1Δ	0.8999947
	WT vs set2Δ	0.8999947		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud1Δ	0.0010298		WT vs jhd1Δ mud1Δ	0.4495094
	mud1Δ vs set2Δ	0.8999947		mud1Δ vs jhd1Δ	0.8999947
	mud1Δ vs set2Δ mud1Δ	0.0026851		mud1Δ vs jhd1Δ mud1Δ	0.7211679
	set2Δ vs set2Δ mud1Δ	0.0014761		jhd1Δ vs jhd1Δ mud1Δ	0.5448331

Mud2 Double Mutants					
Gene Analyzed by PCR	**treatments pair**	**Tukey HSD inferfence**		**treatments pair**	**Tukey HSD inferfence**
SRB2	WT vs mud2Δ	0.0010053		WT vs mud2Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud2Δ	0.0010053		WT vs jhd1Δ mud2Δ	0.0010053
	mud2Δ vs set2Δ	0.0010053		mud2Δ vs jhd1Δ	0.0010053
	mud2Δ vs set2Δ mud2Δ	0.0036492		mud2Δ vs jhd1Δ mud2Δ	0.3569099
	set2Δ vs set2Δ mud2Δ	0.0010053		jhd1Δ vs jhd1Δ mud2Δ	0.0010053
DBP2	WT vs mud2Δ	0.098157		WT vs mud2Δ	0.052297
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0010053
	WT vs set2Δ mud2Δ	0.0010053		WT vs jhd1Δ mud2Δ	0.1307498
	mud2Δ vs set2Δ	0.0010053		mud2Δ vs jhd1Δ	0.0205863

	mud2Δ vs set2Δ mud2Δ	0.0010053		mud2Δ vs jhd1Δ mud2Δ	0.8999947
	set2Δ vs set2Δ mud2Δ	0.8450478		jhd1Δ vs jhd1Δ mud2Δ	0.0087079
RPS21B	WT vs mud2Δ	0.0010053		WT vs mud2Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud2Δ	0.0010053		WT vs jhd1Δ mud2Δ	0.0010053
	mud2Δ vs set2Δ	0.0010053		mud2Δ vs jhd1Δ	0.0010053
	mud2Δ vs set2Δ mud2Δ	0.6953979		mud2Δ vs jhd1Δ mud2Δ	0.1689675
	set2Δ vs set2Δ mud2Δ	0.0010053		jhd1Δ vs jhd1Δ mud2Δ	0.0010053
TEF5	WT vs mud2Δ	0.8653558		WT vs mud2Δ	0.8512294
	WT vs set2Δ	0.8999947		WT vs jhd1Δ	0.8999947
	WT vs set2Δ mud2Δ	0.8999947		WT vs jhd1Δ mud2Δ	0.0166449
	mud2Δ vs set2Δ	0.8999947		mud2Δ vs jhd1Δ	0.8999947
	mud2Δ vs set2Δ mud2Δ	0.7112306		mud2Δ vs jhd1Δ mud2Δ	0.0484181
	set2Δ vs set2Δ mud2Δ	0.8999947		jhd1Δ vs jhd1Δ mud2Δ	0.0241283

Snu66 Double Mutants					
Gene Analyzed by PCR	**treatments pair**	**Tukey HSD inferfence**		**treatments pair**	**Tukey HSD inferfence**
SRB2	WT vs snu66Δ	0.0010053		WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0108833
	WT vs set2Δ snu66Δ	0.0808001		WT vs jhd1Δ snu66Δ	0.0010053
	snu66Δ vs set2Δ	0.0319482		snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs set2Δ mud2Δ	0.0010053		snu66Δ vs jhd1Δ snu66Δ	0.0414419
	set2Δ vs set2Δ snu66Δ	0.0047228		jhd1Δ vs jhd1Δ snu66Δ	0.0010053
DBP2	WT vs snu66Δ	0.0010053		WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0108833
	WT vs set2Δ snu66Δ	0.8999947		WT vs jhd1Δ snu66Δ	0.0010053
	snu66Δ vs set2Δ	0.0010053		snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs set2Δ mud2Δ	0.0010053		snu66Δ vs jhd1Δ snu66Δ	0.0414419
	set2Δ vs set2Δ snu66Δ	0.0010053		jhd1Δ vs jhd1Δ snu66Δ	0.0010053
RPS21B	WT vs snu66Δ	0.0010053		WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ snu66Δ	0.1360852		WT vs jhd1Δ snu66Δ	0.0010053
	snu66Δ vs set2Δ	0.0113201		snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs set2Δ mud2Δ	0.0010053		snu66Δ vs jhd1Δ snu66Δ	0.0010053
	set2Δ vs set2Δ snu66Δ	0.0038235		jhd1Δ vs jhd1Δ snu66Δ	0.0010053
TEF5	WT vs snu66Δ	0.0010053		WT vs snu66Δ	0.0010053
	WT vs set2Δ	0.8999947		WT vs jhd1Δ	0.8999947
	WT vs set2Δ snu66Δ	0.7824861		WT vs jhd1Δ snu66Δ	0.0010053
	snu66Δ vs set2Δ	0.0010053		snu66Δ vs jhd1Δ	0.0010053
	snu66Δ vs set2Δ mud2Δ	0.0010053		snu66Δ vs jhd1Δ snu66Δ	0.7704008
	set2Δ vs set2Δ snu66Δ	0.8999947		jhd1Δ vs jhd1Δ snu66Δ	0.0010053

Isy1 Double Mutants

Gene Analyzed by PCR	treatments pair	Tukey HSD inferfence		treatments pair	Tukey HSD inferfence
SRB2	WT vs isy1Δ	0.0037442		WT vs isy1Δ	0.0014073
	WT vs set2Δ	0.0031746		WT vs jhd1Δ	0.8999947
	WT vs set2Δ isy1Δ	0.0010053		WT vs jhd1Δ isy1Δ	0.0010053
	ist3Δ vs set2Δ	0.8999947		isy1Δ vs jhd1Δ	0.0018982
	isy1Δ vs set2Δ isy1Δ	0.0010053		ist3Δ vs jhd1Δ isy1Δ	0.2494461
	set2Δ vs set2Δ isy1Δ	0.0010053		jhd1Δ vs jhd1Δ isy1Δ	0.0010053
DBP2	WT vs isy1Δ	0.0010053		WT vs isy1Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0010053
	WT vs set2Δ isy1Δ	0.0010053		WT vs jhd1Δ isy1Δ	0.0010053
	ist3Δ vs set2Δ	0.0288801		isy1Δ vs jhd1Δ	0.204883
	isy1Δ vs set2Δ isy1Δ	0.0147621		ist3Δ vs jhd1Δ isy1Δ	0.0172546
	set2Δ vs set2Δ isy1Δ	0.8999947		jhd1Δ vs jhd1Δ isy1Δ	0.001215
RPS21B	WT vs isy1Δ	0.0308132		WT vs isy1Δ	0.037765
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ isy1Δ	0.0010053		WT vs jhd1Δ isy1Δ	0.0010053
	ist3Δ vs set2Δ	0.0031282		isy1Δ vs jhd1Δ	0.0162793
	isy1Δ vs set2Δ isy1Δ	0.0010053		ist3Δ vs jhd1Δ isy1Δ	0.0010053
	set2Δ vs set2Δ isy1Δ	0.0059715		jhd1Δ vs jhd1Δ isy1Δ	0.0010053
TEF5	WT vs isy1Δ	0.0067635		WT vs isy1Δ	0.0032055
	WT vs set2Δ	0.8999947		WT vs jhd1Δ	0.8999947
	WT vs set2Δ isy1Δ	0.0010053		WT vs jhd1Δ isy1Δ	0.0013782
	ist3Δ vs set2Δ	0.0045848		isy1Δ vs jhd1Δ	0.0021983
	isy1Δ vs set2Δ isy1Δ	0.2210652		ist3Δ vs jhd1Δ isy1Δ	0.8672439
	set2Δ vs set2Δ isy1Δ	0.0010053		jhd1Δ vs jhd1Δ isy1Δ	0.0010053

	treatments pair	Tukey HSD inferfence
SRB2	WT vs isy1Δ	0.0263576
	WT vs H3K36Q	0.0010053
	WT vs H3K36Q isy1Δ	0.0010053
	isy1Δ vs H3K36Q	0.0010053
	isy1Δ vs H3K36Q isy1Δ	0.0010053
	H3K36Q vs H3K36Q isy1Δ	0.0057477
DBP2	WT vs isy1Δ	0.0010053
	WT vs H3K36Q	0.044221
	WT vs H3K36Q isy1Δ	0.0010053
	isy1Δ vs H3K36Q	0.0422947
	isy1Δ vs H3K36Q isy1Δ	0.0010053
	H3K36Q vs H3K36Q isy1Δ	0.0010053
RPS21B	WT vs isy1Δ	0.208539
	WT vs H3K36Q	0.0068671

	WT vs H3K36Q isy1Δ	0.0021351
	isy1Δ vs H3K36Q	0.1340588
	isy1Δ vs H3K36Q isy1Δ	0.0340221
	H3K36Q vs H3K36Q isy1Δ	0.7485035
TEF5	WT vs isy1Δ	0.0252424
	WT vs H3K36Q	0.3093574
	WT vs H3K36Q isy1Δ	0.2090694
	isy1Δ vs H3K36Q	0.0023575
	isy1Δ vs H3K36Q isy1Δ	0.4725264
	H3K36Q vs H3K36Q isy1Δ	0.0155053

Ist3 Double Mutants					
Gene Analyzed by PCR	**treatments pair**	**Tukey HSD inferfence**		**treatments pair**	**Tukey HSD inferfence**
SRB2	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.8999947
	WT vs set2Δ ist3Δ	0.0010053		WT vs jhd1Δ ist3Δ	0.0010053
	ist3Δ vs set2Δ	0.0010053		ist3Δ vs jhd1Δ	0.0010053
	ist3Δ vs set2Δ ist3Δ	0.0010053		ist3Δ vs jhd1Δ ist3Δ	0.3523022
	set2Δ vs set2Δ ist3Δ	0.0010053		jhd1Δ vs jhd1Δ ist3Δ	0.0010053
DBP2	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs set2Δ	0.0010053		WT vs jhd1Δ	0.0775604
	WT vs set2Δ ist3Δ	0.0010053		WT vs jhd1Δ ist3Δ	0.0010053
	ist3Δ vs set2Δ	0.0010053		ist3Δ vs jhd1Δ	0.0010053
	ist3Δ vs set2Δ ist3Δ	0.0105361		ist3Δ vs jhd1Δ ist3Δ	0.0038239
	set2Δ vs set2Δ ist3Δ	0.0010053		jhd1Δ vs jhd1Δ ist3Δ	0.0010053
RPS21B	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs set2Δ	0.0012042		WT vs jhd1Δ	0.8999947
	WT vs set2Δ ist3Δ	0.0010053		WT vs jhd1Δ ist3Δ	0.0010053
	ist3Δ vs set2Δ	0.0010053		ist3Δ vs jhd1Δ	0.0010053
	ist3Δ vs set2Δ ist3Δ	0.4150881		ist3Δ vs jhd1Δ ist3Δ	0.8999947
	set2Δ vs set2Δ ist3Δ	0.0010053		jhd1Δ vs jhd1Δ ist3Δ	0.0010053
TEF5	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs set2Δ	0.8999947		WT vs jhd1Δ	0.8999947
	WT vs set2Δ ist3Δ	0.0010053		WT vs jhd1Δ ist3Δ	0.0010053
	ist3Δ vs set2Δ	0.0010053		ist3Δ vs jhd1Δ	0.0010053
	ist3Δ vs set2Δ ist3Δ	0.0017769		ist3Δ vs jhd1Δ ist3Δ	0.0010053
	set2Δ vs set2Δ ist3Δ	0.0010053		jhd1Δ vs jhd1Δ ist3Δ	0.0010053
	treatments	**Tukey HSD**		**treatments**	**Tukey HSD**
	pair	**inferfence**		**pair**	**inferfence**
SRB2	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs H3K36A	0.0010053		WT vs H3K36R	0.0010053

	WT vs H3K36A ist3Δ	0.0010053		WT vs H3K36R ist3Δ	0.0010053
	ist3Δ vs H3K36A	0.0010053		ist3Δ vs H3K36R	0.0010053
	ist3Δ vs H3K36A ist3Δ	0.0015547		ist3Δ vs H3K36R ist3Δ	0.0010053
	H3K36A vs H3K36A ist3Δ	0.0010053		H3K36R vs H3K36R ist3Δ	0.0010053
DBP2	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs H3K36A	0.044451		WT vs H3K36R	0.1887671
	WT vs H3K36A ist3Δ	0.0010053		WT vs H3K36R ist3Δ	0.0010053
	ist3Δ vs H3K36A	0.0010053		ist3Δ vs H3K36R	0.0010053
	ist3Δ vs H3K36A ist3Δ	0.0012431		ist3Δ vs H3K36R ist3Δ	0.0010053
	H3K36A vs H3K36A ist3Δ	0.0010053		H3K36R vs H3K36R ist3Δ	0.0010053
RPS21B	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs H3K36A	0.4415016		WT vs H3K36R	0.4022168
	WT vs H3K36A ist3Δ	0.0010053		WT vs H3K36R ist3Δ	0.0010053
	ist3Δ vs H3K36A	0.0010053		ist3Δ vs H3K36R	0.0010053
	ist3Δ vs H3K36A ist3Δ	0.3698207		ist3Δ vs H3K36R ist3Δ	0.0129043
	H3K36A vs H3K36A ist3Δ	0.0010053		H3K36R vs H3K36R ist3Δ	0.0010053
TEF5	WT vs ist3Δ	0.0010053		WT vs ist3Δ	0.0010053
	WT vs H3K36A	0.865004		WT vs H3K36R	0.1619295
	WT vs H3K36A ist3Δ	0.0010053		WT vs H3K36R ist3Δ	0.0010053
	ist3Δ vs H3K36A	0.0010053		ist3Δ vs H3K36R	0.0010053
	ist3Δ vs H3K36A ist3Δ	0.0062069		ist3Δ vs H3K36R ist3Δ	0.0772512
	H3K36A vs H3K36A ist3Δ	0.0010053		H3K36R vs H3K36R ist3Δ	0.0010053

Bud13 Double Mutants		
Gene Analyzed by PCR	**treatments pair**	**Tukey HSD inferfence**
SRB2	WT vs bud13Δ	0.0010053
	WT vs H3K36Q	0.0010053
	WT vs H3K36Q bud13Δ	0.0010053
	bud13Δ vs H3K36Q	0.0010053
	bud13Δ vs H3K36Q bud13Δ	0.3137478
	H3K36Q vs H3K36Q bud13Δ	0.0010053
DBP2	WT vs bud13Δ	0.0010053
	WT vs H3K36Q	0.3325522
	WT vs H3K36Q bud13Δ	0.0010053
	bud13Δ vs H3K36Q	0.0010053
	bud13Δ vs H3K36Q bud13Δ	0.0010053
	H3K36Q vs H3K36Q bud13Δ	0.0010053
RPS21B	WT vs bud13Δ	0.0010053
	WT vs H3K36Q	0.0158861

	WT vs H3K36Q bud13Δ	0.0010053
	bud13Δ vs H3K36Q	0.0045448
	bud13Δ vs H3K36Q bud13Δ	0.1619283
	H3K36Q vs H3K36Q bud13Δ	0.0010053
TEF5	WT vs bud13Δ	0.0010053
	WT vs H3K36Q	0.1497131
	WT vs H3K36Q bud13Δ	0.0010053
	bud13Δ vs H3K36Q	0.0010053
	bud13Δ vs H3K36Q bud13Δ	0.0038312
	H3K36Q vs H3K36Q bud13Δ	0.0010053

Lea1 Double Mutants

Gene Analyzed by PCR	treatments pair	Tukey HSD inferfence
SRB2	WT vs lea1Δ	0.0010053
	WT vs H3K36R	0.0010053
	WT vs H3K36R lea1Δ	0.0010053
	ist3Δ vs H3K36R	0.0010053
	lea1Δ vs H3K36R lea1Δ	0.0249444
	H3K36R vs H3K36R lea1Δ	0.0010053
DBP2	WT vs lea1Δ	0.0010053
	WT vs H3K36R	0.1529732
	WT vs H3K36R lea1Δ	0.0010053
	ist3Δ vs H3K36R	0.0010053
	lea1Δ vs H3K36R lea1Δ	0.0010053
	H3K36R vs H3K36R lea1Δ	0.0010053
RPS21B	WT vs lea1Δ	0.0021445
	WT vs H3K36R	0.1502983
	WT vs H3K36R lea1Δ	0.0150307
	ist3Δ vs H3K36R	0.0479099
	lea1Δ vs H3K36R lea1Δ	0.4429986
	H3K36R vs H3K36R lea1Δ	0.4038531
TEF5	WT vs lea1Δ	0.0650792
	WT vs H3K36R	0.0465317
	WT vs H3K36R lea1Δ	0.0010053
	ist3Δ vs H3K36R	0.0010872
	lea1Δ vs H3K36R lea1Δ	0.0122669
	H3K36R vs H3K36R lea1Δ	0.0010053

Table S6. Tukey method p-values for Figure 5.

Gene analyzed by RT-qPCR	Treatment Pair	p-value
DBP2	WT vs set2Δ	0.0081695
	WT vs set2- H199L	0.0010053
	WT vs set2-R195C	0.0010053
	WT vs set2-SRIΔ	0.0010053
	WT vs set2-K663L	0.7748568
	WT vs set2-H199L K663L	0.0015543
	set2Δ vs set2- H199L	0.793005
	set2Δ vs set2-R195C	0.0010053
	set2Δ vs set2-SRIΔ	0.0097685
	set2Δ vs set2-K663L	0.0992895
	set2Δ vs set2-H199L K663L	0.8999947
	set2- H199L vs set2-R195C	0.0010053
	set2- H199L vs set2-SRIΔ	0.0010053
	set2- H199L vs set2-K663L	0.0087066
	set2- H199L vs set2-H199L K663L	0.8999947
	set2-R195C vs set2-SRIΔ	0.0010053
	set2-R195C vs set2-K663L	0.0010053
	set2-R195C vs set2-H199L K663L	0.0010053
	set2-SRIΔ vs set2-K663L	0.8260777
	set2-SRIΔ vs set2-H199L K663L	0.0018448
	set2-K663L vs set2-H199L K663L	0.0190103
SRB2	WT vs set2Δ	0.0017351
	WT vs set2- H199L	0.0010053
	WT vs set2-R195C	0.5642598
	WT vs set2-SRIΔ	0.0258012
	WT vs set2-K663L	0.0141136
	WT vs set2-H199L K663L	0.0010053
	set2Δ vs set2- H199L	0.7197977
	set2Δ vs set2-R195C	0.0441594
	set2Δ vs set2-SRIΔ	0.7530135
	set2Δ vs set2-K663L	0.7199518
	set2Δ vs set2-H199L K663L	0.8929803
	set2- H199L vs set2-R195C	0.0029383
	set2- H199L vs set2-SRIΔ	0.8999947
	set2- H199L vs set2-K663L	0.1039273
	set2- H199L vs set2-H199L K663L	0.1788571
	set2-R195C vs set2-SRIΔ	0.0032898
	set2-R195C vs set2-K663L	0.4637204
	set2-R195C vs set2-H199L K663L	0.3005891
	set2-SRIΔ vs set2-K663L	0.1157979
	set2-SRIΔ vs set2-H199L K663L	0.1976973
	set2-K663L vs set2-H199L K663L	0.8999947
RPS21B	WT vs set2Δ	0.6149654
	WT vs set2- H199L	0.0170643
	WT vs set2-R195C	0.1664185

	WT vs set2-SRIΔ	0.8999947
	WT vs set2-K663L	0.0048958
	WT vs set2-H199L K663L	0.0010053
	set2Δ vs set2- H199L	0.3035886
	set2Δ vs set2-R195C	0.8999947
	set2Δ vs set2-SRIΔ	0.552635
	set2Δ vs set2-K663L	0.1032309
	set2Δ vs set2-H199L K663L	0.002552
	set2- H199L vs set2-R195C	0.8231855
	set2- H199L vs set2-SRIΔ	0.0137313
	set2- H199L vs set2-K663L	0.8999947
	set2- H199L vs set2-H199L K663L	0.1558989
	set2-R195C vs set2-SRIΔ	0.1368805
	set2-R195C vs set2-K663L	0.4637287
	set2-R195C vs set2-H199L K663L	0.0158543
	set2-SRIΔ vs set2-K663L	0.0039498
	set2-SRIΔ vs set2-H199L K663L	0.0010053
	set2-K663L vs set2-H199L K663L	0.4238481
TEF5	WT vs set2Δ	0.0219581
	WT vs set2- H199L	0.0129503
	WT vs set2-R195C	0.8999947
	WT vs set2-SRIΔ	0.0010053
	WT vs set2-K663L	0.8999947
	WT vs set2-H199L K663L	0.0680603
	set2Δ vs set2- H199L	0.8999947
	set2Δ vs set2-R195C	0.0214017
	set2Δ vs set2-SRIΔ	0.0010053
	set2Δ vs set2-K663L	0.0082938
	set2Δ vs set2-H199L K663L	0.8999947
	set2- H199L vs set2-R195C	0.012622
	set2- H199L vs set2-SRIΔ	0.0010053
	set2- H199L vs set2-K663L	0.0049074
	set2- H199L vs set2-H199L K663L	0.8999947
	set2-R195C vs set2-SRIΔ	0.0010053
	set2-R195C vs set2-K663L	0.8999947
	set2-R195C vs set2-H199L K663L	0.0663943
	set2-SRIΔ vs set2-K663L	0.0010053
	set2-SRIΔ vs set2-H199L K663L	0.0010053
	set2-K663L vs set2-H199L K663L	0.0261485

Supplemental Data Set information (in separate files):

Sorenson et al Dataset S1: Binned Flow Cytometry Data from Histone Mutants and Deletion Mutants.
The “binned” raw data that are clustered and described in Figures 1 and S1 are available in

this.txt file and can be clustered as described in the caption of Figure S1. Each row contains binned flow cytometry data for an individual deletion or histone point mutant (labeled in first column). Columns represent a bin corresponding to a specific position on the two-dimensional mCherry vs. GFP flow cytometry dot plots. Bins are ordered from the origin to the top right, increasing in y value until the top, then shifting to the next x bin, etc. For further explanation of binning and clustering analysis, please refer to Sorenson and Stevens, RNA 2014; 20(5):732-45.

Sorenson et al Dataset S2: CDT File for Clustered Data in Figures 1 and S1.
This is the .cdt file to be viewed with Java TreeView. To open and retain formatting, file names for Datasets S2-S4 must be renamed with the same name, yet retain extensions.

Sorenson et al Dataset S3: GTR File for Clustered Data in Figures 1 and S1.
This is the .gtr file to be viewed with Java TreeView. To open and retain formatting, file names for Datasets S2-S4 must be renamed with the same name, yet retain extensions.

Sorenson et al Dataset S4: JTV File for Clustered Data in Figures 1 and S1.
This is the .jtv file to be viewed with Java TreeView. To open and retain formatting, file names for Datasets S2-S4 must be renamed with the same name, yet retain extensions.

Figure S1

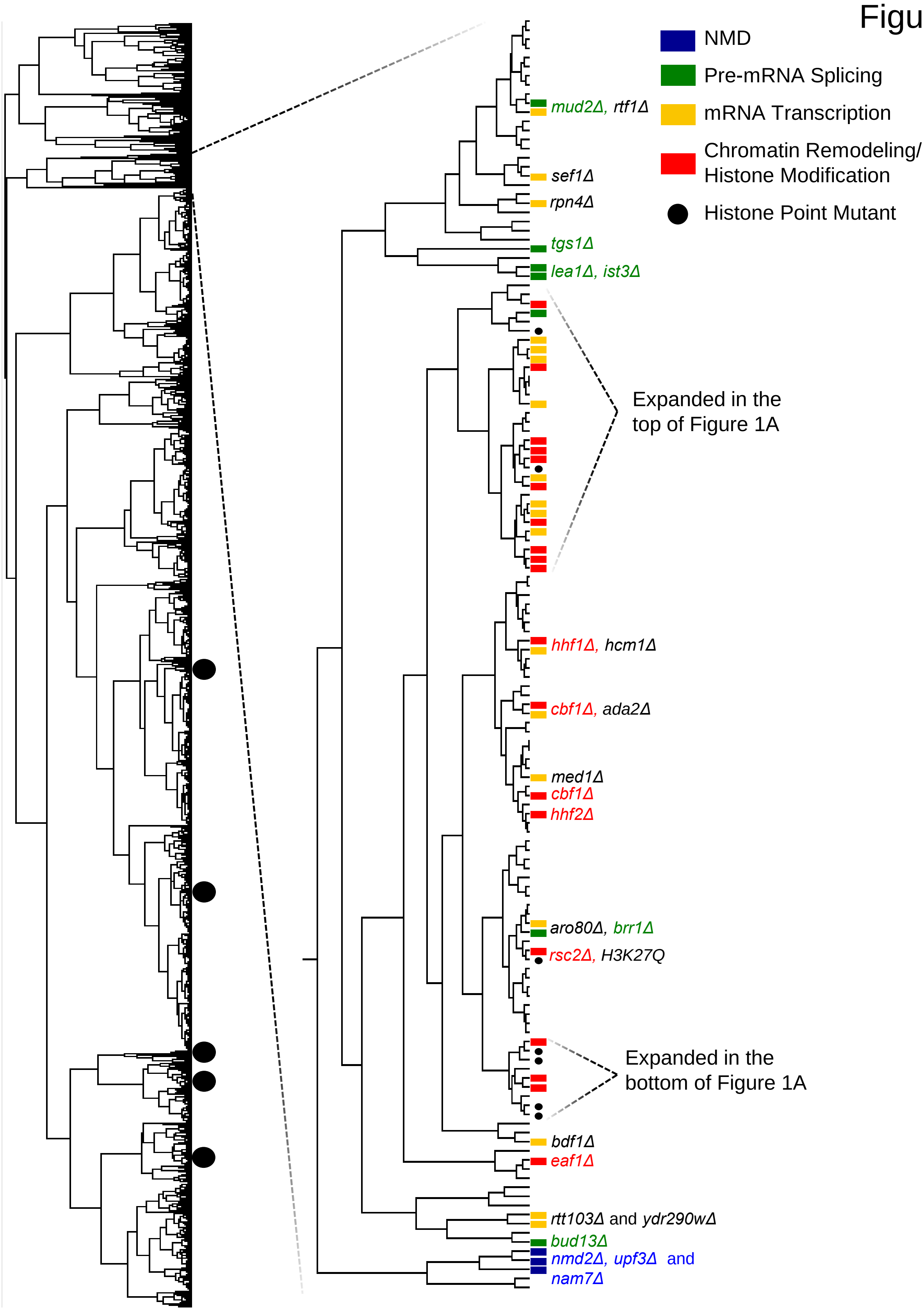

Figure S1: Global view of the clustering of histone point mutants of interest. Clustering behavior of histone point mutants within the deletion collection data set. Binning data for a collection of histone point mutants were integrated with the deletion collection data set, clustered with complete linkage and a centered absolute correlation similarity metric, shown on the left. The large black circles designate the clades that contain the majority of the histone point mutants, which are among wild-type-like mutants, in terms of reporter phenograph. In the middle, a cluster that contains seven specific histone point mutants and many red-shifted gene expression factor mutants is expanded and nodes are color-coded based on function or type of mutant. Highlighted clusters are expanded in Figure 1.

Figure S2

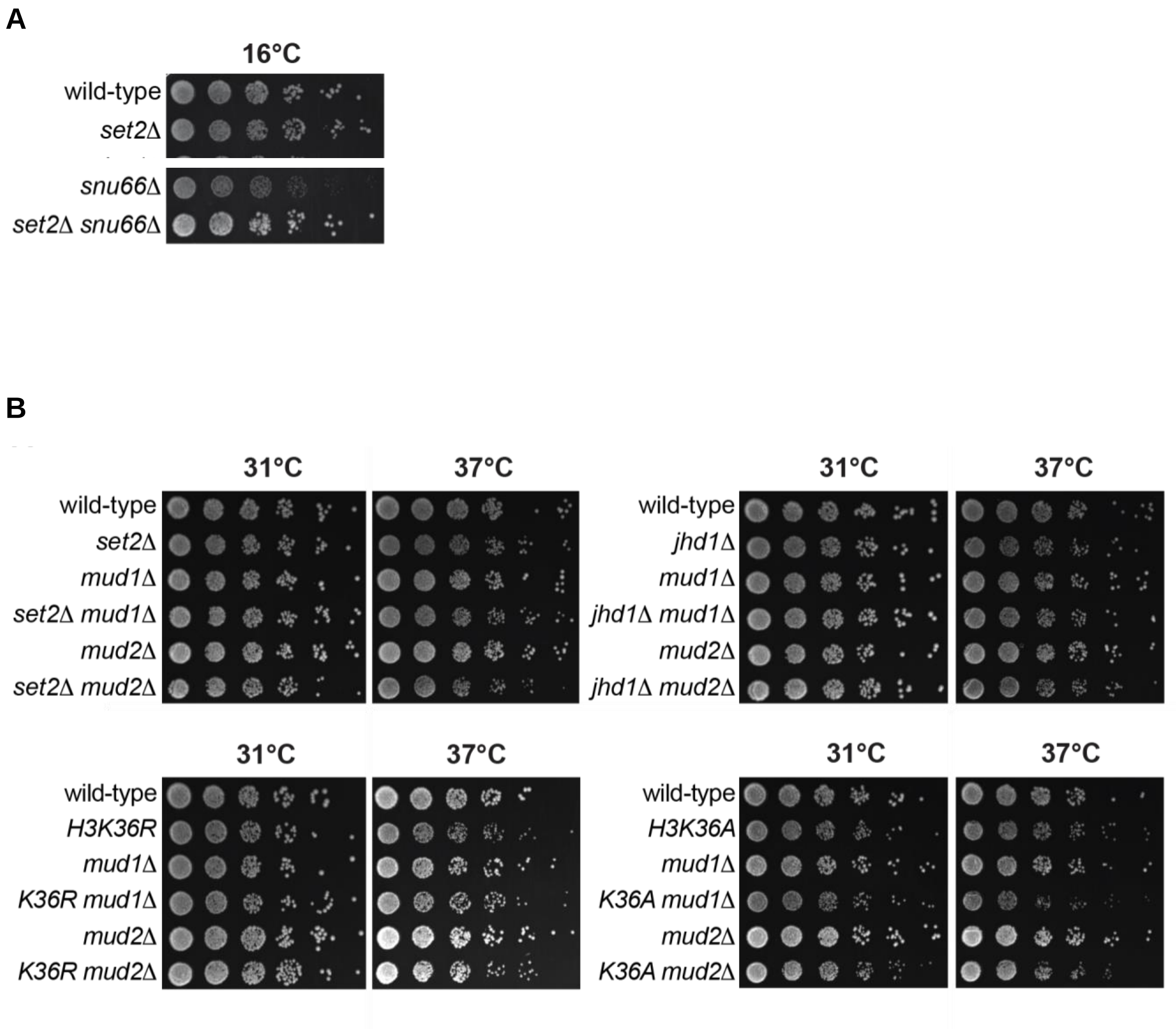

Figure S2. A. Deletion of *SET2* partially suppresses the cold sensitive growth defect the *snu66Δ* strain and range of mild to no genetic interaction observed between mud1*Δ* or mud2*Δ* deletion mutants and mutations that alter H3K36me. Serial dilutions of WT, single and double mutant strains grown on rich media at 16°C. Plates photographed after 10 days of growth**.** **B.** Mutations that alter the methylation state of H3K36 do not display genetic interactions with yeast mutants lacking the *MUD1* gene or the *MUD2* gene. Serial dilutions of WT, single and double mutant strains grown on rich media at 31°C and 37°C. Plates photographed after 48 hours growth.

Figure S3

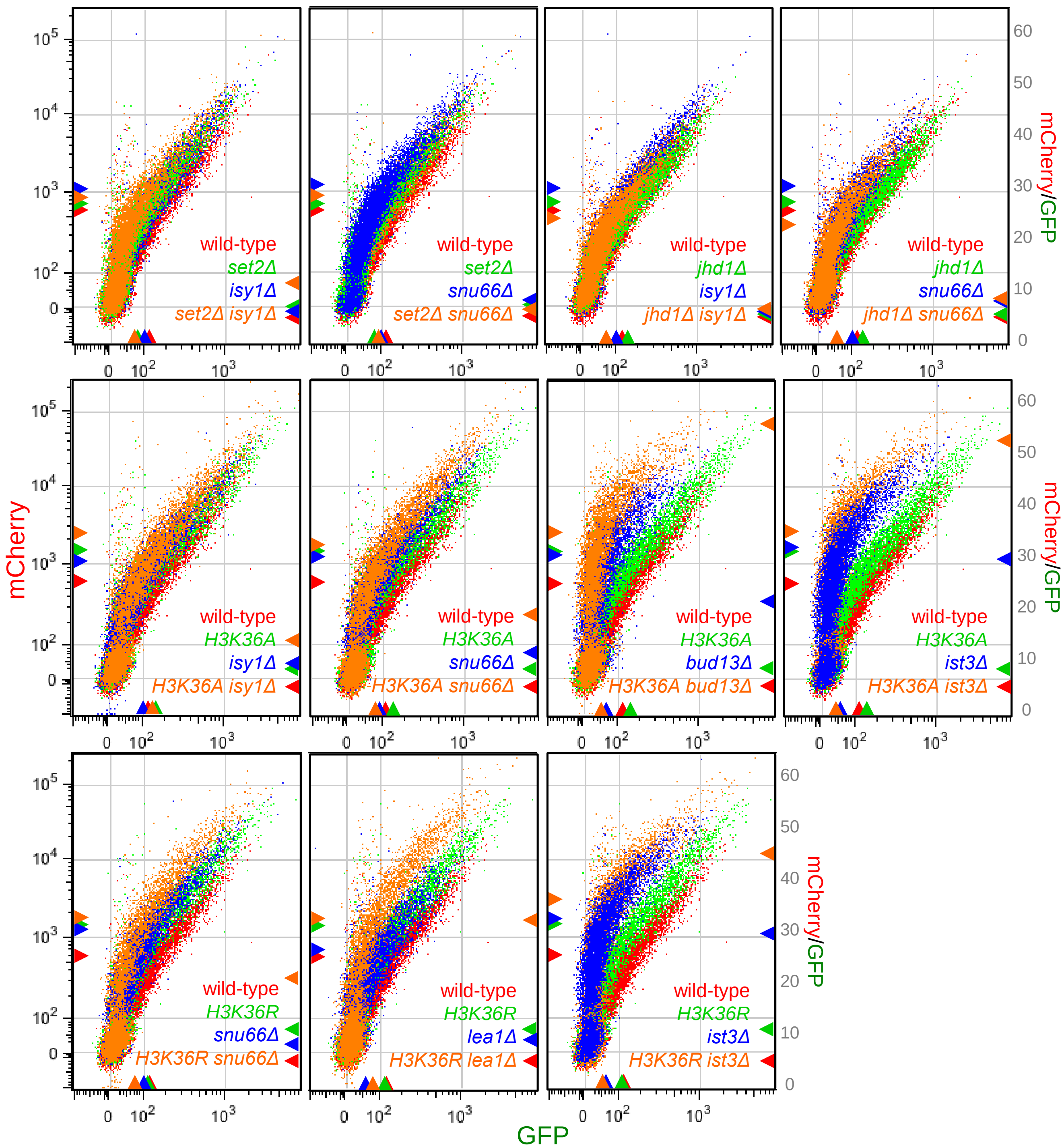

Figure S3. Gene Expression Reporter phenographs for selected single and double mutants. Standard flow cytometry overlays (mCherry versus GFP) are shown in each panel, depicting reporter expression in four yeast strains. Wild-type yeast is shown in red, histone modifier and point mutants are in green, pre-mRNA splicing mutants in blue and the double mutants in orange. To aid in comparing reporter expression levels the mean mCherry and GFP values for 21,000 cells are shown on *y*- and *x*-axis respectively with the colored arrowheads. To add another level means of comparison we show the mCherry/GFP ratio (unspliced/spliced) on the right of each panel (note that this is on a linear scale), serving as a proxy for pre-mRNA splicing efficiency.

Figure S4

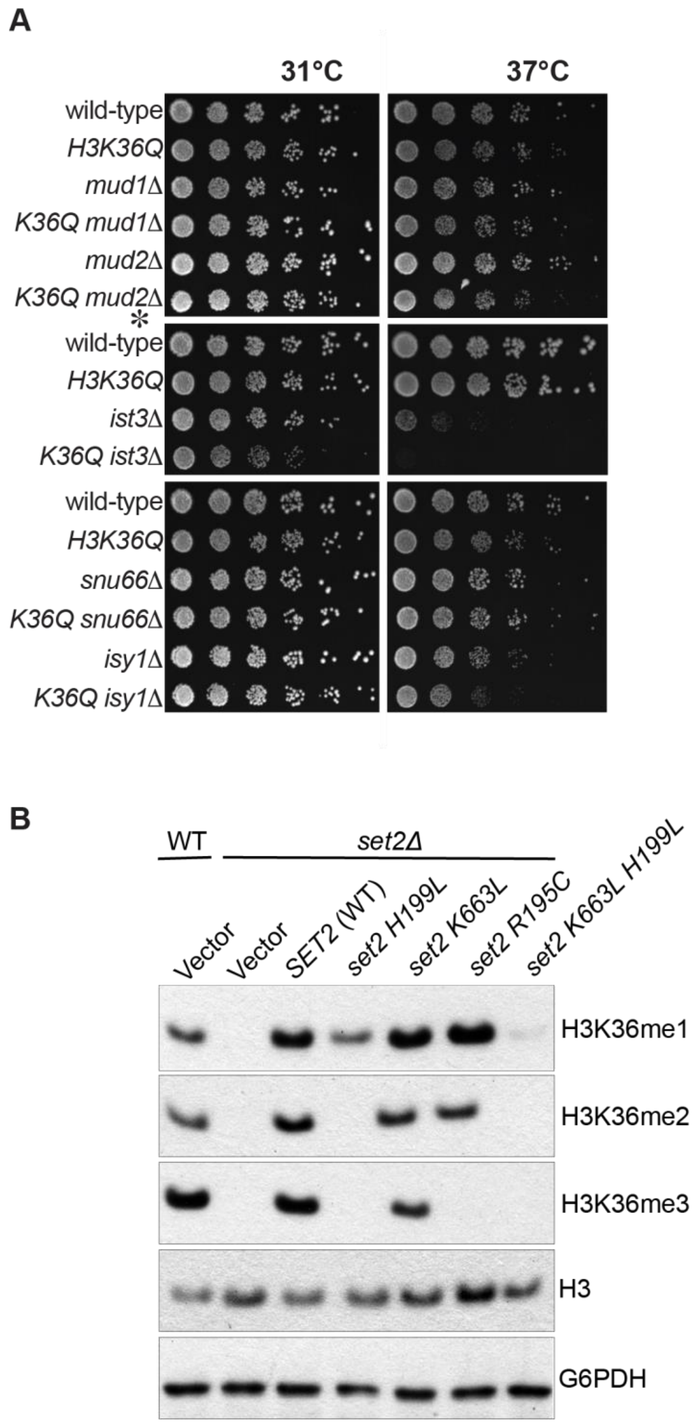

Figure S4: H3K36 is genetically implicated in RNA splicing and effect of *set2* mutations on H3K36 methylation status.(A) H3K36me is genetically implicated in RNA splicing. Serial dilutions of wild-type (WT), single and double mutant strains grown on rich media at 31°C and 37°C. Plates photographed after 48 hours growth (* middle 37C panel photographed after 72 hours growth). (B) Methylation status of various *SET2* constructs used in splicing and transcriptional assays. Log phase growing cells (from indicated strains) were used to prepare whole cell extracts (using SUMEB method) and probed using indicated antibodies.

Figure S5

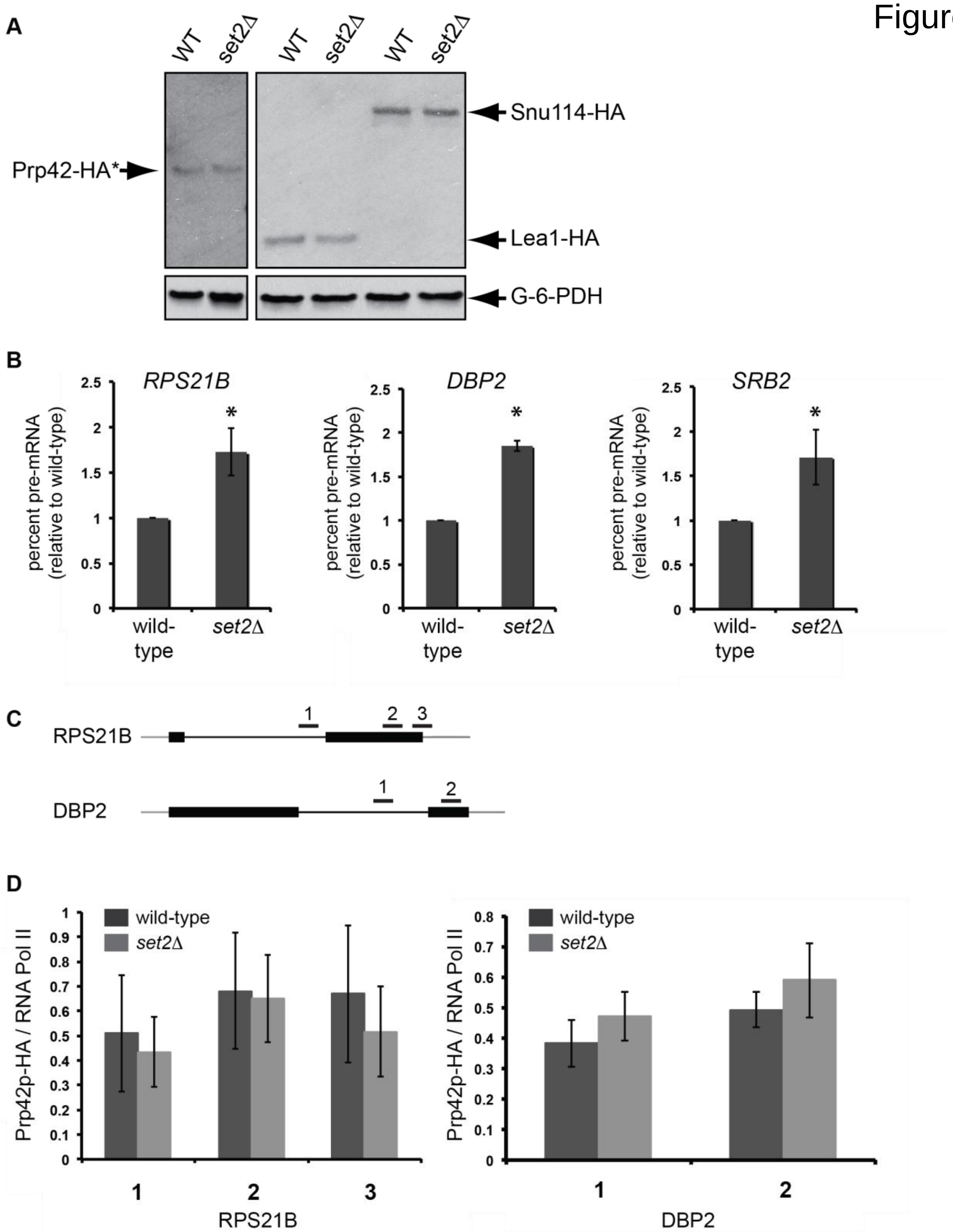

Figure S5: Deletion of *SET2* causes a reduction in RNA splicing but does not impact HA-tagged splicing factor levels or Prp42-HA association with chromatin. (A) Deletion of *SET2* does not alter the protein levels of the HA-tagged splicing factors. Yeast strains indicated were grown to mid-log phase (0.3-0.5), shifted to 37°C for 30 minutes, and total protein was isolated and quantitated using Bradford protein analysis. Total protein (6ug for Snu114-HA and Lea1-HA and 12ug for Prp42-HA; * note that Prp42-HA contains a single HA tag) was run on a 10% agarose gel. Western polyclonal HA antibodies and standard ECL. (B) Deletion of *SET2* results in impaired RNA splicing. Quantitative splicing assay. cDNA generated from total RNA isolated from *set2Δ* (TYK423) and its corresponding WT (BY4741) grown at 37°C was analyzed by QPCR with primers that detect the intron levels or the total levels of the pre-mRNA indicated in the figure. Represented in the bar graph is the percent unspliced RNA (pre-mRNA), which was determined by dividing the relative amounts of the intron product (pre-mRNA) by the relative amount of the total product (total mRNA) and multiplying by 100. Error bars represent SEM; n=3, * indicates $p>0.05$. (C) Schematic of the location of the primer sets used for ChIP analysis. (D) Deletion of *SET2* does not alter U1 snRNP association with chromatin. Chromatin immunoprecipitations were carried out using α-HA or α-RNA pol II antibodies in a Prp42-HA strain grown at 30°C then shifted to 37°C for 30 min. Shown are the average amounts of HA-tagged protein bound relative to RNA pol II bound at the indicated regions. Error bars represent +/- SEM for each strain and primer set, n=3 biological replicates.