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Supplemental Information

H3K36 Methylation and the Chromodomain Protein Eaf3 Are Required for Proper Cotranscriptional Spliceosome Assembly

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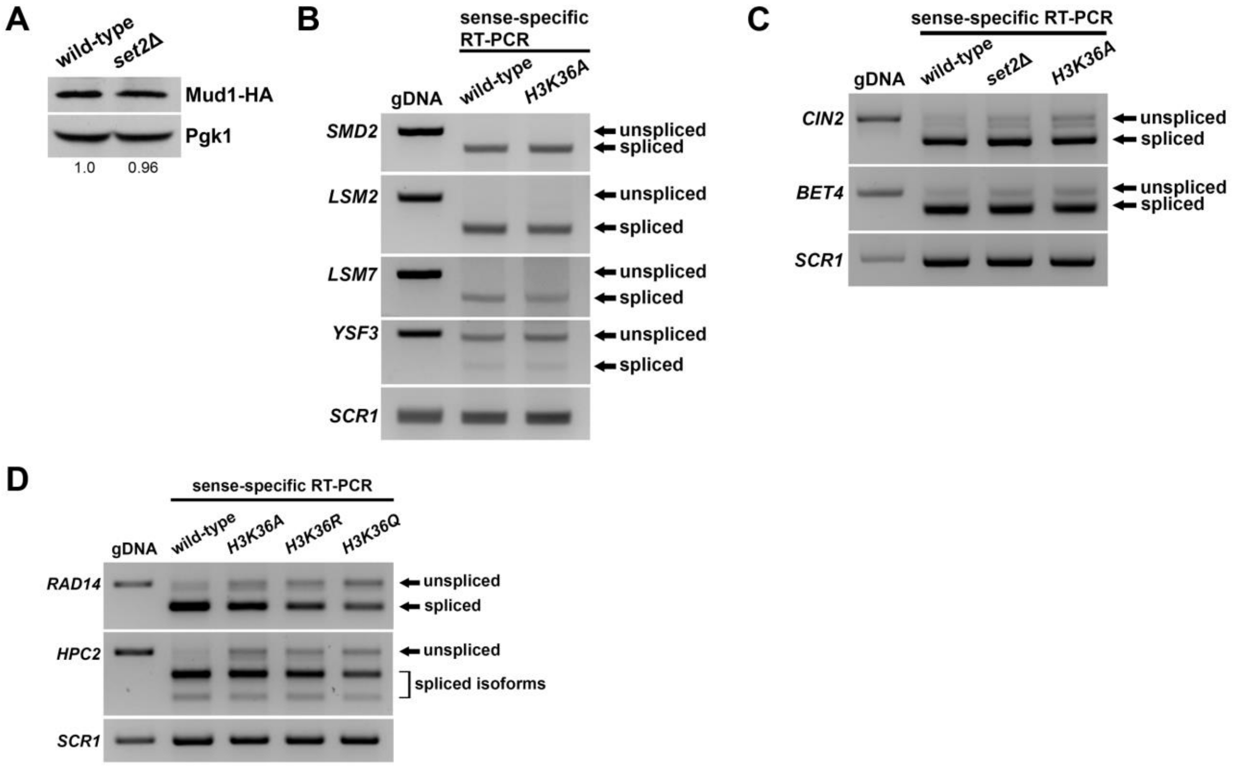

Figure S1. Splicing defects are observed in the absence of H3K36 methylation. Related to Figure 1. A. Western blot analysis of Mud1-HA protein levels in wild-type and *set2*Δ cells. Pgk1 is a loading control. Band intensities were quantified using ImageJ. The intensity of Mud1-HA normalized to Pgk1 in *set2*Δ was compared to the intensity of Mud1-HA normalized to Pgk1 in wild-type (defined as 1.0). **B.** RT-PCR of splicing factor ICGs. Products were analyzed on a 1.8% agarose gel. **C.** RT-PCR of ICGs that are lowly expressed and display a splicing defect in both *set2*Δ and *H3K36A* compared to wild-type. Products were analyzed on a 1.8% agarose gel. **D.** Splicing defects are observed in *H3K36R* and *H3K36Q* mutants. Products were analyzed on a 1.8% agarose gel. gDNA: genomic DNA. *SCR1* is a loading control.

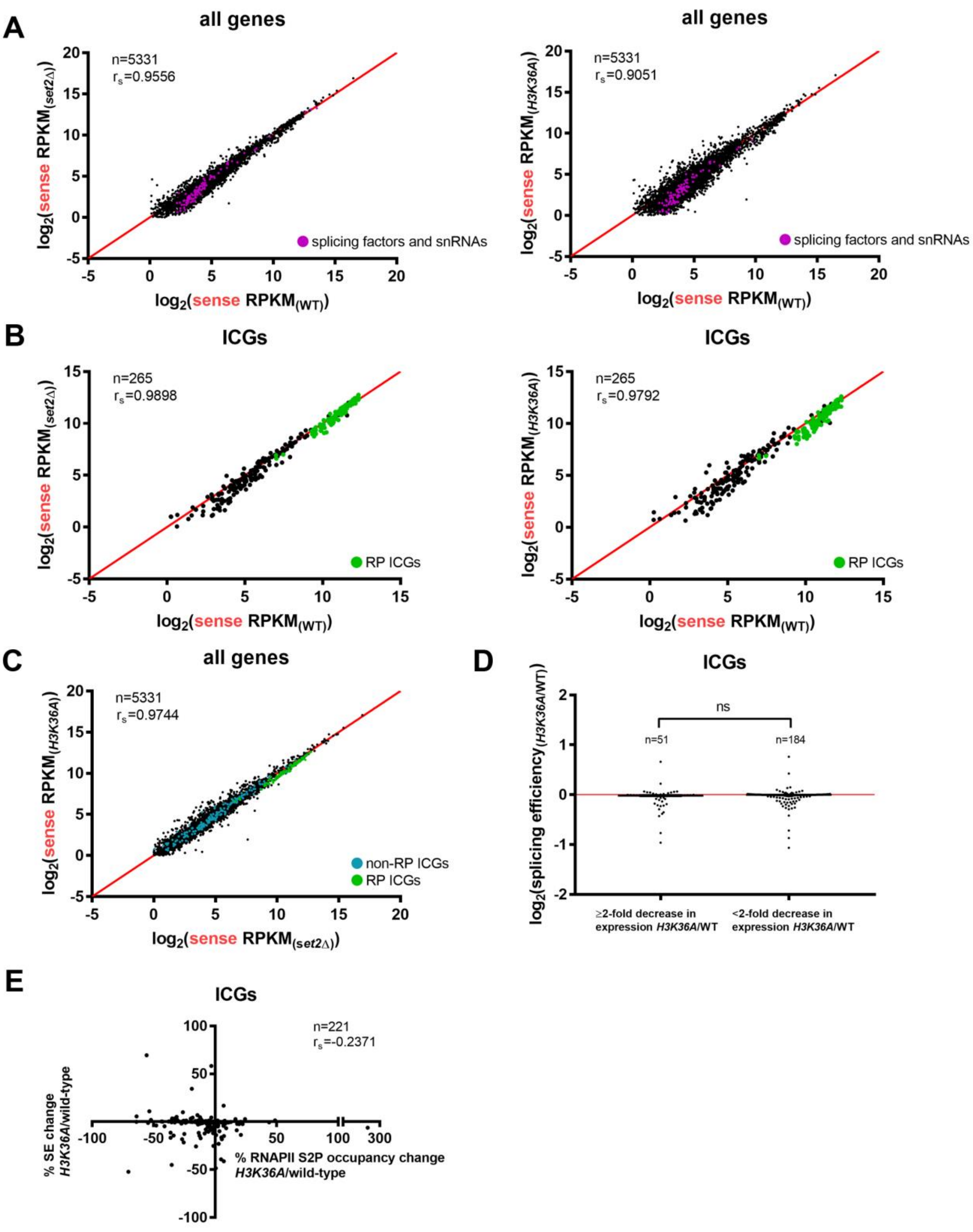
A
all genes
n=5331
r_s=0.9556
$\log_2$(sense RPKM$_{(set2\Delta)}$)
$\log_2$(sense RPKM$_{(WT)}$)
splicing factors and snRNAs
all genes
n=5331
r_s=0.9051
$\log_2$(sense RPKM$_{(H3K36A)}$)
$\log_2$(sense RPKM$_{(WT)}$)
splicing factors and snRNAs
-5
0
5
10
15
20
B
ICGs
n=265
r_s=0.9898
$\log_2$(sense RPKM$_{(set2\Delta)}$)
$\log_2$(sense RPKM$_{(WT)}$)
RP ICGs
ICGs
n=265
r_s=0.9792
$\log_2$(sense RPKM$_{(H3K36A)}$)
$\log_2$(sense RPKM$_{(WT)}$)
RP ICGs
C
all genes
n=5331
r_s=0.9744
$\log_2$(sense RPKM$_{(H3K36A)}$)
$\log_2$(sense RPKM$_{(set2\Delta)}$)
non-RP ICGs
RP ICGs
D
ICGs
$\log_2$(splicing efficiency$_{(H3K36A/WT)}$)
2
1
0
-1
-2
ns
n=51
n=184
≥2-fold decrease in expression H3K36A/WT
<2-fold decrease in expression H3K36A/WT
E
ICGs
n=221
r_s=-0.2371
% SE change H3K36A/wild-type
% RNAPII S2P occupancy change H3K36A/wild-type
100
50
-50
-100
-100
-50
50
100
300

Figure S2. RNA expression profiles in *set2*Δ and *H3K36A* cells. Related to Figure 1. A. XY-scatter plot of wild-type sense RPKM, *set2*Δ sense RPKM (left), and *H3K36A* sense RPKM (right) of all genes. All splicing factors and spliceosomal snRNAs are labeled in purple. Spearman's correlation coefficient indicated. Red line represents the x=y line. **B.** XY-scatter plot of wild-type sense RPKM, *set2*Δ sense RPKM (left), and *H3K36A* sense RPKM (right) of all ICGs. Ribosomal protein intron-containing genes (RP ICGs) are labeled in green. Spearman's correlation coefficient indicated. **C.** XY-scatter plot of *set2*Δ sense RPKM and *H3K36A* sense RPKM of all ICGs. Non-ribosomal protein intron-containing genes (non-RP ICGs) are labeled in blue and ribosomal protein intron-containing genes (RP ICGs) are labeled in green. Spearman's correlation coefficient indicated. **D.** Scatter plot representing change in splicing efficiency (SE) of ICGs that show a ≥2-fold decrease in expression in *H3K36A* compared to wild-type and ICGs that show a <2-fold decrease in expression in *H3K36A* compared to wild-type. Solid black line represents the median. Red line represents the y=0 line. Unpaired t test was used to test significance. **E.** XY-scatter plot of % RNAPII S2P ChIP-seq occupancy change and % SE change of all ICGs in *H3K36A*/wild-type cells.

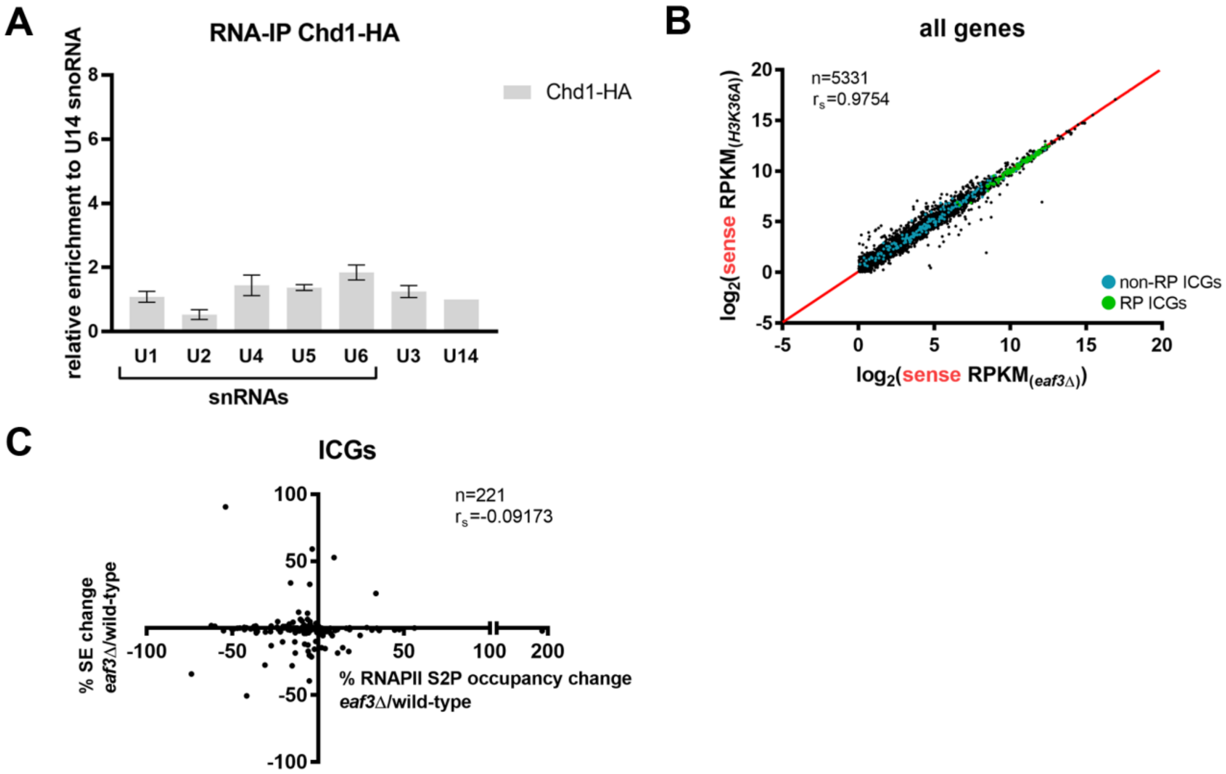

Figure S3. *eaf3*Δ and *H3K36A* cells have similar RNA expression profiles. Related to Figure 2. A. Spliceosomal snRNAs co-immunoprecipitated with Chd1-HA. Bar graph depicting the five spliceosomal snRNAs that are pulled-down with Chd1-HA in wild-type cells under non-crosslinking conditions. U3 and U14 are snoRNAs. Bars represent the average of 3 biological replicates. Error bars represent the standard error of the mean (SEM). **B.** XY-scatter plot of *eaf3*Δ sense RPKM and *H3K36A* sense RPKM of all genes. Non-ribosomal protein intron-containing genes (non-RP ICGs) are labeled in blue and ribosomal protein intron-containing genes (RP ICGs) are labeled in green. Spearman's correlation coefficient indicated. Red line represents the x=y line. **C.** XY-scatter plot of % RNAPII S2P ChIP-seq occupancy change and % SE change of all ICGs in *eaf3*Δ/wild-type cells.

Table S1. Strains used in this study. Related to STAR Methods.

Name	**Genotype**	**Reference**
BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Open Biosystems
TJY7170	MATa *set2*Δ::KANMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	GE Dharmacon
TJY7152	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[*H3K36A*-HHFS]*-URA3	GE Dharmacon
TJY7171	MATa *set2 SRI*Δ::HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7153	MATa *eaf3*Δ::KANMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	GE Dharmacon
TJY7158	MATa EAF3-HA::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7159	MATa *set2*Δ::KANMX4 EAF3-HA:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7214	MATa PRP45-HA::KANMX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7209	MATa PRP45-Myc::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7216	MATa EAF3-HA::HIS3MX6 PRP45-Myc::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7215	MATa *eaf3*Δ::KANMX4 PRP45-HA::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7238	MATa *set2*Δ::KANMX4 PRP45-HA::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7240	MATa PRP19-HA::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7241	MATa *eaf3*Δ::KANMX4 PRP19-HA:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7242	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[*H3K36R*-HHFS]*-URA3	GE Dharmacon
TJY7243	MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[*H3K36Q*-HHFS]*-URA3	GE Dharmacon
TJY7244	MATa MUD1-HA::KANMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7245	MATa *set2*Δ::KANMX4 MUD1-HA::HIS3MX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
TJY7246	MATa CHD1-HA::KANMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study

Table S3. Primer sequences used for RT-PCR, RT-qPCR, and ChIP-qPCR. Related to STAR Methods.

Name	**Sequence (5′ to 3′)**	**Purpose**
HPC2 sense	AACACCACCTCTTTTCATGGTACCA	RT-PCR
HPC2 F	CCTCCACGA CCATATTCAA ACGATTGG	RT-PCR
HPC2 R	GGAACCAGAAATTATAATGGGAGACGG	RT-PCR
RAD14 sense	TGTCAATTTCTTCAGTTTCTAGCCC	RT-PCR
RAD14 F	CGTAGTGAAGGTATCGAACGTAACGC	RT-PCR
RAD14 R	GTGTTAGTGTTAGCAAGCGCAGACG	RT-PCR
DYN2 sense	CGCCAGTGGACCGATATAGAAATAA	RT-PCR
DYN2 F	GGAAAGCCTCCAAAACTACTGCCAG	RT-PCR
DYN2 R	GAAAACTAAAAACGCCAGTGGACCG	RT-PCR
MUD1 sense	GGTAACGTCATTGTTTTGTAGCTTGT	RT-PCR
MUD1 F	CGGCCTCATCAAACCTAAAGAAACC	RT-PCR
MUD1 R	GAAACCGGTCTGCTTCTTCTTGAG	RT-PCR
YCL002C sense	TTATAGTTTTCCTTTTGGCAACCGTG	RT-PCR
YCL002C F	ATGCTTGTTA TTGTTCTGCAGGGC	RT-PCR
YCL002C R	AACTGCCTTAAAACCATCATGCAGC	RT-PCR
TFC3 sense	ACCAATTATGATTGACCCAATAGCC	RT-PCR
TFC3 F	GACGATTTATCCTGACGAACTCGTAC	RT-PCR
TFC3 R	CCTGATTTGGCAACTTCGAGAAGTA	RT-PCR
RPL14A sense	TTAAGCCTTAGCCAAAGCCTTCTTG	RT-PCR
RPL14A F	CAAGGCTTCTAACTGGAGATTAGTCG	RT-PCR
RPL14A R	CAATCTTCTTAGCCCAAGATGAAGC	RT-PCR
SCR1 sense/R	CACAATGTGCGAGTAAATCCTGATG	RT-PCR
SCR1 F	AAGGGATAGTTCTCTATTCCGCACC	RT-PCR
CIN2 sense	CTATAAGTAAGCGCGAAACAACTGC	RT-PCR
CIN2 F	GCTTAAGCATAAATGGACTTTACTGCG	RT-PCR
CIN2 R	GCTGAATCACCCTTCTCCAAGAC	RT-PCR
BET4 sense	CACTTATGCTGCTCCAGGAGATG	RT-PCR
BET4 F	ACTATAAAGCAGTAGGTCAGCAATG	RT-PCR
BET4 R	TCCAATAGACTTTGGGGTAATCCTTC	RT-PCR
YSF3 sense/R	CTACCTCTCTTCTCGTAAGTAGGCTT	RT-PCR
YSF3 F	GGTTATATTACACAATTCGAAACAGTGAAA	RT-PCR
LSM2 sense/R	TCTTTCAGTCATTACCTCCCTTCTGG	RT-PCR
LSM2 F	CAAGACTTTA GTTGACCAAG AAGTGGT	RT-PCR
SMD2 sense/R	ACTCAACAGGGGTTTTTAACACAACG	RT-PCR
SMD2 F	CGCCTTTGACAGTTGATTAGAGGAGT	RT-PCR
LSM7 sense/R	TATAGTACATCAGAACCTTCGGCGG	RT-PCR
LSM7 F	CAAAAAAACATGCATCAGCAACACTCC	RT-PCR
U1 F	ATTGAAGTCATTGATGCAAACTCCT	qPCR
U1 R	GGTGTCAAACTTCTCCAGGCAG	qPCR

U2 F	TATCGATGGGAAGAAATGGTGC	qPCR
U2 R	CTCTTGCAGCGCCACCAG	qPCR
U4 F	CGCATATCAGTGAGGATTCGTC	qPCR
U4 R	CCAAAAATTCCCTACATAGTCTTGAAGTA	qPCR
U5 F	TACAGATCAATGGCGGAGGG	qPCR
U5 R	AAATATGGCAAGCCCACAGTAAC	qPCR
U6 F	AAGTAACCCTTCGTGGACATTTG	qPCR
U6 R	TCTCTTTGTAAAACGGTTCATCCTTAT	qPCR
U3 F	CAAAAGAGCCACTGAATCCAACT	qPCR
U3 R	TAGATGGCCGAACCGCTAAG	qPCR
U14 F	GGTGATGAAAGACTGGTTCCTTA	qPCR
U14 R	AAGGTCTCTAAAGAAGAGCGGTC	qPCR
HPC2 1 F	ACCTCCACGACCATATTCAAACG	ChIP-qPCR
HPC2 1 R	CCCTAACGAAGGGCGGATAATTG	ChIP-qPCR
HPC2 2 F	TTCCTCCAGTACAAACCCGATGG	ChIP-qPCR
HPC2 2 R	ATCACGGGGGATGGTGAATG	ChIP-qPCR
HPC2 3 F	CTCCAGCAAAAAGCCTACGTCTG	ChIP-qPCR
HPC2 3 R	TCTTGGTCGTTGTTGGCTTTGG	ChIP-qPCR
HPC2 4 F	TCTCCGAAGAAGAAGTCGCATCC	ChIP-qPCR
HPC2 4 R	CCGAATCATCAATGAACGGATCTTC	ChIP-qPCR
RAD14 1 F	GTATCGAACGTAACGCTATGACTCC	ChIP-qPCR
RAD14 1 R	ACCCACGGTTAAAATACAAACACAG	ChIP-qPCR
RAD14 2 F	GGCAGCAATCGGGATGATAATG	ChIP-qPCR
RAD14 2 R	TGTTAGTGTTAGCAAGCGCAGACG	ChIP-qPCR
RAD14 3 F	TGCACCTCCTCCAGAGCATATTTC	ChIP-qPCR
RAD14 3 R	CATCATGTAGCACAGGATCCATCTC	ChIP-qPCR
RAD14 4 F	AATGGCAACGTCGTGAAGAAGG	ChIP-qPCR
RAD14 4 R	GCTCTTGTTTTCAGTCGCATTTCC	ChIP-qPCR
ECM33 1 F	AAGAGGAAACGGGTTTCGAG	ChIP-qPCR
ECM33 1 R	ACTCGCCCTAATCCTATGACAG	ChIP-qPCR
ECM33 2 F	ACCAGTGCTTCTTTCGGTTC	ChIP-qPCR
ECM33 2 R	GGTAGAAATGGCAGGCAAAG	ChIP-qPCR
ECM33 3 F	AGGCCGCTTTCAGTAACTTG	ChIP-qPCR
ECM33 3 R	ACTTCAATGGCACCACCAAC	ChIP-qPCR
ECM33 4 F	TAGTGGTGATGCCTCCAATG	ChIP-qPCR
ECM33 4 R	TGGAACAAGTTCTGGAGCAG	ChIP-qPCR
ADH1 1 F	TGGTGTCTGTCACACTGACTTG	ChIP-qPCR
ADH1 1 R	TTCGTGACCACCGACTAATG	ChIP-qPCR
ADH1 2 F	TCACGCTGACTTGTCTGGTTAC	ChIP-qPCR
ADH1 2 R	AATGTGAGCGGCTTGAACAG	ChIP-qPCR
ADH1 3 F	TTGACGGTGGTGAAGGTAAG	ChIP-qPCR
ADH1 3 R	CACCGACAATGTCCTTTTCC	ChIP-qPCR
ADH1 4 F	TCAACCAAGTCGTCAAGTCC	ChIP-qPCR
ADH1 4 R	TCTGGCGAAGAAGTCCAAAG	ChIP-qPCR