Supplemental Figure Legends

Supplemental Figure S1. Positioning by Chd1-Ume6 requires a catalytically active fusion protein.

Difference in nucleosome dyad signal between Δ*ume6* +[$Chd1_{D513N}$-Ume6] and Δ*ume6* cells at permutations of the core *GGCGGC* motif (left) and other known transcription factor motifs (right). Rows are ordered identically to Figure 2B. No significant differences in nucleosome positions were observed when an ATP hydrolysis-deficient $Chd1_{D513N}$-Ume6 fusion was introduced into Δ*ume6* cells.

Supplemental Figure S2. Positioning by Isw2/Ume6 is independent of transcription start site (TSS) location.

A. Positioned nucleosomes at instances of Ume6 binding motifs are compared to movement with respect to associated transcription start sites. If no transcription start site is associated with a motif, or if no directional nucleosome positioning was observed, the direction was "undefined".

B. Comparison of nucleosome positioning within each cluster with respect to motif location (left) or associated transcription start sites (TSS, right). Precise positioning is only observed with respect to Ume6 motif, not TSS.

C. Positioning of nucleosomes for 401 coding region Ume6 binding motifs.

Supplemental Figure S3. Positioning by Chd1-Ume6 at Ume6 sites is independent of Isw2.

(A-C) Same as in Figure 2 but with Chd1-Ume6 introduced into a Δ*isw2* background showing (A) MNase-seq nucleosome dyad signal at known Ume6-regulated genes, (B) difference in nucleosome dyad signals between Δ*isw2* +[Chd1-Ume6] and Δ*isw2* strains at permutations of a core *GGCGGC* motif (left) compared to other transcription factor motifs (right), (C) difference in nucleosome dyad signals between Δ*isw2* and Δ*isw2* +[Chd1-Ume6] strains at all instances of the *WNGGCGGCWW* motif (left) with average

MNase-seq signal for individual strains within each cluster (right). Rows are ordered identically to Figure 2.

Figure S4. Positioning by Isw2/Ume6 is independent of transcription start site (TSS) location.

A. Positioned nucleosomes at instances of Ume6 binding motifs are compared to movement with respect to associated transcription start sites. If no transcription start site is associated with a motif, or if no directional nucleosome positioning was observed, the direction was "undefined".

B. Comparison of nucleosome positioning within each cluster with respect to motif location (left) or associated transcription start sites (TSS, right). Precise positioning is only observed with respect to Ume6 motif, not TSS.

Supplemental Figure S5. Nucleosome positioning at Ume6 sites requires Isw2.

(A) Differences in nucleosome dyad signal between wild type and Δ*isw2* cells at variations of a core *GGCGGC* sequence (left) and yeast transcription factor binding motifs (right).

(B) Differences in nucleosome dyad signal between wild type and Δ*isw2* cells at all intergenic instances of the peak *WNGGCGGCWW* positioning motif (left) clustered by positioning direction with average signal for each individual strain within each cluster (right). Data are ordered identically to Figure 3.

Supplemental Figure S6. Transcriptional repression by Ume6, Isw2, and Rpd3.

(A-D) Differential transcript abundance between wild type and indicated strains showing repressive capacity of (A) Ume6, (B) Isw2, (C) Rpd3 and (D) Isw2+Rpd3. Black dots represent all transcripts, while red dots represent transcripts with proximal URS1 motifs.

Supplemental Figure S7. Disruption of endogenous nucleosome positions by Chd1-Ume6 can lead to cryptic transcription.

(A) Browser shot of nucleosome dyad positions with transparent nucleosome footprint (top) for wild type with and without Chd1-Ume6 and associated RNA abundance for Watson (blue) and Crick (red) strands at Ume6 binding motif for the *ALP1* locus.

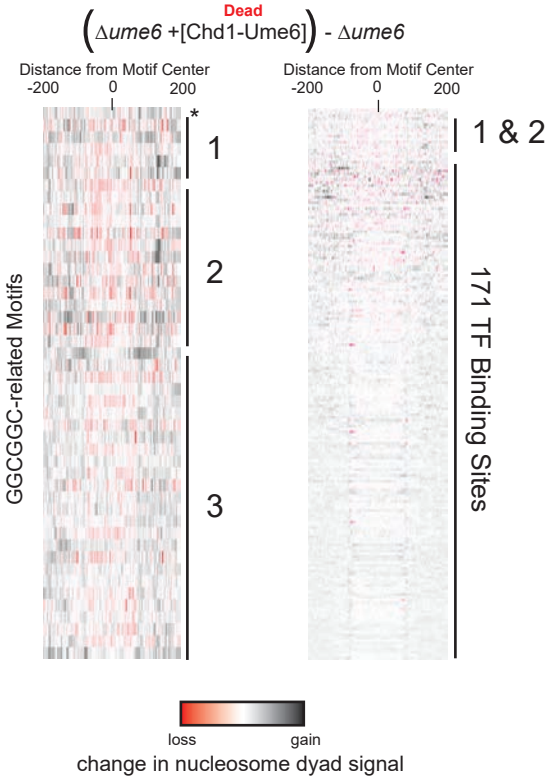
(B) Same as in (A) but at a Ume6 binding motif located at the 3' end of the *HED1* gene. Divergent cryptic transcripts are revealed at each locus due to chromatin disruption by Chd1-Ume6.

Supplemental Figure S8. Nucleosome positioning by Chd1-Ume6 does not expose cryptic unstable transcripts.

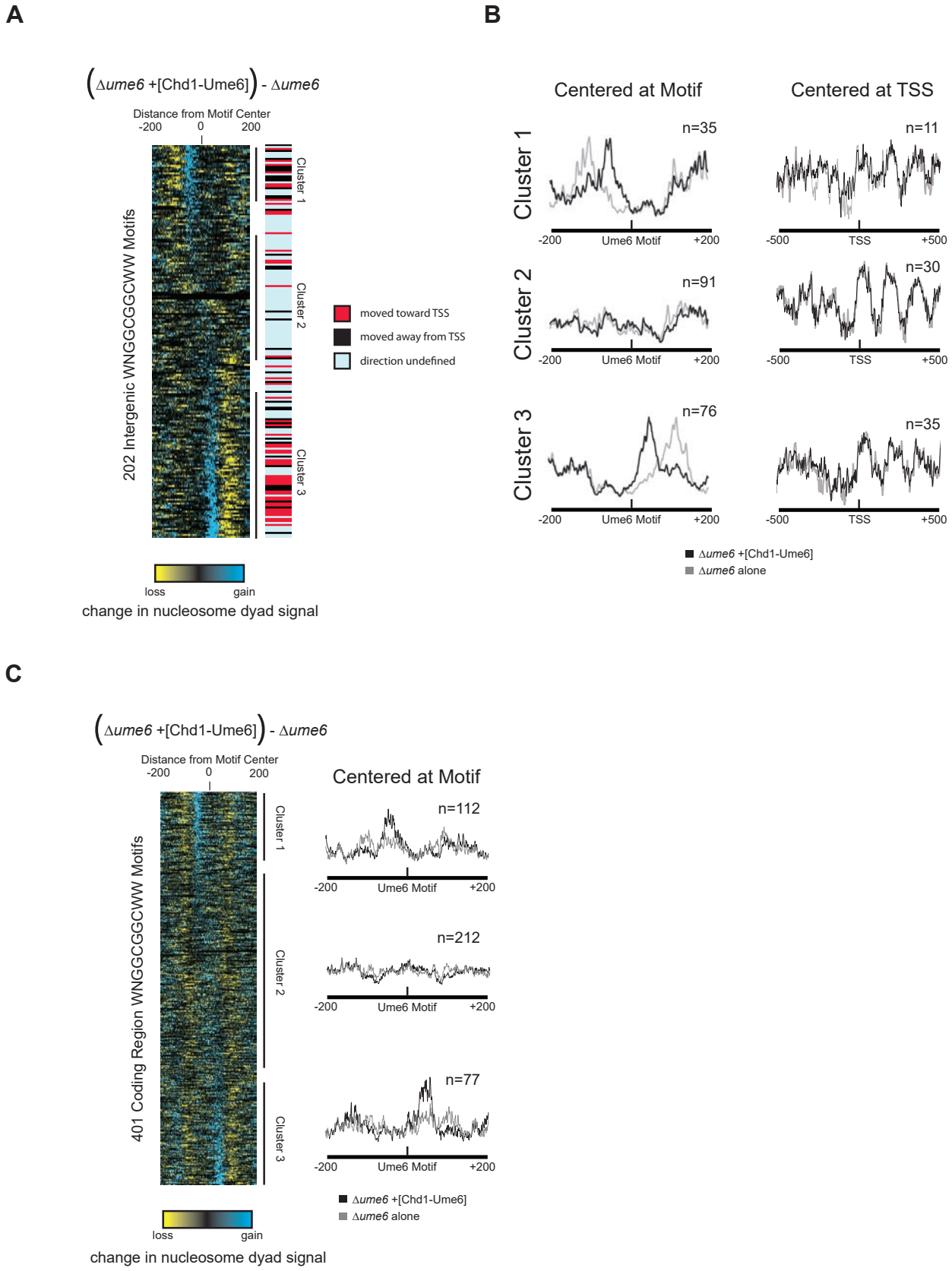
A. Deletion of Rrp6 leads to cryptic unstable transcription as previously described at *LIN1* (left) and at a handful of genes containing Ume6 binding motifs (right).

B. Nucleosome repositioning by Chd1-Ume6 does not lead to appreciable CUT formation at *ALP1* (left) or at any other instance of Ume6 binding motifs (right).

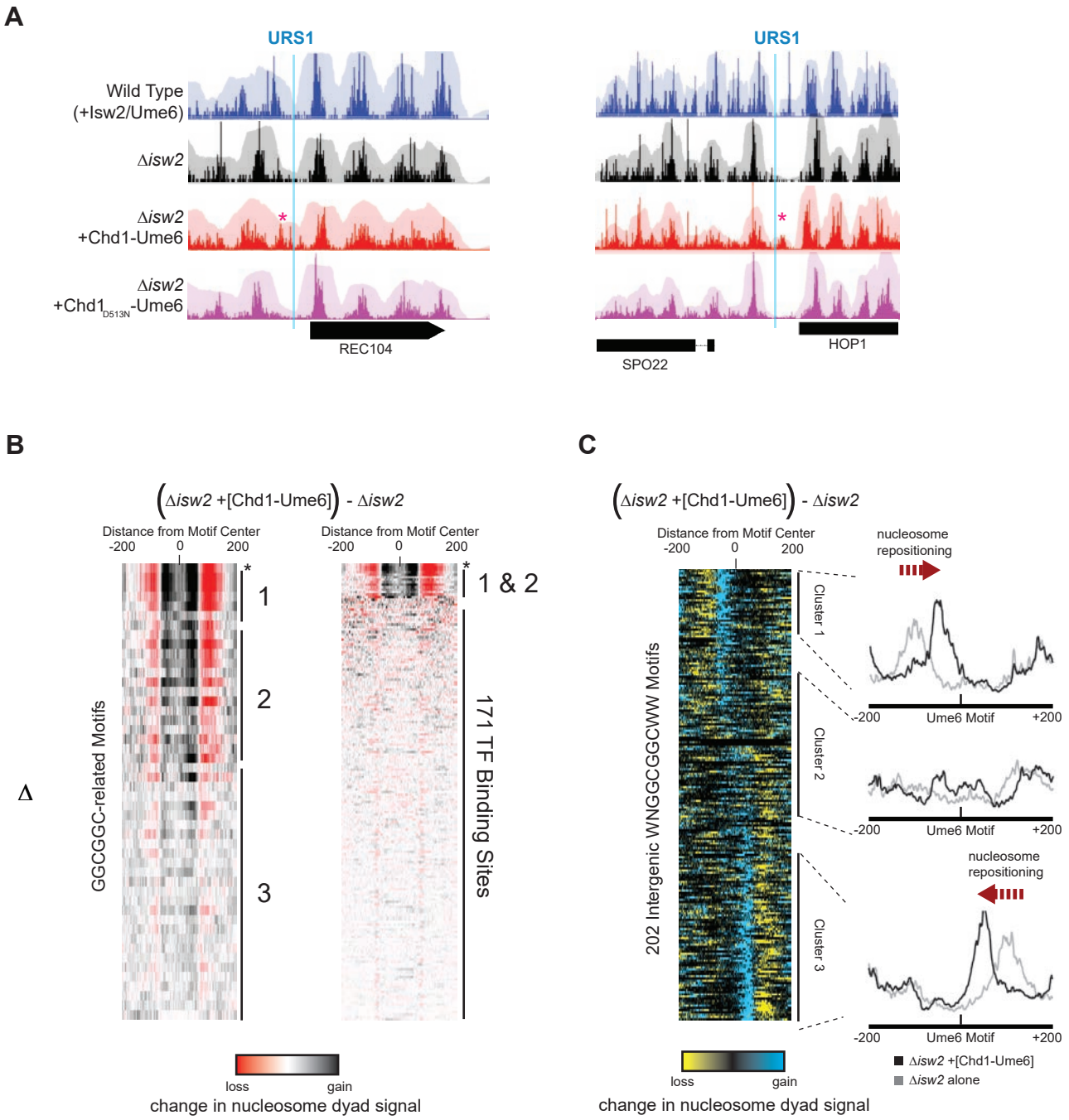
Supplemental Figure S1



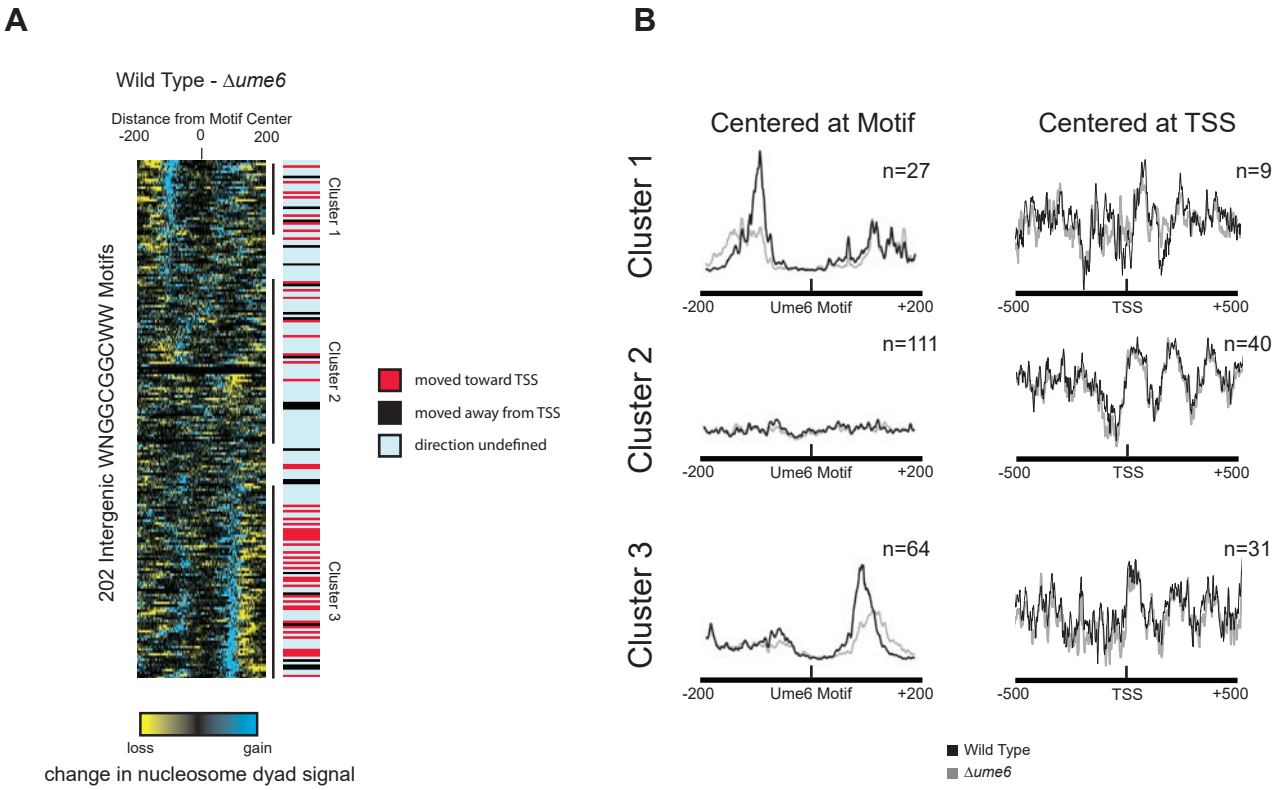
Supplemental Figure S2



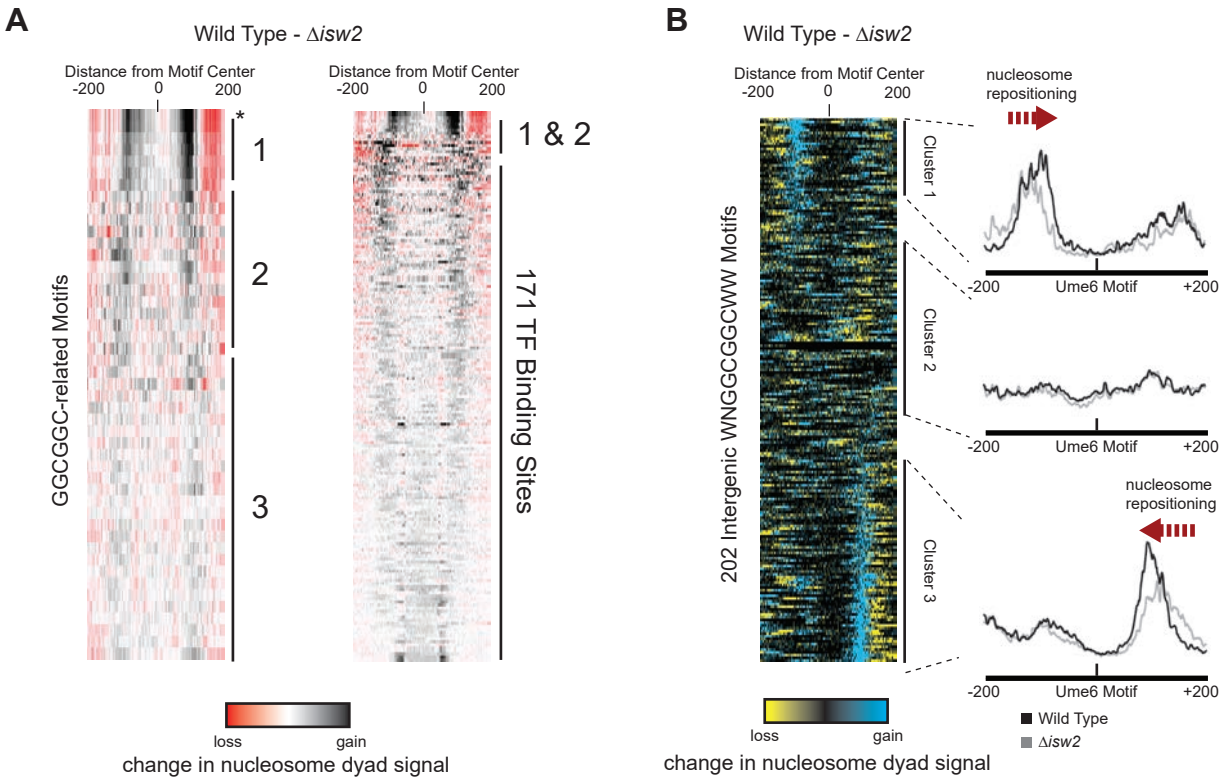
Supplemental Figure S3



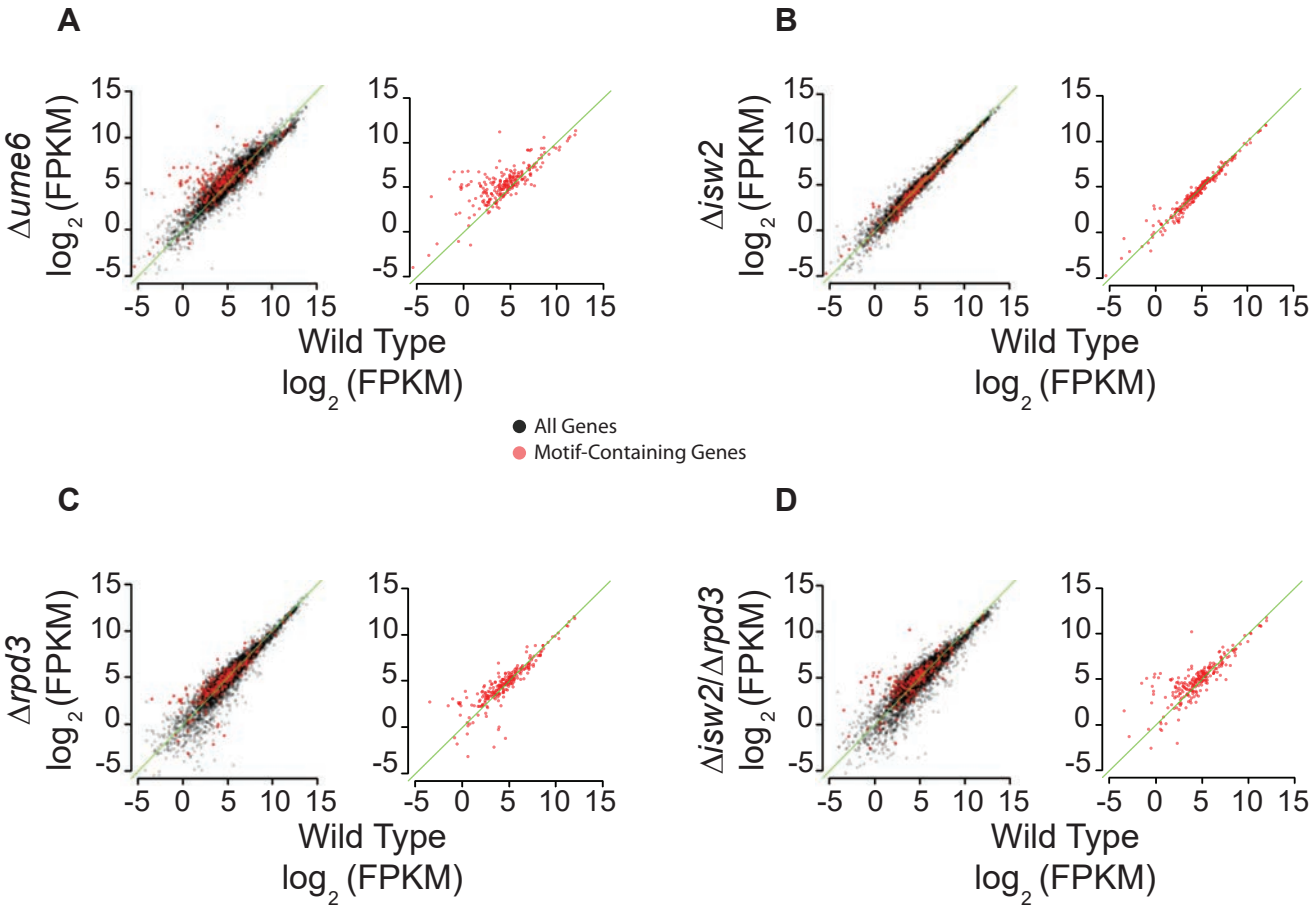
Supplemental Figure S4



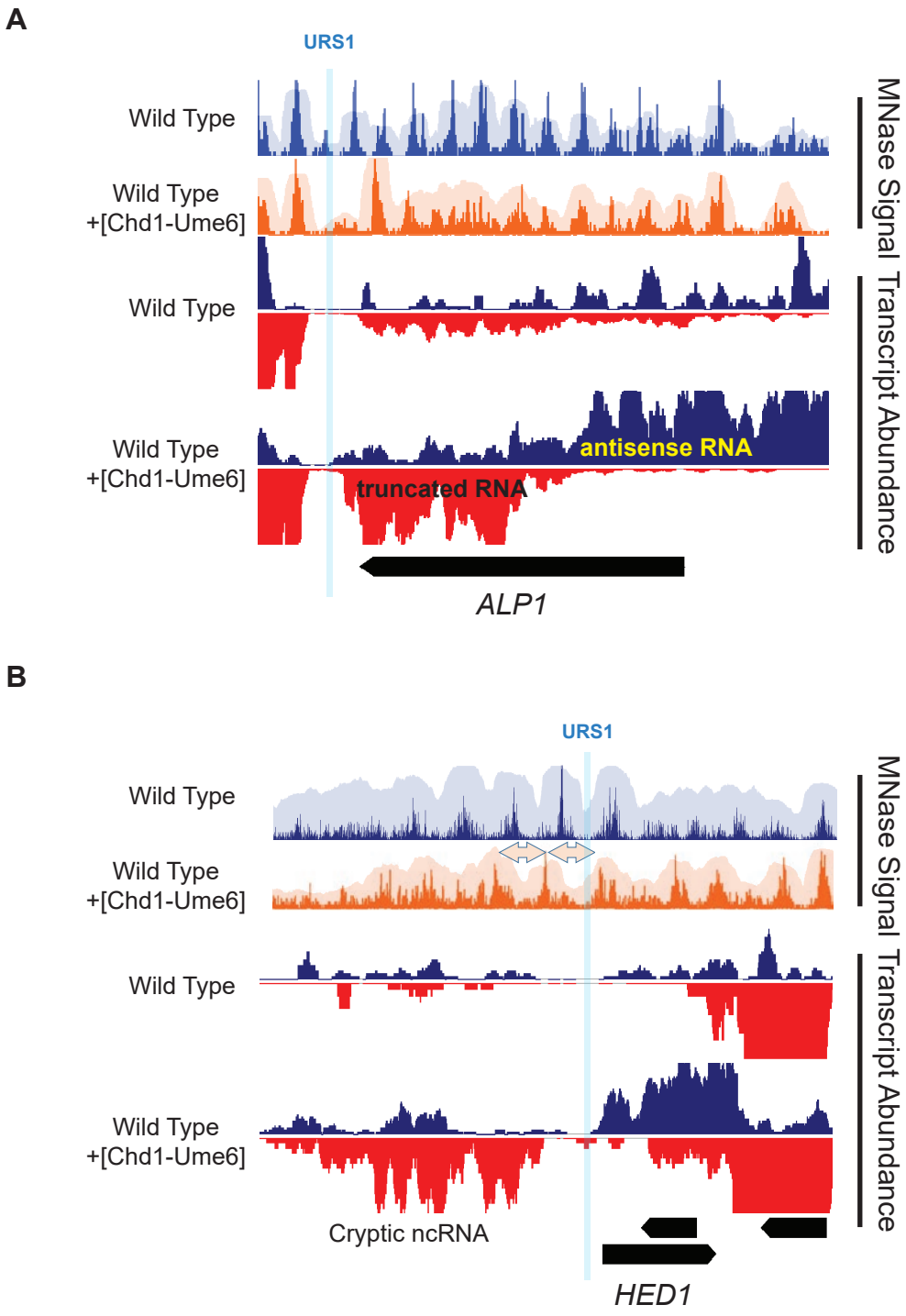
Supplemental Figure S5



Supplemental Figure S6

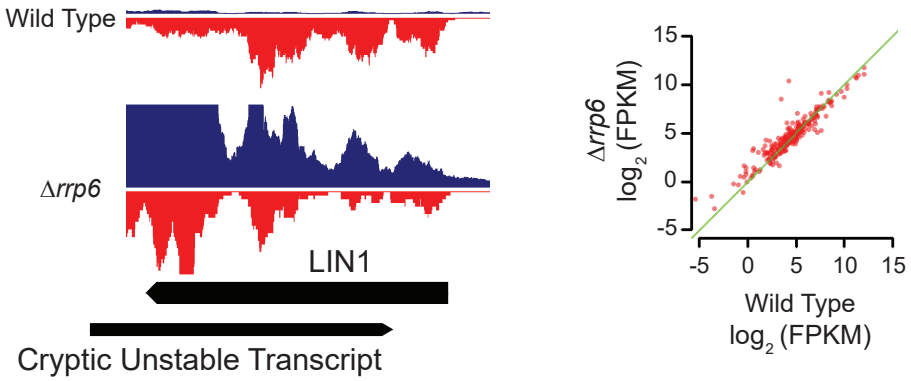


Supplemental Figure S7



Supplemental Figure S8

A



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