

Supplementary information for:

Transcriptome-wide analyses of piRNA binding sites suggest distinct mechanisms regulate piRNA binding and silencing in *C. elegans*

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This supplementary information includes:

Figure S1. *In vivo* miRNA binding sites are enriched at the 3' UTR of mRNAs in *C. elegans*, related to Figure 2.

Figure S2. piRNAs' CDS binding preference cannot be explained by the predicted piRNA targeting sites, related to Figure 3.

Figure S3. The distribution of CSR-1 22G-RNAs, WAGO 22G-RNAs, and piRNA binding sites, related to Figure 5.

Figure S4. The distribution of piRNA binding sites, related to Figure 5.

Figure S1. *In vivo* miRNA binding sites are enriched at the 3' UTR of mRNAs in *C. elegans*, related to Figure 2.

- (A) Metagene trace shows the distribution of miRNA binding events along an mRNA. The solid line indicates the average number of miRNA binding events (read counts) in all mRNAs. The dotted lines indicate the average number plus/minus one standard error.
- (B) The density of miRNA binding events in the indicated regions. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

Figure S2. piRNAs' CDS binding preference cannot be explained by the predicted piRNA targeting sites, related to Figure 3.

- (A) The distribution of predicted piRNA targeting sites around the start codon (left) or stop codon (right). A 200 nucleotide-window centered at start or stop codon is shown. The solid line indicates the average number of predicted piRNA targeting sites in all germline mRNAs. For each position, the average is calculated from all germline mRNAs which possess that position.
- (B) Metagene trace shows the distribution of germline-expressed miRNA binding sites along a germline mRNA (left). The solid line indicates the average number of miRNA binding events (read counts) in germline mRNAs. The dotted lines indicate the average number plus/minus one standard error. The density of experimentally identified germline-expressed miRNA binding sites in the indicated regions of germline mRNAs (right). Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (C) The density of experimentally identified piRNA binding sites in the indicated regions of germline mRNAs that have predicted miRNA binding sites for germline-expressed miRNAs in 3' UTRs (left) or do not (right). Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (D) The density of experimentally identified piRNA binding sites in the indicated regions of germline mRNAs that have experimentally identified miRNA binding sites for germline-expressed miRNAs in 3' UTRs (left) or do not (right). Blue lines represent median

values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

(E) The density of piRNA binding sites in the indicated regions of germline-silenced mRNAs (WAGO targets, left) and germline-expressed mRNAs (CSR-1 targets, right). Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

Figure S3. The distribution of CSR-1 22G-RNAs, WAGO 22G-RNAs, and piRNA binding sites, related to Figure 5

- (A) The density of CSR-1 22G-RNAs in the indicated regions of CSR-1 (top) or WAGO (bottom) targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (B) The density of piRNA binding events in the indicated regions in wild type (orange) or in CSR-1 depleted animals (green) mapped to CSR-1 (top) or WAGO (bottom) targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (C) The density of CSR-1 22G-RNAs mapped to the indicated regions of CSR-1 subgroup genes, including 3' end 22G-RNA enriched (top) or not enriched (bottom) CSR-1 genes. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (D) The density of piRNA binding events mapped to the indicated regions in wild type (orange) or in CSR-1 depleted animals (green) of CSR-1 subgroup genes, including 3' end 22G-RNA enriched (top) or not enriched (bottom) CSR-1 genes. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (E) The density of piRNA binding events (read counts) in the indicated regions of WAGO targets or CSR-1 targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

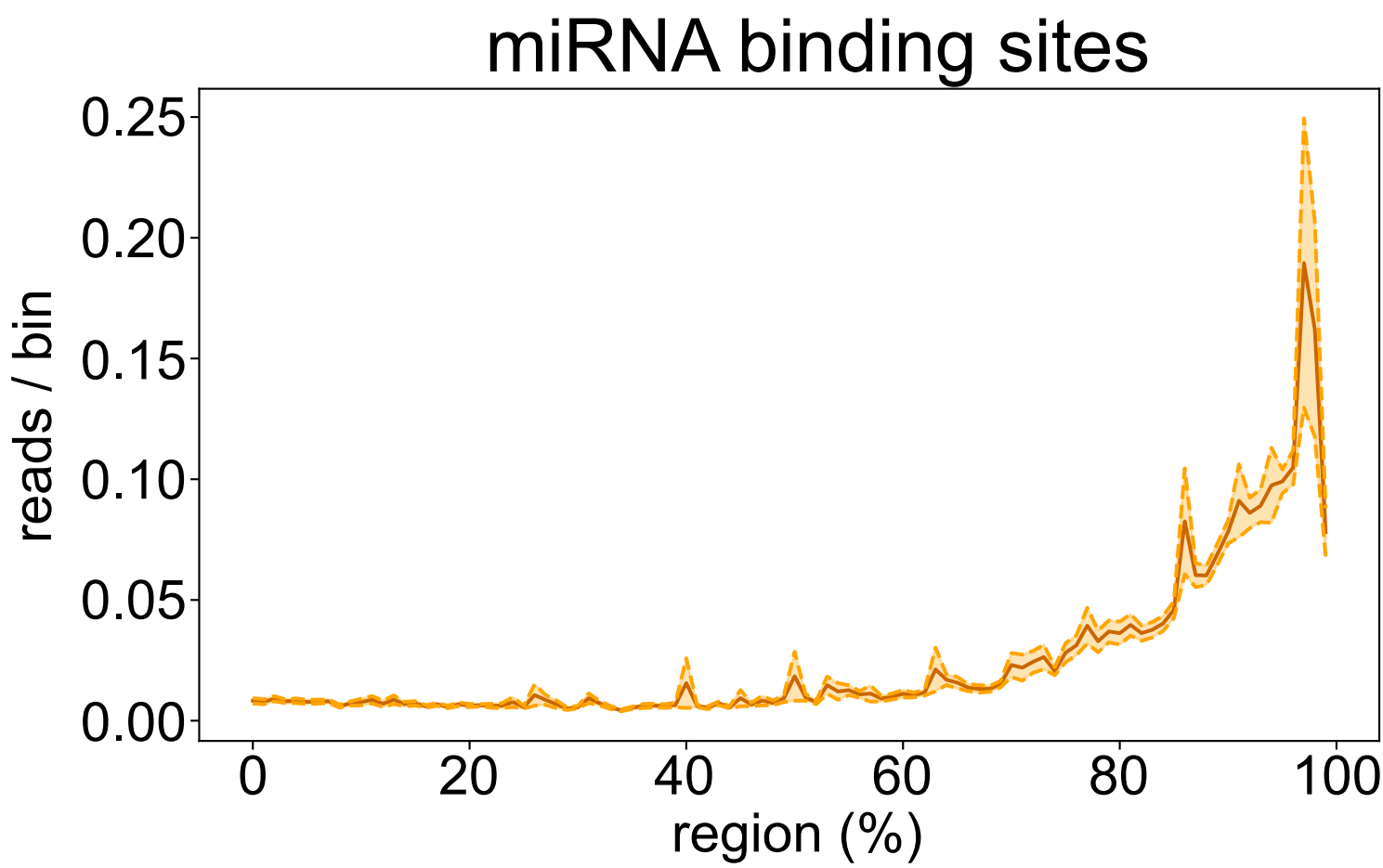
- (F) The density of piRNA binding events (read counts) standardized by normalized mRNA reads (RPKM) in the indicated regions of WAGO targets or CSR-1 targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (G) The density of WAGO-9 (left) or WAGO-1 (right) 22G-RNAs mapped to the regions of WAGO targets or CSR-1 targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (H) The density of WAGO-9 (HRDE-1) 22G-RNAs mapped to the indicated regions in wild type or in CSR-1 depleted animals of CSR-1 (top) or WAGO (bottom) targets. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.
- (I) The density of WAGO-9 (HRDE-1) 22G-RNAs in wild type or in CSR-1 depleted animals mapped to the indicated regions of CSR-1 subgroup genes, including 3' end 22G-RNA enriched (top) or not enriched (bottom) CSR-1 genes. Blue lines represent median values. The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

Figure S4. The distribution of piRNA binding sites, related to Figure 5

- (A) The distribution of piRNA binding events around the start codon (left) or stop codon (right) in wild type or in CSR-1 depleted animals. A 200 nucleotide-window centered at start or stop codon is shown. The solid line indicates the average number of WAGO-9 22G-RNAs read counts in WAGO-1 targets (left) or CSR-1 targets (right).
- (B) The density of piRNA binding events (read counts) in the indicated regions of highly-translated or poorly-translated WAGO targets (left) and highly-translated or poorly-translated CSR-1 targets (right). The statistical significance (P-value) of the difference in density between two regions was calculated by the Mann-Whitney U test.

Figure S1

A



B

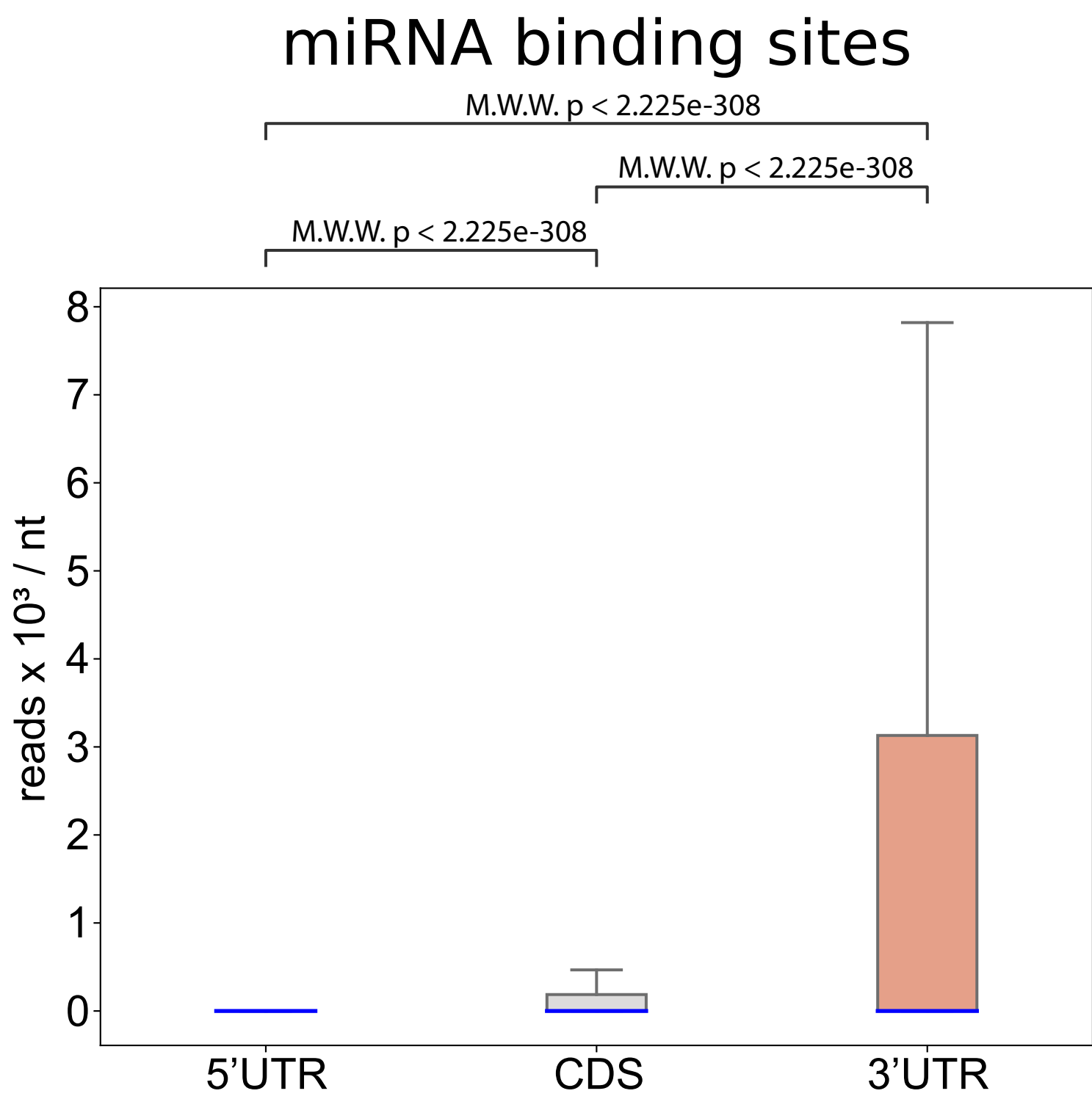
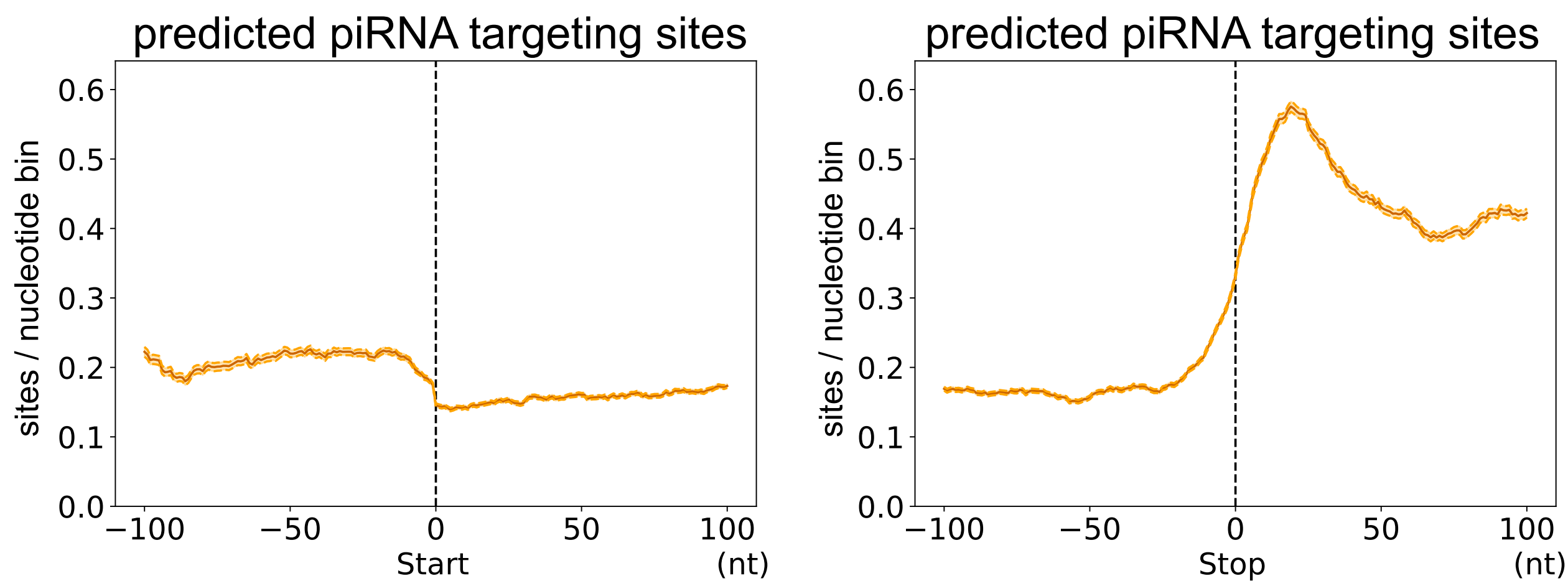
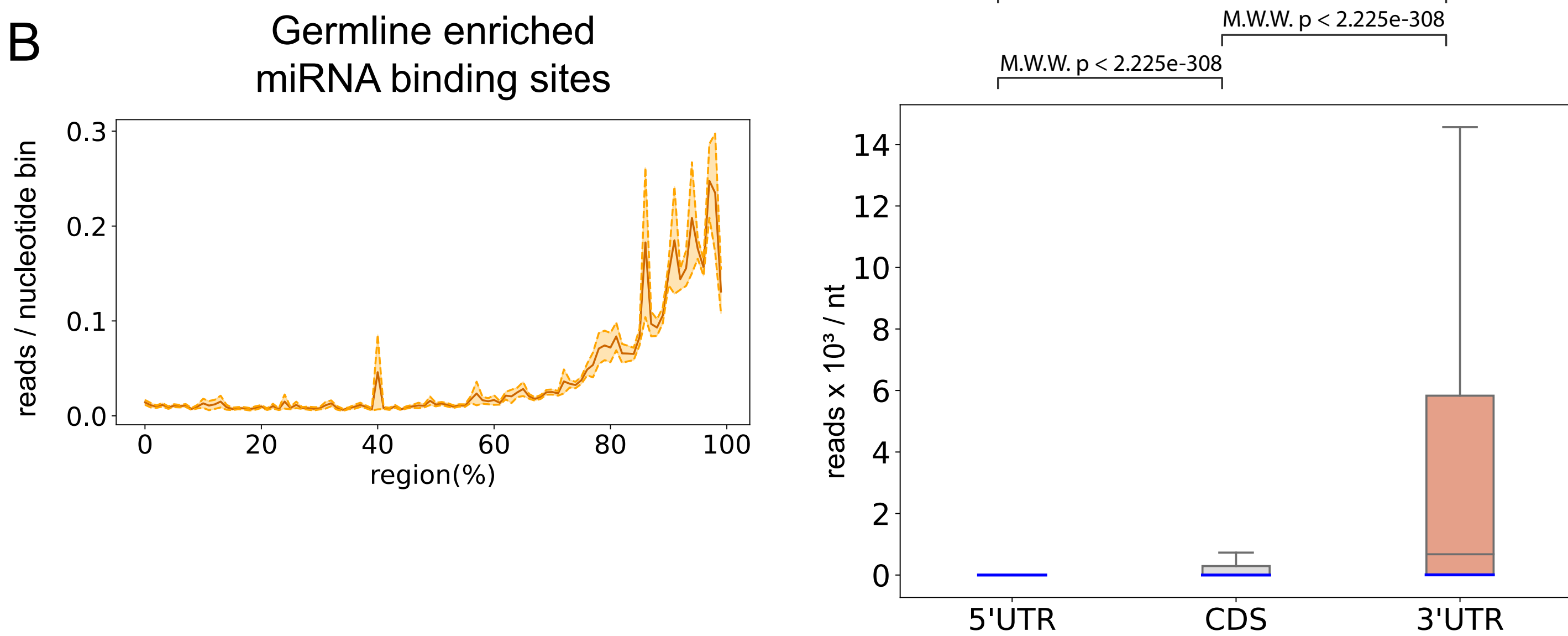


Figure S2

A



B



C

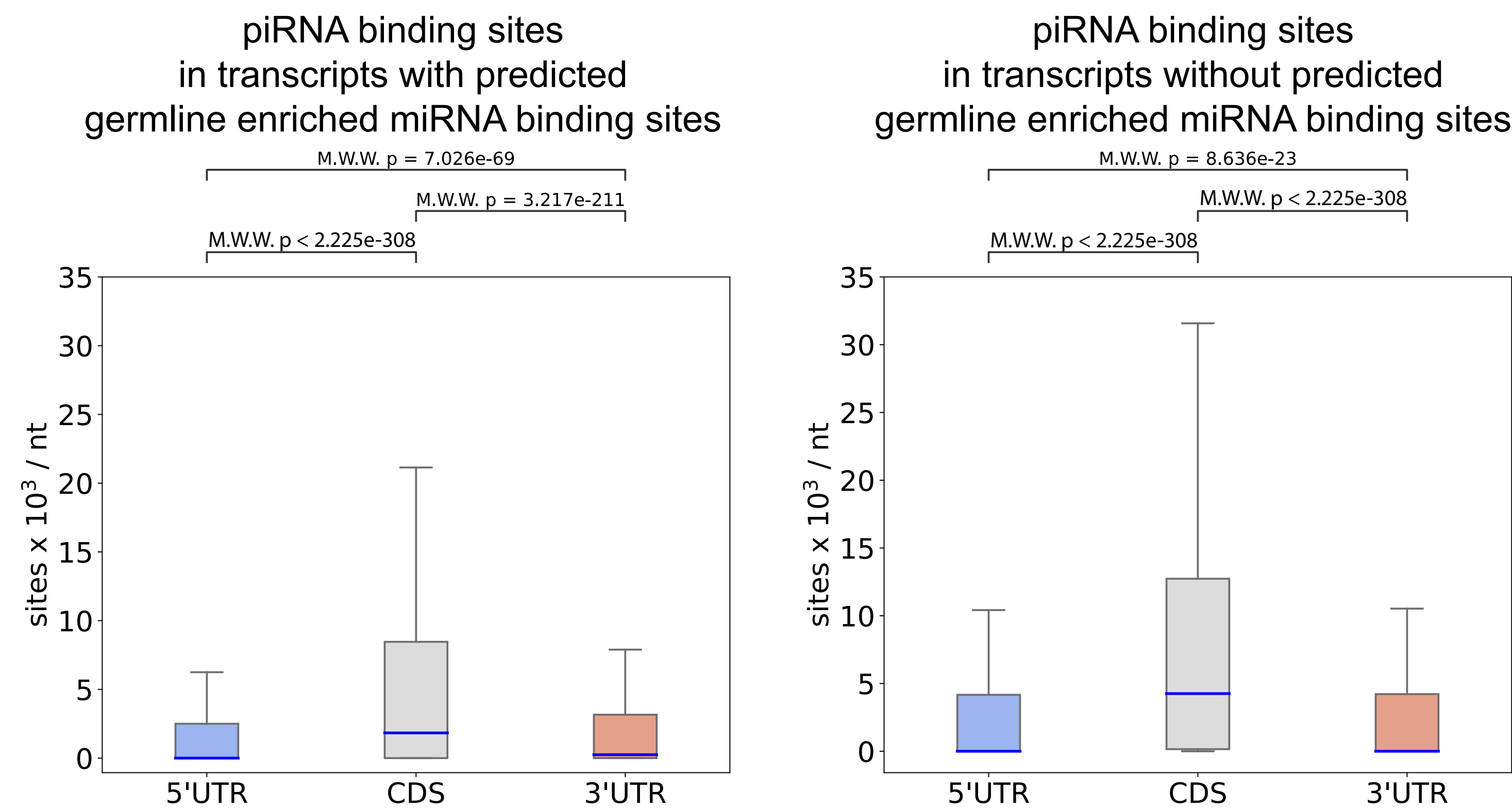
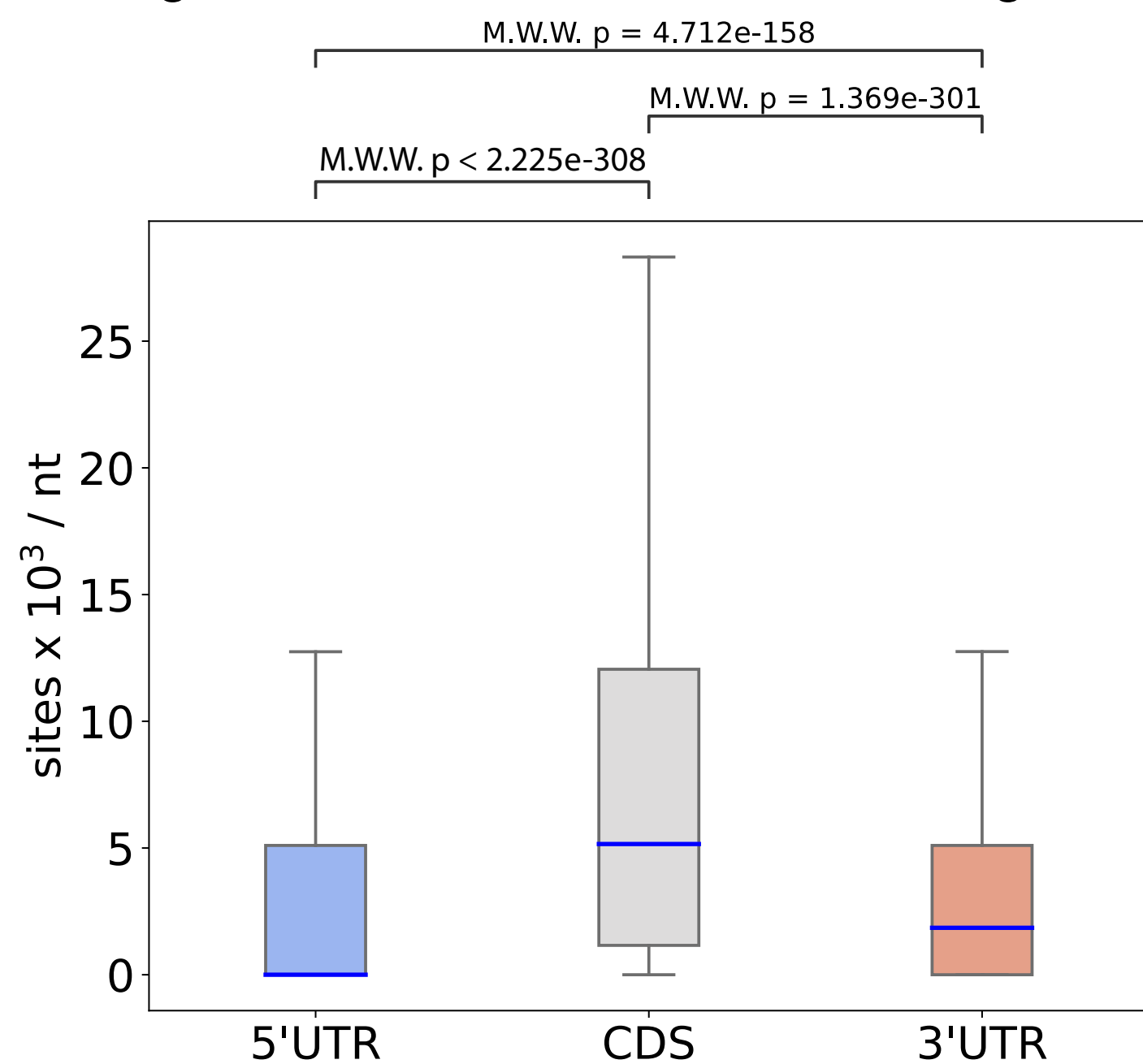


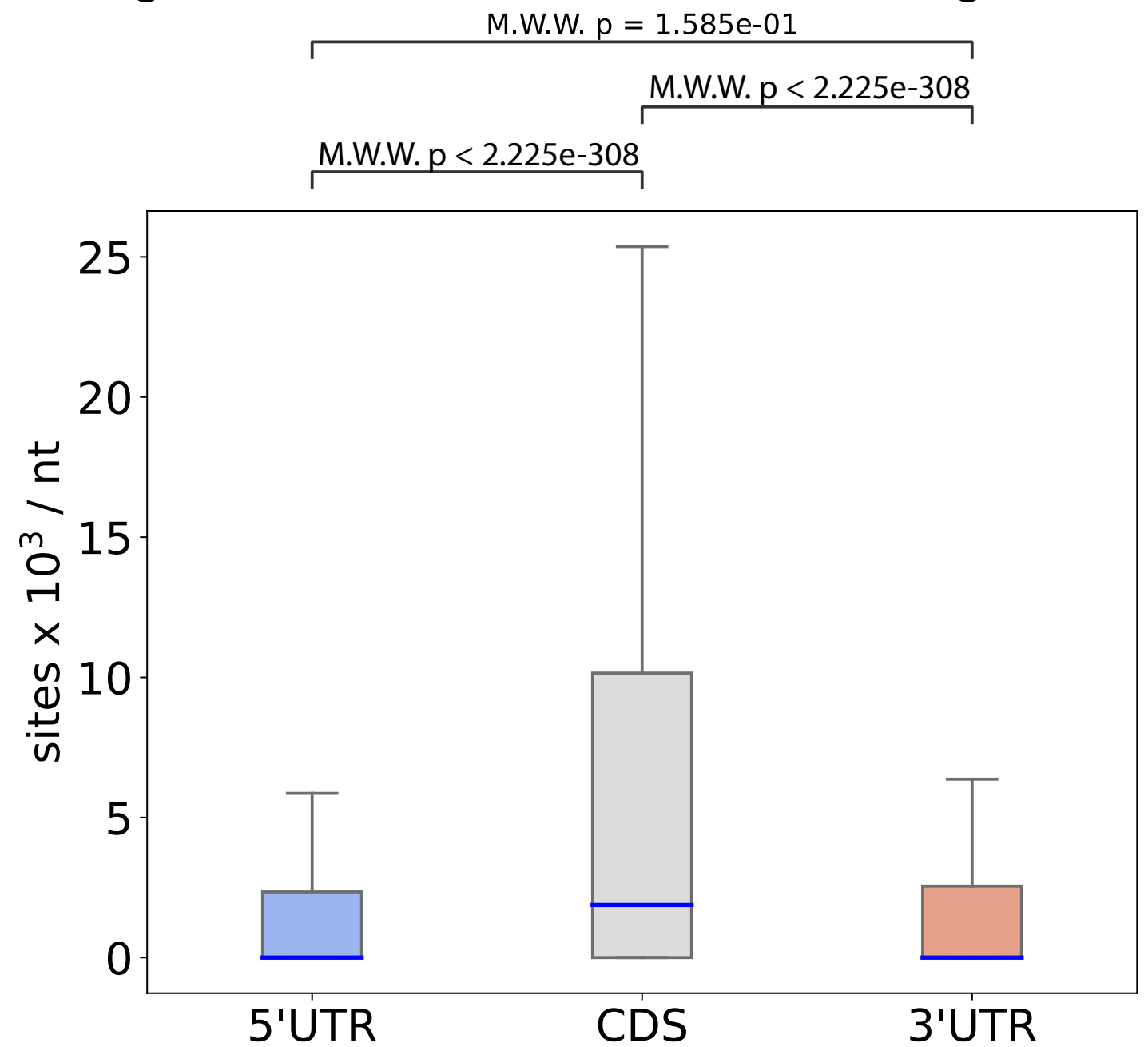
Figure S2, continued

D

piRNA binding sites
in transcripts with
germline enriched miRNA binding sites

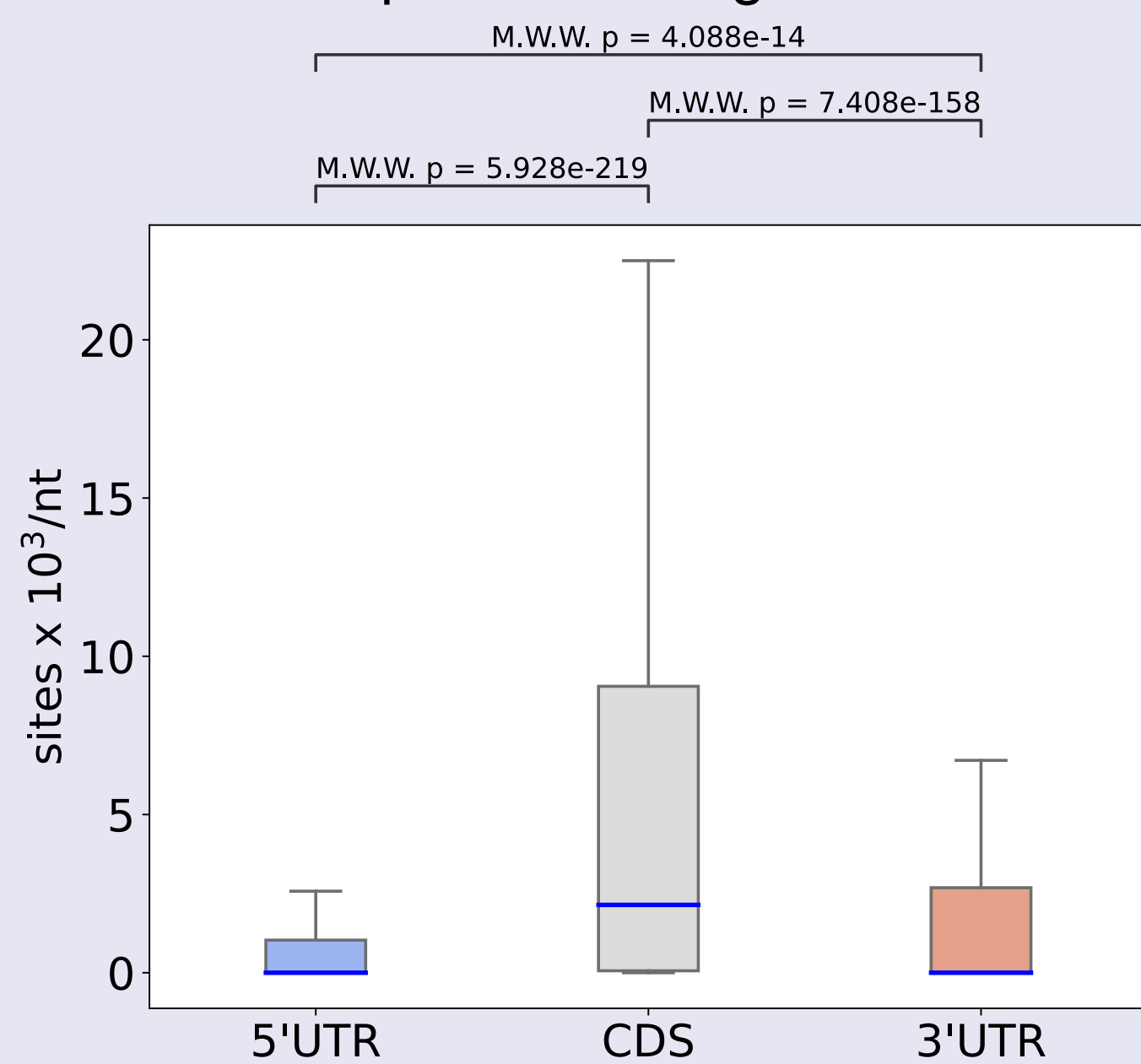


piRNA binding sites
in transcripts without predicted
germline enriched miRNA binding sites



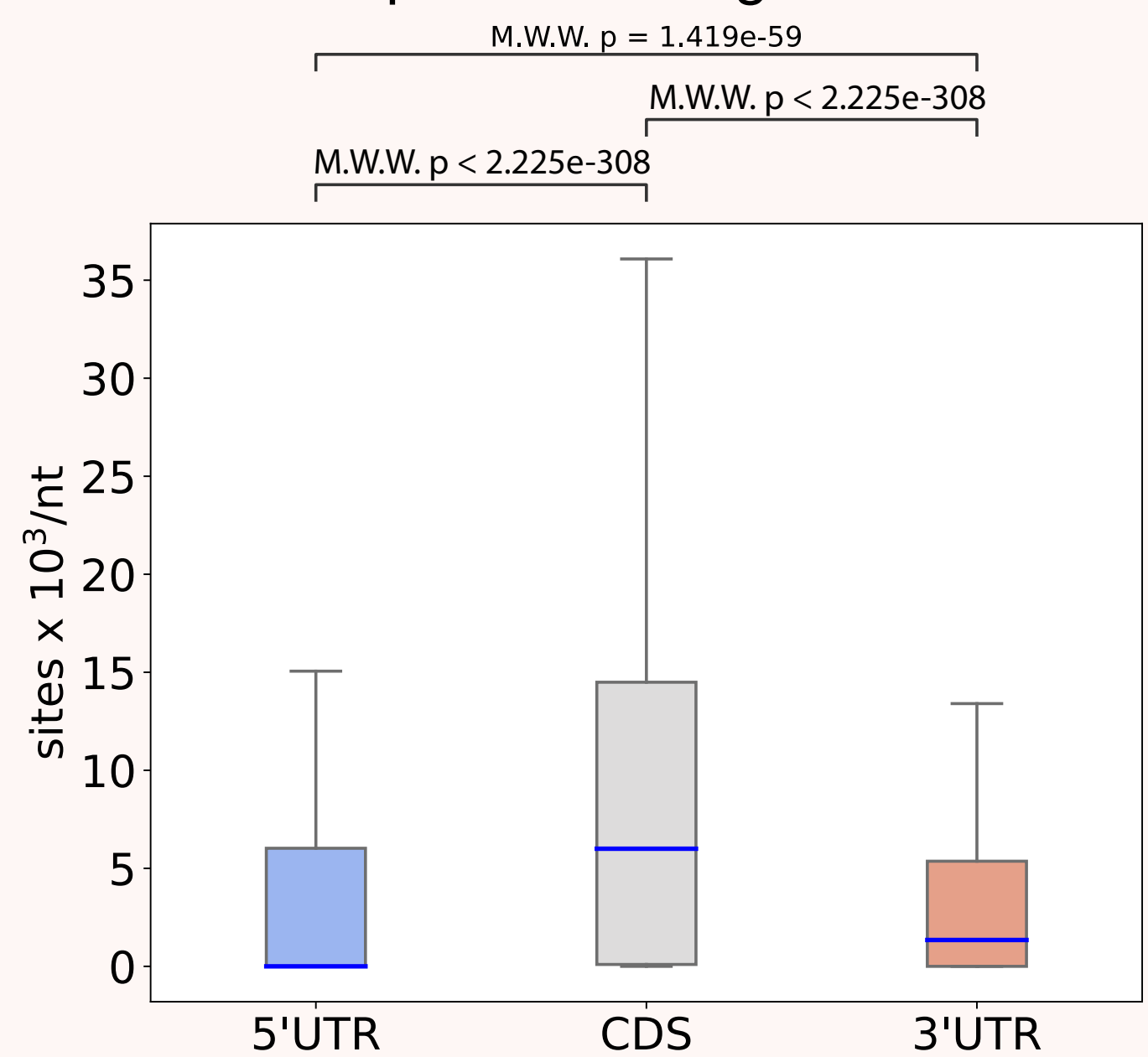
E

piRNA binding sites



WAGO targets

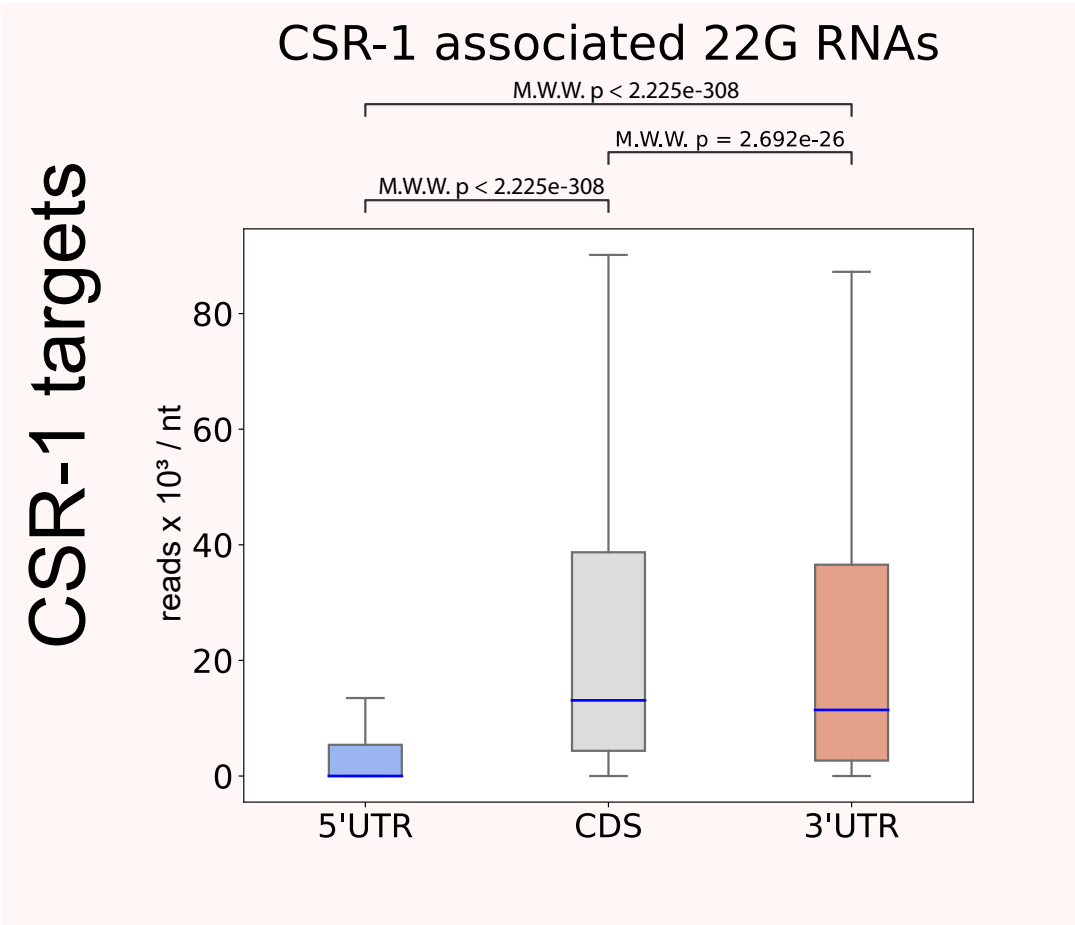
piRNA binding sites



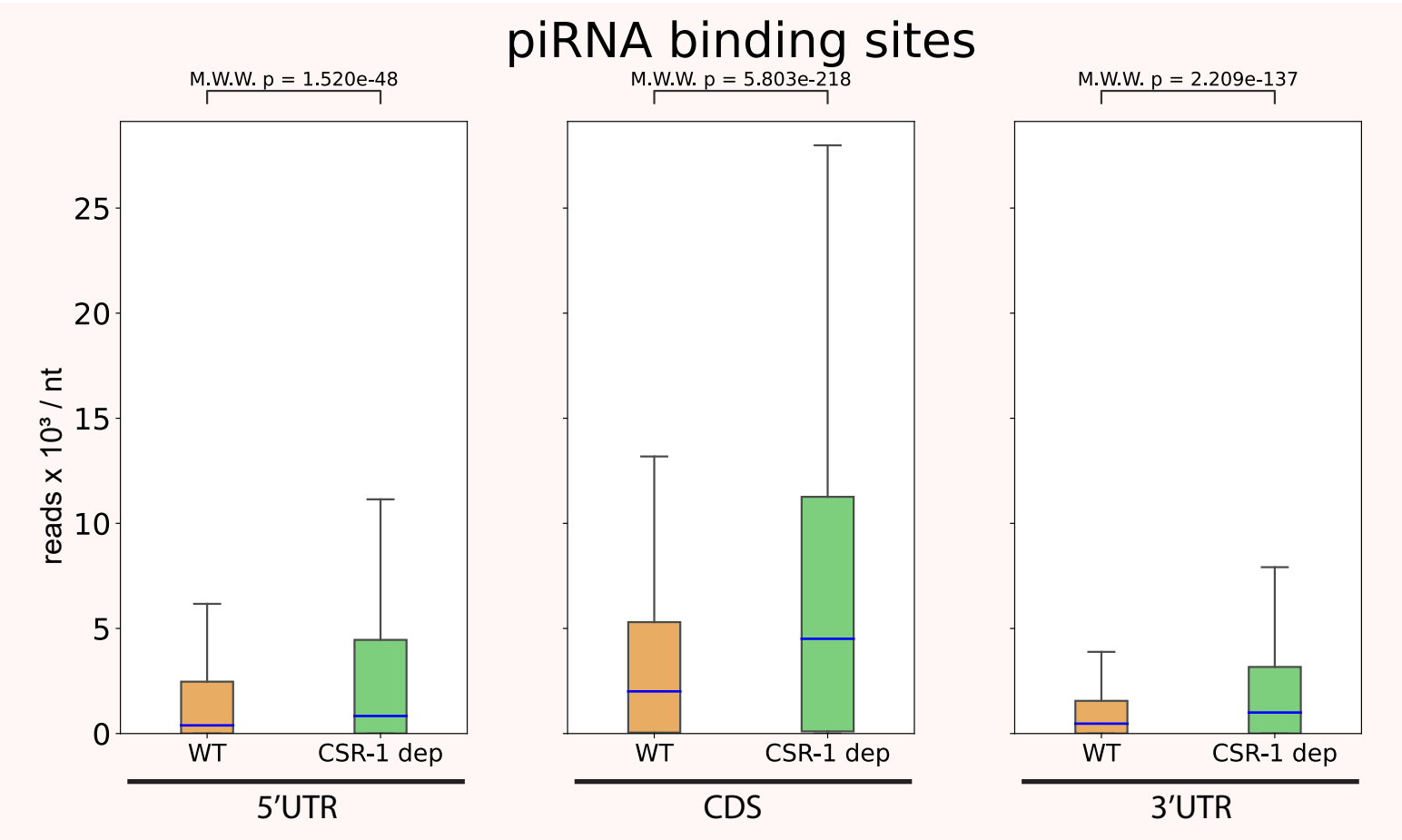
CSR-1 targets

Figure S3

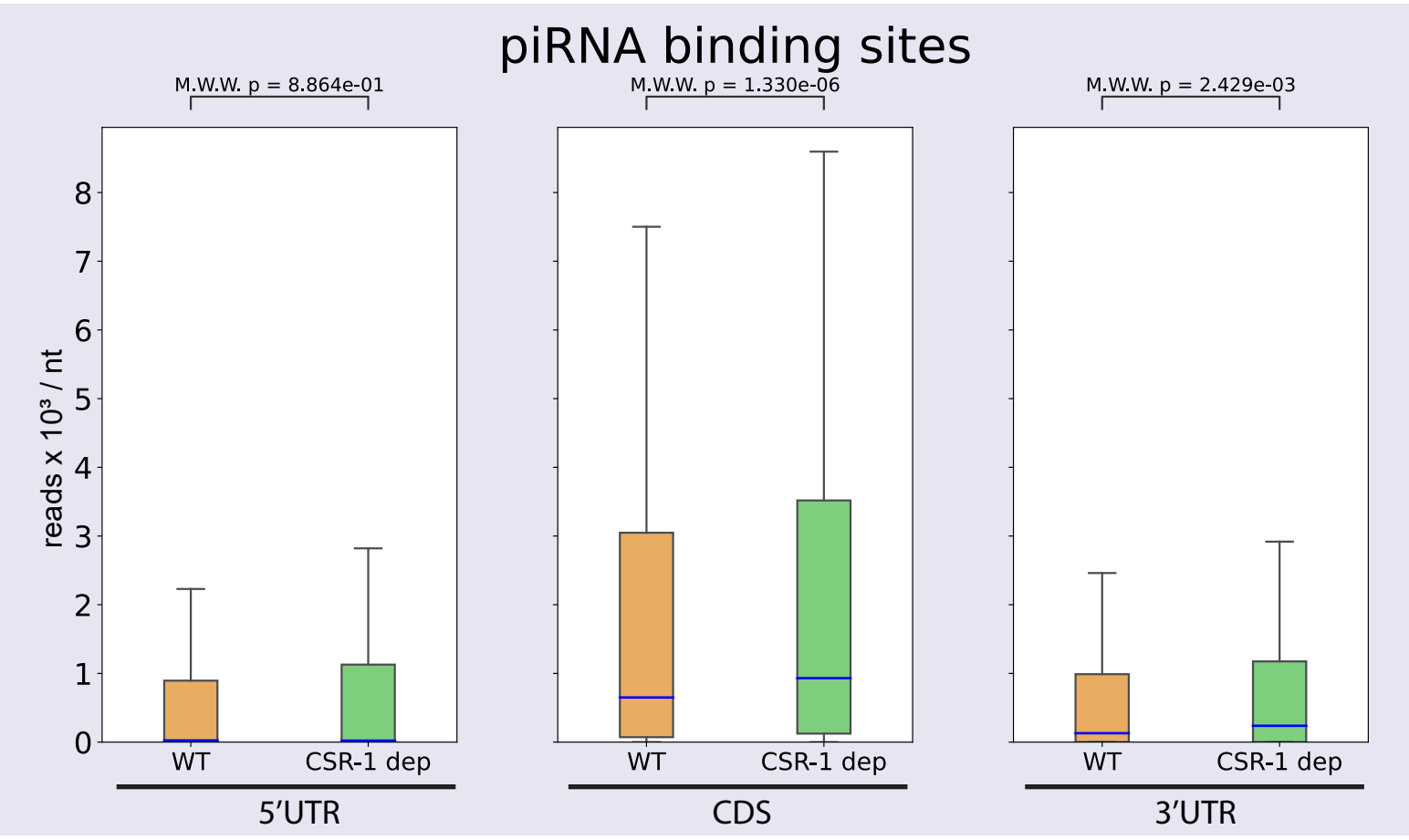
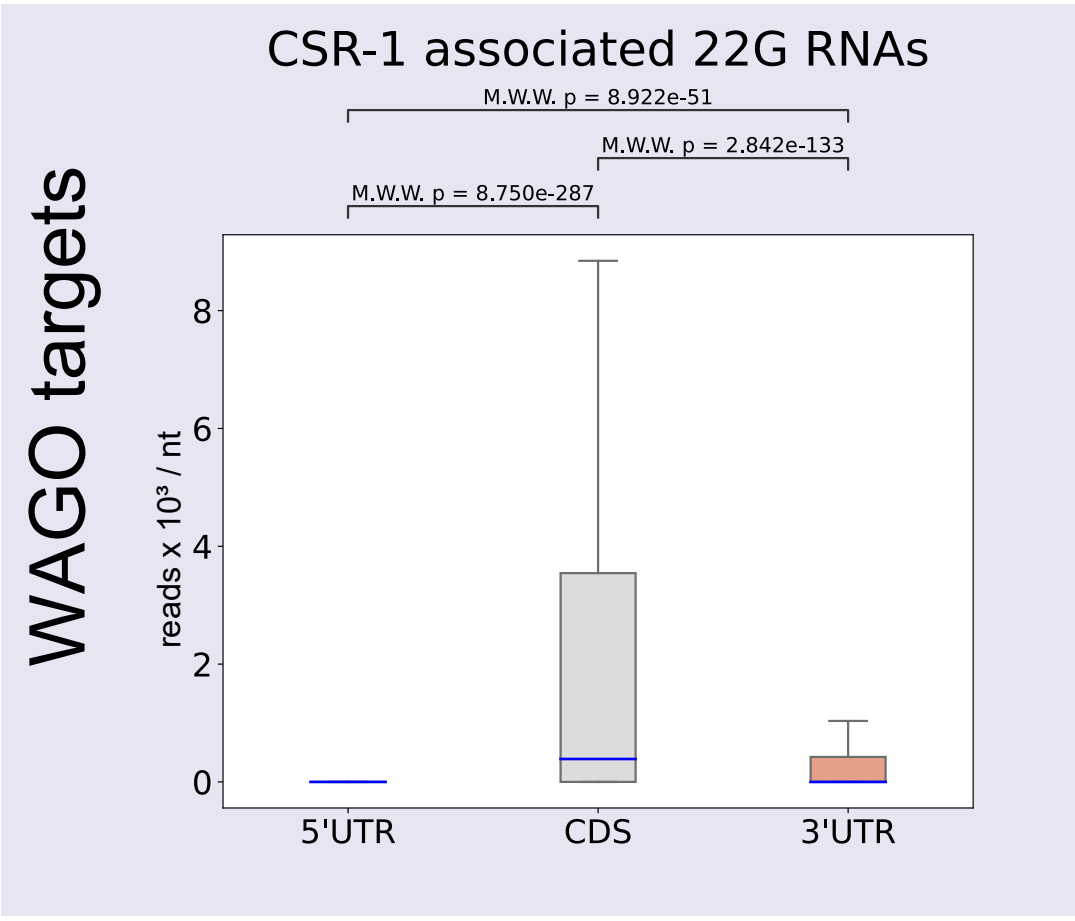
A



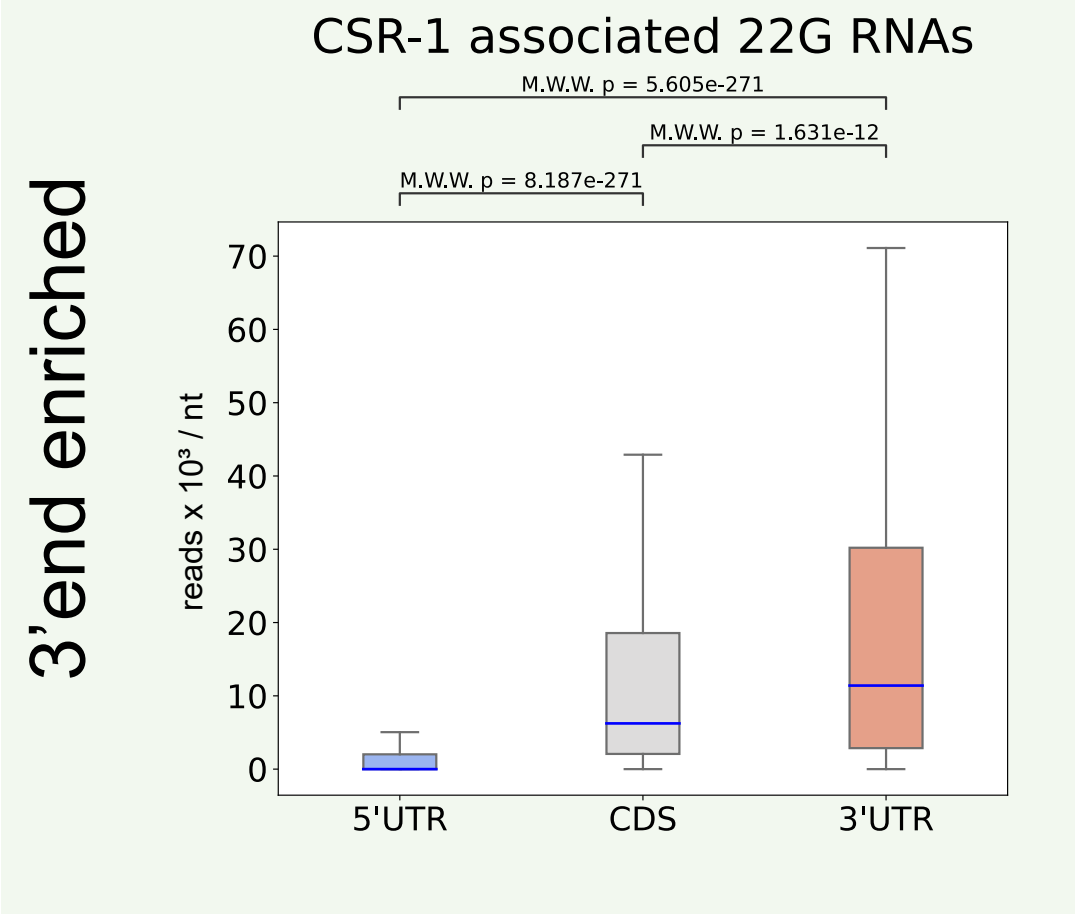
B



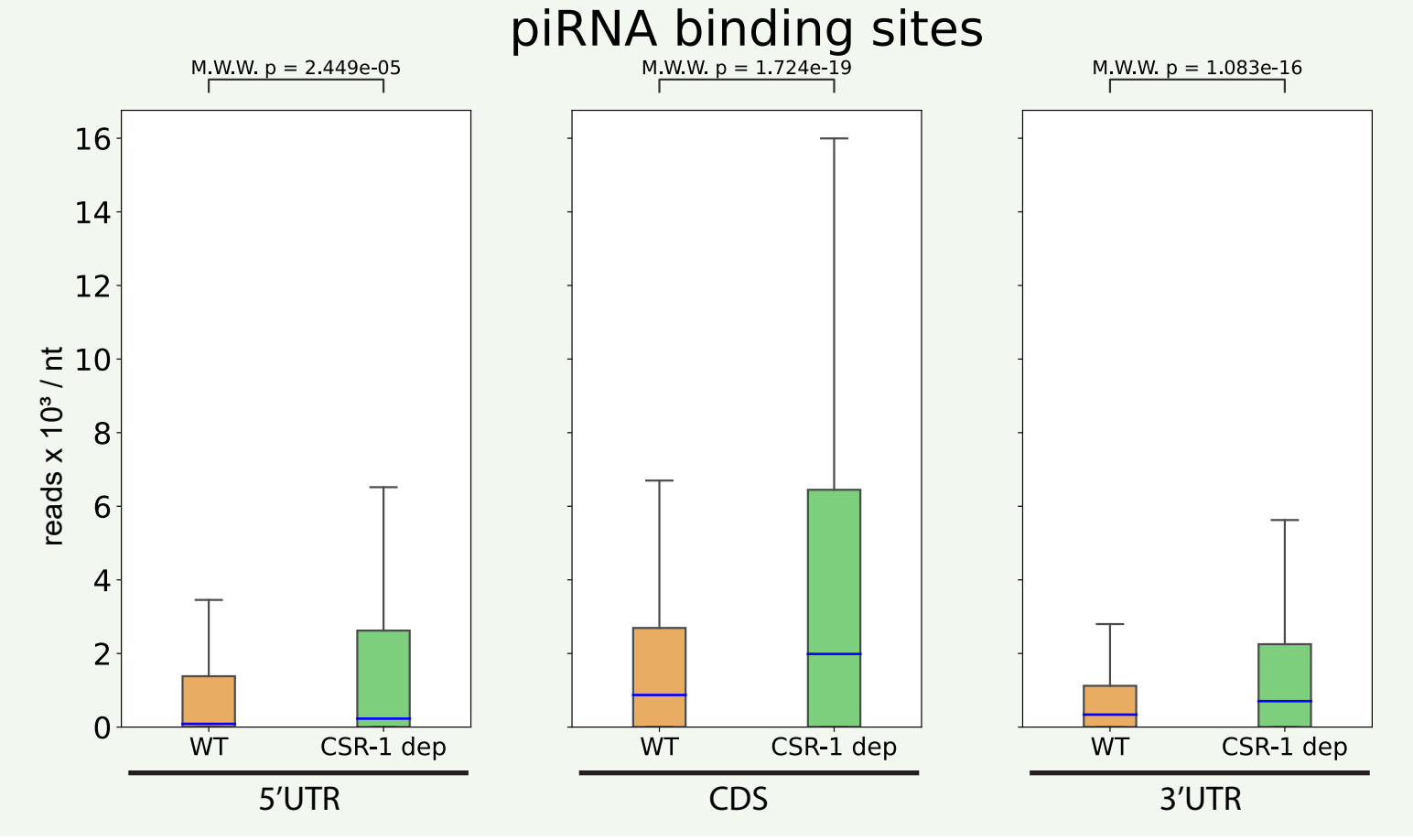
WAGO targets



C



D



Not enriched

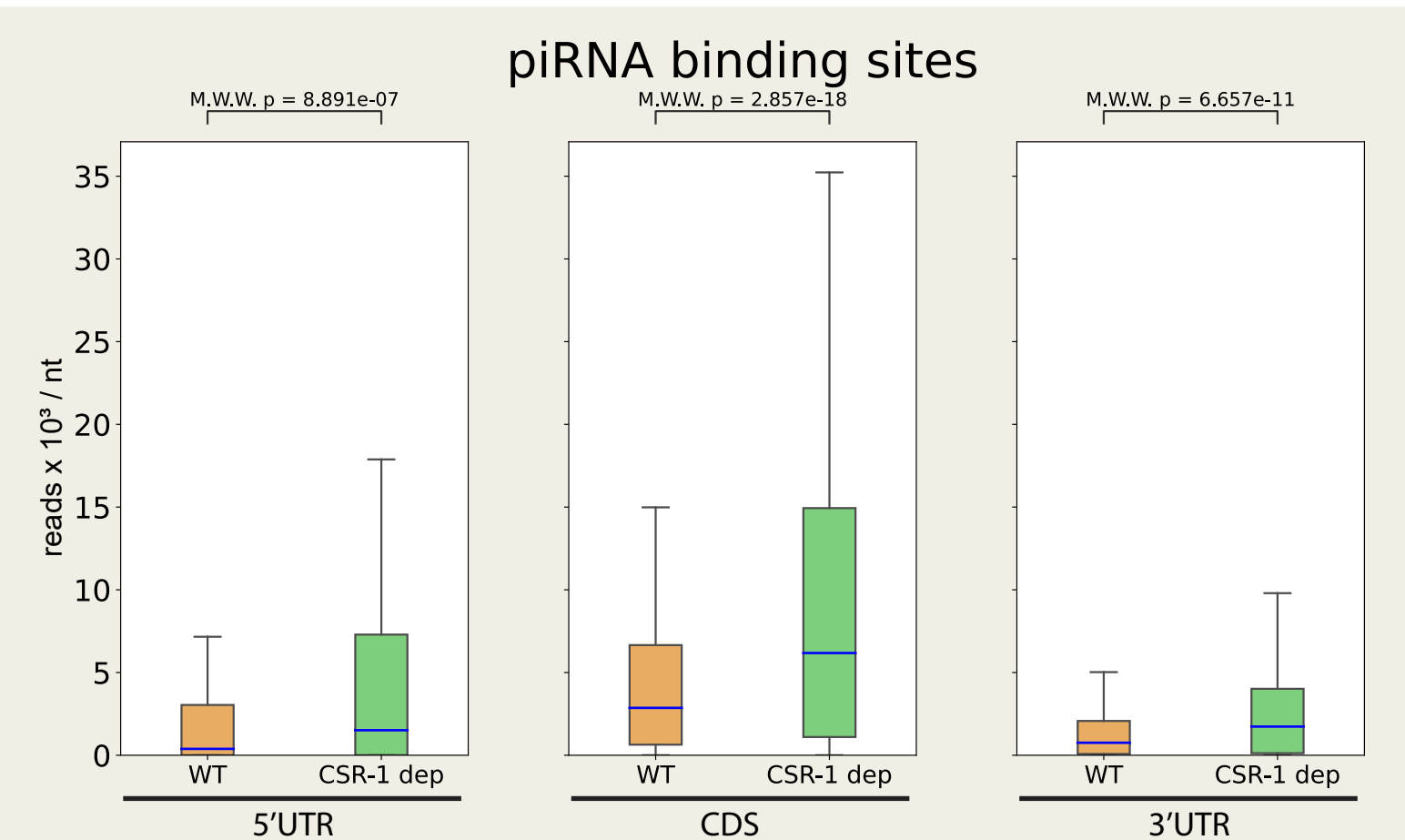
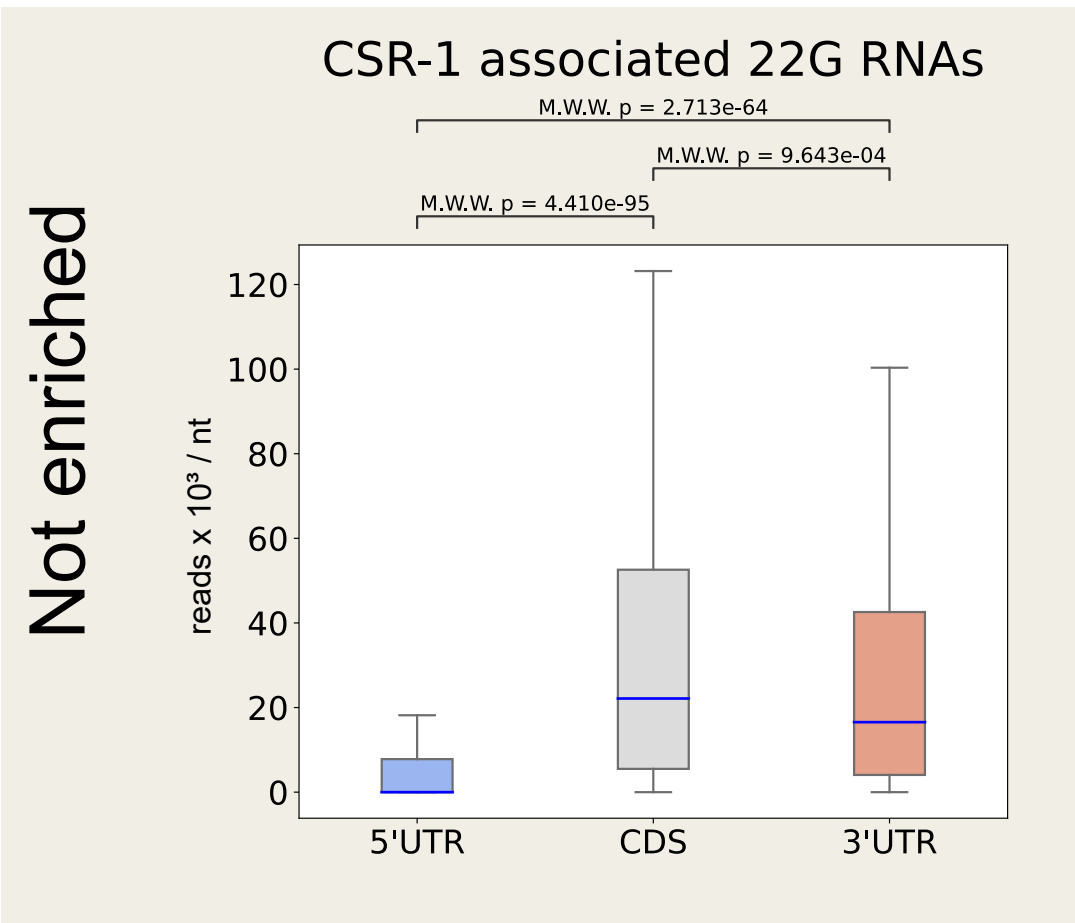


Figure S3, continued

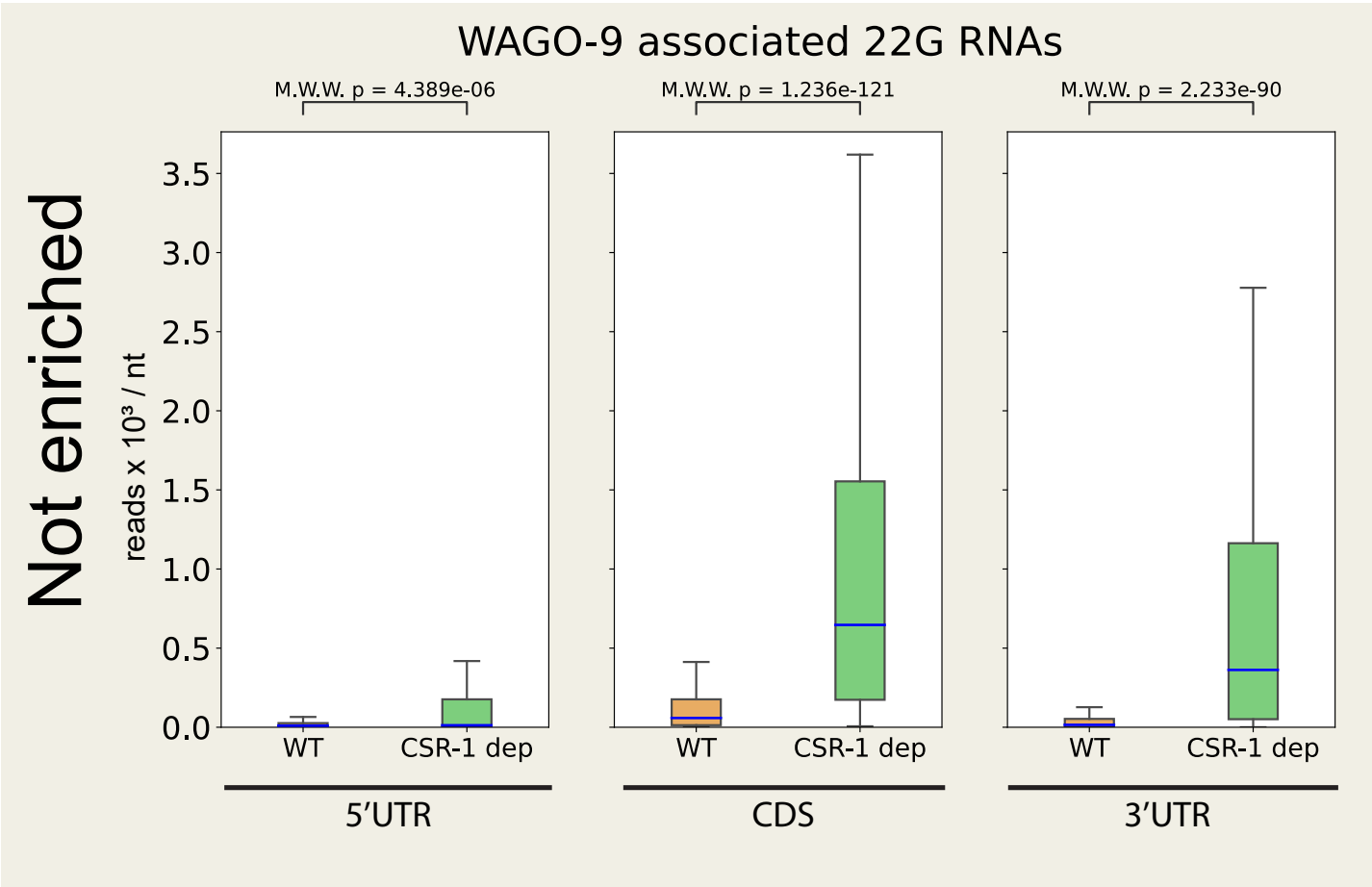
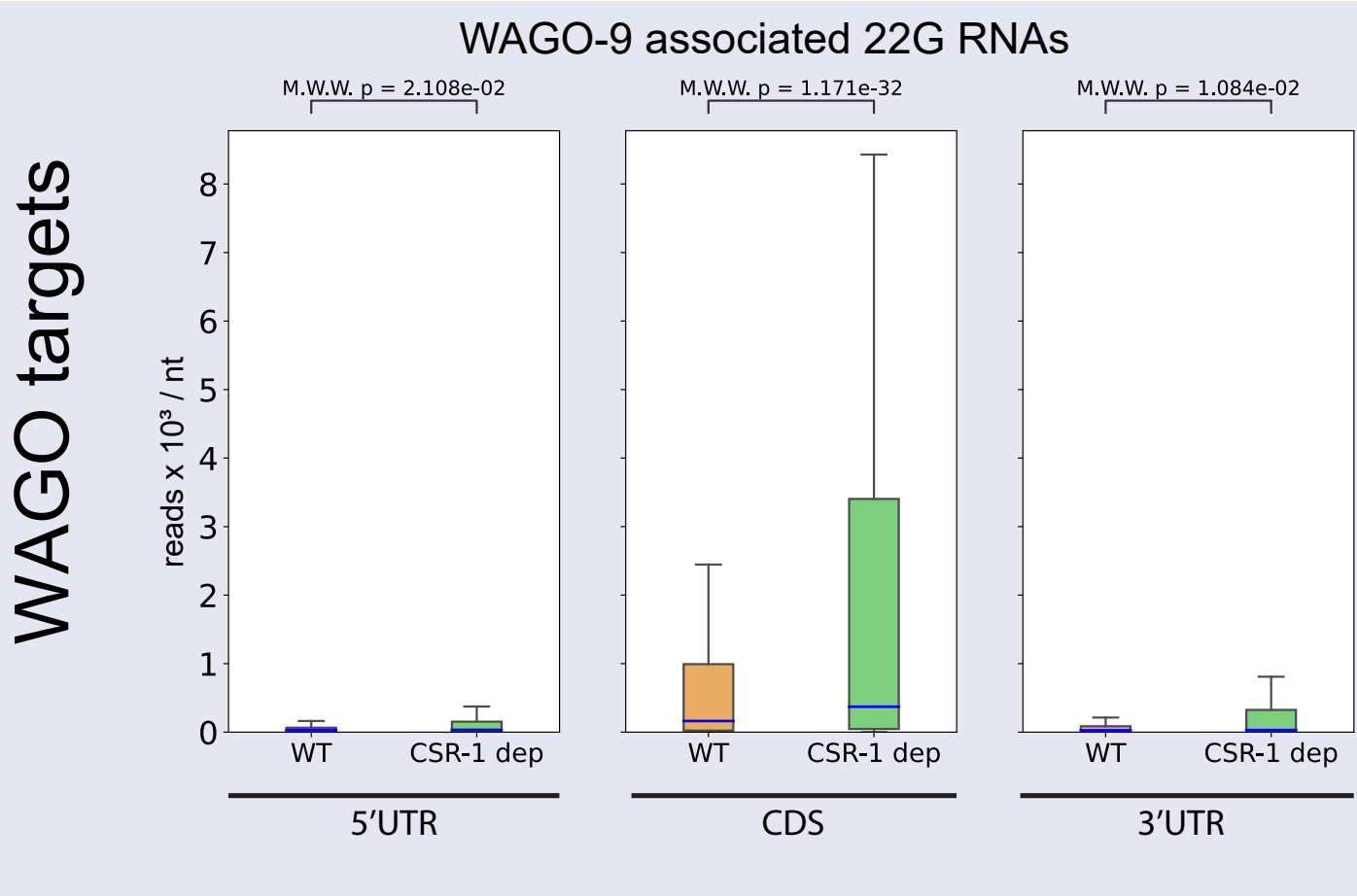
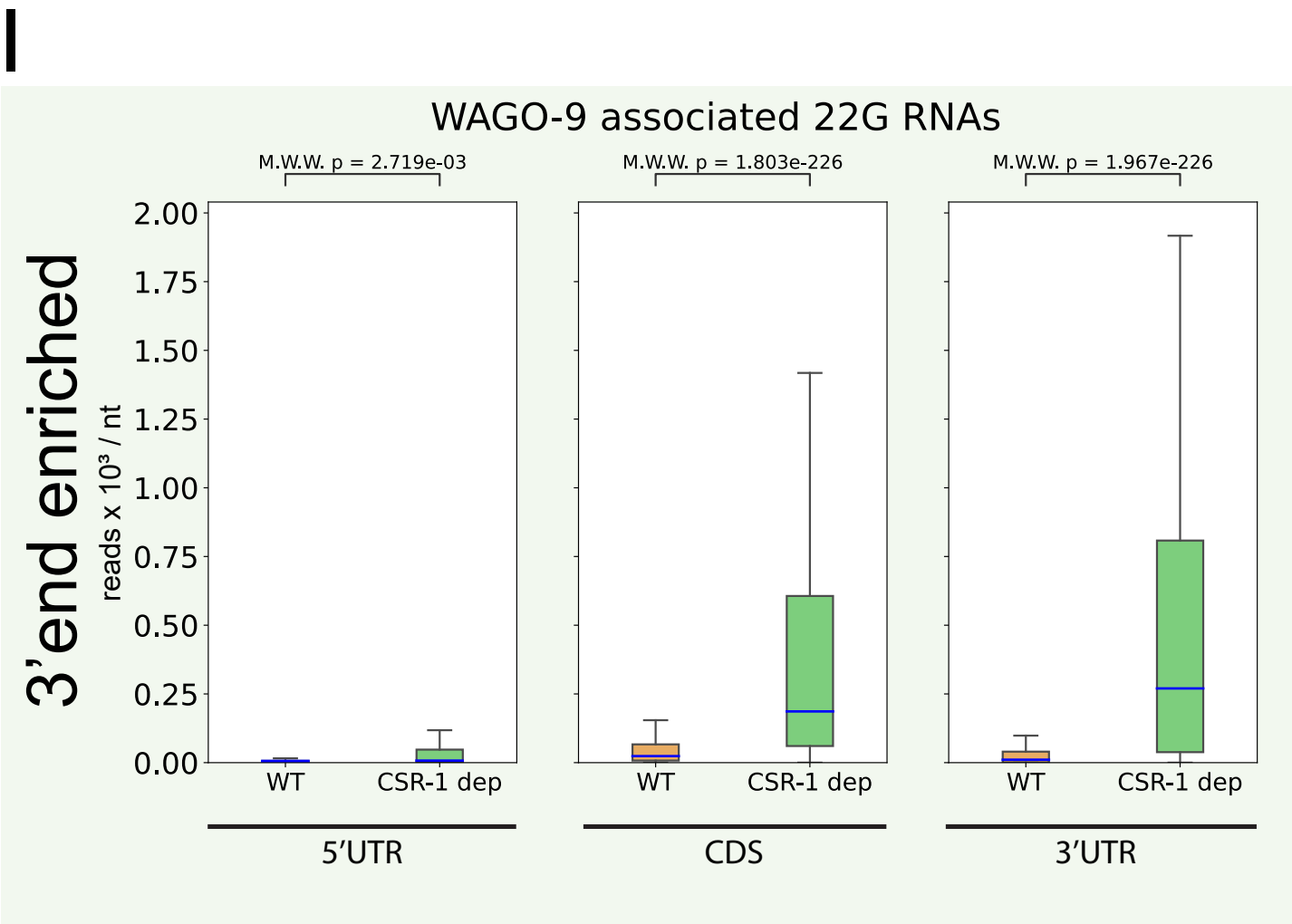
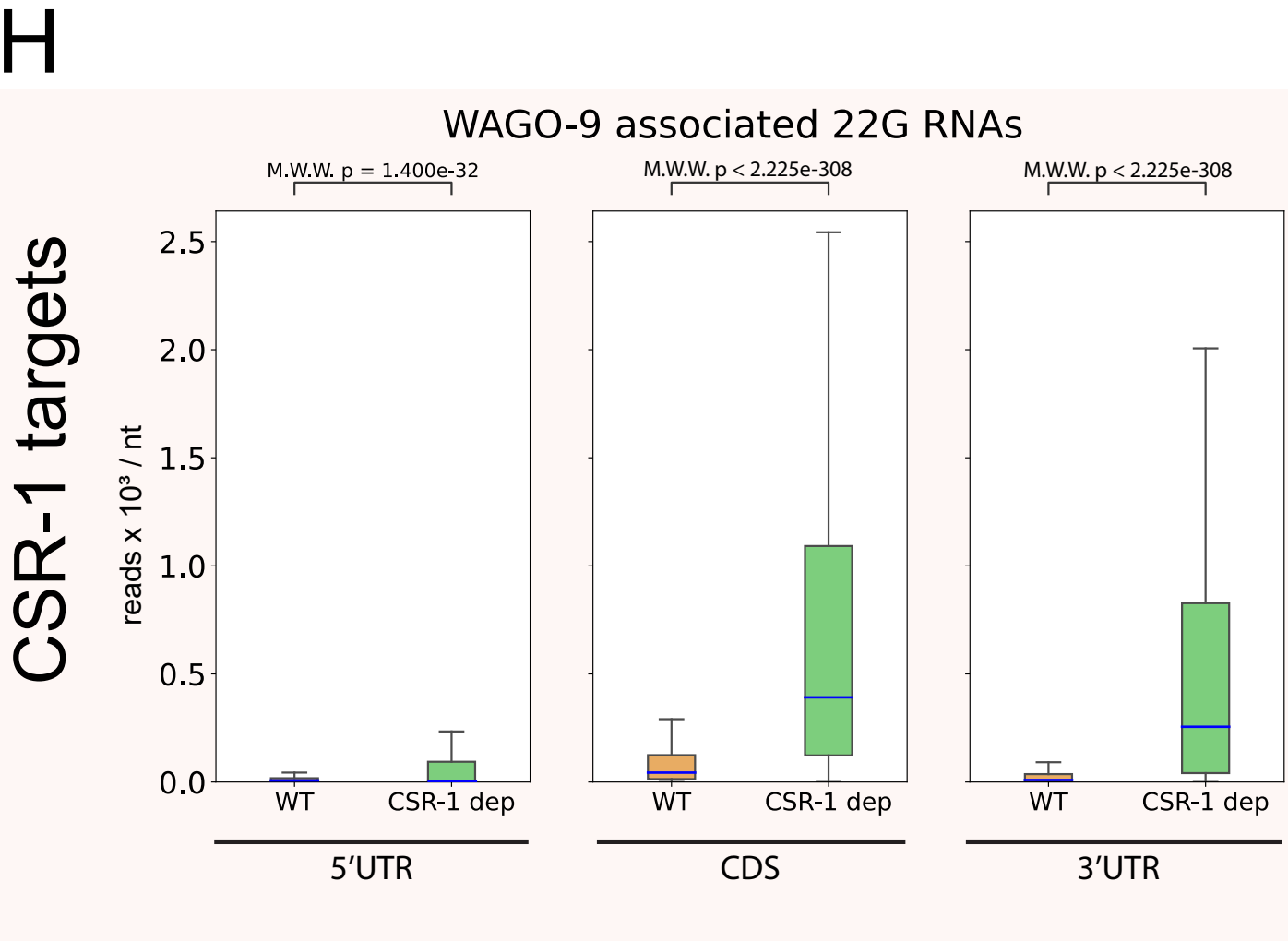
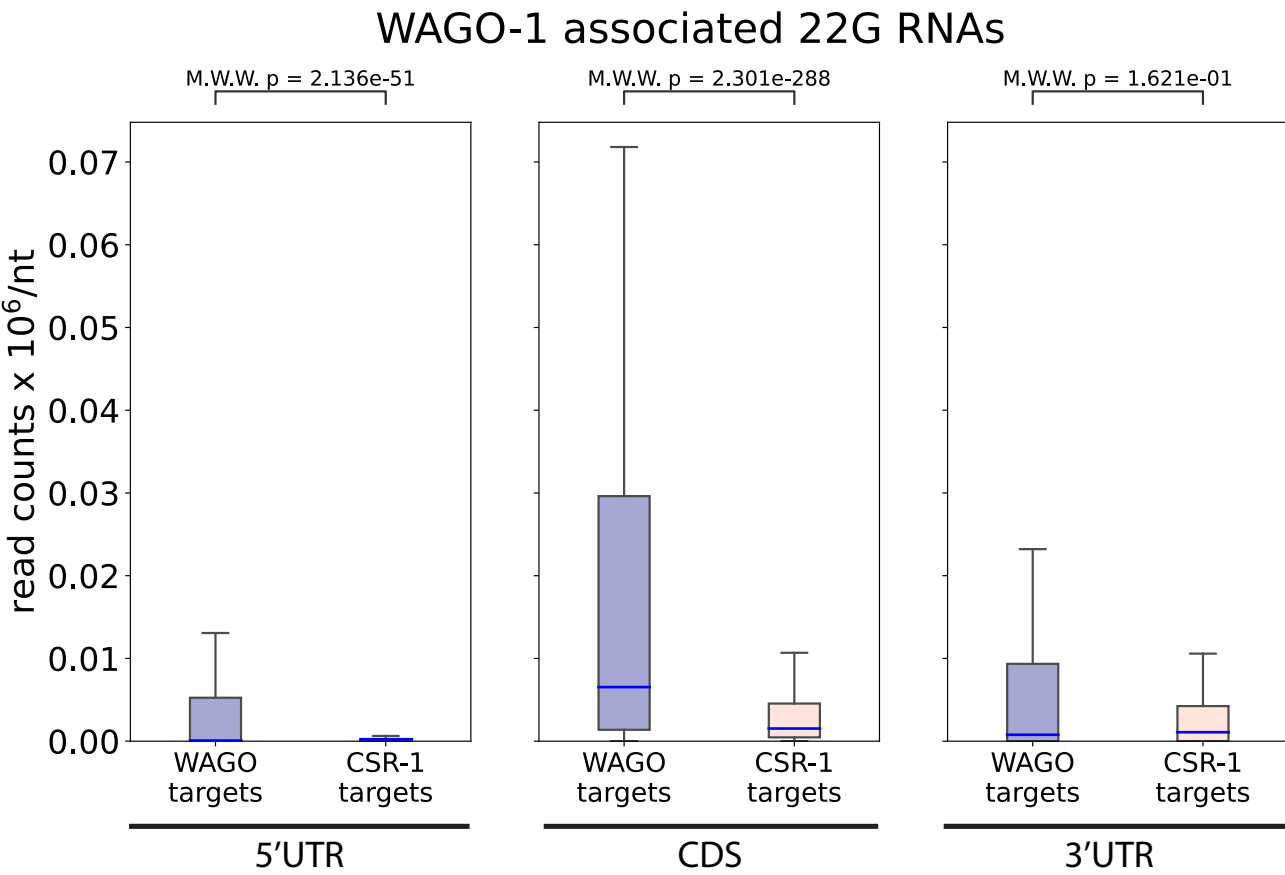
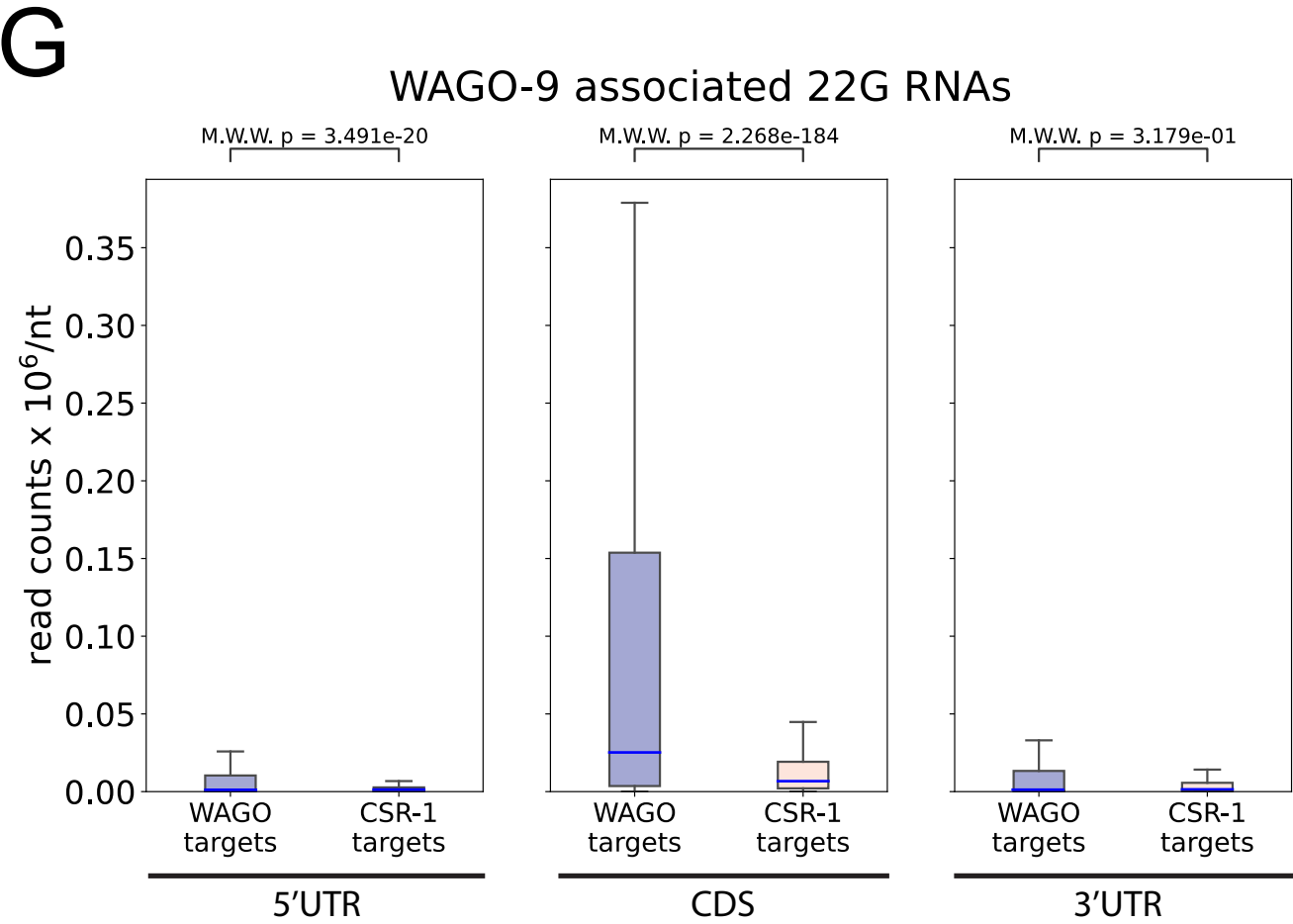
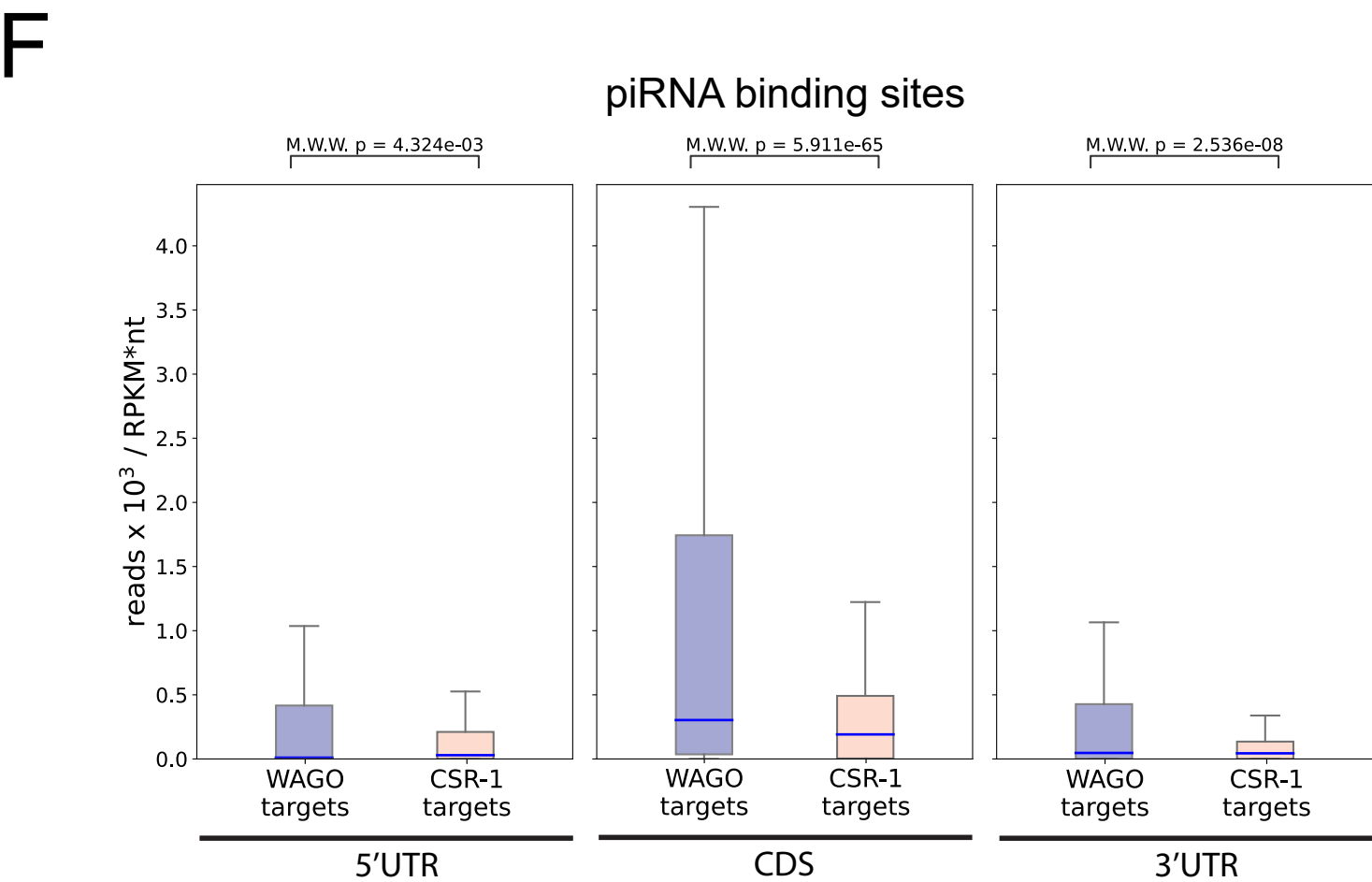
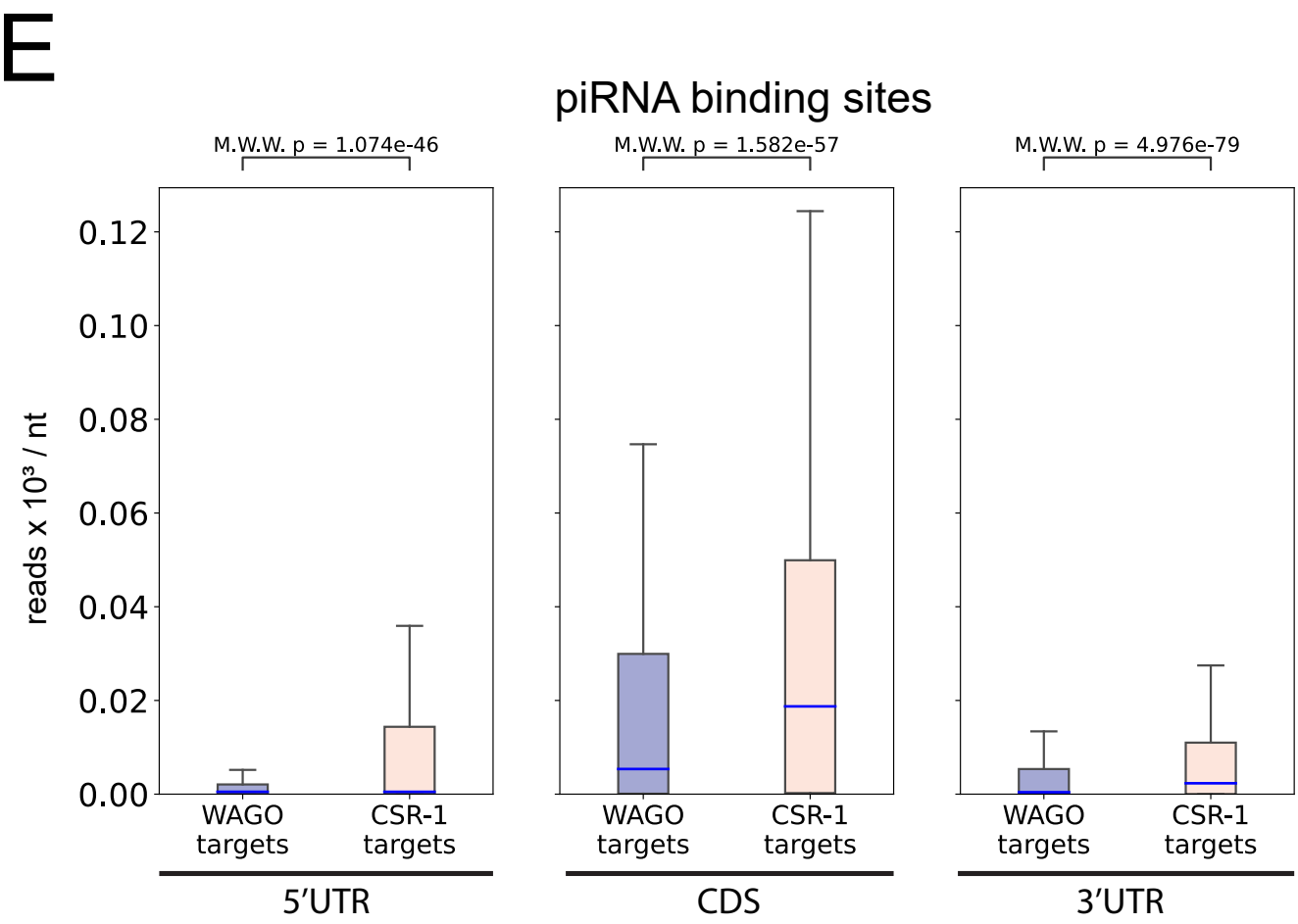
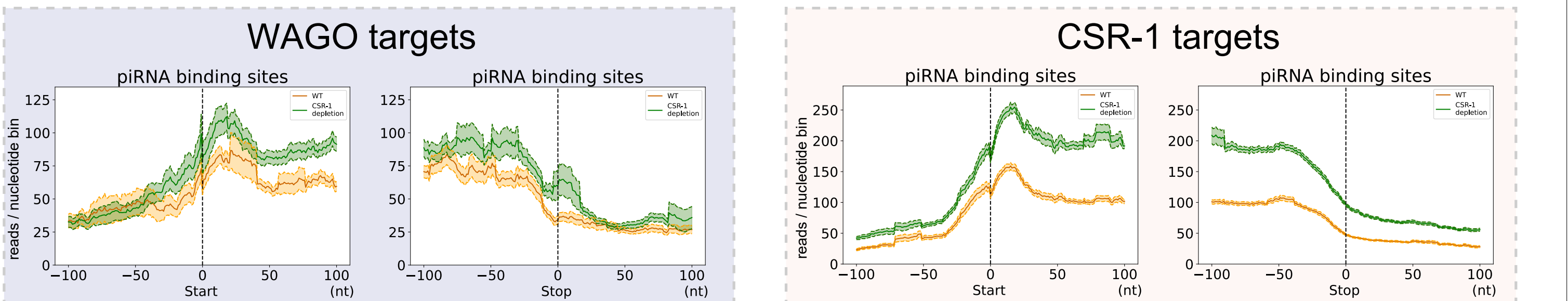


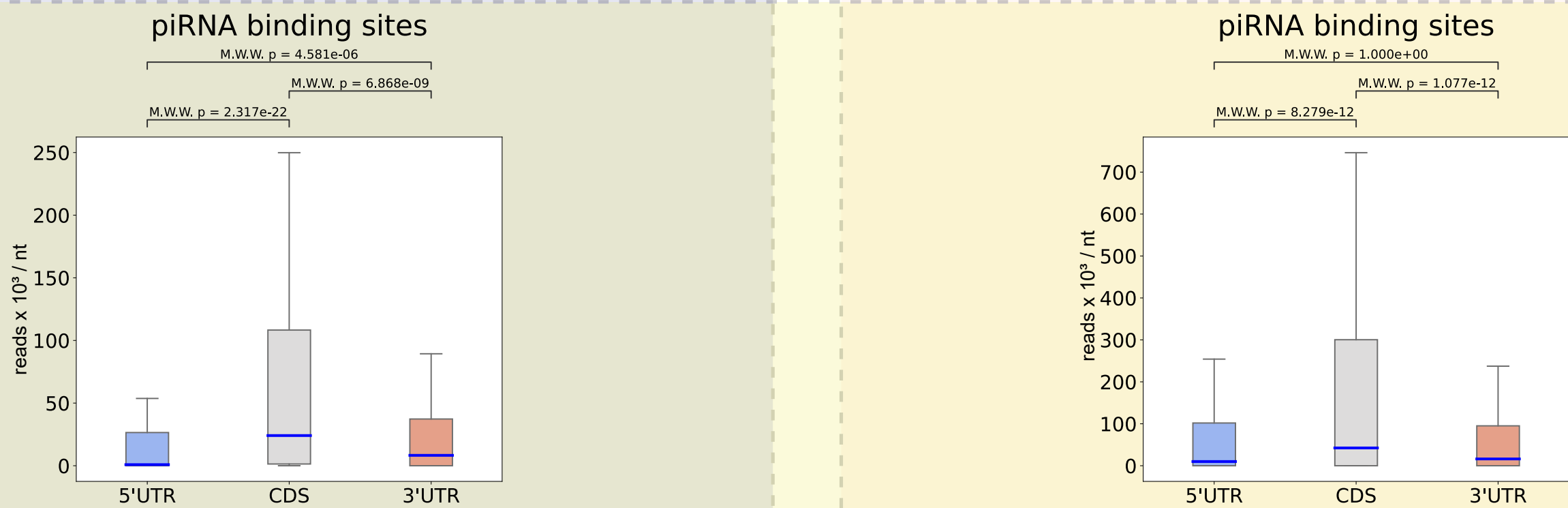
Figure S4

A



B

Highly translated transcripts



Lowly translated transcripts

