Supplemental Figure 1. Purification of polyribosome peaks and troughs using the Gradient Station (Biocomp Corp. New Brunswick, Canada). A representative bioanalyzer electropherogram showing RNA purified from the polyribosome peak and the neighboring trough. This figure illustrates the precision of our polyribosomal extraction method.

Supplemental Figure 2. Scatter plot showing the correlation of biological duplicates using Spearman's rank correlation metric for both the cytoplasmic (A) and polyribosome fractions (B). Each point is the representation of an estimated Ψ value for one event in the MISO database.

Supplemental Figure 3. Plot of the frequency of event types and their corresponding gene locations (3'UTR, 5'UTR, and CDS). Significant events as compared to "no diff" (not significantly different) events show a marked difference in the distribution of events with respect to gene location (χ^2 test p-value 4.2×10^{-49}).

Supplemental Figure 4. Representative gel electrophoresis of endpoint RT-PCR for five exemplar genes. C1 and C2 refer to the cytoplasmic derived replicates, whereas P1 and P2 refer to the polyribosome replicates. Sashimi plots depict RPKM coverage of RNA-seq reads used to estimate Ψ values, with junction reads annotated as connecting lines. (A) *DHX9* (B) *SGOL1* (C) *THOC5* (D) *MOV10* (E) *NDUFS1* (F) Analysis of *RBM41* isoform-specific polyribosome

association. (*Upper panel)* Genome browser snap shot of the alternative last exons in *RBM41* isoforms. RNA-Seq read coverage data from replicate cytoplasmic and polyribosomal fractions are plotted. Both 3'UTRs are well represented in the cytoplasmic fractions. By contrast, the distal 3'UTR is underrepresented in the polyribosome fraction, suggesting that transcripts containing the proximal 3'UTR are preferentially associated with polyribosomes. (*Lower panel)* RT-PCR analysis of *RBM41* mRNA isoforms. The proximal 3'UTR isoform is amplified using the blue and red primer pair (top panel) and the distal 3'UTR isoform is amplified using the blue and gold primer pair (middle panel). Actin mRNA was amplified from both fractions as a loading control (bottom panel).

Supplemental Figure 5. Quantification of RT-PCR by densitomtry. Determined values for Ψ in individual polyribosome fractions for *DGUOK, DDX50, KARS,* and *HNRNPR* are plotted. Experiments were performed in triplicate, and standard error is plotted.

Supplemental Figure 6. Frequency of all hexamers within 50bp from the TSS (Transcriptional Start Site) in (A,B), and the polyadenylation site (C,D) for differential events are compared to a genomic background from gene models. Hexamers from the significantly different associated sequences are binomially distributed with respect to the background probability set, and a Poisson approximated p-value for statistical enrichment of each hexamer was

calculated. P-values are subsequently adjusted (FDR of 1.0%) for multiple comparisons following the method of Benjamini & Hochberg. After significance filtering, we calculate a dissimilarity matrix from pairwise local alignment scores across all remaining hexamers and apply k-medoid clustering where k equals 2 to n. Choosing the value for k which maximizes the average cluster silhouette width, we calculate k position specific weight matricies given pairwise alignments of each cluster member its medoid hexamer. Finally, each position specific weight matrix is plotted with bases of even frequency substituted with their corresponding standard IUPAC character. Exhibited are the corresponding dissimilarity matrices and frequency plots for enriched hexamers. Transparency values for n-mer positions are weighted by both the relative number of contributing n-mers and the maximum frequency at a given position.