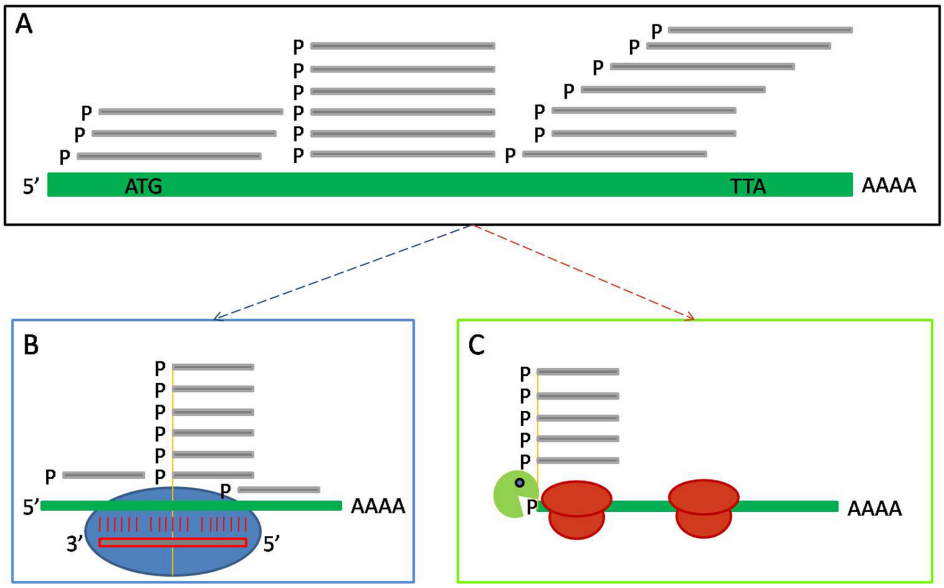
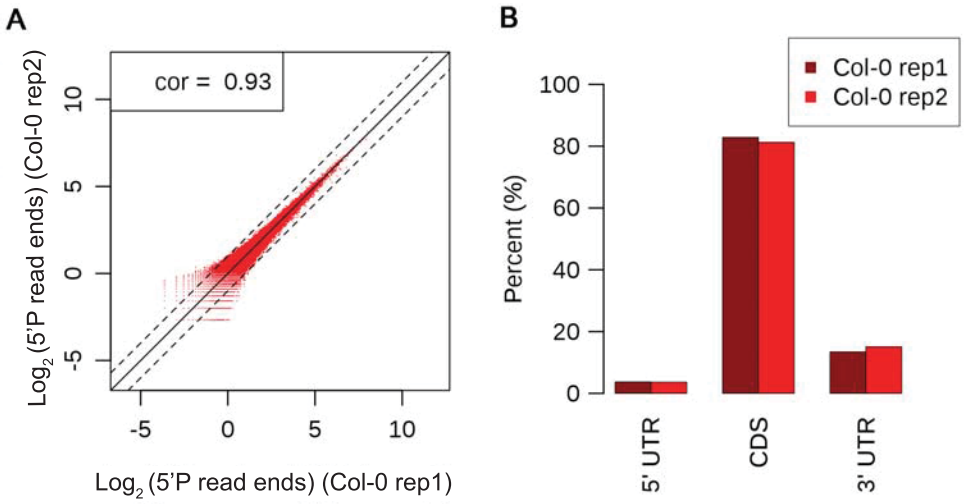


Supplemental Figure 1. The GMUCT Approach Reveals Both miRNA-Directed Cleavage Sites in Target mRNAs And The Features of Co-translational RNA Decay in Plant Transcriptomes.

(A) 5'P reads (gray rectangle) mapping to mature mRNA transcripts (green rectangle).

(B) Accumulation of 5'P read ends precisely at ARGONAUTE (blue ellipse)-mediated miRNA (red rectangle)-directed cleavage sites.

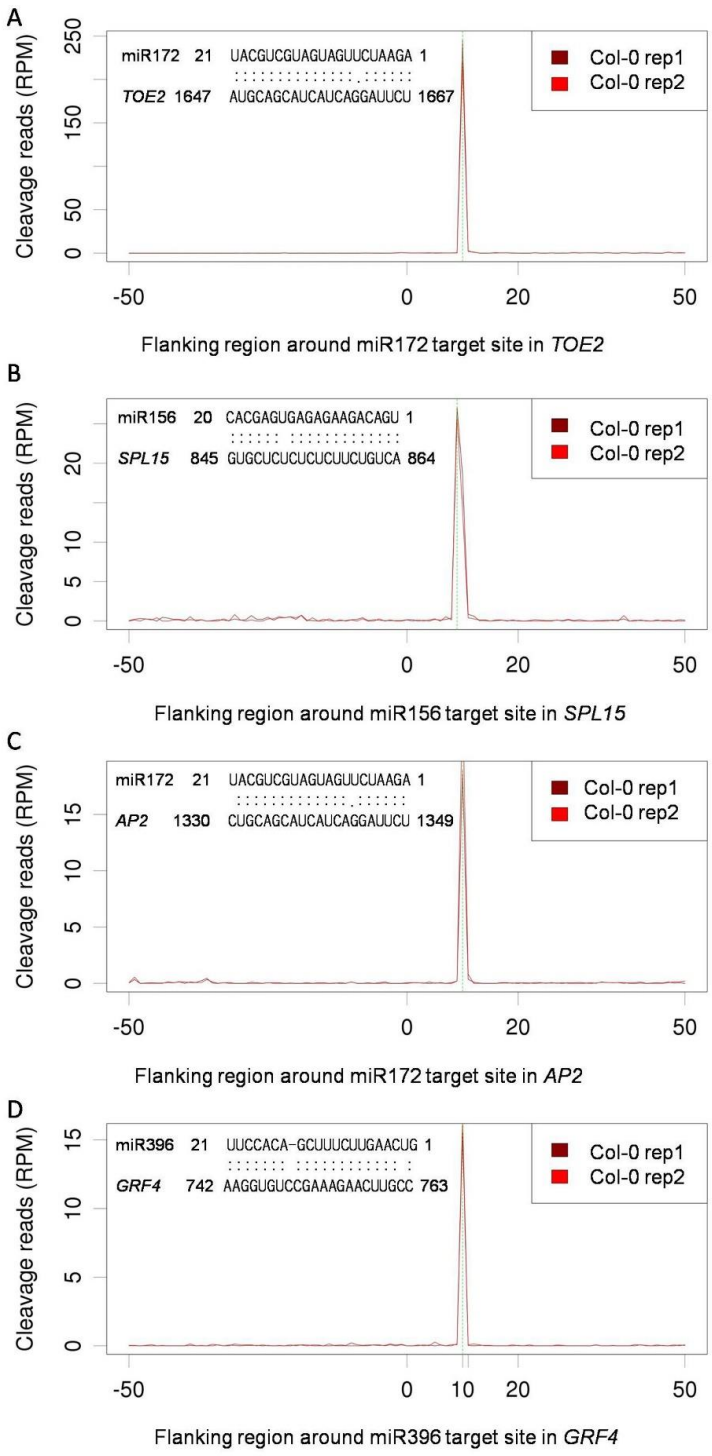
(C) Accumulation of 5'P read ends at the last 5' ribosome boundary due to the process of co-translational RNA decay.



Supplemental Figure 2. GMUCT Is A Highly Reproducible Approach.

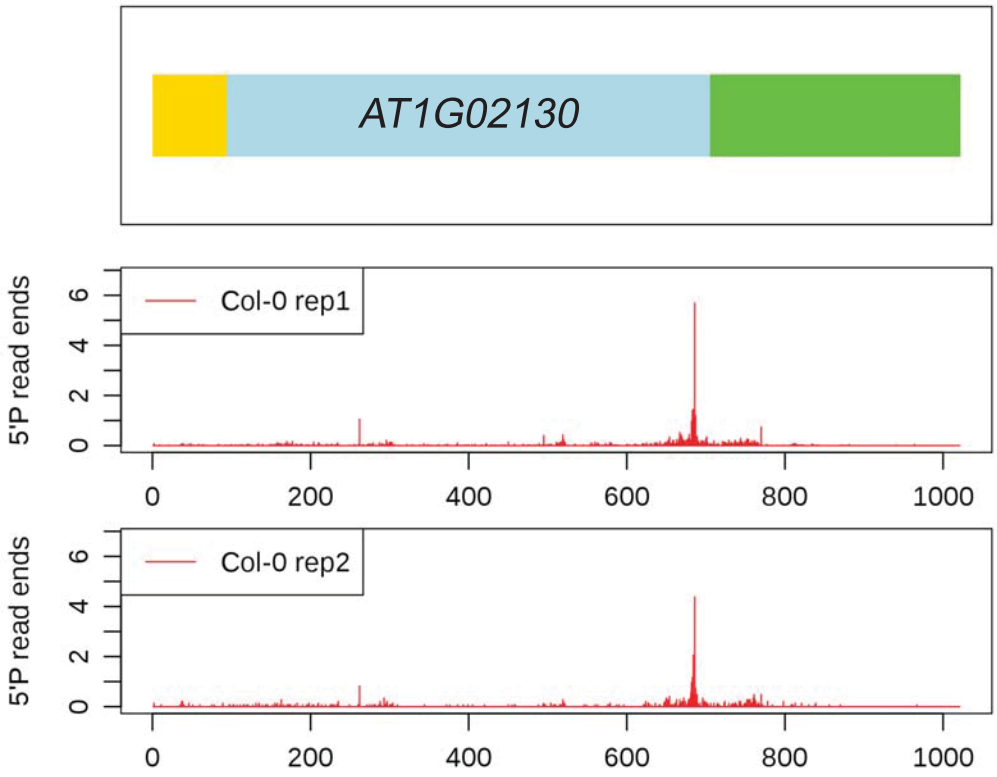
(A) Correlation in GMUCT read counts in a 100-nt sliding window between two biological replicates of Col-0 unopened flower buds.

(B) The percentage of 5'P reads from two Col-0 unopened flower buds that map to mRNA 5' UTRs, CDSs, and 3' UTRs.

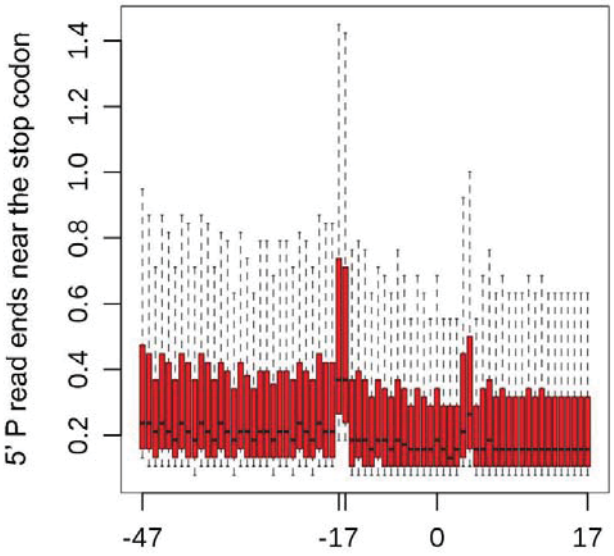


Supplemental Figure 3. GMUCT Identifies miRNA-Directed Cleavage Sites in Target mRNAs.

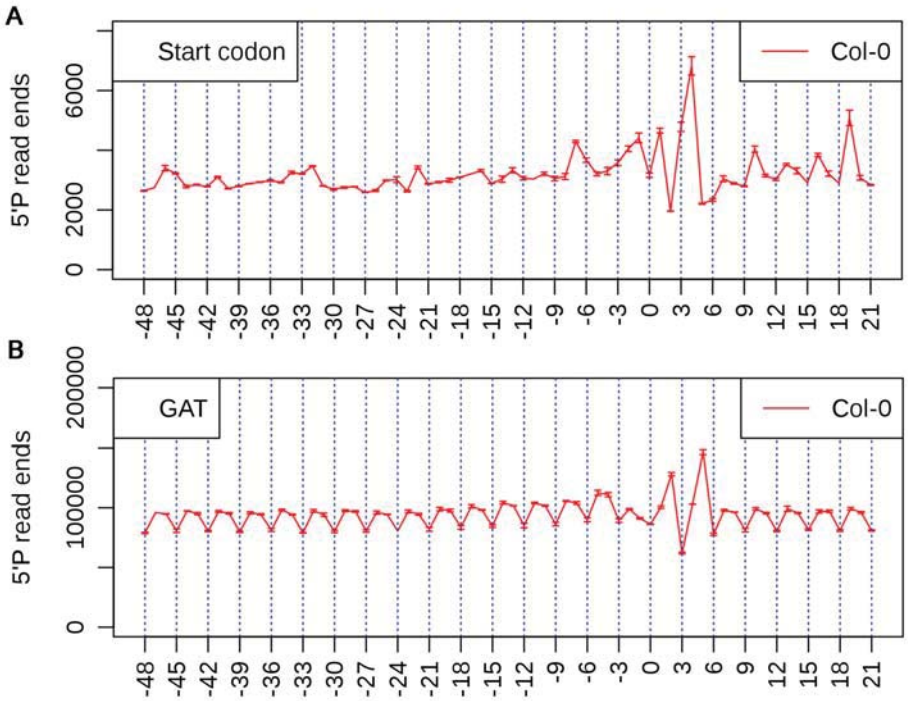
(A) to **(D)** Four of the most efficiently cleaved miRNA target sites in Arabidopsis mRNAs identified by GMUCT. In each figure, the relative positions within the miRNA target sites are denoted by the numbering 0 to 20 (21-nt total length), and the surrounding 50 nt both up and downstream are also shown. The dashed vertical lines mark the region between nt 10 and 11 of the miRNA target site, as this is the precise region where miRNA cleavage should occur.



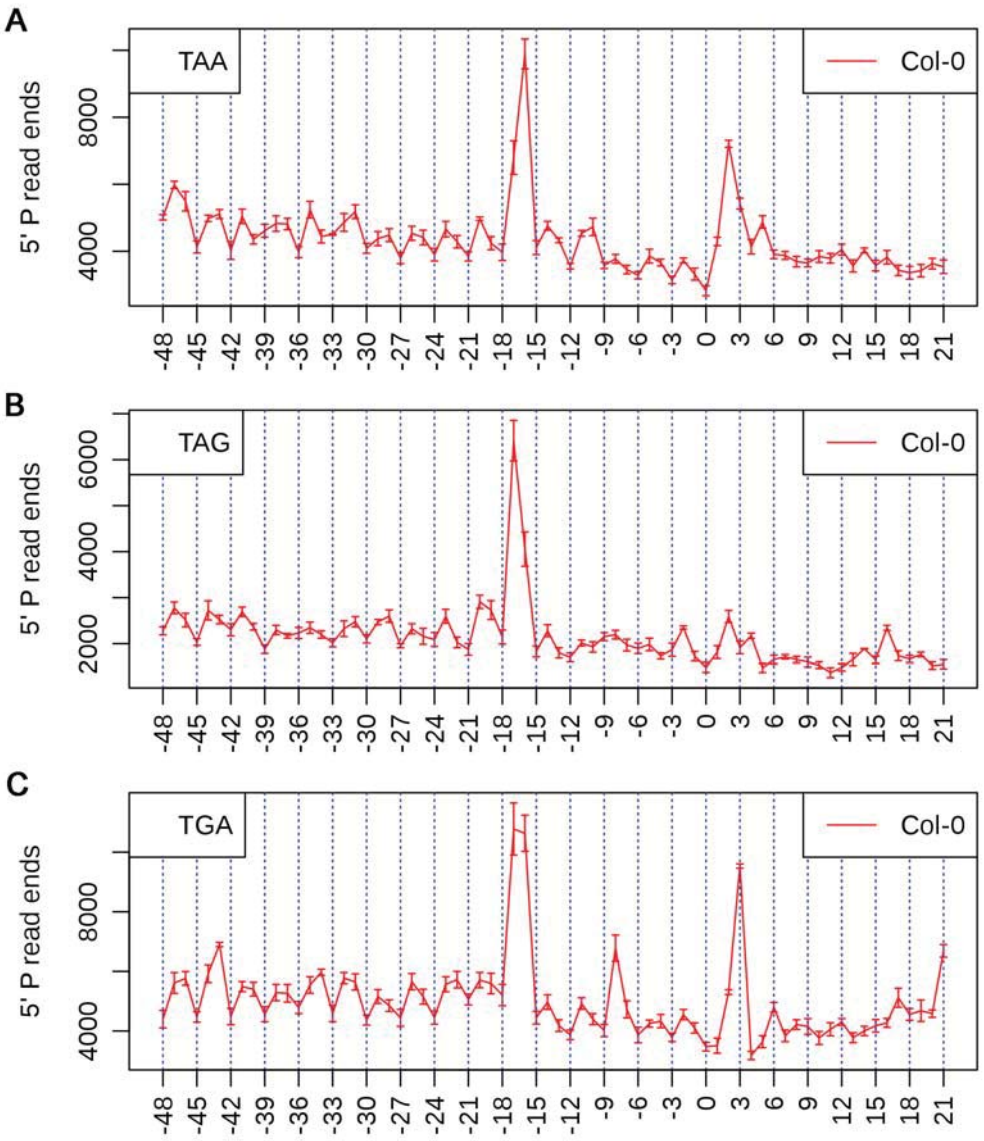
Supplemental Figure 4. GMUCT Reveals Evidence of Co-translational RNA Decay in The Arabidopsis Transcriptome. An example transcript (*AT1G02130*) showing the accumulation of 5'P read ends upstream and nearby its stop codon. In the transcript model, the yellow rectangle represents the 5' UTR, the light blue represents the ORF, and the green box represents the 3' UTR of this transcript.



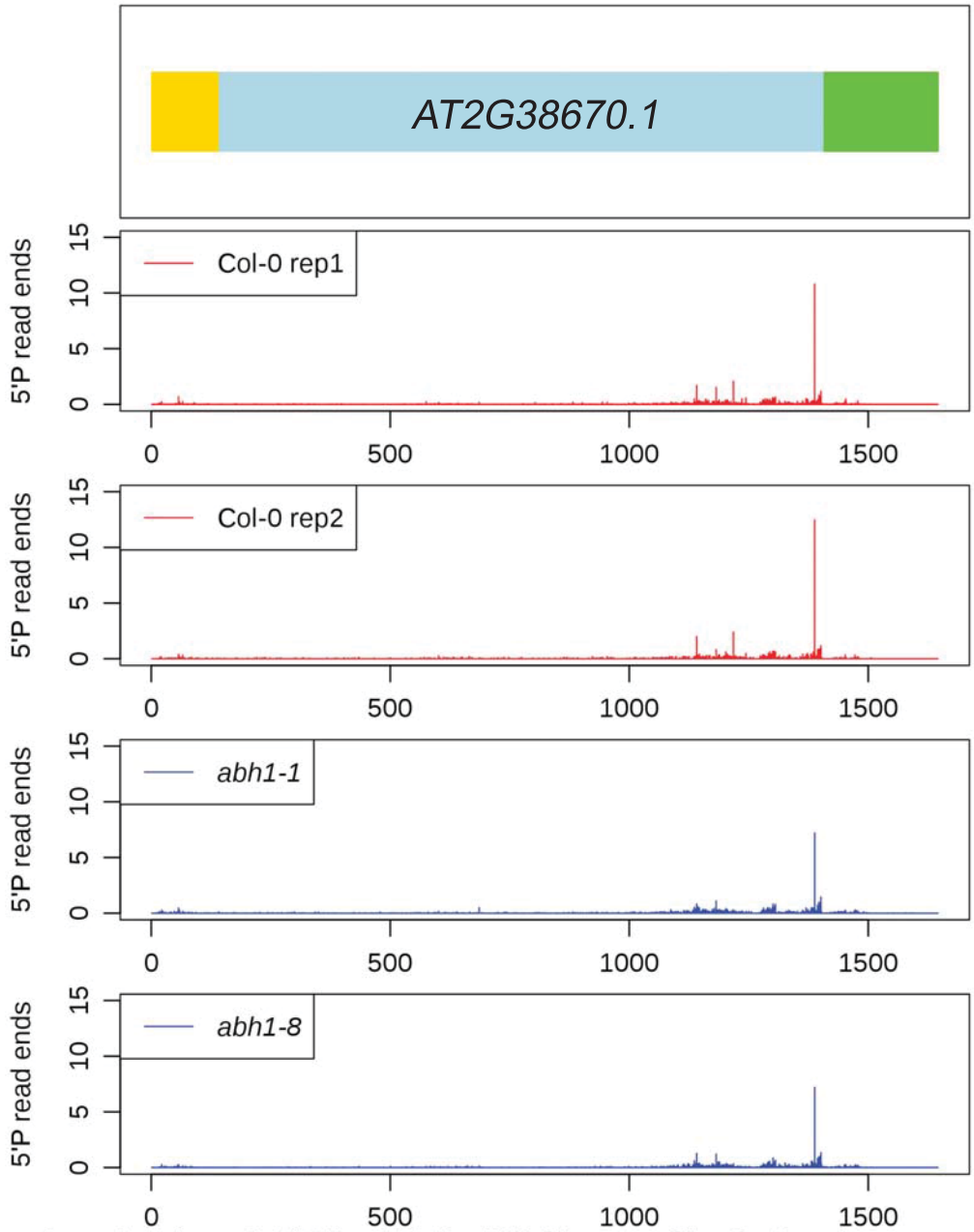
Supplemental Figure 5. Ribosome Pausing at The Stop Codons of mRNA ORFs Provides Evidence for Co-translational RNA Decay in The Arabidopsis Transcriptome. The distribution of 5'P read end abundance within the 2000 transcripts with highest accumulation GMUCT reads near the stop codon. The first nt of the stop codon is denoted as 0. The plot also shows the 47 nt up and 17 nt downstream of the stop codon sequence.



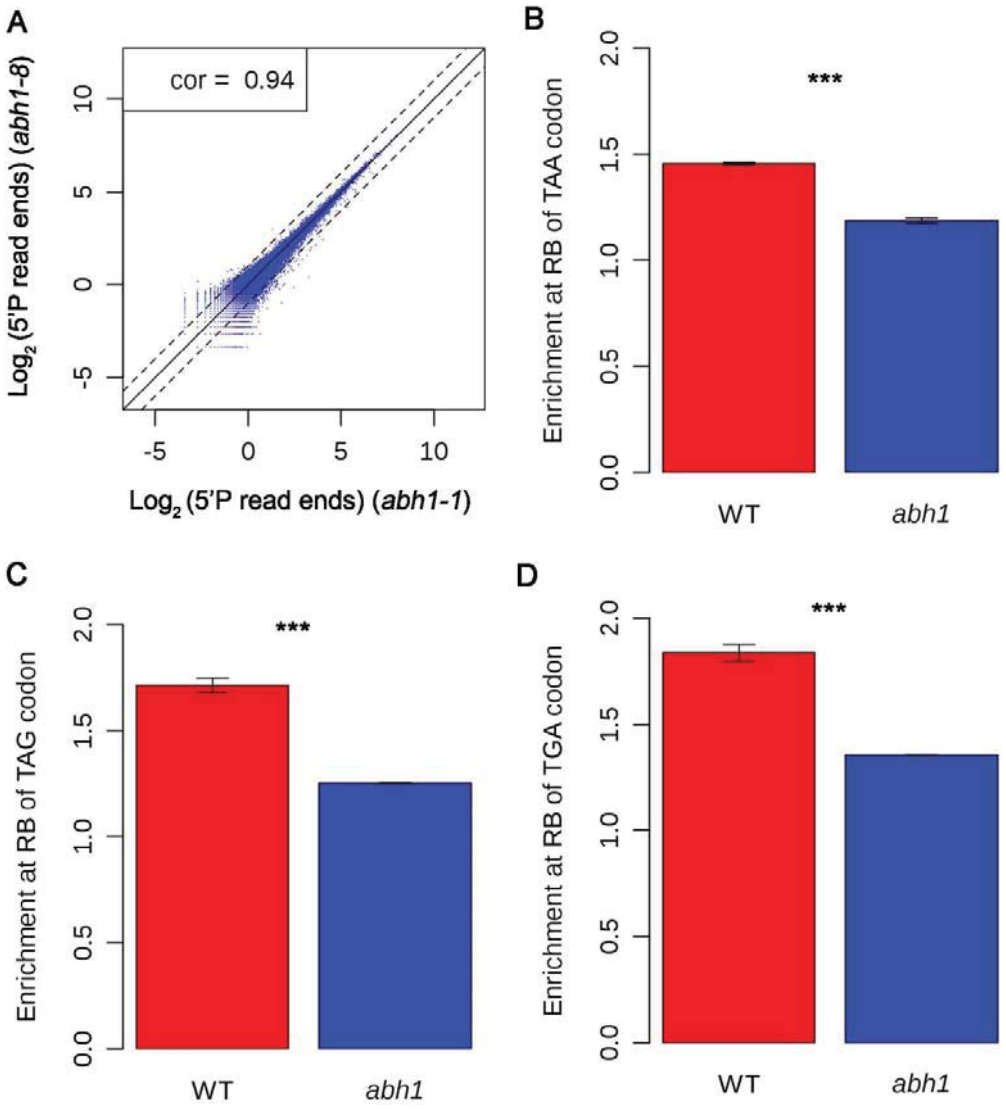
Supplemental Figure 6. The Distribution of 5'P Read Ends around The Start **(A)** and GAT **(B)** Codons in GMUCT Experiments Using Untreated Arabidopsis Unopened Flower Buds.



Supplemental Figure 7. The Distribution of 5'P Read Ends Is Distinct within Transcripts Containing The Three Different Stop Codon Sequences in Arabidopsis. The distribution of 5'P read ends relative to mRNA ORF stop codons TAA **(A)**, TAG **(B)**, and TGA **(C)**.



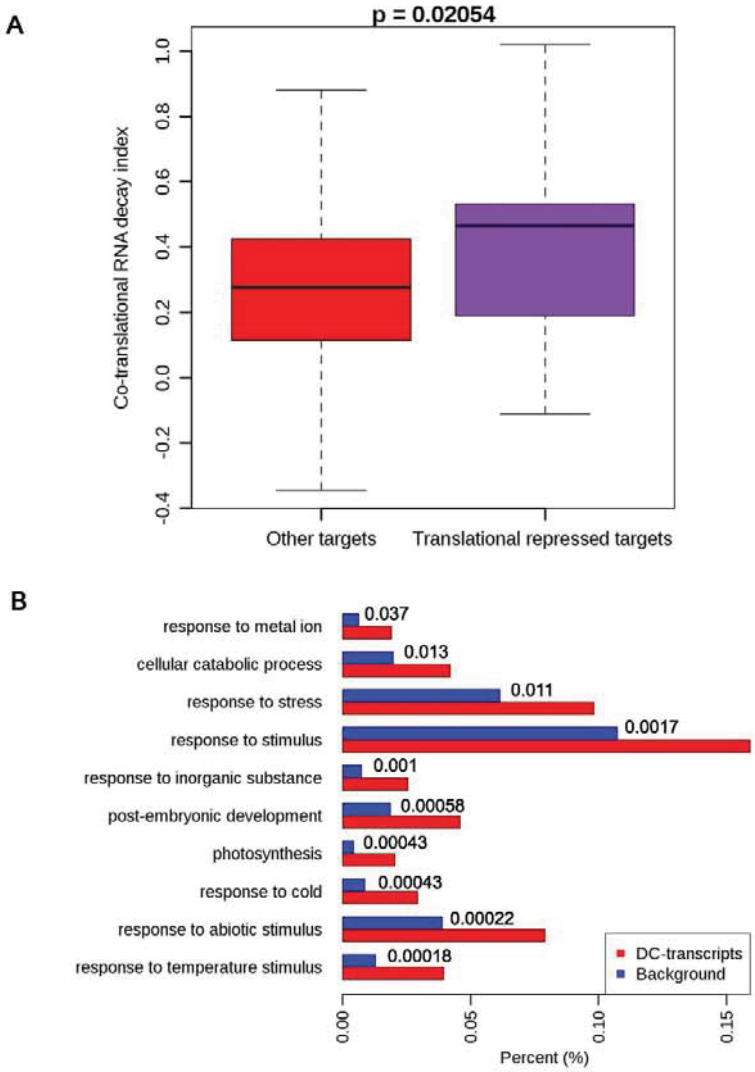
Supplemental Figure 8. 5'P Read Ends at RB Sites near Stop Codons Are Decreased in *abh1* Mutant Plants as Compared to Wild Type Col-0. An example transcript showing the reduced accumulation of 5'P read ends upstream and nearby the stop codon of *AT2G38670* in *abh1* mutants as compared to Col-0 plants. In the transcript model, the yellow rectangle represents the 5' UTR, the light blue represents the ORF, and the green box represents the 3' UTR of this transcript.



Supplemental Figure 9. The Nuclear Cap-Binding Complex of Arabidopsis Functions in Co-translational RNA Decay.

(A) Correlation in GMUCT read counts in a 100-nt sliding window between GMUCT data from *abh1-1* (x-axis) and *abh1-8* (y-axis) unopened flower buds. The very high correlation value between data from these two *abh1* null alleles allowed us to combine the data for comparison with data from Col-0 plants.

(B) to **(D)** Enrichment of 5'P read ends at the ribosome boundary (RB) of the three stop codons TAA **(B)**, TAG **(C)**, and TGA **(D)** as compared to the median coverage within the 50 up and downstream flanking nt for Col-0 as compared to *abh1* mutant plants. *** denotes a significant difference at a p-value < $1x10^{-100}$ as determined by a chi-squared test. Error bars represent standard error of the mean.



Supplemental Figure 10. The Levels of Co-translational RNA Decay Vary Significantly between miRNA Target Transcripts Regulated by Translation Inhibition and Those That Are Not, and GO Enrichment of Genes with Significantly Different CRI Values between Col-0 and *abh1* Mutant Unopened Flower Buds.

(A) Boxplot of co-translational RNA decay index (CRI) values for miRNA target genes that are regulated by translation inhibition (purple box) and those that are not (red box). We found a significant difference between the CRI values of these two collections of miRNA target transcripts. The p-value of the significance test (chi-squared test) is shown at the top of the boxplot. Boxes extend from the 25th to 75th percentiles. Lines in the middle of the box are plotted at the median. Whiskers are drawn down to the 5th percentile and up to the 95th.

(B) Gene Ontology (GO) analysis for the group of transcripts that display significantly different CRI values between Col-0 and *abh1* mutant unopened flower buds. The value on each bar in the graph is the FDR of enrichment for each GO term. DC-transcripts denotes transcripts with significantly different CRI values for *abh1* mutant compared to Col-0 unopened flower bud GMUCT data sets.