Supplemental Figure S1: Replicate Repeatability Correlations
A-C,E-G,I-K,M-O) Whole transcriptome correlations of A/C nucleotide reverse transcriptase stop counts in DMS-treated libraries between biological replicates of the same condition: shoot contol, shoot salt, root control, and root salt respectively. D,H,L,P) Distributions of per transcript correlations of A,C nucleotide reverse transcriptase stop counts between biological replicates of the same condition. DMS modifications are suitably repeatable between our biological replicates on both a whole transcriptome and individual transcript basis. A complete table of all libraries and treatments is included in Supplemental Data 6: Table W1. Numbers of transcripts used in this analysis are higher than those in Fig. 1C due to some transcripts not having the requisite 10 or more As plus Cs before the last 30 bp of the transcript, i.e. correlation of stops could be assessed but a reliable mean reactivity could not be resolved.

Supplemental Figure S2: Salt Stress Induced Average Mean Reactivity Changes
Distributions of mean reactivity are plotted for both control (blue) and NaCl-treated (yellow) transcripts and transcript regions in both shoot (A-D) and root (E-H). Changes in mean reactivity between conditions of each transcript or region are shown by lines between the distributions; strong changes (>= 0.075) have a more intense shading to highlight differences. A statistical summary of these results can be found in Table 1 and Supplemental Data 3: Table S1A. The data used in these analyses can be found in Supplemental Data 1: D1-D8.

Supplemental Figure S3: The Effect of Salt Stress on Intra-Tissue Transcript Reactivity Gini Index
The distributions of the Gini index of reactivity are plotted for both control (navy) and salt treated (orange) transcripts and transcript regions in both shoot (A-D) and root (E-H). Changes in Gini index of reactivity between conditions of each transcript or region are shown by lines between the distributions; stronger changes have a more intense shading to highlight differences. A full statistical summary of these changes can be found in Supplemental Data 3: Table S1C. The data used in these analyses can be found in Supplemental Data 1: D1-D8.

Supplemental Figure S4: The Shoot Structurome is More Reactive than the Root Structurome
Distributions of mean reactivity are plotted for both shoot (blue) and root (yellow) transcripts and transcript regions in both control (A-D) and NaCl-treatment (E-H) conditions. Changes in mean reactivity between conditions of each transcript or region are shown by lines between the distributions; strong changes (>= 0.075) have a more intense shading to highlight differences. A statistical summary of these results can be found in Table 1 and Supplemental Data 3: Table S1A. The data used in these analyses can be found in Supplemental Data 1: D9-D16.

Supplemental Figure S5: The Effect of Salt Stress on Inter-Tissue Transcript Reactivity Gini Index
The distributions of Gini index of reactivity are plotted for both shoot (navy) and root (orange) transcripts and transcript regions in both control (A-D) and salt (E-H) conditions. Changes in Gini index of reactivity between conditions of each transcript or region are shown by lines between the distributions; stronger changes have a more intense shading to highlight differences . A full statistical summary of these changes can be found in Supplemental Data 3: Table S1C. The data used in these analyses can be found in Supplemental Data l: D9-D16.

Supplemental Figure S6: The Effect of Salt Stress on Arabidopsis Transcript Mean Reactivity via ANOVA

A-D) Two-way (mean~treatment*tissue) ANOVAs for transcript and transcript regions (Supplemental Data 3: Table S4). E) Three-way (mean~treatment*tissue*region) ANOVA using region as a factor (Supplemental Data 3: Table S4). Tissue and transcript region are more powerful determinants of mean reactivity than salt stress. The data for these analyses used a common normalization scale across all conditions (Supplemental Data 1: D17-D20.)

Supplemental Figure S7: Change in DMS Reactivity is Inversely Correlated with Change in Abundance and the Correlation is Enhanced by Concordancy, Root

A-D) For each transcript and transcript region in root, we assessed the relationship between change in mean DMS reactivity (Δ mean reactivity, NaCl-treatment mean reactivity – control mean reactivity, x-axis) and change in relative abundance (Δ abundance, $\log_2(\text{TPM}_{\text{NaCl}}) - \log_2(\text{TPM}_{\text{control}})$, y-axis) between control and NaCl treatment. This revealed a strong inverse correlation (Table 2A, Supplemental Data 3: Table S6A). E-H) Transcripts that share the same sign of Δ mean reactivity between their 5'UTR, CDS, and 3'UTR regions were subset as concordant. For each transcript and transcript region of these fully concordant transcripts, we assessed the relationship between the change in mean reactivity (Δ mean reactivity, NaCl mean reactivity – control mean reactivity, x-axis) and the change in relative abundance (Δ abundance, $\log_2(\text{TPM}_{\text{NaCl}}) - \log_2(\text{TPM}_{\text{control}})$, y-axis) between control and NaCl-treatment in root, revealing a strong enhancement of the inverse correlation found among all transcripts and regions (Table 2C, Supplemental Data 3: Tables S6A, S6D).

Supplemental Figure S8: Shoot Region Δ Reactivity Correlations, Concordant vs. Discordant

Column 1. For each two regions of each transcript resolved in our shoot contrast, we performed a Δ mean reactivity vs. Δ mean reactivity correlation to test for a relationship between the regions' structural change; the strength of such relationships is negligible (Supplemental Data 3: Table S6C). We assessed the strength of the Δ reactivity versus Δ abundance relationship between every two pairs of transcript regions (5'UTR:CDS, 3'UTR:CDS, 5'UTR:3'UTR), sub-grouping them into concordant and discordant categories. Concordancy here was defined as the two transcript regions under comparison sharing the direction of reactivity change between control and NaCl stress and discordancy as the two transcript regions under comparison not sharing the direction of reactivity change between conditions. This leads to 24 different combinations, which are presented as Δ abundance versus Δ reactivity plots (Columns 2-5) with all tests and statistics available (Supplemental Data 3: Table S6E). Regions concordant in Δ reactivity with the other region in the pairwise comparison (Columns 2 and 4) have a stronger inverse Δ reactivity-per-region to Δ abundance-per-transcript relationship than regions that are discordant (Columns 3 and 5, Supplemental Data 3: Table S6E). For instance, transcripts with 5'UTRs that are concordant in the direction of reactivity change with their respective CDS have a stronger relationship to abundance change (–0.51, $p<2.20e^{-16}$), than transcripts with 5'UTRs that are discordant in the direction of reactivity change with their respective CDS; the latter even have a slight direct correlation (+0.15, $p<2.20e^{-16}$). These results suggest that interplay between the structuredness of two different transcript regions (5'UTR, CDS, 3'UTR) can contribute to the overall Δ reactivity versus Δ abundance relationship (see Discussion).

Supplemental Figure S9: Root Region Δ Reactivity Correlations, Concordant vs. Discordant

We repeated all pair-wise concordancy analyses with our root data, finding very similar results as for the shoot data (Supplemental Figure 8). Column 1. For each two regions of each transcript resolved in our root contrast, we performed a Δ mean reactivity vs. Δ mean reactivity correlation to test for a relationship between the regions' structural change; no such relationship exists (Supplemental Data 3: Table S6C). Columns 2 and 4. Transcript regions that concordantly change with another region have stronger Δ reactivity to Δ abundance correlations. Columns 3 and 5. Likewise, discordancy tends to decrease the strength of the Δ reactivity to Δ abundance correlation (Supplemental Data 3: Table S6E).

Supplemental Figure S10: Relationship between Reactivity and Abundance with No Contrasts

To comprehensively assess the relationship between mean transcript reactivity and transcript in a manner as close as technically possible, we re-calculated all reactivities for every transcript and transcript region in each condition and treatment individually (Supplemental Data 2: R1-R16), i.e. one structurome at a time. This allowed a greater number of transcripts per individual structurome to investigate this relationship because mutual transcript coverage between conditions did not have to be considered. Additionally, using each structurome's internal normalization scale mitigates some dispersion of reactivity values. A-D) Shoot control transcript regions. E-H) Shoot salt transcript regions. I-L) Root control transcript regions. M-P) Root salt transcript regions. When we assess these datasets for the relationship between whole transcript mean reactivity and transcript abundance, we find a strong positive correlation ($r \geq 0.75$, $p<2.20e^{-16}$, Supplemental Data 3: S8A) as anticipated[1]. This correlation appears driven primarily by the reactivity of the CDS, as it was only weakly observed for either 5' or 3' UTRs (Columns 2 and 4, Supplemental Data 3: Tables S8A). Shoot control whole transcript (A) mirrors Fig. 5A but with non-contrasted data, showing that the positive correlation between mean reactivity and abundance found in contrasted data is also present in each independent steady state structurome and transcriptome. We repeated every test on our contrasted data as well, showing parallel results (Supplemental Data 3: Table S8B).

Supplemental Figure S11: Relationship between Reactivity and Abundance and its Modulation by Salt Stress, Extended

Figs. 5A, 5B, 5C are included as panels A, D, G of this extended figure for a complete comparison; panels B and C show the full set of shoot reactivity increases and decreases, and panels E and F show the full set of shoot abundance increases and decreases. Changes in root data are likewise paralleled in panels H-N. Associated numbers and tests can be found in Supplemental Data 3: Table S8C. All data shown are for transcripts in their unstressed conditions, i.e. reactivity increases are those transcripts that increase in reactivity after salt stress.

Supplemental Figure S12: Downscaling of Reverse Transcriptase Stops does not Greatly Alter Transcript Mean Reactivity

Using the StructureFold2 rtsc_downscale module, we downscaled our RT stops from both (-) and (+) DMS libraries by several ratios (.50, .25, .125), under both fractional and randomread settings. Fractional mode simply multiplies the RT stops each nucleotide position by the given ratio, where under randomread settings each stop has the given ratio chance of being retained. Using these downscaled stops (rtsc files), we recalculated all reactivity values (Supplemental Data 2: R17) for shoot control. A-D) For the top 100 most abundant transcripts we plotted the mean reactivity calculated with all of the RT stops against the mean reactivity calculated with all, half, quarter, and an eighth of the fractional RT stops. E-H) The top 100 most abundant transcripts are shown as red dots on a modified version of Fig. 5A using their reactivity calculated with all, half, quarter, and an eighth of the fractional RT stops. Even when the top 100 most abundant transcripts have lost 87.5% of their reads, they still remain significantly more reactive than the rest of the transcripts (Supplemental Data 3: Table S8D), largely because it is the ratio of (+) to (-) reads (stops) that mostly determines reactivity. I-L) A zoom

in on the top 100 most abundant transcripts of panels E-H. M-P) Mirrors of panels A-D, except using randomread downscaled stops. Q-T) Mirrors of panels E-H, except using randomread stops; the top 100 most abundant transcripts remain more reactive than the rest of the transcripts using randomread downsampling as well (Supplemental Data 3: Table S8D). U-X) Mirrors of panels I-L. Even under random downsampling, the overall relationship is maintained.

Supplemental Figure S13: Gene Ontology Analyses Reveal Enrichment of Stress-related Transcripts

Results from multiple GO analyses (via agriGOv2[2]) are combined and displayed as a heat map, where color indicates the significance of enrichment within an analysis. Categorical enrichment of transcripts and transcript regions within the top (A) and bottom (B) 5% of reactivity differences between shoot and root are displayed. The full results of these GO analyses are found in Supplemental Data 4: G17-G32. The total number of genes classified within a GO category are listed beside each category name.

Supplemental Figure S14: Extended Confirmation of Delta Reactivity Versus Delta Abundance Relationships

We subset our shoot whole transcript Δ reactivity versus Δ abundance data (Fig. 4A) to only include transcripts of genes that had differential expression under salt treatment in the report from Anderson et al.[3] (Table S3 of Anderson et al.[3]); each dot represents a single transcript in our study colored by the fold change of its respective gene in the Anderson et al. study[3]. An overwhelming majority of NaCl-induced fold changes in that study[3] are paralleled in our study, showing parity between the effect of salt treatment on transcript abundance between studies while also highlighting these transcripts' corresponding changes in reactivity in our own study. ($r = -0.84$, p-value < 2.2e-16). Moreover, in general, transcripts that lost abundance gained DMS reactivity in our study, while transcripts that gained abundance lost DMS reactivity in our study; these comparisons support the conclusion that the transcriptome changes we observe typify salinity impacts on the Arabidopsis transcriptome. Beyond validating our expression results and the effectiveness of our salt stress treatments with another study on salinity stress effects on the Arabidopsis transcriptome[3], the congruency between these datasets implicates RNA structure as an essential mechanism that regulates mRNA abundance. Our mutual coverage requirements for comparing the structuromes between conditions (see Methods) may prevent the comparison of extreme changes of abundance by our method, i.e. where one transcript is below the reactivity coverage threshold in one of the conditions.

Supplemental Figure S15: Transcripts Lacking m^{6}A Modification show the Inverse Relationship between Change in Reactivity and Change in Abundance

We extracted the genomic coordinates of high confidence m^{6}A modifications[3] (Table S4 of Anderson et al.[3]), and assigned these peaks to genes. All transcripts of genes with a detected m^{6}A peak in either condition were removed from our shoot data, and the remaining transcripts ($n = 3{,}823$) were plotted exactly as in our Δ reactivity and Δ abundance plots, showing that the inverse correlation of Δ reactivity and Δ abundance is retained ($r = -0.69$, $p < 2.2e\text{-}16$) in transcripts with no detected m^{6}A modifications.

Supplemental Figure S16: Gel-based Assay for Single Hit Kinetics Determination

Time course of *in vivo* DMS modification of Arabidopsis 18S rRNA in root and shoot tissue from control and salt-stressed plants. The extent of DMS modification was read out by gel-based probing. Single hit kinetics, defined as on average not more than one DMS modification per 100-200 nt, was used as the criterion to determine the DMS treatment durations. A modification rate of 10-20% is consistent with the expected Poisson distribution of single-hit modification[4]. For each lane, modification rate was calculated as: [(the signal intensity of the full length band) / (the signal intensity

of whole lane) x 100%]. Resultant optimal durations of DMS treatment (found at the top of the gel) are those that correspond to the percentage modifications labeled in green font below the gel.