

Supplemental figures

Optimized tRNA structure-seq reveals robust tRNA secondary structures in *S. cerevisiae* under mild stress conditions

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Ryota Yamagami

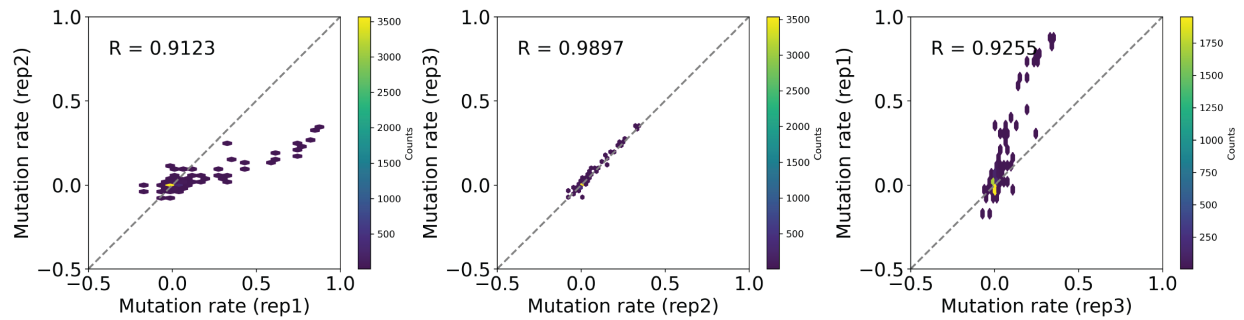
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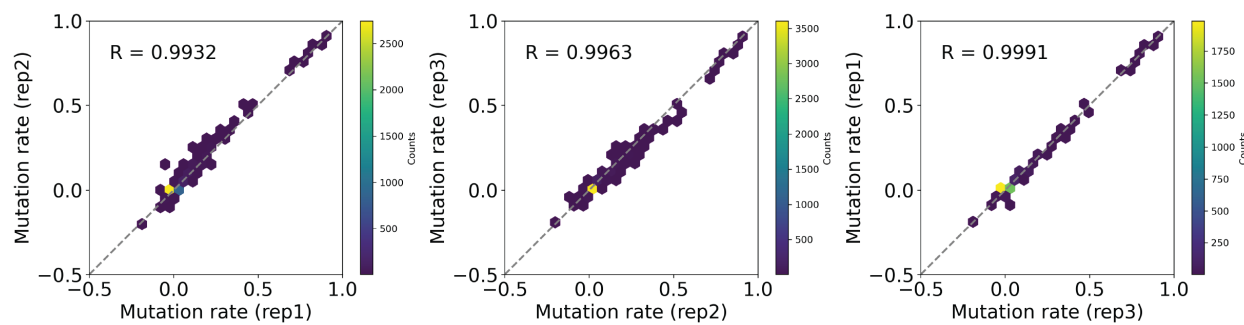
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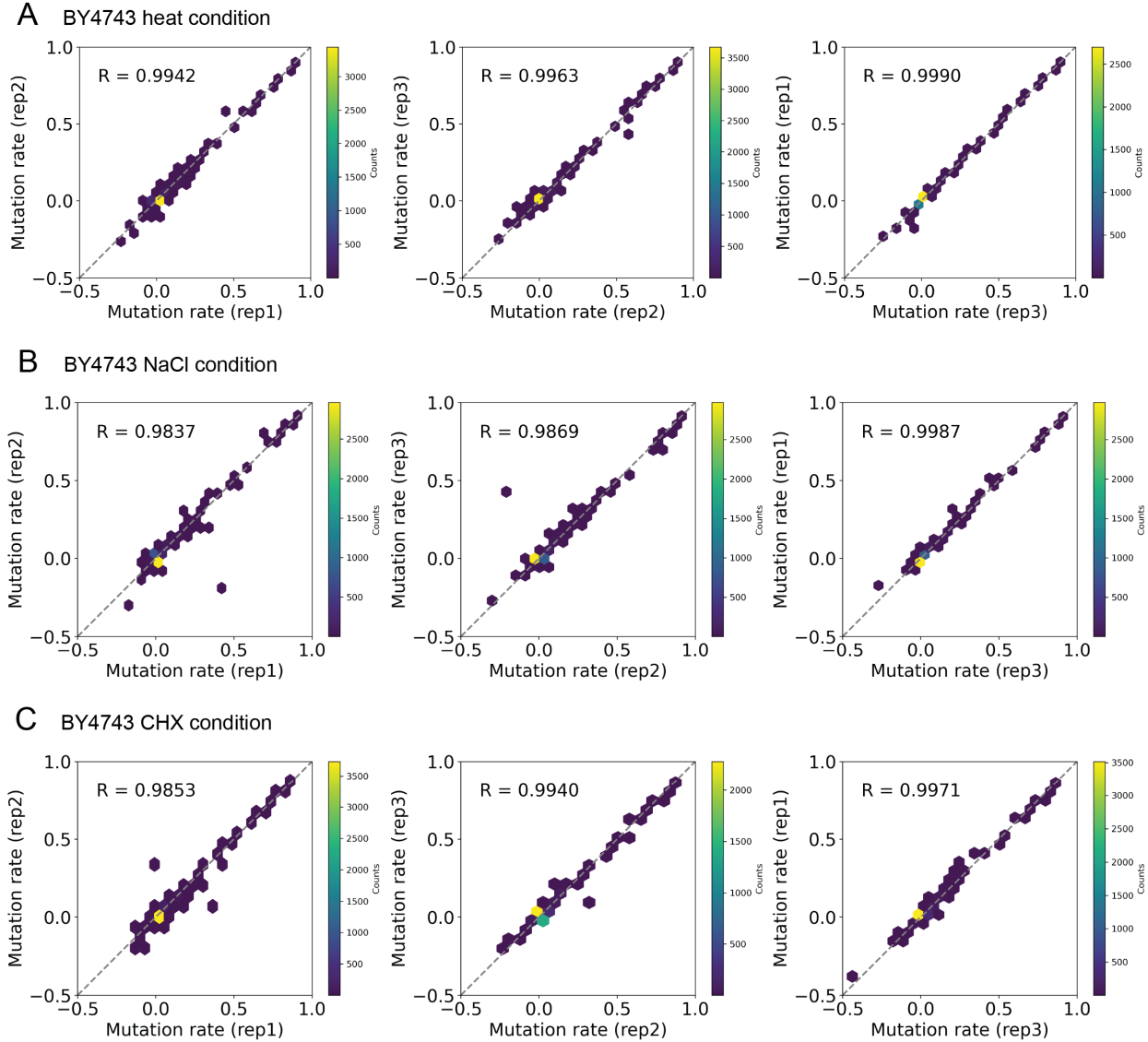
A BY4743 in vitro DMS condition



B BY4743 in vivo DMS condition

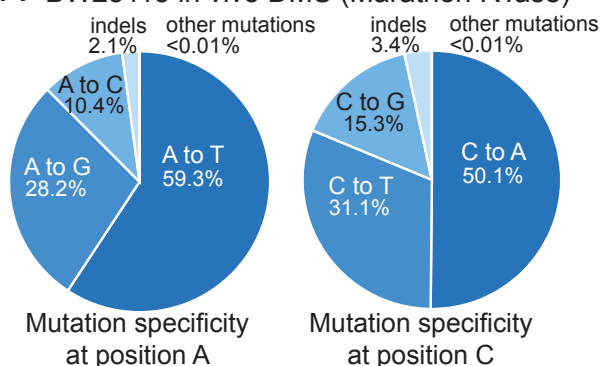


Supplemental Fig. 1 tRNA Structure-seq datasets obtained in the unstressed conditions showed high reproducibility. (A – B) Mutation rates were plotted to assess correlations between biological replicates for (A) *S. cerevisiae* BY4743 in vitro DMS conditions and (B) BY4743 in vivo DMS condition. Pearson correlation coefficient (R) values were determined by linear regression. Correlation between biological replicates is generally high; although replicate 1 shows slightly lower correlation with the other replicates. The R values (0.91–0.93) indicate strong overall reproducibility.

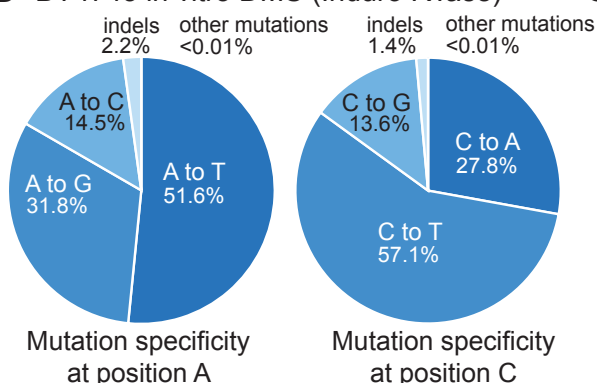


Supplemental Fig. 2 tRNA Structure-seq datasets obtained in the mild stress conditions showed high reproducibility. (A – C) Mutation rates were plotted to assess correlations between biological replicates for (A) *S. cerevisiae* BY4743 heat condition, (B) BY4743 NaCl condition, and (C) BY4743 antibiotic condition where CHX means cycloheximide. Pearson correlation coefficient (R) values were determined by linear regression.

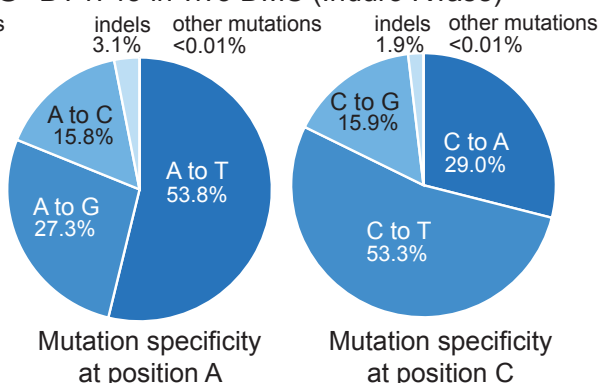
A BW25113 in vivo DMS (Marathon RTase)



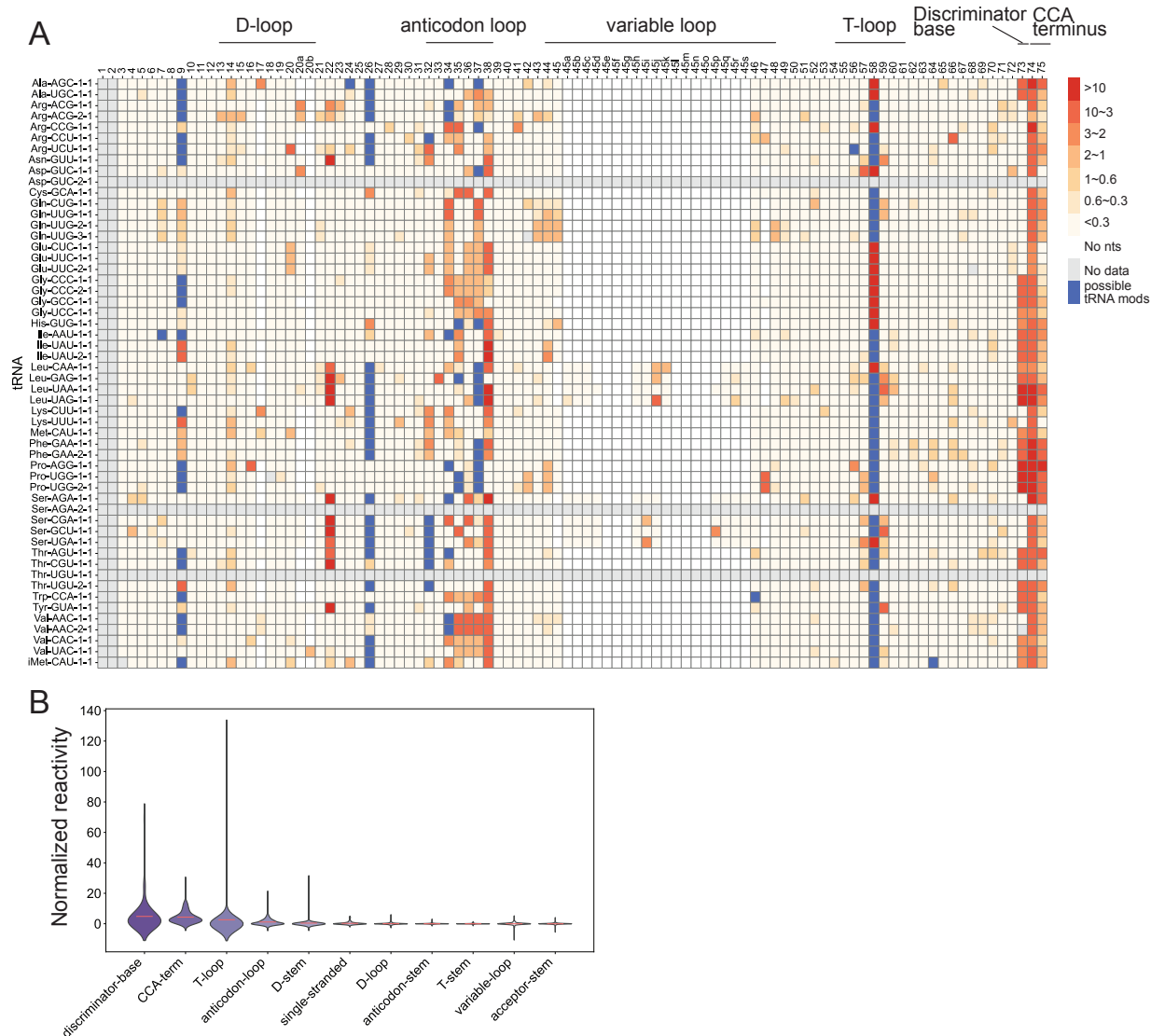
B BY4743 in vitro DMS (Induro RTase)



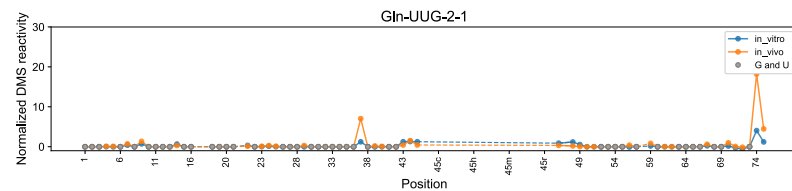
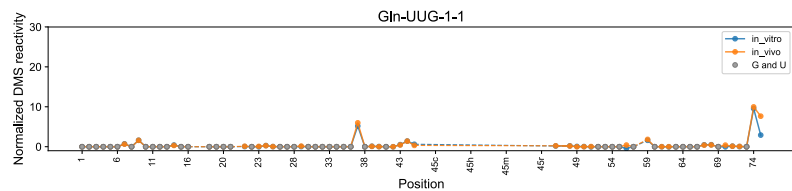
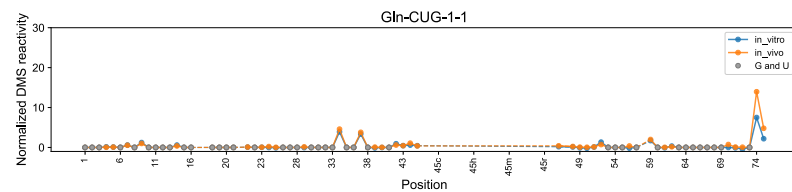
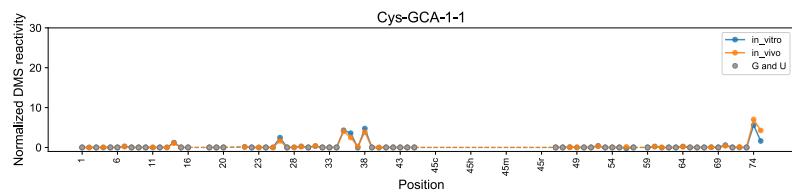
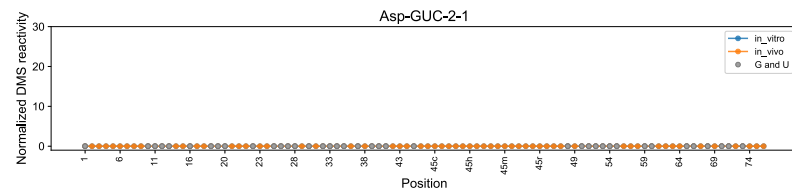
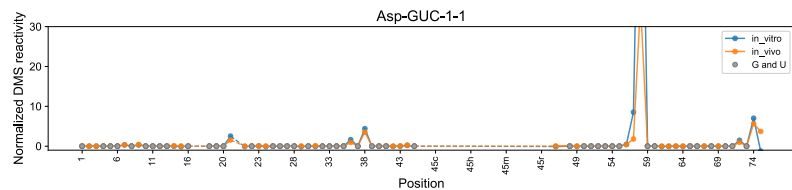
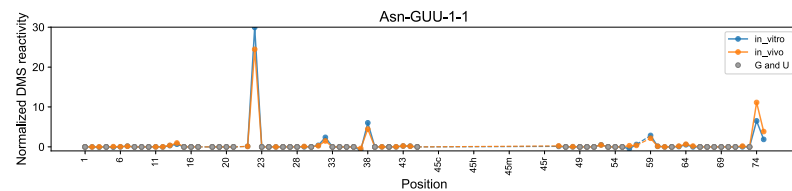
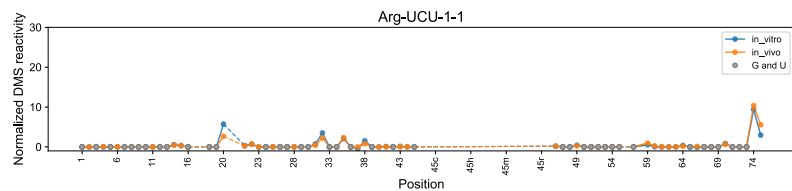
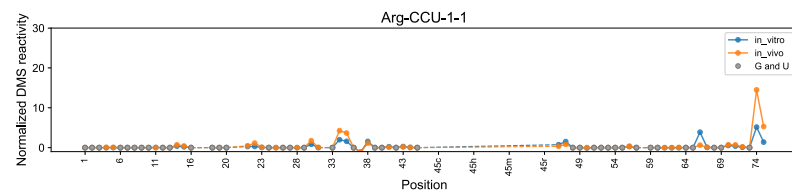
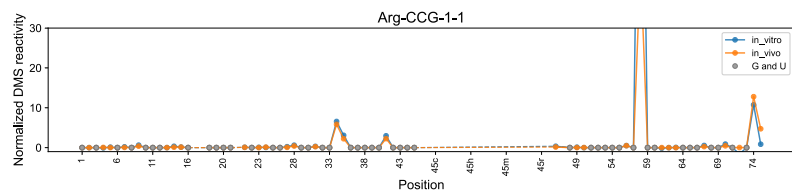
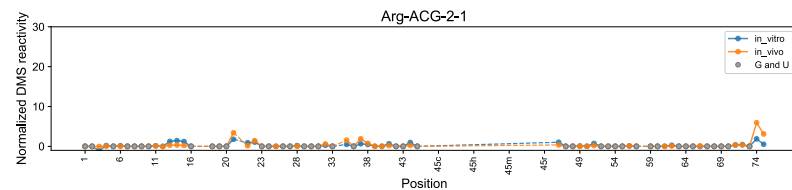
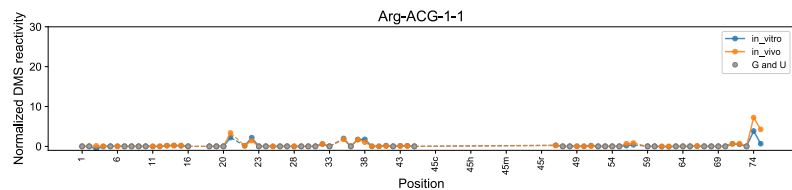
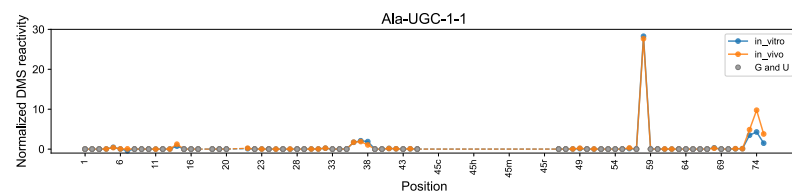
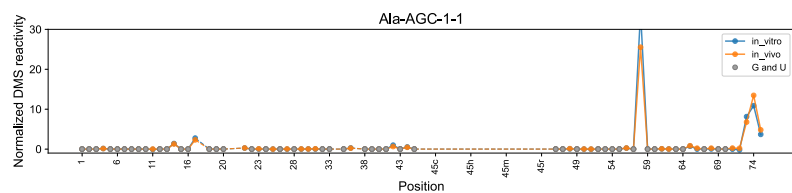
C BY4743 in vivo DMS (Induro RTase)

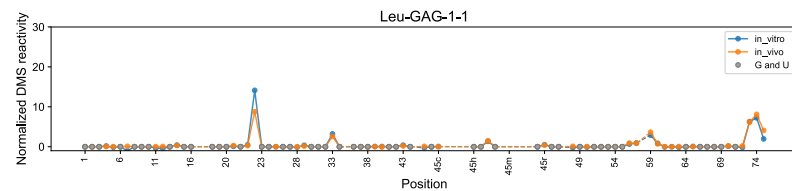
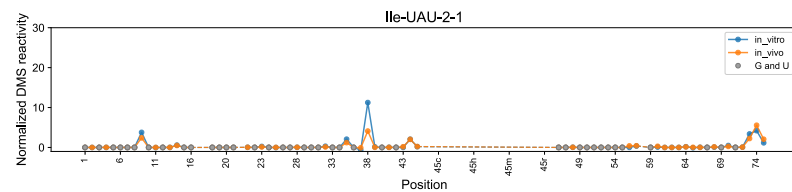
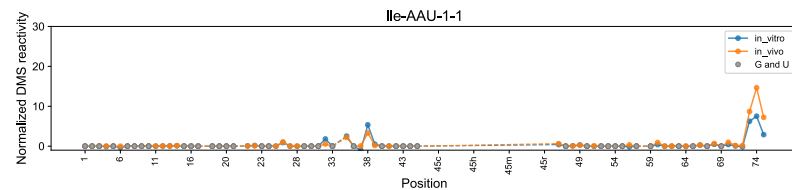
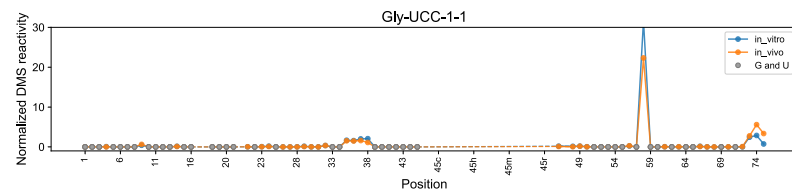
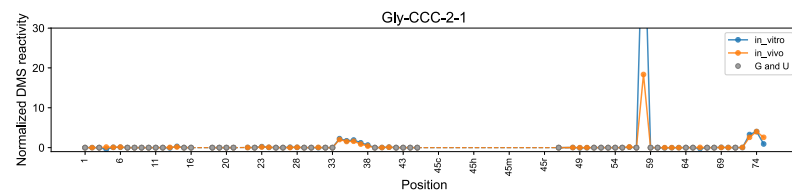
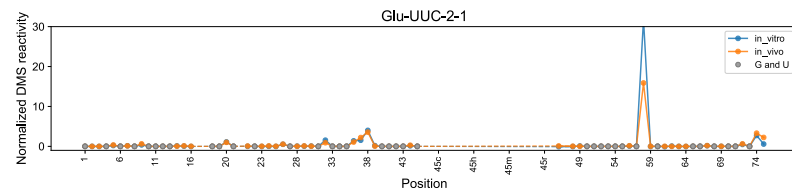
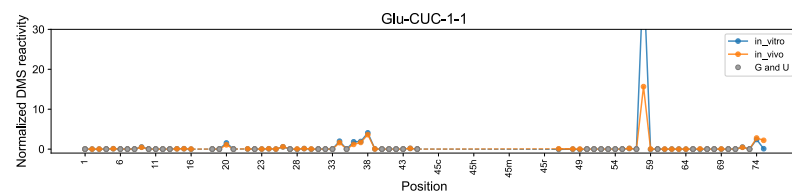
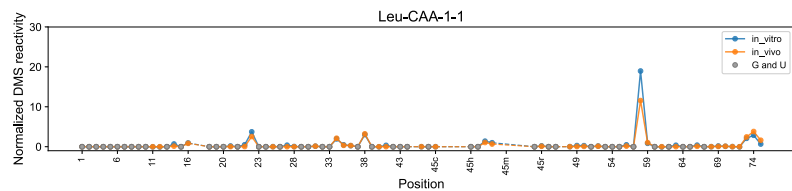
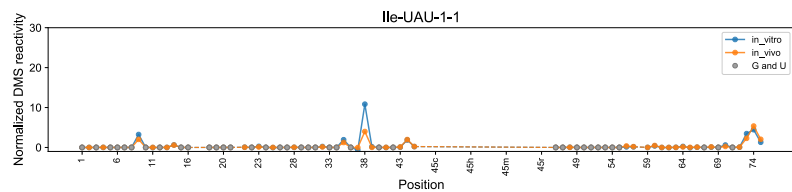
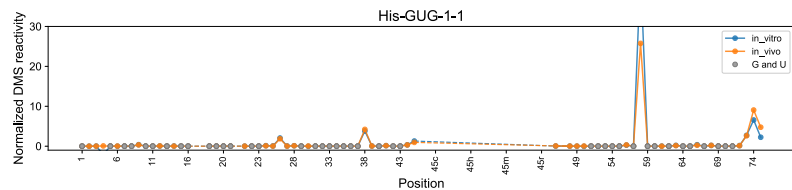
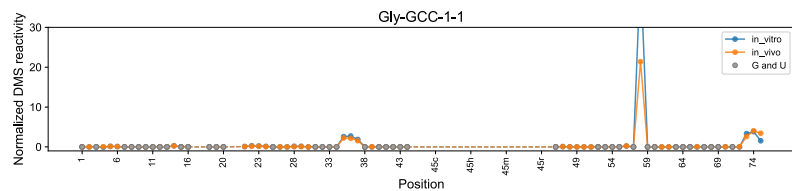
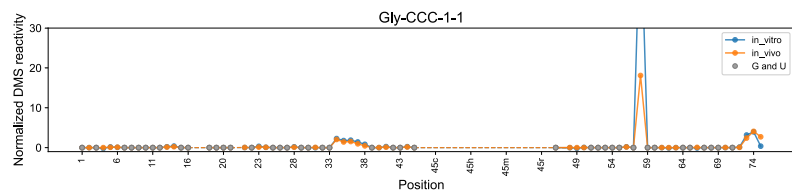
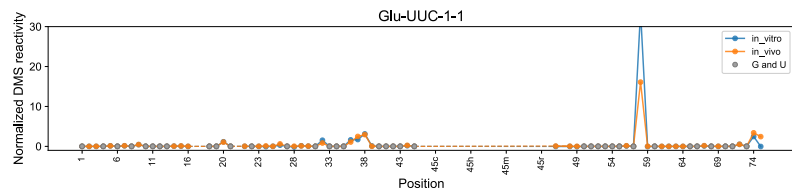
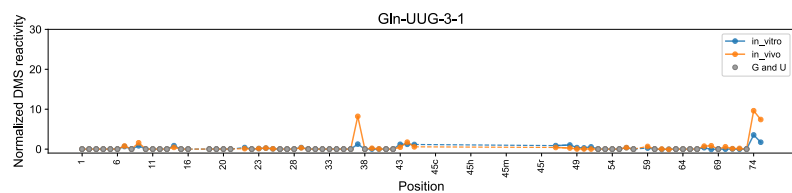


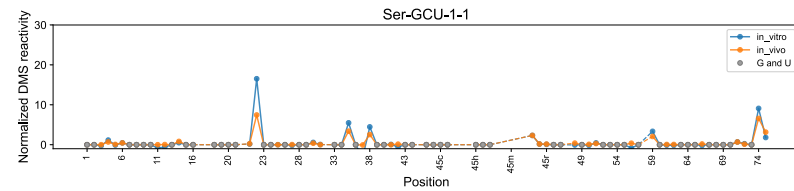
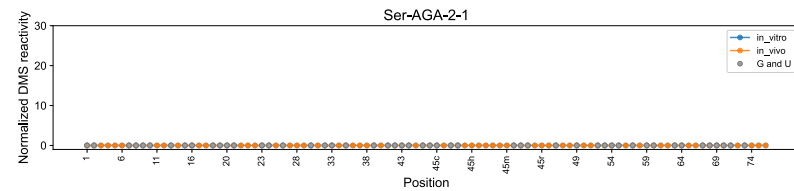
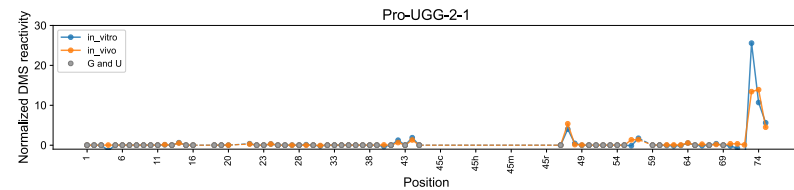
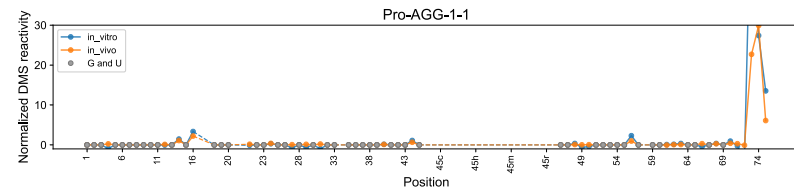
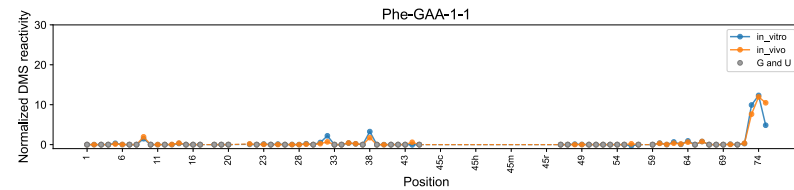
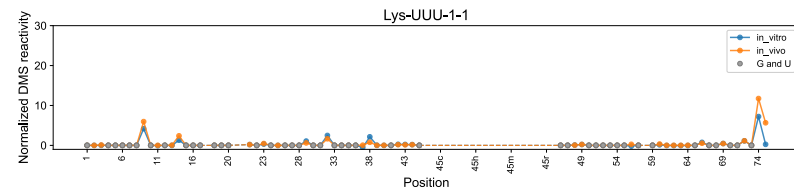
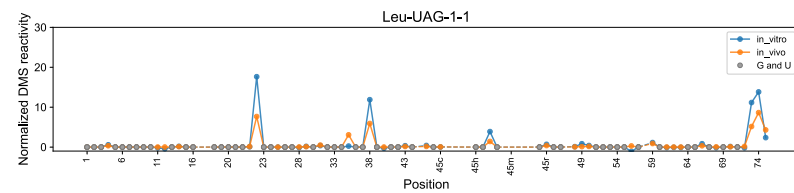
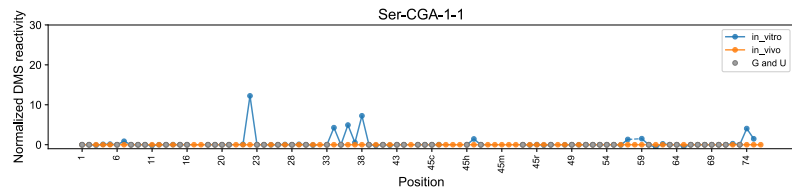
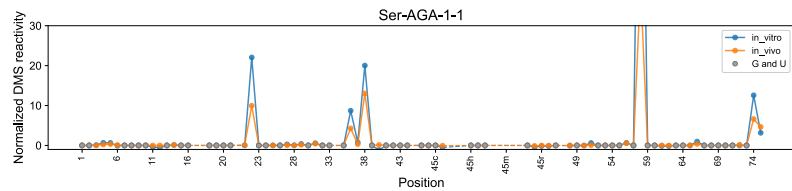
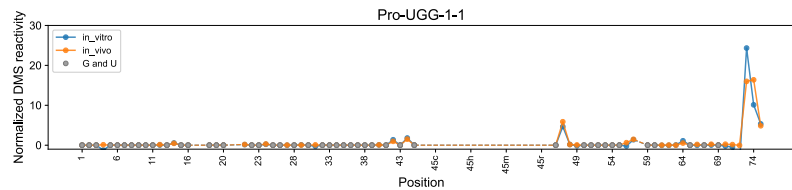
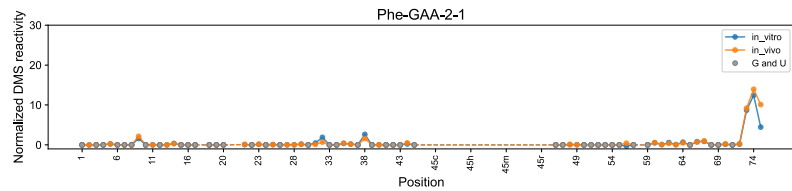
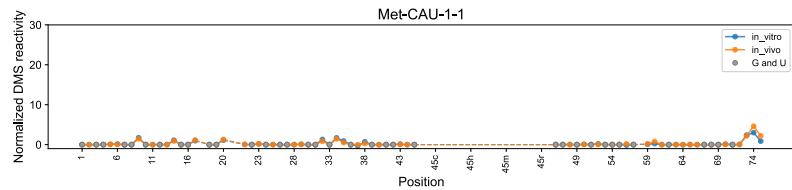
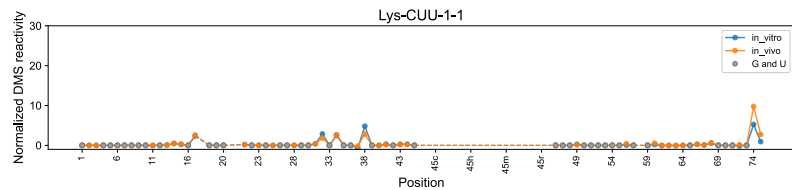
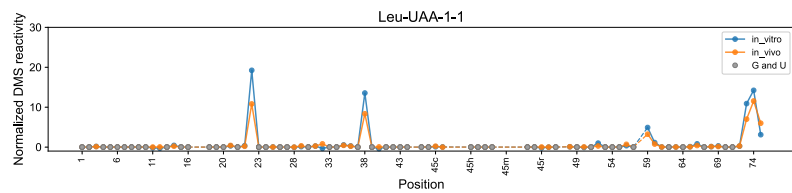
Supplemental Fig. 3 Marathon and Induro reverse transcriptases showed similar mutation specificities. (A – C) Mutation specificities at positions A and C were calculated from mutation profiling data generated by ShapeMapper2 for (A) *E. coli* BW25113 in vivo DMS condition where Marathon RTase was used for reverse transcription, (B) yeast BY4743 in vitro DMS condition, and (C) BY4743 in vivo DMS condition where Induro RTase was used for reverse transcription. The *E. coli* dataset was obtained from source data published in our previous research (Yamagami et al. 2022) and reanalyzed in this study.

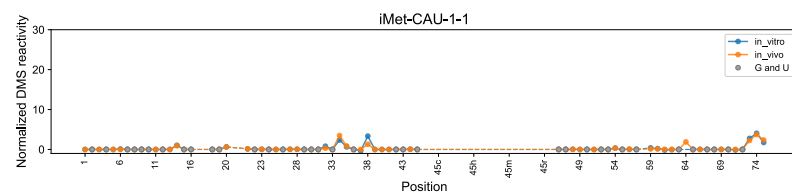
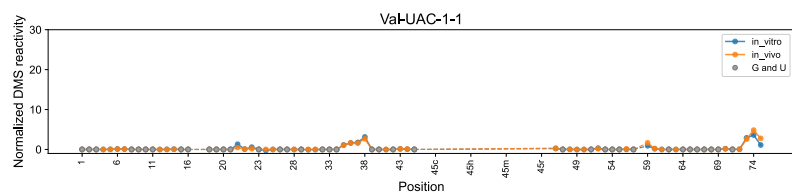
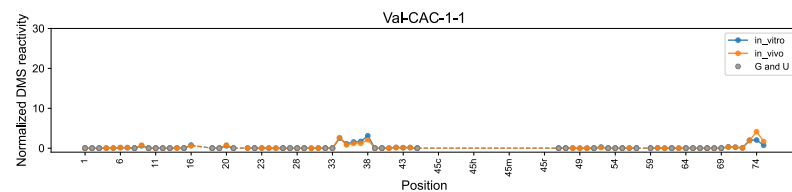
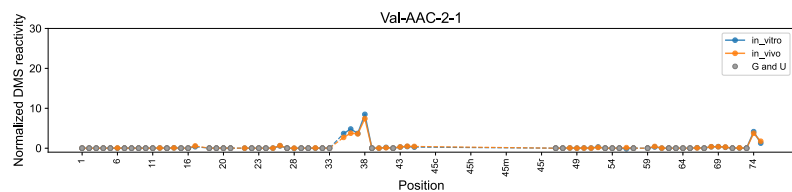
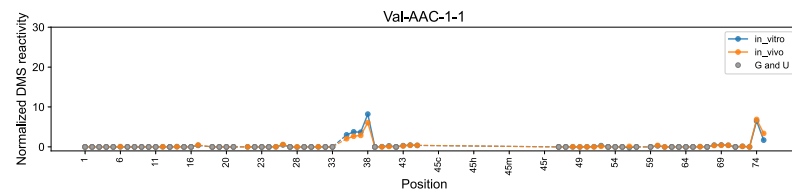
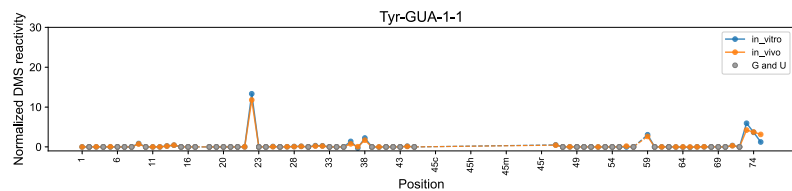
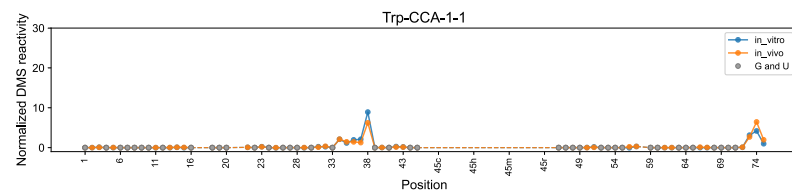
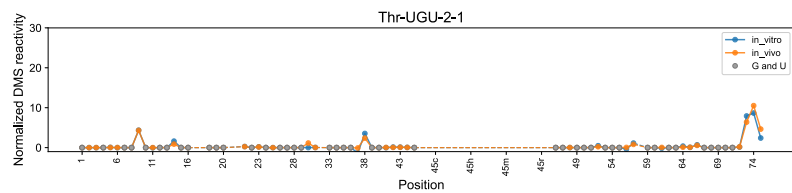
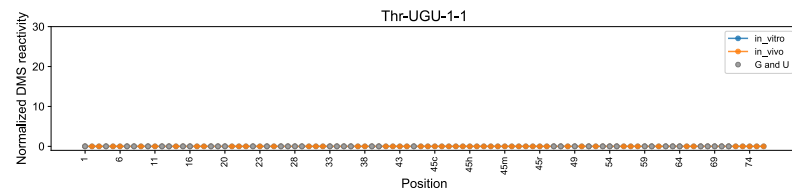
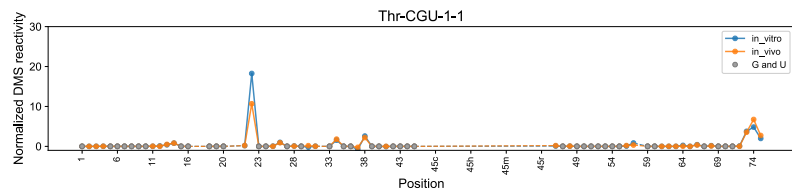
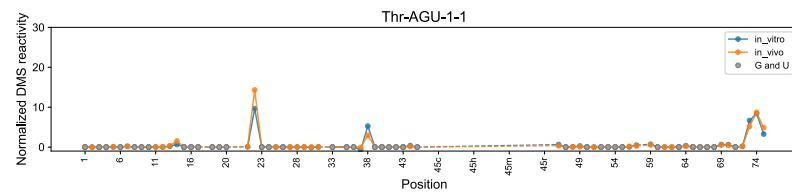
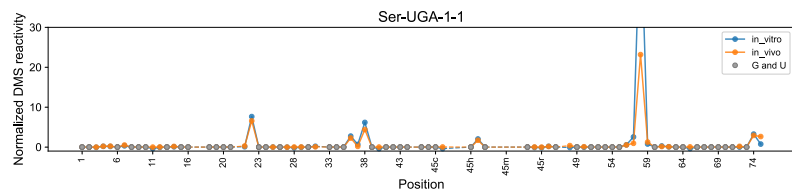


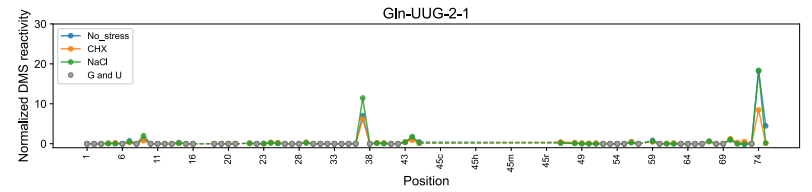
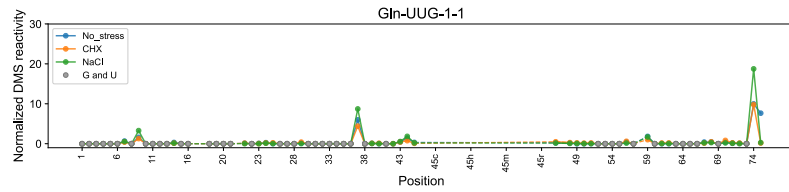
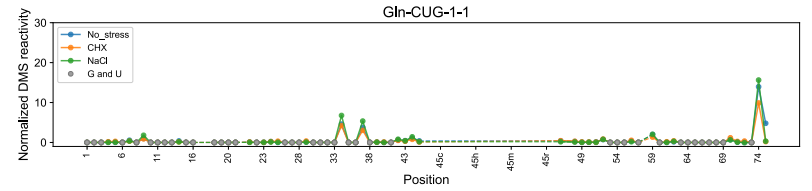
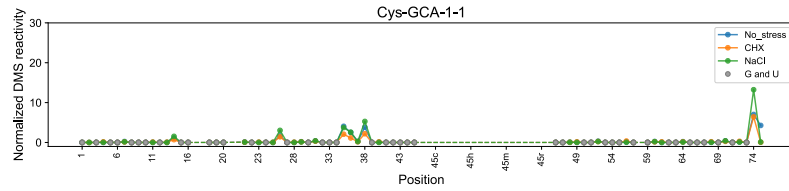
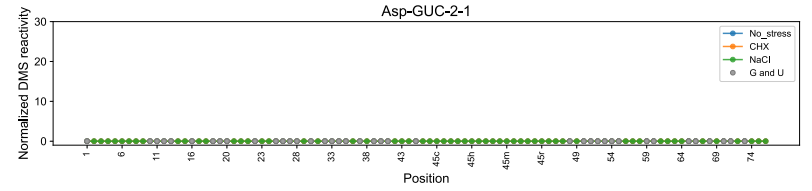
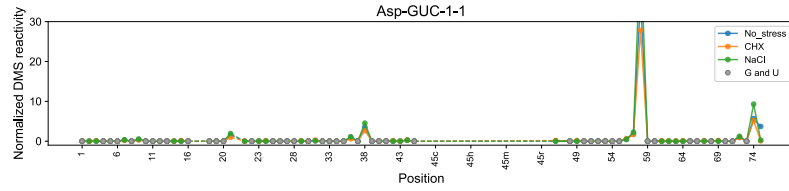
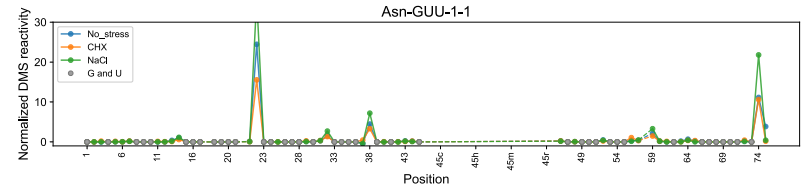
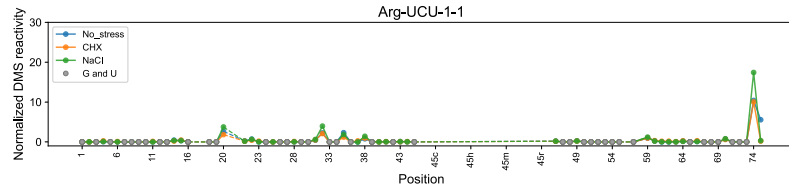
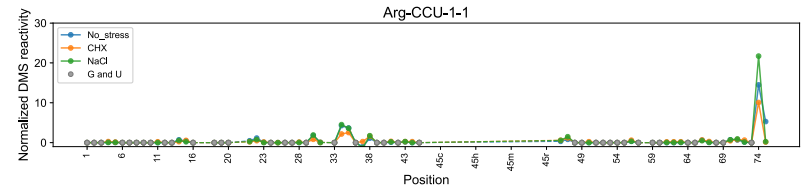
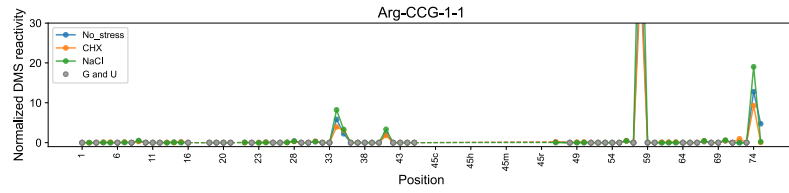
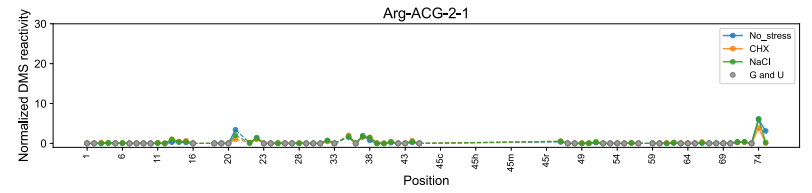
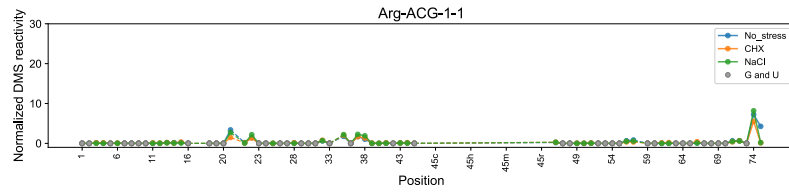
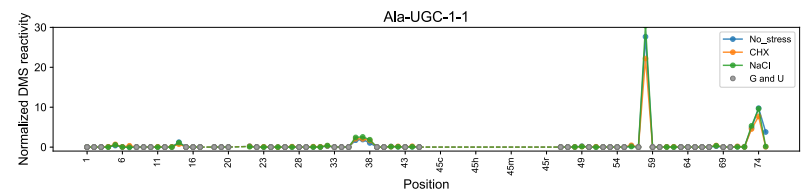
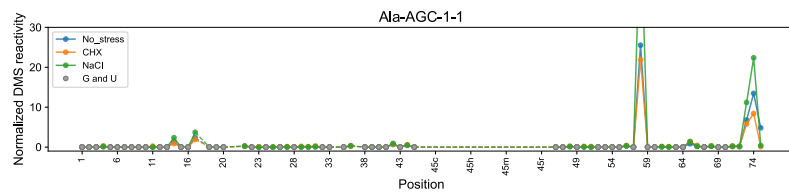
Supplemental Fig. 4 Global DMS profiling data from in vitro DMS condition are consistent with the canonical cloverleaf structure of tRNA. (A) DMS reactivity profiles obtained from in vitro DMS condition were visualized for each tRNA species. Three successive putative native modifications identified by ShapeMapper2 were excluded from the plot, as three adjacent modifications are rarely observed in the Modomics database. The last nucleotide at position 76 is also excluded from the plot, as the nucleotide was not detectable in our MaP analysis. (B) The reactivities are plotted by tRNA structure region where the red line indicates the mean reactivity for each region.

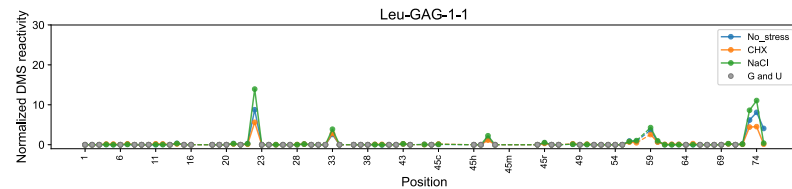
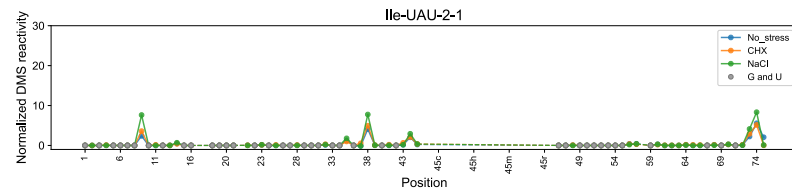
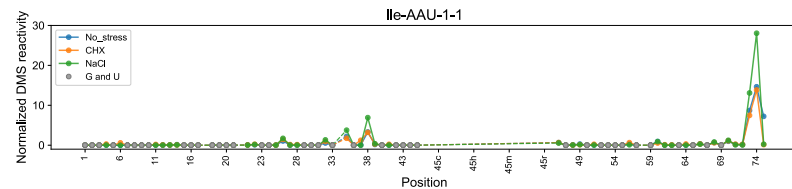
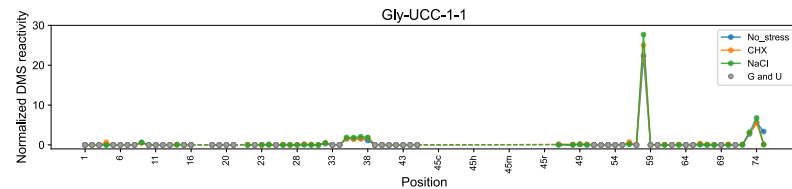
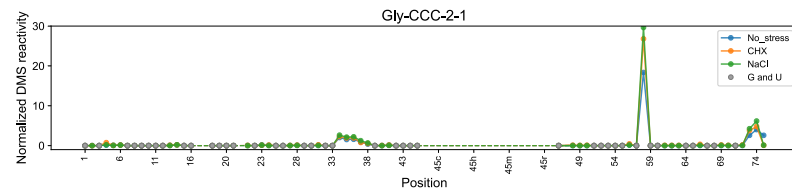
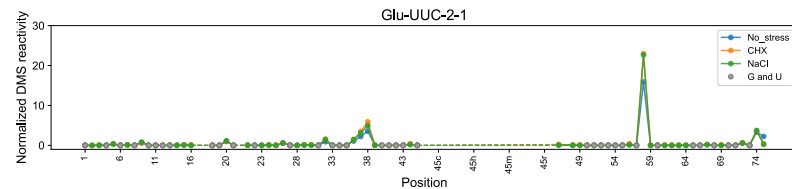
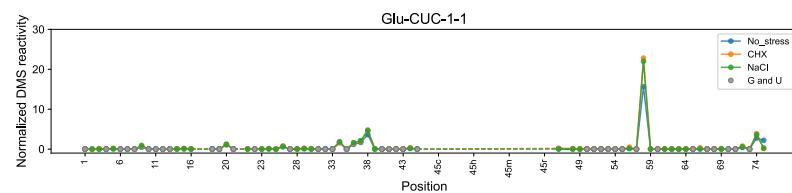
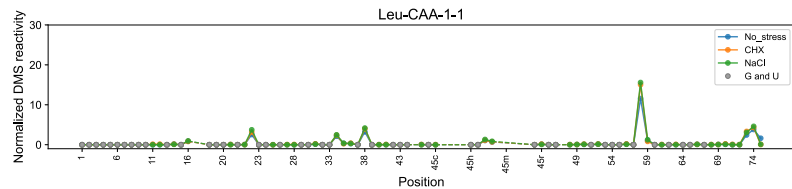
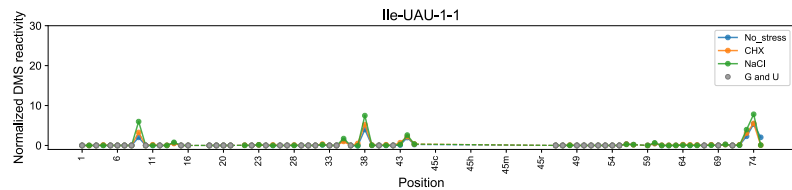
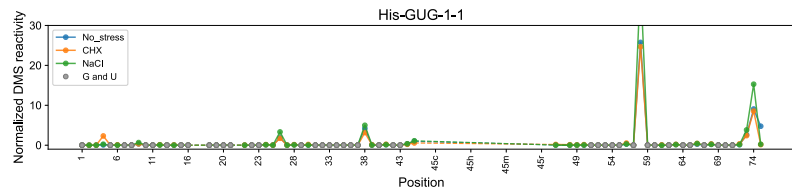
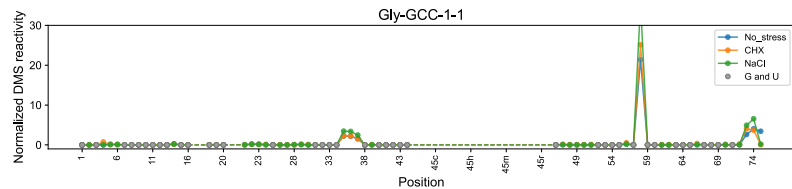
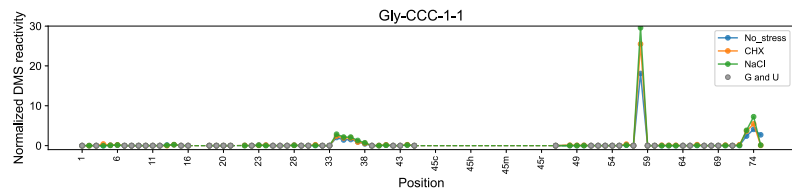
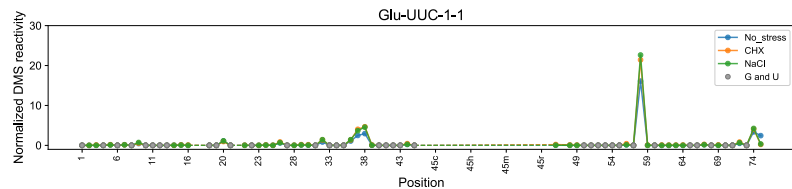
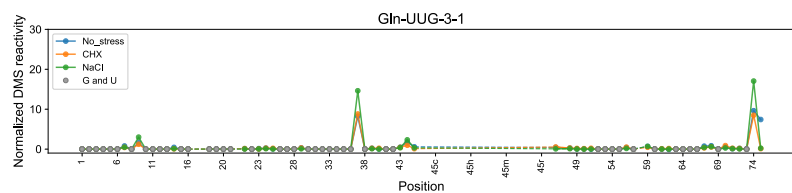


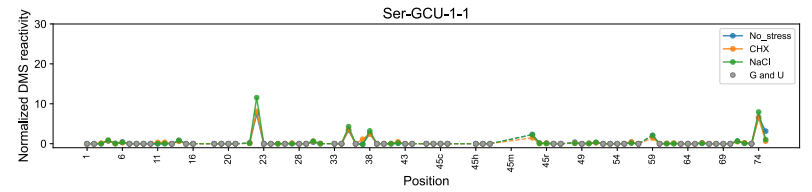
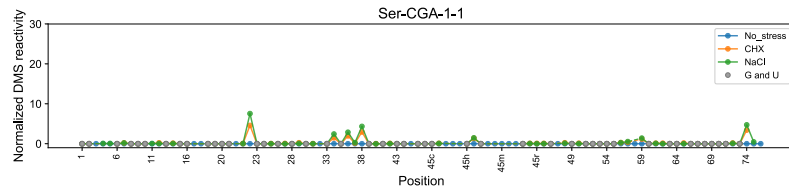
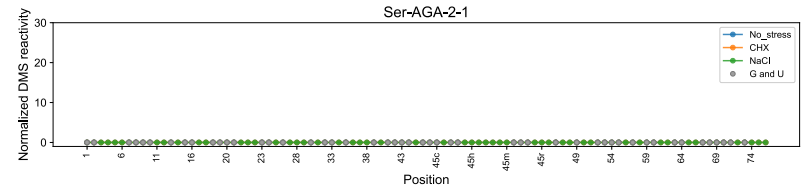
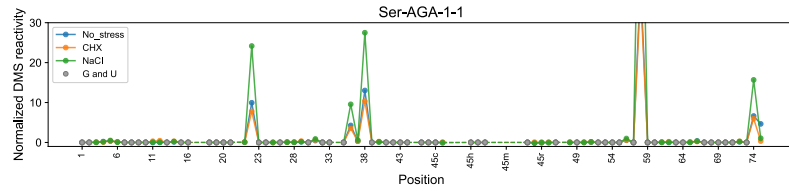
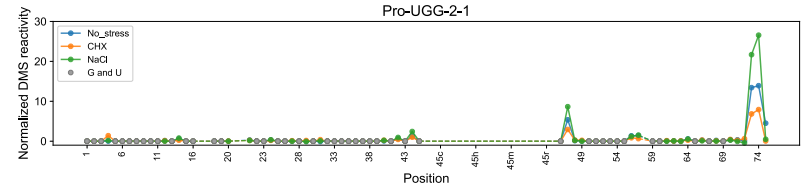
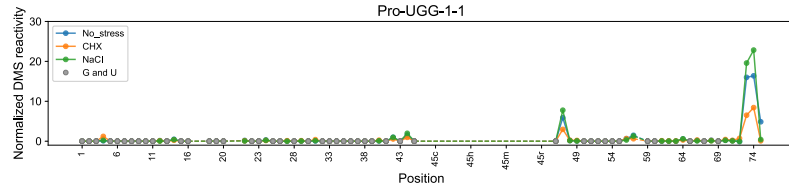
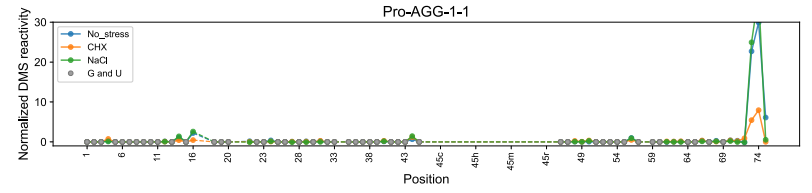
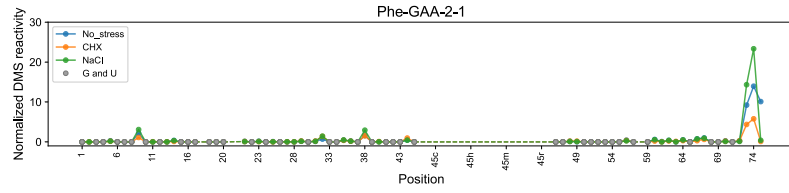
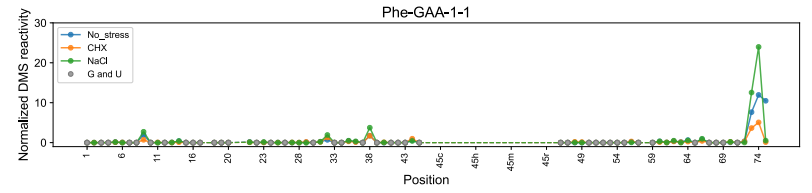
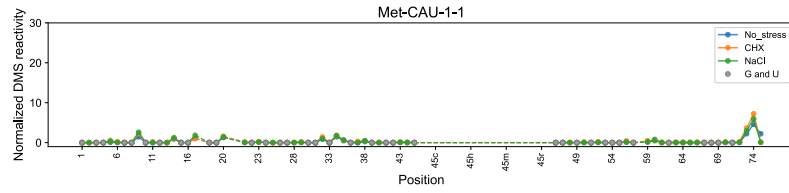
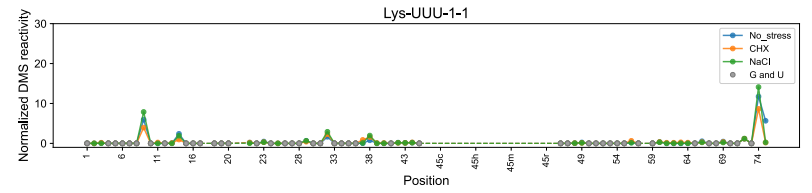
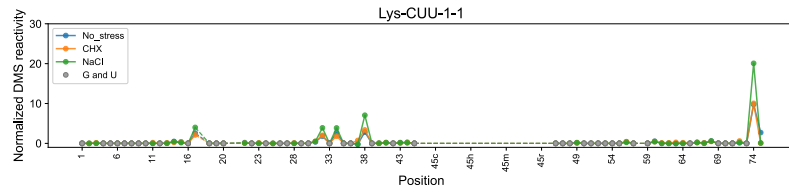
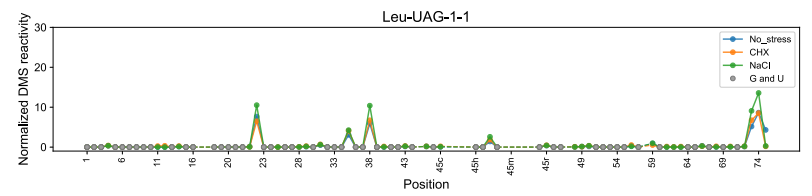
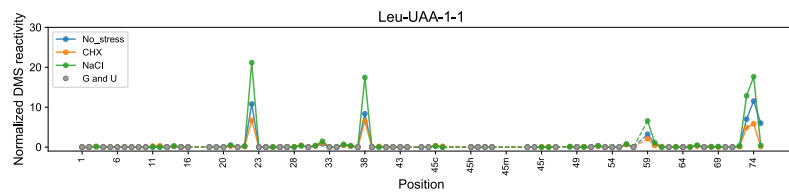


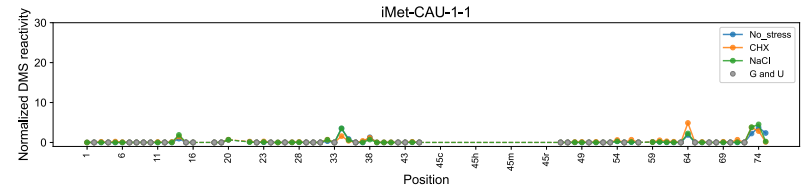
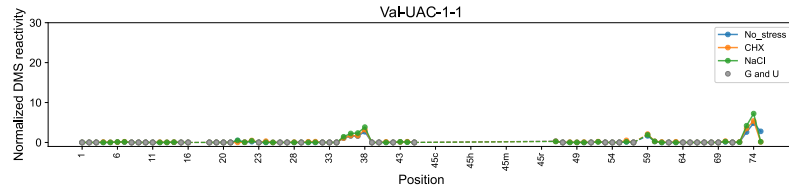
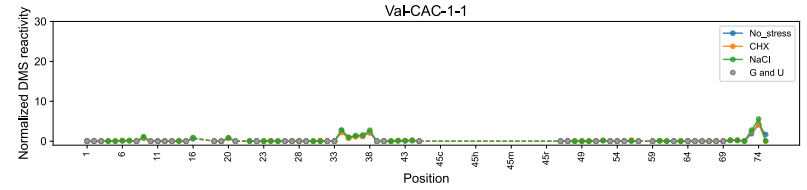
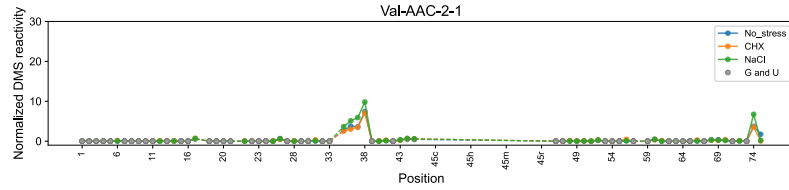
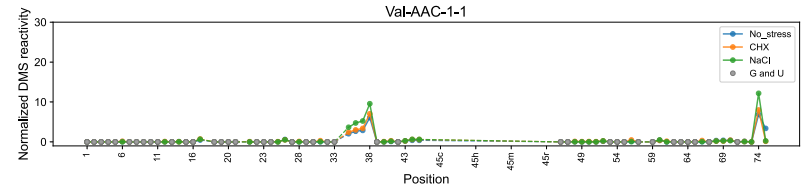
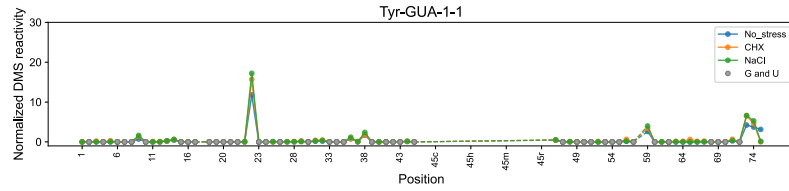
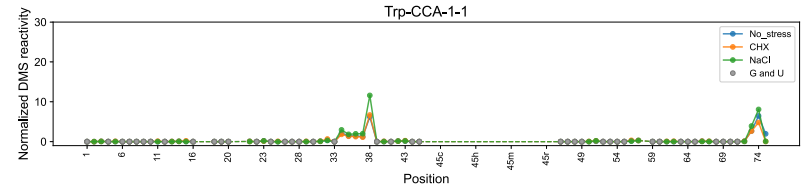
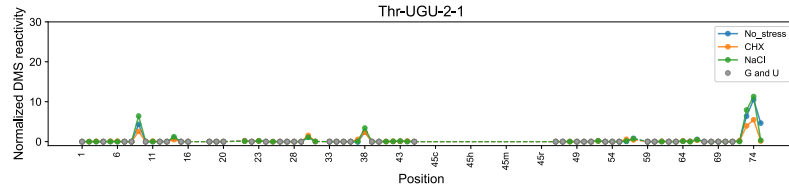
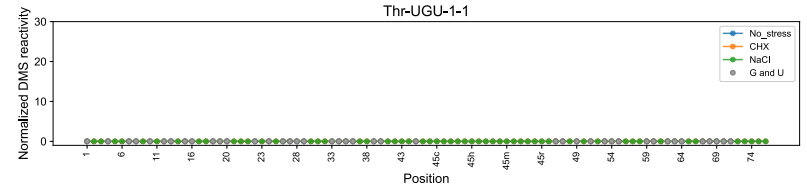
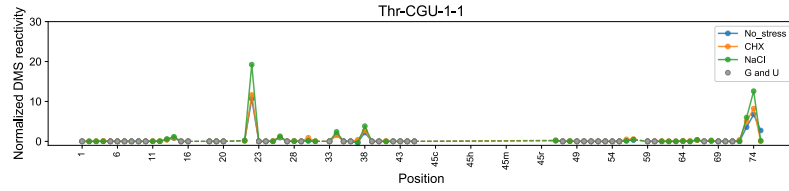
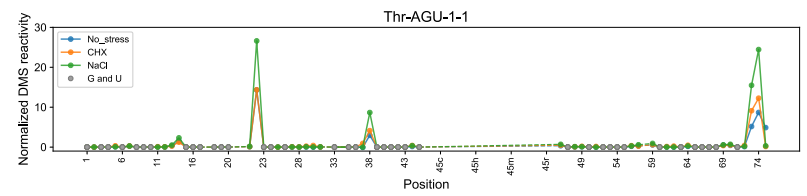
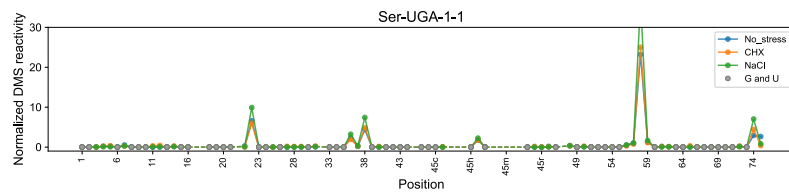


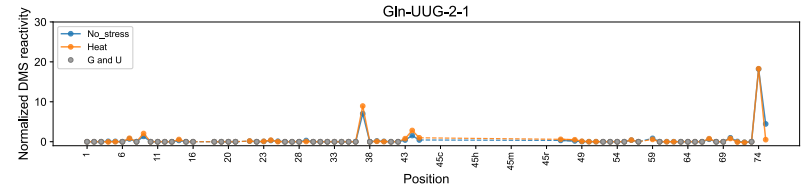
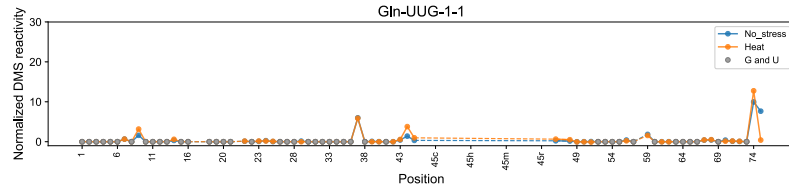
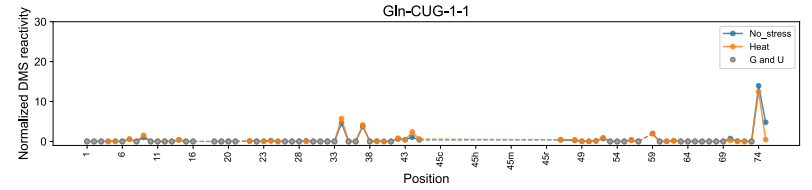
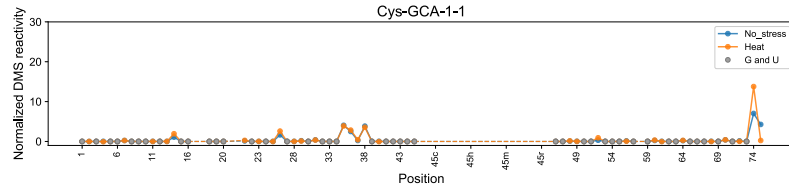
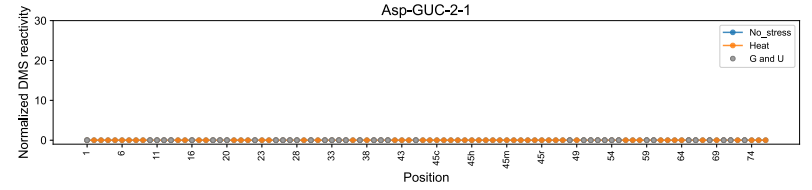
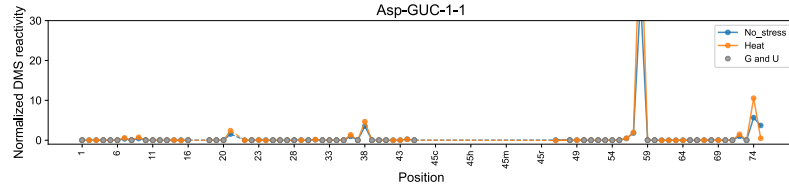
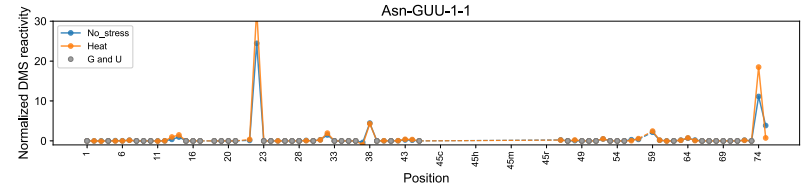
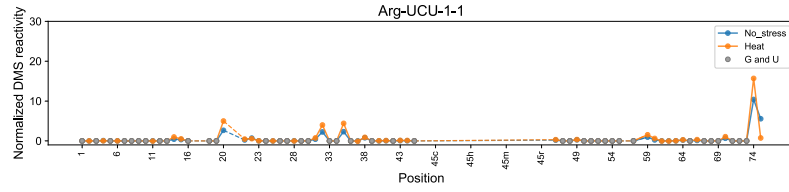
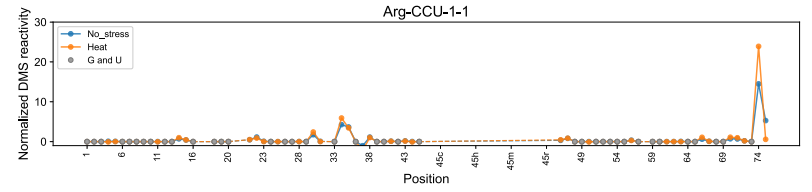
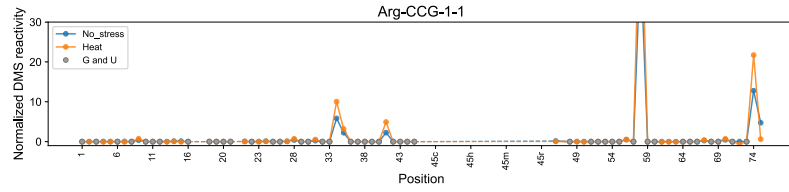
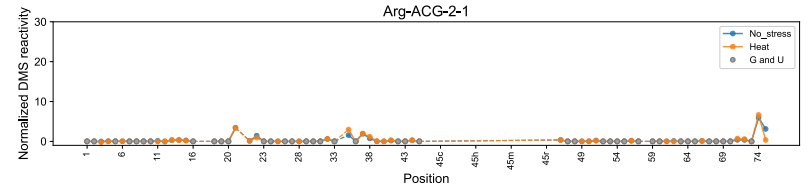
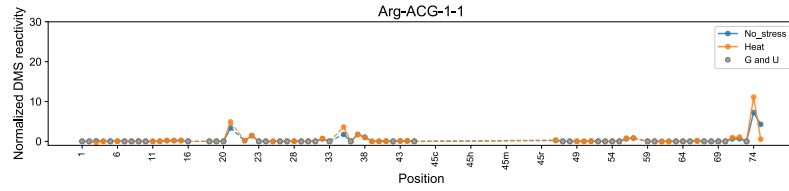
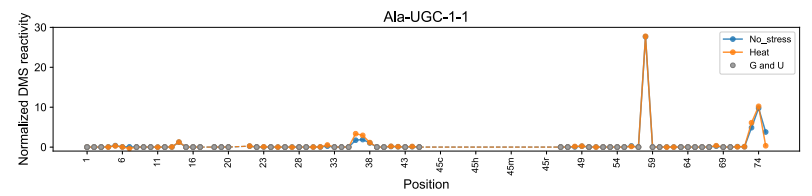
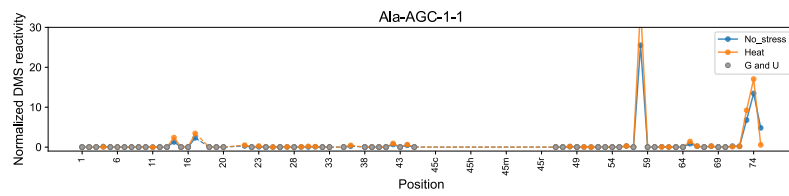


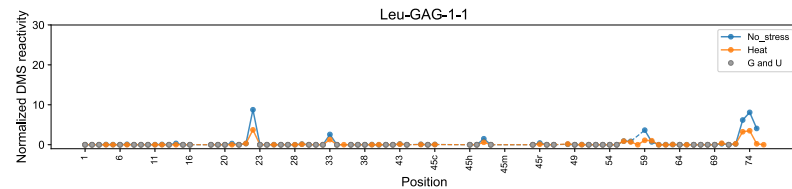
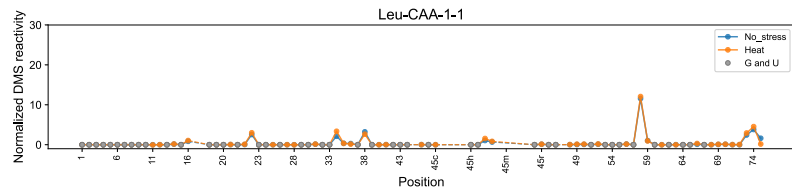
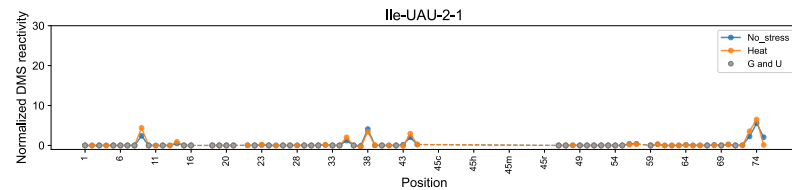
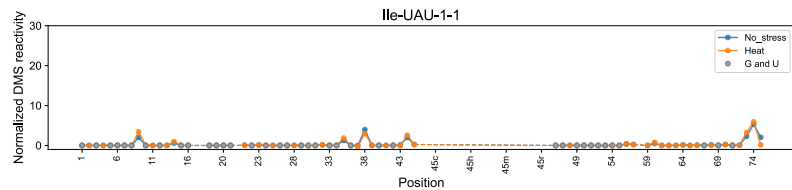
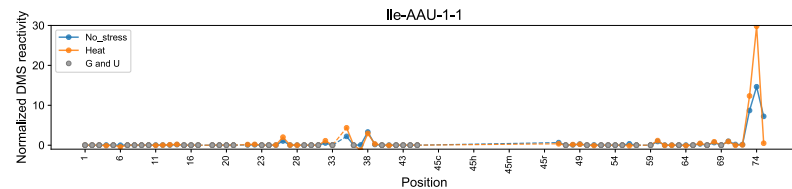
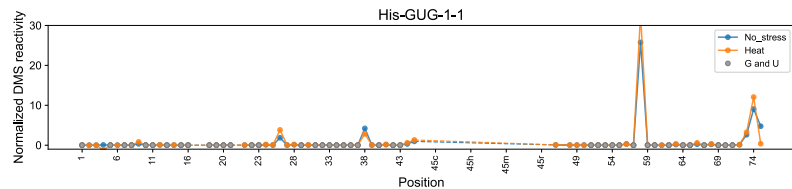
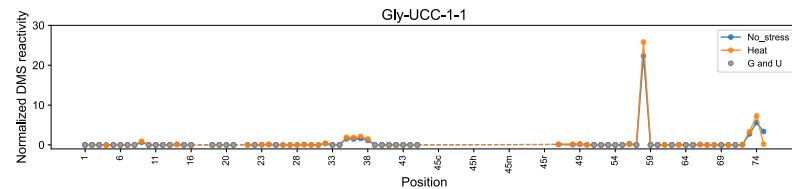
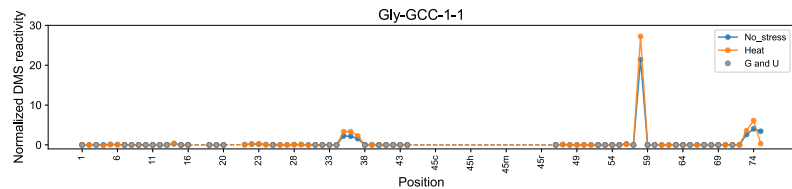
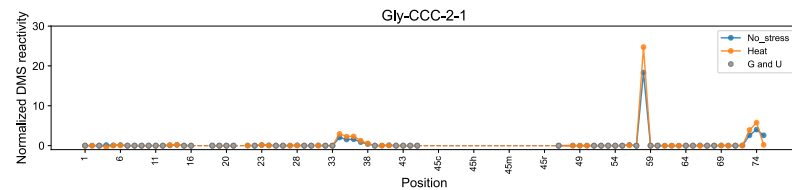
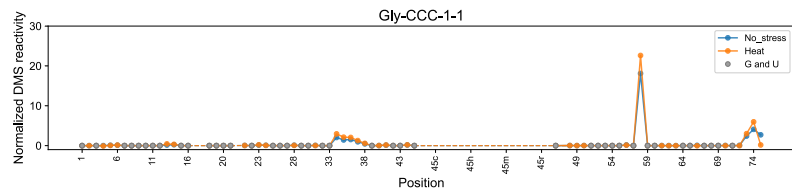
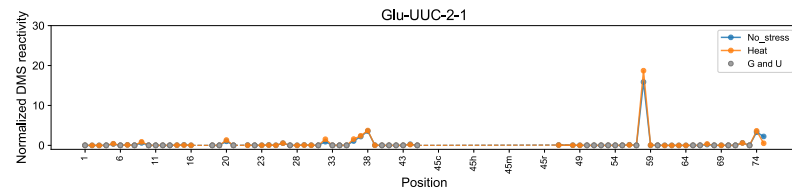
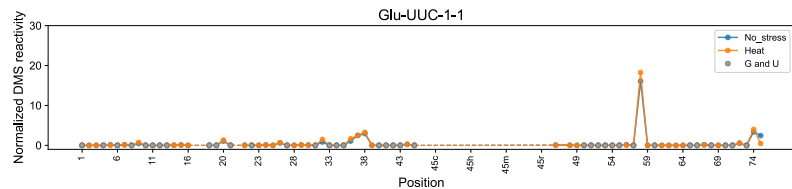
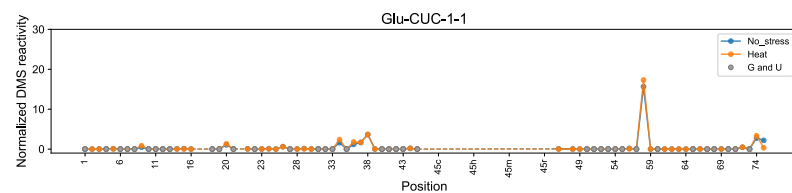
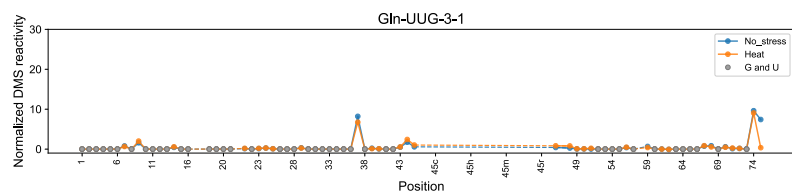


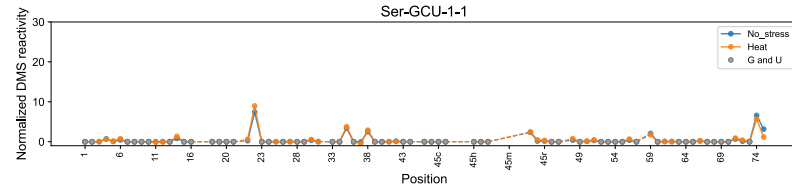
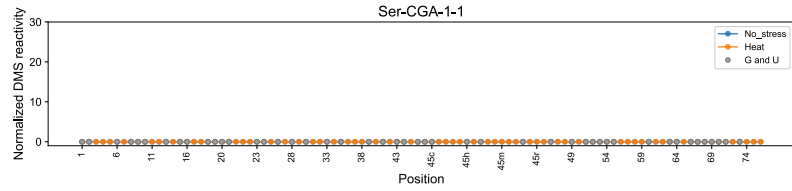
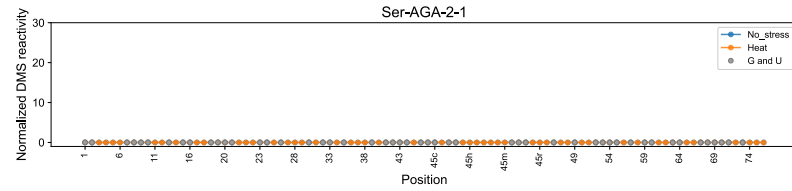
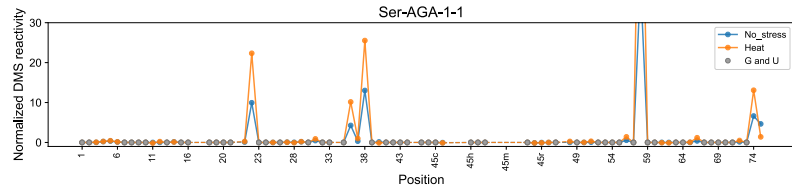
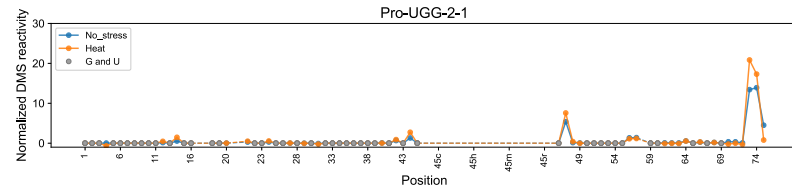
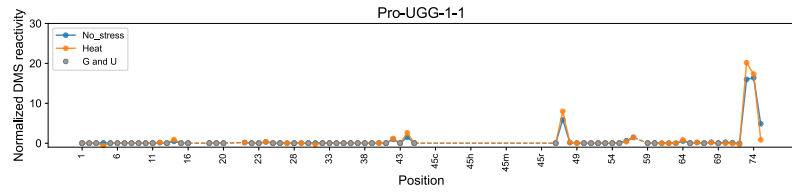
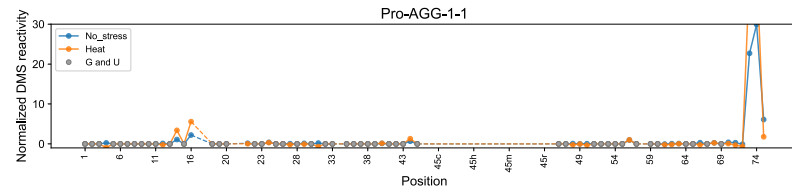
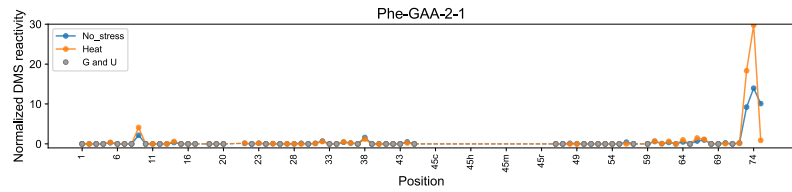
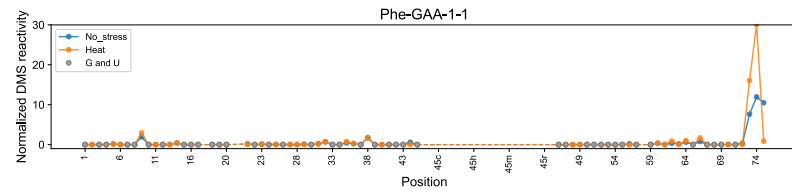
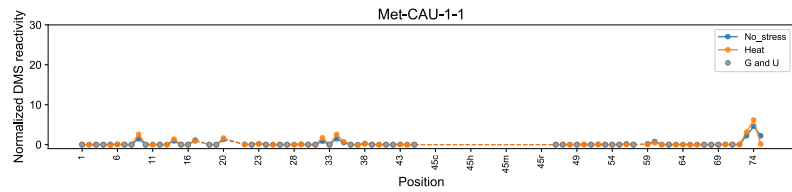
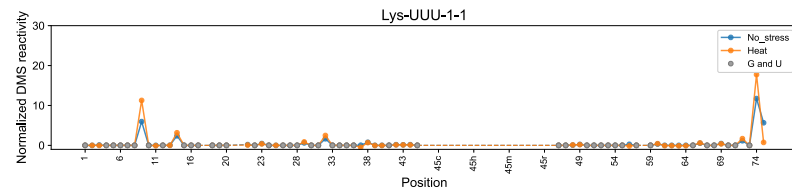
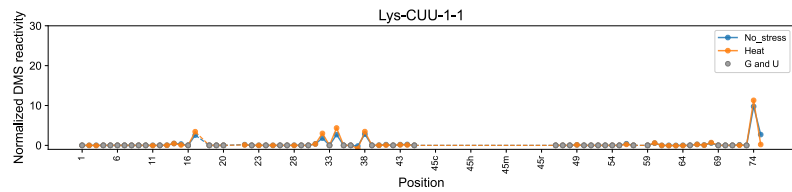
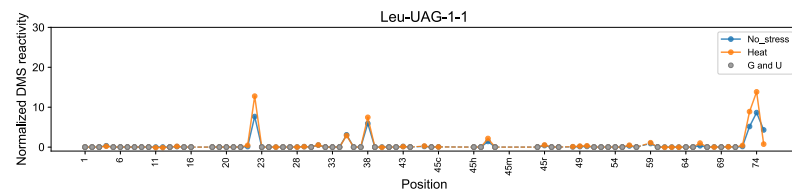
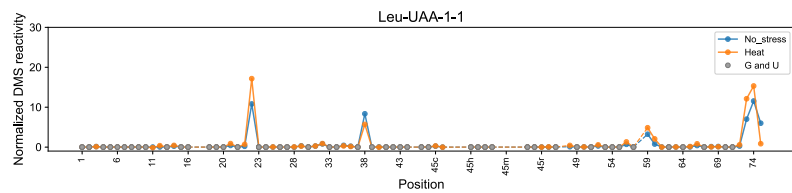


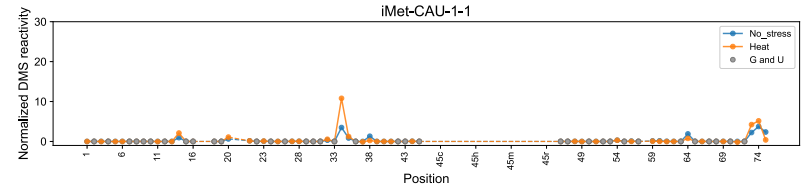
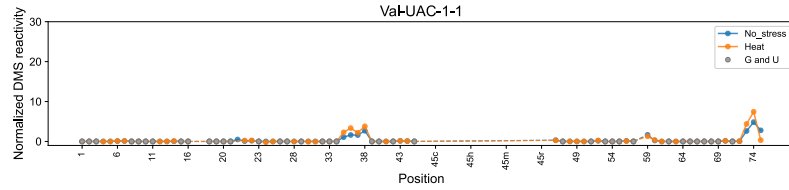
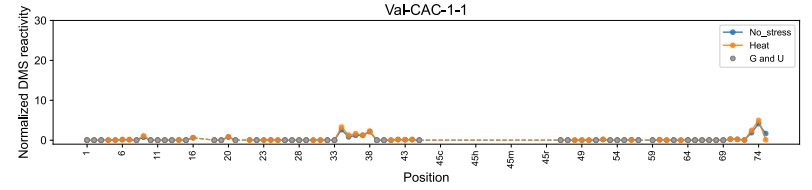
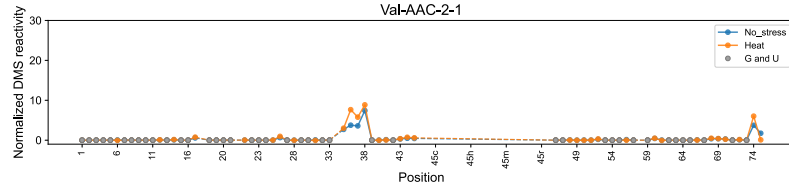
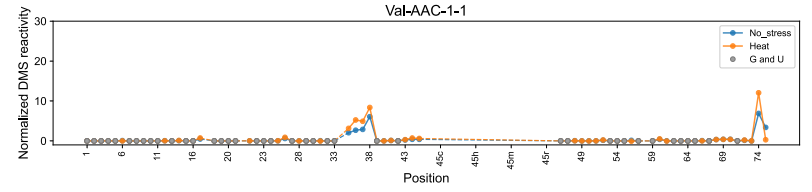
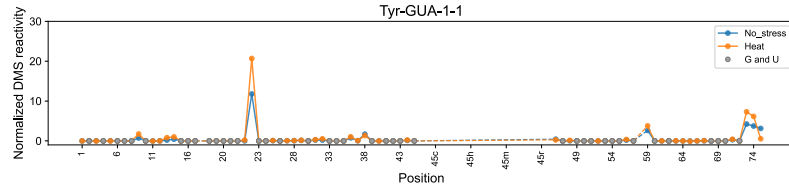
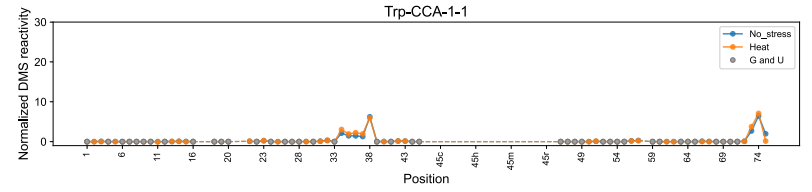
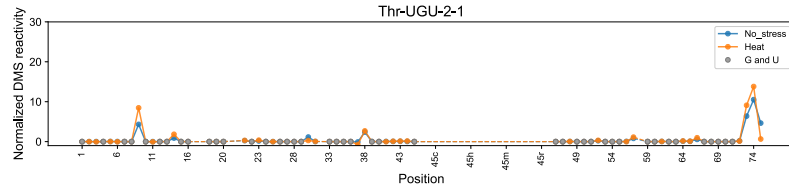
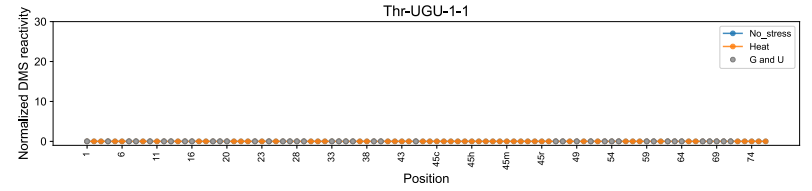
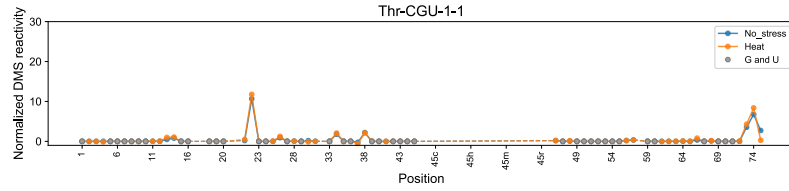
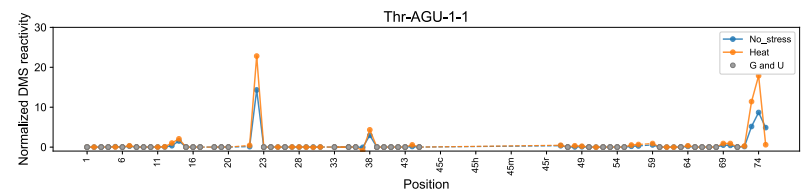
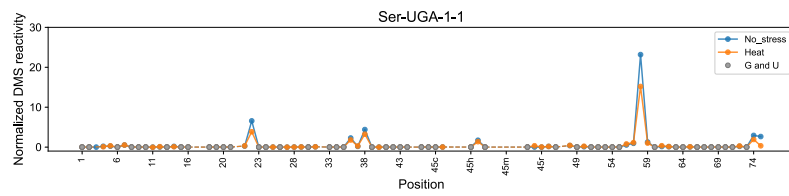




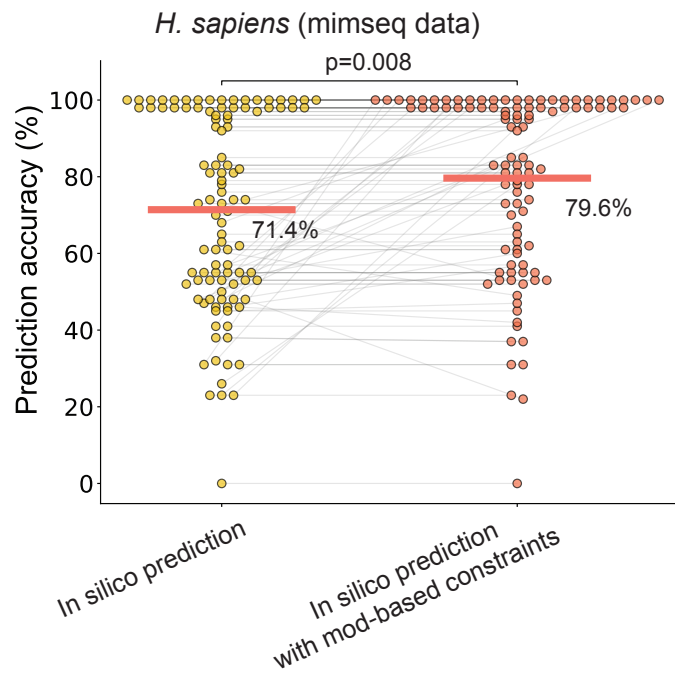




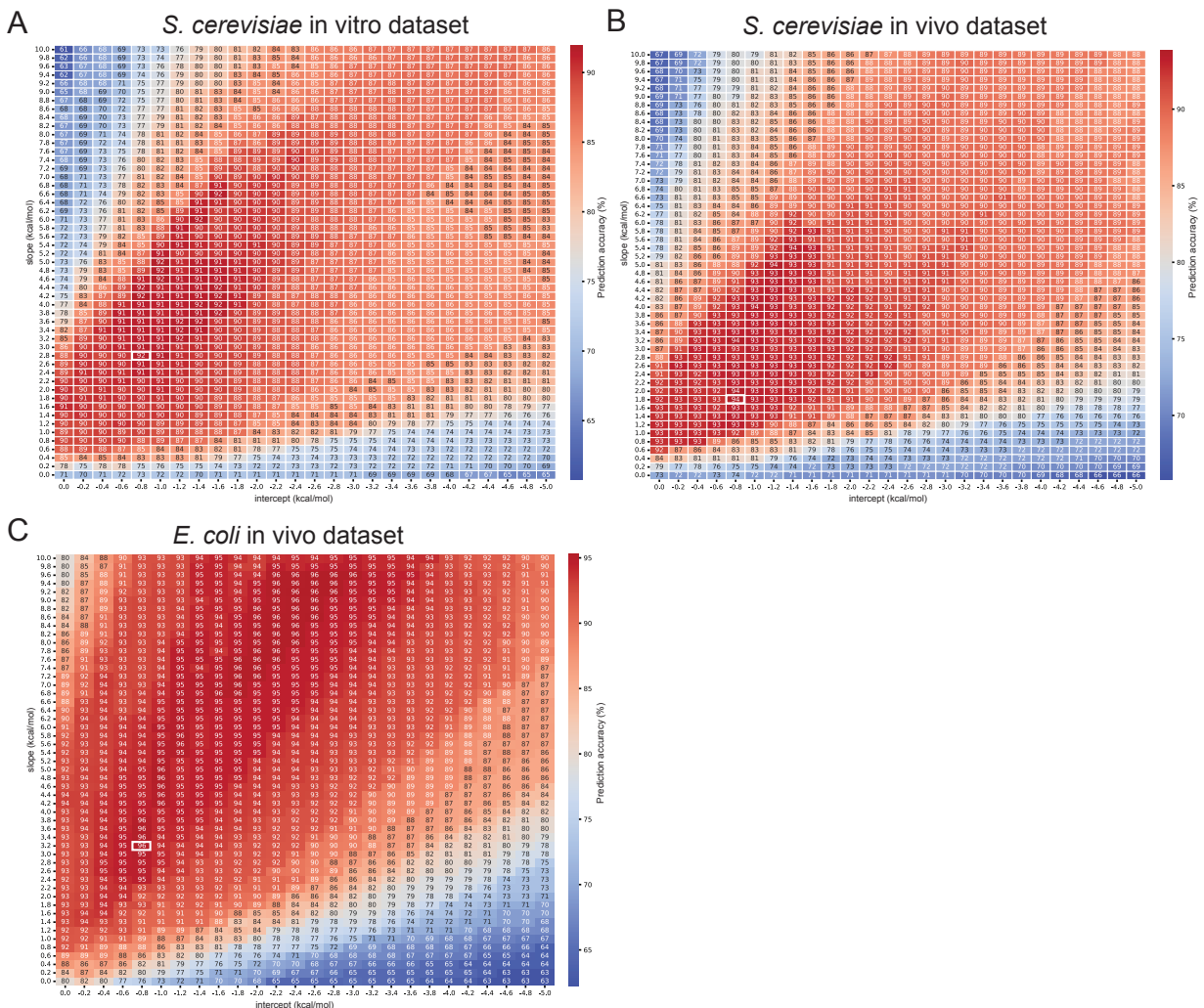




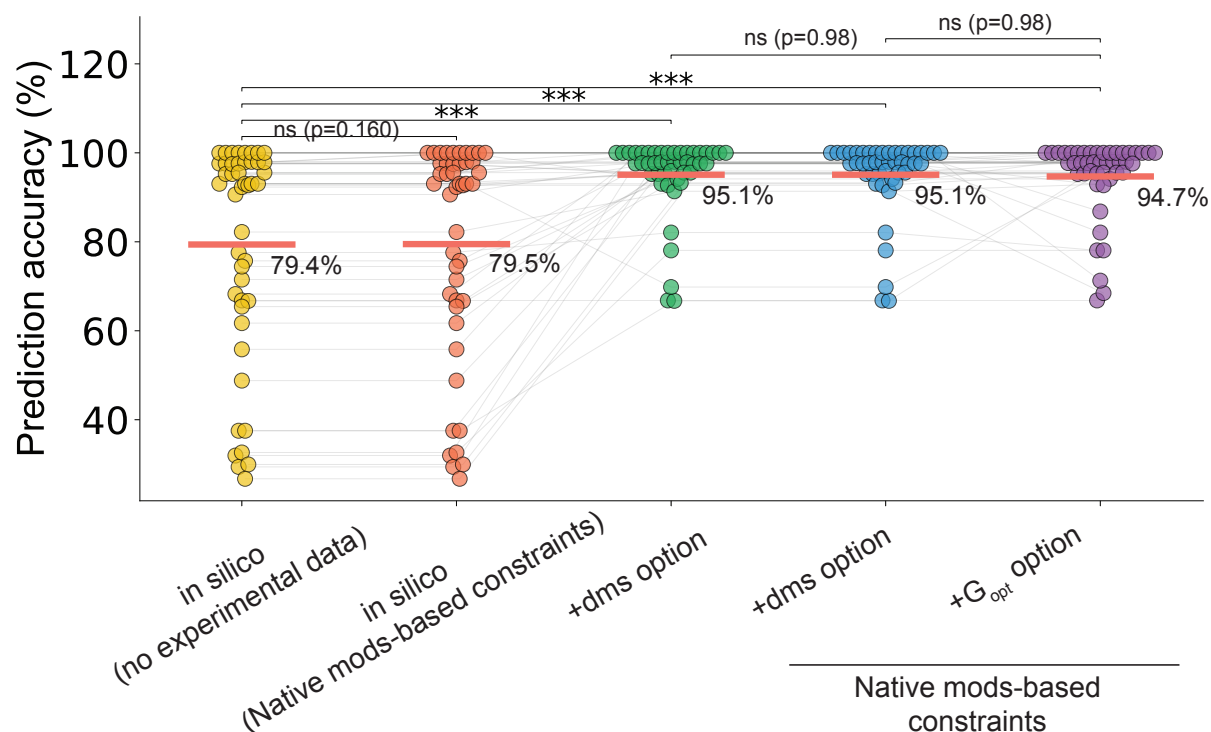
Supplemental Fig. 5 DMS reactivity patterns for each tRNA species. DMS reactivity patterns for each tRNA species under in vitro, in vivo, and stress conditions were provided. Dash lines indicate no nucleotides or DMS reactivity is not available. Grey circles show G/U positions in tRNA. We follow the numbering of Sprinzl found in tRNA alignment in Supplemental File 2.



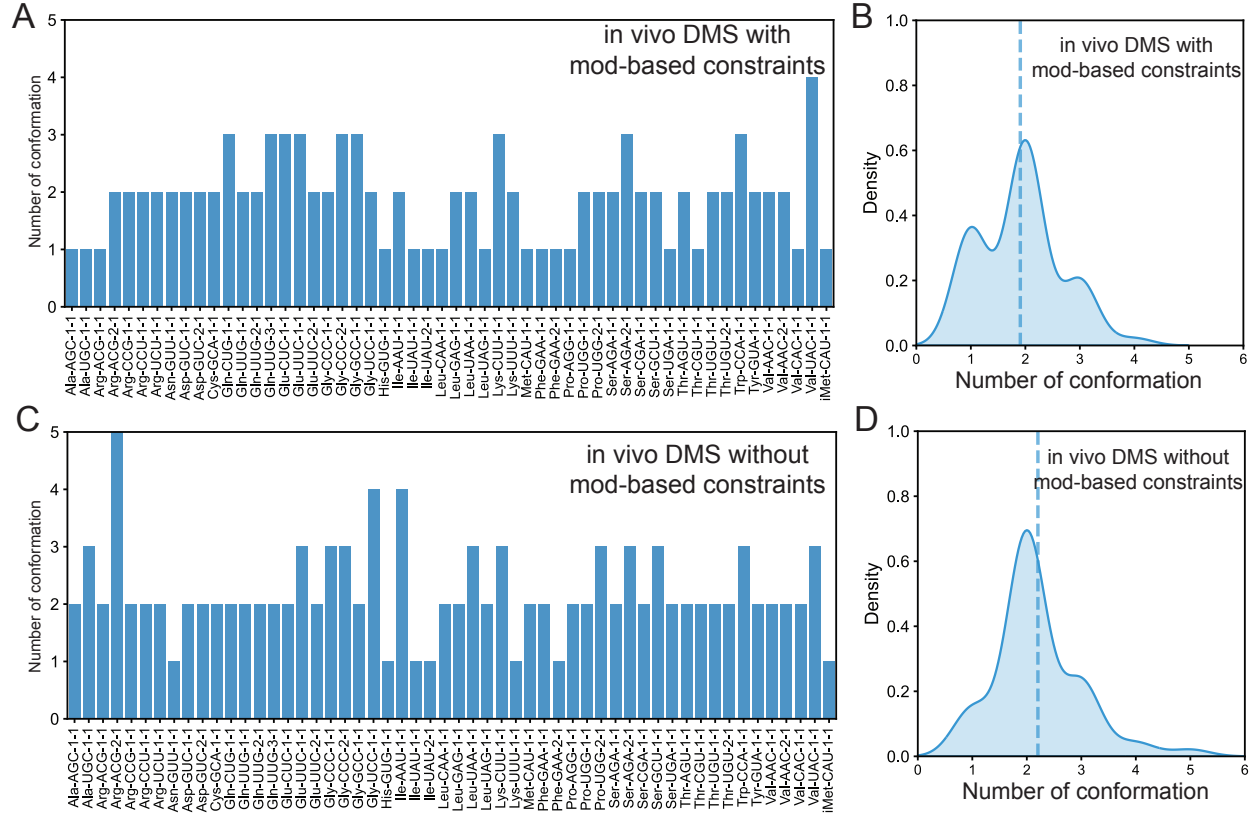
Supplemental Fig. 6 Native modification-based constraints significantly improve prediction accuracy of tRNA secondary structure. Structure prediction was performed with/without the native modification-based constraints for *H. sapiens* using published mimseq data (Behrens et al. 2021). The red line indicates the mean value for prediction accuracy. Statistical significance was calculated using Wilcoxon signed-rank test.



Supplemental Fig. 7 Optimization of pseudo-energy parameters was performed for *S. cerevisiae* and *E. coli* tRNAs. (A–C) Pseudo-free energy parameters were systematically varied, and secondary structure prediction was performed for each parameter set. The parameters can be specified with the SHAPE option in RNAstructure Fold. Prediction accuracies for (A) *S. cerevisiae* in vitro DMS condition, (B) *S. cerevisiae* in vivo DMS condition, and (C) *E. coli* in vivo DMS condition, are shown as a heatmap. White boxes indicate parameter sets that yielded the highest prediction accuracy. The *E. coli* dataset was obtained from our previous report (Yamagami et al. 2022).

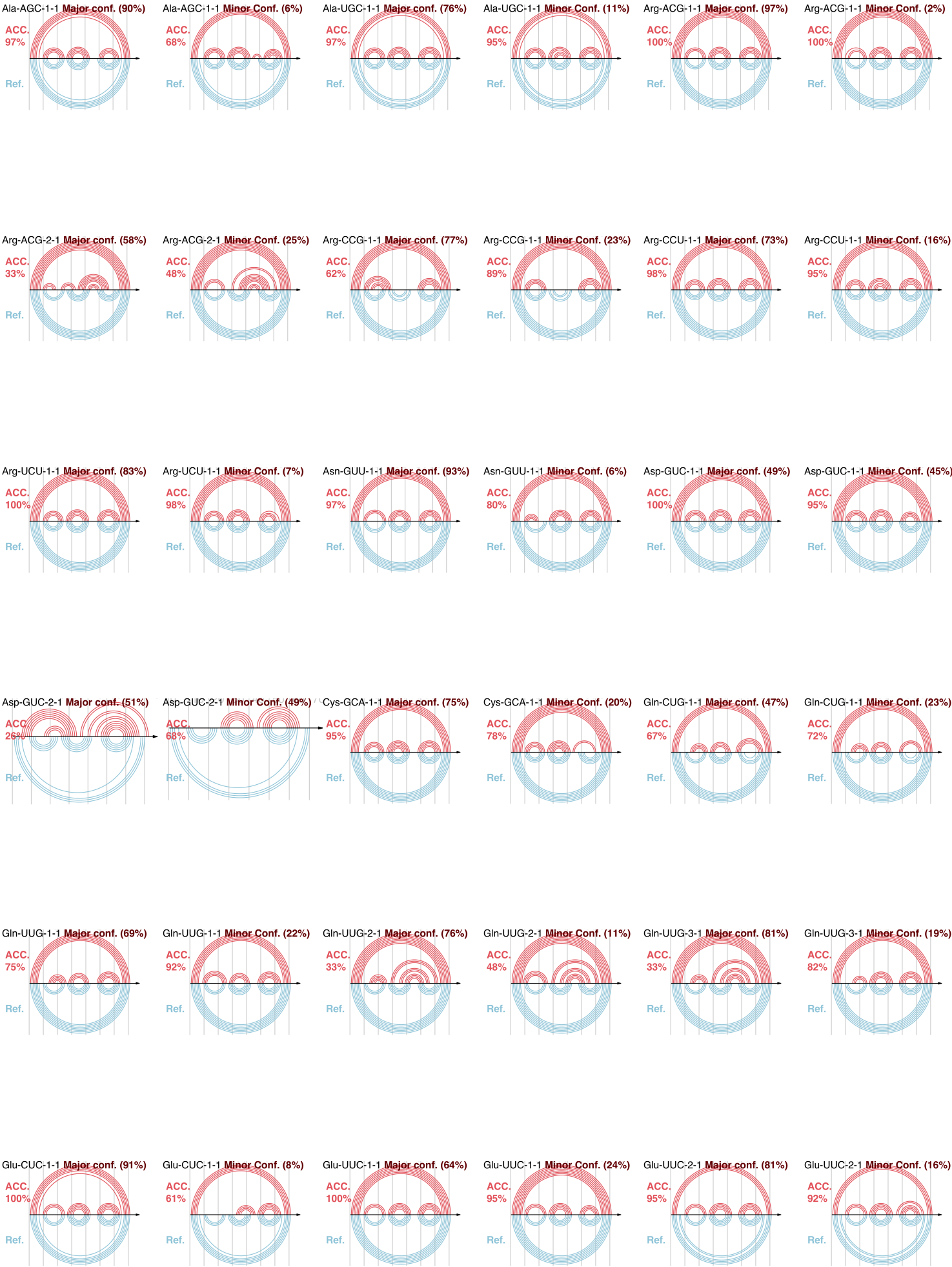


Supplemental Fig. 8 Comparison of secondary structure prediction of tRNAs using in vivo DMS dataset from *E. coli* BW25113 strain. Prediction accuracy was compared across different conditions: in silico prediction without experimental data, prediction with native modification–based constraints, prediction using the dms option, prediction using the dms option combined with native modification–based constraints, and prediction using the G_{opt} option with optimized parameters and native modification–based constraints. The red line indicates the mean prediction accuracy for each condition. tRNA species lacking DMS data were excluded from the analysis. Statistical significance was calculated using Wilcoxon signed-rank test. Asterisk (***) indicates $p < 0.0001$.

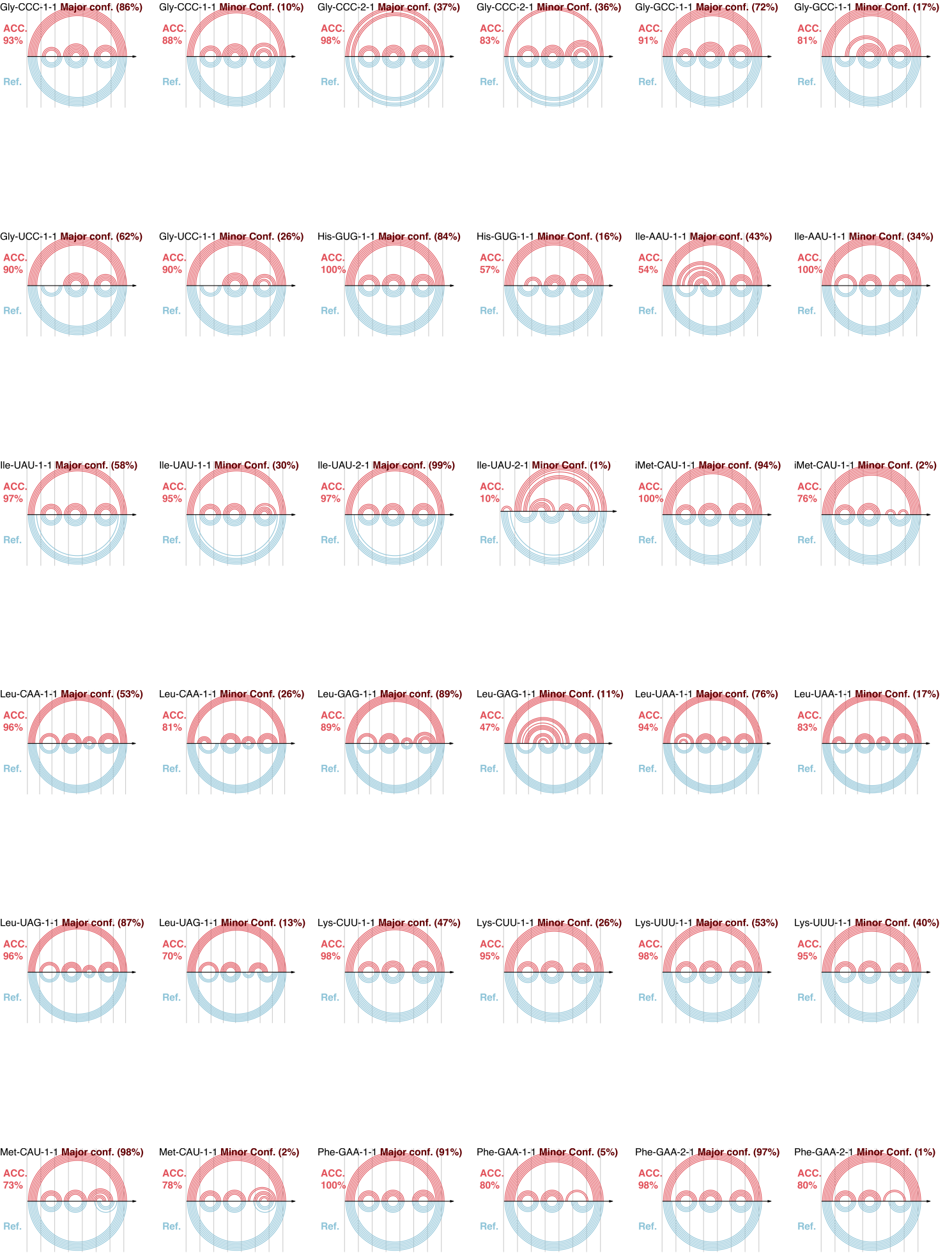


Supplemental Fig. 9 native modification–based constraints increase structural homogeneity of yeast tRNAs. (A–D) Bar plots and histograms show the number of predicted conformations with probabilities greater than 10% for each tRNA species under (A, B) in vivo DMS conditions with modification–based constraints and (C, D) in vivo DMS conditions without native modification–based constraints. The dashed line in each histogram indicates the mean number of conformations across tRNA species.

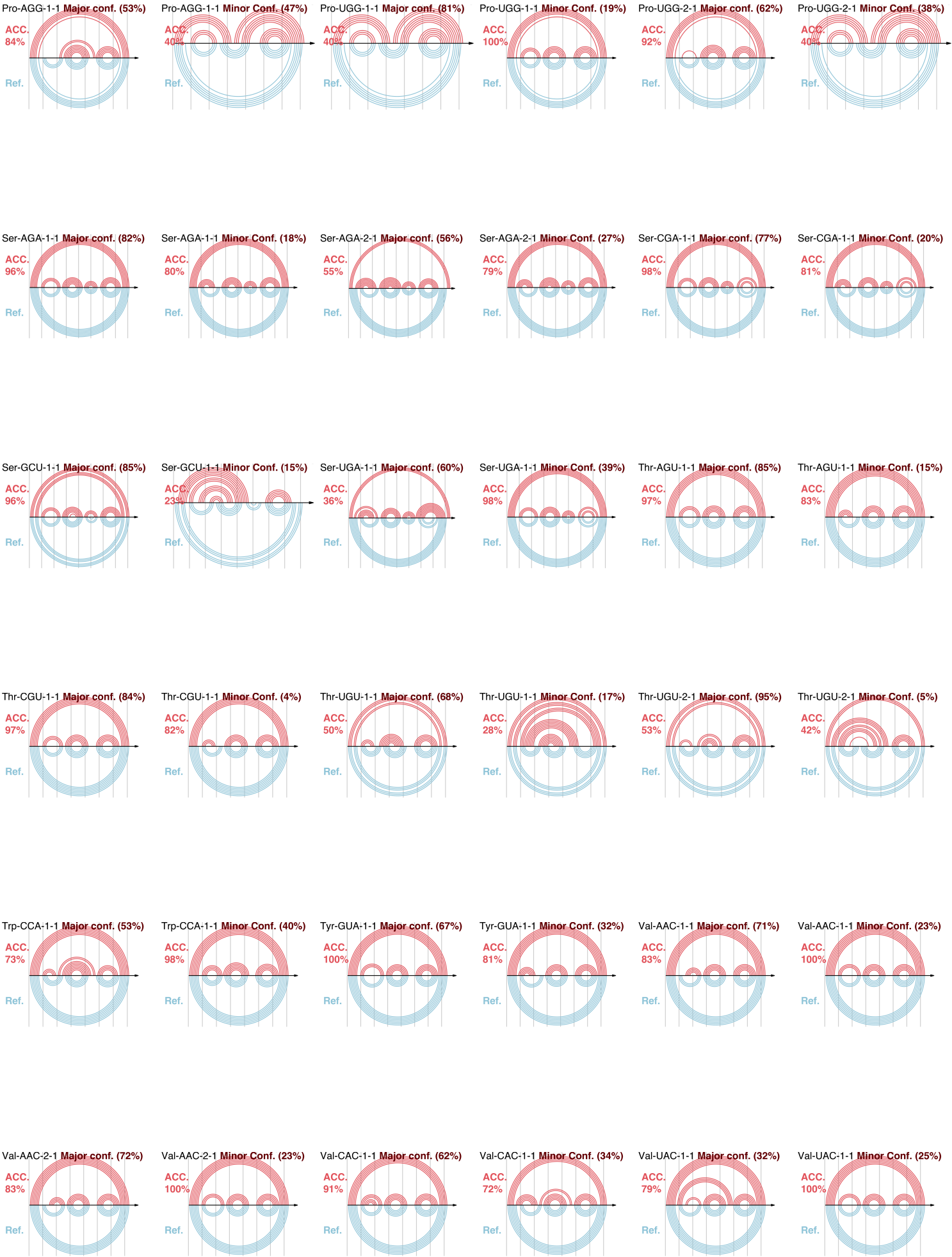
In vitro DMS condition



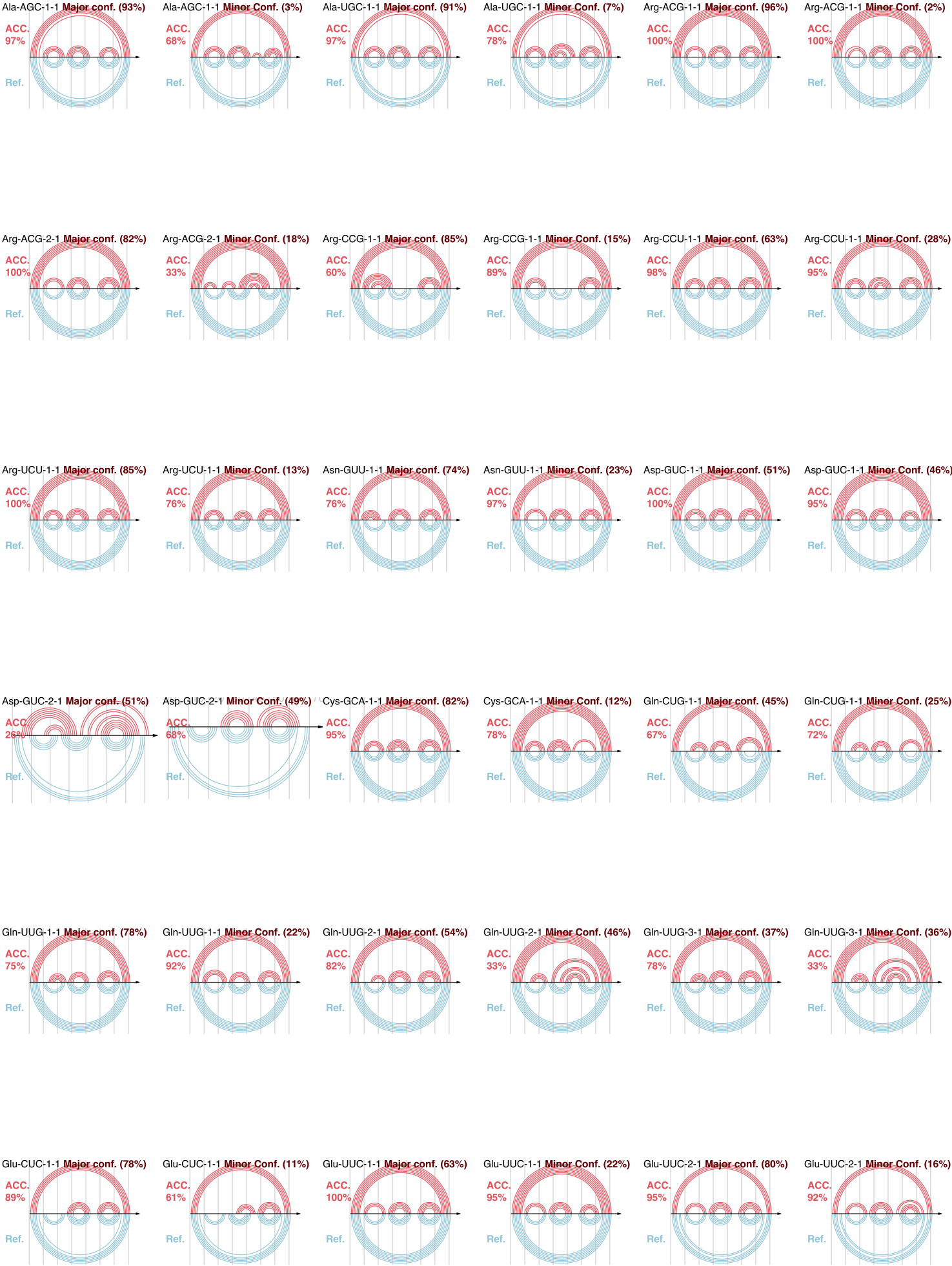
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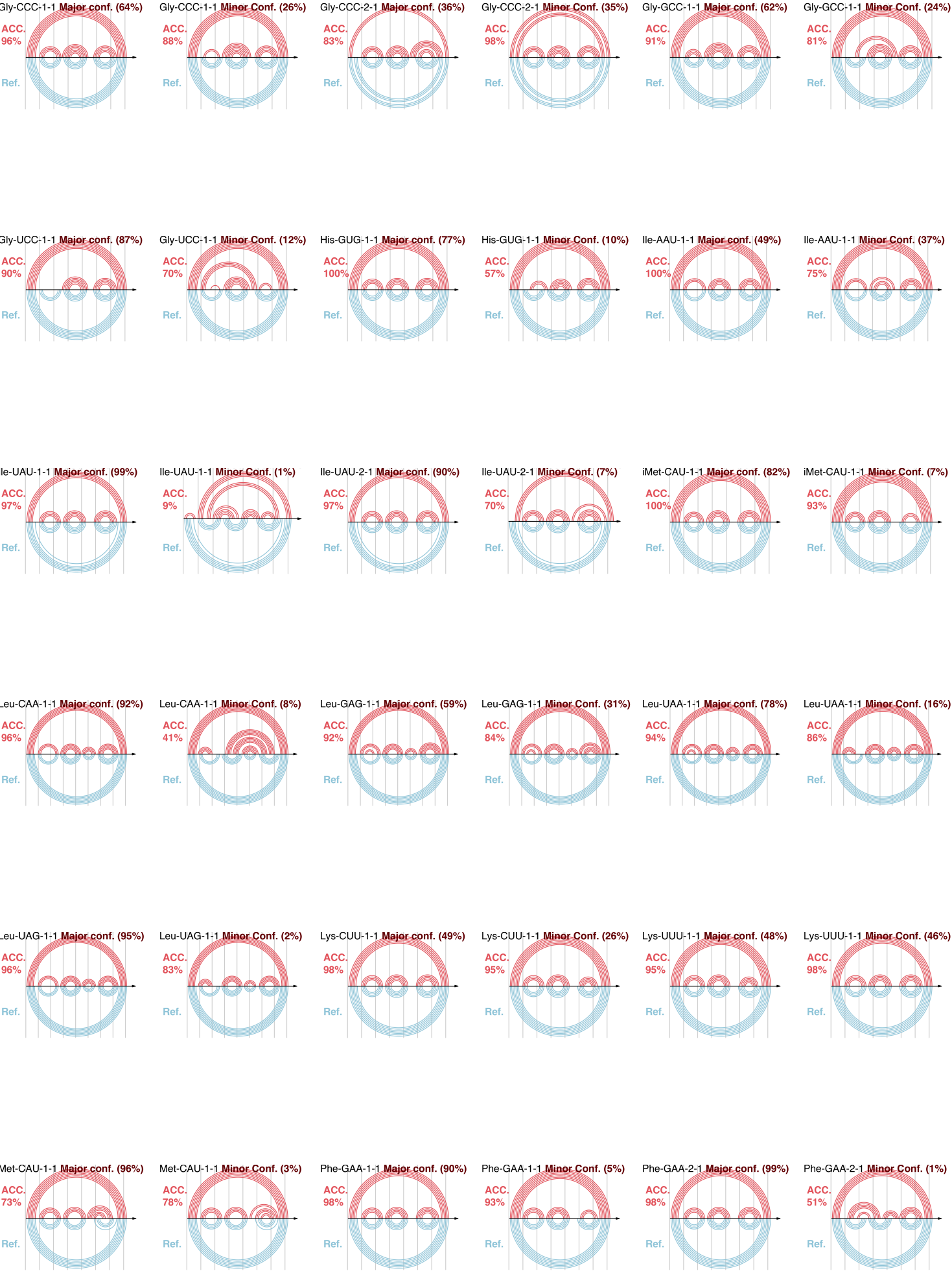
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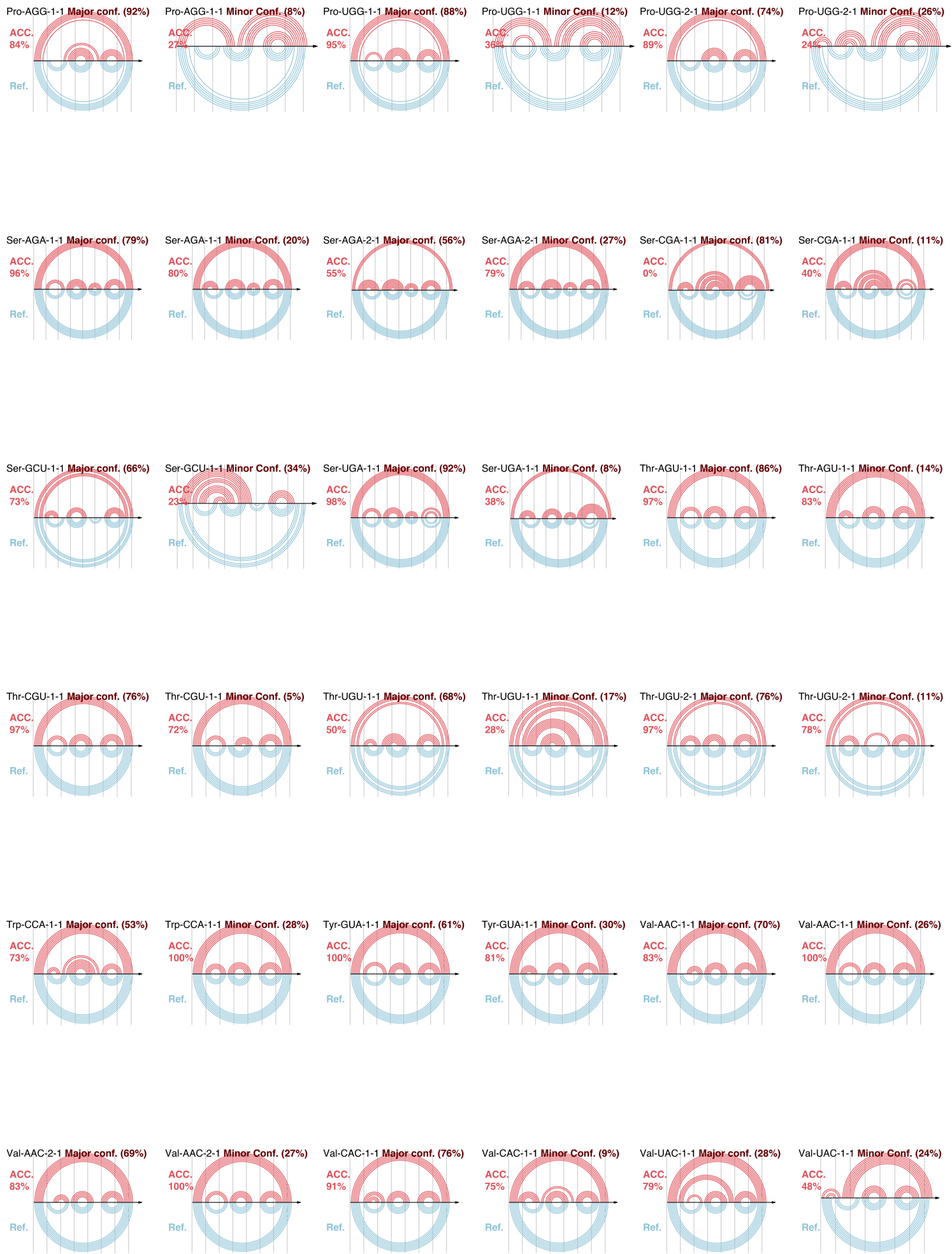
In vivo DMS condition



In vivo DMS condition

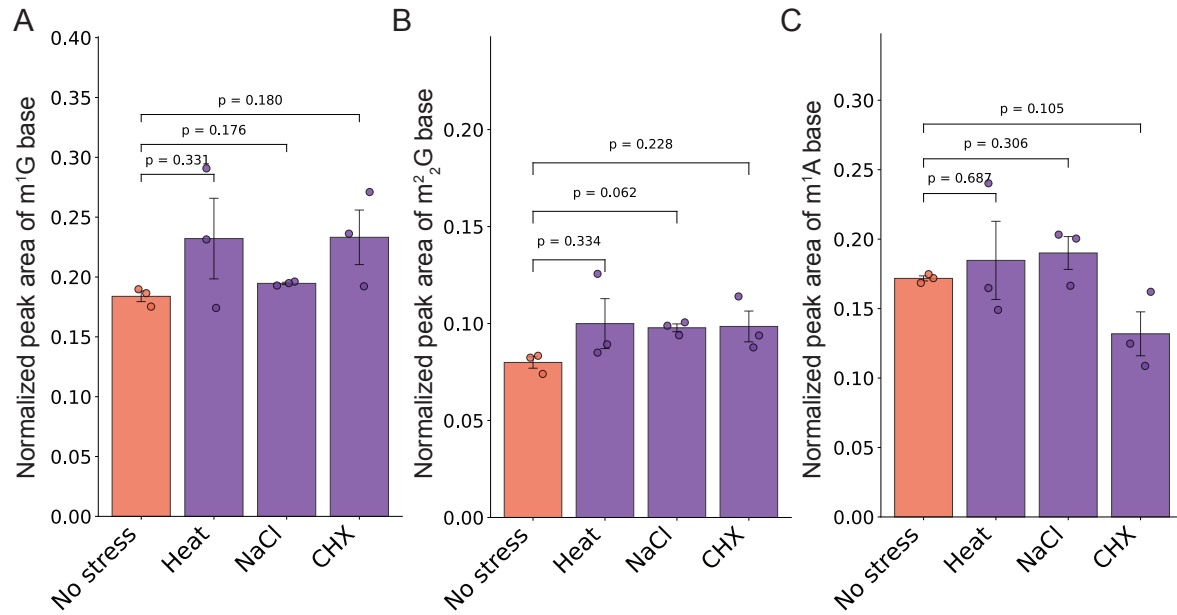


In vivo DMS condition



Supplemental Fig. 10 Two most populated secondary structure of tRNA predicted by Rsample under in vitro and in vivo conditions.

Rsample results were visualized by arc diagrams. The top red lines indicate predicted secondary structures, and bottom blue lines indicate the reference secondary structure.



Supplemental Fig. 11 Nucleoside analysis using LC-MS/MS. (A–C) Relative quantification by mutation profiling was cross-validated by LC-MS/MS experiments. Peak areas of (A) m^1G , (B) m^2G , and (C) m^1A bases were quantified and normalized to the peak area of cytosine. Error bars indicate the standard deviation from three biological replicates ($n = 3$). Wilcoxon signed-rank test was used for the calculation of p-values.

References

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