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Supplemental Material to:

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The absence of A-to-I editing in the anticodon of plant cytoplasmic $tRNA^{Arg}_{ACG}$ demands a relaxation of the wobble decoding rules

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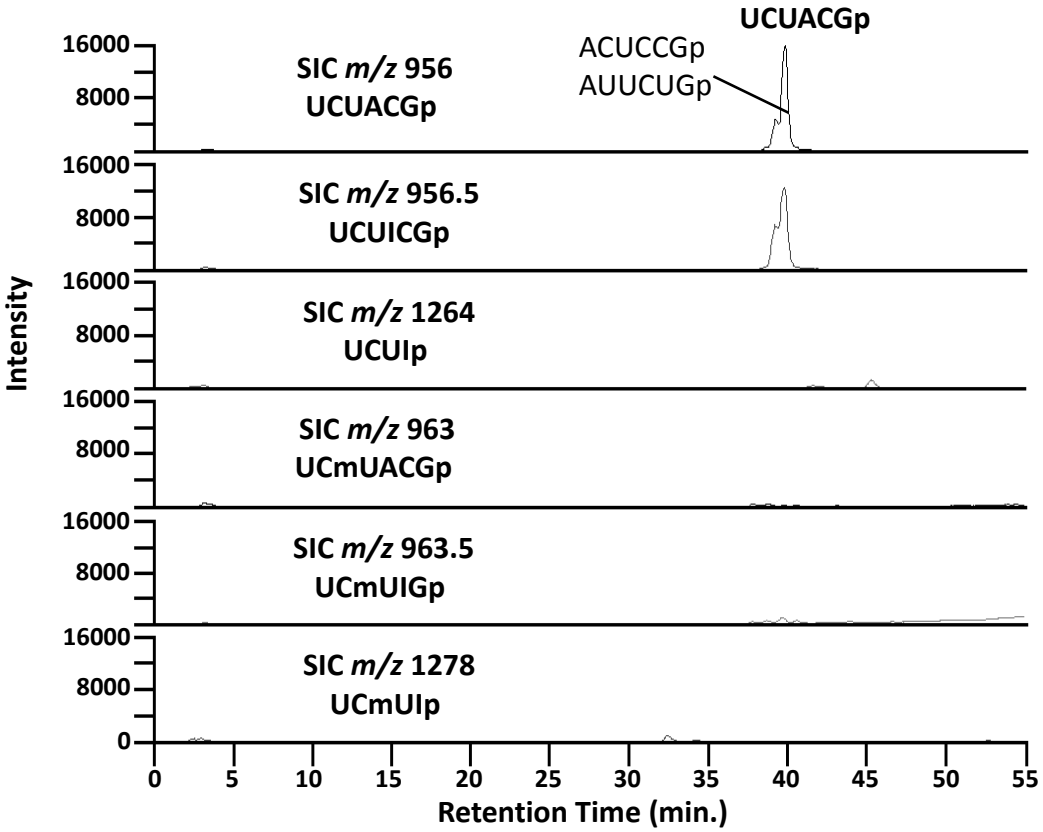
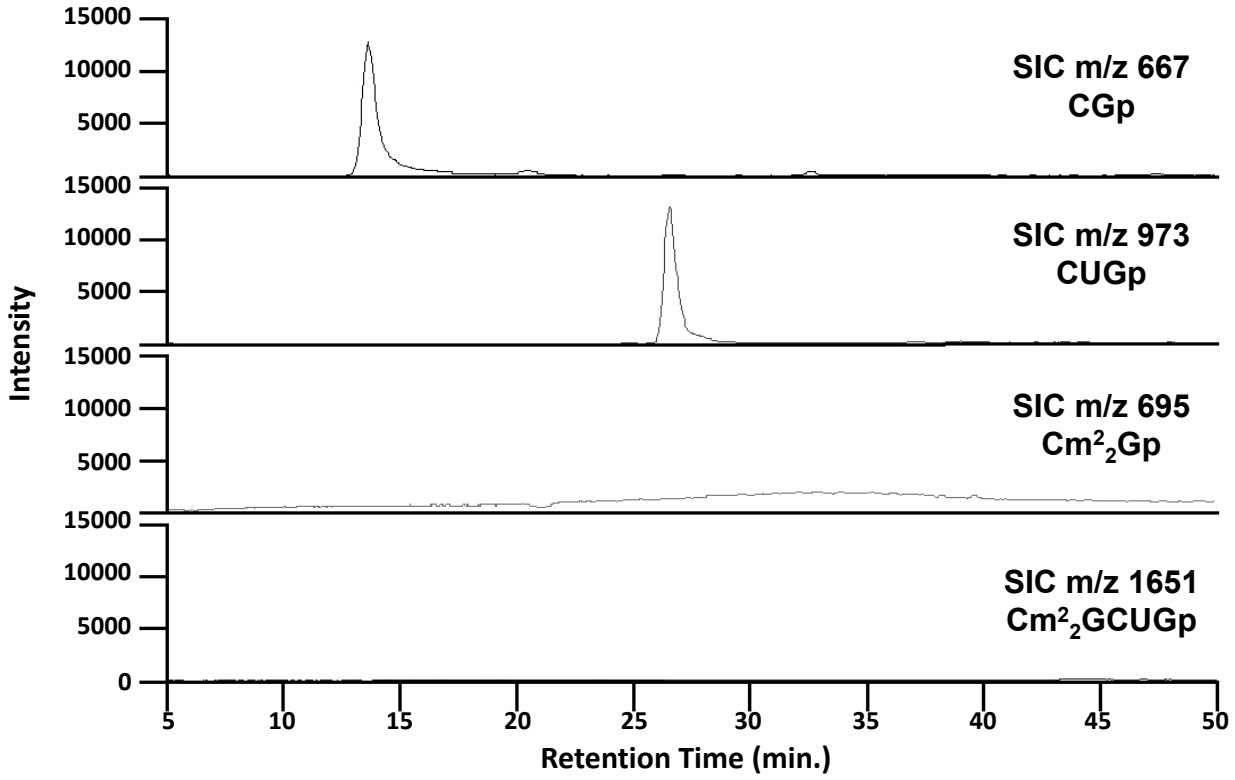
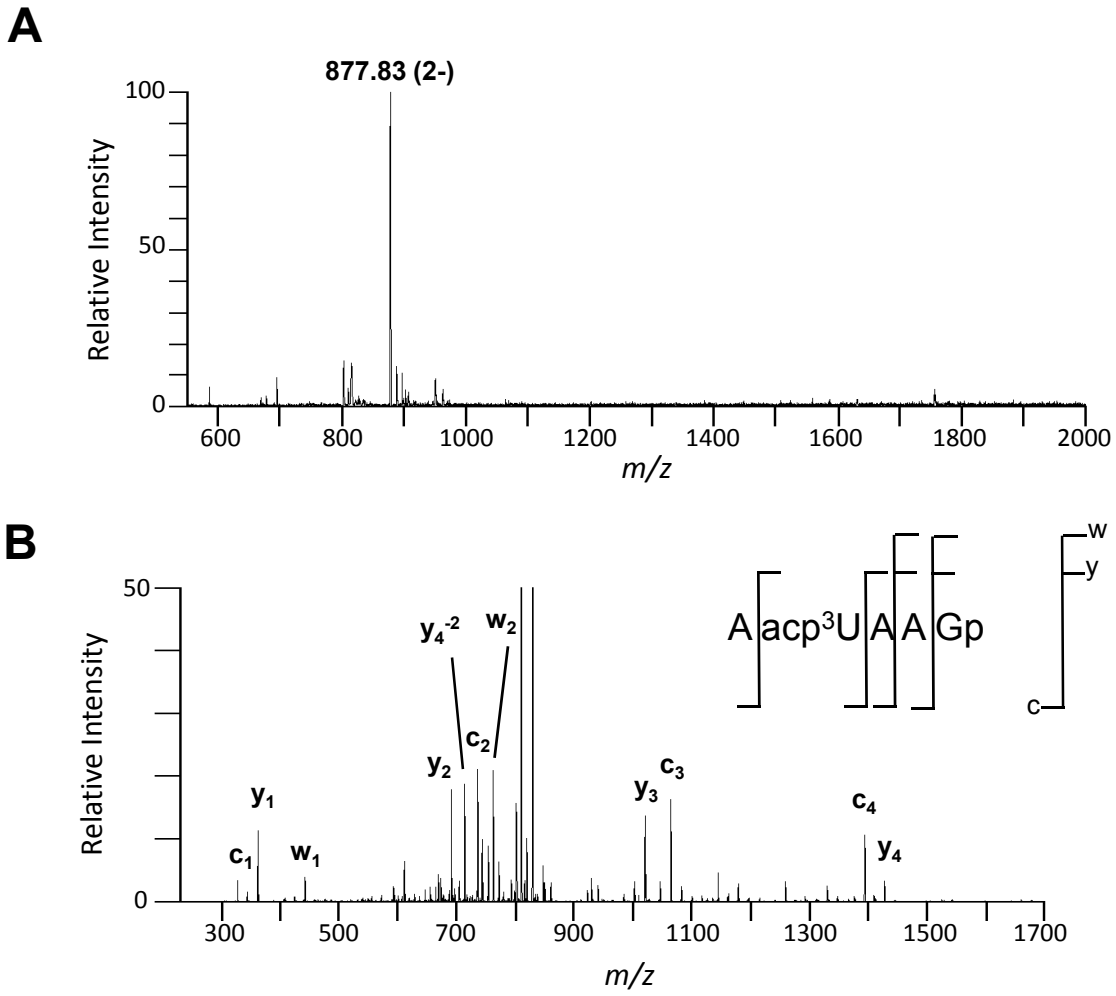


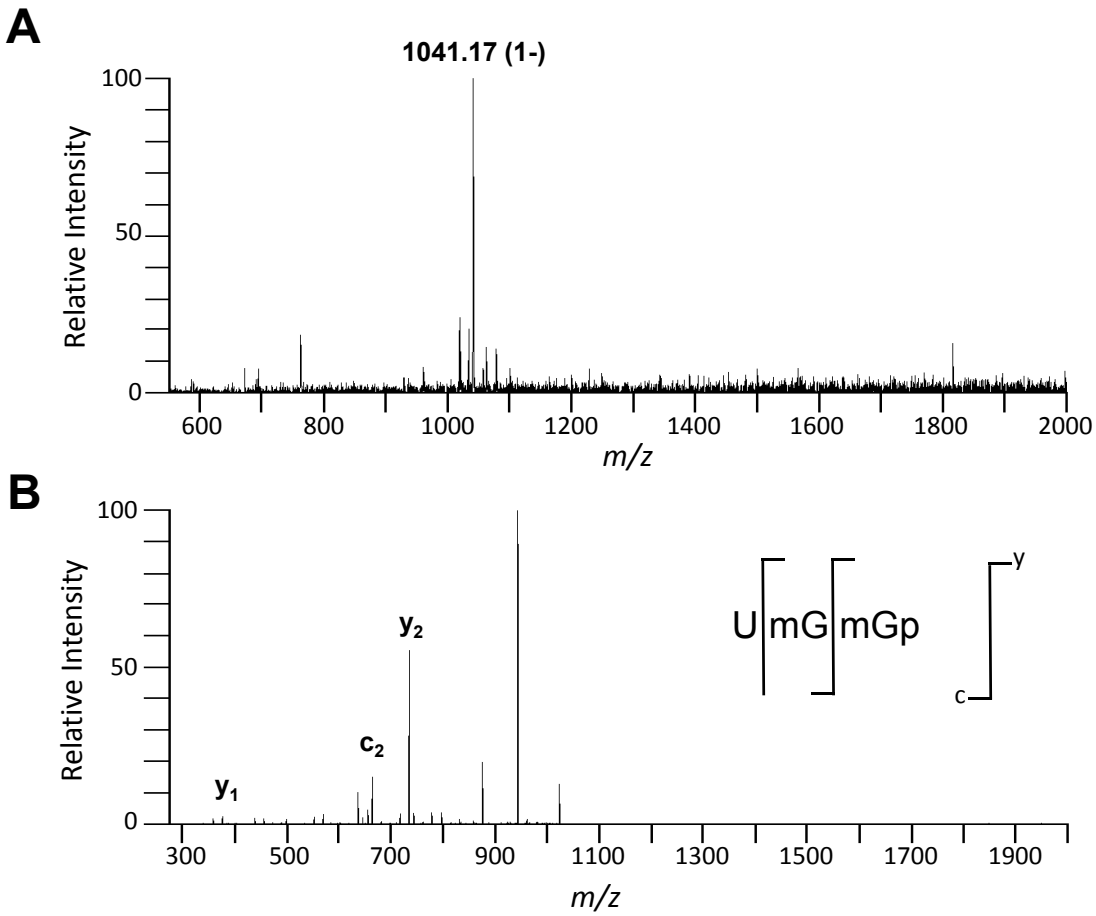
Supplementary Figure 1. Single ion chromatograms (SIC) of the *m/z* values corresponding to the predicted RNase T1 digestion products UCUACGp (*m/z* 956), UCU[I]CGp (*m/z* 956.5), UCU[I]p (*m/z* 1264), UCU[I]CGp + methyl group (14 u) (*m/z* 963.5) and UCU[I]p + methyl group (14 u) (*m/z* 1278). The SIC for *m/z* 956 reveals two peaks that were chromatographically separated. These two peaks correspond to the RNase T1 digestion products UCUACGp (major peak) and a mixture of ACUCCGp and AUUCUGp (minor peak). Significantly, no significant ion signals were detected from RNase T1 digestion products that would correspond to I34 in the anticodon. All SICs scaled at the same absolute peak intensity.



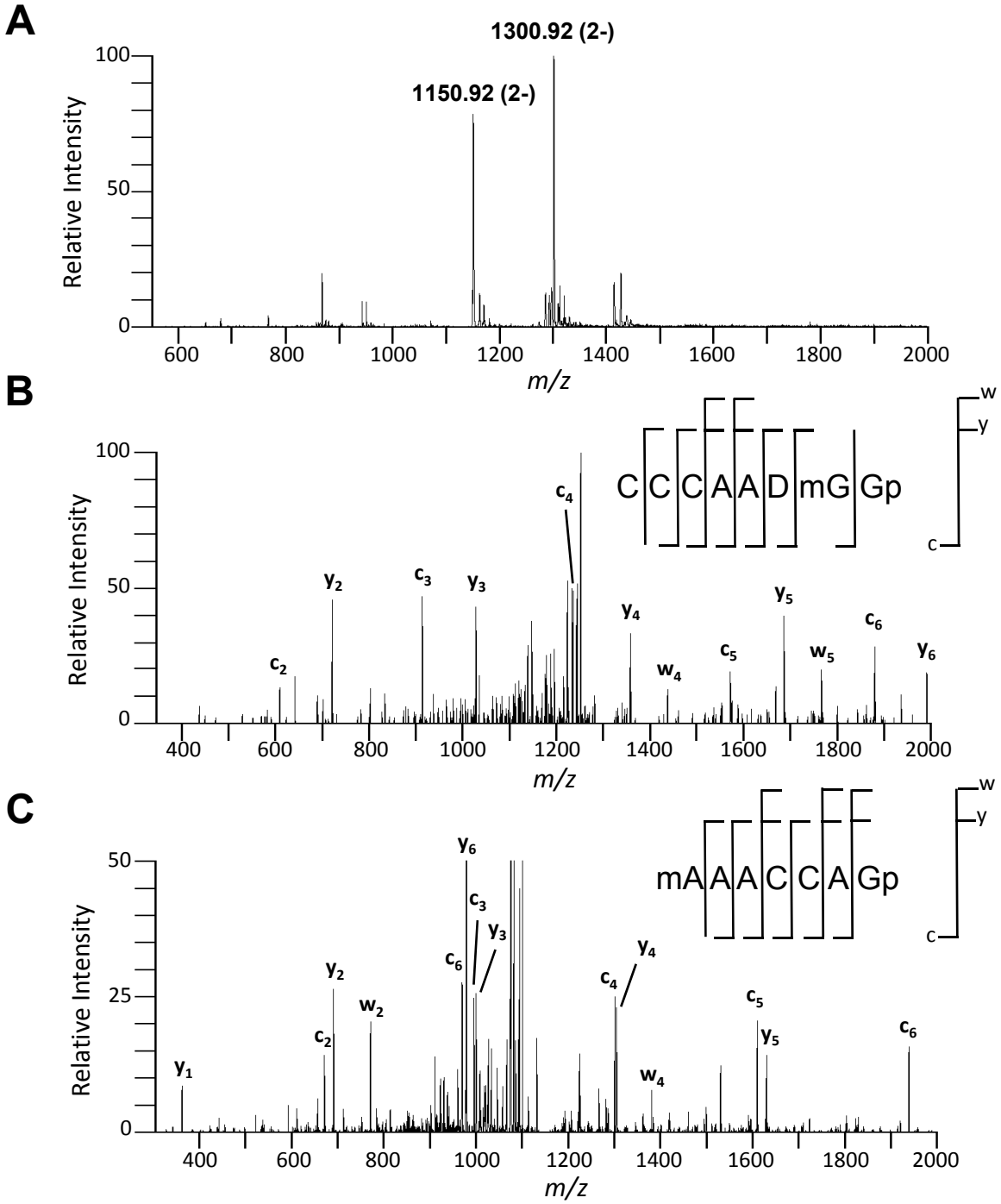
Supplementary Figure 2. Single ion chromatograms (SICs) of the predicted RNAse T1 fragments that could be present with and without m^2_2G26 in the tRNA. These are CGp (*m/z* 667), CUGp (*m/z* 973), C[m^2_2G26]p (*m/z* 695), and C[m^2_2G26]CUGp (*m/z 1651*). No significant signal was detected for those fragments that potentially contain the m^2_2G26 modification. All SICs scaled at the same absolute peak intensity



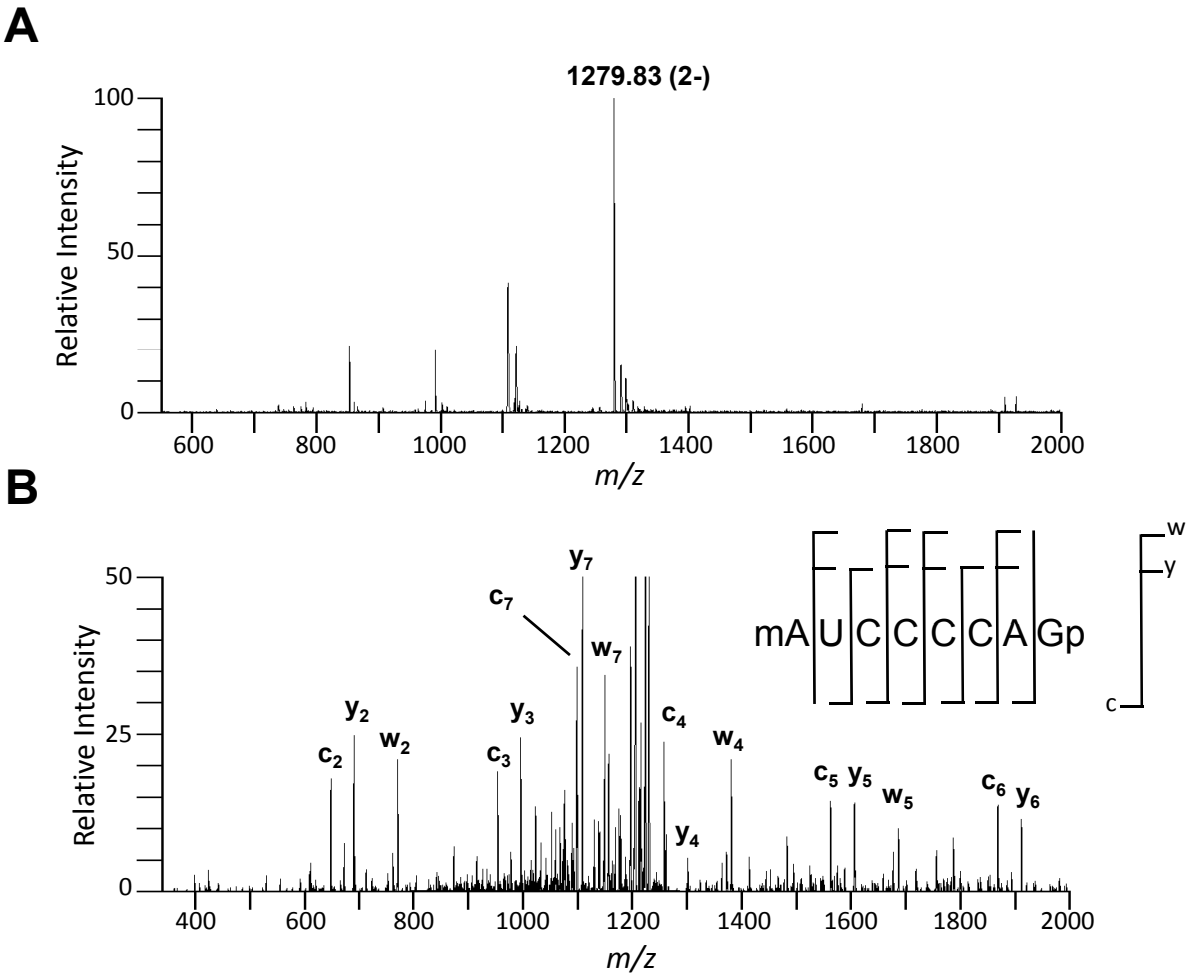
Supplementary Figure 3. (A) Mass spectrum of a doubly charged ion (*m/z* 877.83) that produced the MS/MS spectrum in (B) after collision-induced dissociation. This spectrum contains the majority of c-, y- and w-type ions that are consistent with the sequence A[acp^{3}U]AAGp, where acp^{3}U is 3-(3-amino-3-carboxypropyl)uridine, which is an expected RNase T1 digestion product of the tRNA$^{Arg}_{ACG}$ isoacceptor sequence.



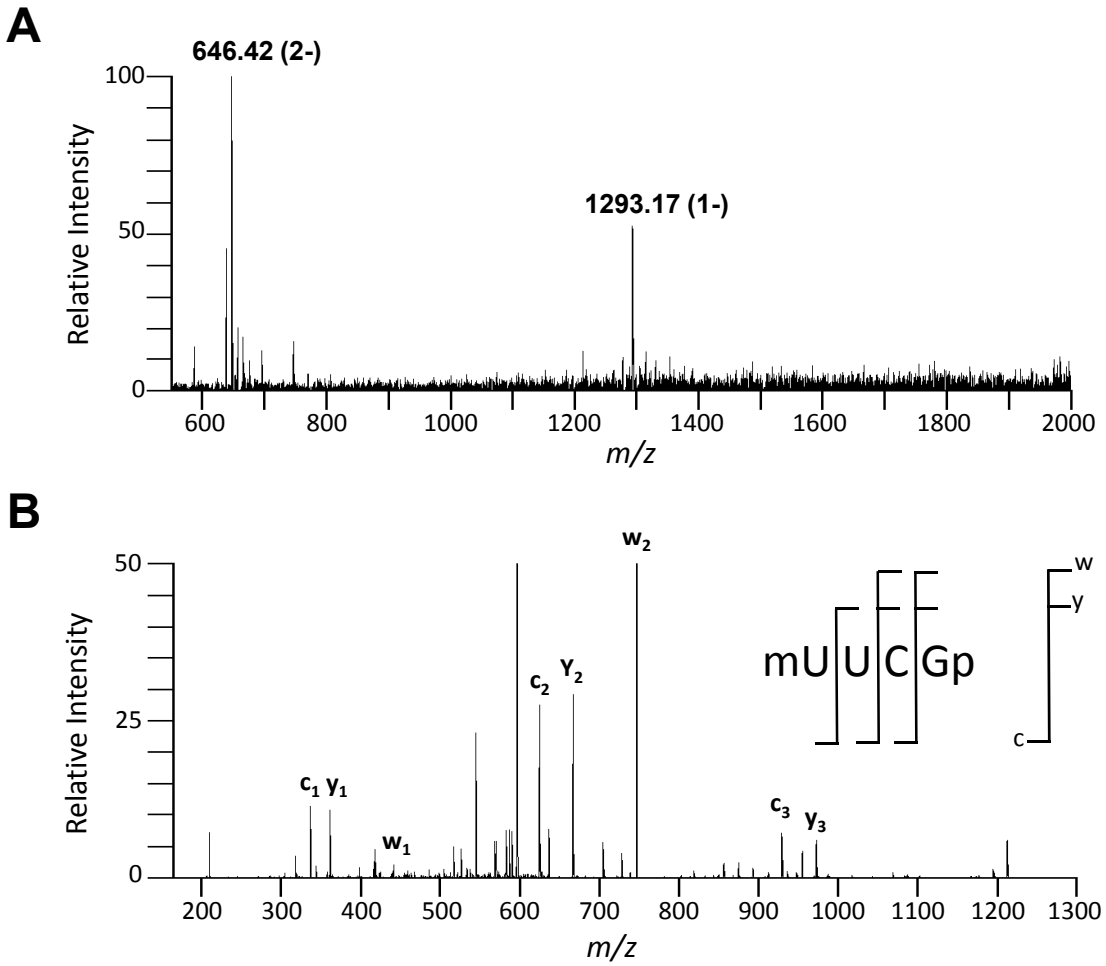
Supplementary Figure 4. (A) Mass spectrum of a singly charged ion (*m/z* 1041.17) that produced the MS/MS spectrum in (B) after collision-induced dissociation. This spectrum contains sufficient c- and y-type ions to be consistent with the sequence U[mG][mG]p, although the MS/MS data cannot identify whether methylation occurs on the nucleobase or sugar. The assigned sequence is an expected RNase T1 digestion product of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence.



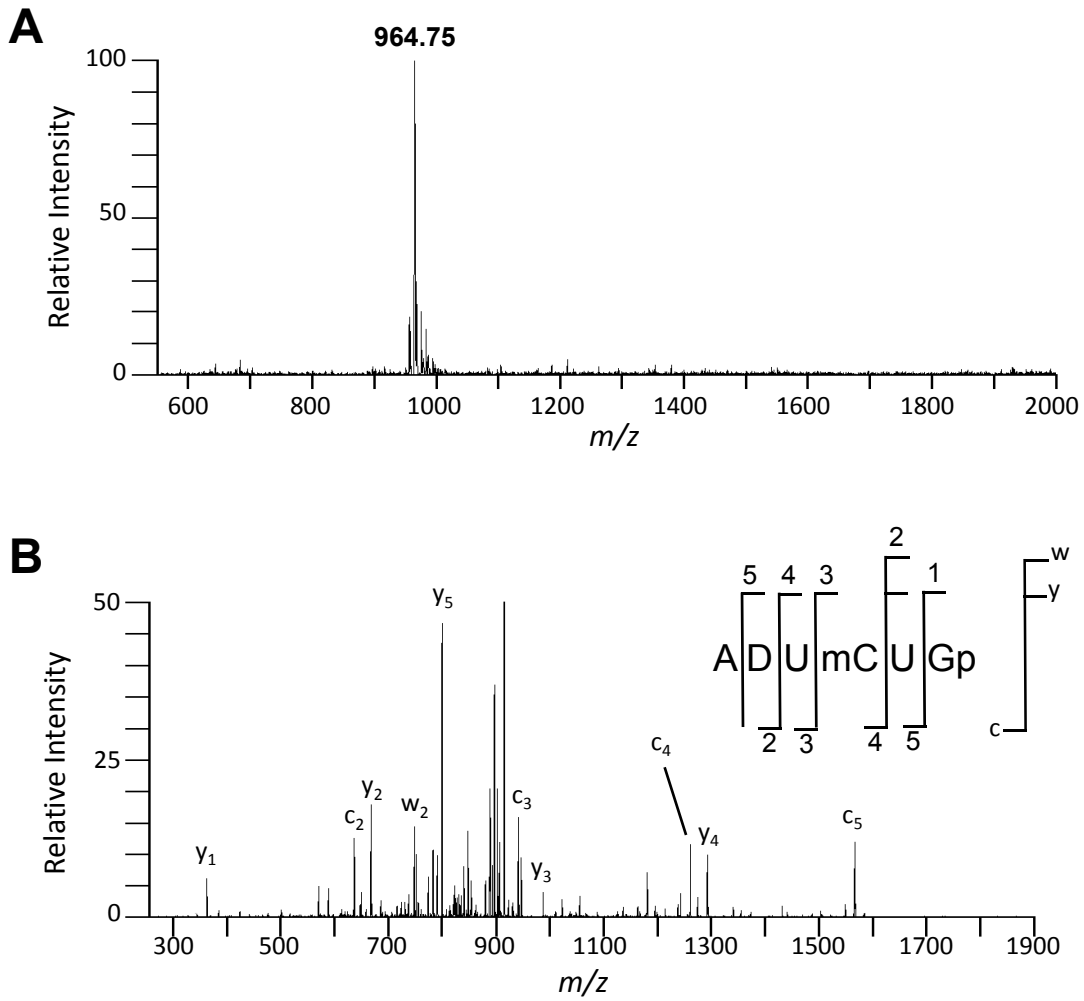
Supplementary Figure 5. (A) Mass spectral data illustrating co-eluting doubly charged peaks (*m/z* 1150.92 and 1300.92). (B) MS/MS data obtained from collision-induced dissociation of *m/z* 1300.9. This data reveals c-, y- and w-type fragment ions consistent with sequence CCCAA[D][mG]Gp, although the MS/MS data cannot identify whether methylation occurs on the nucleobase or sugar. (C) MS/MS data obtained from the collision-induced dissociation of *m/z* 1150.9. This data reveals c-, y- and w-type fragment ions consistent with the sequence [mA]AACCGp, although the MS/MS data cannot identify whether methylation occurs on the nucleobase or sugar. Both sequences assigned from the data in (B) and (C) are expected RNase T1 digestion products of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence.



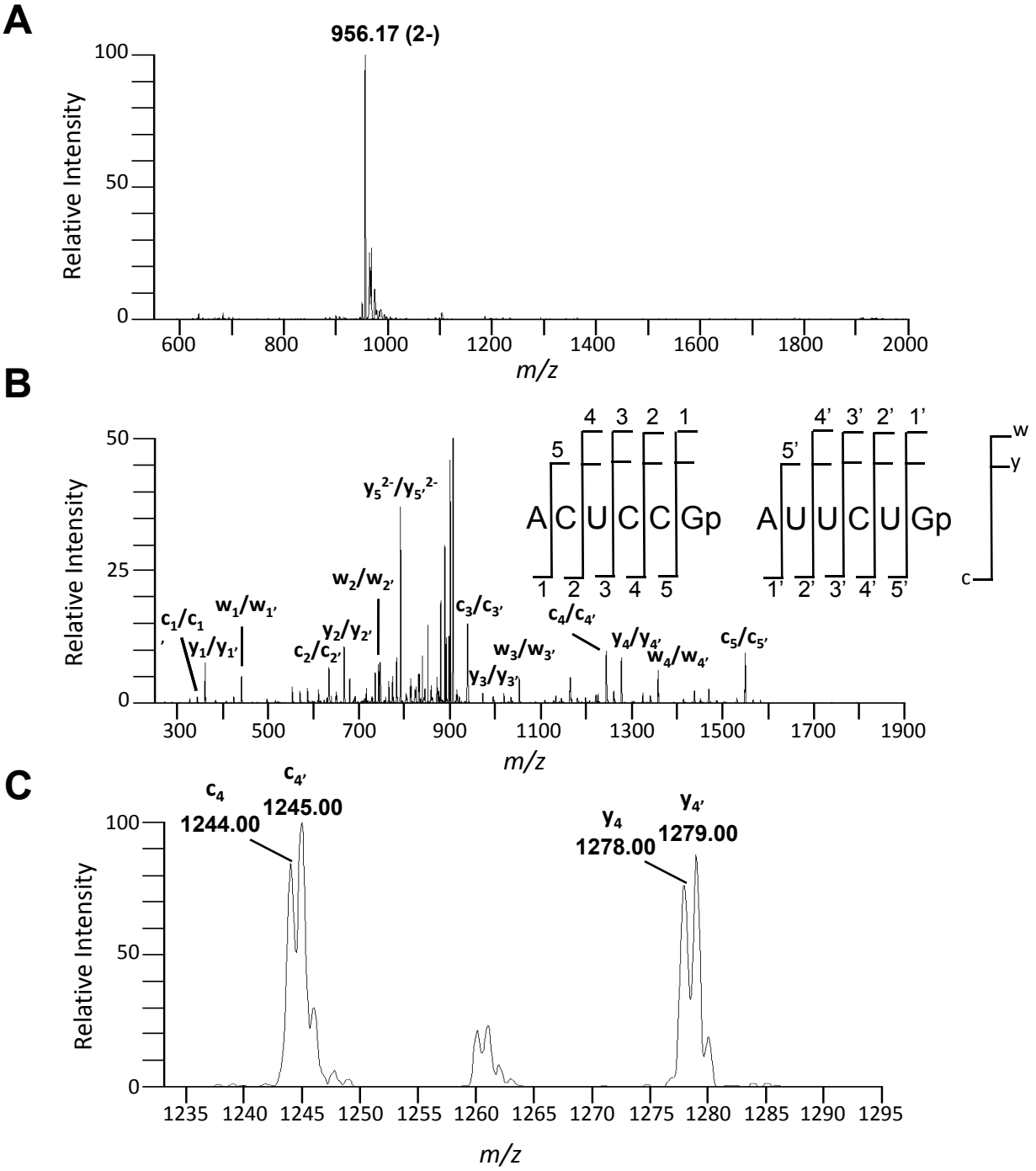
Supplementary Figure 6. (A) The mass spectrum of a doubly charged (*m/z* 1279.83) ion that that produced the MS/MS spectrum in (B) after collision-induced dissociation. This spectrum contains the majority of c-, y- and w-type ions that are consistent with the sequence [mA]UCCCCAGp, although the MS/MS data cannot identify whether methylation occurs on the nucleobase or sugar. The assigned sequence is an expected RNase T1 digestion product of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence.



Supplementary Figure 7. (A) Mass spectrum of a single digestion product producing doubly charged (*m/z* 646.42) and singly charged (*m/z* 1293.17) ions that that produced the MS/MS spectrum in (B) after collision induced dissociation. This spectrum contains the majority of c-, y- and w-type ions that are consistent with the sequence [mU]UCGp, although the MS/MS data cannot identify whether methylation occurs on the nucleobase or sugar. The assigned sequence is an expected RNase T1 digestion product of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence, and would be consistent with the [m^5U][Ψ]CGp digestion product, where m^5U is 5-methyluridine (ribothymidine, rT) and Ψ is pseudouridine.



Supplementary Figure 8. (A) Mass spectrum of a single RNase T1 digestion product that is doubly charged (*m/z* 964.75). These ions were used to produce the MS/MS spectrum after collision induced dissociation (B). The MS/MS spectrum of the *m/z* 964.75 ion produced c, y, and w type ions that are consistent with the sequence A[D]U[mC]UGp. While it was possible to identify a methylated cytidine modification, the location of the modification on the nucleotide can not be determined by this analysis. However, A[D]U[m^5C]UGp is an expected RNase T1 digestion product of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence.



Supplementary Figure 9. (A) Mass spectrum of a doubly charged ion (*m/z* 956.17) that that produced the MS/MS spectrum in (B) after collision-induced dissociation. Evaluation of the data in (B) reveals the presence of two different RNase T1 digestion products (ACUCCGp and AUUCUGp) as can be seen from the representative spectrum of the c_4 and y_4 ion pairs of these two digestion products in (C). Both assigned sequences are expected RNase T1 digestion product of the $tRNA^{Arg}_{ACG}$ isoacceptor sequence.