Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Novel ribonuclease activity of cusativin from *Cucumis sativus* for mapping nucleoside modifications in RNA

Balasubrahmanyam Addepalli, Sarah Venus, Priti Thakur, Patrick A Limbach

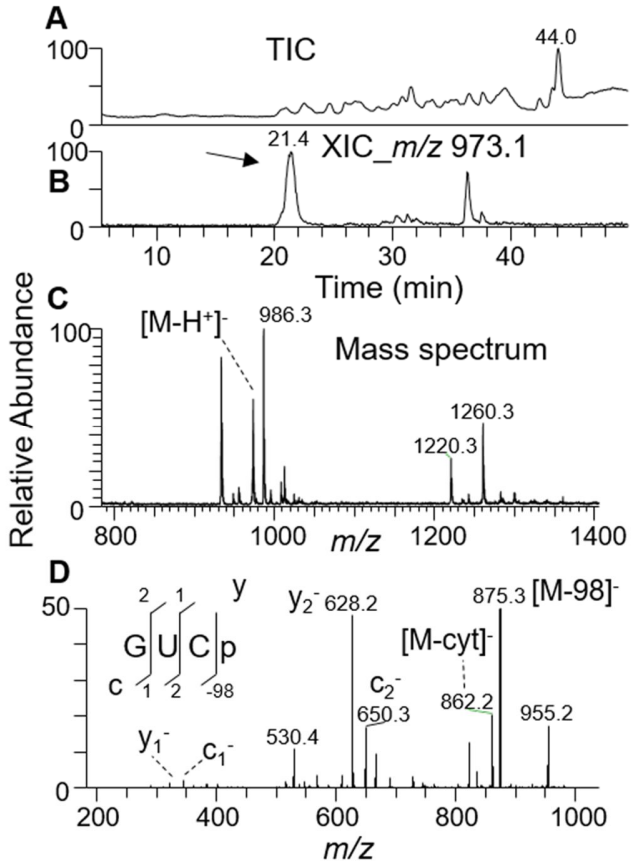

Fig. S1 LC-MS/MS analysis of Cusativin digestion product GUCp from *E. coli* $tRNA^{Tyr\ I}$.
(A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (XIC) for *m/z* 973.4, a peak with retention time of 21.4 min corresponding to a digestion product GUCp (position 44-46) is shown. (C) Mass spectrum associated with the XIC at 21.4 min depicting the presence of deprotonated singly charged anion. (D) Tandem mass spectrum (MS/MS) of the precursor ion *m/z* 973.4 following collision-induced dissociation (CID). The observed sequence informative product ion series, c_n (sharing common 5' end) and y_n (with common 3' end) with a subscript denoting the position of cleavage on phosphodiester backbone, or loss of phosphate (-98 D) are labeled and plotted

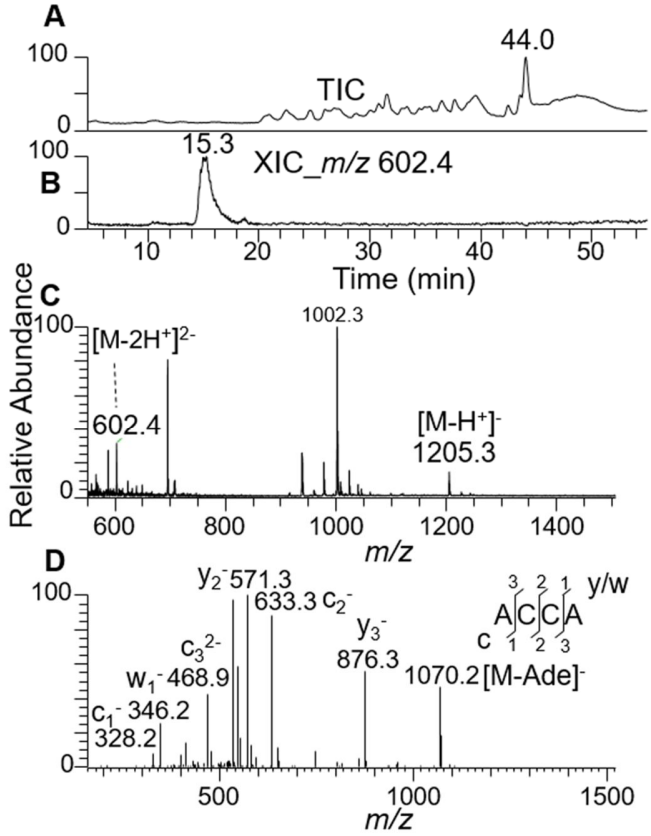

Fig. S2 LC-MS/MS analysis of RNase Cusativin digestion product ACCA from *E. coli* $tRNA^{Tyr\ I}$.

(A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (XIC) for *m/z* 602.4, corresponding to a digestion product ACCA (position 80-83). (C) Mass spectrum of the XIC at 15.3 min. Note the presence of double and singly deprotonated oligonucleotide anions in the spectrum. (D) Tandem mass spectrum (MS/MS) of the precursor ion, *m/z* 602.4, following collision-induced dissociation (CID) is depicted. The observed sequence informative product ion series, c_n and y_n with a subscript denoting the position of cleavage on phosphodiester backbone are labeled and plotted

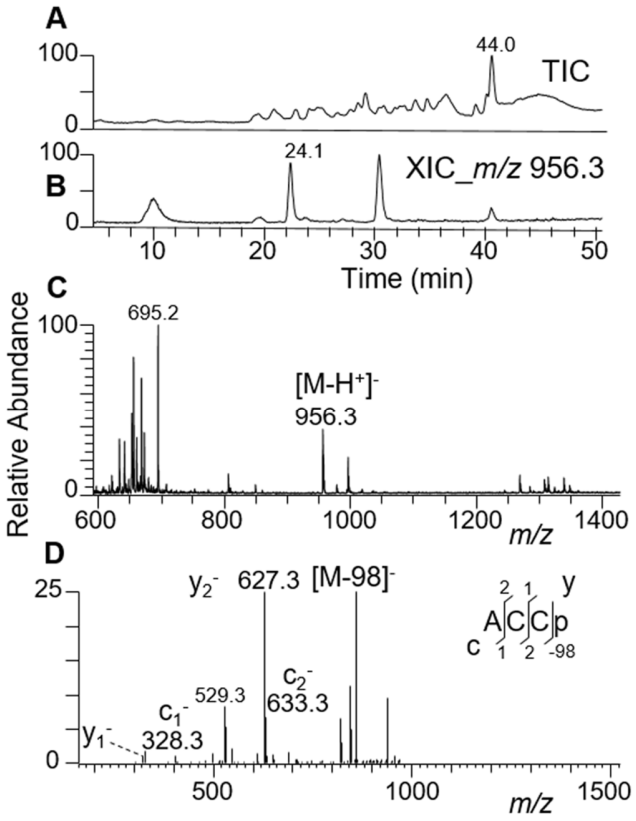

Fig. S3 LC-MS/MS analysis of RNase Cusativin digestion product ACCp from *E. coli* $tRNA^{Tyr\ I}$. (A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (XIC) for *m/z* 956.3, corresponding to a digestion product ACCp (position 77-79). (C) Mass spectrum of the XIC at 24.1 min. Note the presence of singly deprotonated oligonucleotide anions in the spectrum, (D) tandem mass spectrum (MS/MS) of the precursor ion, *m/z* 956.3 following collision-induced dissociation (CID) is depicted. The observed sequence informative product ion series, c_n and y_n with a subscript denoting the position of cleavage on phosphodiester backbone are labeled and plotted

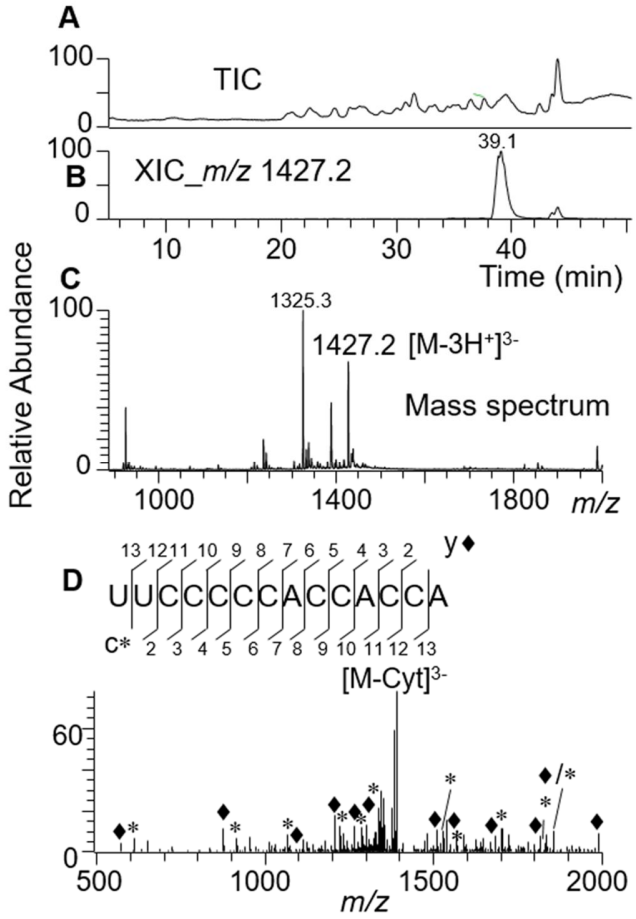

Fig. S4 LC-MS/MS analysis of RNase Cusativin digestion product, UUCCCCCCACCACCA from *E. coli* $tRNA^{Tyr\ I}$.

(A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (XIC) for *m/z* 1427.2, corresponding to a digestion product UUCCCCCCACCACCA (position 70-83) with a 3'-OH. (C) Mass spectrum associated with the XIC at 39.1 min. Note the presence of triply deprotonated oligonucleotide anion in the spectrum. (D) Tandem mass spectrum (MS/MS) of the precursor ion, *m/z* 1427.2, following collision-induced dissociation (CID). The observed sequence informative product ion series, c_n (sharing common 5' end indicated by ∗) and y_n (with common 3' end indicated by ♦) with a subscript denoting the position of cleavage on phosphodiester backbone are labeled and plotted

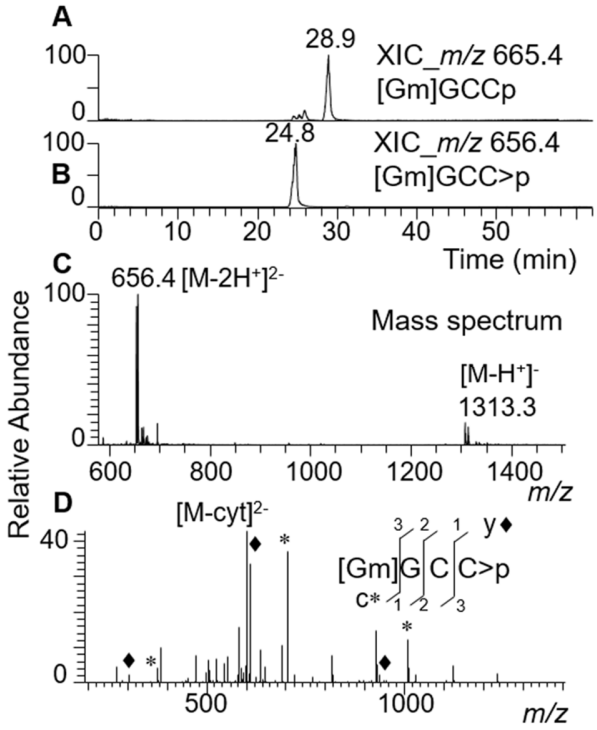

Fig. S5 LC-MS/MS analysis of cusativin digestion product [Gm]GCC>p (position 15-18) from *E. coli* tRNA(Tyr I).

(A) Extracted ion chromatogram (XIC) for *m/z* 665.4, corresponding to [Gm]GCC>p with linear phosphate. (B) Extracted ion chromatogram (XIC) for *m/z* 656.4, corresponding to same digestion product with 2', 3'-cyclic phosphate. (C) Mass spectrum associated with the XIC at 24.8 min depicting the singly and doubly charged molecular ions. (D) Tandem mass spectrum (MS/MS) of the precursor ion *m/z* 656.4 following collision-induced dissociation (CID) is depicted. The observed sequence informative product ion series, c_n (sharing common 5' end and indicated by ∗) and y_n (with common 3' end and indicated by ♦) with a subscript denoting the position of cleavage on phosphodiester backbone, are labeled and plotted

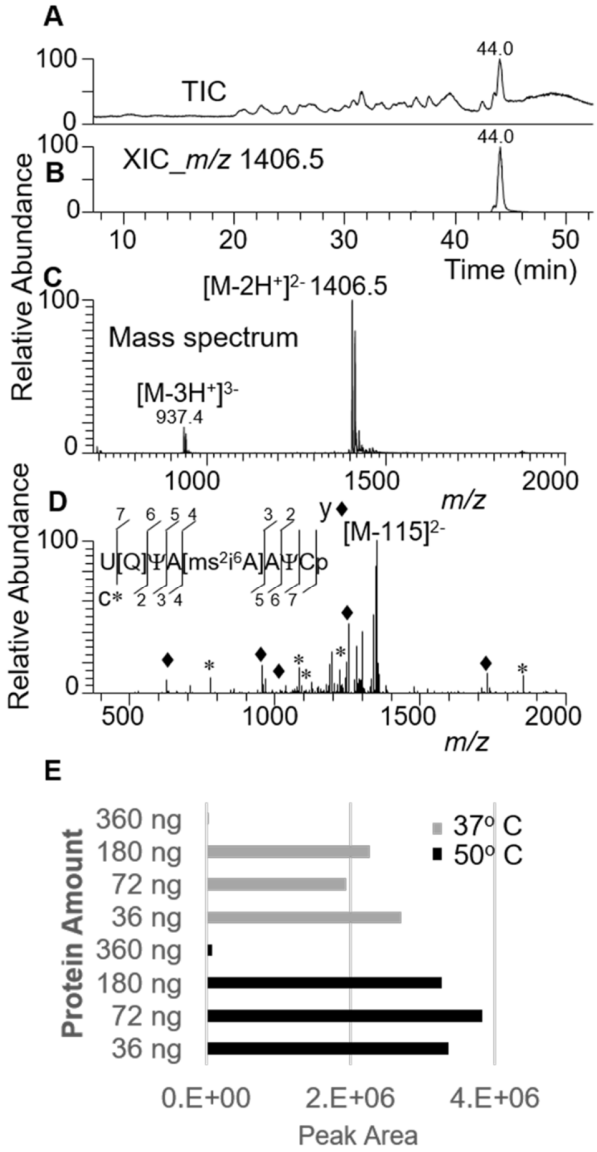

Fig. S6 LC-MS/MS analysis of RNase cusativin digestion product U[Q][Ψ]A[ms^{2}i^{6}A]A[Ψ]Cp from *E. coli* tRNA(Tyr I).

(A) Total ion chromatogram (TIC). (B) Extracted ion chromatogram (XIC) for *m/z* 1406.5, corresponding to U[Q][Ψ]A[ms^{2}i^{6}A]A[Ψ]Cp (position 32-39). (C) Mass spectrum associated with the XIC at 44 min. Note the presence of double and triple deprotonated oligonucleotide anions in the spectrum. (D) Tandem mass spectrum (MS/MS) of the precursor ion, *m/z* 1406.5, following collision-induced dissociation (CID). The observed sequence informative product ion series, c_n (sharing common 5' end and indicated by ∗) and y_n (with common 3' end and indicated by ♦) with a subscript denoting the position of cleavage on phosphodiester backbone, are labeled and plotted. (E) Changes in abundance levels of oligonucleotide, U[Q][Ψ]A[ms^{2}i^{6}A]A[Ψ]Cp, at different protein concentrations and incubation temperature is shown

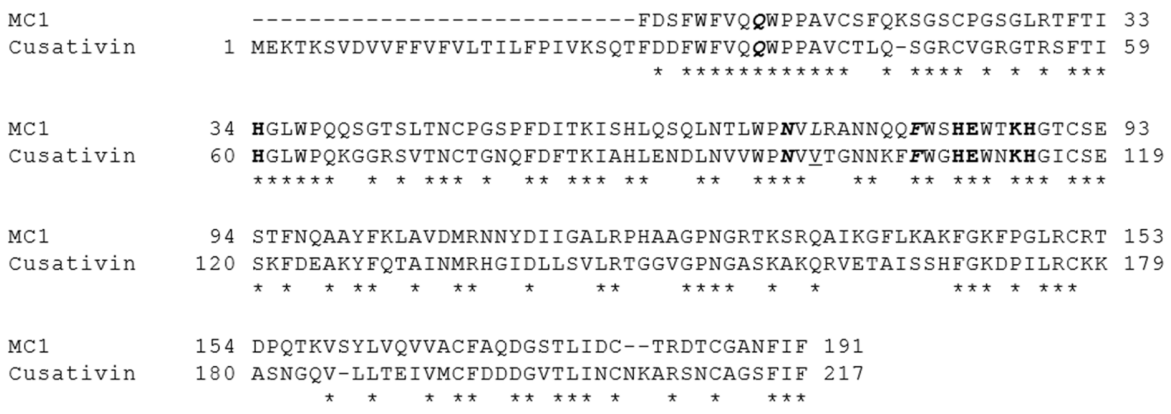

Fig. S7 Homology based alignment of amino acid sequences of Rnase MC1 and cusativin. Identical amino acid residues at respective positions are indicated by *, Substitutions with residues of similar polarity are indicated by ":" or "." symbols. Identical catalytic residues in both sequences are depicted in bold type, whereas the identical nucleobase recognizing residues are represented in bold italics. The nucleobase recognizing residue that is different in cusativin is underlined