Supplementary Information

An RNA-binding peptide consisting of four types of amino acid

by *in vitro* selection using cDNA display

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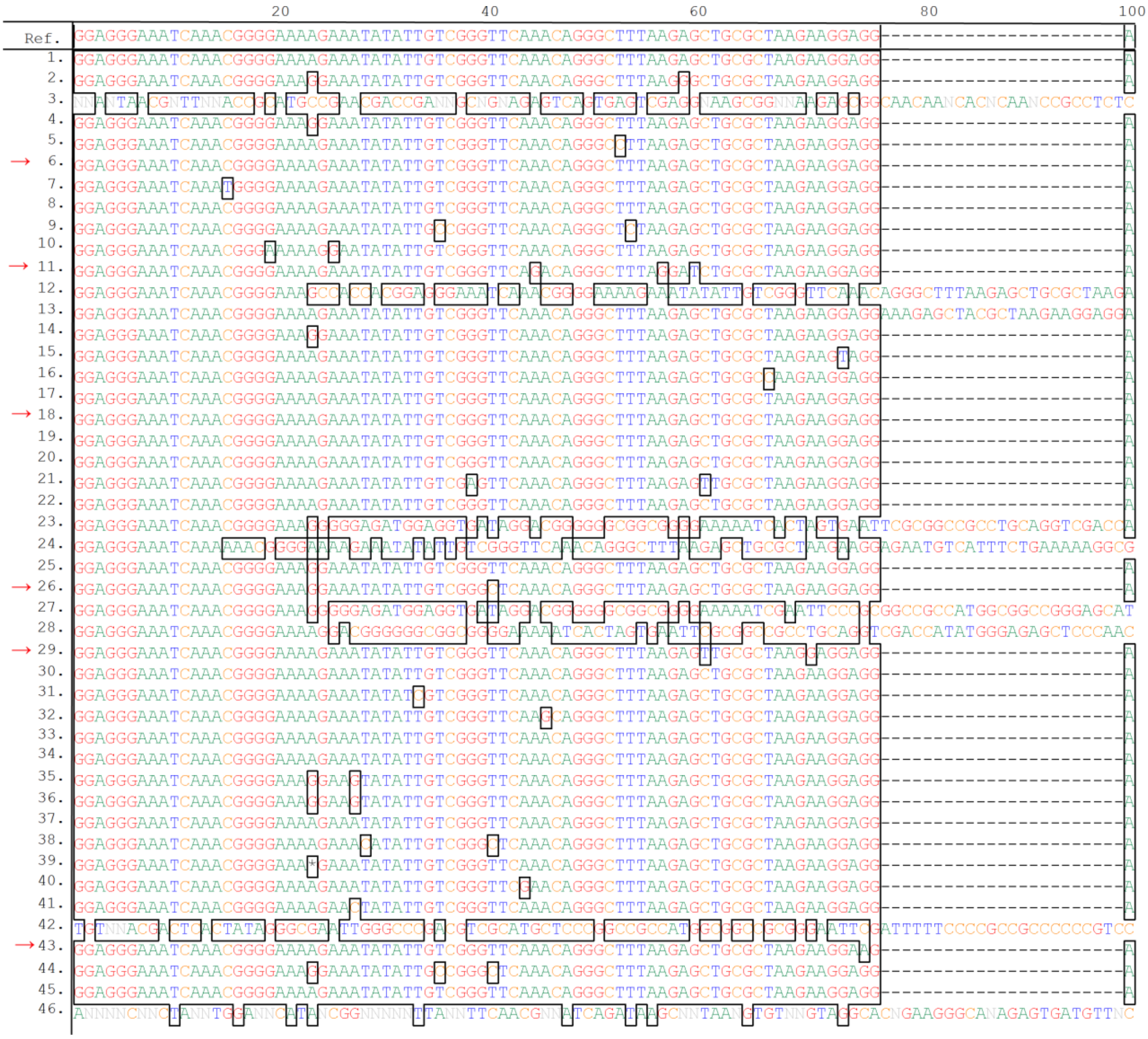
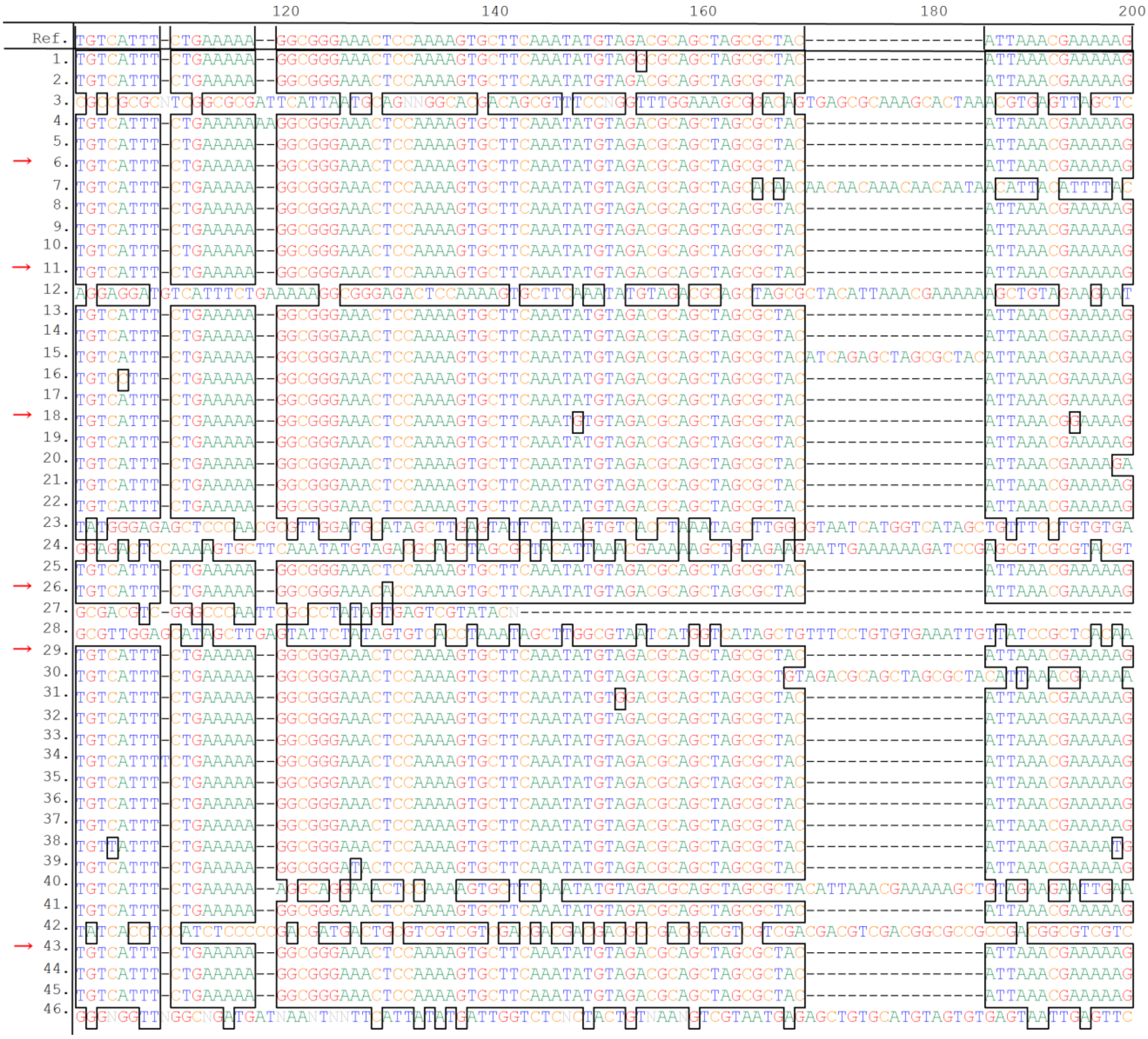
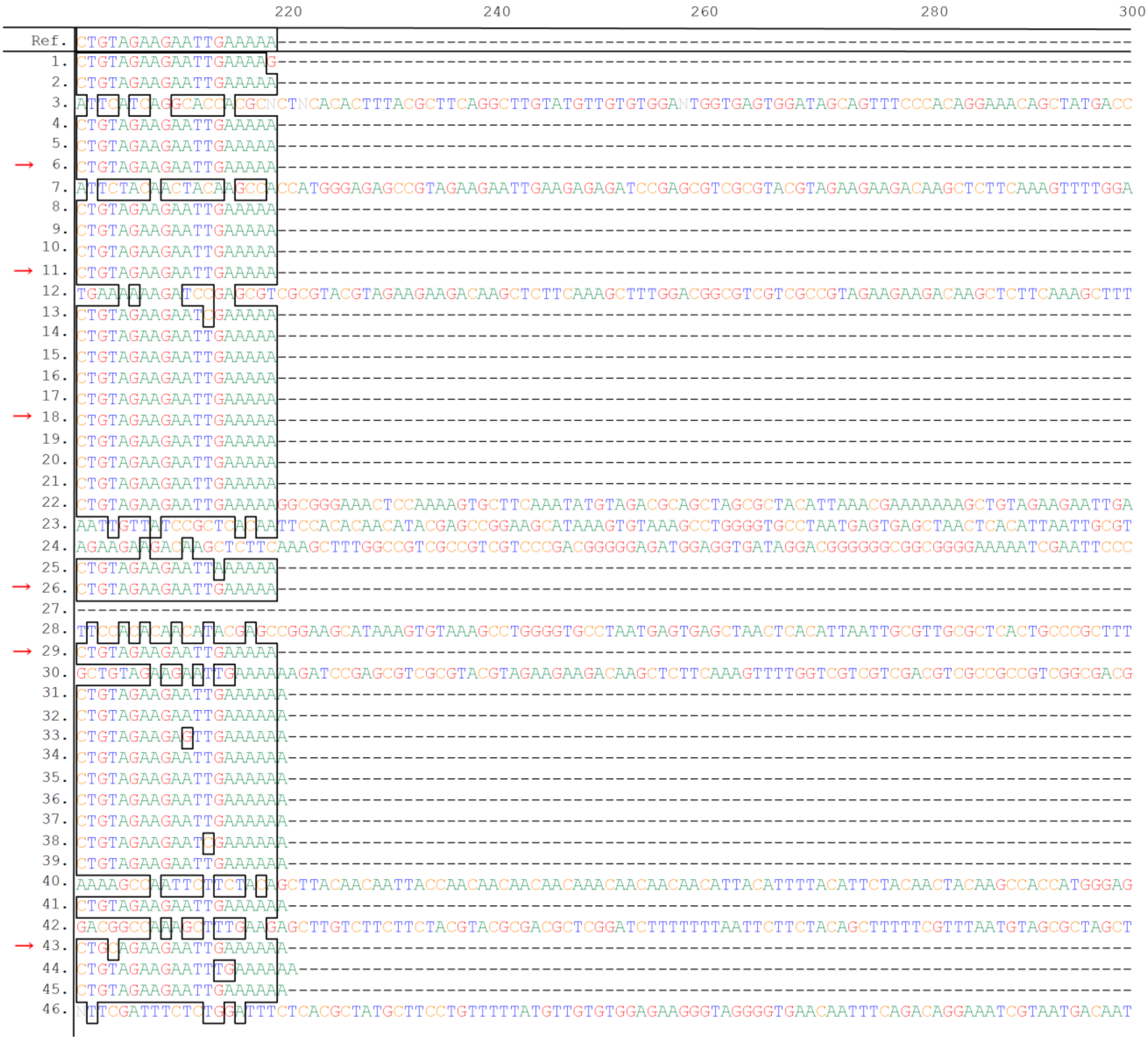
Supplementary Table

Table 1. Selected DNA sequences of eXact tag and GNC random region. eXact tag region




120
140
160
180
200
Ref.


220
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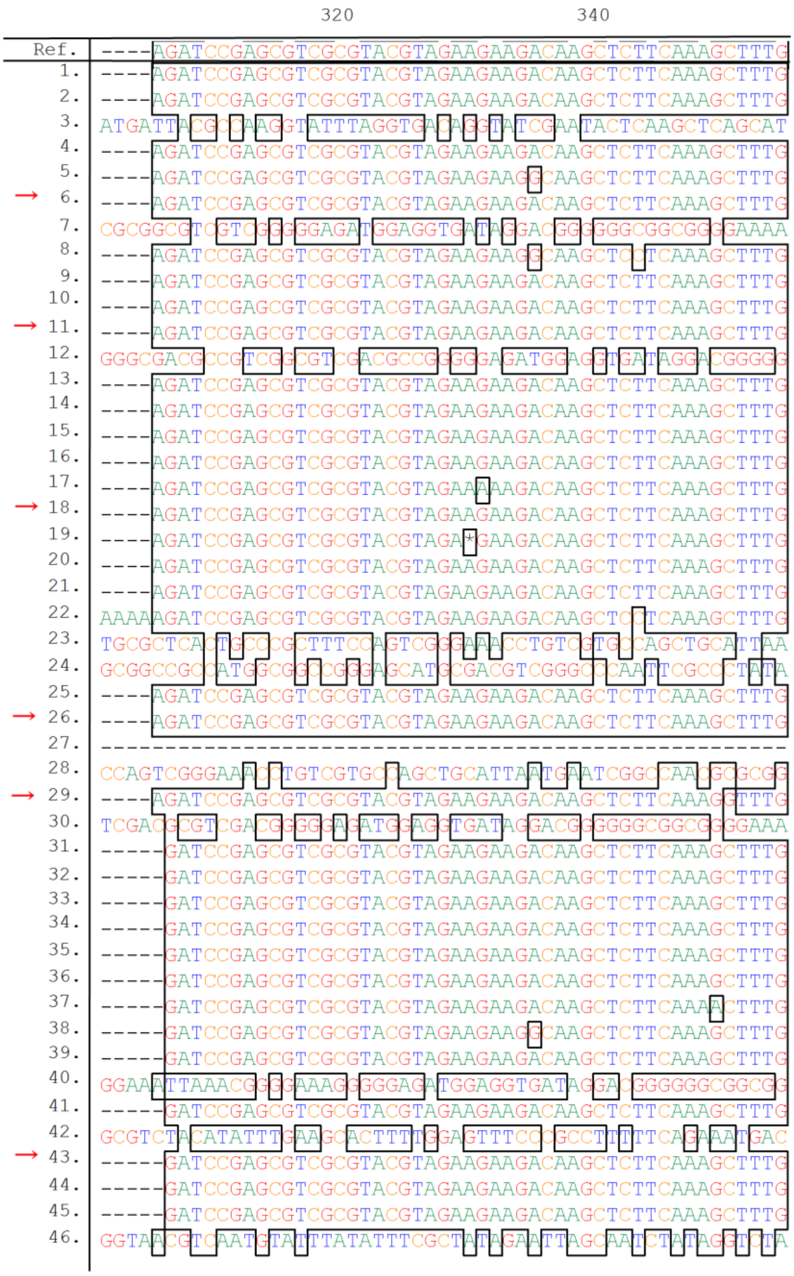
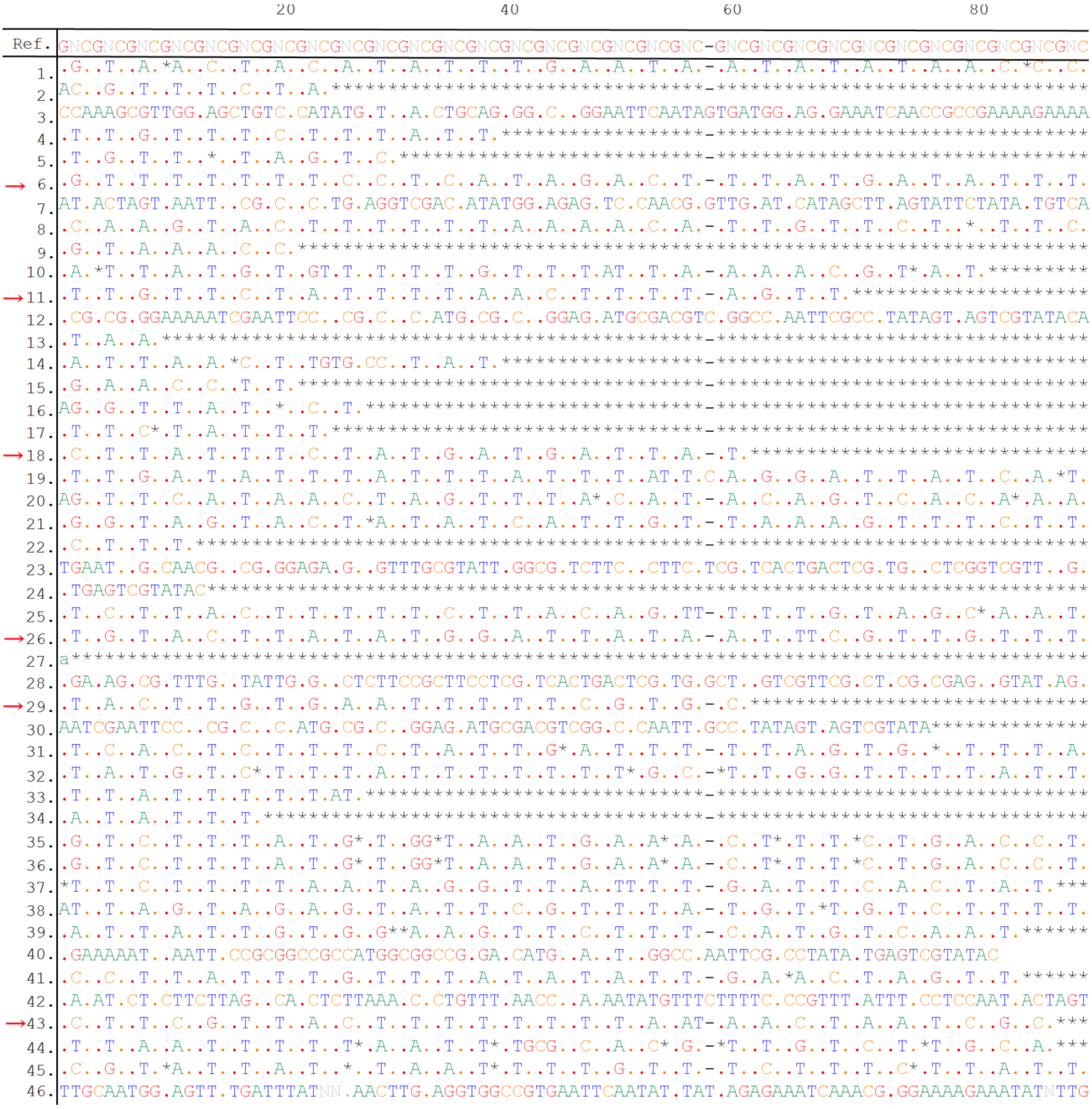
260
280
300
Ref.



GADV random region



Arrows indicate the selected peptides that consisted of four types of amino acid. The sequences 6, 11, 18, 26, 29, and 43 correspond to GADV1, GADV4, GADV6, GADV2, GADV5, and GADV3, respectively.

Supplementary Figures

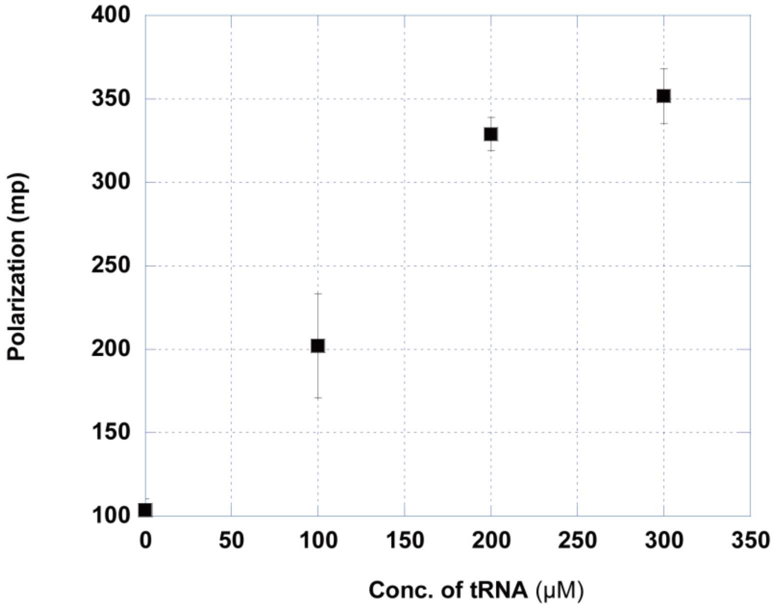


Figure S1. Fluorescent depolarization analysis of the interaction between GADV1 and tRNA. The GADV1 peptide (100 nM) and the tRNA (each conc.) were incubated at 25 °C for 1 h in 100 μL of the selection buffer. The polarization of those solutions was measured using a Beacon 2000 (Invitrogen). The polarization is shown by:

$$\mathrm{mp} = \frac{[\text{Strength of parallelism}]-[\text{strength of verticalism}]}{[\text{Strength of parallelism}]+[\text{strength of verticalism}]} \propto \frac{3\eta V}{RT}, \quad (1)$$

where η denotes viscosity, V the size of the molecules, R the gas constant and T the absolute temperature. The GADV1 peptide-tRNA complex has a higher mp-value than the peptide only, because the peptide-tRNA complex has a larger rotational relaxation time than the peptide. Higher mp value was observed as the concentration of tRNA was increased, indicating that GADV1 peptide interacted with tRNA and/or the viscosity was increased.

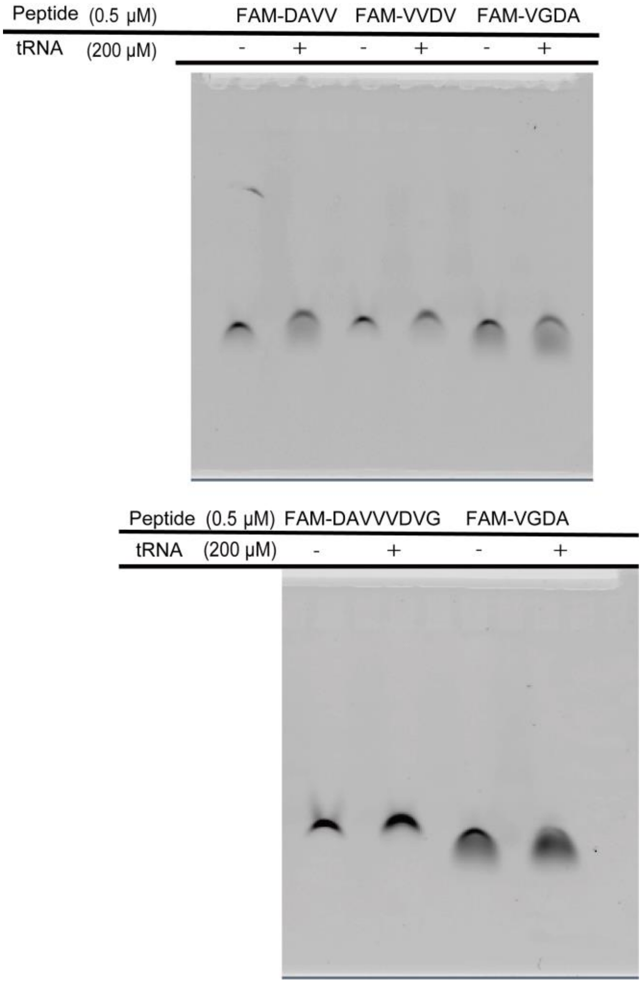


Figure S2. Binding assay of the short consensus peptide sequences against tRNA by an electrophoretic mobility shift assay (EMSA). The peptides were incubated with 200 μM of tRNA at 25 °C for 1 h in the selection buffer (50 mM Tris-HCl, pH 7.0, 467 mM NaCl, 57 mM $MgCl_2$, 13 mM $CaCl_2$, 9 mM KCl, 0.2% Tween20) and loaded onto a 4% native PAGE after performing pre-electrophoresis at 100 V for 1 h. The BPB was loaded to confirm alternate lanes. The electrophoresis was performed at 100 V for 90 min at 4 °C and visualized with a fluorescence image analyzer.

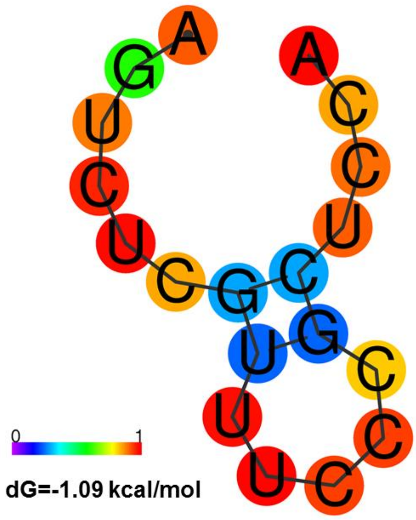


Figure S3. The possible secondary structure of the single strand RNA. The RNA secondary structure was predicted by CentroidFold.[1] The dG at 25°C was predicted by mfold.[2-4] The base paired regions consist of only two or three base pairs, and therefore are unlikely to be stable.

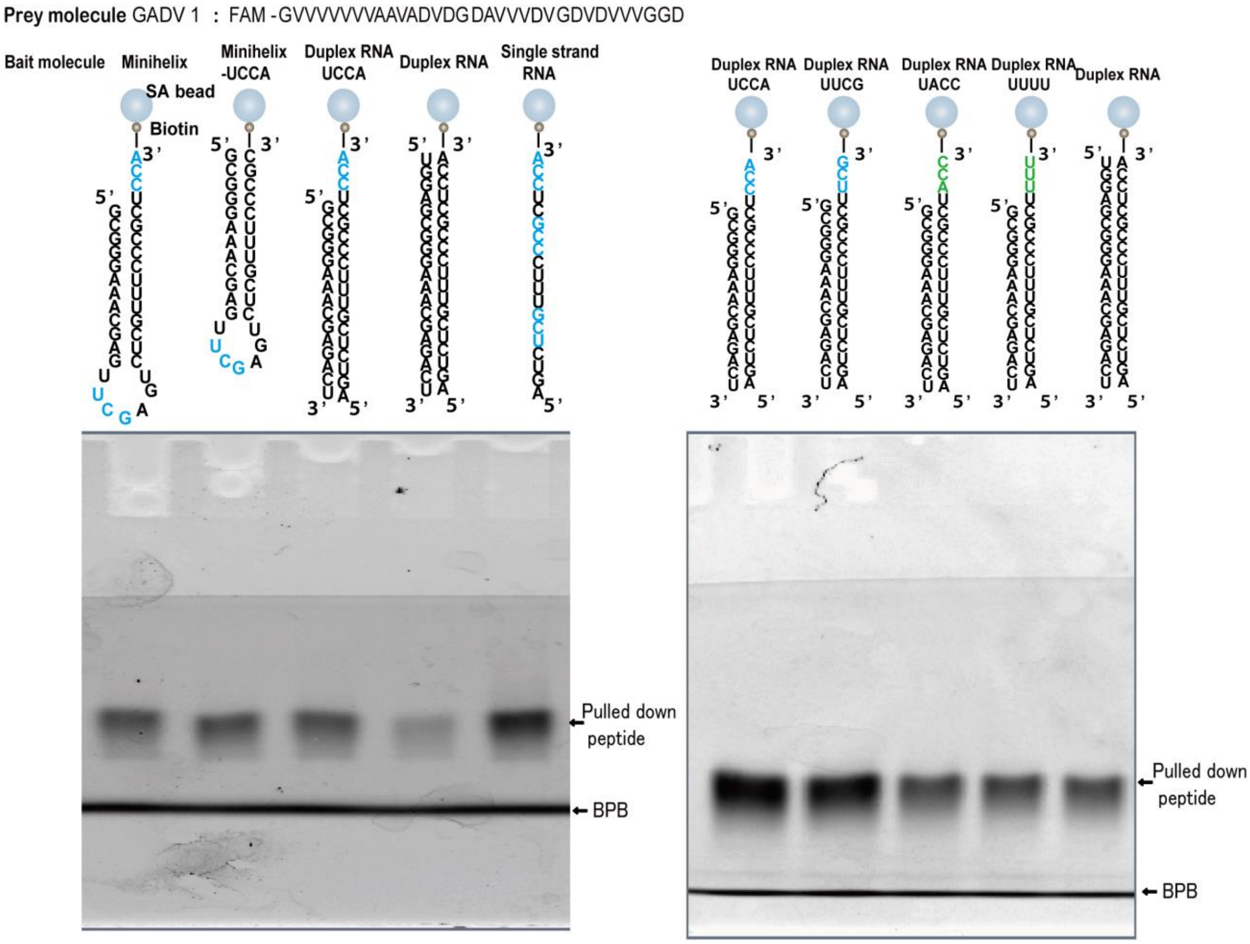


Figure S4 Full gel images for main text figures. The full gels are provided for main text Figure 2a and 2b.

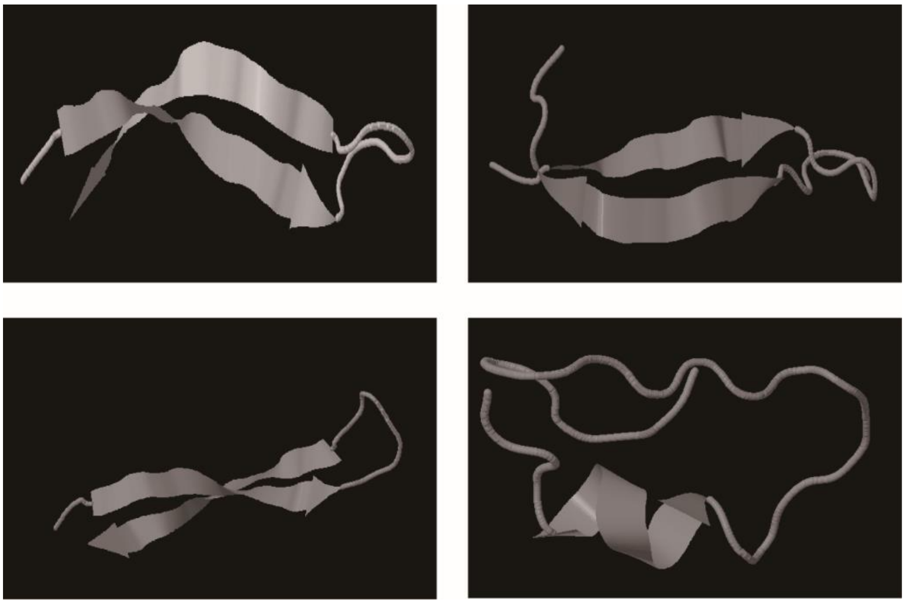


Figure S5. Four possible conformations of the GADV1 peptide obtained by PEP-FOLD.[5-7] Cartoon models are depicted using Jmol software. The peptide was predicted to form both β-sheet and α-helix secondary structures.

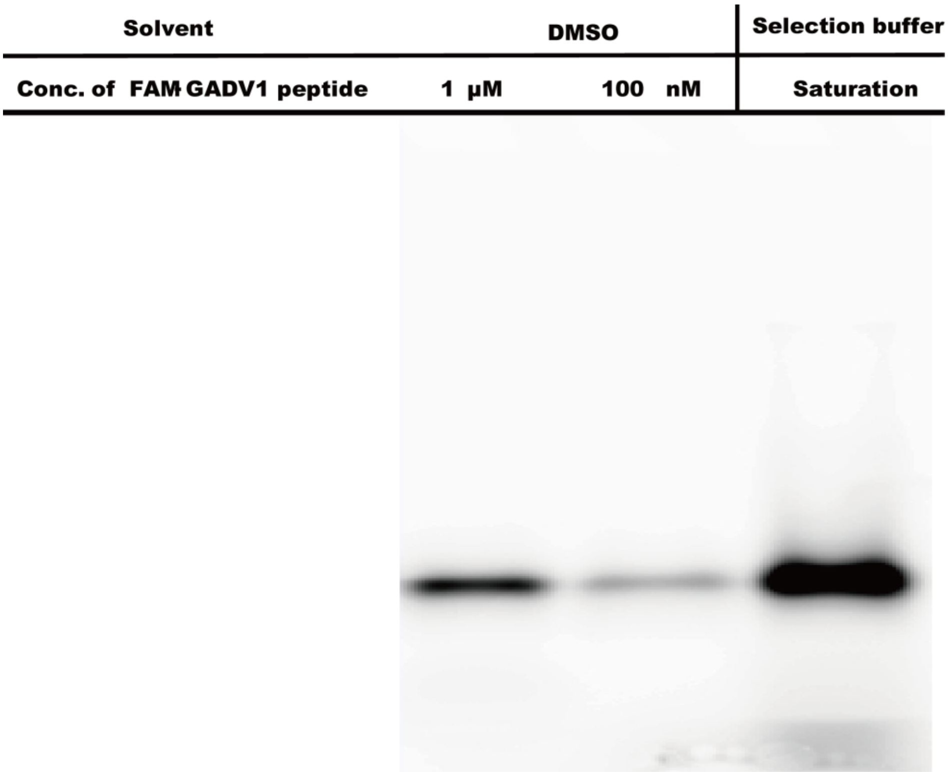
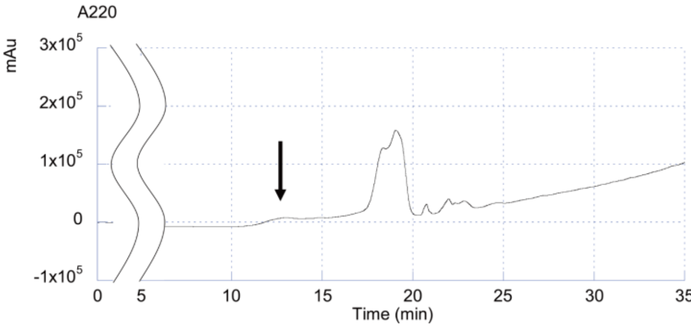
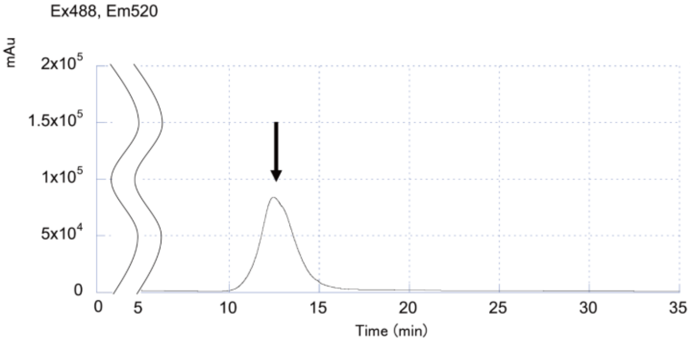


Figure S6. Tricine SDS-PAGE analysis of the GADV1 peptide for the estimation of peptide concentration. The GADV1 peptide was shaken at room temperature for 30 min in DMSO (1 μM and 100 nM) or the selection buffer at saturating concentration. The precipitate of GADV1 peptide formed in the selection buffer was removed by centrifugation and the supernatant was used for Tricine SDS-PAGE. Each GADV1 peptide in the solution at different concentrations was subjected to Tricine SDS-PAGE and visualized with a fluorescence image analyzer. The single bands were detected and the concentration of the GADV1 peptide in the saturated selection buffer solution was estimated to be 3–4 μM based on the band intensity.



Detector A-Ch1(220 nM)				
Peak#	R.Time	I.Time	F.Time	Area
1	7.724	7.408	4892	260
2	13.006	10.458	1144632	10281
3	14.826	13.867	449735	6682
4	15.081	15.033	86018	5951
5	18.366	15.283	5877247	119388
6	19.047	18.517	9085846	148369
7	20.765	20.392	304722	17556
8	21.979	21.158	625940	22129
9	22.335	22.2	246326	13455
10	22.843	22.525	515759	14414



Detector B-Ch1(Ex488, Em: 520)				
Peak#	R.Time	I.Time	F.Time	Area
1	12.45	9.375	18.058	11322785
2	20.458	20.25	21.358	3842

Figure S7. Reversed-phase HPLC analysis of the GADV1 peptide. The fraction corresponding to the peak having absorbance at 220 nm and emission at 520 nm at the same time was defined as the FAM-GADV1 peptide (arrow). The area of FAM-GADV1 peptide (monitored at 220 nm absorbance) represents 3% of the total peptides. If the other 97% was defined as GADV1 without FAM, the total concentration of GADV1 ([FAM-GAVD1] + [GADV1]) was estimated to be 17 μM in binding assay of GADV1 against tRNA by EMSA. Even so, the apparent concentration of the concentration of tRNA at which 50% of the peptide is bound (66 μM) was determined to be higher than that of GADV1 (17 μM).

(1) Sato, K.; Hamada, M.; Asai, K.; Mituyama, T. CENTROIDFOLD: a web server for RNA secondary structure prediction. *Nucleic Acids Res.* ***2009,*** *W277-80,* Doi: 10.1093/nar/gkp367

(2) Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **2003**, *31*, 3406-3415

(3) Waugh,A.; Gendron, P.; Altman, R.; Brown, J. W.; Case, D. ; Gautheret, D.; Harvey, S. C.; Leontis, N.; Westbrook, J.; Westhof, E.; Zuker, M.; Major, F. RNAML: A standard syntax for exchanging RNA information. *RNA* **2002,** *8*, 707-717

(4) Zuker, M.; Jacobson, A. B. Using Reliability Information to Annotate RNA Secondary Structures. *RNA* **1998**, *4*, 669-679

(5) Maupetit, J.; Derreumaux, P.; Tufféry, P. PEP-FOLD: an online resource for de novo peptide structure prediction. *Nucleic Acids Res.* **2009**, *37*, W498-503, Doi: 10.1093/nar/gkp323

(6) Maupetit, J.; Derreumaux, P.; Tufféry, P. A fast method for large-scale de novo peptide and miniprotein structure prediction. *J. Comput. Chem*. **2010**, *31*, 726-738

(7) Thévenet, P.; Shen,Y.; Maupetit, J.; Guyon, F.; Derreumaux, P.; Tufféry, P. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. *Nucleic Acids Res*. **2012**, *40*, W288-293, Doi: 10.1093/nar/gks419