Supplementary Materials for

"Position-dependent Effects of Regioisomeric Methylated Adenine and Guanine Ribonucleosides on Translation"

by

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Figure S1. Schematic diagrams illustrating the preparation of methylated purine ribonucleoside-bearing translation templates for the reconstituted *E. coli* translation reactions **(a)** or the wheat germ extract-mediated translation reactions **(b)**. 'X' indicates an m^1G, m^2G, m^6G or G, and the ribosome binding sites were underlined. Only the RNA templates containing a methylated ribonucleoside at the first position of sixth codon of the mRNA were shown.

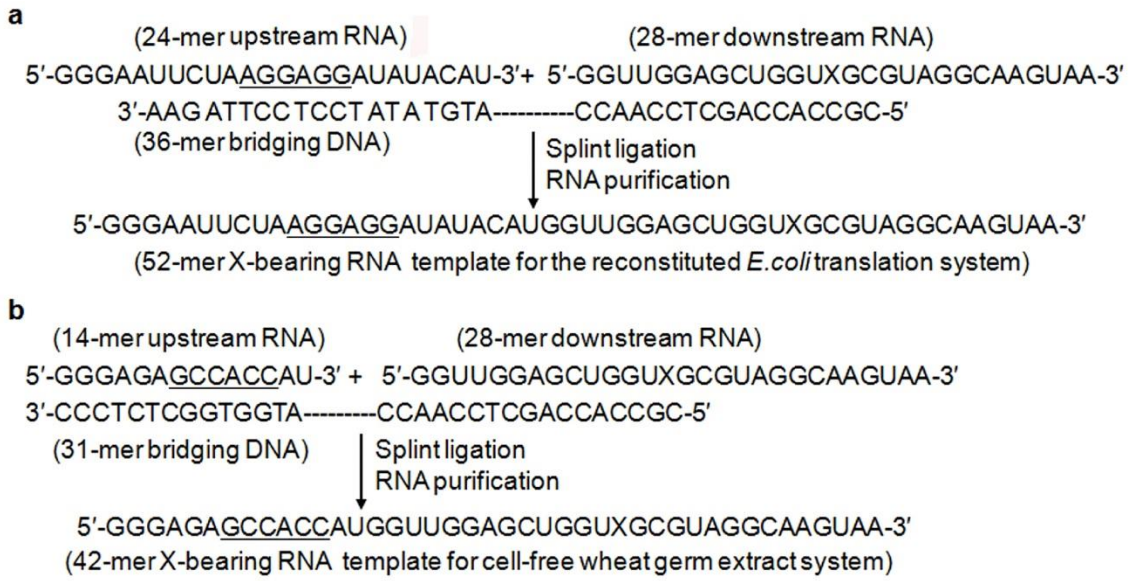


Figure S2. Representative gel image (**a**) and quantification data (**b**) for determining the relative concentrations of mRNA templates carrying a G, m^1G, m^2G and m^6G for the reconstituted *E. coli* translation reactions. The relative concentration was calculated by normalizing the ratio of RNA adduct signal to the reference signal against the ratio of the corresponding competitor (i.e., 'Comp') signal to the reference signal. '1st', '2nd' and '3rd' indicate the first, second and third positions of the codon, respectively. The data represent the mean and standard error of results from three independent experiments.

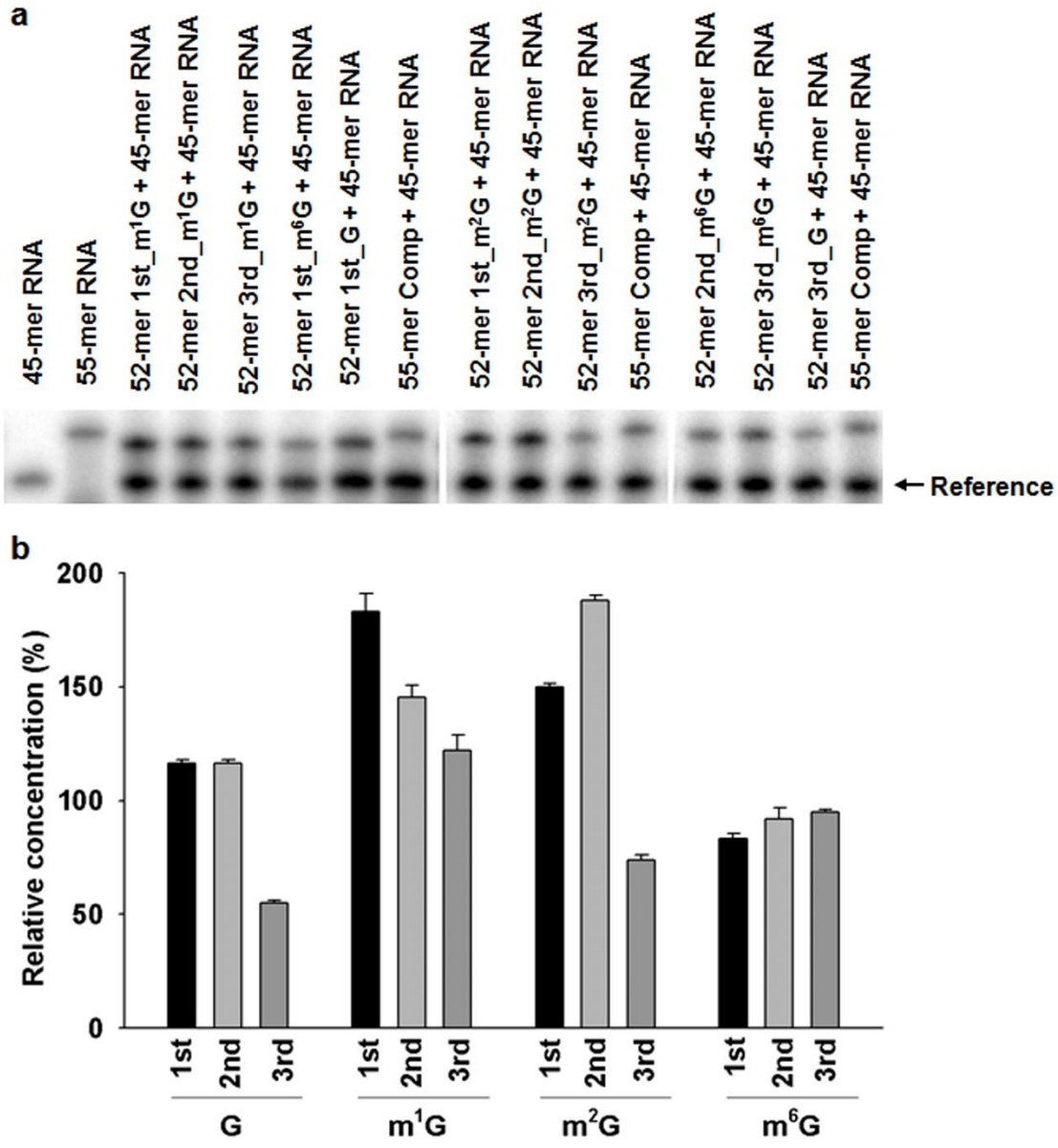


Figure S3. Representative gel image (**a**) and quantification data (**b**) for determining the relative concentrations of mRNA templates carrying an A, m^1A and m^6A for the reconstituted *E. coli* translation reactions. The relative concentration was calculated by normalization of the ratio of RNA adduct signal to the reference signal against the ratio of the corresponding competitor (i.e., 'Comp') signal to the reference signal. '1st', '2nd' and '3rd' indicate the first, second and third positions of the codon, respectively. The data represent the mean and standard error of results from three independent experiments.

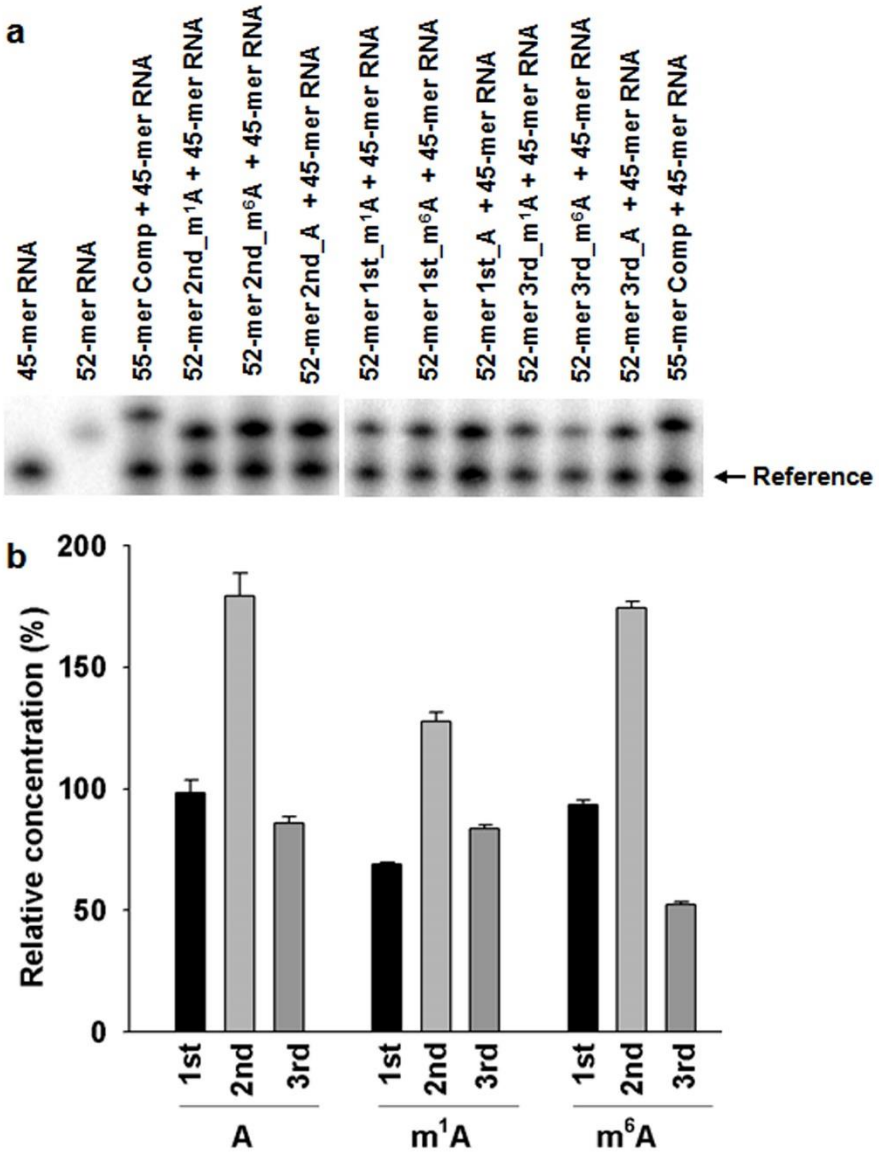


Figure S4. mRNA templates employed in this study. ‘X’ designates an unmodified or methylated purine ribonucleoside. ‘RBS’ and ‘STOP’ indicate the ribosome binding site and the stop codon, respectively.

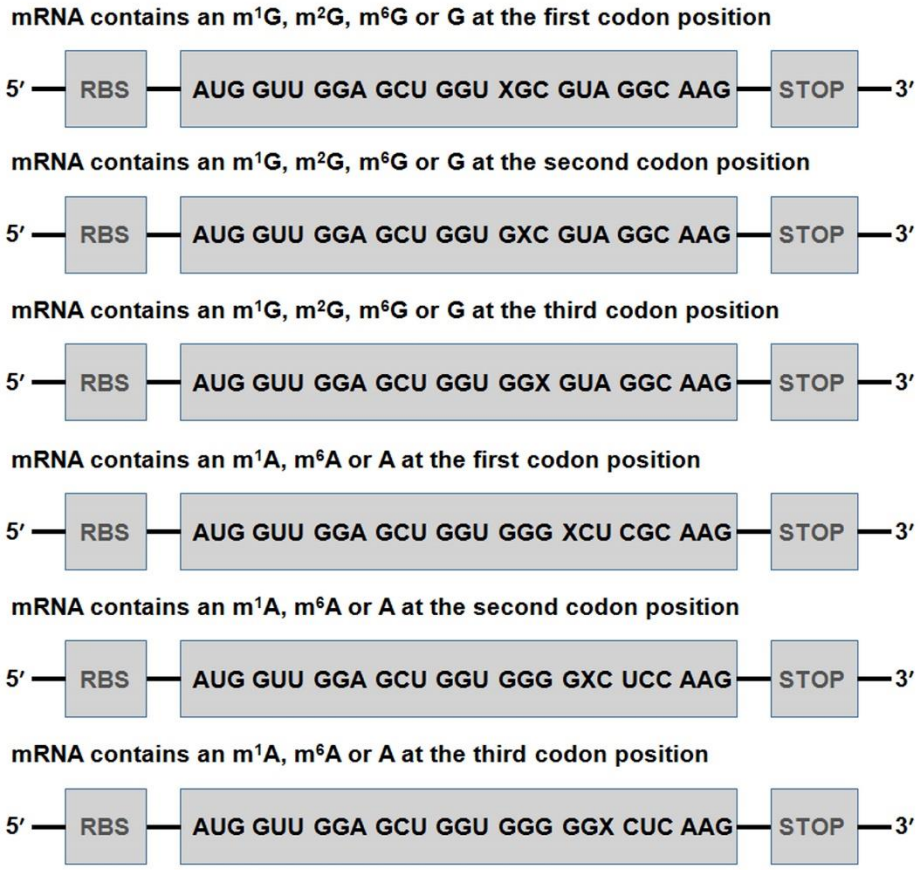


Figure S5. The MS/MS of the $[M+2H]^{2+}$ ion of the wild-type peptide product MVGAGGVGK and the competitor peptide product MVVGAGGVGK from the reconstituted *E. coli* translation reaction, where 'GGC' was used as the sixth codon of the unmodified mRNA template.

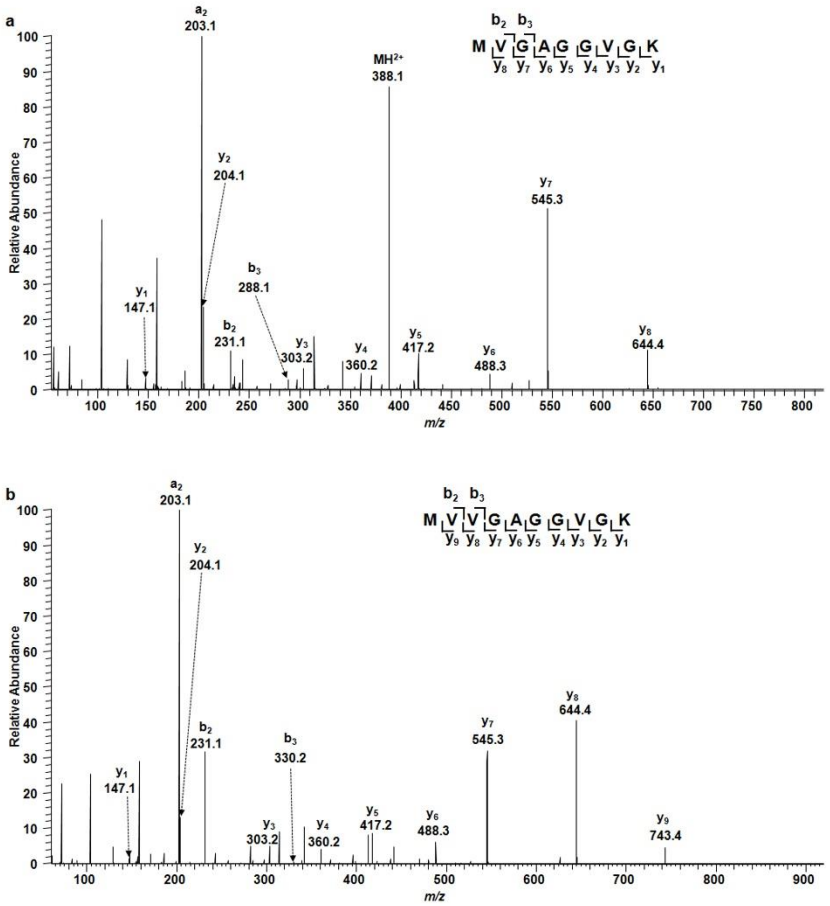


Figure S6. The relative abundances of the wild-type peptide product MVGAGGVGK (i.e., 9AA-WT), the mutant peptide product MVGAGDVGK (i.e., 9AA-Mu), and the competitor peptide product MVVGAGGVGK (i.e., 10AA-Comp) from the reconstituted *E. coli* translation reactions, where GGC (**a**) and Gm^6GC (**b**) were employed as the sixth codon for mRNA templates, respectively. 'MA', peak area found in the selected-ion chromatogram for monitoring the formation of the $[M+2H]^{2+}$ ions for the peptide products. (**c-d**) The MS/MS of the $[M+2H]^{2+}$ ion of wild-type (**c**) and the mutant peptide product MVGAGDVGK (**d**) formed from the reconstituted *E. coli* translation reaction, where Gm^6GC was used as the sixth codon for the mRNA template.

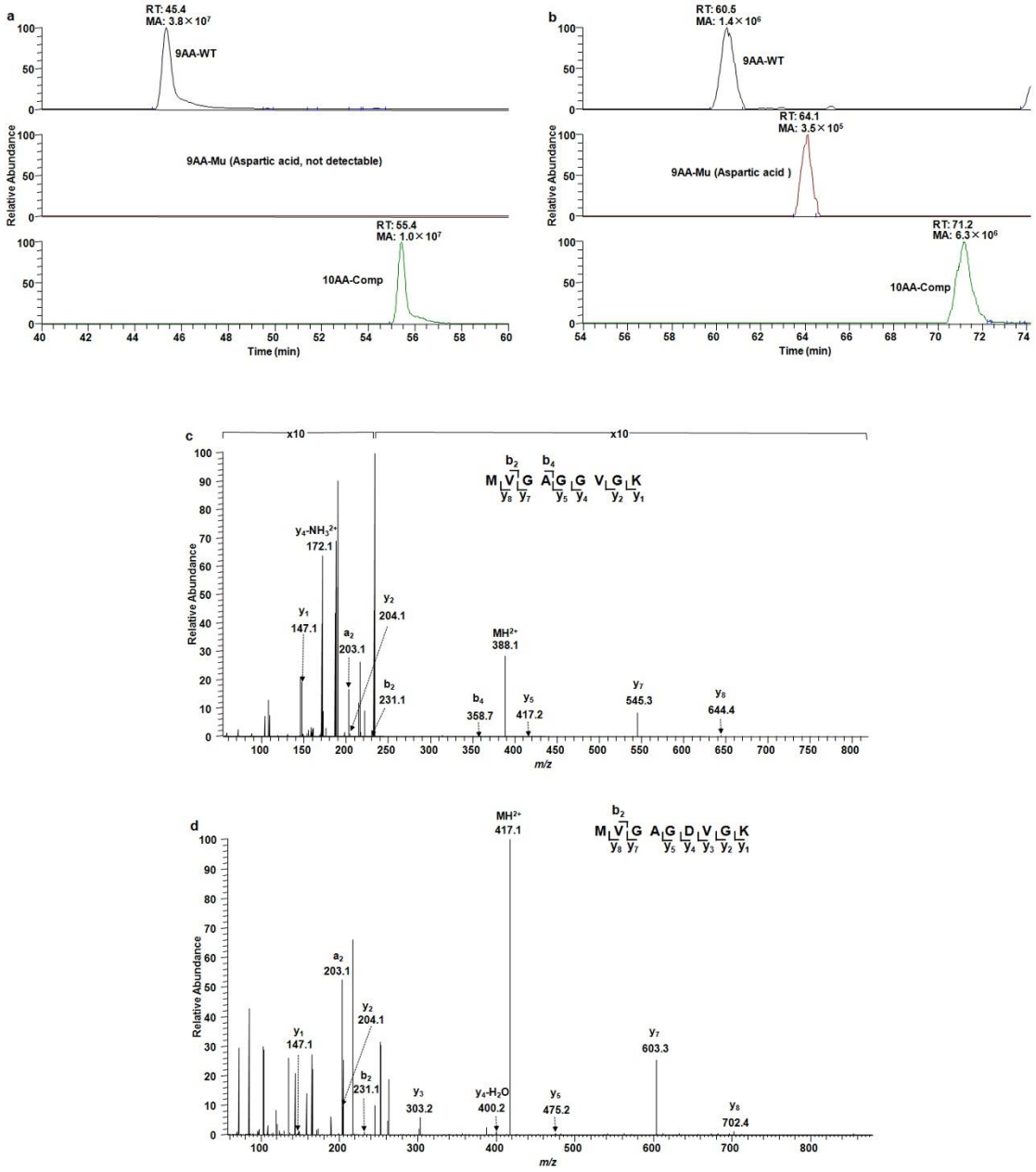


Figure S7. The relative abundance of the wild-type peptide product MVGAGGVGK (i.e., 9AA-WT), the mutant peptide product MVGAGCVGK and MVGAGSVGK (i.e., 9AA-Mu), and the competitor peptide product MVVGAGGVGK (i.e., 10AA-Comp) from the wheat germ extract-mediated translation reactions, where GGC (**a**) and m^6GGC (**b**) were used as the sixth codon for the mRNA templates, respectively. 'MA', peak area found in the selected-ion chromatogram for monitoring the formation of the $[M+2H]^{2+}$ ions for the peptide products. (**c-e**) The MS/MS of the $[M+2H]^{2+}$ ion of wild-type (**c**) and the mutant peptide products MVGAGCVGK (**d**) and MVGAGSVGK (**e**) from the wheat germ extract-mediated translation reaction of the m^6G-bearing mRNA templates.

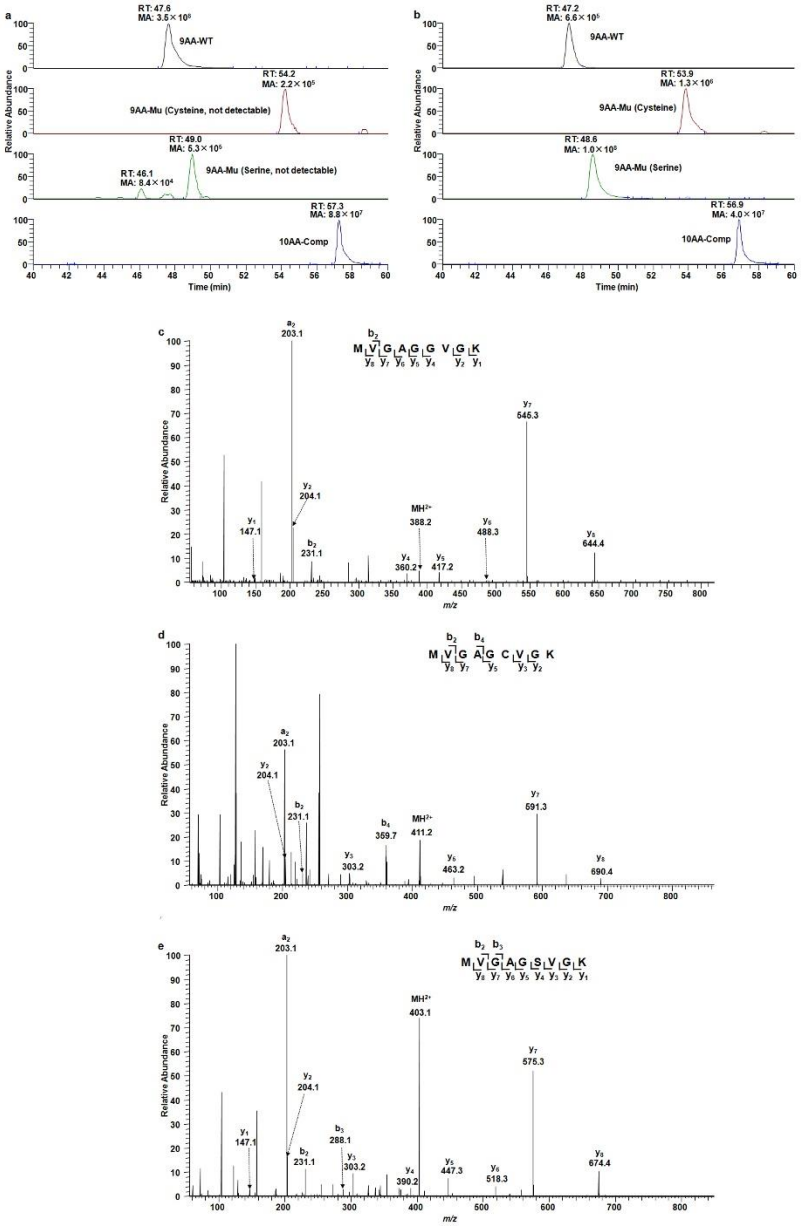
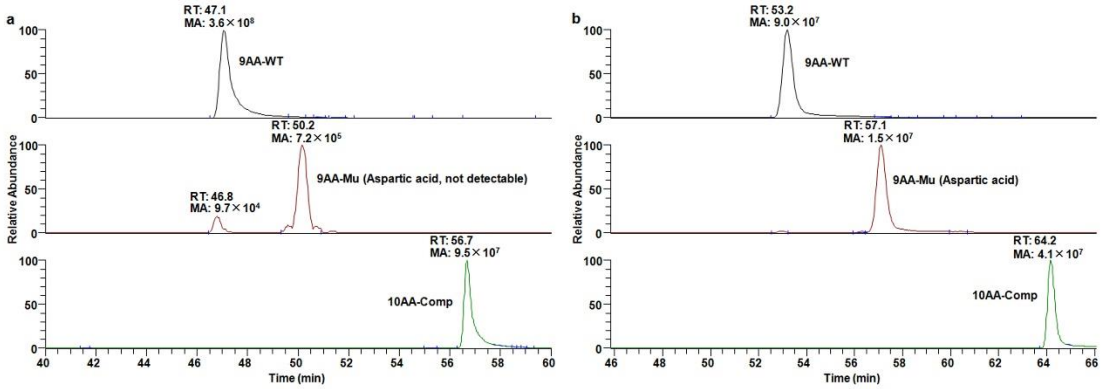
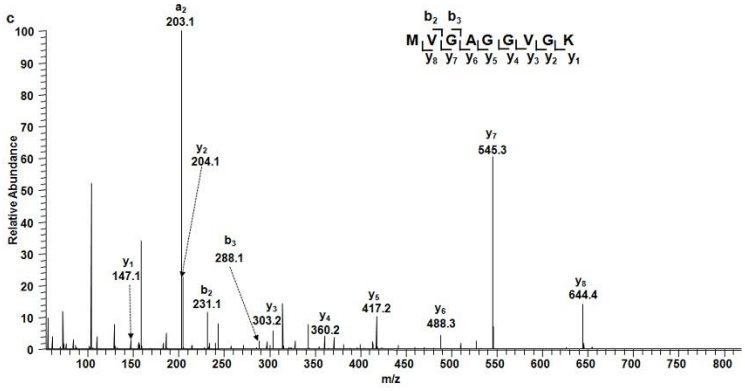


Figure S8. The relative abundance of the wild-type peptide product MVGAGGVGK (i.e., 9AA-WT), the mutant peptide product MVGAGDVGK (i.e., 9AA-Mu), and the competitor peptide product MVVGAGGVGK (i.e., 10AA-Comp) from the wheat germ extract-mediated translation reactions, where GGC (**a**) and Gm^{6}GC (**b**) were used as the sixth codon of the mRNA templates, respectively. 'MA', peak area found in the selected-ion chromatogram for monitoring the formation of the $[M+2H]^{2+}$ ions for the peptide products. (**c-d**) The MS/MS of the $[M+2H]^{2+}$ ion of wild-type (**c**) and the mutant peptide products MVGAGDVGK (**d**) from the wheat germ extract-mediated translation reaction of the m^{6}G-bearing mRNA templates.





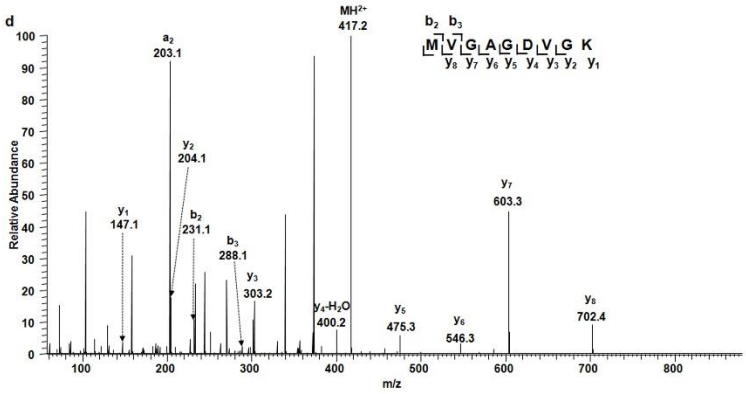


Figure S9. The relative abundances of the wild-type peptide product MVGAGGVGK (i.e., 9AA-WT), the mutant peptide products MVGAGCVGK and MVGAGRVGK (i.e., 9AA-Mu), and the competitor peptide product MVVGAGGVGK (i.e., 10AA-Comp) from the wheat germ extract-mediated translation reactions, where GGC (**a**) and m^1GGC (**b**) were used as the sixth codon for the mRNA templates, respectively. 'MA', peak area found in the selected-ion chromatogram for monitoring the formation of the $[M+2H]^{2+}$ ions for the peptide products. (**c-d**) The MS/MS of the $[M+2H]^{2+}$ ion of the mutant peptide products MVGAGCVGK (**c**) and MVGAGRVGK (**d**) from the wheat germ extract-mediated translation reaction of the m^1G-bearing mRNA templates.