Importance of tRNA anticodon loop modification and a conserved, noncanonical anticodon stem pairing in $tRNA^{Pro}_{CGG}$ for decoding

SUPPORTING INFORMATION

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Running title: Anticodon stem-loop elements affect $tRNA^{Pro}_{CGG}$ decoding

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SUPPLEMENTARY INFORMATION

Table S1. Sequences of RNA used in this study.

Insertions are bolded, the anticodon is underlined, and the N1 methylation at position 37 is noted as m^1G for ASLs.

ASL^{SufA6}	GCU CGU U<u>CG G</u>(m^1G)**G** ACG AGC
ASL^{SufA6} Δm^1G37	GCU CGU U<u>CG G</u>G**G** ACG AGC
ASL^{SufA6} A37.5	GCU CGU U<u>CG G</u>(m^1G)**A** ACG AGC
ASL^{SufA6} A37.5 Δm^1G37	GCU CGU U<u>CG G</u>G**A** ACG AGC
ASL^{Pro}	GCU CGU U<u>CG G</u>(m^1G)A CGA GC
ASL^{Pro} Δm^1G37	GCU CGU U<u>CG G</u>GA CGA GC
mRNA_Asite_CCG	GGC AAG GAG GUA AAA AUG CCG UAC CA
mRNA_Asite_CCCU	GGC AAG GAG GUA AAA AUG CCCU ACCA

Table S2. K_d and B_{max} values (best fit ± SE) for ASL^{Pro}, ASL^{SufA6}, and ASL^{SufA6} A37.5 as determined by filter binding experiments at equilibrium.

Binding constants were obtained by fitting the data from 6 replicates to a one site specific binding model using GraphPad Prism.

	mRNA			
	CCG		CCCU	
	K_d (nM)	B_{max}	K_d (nM)	B_{max}
ASL^{Pro}	284 ± 27	0.69 ± 0.03	351 ± 79	0.20 ± 0.02
ASL^{Pro} Δm^1G37	1795 ± 475	0.42 ± 0.08	274 ± 46	0.18 ± 0.01
ASL^{SufA6}	781 ± 333	0.25 ± 0.06	767 ± 173	0.10 ± 0.01
ASL^{SufA6} Δm^1G37	497 ± 187	0.15 ± 0.03	791 ± 180	0.51 ± 0.06
ASL^{SufA6} A37.5 Δm^1G37	4558 ± 4067	0.27 ± 0.21	212 ± 9	0.81 ± 0.01
ASL^{SufA6} A37.5	1084 ± 188	0.19 ± 0.02	444 ± 98	0.055 ± 0.006

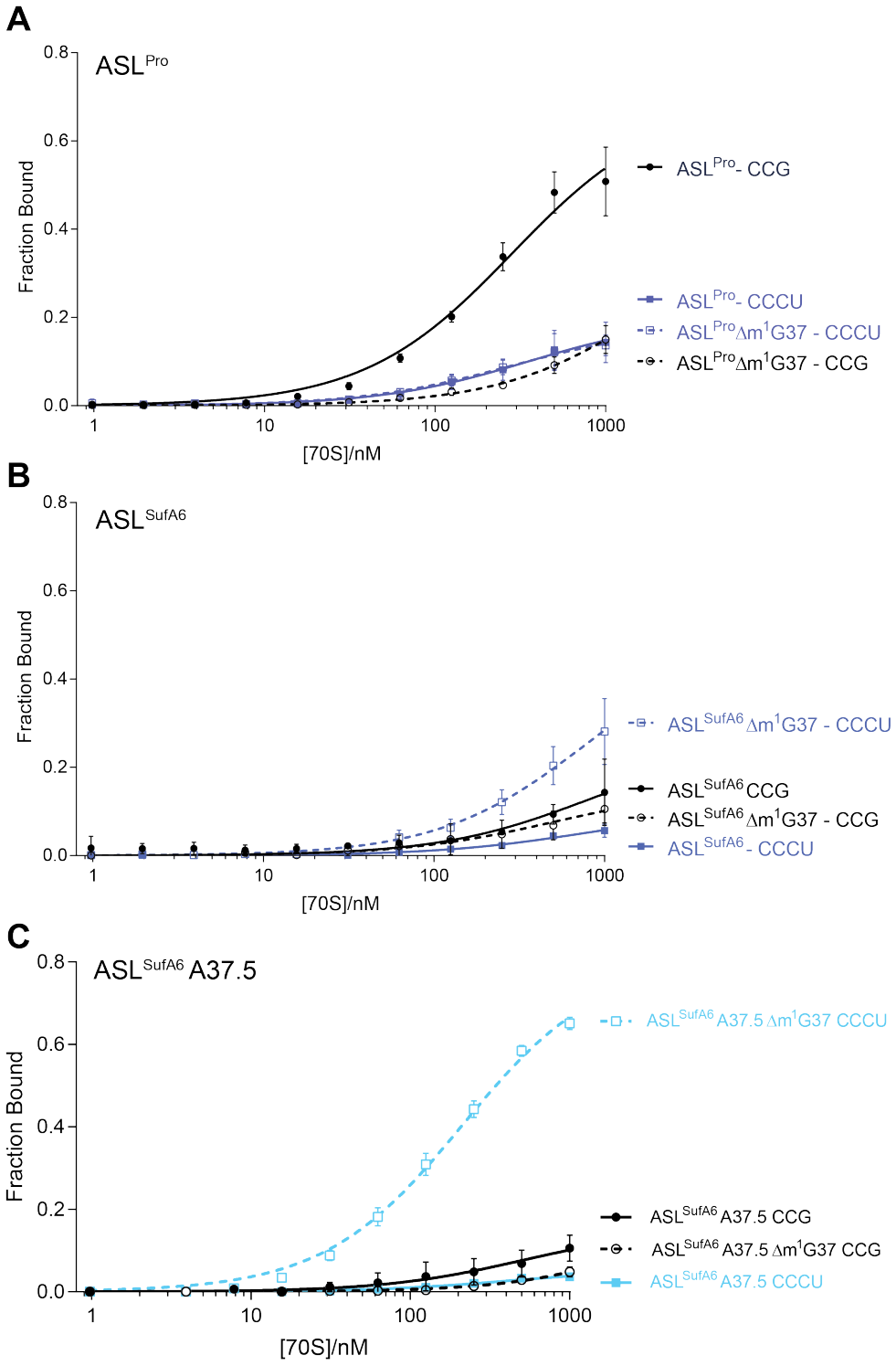

Figure S1. Equilibrium binding curves ASL^{Pro}, ASL^{SufA6}, and ASL^{SufA6} A37.5.
Binding constants were obtained by fitting the data from 6 replicates to a one site specific binding model using GraphPad Prism.

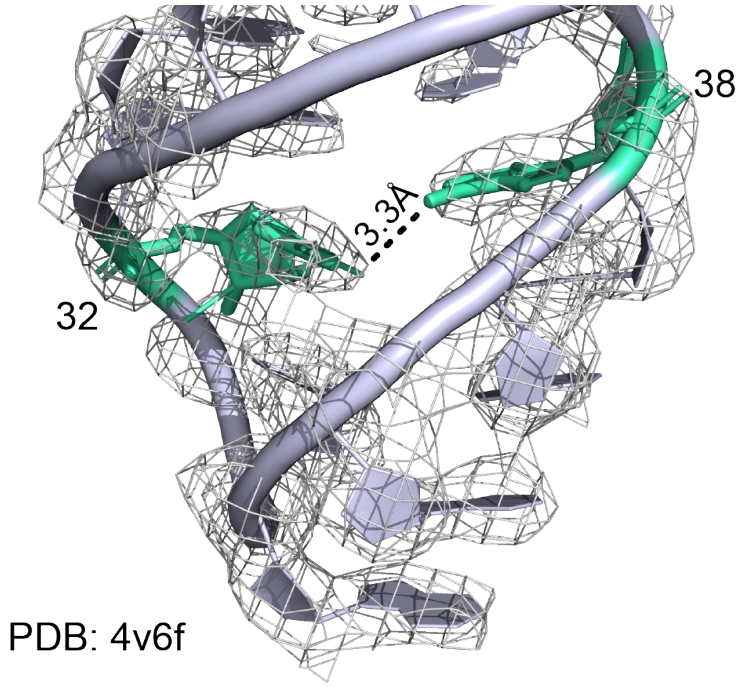

Figure S2. The 32-38 pairing in tRNA^{Phe} shows a conserved hydrogen bond (PDB code 4V6F; (1)) similar to that of modified tRNA^{Pro} binding to a cognate codon.
$2F_o$-F_c electron density map contoured at 1.5σ is shown in gray.

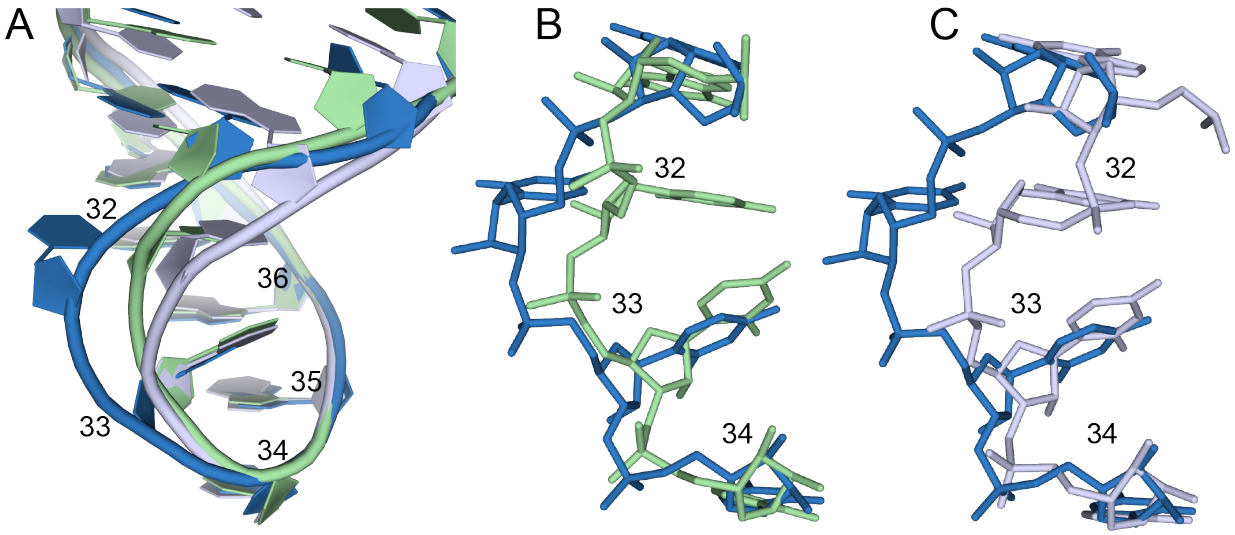

Figure S3. The phosphate backbone of the ASL^{SufA6} is widened in the case of the A37.5 insertion. *A*, Overlay of ASL^{SufA6} A37.5 Δm^{1}G37 bound to CCC-U codon (blue, PDB code 6NDK;(2)), ASL^{Pro} bound to CCG (green, PDB code 4LT8; (2)), and ASL^{SufA6} bound to CCC-U (gray, PDB code 4L47; (2)) showing the reordering of the ASL on the opposite side of the insertion.
B-C, ASL^{SufA6} A37.5 Δm^{1}G37 bound to A-site CCC-U (blue) codon adopts a conformation more similar to ASL^{Pro} on CCG (green) than that of ASL^{SufA6} bound to CCC-U (gray).

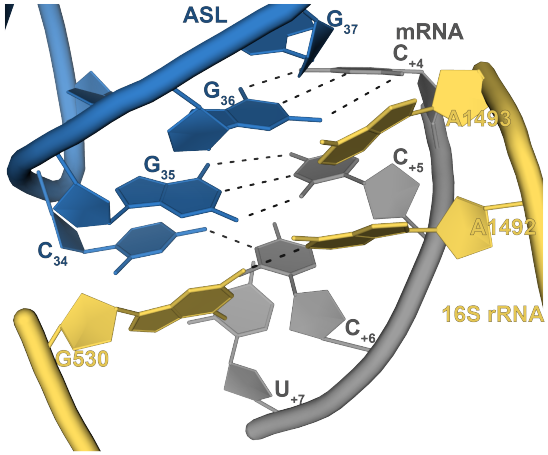

Figure S4. The ASL^{SufA6} A37.5 is accepted by the ribosome.
16S rRNA nucleotides A1492 and A1493 flip from the internal helix 44 and G530 is positioned close to A1492 demonstrating recognition by the ribosome. Paromomycin was not added to the crystallization complex formation.

REFERENCES

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