

**Disruption of evolutionarily correlated tRNA elements  
impairs accurate decoding**

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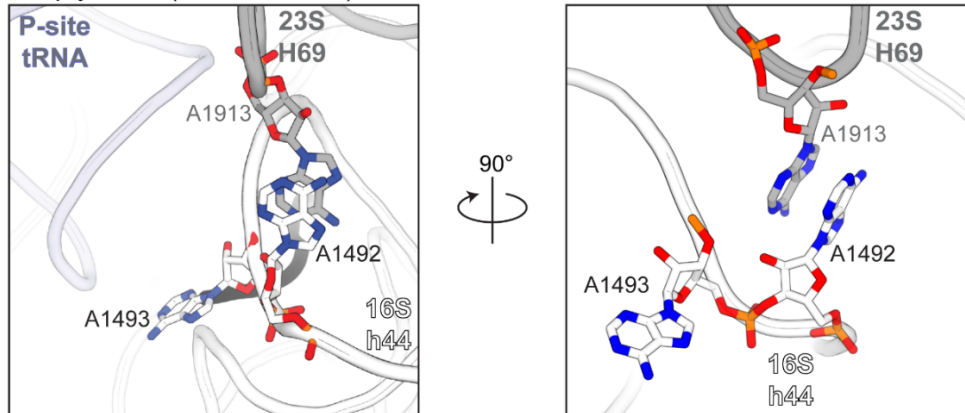
Supporting Information

**This PDF file includes:**

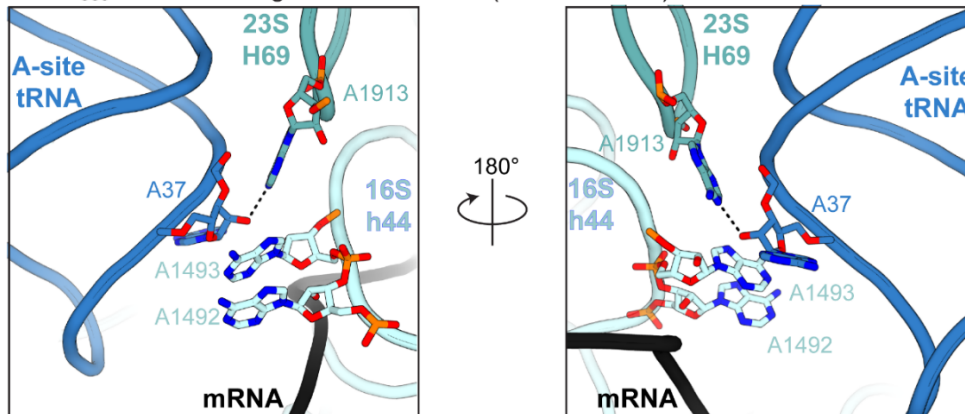
Figures **S1** to **S7**  
SI References

## SUPPLEMENTARY FIGURES

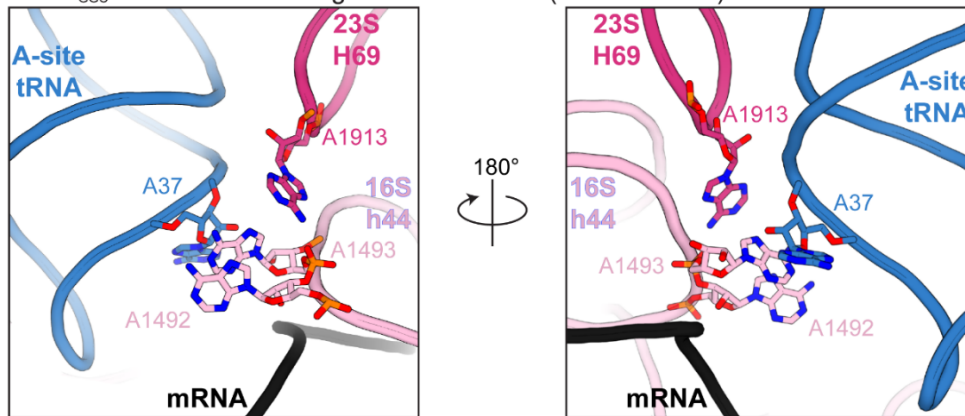
**A** Empty A site (PDB ID 5MDZ)



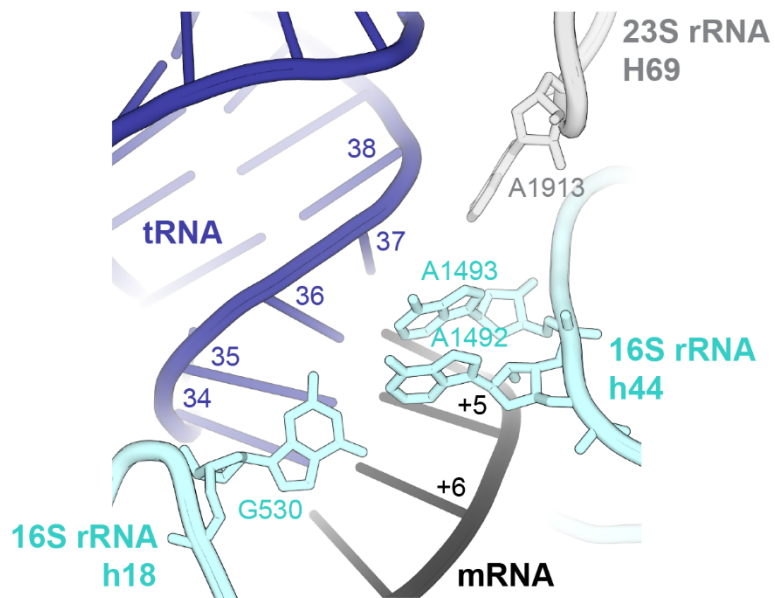
**B** tRNA<sup>Ala</sup><sub>GCC</sub> bound to a cognate GCC codon (PDB ID 6OF6)



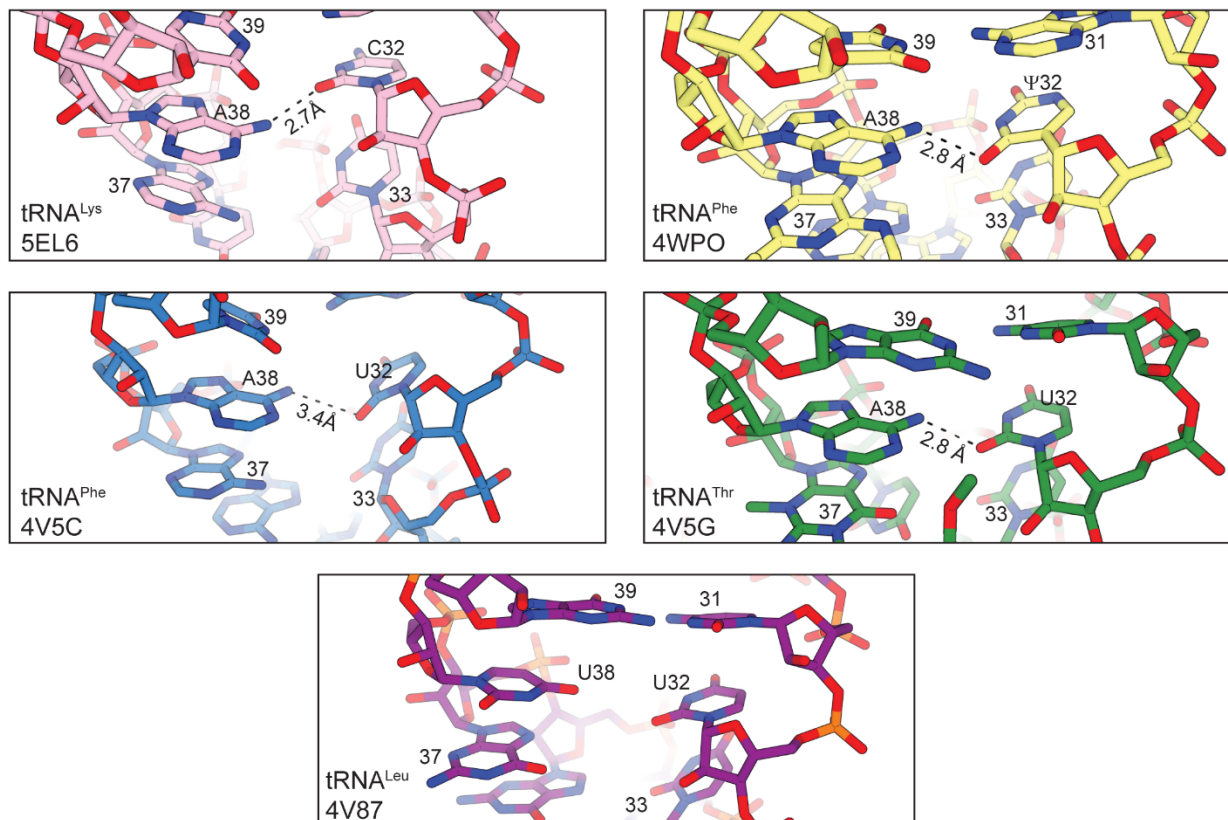
**C** tRNA<sup>Ala</sup><sub>GCC</sub> bound to a near-cognate GCA codon (PDB ID 6OJ2)



**Figure S1. Conformational changes of rRNA nucleotides 23S rRNA A1913 (from Helix 69) and 16S rRNA A1492-1493 (from helix 44) during decoding.** **A**, In the absence of tRNA in the A site, A1913 and A1492 form a stacking interaction (PDB code 5MDZ (1)). **B**, When tRNA<sup>Ala</sup><sub>GCC</sub> binds to a cognate GCC codon in the A site, A1492 flips to form stacking interactions with A1493 replacing A1492's interaction with A1913, and A1913 also stacks against the tRNA backbone. **C**, When tRNA<sup>Ala</sup><sub>GCC</sub> binds to a near-cognate GCA codon in the A site, A1492 flips to stack with A1493, A1913 partially swings towards the A-site tRNA but the nucleobase is not engaged with the tRNA backbone.

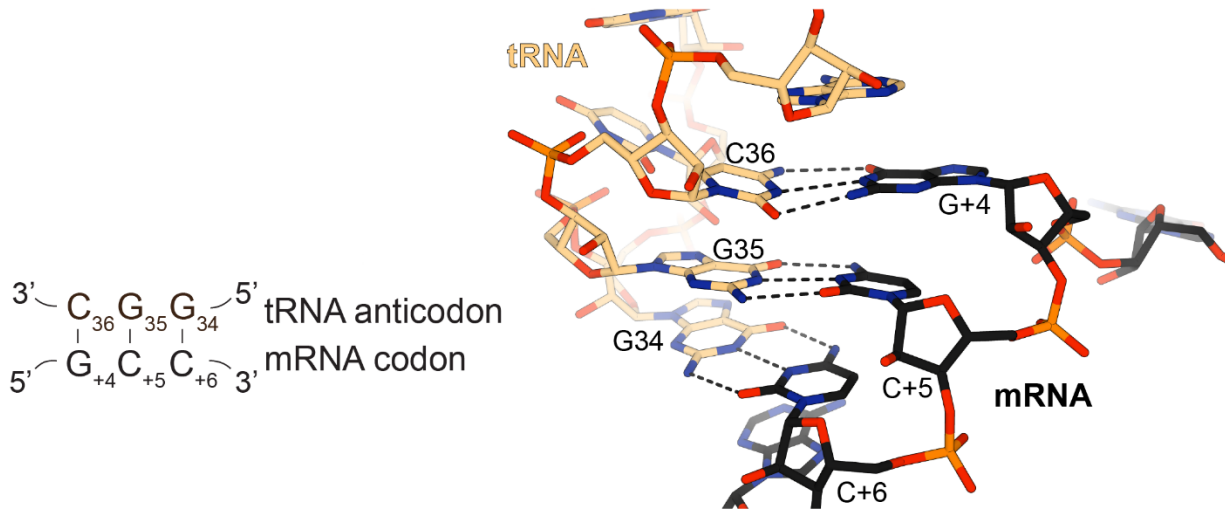


**Figure S2. The decoding center of the ribosome.** 16S rRNA nucleotides G530 (from helix 18), A1492 and A1493 (from helix 44) (teal) inspect the A-site tRNA anticodon (blue) and mRNA codon (black) interaction, while 23S rRNA nucleotide A1913 (from Helix 69) (gray) packs against the tRNA phosphate backbone.

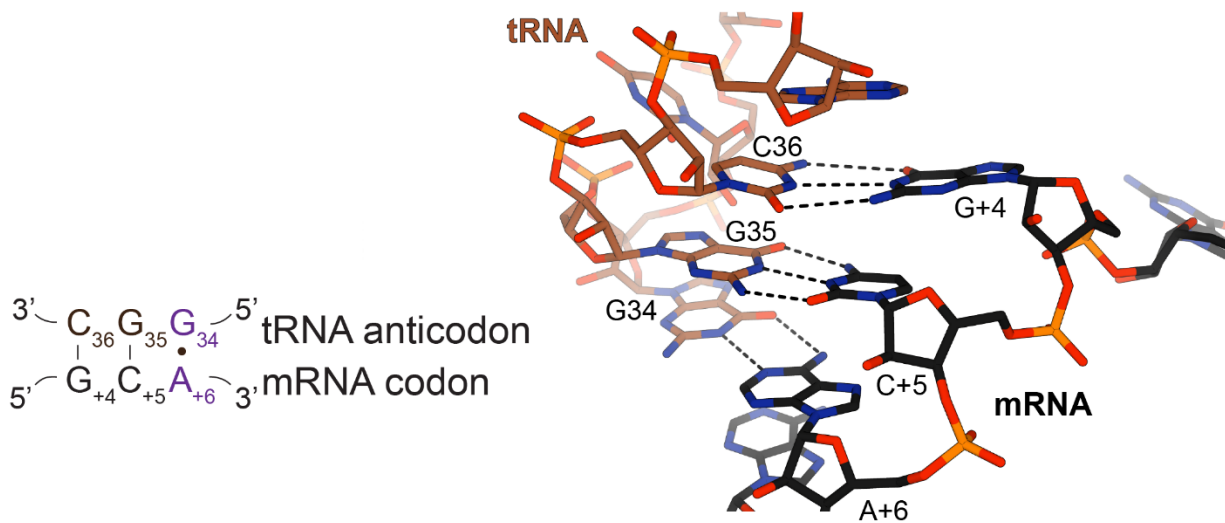


**Figure S3. The 32-38 pair in tRNAs bound to the ribosomal A site.** Structures of different tRNAs bound to the 70S showing an interaction between nucleotides 32 and 38 in the case of C•A, Ψ•A, and U•A. The U32-U38 base pair does not appear to form an interaction (PDB codes 5EL6 (1), 4WPO (2), 4V5C (3), 4V5G (4), 4V87 (5)). Ψ: pseudouridine.

**A** tRNA<sup>Ala</sup><sub>GGC</sub> **U32-A38** bound to a cognate GCC codon (PDB ID 6ORD)

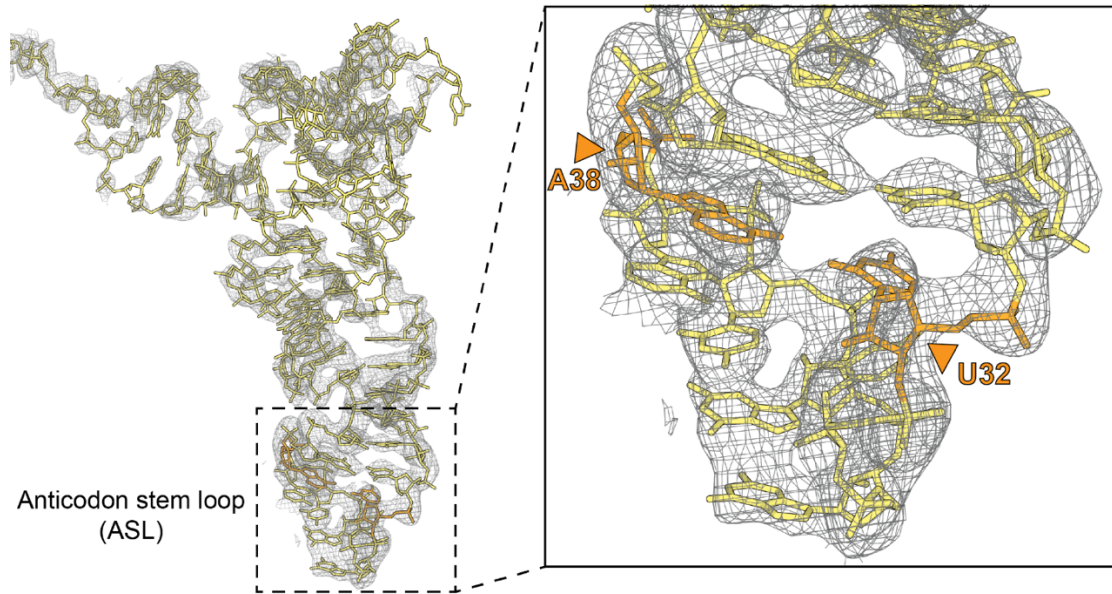


**B** tRNA<sup>Ala</sup><sub>GGC</sub> **U32-A38** bound to a near-cognate GCA codon (PDB ID 6OPE)

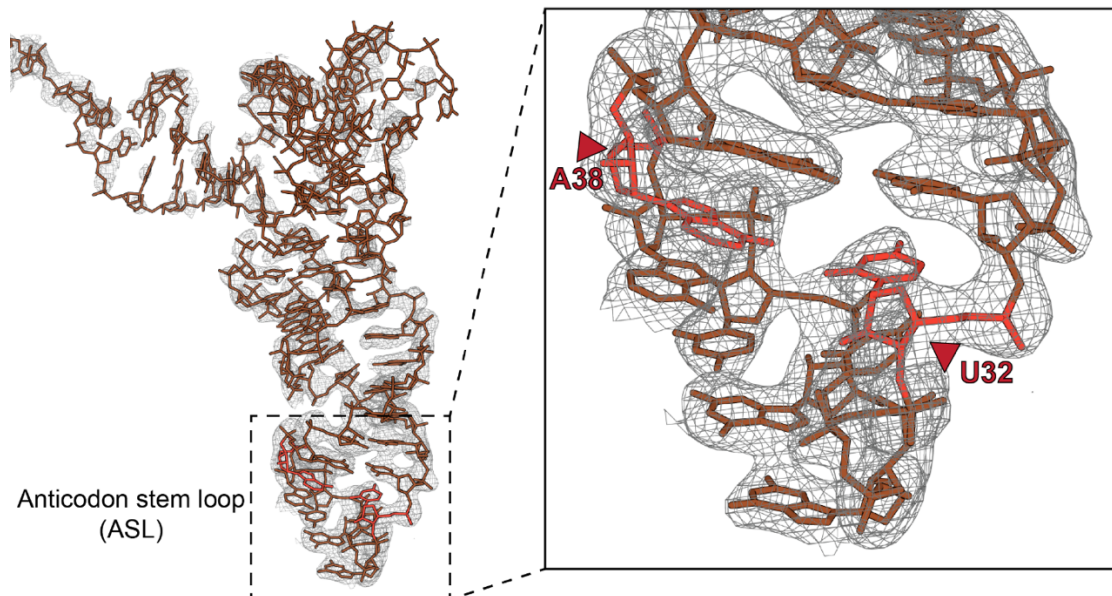


**Figure S4.** The codon-anticodon interactions in structures of the tRNA<sup>Ala</sup><sub>GGC</sub> U32-A38 mutant bound in the A site. The interaction is maintained for the cognate (panel **A**) and near-cognate (panel **B**) codons similar to that in the wild-type tRNA. This is similar to the codon-anticodon interactions shown in Figs. 2,3.

**A** tRNA<sup>Ala</sup><sub>GGC</sub> **U32-A38** bound to a cognate GCC codon (PDB ID 6ORD)

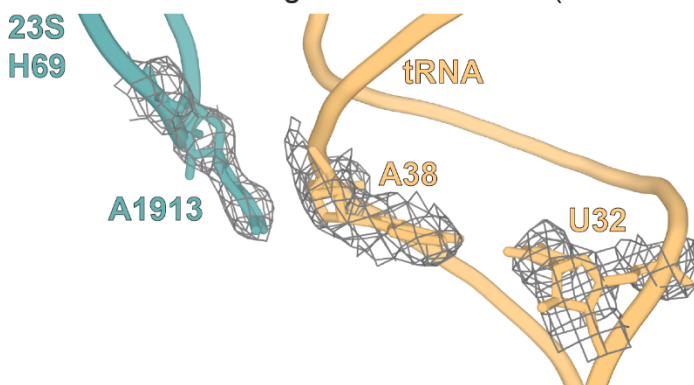


**B** tRNA<sup>Ala</sup><sub>GGC</sub> **U32-A38** bound to a near-cognate GCA codon (PDB ID 6OPE)

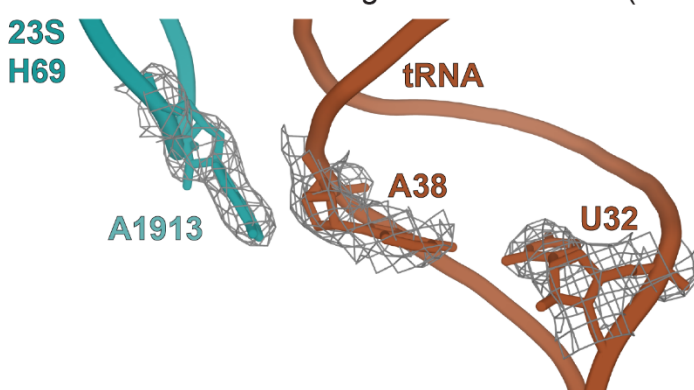


**Figure S5. tRNA<sup>Ala</sup><sub>GGC</sub> with the reversed 32-38 pairing shows good electron density of the pairing even when bound to a near-cognate codon.** **A**, In the structure of tRNA<sup>Ala</sup><sub>GGC</sub> with the reversed 32-38 pairing bound to a cognate codon in the A site, the tRNA shows good electron density for the whole tRNA and, in particular, for the 32-38 pair (inset). **B**, In the structure of 70S-tRNA<sup>Ala</sup><sub>GGC</sub> with the reversed 32-38 pairing bound to a near-cognate codon in the A site, there is good electron density for the whole tRNA and, in particular, for the 32-38 pair (inset). This is in contrast to the structure of 70S with wild-type tRNA<sup>Ala</sup><sub>GGC</sub> where the 32-38 pair shows a lack of electron density in the presence of a near-cognate codon. The 2F<sub>o</sub>-F<sub>c</sub> electron density maps (gray mesh) are contoured at 1 $\sigma$ .

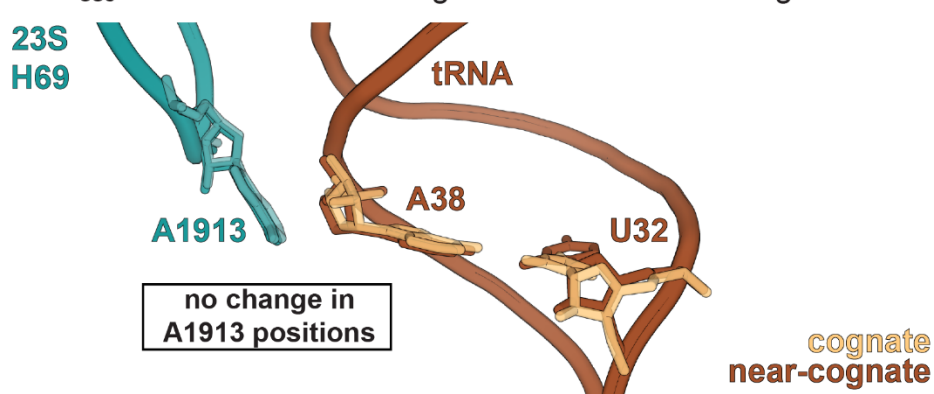
**A** tRNA<sup>Ala</sup><sub>GCC</sub> **U32-A38** bound to a cognate GCC codon (PDB ID 6ORD)



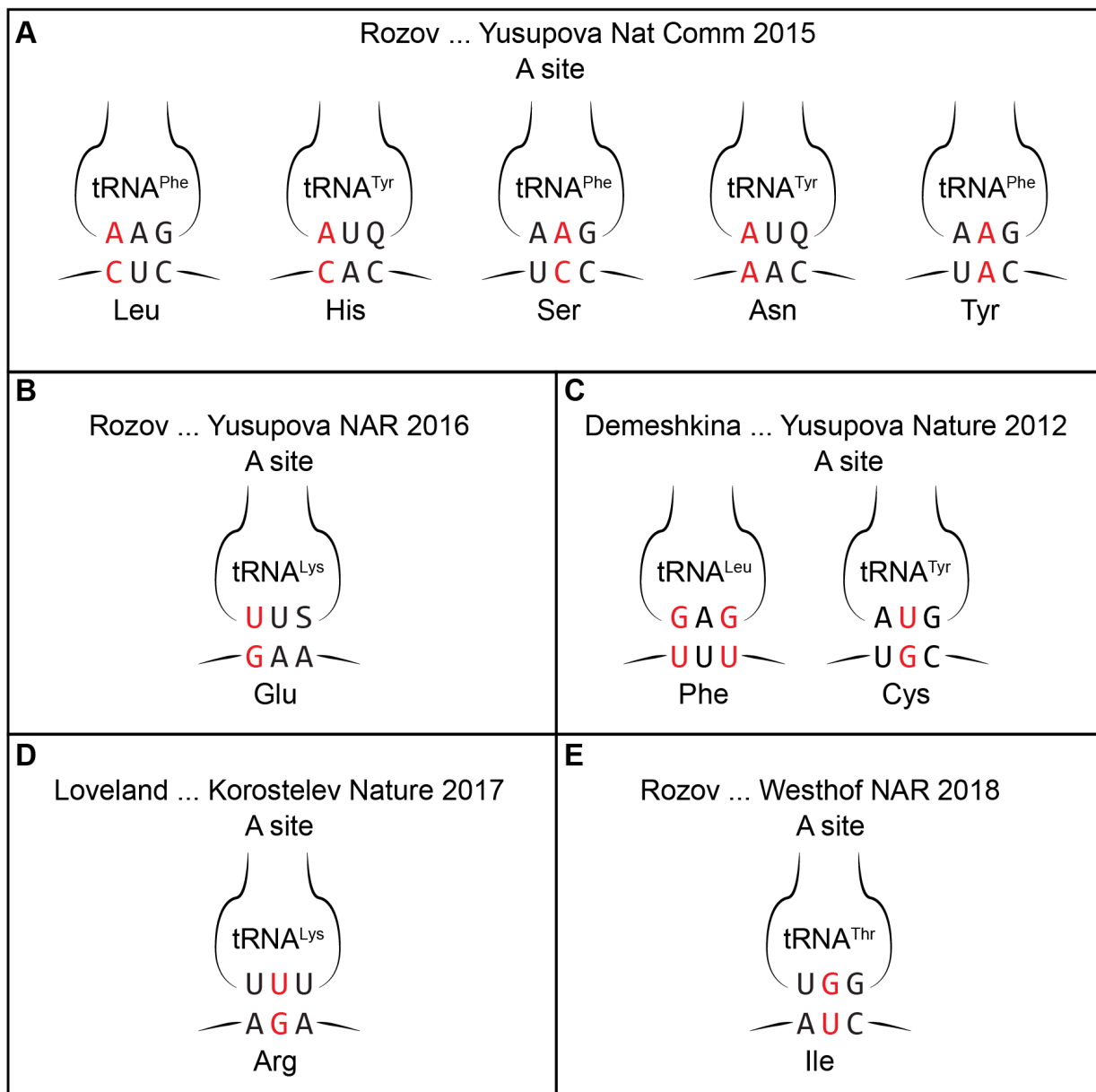
**B** tRNA<sup>Ala</sup><sub>GCC</sub> **U32-A38** bound to a near-cognate GCA codon (PDB ID 6OPE)



**C** Overlay of tRNA<sup>Ala</sup><sub>GCC</sub> **U32-A38** bound to cognate GCC and near-cognate GCA codons



**Figure S6. Representative electron density of 23S rRNA A1913 and the reversed 32-38 pairing when bound to a cognate or near-cognate codon.** **A**, The 70S-tRNA<sup>Ala</sup><sub>GCC</sub> structure containing the reversed 32-38 pairing shows A1913 packing against the tRNA with good electron density when bound to a cognate codon. **B**, When bound to a near-cognate codon, A1913 and the 32-38 pairing also have good electron density. **C**, An overlay of the two structures indicates that the position of A1913 superimposes well. The 2F<sub>o</sub>-F<sub>c</sub> electron density map is contoured at 1 $\sigma$ .



**Figure S7. Examples of previously solved structures of anticodon-codon mismatches bound to the ribosome. A**, A•A and A•C mismatches in the ribosomal A site in the first and second positions do not form stable Watson-Crick base pairs, but H69 A1913 adopts the 'ON' position in all of these cases (6). **B-E**, The G•U mismatches have been extensively investigated at the first, second, and third positions in both the P and A sites, and at all positions, a G•U mismatch adopts a Watson-Crick geometry (5, 7-9). Q: queuosine, S: 5-methylaminomethyl-2-thiouridine, mnm<sup>5</sup>s<sup>2</sup>U



## SUPPLEMENTARY REFERENCES

1. A. Rozov *et al.*, Novel base-pairing interactions at the tRNA wobble position crucial for accurate reading of the genetic code. *Nat Commun* **7**, 10457 (2016).
2. J. Lin, M. G. Gagnon, D. Bulkley, T. A. Steitz, Conformational changes of elongation factor G on the ribosome during tRNA translocation. *Cell* **160**, 219-227 (2015).
3. R. M. Voorhees, A. Weixlbaumer, D. Loakes, A. C. Kelley, V. Ramakrishnan, Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nat Struct Mol Biol* **16**, 528-533 (2009).
4. T. M. Schmeing *et al.*, The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* **326**, 688-694 (2009).
5. N. Demeshkina, L. Jenner, E. Westhof, M. Yusupov, G. Yusupova, A new understanding of the decoding principle on the ribosome. *Nature* **484**, 256-259 (2012).
6. A. Rozov, N. Demeshkina, E. Westhof, M. Yusupov, G. Yusupova, Structural insights into the translational infidelity mechanism. *Nat Commun* **6**, 7251 (2015).
7. A. Rozov, E. Westhof, M. Yusupov, G. Yusupova, The ribosome prohibits the G\*U wobble geometry at the first position of the codon-anticodon helix. *Nucleic Acids Res* **44**, 6434-6441 (2016).
8. A. B. Loveland, G. Demo, N. Grigorieff, A. A. Korostelev, Ensemble cryo-EM elucidates the mechanism of translation fidelity. *Nature* **546**, 113-117 (2017).
9. A. Rozov *et al.*, Tautomeric G\*U pairs within the molecular ribosomal grip and fidelity of decoding in bacteria. *Nucleic Acids Res* (2018).