SUPPLEMENTARY MATERIALS

A new mechanism of post-transfer editing by aminoacyl-tRNA synthetases: Catalysis of hydrolytic reaction by prolyl-tRNA synthetase of bacterial type

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Figure S1. Model of *Enterococcus faecalis* Prolyl-tRNA synthetase (ProRSEf) complexed with tRNA^{Pro} in post-transfer editing conformation (Crepin, T., Yaremchuk, A., Tukalo, M., & Cusack, S., 2006).

Crepin, T., Yaremchuk, A., Tukalo, M. & Cusack, S. (2006) Structures of two bacterial prolyl-tRNA synthetases with and without a cis-editing domain. *Structure*, 14, 1511-1525.

Figure S2. Sequence alignment of bacterial ProRS INS domains. Residues, which change to alanine entails more than 10-fold lost of posttransfer editing activity in ProRSEf colored red, as well their homologs. Positions shown more than 4-fold effect colored yellow. Residues not involved in mutagenesis experiments that are absolutely conserved in this set of sequences market blue on the first line.

Figure S3. Diagram demonstrating energy of ProRSEf INS domain amino acid moieties in the complex with alanyl-tRNA^{Pro}.

Figure S4. Methyl side chain of substrate Ala binding in a hydrophobic pocket of ProRSEf INS domain.