Supplementary Material for

G:U-Independent RNA Minihelix Aminoacylation by *Nanoarchaeum equitans* Alanyl-tRNA Synthetase: An Insight into the Evolution of Aminoacyl-tRNA Synthetases

Misa Arutaki[1,†], Ryodai Kurihara[1,†], Toru Matsuoka[1], Ayako Inami[1], Kei Tokunaga[1], Tomomasa Ohno[1], Hiroki Takahashi[1], Haruka Takano[1], Tadashi Ando[2,3], Hiromi Mutsuro-Aoki[1], Takuya Umehara[1], and Koji Tamura[1,3,*]

[1]Department of Biological Science and Technology, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan

[2]Department of Applied Electronics, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan

[3]Research Institute for Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

[†]Misa Arutaki and Ryodai Kurihara have contributed equally to this work.

[*]To whom correspondence may be addressed. Email: koji@rs.tus.ac.jp

This PDF file includes:

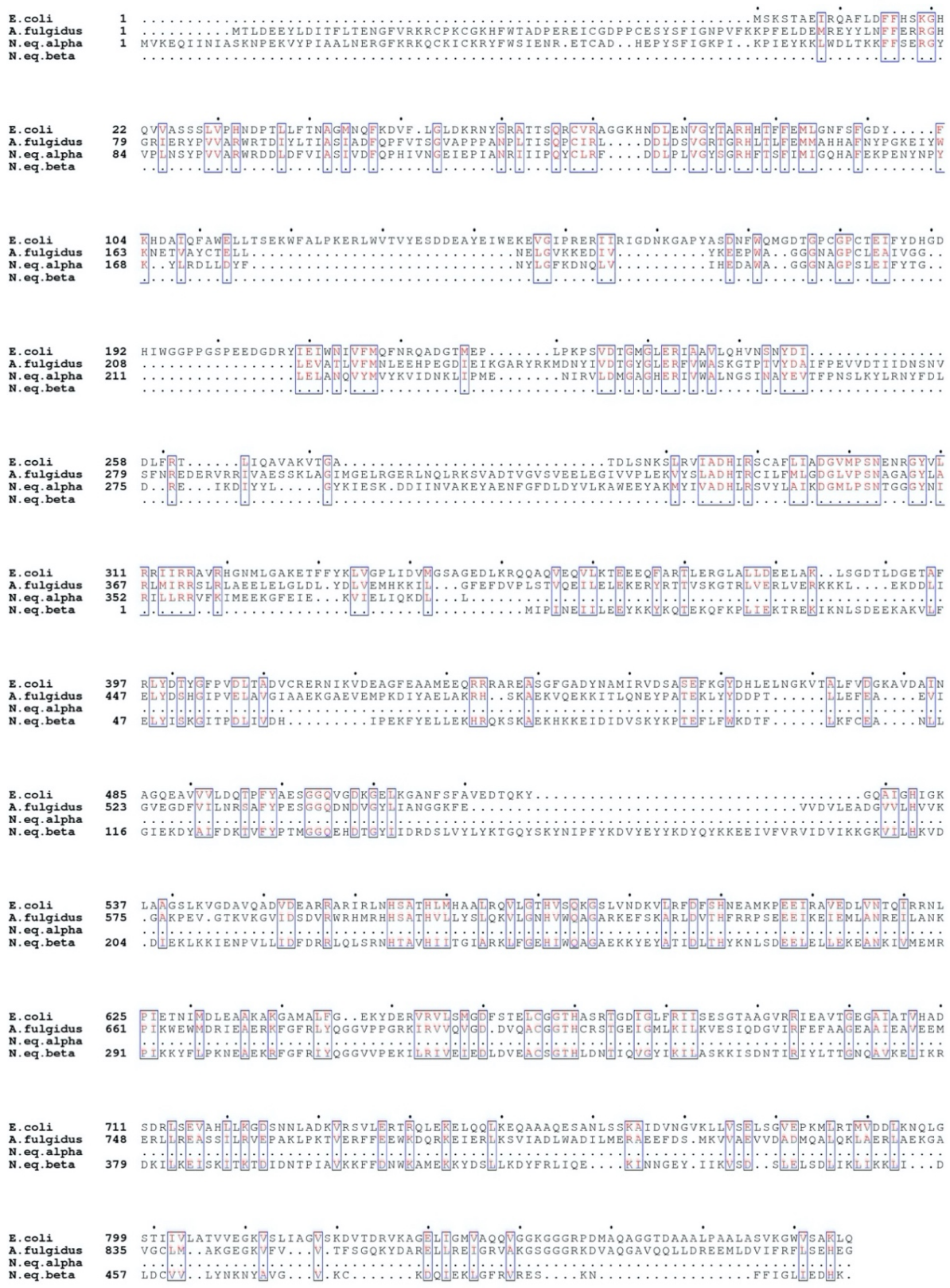

Fig. S1 Multisequence alignment of *N. equitans* AlaRS-α (NEQ547) and AlaRS-β (NEQ211) with *E. coli* AlaRS and *A. fulgidus* AlaRS. Similar and conserved amino acids are labeled in red and boxed in blue, respectively. Alignments were performed using ClustalOmega (Sievers et al. 2011) and figures produced using ESPript (Robert and Gouet 2014).

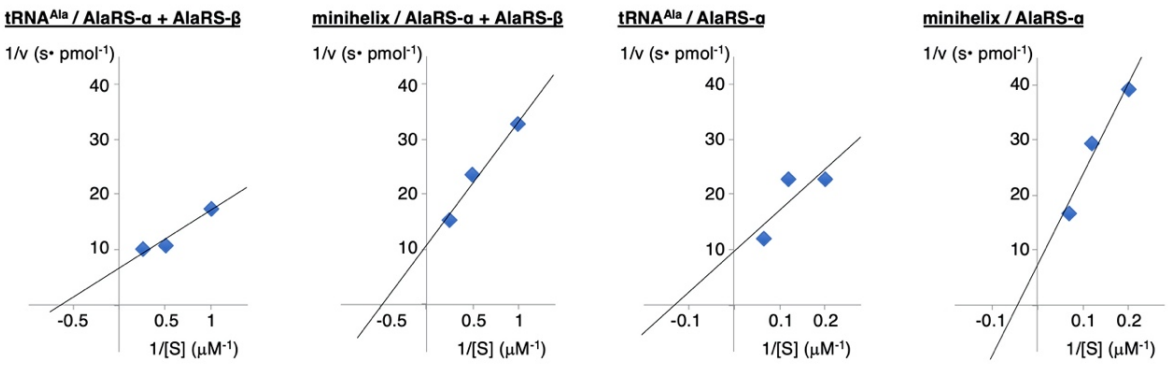

Fig. S2 A Lineweaver-Burk plot of 1/v against 1/[S] ([S] indicates the RNA concentration and v indicates the observed initial velocity of alanylation) in alanylation of $tRNA^{Ala}$ and $minihelix^{Ala}$ by a combination of *N. equitans* AlaRS-α and AlaRS-β, and by AlaRS-α alone.

References

Robert X, Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res 42:W1

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539