Supplementary Table 1. Aminoacylation kinetics of *Nm*LeuRS1 for *Nm*tRNA^{Leu}(GAG) and *Nm*tRNA^{Leu}(GAG)-A47cC at 40 °C^a

tRNA ^{Leu}	$K_{\rm m}(\mu { m M})$	k_{cat} (s ⁻¹)	$k_{cat}/K_{\rm m}$ (mM ⁻¹ s ⁻¹)	Activity Ratio
NmtRNA ^{Leu} (GAG)	7.73±0.22	(6.40±0.19)×10 ⁻²	8.28	1^{b}
NmtRNA ^{Leu} (GAG)-A47cC	20.2±1.1	$(1.24\pm0.03)\times10^{-2}$	0.614	0.07

^a The results are the average of three independent repeats with standard deviations indicated.

^b The activity of *Nm*LeuRS1 for *Nm*tRNA^{Leu}(GAG) is defined as 1.

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1	Ec Tt Bs Hp_B Nm_1 Hh_1 Nm_2	MESYTASTLSELYK	. MQEQYRPEI . MEKYNPHAI . MSFQHKEI . MSFQHKEI MGTRQFDHTBI GMSDAGYDHAAY ERERGFDHTBI . MTNQYDHAQY	ESKVQLHWDE EAKWQFFWBB EKKWQFFWBB EHRWQBAWDD EQRWQAAWDD EQRWQAAWDD EPRWQRTWDE QEFWQYYWER	KRTFEVTED. KGFMKAKDLP NKTFATLDN. ADVFHIPDE. ADAYRTSDD. ADVFRIDDD. DGVABLPDG.	ESKEKY. YCL GGRGKQ. YVL NEKQKF. YAL .ATDPT. YVL .VDDPT. YVL .ESDPE. YVL .AVDPT. YVL	SMLPYPSGR. VMPPYPSGD. DMPPYPSGAG GMPPYTSGQ. GMPPYTSGS. GMPPYTSGS. GMPPYTSGT.	EHMGHVRNYTIG LHMGHLKNYTMG LHVGHPBGYTAT LHMGHVRNYTIT LHMGHVRNYTIT LHMGHVRNYTIT LHMGHVRNYAIT	DVIARYQRMLGKNVLQP DVIABFRRMQGYBVLHP DILSRMKRMQGYDVLHP DAYARYKRMDGBNVLHP DAYARFROMCGDDVLHP DAYARFRMRGBSVLHP DAYARFRMRGBSVLHP
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2	Hh_1 Hh_1 Nm_2 Hh_2 Ph Si_1 Si_2 Hp_A Hv	EAGLVERQAABLINK EAGLVEFTGATVNW DAGLVDYGAATVNW EKGYIVKGAHRVRWI ELGYLVTEDDVVGY BRGLIEKGLHPVGY DRGRIBKGLHPVKY	CPSCBTVLADEQ CPDCBTVLADAQ CPDCBTVLADAQ DPVVGTPLGDHD CPNDNFPVGMHD CPNHFPVGMHD CTNBGNPVTTHD CTNBGNPVTTHD	VBGEB VAVDEGGQAV VETPP IMEGE TRGDI ILEGE LLEGE	TATADEAGDN	GDSAGGAHDE ETTAGTGHSH	SNGNAHVHEH THG.		PIEAREMDQWFFTITDY PVEQRBLDQWFFTITDY GVEQRDLDQWFFTITDY .DVPILDYIIIKFEL .EPBITMNIVILFBG .EPBIGEFVLIYFN. .EAEFQEYTLVKFGW
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Hh 1	AABAP. BDYEMI	BRÂLVENTREDIRDIV	DTVGIEDPQTITLAVAPEWKHRVLDLARNA	DGNVVGTVMQD	EDLREQG
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Ph	EPVEEWWNETIEA	BEEFIRSVMEDIKEII	EVAK.IENAKRAY <mark>I</mark> YTAE <mark>D</mark> WKWKVAEVV:	SE KRDFKSSM	BELMK.D
Si_1	DPNELDLYPDAIL	EISYINEIIENVRELE	DLVHKKAEKVVIY <mark>I</mark> NESK <mark>K</mark> VKELMKNAI:	SAINDGISLR	EFVMKTG
Si_2	BIEGSKIDELTLL	KH BYMKRIVEDIRSIL	WFKGTPKLIKIYALNDSEYVBLLRDAI	EANGQMKKFMDAH	KPKSKBD
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1 Ec	DATEEQVR ERAGQI	BHLVAKYLDGV TVR	KVIYVPGKLLNLVVG		
Tt	DAPLEVAR ABALKY	VRNVRAHLEGK EVV	KBIYVPGKILNLVVRG		
Bs	DATKEQLE QLAQAI	DEKVKEQLEGK TIR	KIIAVPGKLVNIVAN		
2 Hp_B	DDAAKYGQ. BLQQBI	RQSLPETLSPE REQ	VALRRANWLFQREFGADVRELTA	DEADSDVASKARPGR	PAIHID.
Nm_1	DAAANYGQDLQABI	REALSMTLGPD BEH	BALESAAWLLEREFEAPVSVVHA	DEVDESVLKNAEPGR	PAIEIED
Hn_1	EAAADFAK BLAGRA	AQSLDEQLPPEREQ.		ABADPDLVGKAGPGK	PAIDIDE
Nm_2	DVLABFVADQRRIDAG	CACIEDITOCE DEL	TLLEQAAWLLADEFDVTVSWRSATAVGTED	A DODDI AN KAR GR	PALKIU.
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Supplementary Figure 1. Sequence alignment of the N-terminal region (the Rossman fold, CP1, CP2 and α-helix bundle domain) (A) and C-terminal domain (B) of LeuRSs, respectively. Ec, *Escherichia coli*; Tt, *Thermus thermophilus*; Bs, *Bacillus subtilis*; Hp_B & Hp_A, *Haladaptatus paucihalophilus* LeuRS [Bacterial type (accession No. WP_007982262.1) & Archaeal type (accession No. WP_007982263.1)]; Nm_1 & Nm_2, *Natrialba magadii* LeuRS1 & LeuRS2; Hh_1 & Hh_2, *Haloarcula hispanica* LeuRS [LeuRS1 (accession No. YP_004794828.1) & LeuRS2 (accession No. YP_004797622.1)]; Ph, *Pyrococcus horikoshii*; Si_1 & Si_2, *Sulfolobus islandicus* LeuRS [LeuRS1 (accession No. YP_002829648.1) & LeuRS2 (accession No. YP_002829589.1)]; Hv, *Haloferax volcanii*.



Supplementary Figure 2. *Nm*tRNA^{Leu} are typical archaeal tRNA^{Leu} with conserved A47c and G47d bases. (A) Sequence alignment of all five *Nm*tRNA^{Leu} isoacceptors and tRNA^{Leu}(GAG)s from different species. Nm, *Natrialba magadii*; Hh, *Haloarcula hispanica*; Ph, *Pyrococcus horikoshii*; Hv, *Haloferax volcanii*; Si, *Sulfolobus islandicus*; Mj, *Methanocaldococcus jannaschii*; Cs, *Cenarchaeum symbiosum*; Ec, *Escherichia coli*; Tt, *Thermus thermophilus*; Aa, *Aquifex aeolicus*; Bs, *Bacillus subtilis*; Sa, *Staphylococcus aureus*. Acceptor stem, D stem, AC stem (Anti-codon stem), LV stem (Long variable stem), T stem and the site of 47c, 47d in archaeal tRNA^{Leu} are specified. (B) Cloverleaf structure of *Nm*tRNA^{Leu} with conserved bases specified. The site of A47c and G47d, non-conserved (■) and divergent (□) bases were indicated.



Supplementary Figure 3. Optimization of aminoacylation conditions of *Nm*LeuRS2. (A) Relative aminoacylation activity of *Nm*LeuRS2 under different KCl concentrations (•). The activities of *Nm*LeuRS2 in 3.4 M KCl were defined as 100%; (B) Relative aminoacylation activities of *Nm*LeuRS2 under different pH conditions of Tris-HCl (•). The activity of *Nm*LeuRS2 in Tris-HCl pH9.0 was defined as 100%.



Supplementary Figure 4. *Hh*LeuRS1 showed higher amino acid activation, amino-acylation and post-transfer editing activities than *Hh*LeuRS2. (A) The 10% SDS-PAGE analysis of the purified *Hh*LeuRSs from *E. coli*. Lanes: 1, 2, 3 are *Hh*LeuRS1, *Hh*LeuRS2 and molecular markers (Thermo Scientific, #26614), respectively; (B) The amino acid activation activities of 1 μ M *Hh*LeuRS1 (•) and 1 μ M HhLeuRS2 (•) for 5mM Leu in pH 8.5, 3.5 M KCl solution; (C) The amino-acylation activities of 400 nM *Hh*LeuRS1 (•) and 400 nM *Hh*LeuRS2 (•) for 4 μ M *Hh*tRNA^{Leu}(GAG) in pH 8.5, 3.5 M KCl solution; (D) Representative graph showing the post-transfer editing activity of 500 nM *Hh*LeuRS1 and 500 nM *Hh*LeuRS2 in pH 7.5, 3.5 M KCl solution based on TLC assays; (E) Quantification of the de-acylation activities of *Hh*LeuRS1 (•) and *Hh*LeuRS2 (•) in D.



Supplementary Figure 5. The aminoacylation activities of *Nm*LeuRS1-*Ec*CTD for *Nm*tRNA^{Leu} (\blacksquare), *Ec*tRNA^{Leu} (\bullet) and *Ph*tRNA^{Leu} (\blacktriangle). The aminoacylation activity of *Nm*LeuRS1 for *Nm*tRNA^{Leu} (\square) was used as the positive control.



Supplementary Figure 6. The aminoacylation activities of *Nm*LeuRS1 and *Nm*LeuRS1-D354A (A), *Nm*LeuRS2 and *Nm*LeuRS2-D390A (B) for cognate Leu and non-cognate Nva, ABA, Ile and Met based on TLC assays. (C, D) The aminoacylation activities of *Nm*LeuRS1 for Leu and without amino acid (No aa) were used as the positive and negative control, respectively.