

Supplementary Table 1. Aminoacylation kinetics of *NmLeuRS1* for *NmtRNA*^{Leu}(GAG) and *NmtRNA*^{Leu}(GAG)-A47cC at 40 °C^a

tRNA ^{Leu}	K_m (μ M)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1} s^{-1}$)	Activity Ratio
<i>NmtRNA</i> ^{Leu} (GAG)	7.73 \pm 0.22	(6.40 \pm 0.19) $\times 10^{-2}$	8.28	1 ^b
<i>NmtRNA</i> ^{Leu} (GAG)-A47cC	20.2 \pm 1.1	(1.24 \pm 0.03) $\times 10^{-2}$	0.614	0.07

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

^bThe activity of *NmLeuRS1* for *NmtRNA*^{Leu}(GAG) is defined as 1.

A-1

1 Ec ..MQEQYRPEEIESKVVQLHDEKRTFEVTEDESKSEKYVCLSM LPPYPSGR LHMGHVNRNYITGDVIARYQRMLGKNVLP...
TtMEKYNPHAI EAKWQRFWEKGFMKAKDLPPGGRGKQ..YVLVMPFPYPSGD..LHMGHLKKNYITMGDLVLRFRRMGGYEVLELP...
BsMSFQHKBEIEKKWQTYWLENKTFATLDN.NEKQKF.YVALDMFPYPSPGAGLHVGHVPEGYTATDLRSRMKRMGGYDVLLEP...
Hp BMQTRRRFDHTEIEHRWQBAWDDADVFHIDDE..ATDPT.YVLGMFPYPSGQ..LHMGHVRNRYITDARYKRMGGENVLELP...
Nm 1 MESYASTLSLYKQMSDAGYDHAAVQRWQAADDDADAVRTSDD..VDDPT.YVLGMFPYPSGK..LHMGHVRNRYITDARYRQMGGDDVLELP...
Hh 1MTTGEERERGFDDHTEIEPRWQRTWDEADVFRIIDD..ESDPE.YVLAMFPYPSGS..LHMGHVRNRYITDARYFRMRGGESVLELP...
Nm 2MTNQYDHAQVQEFWQYVWERDGVABELPDG..AVDPT.YVLGMFPYPSGT..LHMGHVRNRYITDARYRRLRGDDVLELP...
Hh 2MSRRYDHDARVQBYWQQAWERDGVFPCPTD..AENPT.YVLGMFPYPSGS..LHMGHVRNRYITDARYRRLRGDDVLELP...
2 PhMABLNFKAIEBKWKQRLEAKIFBPNIRDKPKKPKFYITVAPPYLSGH..LHVGHARTYITIPDVIARYFRMRGGYNVLELP...
Si 1MYNDFFNISIAKQWABWEKSKVPBANI..DYSKPKFPI TVPFPYTNSP..MHVGHGRTYVTVADVIARYLRMRGGYNVLELP...
Si 2MNNVAHKWQTKWEBAKVYESNP..NSNRSKPFTTVAPPYNSP..MHI GHARTYVTVADVIARYLRMRGGYNVLELP...
Hp AMDYDPQELBARWRREKSESGRYEADP.DADADDATFVTVFPYPSGG..MHI GHARTYVTVADVIARYLRMRGGYNVLELP...
HvMDYDPQELBARWRREKSESGRYEADP.DADADDATFVTVFPYPSGG..MHI GHARTYVTVADVIARYLRMRGGYNVLELP...

1 Ec I GWDAPGLPAEGA AVKNNTAPAWTYDN IAYMKNQ..LKM LFGFGYDWSRELAATCT..PEYYRWEQRFFTBLY...
Tt MGWDAPGLPAENAL KFGVHPKDWTYANI RQAKES..LRLMGILYDWDREBVTTCB..PEYYRWNQWIFLKMW...
Bs MGWDAPGLPAEQYALDTGNDPAVFTKQNI DNFRRO..IQALGFSYDWDREBINTTD..PEYYKWTQWIFLKLK...
Hp B MGWDSFGLPAENAI ERDTNPRDWTLSCL DTMREQ..MEGMGFGYDWDREBITCQ..PDYYRWNQWLFTRFR...
Nm 1 MGWDAPGLPAENAKDRDTNPRDWFDCI DTMRDQ..MRAMGFGYDWDREBITCTC..PDYYRWNQWLFPSRFH...
Hh 1 MGWDSFGLPAENAEERDTNPRDWTMQCI DSMRDQ..LTEMGFGYDWDREBITACE..PDYYQWNQWLFKQFC...
Nm 2 MGWDAPGLPAENAA YERASDPESWTRACI RMRREE..LET LFGFGYDWSREBITCE..PSYYRWNQWLFKRFH...
Hh 2 MGWDAPGLPAENAA YERDTDPESWTRTCI DQMKAD..LREMFGYDWSREBITCTD..PEYYQWNQWLFQPY...
2 Ph MAWHITGSPVIGIABRIKNRDPKTIWIYRDV..KVPBEILWTFEDPI NIVKYFMKA AKET..FIRAGFSYDWSREBFTTSLFP...
Si 1 PAFQFTGTPVLA IADSI RRGDVDMIBFKSV..GVPBDKI KBLGD PYKLA EYFKBAKMT..AKSIGMSIDWRRSFTTID..TRFEKFIHQWLRLKLK...
Si 2 MAFH YGTPI MAMADAI AKGD KELIETFKDI..EISPDVI PRMSD PFMANYFKIEDIKTS..MREI GLGIDWRRBFTTID..PEFSFVTFWQFHKLK...
Hp A IAWHVTGTPVIGAVERLKRREPEQMSVLDTD..NVSDDELSBEMETPMGFARHFIBDSYKKNMQSLGLS DWRREBFTTND..BRYSKFIWQYBTLK...
Hv IAWHVTGTPVIGAVERLQRREBKQLSVLTD..NVPEDTLSDES PMGAWARYFI BEHYKKGMSLGLS DWRREBFTTANN..BRYSKFIWQYBTLK...

1 Ec K KGLVYKKTSAWVWCPNDQTVLANEQVLDGC.....CWR.CDTKVERKETPQWFITKITAY...
Tt EKGLAYRAKGLVWVWCPKCCQTVLANEQVVEGR.....CWR.HEDTPVKRELEBQWYLRTITAY...
Bs EKGLAYVDEVPVWVWCPALGTVLANEBVIDGK.....SER.GGHPVERPMKQWMLKITAY...
Hp B EQLVEREAAEINWVWCPSECTVLAD EQVEGDA.....ELCWR.CDTPVEQRELDQWFLKITAY...
Nm 1 QEDLVERRDABVWVWCPSECTVLAD EQVEGEA.....BLCWR.CDTPVEQRELDQWFLKITAY...
Hh 1 EAGLVERQAAELWVWCPSECTVLAD EQVEGEE.....BLCWR.CDTPVEQRELDQWFLKITAY...
Nm 2 EAGLVEFTGATVWVWCPDCECTVLADAQVAVDEGGQAVTATADEAGDNGDSAGGAHDESNNAHVHEHSTARV..CWR.CGT PVEQRELDQWFFITIDY...
Hh 2 DAGLV DYGAATVWVWCPDCECTVLADAQVETPP.....BADEGETTAGTGHSHTHG.....GGV.CWR.CGTGVQRELDQWFFITIDY...
2 Ph EKGYIVKGAHRVRWDPVVGTPLDG HDLMEGE.....CWR.CDTPVEQRELDQWFFITIDY...
Si 1 ELGYLVTEDDVWVWCPNDNFPVGMHDTRGDI.....CWR.CDTPVEQRELDQWFFITIDY...
Si 2 SKGYVVKDTHPVWVWCPVHHIIPVGMHDTKGDV.....CWR.CDTPVEQRELDQWFFITIDY...
Hp A BRGLLEKGLHPVWVWCPVCTNEGMPVTT HDLLGGE.....CWR.CDTPVEQRELDQWFFITIDY...
Hv DRGRLEKGLHPVWVWCPVCTNEGMPVTT HDLLGGE.....CWR.CDTPVEQRELDQWFFITIDY...

1 Ec ADELLNDLKDLDHWPDTVKMQRNWIGRSEGVEIT..NVNDYD.....NTLTVYTTTRPDTFMGCTYVLA VAGHP LQAQ..KAAE NNP...
Tt AERLLKDL EGLN.WPEKVKAMQRAWIGRSEGAEIL..PVEGKE.....VRI PVFTTRPDTLFGATFLVLAPEHPLTLELAAP EKRE...
Bs ADRLLEDLEELD.WPESIKDMQRNWI GRSEGAVH..FAIDGHD.....DSPTVFTTRPDTLFGATFLVLAPEHPLTLELAAP EKRE...
Hp B ADELDDLDLDDLGWPSNVRMQRNWIGRQHGATVE..EVS EAAASETSSGKQGSRDYGPEVVFTRLDITIFGVTFALAPGHPIAEB LAEED..D...
Nm 1 ADELLEDIDDLGEPNSVRMQRNWIGRQYGA EVE..EIGG.....HGSVTAFTRLDITIHGATFPALAPGHPIAEB LAEED..D...
Hh 1 ADELLEDLDDLGWPNVREMQRNWIGRQAGASVA..EVDG.....YGEVDITRLDITIHGATFPALAPGHPIAEB LAEED..D...
Nm 2 ADELVDGLDDLDQWPEGVREIQRNWI GRQEGARIT..DVSTAAD ESD.....TAVDVFTSTRS DTVFGATFLVLAPEHPLTLELAAP EKRE...
Hh 2 ADELVDGLDDLDQWPEGVREIQRNWI GRQEGARIT..SIDHDDHDT.....VIAFTLRLDITIHGATFLVLAPEHPLTLELAAP EKRE...
2 Ph RENGE.....VIYLPAAITLRPETVYGVNMMWVNP NATYV KAKVRRKDKBE...
Si 1 SESY.....NFMVATSRBELIFGVVALM VNSDAN YVVVYEGKN...
Si 2 SEKG.....IFPAATLRPETIFGATGLW INPNEMVIANILDKK...
Hp A DDEAQRASESASSAERRSANRSSGSESPR.....AITVPMATLRPETVHGVTNAYIDPEATYVVEA VDGEBT...
Hv B.....RDGGD.....VVVPMATLRPETVHGVTNAYIDPEATYVVEA VDGEBT...

1 Ec ELAA FID ECRNT...KVABEAMATMEKKGVDTGPKAVHPLTGE EIPVWAANFVLM EYGTGAVMAVPGHQRDYEPASKYGLNIKPVILAAD...
Tt EVLAVVEA AAKRK...TEIBRQAGREKTVGFLGAYALNPAATGEERIPITWADYVLFYGYGTGAIMAVPHDQRDYEPARKFGLPKKVI BRPG...
Bs AVEAYIK EIQSK...SDLERTDLAKTKTVGFTGAYAINPVMGEEKLPVIA DYV LASYGTGAVMAVPGHQRDYEPARKFGLPKKVI BRPG...
Hp B DIAHFVEE VAD.....PEGDEP...QGVPTDLTATNPATGE EIPVFPVADFVLSDVGTGALMGPVPHDRDHPAFTSMOVDIVPVVAPS...
Nm 1 DVRHFIEHEAD.....PEGDEP...NGVATHLTATNPTVGE EIPVYVADFVLSDVGTGALMGPVPHDRDHPAFTSMOVDIVPVVAPS...
Hh 1 EVAEYIETAEQ.....ADEDEL DVTSVFTGRYAVNPAATGE EIPVYVADYVLT DVGTGALYAVPAHDERDHPAFAEHDIDIVQVVEPTED...
Nm 2 DVATFVDQARTSAPDTGHAARDDFS.TAGVKT DATABPTVGE ELPVYVAYVLADVGTGAVMGPVPHDRDHPAFAEHDIDIVQVVEPHG...
Hh 2 AVADYVESV.....ASTDDAG.MSGVETDLTATHPTVGE ELPVYVAYVLADVGTGAVMGPVPHDRDHPAFAEHDIDIVQVVEPHG...
2 Ph ETWIVSKBAAYKLSFDREIBVIEBFKGBKLIK YVRRNPSGDEVI ILLPAEFVDFDNATG VVMSVPHAPFDHVALDLKRETEILBK...
Si 1 FIISEKAYKKLSYQDK.MKMIKTISTSDIVKLYGINPVTGKKLEI I KSKHVDLISLGTGVVMSVPAHDPH YLAIAEANK..EF EII P...
Si 2 MILISEKSATKLSFDI DNBIE DKKIKGSKLVGLKVENPITGGKIVVMGADFDASLGTGVVMSVPAHAPFDYYSKILKNNNIGIIP...
Hp A WLVSESAABKLELQAHQVIRDFHSGERLVGEBHVTNPVTGDDVLLVPASFDADNATG VVMSVPAHSDPDWALQEAKAARRABQSSGQSP...
Hv WLVSESAABKLELQAHQVIRDFHSGERLVGEBHVTNPVTGDDVLLVPADFDADNATG VVMSVPAHSDPDYVALQEAKADAD...

1 EcGSEPDLSQALTEKGVLFNSG.....EFGLDHBAAFNATA...
TtEPLPEPLERAYE EPGIMVNSG.....PFDGTES BEGKRKVI...
BsNVEEAYTGDGEHVNSD.....FLNGLHQBAIEKVI...
Hp BBDDVPDVS EAYTEDGVLVNSG.....DIDGLDSBARERLE...
Nm 1EGEPEVPDIS EEAFTEDGVLVNSG.....EYDGLS BTARERLT...
Hh 1ADADPEDIDVQEAAYPEDGLVNSG.....EYDGLSSNEARDR...
Nm 2NGTGSDDGVATDAPMTGEGT LVLES PADRAS...GQPSBDVREHLV...
Hh 2DGSGTD...LPHAPYTDGOMLTDG...EYDGLASSAARERLR...
2 PhDIDPRIVENI TYISLIKLEG...YGDFFPAVEEVNKLGIKSKDKKELBQATKTIYKABYHKVFKVP..PYE GKPVEVLEKBAIA...
Si 1VIRTEEBLBI PGESA VLQT...KNIYALKDFMESIYRTEYKGYMKDILSLVDPFLKQYVKEN...IVGKQVBARKNTI...
Si 2VITVEGLNDELAKDVVEKNN...PKNDEBLLKLTBYRTEYKGIKLSLLENLIEYERNELKN...LGLPVPKGRKELIT...
Hp A RDAADARMEAYGDPEEVAAT EPKAI DVEG...YGFPAEDA VVBHIEDSSDPALEDATQEVYRNFHSGKLRGMYDDVAGEI VEDVRRDLDK...
HvYLFESFGIDPADVADI EPIPI LDIDSBDT YGI PAESA VBNRGI ESSDDPALBKAT KDLYNKEFHAGRLKEFFYGDYAGGLVEVRRDLDK...

A-2

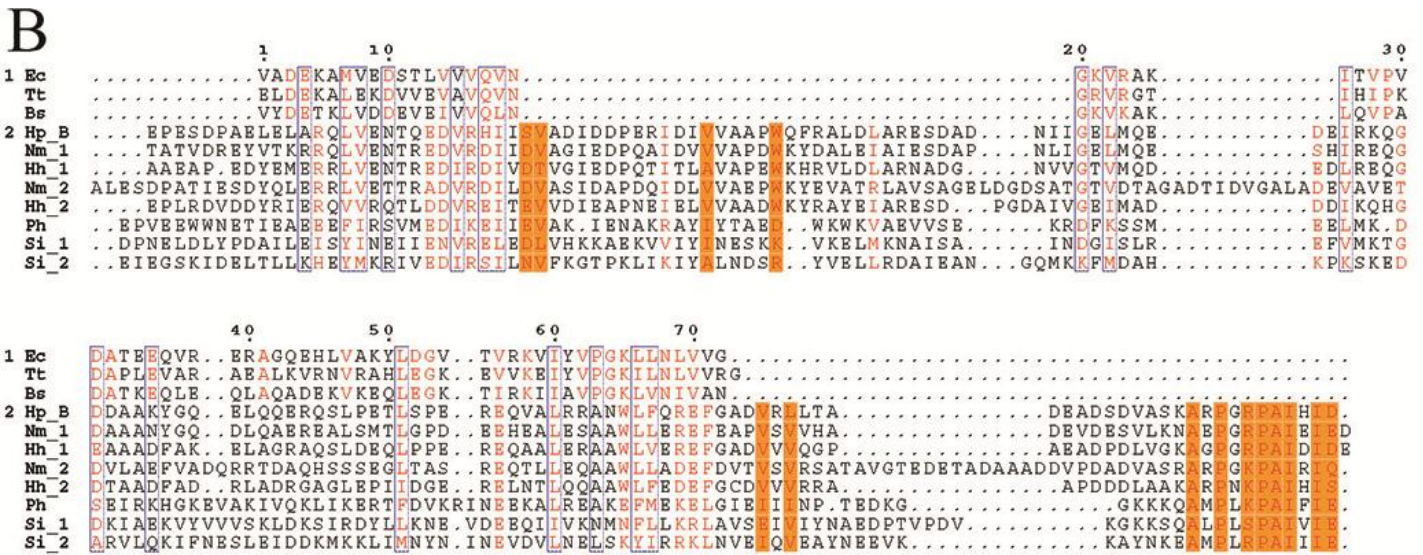
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1 Ec  DKL TAMGVGRKVN YRLRDWGSRQRWGAFTPMVLTLED.GTVMPTDQDLFVILPEDVVMGDGIT.....SPTKADPPEAKTTVN..EMPALRE
Tt  AWLBEKGLGKGRVTYRLRDWLSRQRWGTPIPMVHCEA.CGVPVPEBEEPLVLPDLKDVBDIRP...KGKSPLEAHPFYEYTTCPKCGGPAKRD
Bs  AWLBETKNGEKKVTYRLRDWLSRQRWGEPIPIVHWED.GTSTAVPEBEEPLVLPKTDDEIKPSG...TGESPLANIKBWEVTDPETKKGRRE
Hp_B GAI ES...AAHDVQYRLRDWGSRQRWGTPIPIIHCEB.CGVPVPEDEDLVPELPEFVRT.....TGNPLDAAAEWKHVNCPECGADARRE
Nm_1 ELLKSL...AEHTQYQLRDWGSRQRWGTPIPVVHCHDCS.SVVPPEBEEPLVPELPEFINT.....TGNPLDAAAEWKHTTCEPCGADATRE
Hh_1 BEFD...GEHRTYENLRDWGSRQRWGTPIPMIHCCD.CGVVPEDEDLVPELPEFVHT.....TGNPLDAAAEWKHVDCPCDADAVRE
Nm_2 DDHEA...IDPDVTYRLRDWLSRQRWGTPIPVHCBDD.CGHLVPEDEDLVPELPEFVQT.....TGNPLDAAAEWKETSCDCCGPAERE
Hh_2 .BHGA...ABSAVTYRLRDWLSRQRWGTPIPIVHCBDD.CGHLVPEDEDLVPELPEFVQT.....TGNPLDAAAEWKETTCCDADAVRE
2 Ph  KEMLEK...GIABIMYEFABKNVSRFGNRAVIKIIDQWFDYGNPEWKBKAR KALERMKILPETRRAQFEAIDWLDKKACARKIGLTPPLPVD
Si_1 ELLKSL...NMYDTIYHISNGPIYCRCGAETVPKKIKDQWFIAYDNPKWKASVLKAINNIBLIPNLAKSBLEKTIPNTRREPIGRSRGIVKLPVD
Si_2 NFLISK...GLGRKIFETIMNKPVYCRCGTEIVVKILKDQWFLDYSNEEWKELAR KSLSKMQUIPEESRKDFEFTWLEKRACARTRGLTPPLPVD
Hp_A AEFQEQ...GSFDTMHSES.EEVVCRCG.GAVEVAEQDTWFLRYNDEAWKAKAHEVVAELDAIPENTREQYDHTIDWLNWPCIRNYGLTRLPVD
Hv  ADGVEA...GQFDVMQSES.EVVVCRCG.GDVVVAEQDTWFLSYDDEDDWKELAHDVADGLDAIPENTREBYHHTIDWLNWPCIRNYGLTRLPVD
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          490      500      510      520      530
1 Ec  TD.TFDTFMESWYIARVYTCQYKRGMLD.....SFAANYWLPVDIYIGGIEHAIM
Tt  TD.TMDTFFDSWYIARYTDPHNDRLPFD...PEKANAWMPVDQYIGGVEHAVL
Bs  TN.TMPQWASCSWYFLRYIDPHNDQLAS...PEKLEKWLVDMYIGGAEHAVL
Hp_B TD.TMDTFFDSWYFLRYVSPHLDAPFD...TEQADDWMPVDQYVGGIEHAVM
Nm_1 TD.TMDTFFDSWYFLRYVSPDLBAPFD...LARANDWMPVDQYVGGIEHAVM
Hh_1 TD.TMDTFFDSWYFLRYTSPEMADAPFD...ABRASDWMPVDQYVGGIEHAVM
Nm_2 TD.TMDTFFDSWYFLRYFLSPLDADAPFD...TELANEWMPVDQYVGGIEHAIL
Hh_2 TD.TMDTFFDSWYFLRYLSPHFDAPFD...QETADEWLPVDQYVGGIEHAVL
2 Ph  PEWVIESLSDSTIYMYVYTI SRHINKLRQEGKLDPEKLTPEFFDYIFLEEFSEBDEKELEKKTGIPABIHEMKBEFEYWPVDWRCSGKDLIPN
Si_1 BSQIVESLSDSTLYTVLYTIIYKM.....PVDINDELDFMFLGKGD...TKELEKKGAD...LVQLREBFLYWPVDQRHTGRDLIPN
Si_2 KKWVIESLSDSTIYMYVYTI SHKIKQY...KISPSKLTQEFWDVYMLGIGN...LEDVSKNTGIPSNIKELREBFLYWPVDIRHSGKDLIPN
Hp_A DEFWIEPLSDSTIYMYVYTI IAHRI SDVP...VEBLDKBFFDLPFYGSBA...VDDPNBTALALREBWDYWPVDYRCSANDLVSN
Hv  DQFVIEPLSDSTIYMYVYTI IAHRLQBIIP...TEBLTQEFFDALFYGPDA...VDDAPEKALDLREBWDYWPVDYRFSNDLIPN
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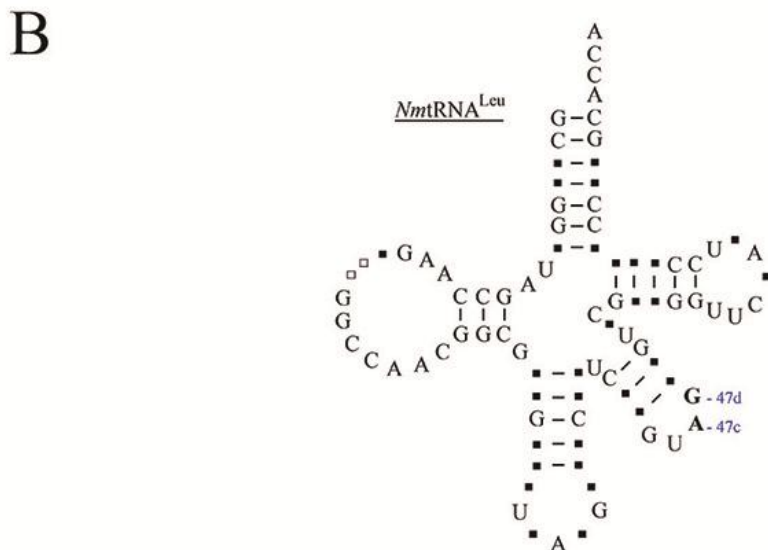
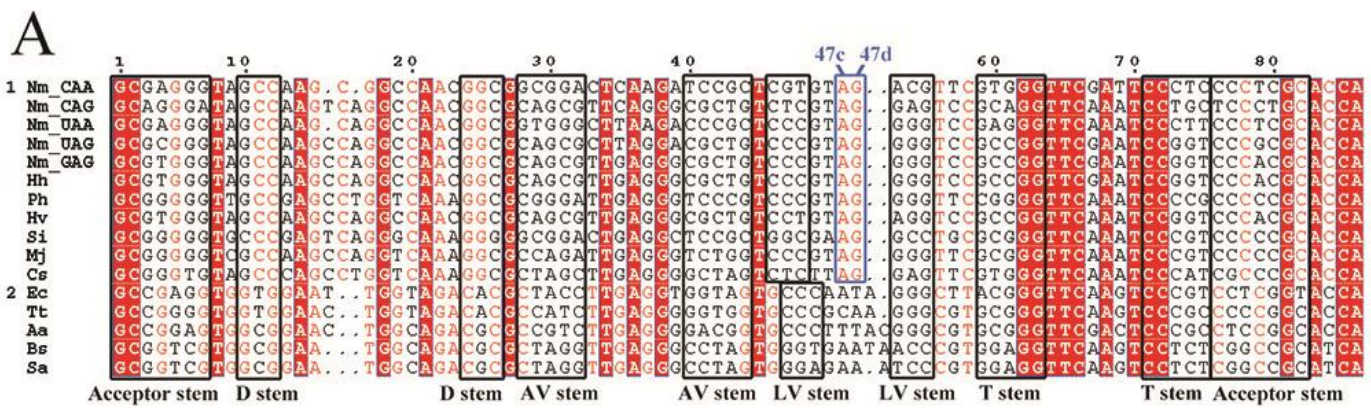
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          540      550      560      570      580      590      600      610      620
1 Ec  HLLYFRFFPKLMDRAGMVN.SDEPAKQLLCCGMVLADAFYVVGenger.....NwvSPDAIVERDEKGRIVKAKDAAGHBLVYTGMSKM
Tt  HLLYSRFFPKLHLDLMVK.VBEPFQGLFTCGMVLAWTDGFPVVEGSSVRLPEPTRIRLEIPESALSLEBDRKMGAEALRPHEDGTLHLWKPAM
Bs  HLLYARFFPKFLYDIDVVP.TKEFPQKLYNCGMVLGENNE.....K
Hp_B HLLYSRFFPKVISDMOMLD.HREPFNTLVT CGMVL.....LDGD KM
Nm_1 HLLYSRFFPKVLADHGLE.HREPFNTLVLACGMVQ.....LDGE KM
Hh_1 HLLYARFFPKVLDDLDMVDGVRPFNTLVTNCGMVLG.....LDGN KM
Nm_2 HLLYIRFFVTRALADLGLD.QREPVERLVSQGTVL.....YBGE KM
Hh_2 HLLYIRFFVTRALSIDIGLD.RBEPVERLINCQTVL.....HSGE KM
2 Ph  HLTFFIFNHVAIFREHWP.....KGIAVNGFT.....LEQG KM
Si_1 HLTFFIYNHLAIFGKYLP.....KRIVINGFVR.....VGG KM
Si_2 HLTFFIFNHAAIFQBNLWP.....KAIAVNLVL.....YEG KM
Hp_A HLTFFFLFHABLFDPANWP.....QGITVGMGL.....LEGE KM
Hv  HLTFFYQFHGELFDBPQNP.....RGIIVIMGMGL.....LEGE KM
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          630      640      650      660      670      680      690      700      710
1 Ec  SSKKNGITDPQVMVERGADTVLRFMMFASPADMTLEWQESGVEGANRFLKRVWKLVEHTAKG...DVAALNVDALTENQKALRRDVKHTIAKVT
Tt  SSKKNGVMVGPVFKGADIARITILFAAPPENEMVTEBEGQAWRFLNRIYRRVADREBALLETSGVFQABALEGKDRILYGLHETLKKVT
Bs  SSKKNGVNPDEIVASEGADTLRLYEMFMGLDASIAWSESGLDGARRFLDRVWRLIED.....SGELNGKIVEGAGETLERVYHBTVMKVT
Hp_B SSKKNGVNSPQRIVEEYGADTARLFTMQAAQPEKDFDWTBEGVKSSEYFLQRLVAGAVESFABS.TPD...GDRDEIABYVERBIDATVIAIAN
Nm_1 SSKKNGNTVSPQRIVEEYGADTARLFTMQAAQPEKDFDWTBEGVKSSEYFLQRLVAGAVESFATE..QPA...GEDDATARYVANEIDATI IAIAG
Hh_1 SSKKLDNGVNSPQRIIEEYGADTARLFIMEAAQPEKEFWASPEGVQSAHSFLQNVYTLVSEYANG..EABTATDLANGDDIADYVAREIDATAANAT
Nm_2 SSKSKGNVVT...DEYGAETTLFVLSAAHPEQDFEWANDVRGAYDLQALYSMATFVFE...GETR...VERVSHDEPVDREIDRTI IAAVT
Hh_2 SSKKGNDIAP...HEVGAETTLFVLSAAHPSQDFEWTVKDVSAYDFQOTLYRLVTEYTD...TETR...TESTDHDAYLREIDRTI IAAVT
2 Ph  SSKKGNVNLNFI DAIEEN GADVVRLYIMSLAEHDSDFDWRKKEVGLKRLKQIERFPEYELISQFAE...YEVKGNVBLKDI DRWMLHRLNKA I KETT
Si_1 SSKLKNIIYTLSKAIKEP GVDVPRITLTSTSDLLQDLDFNENLVNPIAEQLKVVYDLIDRLLS...INTE.IKGLRTADEWLLSKIRBIVEKVT
Si_2 SSKLRNIIPLRKGKLMV GVDVPRITAVSSTADMGSDVNFSBSLKTVGETLRMYELFKSLDN...YTED.TGFPF...BKWLLSRIYBITTNTT
Hp_A SSKKHVVLPENAI BKYGADTVRFPLMNSAEPWQDYDWRSEQVSTGNQLRFPWNRANEIIS...APEGBRDLQRI DRMLLSKLGQTVREV
Hv  SSKKHVVVLPGEAIGYGADTVRFPLLNSAEPWQDYDWRDLDVGSVHDLRFPWNRANQBIITD...PGPDDQPDLETVDRMLLSKLGQTVRDVT
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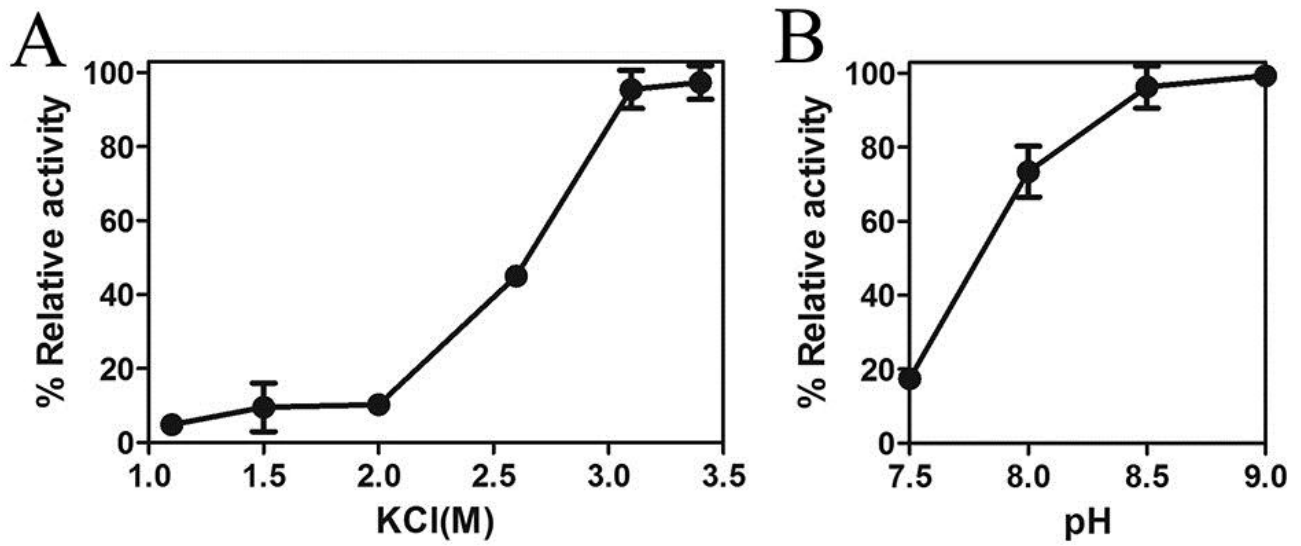
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          720      730      740      750      760      770      780
1 Ec  DLTGRRQT FN TAI AALME L MNK L A K A P . . . . . T D G B Q D R A L M Q E A L L A V V R M L N P F T P H I C F T L W Q E B K G E G D . . . . . I D N A P W P
Tt  E D L E A L R . F N T A I A A L M E F L N A L Y E Y R . . . . . K D . R P V T P V Y R T A I R Y I L Q M L F P F A P H L A E B L W H W F . W P D S . . . . . L F E A G W P
Bs  D H Y E G L R . F N T G I S Q L M V F I N E A Y K . . . . . A T E L P K E Y M E G F V K L L S P V A P H L A E B L W E K L G H S G T . . . . . I A Y B A W P
Hp_B D E F E S L T . F N G A L R E T Q E L T S L L A R Y R . . . . . E E T S P H G T L E R G L R T V V K L L A P V T P H I C E L E W S E L G . G E G . . . . . F V V B A E W P
Nm_1 G E Y D D L T . F N R A L R E T Q D L V Q T L R Q Y A . . . . . A Y T E P H A B T Y E R G L S A V V R L L A P V A P H L A E B L Y A E L D T G E D G D E T E F V V D A P W P
Hh_1 V E F E D F R . F N H A L Q A V R E L V S L L R R Y Q . . . . . E A T T P D A D T F E R G L A T A A K L L A P V A P H A E E M W A B L G H D D . . . . . L I A B A E W P
Nm_2 T E F E R F R . F H R V V T E V Q E F A R L L G R Y A . . . . . G Y D R I H G E V Y R G R L T I A A L T S P I A P H L G E B L W N K L R G D G . . . . . L V B A D W P
Hh_2 B E Y D R F R . F H R V I G E L Q R F A R L L G R Y A . . . . . G Y D R P Y Q F A Y G R G L R V L A G L V A P I A P P L A E E M W Q L D E D G . . . . . L V A E S R W P
2 Ph  N A L B E F R T R A V Q W A P Y S I M N D L R W Y L R R T E G R D D E A K R Y V L R T L A D V V R L M A P F T P H I C E L E W E K L G . G E G . . . . . F V S L A K W P
Si_1 N D I T S P E P R D A V N L L Y E M Y D V L R D Y F D L I E I P . . . . . N Q E V I R K I S I W I R A L A P F A P H I A E B L W H K I S . S T . . . . . F V S L E K Y P
Si_2 K H M A L E L R D A A N E L L Y F S S D L D E Y F G M V N A E G R R A N N K L R E V L T I W K L L I T P F A P H L A E B I W H E I L K Q T T . . . . . F I V N E K W P
Hp_A D A M D A L F E T R T A S Q D A F Y G F E E H L K W Y R K R T D T D . R S G A R W T L R T V L N A R L R L L A P F I P F M A T E L H E R I T . G N . . . . . P I A B A E W P
Hv  T A M D E A B T S A S Q A A F Y N F E E S L R W Y R R R T D L D . R P A A K W T L R T V L E T R L R L L A P F I P F M T N E L H B Q L T . . . . . P A B D A P W P
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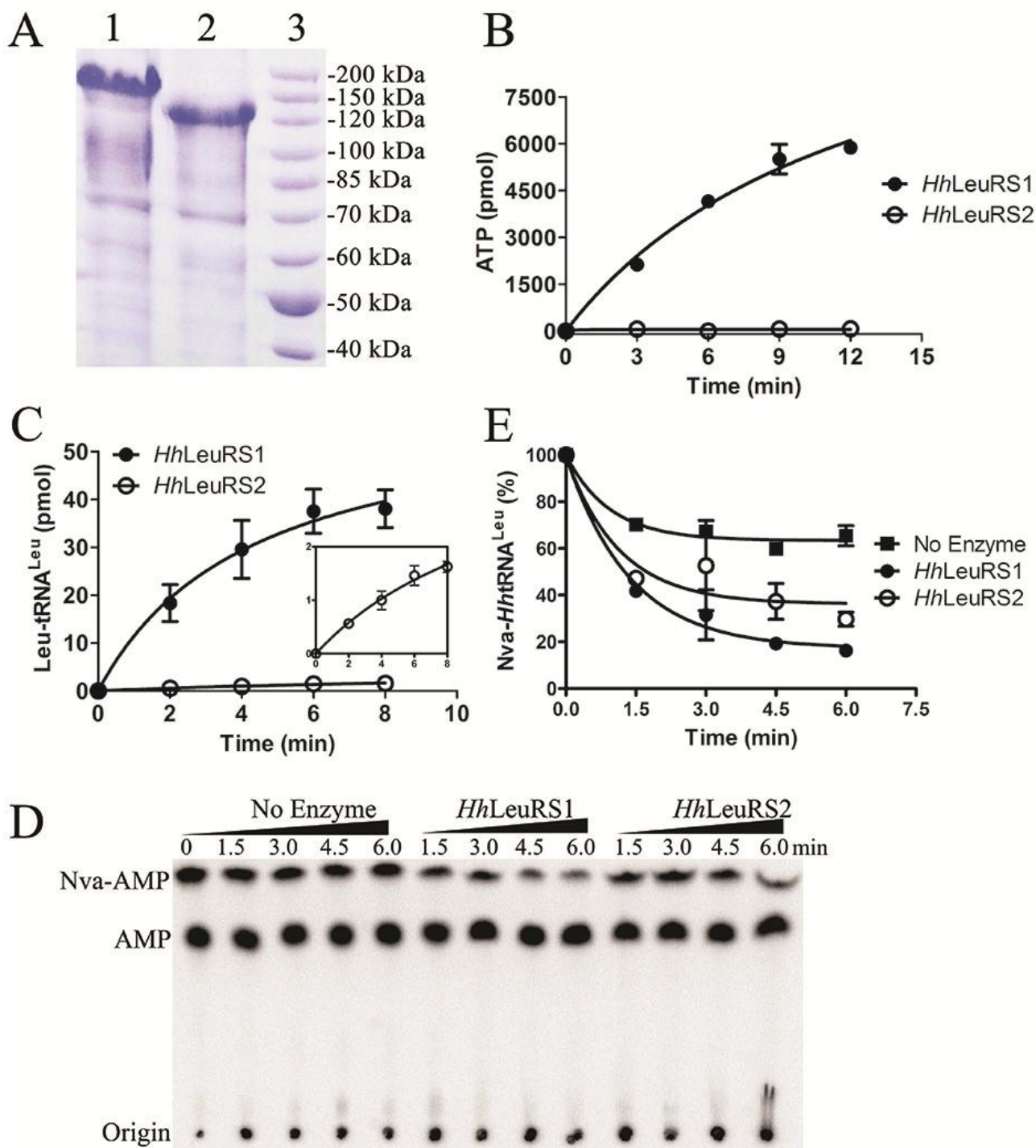
Supplementary Figure 1. Sequence alignment of the N-terminal region (the Rossmann fold, CP1, CP2 and α -helix bundle domain) (A) and C-terminal domain (B) of LeuRSs, respectively. Ec, *Escherichia coli*; Tt, *Thermus thermophilus*; Bs, *Bacillus subtilis*; Hp_B & Hp_A, *Haladaptatus paucihalophilus* 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*.



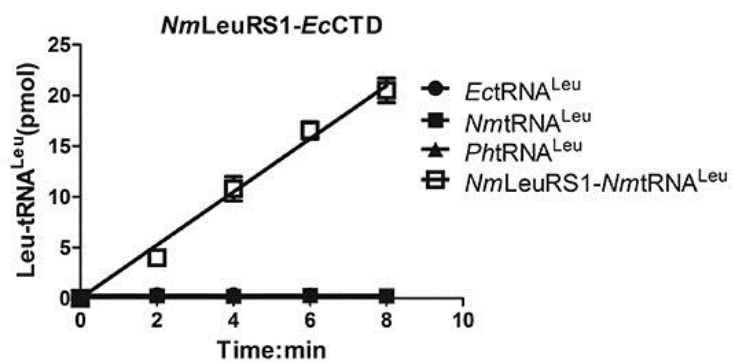
Supplementary Figure 2. *NmtRNA^{Leu}* are typical archaeal tRNA^{Leu} with conserved A47c and G47d bases. (A) Sequence alignment of all five *NmtRNA^{Leu}* isoacceptors and tRNA^{Leu}(GAG)s from different species. Nm, *Natrialba magadii*; Hh, *Haloarcula hispanica*; Ph, *Pyrococcus horikoshii*; Hv, *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 *NmtRNA^{Leu}* with conserved bases specified. The site of A47c and G47d, non-conserved (■) and divergent (□) bases were indicated.



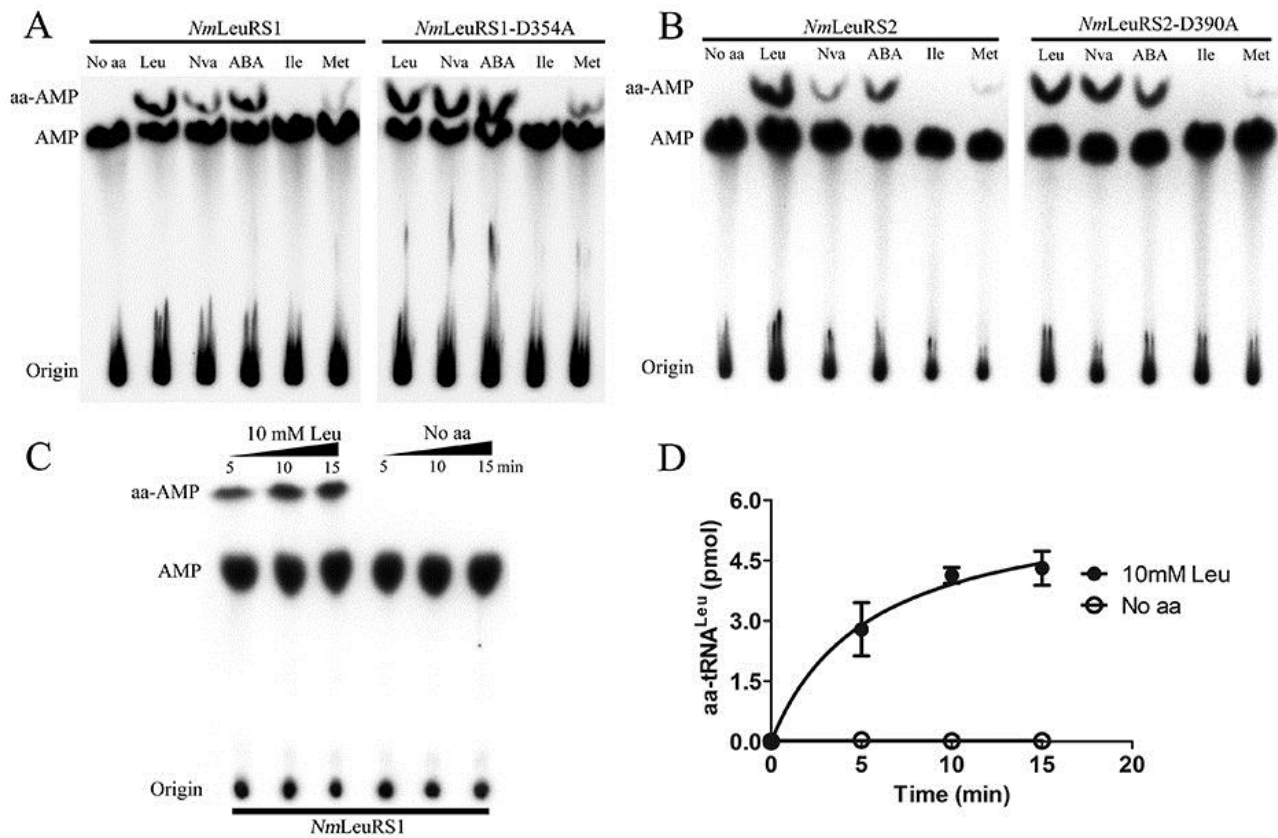
Supplementary Figure 3. Optimization of aminoacylation conditions of *NmLeuRS2*. (A) Relative aminoacylation activity of *NmLeuRS2* under different KCl concentrations (●). The activities of *NmLeuRS2* in 3.4 M KCl were defined as 100%; (B) Relative aminoacylation activities of *NmLeuRS2* under different pH conditions of Tris-HCl (●). The activity of *NmLeuRS2* in Tris-HCl pH9.0 was defined as 100%.



Supplementary Figure 4. *HhLeuRS1* showed higher amino acid activation, amino-acylation and post-transfer editing activities than *HhLeuRS2*. (A) The 10% SDS-PAGE analysis of the purified *HhLeuRSs* from *E. coli*. Lanes: 1, 2, 3 are *HhLeuRS1*, *HhLeuRS2* and molecular markers (Thermo Scientific, #26614), respectively; (B) The amino acid activation activities of 1 μ M *HhLeuRS1* (●) 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 *HhLeuRS1* (●) and 400 nM *HhLeuRS2* (○) for 4 μ M *HhtRNA*^{Leu}(GAG) in pH 8.5, 3.5 M KCl solution; (D) Representative graph showing the post-transfer editing activity of 500 nM *HhLeuRS1* and 500 nM *HhLeuRS2* in pH 7.5, 3.5 M KCl solution based on TLC assays; (E) Quantification of the de-acylation activities of *HhLeuRS1* (●) and *HhLeuRS2* (○) in D.



Supplementary Figure 5. The aminoacylation activities of *NmLeuRS1-EcCTD* for *NmtRNA*^{Leu} (■), *EctRNA*^{Leu} (●) and *PhtRNA*^{Leu} (▲). The aminoacylation activity of *NmLeuRS1* for *NmtRNA*^{Leu} (□) was used as the positive control.



Supplementary Figure 6. The aminoacylation activities of *NmLeuRS1* and *NmLeuRS1-D354A* (A), *NmLeuRS2* and *NmLeuRS2-D390A* (B) for cognate Leu and non-cognate Nva, ABA, Ile and Met based on TLC assays. (C, D) The aminoacylation activities of *NmLeuRS1* for Leu and without amino acid (No aa) were used as the positive and negative control, respectively.