a) Aminoacylation conformation



Supplementary Figure 1. Contacts with *E. coli* $tRNA_5^{Leu}(UAA)$ in LeuRSEC-tRNA functional complexes. (a) Nucleotides that interact with LeuRS in the aminoacylation state and (b) in the editing state are indicated by boxes using the domain color code introduced in Figure 1.

c) Electron density of the 3' end tRNA-LeuAMS



d) 3' end tRNA in bacterial and archaeal LeuRS



Supplementary Figure 1(c) Stereo diagram showing unbiased m(Fo-Fc) difference electron density (contoured at 3 σ) for bases Cyt75 and Ade76 of the tRNA and LeuAMS in the *E. coli* aminoacylation complex. The density was obtained by rebuilding and refinement of the molecular replacement model without inclusion of 75-Cyt-Ade-76 and LeuAMS. **(d)** The tRNA 3' end conformation in the aminoacylation states of LeuRSEC (left, this work) and LeuRSPH (right, PDB 1WZ2) after superposition of the bodies of the enzymes.

Supplementary Figure 2 Phylogenetic analysis of bacterial LeuRS. a)



Supplementary Figure 2. Phylogenetic analysis of bacterial LeuRS. (a) Circular phylogenetic tree showing the main bacterial clades (in bold letters) and subclades in the prokaryotic kingdom. Representative members for each bacterial subclade (in regular letters) were used for the sequence alignments in b-e). Sequences for each species are ordered from top to down according to the identity to *E. coli* LeuRS, and the corresponding secondary structure elements in *E. coli* LeuRS are coloured as in Figure 1. Some of the key residues for aminoacylation/proof-reading and for tRNA recognition are highlighted by stars.

c) Sequence alignment of bacterial LeuRS

,	1 0		Editing			Cata	alytic	
	E.coli	- 2000000000	·	\rightarrow \rightarrow				· eee
100		400	410	420	430		440	450
	E.coli	FNAIADKLTAMGVG	ERKVNY	(RLRDWGV <mark>SRQ</mark>)	YW G A P I	PMVTLED	GTV <mark>M</mark> P	TPDDQ <mark>LP</mark> VIL
	Salmonella.E	FNAIADKLAEKGVG	ERKVNY	(<mark>rlrd</mark> wgv <mark>srq</mark>)	YW G API	PMVTLED	GTVLP	TPEDQ <mark>LP</mark> VIL
	H.influenzae	FNGIADKLEKLGVG	KRQVNY	(<mark>RLRD</mark> WGV <mark>SRQ</mark>]	RYW <mark>G</mark> API	PMLTLEN	GDV <mark>V</mark> P	APMED <mark>LP</mark> IIL
	Xanthomonas.C	FEALAERFERKGQG	QRRVNY	[<mark>RLRD</mark> WGV <mark>SRQ</mark>]	RYWGCPI	PVIYCAK	CGA <mark>V</mark> P	V P E D Q <mark>L P</mark> V V L
	Neisseria.M	FDAVAAKLQNQGAG	EPKTQY	(<mark>RLRD</mark> WGI <mark>SRQ</mark>]	RYW <mark>G</mark> CPI	PIVHCEQ	CGD <mark>V</mark> P	V P A D Q L P V V L
	P.aeruginosa	FDAIGADLEAKGLG	QARTQE	` <mark>RL<mark>RD</mark>WGI<mark>SRQ</mark>]</mark>	RYW <mark>G</mark> C <mark>P</mark> I	PIIHCDA	CGD <mark>V</mark> P	V P A E Q <mark>L P</mark> V V L
	Buchnera.A	TEKIKKILYKKKIL	K <u>EKINY</u>	KLQDWCI SRQ I	RYW <mark>G</mark> TPI	ΡΜΑΚΓΚΝ	GKMIP	IPENQ <mark>LP</mark> VVL
2	Desulfovibrio.V	KQKIADSLETSGKG	R RTVN Y	RLRDWN I SRQI	YW G API	PVVYCDA	CGV <mark>V</mark> A	EKDEN <mark>LP</mark> VLL
	Clostridium.T	IGKIIDYIEQKGYG	K <u>RKIN</u> Y	RLRDWLISRQI	RYW <mark>G</mark> API	PIIYCDD	CGA <mark>V</mark> P	V P E E D <mark>L P</mark> V I L
	Agrobacterium.T.C58	FEAVVQKLSAQSLG	NAPQAE <mark>RKVN</mark> E	' <mark>RLRD</mark> WGI SRQ I	XYW <mark>G</mark> C <mark>P</mark> I	PVIHCEV	CGV <mark>V</mark> P	V P K K D <mark>L P</mark> V K L
d	P.gingivalis	IAQTKEYIREKHLG	CVKVNY	(RLRDAIFSROI	RYWGEPF	ΡΙΥΥΚΕG	MP H A	LDEDR <mark>LP</mark> LRL
6	T.thermophilus	KRKVIAWLEEKGLG	K <mark>GRVT</mark>	(RLRDWLISRQ)	XYW <mark>G</mark> T P I	PMVHCEA	CGV <mark>V</mark> P	V P E E E <mark>L P</mark> V L L
	T.maritima	IEKVINWLEEKGIG	K <u>RSVQ</u> Y	KLRDWLISRQI	YW G API	PIIYCEK	CGV <mark>V</mark> P	V P E E D <mark>L P</mark> V R L
	R.palustris	KEDVAKRLETATLG	NAPVGE <mark>RKVN</mark> E	' <mark>RLRD</mark> WGI SRQ I	YWGCPI	PVIHCEV	CGV <mark>V</mark> P	V P D K D <mark>L P</mark> V V L
0	P.marinus	KLKISEEGVNNGWA	E <mark>NKIO</mark> Y	RLRDWLISROI	X Y W G C P I	ΡΙνΝΟΚΚ	CGS <mark>V</mark> P	LNOSELPVAL
	Bacillus.S	IEKVIAWLEETKNG	E <u>KKVŤ</u> Y	RLRDWLFSROI	RYW G E P I	PVIHWED	GISTA	V P E E E <mark>L P</mark> L I L
	C. jejuni	RLKIISQFEAKNIG	K rvvn e	KIRDWGVSRQI	YW G API	PMIKCOS	CGI <mark>V</mark> P	Q K L E N <mark>L P</mark> I T L
	Streptococcus.N	IAKIVACLEEKGCG	Q <u>ekvt</u> y	RLRDWLFSRQI	YWGEPI	PIIHWED	GISTA	VPETE LP LVL
	H.pylori	REKIIAYFEKENLG	KRVINY	RLQDWGV SRQ	RYW <mark>G</mark> API	РМІНСКН	CGI <mark>V</mark> P	ETQ <mark>LP</mark> VTL
	Wolbachia.Sp	KEVIIKKLKEKGIG		RLHDWGVSROI	YWGCPI	PIIYCKD	CGT <mark>V</mark> P	VPEKDLPVIL
	C.pneumoniae	KDYVINYLEMRSLG	RAKTMY	RLRDWLFSROI	RYW G E P I	PIIHFED	GTH <mark>R</mark> P	LEDDE <mark>LP</mark> LLP
	S.aureus	ITKAIQLLEQKGAG	E KKVNY	KLRDWLFSRQI	XYW G E P I	PVIHWED	GTM T T	V P E E E <mark>L P</mark> L L L
	L.casei	IDRMIKWLEDKGIG	SAKINY	KLRDWVFSROI	YWGEPI	PVIHWED	GET <mark>T</mark> L	VPEDE <mark>LP</mark> LTL
	Borrelia.B	KDKVIKWLTKNKKG	K <u>ekva</u> y	KLRDWIFSRQI	RYWGEPI	PILFDKL	GNAIP	LEENDLPLKL
	R.baltica	KAALTAELAKQGLA	CEAVNY	KLRDWLFSROI	RFWGEPF	PVLHEIDSEG	NATGVR <mark>R</mark> L	V P D D Q <mark>L P</mark> V T L
	M.mycoides	LQVIHDYVEKNNLG	KRKINY	KLRDWLFSRQI	RFYGEPF	PVLYDKD	NNIVL	IEDNN <mark>LP</mark> ITL
40	M.pneumoniae	NTQITQMLVERQKA	K KTTV Y	KLRDWIFSRQI	YWGEPF	PILFDE	NNQP	HLVKE <mark>LP</mark> VTL

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E.coli	200	20000	معععه	2000	فعف	eee	4720	مععد	22222	معع	2000
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E.COII	NVDAL	IENQKAL	RRDVI	IKIIA	V I	JUIG	· RRQ		AT AAT ME	LML	KLAKAP
Salmonella.E	NVDAL	SEDQKAL	RRDVI	IKTIA	VII	DDVG	. RRQ	TFNTA	AIAAIME	LMN	KLAKAP
H.influenzae	DLTAL	SAEQKVL	RREVE	IKTIA	VSI	DDIG	. RRQ	TFNTA	ALAAVME	LMN	KLTKAP
Xanthomonas.C	DVAAL	SAEQKAV	RRKTH	IETIG	VSI	DDYG	. RRH	SFNTA	AIAAVME	LSN	ALAKFD
Neisseria.M	AGSQDGL	SKELKDL	RHKLH	ISTIA	VS	DDYG	. <mark>RR</mark> Q	QFNTA	AIAAVME	LLN	QYDKTD
P.aeruginosa	DGASL	SDAQKQV	RRAIH	ILAIR	QAS (QDVG	.QHH	K F N T F	AIAAVMT	LMN	VLEKAP
Buchnera.A	NFDFL	NHQQSEL	RYQLH	IKTIA	VS	DDIG	. RRQ	TFNTZ	AISEIMK	LVN	QLSKAP
Desulfovibrio.V	ATAADAA	TPQGKD <mark>L</mark>	RNKEF	HATVR	AGZ	ADISD.	RF	QFNTA	AIAAVME	LVN	ALYLAK
Clostridium.T	NIDTSTF	T K A D K E <mark>L</mark>	WYMLN	JNTLK	VTI	DDISQ.	RF	NFNT F	AISAVME	ΓΛΝ	SLYYYK
Agrobacterium.T.C58	ATEG	EGLA <mark>A</mark>	SKAAH	4KTLK	VQ	DLD	KL.	AFNK <i>F</i>	AIARIYE	ΓΛΝ	ALAGPLADVA
P.gingivalis	DTAP	TKE <mark>E</mark>	LKSLH	IKLIK	VGς	Q <mark>D</mark> IE	SF	SFNTS	SIPAFMI	CVN	ELTAAK
T.thermophilus	VFQAEAL	EGKDRE <mark>L</mark>	YGKLH	HETLK	VT	DLEA.	L	R F N T A	AIAALME	FLN	ALYEYR
T.maritima	TENLVLK	NSTEKE <mark>L</mark>	RRKLF	ISIIK	ITI	DIEGG	F	K F N T I	\ISGLME	ΓΛ	HLSQYL
R.palustris	DAFGP	EALAV	RKAAF	IGALD	VLS	SGIE	R L.	AFNVS	SLAHIRE	FSN	SLGEALARPG
P.marinus	Y	PNKEKT	IKSMN	JIAIKI	ITI	NDIS	N N	QFNT <i>F</i>	AISELMK	FΥΝ	SLSNNI
Bacillus.S	VEGA	GET <mark>L</mark>	ERVYF	HE TVM	VT	HYE	GL	RFNT0	JISQLMV	FIN	EAYKAT
C.jejuni	QENL	NKEEKY <mark>A</mark>	RLKVY	ΎΕΑΙΚ	SFE	VY	HQSF.	AFNTI	JIAACME	ALN	ALALC.
Streptococcus.N	LAEN	NGAL	DKAYN	JETVKZ	VT	QIE	SL	KFNTA	AIAQLMV	FVN	AANKED
H.pylori	EVGL	NEAQKL <mark>A</mark>	RKKVY	ΎΕΑΙΚ	SH	IFNKA	ESAY	AFNTI	IASCME	ALN	ALNAQ.
Wolbachia.Sp	SVTG	GLLE <mark>Y</mark>	RKKIH	IKLLH	LT	DLE	NC	RLNCV	/VAKFRE	ΜTΝ	LIAEID
C.pneumoniae	QDIE	DRD <mark>G</mark>	LVLAH	IKLVF	ITI	HIE	<mark>K</mark> M	SLNTI	PSSFME	FLN	DFSKLP
S.aureus	VTTN	NKSL	DKVYN	JQTVK	VT	DFE	T L	GFNT#	AISQLMV	FIN	ECYKVD
L.casei	TTIN	DGR <mark>L</mark>	DKVYN	JETVK	VT	DYD	AL	HFNT7	ISQMMV	FIN	SARKDD
Borrelia.B	KENP	PRE <mark>I</mark>	ISELH	IKVIKI	VTI	DTE	KL	NFNT7	AISAMMI	FIN	ELLKYE
R.baltica	DTAC	DEE <mark>Q</mark>	LRVLH	IQTIR	VT	DNE	AM	SFNTA	AIAKMME	FTN	HFTRCE
M.mycoides	SNTN	NHKL	DYVYN	JDVVK	VTC	ZMIQ	E L	K F N T A	ISQLMV	LVN	AIYKEE
M.pneumoniae	KETV	DQE <mark>T</mark>	VYGYN	JL <mark>FL</mark> K	SF	HLE	<mark>K</mark> Q	ELNLV	/ISQMMI	FLN	LLYKTK

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E.coli				_	-							_	•				٤	٥٥	e	٥	يە	٩	٤	1	وه	Q	2		_		1	_			-		-			-	
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E.coli	1	Ľ	٧V	7V	۷Q	7 N	GI	ΚV	RZ	Δĸ	I.	ΓV	Ρ	VI	A	ΤI	ΕE	Q	V.	RΕ	R	AG	Q	ΕH	ΙL	V A	ĸ	ΥL	D	ΞV	ΤV	7R	٢V	IΥ	VI	? G	КL	LN	ΓV	7 V G	
Salmonella.E	1	Ľ	٧V	7V	٧Q	7 N	GI	ΚV	R	GΚ	I.	ΓV	Α	VI	A	Τ1	ΕĒ	Q	V.	RΕ	R	AG	Q	ΕH	ΙL	VA	ĸ	ΥL	D	ΞV	ΤV	/R	۲V	ΙY	VI	? G	ΚL	LN	$\Gamma \Lambda$	1 V G	
H.influenzae	F	ίL	ΙV	7 V	٧Q	7 N	GI	ΚV	R	ΞK	V.	ГV	Α	ΑI	A	D 1	ΕĽ	Т	V.	KΤ	I	ΑF	'A	DE	Ν	VΚ	ĸ	F I	DI	QV	ΗI	I V I	۲V	ΙY	V	ΙG	ΚL	LN	٧V	7 V K	
Xanthomonas.C	I	Т	LΡ	۱V	QI	ΕN	GI	ΚL	R	GΤ	ΙI	V	Α	ΑI	A	Τ1	R E	Q	I	ΞA	. Ц	ΑÇ	<u>)</u> A	ΕP	Ν	ΑA	ĸ	ΓL	D	ΞL	SV	7R	۲N	ΙI	VI	? G	ΚI	VN	ΙV	/AG	
Neisseria.M	1	E	V٢	1 V	٧Q	7 N	GI	ΚL	R	βK	I.	Г٧	Α	ΑI	A	SI	ΚÆ	1D	\mathbf{L}	ΞA	A	ΑI	A	ΤE	G	ΑV	ĸ	T M	Ε (ΞK	PF	λK	<i.< th=""><th>ΙV</th><th>VI</th><th>? G</th><th>RL</th><th>VN</th><th>ΙV</th><th>/V.</th><th></th></i.<>	ΙV	VI	? G	RL	VN	ΙV	/V.	
P.aeruginosa	I	Q	Γ	7 V	٧Q	7 N	GI	ΚL	R	GΗ	ΙI	V C	Α	AS	5 A	SI	RΕ	D	V	ΞA	A	ΑR	١A	ΝE	Ν	VI	R	Τ	Е (GL	SJ	I R	۷V	ΙV	VI	? G	ΚL	VN	ΙV	/ A N	1
Buchnera.A	5	зT	IV	7 A	QI	ΕN	GI	ΚK	R	CA	ΤI	ΚI	S	DS	δL	ΤI	ΚE	ΞE	I	FL	Υ	ΙÇ	2 N	QP	Ι	ΙK	ĸ	ΥL	ΕI) V	DJ	ΓK	ΚI	ΙY	II	? K	ΚI	IN	FΛ	/Т.	
Desulfovibrio.V	E	Т	IV	7 V	01	ΕN	GI	ΚL	R	3 R	V	sν	Р	AO	ΞA	Di	Αk	٢A	I	ΞA	A	ΑI	S	ΕP	Ν	VA	R	L	ΕI) V	ΤN	/R	(V	VV	ΊI	9 G	ΚL	VN	٧V	/ V G	ć
Clostridium.T	1	E	ΙV	7 V	01	ΕN	G	ΚV	R) <mark>K</mark>	I١	νV	Р	SI	L	ΤI	ΚE	z o	V	ΕE	R	ΑI	N	SE	K	ΙK	A	ΕТ	A (GΚ	N	7 V I	۲V	ΙS	V	? G	КL	VN	ΙV	∕ V K	
Agrobacterium.T.C58	1	7V	ΜF	v	٥ı	7 N	GI	ΚK	R	ΞE	L.	ГΙ	Α	RI	A	D	QE	A	V	RΤ	А	ΑI	E	LD	A	VK	S	ΙL	A (ΞG	ΕF	P K	۷V	ΙV	VI	20	RΙ	VN	ΙV	7V.	
P.gingivalis	7	/R	ΥF	v	SI	ΓN	GI	ΚV	R	ΓN	II	ΞL	P	ΑI	M	SI	Κŀ	(D	V	ΞQ	А	ΑI	г	ΑP	E	AA	R	ΝL	Е (GΚ	SF	P K	۷V	ΙV	V	P G	RΙ	VN	٧V	/V.	
T.thermophilus	7	ΤE	VP	١V	ZQ	7 N	GI	R V	R	ЭT	ΙJ	Ι	Ρ	ΚI	A	Р:	LΕ	v	A	RA	E	ΑI	ΓK	VR	N	VF	А	: L	ΕO	ΞK	ΕV	7 V I	٢E	ΙY	V	? G	ΚI	LN	LΙ	/ V R	
T.maritima	7	ΤE	ΙÆ	ΙI	Q1	ΕN	GI	ΚV	RI	K	٧V	νV	Ρ	VI	I	SI	ΕĒ	D	L	ΚR	I	VI	ιE	RE	R	VK	Е	ΥV	D	GΚ	ΡJ	I R	٢F	ΙY	V	ΚG	RΙ	VN	ΙV	JV.	
R.palustris	1	Т	LΡ	v	٥١	7 N	GI	ĸκ	R	ΞE	V:	ΓV	A	RI	A	ΝI	N F	E	I	ΞA	A	VI	A	LD	A	VF	0	ΑL	D	GΚ	ΡN	7R	۷	ΙV	VI	2 0	RΙ	VN	٧V	/G.	
P.marinus	3	ΖV	Γ.	7 I	γQ	7 N	GI	ΚV	R	ΣK	Ιl	ΙR	Ν	ΝE	C M	NI	ΕĽ	Q	I	ΚE	L	ΤI	ĸ	RΡ	Ν	ΙI	ĸ	Ϋ́Τ	QI	ΟK	ΕJ	I R	<i.< th=""><th>ΙI</th><th>V</th><th>ΚG</th><th>ΚI</th><th>ΜN</th><th>ΙV</th><th>7V.</th><th></th></i.<>	ΙI	V	ΚG	ΚI	ΜN	ΙV	7V.	
Bacillus.S	7	ΓE	IV	7 V	QI	LΝ	GI	ΚV	K Z	ΑK	Гò	Ω	Ρ	ΑI	A	ΤI	ΚE	Q	L	ΞQ	L	AÇ) A	DE	K	VK	Е	QΓ	ΕO	ΞK	ΤJ	I R	<i.< th=""><th>ΙA</th><th>V</th><th>P G</th><th>ΚL</th><th>VN</th><th>ΙV</th><th>/ A N</th><th>1</th></i.<>	ΙA	V	P G	ΚL	VN	ΙV	/ A N	1
C.jejuni	I	N	LΡ	١V	S1	ΕN	GI	ΚK	R Z	ΑE	F]	ΞI	S	SS	5 A	SI	ΚĒ	ΞE	I	LΑ	F	ΑK	Έ	Ν.		ΤA	ĸ	ΝL	Е (GΚ	SJ	[V]	٢E	ΙY	VI	ΞG	ΚL	VN	ΓV	ΙIΚ	
Streptococcus.N	1	ΞE	IV	7 V	QI	ΓK	GI	ΚV	R7	ΑK	Lľ	٧N	A	ΚI	L	SI	RE	ΕE	L	QΕ	Ι	ΑI	A	DE	K	VK	A	ΕI	D	ΞK	ΕJ	[V]	۷	ΙS	V	P N	ΚL	VN	ΙV	<mark>i v</mark> k	
H.pylori	Þ	1 T	ΓC	ΓL	Τ1	ΕN	G	ΚR	R /	ΑE	L	ΧV	N	Ιľ	JΑ	SI	ΚE	Ε	I	ΙI	L	ΑK	Κ	Ε.		LΕ	ĸ	ΥL	ΕI	NΑ	S	7 K	٢E	ΙY	V	P N	ΚL	VN	FΛ	ΙTΑ	
Wolbachia.Sp	7	/Т	ΙÆ	١V	QI	ΕN	GI	ΚL	R7	ΑT	II	ΞV	A	ΙN	JΓ	Р (QE	ΕE	L	κ.		ΚI	A	ΙD	S	VS	S	ΚI	D	ΩN	Κ	/R	C V	ΥA	V	P N	ΚI	VN	ΙV	/Ι.	
C.pneumoniae	7	7Т	FΝ	7 V	٧Q	7 N	GI	ΚL	R	GR	LI	ΞV	A	ΚB	ΞA	Ρ1	ΚĒ	ΕE	V	LS	L	SR	٢S		V	VA	ĸ	ΥL	ΕI	NΑ	QJ	I R	٢E	ΙY	V	P N	ΚL	VN	FΛ	/Ι.	
S.aureus	7	ΤE	IV	7 V	٧Q	7 N	GI	ΚL	R Z	ΑK	ΙJ	ΚI	Α	ΚI	Т	SI	ΚE	ΞE	М	QΕ	I	ΑI	S	ND	N	VK	А	SΙ	E (GΚ	DJ	ΙM	۷V	ΙA	V	? Q	ΚL	VN	ΙV	ΙAΚ	
L.casei	2	Δ	ММ	1 I	10	7 N	GI	ΚL	R	3 S	I:	ΓА	D	٧N	JΕ	SI	DE	E	I	κo	А	ΑI	A	ND	N	vc	ĸ	T T	A (ΞK	DJ	ΙK	<i< th=""><th>ΙV</th><th>VI</th><th>R</th><th>ΚI</th><th>VN</th><th>ΙV</th><th>ΙAΚ</th><th></th></i<>	ΙV	VI	R	ΚI	VN	ΙV	ΙAΚ	
Borrelia.B	P	ξE	IV	7L	Q1	ΕN	GI	ΚI	κI	K	II	LΙ	N	ΚE	ΞТ	GI	Εŀ	ΚE	L	ΚĒ	Ι	ΑM	1 E	ΝS	K	ΙK	S	ΝL	ГI	ΝK	ΚJ	I V	< I	ΙV	ΊI	ΚN	ΚL	VN	ΙV	ΙIΚ	
R.baltica	1	ΞE	ΙF	V	Q1	ΕN	G	κv	K Z	ΔK	IS	SI	S	ΡI	A	ΚI	ΡN	JΕ	М	ΞE	А	ΑI	A	DP	А	VÇ	Ν	ΑI	GΙ	ΟK	Κ	7 V I	۲	ΙA	VI	G	RМ	VN	LΙ	∕ V K	
M.mycoides	7	7V	ΙP	ΙL	2V	7 N	G	КL	RS	5 T	IJ	ΞV	Е	ΚC	ЗT	DI	ΚE	Т	L	ΙN	L	ΑE	K	ΝE	Ν	ΙÏ	ĸ	T I	ΚI	ΟH	ΚJ	ΓL	۲R	ΙA	V.	I D	RΙ	VN	ΙV	ΙE	
M.pneumoniae	V	ΙK	ΙI	L	SI	7 N	G	RΕ	K	GΤ	K	ΞF	Α	ΚC	ΞV	DI	ΕÇ	Σg	V	LΚ	А	FK	Т	DP	Е	FÇ	А	. L	Γl	QC	ΡI	ΊA	R V	VF	'VI	P N	RΙ	ΙN	VΙ	LK	1

Supplementary Figure 3. Comparison of LeuRSEC and LeuRSTT.

a) LeuRSEC:tRNA^{Leu} editing complex



Supplementary Figure 3. Comparison between (a) *E. coli* and (b) *T. thermophilus* **LeuRS:tRNA**^{Leu} editing complexes. For each complex, two orientations with a rotation about the vertical axis by 90° clockwise are shown. Domains are colour coded as in Figure 1. The main differences between the two complexes are highlighted on the *T. thermophilus* editing complex. The inset panels show tRNA^{Leu} shape recognition by the two LeuRSs. tRNA^{Leu} (grey surface) is grasped between the anticodon-binding (red) and the C-terminal (orange) domains. The loop 595-600 in the leucine-specific domain (pink) and specific bases of the variable arm of the tRNA (blue surface) participate in the interactions in the *E. coli* complex, but not in the *T. thermophilus* complex.



Supplementary Figure 3c-e. Comparison between the ZN1 domain orientation and interface with the editing domain in the (c) *T. thermophilus* LeuRS:tRNA^{Leu} editing complex and (d) *E. coli* LeuRS:tRNA^{Leu} aminoacylation complex. Different interactions with the editing domain loop 277-303 stabilise the ZN1 domain in the closed/down position (c) in the LeuRSTT editing complex or in the open/up position (d) in the LeuRSEC aminoacylation state. Both figures are drawn with the same view after superposition of the enzyme bodies The last acceptor stem base-pair and 3' end of the tRNA is shown. In the closed position (d), the ZN1 domain stabilises the stacking of Tyr43 over the leucine substrate. With the ZN1 domain in this position, binding of the 3' end of the tRNA in the functional aminoacylation configuration is impossible. The closed configuration has not so far been observed in the *E. coli* system where the ZN1 domain is disordered, presumably due to flexibility in the LeuRSEC editing complex and there is no LeuRSEC structure in the absence of tRNA. (e). Schematic view of the rotation of the flexibly linked ZN1 domain between the editing (red) and aminoacylation states (blue), after superposition of the enzyme bodies (*E. coli* LeuRS is represented in grey). The rotation to the open position allows 3' end binding for aminoacylation.

Supplementary Figure 4. Mutational analysis of LeuRSEC.

a)



b)



Supplementary Figure 4a,b. Ura16 is a new tRNA identity element in *E. coli.* (a) Specific recognition of Ura16 by LeuRSEC as observed in both the editing and aminoacylation LeuRS:tRNA^{Leu} complexes. The residues of the anticodon-binding domain (red) involved in the recognition of Ura16 are shown as sticks. (b) Effects of anticodon-binding domain mutations (left) on the kinetic parameters of LeuRSEC and effects of Ura16 mutations (right) on the aminoacylation of tRNA^{Leu}. Error bars represent standard deviations for three independent reactions.

c) Arrhenius plots



Supplementary Figure 4c,d. Glu532 is involved in ATP-activation. (c) Arrhenius plots showing the leucine-dependent PPi-ATP exchange activity (left) and overall aminoacylation (right) measured at 20, 25, 30, 35 and 40 °C for the wild-type (diamonds) and the E532Q mutant (squares). (d) Free energy diagrams representing the ATP-activation energies (left) and overall aminoacylation energies (right) for the wild type (continuous line) and the mutant E532Q (dashed line) *Ec*LeuRS.

	Buried surface (Ų)ª										
	E. coli	E. coli	T. thermophilus								
	aminoacylation	editing	editing								
Total	5 759	4 830	4 144								
Catalytic	1 867	559	610								
ZN1	456	-	110								
Editing	416	1 065	1 210								
Leucine-specific (including KMSKS)	433	368	0								
Anticodon-binding	1 716	1 760	1 390								
C-terminal	1 171	1 151	894								

Supplementary Table 1 Buried surface area due to tRNA binding

^aSurfaces were calculated using NACCESS (http://www.bioinf.manchester.ac.uk/naccess/). Accessible surface area buried upon complex formation (S) was obtained using the formula: $S = S_{prot} + S_{tRNA} - S_{complex}$.

Buried surface normalized											
	E. coli	E. coli	T. thermophilus								
	aminoacylation	editing	editing								
Total	1	1	1								
Catalytic	0.3	0.114	0.144								
ZN1	0.075	-	0.026								
Editing	0.068	0.217	0.287								
Leucine-specific (including KMSKS)	0.071	0.075	0								
Anticodon-binding	0.283	0.359	0.33								
C-terminal	0.193	0.234	0.212								
Domains limits											
	Е. са	li	T. thermophilus								
	1-14	8	1-148								
Catalytic	194-2	24	195-223								
	416-5	68	148-576								
ZN1	149-1	93	149-194								
Editing	225-4	15	224-417								
Leucine-specific (includin KMSKS)	g 569-6	29	577-646								
Anticodon-binding	630-7	95	647-812								
C-terminal	796-8	60	813-878								