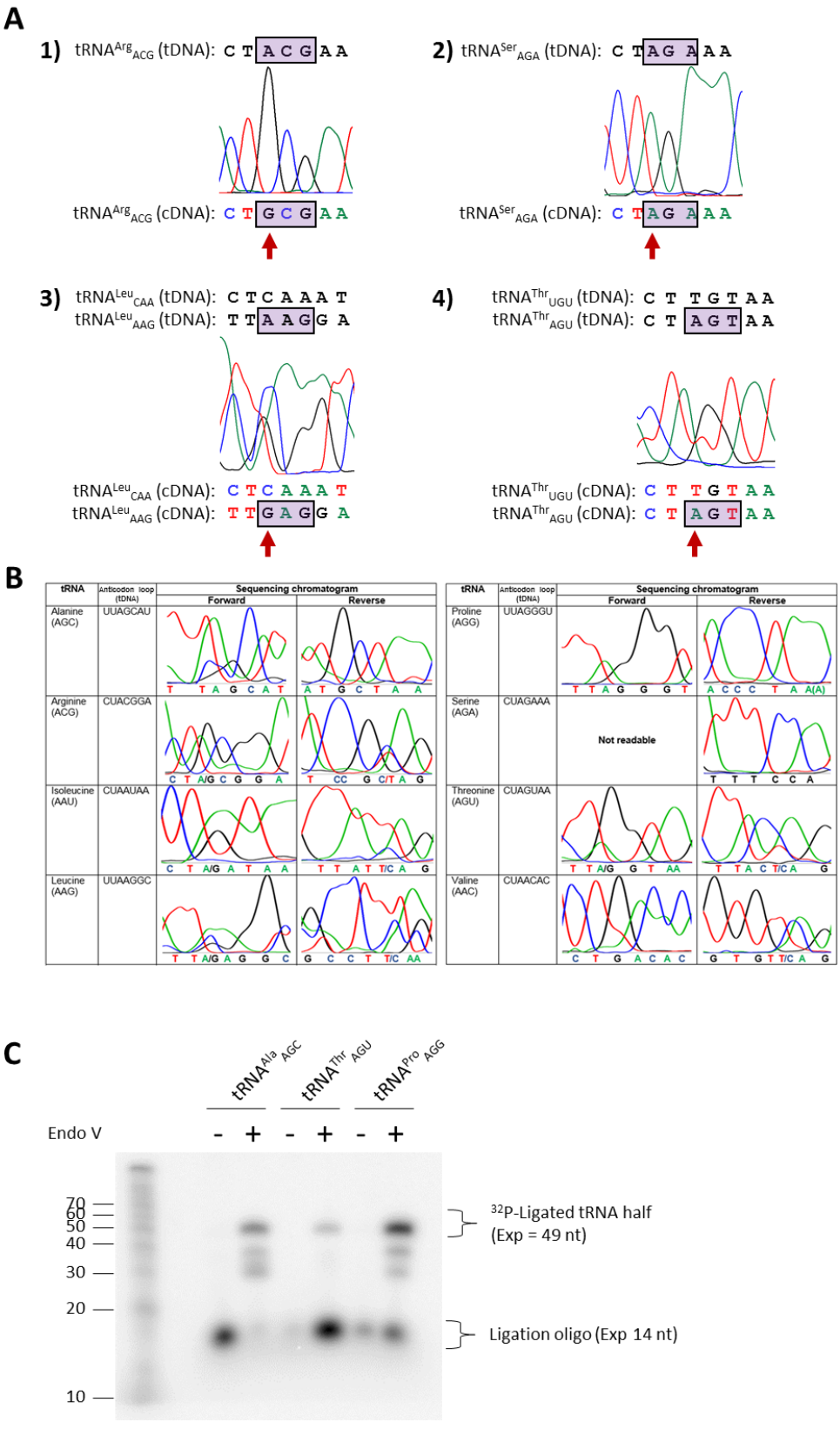

Figure S1: (A) Sequencing spectra of RT-PCR amplicons derived from *O. oeni* tRNA$^{Arg}_{ACG}$ (1), tRNA$^{Ser}_{AGA}$ (2), tRNA$^{Leu}_{AAG}$ (3), and tRNA$^{Thr}_{AGU}$ (4). The anticodon sequence is highlighted, the 'wobble' position is indicated (arrow). Inosine at this position should be detected as a Guanosine, as opposed to the unmodified residue which is detected as Adenosine (tDNA sequences are shown for reference). Note that due to sequence similarities, sequencing of RT-PCR amplicons derived from tRNA$^{Leu}_{AAG}$ are partially masked by amplicons derived from tRNA$^{Leu}_{CAA}$; and those derived from tRNA$^{Thr}_{AGU}$ are partially masked by amplicons derived from tRNA$^{Thr}_{UGU}$. **(B)** Sequencing spectra of RT-PCR amplicons derived from *T. thermophila* tRNA$^{Ala}_{AGC}$, tRNA$^{Arg}_{ACG}$, tRNA$^{Ile}_{AAU}$, tRNA$^{Leu}_{AAG}$, tRNA$^{Pro}_{AGG}$, tRNA$^{Ser}_{AGA}$, tRNA$^{Thr}_{AGU}$, and tRNA$^{Val}_{AAC}$. Sequencing spectra using Forward and Reverse primers are shown. Inosine at the anticodon 'wobble' position should be detected as a Guanosine (Cytosine in the reversed strand), as opposed to the unmodified residue which is detected as Adenosine (Thymine in the reversed strand). **(C)** Evaluation of I34 presence/absence on *T. thermophila* tRNA$^{Ala}_{AGC}$, tRNA$^{Thr}_{AGU}$, and tRNA$^{Pro}_{AGG}$ by SL-ID.

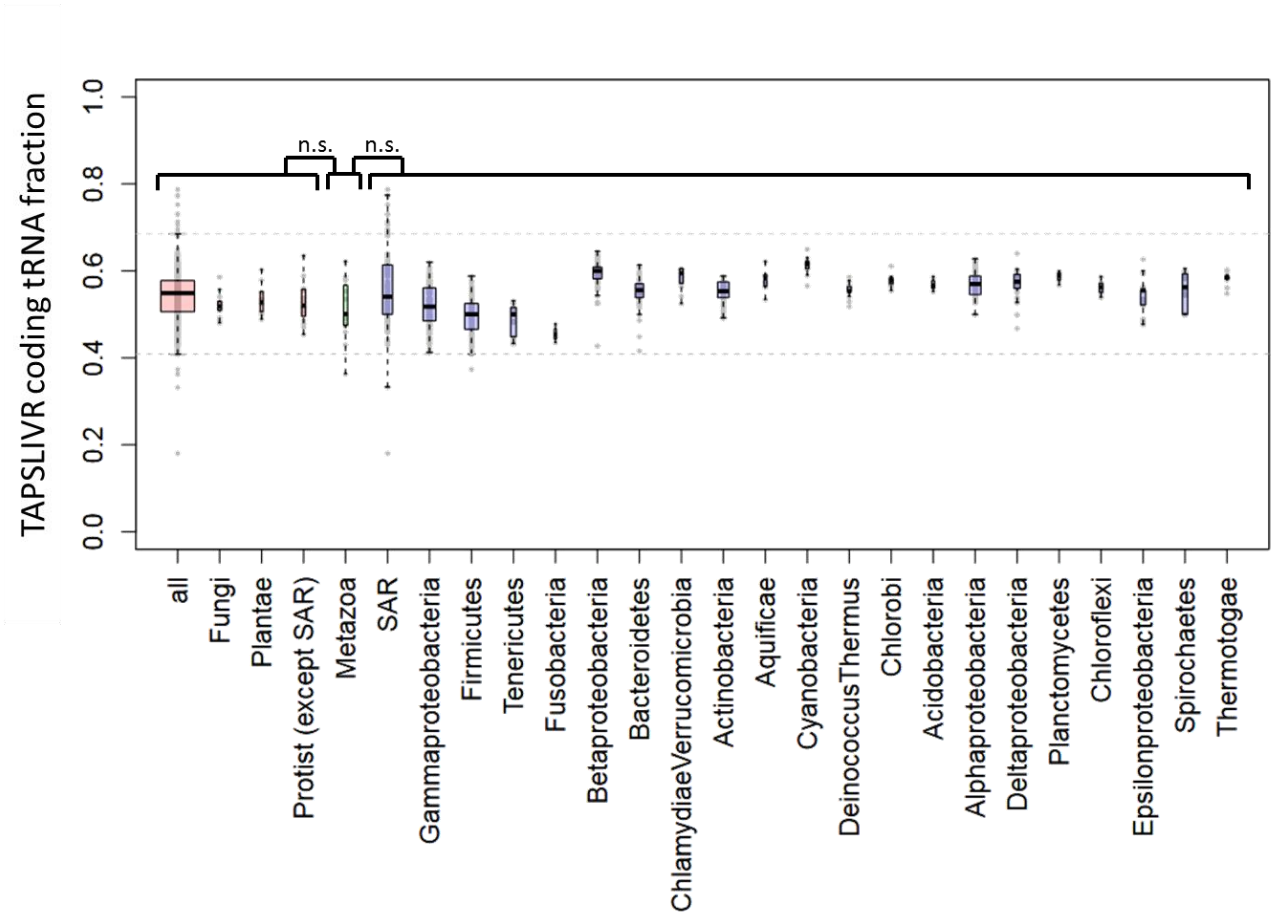

Figure S2: tRNA quantification boxplot for eukaryotic kingdoms (red), the eukaryotic superphylum *Heterokonta* (green) and different bacterial phyla (blue). The y-axis represents the fraction of all the isoacceptors for TAPS and LIVR amino acids among all the isoacceptors for all the amino acids. The width of each boxplot is proportional to the number of organisms analyzed. 'all' boxplot represents all the data together. Horizontal dashed lines are placed in Q1 = 0.41 and Q4 = 0.68 from 'all' boxplot.

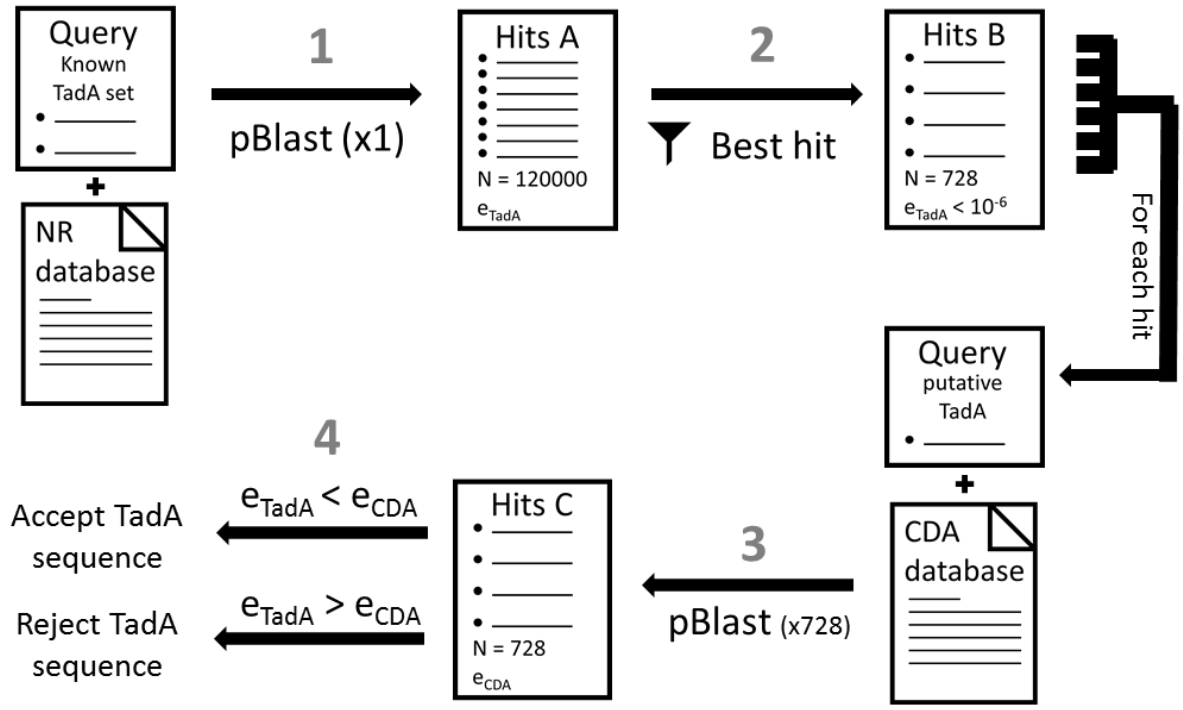

Figure S3: Identification of TadA sequences scheme. NR: non-redundant NCBI database. e_x: BLAST e-value from X query. CDA: cytidine deaminase. pBLAST: protein BLAST. N: number of hits. The scheme is analogous to find the ADAT2 and ADAT3 protein sequences.

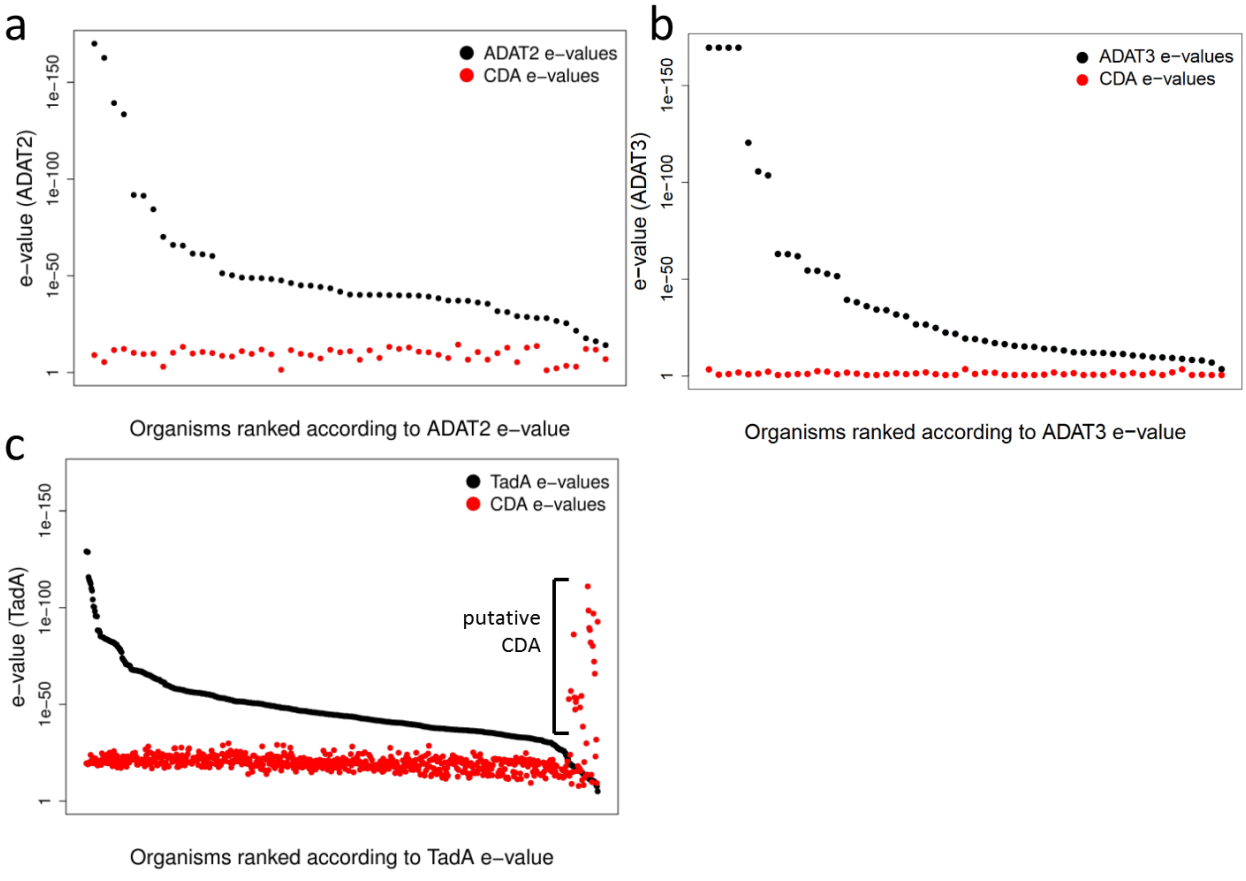

Figure S4: comparison of e-values between the Adenosine deaminase (black circles) and the cytidine deaminases (grey circles). The values are ranked for ADAT2 (a), ADAT3 (b) and TadA (c).

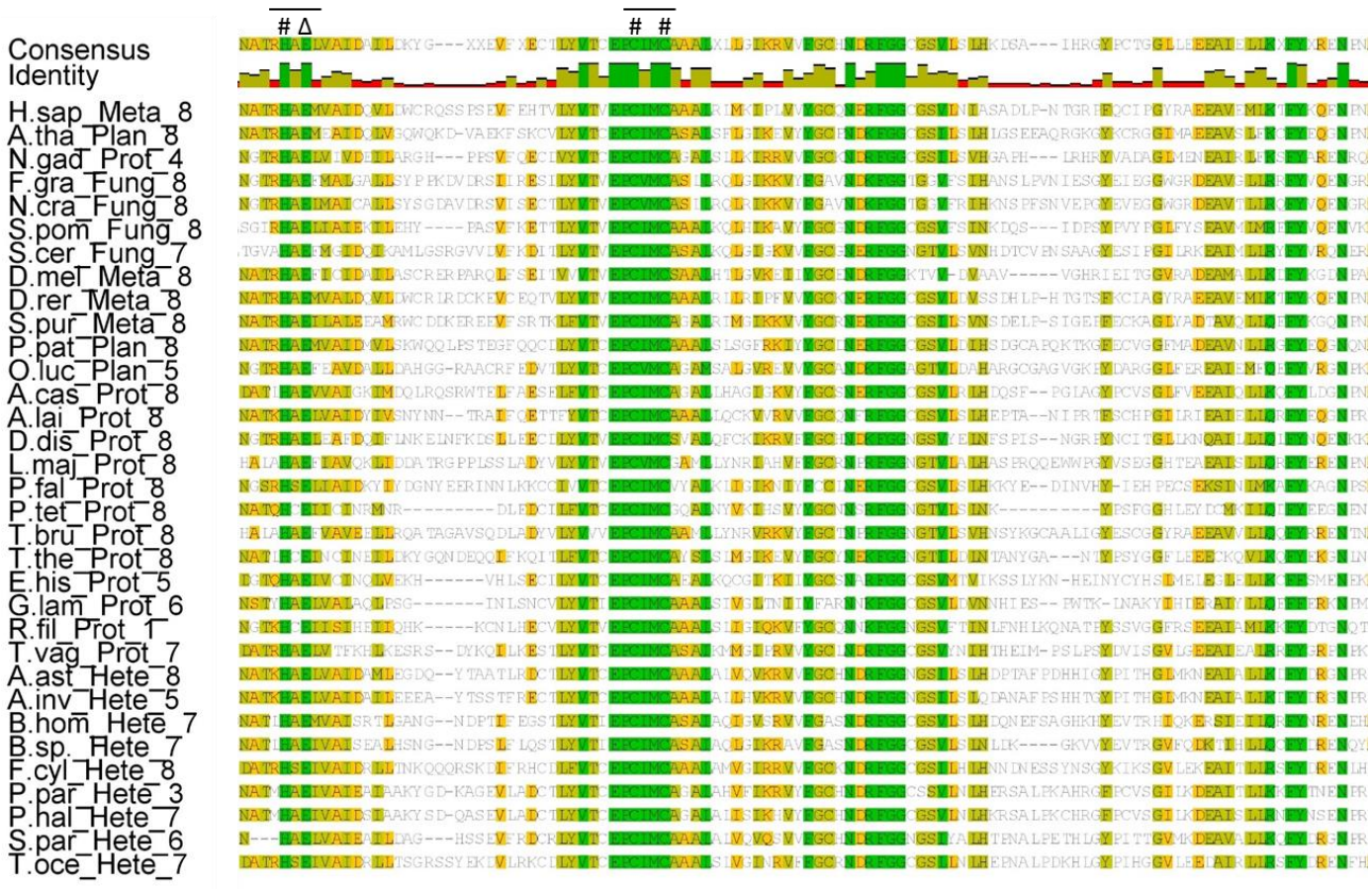

Figure S5: Multiple sequence alignments of ADAT2 deaminase domains. Residues with 100%, 80-100%, 60-80% and <60% of similarity are respectively framed with green, olive, orange and white. The deaminase domains are overlined, where the residues that coordinates with Zn (#) and the glutamic acid that allows the nucleophilic attack (Δ) are depicted. The organisms names are displayed as *X.yyy_zzzz_n* where *X* is the first letter of the Genre, *yyy* the first 3 letters of the specie, *zzzz* the 4 first letters of the phylum and *n* the number of different A34 tRNAs.

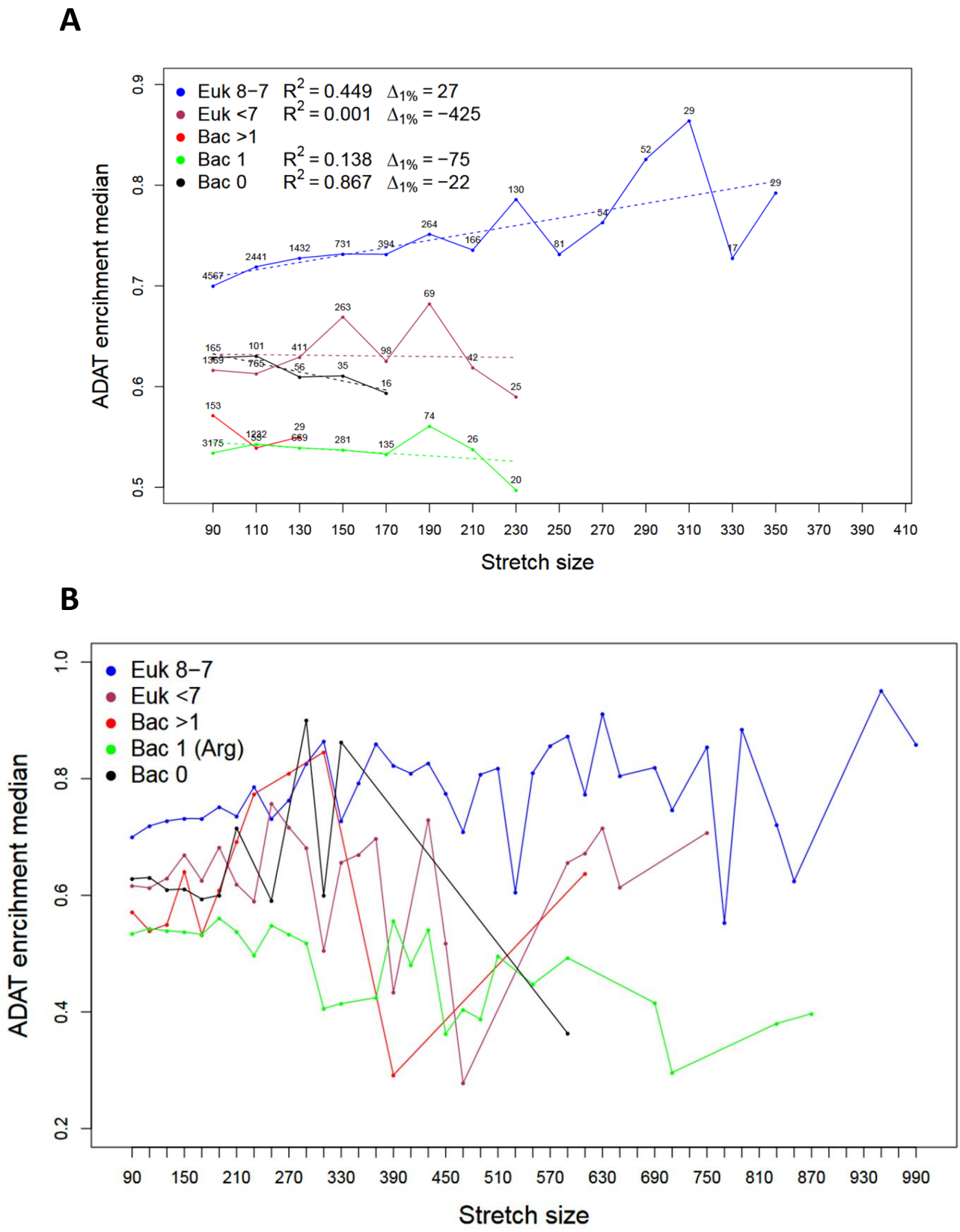

Figure S6: (A) ADAT stretch distribution. Median values of ADAT-sensitive codon enrichment as a function of ADAT amino acid stretch length, plotted for different groups based on A34-tRNA diversity values (color-coded). Linear regressions (colored dashed lines) were calculated for all groups with more than 5 data points. Numbers on top of each data point refer to the number of samples for each data point. Data points with less than 15 samples were omitted. $\Delta_{1\%}$ represents the number of codons needed to increase ADAT enrichment by 1% based on the slope of the linear model. **(B)** Graph as in **(A)** showing the full ADAT stretch size distribution.

Table S1: oligos used for *T. thermophila* and *O. oeni* PCR amplification.

Name	Sequence	Details
oTFW_94	gaatgtcataagcgCCAAGCGAGCGCTCTACCATTTG	Bridge oligo T. the/H. sapiens tRNA AlaAGC - Splinted Ligation
oAGT_230	gaatgtcataagcgCCAGGCGAATGCTCTAACCACTG	Bridge oligo T. the tRNA ThrAGT - Splinted Ligation
oAGT_231	gaatgtcataagcgCCAAACGAGAATCATGCCACTAG	Bridge oligo T. the tRNA ProAGG - Splinted Ligation
oAGT_312	gaatgtcataagcgGCAGTCAGACGCTCTATCCAATTG	Bridge oligo O. oeni tRNA ArgACG - Splinted Ligation
oAGT_313	gaatgtcataagcgTCAATCCTGCGCGTCTGCCAATTC	Bridge oligo O. oeni tRNA LeuAAG - Splinted Ligation
oAGT_314	gaatgtcataagcgCCAGACCGACCCCTTCAGCCAC	Bridge oligo O. oeni tRNA SerAGA - Splinted Ligation
oAGT_315	gaatgtcataagcgCCAGTGAAGTGCTCTAGCCAAC	Bridge oligo O. oeni tRNA ThrAGT - Splinted Ligation
oTFW-31	AGCTTAATACGACTCACTATAGGGGATCTAGCTCA	FWD primer T. thermophila PCR Ala
oTFW-36	GATCCACATGTTGGTGGAGAACCTGGGCATT	RVR primer T. thermophila PCR Ala
oTFW-37	AGCTTAATACGACTCACTATAGGGGTGATGG	FWD primer T. thermophila PCR Arg
oTFW-42	GATCCCCTGGCGAGATGAGCAGGACTCGAAC	RVR primer T. thermophila PCR Arg
oTFW-43	AGCTTAATACGACTCACTATAGCTCGGGTAGCTCAG	FWD primer T. thermophila PCR Ile
oTFW-48	GATCCCCTGGTGCTCCGGGAGGGGCTTGAAC	RVR primer T. thermophila PCR Ile
oTFW-49	AGCTTAATACGACTCACTATAGATGAAGTGGCCGAG	FWD primer T. thermophila PCR Leu
oTFW-54	GATCCCCTGGTGATGAAGGCGAGATTCGAACT	RVR primer T. thermophila PCR Leu
oTFW-55	AGCTTAATACGACTCACTATAGGGTTGTTGGTC	FWD primer T. thermophila PCR Pro
oTFW-60	GATCCCCTGGGGGGTCGTCCGAGAATCGA	RVR primer T. thermophila PCR Pro
oTFW-61	AGCTTAATACGACTCACTATAGACAATTTGTCCGAG	FWD primer T. thermophila PCR Ser
oTFW-66	GATCCCCTGGCGACAACTGCAGGATTCGA	RVR primer T. thermophila PCR Ser
oTFW-67	AGCTTAATACGACTCACTATAGCCGCTTTAGCTC	FWD primer T. thermophila PCR Thr
oTFW-72	GATCCCCTGGAGCCACTTGGCGGGATTG	RVR primer T. thermophila PCR Thr
oTFW-73	AGCTTAATACGACTCACTATAGATTCCTTAGTG	FWD primer T. thermophila PCR Val
oTFW-78	GATCCCCTGGTGATTCCTCCGAGGTTTGA	RVR primer T. thermophila PCR Val
oAGT_296	CAGGAAACAGCTATGACCCACCATTAGCGCAATTGG	FWD primer tRNA Arg ACG Oenococcus oeni with M13-RP adaptor for sequencing
oAGT_297	GCACCATGTAGGAGTCGAAC	RVR primer tRNA Arg ACG Oenococcus oeni
oAGT_298	CAGGAAACAGCTATGACCGACGTGGCGGAATTGGCAG	FWD primer tRNA Leu AAG Oenococcus oeni with M13-RP adaptor for sequencing
oAGT_299	GGCGATGGGAGTCGAACCCATAC	RVR primer tRNA Leu AAG Oenococcus oeni
oAGT_300	CAGGAAACAGCTATGACCGATGGATACCCAAGTGGC	FWD primer tRNA Ser AGA Oenococcus oeni with M13-RP adaptor for sequencing
oAGT_301	GAGAGATTCGAACTCTCG	RVR primer tRNA Ser AGA Oenococcus oeni
oAGT_302	ATTTAGGTGACACTATAGAATAGCTCAGTTGGCTAGAGCAC	FWD primer tRNA Thr AGT Oenococcus oeni with Sp6 adaptor for sequencing
oAGT_303	TGCCGACTAGAGGATTCG	RVR primer tRNA Thr AGT Oenococcus oeni

Table S2: The Universal Genetic Code. ADAT codons are colored in blue (dark and soft). ADAT-sensitive codons are colored in dark blue. For each codon, the amino acid that decodes is depicted in one-letter and three-letter convention.

Second Codon Letter

First Codon Letter	U		C		A		G		Third codon Letter
U	F	Phe	S	Ser	Y	Tyr	C	Cys	U
	F	Phe	S	Ser	Y	Tyr	C	Cys	C
	L	Leu	S	Ser	Stop		Stop		A
	L	Leu	S	Ser	Stop		W	Trp	G
C	L	Leu	P	Pro	H	His	R	Arg	U
	L	Leu	P	Pro	H	His	R	Arg	C
	L	Leu	P	Pro	Q	Gln	R	Arg	A
	L	Leu	P	Pro	Q	Gln	R	Arg	G
A	I	Ile	T	Thr	N	Asn	S	Ser	U
	I	Ile	T	Thr	N	Asn	S	Ser	C
	I	Ile	T	Thr	K	Lys	R	Arg	A
	M	Met	T	Thr	K	Lys	R	Arg	G
G	V	Val	A	Ala	D	Asp	G	Gly	U
	V	Val	A	Ala	D	Asp	G	Gly	C
	V	Val	A	Ala	E	Glu	G	Gly	A
	V	Val	A	Ala	E	Glu	G	Gly	G