

# **Codon bias coevolves with longevity**

## **Supplementary material**

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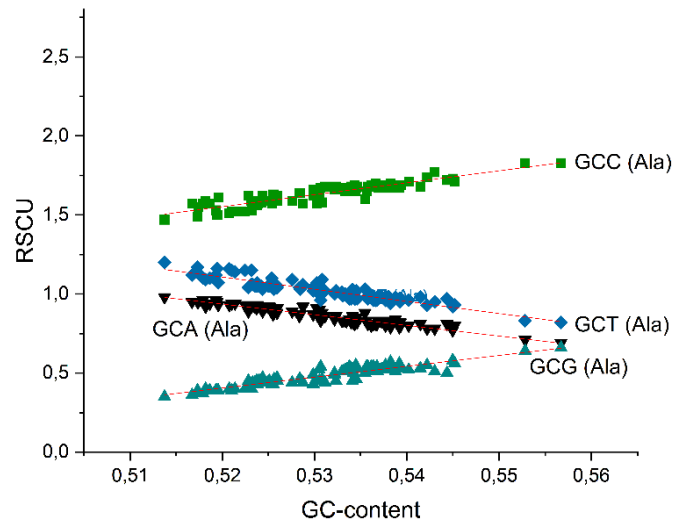
## **Impact of GC content of coding genomes on codon bias of mammalian species**

The file presents the plots reflecting the impact of GC content of coding genomes on codon bias of mammalian species. The parameters of the regression analyses of these plots are presented in Supplementary file 2.

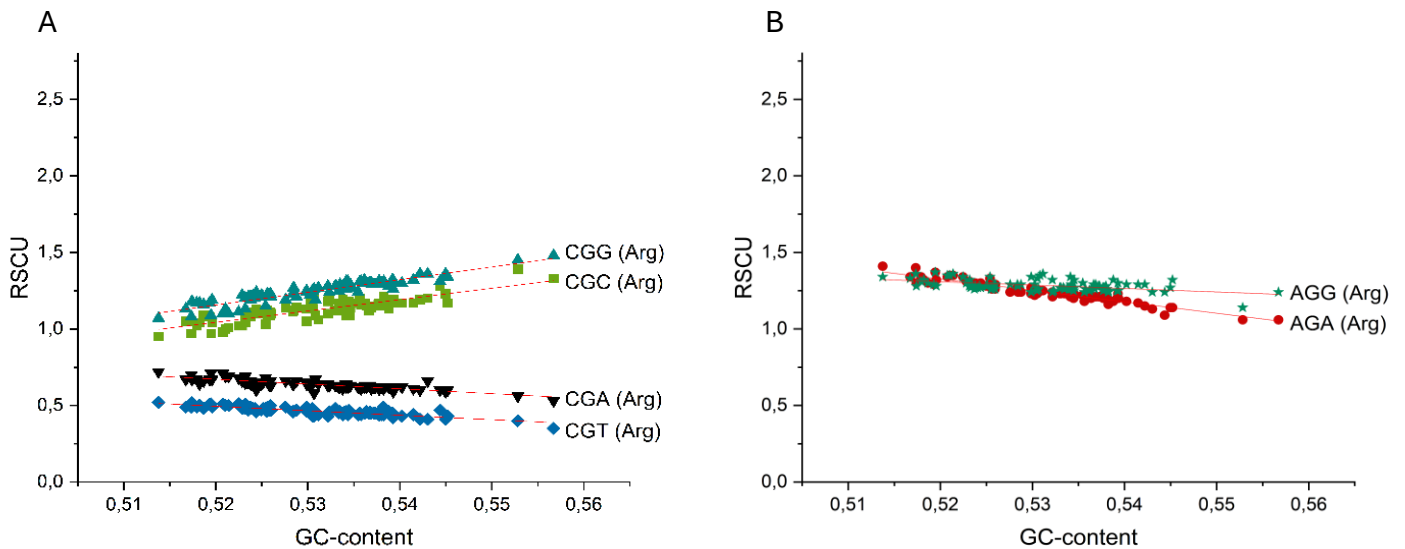
The plots are presented in alphabetical order of the names of the amino acids; data for amino acids Met and Trp that are encoded by a single codon are not shown.

We also present the data for the three stop codons at the end of the list of the 18 amino acids encoded by more than one synonymous codon.

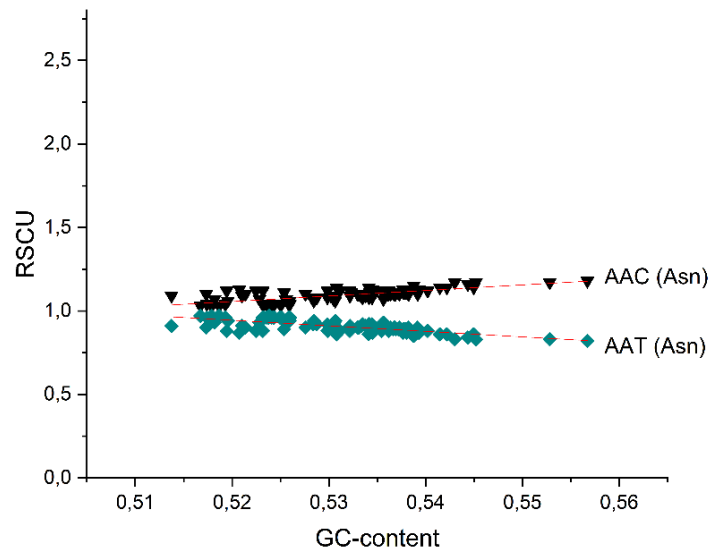
In the case of the 18 amino acids and the termination codons the figures present the plots reflecting the impact of the GC content of the coding genome of mammalian species on the relative synonymous codon usage (RSCU) of the given amino acid or termination codons. In these analyses we have plotted the RSCU values as a function of the GC-content of the coding genomes of the 96 mammalian species included in the present analysis.



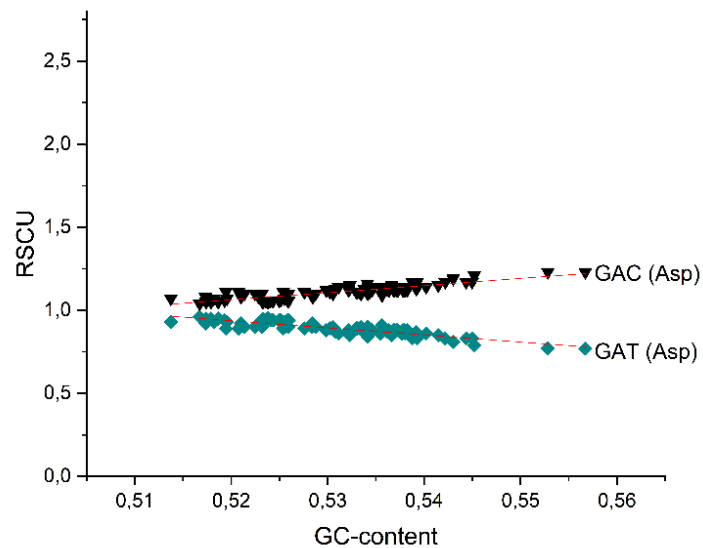
**Supplementary figure 1.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the four **alanine** codons.



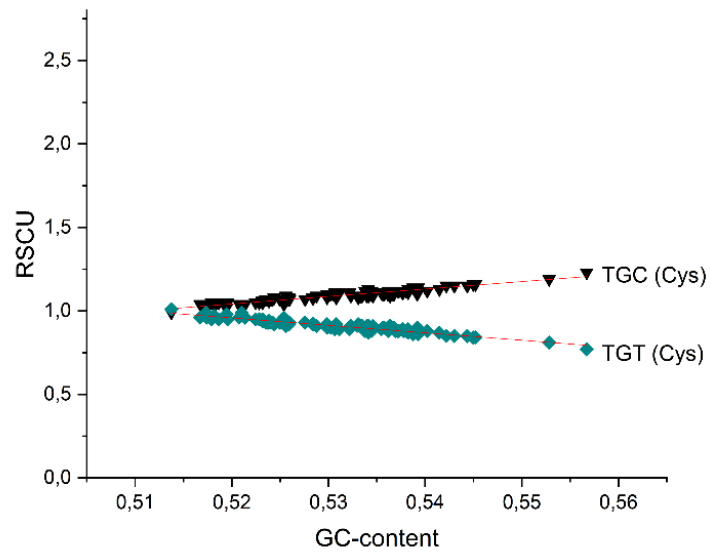
**Supplementary figure 2.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the six **arginine** codons.



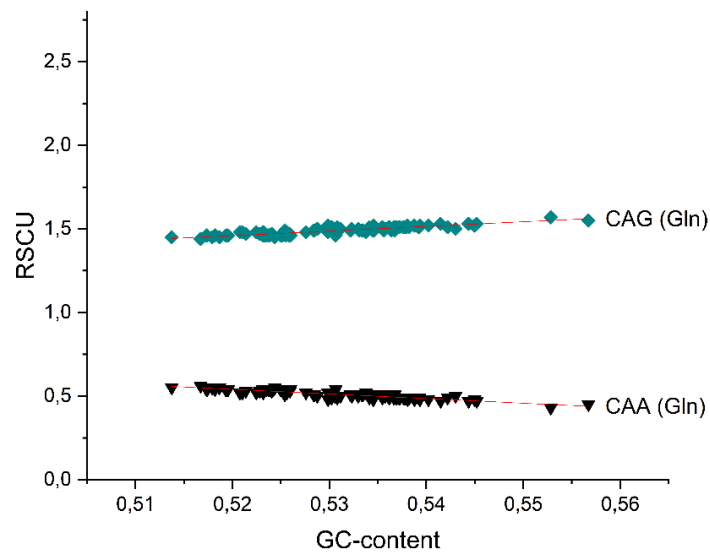
**Supplementary figure 3.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **asparagine** codons.



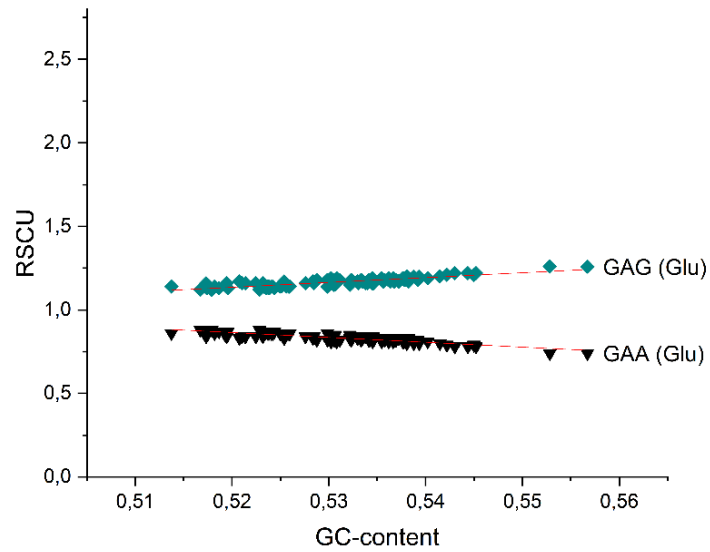
**Supplementary figure 4.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **aspartic acid** codons.



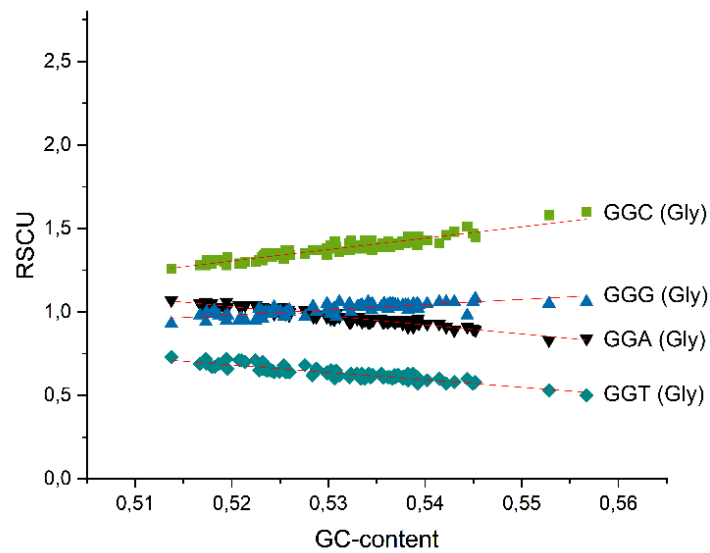
**Supplementary figure 5.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **cysteine** codons.



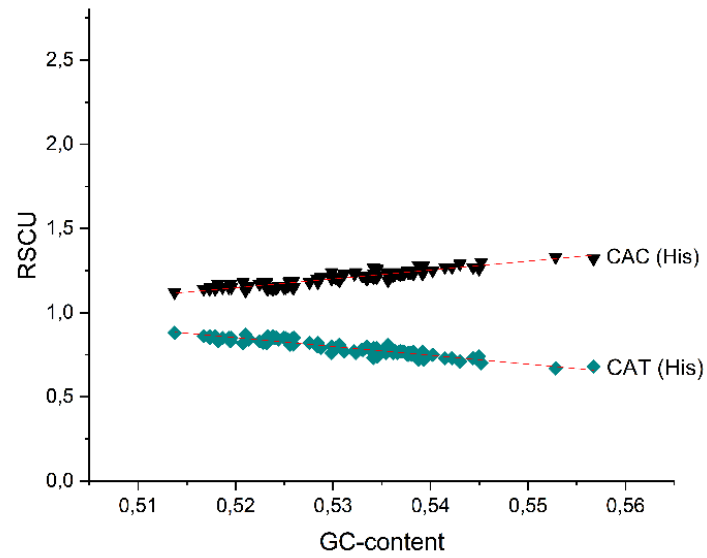
**Supplementary figure 6.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **glutamine** codons.



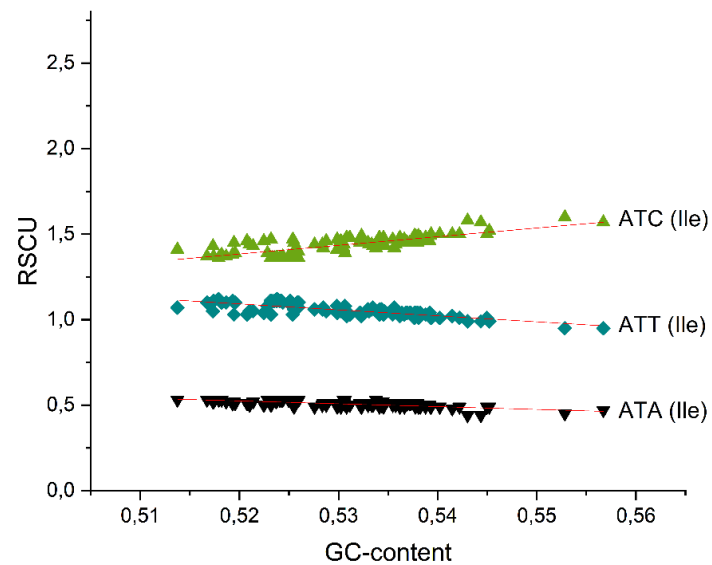
**Supplementary figure 7.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **glutamic acid** codons.



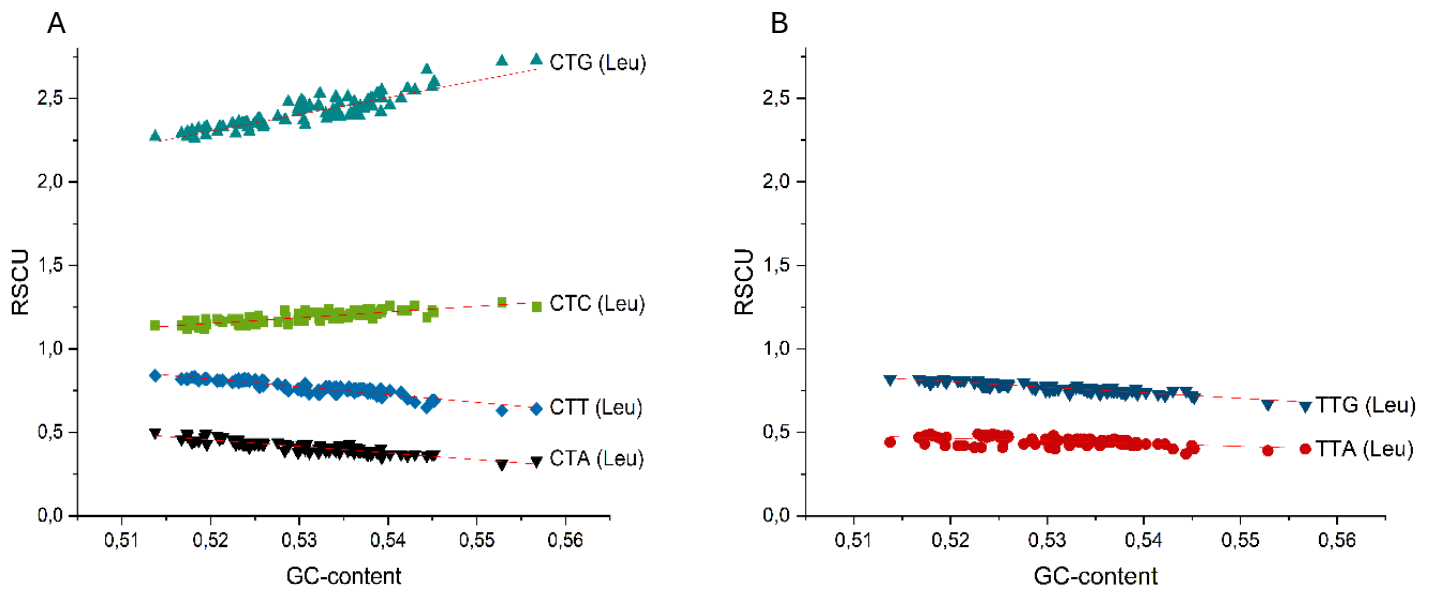
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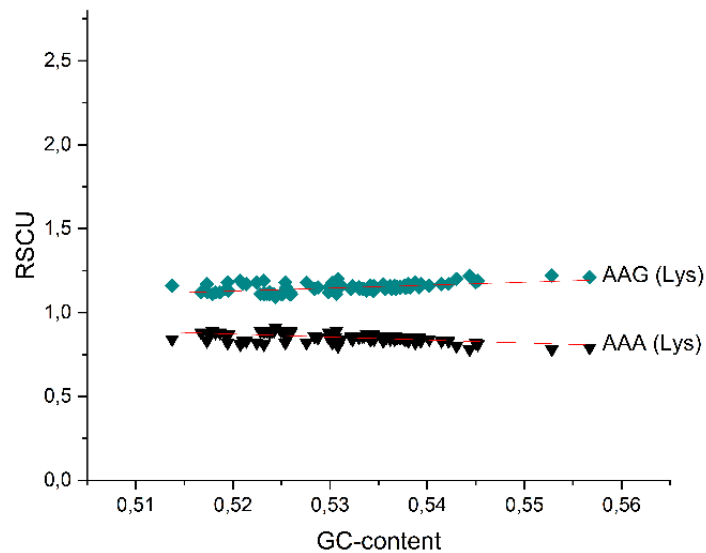
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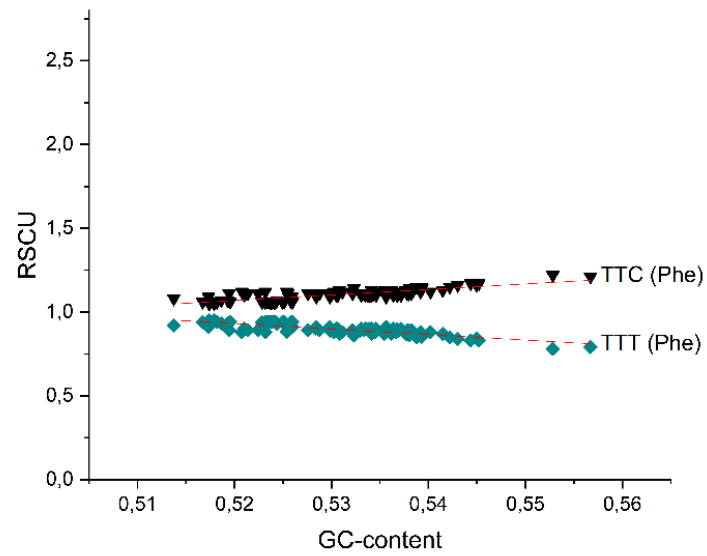
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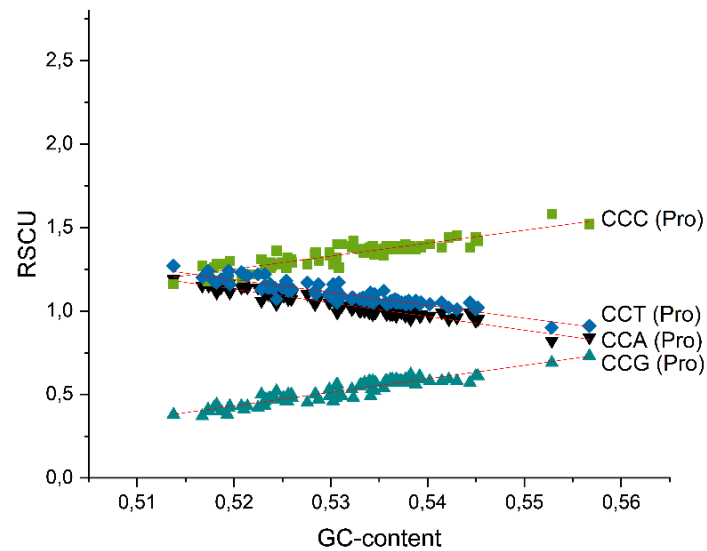
**Supplementary figure 11.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the six **leucine** codons.



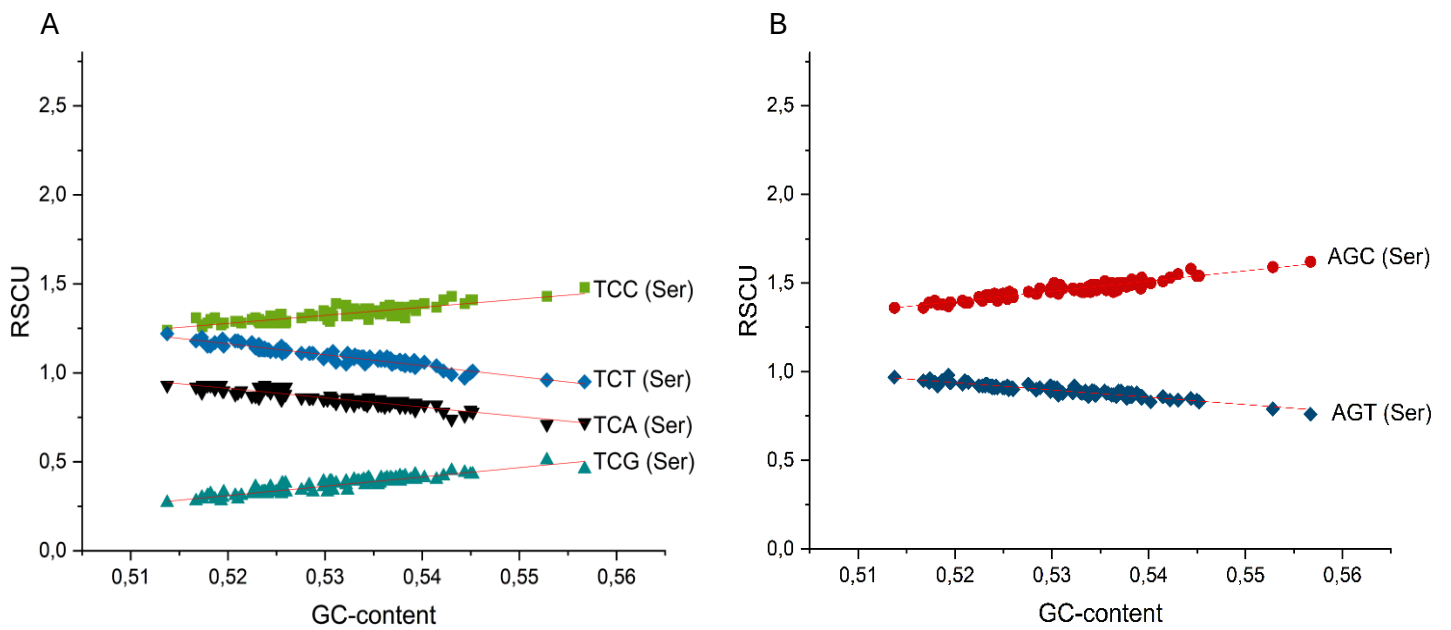
**Supplementary figure 12.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **lysine** codons.



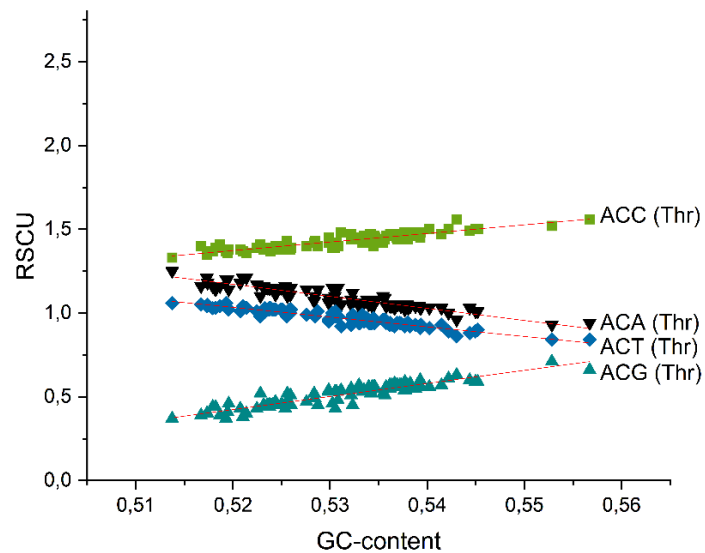
**Supplementary figure 13.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **phenylalanine** codons.



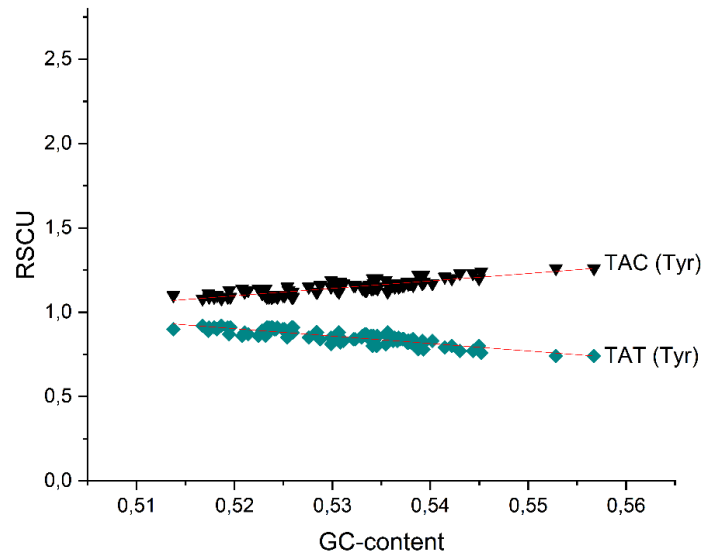
**Supplementary figure 14.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the four **proline** codons.



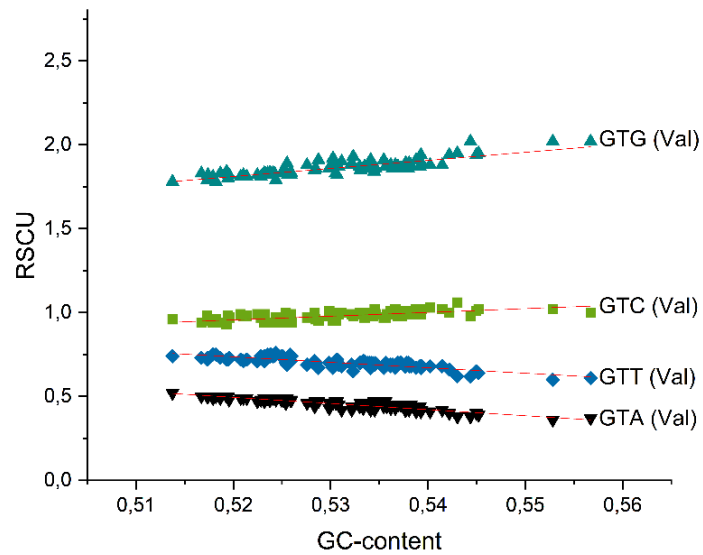
**Supplementary figure 15.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the six **serine** codons.



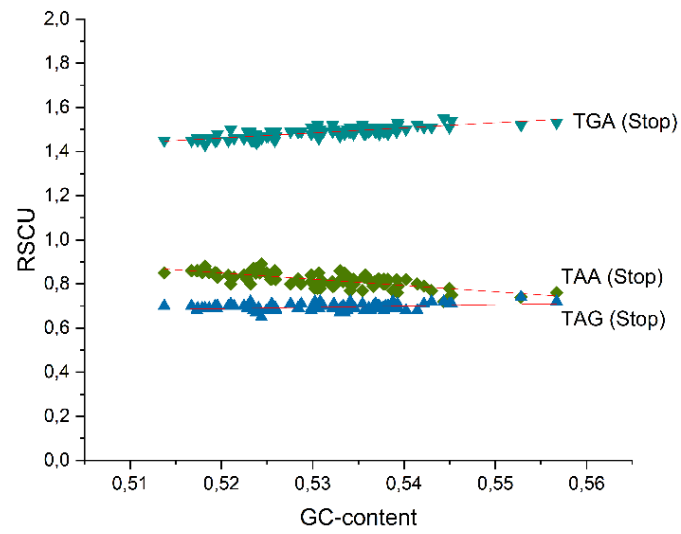
**Supplementary figure 16.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the four **threonine** codons.



**Supplementary figure 17.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the two **tyrosine** codons.



**Supplementary figure 18.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the four **valine** codons.



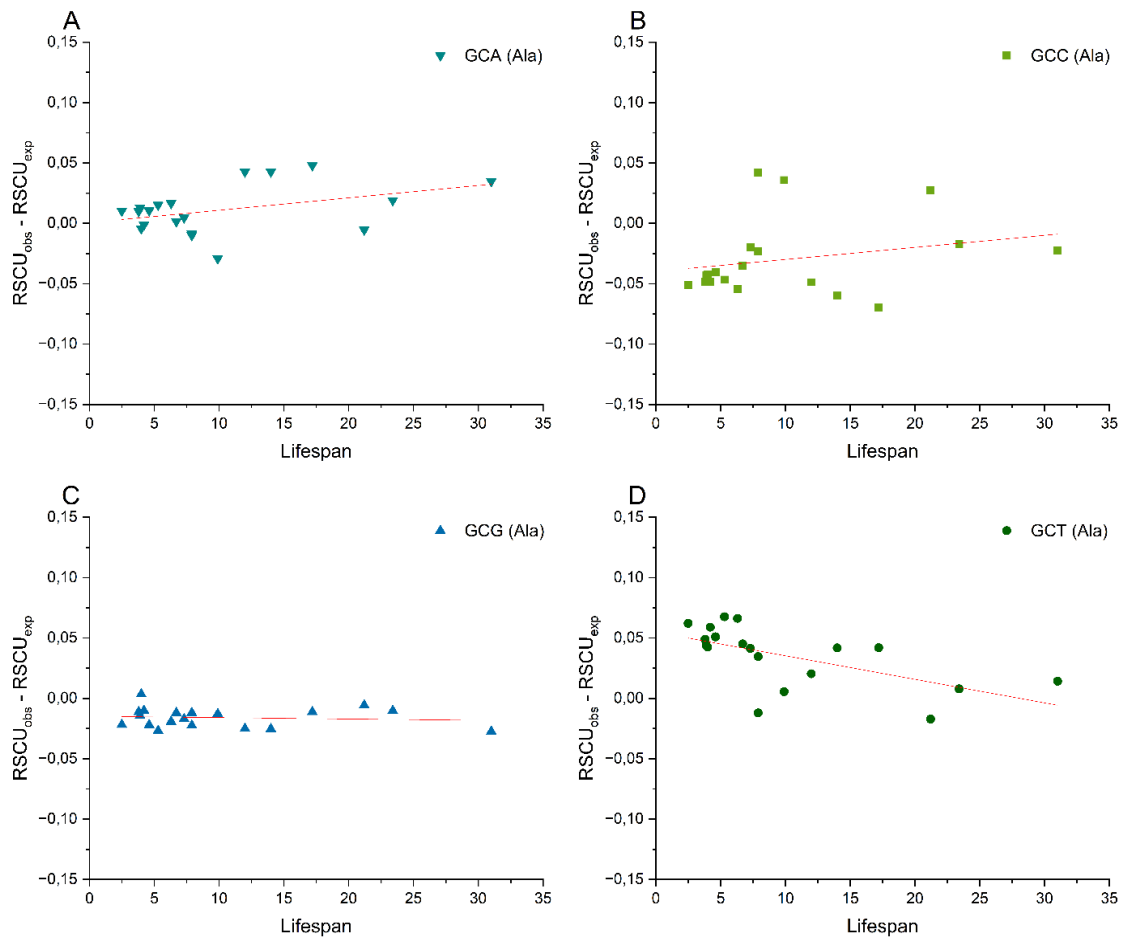
**Supplementary figure 19.a.** Impact of the GC content of coding genomes of mammalian species on their relative synonymous codon usage (RSCU) of the three **stop** codons.

## Changes in codon usage and evolution of longevity in mammalian species

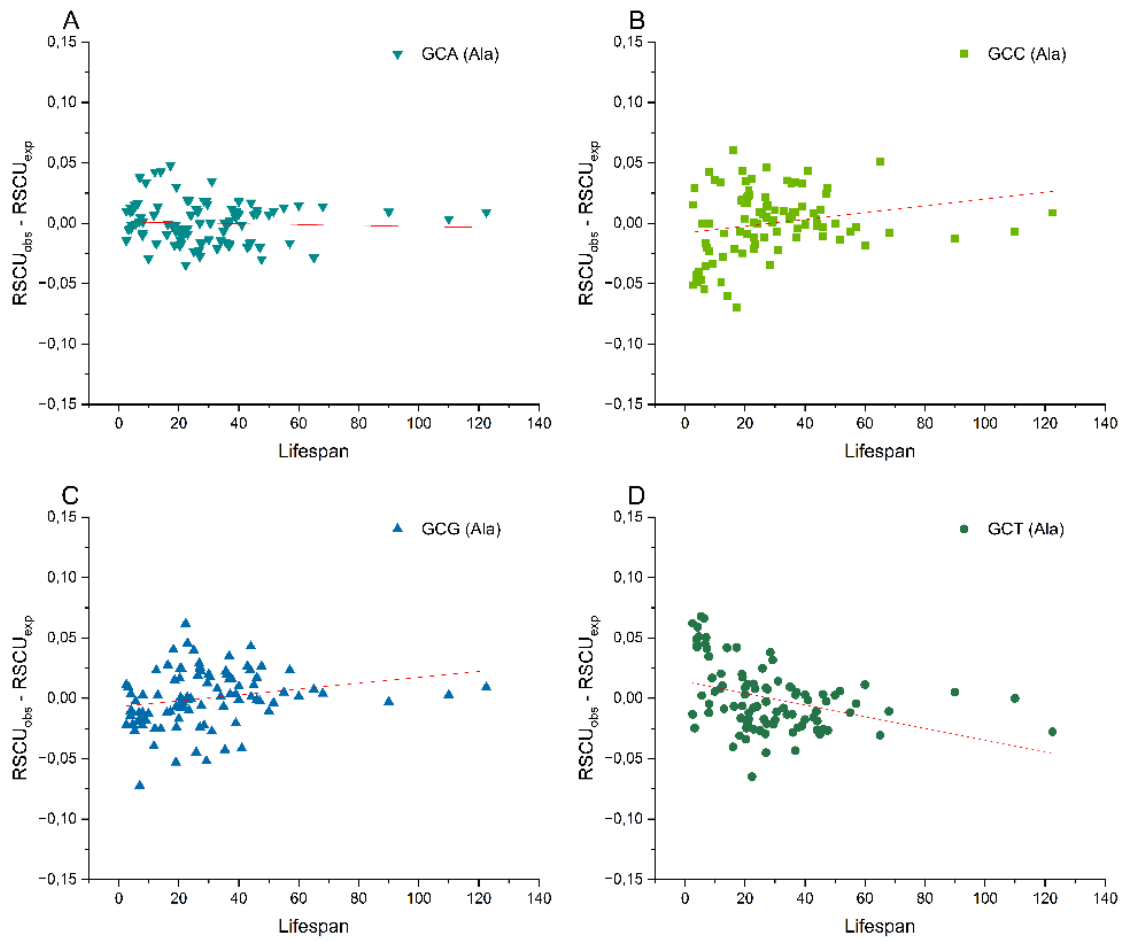
The file presents the plots reflecting changes in codon usage as a function of the lifespan of mammalian species. Note that in these analyses we have subtracted the RSCU values expected at the given GC content ( $RSCU_{exp}$ ) from the actual RSCU values ( $RSCU_{obs}$ ) and plotted these deviations as a function of the known lifespan (years) of the species. The parameters of the regression analyses of these plots are presented in Supplementary file 2.

The plots are presented in alphabetical order of the names of the amino acids; data for amino acids Met and Trp that are encoded by a single codon are not shown. We also present the data for the three stop codons at the end of the list of the 18 amino acids encoded by more than one synonymous codon.

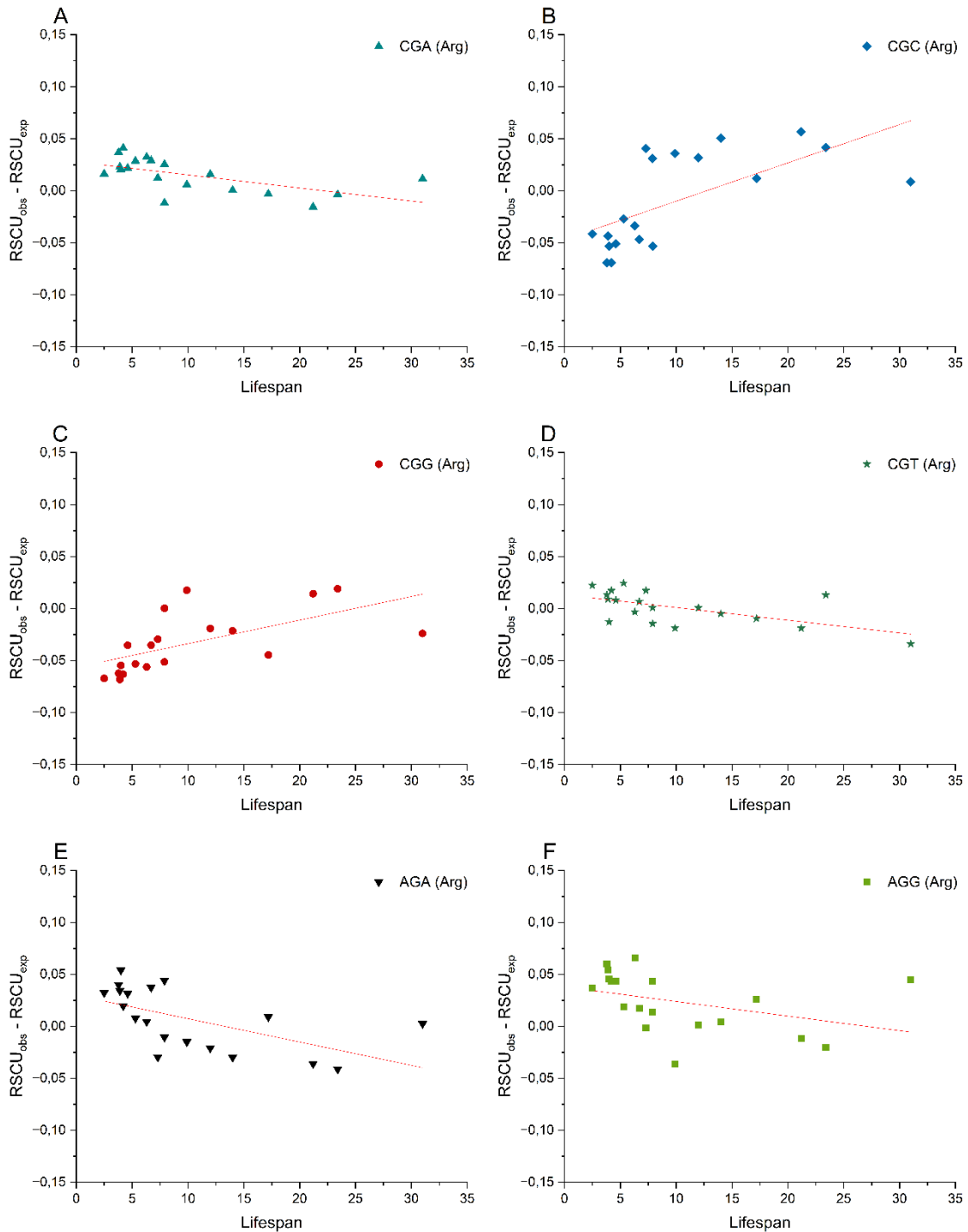
In the case of the 18 amino acids and the termination codons the first figures (**Supplementary figures x.a**) present the plots reflecting changes in codon usage as a function of the lifespan of 19 rodent species. The second figures (**Supplementary figures x.b**) present the plots reflecting changes in codon usage as a function of the lifespan of the 96 mammalian species included in the present analysis.



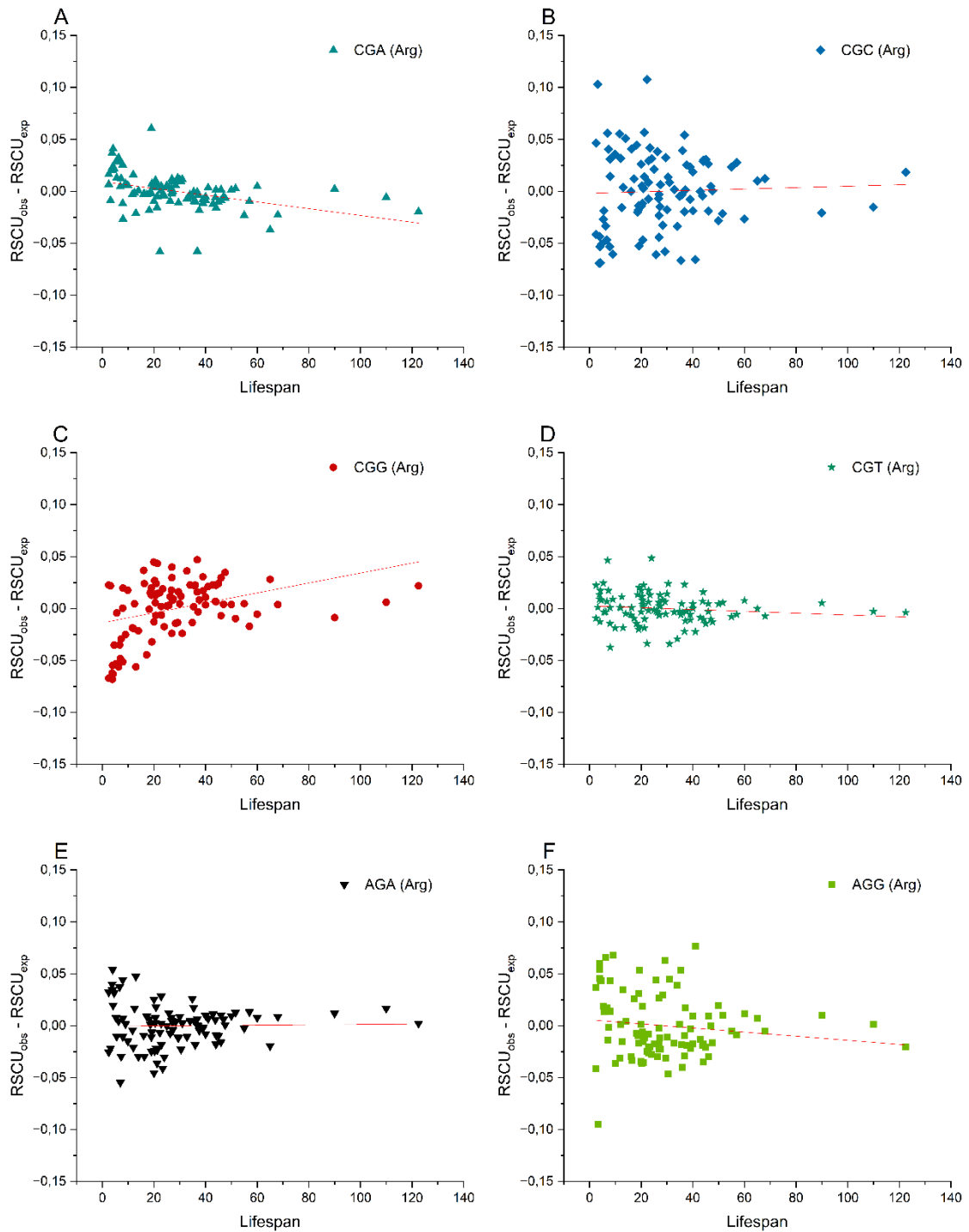
**Supplementary figure 1.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **alanine**, plotted against maximum lifespan (years) of 19 **rodent** species.



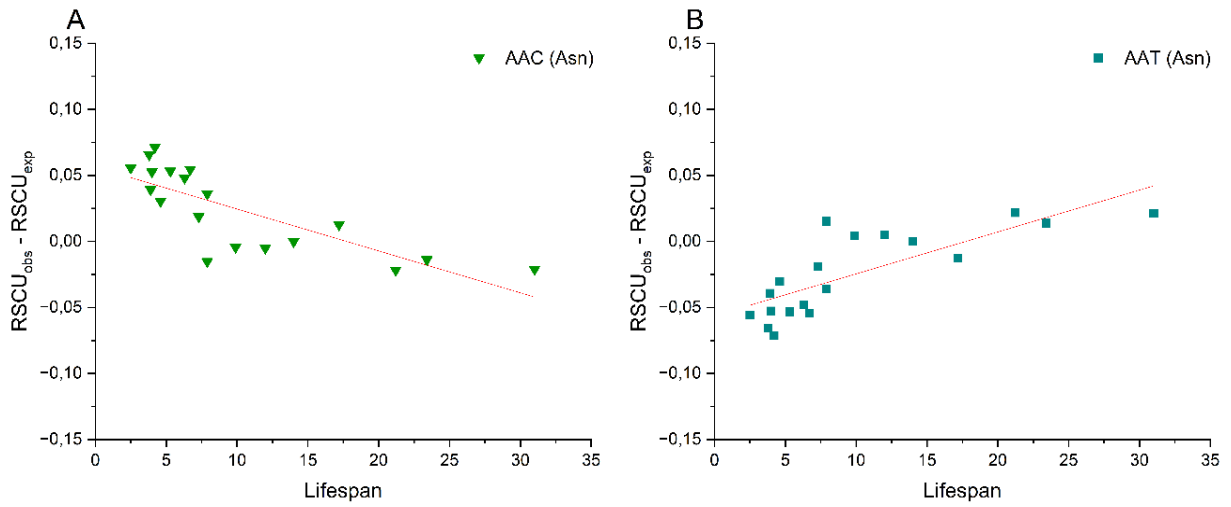
**Supplementary figure 1.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **alanine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



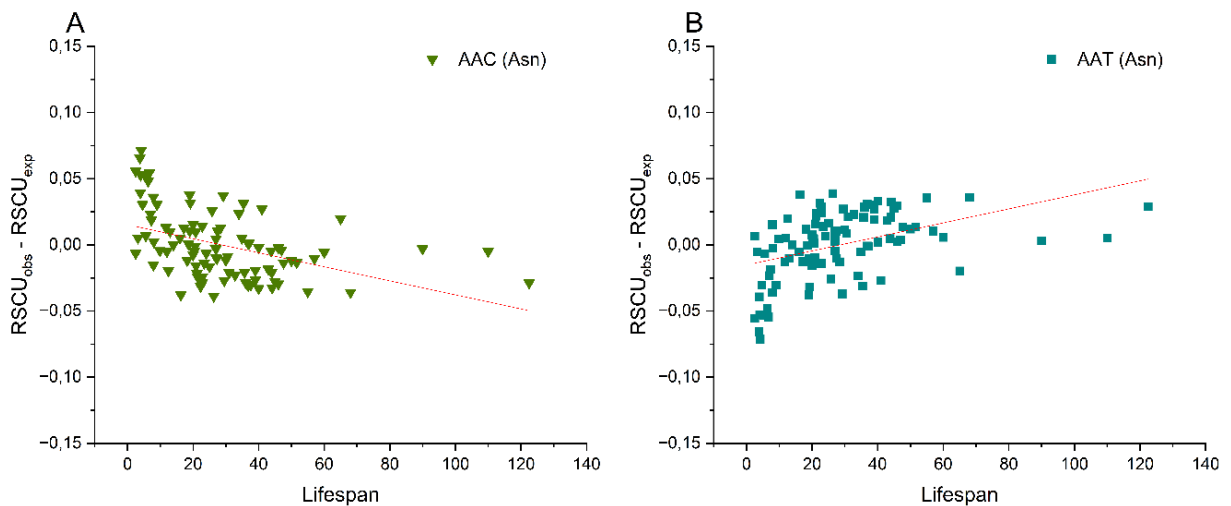
**Supplementary figure 2.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **arginine**, plotted against maximum lifespan (years) of 19 **rodent** species.



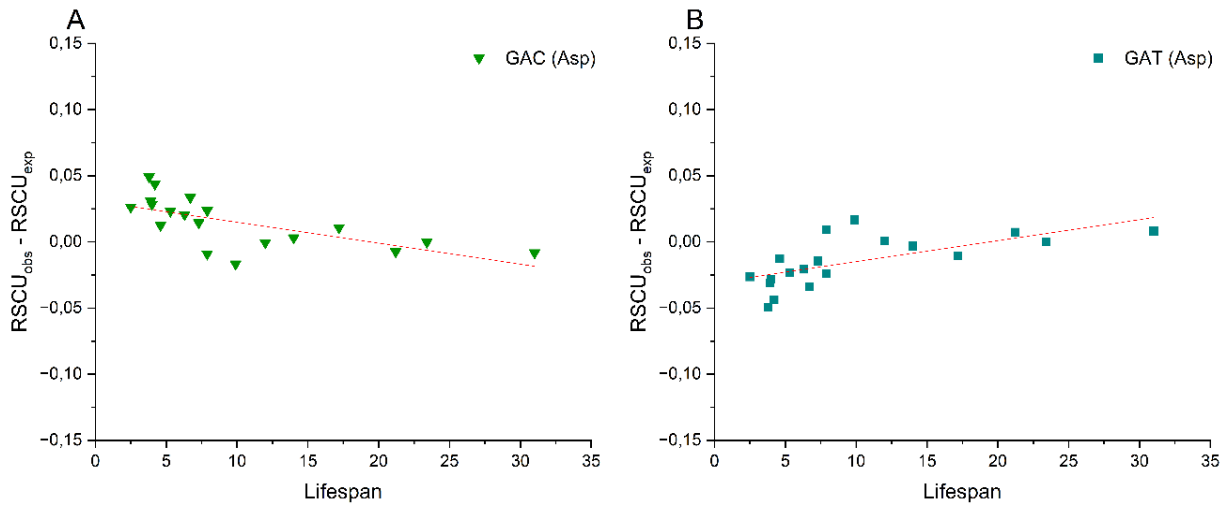
**Supplementary figure 2.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **arginine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



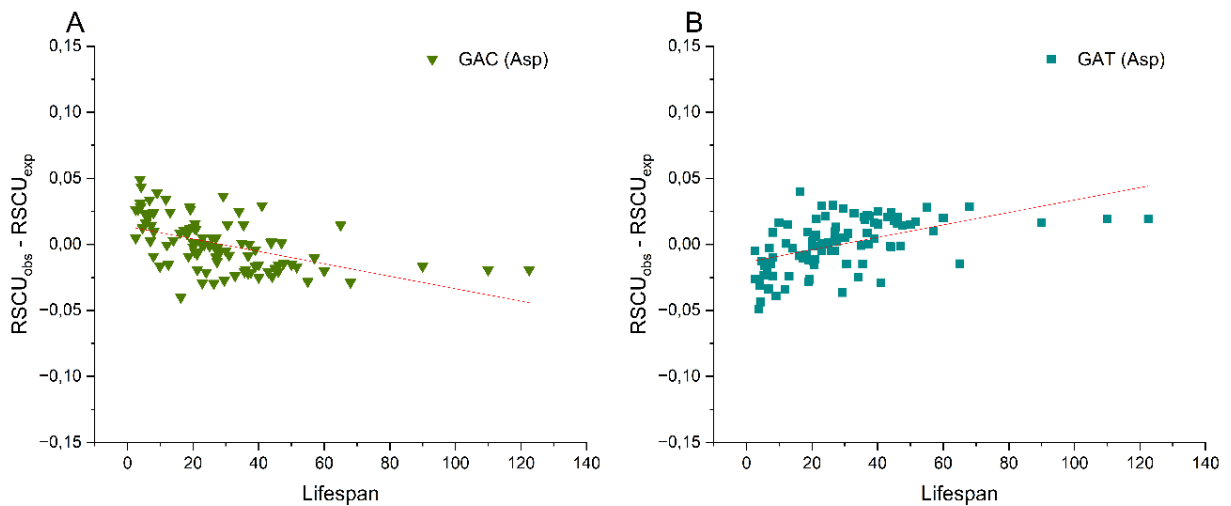
**Supplementary figure 3.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **asparagine**, plotted against maximum lifespan (years) of 19 **rodent** species.



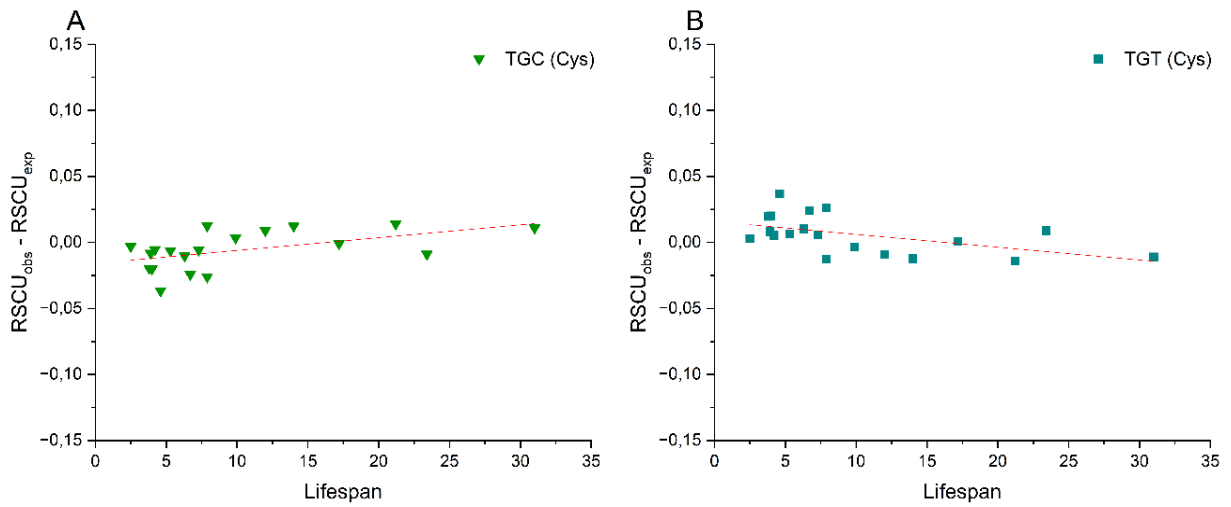
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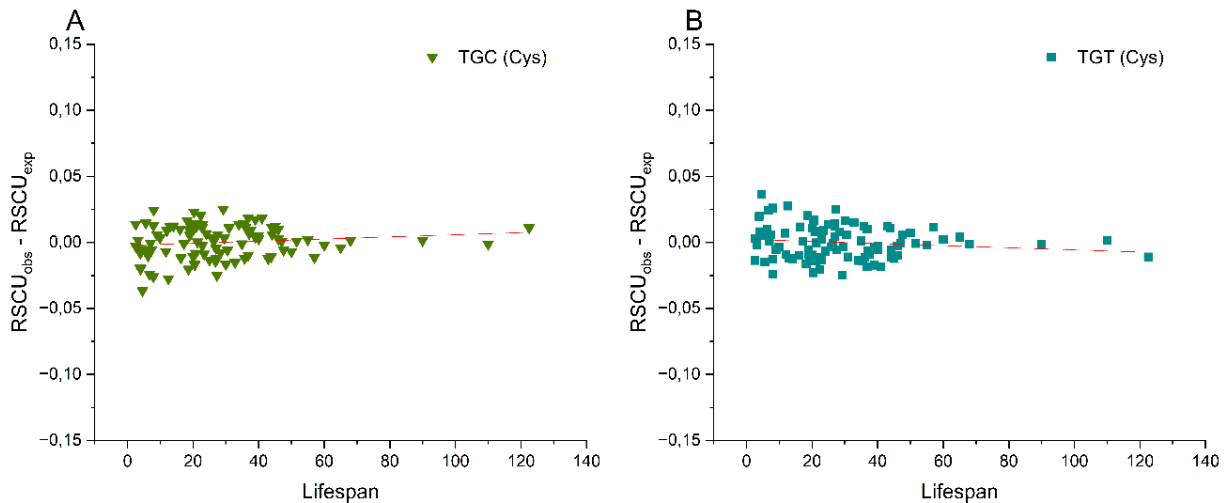
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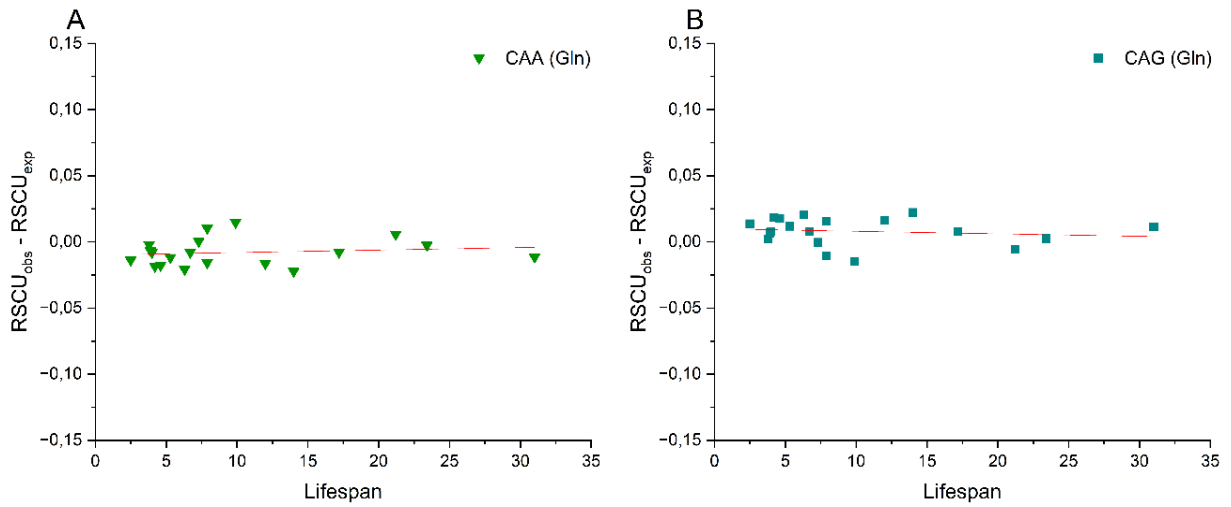
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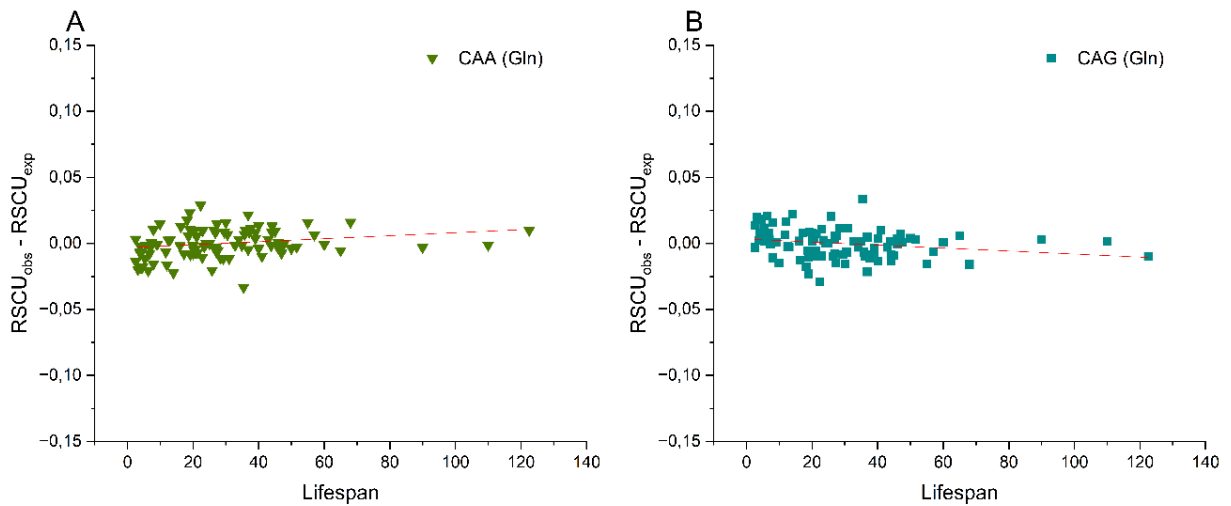
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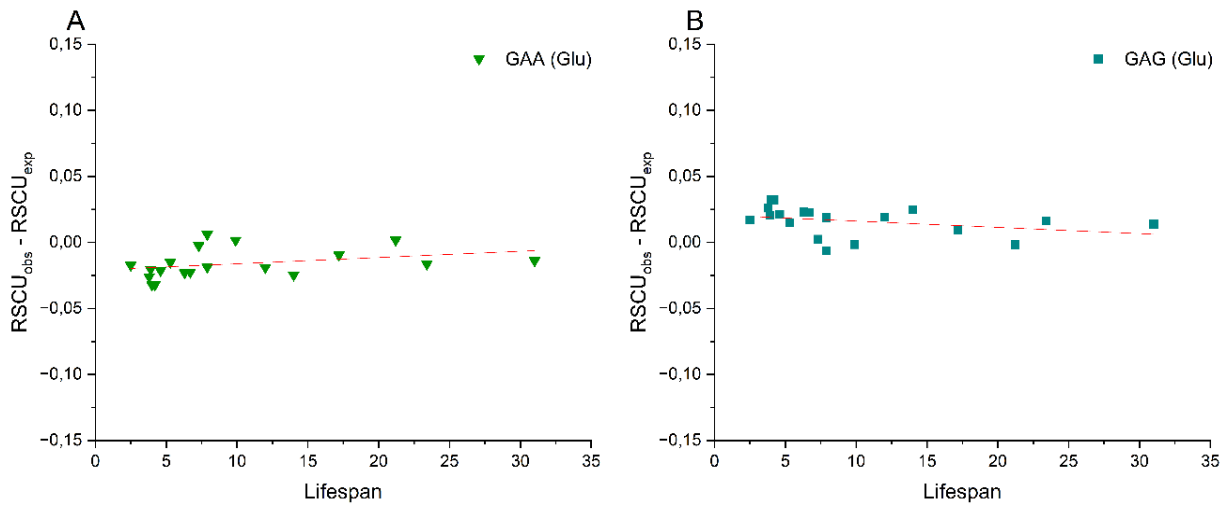
**Supplementary figure 5.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **cysteine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



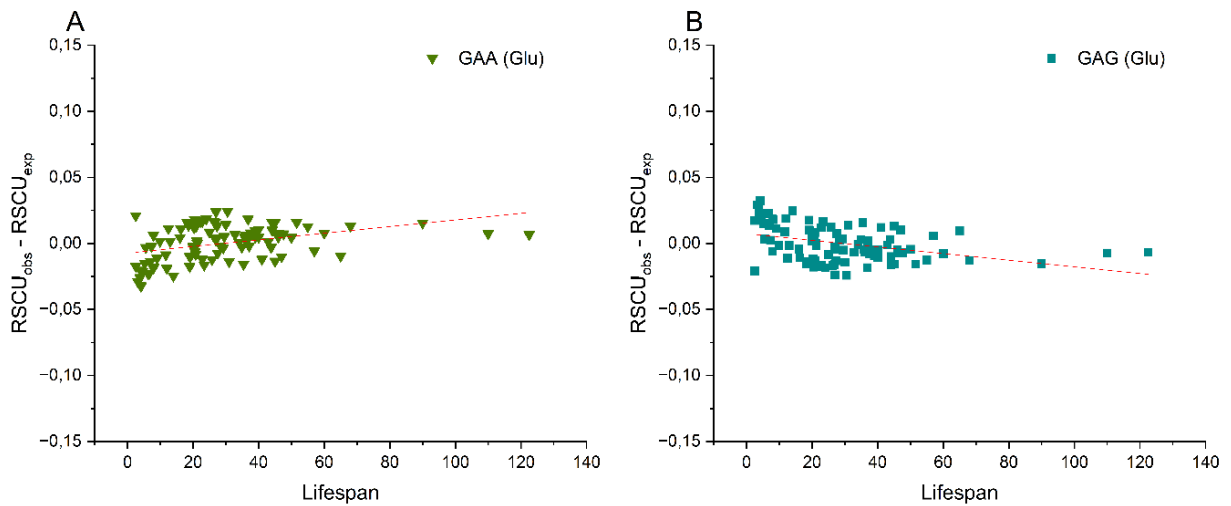
**Supplementary figure 6.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **glutamine**, plotted against maximum lifespan (years) of 19 **rodent** species.



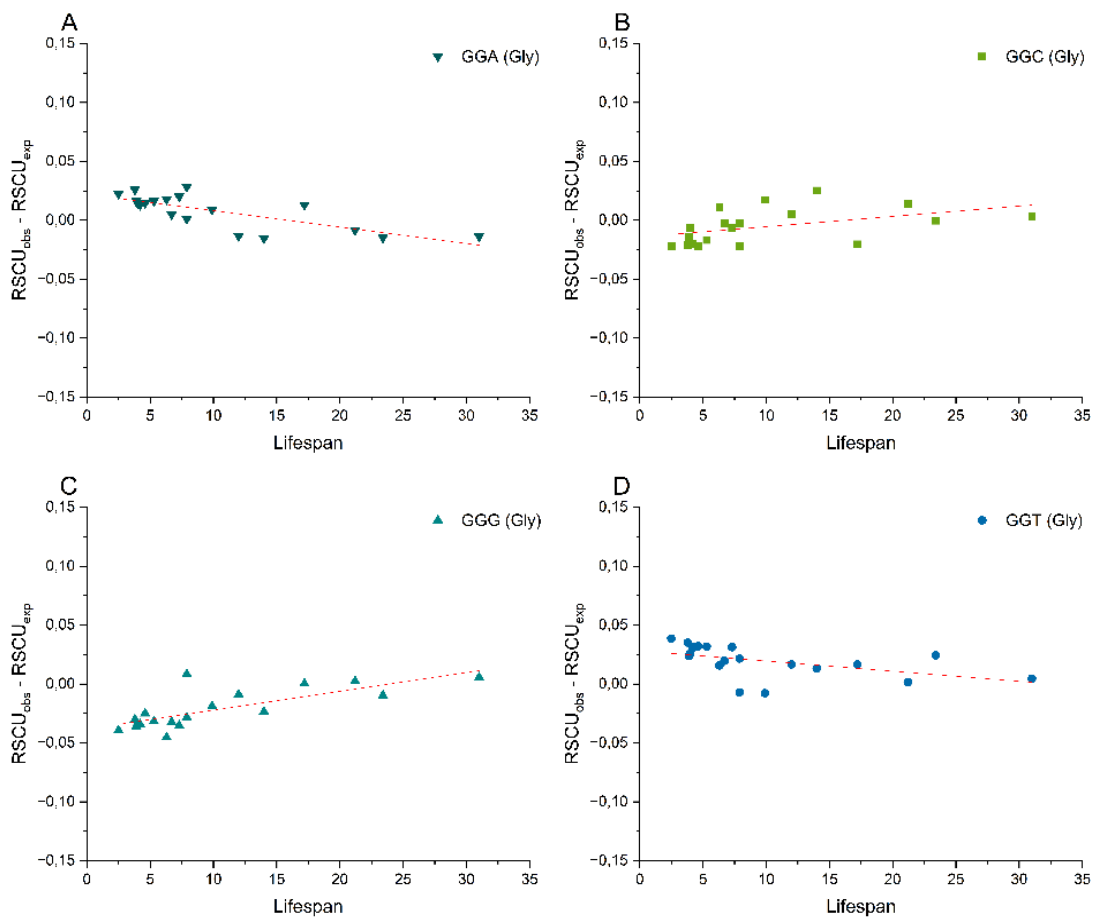
**Supplementary figure 6.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **glutamine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



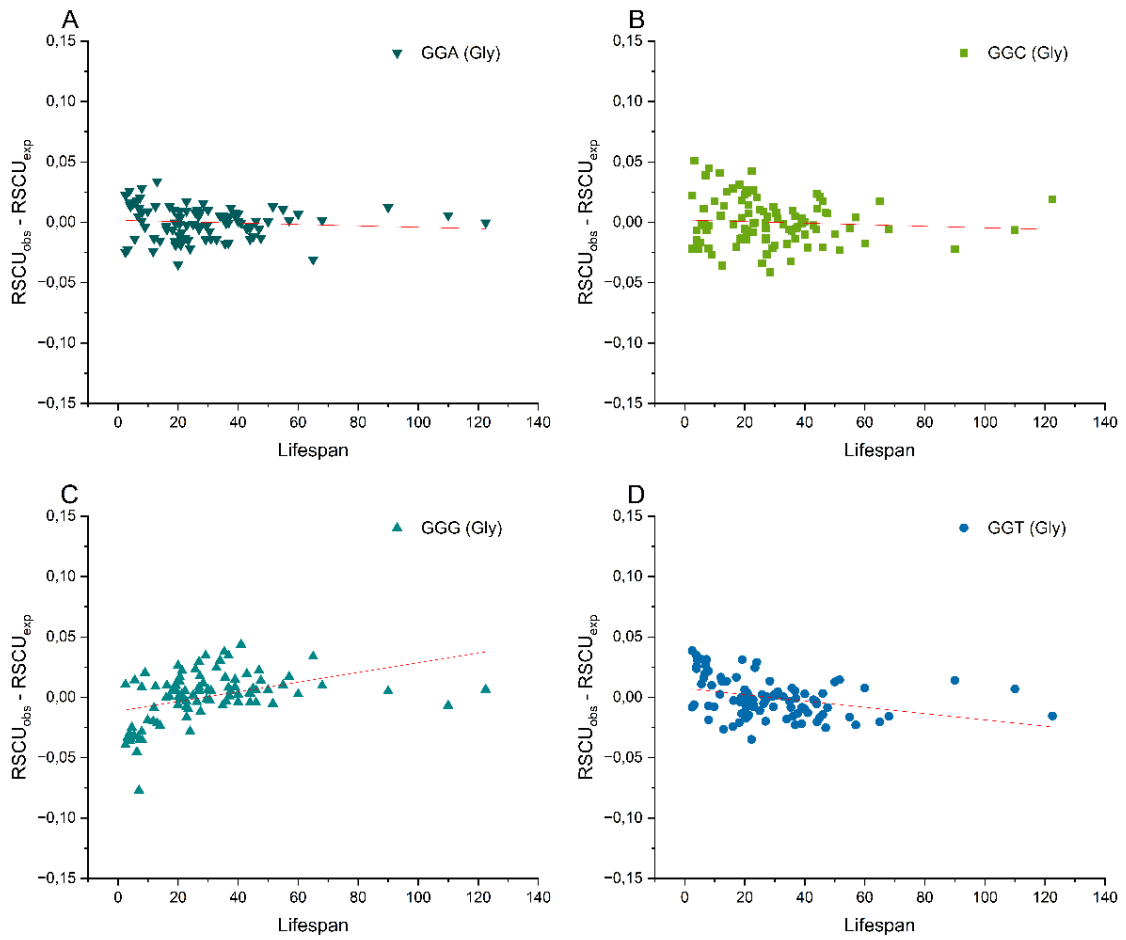
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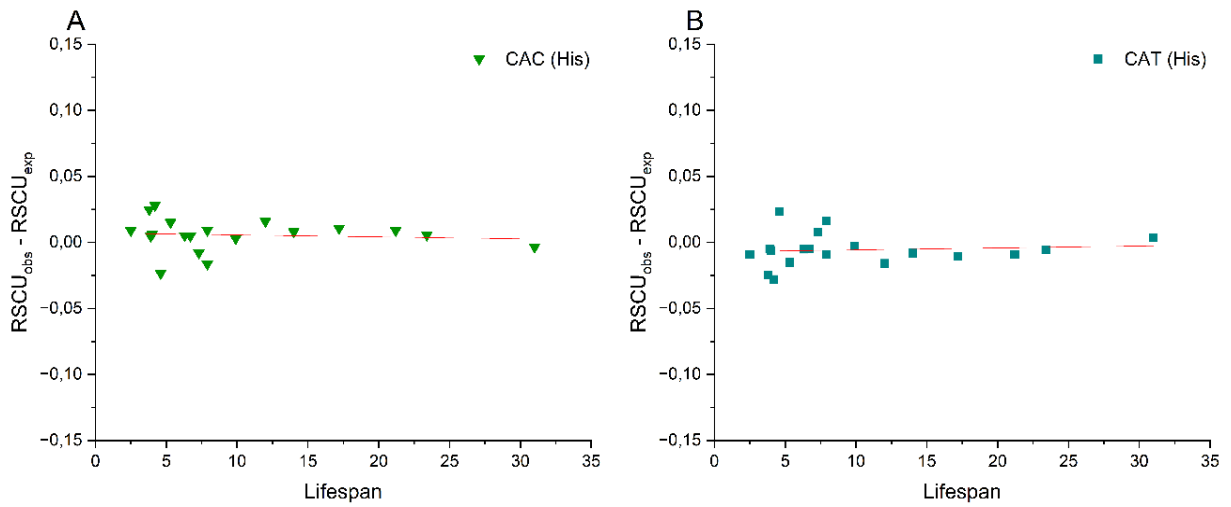
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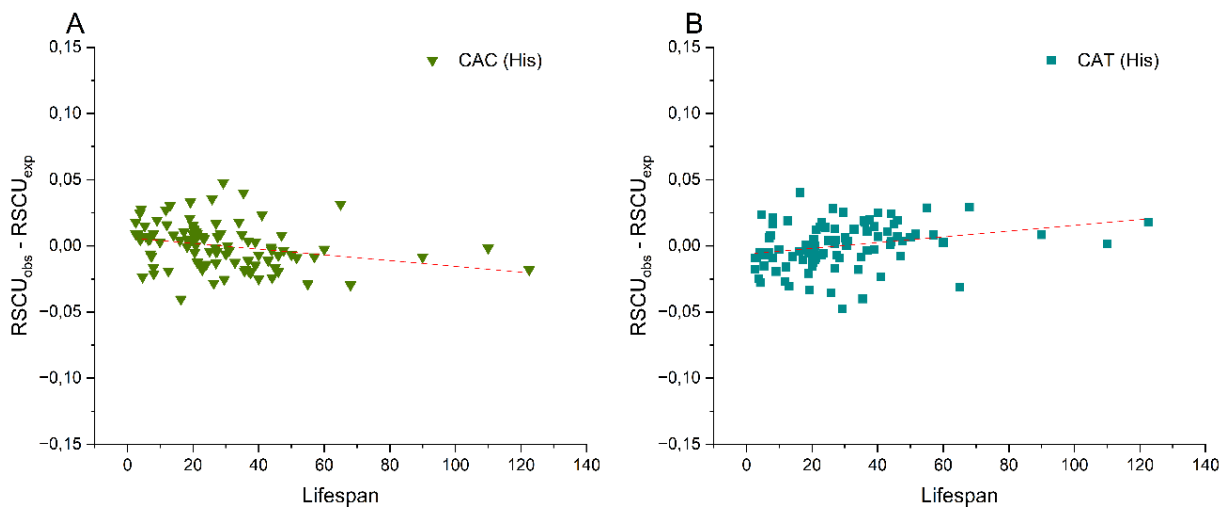
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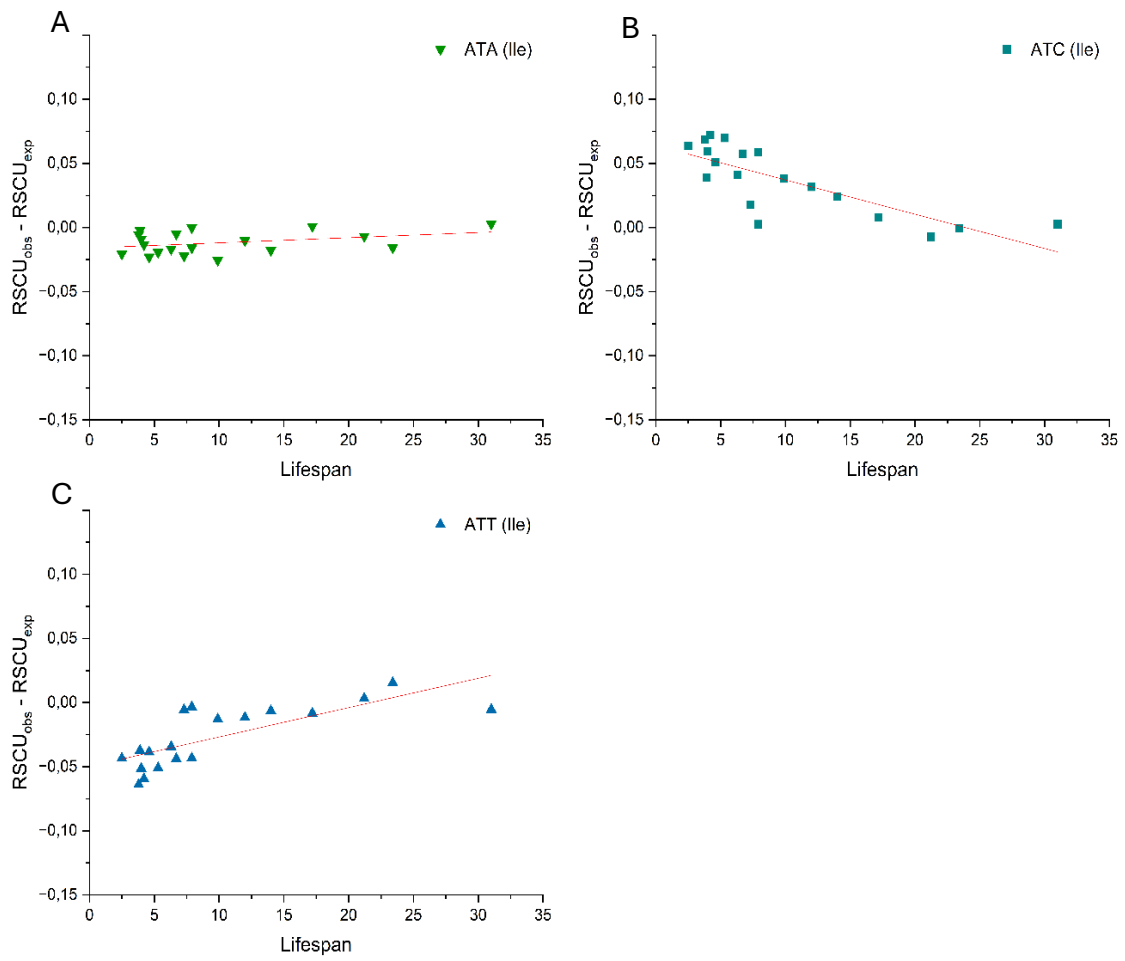
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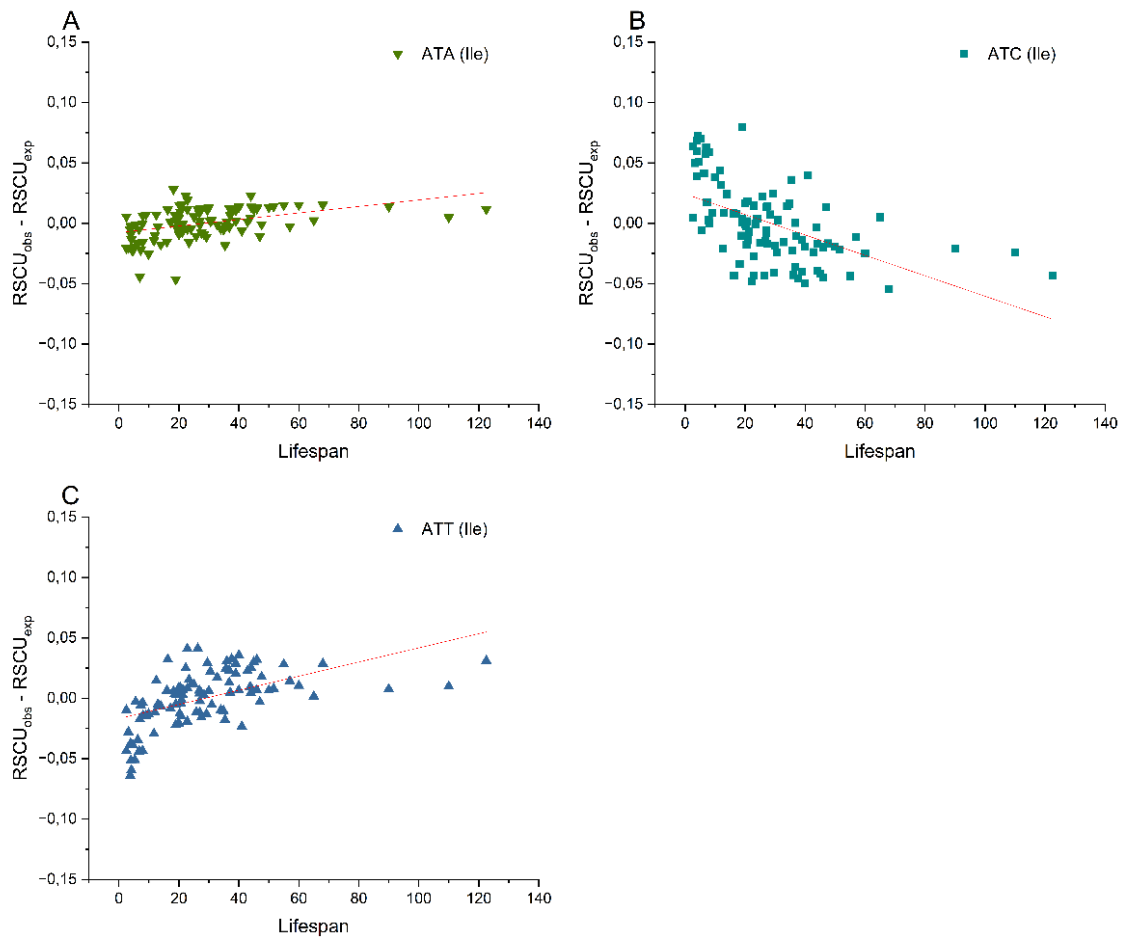
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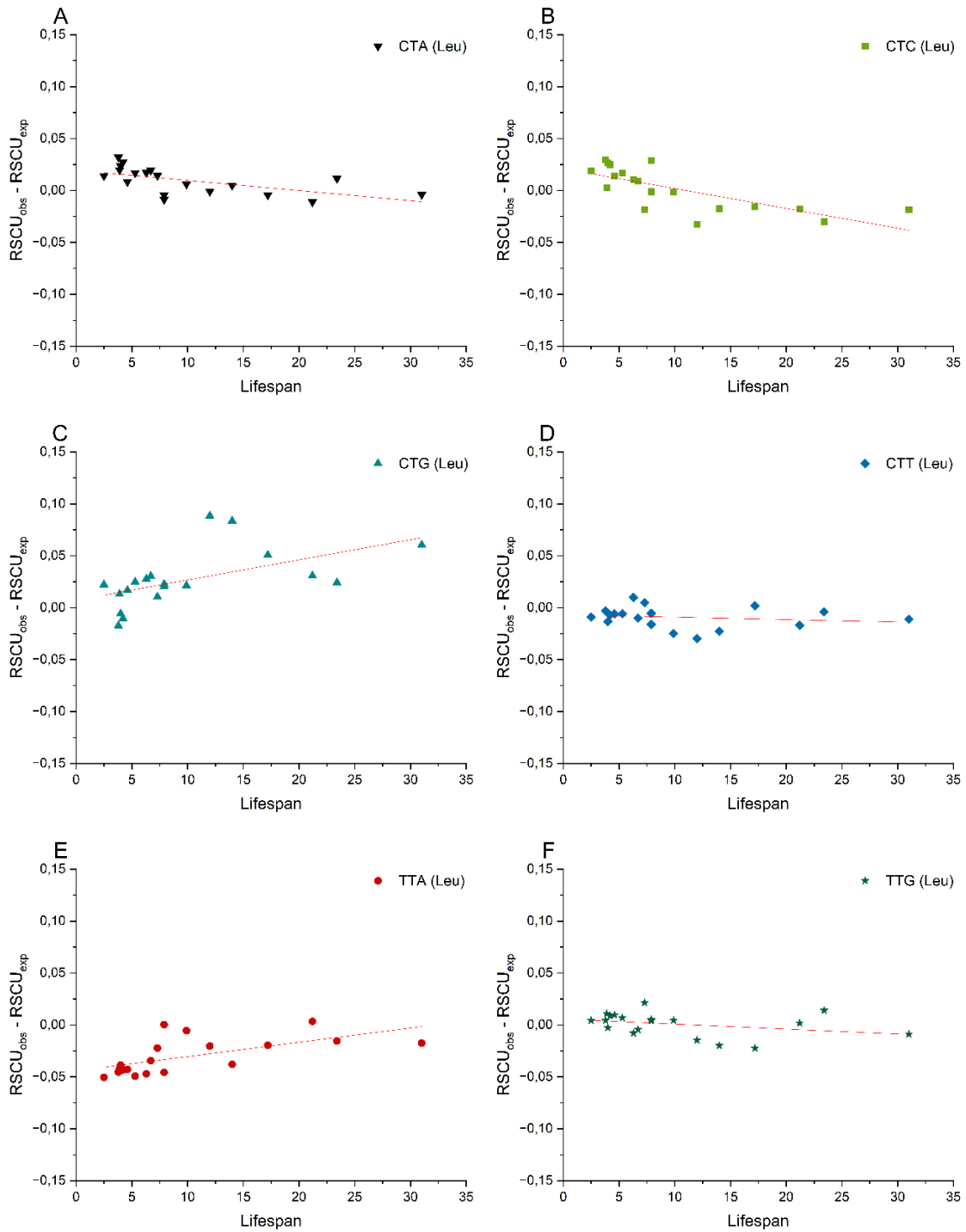
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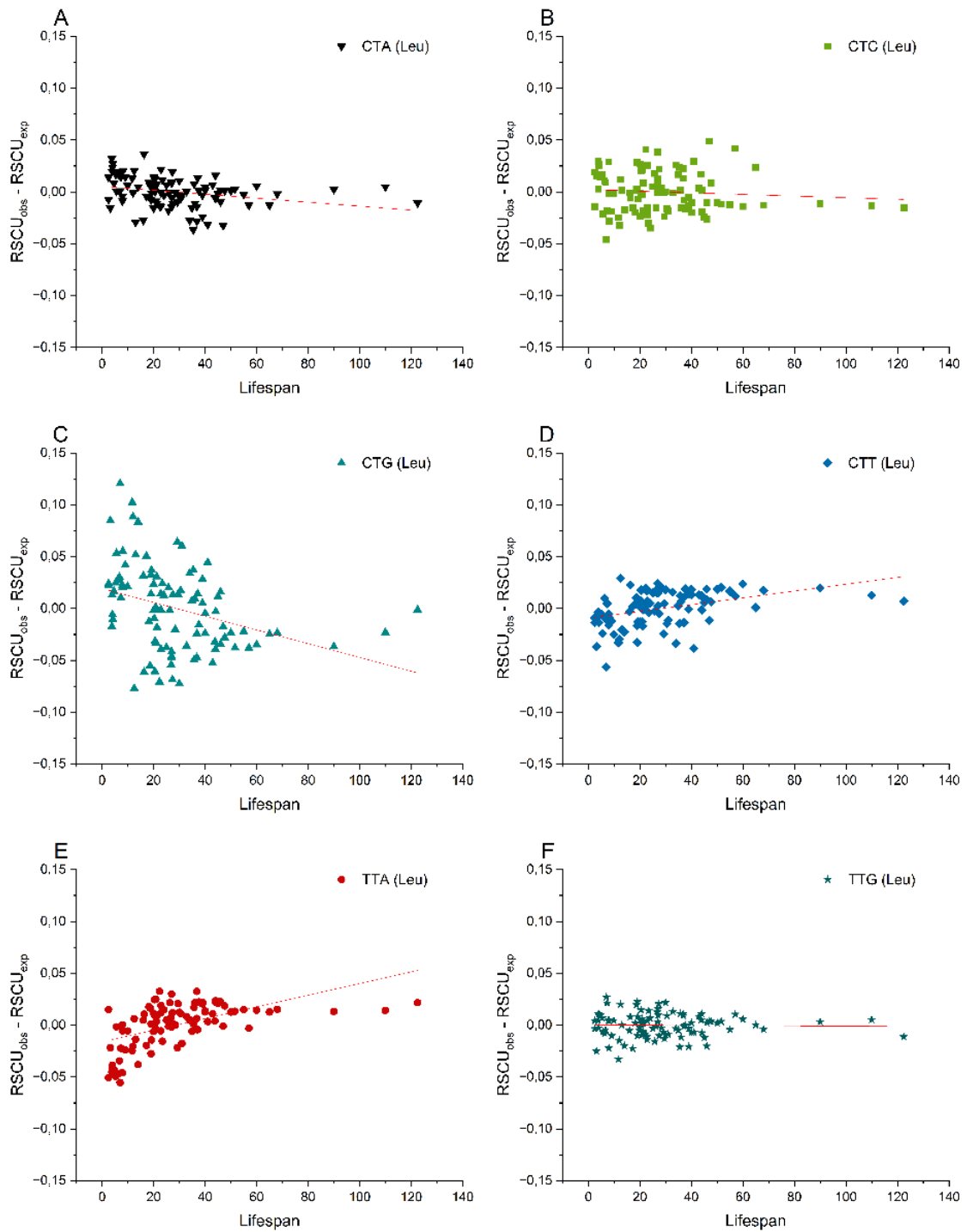
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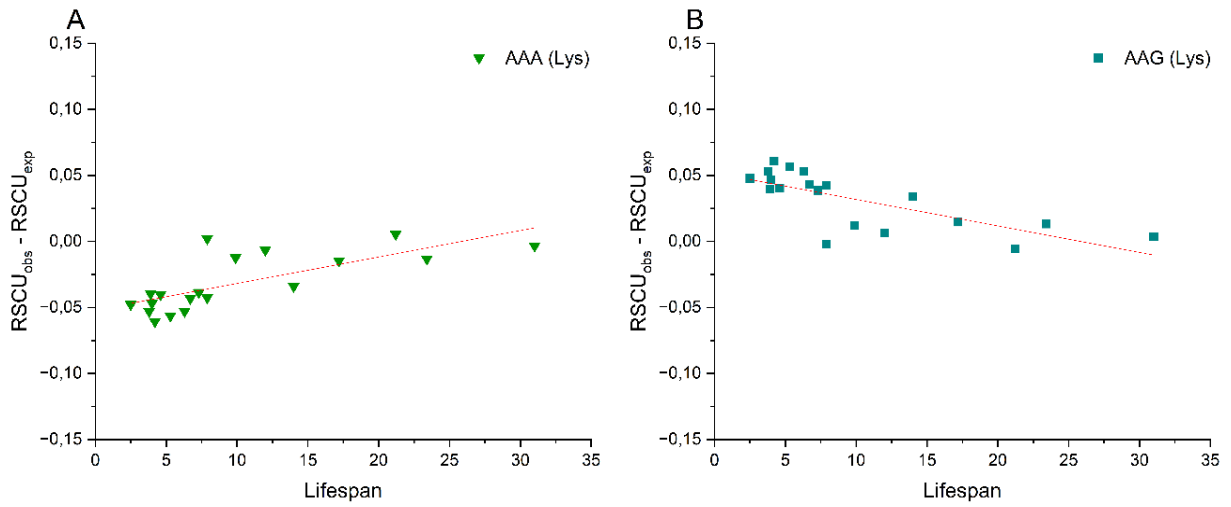
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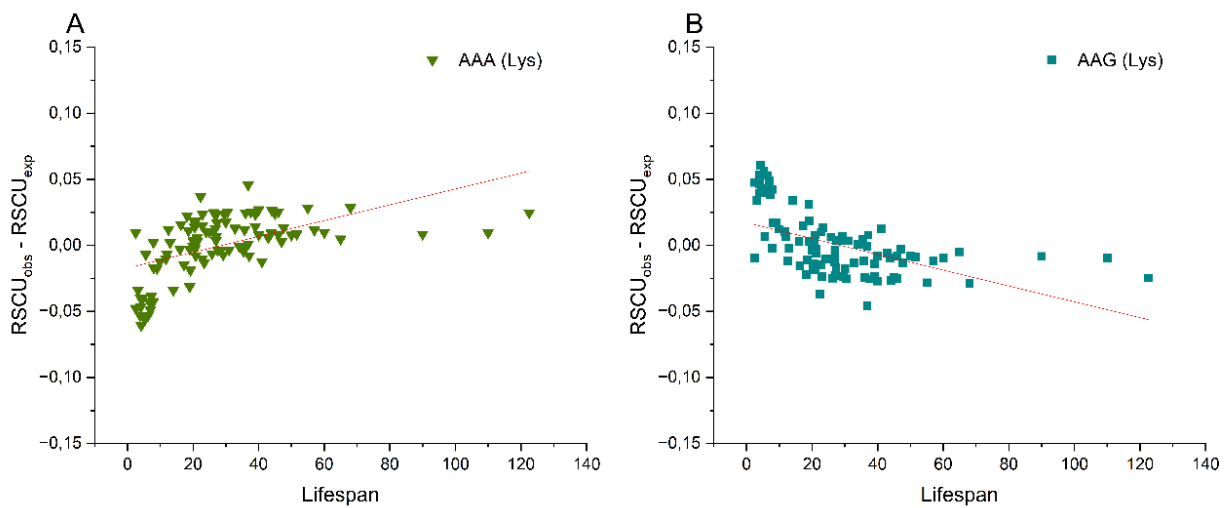
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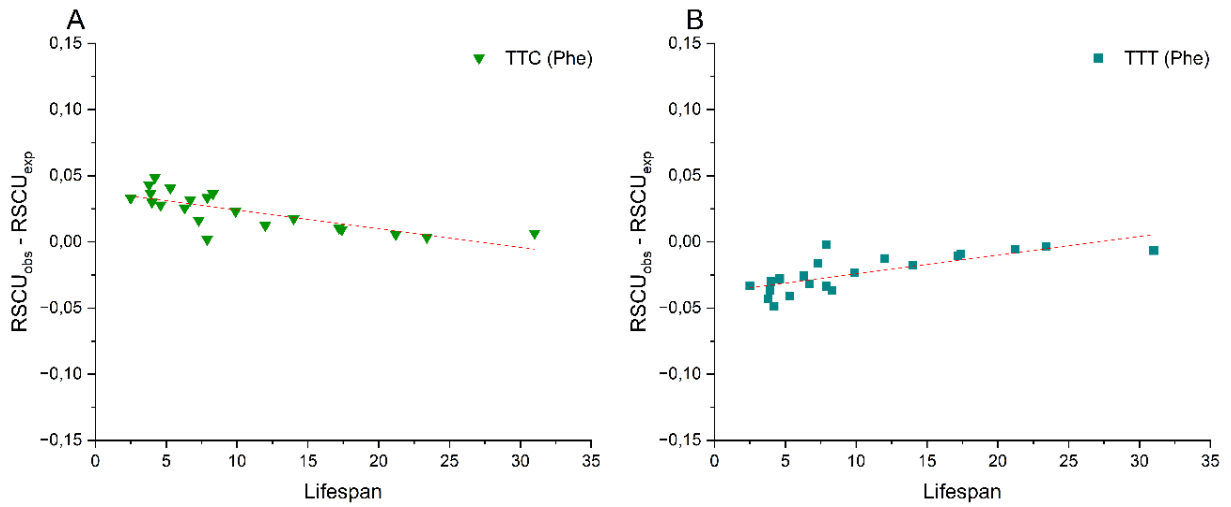
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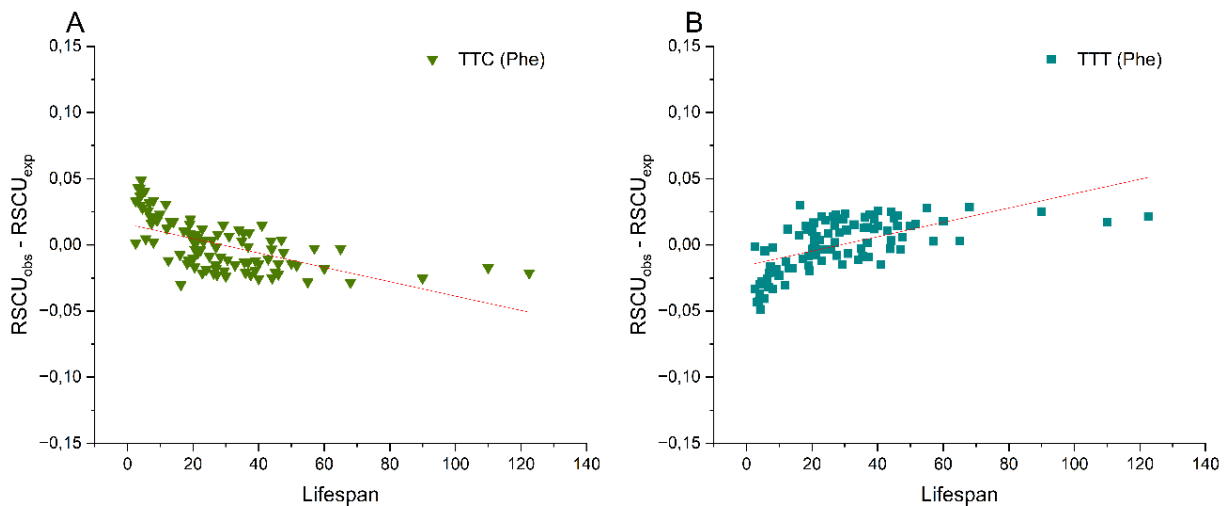
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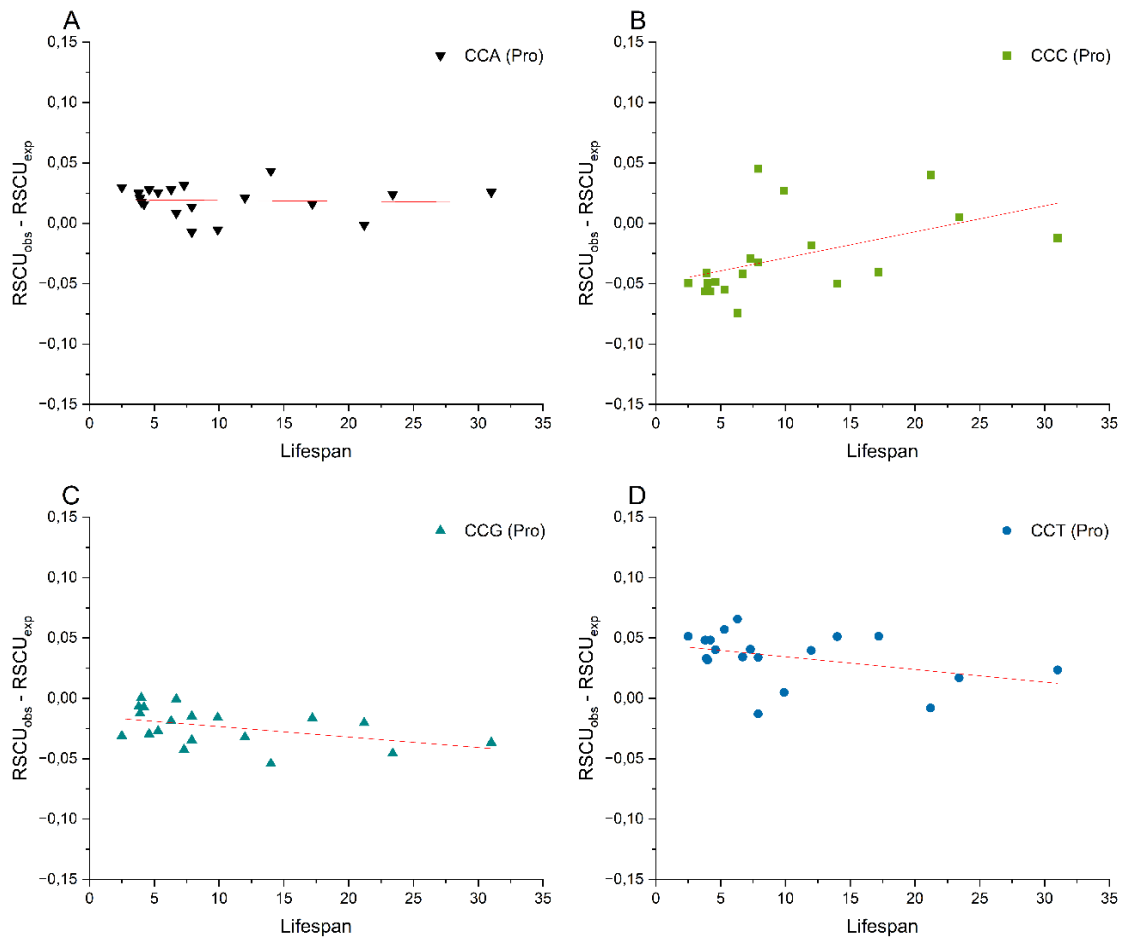
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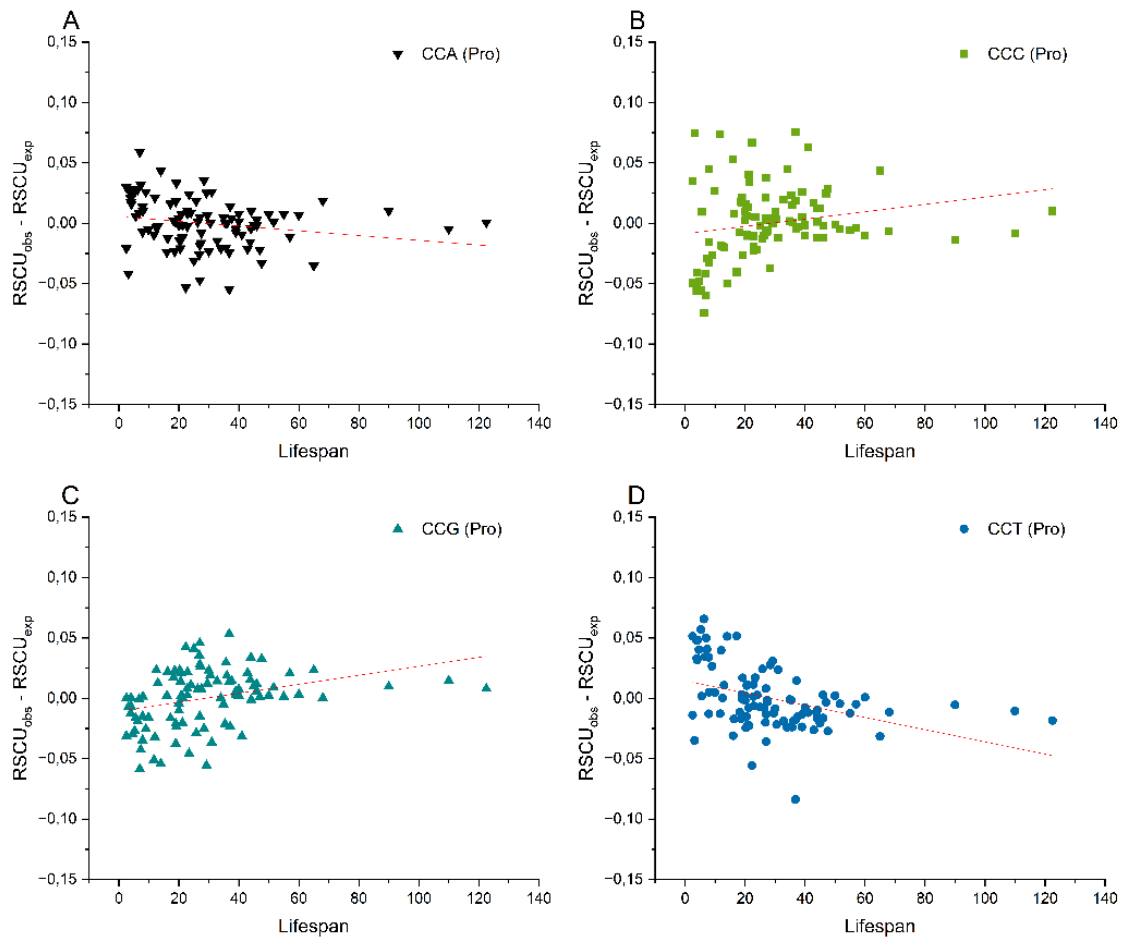
**Supplementary figure 13.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **phenylalanine**, plotted against maximum lifespan (years) of 19 **rodent** species.



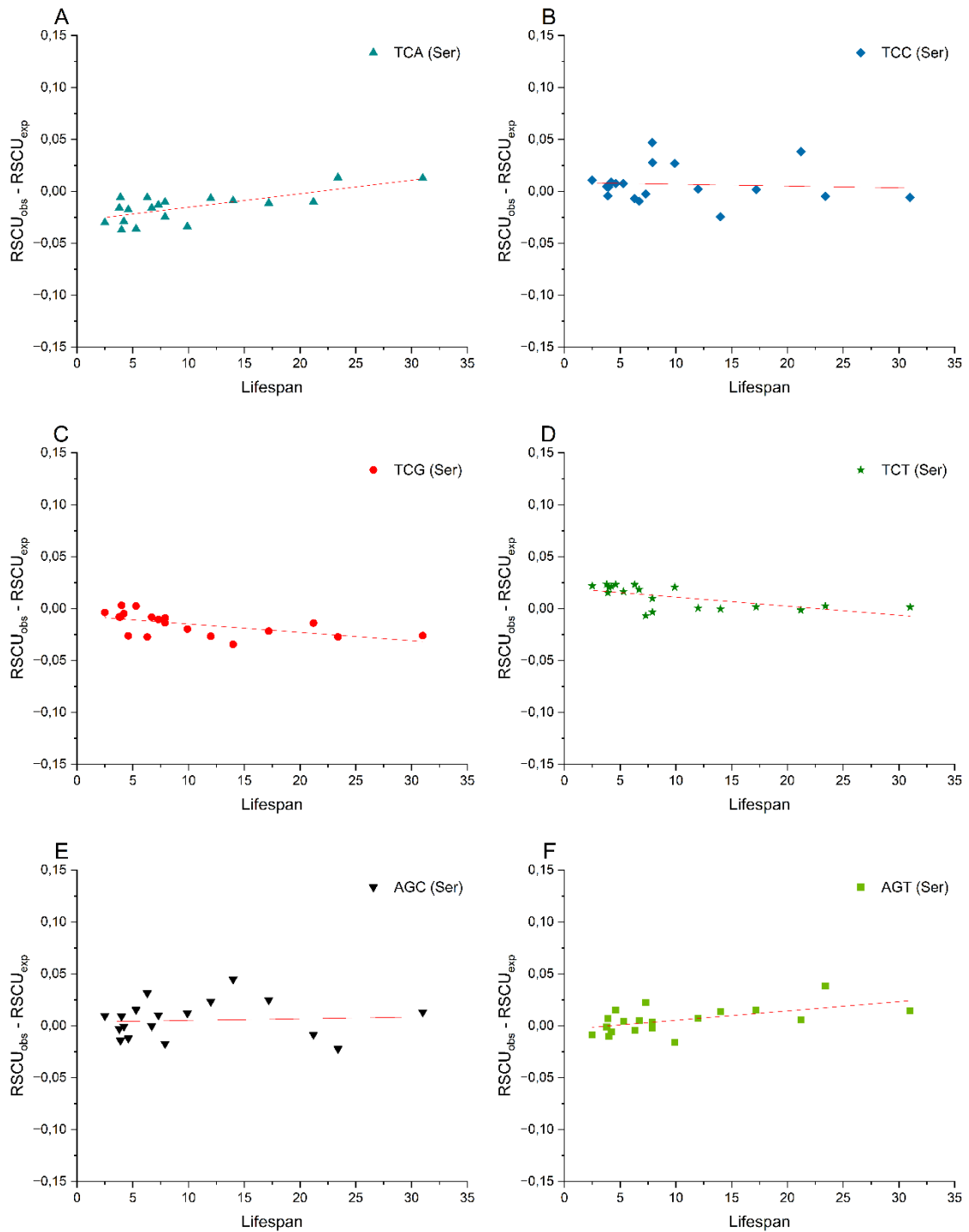
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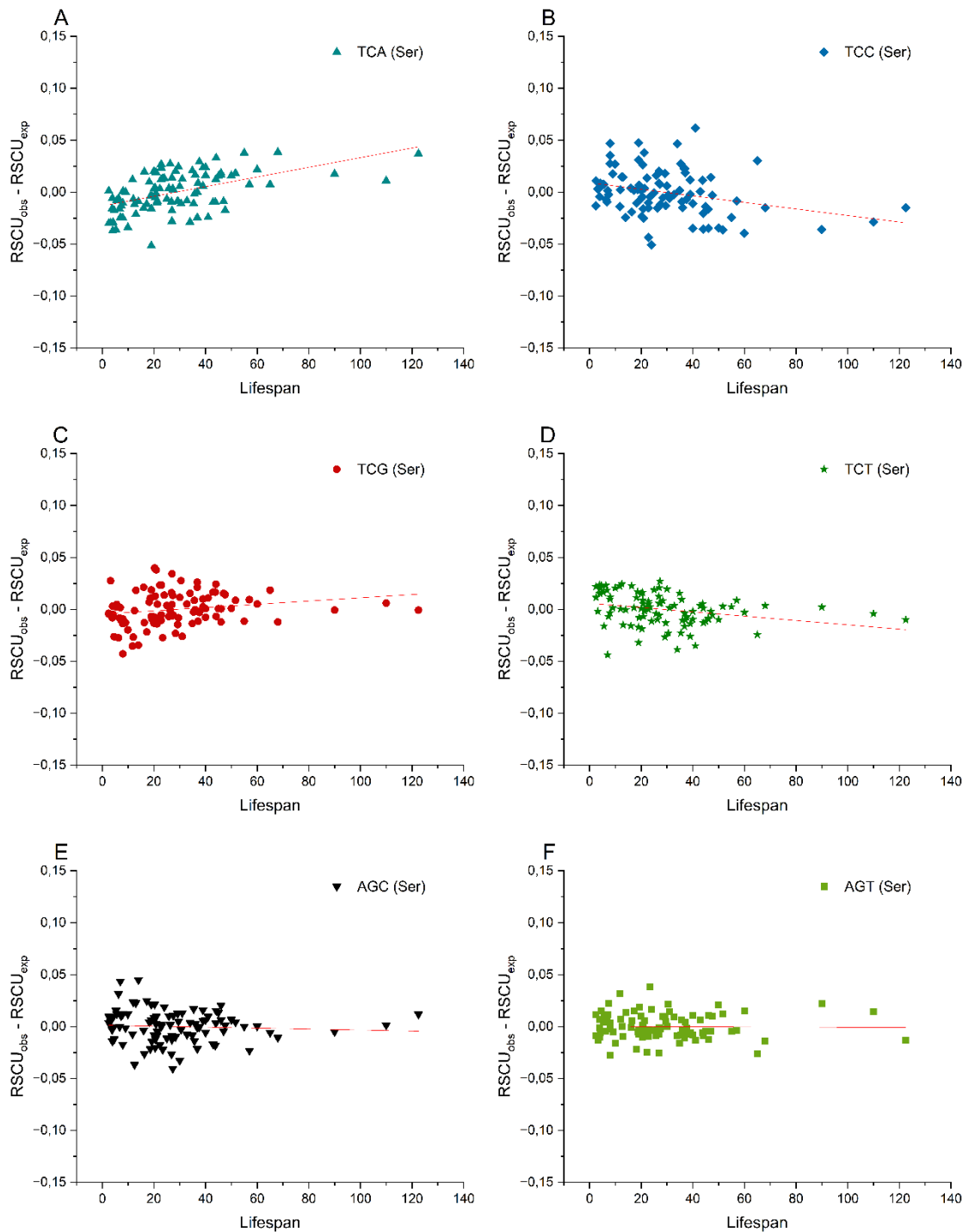
**Supplementary figure 14.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **proline**, plotted against maximum lifespan (years) of 19 **rodent** species.



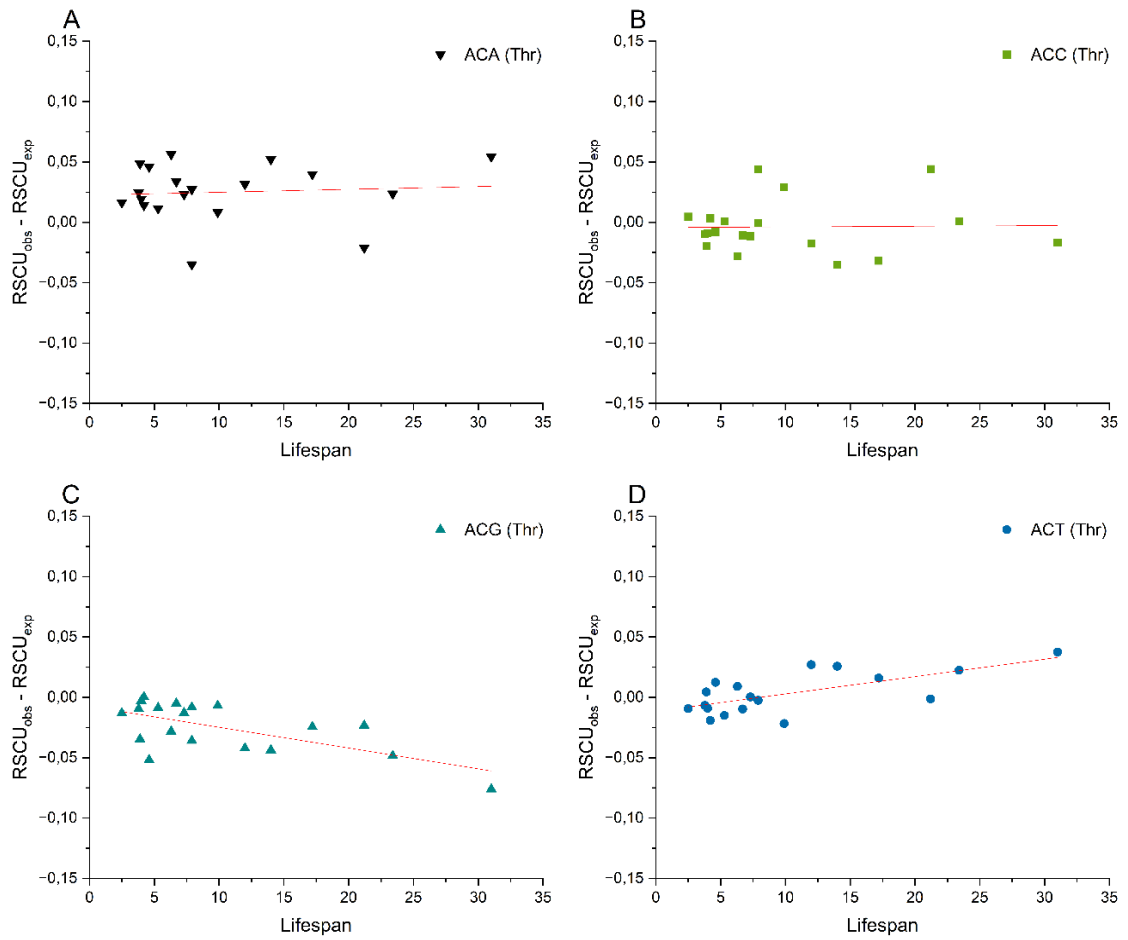
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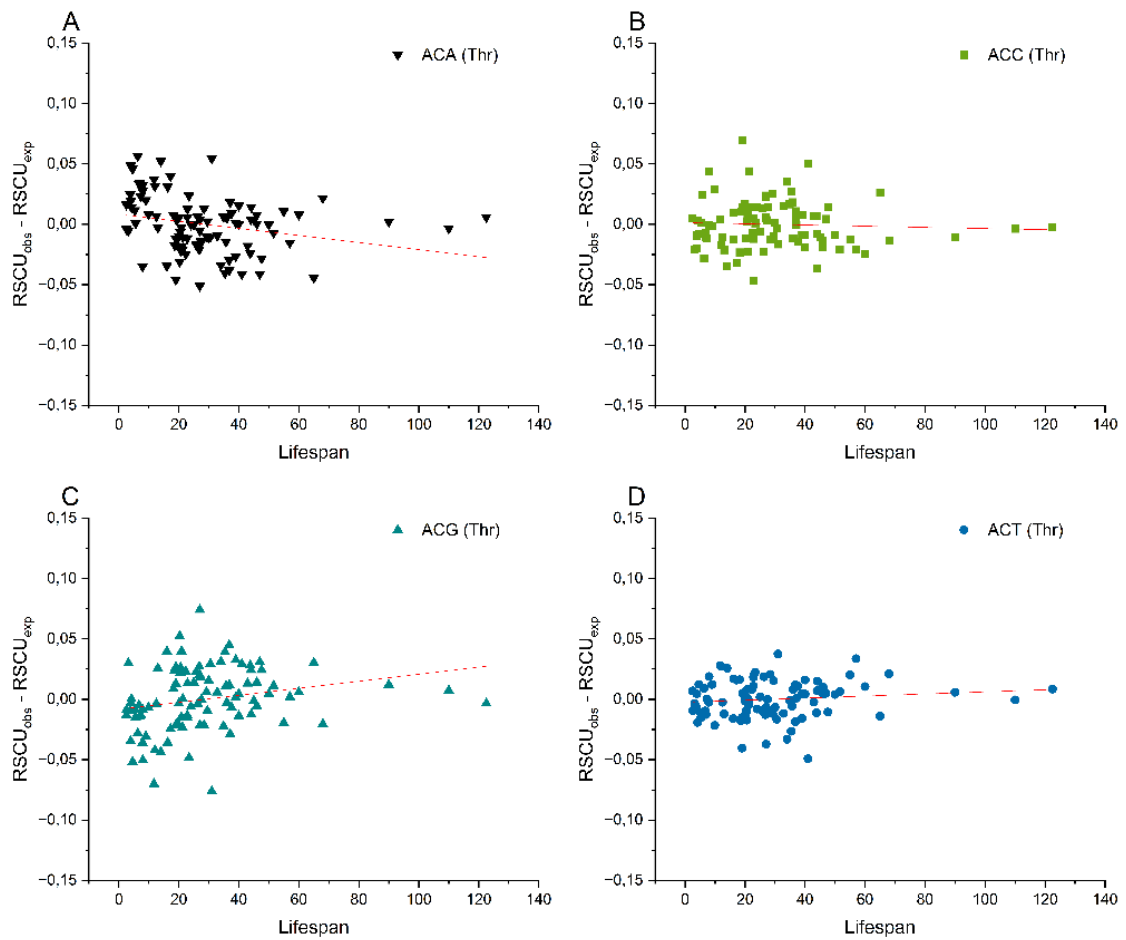
**Supplementary figure 15.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **serine**, plotted against maximum lifespan (years) of 19 **rodent** species.



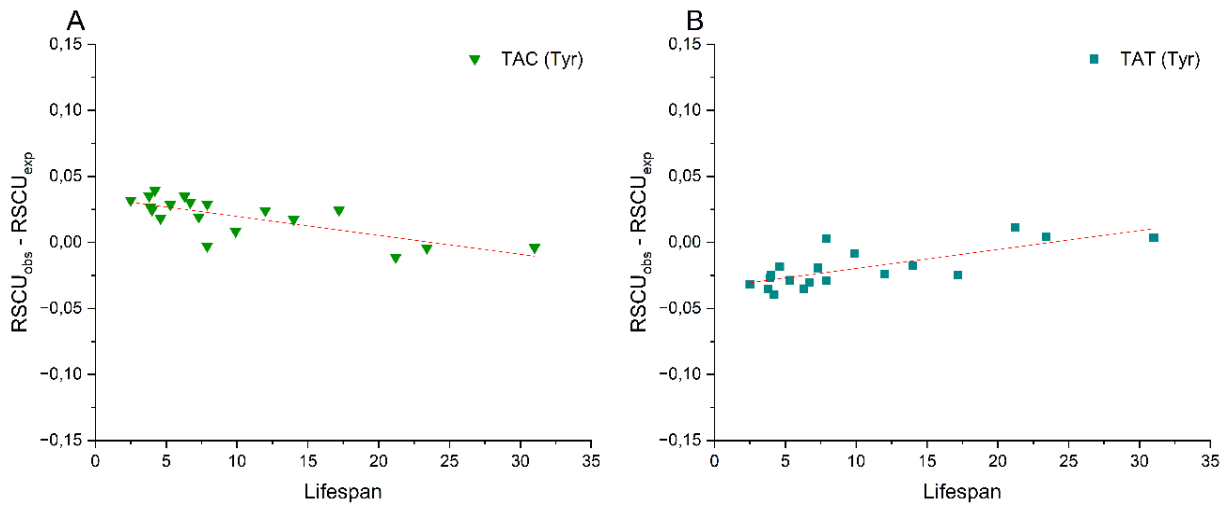
**Supplementary figure 15.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **serine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



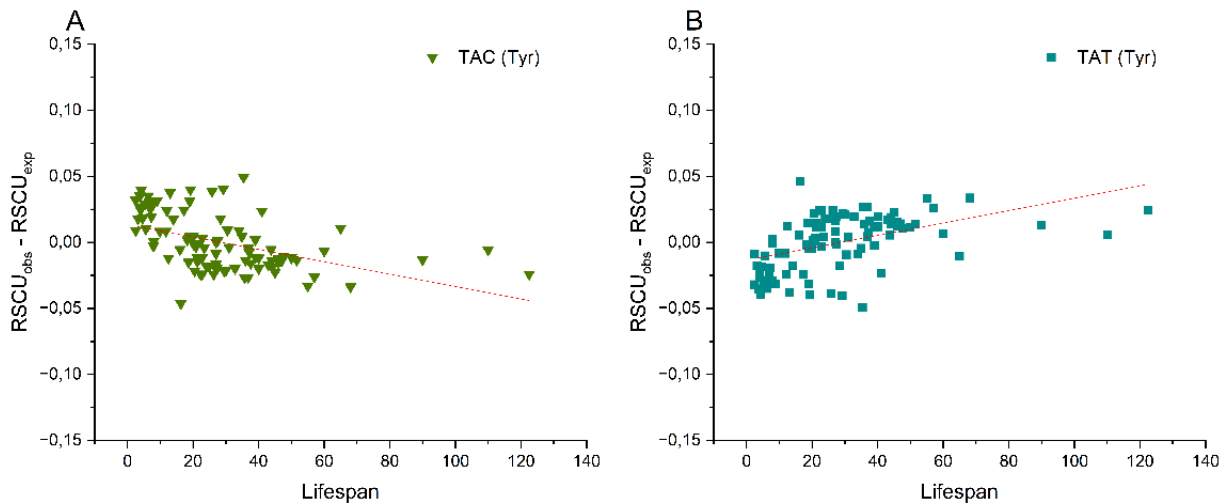
**Supplementary figure 16.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **threonine**, plotted against maximum lifespan (years) of 19 **rodent** species.



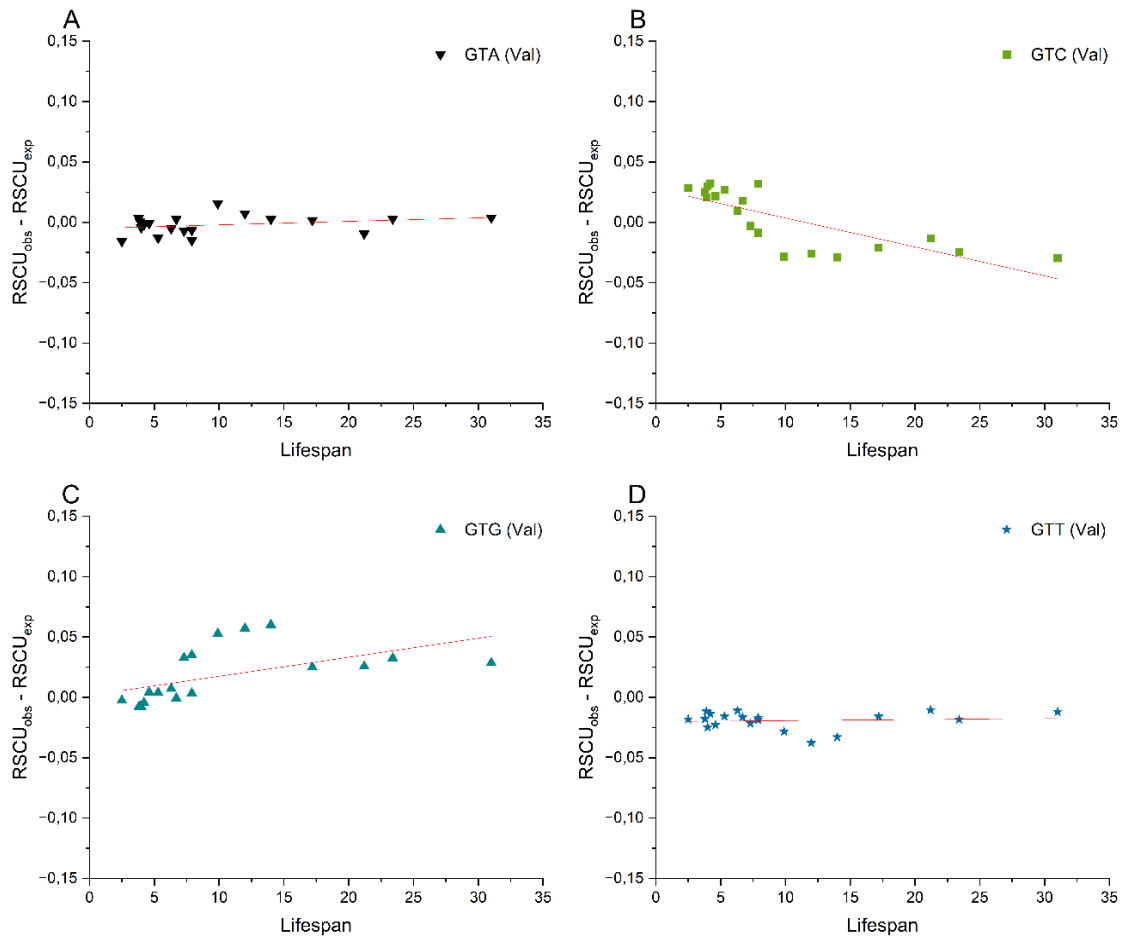
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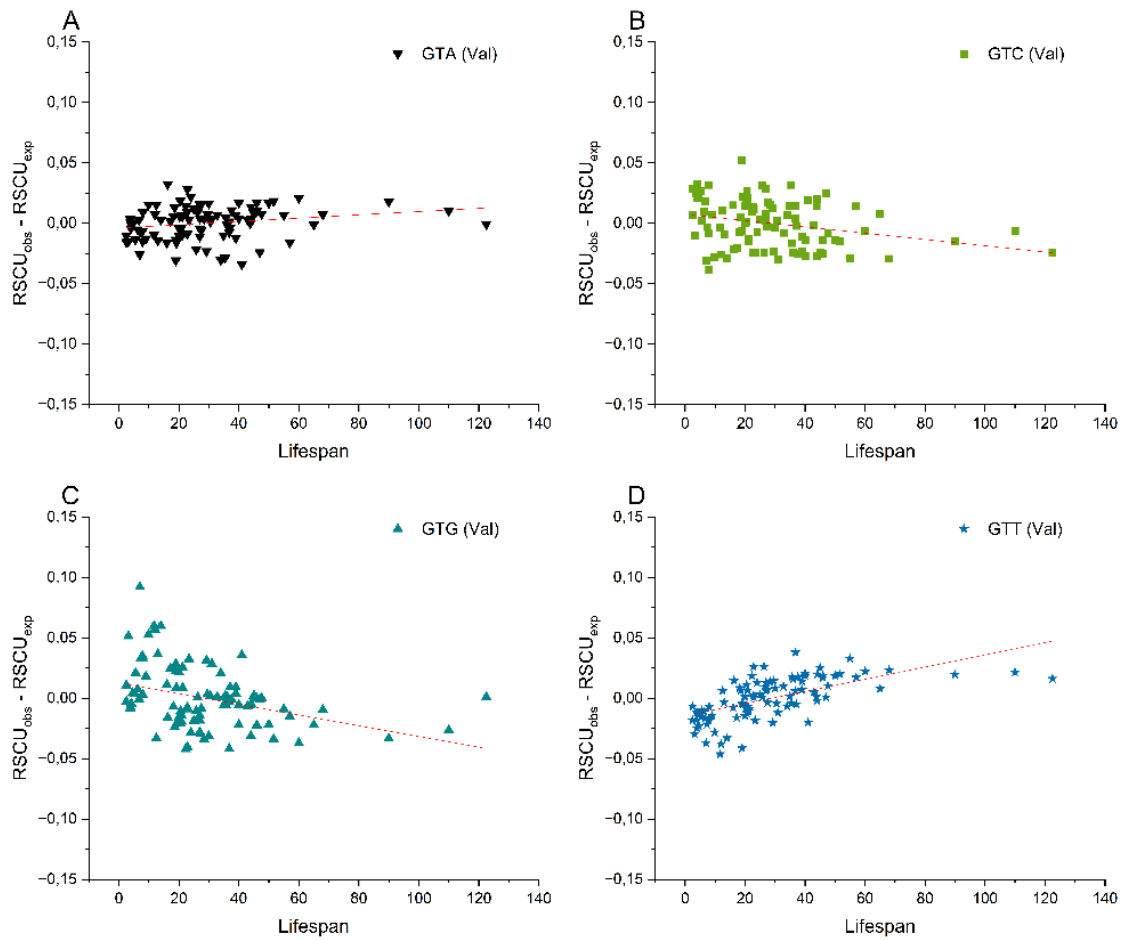
**Supplementary figure 17.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **tyrosine**, plotted against maximum lifespan (years) of 19 **rodent** species.



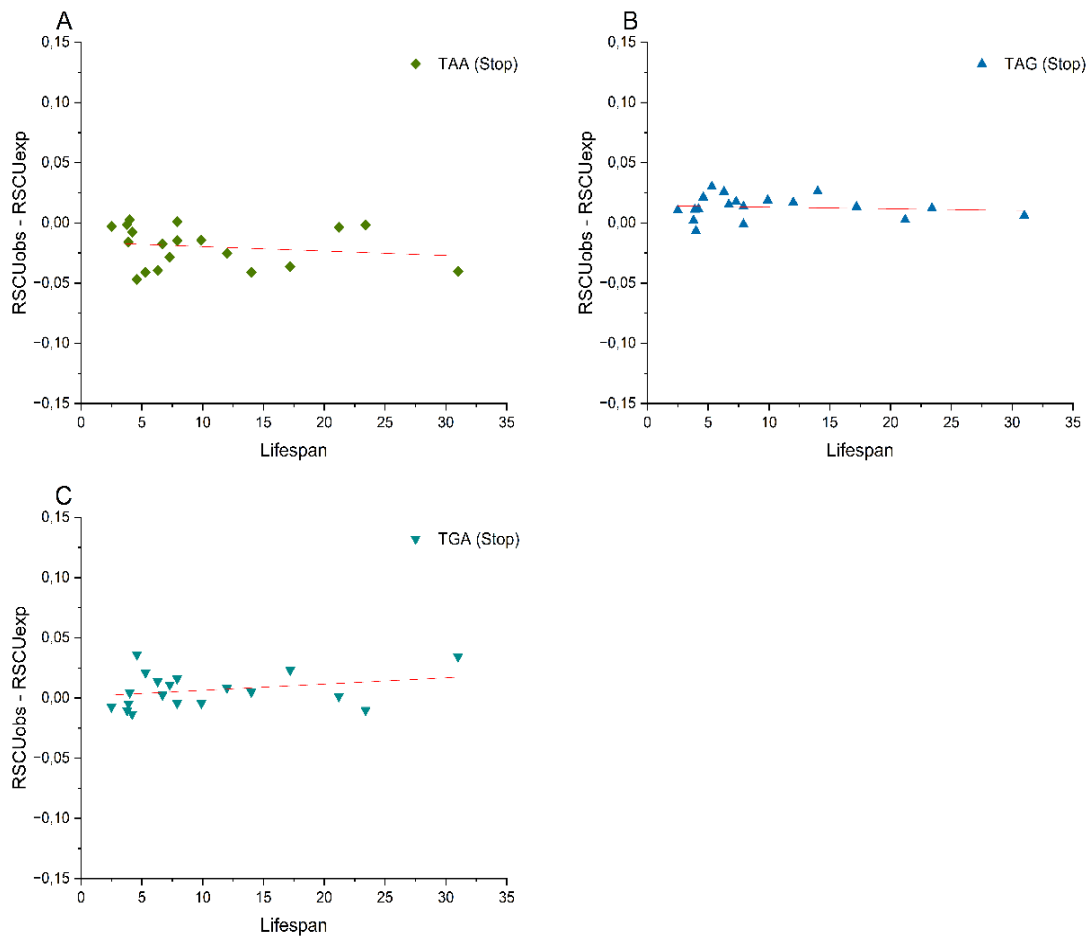
**Supplementary figure 17.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **tyrosine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



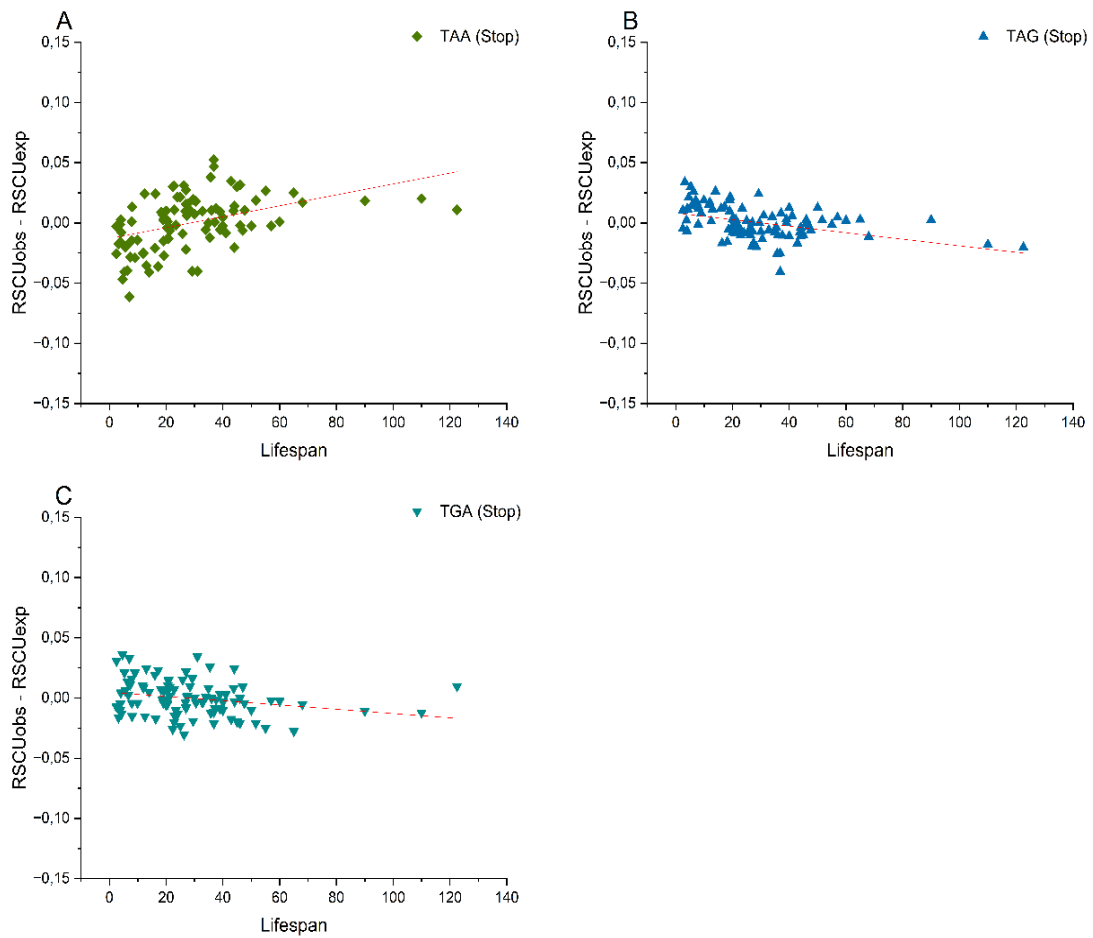
**Supplementary figure 18.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **valine**, plotted against maximum lifespan (years) of 19 **rodent** species.



**Supplementary figure 18.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for codons encoding **valine**, plotted against maximum lifespan (years) of 96 **mammalian** species.



**Supplementary figure 19.a.** Differences between observed and expected relative synonymous codon usage (RSCU) values for **stop** codons, plotted against maximum lifespan (years) of 19 **rodent** species.



**Supplementary figure 19.b.** Differences between observed and expected relative synonymous codon usage (RSCU) values for **stop** codons, plotted against maximum lifespan (years) of 96 **mammalian** species.

# Copy number of tRNA genes in metazoa and impact of GC content of coding genomes and lifespan on relative synonymous codon usage (RSCU) of mammalian species

Note: Data for synonymous codons with the highest and lowest numbers of matching isodecoder tRNA genes in green and red, respectively

Codon	RSCU <sub>met</sub>	Copy number of tRNA genes in metazoa					Copy number of tRNA genes in mammals					Copy number of tRNA genes in mammalian species analyzed in this study					Impact of GC content of coding genomes on codon usage (RSCU) of mammalian species					Correlation between changes in codon usage and lifespan in rodent species					Correlation between changes in codon usage and lifespan in mammalian species										
		Amino acid	Anticodon (tRNA)	Anticodon (RNA)	Fraction of species lacking the tRNA gene	Mean of tRNA gene copy number	Median of tRNA gene copy number	Anticodon (tRNA)	Anticodon (RNA)	Fraction of species lacking the tRNA gene	Mean of tRNA gene copy number	Median of tRNA gene copy number	Anticodon (tRNA)	Anticodon (RNA)	Fraction of species lacking the tRNA gene	Mean of tRNA gene copy number	Median of tRNA gene copy number	Codon	Number of points (species)	Pearson's r	R-Square (COD)	Slope value	Standard error of slope analysis	Adj. R-square of slope analysis	Codon	Number of points (species)	Pearson's r	R-Square (COD)	Slope value	Standard error of slope analysis	Adj. R-square of slope analysis	Codon	Number of points (species)	Pearson's r	R-Square (COD)	Slope value	Standard error of slope analysis
Ala	1.071	Alanine	UGC	GCA	0	13.32	UGC	GCA	0	13.62	9	UGC	GCA	0	16.30	9	GCA (Ala)	96	-0.9540	0.9101	-6.7442	0.2196	0.9092	GCA (Ala)	19	0.4012	0.1610	0.0010	0.0006	0.1116	GCA (Ala)	96	-0.4460	0.0021	0.0000	0.0001	-0.0085
Ala	1.401	Alanine	GCC	GCC	0.794	2.77	GCC	GCC	0.759	3.34	0	GCC	GCC	0.727	5.70	0	GCC (Ala)	96	0.9165	0.8399	7.9626	0.3405	0.8382	GCC (Ala)	19	0.2447	0.0599	0.0010	0.0010	0.0046	GCC (Ala)	96	0.2187	0.0478	0.0003	0.0001	0.0377
GCG	0.267	Alanine	CGC	GCG	0	7.45	GCG	GCG	0	7.24	5	GCG	GCG	0	8.64	5	GCG (Ala)	96	0.9249	0.8555	6.8735	0.2914	0.8540	GCG (Ala)	19	-0.1078	0.0116	-0.0001	0.0002	-0.0465	GCG (Ala)	96	0.2218	0.0492	0.0002	0.0001	0.0391
GCT	1.261	Alanine	AGC	GCU	0	19.49	AGC	GCU	0	20.10	15	AGC	GCU	0	23.67	16	GCT (Ala)	96	-0.9187	0.8440	-7.6837	0.3947	0.8424	GCT (Ala)	19	-0.6105	0.3727	-0.0020	0.0006	0.3358	GCT (Ala)	96	-0.3779	0.1428	-0.0005	0.0001	0.1337
Arg	1.475	Arginine	UCU	AGA	0	10.51	UCU	AGA	0	8.60	6	UCU	AGA	0	8.06	6	AGA (Arg)	96	-0.9504	0.9033	-7.4449	0.2513	0.9022	AGA (Arg)	19	-0.5877	0.3454	-0.0023	0.0008	0.3069	AGA (Arg)	96	0.0183	0.0003	0.0000	0.0001	-0.0103
Arg	1.354	Arginine	CCU	AGG	0	9.97	CCU	AGG	0	8.76	7	CCU	AGG	0	8.70	7	AGG (Arg)	96	-0.9135	0.8937	-7.2483	0.3075	0.8558	AGG (Arg)	19	-0.3852	0.1464	-0.0014	0.0006	0.0983	AGG (Arg)	96	-0.1351	0.0152	-0.0002	0.0001	0.0076
CGA	0.734	Arginine	UCG	CGA	0	9.15	UCG	CGA	0	6.84	5.5	UCG	CGA	0	5.82	5	CGA (Arg)	96	-0.8539	0.8954	-3.1243	0.2153	0.8921	CGA (Arg)	19	-0.6096	0.3716	-0.0013	0.0004	0.3347	CGA (Arg)	96	-0.4954	0.1641	-0.0003	0.0001	0.1552
CGC	0.895	Arginine	GCC	COC	0.928	0.19	GCC	COC	0.828	0.36	0	GCC	COC	0.758	0.52	0	CGC (Arg)	96	0.8586	0.7372	7.4179	0.4568	0.7344	CGC (Arg)	19	0.6438	0.4145	0.0037	0.0011	0.3801	CGC (Arg)	96	0.0396	0.0016	0.0001	0.0002	-0.0091
CGG	0.992	Arginine	CCG	CGG	0.354	2.55	CCG	CGG	0	4.05	3.5	CCG	CGG	0	3.67	3	CGG (Arg)	96	0.9270	0.8893	8.2658	0.3449	0.8578	CGG (Arg)	19	0.6151	0.3783	0.0023	0.0007	0.3417	CGG (Arg)	96	0.3681	0.1355	0.0005	0.0001	0.1263
CGU	0.554	Arginine	ACG	CGU	0	12.01	ACG	CGU	0	7.05	7	ACG	CGU	0	6.79	6	CCT (Arg)	96	-0.8524	0.7266	-2.9289	0.1853	0.7237	CCT (Arg)	19	-0.6007	0.3608	-0.0012	0.0004	0.3232	CCT (Arg)	96	-0.1258	0.0158	-0.0001	0.0001	0.0054
Asn	0.99	Asparagine	GUU	AAC	0	18.61	GUU	AAC	0	15.48	15	GUU	AAC	0	15.36	15	AAC (Asn)	96	0.7319	0.5357	3.3086	0.3177	0.5338	AAC (Asn)	19	-0.7887	0.6189	-0.0032	0.0006	0.5984	AAC (Asn)	96	-0.4419	0.1953	-0.0005	0.0001	0.1867
Asn	1.01	Asparagine	AUU	AUU	0.785	0.65	AUU	AUU	0.603	0.86	0	AUU	AUU	0.576	1.03	0	AAT (Asn)	96	-0.7319	0.5357	-3.3086	0.3177	0.5338	AAT (Asn)	19	0.7887	0.6189	0.0032	0.0006	0.5984	AAT (Asn)	96	0.4419	0.1953	0.0005	0.0001	0.1867
Asp	0.978	Aspartic Acid	GUC	GAC	0	20.25	GUC	GAC	0	13.62	12	GUC	GAC	0	13.39	12	GAC (Asp)	96	0.8756	0.7667	4.2804	0.2706	0.7642	GAC (Asp)	19	-0.6639	0.4407	-0.0016	0.0004	0.4078	GAC (Asp)	96	-0.5123	0.2625	-0.0005	0.0001	0.2546
GAT	1.023	Aspartic Acid	AUC	GAU	0.897	0.68	AUC	GAU	0.793	1.08	0	AUC	GAU	0.848	3.12	0	GAT (Asp)	96	-0.8756	0.7667	-4.2804	0.2436	0.7642	GAT (Asp)	19	0.6639	0.4407	0.0016	0.0004	0.4078	GAT (Asp)	96	0.5123	0.2625	0.0005	0.0001	0.2546
Cys	0.953	Cysteine	AUC	UGC	0	17.85	AUC	UGC	0	25.71	22	AUC	UGC	0	26.45	22	UGC (Cys)	96	0.9438	0.8907	4.4568	0.1610	0.8895	UGC (Cys)	19	0.5259	0.2786	0.0010	0.0004	0.2340	UGC (Cys)	96	0.1326	0.0176	0.0001	0.0001	0.0071
TCU	1.047	Cysteine	ACA	UGU	0.789	0.49	ACA	UGU	0.672	0.63	0	ACA	UGU	0.636	0.52	0	TGU (Cys)	96	-0.8438	0.8907	-4.4568	0.1610	0.8895	TGU (Cys)	19	-0.5259	0.2786	-0.0010	0.0004	0.2340	TGU (Cys)	96	-0.1326	0.0176	-0.0001	0.0001	0.0071
Gln	0.596	Glutamine	UUG	CAA	0	9.78	UUG	CAA	0	6.28	5	UUG	CAA	0	5.21	5	CAA (Gln)	96	-0.9006	0.8110	-2.7653	0.1377	0.8090	CAA (Gln)	19	0.1447	0.0209	0.0002	0.0003	-0.0367	CAA (Gln)	96	0.2191	0.0480	0.0001	0.0001	0.0379
CAU	1.404	Glutamine	CUU	CAG	0	13.44	CUU	CAG	0	11.33	10.5	CUU	CAG	0	10.82	10	CAG (Gln)	96	0.9006	0.8110	2.7653	0.1377	0.8090	CAG (Gln)	19	-0.1447	0.0209	-0.0002	0.0003	-0.0367	CAG (Gln)	96	-0.2191	0.0480	-0.0001	0.0001	0.0379
Glu	0.922	Glutamic Acid	UUC	GAA	0	16.17	UUC	GAA	0	15.62	9	UUC	GAA	0	14.00	9	GAA (Glu)	96	-0.8659	0.7497	-2.8998	0.1728	0.7470	GAA (Glu)	19	0.3372	0.1137	0.0005	0.0003	0.0616	GAA (Glu)	96	0.3976	0.1581	0.0003	0.0001	0.1491
GAG	1.078	Glutamic Acid	CUU	GAG	0	16.78	CUU	GAG	0	16.55	13	CUU	GAG	0	14.09	12	GAG (Glu)	96	0.8659	0.7497	2.8998	0.1728	0.7470	GAG (Glu)	19	-0.3372	0.1137	-0.0005	0.0003	0.0616	GAG (Glu)	96	-0.3976	0.1581	-0.0003	0.0001	0.1491
Gly	1.14	Glycine	UCC	CGA	0	16.55	UCC	GGA	0	17.67	9	UCC	GGA	0	16.64	9	GGA (Gly)	96	-0.9588	0.9193	-5.4366	0.1661	0.9185	GGA (Gly)	19	-0.7540	0.5685	-0.0014	0.0003	0.5431	GGA (Gly)	96	-0.0821	0.0085	-0.0001	0.0001	-0.0021
GCC	1.169	Glycine	GCC	GGC	0	18.86	GCC	GGC	0	14.69	13	GCC	GGC	0	13.85	12	GCC (Gly)	96	0.9466	0.8960	6.8201	0.2397	0.8949	GCC (Gly)	19	0.4576	0.2094	0.0009	0.0004	0.1629	GCC (Gly)	96	-0.0711	0.0051	-0.0001	0.0001	-0.0055
GGG	0.92	Glycine	CCC	GGG	0.054	6.76	CCC	GGG	0	10.95	5.5	CCC	GGG	0	9.21	6	GGG (Gly)	96	0.7758	0.6019	3.0929	0.2594	0.5977	GGG (Gly)	19	0.7575	0.5737	0.0016	0.0003	0.5487	GGG (Gly)	96	0.4099	0.1080	0.0004	0.0001	0.1592
GGT	0.772	Glycine	ACC	GGU	0.870	8.01	ACC	GGU	0.741	2.66	0	ACC	GGU	0.758	4.21	0	GTT (Gly)	96	-0.9317	0.8349	-4.4586	0.2045	0.8332	GTT (Gly)	19	-0.4979	0.2479	-0.0009	0.0004	0.2037	GTT (Gly)	96	-0.3393	0.1151	-0.0003	0.0001	0.1057
His	1.043	Histidine	AUG	CAU	0	12.80	AUG	CAC	0	8.74	9	AUG	CAC	0	8.61	8	CAC (His)	96	0.9307	0.8662	5.2582	0.2131	0.8648	CAC (His)	19	-0.0950	0.0090	-0.0001	0.0004	-0.0493	CAC (His)	96	-0.2703	0.0731	-0.0002	0.0001	0.0632
CAT	0.957	Histidine	AUG	CAU	0.928	0.10	AUG	CAU	0.898	0.14	0	AUG	CAU	0.848	0.24	0	CAT (His)	96	-0.9307	0.8662	-5.2582	0.2131	0.8648	CAT (His)	19	0.0950	0.0090	0.0001	0.0004	-0.0493	CAT (His)	96	0.2703	0.0731	0.0002	0.0001	0.0632
Ile	0.558	Isoleucine	UAU	AUA	0.004	7.05	UAU	AUA	0	5.81	5	UAU	AUA	0	6.24	5	AUA (Ile)	96	-0.7112	0.9058	-1.6556	0.1888	0.9006	AUA (Ile)	19	0.3625	0.1314	0.0004	0.0003	0.0803	AUA (Ile)	96	0.4243	0.1801	0.0003	0.0001	0.1713
ATC	1.283	Isoleucine	AUC	AUC	0.780	2.41	AUC	AUC	0.690	0.72	0	AUC	AUC	0.667	1.03	0	ATC (Ile)	96	0.7873	0.6198	5.0612	0.4089	0.6158	ATC (Ile)	19	-0.7885	0.6217	-0.0027	0.0005	0.5994	ATC (Ile)	96	-0.5485	0.3008	-0.0008	0.0001	0.2934
ATT	1.162	Isoleucine	AUU	AUU	0	15.97	AUU	AUU	0	12.55	11	AUU	AUU	0	13.38	11	ATT (Ile)	96	-0.7806	0.6993	-3.4800	0.2874	0.6951	ATT (Ile)	19	0.7590	0.5789	0.0023	0.0005	0.5511	ATT (Ile)	96	0.5407	0.2524	0.0002	0.0001	0.2848
Leu	0.535	Leucine	AUG	CUA	0.013	6.11	UAG	CUA	0	3.98	4	UAG	CUA	0	4.00	4	CUA (Leu)	96	-0.9140	0.8354	-3.9660	0.1816	0.8337	CUA (Leu)	19	-0.6087	0.3705	-0.0010	0.0003	0.3335	CUA (Leu)	96	-0.2806	0.0787	-0.0002	0.0001	0.0689
CTC	1.084	Leucine	GAG	CUC	0.946	0.07	GAG	CUC	0.983	0.02	0	GAG	CUC																								

