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Supplemental Information for:

Individual variation in early-life telomere length and survival in a wild mammal

Sil H.J. van Lieshout, Amanda Bretman, Chris Newman, Christina D. Buesching, David W. Macdonald & Hannah L. Dugdale

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This supplementary materials document includes a description of the quality control for the monochrome multiplex quantitative PCR method and supplementary results, tables and figures.

Supplementary methods

Quality control of telomere length estimation through quantitative PCR

LinRegPCR 2017.1 (Ruijter *et al.* 2009) was used to correct for baseline fluorescence, to determine the window of linearity for each amplicon (i.e. separate windows for IRBP and telomere reactions) and to calculate amplification efficiencies for each well. Subsequently, Cq-values for each sample were calculated in R 3.3.1 (R Development Core Team 2019). Across plates (n = 34), fluorescence thresholds (Nq) were set to a constant value within the window of linearity for the amplification curves: 0.432 for IRBP and 0.694 for telomeres. Mean amplification efficiency across wells for each amplicon group per plate, excluding outliers (outside the 5th and 95th percentiles), were used as our estimates of reaction efficiency (as recommended by Ruijter *et al.* 2009).

Further quality control was applied, where samples were excluded from further analyses if the standard deviation across their duplicate Cq values for either amplicon group was greater than 5% of the mean Cq for that sample (n = 25). We also excluded any sample if the standard deviation across the duplicate well-specific efficiencies for either amplicon was greater than 5% of the overall mean efficiency for that amplicon group (n = 44). Lastly, samples with a Cq-value >28 for telomere, or >29 for IRBP, were excluded from the analysis (n = 24), assuming that these were failed reactions. In order to determine failed reactions for control samples, we applied a similar rule where samples with a standard deviation of the duplicate T/S ratios >8% of the mean T/S ratio for that sample were excluded, as at least one of the duplicate samples was assumed to have failed (n = 19; <12% of samples).

Reaction efficiencies differed between our IRBP and telomere reactions (mean efficiencies across all samples on all plates run: IRBP = 1.793 ± 0.004 SE; Telomere: 1.909 ± 0.004 SE). Assuming constant amplification efficiencies across plates can bias qPCR results when these actually differ, we therefore calculated relative leukocyte telomere length (RLTL) using a method that does not assume consistent efficiencies across plates (Pfaffl 2001):

$$RLTL = \frac{(E_{tel}^{(cq_{tel(calibrator)} - Cq_{tel(sample)}))}}{(E_{IRBP}^{(cq_{IRBP(calibrator)} - Cq_{IRBP(sample)}))}}$$

In this equation, E_{tel} and E_{IRBP} represent the mean well efficiencies for each of the amplicons, calculated in LinRegPCR, $Cq_{tel(calibrator)}$ and $Cq_{IRBP(calibrator)}$ are the mean Cq-values for the calibrators (20 ng/ μ l) of the reference sample for each amplicon and $Cq_{tel(sample)}$ and $Cq_{IRBP(sample)}$ are the mean Cq-values for both amplicons in each sample.

Only 69 of 1324 samples did not pass the initial quality control and these samples were repeated. 17 repeated samples passed the quality control, meaning that 52 samples (<4%) were excluded. Additionally, as 24 samples had a Cq-value >28 for TL or Cq-value >29 for IRBP, which we considered to be failed reactions, these were excluded from the analyses. This resulted in a total of 1248 RLTL measurements from 612 individuals (308 males and 304 females), with 163 individuals having 1 sample (early-life samples to reduce bias from viability selection), 408

individuals with 2 samples, 5 individuals with 5 samples, 17 individuals with 6 samples, 12 individuals with 7 samples, 5 individuals with 8 samples and 2 individuals with 9 samples.

Inter-plate repeatability (intraclass correlation coefficient), calculated with rptR 0.9.2 (Stoffel *et al.* 2017), was 0.82 (95% CI = 0.76-0.87). Intra-plate repeatability was 0.90 (95% CI = 0.86-0.93) and 0.84 (95% CI = 0.79-0.90) for IRBP and telomere Cq values, respectively, with an intra-plate repeatability of 0.87 (95% CI = 0.82-0.91; n=1248 samples; 34 plates) for RLTL measurements.

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Results S1 - summary of analyses excluding individuals aged based on tooth wear

The following summary results represent the exact same analyses, but without the 67 individuals whose age was determined based on tooth wear.

Individual repeatability was 0.014 (95% CI = 0.001 - 0.100) including cub and adult RLTL and 0.026 (95% CI = 0.001 - 0.158) with only RLTL measurements in adulthood (marginal $R^2 = 0.072$; $X^2 = 0.930$, P = 0.335). These repeatabilities changed to 0.020 (95% CI = 0.001 - 0.101) and 0.032 (95% CI = 0.001 - 0.156), respectively, when plate variance was removed. This did not differ from the estimates including the 67 individuals (0.017, 95% CI = 0.001 - 0.098, all samples; 0.026, 95% CI = 0.001 - 0.143, adult samples).

Telomere elongation was observed in 60.9% of within-individual changes (versus 61.2% including the 67 individuals). The random effect estimate for individual ID with technical replicates was 0.0369 (95% CI = 0.0320 – 0.0429), whereas for within-individual samples the random effect estimate was 0.0009 (95% CI = 0.0003 – 0.0045) without overlapping 95% credible intervals. For the group that increased in RLTL, the random effect estimate for technical replicates was 0.0417 (95% CI = 0.0345 – 0.0516) and for within-individual samples the estimate was 0.0012 (95% CI = 0.0002 – 0.0054). For individuals that exhibited decreases in RLTL the random effect estimate for technical replicates was 0.0392 (95% CI = 0.0317 – 0.0470) and for within-individual samples the estimate was 0.0014 (95% CI = 0.0003 – 0.0065), where neither group showed overlapping 95% credible intervals. Residual variance ($\sigma_{\varepsilon}^{-2}$) was smaller (0.041) than the overall change ($\sigma_{\varepsilon}^{'2}$) in RLTL (0.922; F_{31,40} = 22.48, P < 0.001). Removing the 67 individuals therefore did not alter our conclusions on telomere elongation.

Removing the 67 individuals also did not alter our conclusions on the relationship between RLTL and survival/lifespan: Early-life RLTL predicted lifespan (β = 0.132, 95% CI = 0.028 – 0.236; n = 424), while early-life RLTL also showed a positive relationship with survival to adulthood (β = 0.434, 95% CI = 0.132 – 0.734; n = 424). Early-life RLTL (β = 0.043, SE = 0.350, P = 0.900; n = 325) and corresponding RLTL (β = -0.028, 95% CI = -0.329 – 0.276; n = 663) did not predict adult survival probability.

The addition of individuals aged using tooth wear did therefore not alter our results or conclusions.

Results S2 – summary of analyses when first cohorts (1987 – 1992) are omitted

The following summary results represent the exact same analyses, but without the six (1987–1992) cohorts where early-life RLTL was lower and more variable than other cohorts.

Individual repeatability was 0.030 (95% CI = 0.001 – 0.123; including all samples) and 0.052 (95% CI = 0.001 – 0.206) with only RLTL measurements in adulthood, (marginal R^2 = 0.060; X^2 = 1.501, P = 0.220). These repeatabilities changed to 0.035 (95% CI = 0.001 – 0.126) and 0.063 (95% CI = 0.001 – 0.220), respectively, when plate variance was removed.

Telomere elongation was observed in 59.2% of within-individual changes. The random effect estimate for individual ID with technical replicates was 0.0340 (95% CI = 0.0288 - 0.0388), whereas for within-individual samples the random effect estimate was 0.0018 (95% CI = 0.0002 - 0.0044) without overlapping 95% credible intervals. For the group that increased in RLTL, the

random effect estimate for technical replicates was 0.0410 (95% CI = 0.0328 – 0.0515) and for within-individual samples the estimate was 0.0008 (95% CI = 0.0003 – 0.0055). For individuals that exhibited decreases in RLTL the random effect estimate for technical replicates was 0.0315 (95% CI = 0.0264 – 0.0413) and for within-individual samples the estimate was 0.0012 (95% CI = 0.0002 – 0.0059), where neither group without overlapping 95% credible intervals. Residual variance ($\sigma_{\varepsilon}^{-2}$) was smaller (0.038) than the overall change ($\sigma_{\varepsilon}^{'2}$) in RLTL (0.872; F_{20,27} = 23.01, P < 0.001).

Early-life RLTL predicted lifespan (β = 0.119, 95% CI = 0.019 – 0.219; n = 389), while early-life RLTL also showed a positive relationship with survival to adulthood (β = 0.430, 95% CI = 0.118 – 0.742; n = 389). Early-life RLTL (β = -0.125, SE = 0.369, P = 0.730; n = 299) and corresponding RLTL did not predict adult survival probability (β = -0.070, 95% CI = -0.363 – 0.221; n = 594).

The addition of individuals from cohorts 1987 - 1992 did not alter any results or conclusions.

Supplementary tables

Table S1: The different survival probabilities of cubs and adults, based on age at last capture and when correcting the lifespan model for the different survival probabilities between cubs and adults (+12 months last captured as cubs, +24 months for adults). Model averaged parameters of models. Σ = relative variable importance, β = direction and magnitude of effect, S.E. = standard error, 95% CI = 95% confidence interval; with reference terms in brackets = reference level for factors. Parameters where the 95% CI do not overlap zero are italicised and underlined.

Parameter (reference level)	Σ	β	S.E.	95% CI
Lifespan (corrected for differe	ntial survival	probability)		
Intercept		4.059	0.469	3.141 to 4.984
Early-life RLTL	<u>0.86</u>	<u>0.084</u>	<u>0.034</u>	<u>0.016 to 0.152</u>
Sex (female)	0.27	-0.031	0.065	-0.158 to 0.097
<u>Cohort</u>	<u>1.00</u>			
Lifespan (based on age of last	capture)			
Intercept		3.531	0.702	2.158 to 4.916
Early-life RLTL	<u>0.86</u>	<u>0.125</u>	<u>0.052</u>	0.024 to 0.227
Sex (female)	0.25	-0.030	0.097	-0.220 to 0.161
<u>Cohort</u>	<u>1.00</u>			

Table S2: Comparison of models describing the relationship between relative leukocyte telomere length and age, with a variety of age functions (0 = no age function, 1 = linear age function, 2 = log age function, 3 = quadratic age function, 4 = cubic age function, F = factorial age function, T1 = single threshold, T2 = double threshold, T3 = triple threshold) and cohort, including the interaction of cohort with age (cohort * age), with plate, year and individual ID as random effects and sex as a fixed effect. Models were ordered and numbered by AICc, and the difference from the top model (lowest AICc) is stated in the column termed ' Δ AICc', with models within Δ AICc <7 in bold.

Model	Function	Threshold 1	Threshold 2	Threshold 3	Cohort	Cohort*Age	Degrees of freedom	AICc	ΔΑΙС
1	T3	29	65	112	Yes	No	34	-1704.412	
2	T2	65	112		Yes	No	33	-1701.832	2.580
3	T2	29	65		Yes	No	33	-1695.173	9.239
4	T1	65			Yes	No	32	-1694.749	9.663
5	1				Yes	No	31	-1691.114	13.298
6	3				Yes	No	32	-1690.745	13.667
7	2				Yes	No	31	-1689.680	14.732
8	T1	112			Yes	No	32	-1689.574	14.838
9	T1	29			Yes	No	32	-1689.344	15.068
10	4				Yes	No	33	-1689.256	15.156
11	T3	29	65	112	No	No	11	-1688.943	15.469
12	T2	29	112		Yes	No	33	-1688.306	16.106
13	0				Yes	No	30	-1684.658	19.754
14	T2	65	112		No	No	10	-1682.605	21.807
15	T2	29	65		No	No	10	-1681.564	22.848
16	1				No	No	8	-1679.276	25.136
17	T1	112			No	No	9	-1679.217	25.195
18	T1	29			No	No	9	-1678.154	26.258
19	T1	65			No	No	9	-1678.106	26.306
20	0				No	No	7	-1678.082	26.330
21	2				No	No	8	-1677.796	26.616
22	T2	29	112		No	No	10	-1677.428	26.984
23	3				No	No	9	-1677.272	27.140
24	4				No	No	10	-1675.252	29.160
25	2				Yes	Yes	54	-1663.010	41.402
26	1				Yes	Yes	54	-1658.236	46.176
27	T2	65	112		Yes	Yes	74	-1656.381	48.031
28	T1	112			Yes	Yes	62	-1655.049	49.363
29	T1	65			Yes	Yes	67	-1649.490	54.922
30	T1	29			Yes	Yes	75	-1639.517	64.895
31	T3	29	65	112	Yes	Yes	95	-1635.637	68.775
32	T2	29	112		Yes	Yes	83	-1633.420	70.992
33	3				Yes	Yes	78	-1632.109	72.303
34	T2	29	65		Yes	Yes	88	-1630.728	73.684
35	4				Yes	Yes	102	-1623.823	80.589
36	F				Yes	No	118	-1620.230	84.182
37	F				No	No	95	-1609.840	94.572
38	F				Yes	Yes	380	-1219.151	485.261

Table S3: Model selection of factors linked to lifespan. Models retained for subsequent model averaging are in bold. $\sqrt{\ }$ = categorical term included in the model, Int = intercept (± SE), RLTL = relative leukocyte telomere length (± SE), d.f. = degrees of freedom, \triangle AlCc = change in Akaike Information Criterion (AlCc) relative to best supported model, ω = adjusted weight based on AlCc.

Model	Int	RLTL	Sex	Cohort	d.f.	ΔΑΙС	ω
1	3.534 ± 0.701	0.126 ± 0.052		√	24	0.00	0.641
2	3.547 ± 0.703	0.125 ± 0.052	✓	✓	25	2.16	0.218
3	3.491 ± 0.706			✓	23	3.63	0.105
4	3.506 ± 0.707		✓	✓	24	5.75	0.036
5	3.499 ± 0.088				4	43.02	0.000
6	3.497 ± 0.088	0.062 ± 0.053			5	43.68	0.000
7	3.522 ± 0.104		\checkmark		5	44.91	0.000
8	3.519 ± 0.105	0.062 ± 0.053	\checkmark		6	45.59	0.000

Table S4: Model averaged parameters. Σ = relative variable importance, β = direction and magnitude of effect, S.E. = standard error, 95 % CI = 95 % confidence interval; reference terms in brackets = reference level; for cohort effect estimates see Figure S2. * = interaction. Parameters where the 95% CI do not overlap zero are italicised and underlined.

Parameter (reference level)	Σ	β	S.E.	95% CI
Lifespan model [†]				
Intercept		3.531	0.702	2.158 to 4.916
<u>Early-life RLTL</u>	<u>0.86</u>	<u>0.125</u>	<u>0.052</u>	<u>0.024 to 0.227</u>
Sex (female)	0.25	-0.030	0.097	-0.220 to 0.161
<u>Cohort</u>	1.00			
Survival to adulthood model [‡]				
Intercept		0.053	1.478	-2.847 to 2.965
Early-life RLTL	<u>0.95</u>	<u>0.408</u>	<u>0.153</u>	0.108 to 0.708
Sex (female)	0.25	0.102	0.264	-0.417 to 0.622
<u>Cohort</u>	1.00			
Parameter (reference level)		β	S.E.	P-value
Annual adult survival model§				
Early-life RLTL		0.032	0.346	0.930
<u>Sex (female)</u>		<u>0.352</u>	<u>0.127</u>	<u>0.006</u>
Parameter (reference level)	Σ	β	S.E.	95% CI
Adult survival to following year mode	el¶			
Intercept		2.325	0.466	1.400 to 3.235
Adult RLTL	0.37	-0.026	0.135	-0.290 to 0.242
<u>Sex (female)</u>	<u>0.94</u>	<u>-0.664</u>	<u>0.280</u>	-1.214 to -0.114
Age (≤ 29 months)	0.28	0.084	0.160	-0.227 to 0.411
(>29 and ≤ 65 months)	0.31	-0.131	0.191	-0.528 to 0.246
<u>(>65 and ≤ 112 months)</u>	<u>0.99</u>	<u>-0.501</u>	<u>0.171</u>	<u>-0.836 to -0.158</u>
(> 112 months)	0.32	-0.089	0.125	-0.344 to 0.156
Age (≤ 29 months*RLTL)	0.01	-0.064	0.129	-0.320 to 0.225
(>29 and ≤ 65 months)*RLTL	0.03	-0.107	0.091	-0.357 to 0.133

(>65 and \leq 112 months)*RLTL	0.08	-0.070	0.115	-0.310 to 0.176
(> 112 months)*RLTL	0.04	0.130	0.143	-0.145 to 0.428

Model averaged random effect estimates (variance): † Observation (0.8730), Plate (4.330*10⁻¹⁵), Natal social group (0.1751); ‡ Plate (0.000), Natal social group (0.3193); § Cox mixed model random effect estimates: Cohort (0.2816), Plate (0.0481), Natal social group (0.3963); ¶ Individual ID (0.4620), Cohort (0.1415), Year (0.8757), Plate (0.2894), Natal social group (1.919*10⁻⁶), Current social group (1.2300).

Table S5: Model selection of factors linked to survival to adulthood. Models retained for subsequent model averaging are in bold. \checkmark = categorical term included in the model, Int = intercept (± SE), RLTL = relative leukocyte telomere length (± SE), d.f. = degrees of freedom, \triangle AICc = change in Akaike Information Criterion (AICc) relative to best supported model, ω = adjusted weight based on AICc.

Model	Int	RLTL	Sex	Cohort	d.f.	ΔΑΙС	ω
1	0.072 ± 1.476	0.407 ± 0.153		√	23	0.00	0.694
2	0.022 ± 1.484	0.410 ± 0.153	√	✓	24	2.09	0.244
3	-0.064 ± 1.471			✓	22	5.42	0.046
4	-0.099 ± 1.478		\checkmark	\checkmark	23	7.57	0.016
5	1.271 ± 0.140	0.254 ± 0.122			4	16.18	0.000
6	1.201 ± 0.185	0.256 ± 0.122	\checkmark		5	17.89	0.000
7	1.254 ± 0.138				3	18.62	0.000
8	1.189 ± 0.184		\checkmark		4	20.37	0.000

Table S6: Model selection of factors linked to adult survival probability. Models retained for subsequent model averaging are in bold. $\sqrt{}$ = categorical term included in the model, * interaction between two terms, Int = intercept (± SE), RLTL = relative leukocyte telomere length (± SE), Age parameters (± SE) refer to threshold model where Age 1 ≤ 29 months old, Age 2 > 29 months and ≤ 65 months old, Age 3 > 65 months and ≤ 112 months old and Age 4 > 112 months old, d.f. = degrees of freedom, ΔAICc = change in Akaike Information Criterion (AIC) relative to best supported model, ω = adjusted weight based on AICc. Table only includes models with ΔAICc ≤ 7 (not showing 159 models).

Model	Int	RLTL	Age 1	Age 2	Age 3	Age 4	Sex	Age 1*RLTL	Age 2*RLTL	Age 3*RLTL	Age 4*RLTL	d.f.	ΔΑΙСα	ω
1	2.342		-	-	-0.532	-	√	-		-	-	9	0.00	0.166
	±				±									
	0.461				0.157									
2	2.362				-0.488	-0.078	✓					10	1.62	0.074
	±				±	±								
	0.465				0.170	0.119								
3	2.348			-0.097	-0.489		✓					10	1.75	0.069
	±			±	±									
	0.464			0.176	0.175									
4	2.338		0.053		-0.537		✓					10	1.92	0.064
	±		±		±									
	0.459		0.143		0.157									
5	2.348	-0.034			-0.537		✓					10	1.99	0.061
	±	±			±									
	0.464	0.136			0.160									
6	2.341		0.141	-0.188	-0.459		✓					11	3.15	0.034
	±		±	±	±									
	0.459		0.174	0.209	0.176									
7	2.371			-0.114	-0.432	-0.088	✓					11	3.27	0.032
	±			±	±	±								

	0.469			0.178	0.191	0.121						
8	2.358		0.049		-0.494	-0.076	✓			11	3.56	0.028
	±		±		±	±						
	0.462		0.143		0.170	0.119						
9	2.366	-0.026			-0.493	-0.076	✓			11	3.64	0.027
	±	±			±	±						
	0.467	0.137			0.173	0.120						
10	2.362	-0.023			-0.531		✓	-0.059		11	3.72	0.026
	±	±			±			±				
	0.465	0.137			0.160			0.105				
11	2.354	-0.030		-0.094	-0.494		✓			11	3.76	0.025
	±	±		±	±							
	0.467	0.137		0.177	0.178							
12	2.344	-0.032	0.052		-0.542		✓			11	3.92	0.023
	±	±	±		±							
	0.461	0.135	0.143		0.159							
13	2.003				-0.504					8	4.16	0.021
	±				±							
	0.413				0.153							
14	2.364		0.146	-0.208	-0.399	-0.091	✓			12	4.64	0.016
	±		±	±	±	±						
	0.463		0.174	0.210	0.192	0.120						
15	2.349	-0.032			-0.488	-0.132	✓		0.104	12	5.00	0.014
	±	±			±	±			±			
	0.465	0.137			0.173	0.138			0.128			
16	2.345	-0.020	0.139	-0.185	-0.463		✓			12	5.20	0.012
	±	±	±	±	±							
	0.461	0.136	0.175	0.210	0.179							

17	2.374	-0.019		-0.112	-0.436	-0.087	✓					12	5.31	0.012
	±	±		±	±	±								
	0.470	0.138		0.179	0.195	0.122								
18	2.368	-0.017		-0.099	-0.485		✓			-0.062		12	5.48	0.011
	±	±		±	±					±				
	0.468	0.139		0.177	0.179					0.106				
19	2.063	-0.019		-0.085	-0.464		✓		-0.108			12	5.52	0.010
	±	±		±	±				±					
	0.001	0.001		0.001	0.001				0.001					
20	2.373	-0.019			-0.500	-0.062	✓			-0.045		12	5.54	0.010
	±	±			±	±				±				
	0.467	0.138			0.174	0.124				0.109				
21	2.362	-0.025	0.048		-0.500	-0.074	✓					12	5.59	0.010
	±	±	±		±	±								
	0.464	0.136	0.143		0.173	0.120								
22	2.358	-0.021	0.054		-0.535		\checkmark			-0.060		12	5.65	0.010
	±	±	±		±					±				
	0.463	0.137	0.143		0.159					0.105				
23	2.352	-0.035	0.052		-0.539		✓	-0.064				12	5.73	0.009
	±	±	±		±			±						
	0.463	0.136	0.143		0.159			0.129						
24	2.009		0.090		-0.514							9	5.79	0.009
	±		±		±									
	0.411		0.137		0.152									
25	2.019				-0.461	-0.076						9	5.79	0.009
	±				±	±								
	0.416				0.167	0.118								
26	2.352	-0.017			-0.487	-0.142	✓			-0.152	0.202	13	5.84	0.009
	±	±			±	±				±	±			

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	27	0.463 2.001 ±	0.137		-0.062 ±	0.173 -0.474 ±	0.139			0.139	0.157	9	6.07	0.008
29	28	2.009 ±	±		0.169	-0.509 ±						9	6.11	0.008
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	29	2.368 ±	-0.007 ±	±	±	-0.471 ±		√	±			13	6.45	0.007
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	30	2.356	-0.025	0.175	-0.111	-0.431		✓	0.122		±	13	6.68	0.006
32 2.391 -0.009	31	2.366	-0.009		-0.207	-0.401	-0.090	✓			0.128	13	6.70	0.006
33 2.360 -0.006 0.146 -0.194 -0.452	32	2.391	-0.009	0.175	-0.096	-0.453	-0.072	✓				13	6.75	0.006
34 2.264 -0.307 -0.218 ✓ 10 6.93 0 ± ± ±	33	2.360	-0.006		-0.194	-0.452	0.123	✓	0.126			13	6.86	0.005
	34	2.264	0.138	0.175	-0.307	0.179		✓		0.105		10	6.93	0.005
	35	±	±	±	0.153	±	±	✓			±	13	6.98	0.005

Supplementary figures

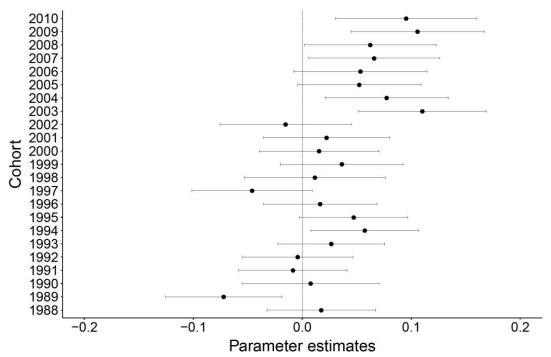


Figure S1: Parameter estimates (± SE) for cohort effects from the best-fitting age model (Table 1; main text), with telomere length across all ages as the response variable, relative to 1987.

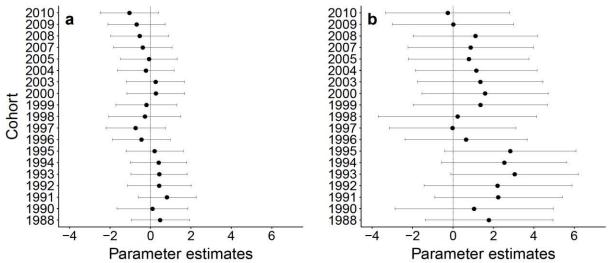


Figure S2: Parameter estimates and associated 95% confidence intervals for cohort effects (relative to 1987) in models of: a) Lifespan – early-life RLTL (relative leukocyte telomere length) and b) Survival to adulthood – early-life RLTL mixed models.