1 Electronic supplementary material

2

3 Electronic supplementary material S1

4 **Table S1. Comparison of the results of univariate modelling of the factors influencing IFNγ response magnitude using baseline corrected or non-corrected**
5 **values and including or excluding the ELISA bTB test**. Where: Int = Intercept; Δ Age = within-individual age term; Mean Age = between-individual age term;
6 bTB = bovine tuberculosis status ('-ve' = negative, '+ve' = positive, 'Ex' = Excretor); Cond = Condition; df= degrees of freedom; ΔAICc = deviation in AICc from
7 the best supported model; AW = Adjusted model weight for models included in the top model set; ✓ = factors level included in the model with their
8 corresponding contrasts adjacent to them; terms in bold denote the best supported model.

9

Int	Δ Age	Mean Age	Sex		bTB			Cond	df	ΔAICc	AW
			Female	Male	-ve	+ve	Ex				
Baseline corrected, ELISA excluded											
0.323	**-0.033**	**-0.017**	✓	**-0.025**	✓	**-0.013**	**-0.057**		**10**	**0.00**	**0.67**
0.358	-0.032	-0.017			✓	-0.014	-0.062	-0.006	10	3.19	0.13
0.319	-0.036	-0.018	✓	-0.027					8	3.82	0.10
0.311	-0.033	-0.016			✓	-0.014	-0.060		9	3.83	0.10
Baseline Corrected, ELISA included											
0.322	**-0.033**	**-0.017**	✓	**-0.025**	✓	**-0.007**	**-0.056**		**10**	**0.00**	**0.64**
0.319	-0.036	-0.018	✓	-0.027					8	2.84	0.15
0.356	-0.032	-0.016			✓	-0.009	-0.061	-0.006	10	3.34	0.12
0.309	-0.033	-0.016			✓	-0.008	-0.059		9	3.96	0.09
Non-Baseline corrected, ELISA Included											
0.335	**-0.033**	**-0.016**	✓	**-0.024**	✓	**-0.012**	**-0.055**		**10**	**0.00**	**0.65**
0.368	-0.032	-0.016			✓	-0.014	-0.060	-0.006	10	3.00	0.15
0.323	-0.033	-0.016			✓	-0.013	-0.058		9	3.60	0.11
0.331	-0.036	-0.017	✓	-0.026					8	3.73	0.10

10
11

12 ## Electronic supplementary material S2

13 *Complete methodology for absolute telomere length measurements*

14 Information taken from Beirne C, Delahay RJ, Hares M, Young A (2014) Age-Related Declines and
15 Disease-Associated Variation in Immune Cell Telomere Length in a Wild Mammal. *PloS one*, 9,
16 e108964.

17

18 *Sample acquisition*

19 Blood samples were collected from individually-marked badgers of known-age (ranging from
20 0.3 to 10.3 years) routinely trapped as part of a long-term study at Woodchester Park,
21 Gloucestershire, UK (see [1] for methods). A 4ml heparinised blood sample was obtained by
22 venipuncture from each captured badger and spun at 3000 rpm for 10 minutes within 30
23 minutes of the sample being taken. A 40µl aliquot of the resultant buffy coat (immune cell
24 layer) was stored at -80°C until DNA extraction. Buffy coat samples were gently thawed on ice
25 then DNA was extracted using Fermentas Whole Blood DNA extraction spin columns
26 according to the manufacturer's protocol. DNA was eluted in 100µl of low EDTA TE buffer and
27 stored at -20°C until qPCR analysis. DNA was successfully extracted from 361 buffy coat
28 samples collected from 173 badgers captured and sampled on 1-7 (median=2) separate
29 occasions between May 2012 and October 2013. Average DNA yield (ng/ul) was 57.1 (SD±29)
30 and average DNA purity (A_{260}/A_{280}) was 1.83 (SD±0.16). DNA integrity was validated by
31 electrophoresis on a 0.7% agarose gel. No evidence of sample degradation was detected. All
32 work was approved by the Food and Environment Research Agency Ethical Review Committee
33 and carried out under licence granted by the Home Office under the 1986 Animal (Scientific
34 Procedures) Act.

35 *Relative telomere length qPCR method*

36 In order to measure mean immune cell telomeres in the European badger we used a
37 quantitative PCR (qPCR) approach. This assay has advantages and disadvantages over other
38 methods available which are reviewed comprehensively in [2–5] and are not discussed
39 further. Briefly, two sets of primers are designed: one to target repeat sequences associated
40 with telomeric regions (TTAGGG) and a second to a non-variable copy number control gene
41 (we used inter-photoreceptor retinoid-binding protein (IRBP): Ascension number AB082979).
42 Target sequences are amplified using realtime qPCR in the presence of either a fluorescent
43 nonspecific intercalating dye (used here) or a sequence specific fluorescent reporter
44 molecule. Relative telomere length is calculated as the ratio of fluorescence from the
45 telomeric amplicon compared to that from the non-variable copy gene standardised to a
46 common sample ('gold sample') run on all plates (see equations below).

47 *Primers*

48 During assay development seven primer pairs targeting four control genes were designed
49 from *Meles meles* sequences available in the GenBank database: Inter-photoreceptor
50 retinoid-binding protein (IRBP), Actin alpha cardiac muscle protein (ACTC), Transthyretin
51 protein (TTR) and cystic fibrosis transmembrane conductance regulator (CFTR). Primer pairs
52 which showed non-specific amplification or primer-dimer formation were discarded. A primer
53 pair targeting the IRBP region was chosen (see Table S2A.) owing to its superior performance
54 and previous use in telomere assays [6] and phylogenetic work [7] (owing to it being non-
55 variable in copy number).

56 *Plate setup*

57 High-purity salt-free primers were synthesised by Eurofins (see Table S2A), diluted and stored
58 at -20°C until use. Non-skirted 96-well Polypropylene qPCR plates were loaded manually and
59 sealed with Mx3000P/Mx3005P Optical Strip Caps (Agilent). Control gene (IRBP) and telomere

60 reactions were run on separate plates owing to differing optimal reaction temperatures (data
61 not shown). Telomere and IRBP primer concentrations were optimised to 400nM and 200nM
62 respectively. All reactions were run in triplicate (technical replicates) and averaged prior to
63 analysis. In order to account for differences in amplification efficiencies between different
64 plates, a standard curve was run on all plates comprising 1:2 serial dilutions of a pool of DNA
65 from 10 individuals of unknown age (resulting in total DNA concentrations of 20, 10, 5, 2.5
66 and 1.25ng). In order to calculate a relative telomere length value for all samples, the 5ng
67 dilution of the standard curve was used as the 'golden sample' to which all other samples
68 were compared. See Table S2B for a schematic representation of the plate setup.
69
70 Individuals were randomly allocated to qPCR plates (all samples from a given individual were
71 run on the same plate in order remove the impact of inter-plate variation on within-individual
72 telomere length comparisons). The final reaction volume was 20ul containing 10ul of Brilliant
73 II SYBR® Green Low ROX QPCR Master Mix (Agilent), 4ul nuclease free water (Fisher), 1ul each
74 of forward and reverse primers (see Table S2A) and 4ul of 1.25ng/ul DNA sample (or 4ul
75 nuclease free water for the no template control). Reactions were run on the Stratagene
76 Mx3000P qPCR system using a two-step reaction profile (Control Gene: 10 mins at 95°C,
77 followed by 40 cycles of 30s at 95°C and 1min at 60°C, Telomere: 10 mins at 95°C, followed
78 by 40 cycles of 30s at 95°C and 1min at 56°C). Fluorescence was recorded at the end of the
79 low temperature annealing/extension step. LinRegPCR (v2013.0) was used to correct for
80 baseline fluorescence and determine the window of linearity per amplicon. The threshold
81 values (Nq) were set at the centre of the window in linearity for each amplicon (Nq = 0.22 and
82 0.17 (log fluorescence units) for IRBP and telomere reactions respectively). Threshold cycle
83 values (Cq) for each sample were then determined as the cycle at which the amplification plot

84 crossed the Nq. Primer specificity was confirmed through melt curve analysis (see Figure S2A)
85 and observation of a single band of the expected size after electrophoresis on a 3% agarose
86 gel.

87 *Calculations*

88 Cq values were plotted against log concentration in order to determine the amplification
89 efficiency of both IRBP and Telomere primers for each plate pair run. Across all plates, the
90 mean standard curve amplification efficiencies were 99.9% (SE ± 1.5) for IRBP primers and
91 99.2% (SE ± 0.9) for the telomere primers. The R^2 for each standard curve was >0.99.

92 First, two initial starting quantities (X_0) were calculated for each sample, one from its telomere
93 plate (X_0 TEL) and one from its IRBP plate (X_0 IRBP). To reconcile for amplification efficiency
94 differences between plate runs we used the following equation:

95 $X_0 = 10^{(Cq-b)/m}$

96 Where Cq = Cycle at which the focal sample crosses the threshold (Nq), b = plate specific
97 intercept of the log of the standard curve and m = plate specific slope of the log of the
98 standard curve.

99 The amount of telomere in the focal sample was then normalised to the initial quantity of
100 DNA in the sample by calculating:

101 X_0 sample = X_0 TEL / X_0 IRBP

102 Finally, relative telomere length (RTL) was calculated by normalising the focal sample to the
103 golden sample:

104 RTL = X_0 sample / X_0 golden sample

105 *Repeatability*

106 Amplicon specific within-plate variability was determined by examining the standard
107 deviation of the triplicate Cq values for each sample across each plate. The median and inter-

108 quartile range of the standard deviations across all samples (n=361) was 0.054 (0.036-0.082)
109 for the IRBP primers and 0.097 (0.059- 0.14) for the telomere primers. In order to determine
110 between-plate repeatability, 21 randomly selected samples (a single plate) were each run
111 three times (each run once for telomere and once for IRBP, totalling 6 plates). The coefficient
112 of variation in the relative telomere length estimates across all samples was 7.5%.

113

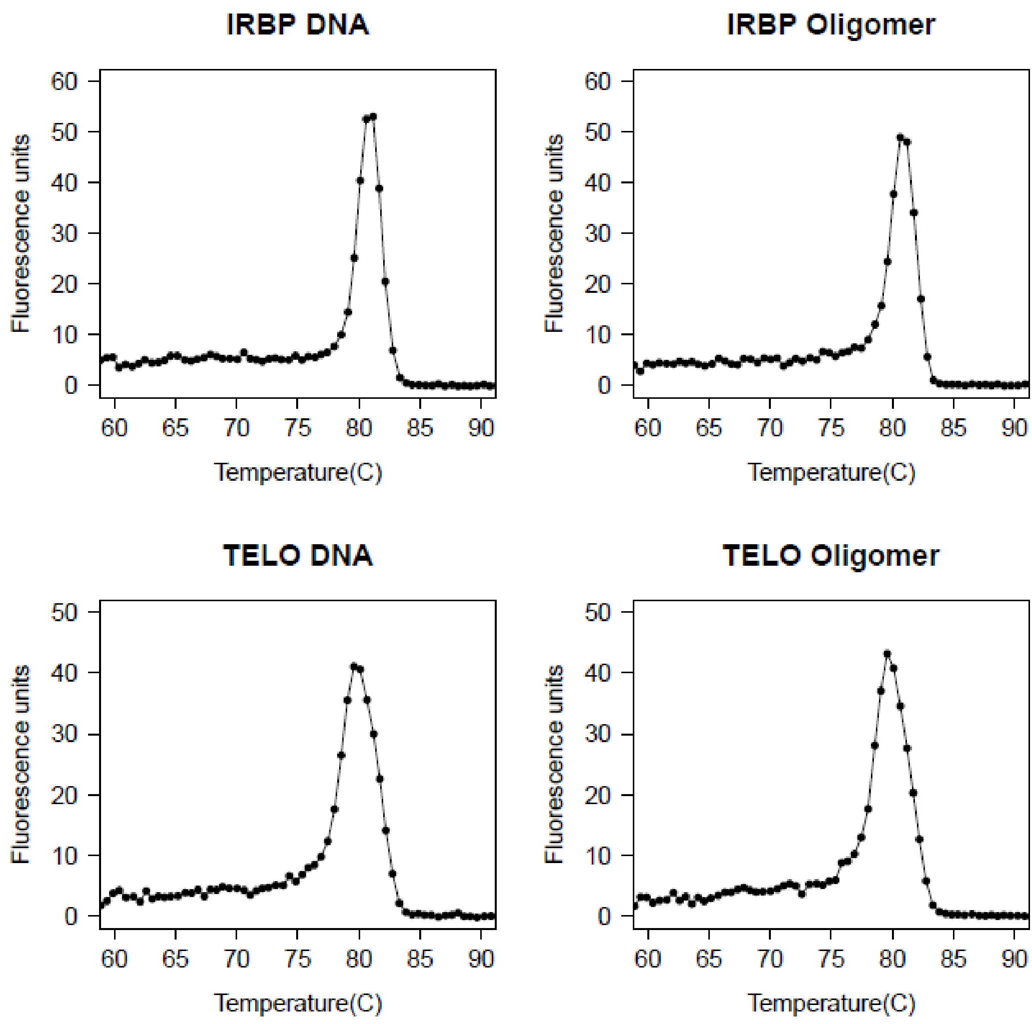
114 *Absolute Telomere Length Estimation*

115 The 21 samples selected for between-plate repeatability analysis (see above) were also
116 subjected to the absolute telomere estimation method described by O'Callaghan & Fenech
117 (2011). This method allows the calibration of relative telomere length estimates obtained
118 using qPCR to known quantities of synthetic telomere and control gene oligomers (see Table
119 S2A for sequences used here). In addition to the standard curve required for the relative
120 method (see above) we included ten-fold dilutions of known concentrations of each synthetic
121 oligomer on the same plate. Determining where each DNA sample crosses the synthetic
122 standard curve for each amplicon can be used in order to determine the absolute quantity of
123 telomere in each sample. Melt-curve analysis showed that primer products from both DNA
124 and oligomer templates were specific (single peak) and had the same melt temperatures
125 (Figure S2A). As synthetic oligomers may have different amplification efficiencies to
126 biologically extracted DNA (leading to bias in absolute telomere quantities), we calculated
127 absolute starting quantities for each amplicon (A_0: kb for telomere amplicon and diploid
128 genomes for IRBP amplicon) whilst reconciling for differences in amplification efficiency
129 between synthetic oligomers and extracted DNA samples as follows:

130 $$A_0 = {E_{DNA}}^{b_{Oligo} \log_{E_{DNA}}(E_{Oligo}) - Cq_{sample}}$$

131 Where DNA = Biological extracted DNA, Oligo = synthetic oligomer, Cq = Cycle at which the
132 focal sample crosses the threshold (Nq), b = intercept of the log of the standard curve, E =
133 Efficiency of standard curve $10^{(1/-m)}$ and m = slope of the log of the standard curve.
134 We then standardised the absolute amount of telomere to the number of diploid genomes
135 contained in the sample as follows:
136 Absolute TL = A_0 TEL / A_0 IRBP
137
138 *Relative to Absolute Conversion*
139 The estimates derived from the relative and absolute methods for these 21 samples were
140 linearly related and highly correlated (R^2 >0.99, Figure S2B). Given that badgers have 22
141 chromosome pairs (44 chromosomes = 88 telomeres) and that the IRBP is a single copy gene
142 [7], it was possible define an equation for the conversion of the relative telomere lengths into
143 absolute telomere length per chromosome end:
144 Absolute TL Estimate (Kb) = (8.5 + 932.9*(RTL Estimate))/88
145 We estimated the average immune cell telomere length in our population of European
146 badgers to be ~10kb, which is similar to immune cell telomere length estimates in humans
147 (~15kb in young individuals [9]). It is important to note that the average immune cell telomere
148 length estimate quoted here must be treated with caution. This estimate has not been
149 validated a secondary direct methodology (such as TRF) [2,4].

150 Figure S2A. Shows the melt curve analysis of both DNA and oligomer templates.



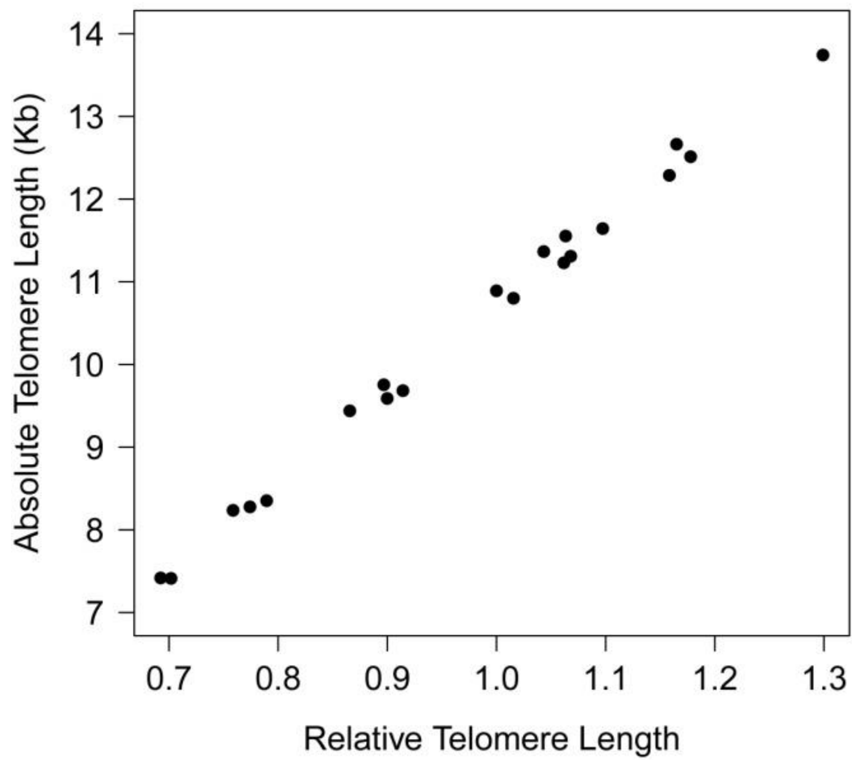
151

152

153 Figure S2B. shows the correlation between absolute telomere length estimates and relative

154 telomere length estimates for the 21 randomly selected samples.

155



156

157 **Table S2A.** Details the primer and oligonucleotide sequences used in the qPCR experiment.

Name	Target	Sequence
Tel1b	Telomeric Region	5’-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3’
Tel2b	Telomeric Region	5’-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3’
IRBP-F	Inter-photoreceptor retinoid-binding protein (Ascension# AB082979)	5’-GCCACATTTCTGGTATCCCCT-3’
IRBP-R	Inter-photoreceptor retinoid-binding protein (Ascension# AB082979)	5’-GGGCGGTCGTAGATGGTATC-3’
Oligo-IRBP	NA	GCCACATTTCTGGTATCCCCTACTTCATCTCCTACCTGCACC CAGGGAACACAGTCCTGCACGTGGATACCATCTACGACCG CCC
Oligo-Telo	NA	$(TTAGGG)_{14}$

158

159

160 **Table S2B.** Shows a schematic of the standardised plate qPCR plate set-up. All numbers refer
161 to the replicate of an individual sample, NTC = No Template Control, GS = Gold Sample,
162 numbers preceded by S denote standard curve dilutions whereby: S1 = 20ng, S2 = 10ng, S3 =
163 5ng, S4 = 2.5ng, and S5 = 1.25ng. For the absolute qPCR telomere length estimation and inter-
164 plate variation experiment, samples 3-7 were replaced by one in ten dilutions of known
165 concentrations of synthesised oligomers (see table S2A).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S1	S1	3	3	3	11	11	11	19	19	19
B	S2	S2	S2	4	4	4	12	12	12	20	20	20
C	S3/GS	S3/GS	S3/GS	5	5	5	13	13	13	21	21	21
D	S4	S4	S4	6	6	6	14	14	14	22	22	22
E	S5	S5	S5	7	7	7	15	15	15	23	23	23
F	1	1	1	8	8	8	16	16	16	24	24	24
G	2	2	2	9	9	9	17	17	17	25	25	25
H	NTC	NTC	NTC	10	10	10	18	18	18	26	26	26

166

167

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195

196

197 ## Electronic supplementary material S3

198 **Table S3. – Full unabridged univariate model selection table**. Where: Int = Intercept; bTB = Current
199 bovine tuberculosis status; Cond = Condition (scaled mass index); df = Degrees of freedom; AICc =
200 Akaike's Information Criterion corrected for small sample size; ΔAICc = deviation in AICc from the best
201 supported model; W = Model weight; AW = Adjusted model weight after exclusion of models with
202 ΔAICc < 6 from the top model and models with a simpler, nested version with more model weight
203 (support); the grey area denotes the models included in the top set; ✓ = terms included in the model;
204 and * = interactions.

Int	Δ Age	Mean Age	Sex	bTB	Cond	Δ Age * Mean Age	Δ Age * Sex	Δ Age *bTB	df	logLik	AICc	ΔAICc	W	AW
✓	**✓**	**✓**	**✓**	**✓**					**10**	**737.4**	**-1454.5**	**0.00**	**0.24**	**0.67**
✓	✓	✓	✓	✓	✓				11	738.3	-1454.4	0.12	0.23	0.00
✓	✓	✓	✓	✓		✓			11	737.5	-1452.7	1.76	0.10	0.00
✓	✓	✓	✓	✓	✓	✓			12	738.5	-1452.7	1.82	0.10	0.00
✓	**✓**	**✓**		**✓**	**✓**				**10**	**735.8**	**-1451.3**	**3.19**	**0.05**	**0.14**
✓	**✓**	**✓**	**✓**						**8**	**733.4**	**-1450.7**	**3.82**	**0.04**	**0.10**
✓	**✓**	**✓**		**✓**					**9**	**734.4**	**-1450.7**	**3.83**	**0.04**	**0.10**
✓	✓	✓	✓	✓			✓	✓	13	738.3	-1450.3	4.21	0.03	0.00
✓	✓	✓	✓	✓	✓		✓	✓	14	739.3	-1450.2	4.35	0.03	0.00
✓	✓	✓	✓		✓				9	734.1	-1450.0	4.47	0.03	0.00
✓	✓	✓		✓	✓	✓			11	736.0	-1449.6	4.86	0.02	0.00
✓	✓	✓	✓			✓			9	733.6	-1449.1	5.40	0.02	0.00
✓	✓	✓		✓	✓			✓	12	736.7	-1449.0	5.48	0.02	0.00
✓	✓	✓		✓		✓			10	734.6	-1448.9	5.57	0.02	0.00
✓	✓	✓	✓				✓		9	733.4	-1448.7	5.82	0.01	0.00
✓	✓	✓	✓		✓	✓			10	734.4	-1448.5	5.97	0.01	0.00
✓	✓	✓		✓				✓	11	735.4	-1448.4	6.07	0.01	0.00
✓	✓	✓	✓		✓		✓		10	734.1	-1448.0	6.50	0.01	0.00
✓	✓	✓			✓				8	731.1	-1446.1	8.44	0.00	0.00
✓	✓	✓							7	730.1	-1446.0	8.48	0.00	0.00
✓	✓	✓							7	730.1	-1446.0	8.48	0.00	0.00
✓	✓	✓				✓			8	730.3	-1444.5	10.02	0.00	0.00
✓			✓	✓	✓				9	703.6	-1389.1	65.40	0.00	0.00
✓				✓	✓				8	702.4	-1388.6	65.93	0.00	0.00
✓			✓	✓					8	702.2	-1388.2	66.30	0.00	0.00
✓				✓					7	700.6	-1387.1	67.44	0.00	0.00
✓			✓		✓				7	693.2	-1372.4	82.14	0.00	0.00
✓			✓						6	692.2	-1372.3	82.20	0.00	0.00
✓					✓				6	691.5	-1370.8	83.65	0.00	0.00
✓									5	690.1	-1370.2	84.29	0.00	0.00

206

Electronic supplementary material S4

207 **Table S4. Model selection table output after exclusion of all known infected individuals (those classed as 'exposed' or 'excretor').** Where: Int = Intercept;
208 Cond = Condition (scaled mass index); df = Degrees of freedom; AICc = Akaike's Information Criterion corrected for small sample size; ΔAICc = deviation in
209 AICc from the best supported model; W = Model weight; AW = Adjusted model weight after exclusion of models with ΔAICc<6 from the top model and models
210 with a simpler, nested version with more model weight (support); ✓ = terms included in the model; * = interactions.

211

Int	Δ Age	Mean Age	Sex	Cond	Δ Age * Mean Age	Δ Age * Sex	df	logLik	AICc	ΔAICc	W	AW
0.32	**-0.04**	**-0.02**	**✓**				**8**	**733.4**	**-1450.7**	**0.00**	**0.29**	**0.84**
0.35	-0.04	-0.02	✓	0.00			9	734.1	-1450.0	0.64	0.21	0.00
0.32	-0.04	-0.02	✓		0.00		9	733.6	-1449.1	1.58	0.13	0.00
0.32	-0.03	-0.02	✓			✓	9	733.4	-1448.7	1.99	0.11	0.00
0.35	-0.04	-0.02	✓	0.00	0.00		10	734.4	-1448.5	2.15	0.10	0.00
0.35	-0.03	-0.02	✓	0.00		✓	10	734.1	-1448.0	2.67	0.08	0.00
0.35	**-0.04**	**-0.02**		**-0.01**			**8**	**731.1**	**-1446.1**	**4.62**	**0.03**	**0.08**
0.31	**-0.04**	**-0.02**					**7**	**730.1**	**-1446.0**	**4.65**	**0.03**	**0.08**
0.31	-0.04	-0.02			0.00		8	730.3	-1444.5	6.19	0.01	0.00
0.31			✓	-0.01			7	693.2	-1372.4	78.32	0.00	0.00
0.27			✓				6	692.2	-1372.3	78.37	0.00	0.00
0.31				-0.01			6	691.5	-1370.8	79.83	0.00	0.00
0.26							5	690.1	-1370.2	80.47	0.00	0.00

212

213 ## Electronic supplementary material S5

214 **Table S5. The unabridged model output from the multivariate modelling process.** The table shows
215 relevant information for the random effects, fixed effects and the within-and among-individual
216 posterior correlations for the two traits. Where: G-structure = covariance between the random
217 effects; R-structure = co-variance of the residuals; Posterior Correlation = denotes the correlation
218 within- and among-individuals between the two traits; and CI = 'Credibility Interval'.

219

220

Random Effects

G-structure	Mean	Lower 95% CI	Upper 95% CI
Telomere Variance	0.857	0.534	1.202
Telomere/IFNγ Covariance	0.004	-0.012	0.022
IFNγ Variance	0.012	0.009	0.014
Plate (Telomere)	0.857	0.248	1.751
Plate (IFN)	0.005	0.004	0.007

R-structure:	Mean	Lower 95% CI	Upper 95% CI
Telomere Variance	1.217	0.977	1.473
Telomere/IFNγ Covariance	0.002	-0.011	0.015
IFNγ Variance	0.009	0.008	0.010

Fixed Effects

	Mean	Lower 95% CI	Upper 95% CI
Intercept (IFNγ)	0.267	0.242	0.294
Sex (IFNγ: Female)	0.022	-0.010	0.047
bTB(IFNγ: Exposed)	-0.029	-0.050	-0.005
bTB(IFNγ: Excretor)	-0.092	-0.138	0.046
Intercept (Telomere)	9.791	9.21	10.349
bTB (Telomere: Exposed)	-0.159	-0.022	0.539
bTB(Telomere: Excretor)	-0.670	-1.447	-0.020

Posterior Correlation

	Mean	Lower 95% CI	Upper 95% CI
Within: Telomere/IFNγ	-0.021	-0.101	0.149
Among: Telomere/IFNγ	0.015	-0.143	0.203

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