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1 **Age-related response to an acute innate immune challenge in mice: proteomics reveals a**
2 **telomere maintenance-related cost.**

3

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16

17 **Abstract**

18 Ageing is characterized by the impairment of the acute innate immune response and the up-regulation
19 of low-grade inflammation, i.e. inflammaging. At the cellular level, telomeres are considered as a
20 marker of biological ageing as their length is progressively eroded in the absence of repair mechanisms.
21 However, the link between telomeres and inflammaging remains underexplored. We aimed to identify
22 proteins that are differentially expressed between age classes in response to an acute inflammatory
23 challenge. We challenged young (2 months) and old (12 months) C57BL/6 mice using bacterial
24 lipopolysaccharide (LPS) and measured telomere length and proteomic profiles in splenocytes. 233 out
25 of the 1966 proteins we quantified differed among experimental groups. A hierarchical clustering
26 analysis revealed that nine of those 233 proteins highly affected experimental groups differentiations.
27 Young mice responded to LPS by increasing the expression of proteins involved in the innate immune
28 response, and interestingly, in telomere length maintenance. However, this regulation was impaired
29 at older ages. These results are in accord with the assumption that the strength of selection declines
30 with age, potentially explaining the maintenance of costly, dysregulated, immune responses at old age.
31 We establish that the immune response is competing with telomere maintenance process, highlighting
32 how telomeres reflect quality of ageing even in a species where telomere length is not related to
33 lifespan.

34

35 **Introduction**

36 The immune system is usually depicted as a two edged sword, with conferring benefits but also
37 generating costs. While the benefits of immune protection are obvious [1], the immune response has
38 been shown to incur energy-dependent and independent costs. Constitutive and induced immune
39 responses draw host resources away from other physiological functions and this can generate trade-
40 offs between immunity and life-history traits such as growth or reproduction [2, 3]. In addition to these
41 resource-based trade-offs, immunity can incur costs if immune effectors target host tissues and organs
42 [4]. These costs are referred to as immunopathology. Although immune-mediated diseases have been
43 identified for quite a long time, the importance of immunopathology in the context of the ecology and
44 evolution of host-parasite interactions has emerged as a new concept during the last two decades [5].
45 The risk of immune-induced damage is particularly strong during the process of acute and chronic
46 inflammation. Broadly speaking, infection induces the activation of innate immunity effectors within
47 minutes to hours [6]. Such rapid response and the associated local inflammation then favours
48 mounting of the adaptive immune response (*i.e.* via lymphocyte recruitment, [7]) within days and
49 weeks. Innate and adaptive responses not only differ in terms of rapidity, but also in terms of
50 specificity. While adaptive response is highly specific, innate immunity is essentially based on the
51 recognition of conserved pathogen molecular patterns such as structural components of the bacterial
52 wall (lipopolysaccharide in gram-negative bacteria or peptidoglycan in gram-positive bacteria), by
53 pattern recognition receptors (such as Toll-like receptors) and the induction of cytotoxic effectors
54 (oxidative burst) [7, 8]. By their inherent function/nature, these effectors cannot discriminate invading
55 pathogens from host cells and tissues and therefore can induce substantial damage if they get out from
56 control [9].

57 Failure in the resolution of acute and chronic inflammation can induce life-threatening
58 diseases [10, 11]. Indeed, inflammation and activation of oxidative attacks on intruders may have many
59 collateral negative impacts on the host itself, including ultimate costs on life history traits [12]. Ageing
60 has been shown to be associated with the up-regulation of low grade inflammation, *i.e.* inflammaging

61 [13, 14], which makes elderly more susceptible to many degenerative age-associated pathologies such
62 as cancer, diabetes, cardio-vascular diseases [15], and ultimately impairs lifespan of individuals. Old
63 individuals have also been shown to pay disproportionately high costs of inflammatory activation when
64 regulatory mechanisms are experimentally broken-down, suggesting that ageing individuals are more
65 prone to immunopathology [16]. In a companion study, we notably reported that LPS-induced
66 oxidative stress in the liver induces immunopathology costs in old mice [17]. Accumulation of
67 senescent cells in old organisms may be one of the mechanisms leading to chronic inflammation [18].

68 Telomeres, which are repeated DNA sequences that protect chromosome extremity, are one
69 of the structures sensitive to the mechanisms involved in the inflammatory process. Telomeres. The
70 length of these repeated motifs are supposed to reflect biological age, since cellular replication and
71 stress erodes them (although mechanisms that restore telomere caps exist) [19]. Telomere length is
72 thus used as a marker of cell senescence [20] as well as the whole organism's survival [21-24].
73 Telomeres have been previously linked with the immune cell response to pathogens [25]. They are
74 particularly susceptible to erosion during infection and the associated inflammatory response. This was
75 shown for instance in a wild passerine, where a mild malaria infection over life induces an accelerated
76 erosion of blood cell telomere, resulting in a subsequent significant reduction in lifetime reproduction
77 output and adult lifespan [26]. It is therefore possible that up-regulated chronic inflammation at old
78 age (inflammaging) and the lower ability of old individuals to cope with acute inflammatory responses
79 are accelerating factors promoting telomere attrition [27], leading to a vicious circle between telomere
80 attrition, emergence of senescent cells and inflammaging.

81 The aim of this study was twofold. First, we wished to explore if an acute inflammatory
82 challenge induces age-dependent variation in protein expression in an immune organ, the spleen,
83 using a quantitative proteomic approach. Second, we investigated whether telomere length differed
84 with age and whether the proteomic profile activated by the immune challenge could reveal
85 mechanisms involved in telomere attrition. In doing so, we wanted to test if the cost of an immune
86 challenge is mirrored in telomere malfunction even in a species where telomerase is active at adulthood.

87

88 **Materials and methods**

89 *Ethical statement*

90 The present experiment has been conducted under the permit n° AL/05/34/12/12 delivered
91 by the 'Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg'. Animals
92 were maintained in captivity within the IPHC-DEPE animal husbandry (French Department of
93 Veterinary Services n° E67-482-18) and under standardized rearing conditions previously indicated in
94 [17]. F. Criscuolo is the holder of an animal experimentation licence granted by French authorities (no.
95 67-78).

96 *Experimental design*

97 The experiment was conducted on 26 C57BL/6J male mice from our own colony and originated
98 from Elevage-Janvier laboratory [12 mice were 2 months old (hereafter young), and 14 mice were 12
99 months old (hereafter old)], reared in age-separated cages of 6-7 animals in our laboratory under
100 constant temperature (24 ± 2 °C), humidity (50-55%) and photoperiod (13:11 L:D cycle) and fed *ad*
101 *libitum* on a standard diet (SAFE A03). Mice in each age class were randomly allocated to the two
102 experimental groups: 6 young and 8 old mice received an intraperitoneal injection of 25 µg/kg body
103 mass of lipopolysaccharide [LPS; *Escherichia coli*, Sigma Aldrich, a non-lethal dose based on a previous
104 study [17]], and the other mice were kept as controls and injected with sterile phosphate buffered
105 solution (PBS). LPS is a TLR4 ligand that stimulates neutrophils and macrophages, induces the release
106 of pro-inflammatory cytokines (IL-6, IFN- γ , TNF- α , IL-1 β), cytotoxic reactive oxygen and reactive
107 nitrogen species (ROS, RNS) (e.g. [28]). As previously observed, the LPS-injected mice stopped eating
108 and remained motionless for up-to 24h after injection, after what they started gaining body mass [17]
109 One old mouse died in the LPS group, reducing the sample size of this group to seven.

110 Twenty-four hours post-challenge, all mice were euthanized by cervical dislocation and two
111 aliquots of the spleen were immediately snap-frozen in liquid nitrogen before storage at -80°C, until
112 telomere length measurements by qPCR, proteomic and Western blot analyses (Methodological

113 details are available in the electronic supplementary material, ESM 1). Body mass (± 0.1 g) was also
114 measured just before the intraperitoneal injection and 24h post-challenge (ESM3). We chose this
115 short-term measurements (and age classes) based on previous LPS-injection experiments conducted
116 on the same mouse line [17] showing that body mass loss and oxidative costs was maximal at that time
117 (i.e. the putative cost being easier to measure at the telomere level) and will not be compensated by
118 the triggering of telomerase activity during recovery.

119 *Statistical analyses*

120 We selected a three-step analysis for the proteomic and molecular data obtained on spleen
121 tissues. As we did in previous studies [17, 29], we first sorted the significant effects of age (young and
122 old) and treatment (PBS and LPS) as fixed factors using ANOVA for each of the 1966 quantified proteins
123 (Electronic Supplementary Material, Table S2). We thus selected the 233 proteins for which i) ANOVA
124 p-values were below 0.05, and ii) the post-hoc Tukey test p-value (with Bonferroni correction) was
125 below 0.05 and the absolute fold change was > 1.5 fold for at least one pairwise comparison. In a
126 second step, we used this subsample to run a hierarchical clustering on principal component analysis
127 (PCA) to obtain a dendrogram that assembles proteins based on similarities of variation according to
128 experimental groups [30]. This second step was done to further reduce the number of variables to
129 avoid multicollinearity among a too large number of variables. We chose to set-up the hierarchical
130 clustering analysis with four clusters, based on inertia gain choice (4 clusters explained a total of 82%
131 of the total variance while 3 clusters explained 74%), and because two of these clusters (3 and 4)
132 explained a large fraction of the total variance in both axes (81.1% and 6.1%, respectively figure 1).
133 These clusters (3 and 4) encompassed 9 proteins. The final step of analysis consisted of merging the
134 proteomic variables so far selected (9 proteins) and the physiological and molecular data (body mass
135 and telomere length) to run a Principal Component Analysis (PCA) with varimax rotation (determinant
136 of 0.001). PCA resulted in three axes (PCA1, PCA2 and PCA3) explaining 36.2%, 24.4% and 13.9% of the
137 variance, respectively (total 74.5%). Kaiser-Meyer-Olkin (KMO) value was of 0.66 and Bartlett's test of
138 sphericity confirmed an adequate correlation among variables for PCA (χ^2 (55) = 140.9, $P < 0.001$). Given

139 the number of variables, only components of the axis with an eigenvalue over 0.4 were retained
140 (Kaiser's criterion). PCA scores of each individual were then used as the dependent variable in a
141 Generalized Linear Model (GLM) with age (young vs. old), immune challenge (LPS vs. PBS) and their
142 interaction. To confirm the significance of the relationship between individual telomere length and the
143 protein signals, we chose to run an additional analysis on the raw values (not on the PCA values). To
144 do so, we finally investigated the association between telomere length and the 9 proteins (among
145 which some were previously known to be involved in telomere dynamics, *e.g.* chitinase-like protein 3)
146 using a model selection after testing for absence of multicollinearity using variance inflation factor
147 (minimum Tolerance index > 0.19). For western-blot analysis of shelterin proteins, we performed one-
148 way ANOVA with Tukey posthoc tests. Adjustment of p values was done according to Bonferroni-Holm
149 ($p < 0.05$), after checking for normality distribution (Shapiro-Wilk test) and homoscedasticity (Bartlett
150 test). All statistics were run on the program R using RStudio (version 1.1.414). Hierarchical clustering
151 and PCA were conducted using FactoMineR R package [31]. We used the dredge function of the MuMIn
152 package (version 1.15.1) to run model selection. Normality of the residuals was checked using QQ plots
153 and Kolmogorow-Smirnov tests. Significance threshold used is $P \leq 0.05$.

154

155 **Results**

156 The proteomics analysis yielded the robust identification of 2788 proteins, of which 1966
157 fulfilled the criteria for quantification (ESM 2, table S2). A total of 285 proteins were differentially
158 expressed as they exhibited ANOVA p-values below 0.05, and this number dropped to 233 when
159 considering Tukey p-values below 0.05 and absolute fold changes above 1.5 for at least one pairwise
160 comparison. Hierarchical clustering pointed to nine of these proteins as major contributors belonging
161 to two different clusters (3 and 4) that explained most of the variation on both axes of the dendrogram
162 (figure 1A) and which are well characterizing the young PBS and young LPS groups (figure 1B). To test
163 how these proteomic data, once added to biological variables, vary with age and treatment, we used

164 these nine proteins, changes in body mass during the 24h post-challenge and telomere length to run
165 a principal component analysis. The contribution of the different variables to the three principal
166 components (PCA1, 2 and 3) are reported in table 1. PCA1 (36.2% of variance explained) positively
167 loaded with protein S100-A9, lysozyme C-2, and annexin A1; PCA2 (24.4% of variance explained) had
168 positive loads with coronin 1A and RAS-C3, and negative loads with endoplasmin HSP90b1, GRP-78,
169 carbonic-anhydrase 1, and changes in body mass; finally PCA3 (13.9% of variance explained) positively
170 loaded with endoplasmin HSP90b1, GRP-78 and chitinase-like protein 3, and negatively with telomere
171 length. Based on Gene Ontology annotations and literature analysis, the three principal component
172 axes may be attributed to the following biological functions: Immune response and inflammation
173 (PCA1), response to stress and ageing (PCA2) and stress and telomere function/length regulation
174 (PCA3).

175 We then used the individual PCA values on each of the three axes as the dependent variables
176 in a general linear model to investigate how these values varied as a function of age and immune
177 treatment. The results showed that PCA1 was significantly affected by the interaction age x immune
178 treatment. In particular, young LPS-treated mice had higher PCA1 values compared to the other
179 experimental groups, suggesting an up-regulated expression of protein S100, annexin A1 and lysozyme
180 (table 2). Old mice had similar PCA1 values, whatever their immune treatment (LPS vs. PBS; estimates
181 0.267 ± 0.317 , NS.).

182 The PCA2 axis was only affected by age (table 2), with old mice having lower values than young
183 mice. This suggests an age-dependent up-regulation of carbonic-anhydrase 1, GRP 70 kDa and Hsp 90,
184 and a down-regulation of Coronin 1A and RAS-C3.

185 As for PCA1, the PCA3 axis was also affected by the interaction between age and the immune
186 treatment (table 2): while young mice significantly increased their PCA3 values in response to LPS, old
187 mice presented unchanged PCA3 values after the immune treatment (and had mean overall higher
188 values throughout the study). This suggests an up-regulated expression of GRP 70 kDa, Hsp 90 and
189 chitinase-like protein 3, and shorter telomeres in old mice compared to young mice.

190 Given that chitinase-like protein 3 and Hsp 90 (over-expressed in old mice), and the protein
191 S100-A9 (over-expressed in young mice in the LPS group) have been shown to have a function in the
192 regulation of telomere length, we further investigate the relationship between telomere length and
193 the proteomic profile at the individual level. Model selection indicates that chitinase-like protein 3 and
194 Hsp 90 proteins were the best predictors of telomere length independently of the treatment or age
195 (table 3). In addition, we found that chitinase-like protein 3 was negatively correlated with telomere
196 length, both when the regression was conducted using the entire dataset or separated by treatments
197 (PBS and LPS, figure 2). The regression was not significant when the dataset is separated by age (young
198 mice only, $r = 0.373$, $r^2 = 0.139$, $F_{1, 11} = 1.619$, $P = 0.232$ and old mice only $r = 0.530$, $r^2 = 0.281$, $F_{1, 11} =$
199 3.903 , $P = 0.076$).

200 Finally, of the different shelterin proteins implicated in telomere length regulation, western-
201 blot analysis highlighted that only TRF1-interacting nuclear factor 2 (TIN2) expression levels exhibited
202 significant differences among mice groups: a non-significant increase following LPS injection was
203 observed especially in young mice, and there was a significantly higher level of expression for LPS-
204 injected younger mice compared to old ones (figure 3, see ESM4 for statistics and ESM5 for additional
205 representative blots).

206

207 **Discussion**

208 Our results show that the induction of an acute inflammatory response produced age-
209 dependent changes in the proteomic profile of mouse splenocytes. LPS challenge induced an up-
210 regulated expression of protein S100-A9, lysozyme C-2 and annexin A1 in young mice compared to the
211 other groups, while in old mice, this proteomic profile of did not change in relation to LPS.. Similarly,
212 over the whole experiment old mice presented a significantly higher level of expression of proteins
213 associated with immune response and oxidative stress, as well as shorter telomeres compared to
214 young individuals.

215 Analysis of the PCA1 axis which contained protein S100-A9, lysozyme and annexin A1 showed
216 higher values in LPS treated young mice compared to control mice. These proteins play key roles as
217 anti-bacterial effectors and in the regulation of the inflammatory response. For instance, lysozyme
218 degrades the membrane of gram positive bacteria [32], and protein S100-A9, released by apoptotic
219 neutrophils, favors the resolution of the inflammatory response by promoting their elimination by
220 macrophages, and down-regulate pro-inflammatory signals [33]. Similarly, annexin A1, a protein
221 expressed in innate immune cells, has an anti-inflammatory effect [34] and mice that do not express
222 annexin A1 (KO) suffer from fatal septic shock following a LPS injection [35]. Therefore, young mice
223 challenged with LPS had a proteomic signature in splenocytes indicating both the up-regulation of anti-
224 bacterial effectors, and proteins involved in the fine-tuned control of the acute inflammatory response.

225 In a companion study, we showed that the liver proteome of C57BL/6 old mice, exposed to a
226 24h-LPS challenge, was characterized by the up-regulation of proteins involved in the oxidative balance
227 compared to young mice; in the same study, the expression of proteins with specific anti-bacterial
228 functions was not affected by the immune challenge [17]. Using spleen tissue, we found a comparable
229 pattern of variation for PCA2, old mice being similar in their protein profiles before and after injection,
230 but being different from young mice in that they expressed more HSP90, HSP70 (GRP-78 kDa) and
231 carbonic anhydrase 1. Carbonic anhydrase 1 has been previously reported to be over-expressed in old
232 mice [36]. Of particular interest is the up-regulated expression of HSP90 and HSP70 in old mice,
233 because these proteins have pleiotropic functions, with both protective [37] and potentially damaging
234 properties [38-40]. Compared to young mice, old mice also had a lower expression of RAS3 and coronin
235 1A. These proteins are involved in the regulation of the stress response [41] and immunodeficiency
236 [42].

237 *Telomere maintenance as a cost of innate immunity*

238 Mice have highly active telomerase [43, 44], and as a consequence, it is generally assumed that
239 telomere length does not decrease with age in laboratory mice [45]. However, this does not mean that
240 mice are not exposed to telomere erosion when facing stressful events, *i.e.* when telomerase activity

241 is potentially inhibited for instance by increased corticosterone levels [46]. Since marginal telomere
242 shortening with age in some mice tissue has been reported (see [43]), we do know that telomere
243 shortening over generations in telomerase knock-out mice does negatively affect organ functioning
244 and lifespan [47]. This particularly affects highly proliferative tissues which are lined by the
245 hematopoietic cells [48]. In agreement with this, our finding that old mice had shorter telomeres
246 compared to young mice (see ESM, Table S3) could be explained by a transient inhibition of telomerase
247 activity due to the trade-off between telomere maintenance and the immune response. This is in
248 accordance with the recent idea that telomere maintenance *per se* could be energy costly [49].
249 Telomere shortening in old mice was concomitant with over-expression of HSP90, HSP70, and
250 chitinase-like protein 3. Interestingly, HSP90 binds to the catalytic subunit of the telomerase, allowing
251 the assembly of the whole enzyme and maintenance of its activity [50]. Immune cells are quite diverse
252 in function, from hematopoietic stem cells to peripheral blood cells, all which have different
253 proliferation capacities and telomerase activity [51]. The dynamics of telomere length in white blood
254 cells during infection and particularly after mitogen and LPS injections was previously studied [52].
255 Subsequent results provided evidence for an increased telomerase activity in response to infection,
256 mostly restricted to lymphocyte cell lines. However, mice lacking the telomerase catalytic subunit
257 *TERC*^{-/-} also showed higher inflammation responses [53] and we detected the chitinase-like protein 3
258 in mice spleen, which is highly expressed in activated macrophages [54]. This suggests that (i)
259 macrophages may indeed be largely present in our sampled cell population, and (ii) in old mice, HSP90
260 expression may be linked to an (unsuccessful) response to save telomeres *via* telomerase activity.
261 Chitinase-like protein 3 has a dual key role for the immune system (i) by inducing macrophage
262 activation *via* the degradation of bacterial chitin, which triggers pro-inflammatory cytokine secretion,
263 and (ii) by insuring a negative feedback loop on the inflammation process necessary to the remodeling
264 of injured tissues [55]. The fact that chitinase-like protein 3 is more highly expressed in old mice as well
265 as in young mice after LPS injection is in accordance with a lower capacity to control inflammation at
266 old ages. In addition, chitinase-like protein 3 expression and telomere ends and telomerase

267 dysfunction have been previously linked [56, 57], suggesting that chitinase-like protein 3 is a robust
268 ageing biomarker. Again, our results are in accordance with a direct or indirect implication of chitinase-
269 like protein 3 in telomere maintenance, since we highlighted that chitinase-like protein 3 expression
270 levels are negatively linked with telomere lengths irrespectively of age but in relation to LPS treatment
271 (figure 3). As a consequence, chitinase-like protein 3 may be an element explaining the immune trade-
272 off faced by young mice injected with LPS, independently of previously suggested pathways (*e.g.*
273 collateral direct oxidative stress). Chitinase-like protein 3 may be one of the links between
274 inflammatory reaction and telomere shortening. Although the nature of such a mechanism remains to
275 be established, macrophages functioning is likely to be implicated. Another pathway that may be
276 implicated concerns the shelterin TIN2 expression, which clearly tended to be up-regulated in young-
277 LPS mice. TIN2 is regulating the access of telomerase to telomere ends and is necessary for telomere
278 maintenance and telomere end adequate functioning [58, 59]. In addition, TIN2 has been shown to
279 insure cell viability independently of any telomere length regulation [60], underlying that its expression
280 may also participate to the mitigation of negative side-effects of inflammation.

281 Previous studies suggested that telomere length is involved in the capacity of individuals to respond
282 to infection [61, 62]. Based on our data, we hypothesize here, using a proteomic exploratory approach,
283 that the age-related costs of the innate response to LPS implies at least partly a telomere and/or
284 telomerase-related pathway. In mice, even if telomere length is not related to lifespan [63], transient
285 telomere shortening may be the outcome of life-history trade-offs *via* the direct competition for
286 energy of telomere maintenance pathways or due to the indirect effect of stress hormones.

287

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290 husbandry.

291 **Competing interests**

292 We declare no competing interests.

293 **Author contributions**

294 F.C. conceived the study and carried out the experimental work, F.B. and M. B.-D. conducted proteomic
295 and Western Blot analyses while S.Z. conducted telomere length measurements, F.C. ran the statistical
296 analyses and F.C., G.S., F.B. and B.F. drafted the manuscript. All authors gave final approval for
297 publication.

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303 **Data accessibility**

304 Raw data are given in the supplementary material files (table S3).

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476

477 **Figure legends**

478 *Figure 1*

479 **A.** Hierarchical dendrogram built on the 233 proteins detected in mice spleen (n=24). The number of
480 protein clusters selected (k = 4, indicated by the arrow in dots) was chosen because it increased the
481 percentage of total variance explained by the classification by 7.4% and then thereafter only by 4%.
482 Among the four clusters thereby selected, two were explaining more the total variation of axes 1 and
483 2 (cluster 3 and 4 indicated in shadow), which gathered 9 proteins (cluster 3, ID 2156, 1548, 277, 2242,
484 656; cluster 4, ID 928, 471, 132,546). Numbers refer to identification N° in Table S2. Height represents
485 the numbers of mice present in each cluster.

486 **B.** Hierarchical dendrogram built using mice spleen proteomic profiles (based on the 233 proteins
487 quantified), showing mice' distribution (n=24) among clusters (k = 3, separating old mice, and young
488 PBS (dark shadow) and young LPS (grey shadow) groups. Setting the number of clusters to 4 or more
489 did not show any sharp increase in the total variance explained (9% for 4 clusters, and 8% for 5
490 clusters...). Clusters of mice correspond to the protein clusters represented in figure 1A. Height
491 represents the numbers of proteins present in each cluster.

492

493 *Figure 2*

494 Regression checking for linear relationship between individual telomere length and proteomic levels
495 of expression of the chitinase-like 3 protein. Lines represent 95% confidence interval. $\text{Log}_{10}(\text{T/S ratio})$
496 $= 0.10 - 7.95 \times 10^{-10} (\text{chitinase-like 3 protein})$, $r = 0.636$, $r^2 = 0.404$, $F_{1,23} = 14.934$, $P = 0.001$. Dispersion
497 of the experimental groups is shown by different colors, filled circles indicate young-PBS mice, open
498 circles indicate young-LPS mice, dark-grey circles indicate old-PBS mice and light-grey circles indicate
499 old-LPS mice. Regressions tested by treatment are also both significant (PBS: $r = 0.635$, $r^2 = 0.403$, $F_{1,11}$
500 $= 6.763$, $P = 0.026$, LPS: $r = 0.623$, $r^2 = 0.389$, $F_{1,11} = 6.357$, $P = 0.030$).

501

502 *Figure 3*

503 TIN2 protein levels in mice spleen.

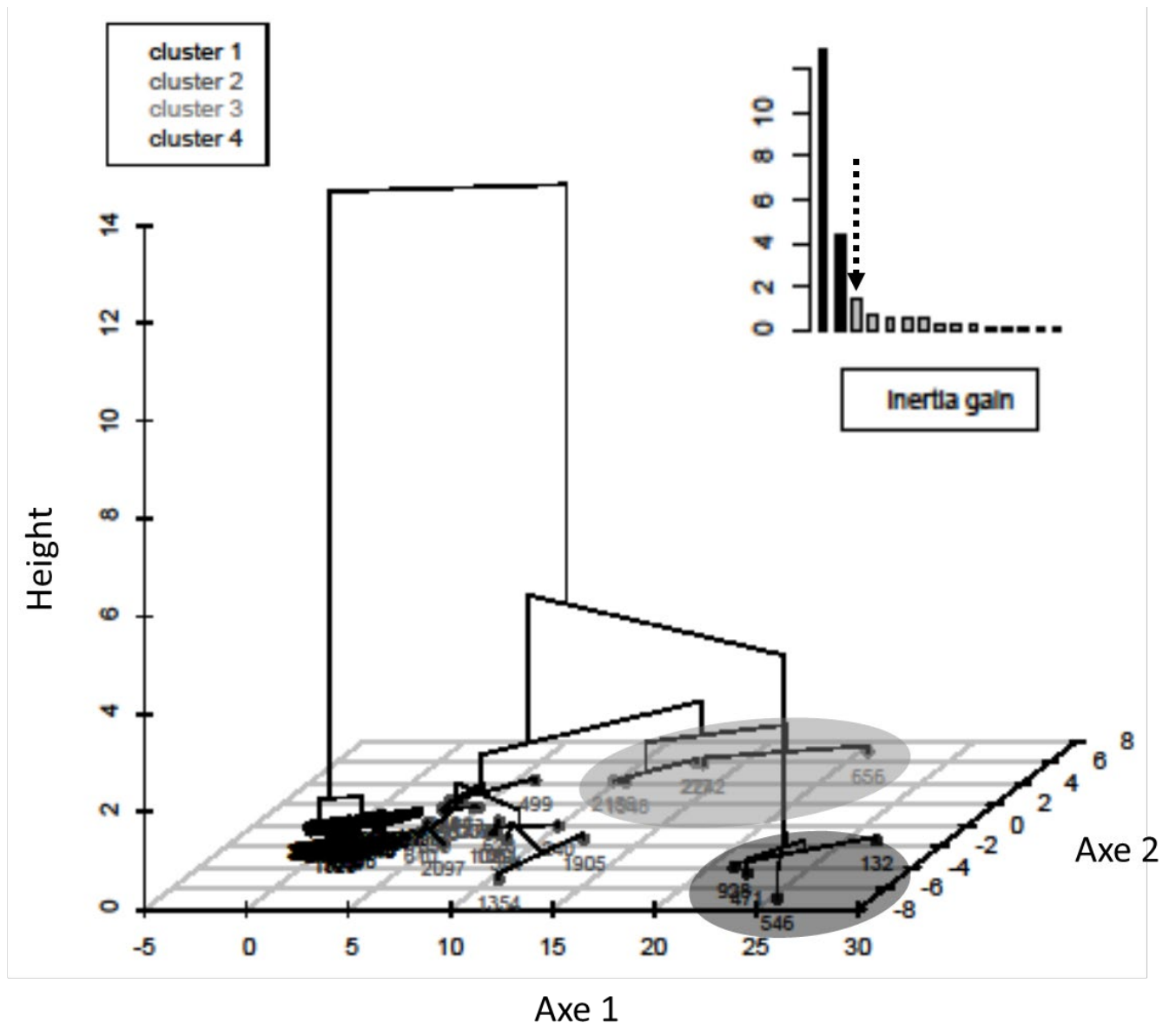
504 Representative western blots for protein levels of TERF1-interacting nuclear factor 2 (TIN2) in the
505 spleen of mice are shown in the upper-panel. Fold changes in young mice injected with LPS and old
506 mice injected with PBS or LPS relative to the mean value in young mice injected with PBS (which was
507 arbitrarily set to 1) are shown in the lower-panel. Values that do not share the same superscript letter
508 are significantly different (see ESM4 and ESM5).

509

510

511 **Figures**

512 *Figure 1A*



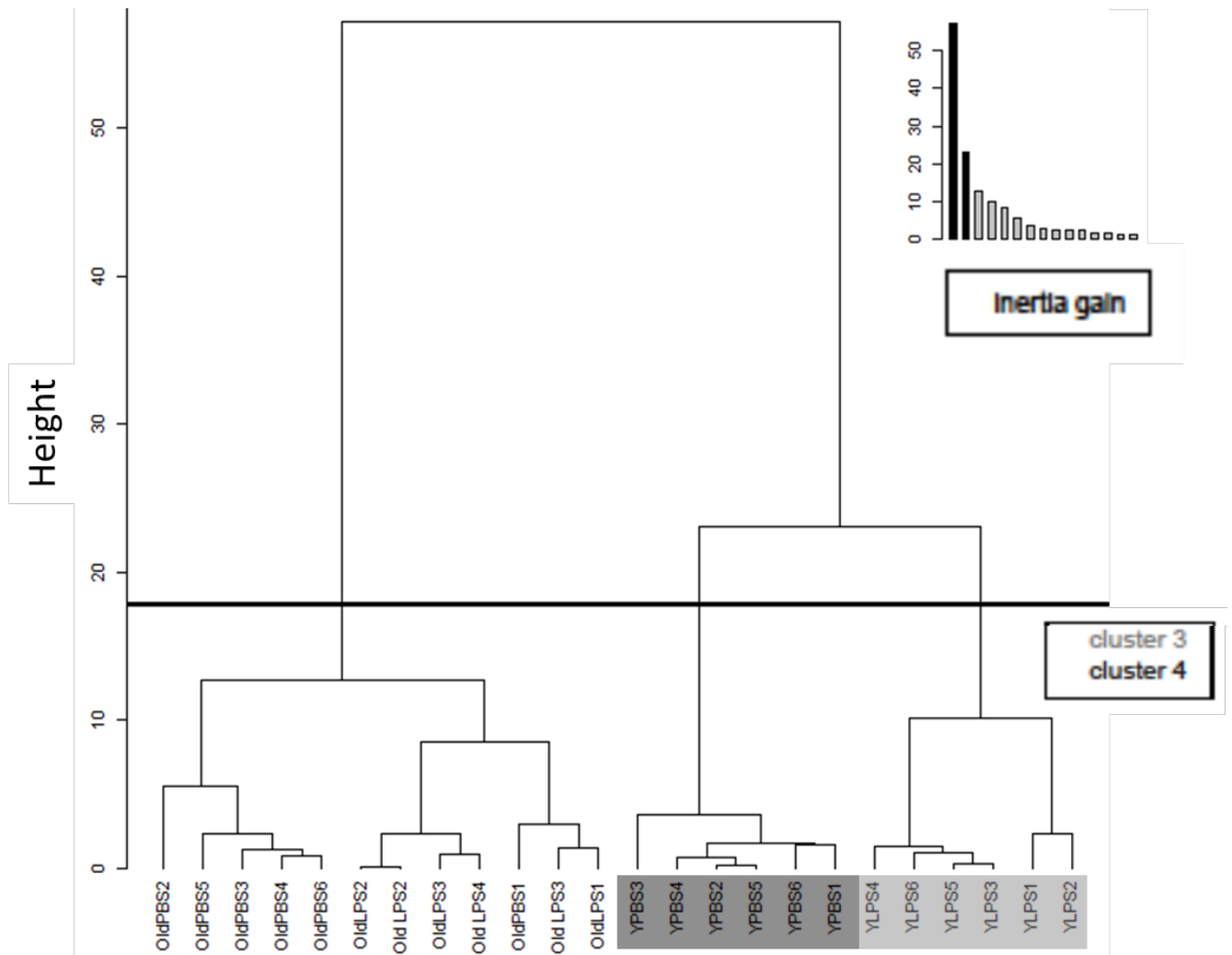
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516 Figure 1B

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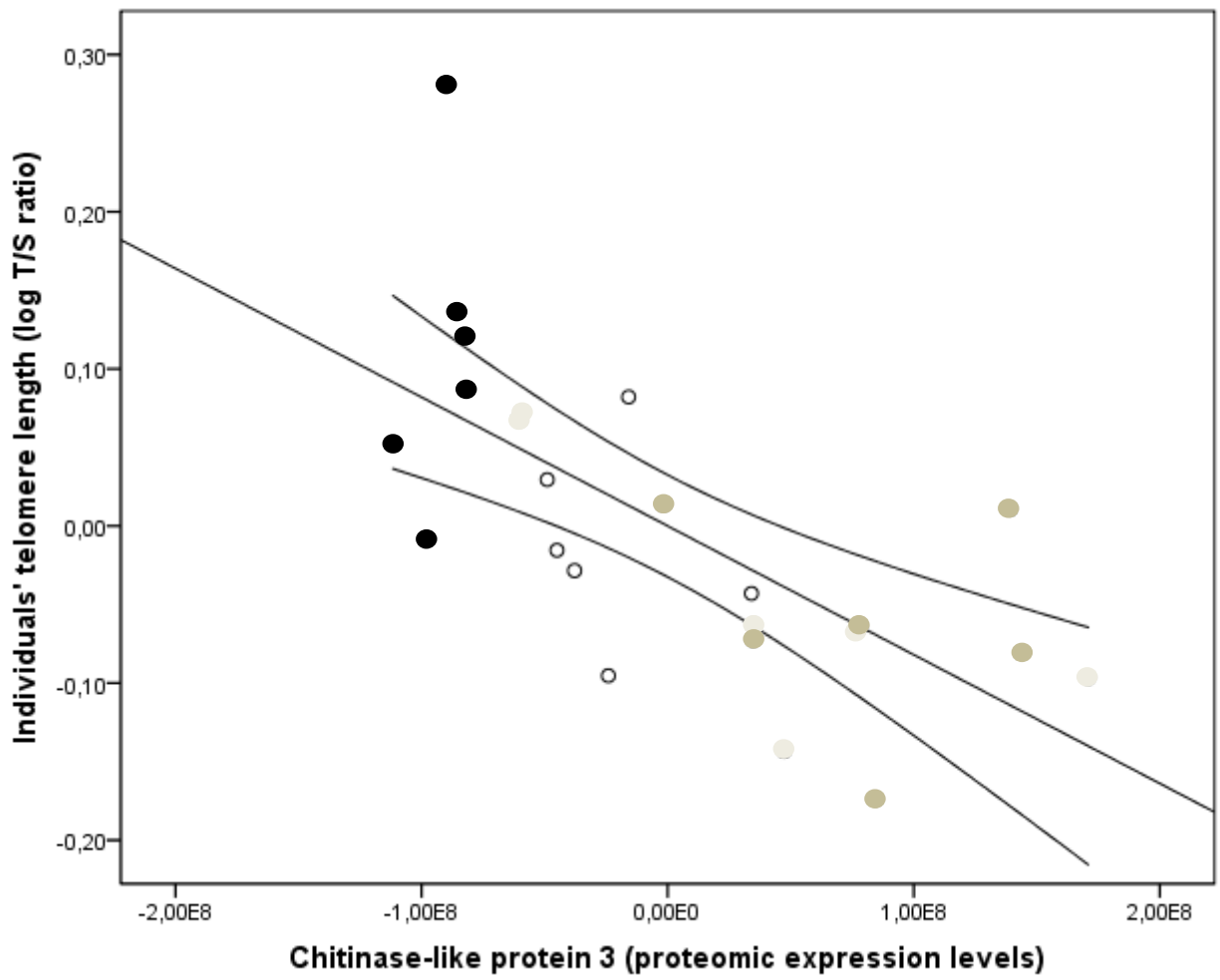
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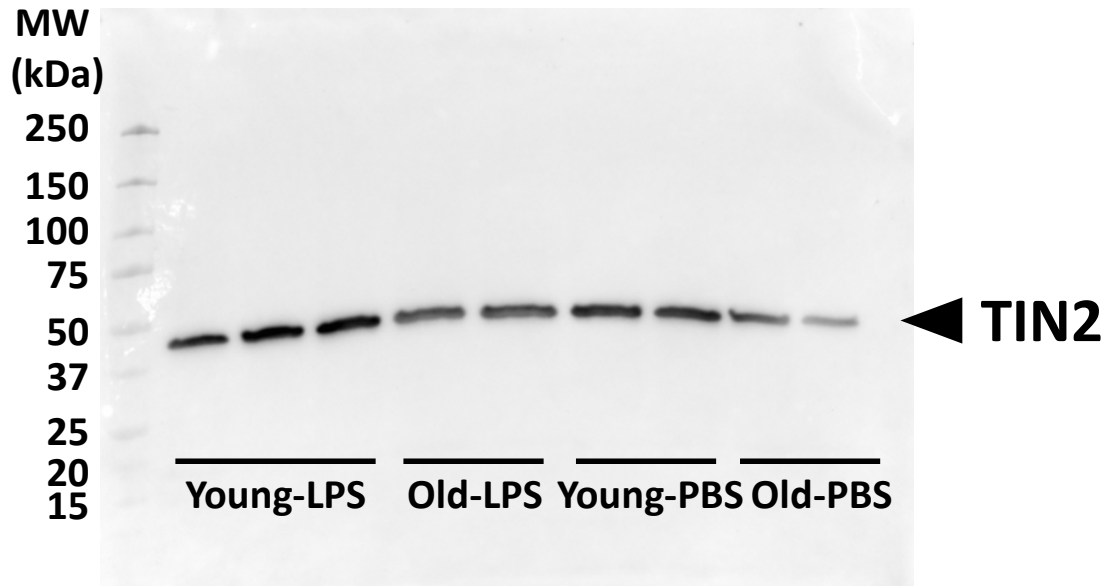
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520 *Figure 2*

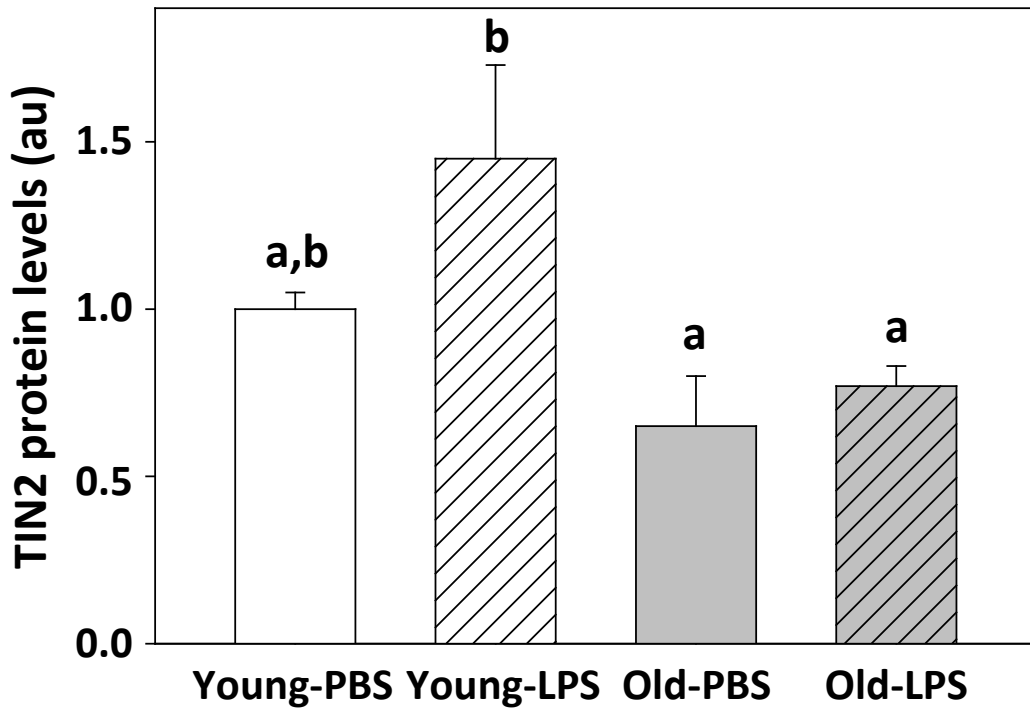
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529 **Tables**530 *Table 1*

		PCA1	PCA2	PCA 3
Proteomic signals				
	ID N°			
Protein S100-A9	2156	0.922		
Lysozyme C-2	1548	0.819		
Annexin A1	277	0.882		
Endoplasmin Hsp90b1	928		-0.441	0.742
GRP-78 kDa	132		-0.463	0.588
Carbonic-anhydrase 1	471		-0.853	
Coronin 1A	656		0.865	
RAS-C3	2242		0.817	
Chitinase-like protein 3	546			0.818
Physiological signals				
<i>Body mass loss</i>			-0.535	
Telomere length				-0.892
% variance explained		36.2	24.4	13.9

531 Nature of the variables forming the main axes (PCA1, PCA2 and PCA3) of the Principal Components

532 Analysis conducted on proteomic and physiological variables describing spleen cellular responses to

533 an immune challenge (LPS) among old and young mice. Only eigenvalues over 0.4 were taken into

534 account and are indicated (see text for details). Total variance explained by the model: 74.5%. ID N°

535 refers to "Identification N°" of proteins that are listed in Table S2.

536

Variables : physiological and proteomic signals	Estimates	D.F	F	P
(a) PCA1				
Age (Old vs. young)	0.319 ± 0.317	1, 23	7.208	0.007
Immune Treatment (LPS vs. PBS)	2.110 ± 0.317	1, 23	28.047	<0.001
Age x Immune Treatment		1, 23	16.867	<0.001
Young LPS vs. Old LPS	1.524 ± 0.317			<0.001
vs. Old PBS	1.791 ± 0.408			<0.001
vs. Young PBS	2.110 ± 0.419			<0.001
(b) PCA2				
Age (Old vs. young)	-1.172 ± 0.466	1, 23	10.846	0.001
Immune Treatment (LPS vs. PBS)	-0.276 ± 0.386	1, 23	0.332	0.565
Age x Immune Treatment		1, 23	0.068	0.794
(c) PCA3				
Age (Old vs. young)	1.900 ± 0.368	1, 23	27.656	<0.001
Immune Treatment (LPS vs. PBS)	0.780 ± 0.517	1, 23	0.907	0.341
Age x Immune Treatment		1, 23	4.217	0.040
Young LPS vs. Old PBS	-1.119 ± 0.368			0.014
vs. Young PBS	0.781 ± 0.370			0.034
Young PBS vs. Old PBS	-1.614 ± 0.408			<0.001
vs. Old LPS	-1.190 ± 0.419			<0.001

538 Generalized linear models explaining the variability in the components axes (PC1, PC2, PC3) of proteomic and
539 physiological signals in old and young mice challenged with PBS or LPS (n=6 in each group). Generalized linear
540 model analysis separated by treatment (a) was used to assess Interaction Age x Immune treatment
541 differences among groups (indicated in bold). Estimates and mean values are given ± SE and only significant
542 differences among Age x Treatment interactions are indicated (using Bonferroni correction). Residuals of
543 each models followed a normal distribution (checked using Kolmogorov-Smirnov test and QQ plot).

Fixed effect	Estimates	Standard error	z-value	P-value
Intercept	0.105	0.089	1.130	0.259
Chitinase-like protein 3	-7.926⁻¹⁰	2.535⁻¹⁰	2.967	0.003
<i>Annexin A1</i>	7.587⁻¹⁰	5.000⁻¹⁰	1.439	0.150
<i>Lysozyme C-2</i>	-1.238 ⁻⁹	8.560 ⁻¹⁰	1.380	0.168
<i>Endoplasmin Hsp90b1</i>	-1.087 ⁻⁹	8.788 ⁻¹⁰	1.194	0.232
<i>Protein S100-A9</i>	1.051 ⁻⁹	9.059 ⁻¹⁰	1.160	0.250
<i>Carbonic-anhydrase 1</i>	3.030 ⁻¹⁰	3.610 ⁻¹⁰	0.915	0.360
RAS-C3	-3.124 ⁻¹⁰	7.753 ⁻¹⁰	0.403	0.687
GRP-78 kDa	3.365 ⁻¹⁰	6.524 ⁻¹⁰	0.490	0.624
Coronin 1A	-1.344 ⁻¹⁰	6.473 ⁻¹⁰	0.196	0.845

546 Model of the relationship between (i) the spleen proteomic expression levels of 9 proteins and (ii) the spleen
547 telomere length (log-transformed). The estimates and P-values for the fixed effects are averaged over the 7
548 best general linear models for which the $\Delta AIC < 2$ in comparison to the best model (AIC=-47.6, which used
549 Annexin A1 and chitinase-like 3 as fixed factors (indicated in bold)). Fixed factors used in those 7 best models
550 are indicated in italic.