1

# Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice

Clea Bárcena[1], Rafael Valdés-Mas[1], Pablo Mayoral[1], Cecilia Garabaya[1], Sylvère Durand[2,3,4,5], Francisco Rodríguez[1], María Teresa Fernández-García[6], Nuria Salazar[7,8], Alicja M. Nogacka[7,8], Nuria Garatachea[9,10], Noélie Bossut[2,3,4,5], Fanny Aprahamian[2,3,4,5], Alejandro Lucia[11,12], Guido Kroemer[2,3,4,5,13,14,15], José M. P. Freije[1,16], Pedro M. Quirós[1,16]*, and Carlos López-Otín[1,16]*

*[1]Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Oviedo, Spain; [2]Cell Biology and Metabolomics platforms, Gustave Roussy Cancer Campus; Villejuif, France; [3]Equipe 11 labellisée par la Ligue contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; [4]INSERM, U1138, Paris, France; [5]Université Paris Descartes, Sorbonne Paris Cité; Paris, France; [6]Unidad de histopatología molecular, IUOPA, Universidad de Oviedo, Oviedo, Spain; [7]Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Villaviciosa, Spain; [8]Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA); [9]Faculty of Health and Sport Sciences, Department of Physiatry and Nursing, University of Zaragoza, Huesca, Spain; [10]GENUD (Growth, Exercise, NUtrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain; [11]Faculty of Sport Science, Universidad Europea de Madrid, Madrid, Spain; [12]Instituto de Investigación Hospital 12 de Octubre (i+12) y Centro de Investigación Biomédica en Red de Fragilidad y Enjevecimiento Saludable (CIBERFES), Spain; [13]Université Pierre et Marie Curie, Paris, France; [14]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP; Paris, France; [15]Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden; [16]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Spain.*

***Send correspondence to:**
Carlos López-Otín (clo@uniovi.es) or Pedro M. Quirós (pmquiros@gmail.com)
Departamento de Bioquímica y Biología Molecular
Facultad de Medicina, Universidad de Oviedo
33006 Oviedo-SPAIN
Tel. 34-985-104201; Fax: 34-985-103564

The gut microbiome is emerging as a key regulator of several metabolic, immune and neuroendocrine pathways[1,2]. Gut microbiome deregulation has been implicated in major conditions such as obesity, type-2 diabetes, cardiovascular disease, non-alcoholic fatty acid liver disease and cancer[3-6], but its precise role in aging remains to be elucidated. Here, we find that two different mouse models of progeria are characterized by intestinal dysbiosis with alterations that include an increase in the abundance of proteobacteria and cyanobacteria, and a decrease in the abundance of verrucomicrobia. Consistent with these findings, we found that human progeria patients also display intestinal dysbiosis and that long-lived humans (*i.e.*, centenarians) exhibit a substantial increase in verrucomicrobia and a reduction in proteobacteria. Fecal microbiota transplantation from wild-type mice enhanced healthspan and lifespan in both progeroid mouse models and transplantation with the verrucomicrobia *Akkermansia muciniphila* was sufficient to exert beneficial effects. Moreover, metabolomic analysis of ileal content points to the restoration of secondary bile acids as a possible mechanism for the beneficial effects of reestablishing a healthy microbiome. Our results demonstrate that correction of the accelerated aging-associated intestinal dysbiosis is beneficial, suggesting the existence of a link between aging and the gut microbiota that provides a rationale for microbiome-based interventions against age-related diseases.

**Keywords:** aging, centenarians, fecal transplants, dysbiosis, longevity, metagenomics, microbiome, progeria.

61 Traditionally seen as detrimental, the pathophysiological implications of the
62 microbiota have considerably expanded in the last years. It is now known that the
63 microbiota has essential metabolic and immunological functions conserved from worms[7]
64 to humans[1,2]. In mammals, the gut microbiota is involved in food processing, activation
65 of satiety pathways, protection against pathogens, and production of metabolites
66 including vitamins, short chain fatty acids and secondary bile acids[8-10]. The gut
67 microbiota also signals to distant organs, contributing to the maintenance of host
68 physiology[11]. Intestinal microbiota alterations are associated with major conditions like
69 obesity, type-2 diabetes, cardiovascular disease, non-alcoholic fatty acid liver disease,
70 cancer, and the response to antineoplastic therapy[3-6].

71 Although some works have explored the microbiome profile of long-lived
72 humans[12,13], no alterations have been described in accelerated aging syndromes. In this
73 work, we studied the gut microbiome of two mouse models of Hutchinson-Gilford
74 progeria syndrome (HGPS), patients with HGPS[14] and Nestor-Guillermo progeria
75 syndrome (NGPS)[15], as well as human centenarians and their controls. We found
76 intestinal dysbiosis in both mouse models and progeria patients. In turn, the microbiota
77 of centenarians is characterized by the presence of both pathological- and health-
78 associated bacterial genera. We show that fecal microbiota transplantation (FMT) from
79 wild-type (WT) donors to progeroid recipients attenuates the accelerated-aging
80 phenotype and increases survival, whereas FMT from progeroid donors to WT recipients
81 induces metabolic alterations. Analysis of centenarians and progeria mouse models point
82 to a beneficial role for the genus *Akkermansia,* as oral gavage of *Akkermansia muciniphila*
83 extends the lifespan of progeroid mice.

84 To explore the relevance of microbiome in progeria, we first studied the gut
85 metagenome profile of the *Lmna*$^{G609G/G609G}$ mouse model of HGPS[16], by comparing WT

86 and *Lmna*$^{G609G/G609G}$ mice at three different ages: 1 month (WT 1mo and *Lmna*$^{G609G/G609G}$
87 1mo), 4 months –when *Lmna*$^{G609G/G609G}$ mice exhibit a progeroid phenotype – (WT 4mo
88 and *Lmna*$^{G609G/G609G}$ 4mo), and 22 months (for WT mice only; WT 22mo; Extended Data
89 Fig. 1a). To assess how progeria affects the gut microbial community structure, we
90 studied the alpha- and beta-diversity associated with each genotype, and compared the
91 microbial diversity within and between communities. Alpha-diversity was analyzed by
92 calculating the Chao1 (a proxy for community richness) and Shannon's index (a proxy
93 for diversity, taking into account both richness and evenness). We did not observe
94 differences in bacterial diversity or richness within any of the mouse groups (Extended
95 Data Fig. 1b,c and Supplementary Table 1). Next, we evaluated the beta-diversity across
96 mouse groups, identifying a differential clustering of *Lmna*$^{G609G/G609G}$ 4mo mice in a
97 principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity (qualitative
98 measure) (Fig 1a) and the Jaccard distances (quantitative measure) (Extended Data Fig.
99 1d). Similar differences were revealed by hierarchical clustering, where *Lmna*$^{G609G/G609G}$
100 4mo mice were grouped together and separated from all other groups (Extended Data Fig.
101 1e,f).

102 Next, we calculated the percentage of bacterial taxa in each group (Fig. 1b and
103 Extended Data Fig. 2a), applying a linear discriminant analysis (LDA) effect size (LEfSe)
104 method (see Methods; Supplementary Table 2). We noted a similar profile for WT and
105 *Lmna*$^{G609G/G609G}$ mice at 1 month of age (Fig. 1b and Extended Data Fig. 2a), observing
106 solely an increment in the genera *Allobaculum* from the family Erysipelotrichaceae,
107 *Desulfovibrio* (class deltaproteobacteria) and Clostridiales of the families
108 Ruminococcaceae and Lachnospiraceae, in *Lmna*$^{G609G/G609G}$ 1mo (Extended Data Fig. 2b).
109 As WT mice aged (from 1 to 4 months), we noted an increment in genera such as
110 *Allobaculum*, *Ruminococcus*, *Coprococcus*, *Turicibacter* or *Parabacteroides* (Extended

Data Fig. 2c); however, the changes from 1 to 4 months were more profound in progeroid mice, displaying a loss in *Akkermansia* and *Dehalobacterium* and an enrichment in *Parabacteroides*, *Prevotella* and the Enterobacteriaceae, among other differences (Extended Data Fig. 2d). When comparing WT and progeroid mice at 4 months of age, the pattern was substantially different (Fig. 1b,c and Extended Data Fig. 2a), in agreement with the progression of the aging phenotype. The main differences were a reduction in abundance of Erysipelotrichales (phylum Firmicutes), Burkholderiales (class Betaproteobacteria) and Verrucomicrobiales (phylum Verrucomicrobia) in progeroid mice, together with an increase in Bacteroidales (phylum Bacteroidetes), Deferribacterales (phylum Deferribacteres), YS2 (phylum Cyanobacteria) and the proteobacteria Enterobacteriales and Pseudomonadales (class Gammaproteobacteria) (Fig. 1c and Supplementary Table 2). At a lower taxonomical level, *Lmna*$^{G609G/G609G}$ 4mo mice showed a loss in Clostridiaceae, *Allobaculum*, *Sutterella*, *Dehalobacterium*, *Rikenella* and *Akkermansia* (Fig. 1d). Of note, high abundance of *Akkermansia* has been associated with improved immunomodulation and metabolic homeostasis, reduced inflammation, and protection against atherosclerosis[17-19]. By contrast, *Lmna*$^{G609G/G609G}$ 4mo exhibited an increment in *Mucispirillum*, *Enterococcus*, *Acinetobacter*, *Staphylococcus*, *Parabacteroides*, *Bacteroides*, *Prevotella* and Enterobacteriaceae, which contains the genus *Escherichia* and has been associated with dysbiosis and intestinal inflammation[20] (Fig. 1d). Changes in *Escherichia* and *Akkermansia* were validated afterwards at the species level by qPCR in a second, independent group of *Lmna*$^{G609G/G609G}$ mice (Fig. 1e). The gut microbiome of a fraction of WT 22mo exhibited a tendency towards intestinal dysbiosis (Extended Data Fig. 2a); however, the only significant shift consisted in the loss in *Rikenella* (Extended Data Fig. 2e), a change also found when comparing *Lmna*$^{G609G/G609G}$ 4mo to WT 4mo mice (Fig. 1d). To explore the

136 functional implications of the microbiome shift in progeroid mice, we then investigated
137 the metagenome data with PICRUSt and HUMAnN2 (see Methods). LEfSe analysis
138 detected an increase in KEGG pathways related to pathological bacteria such as ‘Bacterial
139 invasion of epithelial cells’ (ko05100) and ‘Flagellar assembly’ (ko02040), and a
140 differential enrichment in multiple metabolic pathways (Extended Data Fig. 2f and
141 Supplemental Table S3).

142 To confirm the association of gut dysbiosis with progeria, we analyzed the gut
143 microbiota of a different progeria model, *Zmpste24*$^{-/-}$ mice[21] (Extended Data Fig. 3a).
144 Although *Zmpste24*$^{-/-}$ mice showed no differences in bacterial diversity (Extended Data
145 Fig. 3b), they exhibited higher bacterial richness (Extended Data Fig. 3c). Like
146 *Lmna*$^{G609G/G609G}$ mice, *Zmpste24*$^{-/-}$ animals showed differences in beta diversity at the
147 quantitative (Fig. 1f) and qualitative level (Extended Data Fig. 3d), clustering differently
148 from WT mice, and exhibited signs of dysbiosis (Fig. 1g, Extended Data Fig. 3e) with a
149 high abundance of Proteobacteria (class Alphaproteobacteria) and Cyanobacteria (Fig.
150 1g, Extended Data Fig. 3e and Supplementary Table 2). *Zmpste24*$^{-/-}$ mice also showed a
151 tendency to a lower amount of Verrucomicrobia (Extended Data Fig. 3e), although this
152 trend was less marked than in *Lmna*$^{G609G/G609G}$ animals, perhaps due to the later onset of
153 the progeroid phenotype in *Zmpste24*$^{-/-}$ mice. At a lower taxonomic level, we found,
154 among other differences, an enrichment in the order YS2 (phylum Cyanobacteria) and
155 the genera *Bacteroides*, *Parabacteroides* and *Prevotella* (Fig. 1h), similarly to
156 *Lmna*$^{G609G/G609G}$ 4mo mice.

157 To investigate the possible existence of gut dysbiosis in human progeria, we
158 obtained fecal samples from four children with HGPS and their healthy siblings, as well
159 as from a patient with NGPS and his healthy sibling and mother (Extended Data Fig. 4a).
160 When comparing progeria patients with their healthy controls, we found no differences

161 in alpha-diversity (Extended Data Fig. 4b,c) or beta-diversity indices (Fig. 2a and
162 Extended Data Fig. 4d). However, when samples were compared by geographical
163 location (*i.e.*, comparing families), a differential clustering was observed at the
164 quantitative (Fig. 2b) and qualitative levels (Extended Data Fig. 4e). Despite the low
165 number of samples, these results suggest that the environment has more influence on the
166 gut microbiome than the pathological condition, which is in accordance with recent
167 findings[22,23]. Yet, in each of the studied families, the progeria patients had a different
168 profile compared to their healthy siblings. This was particularly evident in families where
169 more than one healthy member was studied (Extended Data Fig. 4f). LEfSe analysis
170 pointed to a loss in *Gemmiger* –family Ruminococcaceae– and an enrichment in
171 *Clostridium* from families Erysipelotrichaceae and Lachnospiraceae in progeria patients
172 (Fig. 2c).

173 Considering the gut microbiome alterations observed in mice and progeria
174 patients, we hypothesized that individuals with exceptionally long lifespans (*i.e.*,
175 centenarians) might possess a health-promoting microbiome. Therefore, we performed a
176 metagenomic analysis of a centenarian cohort and ethnically-matched healthy adult
177 controls (Extended Data Fig. 5a). Centenarians showed lower alpha-diversity compared
178 to their controls (Extended Data Fig. 5b), and a considerably lower bacterial richness
179 (Extended Data Fig. 5c). Also, both groups clustered differentially, based on quantitative
180 (Fig. 2d) and qualitative (Extended Data Fig. 5d) beta-diversity indexes, indicating that
181 centenarians had a different microbial community structure than controls. Indeed, we
182 encountered distinct microbial profiles in both groups (Fig. 2e and Extended Data Fig.
183 5e). LEfSe analysis showed that centenarians presented less Betaproteobacteria and more
184 Synergistia and Verrucomicrobiae, among others (Fig. 2e and Supplementary Table 2).
185 At a lower taxonomic level, centenarians were characterized by a lower abundance of

186 Desulfovibrionaceae, Lachnospiraceae and Erysipelotrichaceae – the last two enriched in
187 progeria patients – and *Prevotella*, *Sutterella*, *Roseburia*, *or Butyricicoccus,* among other
188 differences (Fig. 2f). Notably, centenarians exhibited a higher abundance of
189 Enterobacteriaceae, Ruminococcaceae and Christensenellaceae and the genera
190 *Klebsiella*, *Lactobacillus*, *Parabacteroides* and *Akkermansia* (Fig. 2f). These results are
191 in line with previous studies reporting high levels of *Akkermansia* and
192 Christensenellaceae and low levels of *Roseburia* in centenarians[12,24].

193 Based on the aforementioned results, we hypothesized that changes in the gut
194 microbiota might accompany the accelerated aging of HGPS mice. To explore this
195 possibility, we performed FMT in four different mouse groups. First, we used WT control
196 mice as microbiota donors (herein referred to as WTmic, *WT microbiota*) and
197 $Lmna^{G609G/G609G}$ mice as recipients. We also transplanted $Lmna^{G609G/G609G}$ mice with
198 microbiota from older $Lmna^{G609G/G609G}$ mice, that is, progeroid mice with a more advanced
199 phenotype (oG609Gmic, *old* $Lmna^{G609G/G609G}$ microbiota) (Fig. 3a). FMT effectiveness
200 was evaluated by comparing the gut metagenomic profiles of control, transplanted and
201 donor mice, showing that transplanted progeroid mice acquired the donor microbiota
202 (Extended Data Fig. 6a,b). Additionally, we performed microbiome ablation and sham-
203 transplanted $Lmna^{G609G/G609G}$ mice (herein referred to as EmptyT, *empty transplant*) (Fig.
204 3a). $Lmna^{G609G/G609G}$-WTmic manifested a delayed loss of body weight and temperature
205 (Fig. 3b,c). Phenotype-dependent hypoglycemia was avoided and renal perivascular
206 fibrosis was attenuated in $Lmna^{G609G/G609G}$-WTmic (Fig. 3d,e). Spleen weight, typically
207 reduced in progeroid mice, was similar in $Lmna^{G609G/G609G}$-WTmic and $Lmna^{G609G/G609G}$
208 controls, but lower in $Lmna^{G609G/G609G}$-oG609Gmic (Extended Data Fig. 6c). We also
209 noted an increase in intestinal inflammation markers in $Lmna^{G609G/G609G}$ mice that was
210 recovered in $Lmna^{G609G/G609G}$-WTmic (Extended Data Fig. 6d). Surprisingly, some

211 markers were also decreased in *Lmna*$^{G609G/G609G}$-oG609Gmic, pointing to a possible
212 beneficial effect of the FMT protocol *per se* (Extended Data Fig. 6d). Most importantly,
213 *Lmna*$^{G609G/G609G}$-WTmic showed improved survival compared to control *Lmna*$^{G609G/G609G}$
214 mice ($P$ = 0.0029), with a 13.5% increase in median lifespan (160 vs 141 days,
215 respectively) (Fig. 3f). *Lmna*$^{G609G/G609G}$-WTmic also exhibited an extended maximal
216 survival (9% increment, $P$ = 0.04) (Fig. 3f and Extended Data Fig. 6e). In contrast,
217 *Lmna*$^{G609G/G609G}$-oG609Gmic showed reduced survival compared to control
218 *Lmna*$^{G609G/G609G}$ mice ($P$ = 0.045), with a reduction in median lifespan (129 days) (Fig.
219 3f). *Lmna*$^{G609G/G609}$ -EmptyT did not show survival differences when compared to control
220 *Lmna*$^{G609G/G609G}$ mice (Extended Data Fig. 6f). Lastly, to evaluate the potential
221 pathogenicity of progeroid microbiota, we also performed FMT from old *Lmna*$^{G609G/G609G}$
222 donors into WT mice (WT-oG609Gmic). Effective gut colonization was validated by
223 comparing the metagenomic profiles of control, transplanted and donor mice (Extended
224 Data Fig. 6a,b). We did not observe progeria-related features in WT-oG609Gmic mice.
225 However, this maneuver caused metabolic alterations, including a higher body weight,
226 higher glucose levels, lower $O_2$ consumption, lower $CO_2$ production and reduced energy
227 expenditure (Fig. 3g-k and Extended Data Fig. 7a-d).

228 To validate these results, we also performed FMT in *Zmpste24*$^{-/-}$ mice using
229 microbiota from WT mice (*Zmpste24*$^{-/-}$-WTmic). This manipulation caused *Zmpste24*$^{-/-}$
230 mice to manifest a less pronounced cervicothoracic lordokyphosis, larger body size and
231 maintained grooming (Fig. 3l). Body weight loss (Fig. 3m and Extended Data Fig. 8a)
232 and hypoglycemia (Fig. 3n) were avoided in *Zmpste24*$^{-/-}$-WTmic mice. Like
233 *Lmna*$^{G609G/G609G}$ mice transplanted with WT microbiota, *Zmpste24*$^{-/-}$-WTmic mice also
234 exhibited enhanced survival ($P$ = 0.0092), with a median lifespan increase of 13.4% (279

235 vs 246 days) (Fig. 3o). Maximum survival was increased by almost 8% ($P$ = 0.09) (Fig.
236 3o and Extended Data Fig. 8b).

237 The results above in both human and murine gut metagenomic profiles described
238 a lost in *A. muciniphila* in *Lmna*$^{G609G/G609G}$ 4mo mice and an increase in centenarians'
239 samples. Since this bacterium exerts beneficial effects in other models[17,19,25,26], we tested
240 if external supply of *A. muciniphila* to *Lmna*$^{G609G/G609G}$ mice would improve their
241 healthspan or lifespan. By supplementing *Lmna*$^{G609G/G609G}$ mice with *A. muciniphila*
242 through oral gavage, we obtained a modest lifespan extension ($P$ = 0.016) (Fig. 4a),
243 suggesting a protective role of this microorganism against accelerated aging
244 manifestations. As previously described[19], *A. muciniphila* supplementation induced ileal
245 expression of *Reg3g* (Fig. 4b) and favored the thickening of the intestinal mucosa layer
246 (Fig. 4c). *Lmna*$^{G609G/G609G}$ mice receiving *A. muciniphila* also showed an increment in the
247 intestinal trefoil factor *Tff3* (Fig. 4b), which might promote wound healing and repair of
248 the mucosa layer[27].

249 Finally, to investigate the potential mechanisms accounting for the healthspan and
250 lifespan extension of *Lmna*$^{G609G/G609G}$ mice after FMT, we performed metabolome
251 profiling of ileal content from WT, *Lmna*$^{G609G/G609G}$ and *Lmna*$^{G609G/G609G}$-WTmic mice.
252 Significant changes were analyzed by metabolite set enrichment analysis using KEGG
253 pathways, detecting an enrichment in 'secondary bile acid biosynthesis' (Fig. 4d). As
254 recently described[28], different bile acids were decreased in *Lmna*$^{G609G/G609G}$ compared to
255 WT mice, and recovered in *Lmna*$^{G609G/G609G}$-WTmic (Fig. 4e). Moreover, *Lmna*$^{G609G/G609G}$
256 mice ileal content exhibited a depletion of the monosaccharides arabinose and ribose, the
257 nucleoside inosine, and the ether-phospholipid PCae (18:0) that was reversed upon FMT
258 with WT microbiota (Fig. 4f).

259 FMT might modulate obesity and metabolism in humans and mice[29,30], ameliorate
260 metabolic syndrome in patients[31], and contribute to the treatment of refractory immune
261 checkpoint inhibitor (ICI)-associated colitis[32] and recurrent infections by *Clostridium*
262 *difficile*[33]. The promising *in vivo* results obtained in this study suggest that therapeutic
263 interventions on the intestinal microbiome may lead to healthspan end even lifespan
264 improvements. In this regard, we show that *A. muciniphila* administration lead to a
265 lifespan enhancement in progeria, thus extending previous findings on pro-health
266 activities of *Akkermansia* spp. in the intestinal tract of mammals[17-19] and in aged mice[34].
267 Previous studies have described that successful FMT treatment of recurrent infection by
268 *C. difficile* relies on restoration of correct bile acid metabolism[35,36], and that antibiotic-
269 induced microbiome depletion reduces the secondary bile acid pool[37]. Of note, secondary
270 bile acids are produced by the gut microbiota[38] and positively correlate with *Akkermansia*
271 levels in mice[39]. Considering that bile acids regulate metabolism and anti-inflammatory
272 signals[40] and that they are depleted in *Lmna*$^{G609G/G609G}$ mice[28], the restoration of
273 secondary bile acids – and other metabolites (arabinose, ribose, inosine) – by FMT might
274 contribute to extend healthspan and lifespan in progeroid mice (Fig. 4g). Future work
275 might identify the functional mechanisms by which some bacterial species and
276 metabolites are responsible for the healthspan and lifespan extension conferred by FMT,
277 and explore the applicability of FMT in normal or accelerated aging.

278

279 **Acknowledgements.** We thank M. Stamsnijder from the Progeria Family Circle, and the
280 families that have kindly participated in our study. We also thank G. Velasco, Y. Español,
281 A.R. Folgueras, X.M. Caravia, J.M. Fraile, I. Varela, M. Mittelbrunn and K. Iribarren for
282 helpful comments and advice; R. Feijoo, A. Moyano, D.A. Puente, S.A. Miranda, M.S.
283 Pitiot, V. García de la Fuente and M.C. Muñiz for excellent technical assistance; M.
284 Gueimonde and C.G. de los Reyes-Gavilán (IPLA-CSIC) for providing *A. muciniphila*;

285 and C. Mayolas for help with logistic organization. We also acknowledge the generous
286 support by J.I. Cabrera. A.L. research on aging is funded by Fondo de Investigaciones
287 Sanitarias and Fondos FEDER (PI15/00558). Metabolomics platform is supported by
288 H2020 European Union project OncoBiome. G.K. is supported by the Ligue contre le
289 Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs;
290 ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases;
291 Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France;
292 Chancelerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale
293 (FRM); a donation by Elior; European Research Area Network on Cardiovascular
294 Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea; the European Union
295 Horizon 2020 Project Oncobiome; Fondation Carrefour; Institut National du Cancer
296 (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx
297 Immuno-Oncology; the RHU Torino Lumière; the Seerave Foundation; the SIRIC
298 Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and
299 the SIRIC Cancer Research and Personalized Medicine (CARPEM). The Instituto
300 Universitario de Oncología is supported by Fundación Bancaria Caja de Ahorros de
301 Asturias. J.M.P.F. is supported by Ministerio de Economía y Competitividad-FEDER and
302 Gobierno del Principado de Asturias. C.L-O. is supported by grants from European
303 Research Council (DeAge, ERC Advanced Grant), Ministerio de Economía y
304 Competitividad-FEDER, Instituto de Salud Carlos III (RTICC) and Progeria Research
305 Foundation.

306

307 **Author contributions.** C.B., J.M.P.F., P.M.Q. and C.L.-O. conceived and designed
308 experiments. R.V.-M., P.M.Q. and C.B. performed bioinformatics analysis and results
309 interpretation. C.B., P.M., C.G., F.R. and P.M.Q. performed experiments and analyzed
310 data. M.T.F.-G. performed histopathological analysis. N.S. and A.M.N. performed the
311 culturing and bacterial solutions of *A. muciniphila*. N.G. and A.L. organized and carried
312 out pickup of human centenarian samples. S.D., N.B., F.A. and G.K. performed LC-MS-
313 based metabolomics. C.B., J.M.P.F., P.M.Q. and C.L.-O. wrote the manuscript. A.L. and
314 G.K. assisted with manuscript edition and all authors revised the manuscript and provided
315 input.

316

317

318 **Competing interests statement.** GK is one of the scientific co-founders of everImmune.

319
320 **References**
321

322 1. Thaiss, C.A., Zmora, N., Levy, M. & Elinav, E. The microbiome and innate
323 immunity. *Nature* **535**, 65-74 (2016).

324 2. Leulier, F*., et al.* Integrative physiology: at the crossroads of nutrition,
325 microbiota, animal physiology, and human health. *Cell Metab* **25**, 522-534 (2017).

326 3. Koeth, R.A*., et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in
327 red meat, promotes atherosclerosis. *Nat Med* **19**, 576-585 (2013).

328 4. Loomba, R*., et al.* Gut microbiome-based metagenomic signature for non-
329 invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell*
330 *Metab* **25**, 1054-1062 e1055 (2017).

331 5. Qin, J*., et al.* A metagenome-wide association study of gut microbiota in type 2
332 diabetes. *Nature* **490**, 55-60 (2012).

333 6. Zitvogel, L., Daillere, R., Roberti, M.P., Routy, B. & Kroemer, G. Anticancer
334 effects of the microbiome and its products. *Nat Rev Microbiol* **15**, 465-478 (2017).

335 7. Cabreiro, F. & Gems, D. Worms need microbes too: microbiota, health and
336 aging in Caenorhabditis elegans. *EMBO Mol Med* **5**, 1300-1310 (2013).

337 8. Rios-Covian, D*., et al.* Intestinal short chain fatty acids and their link with diet
338 and human health. *Front Microbiol* **7**, 185 (2016).

339 9. Breton, J*., et al.* Gut commensal E. coli proteins activate host satiety pathways
340 following nutrient-induced bacterial growth. *Cell Metab* **23**, 324-334 (2016).

341 10. Wahlstrom, A., Sayin, S.I., Marschall, H.U. & Backhed, F. Intestinal crosstalk
342 between bile acids and microbiota and its impact on host metabolism. *Cell Metab* **24**,
343 41-50 (2016).

344 11. Schroeder, B.O. & Backhed, F. Signals from the gut microbiota to distant organs
345 in physiology and disease. *Nat Med* **22**, 1079-1089 (2016).

346 12. Biagi, E*., et al.* Gut microbiota and extreme longevity. *Curr Biol* **26**, 1480-1485
347 (2016).

348 13. O'Toole, P.W. & Jeffery, I.B. Gut microbiota and aging. *Science* **350**, 1214-1215
349 (2015).

350 14. Gordon, L.B., Rothman, F.G., Lopez-Otin, C. & Misteli, T. Progeria: a paradigm
351 for translational medicine. *Cell* **156**, 400-407 (2014).

352 15. Puente, X.S., *et al.* Exome sequencing and functional analysis identifies BANF1
353 mutation as the cause of a hereditary progeroid syndrome. *Am J Hum Genet* **88**, 650-
354 656 (2011).

355 16. Osorio, F.G., *et al.* Splicing-directed therapy in a new mouse model of human
356 accelerated aging. *Science translational medicine* **3**, 106ra107 (2011).

357 17. Plovier, H., *et al.* A purified membrane protein from Akkermansia muciniphila
358 or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med*
359 **23**, 107-113 (2017).

360 18. Schneeberger, M., *et al.* Akkermansia muciniphila inversely correlates with the
361 onset of inflammation, altered adipose tissue metabolism and metabolic disorders
362 during obesity in mice. *Sci Rep* **5**, 16643 (2015).

363 19. Everard, A., *et al.* Cross-talk between Akkermansia muciniphila and intestinal
364 epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* **110**, 9066-9071
365 (2013).

366 20. Maharshak, N., *et al.* Altered enteric microbiota ecology in interleukin 10-
367 deficient mice during development and progression of intestinal inflammation. *Gut*
368 *Microbes* **4**, 316-324 (2013).

369 21. Varela, I., *et al.* Accelerated ageing in mice deficient in Zmpste24 protease is
370 linked to p53 signalling activation. *Nature* **437**, 564-568 (2005).

371 22. Rothschild, D., *et al.* Environment dominates over host genetics in shaping
372 human gut microbiota. *Nature* **555**, 210-215 (2018).

373 23. Lloyd-Price, J., *et al.* Strains, functions and dynamics in the expanded Human
374 Microbiome Project. *Nature* **550**, 61-66 (2017).

375 24. Goodrich, J.K., *et al.* Human genetics shape the gut microbiome. *Cell* **159**, 789-
376 799 (2014).

377 25. Gopalakrishnan, V., *et al.* Gut microbiome modulates response to anti-PD-1
378 immunotherapy in melanoma patients. *Science* **359**, 97-103 (2018).

379 26. Routy, B., *et al.* Gut microbiome influences efficacy of PD-1-based
380 immunotherapy against epithelial tumors. *Science* **359**, 91-97 (2018).

381 27. Kalabis, J., Rosenberg, I. & Podolsky, D.K. Vangl1 protein acts as a
382 downstream effector of intestinal trefoil factor (ITF)/TFF3 signaling and regulates
383 wound healing of intestinal epithelium. *J Biol Chem* **281**, 6434-6441 (2006).

384 28. Barcena, C., *et al.* Methionine restriction extends lifespan in progeroid mice and
385 alters lipid and bile acid metabolism. *Cell Rep* **24**, 2392-2403 (2018).

386 29. Ridaura, V.K., *et al.* Gut microbiota from twins discordant for obesity modulate
387 metabolism in mice. *Science* **341**, 1241214 (2013).

388 30. Cox, L.M., *et al.* Altering the intestinal microbiota during a critical
389 developmental window has lasting metabolic consequences. *Cell* **158**, 705-721 (2014).

390 31. Vrieze, A., *et al.* Transfer of intestinal microbiota from lean donors increases
391 insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913-
392 916 e917 (2012).

393 32. Wang, Y., *et al.* Fecal microbiota transplantation for refractory immune
394 checkpoint inhibitor-associated colitis. *Nat Med* **24**, 1804-1808 (2018).

395 33. van Nood, E., *et al.* Duodenal infusion of donor feces for recurrent Clostridium
396 difficile. *N Engl J Med* **368**, 407-415 (2013).

397 34. Bodogai, M., *et al.* Commensal bacteria contribute to insulin resistance in aging
398 by activating innate B1a cells. *Science translational medicine* **10**(2018).

399 35. Weingarden, A.R., *et al.* Microbiota transplantation restores normal fecal bile
400 acid composition in recurrent Clostridium difficile infection. *Am J Physiol Gastrointest*
401 *Liver Physiol* **306**, G310-319 (2014).

402 36. Buffie, C.G., *et al.* Precision microbiome reconstitution restores bile acid
403 mediated resistance to Clostridium difficile. *Nature* **517**, 205-208 (2015).

404 37. Zarrinpar, A., *et al.* Antibiotic-induced microbiome depletion alters metabolic
405 homeostasis by affecting gut signaling and colonic metabolism. *Nat Commun* **9**, 2872
406 (2018).

407 38. de Aguiar Vallim, T.Q., Tarling, E.J. & Edwards, P.A. Pleiotropic roles of bile
408 acids in metabolism. *Cell Metab* **17**, 657-669 (2013).

409 39. Pierre, J.F., *et al.* Activation of bile acid signaling improves metabolic
410 phenotypes in high-fat diet-induced obese mice. *Am J Physiol Gastrointest Liver*
411 *Physiol* **311**, G286-304 (2016).

412 40. Postler, T.S. & Ghosh, S. Understanding the holobiont: how microbial
413 metabolites affect human health and shape the immune system. *Cell Metab* **26**, 110-130
414 (2017).

415

416

417

418

419

420

421

422 **Figure legends**

423 **Figure 1. Gut dysbiosis in *Lmna*$^{G609G/G609G}$ and *Zmpste24*$^{-/-}$ progeroid mice.** (**a**)
424 Principal coordinates analysis (PCoA) of beta-diversity using the Bray-Curtis
425 dissimilarity metric among samples of the five groups of mice analyzed ($P$ = 0.001;
426 PERMANOVA). G609G 4mo mice show statistical differences with each of the other
427 groups (see **Supplementary Table 1**). Each dot represents an individual mouse. PCo1
428 and PCo2 represent the percentage of variance explained by each coordinate (WT 1mo,
429 n = 6; G609G 1mo, n = 5; WT 4mo, n = 8; G609G 4mo, n = 9; WT 22mo, n = 8). (**b**)
430 Average relative abundance of prevalent microbiota at the class level in the 5 groups
431 studied: 1-month-old wild-type (WT 1mo, n = 6), 1-month-old *Lmna*$^{G609G/G609G}$ (G609G
432 1mo, n = 5), adult 4-month-old wild-type (WT 4mo, n = 8), 4-month-old *Lmna*$^{G609G/G609G}$
433 (G609G 4mo, n = 9) and 22-month-old wild-type (WT 22mo, n = 8). The low abundance
434 bacteria group includes all bacterial classes with less than 0.5% of total abundance. (**c**)
435 Taxonomic cladogram obtained from LEfSe analysis showing bacterial taxa (phylum,
436 class and order) that were differentially abundant in progeroid and WT mice at 4 months
437 of age. Red indicates increased abundance in progeroid mice, grey indicates increased
438 abundance in WT mice. Def: Deferribacteres (phylum), Deferribacteres (order),
439 Deferribacterales (class); Cya: Cyanobacteria (phylum); Pseudo: Pseudomonadales;
440 Entero: Enterobacteriales; Burkho: Burkholderiales. (**d**) Results of LEfSe analysis
441 showing bacterial taxa that were significantly different in abundance between G609G and
442 WT mice at 4 months of age. For c and d: WT 4mo, n = 8; G609G 4mo, n = 9. (**e**)
443 Validation using qPCR of the differences in abundance of *E. coli* (W = 6, $P$ = 0.0046)
444 and *A. muciniphila* (W = 52, $P$ = 0.03) between WT (n = 8) and G609G mice (n = 8) at
445 10 weeks of age. Two-tailed unpaired Wilcoxon rank-sum test. Each dot represents an
446 individual mouse. In the box plots, upper and lower hinges correspond to the first and
447 third quartiles, center line represents the median, whiskers indicate the highest and lowest
448 values that are within 1.5 * IQR, and data beyond the end of the whiskers are outliers and
449 plotted as points. (**f**) PCoA of beta-diversity using the Bray-Curtis dissimilarity metric
450 between *Zmpste24*$^{-/-}$ and WT mice ($P$ = 0.003, PERMANOVA). Each dot represents an
451 individual mouse. PCo1 and PCo2 represent the percentage of variance explained by each
452 coordinate. (**g**) Average relative abundance of prevalent microbiota at the class level in
453 WT and *Zmpste24*$^{-/-}$ mice. (**h**). LEfSe analysis showing bacterial classes that were
454 significantly different in abundance between *Zmpste24*$^{-/-}$ and WT mice. For f-h: 4-month-

455 old WT mice, n = 8 (same as panel a-d); 4-month-old *Zmpste24*$^{-/-}$ mice, n = 4. p: phylum;
456 c: class; o: order; f: family; g: genus.

457

458 **Figure 2. Alterations in the gut microbiome in progeria patients and long-lived**
459 **humans.** (**a,b**) PCoA of beta-diversity using the Bray-Curtis dissimilarity metric among
460 fecal samples analyzed by (**a**) health status (*P* = 0.5, PERMANOVA) (Control, n = 9
461 individuals; Progeria, n = 5 individuals) and (**b**) family (*P* = 0.001, PERMANOVA)
462 (Family A, n = 3; Family B, n = 3; Family C, n = 3; Family D, n = 2; Family E, n = 3).
463 Each dot represents one person. PCo1 and PCo2 represent the percentage of variance
464 explained by each coordinate. (**c**). Results of LEfSe analysis showing bacterial genera
465 whose abundance significantly differed between progeria patients and their healthy
466 siblings (Control=9 individuals; Progeria=5 individuals). (**d**) PCoA of beta-diversity
467 using the Bray-Curtis dissimilarity metric of healthy controls (HC; n = 14 individuals)
468 and centenarians (Ce; n = 17 individuals) (*P* = 0.001, PERMANOVA). Each dot
469 represents one person. (**e**) Average relative abundance of prevalent microbiota at the class
470 level in centenarians (Ce; n = 17 individuals) and healthy controls (HC; n = 14
471 individuals). The low abundance bacteria group includes all bacterial classes with less
472 than 0.5% of total abundance. (**f**) Results of LEfSe analysis showing bacteria, at the
473 lowest taxonomic level, that were significantly different in abundance in centenarians
474 (Ce; n = 17 individuals) vs healthy controls (HC; n = 14 individuals). p: phylum; c: class;
475 o: order; f: family; g: genus.

476

477 **Figure 3. Effects of fecal microbiota transplantation in progeroid and WT mice** (**a**)
478 Scheme of the experimental design, in which the effects of FMT were assessed using 4
479 different groups of progeroid mice: control untransplanted *Lmna*$^{G609G/G609G}$ mice (n = 11),
480 *Lmna*$^{G609G/G609G}$ mice transplanted with fecal microbiota from WT mice (*Lmna*$^{G609G/G609G}$-
481 WTmic; n = 11), *Lmna*$^{G609G/G609G}$ mice transplanted with microbiota from older
482 *Lmna*$^{G609G/G609G}$ mice (*Lmna*$^{G609G}$-oG609Gmic; n = 11) and *Lmna*$^{G609G/G609G}$ mice
483 subjected to ablation of their own microbiota and transplanted with empty buffer
484 (*Lmna*$^{G609G}$-EmptyT; n = 8). Transplants were carried on at ~8-10 weeks of age, using as
485 donors 4 months-old WT and 4 months-old *Lmna*$^{G609G/G609G}$ mice. (**b**) Comparison of the
486 percentage of initial body weight between *Lmna*$^{G609G/G609G}$ (n = 11) and *Lmna*$^{G609G/G609G}$-
487 WTmic (n = 11) mice over the indicated time period. Differences in body weight over

488 time were assessed with a linear mixed model and analyzed with an Anova Type II Wald
489 Chi-square test ($X^2$ = 8.06, df = 1, $P$ = 0.0045). Data are presented as mean ± SEM. (**c**)
490 Box plots showing differences in the body temperature between WT (n = 4),
491 *Lmna*$^{G609G/G609G}$ (n = 6) and *Lmna*$^{G609G/G609G}$-WTmic (n = 5) mice. One-way ANOVA
492 with Tukey's correction (F = 20.45, df = 2, $P$ = 0.0001). Exact adjusted p-values are
493 reported within the plot. (**d**) Comparison of the glucose levels between *Lmna*$^{G609G/G609G}$
494 (n = 6) and *Lmna*$^{G609G/G609G}$-WTmic (n = 5) mice over the indicated time period. Unpaired
495 two-tailed Student's *t*-test (for week 5: t = 2.74, df = 36, $P$ = 0.009). (**e**) Representative
496 histological images of renal vasculature, showing increased perivascular fibrosis in
497 *Lmna*$^{G609G/G609G}$ mice (n = 10) (blue staining indicated with an arrow) compared to WT
498 (n = 10) and *Lmna*$^{G609G/G609G}$ mice transplanted with WT microbiota (*Lmna*$^{G609G}$-WTmic;
499 n = 8). Renal fibrosis scores were analyzed with a Kruskall-Wallis test with Dunn's
500 correction. Exact adjusted p-values are reported within the plot. Each dot represents a
501 single mouse. The horizontal line represents the mean ± 95% of confidence interval (CI).
502 Scale bar = 100 μm. (**f**) Percentage survival of *Lmna*$^{G609G/G609G}$ (n = 11), *Lmna*$^{G609G/G609G}$-
503 WTmic (n = 11) and *Lmna*$^{G609G/G609G}$ mice transplanted with old *Lmna*$^{G609G/G609G}$
504 microbiota (*Lmna*$^{G609G}$-oG609Gmic; n = 11). Differences were analyzed with the Log-
505 rank Mantel-Cox test and BH correction was applied after pairwise comparisons between
506 all experimental groups, including Empty transplant (Extended Data Fig. 6f). Hazard ratio
507 (HR) was calculated using a Cox proportional model. For *Lmna*$^{G609G/G609G}$-WTmic vs
508 *Lmna*$^{G609G/G609G}$, HR of 0.2 [95% confidence interval (CI) 0.07–0.53], $P$ = 0.0012; for
509 *Lmna*$^{G609G/G609G}$-oG609Gmic vs *Lmna*$^{G609G/G609G}$, HR of 4.1 [95% CI 1.5–11.1], $P$ =
510 0.005. Median and maximal survival, percentage of median and maximal lifespan
511 extension and log-rank test adjusted p-values are indicated in the Kaplan-Meier plot.
512 Transplantation was performed starting at ~8-10 weeks of age. (**g**) Comparison of body
513 weight between male WT mice transplanted with progeroid microbiota (WT-
514 oG609Gmic; n = 7) and male WT controls (n = 7) over the indicated time period.
515 Differences of body weight over time were assessed with a linear mixed model and
516 analyzed with an Anova Type II Wald Chi-square test ($X^2$ = 5.49, df = 1, $P$ = 0.019). Data
517 is presented as mean ± SEM. (**h**) Blood glucose levels of WT mice (n = 14; 7 males and
518 7 females) and WT-oG609Gmic (n = 14; 7 males and 7 females). Unpaired two-tailed
519 Student's *t*-test (t = 4.56, df = 26, $P$ = 0.0001). Each dot represents a single mouse. The
520 horizontal line represents the mean ± 95% CI. (**i-k**) Metabolic parameters, measured with

521 an Oxymax system, of WT (n = 14; 7 males and 7 females) and WT-oG609Gmic (n = 14;
522 7 males and 7 females) mice. Differences were analyzed with an unpaired two-tailed
523 Welch's *t*-test. (**i**) Volume of $O_2$ consumed ($VO_2$) (t = 2.71, df = 28, *P* = 0.011). (**j**)
524 Volume of $CO_2$ produced ($VCO_2$) (t = 3.94, df = 23, *P* = 0.0006). (**k**) Energy expenditure
525 (EE) (t = 3.15, df = 25, *P* = 0.0043). (**l**) Representative pictures of control *Zmpste24*$^{-/-}$
526 mice and *Zmpste24*$^{-/-}$ mice transplanted with WT microbiota (*Zmpste24*$^{-/-}$-WTmic).
527 Transplanted mice appear to be healthier, as manifested by an ameliorated cervicothoracic
528 lordokyphosis, a larger size and better grooming. (**m**) Body weight at 35 weeks of life of
529 *Zmpste24*$^{-/-}$ mice (n = 4) and *Zmpste24*$^{-/-}$-WTmic mice (n = 6). Unpaired two-tailed
530 Student's *t*-test (t = 4.31, df = 8, *P* = 0.0026). (**n**) Blood glucose levels of *Zmpste24*$^{-/-}$ mice
531 (n = 5) and *Zmpste24*$^{-/-}$-WTmic mice (n = 7) at 30 weeks. Unpaired two-tailed Student's
532 *t*-test (t = 5.47, df = 11, *P* = 0.0002). (**o**) Percentage survival of *Zmpste24*$^{-/-}$ (n = 7) and
533 *Zmpste24*$^{-/-}$-WTmic female mice (n = 7). Differences were performed with the Log-rank
534 Mantel-Cox test (*P* = 0.0092). HR of 0.15 [95% CI 0.03-0.75], *P* = 0.021. Median and
535 maximal survival, percentage of median and maximal lifespan extension and exact p-
536 value are indicated in the Kaplan-Meier plot. Transplantation was performed starting at
537 ~7-10 weeks of age. For c, d, i-k, m and n: box plots show upper and lower hinges
538 corresponding to the first and third quartiles, center line represents the median, whiskers
539 indicate the highest and lowest values that are within 1.5 * IQR, and data beyond the end
540 of the whiskers are outliers and plotted as points. Each dot represents a single mouse.

541

542 **Figure 4. *Akkermansia muciniphila* supplementation in progeroid mice and**
543 **metabolomic analysis of ileal content.** (**a**) Percent survival of *Lmna*$^{G609G/G609G}$ mice
544 receiving *A. muciniphila* by oral gavage (AKK; n = 9) compared to *Lmna*$^{G609G/G609G}$ mice
545 (G609G; n = 12). *A. muciniphila* transplantation was performed starting at 7-8 weeks of
546 age. Differences in survival were analyzed with Log-rank Mantel-Cox test (*P* = 0.0163).
547 Hazard ratio (HR) was calculated using a Cox proportional model (HR of 0.31 [95%
548 confidence interval (CI) 0.11–0.86], *P* = 0.0244). (**b**) Relative expression levels of *Reg3g*
549 and *Tff3* in G609G (n = 8) and AKK (n = 7) mice. Differences were analyzed with an
550 unpaired two-tailed Student's *t*-test. For *Reg3g*, t = 2.33, df = 13, *P* = 0.0365; for *Tff3*, t
551 = 2.42, df = 13, *P* = 0.0306. Data are represented as dots (one per mouse) with mean ±
552 95% of CI. (**c**) Left, representative histological images of the intestinal mucosa layer of
553 G609G and AKK mice. Arrow indicates the thickness of the mucosa layer. Scale bar =

554 100 μm. Right, comparison of mucosa layer thickness of G609G (n = 8) and AKK (n =
555 7) mice. Differences were calculated using an unpaired two-tailed Welch's *t*-test (t = 2.72,
556 df = 12, *P* = 0.018). Data are represented as dots (one per mouse) with mean ± 95% of
557 CI. For b and c, G609G group was composed by 4 males and 4 females, and AKK group
558 was composed by 4 males and 3 females. (**d**) Metabolic set enrichment analysis of all
559 metabolites with statistically significant differences between the three groups: WT (n =
560 8), $Lmna^{G609G/G609}$ (n = 8) and $Lmna^{G609G/G609G}$-WTmic (n = 8), using all annotated KEGG
561 pathways (see Methods). (**e**) Box plots showing the relative levels of different bile acids
562 in WT (n = 8), $Lmna^{G609G/G609}$ (n = 8), and $Lmna^{G609G/G609G}$-WTmic (n = 8). CA: cholic
563 acid; CDCA: chenodeoxycholic acid; βMCA: beta-muricholic acid; DCA: deoxycholic
564 acid; HCA: hyocholic acid; ωMCA: omega-muricholic acid; UDCA: ursodeoxycholic
565 acid; 12-KCDCA: 12-ketochenodeoxycholic acid. (**f**) Box plots showing the relative
566 levels of selected metabolites between WT (n = 8), $Lmna^{G609G/G609G}$ (n = 8) and
567 $Lmna^{G609G/G609G}$-WTmic (n = 8). For e and f, each group of 8 mice was composed by 4
568 males and 4 females. In both panels, differences were analyzed using a one-way ANOVA
569 with multiple comparison test with one-side relative to control $Lmna^{G609G/G609G}$ mice.
570 Exact adjusted p-values are reported in each plot. In the box plots, upper and lower hinges
571 correspond to the first and third quartiles, center line represents the median, whiskers
572 indicate the highest and lowest values that are within 1.5 * IQR, and data beyond the end
573 of the whiskers are outliers and plotted as points. Each dot represents a single mouse. (**g**)
574 Schematic representation of the model proposed in this work. WT mice are characterized
575 by a gut symbiosis in which bacterial metabolites participate in systemic homeostasis. By
576 contrast, progeroid mice are characterized by a gut dysbiosis, leading to decreased bile
577 acid levels and a reduced healthspan and lifespan. FMT with WT microbiota (WT mic)
578 into progeroid mice raises bile acids levels and improves healthspan and lifespan.

579

580

581 **Methods**

582 *Mouse models*

583 Both *Lmna*$^{G609G/G609G}$ and *Zmpste24*$^{-/-}$ mice were generated by crossing
584 *Lmna*$^{G609G/+}$ and *Zmpste24*$^{+/-}$ mice and genotyped in our laboratory as previously
585 described[16,21]. All mice used in this study were in C57BL/6N background. Mice were
586 caged separately by sex and transplantation group and checked daily for water and food
587 availability, as well as for good physical condition. Mice were housed in cages with solid
588 floors, sawdust and nests. Mice in all groups were given every day pellets of food
589 previously softened in water for 1–2 hours to facilitate the feeding of progeroid mice. All
590 components of the cages, including food, had been previously autoclaved. For glucose
591 determination, blood samples were obtained from the tail vein and measured with Accu-
592 Chek glucometer (Roche Diagnostics; Mannheim, Germany). Body temperature was
593 measured by rectal probe (Acorn® Temp TC Thermocouple Thermometer, Fisher
594 Scientific; Hampton, NH). Transplantation experiments in progeroid mice began at ~6-
595 10 weeks of age. In the *Lmna*$^{G609G/G609G}$ survival experiments, 11 mice were analyzed in
596 control (7 males and 4 females), transplanted with WT (5 males and 6 females) and
597 transplanted with *Lmna*$^{G609G/G609G}$ groups (5 males and 6 females), whereas 8 mice (3
598 males and 5 females) were analyzed in the empty transplant group. *Zmpste24*$^{-/-}$
599 transplantation experiments were performed with 7 females per group. In the WT
600 transplantation experiments, 14 animals per group were used (7 males and 7 females). In
601 the WT and *Lmna*$^{G609G/G609G}$ validation experiment, 8 mice (4 males and 4 females) were
602 used in each group of transplanted mice, and pooled samples from 12 WT (6 males and 6
603 females) and 15 *Lmna*$^{G609G/G609G}$ mice (8 males and 7 females) were used as donors.
604 Survival curves were analyzed with Log-rank (Mantel-Cox) test. Maximum survival was
605 analyzed by Fisher's exact test at 80$^{th}$ percentile[41]. All animal experiments were approved

606 by the Committee for Animal Experimentation of the Universidad de Oviedo (Spain) and
607 performed in accordance with the European and Spanish legislative and regulatory
608 guidelines (European convention ETS 1 2 3, on the use and protection of vertebrate
609 mammals in experimentation and for other scientific purposes, and Spanish Law 6/2013,
610 and R.D. 53/2013 on the protection and use of animals in scientific research), making
611 every effort to minimize mouse discomfort.

612 *Human samples*

613 We obtained samples from four HGPS families and one NGPS family that include
614 individuals affected with progeria and their healthy siblings. Additionally, we collected
615 samples from a Spanish cohort composed by 17 centenarians, independently of their
616 health status, and 14 healthy ethnically-matched adults, aged 30–50 years and with no
617 history of any major disease. Research involving humans was approved by the Ethical
618 Committee of Regional Clinical Research of the Principality of Asturias, project
619 no.105/16. All participants read and signed an informed consent.

620 *Preparation of 16S DNA for metagenome profiling*

621 Mouse feces were collected for 4 h and immediately kept at -80 °C until DNA
622 extraction. For human studies, samples were picked and stored at –20 ºC in OmniGene
623 Gut kits (Ref. OMR-200, DNA Genotek; Ora Sure Techonologies; Bethlehem, PN). In
624 all cases (mice and human samples), DNA was extracted using the PowerSoil DNA
625 Isolation kit (MO BIO Laboratories, Quiagen N.V.; Hilden, Germany). DNA quality and
626 quantification were assessed with Qubit fluorometer (Thermo Fisher Scientific; Waltham,
627 MA). Libraries were prepared following the 16S Metagenomic Sequencing Library
628 Preparation protocol from Illumina. Briefly, the region V3-V4 from 16S rRNA was
629 amplified using the primers 341F/805R to which Illumina Sequencing adapters and dual-

630 index barcodes of the Nextera XT kit were added (FWD 5'-
631 TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCCTACGGGNGGCWGCAG-
632 3’ and REV 5'-GTCTCGTGGGCTCGGAGA
633 TGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3’). Sequencing was
634 performed in a MiSeq platform (IMEGEN, Valencia, Spain) using 2 x 300 bp-end
635 protocol.

636 *Metagenome profiling*

637 Raw paired-end reads were processed with QIIME 2 (version 2018.6.0). Sequence
638 quality controls were performed with DADA2 (qiime dada2 denoise-paired, with a
639 number of expected errors higher than 6): reads were filtered, trimmed, denoised,
640 dereplicated, forward and reverse sequences were merged, and chimeras were removed.
641 Taxonomy was assigned using a pre-trained Naïve Bayes classifier, with a trimmed
642 version of Greengenes 13_8 99% OTUs, which includes the V3-V4 regions, bounded by
643 the 341F/805R primer pair. For *Lmna*$^{G609G/G609G}$ model, we obtained 3,071,354 paired-
644 end reads of 300 nucleotides each, with 1,084 OTUs identified after quality filtering
645 (Extended Data Fig 1a), and for *Zmpste24*$^{-/-}$ model, we obtained 1,149,187 paired-end
646 reads of 300 nucleotides each, with 882 OTUs identified after quality-filtering (Extended
647 Data Fig 3a). 4-month-old C57BL/6N WT mice in experiments from Figure 1 where used
648 as controls for both *Lmna*$^{G609G/G609G}$ and *Zmpste24*$^{-/-}$ mice. For human samples in progeria
649 patients, we obtained 1,709,578 paired-end reads of 300 nucleotides each, with 691 OTUs
650 identified after quality filtering (Extended Data Fig 4a), whereas for centenarians and
651 healthy controls we obtained 6,196,891 paired-end reads of 300 nucleotides each, with
652 1,761 OTUs identified after quality-filtering (Extended Data Fig 5a). For validating FMT
653 experiments 8 mice per group and condition were used, obtaining 8,761,200 paired-end
654 reads of 300 nucleotides each, with 18,402 OTUs identified after quality filtering. Alpha

655 diversity and statistical analysis were calculated based on different metrics (shannon,
656 chao1). Beta diversity was measured using Bray-Curtis dissimilarity and Jaccard
657 similarity index (braycurtis, jaccard). Samples were hierarchical clustered with the
658 Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and using
659 different beta diversity metrics (braycurtis, jaccard). Differences in bacteria abundance
660 were calculated using LEfSe[42]. Metagenome functional content prediction was performed
661 using PICRUSt[43] and HUMAnN2 (v0.11.1)[44], and analyzed with LEfSe.

662 *Microbiome transplants*

663 Prior to transplantation, mice were treated for three consecutive days with 200 μL
664 of an antibiotic-cocktail (with each daily dose being administered by oral gavage after a
665 6-hour fast) which contained 1 g/L ampicillin, 0.5 g/L neomycin, 0.5 g/L vancomycin and
666 1 g/L metronidazole. Thereafter mice were given 100 μL of the microbiome suspension
667 twice a week for 2 weeks, starting the first day after the antibiotic cycle. After this 2-week
668 period mice received the microbiome suspension once a week until natural death or
669 sacrifice. For the microbiome suspension preparation, 2-5 fresh feces pellets (80-100 mg)
670 were resuspended with a vortex in 600 μL of reduced PBS (PBS with 0.5 g/L cysteine
671 and 0.2 g/L $Na_2S$). After resuspension, tubes containing the feces in reduced PBS were
672 centrifuged at 2,500 rpm (500 g) for 1 min to remove insolubilized material, and 100 μL
673 of supernatant were administered to the mice by oral gavage. Empty transplant group
674 received the same antibiotics treatment and were transplanted only with reduced PBS.

675 *Calorimetry measurements*

676 Metabolic parameters (oxygen consumption, carbon dioxide production and total
677 energy expenditure), were obtained using the comprehensive lab animal monitoring
678 system (Oxymax CLAMS, Columbus Instruments; Columbus, OH) and analyzed

679 following the manufacturer's instructions. Mice were monitored for 48 h and the first 24
680 h were discarded in the analysis, considering them as acclimation period.

681 *Akkermansia muciniphila culture and oral supplementation*

682 Cultures of the strain *Akkermansia muciniphila* CIP107961 grown for 24 h in
683 GAM medium (Nissui Pharmaceutical Co; Tokyo, Japan) supplemented with 0.25%
684 (w/v) L-cysteine (Sigma Chemical Co.; St. Louis, MO) (GAMc) in anaerobic conditions
685 were used to inoculate (2% v/v) fresh pre-reduced GAMc broth which was incubated for
686 24 h. Afterwards, cultures were washed with PBS and concentrated in anaerobic PBS that
687 included 25% (v/v) glycerol to a concentration of about $1x10^{10}$ cfu/mL under strict
688 anaerobic conditions and stored at −80 °C until use. To test the viability of glycerol
689 stocks, serial dilutions in PBS were made and deep plated on agar-GAMc. Plates were
690 incubated under anaerobic conditions for 5 days to determine the *Akkermansia* counts
691 (cfu/ml). Before administration by oral gavage, the glycerol stocks were thawed under
692 anaerobic conditions and diluted with anaerobic PBS to a final concentration of $2x10^8$
693 viable cfu/0.1 mL. *Lmna*$^{G609G/G609G}$ mice were treated by oral gavage with 100 µL of either
694 *Akkermansia* suspension (AKK group, n = 9) or anaerobic PBS (control group, n=12)
695 three days a week beginning at 12 weeks of age and until decease.

696 *Quantitative polymerase chain reaction*

697 For RNA expression analysis, total RNA from about 30 mg of frozen ileon
698 samples was extracted using Trizol (Life Technologies) and resuspended in nuclease-free
699 water (Life Technologies). 1–2 µg of total RNA was used for reverse transcription using
700 the QuantiTect Reverse Transcription kit (Quiagen N.V.). 10× diluted cDNA was used
701 for quantitative polymerase chain reaction (qPCR) using Power SYBR Green PCR
702 Master Mix (Life Technologies; Carlsbad, CA) and Real-Time PCR (7300 HT, Applied

703 Biosystems; Foster City, CA). Gene expression was normalized to the GAPDH
704 expression. For bacterial quantification, DNA from mouse feces was extracted as
705 described above. 1– 4 ng of DNA were used for qPCR reactions using specific primers to
706 amplify bacterial 16S rDNA. Bacterial abundance was assessed by normalizing with the
707 abundance of total bacteria in feces using the conserved eubacterial 16S rDNA primer
708 pair UniF340/UniR514. Results are represented as relative quantification using RQ value
709 ($RQ=2^{-\Delta\Delta Ct}$). Primer sets for qPCR analyses are shown in Supplementary Table 4.

710 *Metabolomic analysis*

711 Prior to sacrifice for sample collection, mice were starved overnight and thereafter
712 allowed to eat for 4 h. 30 mg of ileum content for each condition were first weighted and
713 solubilized into 1 mL polypropylene Precellys lysis tubes, with 1 mL of cold lysate buffer
714 (MeOH/Water/Chloroform, 9/1/1, -20 °C). After being vortexed for 10 min, samples were
715 centrifuged (10 min at 15,000 g, 4 ºC), and the upper phase was collected and split in two
716 parts: the first 270 µL used for the Gas Chromatography coupled to Mass Spectrometry
717 (GC-MS) and 250 µL used for the Ultra High Pressure Liquid Chromatography coupled
718 to Mass Spectrometry (UHPLC-MS). For GC-MS measurements, 150 µL from the
719 aliquot were transferred to a glass tube and evaporated. Then, 50 µL of methoxyamine
720 (20 mg/mL in pyridine) was added to dried extracts and samples were then stored at room
721 temperature in the dark for 16 h. The day after, 80 µL of MSTFA was added and final
722 derivatization carried out at 40 ºC during 30 min. Samples were then transferred in vials
723 and directly injected into GC-MS. For LC-MS measurements, the collected supernatant
724 was evaporated in microcentrifuge tubes at 40 ºC in a pneumatically-assisted concentrator
725 (Techne DB3, Techne; Staffordshire, UK). Dried extracts were solubilized with 450 µL
726 of MilliQ water and aliquoted in 3 microcentrifuge tubes (100 µL) for each LC method

727 and one microcentrifuge tube for safety. Aliquots were transferred to LC vials and
728 injected into LC-MS or kept at -80 ºC until injection. A daily qualification of the
729 instrumentation was set up with automatic tune and calibration processes. These
730 qualifications were completed with double injections of standards mixes, at the beginning
731 and at the end of the run, as for a blank extracted sample to control the background
732 impurities. Mixtures were adapted for each chromatographic method. After the
733 extraction, fractions of each biological sample were pooled to create a Quality Control
734 (QC) sample, use to passivate the column before the analysis with the proper biological
735 matrix. This QC sample was re-injected in each batch to monitor and correct analytical
736 bias. Analytical methods and data processing were performed as previously described[45].
737 Results were represented as the normalized area of the MS picks in log2 scale using
738 arbitrary units. Normalization was performed by correcting the area of the MS picks
739 across the batches using the QC pooled samples and by centering their values around the
740 mean of the QC areas. Standard reagents (acetonitrile, methanol, chloroform, acetic acid
741 and dibutylamine acetate concentrate) were acquired from Sigma Aldrich (Saint Luis,
742 MO). Differentially expressed metabolites in each condition were identified using
743 moderate t-statistic implemented in the R/Bioconductor package limma[46], using sex as a
744 covariate. Metabolites with a nominal $P$-value $< 0.05$ and q-value $< 0.25$ were selected
745 for metabolic set enrichment analysis using one-sided Fisher's exact test against all
746 metabolites annotated in each KEGG pathway. Metabolomic results are provided in
747 Supplementary Table 5.

748 *Histological analysis*

749 Kidneys and intestines were fixed in 4% paraformaldehyde in PBS and stored in
750 50% ethanol. Fixed tissues were embedded in paraffin by standard procedures. Blocks
751 were sectioned (5 µm) and stained with hematoxylin and eosin and Masson trichrome

752 (H&E, MT, kidney) and periodic acid Schiff-alcian blue (PAS-AB, intestine). Renal
753 perivascular fibrosis was analyzed/graded from 0 to 4, by using a histology damage score
754 (0: no lesion; 1: focal lesion; 2: multifocal mild lesion; 3: multifocal moderate lesion; 4:
755 diffuse, moderate or severe damage). Five fields were scored from each slide.

756 *Statistical analysis*

757 Number of mice allocated per group was based on previous experiments and their
758 distribution was randomized, being indicated in each Figure legend. Comparisons
759 between two groups following normal distribution were performed using a two-tailed
760 Student's *t*-test, while one-factor analysis of variance (ANOVA) was used for
761 comparisons of three or more groups. Unless specified in the Figure legends, adjusted *p*-
762 values were obtained using Tukey's correction. For non-parametric distributions, the
763 Wilcoxon rank-sum and Kruskal-Wallis test (the latter followed by the Dunnett *post-hoc*
764 test) were performed for comparisons between two groups or three or more groups,
765 respectively. Survival analysis was performed by using the Kaplan-Meier method and
766 statistical differences were analyzed with the Log-rank (Mantel-Cox) test (GraphPad
767 Prism 6.0 and survival R package). Body weight curves were analyzed using a linear-
768 mixed effect model (lme4 R package). The hazard ratio was calculated using a Cox
769 proportional hazards regression model (survival R package). Sample sizes for lifespan
770 experiments were chosen with a power of 80%, based on our previous studies[16,28,47].
771 Maximal survival was calculated using Fisher's test at $80^{th}$ percentile. Plots were
772 generated with GraphPad Prism 6.0 and RStudio (using ggplot2 R package), and edited
773 with Illustrator CC (21.0.0). Statistical analysis was performed using RStudio and
774 GraphPad Prism 6.0. Exact P-values are indicated in each figure.

775 *Reporting summary*

Further information on research design is available in the Nature Research Life Sciences Reporting Summary linked to this article.

## Data availability statement

Sequence data supporting these findings have been deposited in EGA under with accession number EGAS00001003656. Metabolomics data are provided in the Supplementary Table 5. Any additional data generated and analyzed in this study are available from the corresponding authors upon reasonable request.

## Methods-only references


41. Wang, C., Li, Q., Redden, D.T., Weindruch, R. & Allison, D.B. Statistical methods for testing effects on "maximum lifespan". *Mech Ageing Dev* **125**, 629-632 (2004).

42. Segata, N., *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* **12**, R60 (2011).

43. Langille, M.G., *et al.* Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* **31**, 814-821 (2013).

44. Abubucker, S., *et al.* Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol* **8**, e1002358 (2012).

45. Enot, D.P., *et al.* Metabolomic analyses reveal that anti-aging metabolites are depleted by palmitate but increased by oleate in vivo. *Cell cycle* **14**, 2399-2407 (2015).

46. Ritchie, M.E., *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic acids research* **43**, e47 (2015).

47. Osorio, F.G., *et al.* Nuclear lamina defects cause ATM-dependent NF-kappaB activation and link accelerated aging to a systemic inflammatory response. *Genes Dev* **26**, 2311-2324 (2012).