

1 Gut Microbiome Pattern Reflects Healthy Aging and Predicts Extended Survival in 2 Humans

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20 **Abstract:** The gut microbiome has important effects on human health, yet its importance in
21 human aging remains unclear. Using two independent cohorts comprising 4582 individuals
22 across the adult lifespan we demonstrate that, starting in mid-to-late adulthood, gut microbiomes
23 become increasingly unique with age. This uniqueness pattern is strongly associated with gut
24 microbial amino acid derivatives circulating within the bloodstream, many of which have been
25 previously identified as longevity biomarkers. At the latest stages of human life, two distinct
26 patterns emerge wherein individuals in good health show continued microbial drift toward a
27 unique compositional state, while the same drift is absent in individuals who perform worse on a
28 number of validated health measures. The identified healthy aging pattern is characterized by an
29 overall depletion of core genera found across most humans - primarily a depletion in the nearly
30 ubiquitous genus *Bacteroides*. Consistently, retaining a high *Bacteroides* dominance into
31 extreme age, or, equivalently, having a low gut microbiome uniqueness score, predicts decreased
32 survival in a four-year follow-up. Our comprehensive analysis identifies the gut microbiome as a
33 novel component of healthy aging, with important implications for the world's growing older
34 population.
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44 Introduction

45 The human gut harbors a diverse microbial ecosystem that has increasingly been shown to play
46 an important role in host health¹⁻³. Despite considerable progress in our understanding of the gut
47 microbiome, very little is known about how it changes with age and how these changes interact
48 with host physiology. Furthermore, there is no consensus on whether or not age-associated
49 changes in the gut microbiome are related to the health state of the host. Importantly,
50 identification of aging patterns within the gut microbiome could have major clinical implications
51 for both monitoring and modifying gut microbiome health throughout life.

52 Several studies conducted on centenarian populations provided potential insight into gut
53 microbial trajectories associated with aging. Biagi *et al.*⁴ demonstrated that gut microbiomes of
54 centenarians (≤ 104 years of age) and supercentenarians (104+ years) show a depletion in core
55 abundant taxa (*Bacteroides*, *Roseburia* and *Faecalibacterium*, among others), complemented by
56 an increase in the prevalence of rare taxa. Similar findings have since been reported in other
57 centenarian populations across the world, such as in Sardinian, Chinese and Korean centenarians,
58 relative to healthy, younger controls⁵⁻⁷. Some studies have also reported higher α -diversity in
59 centenarians compared to younger individuals⁶⁻⁸, suggesting that the gut microbiome continues
60 to develop within its host even in the latest decades of human life.

61 Gut microbial associations reported in centenarians often contradict findings reported in
62 younger elderly populations. In particular, studies on the ELDERMET cohort (i.e. the most
63 extensively studied cohort of older persons with gut microbiome data to date) reported an
64 increased dominance of the core genera *Bacteroides*, *Alistipes* and *Parabacteroides* in those 65+
65 years old compared to healthy, younger controls⁹. Studies on older long-term care residents
66 further demonstrated a gradual change in microbiome composition associated with duration of
67 stay in the care facility, which has been attributed to changes in diet and lifestyle^{10,11}.
68 Collectively, these and other studies^{12,13} provide a view of the human gut microbiome as
69 relatively stable up until old age, at which point gradual compositional shifts occur that are
70 driven by dietary and lifestyle changes, as well as declining health.

71 The often-contradictory findings in elderly and centenarian populations indicate there
72 may exist multiple gut microbiome patterns of aging, some of which reflect better health and life
73 expectancy outcomes than others. Although recent analyses have demonstrated a link between
74 gut microbiome composition and long-term health outcomes^{3,14}, the scarcity of elderly cohorts
75 with longitudinal follow-up data, the lack of detailed molecular phenotyping and health metrics,
76 and the relatively small sample sizes of existing studies on aging limit our understanding of gut
77 microbial changes seen across the human lifespan. In the present study, we overcome these
78 limitations and present an analysis of the gut microbiome and phenotypic data from 4582
79 individuals spanning 18 to 98 years of age, with longitudinal follow-up data in an older cohort
80 that allowed us to track survival outcomes.

81 Results

82 We studied two distinct cohorts: a deeply phenotyped population of individuals who self-
83 enrolled in a scientific wellness company (the ‘Arivale cohort’, ages 18-87) (Table S1) and the
84 Osteoporotic Fractures in Men (MrOS) cohort (ages 78-98)¹⁵⁻¹⁷ (Table S2)(Fig. 1). These
85 cohorts further subdivide into two groups each. The MrOS cohort separates into a discovery
86 cohort (N=599) and a validation cohort (N=308), because stool samples from this population
87 were processed in two separate batches several years apart. The Arivale cohort separates into
88 Group A (N=2539) and Group B (N=1114), where the distinguishing factor is the use of
89

90 different vendors for the collection and processing of stool samples (see Methods). We began by
91 analyzing baseline data from the Arivale cohort to identify gut microbial aging patterns across
92 most of the adult human lifespan, and investigate how these patterns correspond to host
93 physiology. We then extended our analysis to the MrOS cohort, where we had detailed health
94 metrics and follow-up data on mortality, to evaluate how the patterns identified within the
95 Arivale cohort correspond to health and survival in the latter decades of human life.

96 97 ***A gut microbiome aging pattern that spans much of the adult lifespan***

98 To characterize gut microbial patterns associated with aging, we initially performed a β -diversity
99 analysis comparing all available baseline microbiome samples from a heterogeneous, and
100 relatively healthy Arivale population (**Fig.1** and **Table S1**). Our analysis involved extracting the
101 minimum value for each individual from a calculated Bray-Curtis dissimilarity matrix. This
102 value reflects how dissimilar an individual is from their nearest neighbor, given all other gut
103 microbiome samples in the cohort. We refer to this as a measure of ‘uniqueness’: the higher the
104 value, the more distinct the gut microbiome is from everyone else’s in the studied population.
105 Arivale participants showed initial drift toward an increasingly unique gut microbiome
106 composition starting between 40-50 years of age, and this continued to increase with every
107 passing decade (linear models adjusted for age, body mass index (BMI), sex and Shannon
108 diversity) (**Fig. 2A**). We replicated our analysis using additional β -diversity metrics. Uniqueness
109 based on Weighted UniFrac demonstrated a similar positive association with age across both
110 vendors, while Jaccard and Unweighted UniFrac metrics resulted in either a weaker association
111 (vendor B) or no association (vendor A) with age (**Fig. 2B**). These results indicate that the
112 observed age-related increase in uniqueness is likely not a result of the loss or acquisition of
113 microbial genera in older individuals, which would increase unweighted β -diversity and Jaccard
114 distance measures, but rather is driven more by shifts in relative abundance of microbes already
115 present in the ecosystem.

116 To further characterize the observed gut microbiome aging pattern, and understand how it
117 is reflected in host physiology and health, we combined data from both Arivale vendors (**Fig.**
118 **2C**) and tested the correspondence between Bray-Curtis uniqueness and a wide variety of clinical
119 laboratory tests, demographic information, and self-reported lifestyle/health measures, adjusting
120 for microbiome vendor (**Fig. 2D, Table S3**). Of all the factors tested, age demonstrated the
121 strongest association with gut microbial uniqueness. Several other factors were significantly
122 associated with uniqueness, but many of them were no longer significant after adjusting for age.
123 In fact, after adjusting for age, essentially only lipid markers and BMI remained significantly
124 associated with gut microbial uniqueness, with the direction of association indicating healthier
125 metabolic and lipid profiles in individuals with more unique gut microbiomes: e.g. lower BMI,
126 lower n6/n3 fatty acid ratio, higher high-density lipoprotein (HDL) cholesterol, lower low
127 density lipoprotein (LDL) cholesterol, higher vitamin D, and lower triglycerides in individuals
128 with more unique microbiomes (**Fig. 2D**). Interestingly, self-reported dietary measures showed
129 no association with our gut microbiome uniqueness score, suggesting that the identified gut
130 microbial aging pattern is not driven by self-reported differences in dietary habits.

131 Women have an extended average lifespan compared to men¹⁸, with previous studies
132 also indicating varying aging patterns across sex^{19,20}. To evaluate whether the observed
133 increased uniqueness with age is sex-dependent, we investigated the association of age with
134 Bray-Curtis uniqueness independently in men and women, adjusting for age, BMI, Shannon
135 diversity and microbiome vendor. Although both sexes showed a significant positive association

136 between age and our gut microbiome uniqueness score, women showed a nearly 50% greater β -
137 coefficient compared to men (adj. β (95%CI): men= 0.0088 (0.006, 0.012), women= 0.013
138 (0.010, 0.015), interaction term P -Value= 0.011), indicating that women's microbiomes become
139 more unique with age at a significantly faster rate.

140 141 ***Reflection of gut microbial uniqueness in the host metabolome***

142 Our research group has previously demonstrated a strong reflection of gut microbiome
143 community structure in the human plasma metabolome²¹. In order to better understand how host
144 physiology reflects the increasingly unique composition of the gut microbiome seen with aging,
145 and to gain potential mechanistic insight into the functional changes that take place in the
146 microbiota, we regressed our uniqueness measure against each of the 652 plasma metabolites
147 measured in the Arivale cohort, adjusting for age, age squared, sex, a sex*age interaction term,
148 BMI, vendor and Shannon diversity. A total of eight metabolites, all microbial in origin,
149 remained significantly associated with uniqueness after multiple hypothesis correction
150 (Bonferroni P -Value<0.05) (**Fig. 3A&B**). These metabolites fell primarily into one of two
151 classes: phenylalanine/tyrosine metabolites (phenylacetylglutamine, p-cresol glucuronide, p-
152 cresol sulfate) and tryptophan metabolites (3-indoxyl sulfate, 6-hydroxyindole sulfate and
153 indoleacetate). Interestingly, significant changes in both tryptophan and phenylalanine pathways
154 have been previously reported in centenarians relative to younger controls, with centenarians
155 showing greater activation of these pathways in the gut microbiome^{22,23}. The previously
156 identified longevity biomarker, phenylacetylglutamine²³, demonstrated the strongest
157 correspondence with gut microbial uniqueness in our analysis, explaining 8.4% of the variance
158 (adj. β (95%CI) = 0.015 (0.012,0.018), P -Value= 3.65e-20) (**Fig. 3C, Table S4**). These findings
159 indicate that the observed gut microbial drift towards a more unique compositional state seen
160 with age is characterized by alterations in microbial amino acid metabolism, which may serve as
161 a useful biomarker for gut microbiome shifts across the human lifespan.

162 163 ***Gut microbial pattern of healthy aging in latest decades of human life***

164 To better understand the long-term health implications of the identified aging dynamics of the
165 gut microbiome, we extended our analysis into a separate cohort of older men with paired health
166 and longitudinal follow-up data (the MrOS cohort). The MrOS study recruited older male
167 participants across the United States. At the fourth follow-up visit, a subset of the participants
168 provided stool samples for 16S rRNA sequencing of their gut microbiome (discovery cohort
169 N=599, validation cohort N=308)¹⁷. All participants who provided a stool sample exceeded 78
170 years of age at the time of sampling, allowing us to gain insight into the relationship between the
171 gut microbiome and host health at the latest decades of human life (**Fig.1 & Table S2**). Once
172 again, we calculated a uniqueness score for each individual using the Bray-Curtis dissimilarity
173 metric. Projecting MrOS microbiome data onto the first two Principal Coordinates revealed that
174 samples with the highest Bray-Curtis uniqueness tended to fall away from common microbiome
175 profiles, i.e. *Bacteroides* or *Prevotella* dominated ecosystems (**Fig. 4A-C**). In fact, the relative
176 abundance of *Bacteroides* showed a strong negative association with gut microbiome uniqueness
177 (Spearman Rho=0.73, **Fig. 4D**). The association was even more pronounced when the sum of
178 both *Bacteroides* and *Prevotella* abundances for each individual was compared to gut
179 microbiome uniqueness (Spearman Rho=0.80, **Figure S1A**).

180 Consistent with our initial analysis, age showed a trending positive association with our
181 uniqueness score in the MrOS cohort (Pearson's r =0.075, P -Value= 0.065). Unlike the Arivale

182 cohort, MrOS participants were considerably more health heterogenous at time of sampling, with
183 a large proportion of participants reporting chronic conditions (**Table S2**). The large health
184 heterogeneity of MrOS participants provided an opportunity to better understand whether the
185 observed increase in microbiome dissimilarity with age depends on host health. Hence, we re-ran
186 the above analysis under four different stratifications based on: medication use, self-perceived
187 health, life-space score (LSC), and walking speed. We chose these four health metrics because
188 collectively they encompass a diverse repertoire of health in older populations (**Table 1**).

189 Under all stratifications considered, we observed a stronger positive association between
190 age and microbiome uniqueness in healthier individuals, while the association was absent
191 altogether in individuals demonstrating worse health (**Fig. 4E**). We further generated a
192 composite stratification (composite healthy), where MrOS participants had to meet at least three
193 of the four criteria outlined above to be classified as healthy (**Table 1 & Table S2**). In this
194 limited group of 133 individuals we observed an even stronger association between gut microbial
195 uniqueness and age than under any individual stratification. We replicated the analysis on the
196 second batch of MrOS gut microbiome samples, which were processed independently several
197 years apart (validation cohort, N=308), demonstrating very similar results (**Fig. 4E**). We also ran
198 the same analysis using Weighted UniFrac dissimilarity, and observed high level of congruence
199 between results (**Fig. S1B**). In contrast, measures of α -diversity were not significantly associated
200 with age under any stratification considered (**Fig. S1B**).

201

202 ***Gut microbiome and mortality in extreme aging***

203 Next, we focused exclusively on community-dwelling individuals (i.e. excluding participants in
204 assisted living, nursing homes, and/or who have been hospitalized in the past 12 months) from
205 the two MrOS data sets, combined together for increased power (N=706) (**Fig. 1**). We performed
206 genus-level differential abundance analysis to identify genera associated with age in healthy
207 composite individuals (N=173) and the remainder of the cohort (N=533), separately, adjusting
208 for batch (discovery/validation) and BMI. In healthy composite individuals, only the genus
209 *Bacteroides* (adj. β (s.e.): -0.062 (0.017), *P-Value*=0.0006) demonstrated a significant negative
210 association with age after multiple hypothesis correction (**Fig. 5A**). These findings are consistent
211 with our gut dissimilarity analysis, where the uniting feature of unique microbiomes is the
212 depletion of the most common and dominant genera. Consistently, there was no significant
213 association between age and *Bacteroides* in participants who did not meet our health criteria (adj.
214 β (s.e.): -0.008 (0.009), *P-Value* =0.37) (**Fig. 5A**). In contrast, individuals in worse health
215 demonstrated a distinct gut microbiome aging pattern characterized by a decline in the genera
216 *Lachnospirillum* (adj. β (s.e.): -0.035 (0.0091), *P-Value* =0.0002) and the *Ruminococace*
217 family genus *UBA1819* (adj. β (s.e.): -0.074 (0.015), *P-Value* =2.57e-06) with age. These results
218 provide further evidence for the existence of multiple gut microbiome aging patterns in the later
219 stages of human life.

220 Given that our findings from both β -diversity and differential abundance analysis of
221 healthy elderly are consistent with observations previously reported in centenarians⁴, we utilized
222 longitudinal data from the MrOS cohort to investigate whether the observed gut microbiome
223 pattern of healthy aging is predictive of mortality. We performed the analysis in two steps: 1) on
224 all community-dwelling participants (N=706) and 2) only on community-dwelling participants in
225 the top age tertile (85+ years of age, N=257) at time of gut microbiome sampling, because these
226 participants were the closest to achieving extreme age in the course of the study's follow-up
227 period (~4 years). When focusing on all individuals in the cohort, we identified a significant

228 positive association between relative *Bacteroides* abundance and increased risk of all-cause
229 mortality, independent of age, BMI, clinical site, self-perceived health, diagnosis of congestive
230 heart failure, and batch in which stool samples were processed. Replicating the analysis in the
231 oldest individuals (85+ years old) revealed a stronger association and higher Hazard Ratios
232 compared to the whole cohort (**Fig. 5B-C**). Using the participants' calculated Bray-Curtis
233 uniqueness score yielded comparable results in 85+ year olds, where mortality risk decreased in
234 individuals with more unique gut microbiomes independent of the same covariates. In contrast,
235 the same association between Bray-Curtis uniqueness and mortality was not present when
236 younger participants were included in the analysis (**Fig. 5C**).

237

238 **Discussion**

239 There is a limited understanding of how the human gut microbiome changes throughout
240 adulthood and how these changes influence host physiology. Here, we evaluated gut microbial
241 patterns associated with aging across 4582 individuals from two distinct study populations
242 spanning 18-98 years of age. The major findings of our analysis were: 1) individual gut
243 microbiomes became increasingly more unique with age, starting in mid-to-late adulthood, and
244 this uniqueness was positively associated with known microbial metabolic markers for health
245 and longevity; 2) the increase in microbiome uniqueness with age occurred in both males and
246 females, but was 50% more pronounced in females; 3) in the later decades of human lifespan,
247 healthy individuals continued to show an increasingly unique gut microbial compositional state
248 (associated with a decline in core taxa) with age, while that pattern was absent in those in worse
249 health; 4) in individuals approaching extreme age (85+ years old), retaining high relative
250 *Bacteroides* abundance and having a low gut microbiome uniqueness score were both associated
251 with decreased survival in the course of 4 year follow-up. These observations are strengthened
252 by the presence of similar age-related trends in two separate cohorts, and the replication of
253 associations with health and longevity in a validation cohort.

254 Our findings indicate that healthy aging of the gut microbiome involves depletion of core
255 microbes and their replacement by less common taxa, resulting in increasingly distinct
256 microbiomes. These findings are consistent with patterns previously reported in centenarians
257 across the world^{6,7}, despite the fact that dominant genera (i.e. core microbiota) often vary across
258 cultures and geographic locations²⁴. Using our alternative beta-diversity approach, we provide
259 novel insight into the aging gut microbiome that a) validates across different vendors and cohorts
260 and b) is consistent with previous longevity research. It is quite possible that becoming
261 increasingly dissimilar as you age is a universal characteristic, independent of the variability in
262 core gut microbes observed across the world (e.g. *Bacteroides* vs. *Prevotella*). This would make
263 gut microbiome uniqueness an intriguing new dimension of healthy aging, and a critical new
264 component for personalized medicine and precision health.

265 The reflection of gut microbial uniqueness in plasma phenylalanine/tyrosine and
266 tryptophan microbial metabolites is consistent with our recent work showing a robust
267 relationship between the host blood metabolome and gut microbial diversity²¹. Both tryptophan
268 and phenylalanine metabolism have been implicated in longevity^{22,23,25}. Phenylacetylglutamine
269 and p-cresol sulfate demonstrated some of the strongest associations with gut microbial
270 uniqueness, independent of age and other covariates. These same metabolites were previously
271 proposed as biomarkers for healthy aging and longevity²³. Additional metabolites associated
272 with our observed gut microbial pattern were dominated by indoles, which are gut microbiome
273 degradation products of tryptophan. Indoles have been shown to increase healthspan and extend

274 survival in a number of animal models²⁶. Their most characterized mechanism of action is
275 mediating inflammation through binding the aryl hydrocarbon receptor²⁷. While further studies
276 are needed to establish a direct link between these microbial compounds and longevity in
277 humans, the elevated levels of these metabolites in circulation in individuals whose microbiomes
278 are more unique opens promising new leads into the role of the gut microbiome in aging.

279 Previous studies in older populations have suggested that gut microbial composition and
280 structure is generally stable throughout adulthood and into old age¹², at which point changes are
281 observed and further accelerated due to adverse health events and lifestyle changes (i.e. entering
282 long-term care facilities)^{10,11,13}. In sharp contrast, our findings suggest that gut microbiomes of
283 healthy individuals continue to develop throughout aging, and that it is the lack of this
284 development that appears to be associated with worse health and prognosis. As our
285 understanding of the aging microbiome increases, monitoring and identifying modifiable features
286 that may promote healthy aging and longevity will have important clinical implications for the
287 world's growing older population.

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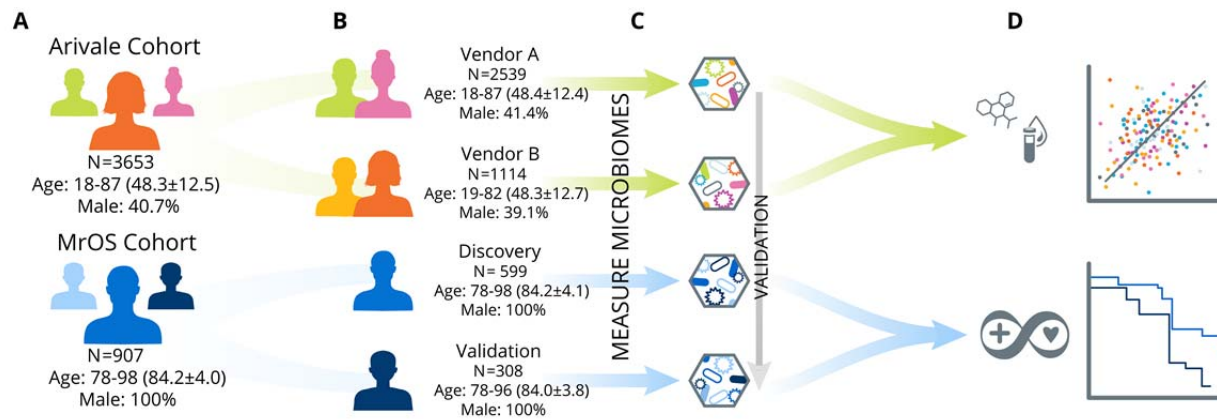
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Health Metric	Description	Stratification
Medication use	Medication use is associated with chronic diseases and comorbidities, and is an important modulator of the gut microbiome ²⁸ . High medication use is particularly prevalent in older populations, with nearly 40% of individuals 65+ years old reporting taking ≥ 5 medications ²⁹ .	High: >8 , Low: ≤ 8 medications. This allowed us to generate two groups of participants with similar age distribution but very different pharmacological profiles (low-med: N=292; high-med: N=307). Despite no significant differences in age between these two groups (student's t-test, P -Value=0.33), the prevalences of several diseases, including diabetes, chronic obstructive pulmonary disease, and congestive heart failure, were significantly higher in individuals reporting high number of medications (Table S5).
Self-perceived health	Self-perceived health has been previously shown to be an independent predictor of earlier mortality in older populations ³⁰⁻³² .	In the MrOS cohort, individuals chose one out of five possible responses (excellent, good, fair, poor, very poor). We stratified the cohort into individuals who reported excellent health (N=205) and those who reported anything less than excellent (N=394).
Life-space Score (LSC)	LSC is an indicator of mobility, i.e. how often an individual leaves their room, house, or neighborhood and has been previously associated with risk of mortality in MrOS participants ³³ . Its strength as a measure lies in that it not only provides insight into whether an individual is physically <i>capable</i> of performing activities, but also whether that individual <i>actually</i> performs these activities ³⁴ .	For both the LSC and walking speed, we stratified the cohort into tertiles and defined the top tertile as the healthy group (High), while the bottom two tertiles were combined into the less healthy group (low).
Walking Speed	Walking speed is a validated measure used to assess functional status and overall health ³⁵ , and had been previously shown to be associated with executive function, and predictive of cognitive decline ³⁶ .	
Composite	A composite of all 4 of the above measures	Healthy - individuals who met 3+ of the above criteria

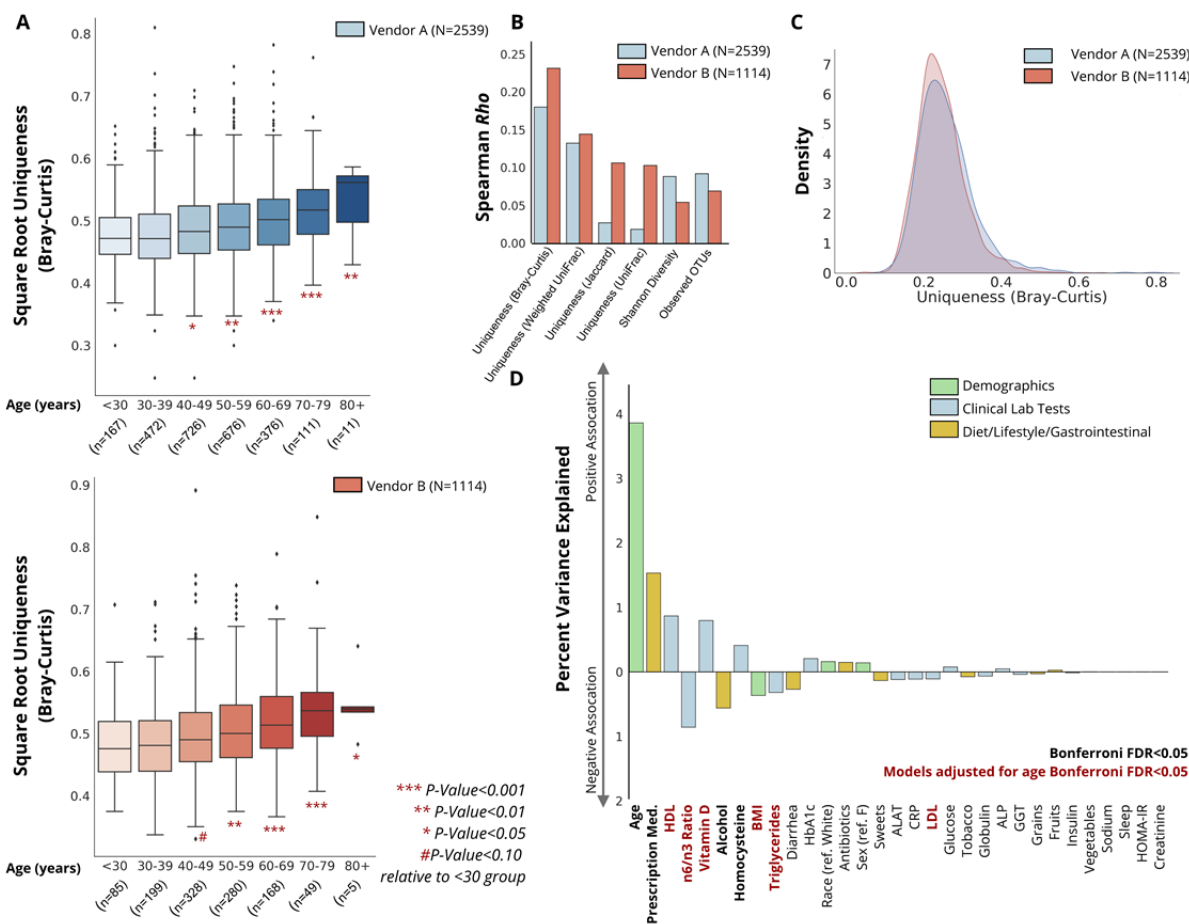
398 **Table 1: Description of health metrics used for stratification in the MrOS cohort.**



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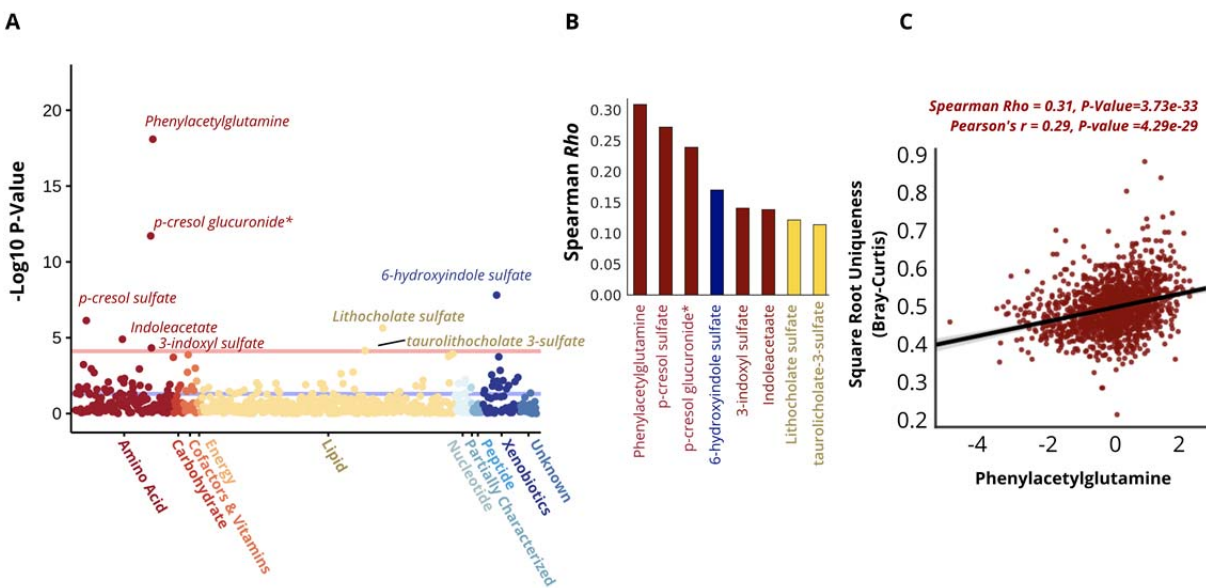
400 **Fig. 1. Conceptual outline of study and analysis workflow.** (A) Two different study
401 populations were used: the Arivale cohort and the Osteoporotic Fractures in Men (MrOS) cohort.
402 (B) Each of these two study populations were further subdivided into two groups; the Arivale
403 cohort was split based on the microbiome vendor used to collect and process samples while the
404 MrOS cohort separated into Discovery and Validation groups based on the batch in which the
405 samples were run (discovery samples were processed in the initial batch, validation samples were
406 processed several years later). (C) We profiled the microbiomes from these four study
407 populations beginning with the Arivale cohort and validating our findings across the three
408 additional populations. (D) Our analysis pipeline further explored associations between the
409 identified gut microbial aging pattern, lifestyle factors, and host physiology in the combined
410 Arivale cohort, as well as health metrics and mortality in the combined MrOS cohort.

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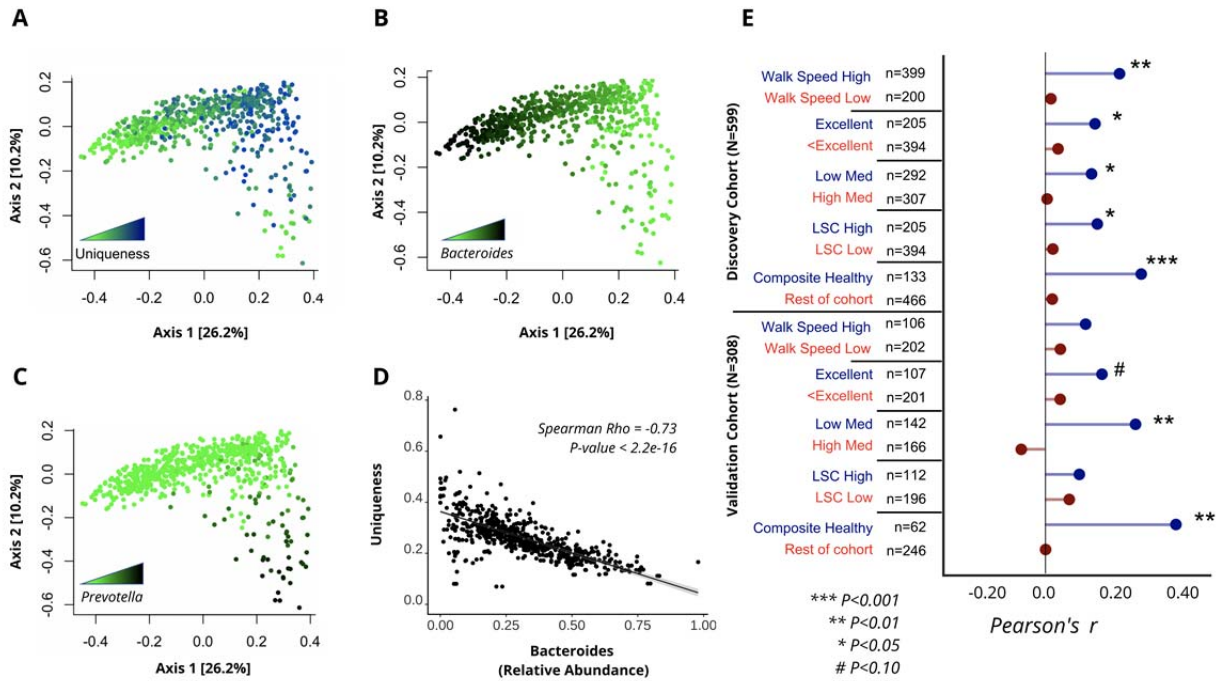
Fig. 2. Associations between gut microbial uniqueness and age across the Arivale cohort. (A) Boxplots showing gut microbiome uniqueness scores calculated using the Bray-Curtis dissimilarity metric across the adult lifespan in individuals whose stool samples were collected and processed by vendor A (blue) or B (red). Asterisks indicate significant differences relative to the youngest <30 group, from a linear regression model adjusted for sex, BMI, and Shannon diversity. Box plots represent the interquartile range (25th to 75th percentile, IQR), with the middle line demarking the median; whiskers span $1.5 \times$ IQR, points beyond this range are shown individually. (B) Spearman correlation coefficients for measures of uniqueness (β -diversity) and α -diversity with age in individuals whose stool samples were processed by vendor A or B. (C) Distribution of uniqueness calculated using the Bray-Curtis metric in each of the two vendors. (D) Percent of variance explained in Bray-Curtis uniqueness by a diverse number of demographic and lifestyle factors, as well as a subset of clinical laboratory tests.



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Fig. 3. Reflection of gut microbiome uniqueness in plasma metabolites. (A) A plot of $-\log_{10}$ p-values for each of the 652 plasma metabolites measured in the Arivale cohort, from OLS regression models predicting Bray-Curtis uniqueness adjusted for age, age squared, sex, an age*sex interaction term, BMI, Shannon diversity and microbiome vendor. Metabolites are color-coded by their super-family. All metabolites above the red line are significant after multiple-hypothesis correction (Bonferroni $P < 0.05$). * indicates metabolites that were confidently identified on the basis of mass spectrometry data, but for which no reference standards are available to verify the identity. (B) Spearman correlation coefficients for each of the metabolites significantly associated with Bray-Curtis uniqueness after adjusting for covariates and multiple-hypothesis correction (Bonferroni $P < 0.05$). Bars are color-coded as in A. (C) Scatter plot of Bray-Curtis Uniqueness and the strongest metabolite predictor, phenylacetylglutamine. The line shown is a $y \sim x$ regression line, and the shaded regions are 95% confidence intervals for the slope of the line.

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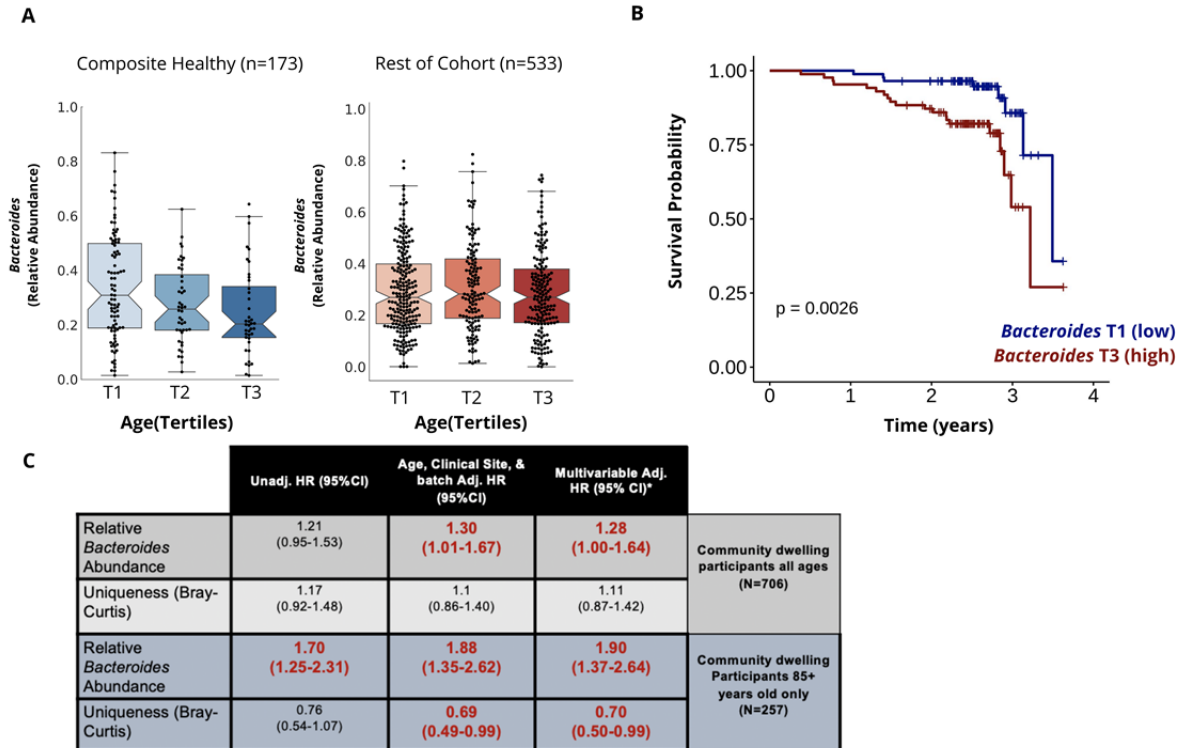
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Fig. 4. Increased dissimilarity of the gut microbiome as a function of healthy aging in the MrOS Cohort. (A-C) PCoA of the MrOS discovery cohort color-coded by (A) Bray-Curtis uniqueness, (B) relative *Bacteroides* abundance, and (C) relative *Prevotella* abundance. (D) Scatter plot demonstrating the negative association of the relative abundance of the most dominant genus *Bacteroides* and gut microbial uniqueness in the discovery cohort. The line shown is the $y \sim x$ regression line, while the shaded region corresponds to the 95% confidence intervals for the slope of the line. (E) Correlation of Bray-Curtis uniqueness scores with age across the MrOS discovery and validation cohorts under different health stratifications. ‘Excellent’ corresponds to individuals who self-reported their health to be excellent, while ‘<Excellent’ incorporates all individuals who self-reported their health being anything less than excellent (good, fair, poor, or very poor). ‘Composite Healthy’ refers to individuals who fell into the healthy sub-group in at least 3 of the 4 stratifications performed. LSC: Life-Space Score.



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458 **Figure 5. Association of *Bacteroides* abundance and survival in latter decades of human**
459 **lifespan. (A)** Boxplots demonstrating the relative abundance of the genus *Bacteroides* across
460 tertiles of age in community-dwelling individuals identified as healthy on 3+ criteria specified
461 (composite healthy) (n=173) and the remainder of the cohort (n=533). **(B)** Kaplan Meier Curve
462 demonstrating the association between overall survival and relative *Bacteroides* abundance
463 grouped into tertiles in community-dwelling MrOS participants who were 85+ years at time of
464 sampling (N=257). **(C)** Unadjusted, age, clinical site and batch adjusted and multivariable
465 adjusted Hazard Ratios (HR) of relative *Bacteroides* abundance and Bray-Curtis Uniqueness
466 scores from Cox Proportional Hazard Regression models evaluating mortality risk in all
467 community-dwelling MrOS participants and exclusively community-dwelling MrOS participants
468 85+ years old. Multivariable models were adjusted for age, clinical site, BMI, self-perceived
469 health, diagnosis of congestive heart failure, and batch in which stool samples were processed.
470 Both relative *Bacteroides* abundance and The Bray-Curtis uniqueness measures were scaled and
471 centered prior to mortality analysis. Significant HRs are bolded and colored in red ($P \leq 0.05$).
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496 participated in study design. T.W., C.D., N.R., S.P., J.W., J.L., J.C.E., A.Z., and J.T.Y.
497 performed data analysis and figure generation. G.G. and M.R. aided in dissimilarity analysis.
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499 managed the logistics of data collection and integration. T.W., S.M.G., E.S.O and N.P. were the
500 primary writers of the paper, with contributions from all authors. All authors read and approved
501 the final manuscript. **Competing interests:** Authors declare no competing interests. **Data and**
502 **materials availability:** Qualified researchers can access the full Arivale deidentified dataset
503 analyzed in this study for research purposes. Requests should be sent to
504 nathan.price@isbscience.org. The MrOS data set is available to researchers through the
505 following website: <https://mrosdata.sfcc-cpmc.net>.

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508 **Methods:**

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509 **Cohorts:** The Arivale cohort consists of individuals over 18 years of age who between 2015 and
510 2019 self-enrolled in a now closed scientific wellness company. The cohort has been described
511 in detail previously¹⁵. For this study, only baseline measurements were considered for each
512 participant. The only inclusion criterion was the availability of gut microbiome data in order to
513 maximize the number of gut microbiomes to which each sample is compared. Demographic
514 information on the cohort is provided in Table S1.

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515 The MrOS study is an ongoing prospective study of close to 6000 men recruited across six
516 clinical U.S. sites. The cohort, recruitment criteria, and stool sample collections have been
517 previously described in detail^{16,17}. Briefly, during the fourth follow-up visit of the original study,
518 a subset of participants across all six clinics was asked if they would consent to have their stool
519 sampled for microbiome analysis. Participants who agreed were given the OMNIgene-GUT
520 stool/feces collection kit (OMR-200, DNA Genotek, Ottawa, Canada) and collected the fecal
521 sample at their homes. Demographic information on MrOS participants is provided in Table S2.
522 In the initial uniqueness analysis, all participants with available high-quality microbiome data
523 were used for analysis (N=907). Subsequent differential abundance analysis focused exclusively
524 on community-dwelling individuals (N=706) (excluding individuals in assisted living, nursing
525 homes and who have been hospitalized in the past 12 months). Finally, survival analysis was
526 conducted on all community dwelling individuals as well as specifically on community dwelling
527 individuals in the latest stages of aging (85+ years old, N=257). The number of deaths in the

528 whole community dwelling group and in 85+ year old community dwelling group was 66 and
529 41, respectively.

530 **Microbiome Analysis:**

531 **Arivale cohort:** Analysis of gut microbiome data from the Arivale cohort has been described in
532 detail elsewhere^{21,37}. Briefly, independent of the vendor used, stool samples were collected at
533 the participants' homes using DNA collection kits with a proprietary chemical DNA stabilizer to
534 maintain DNA integrity at ambient temperatures following collection. Gut microbiome
535 sequencing data in the form of FASTQ files were provided on the basis of either the 300-bp
536 paired-end MiSeq profiling of the 16S V3 + V4 region (DNAgenotek, vendor A) or 250-bp
537 paired-end MiSeq profiling of the 16S V4 region (Second Genome, vendor B). Further analysis
538 was performed using the denoise workflow from mbtools ([https://github.com/gibbons-](https://github.com/gibbons-lab/mbtools)
539 [lab/mbtools](https://github.com/gibbons-lab/mbtools)) that wraps DADA2. In summary, we first trained DADA2³⁸ error models
540 separately for each sequencing run and used those to obtain sequence variants for each sample.
541 This was followed by de novo chimera removal which removed ~17% of all reads as chimeric
542 and resulted in about 89,000 final sequence variants across all samples. Taxonomy assignment
543 was performed using the RDP classifier with the SILVA database (version 132). Here 99% of the
544 reads could be classified on the family level, 89% on the genus level and 32% on the species
545 level. Species level taxonomy was identified by exact alignment to the SILVA reference
546 sequences. Sequence variants were aligned to each other using DECIPHER³⁹ and the multiple
547 sequence alignment was trimmed by removing each position that consisted of more than 50%
548 gaps. The resulting core alignment had a length of 420 base pairs and was used to reconstruct a
549 phylogenetic tree using FastTree⁴⁰. Downstream gut microbiome data analysis was conducted
550 using the *Phyloseq* Package⁴¹. In two separate analyses, gut microbiome samples were rarefied
551 to 13703 (vendor A, DNAgenotek) and 39810 (Vendor B, Second Genome) reads, the minimum
552 number of reads per sample for each vendor. For uniqueness analysis, the Bray-Curtis⁴²,
553 Unweighted and Weighted UniFrac⁴³, and Jaccard matrices were calculated for all samples
554 within each vendor using the rarefied Genus table. The minimum value for each row,
555 corresponding to the dissimilarity of each sample to their nearest neighbor, was then extracted
556 from the matrix and used for downstream analysis.

557 **MrOS cohort:** Stool samples were processed at the Alkek Center for Metagenomics and
558 Microbiome Research (CMMR) at Baylor College of Medicine using their custom analytic
559 pipeline in two separate batches (Discovery N=599, Validation N=320). 16Sv4 rDNA amplicon
560 sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value
561 of 97% using the UPARSE algorithm⁴⁴. OTUs were then mapped to an optimized version of the
562 SILVA Database⁴⁵ containing only the 16S V4 region to determine taxonomies. Abundances
563 were recovered by mapping the demultiplexed reads to the UPARSE OTUs⁴⁴. Preliminary
564 microbiome data analysis was conducted using the *Phyloseq* Package. For α -diversity and
565 uniqueness analysis, OTUs were rarefied to 9424 reads, which is the minimum number of
566 OTUs/sample in the discovery cohort. The same rarefaction number (9424) was used in the
567 Validation cohort (N=320). A total of 12 samples had less reads than the specified cut-off, and
568 hence were excluded from the analysis (Validation N=308). α -diversity measures were
569 calculated at the OTU level using the *Phyloseq* package⁴¹. For β -diversity analysis, OTUs were
570 collapsed into genera. Uniqueness was calculated as described for the Arivale cohort. The
571 calculated uniqueness measure for each participant was then used for downstream analysis. As
572 part of our analytical pipeline, we also performed differential abundance analysis assessing the
573 relationship of individual genera with age in individuals defined as healthy and unhealthy,

574 separately. Analysis was performed in R (version 3.44) using beta-binomial regression through
575 the Corncob package (version 1.0)⁴⁶. Models were adjusted for BMI, and batch
576 (discovery/validation). Type 1 error was controlled using the Bonferroni method ($P < 0.1$).

577 **Plasma Metabolomics & Clinical Laboratory Tests:**

578 Blood draws for all assays were performed by trained phlebotomists at LabCorp or Quest service
579 centers. For the 24-hour period leading up to the blood draw, Arivale participants were required
580 to avoid alcohol, vigorous exercise, aspartame and monosodium glutamate, and to begin fasting
581 12 hours in advance. Plasma metabolomics assays were conducted on the samples by Metabolon,
582 Inc. Sample handling, quality control and data extraction, along with biochemical identification,
583 data curation, quantification and data normalizations have been previously described³⁷. For
584 analysis, the raw metabolomics data were median scaled within each batch, such that the median
585 value for each metabolite was one. To adjust for possible batch effects, further normalization
586 across batches was performed by dividing the median-scaled value of each metabolite by the
587 corresponding average value for the same metabolite in quality control samples of the same
588 batch. In this study, we analyzed participants' baseline plasma metabolomics data. A 10%
589 missing value threshold was set, which was passed by 652 metabolites. Missing values for
590 metabolites were imputed to be the minimum observed value for that metabolite. A total of 1476
591 Arivale participants had paired gut microbiome-plasma metabolome data. Values for each
592 metabolite were log transformed prior to analysis. Clinical laboratory tests were conducted by
593 either Quest or LabCorp. A 10% missing value threshold was set for each clinical laboratory test
594 used in the analysis. All but 104 participants ($N=3549$) had paired clinical laboratory-gut
595 microbiome data. Both metabolomics and clinical laboratory tests were scaled and centered prior
596 to analysis and only baseline measures for each individual were used.

597 **Lifestyle/Health Questionnaires in the Arivale Cohort:**

598 Data on lifestyle, diet and health were obtained through self-administered questionnaires
599 completed by Arivale participants during their initial assessment. For reporting antibiotic use,
600 participants chose from three possible responses ('not in the past year', 'in the past year' and 'in
601 the past three months') which were recoded into ordinal variables 0, 1 and 2 respectively.
602 Participants chose one of several possible frequencies in response to how often they experience
603 diarrhea, that were recoded as follows: 'infrequently/never' = 0, 'once a week or less' = 1, 'more
604 than once a week' = 2 and 'daily' = 2. Similarly, alcohol use (no. of drinks per day) was reported
605 on the following scale which was recoded into corresponding ordinal variables: (0) 'I do not
606 drink', (1) '1-2 drinks': (2) '3-4 drinks': (3) '5-6 drinks': (4) 'More than 6 drinks'. Current tobacco
607 use and prescription medication were both modelled as binary variables (yes/no). Finally, for
608 dietary variables (fruit, vegetables, grains, and sweets intake), participants chose one of the
609 following responses, which were then recoded to the corresponding ordinal variables: (grains):
610 (0) 'Zero/less than 1 per day': (1) '1-2': (2) '3-4': (3) '5-6': and (4) '7 or more'. (fruits,
611 vegetables): (0) 'Zero/less than 1 per day': (1) '1': (2) '2-3': (3) '4-5': (4) '6 or more'.
612 (chocolates/sweets): (0) 'Less than once per month': (1) '1-3 times per month': (2) 'Once per
613 week': (3) '2-4 times per week': (4) '5-6 times per week': (5) 'Once per day': (6) '2-3 times per
614 day': (6) '4-5 times per day': (6) '6+ times per day'. Sleep was reported as the average amount of
615 sleep you get a day on a three-point scale: (0) 'Less than 6 hours': (1) '7 to 9 hours': (2) 'More
616 than 9 hours'. As the Arivale cohort consists of self-enrolled participants, the response rates for
617 different questionnaires vary. The number of missing values for each response is reported in
618 Table S2.

619 **Health Measures in the MrOS Cohort:**

620 We utilized four different health measures that were collected on MrOS participants during their
621 fourth follow-up visit. Medication use, self-perceived health, and the Life-Space score (LSC)
622 were all self-reported. Self-perceived health captured each individual's rating of their own health
623 compared to other individuals their own age. The implementation of the LSC in the MrOS cohort
624 has been described in detail previously³³. Briefly, LSC can range from 0 (restricted to one's
625 bedroom) to 120 (traveled outside one's town daily without assistance). We defined healthy
626 individuals as those in the top tertile of the LSC cohort distribution. This corresponded to an
627 LSC value of ≥ 96 . Walking speed was calculated based on the time it took each participant to
628 walk 6 meters (m/s). Like with the LSC, we defined healthy individuals based on walking speed
629 if their speed was in the top tertile (≥ 1.17). A total of 7 MrOS participants did not have available
630 walking speed data. This is due to either the participants not coming to the clinic, or not being
631 able to attempt the test. These individuals were classified in the walking speed low group in our
632 analysis.

633 **Statistical Analysis:**

634 Depending on the statistical approach, analysis was conducted using either R (v 3.6) or Python (v
635 3.7). The relationship between the calculated uniqueness measure and age in the Arivale cohort
636 was modeled using Ordinary Least Square (OLS) linear regression (Python) where square root
637 transformed Bray-Curtis uniqueness was modeled as the dependent variable and each age decade
638 was compared to the youngest reference group (<30 years), adjusting for sex, BMI, and Shannon
639 diversity. We chose to adjust for Shannon diversity because, in our analysis, it was associated
640 with both age and microbiome uniqueness (higher alpha diversity makes you more likely to be
641 unique). We wanted to assess the significance of our dissimilarity pattern independent of changes
642 in alpha diversity seen with age and previously reported in literature. The same adjustment was
643 not made for MrOS participants, since Shannon diversity showed no association with age in that
644 cohort. Pearson/Spearman correlations were also used to assess the strength of the relationship
645 between different measures of β - and α -diversity and age across all cohorts using the Python
646 statistical functions package (scipy.stats). When assessing the relationship between clinical,
647 lifestyle, and demographic variables with gut microbial uniqueness, Bray-Curtis uniqueness
648 values greater or less than 3 standard deviations from the mean were removed. OLS linear
649 regression was then used to assess the individual relationship between each factor and square
650 root transformed Bray-Curtis gut microbial uniqueness, with microbiome vendor included as a
651 covariate. Percent variance explained by each factor was calculated by taking the percent
652 variance explained by the complete OLS model (variable of interest and microbiome vendor) and
653 subtracting the percent variance explained by microbiome vendor alone. The same analysis was
654 then repeated with age included as a covariate (age-adjusted models). To investigate potential
655 effect modification of sex on the identified gut microbiome aging pattern, an OLS model was
656 generated with a sex*age interaction term predicting square root transformed Bray-Curtis
657 uniqueness, adjusted for sex, age, BMI, microbiome vendor and Shannon diversity. Sex-specific
658 β -coefficients were estimated by first stratifying the cohort by sex and then fitting OLS models
659 for men and women separately, adjusting for the same covariates as the combined model. Age
660 was scaled and centered prior to this analysis. When investigating the relationship between
661 plasma metabolite concentrations and gut microbial uniqueness, each metabolite was log
662 transformed and subsequently scaled and centered. The square root transformed Bray-Curtis
663 uniqueness score was then regressed against each metabolite individually, adjusting for
664 microbiome vendor, sex, age, age², a sex*age interaction term, BMI, and Shannon diversity
665 using OLS regression. In each instance where multiple hypotheses were tested, type I error was

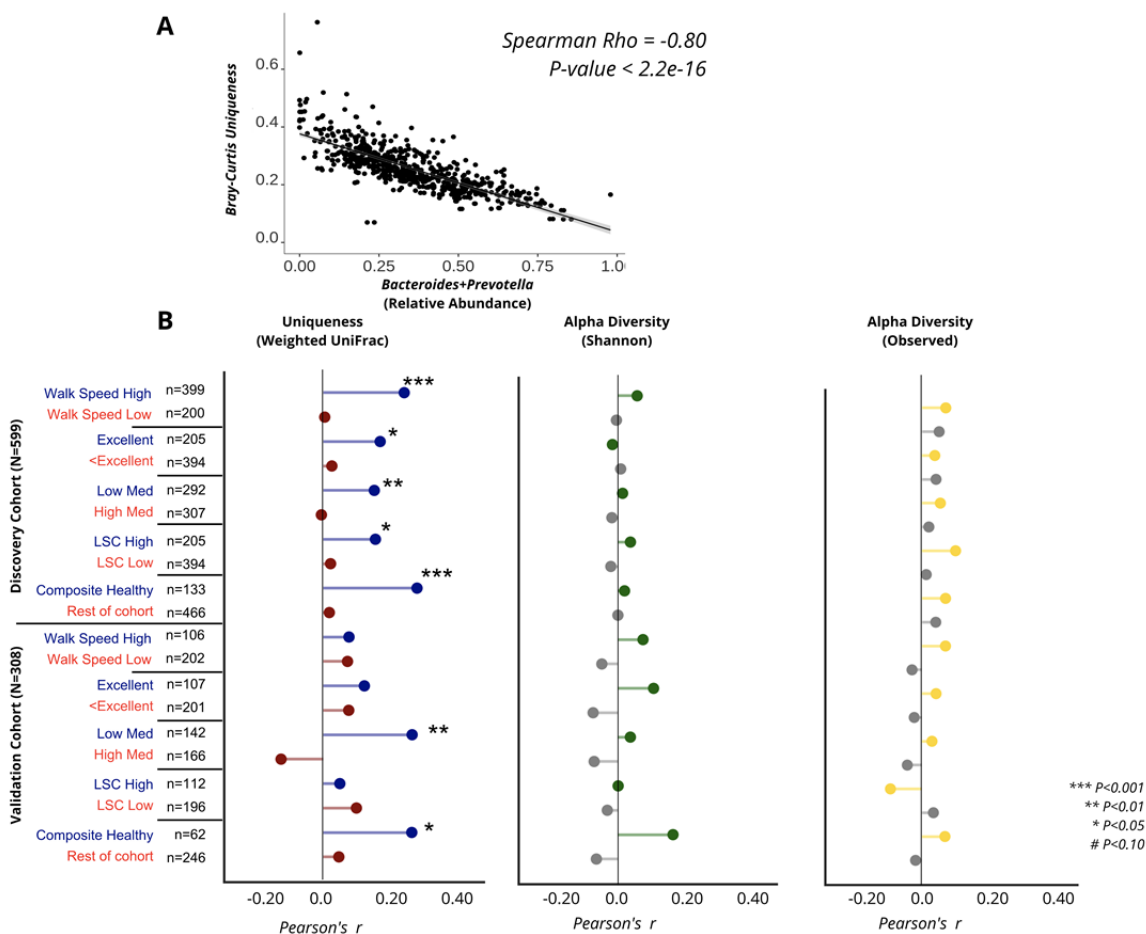
666 controlled for using the Bonferroni method ($P < 0.05$). In the MrOS Cohort, correlation between
 667 Bray-Curtis Uniqueness and age was calculated using the Python statistical functions package
 668 (scipy.stats) using the square root transformed uniqueness score. Mortality analysis was
 669 conducted in R using the package survival (v 2.44-1.1). Relative *Bacteroides* abundance (after
 670 rarefaction) and uniqueness scores were scaled and centered prior to survival analysis. Cox-
 671 proportional hazard regression models were generated assessing the relationship between
 672 survival and Relative *Bacteroides* abundance or Bray-Curtis uniqueness independently, adjusting
 673 for clinical site, batch (discovery/validation) and age, and adjusting for clinical site, age, BMI,
 674 self-perceived health (excellent, good, <good), diagnosis of congestive heart failure, and batch in
 675 which stool samples were processed (discovery/validation).
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681 Supplementary Information:

682 Figure S1

683 Tables S1-S5

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Fig. S1. Associations between age and gut microbiome measures across health stratifications in the MrOS cohort. (A) Scatter plot demonstrating the negative association of the relative abundance of the sum of *Bacteroides+Prevotella* and gut microbial uniqueness. The line shown is the $y \sim x$ regression line, while the shaded region corresponds to the 95% confidence intervals for the slope of the line. **(B)** Plots demonstrating the strength of correlation between age and gut microbiome measures. The blue/red panel corresponds to the calculated Weighted UniFrac (β -diversity) uniqueness score, while the grey/green and grey/yellow panels correspond to Shannon diversity and Observed species (α -diversity measures), respectively. Significant correlations are indicated with pound signs and asterisks.

	Vendor A (N=2539)	Vendor B (N=1114)	P-Value
Mean age (range)	48.4 (18-87)	48.3 (19-82)	NS
Mean BMI (s.d.)	27.1 (5.9)	27.3 (6.1)	NS
Sex (% female)	58.60%	60.90%	NS
Non-white (%)	20.40%	22.00%	NS
Mean HDL (mg dl ⁻¹) (s.d.)	61.8 (18.8)	61.7 (18.4)	NS
Mean LDL (mg dl ⁻¹) (s.d.)	114.0 (33.4)	114.0 (34.7)	NS
Mean blood triglycerides (mg dl ⁻¹) (s.d.)	104.9 (59.6)	105.3 (58.0)	NS
Median Shannon diversity [IQR]	4.34 [4.04-4.60]	4.31 [3.99-4.57]	0.032
Median Observed OTUs [IQR]	276 [217-247]	287.5 [226-371]	4.60E-05
Mean Bray-Curtis uniqueness (s.d.)	0.24 (0.06)	0.26 (0.07)	3.94E-08

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Table S1. Arivale cohort characteristics stratified by microbiome vendor

Statistical tests used to compare groups are as follows: independent samples t-tests were used for comparing age, body mass index (BMI), high density lipoprotein (HDL), low density lipoprotein (LDL), blood triglycerides and Bray-Curtis Uniqueness; nonparametric Mann-Whitney U tests were used to compare Shannon diversity and Observed OTUs; χ^2 tests were used to compare sex (percentage female) and race (percentage non-white). *P-Values* <0.05 are colored in red.

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	Composite Healthy (n=133)	Rest of Cohort (n=466)	Whole Cohort (N=599)	P-Value
Median age (s.d.)	83.5 (3.6)	84.4 (4.2)	84.2 (4.1)	0.013
Mean BMI (s.d.)	26.6 (3.6)	27.1 (3.8)	27.0 (3.8)	0.17
Hispanic (%)	3.8	1.5	2.0	0.15
Mean Shannon diversity (s.d.)	3.6 (0.6)	3.5 (0.6)	3.5 (0.6)	0.58
Mean Observed Species (s.d)	161.0 (50.7)	155.6 (53.1)	156.8 (52.5)	0.29
Diabetes (%)	6.0	17.8	15.2	0.001
Congestive heart failure (%)	0.0	10.5	8.2	<0.001
Hypertension/high blood pressure (%)	41.4	56.7	53.3	0.003
COPD (%)	6.0	12.0	10.7	0.069
Depression (%)	8.3	9.9	9.5	0.70

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Table S2. MrOS discovery cohort characteristics stratified into composite healthy and remainder of cohort

Statistical tests used to compare groups are as follows: independent samples t-tests were used for comparing age, body mass index (BMI), Shannon diversity and Observed Species; χ^2 or Fisher's exact (if assumptions of χ^2 were not met) tests were used to compare ethnicity (percentage Hispanic), and prevalence of each of the specified diseases. P-values <0.05 are colored in red.

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Analyte	P-Value	r squared	B-coefficient	Missing	Age adj. B-coefficient	Age adj. P-Value
Age	5.89E-33	3.85901529	0.00093261	0	NA	NA
Prescription Med	0.00017481	1.53219831	0.01510618	914	0.00341449	0.08545254
HDL	3.03E-08	0.8659935	0.00549509	104	0.003964398	5.47E-05
n6/n3	3.66E-08	0.855769	-0.0054647	104	-0.003148386	0.00156995
Vitamin D	1.08E-07	0.79702949	0.00527528	104	0.003159677	0.00144512
Alcohol	1.36E-05	0.5608151	-0.0070819	3349	-0.002982965	0.0028601
Homocysteine	0.00014395	0.4087152	0.00377064	104	0.001589201	0.10941824
BMI	0.00044766	0.36457364	-0.0005975	260	-0.003869322	0.00010072
Triglycerides	0.00077075	0.32005925	-0.0033549	104	-0.004164096	1.97E-05
Diarrhea	0.00238587	0.26867461	-0.0040083	3415	-0.001701617	0.08690565
HbA1c	0.00715616	0.204807	0.00272545	104	6.97E-05	0.94455438
Race(ref.white)	0.01700183	0.16061072	0.0058388	88	0.000687553	0.48477659
Antibiotics	0.05402505	0.14773123	0.00642737	2500	0.001844306	0.10335017
Sex	0.02355609	0.14101656	0.00452064	0	0.00236889	0.01379305
Sweets	0.27161453	0.13414917	-0.0012896	902	-4.21E-05	0.98258201
ALAT	0.04087102	0.11844347	-0.0020499	104	-0.002165898	0.02620547
CRP	0.04604621	0.11274027	-0.0019787	104	-0.001966477	0.04354322
LDL	0.04967172	0.10913342	-0.0019505	104	-0.003130326	0.00137829
Glucose	0.10416215	0.07481646	0.00163161	104	-0.000440919	0.6562693
Tobacco	0.11771405	0.07460265	-0.0077207	3264	-0.00115745	0.2504751
Globulin	0.13060992	0.06475052	-0.0015033	104	-0.000324745	0.74022842
ALP	0.18418122	0.0499728	0.00133152	104	-0.000589978	0.55024706
GGT	0.25345563	0.03695739	-0.0011321	104	-0.0023493	0.01643053
Grains	0.32021289	0.029105	-0.0012634	3379	-0.000165682	0.86721005

Fruits	0.34161948	0.02630667	0.00113363	3422	-0.000172467	0.86142995
Insulin	0.48819587	0.0136181	-0.0006939	104	-0.001210903	0.21445978
Vegetables	0.84610634	0.0010959	0.00022377	3422	-0.000193127	0.84450268
Sodium	0.89643796	0.0004802	0.00012949	104	-0.000934367	0.33958969
Sleep	0.93138998	0.000316	0.00021369	2335	0.000427359	0.71350207
HOMA-IR	0.95318368	9.77E-05	-5.85E-05	104	-0.000840639	0.38949581
Creatinine	0.98900622	5.38E-06	-1.37E-05	104	-0.000570523	0.55888158

768 **Table S3. Associations between Bray-Curtis gut microbiome uniqueness and clinical,**
769 **demographic, and diet/lifestyle/health measures.** ‘P-Value’ corresponds to the unadjusted P-
770 Value of the β -coefficient (B-Coefficient column) for each analyte from an OLS model adjusted
771 for gut microbiome vendor. ‘r_squared’ reflects the percent of variance explained beyond
772 microbiome vendor for each analyte independently. ‘Missing’ shows the number of missing
773 observations for each analyte. ‘Age adj. B-coefficient’ and ‘Age adj. P-value’ correspond to the
774 β -coefficient and P-Value for each analyte adjusting for gut microbiome vendor and age. Values
775 highlighted in red are statistically significant after multiple-hypothesis correction (Bonferroni P-
776 Value<0.05).

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Metabolite	P-Value	Corrected P-Value	Adj. B-coefficient
phenylacetylglutamine	3.65E-20	2.38E-17	0.015774767
p-cresol glucuronide*	6.76E-13	4.40E-10	0.011978401
6-hydroxyindole sulfate	7.06E-09	4.60E-06	0.009597508
3-indoxyl sulfate	5.24E-07	0.00034152	0.008269926
lithocholate sulfate	7.39E-07	0.00048191	0.008153816
p-cresol sulfate	5.06E-06	0.00329597	0.007949108
indoleacetate	9.55E-06	0.00622582	0.007363627
taurolithocholate 3-sulfate	6.67E-05	0.04347711	0.006750892
trimethylamine N-oxide	8.02E-05	0.0522833	0.006676801
glycodeoxycholate 3-sulfate	0.00011043	0.07199921	0.006384711
1,5-anhydroglucitol (1,5-AG)	0.00019356	0.12620424	-0.006192382
biliverdin	0.00026726	0.17425604	-0.006295182
carotene diol (1)	0.00048694	0.3174862	-0.006178362
4-ethylcatechol sulfate	0.00059272	0.38645107	0.005838135
threonate	0.00095377	0.6218559	-0.005560642
dodecanedioate (C12-DC)	0.00121002	0.78893024	0.005391288
N-acetylputrescine	0.00448236	1	0.004753617
carotene diol (3)	0.00272636	1	-0.005152625
ergothioneine	0.00708238	1	-0.004510327
androstenediol (3alpha, 17alpha) monosulfate (2)	0.00935419	1	-0.004964594
3-hydroxy-2-ethylpropionate	0.00904741	1	0.004420961
4-ethylphenylsulfate	0.00586104	1	0.004664238
propionylglycine	0.00191267	1	0.005169723
isobutyrylcarnitine (C4)	0.00383686	1	0.004969195
tartronate (hydroxymalonnate)	0.00388	1	-0.004950944
cys-gly, oxidized	0.00874903	1	0.004322096
4-acetamidobutanoate	0.0028892	1	0.00512429
bilirubin (Z,Z)	0.00233088	1	-0.005203709
cortisol	0.00512227	1	0.004670237
5-methylthioadenosine (MTA)	0.00432121	1	0.00487853
androstenediol (3beta,17beta) disulfate (2)	0.0078715	1	-0.005096967
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)*	0.00233228	1	0.005882396

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Table S4 Associations between Bray-Curtis gut microbiome uniqueness and plasma metabolites. Only metabolites with P-value<0.01 are shown. ‘P-Value’ corresponds to the

804 covariate adjusted P-value of the β -coefficient (Adj. B-coefficient). ‘Corrected P-Value’
 805 corresponds to the Bonferroni corrected P-values. Metabolites significantly associated with
 806 Bray-Curtis uniqueness after adjusting for covariates and multiple hypothesis correction are
 807 highlighted in red. * indicates metabolites that were confidently identified on the basis of mass
 808 spectrometry data, but for which no reference standards are available to verify the identity.

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	>8 Meds (N=307)	≤8 Meds (N=292)	P-Value
Median age (s.d.)	84.1 (4.0)	84.4 (4.2)	0.33
Mean BMI (s.d.)	27.4 (3.9)	26.6 (3.7)	0.015
Hispanic (%)	2.0	2.1	0.84
Mean Shannon diversity (s.d.)	3.5 (0.6)	3.6 (0.6)	0.021
Mean Observed Species (s.d.)	150.4 (50.8)	163.5 (53.6)	0.002
Diabetes (%)	21.5	8.6	<0.001
Congestive heart failure (%)	13.4	2.7	<0.001
Hypertension/high blood pressure (%)	62.5	43.5	<0.001
COPD (%)	16.6	4.5	<0.001
Depression (%)	11.1	7.9	0.23

813 **Table S5. MrOS discovery cohort characteristics stratified by medication use**

814 Statistical tests used to compare groups are as follows: independent samples t-tests were used for
 815 comparing age, body mass index (BMI), Shannon diversity and Observed species; χ^2 or Fisher’s
 816 exact (if assumptions of χ^2 were not met) tests were used to compare ethnicity (percentage
 817 hispanic) and prevalence of each of the specified diseases. *P-Values* <0.05 are colored in red.

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