

# Causal Epigenetic Age Uncouples Damage and Adaptation

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16 **Abstract**

17 Machine learning models based on DNA methylation can be used to predict the age of biological  
18 samples, but their interpretability is limited due to the lack of causal inferences. Here, we lever-  
19 aged large-scale genetic data and performed epigenome-wide Mendelian Randomization to iden-  
20 tify CpG sites causal to aging-related traits. We show that neither the existing epigenetic clocks  
21 nor DNA methylation changes are enriched in causal CpG sites. Causal CpGs include similar  
22 numbers of sites that contribute to aging and protect against it, yet their combined contribution  
23 negatively affects age-related traits. We developed a framework for integrating causal knowledge  
24 into epigenetic clock models and constructed *DamAge* and *AdaptAge* that measure age-related  
25 damaging and adaptive changes, respectively. *DamAge* acceleration is associated with various  
26 adverse conditions (e.g., mortality risk), whereas *AdaptAge* acceleration is related to beneficial  
27 adaptations. Only *DamAge* is reversed upon cell reprogramming. Our results offer a comprehen-  
28 sive map of CpG sites causal to lifespan and healthspan, allowing to build causal biomarkers of  
29 aging and rejuvenation and assess longevity interventions, age reversal, and aging-accelerating  
30 events.

## 31 **Introduction**

32 Aging is a complex biological process characterized by a buildup of deleterious molecular  
33 changes that result in a gradual decline of function of various organs and systems and ultimately  
34 lead to death <sup>1</sup>. Although the underlying mechanisms of aging are not well understood, various  
35 studies indicate that aging is strongly associated with changes in the epigenome, quantified as a  
36 set of chemical modifications to DNA and histones that affect gene expression and chromatin  
37 structure <sup>2</sup>. DNA methylation is one of the best studied epigenetic modifications. In mammals, 5-  
38 methylcytosine (5mC) is the most common form of DNA methylation, which is achieved by the  
39 action of DNA methyltransferases (DNMTs) <sup>3,4</sup>. Studies have shown that DNA methylation pat-  
40 terns change with age, wherein the global level of DNA methylation decreases slightly during  
41 adulthood, while some local areas may be hypomethylated or hypermethylated <sup>2,5-8</sup>. Furthermore,  
42 the level of methylation of some specific CpG sites shows a strong correlation with age, which  
43 can be used to build machine learning-based models that can accurately predict the age of bio-  
44 logical samples <sup>7,9</sup>. As models can quantify age with very high accuracy, researchers termed  
45 these models epigenetic aging clocks (e.g., Horvath pan tissue epigenetic clock and Hannum  
46 blood based epigenetic clock) <sup>10,11</sup>. The predicted age based on various epigenetic aging clocks  
47 appears to have a higher association with health-related measurements than chronological age.  
48 Therefore, it is believed that they could be used to better represent the biological age of samples  
49 than chronological age <sup>12</sup>.

50 Although epigenetic aging clocks provide a useful tool for profiling biological aging, they should  
51 be used with caution, as they are built based on pure correlations <sup>13</sup>. It is unclear whether the DNA  
52 methylation changes that are used to predict age are causal to aging-related phenotypes or are  
53 simply byproducts of the aging process that does not influence aging themselves. To establish a  
54 causal relationship, the gold standard approach is the application of randomized controlled trials  
55 (RCT), where participants are randomly assigned to the intervention arm that receives the treat-  
56 ment or the control arm. As the randomization step balances all confounding factors between two  
57 arms, the differences observed in the outcome between two groups are purely driven by the inter-  
58 vention; thus, the causal effect can be estimated <sup>14</sup>. However, given the large number of CpG sites  
59 across the genome, it is inefficient and infeasible to perform the perturbation on each of them and  
60 assess the aging-related outcomes.

61 Mendelian randomization (MR) is a genetic approach to causal inference that recapitulates the  
62 principle of RCT. Instead of perturbing an exposure through treatment, the MR uses the genetic  
63 variants that are robustly associated with the exposure as instrumental variables <sup>15,16</sup>. As genetic  
64 variants of parental DNA are naturally randomly passed on to the offspring, the effect estimated  
65 by MR is not affected by environmental confounders and thus can be considered as an estimation  
66 of a causal effect, similar to the RCTs. In recent years, several studies have shown that MR can be  
67 applied to molecular traits by using the genetic variants associated with molecular levels as instru-  
68 ments (also known as molecular quantitative trait loci, molQTL) <sup>17</sup>. These molecular QTLs include  
69 gene expression (eQTL) <sup>18</sup>, RNA splicing (sQTL) <sup>19</sup>, plasma protein (pQTL) <sup>20</sup>, metabolites  
70 (mQTL) <sup>21</sup>, as well as DNA methylation (meQTL) <sup>22</sup>. A previous study showed that it is feasible  
71 to use meQTLs as instruments to identify causal CpG sites for diseases <sup>23</sup>. By integrating molQTLs  
72 with genome-wide association studies for traits such as lifespan, healthspan, extreme longevity,  
73 and other measurements related to aging, it is biologically plausible to perform two-sample MR to  
74 estimate the causal effects of molecular changes on the aging process.

75 Here, we leveraged large-scale genetic data and performed epigenome-wide Mendelian Random-  
76 ization (EWMR) on 420,509 CpG sites to identify CpG sites that are causal to twelve aging-related  
77 traits. We found that none of the existing clocks are enriched for causal CpG sites. We further  
78 constructed a causality-informed clock based on this inferred causal knowledge, as well as clocks  
79 that separately measure damaging and protective changes. Their applications provide direct in-  
80 sights into the aging process. Thus, our results offer a comprehensive map of human CpG sites  
81 causal to aging traits, which can be used to build causal biomarkers of aging and assess novel anti-  
82 aging interventions and aging-accelerating events.

## 83 **Results**

### 84 **Epigenome-wide Mendelian Randomization on aging-related phenotypes**

85 MR is an established genetic approach for causal inference that utilizes natural genetic variants  
86 as instrument variables. Since the allocation of genetic variants is a random process and is deter-  
87 mined during conception, the causal effects estimated using MR are not biased by environmental  
88 confounders. Therefore, it could be used as a tool for investigating causal relationships between  
89 the DNA methylation and aging-related phenotypes (Fig. 1a). To identify CpG sites causal to

90 aging, we used 420,509 CpG sites with meQTLs available (GoDMC, whole blood samples from  
91 36 cohorts, 27,750 European subjects) as exposures and selected twelve aging-related pheno-  
92 types as outcomes (Fig. 1a, Methods), including two lifespan-related traits (lifespan and extreme  
93 longevity)<sup>24</sup>, three health-related traits (healthspan, frailty index, and self-rated health)<sup>25,26</sup>, four  
94 epigenetic age measurements (Horvath age, Hannum age, PhenoAge, and GrimAge)<sup>25</sup>, and three  
95 summary-level aging-related traits (Aging-GIP1, socioeconomic traits-adjusted Aging-GIP1, and  
96 healthy aging)<sup>25</sup>. Aging-GIP1 is the first genetic principal component that captures both the  
97 length of life and age-related health status<sup>27</sup>, which can be considered as a genetic representation  
98 of healthy longevity. It also shows the strongest genetic correlation with all other traits related to  
99 lifespan<sup>25</sup>. Therefore, we further used Aging-GIP1 as the primary aging-related trait to investi-  
100 gate CpG sites causal to the aging process. A genetic correlation analysis showed that all eight  
101 lifespan- and health-related traits are genetically correlated and clustered with each other, while  
102 the four epigenetic age measurements clustered with each other. GrimAge and PhenoAge  
103 showed significant genetic correlations with other health and lifespan-related traits, while Han-  
104 num age and Horvath age did not (Extended Data Fig. 1).

105 We then applied generalized inverse-variance weighted MR (gIVW) and MR-Egger (gEgger) on  
106 each exposure-outcome pair (Fig. 1b). We only included cis-meQTLs (meQTLs located within 2  
107 MB of target CpG sites) in our analysis to avoid pleiotropic effects, as they are more likely to af-  
108 fect DNA methylation via direct mechanisms. To remove additional pleiotropic effects, we used  
109 the results of gEgger, whose estimate is robust to directional pleiotropic effects if the significant  
110 intercept is detected by gEgger regression ( $P < 0.05$ ). After adjusting for multiple tests using  
111 Bonferroni correction, we discovered more than 6,000 CpG sites with significant causal effects  
112 on each trait, ranging from 5,507 (for GrimAge) to 8,341 (for self-rated health) (Fig. 1c).

113 Genetic colocalization is a Bayesian approach that estimates the probability (PP.H4) of overlap-  
114 ping genetic signals between molecular traits and outcome is due to both traits sharing a causal  
115 variant<sup>28</sup>. It is an important method to control false positive results from MR and filter out the  
116 MR signals purely driven by LD or pleiotropy. We then performed a pairwise conditional and  
117 colocalization (PWCoCo) analysis of all conditionally independent instruments against all condi-  
118 tionally independent association signals for the outcome phenotypes<sup>29</sup>. We used the conditional  
119 H4 threshold of 0.7 to identify colocalized signals and detected such signals for more than half of

120 the CpG sites identified by MR for each trait, ranging from 2,943 (for GrimAge) to 4,495 (for  
121 self-rated health).

122 Since we could only perform MR and colocalization analysis on 420,509 CpG sites, the role of  
123 unmeasured CpG sites on a tested trait could not be differentiated from the measured ones. To  
124 further validate whether the effect estimated by MR can be attributed to a single CpG site, we  
125 utilized the point mutation that naturally occurs on the causal CpG sites (C to A or C to T), also  
126 known as meSNP. For the human methylation array, nearly 10% of CpG sites have an meSNP  
127 available. We found that the meSNPs that occur at causal CpG sites have lower allele frequency  
128 in the population compared to noncausal CpG sites (Extended Data Fig. 2). Furthermore, the  
129 meSNPs were significantly depleted at causal CpG sites, suggesting that there is a negative se-  
130 lection against loss-of-function mutations at causal CpG sites (Extended Data Fig. 2). Among  
131 causal CpG sites with meSNPs available, we examined the correlation between the effects on the  
132 outcome trait estimated using a single meSNP and the effect estimated by MR. We observed a  
133 significant positive correlation between the two estimates ( $P = 1e-4$ , Pearson's  $R = 0.4$ , Extended  
134 Data Fig. 2). These results suggest that the causal effect estimated by MR can be partially at-  
135 tributed to a single CpG site, at least in the causal CpG sites with available meSNPs. Yet, consid-  
136 ering many CpG sites do not have meSNPs available and the methylation level of individual  
137 CpG site tends to be highly correlated with neighboring CpG sites<sup>30-32</sup>, we believe the causal  
138 CpG sites we identified also serve as tagging CpG sites for the causal regulatory region, and the  
139 causal effect size we estimated can be interpreted as the causal effect size of the tagged regula-  
140 tory region.

141 Interestingly, the Spearman correlation of the estimated effect size of CpGs across twelve traits  
142 formed two distinct clusters, with the first cluster containing eight lifespan- and health-span-related  
143 traits, and the second all four epigenetic age measurements (Fig. 1d). This observation suggests  
144 that, although all these twelve traits are genetically correlated with each other, causal CpGs do not  
145 have proportional effect sizes – the CpGs with large effects on lifespan and healthspan do not have  
146 a proportional effect size on epigenetic age measurements and *vice versa*.

147 To prioritize CpG sites with the potential causal effect on Aging-GIP1, we first filtered MR signals  
148 based on the  $P$  value threshold after Bonferroni correction. The CpG sites were then ranked ac-  
149 cording to the magnitude of the causal effect, adjusted by the colocalization probability (PP.H4).

150 The top CpG sites whose methylation was observed to promote healthy longevity (Aging-GIP1)  
151 included cg12122041 at the *HTT* locus, which is associated with bone mineral density and age,  
152 cg02613937 at the *TOMM40* locus, which is associated with Alzheimer's disease and age, and  
153 cg19047158 at the non-coding region, which is associated with gestational age and rheumatoid  
154 arthritis. The top CpG sites whose methylation was found to inhibit healthy longevity included  
155 cg04977528 at the *HEYL* locus, which is associated with sex and age, cg06286026 at the *GRK4*  
156 locus (associated with age), cg27161488 at the *C4orf10* locus (associated with rheumatoid arthritis  
157 and age), and cg18744360 at the *MAD1L1* locus (associated with hypotensive disorder, Fig. 1e).  
158 Furthermore, cg19514613 at the *APOE* locus is also among the top sites that limit longevity. Ge-  
159 netic variants near *HTT* and *MAML3* were also shown to significantly affect lifespan in Finnish  
160 and Japanese cohorts in a previous study<sup>33</sup>. Both *TOMM40* and *APOE* are known to contribute to  
161 the risk of Alzheimer's disease and are associated with human lifespan<sup>34,35</sup>. Our results suggest  
162 that the known lifespan-related effect at these loci may be mediated by DNA methylation. More-  
163 over, we also used adjusted Aging-GIP1, where the effects on human lifespan and healthspan that  
164 are correlated with socioeconomic status are removed. We showed that after adjusting for socio-  
165 economic status, the CpG site with the top pro-longevity effect is cg06636172 at the *FOXO* locus,  
166 which is a major longevity locus<sup>36,37</sup>.

167 To further understand the properties of the CpG sites identified as causal to each aging-related  
168 trait, we performed an enrichment analysis using 14 Roadmap annotations<sup>38</sup>. We found that the  
169 causal CpGs for most traits are enriched in promoters and enhancers while depleted in quiescent  
170 regions (Fig. 2a). Furthermore, the causal CpG sites were enriched in CpG shores (Extended Data  
171 Fig. 3). We observed that the causal CpG sites for Aging-GIP1 are significantly more evolutionally  
172 conserved compared to non-causal CpGs, based on both functional genomic conservation scores  
173 (Learning Evidence of Conservation from Integrated Functional genomic annotations, LECIF) and  
174 the phastCons/phyloP scores across 100 vertebrate genomes<sup>39</sup> (Fig. 2b, c, Extended Data Fig. 4).  
175 Moreover, the absolute value of the estimated causal effect sizes showed significant positive cor-  
176 relations between all three conservative scores. These results suggest that the CpG sites identified  
177 as causal for aging-related traits are more likely to be located in functional genomic elements and  
178 more evolutionarily conserved.

179 It is well known that DNA methylation status may affect the binding of transcription factors (TFs)  
180 <sup>40</sup>. To understand the relationship between causal CpG sites and TFs, we performed a transcription  
181 factor binding site enrichment analysis (Fig. 2d). The CpG sites causal to Aging-GIP1 were sig-  
182 nificantly enriched in the binding sites of 63 TFs, including *POLR2A*, *ZNF24*, *MYC*, and *HDAC1*;  
183 while depleted in the binding sites of 19 TFs, including *CTCF*, *CHD4*, and *BRD9* (Fig. 2d). In  
184 particular, *POLR2A* was among the top enriched TFs in 9 of 12 traits. *POLR2A* is the *POLR2*  
185 subunit (RNA polymerase II), and previous research shows that epigenetic modifications can mod-  
186 ulate its elongation and affect alternative splicing. Our results imply that this mechanism is poten-  
187 tially a major contributor that mediates the effects of DNA methylation on aging <sup>10,11,41</sup>. We further  
188 found that there were 3 TF-binding sites (*BRD4*, *CREB1*, and *E2F1*) enriched with CpG sites  
189 whose methylation levels promote healthy longevity (Aging-GIP1), and 4 TF-binding sites  
190 (*HDAC1*, *ZHX1*, *IKZF2*, and *IRF1*) enriched with CpG sites whose methylation levels decrease  
191 healthy longevity (Extended Data Fig. 5). *BRD4* contributes to cellular senescence and promotes  
192 inflammation <sup>42</sup>. Therefore, our findings suggest that higher DNA methylation at *BRD4* binding  
193 sites may inhibit the downstream effects of *BRD4* and promote healthy longevity. Similarly, pre-  
194 vious studies showed that *CREB1* is related to type II diabetes and neurodegeneration <sup>43</sup>, and me-  
195 diates the effect of calorie restriction <sup>44</sup>. However, how DNA methylation may affect *CREB1* bind-  
196 ing is not well studied. Our data suggest that higher methylation at *CREB1*-binding sites may  
197 promote its longevity effects. *HDAC1* is a histone deacetylase, and its activity increases with aging  
198 and may promote age-related phenotypes <sup>45,46</sup>. *HDAC1* has been shown to specifically bind to  
199 methylated sites. Our data, therefore, support the hypothesis that *HDAC1* plays a damaging role  
200 during aging, as increased DNA methylation at *HDAC1* binding sites may causally inhibit healthy  
201 longevity.

202 We also checked the enrichment of causal CpG sites in phenome-wide EWAS signals obtained  
203 from the EWAS catalog <sup>11</sup>. The top enriched phenotypes included rheumatoid arthritis, HIV infec-  
204 tion, nitrogen dioxide exposure, and maternal obesity (Fig. 2e). Interestingly, none of these condi-  
205 tions is primarily caused by aging. On the contrary, both rheumatoid arthritis and HIV infection  
206 are the conditions that have been suggested to accelerate aging and immunosenescence <sup>41</sup>. Addi-  
207 tionally, maternal obesity is associated with accelerated metabolic aging in offspring <sup>47</sup>, and nitro-  
208 gen dioxide exposure is also shown to be associated with an increased risk of mortality <sup>48</sup>. Among  
209 the 12 traits tested, only the causal CpG sites for GrimAge and Hannum age (both are epigenetic



210 biomarker traits) were significantly enriched in the change of the CpG sites with aging, both epi-  
211 genetic biomarker traits (Fig. 2e). Therefore, our results suggest that the causal CpG sites for aging  
212 are enriched in conditions that cause accelerated aging, but not in conditions that are caused by  
213 aging. This is consistent with the previous study, which suggests that differentially expressed genes  
214 reflect disease-induced rather than disease-causing changes <sup>49</sup>.

### 215 **MR on epigenetic age measurements successfully recovers clock sites as causal CpG sites**

216 For epigenetic age measurements, the causal CpG sites were the clock sites and the sites up-  
217 stream of clock sites (Fig. 3a). To validate our EWMR approach for discovering causal CpG  
218 sites, we used clock sites for each clock as ground truth and investigated whether MR could re-  
219 cover the clock sites as causal CpG sites with the correct estimated effects.

220 We first examined the identified causal CpG sites for three epigenetic age measurements with the  
221 clock models publicly available, namely HannumAge, HorvathAge, and PhenoAge <sup>7</sup>. We observed  
222 that the causal CpGs identified by EWMR for each epigenetic age measurement were significantly  
223 enriched with the corresponding clock sites (Fig. 3b; HannumAge  $P = 9.4e-9$ , HorvathAge  $P =$   
224  $1.2e-12$ , PhenoAge  $P = 2.7e-6$ ). Furthermore, EWMR predicted causal effect sizes of causal CpGs  
225 with the correct direction and relative magnitude; as for the three epigenetic age measurements,  
226 the estimated causal effect of MR showed a high and significant linear relationship with the actual  
227 causal effect sizes denoted by the coefficients of the clock model (Fig. 3c-e). Notably, the enrich-  
228 ment and correlation we described were also robust to the choice of threshold (Fig. 3b-e).

229 In MR studies, the P value is not a reliable ranking metric, as it is largely related to the number of  
230 instruments available for the exposure traits <sup>50</sup>. As the epigenetic age GWAS provided a unique  
231 opportunity where a part of the real causal CpG sites was already known, we applied four different  
232 ranking metrics to identify an ideal ranking metric to rank causal CpG sites. We calculated the  
233 area under the receiver operating curve (ROC, AUROC) using the clock sites as ground truth. The  
234 AUROC measures the accuracy of binary classification, where an AUROC of 0.5 corresponds to  
235 a random classification, and an AUROC of 1 corresponds to a perfect classification. Note that  
236 since some causal CpGs are unknown (regulatory CpGs upstream to clock sites, Fig. 3a), the AU-  
237 ROC we calculated underestimated the real accuracy. However, we found that when ranking with  
238 PP-H4 weighted effect size, strikingly higher AUROCs were achieved compared to all other rank-  
239 ing metrics (0.99 for HannumAge, 0.83 for HorvathAge, and 0.73 for PhenoAge, Fig. 3f, and

240 Extended Data Fig. 6). As far as we know, the colocalization probability-weighted effect size has  
241 never been used for ranking MR hits. Therefore, our findings provide novel metrics that could be  
242 reliably used to prioritize MR results of molecular traits and facilitate downstream analyses.

### 243 **Existing epigenetic clocks are not enriched with CpG sites causal to aging**

244 One open question for epigenetic clocks is whether their clock sites are causal to aging and age-  
245 related functional decline. To answer this question, we collected six epigenetic age models in hu-  
246 mans with the clock sites publicly available, namely, the Zhang clock, PhenoAge, PedBE,  
247 HorvathAge, HannumAge, and Dunedin-PACE. We then performed an enrichment analysis of  
248 causal CpGs for all eight lifespan/healthspan-related traits for each clock. After correcting for  
249 multiple testing, none of the existing clocks showed significant enrichment for causal CpGs of  
250 any of the lifespan/healthspan-related traits (Fig. 3g). PhenoAge showed a nominal significant  
251 enrichment with CpGs causal to healthspan and healthy aging, but it was not robust to the choice  
252 of thresholds. This finding suggests that, although some clocks contain CpGs causal to aging  
253 (Table 1), they, by design, favor CpG sites with a higher correlation with age and thus are not en-  
254 riched with causal CpGs.

255 In contrast, even though different clocks were trained on different datasets with different methods,  
256 the causal sites identified for one clock were usually also enriched with the clock sites for other  
257 clocks, suggesting that there is a subset of CpG sites that contribute to the epigenetic age estimate  
258 of all existing epigenetic clocks, which could potentially introduce systemic bias.

### 259 **Integration of MR results and age-related changes reveals protective and deleterious epige- 260 netic changes during aging**

261 Another important question in epigenetic aging is the identity and number of epigenetic changes  
262 that (i) contribute to age-related damage and (ii) respond to it. We approached this question by  
263 integrating information on the causal effect and age-related change for each CpG. The protective  
264 or damaging nature of the age-related methylation change in each CpG is indicated by the prod-  
265 uct of the causal effect and age-related change ( $b_{age} \times b_{MR}$ , Fig. 4a). For example, if a higher  
266 methylation level of a certain CpG site leads to a longer lifespan or healthspan, then during ag-  
267 ing, a decrease of the methylation level at that site would be considered as having a damaging  
268 effect, whereas an increased methylation level would be considered as having a protective effect.

269 The effect of DNA methylation estimated by MR is estimated through linear regression, which  
270 assumes that the relationship between DNA methylation level and lifespan-related outcome is lin-  
271 ear. To annotate protective and damaging CpGs, it is important to understand whether the effect  
272 size of genetic instruments on DNA methylation levels is comparable with the effect of aging. If  
273 they are not at the same scale, the annotation could be inaccurate as age-related methylation  
274 changes may fall outside of the linear regions. We show that the effect of genetic instruments is  
275 comparable with the effect of aging by calculating the ratio between the effect of strongest meQTL  
276 and age-related methylation change (Extended Data Fig. 7). The median ratio is 21.8, suggesting  
277 that the median effect of genetic instruments is roughly equivalent to the effect of 21.8 years of  
278 aging.

279 Therefore, using the age-related blood DNA methylation change data estimated from 7,036 indi-  
280 viduals (ages of 18 and 93 years, Generation Scotland cohort)<sup>50</sup>, we separated the CpG sites causal  
281 to eight traits related to lifespan into four different categories: protective hypermethylation, dele-  
282 terious hypermethylation, protective hypomethylation, and deleterious hypomethylation (Fig. 4b,  
283 Extended Data Fig. 8). Among the top 10 CpG sites whose methylation changes during aging have  
284 a relatively large impact on healthy longevity, we showed that six hypermethylated CpG sites  
285 during aging exhibit strong protective effects, including cg18327056, cg25700533, cg19095568,  
286 cg17227156, cg17113968, and cg07306253; while one hypomethylated CpG site (cg04977528)  
287 also has a protective effect. In contrast, 1 hypermethylated CpG sites (cg26669793) and 2 hypo-  
288 methylated CpG sites (cg25903363 and cg26628907) show damaging effects (Fig. 4b).

289 Contradicting the popular notion that most age-related changes are bad for the organism, our find-  
290 ings revealed that, in terms of the number of CpGs, there was no enrichment for either protective  
291 or damaging methylation changes during aging (Extended Data Fig. 9). We also found that there  
292 was no significant correlation between the size of the causal effect and the magnitude of age-  
293 related changes (Fig. 4b, Extended Data Fig. 9), suggesting that CpG sites with a greater effect on  
294 healthy longevity do not necessarily change their level of methylation during aging. This result is  
295 consistent with our findings discussed above and explains the lack of enrichment of causal sites in  
296 existing epigenetic clocks.

297 As the product of the causal effect and age-related change ( $b_{age} \times b_{MR}$ ) provides an estimate of  
298 the cumulative effect of age-related changes on aging-related phenotypes in a unit of time, we

299 calculated the cumulative effect of age-related changes on Aging-GIP1 (Fig. 4c). Importantly, we  
300 discovered that although the number of protective and damaging CpG sites was similar, the cumu-  
301 lative effect of combined age-related DNA methylation changes was detrimental to age-related  
302 phenotypes, consistent with the overall damaging nature of aging.

### 303 **Algorithms for developing causality-informed epigenetic clocks**

304 Although various existing epigenetic aging clock models can accurately predict the age of bio-  
305 logical samples, they are purely based on correlation. This means that the reliability of existing  
306 clock models is highly dependent on the correlation structure of DNA methylation and pheno-  
307 types. This may result in unreliable estimates when extrapolating the model to predict the age of  
308 novel biological conditions (i.e., applying clocks to interventions that do not exist in the training  
309 population), as the correlation structure may be corrupted by the new intervention.

310 To overcome this problem, we developed novel epigenetic clocks that are based on causal CpG  
311 sites identified by EWMR (Fig. 5a). Specifically, we trained an elastic net model on whole blood  
312 methylation data from 2,664 individuals<sup>51,52</sup>, using CpG sites identified as causal to Aging-GIP1  
313 by EWMR (adjusted  $P < 0.05$ ). In regular epigenetic clock models, the penalty weight is defined  
314 to be 1 for all CpG sites, which produces models that are purely based on correlation. Instead, we  
315 introduced a novel causality-informed elastic net model, where we assigned the feature-specific  
316 penalty factor based on the causality score for each CpG site (Method). The influence of the cau-  
317 sality score on the feature-specific penalty factor is controlled by the causality factor  $\tau$ , which is  
318 an adjustable parameter. If  $\tau = 0$ , the whole model is reduced to a regular elastic net regression,  
319 where the penalty factor equals one for all features. When  $\tau$  becomes large, the model is more  
320 influenced by the causality score and tends to assign larger coefficients to the features with a higher  
321 causality score (Fig. 5A, Method).

322 Using this method, we trained the model to build the causality-informed epigenetic clock *CausAge*  
323 using 2,664 blood samples. We show that the model's accuracy decreased as the causality factor  
324  $\tau$  increased (Fig. 5b, c). This is because the causality factor  $\tau$  controls the trade-off between the  
325 correlation and causality score-weighted penalty factor, and the causality score is not always cor-  
326 related with the predictive power of age. For example, a CpG site with a high correlation with age  
327 may not be causal to aging, and *vice versa*. To balance clock accuracy and causality, we used the  
328 *CausAge* with the causality factor  $\tau$  of 0.3 in the downstream analysis (Fig. 5c).

329 To separately measure adaptive and damaging DNA methylation changes during aging, we further  
330 separated causal CpG sites into two groups based on the causal effect size from MR and the direc-  
331 tion of age-related change (Fig. 4b). We then built *DamAge*, the damaging clock, which contains  
332 only the damaging CpG sites, and *AdaptAge*, the protective clock, which contains only the adap-  
333 tive/protective CpG sites (Fig. 5a). We show that both *DamAge* and *AdaptAge* can predict the age  
334 of blood samples with similar accuracy. And similar to the *CausAge*, the accuracy of *DamAge* and  
335 *AdaptAge* decreases as the causality factor  $\tau$  increases (Fig. 5c).

### 336 ***DamAge* and *AdaptAge* clocks uncouple aging-related damage and adaptation**

337 By design, *AdaptAge* contains only the CpG sites that capture protective effects against aging.  
338 Therefore, in theory, the subject predicted to be older by *AdaptAge* may be expected to accumu-  
339 late more protective changes during aging. On the contrary, *DamAge* contains only the CpG sites  
340 that exhibit damaging effects, which may be considered as a biomarker of age-related damage.  
341 Therefore, we hypothesized that *DamAge* acceleration may be harmful and shorten life expect-  
342 tancy, whereas *AdaptAge* acceleration would be protective or neutral, which may indicate  
343 healthy longevity.

344 To test this hypothesis, we first analyzed the associations between human mortality and epigenetic  
345 age acceleration quantified by causality-informed clocks using 4,651 individuals from the Fram-  
346 ingham Heart Study, FHS offspring cohort ( $n = 2,544$  Caucasians, 54% females) and Women's  
347 Health Initiative cohort (WHI,  $n = 2107$  postmenopausal women, Methods). Among the three cau-  
348 sality-informed clocks, *DamAge* acceleration showed the strongest positive association on mortal-  
349 ity and outperformed *CausAge* and Hannum clock, both of which exhibited a weaker positive as-  
350 sociation with mortality (Fig. 5d, e). This finding supports the notion that age-related damage con-  
351 tributes to the risk of mortality. In contrast, *AdaptAge* acceleration showed a significant negative  
352 association with mortality, suggesting that protective adaptations during aging, measured by  
353 *AdaptAge*, are associated with longer lifespan. In addition, epigenetic age accelerations measured  
354 by *DamAge* and *AdaptAge* were only weakly associated (Pearson's  $R = 0.14$ , Extended Data Fig.  
355 10). These findings highlight the importance of separating adaptive and damaging age-related  
356 changes when building aging clock models.

357 Interestingly, although the clock accuracy monotonically decreased as the causality factor  $\tau$  in-  
358 creased, the association between mortality and epigenetic age acceleration did not follow the same

359 trend (Fig. 5e). Especially for *DamAge* and *CausAge*, the mortality association increased as the  $\tau$   
360 increased and peaked when  $\tau$  was around 0.6. Also, *DamAge* consistently outperformed *CausAge*  
361 in predicting mortality risk, even though *CausAge* was more accurate in age prediction (Fig. 5b-  
362 e). This suggests that although the introduction of the causality score and separation of damaging  
363 CpGs may decrease the accuracy of the clock in terms of predicting chronological age, it improves  
364 the prediction of aging-related phenotypes.

365 Induced pluripotent stem cell (iPSC) reprogramming is one of the most robust rejuvenation mod-  
366 els, which was shown to be able to strongly reverse the epigenetic age of cells <sup>46</sup>. We applied the  
367 causality-informed clock models to iPSC reprogramming <sup>53</sup>. For comparison, we also included  
368 three blood-based epigenetic models, namely Hannum clock, PhenoAge, and DunedinPace. The  
369 Hannum clock was trained on chronological age <sup>54,55</sup>, PhenoAge was trained on the age adjusted  
370 by health-related phenotypes <sup>56,57</sup>, and Dunedin-PACE was trained to predict the pace of aging <sup>56</sup>.  
371 Consistent with Hannum clock and PhenoAge, *DamAge* revealed that epigenetic age decreased  
372 during iPSC reprogramming, but with a stronger negative correlation with the time of reprogram-  
373 ming and higher statistical significance (Fig. 5f). This observation suggests that *DamAge* may  
374 better capture the damage-removal effect of iPSC reprogramming. On the contrary, *AdaptAge* in-  
375 creased significantly during the reprogramming process, suggesting that protective age-related  
376 changes do not capture the rejuvenation effect and that in fact cells may acquire even more pro-  
377 tective changes during iPSC reprogramming.

### 378 **Causality-informed epigenetic clocks capture damage and aging-related effects in the early** 379 **stages**

380 To further examine how *DamAge* and *AdaptAge* capture age-related damage and protective adap-  
381 tations, respectively, we analyzed conditions that specifically promote age-related damage.

382 Paraoxonase 1 (*PON1*) is one of most studied genes associated with cardiovascular disease, oxi-  
383 dative stress, inflammation, and healthy aging <sup>58</sup>. Specifically, *PON1* plays an important role in  
384 detoxifying organophosphorus compounds and removing harmful oxidized lipids <sup>6</sup>. The genetic  
385 variant of *PON1* (R192Q) significantly decreases PON1 activity and is known to be associated  
386 with an increased risk of cardiovascular disease and neurodegenerative diseases <sup>59</sup>. Interestingly,  
387 the *PON1* Q allele is significantly depleted in centenarians <sup>60</sup>. We analyzed the relationship be-  
388 tween *PON1* activity and epigenetic age in 48 whole blood samples (Fig. 6a) <sup>61</sup>. *DamAge* shows

389 a significant negative correlation with *PONI* activity ( $R = -0.55$ ,  $p = 0.0062$ ), whereas *AdaptAge*  
390 showed a significant positive correlation with *PONI* activity ( $R = 0.69$ ,  $p = 0.0003$ ). Again, this  
391 association was not observed by other epigenetic clocks, except for Horvath age, but with a less  
392 significant negative correlation.

393 By definition, causal epigenetic changes occur prior to downstream methylation changes and the  
394 associated phenotypes (which are caused by upstream causal epigenetic changes). Therefore, we  
395 hypothesized that the causality-informed clock models may be able to capture aging-related events  
396 in the early stages, before downstream epigenetic mechanisms are triggered. Previous studies have  
397 shown that anti-aging interventions during development could prolong lifespan and healthspan in  
398 later life, including calorie restriction (CR)<sup>62</sup>, and rapamycin treatment<sup>63</sup>. Small for gestational  
399 age (SGA) is a condition defined as a birth weight less than the 10th percentile for gestational age  
400<sup>64</sup>. SGA is usually caused by nutritional deficiency during pregnancy; therefore, it can be consid-  
401 ered a model of early life CR. We show that children with SGA have a significantly lower *DamAge*  
402 and a higher *AdaptAge* than children with normal birth weight. This observation suggests that  
403 *DamAge* and *AdaptAge* may be able to capture early-life CR effects, which are associated with  
404 decreased damage accumulation and increased protective adaptations. These effects are not cap-  
405 tured by the other epigenetic clocks tested. SGA is usually considered a pathological condition;  
406 some studies suggest that this may be because early life benefits can be reversed in later life by  
407 exposure to excessive nutrients<sup>65</sup>. The different roles of SGA in the early and late stages of life  
408 may need to be further investigated in future studies.

409 In vitro fertilization (IVF) is a common method of treating infertility. Yet, previous studies have  
410 shown that IVF may increase the risk of perinatal morbidity and mortality<sup>66</sup>. It has recently been  
411 proposed that embryos undergo a rejuvenation event shortly after conception to remove age-related  
412 damage<sup>67,68</sup>. Whether the *in vitro* environment of IVF affect this rejuvenation process is unknown.  
413 We analyzed the DNA methylation data from neonatal blood spots of 137 newborns conceived  
414 unassisted (NAT), through intrauterine insemination (IUI), or through IVF using fresh or cryo-  
415 preserved (frozen) embryo transfer<sup>69</sup>. We found that IVF-conceived newborns using fresh or cryo-  
416 opreserved embryos have significantly higher *DamAge* acceleration and lower *AdaptAge* than  
417 NAT-conceived newborns (Fig. 6b). On the other hand, IUI-conceived newborns show no

418 significant differences in both *DamAge* and *AdaptAge* compared to the control (Fig. 6b). This ef-  
419 fect could not be observed by the other five epigenetic clocks tested, except for the Horvath age.

420 Genomic imprinting is an epigenetic mechanism that controls the expression of parent-of-origin-  
421 dependent gene, which plays an important role in embryonic development and has a lifelong im-  
422 pact on health<sup>70</sup>. Some imprinting genes are known to be associated with metabolic disorders and  
423 aging (e.g., IGF2-H19)<sup>71,72</sup>. DNA methylation at imprinting loci is maintained during epigenetic  
424 reprogramming in embryonic development, which coincides with the period of embryonic rejuve-  
425 nation<sup>67,68</sup>. We analyzed the peripheral blood DNA methylation data from patients with single-  
426 locus or Multi-loci imprinting disturbances (SLID or MLID), which is the condition of losing  
427 methylation at single or multiple imprinting centers<sup>73</sup>. Similar to IVF, we found that patients with  
428 imprinting disorders showed significantly higher *DamAge* and lower *AdaptAge* (Fig. 6b). To-  
429 gether, these results suggest that *DamAge* and *AdaptAge* can serve as better biomarkers for events  
430 affecting aging traits already during development.

431 Causality-informed clocks could also capture the aging-related effects of short-term interventions.  
432 For example, we found that short-term treatment with cigarette smoke condensate in bronchial  
433 epithelial cells significantly accelerated *DamAge* but did not affect other tested clocks (Fig. 6c).  
434 Additionally, a 6-week omega-3 fatty acid supplementation in overweight subjects<sup>74</sup>, which has  
435 been shown to be protective against age-related cardiovascular diseases, significantly increased  
436 *AdaptAge* and reduced *DamAge* (Fig. 6c). Together, our data demonstrate the importance of sep-  
437 arating damage and adaptation when building biomarkers of aging and provide novel tools to quan-  
438 tify aging and rejuvenation.

## 439 Discussion

440 Many existing epigenetic aging clock models accurately predict the age of samples<sup>7</sup>, and there  
441 are numerous CpG sites that are differentially methylated during aging<sup>50</sup>. DNA methylation lev-  
442 els affect the structure of chromatin and the expression of neighboring genes<sup>51,52</sup>, through which  
443 they can causally affect aging-related phenotypes. A recent study also suggested that DNA meth-  
444 ylation may play a causal role in the rejuvenation effect observed during iPSC reprogramming<sup>46</sup>.  
445 It is important to understand whether and which DNA methylation changes during aging cause  
446 aging-related phenotypes. A previous transcriptome-wide MR study suggests that differentially



447 expressed genes in human diseases reflect mainly gene expression caused by disease rather than  
448 disease-causing genes<sup>53</sup>. Similarly, DNA methylation changes during aging may primarily re-  
449 flect the downstream effects of aging phenotypes rather than causing them. Our EWMR findings  
450 further support this notion as we found no significant overlap between CpG sites causal to  
451 healthy longevity and those differentially methylated during aging.

452 MR is a powerful method to identify causal relationships between exposure traits and phenotypes  
453<sup>75</sup>. However, it is limited by the availability of genetic instruments for the exposure traits. In our  
454 study, we utilized the DNA meQTLs of 420,509 CpG sites from the Illumina 450K methylation  
455 array as instrumental variables to infer their causal relationship with aging-related phenotypes.  
456 However, there are many unmeasured CpG sites across the genome, and the methylation patterns  
457 of nearby CpG sites are highly correlated<sup>51</sup>. Therefore, it is not possible to fully separate the causal  
458 effect of a single CpG and its neighbors. Analysis of point mutations at causal CpG sites (meSNPs)  
459 suggests that the epimutation of the single causal CpG site identified by MR may be sufficient to  
460 alter the phenotype (Extended Data Fig. 2). However, due to the lack of abundance of meSNPs on  
461 causal CpG sites, this hypothesis is difficult to test across all causal CpG sites we identified. There-  
462 fore, we tend to reach a more conservative conclusion and believe that the causal CpG sites iden-  
463 tified in our study serve as tagging CpG sites for the causal regulatory regions in aging-related  
464 phenotypes. The genome-wide meQTL studies in the future may facilitate further refining of the  
465 causal effects of CpG sites at the base-pair resolution.

466 The genetic instruments of CpG sites were selected from the currently largest meQTL study in  
467 whole blood (GoDMC, 36 cohorts, including 27,750 European subjects). Therefore, the causal  
468 CpG sites we identified are primarily valid in blood. However, a previous study showed that up to  
469 73% cis-meQTLs are shared across tissues<sup>76</sup>. This suggests that the identified causal CpG sites  
470 may also act in other tissues to affect lifespan and healthspan. Future large-scale meQTL studies  
471 across tissues may facilitate the identification of tissue-specific epigenetic effects on aging.

472 We found that TF-binding sites of *BRD4* and *CREB1* are enriched with CpG sites whose methyl-  
473 ation levels promote healthy longevity, and TF-binding sites for *HDAC1* are enriched with CpG  
474 sites whose methylation levels decrease healthy longevity. *BRD4* contributes to cell senescence  
475 and promotes inflammation<sup>42</sup>. Therefore, our findings suggest that higher DNA methylation at  
476 *BRD4* binding sites may inhibit the downstream effects of *BRD4* and promote healthy longevity.

477 Similarly, previous studies showed that *CREB1* is related to type II diabetes and neurodegeneration  
478 <sup>43</sup>, and mediates the effect of calorie restriction <sup>44</sup>. However, how DNA methylation may affect  
479 CREB1 binding is not well studied. Our data suggest that higher methylation at CREB1-binding  
480 sites may support its longevity effects. *HDAC1* is a histone deacetylase, and its activity increases  
481 with aging and may promote age-related phenotypes <sup>45,46</sup>. *HDAC1* has been shown to specifically  
482 bind to methylated sites. Our data, therefore, support the hypothesis that *HDAC1* plays a damaging  
483 role during aging, as increased DNA methylation at *HDAC1* binding sites may causally inhibit  
484 healthy longevity.

485 One general approach for developing anti-aging interventions is to identify molecular changes  
486 during aging and use these changes as targets to modulate the aging process <sup>54,55</sup>. A similar idea  
487 has also been applied to evaluate potential longevity interventions. However, this logic is intrinsi-  
488 cally flawed, as correlation does not imply causation and age-related changes are not necessarily  
489 causal to age-associated declines. As living organisms are complex systems with various adaptive  
490 mechanisms, many molecular changes during aging are potentially neutral downstream effects of  
491 fundamental damaging changes or even adaptive mechanisms that protect against aging pheno-  
492 types. This notion is usually underappreciated as age-related changes are generally assumed to be  
493 damaging. As a result, adaptive mechanisms of aging are largely understudied. However, there is  
494 evidence to suggest that at least some age-related changes are protective against aging phenotypes.

495 An example of age-related protective changes is the Insulin and IGF-1 signaling (IIS) pathway.  
496 Attenuation of IIS signaling intensity through multiple genetic manipulations has been shown to  
497 consistently extend the lifespan of worms, flies, mice, and potentially humans <sup>56,57</sup>. This pathway  
498 also mediates pro-longevity effects of dietary restriction <sup>56</sup>. Growth hormone is produced by the  
499 anterior pituitary gland and can induce the production of IGF-1, thus increasing IIS signaling. Both  
500 growth hormone and IGF-1 levels decline during aging <sup>58</sup>, which is considered to be a defensive  
501 response that extends lifespan <sup>6</sup>. Another example of an age-related adaptation is protein aggrega-  
502 tion. It has been shown in *C. elegans* that the protein aggregation events are increased during aging.  
503 Although it may look like a result of losing proteostasis, it turns out to be a protective mechanism  
504 that drives aberrant proteins into insoluble aggregates to improve overall proteostasis, and has been  
505 observed in long-lived mutants <sup>59</sup>. Similar protective mechanisms are also observed in mouse  
506 nerves at the transcriptomic level <sup>60</sup>.

507 Our results suggest that adaptive mechanisms at the epigenetic level are nearly as common as  
508 damaging changes and that simply following age-related changes in DNA methylation does not  
509 allow us to infer positive, neutral, or negative effects on age-related traits. However, the identified  
510 damaging and protective CpG sites are extremely useful both for understanding aging and quanti-  
511 fying it, and the same applies to rejuvenation. Together, the identified CpGs represent causal epi-  
512 genetic changes, and their combined effect on health-related phenotypes is negative.

513 The framework we described for epigenetic changes in this study may be applied to any other age-  
514 related change, e.g., changes in the transcriptome, metabolome, and proteome. While all age-re-  
515 lated features may be used to construct aging clocks, some of them are expected to be negative,  
516 some neutral, and some protective. Neither the direction nor the degree of change of age-related  
517 changes is important, and inferring the need to bring these changes to those observed in the young  
518 state as a way to rejuvenate an organism is equally incorrect. Instead, the focus should be on the  
519 causal effects of age-related changes, as well as on the direction of their effect.

520 The causal epigenetic clock models, *CausAge*, *AdaptAge*, and *DamAge*, could help separate pro-  
521 tective changes from damaging events. We also showed that by preselecting the CpG sites that  
522 show protective adaptation during aging, it is possible to build an aging clock showing an inverse  
523 relationship with mortality. Specifically, subjects with elevated protective adaptation are predicted  
524 to be age-accelerated by *AdaptAge* and have a lower risk of mortality (Fig. 5c). Similarly,  
525 *AdaptAge* shows an inverse relationship with rejuvenation (e.g., iPSC reprogramming) and aging  
526 acceleration. Note that both *DamAge* and *AdaptAge* show similar accuracy in predicting chrono-  
527 logical age, but their delta-age term shows an opposite biological meaning. This finding suggests  
528 that we should reconsider the way we interpret “epigenetic age acceleration” in clinical settings,  
529 especially for the clocks that are trained in a regular way, which contain a mixture of adaptative  
530 and damaging CpG sites. Together, our finding highlights the importance of pre-selecting deleter-  
531 ious CpG sites when building aging clock models, and our causality-informed clock models pro-  
532 vide novel insights into the aging mechanisms and testing interventions that delay aging and re-  
533 verse biological age.

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539 **Author contributions**

540 K.Y. initiated the study; K.Y. collected and analyzed the data; V.N.G. supervised the study.  
541 K.Y., H.L., and A.T. performed data analyses; All authors contributed to paper writing.

542 **Competing interest statement**

543 The authors declare no competing financial interests.

544 **Data availability statement**

545 Data generated in this study will be publicly available upon publication.

546 **Code availability statement**

547 Code used will be publicly available upon publication.

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710

## 711 **Figure legends**

712 **Fig. 1. Epigenome-wide Mendelian Randomization on various aging-related phenotypes. a.**  
713 Schematic diagram shows the principle of MR using meQTLs as exposures and aging-related  
714 traits as outcomes to identify causal CpG sites. **b.** The flow chart shows the procedure for epige-  
715 nome-wide MR and sensitivity analysis. **c.** Number of significant causal CpG sites identified for  
716 each trait after adjusting for multiple tests using the Bonferroni correction. The darker regions of  
717 the bars indicate the number of causal CpG sites supported by the colocalization analysis with  
718 conditional  $PP-H4 > 0.7$ . **d.** Spearman correlation of the estimated causal effects of CpGs in  
719 twelve traits. Only CpGs with significant MR signals across at least six traits are included in the  
720 analysis. The color scheme corresponds to the Spearman correlation coefficient, \* adjusted  $P <$   
721  $0.05$ , \*\* adjusted  $P < 0.01$ , \*\*\* adjusted  $P < 0.001$ . **e.** The modified Mississippi plot shows sig-  
722 nificant MR signals for Aging-GIP1. The X-axis corresponds to the genomic positions of CpG  
723 sites; Y-axis represents the size of the causal effect adjusted by colocalization probability ( $PP-$   
724  $H4$ ). CpG sites with top adjusted causal effects are annotated with the name and nearest gene.  
725 Only CpG sites with adjusted  $P < 0.05$  are included in the plot.

726 **Fig. 2. CpG sites causal to aging are enriched in specific genetic regulatory regions. a.** The  
727 bar plot shows the enrichment of causal CpG sites in 14 Roadmap genomic annotations. The Y  
728 axis shows  $-\log_{10}$  (FDR) based on Fisher's exact test, signed by  $\log_2$  (Odds ratio). Causal CpG  
729 sites identified for different traits are annotated with different colors. Two dotted horizontal lines  
730 show the FDR threshold of 0.05. TssA, active transcription start site. Prom, upstream/downstream  
731 TSS promoter. Tx, actively transcribed state. TxWk, weak transcription. TxEn, transcribed and  
732 regulatory Prom/Enh. EnhA, active enhancer. EnhW, weak enhancer. DNase, primary DNase.  
733 ZNF/Rpts, state associated with zinc finger protein genes. Het, constitutive heterochromatin.  
734 PromP, Poised promoter. PromBiv, bivalent regulatory states. ReprPC, repressed polycomb states.  
735 Quies, quiescent state. **b, c.** The box plot shows the distribution of conservation scores in causal  
736 and non-causal CpG sites. The conservation scores are obtained by Learning Evidence of Conser-  
737 vation from Integrated Functional genomic annotations (LECIF, **b**) and phastCons (**c**). \*  $P < 0.05$ ,  
738 \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ . **d, e.** Enrichment of causal CpG sites for 12 aging-  
739 related traits against transcription-factor-binding sites (**d**) and EWAS hits (**e**). Each horizontal bar

740 represents an enriched term. The X-axis shows the  $-\log_{10}(P\text{-value})$ , signed by  $\log_2$  (Odds ratio).  
741 The top 10 enriched terms that passed the FDR threshold of 0.05 for each direction are annotated.

742 **Fig. 3. MR on epigenetic age successfully recovers clock sites as causal CpG sites. a.** For epi-  
743 genetic age measurements, true causal sites are the clock sites and the sites upstream of clock sites.  
744 We used these traits as a positive control to validate the MR approach for identifying causal CpGs.  
745 **b.** The forest plot shows the enrichment of clock sites for each model in causal CpG sites identified  
746 by MR for each trait. The X-axis shows the  $\log_2(\text{Odds Ratio})$ .  $P$ -values calculated by Fisher's  
747 exact test are annotated. Error bars show 95% confidence intervals. Different colors represent dif-  
748 ferent thresholds for causal CpGs. **c-e.** Correlation between ground truth causal effects (clock co-  
749 efficients, X-axis) and causal effects estimated by MR (Y-axis) for Hannum age (**c**), Horvath age  
750 (**d**) and PhenoAge (**e**). Different colors represent different thresholds for causal CpGs. Pearson's  
751 correlation coefficients and  $P$ -values are annotated. **f.** The receiver operating characteristic (ROC)  
752 curves show the sensitivity (Y-axis) and the 1-specificity (X-axis) of MR in identifying causal  
753 CpG sites for clock traits, with the area under the ROC curve (AUC) annotated. **g.** The forest plot  
754 shows the enrichment of clock sites for six aging clock models in causal CpG sites identified by  
755 MR for each trait. The X-axis shows the  $\log_2(\text{Odds Ratio})$ .  $P$ -values calculated by Fisher's exact  
756 test are annotated if  $P < 0.05$ . Error bars show 95% confidence intervals. Different colors represent  
757 the different thresholds for causal CpGs.

758 **Fig. 4. Integration of causal information and age-related changes to separate protective and**  
759 **damaging epigenetic changes. a.** Schematic diagram showing the method to identify protective  
760 and damaging epigenetic changes by integrating MR results and age-related differential methyl-  
761 ation. **b.** Relationship between MR-estimated causal effects (X-axis) and age-related methylation  
762 change (Y-axis) for each significant causal CpG identified in Aging-GIP1. The color scheme high-  
763 lights the expected impact of age-related methylation change on aging. Error bars show the stand-  
764 ard error of  $b$ . The size reflects the PP-H4. Only CpG sites with adjusted  $P$ -values  $< 0.05$  and  
765 relative PP-H4  $> 0.7$  are plotted. The CpG sites with the top 10 largest effect sizes are annotated.  
766 **c.** Area plots show the total cumulative effect of changes in DNA methylation on Aging-GIP1.  
767 The X-axis shows the rank of the CpG sites based on the impact of age-related changes  
768 ( $b_{\text{age:CpG}} \times b_{\text{CpG:MR}}$ ). The Y-axis and the color scheme show the cumulative sum of impacts.

769 **Fig. 5. Construction and application of causality-informed epigenetic clocks.** **a.** Schematic  
770 diagram shows the procedure of constructing causality-informed epigenetic clocks. **b.** Scatter plots  
771 show the accuracy of causal clocks on the test set. The X-axis shows the real age of each sample,  
772 and the Y-axis shows the predicted age of the same sample based on each clock model. Median  
773 absolute error (MAE) and Pearson's R are annotated. **c.** Line plot showing the relationship between  
774 causality factor ( $\tau$ ) and clock accuracy measured by MAE and Pearson's R. **d.** The forest plot  
775 shows the Log2 hazard ratio of mortality risk for every 10-year increase in age for each clock  
776 model. The *P* values are annotated if  $P < 0.05$ . The error bars show the standard error of the log2  
777 hazard ratio. **e.** The line plot shows the relationship between the causality factor ( $\tau$ ) and  $-\log_{10}(p)$   
778 for the association with mortality risk (signed by  $\log_2(\text{hazard ratio})$ ). The yellow dashed line shows  
779 the *P* threshold of 0.05. The orange dotted line shows the significance score for Hannum age ac-  
780 celeration **f.** The scatter plots show the application of causal clocks and three other blood-based  
781 clocks to iPSC reprogramming. The X-axis shows the days after initiating reprogramming. Pear-  
782 son's R and *P* values are annotated.

783 **Fig. 6. Causality-informed epigenetic clocks capture aging-related effects in the early stages.**  
784 **a.** Scatter plots show the correlation between epigenetic age and blood *PONI* activity. Epigenetic  
785 age prediction is rescaled to a 0-1 scale for better comparison. The color shows the *PONI* genotype  
786 in subjects. Linear regression is performed, and Pearson's R and *P* values are annotated. **b.** Box  
787 plots show the association between the epigenetic age and the conditions at early developmental  
788 stages, including the small for gestational age (SGA), the in vitro fertilization, and imprinting dis-  
789 turbances. IUI, intrauterine insemination; IVF, in vitro fertilization; SLID, single locus imprinting  
790 disorder; MLID, multiple loci imprinting disorder. **c.** Box plots show the association between the  
791 epigenetic age and short-term treatments, including the 15 months of cigarette smoke condensate  
792 (CSC) treatment and the 6-week supplementation of omega-3 fatty acid supplementation in over-  
793 weight subjects. For all box plots, the significant pairs based on the two-tail t-test are annotated  
794 with stars, and the *P* values from the ANOVA test are annotated. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P <$   
795  $0.001$ , \*\*\*\*  $P < 0.0001$ .

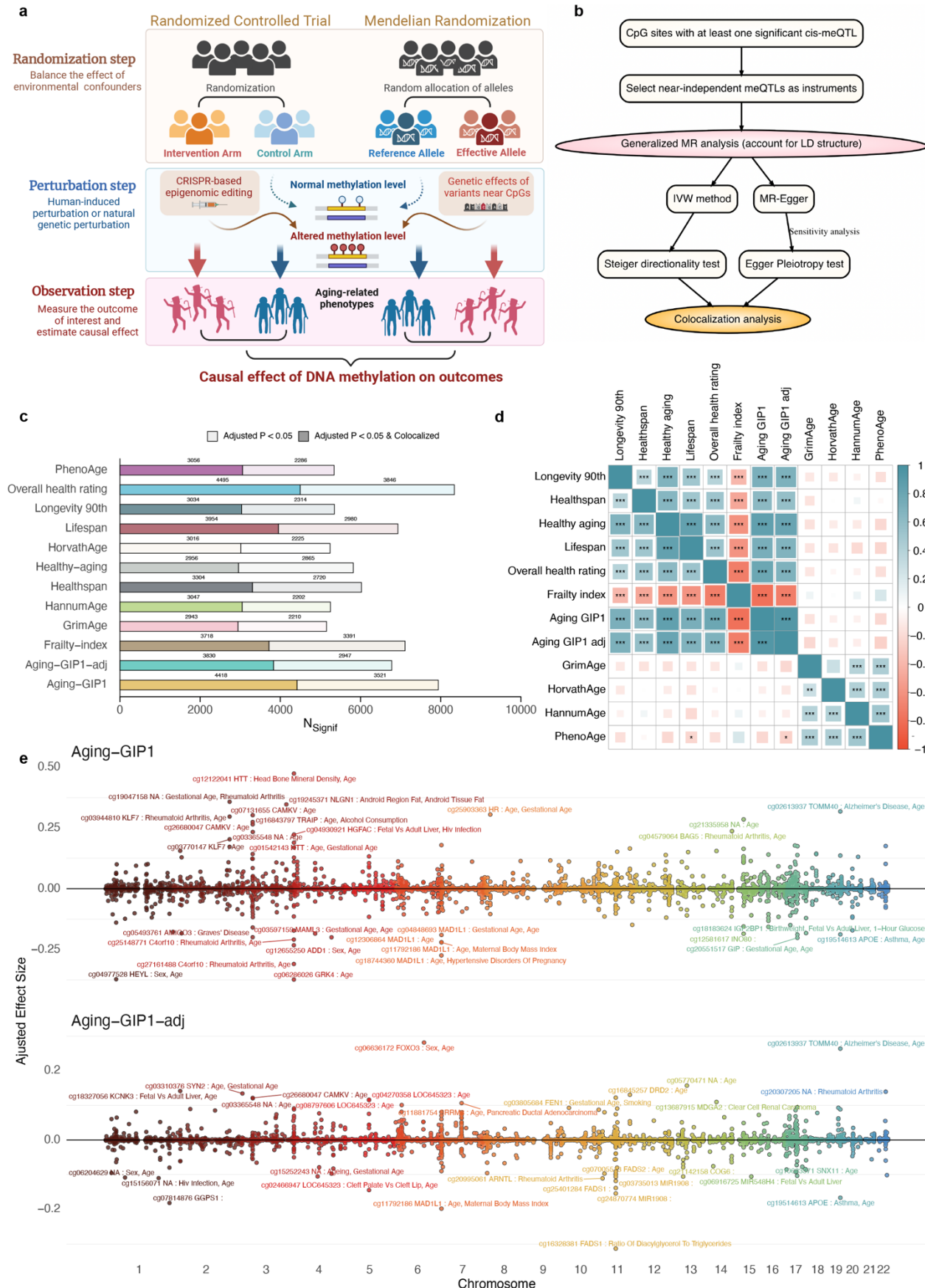
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**Table 1.** Causal CpG sites in existing epigenetic clocks

	Position	Weight	outcome	Beta	SE	P	H4	role
HorvathAge (353)	cg06557358	-0.14	Overall_health_rating	-0.04	0.008	1.96E-07	0.89	Protective
	cg09509673	0.01	Healthy-aging	0.02	0.003	3.86E-13	0.85	Protective
	cg09509673	0.01	Lifespan	0.05	0.006	9.92E-20	0.83	Protective
	cg11299964	-0.16	Aging-GIP1	0.08	0.012	5.42E-12	0.86	Deleterious
	cg16744741	-0.35	Aging-GIP1	0.09	0.017	1.86E-08	0.89	Deleterious
	cg16744741	-0.35	Overall_health_rating	0.06	0.008	6.10E-14	0.86	Deleterious
PhenoAge (513)	cg05087948	-6.99	Aging-GIP1-adj	-0.08	0.013	7.30E-10	1.00	Protective
	cg21926612	-2.15	Overall_health_rating	0.01	0.002	3.27E-11	0.94	Deleterious
	cg11896923	-1.38	Healthspan	0.17	0.024	4.64E-12	0.90	Deleterious
	cg11896923	-1.38	Healthy-aging	0.05	0.008	5.63E-10	0.86	Deleterious
	cg00862290	-0.23	Healthy-aging	0.00	0.001	1.28E-08	0.85	Protective
	cg00862290	-0.23	Lifespan	-0.02	0.003	0	0.94	Protective
Zhang (514)	cg24987259	-1.33	Overall_health_rating	-0.04	0.007	8.25E-09	0.95	Protective
	cg05310309	0.18	Aging-GIP1	0.03	0.003	1.13E-32	0.96	Protective
	cg05310309	0.18	Overall_health_rating	0.01	0.002	2.64E-12	0.92	Protective
	cg06672696	0.02	Frailty-index	0.05	0.010	1.74E-07	0.82	Protective
PedBE (94)	cg04221461	0.03	Frailty-index	0.04	0.008	1.25E-07	0.95	Protective
	cg19381811	-0.08	Aging-GIP1	-0.04	0.004	3.26E-21	0.929544	Protective
	cg19381811	-0.08	Overall_health_rating	-0.03	0.002	8.80E-37	0.955032	Protective

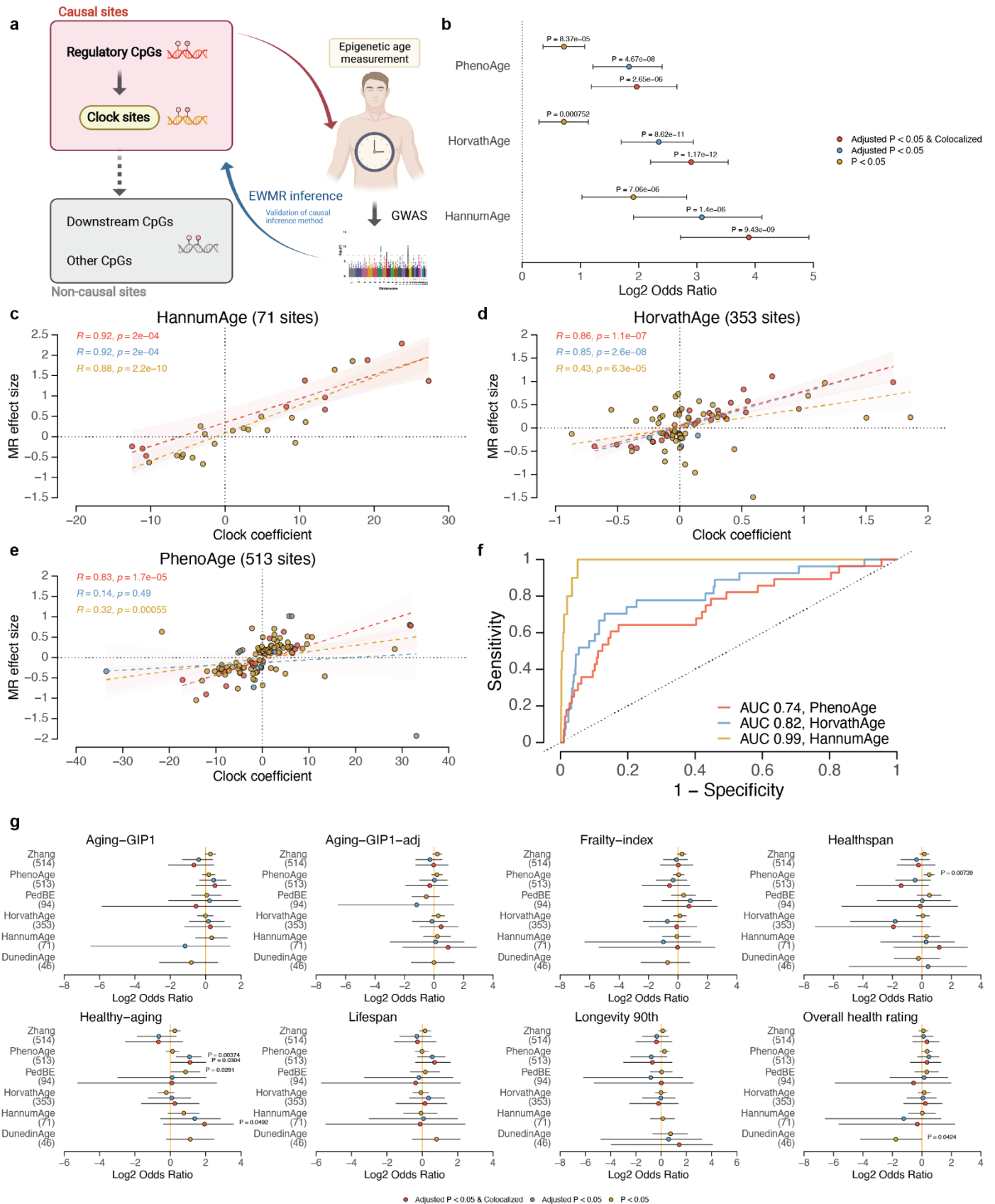
798 **Fig. 1**



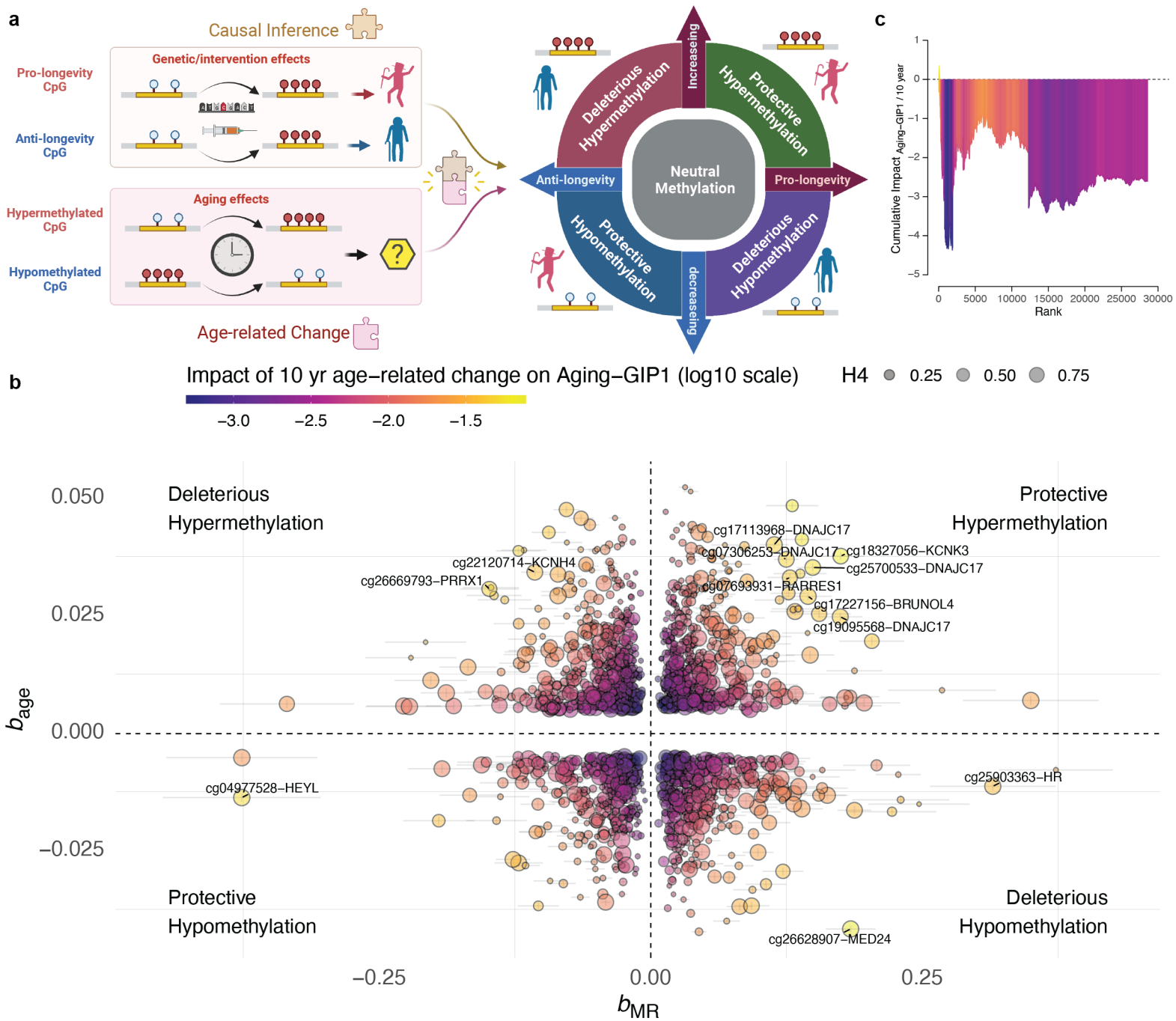




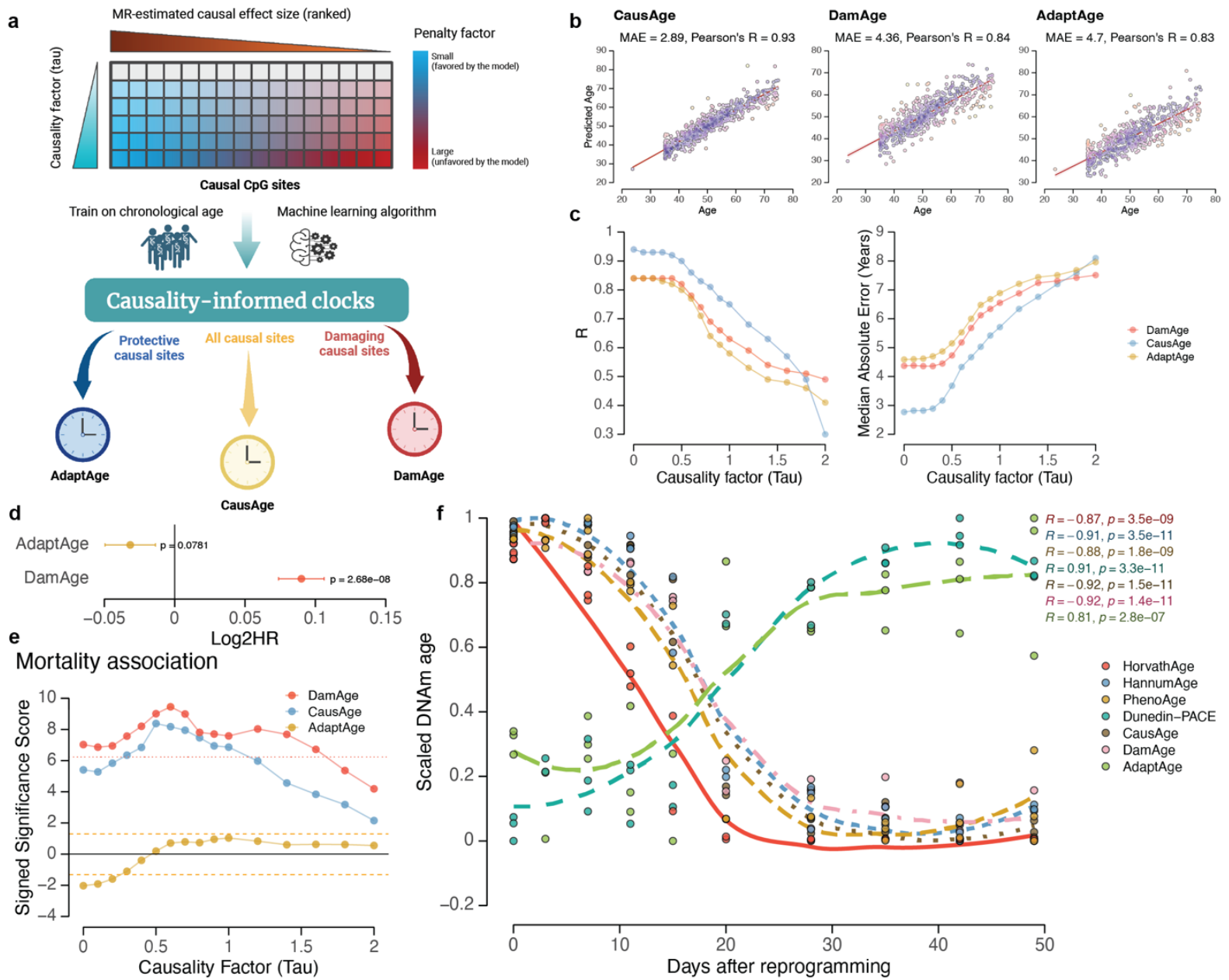
801 **Fig. 3**



802 **Fig. 4**



803 **Fig. 5**



804 **Fig. 6**

