

***ClockBase*: a comprehensive platform for biological age profiling in human and mouse**

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ABSTRACT

Aging represents the greatest risk factor for chronic diseases and mortality, but to understand it we need the ability to measure biological age. In recent years, many machine learning algorithms based on omics data, termed aging clocks, have been developed that can accurately predict the age of biological samples. However, there is currently no resource for systematic profiling of biological age. Here, we describe *ClockBase*, a platform that features biological age estimates based on multiple aging clock models applied to more than 2,000 DNA methylation datasets and nearly 200,000 samples. We further provide an online interface for statistical analyses and visualization of the data. To show how this resource could facilitate the discovery of biological age-

24 modifying factors, we describe a novel anti-aging drug candidate, zebularine, which reduces the
25 biological age estimates based on all aging clock models tested. We also show that pulmonary
26 fibrosis accelerates epigenetic age. Together, *ClockBase* provides a resource for the scientific
27 community to quantify and explore biological ages of samples, thus facilitating discovery of new
28 longevity interventions and age-accelerating conditions.

29 INTRODUCTION

30 Aging is an extremely complex biological process that represents the greatest risk factor for
31 chronic diseases^{1,2}. This makes the aging process a desirable target for preventing age-related
32 diseases and reducing their global burden³⁻⁵. However, to associate aging with diseases and in-
33 terventions that target aging, it is important to be able to measure the rate of aging^{6,7}. In recent
34 years, various machine-learning models based on omics data have emerged (also known as aging
35 clocks), which can accurately predict the age of samples derived from different tissues, cell
36 types, and even single cells^{6,8,9}. Various molecular markers have been shown to have the poten-
37 tial to profile the rate of aging, including DNA methylation, transcriptome, proteome,
38 metabolome, microbiome, and other types of omics data⁶. In addition to assessing chronological
39 age, many aging clock models were trained to reveal associations with various aging-related
40 phenotypes and mortality¹⁰.

41 Aging clocks based on the methylation levels of CpGs are the earliest and some of the most ac-
42 curate age predictors^{11,12}. Such clocks are represented by the blood biomarker developed by
43 Hannum and colleagues¹² and the human pan-tissue epigenetic clock developed by Horvath, the
44 latter trained on 51 different tissue types¹¹. These epigenetic clocks could accurately predict the
45 chronological age of samples, but since they are trained based only on age, only a fraction of the
46 biological variation of the sample could be captured by them. Subsequently, the “second-
47 generation” epigenetic clocks emerged: instead of training solely on chronological age, these
48 clocks incorporated health-related phenotypic information and therefore could reveal a stronger
49 association with aging-related phenotypes. For example, PhenoAge was trained based on the
50 phenotypic age score, which was derived from chronological age and certain mortality-related
51 blood test parameters¹³. Additionally, GrimAge emerged as a robust predictor that is based on
52 multiple phenotypes and the remaining time to death¹⁴. More recently, DunedinPOAm and

53 DunedinPACE biomarkers were reported that are trained based on the pace of biological aging
54 that was derived from multiple clinical biomarkers measured in the Dunedin longitudinal cohort
55 ^{15,16}. We also developed DamAge and AdaptAge, which are causality-informed clock models
56 that could separately measure age-related damage and adaptation ¹⁷. Similar to humans, multiple
57 mouse epigenetic clocks were developed and shown to be able to robustly predict the chronolog-
58 ical age of mice ^{18,19}.

59 One of major applications of aging clocks is the identification of conditions or treatments that
60 modify the aging rate of individuals or reduce their biological age, which could potentially lead
61 to the development of anti-aging therapies ^{20,21}. For example, parabiosis and iPSC reprogram-
62 ming were shown to be associated with the decrease in epigenetic age ²²⁻²⁴, and unhealthy life-
63 styles such as smoking and stress could accelerate epigenetic aging ^{10,25}. Although this type of
64 research has been described in many publications, there has been no systematic effort to identify
65 the impact of available interventions on biological age. One reason is that various clock models
66 require different transformations and pre-processing of omics data, making it difficult to use
67 them and compare them across studies. Some tools exist that try to tackle this problem, but they
68 typically only utilize a small subset of human methylation clocks ²⁶. Moreover, although a large
69 amount of omics data has been acquired by the scientific community that is publicly available
70 through the databases such as Gene Expression Omnibus (GEO) ²⁷, there is currently no public
71 resource that could uniformly process them for biological age profiling.

72 To address this problem, we created *ClockBase*, a comprehensive platform for biological age
73 profiling in humans and mice. We curated 11 best-performing aging clock models, including ep-
74 igenetic clocks for humans and mice, and used them to profile the biological age of samples
75 (Figure 1). We re-processed over 2,000 publicly available DNA methylation datasets from GEO.
76 In total, *ClockBase* contains the biological age information for around 200,000 samples in both
77 mice and humans under various experimental settings. Besides preprocessed data, users can up-
78 load their data to *ClockBase* for biological age calculation. *ClockBase* provides an interactive
79 analysis tool to allow users to perform statistical analyses and visualization of biological age
80 online. We believe that *ClockBase* may provide a valuable resource for the scientific community
81 to explore the biological age of samples, and thus facilitate the discovery of new longevity inter-
82 ventions and age-accelerating conditions.

83 **Results**

84 **Overview of *ClockBase***

85 To develop *ClockBase*, we processed over 2,000 publicly available DNA methylation datasets
86 from GEO and calculated biological age based on multiple aging clocks (Figure 1). In total,
87 *ClockBase* contains biological age information for ~200,000 human and mouse samples (Figure
88 1). We standardized metadata for each experiment, which allows users to search for diseases and
89 treatments of interest and examine biological age under a variety of experimental conditions. All
90 data are available for download. Besides preprocessed data, users can upload their data to
91 *ClockBase* and calculate predicted biological age.

92 *ClockBase* provides an interactive analysis tool that allows users to perform statistical analyses
93 and visualization of biological age online. We also embedded each sample into a low-
94 dimensional space which allows users to explore data interactively. Our toolkit includes group
95 comparison, which allows users to compare biological age across different experimental groups;
96 correlation analysis, which allows users to explore the relationship between biological age and
97 other numeric variables, or the correlation across different clock models; and accuracy analysis,
98 which allows users to explore the accuracy of clock models. All plots and statistical results are
99 available for download. We also created a companion R package called *ClockBasis*, that allows
100 users to calculate biological age of their samples. *ClockBase* is available at <https://clockbase.org>

101 ***ClockBase* offers insights into the relationship among clock models**

102 To understand the biological meaning and relationship among aging clocks, it is important to
103 compare different clocks and have information on their correlation. Although several studies re-
104 ported on this topic, all were performed with established human cohorts and biobanks^{35,36}, which
105 contain only a limited number of interventions and biological variables. As *ClockBase* consists
106 of a large number of samples with highly diverse biological statuses, it provides a unique oppor-
107 tunity for exploring the relationship among different clock models in a much more diverse sam-
108 ple population. We first explored the distribution of biological age measurement across 192,635
109 highly diverse human samples (Figure 2a). Among them, 80,346 samples also had age infor-
110 mation. We, therefore, calculated biological age acceleration for the samples based on each clock
111 (delta age, which is calculated as predicted age minus real age). Note that DunedinPoAm and
112 DunedinPACE are predictors of the pace of age that is independent of the age of samples and is

113 centered at 1. We then examined the distribution of biological age acceleration across the sam-
114 ples (Figure 2b). chi-square test was performed to determine whether there are significantly more
115 samples with accelerated biological age or decelerated biological age. Interestingly, while
116 DunedinPACE, HannumAge, HorvathAge, and ZhangAge clocks showed that there are signifi-
117 cantly more age-accelerated samples, DunedinPoAm and PhenoAge revealed the opposite effect
118 (i.e. there are significantly more age-decelerated samples), whereas PedBE clock showed no sig-
119 nificant difference. This suggests that different clocks may measure different aspects of aging
120 and therefore have a disagreement on the biological age of samples.

121 We further analyzed correlation across biological age prediction based on seven aging clock
122 models (Figure 2c, d). Prior to adjusting for age, PedBE, Horvath Clock, Zhang clock, Hannum
123 Clock, and PhenoAge showed strong correlation with one another, with Pearson's correlation
124 coefficients ranging from 0.59 (PhenoAge and PedBE) to 0.85 (Zhang clock and Hannum
125 Clock). Correlations between DunedinPoAm/DunedinPACE and other clocks were low. This is
126 expected as both DunedinPoAm and DunedinPACE measure the rate of aging, which shows only
127 a weak correlation with chronological age¹⁶. Yet surprisingly, Pearson's correlation coefficient
128 between DunedinPoAm and DunedinPACE was -0.05.

129 After adjusting for age, the five epigenetic age clocks (Horvath Clock, Zhang clock, Hannum
130 Clock, PedBE, and PhenoAge) still showed a significant, yet weaker, positive correlation (Figure
131 2d). Pearson's correlation coefficients ranged from 0.31 (HorvathAge and PhenoAge) to 0.89
132 (ZhangAge and PedPE). DunedinPoAm and DunedinPACE still showed a weak correlation with
133 all other clocks. Notably, DunedinPACE has a significant negative correlation with all other
134 clocks except HorvathAge. These findings reveal the internal discrepancy among different aging
135 clocks when applied to diverse biological samples.

136 To better visualize inconsistency among different aging clocks, we embedded each sample into
137 two-dimensional space by performing UMAP on biological age predictions from each clock
138 model (Figure 3a, b). Locations of the samples on UMAP embedding indicated the relationship
139 among biological age prediction for different aging clocks.

140 As a demonstration, we show that although DunedinPACE has a very weak correlation with oth-
141 er aging clock models, it predicts iPSCs and ESCs to have extremely slow rates of aging, which

142 is related to other clock models that revealed consistently low ages of these cells following long-
143 term maintenance in culture. Therefore, iPSCs/ESCs form a unique cluster in the UMAP space.
144 Similarly, cells overexpressing DNA methyltransferases (DNMTs) are predicted to be relatively
145 young based on Horvath Clock and PhenoAge, and also have a very slow rate of aging based on
146 DunedinPACE³⁷. In contrast, during induced differentiation *in vitro*, hepatocytes appear to be
147 more than 200 years old based on Horvath Clock and PhenoAge and also exhibit an extremely
148 fast rate of aging³⁸.

149 In general, samples form a trajectory in the UMAP space, where the upper left corner represents
150 biologically older samples and the lower right and lower left corners younger samples (Figure
151 3a). The branching of the trajectory indicates disagreement among different aging clocks. For
152 example, the lower left branch has low biological age prediction based on HannumAge,
153 HorvathAge, PhenoAge, and ZhangAge clocks. Yet PedBE shows a moderate biological age
154 prediction, and DunedinPoAm shows that this region contains samples with an accelerated pace
155 of aging. The discrepancy becomes even more obvious when we used biological age acceleration
156 (delta age) as the attribute for t-SNE embedding (Figure 3c). The interactive three-dimensional
157 UMAP and t-SNE embedding are available in the ClockBase online analysis tool.

158 ***ClockBase* facilitates the discovery of longevity interventions and age-accelerating condi-** 159 **tions**

160 To demonstrate the utility of *ClockBase* for identifying novel longevity interventions and age-
161 accelerating conditions, we show two datasets that to our knowledge have not been studied in the
162 context of biological aging. In the first dataset (GSE60446), two different cholangiocarcinoma
163 cell types, TFK-1 and HuCCT1, were treated with a DNA methyltransferase inhibitor zebularine
164 (1-(β -D-ribofuranosyl)-1,2-dihydropyridine-2-one)³⁹. Through only a few clicks on the
165 *ClockBase* online statistical analysis tool, we found that the zebularine treatment significantly
166 reduces the epigenetic age based on almost all clock models and in both cell lines (Figure 4a). In
167 addition, both DunedinPoAm and DunedinPACE showed that the zebularine-treated cells exhib-
168 ited a slower pace of aging. Zebularine has never been studied for its role in rejuvenation, and
169 our results suggest that this compound is a potential longevity intervention, which may be further
170 studied in future studies.

171 The second example is from GSE63704, which includes 204 plasma DNA methylation samples
172 representing healthy controls and lung cancer, pulmonary fibrosis, and chronic obstructive pul-
173 monary disease (COPD) patients⁴⁰. We observed that pulmonary fibrosis patients exhibit a sig-
174 nificantly higher epigenetic age compared to control patients, based on Horvath Clock, PedBE,
175 Zhang clock, and Hannum Clock models (Figure 4b). Additionally, DunedinPACE showed that
176 pulmonary fibrosis patients had a significantly faster pace of aging. Notably, both of these exam-
177 ples were semi-randomly selected for demonstration purposes, suggesting that there are many
178 other potential associations that remain to be explored by future *ClockBase* users.

179 Discussion

180 The emergence of aging clocks provided researchers with promising tools to estimate the age of
181 biological samples and shed light on the associated biology. However, there are currently multi-
182 ple dozens of aging clocks that have been created, making it increasingly important to under-
183 stand the relationship between different aging clocks^{8,41-43}. There have been some efforts to
184 compare clocks based on established human cohorts and biobanks^{35,36}, but these studies are lim-
185 ited in both clocks examined and the dataset used. *ClockBase* currently contains DNA methyla-
186 tion for both mice and humans, with much more diverse sample coverage compared to human
187 biobanks. We believe that this resource can be used to help researchers to understand the rela-
188 tionship between clocks in different experimental settings.

189 Another challenge is that it is currently hard for non-computational experts in the field to use ag-
190 ing clocks, as they usually require different transformations and data preprocessing. Even for
191 computational biologists, downloading individual datasets from GEO and preprocessing each of
192 them is a time-consuming task. *ClockBase* is designed to provide a simple and easy-to-use inter-
193 face for biologists to perform statistical analyses and visualization of biological age. Only a GSE
194 accession identifier and a few clicks are required for analyzing a dataset from GEO. This could
195 remove the barrier for researchers and domain experts to use and understand the aging clocks.

196 We illustrated the utility of *ClockBase* by discovering zebularine, a potent DNMT inhibitor,
197 which affects the methylation status of the samples by directly targeting the DNA methylation
198 machinery⁴⁴. Our data suggest that zebularine is a candidate longevity intervention, as it signifi-
199 cantly reduced the epigenetic age of cultured cells based on almost all clock models. However,

200 as zebularine affects the DNA methylation machinery, DNA methylation clocks should be used
201 with caution. Further investigation and *in vivo* studies are required to understand the role of
202 zebularine in the aging process.

203 We believe many other potential anti-aging interventions are hidden in a large number of availa-
204 ble experimental conditions, that could be explored and explained by domain experts.

205 **MATERIALS AND METHODS**

206 **Data collection**

207 The data used in this study were downloaded before July 30th, 2022, from Gene Expression
208 Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo>). Raw data were downloaded using the R
209 package GEOquery (<https://bioconductor.org/packages/release/bioc/html/GEOquery.html>), and
210 metadata were extracted using the R package GEOmetadb
211 (<https://www.bioconductor.org/packages/release/bioc/html/GEOmetadb.html>). For mouse DNA
212 methylation analyses, all GEO entries associated with “Methylation profiling by high throughput
213 sequencing” were collected; for human DNA methylation analyses, all GEO entries associated
214 with “Methylation profiling by (genome tiling, SNP, or other) array” were collected. Methylation
215 level data were then downloaded from supplementary files of each GEO entry. Only the datasets
216 with at least 6 samples were used for downstream analyses.

217 **Methylation data preprocessing**

218 Existing mouse DNA methylation data are not uniformly structured. A custom R script was used
219 to identify CpG sites and methylation levels of each sample and then standardize data format.
220 Metadata are standardized based on the custom pipeline aspired by refine.bio²⁸. Datasets with
221 missing information or in unrecognized format were excluded. Then, for both mouse and human
222 DNA methylation data, the range of methylation levels was standardized to the 0-1 scale. The
223 data with out-of-range values were replaced with missing values. We impute missing methyla-
224 tion level data using mean methylation for the reference dataset. For humans, we used 2,664
225 blood samples measured using the 450k Human Methylation Beadchip as a reference²⁹. For
226 mice, since sequencing-based methods were used, DNA methylation data were more sparse
227 compared to array-based data. Therefore, we first imputed missing values based on mean meth-
228 ylation levels within 100 base-pair regions, as it was reported in a previous study that the nearby

229 sites tend to exhibit a high correlation with regard to methylation levels³⁰. For the sites still hav-
230 ing missing values, we imputed missing values based on the mean methylation levels of the ref-
231 erence dataset from Petkovich et al.¹⁸. We report the ratio of missingness for each clock model.
232 In general, samples with more than 20% missing values were considered unreliable for biological
233 age prediction; however, we included them in the database with a warning message as they may
234 still provide information.

235 The code for all the preprocessing steps is included in the ClockBasis R package
236 (<https://github.com/albert-ying/ClockBasis>).

237 **Aging clock models implementation**

238 Aging clock models were implemented on the web server and precalculated for all datasets, in-
239 cluding 4 mouse epigenetic clocks and 7 human epigenetic clocks. The following mouse epige-
240 netic clocks were included: Petkovich blood clock (90 sites)¹⁸, Meer multi-tissue clock (435
241 sites)¹⁹, Thompson multi-tissue clock (582 sites)³¹, and Wang liver clock (148 sites)³². The fol-
242 lowing human epigenetic clocks were included: Horvath multi-tissue clock (353 sites)¹¹,
243 Hannum clock (71 sites)¹², PhenoAge (513 sites)¹³, PedBE pediatric buccal clock (94 sites)³³,
244 Zhang blood clock (514 sites)³⁴, DunedinPOAm (46 sites)¹⁵, and DunedinPACE (173 sites)¹⁶.

245 All clock models are publicly available and could be downloaded from the original source. All
246 epigenetic clocks are also available as functions in the ClockBasis R package.

247 **Online statistical analysis**

248 Three types of statistical analysis were implemented in the *ClockBase* online interface.

249 (1) Group comparison: the group comparison function allows users to compare the biological
250 age or another numeric variable across different experimental groups in the dataset. The
251 pairwise T-test is performed across each group and p-value is adjusted by the number of
252 comparisons using the Benjamini-Hochberg procedure. p-value for ANOVA across all
253 groups is also reported. The result is an output in the form of a boxplot followed by the
254 result table.

255 (2) Correlation: the correlation function allows users to calculate Pearson's correlation across
256 two numeric variables in the dataset. The result is an output in the form of a scatter plot
257 with regression lines. Pearson's correlation coefficient and p-value are also reported. Us-
258 ers can further calculate correlations within each subgroup of the dataset and report statis-
259 tics separately. Notably, this function is also useful for quality control by visualizing cor-
260 relation between biological age prediction and percentage missingness of the data. This

261 could avoid reporting false positive results due to imbalanced missingness across experi-
262 mental groups.

263 (3) Accuracy: the accuracy function allows users to calculate accuracy of biological age pre-
264 diction when the true age is given in the dataset. Pearson's R, RMSE, MAE, and p-value
265 are reported.

266

267 **Data availability**

268 All data are available on the *ClockBase* online resource (<https://clockbase.org>) and GEO
269 (<https://www.ncbi.nlm.nih.gov/geo/>).

270 **Code availability**

271 All codes are available in the ClockBasis R package (<https://github.com/albert-ying/ClockBasis>)

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276 **Author contributions**

277 K.Y. and A.T. initiated the study; K.Y. collected the data; V.N.G supervised the study. K.Y.,
278 A.T., and H.L. performed data analyses; All authors contributed to paper preparation and data
279 interpretation.

280 **Competing interest statement**

281 The authors declare no competing financial interests.

282 **Figure legends**

283 **Figure 1. *ClockBase* overview.** Schematic diagram shows data sources and main functionalities
284 of the *ClockBase* online resource.

285 **Figure 2. Distribution and correlation of biological age in human samples. a.** Density plot
286 shows the distribution of biological age measurements across 192,635 samples based on 7 human
287 DNAm aging clocks. **b.** Density plot shows the distribution of biological age acceleration across
288 80,346 samples with chronological age annotation. Dashed line shows the boundary between
289 samples with accelerated and decelerated ages. chi-square test was performed, and the ratio and
290 p-value are shown above the plots **c,d.** Correlation plot shows Pearson's correlation across dif-
291 ferent biological age measurements (**c**) and biological age acceleration (**d**). Upper triangle: Pear-
292 son's correlation plot; lower triangle: Pearson's correlation coefficient. Areas of the squares rep-
293 resent the absolute value of corresponding Pearson's correlations. P values are corrected using
294 Bonferroni correction for 21 tests with $P_{\text{adjusted}} < 0.05$.

295 **Figure 3. ClockBase reveals discrepancy across different biological age measurements. a, b.**
296 The UMAP plot of 192,635 human DNA methylation samples. Colors of the dots represent bio-
297 logical age prediction based on HorvathAge (**a**) and other human DNAm clocks (**b**). **c.** t-SNE
298 plot of 80,346 human DNA methylation samples with chronological age annotation. Colors of
299 the dots represent biological acceleration based on each clock.

300 **Figure 4. Identifying novel biological age-modifying conditions using ClockBase. a.** Epige-
301 netic age comparison between zebularine-treated and untreated cells. **b.** Epigenetic age compari-
302 son across healthy control, lung cancer, pulmonary fibrosis (fibrosis), and COPD patients. Boxes
303 indicate 25–75% interquartile ranges, and whiskers indicate minimum to maximum. * $P_{\text{adjusted}} <$
304 0.05, ** $P_{\text{adjusted}} < 0.01$, *** $P_{\text{adjusted}} < 0.001$, **** $P_{\text{adjusted}} < 0.0001$. COPD: chronic obstructive
305 pulmonary disease.

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381 **Figures**

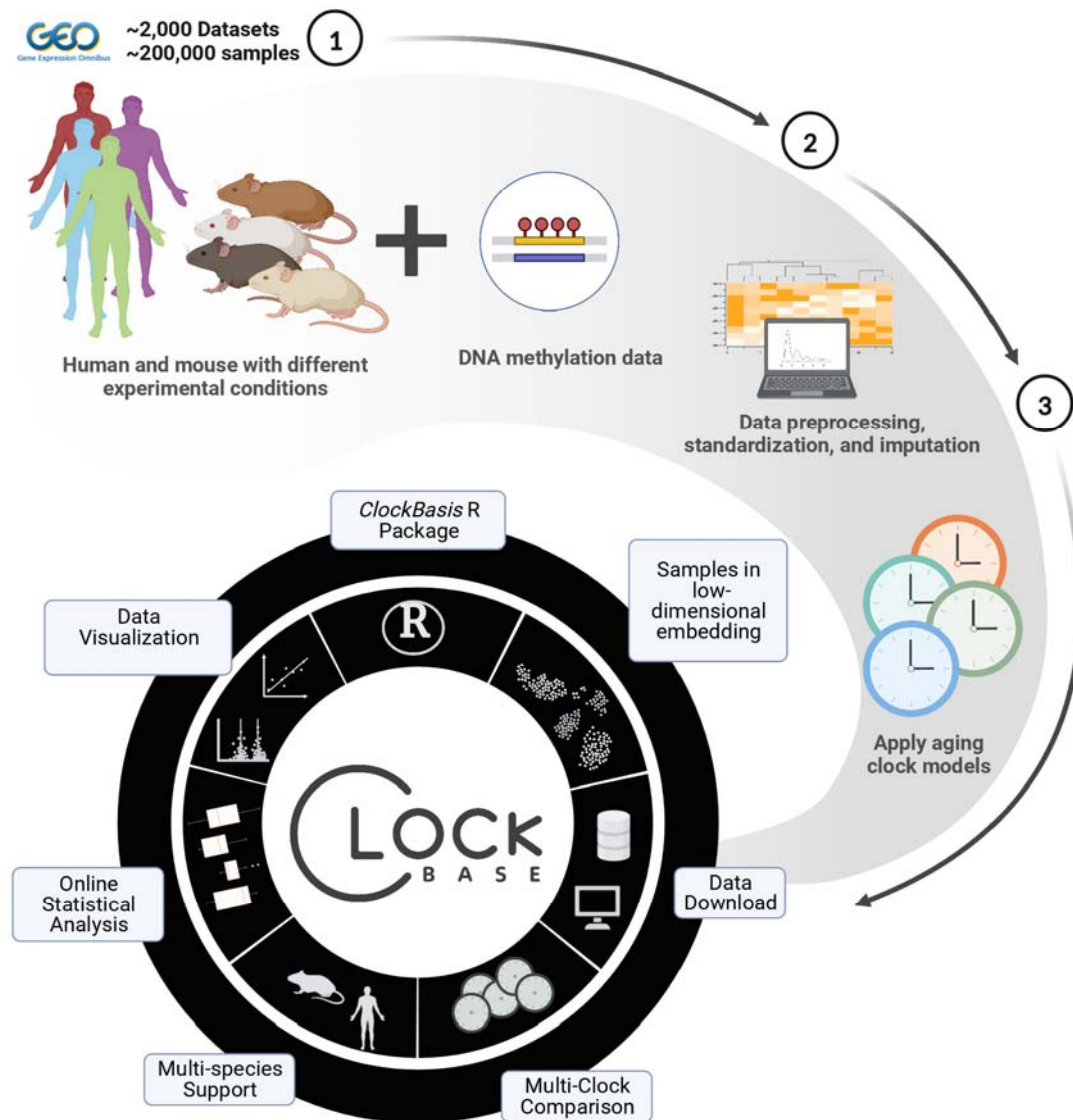


Figure 1

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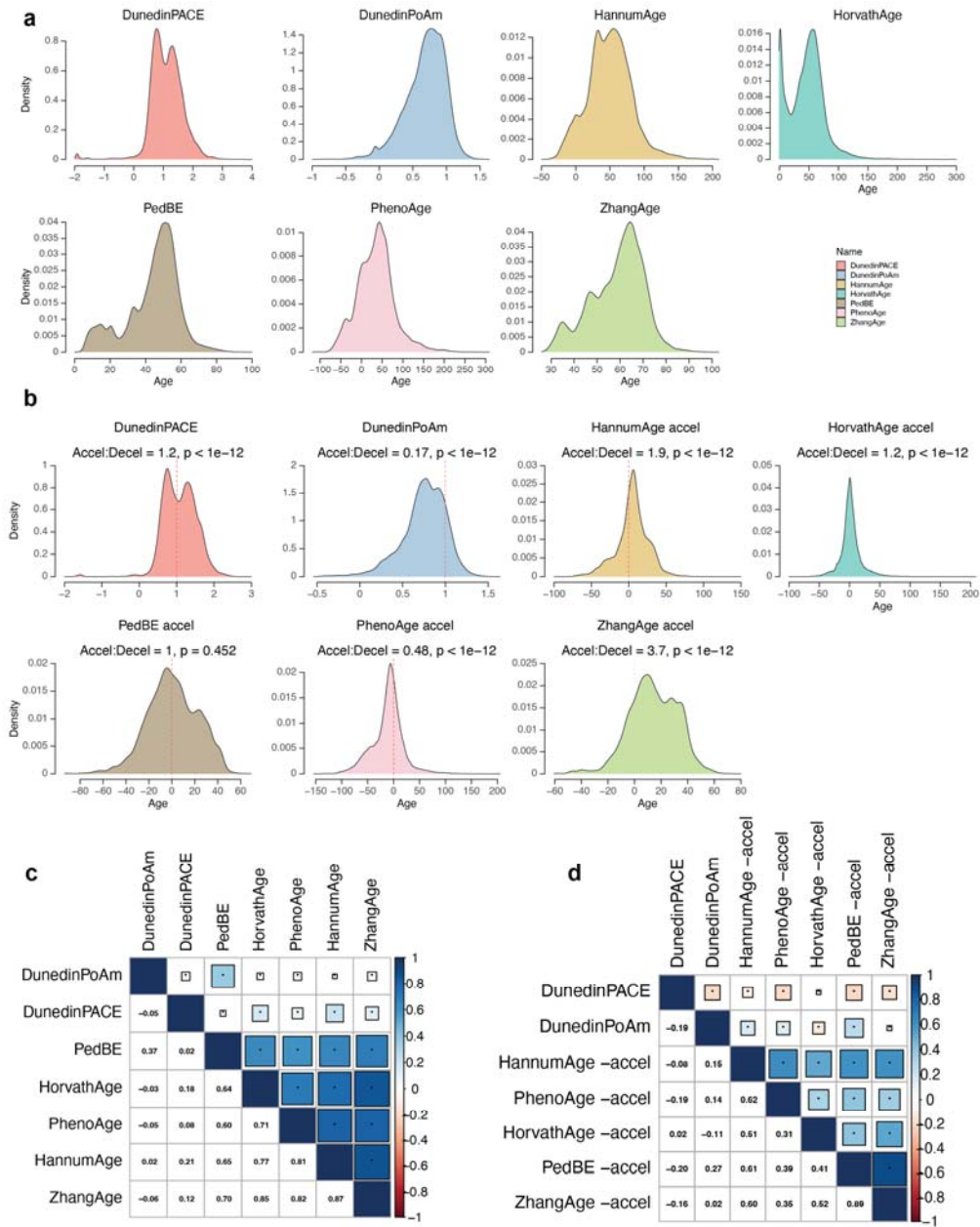


Figure 2

383

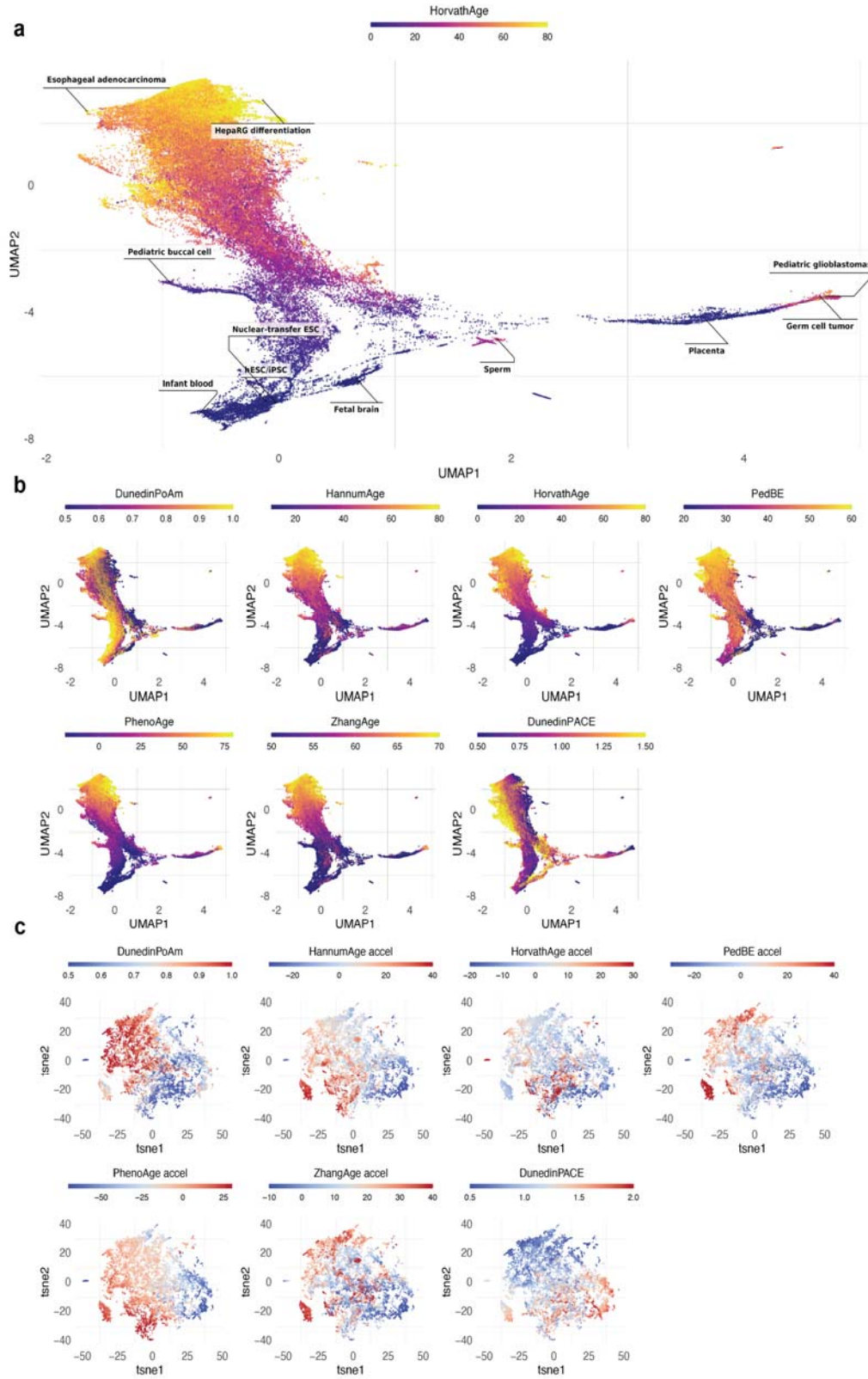


Figure 3

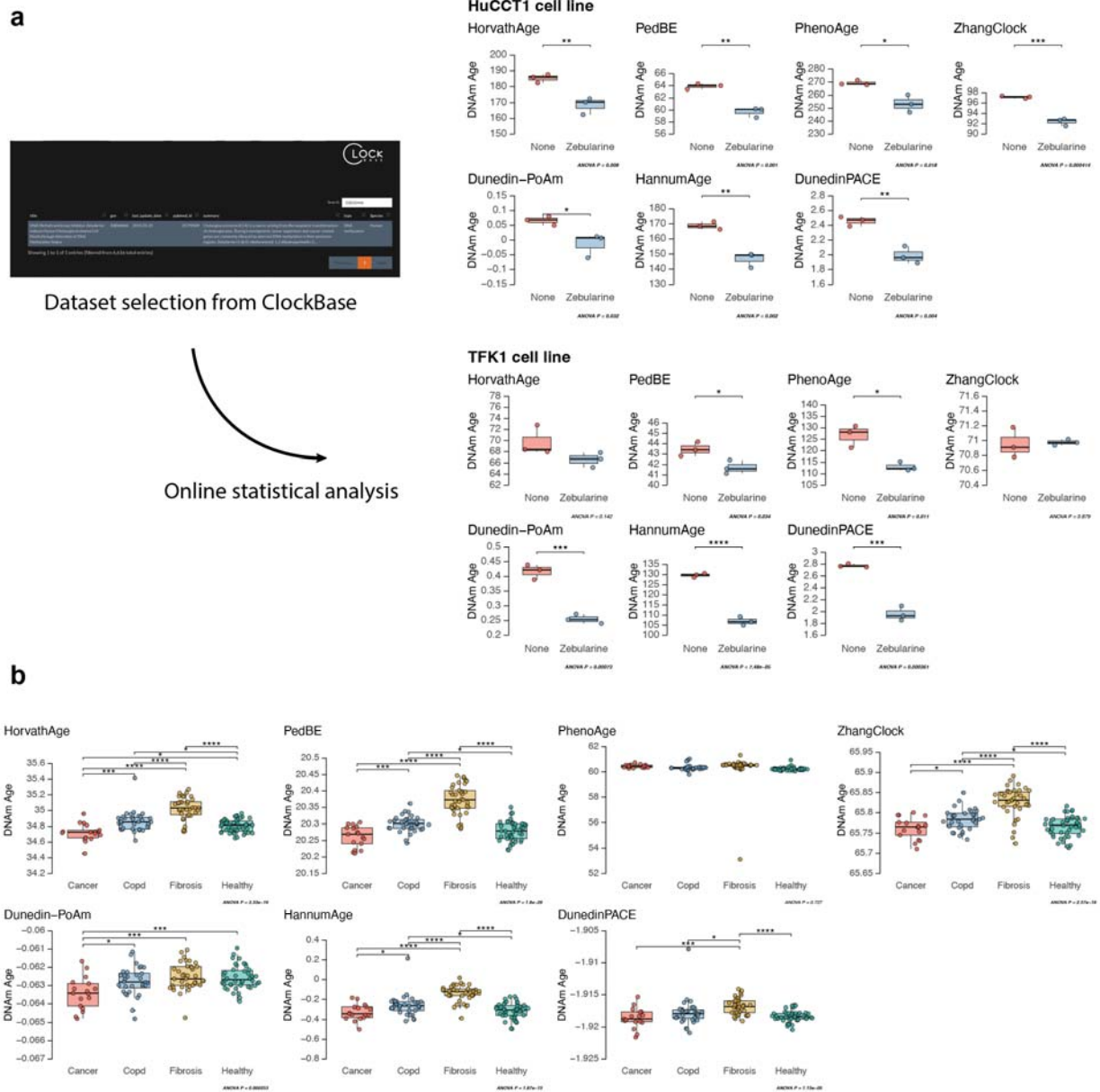


Figure 4

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