Supplementary Material

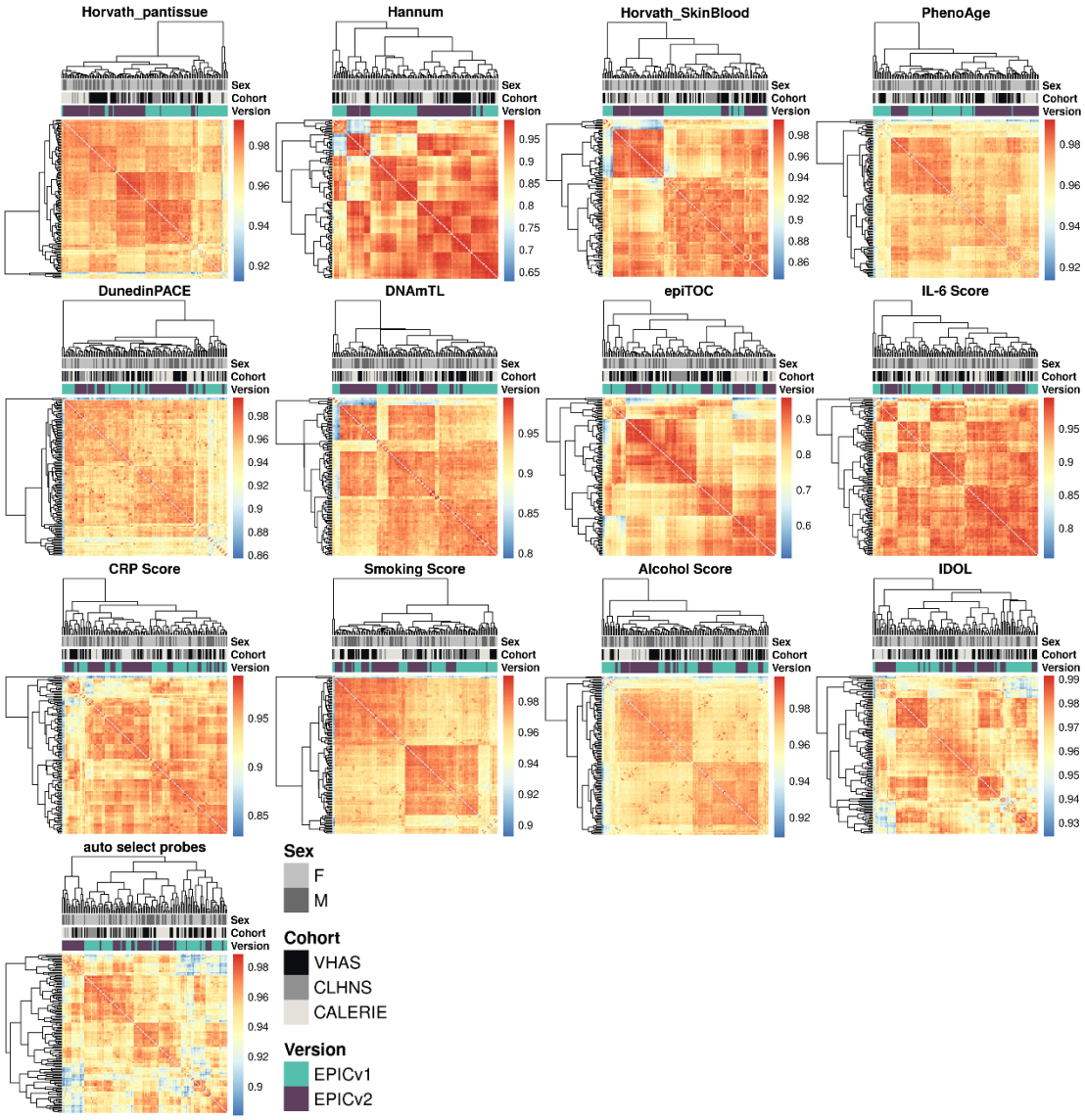
1. Characterization of replicate probes in EPICv2 and Comparing strategies for collapsing EPICv2 replicate probes

In total, there are 11,529 replicate probes on EPICv2 after excluding probes on chromosomes 0 and M. These replicates are distributed across chromosomes 1-22 and X, and are predominantly located within CpG islands, consistent with the intended increased coverage of these genomic regions on EPICv2 (Supplementary Figure 22). Over 80% (4,174) of these probes have two replicates each, with the largest number of replicates being ten (1 in 5,190, 0.02%).

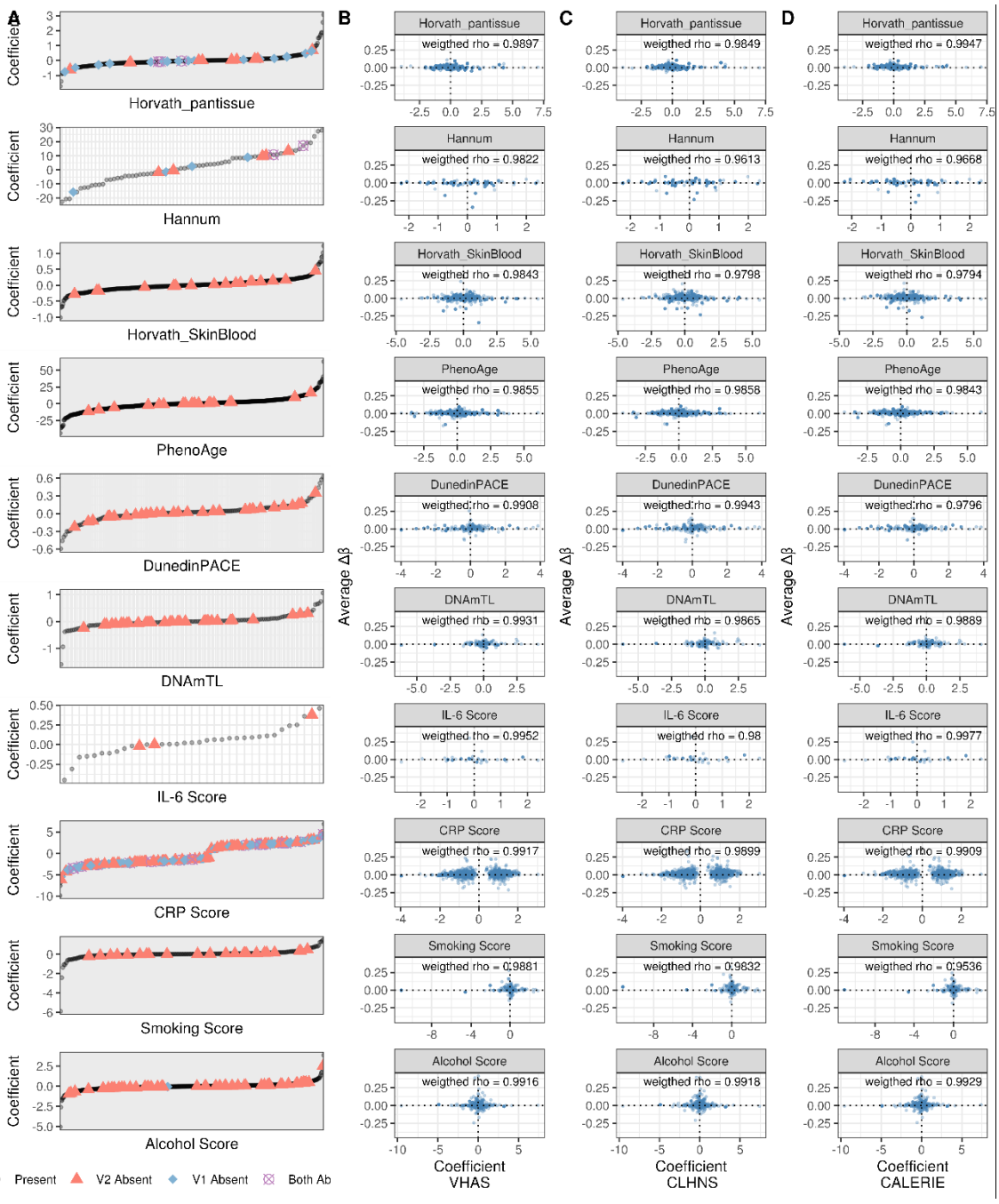
Given that predictive CpGs of several DNA methylation-based tools include EPICv2 replicate probes to varying extent, even some with large coefficient weights contributing substantially to the generated estimates, there is a need to collapse replicates to a single representative beta value analogous to corresponding probes on EPICv1. While significantly high correlation among replicate probes is expected, residual methylation differences between replicate probes of varying designs have been reported[1]. Choosing the replicate with lowest detection p-value as the representative probe has been suggested as a way to collapse replicates[1], however this strategy has not been compared to other methods. To that end, we compared three strategies to collapse replicates into a single β value per locus: (i) previously suggested method of choosing the replicate with lowest detection *p*-value[1], (ii) estimating mean of all replicates mapping to a genomic locus, and (iii) estimating median of all replicates mapping to a genomic locus, and compared representative EPICv2 replicate beta values (3602 probes) thus obtained to respective EPICv1 probes. We also noted that all three methods showed significantly high correlation with EPICv1 in all four cohorts: VHAS (Spearman *rho* values by strategy- detection *p*-value-based: 0.9886, mean-based: 0.9893, median-based: 0.9899), CLHNS (Spearman *rho* values by strategy-detection P-value-based: 0.9850, mean-based: 0.9855, median-based: 0.9859), and CALERIE (Spearman *rho* values by strategy-detection *p*-value-based: 0.9869, mean-based: 0.9856, median-based: 0.9861) (Supplementary Figure 23). Comparing the three strategies to one another, we observed negligible absolute mean differences in average beta values of EPICv2 replicate probes across samples, ranging from 0.0002 - 0.0078 across the four cohorts.

1. Kaur, D. *et al.* Comprehensive evaluation of the Infinium human MethylationEPIC v2 BeadChip. *Epigenetics Commun.* **3**, 6 (2023).

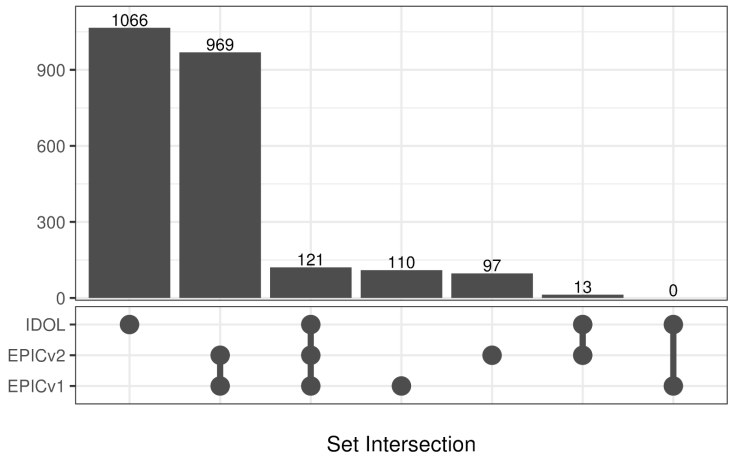
Supplementary Figures



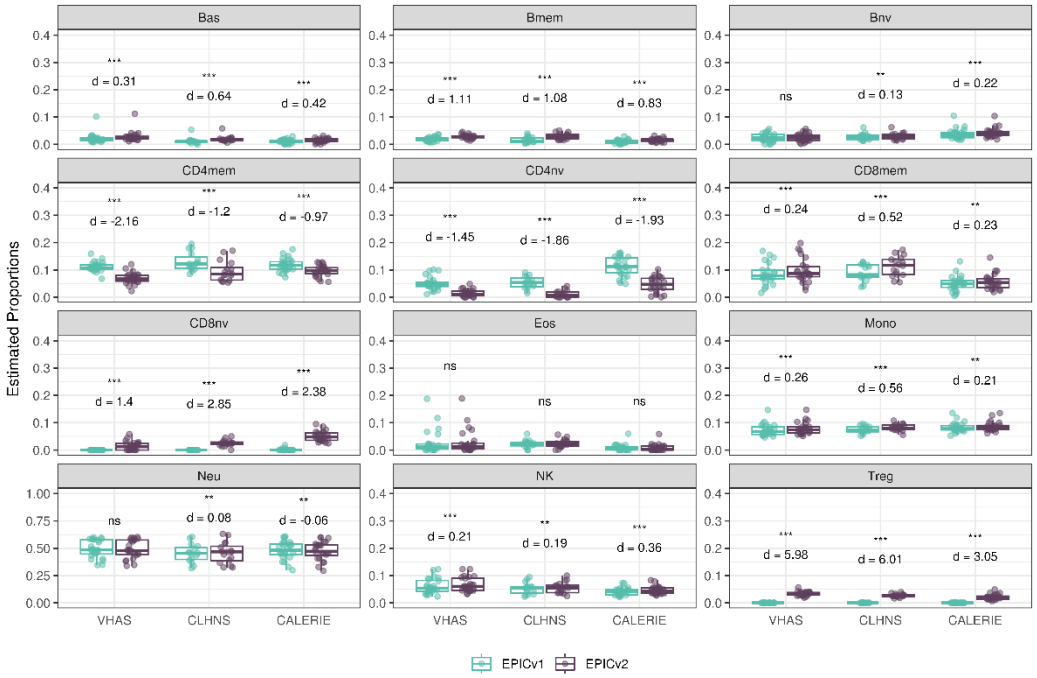
Supplementary Figure 1. Unsupervised hierarchical clustering of matched VHAS, CLHNS, and CALERIE samples by EPIC version, cohort, and sex. Heatmap of Spearman correlation of common predictive CpGs employed by clocks, biomarker predictors, and cell type deconvolution algorithms between EPICv1 and EPICv2; blue to red color range denotes Spearman *rho* correlation from low to high.



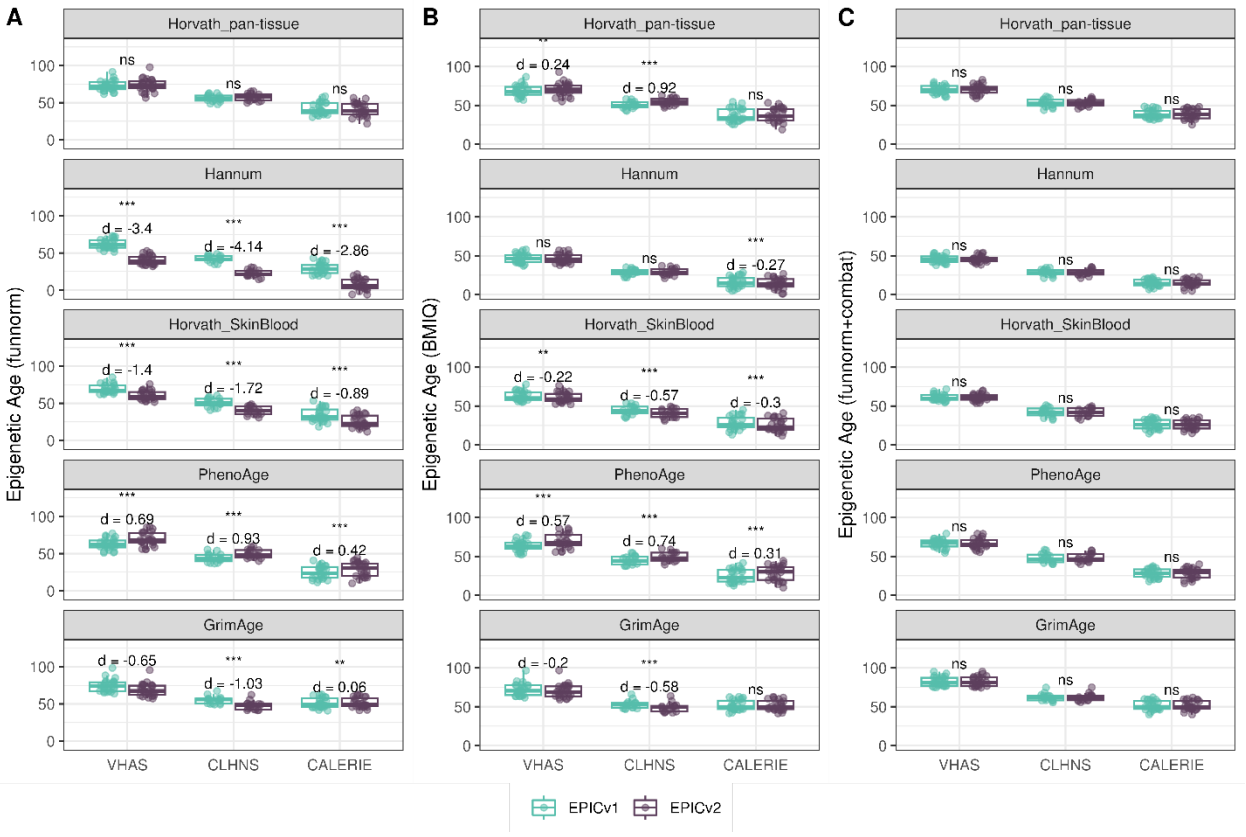
Supplementary Figure 2. (A) Clock and biomarker predictor CpGs absent in EPICv1 and EPICv2 and their corresponding coefficients. Both Ab: probe are absent in both EPICv1 and EPICv2. **(B-C)** Average difference in beta values (EPICv2 β - EPICv1 β) of clock and biomarker predictor CpGs coefficients in **(B)** VHAS, **(C)** CLHNS, **(D)** CALERIE. Spearman correlation between EPICv1 and EPICv2 beta values of clock and predictor CpGs, weighted by corresponding coefficients are provided. IDOL CpGs, which do not have coefficients, and epiTOC CpGs, which have equal coefficients are not shown here.



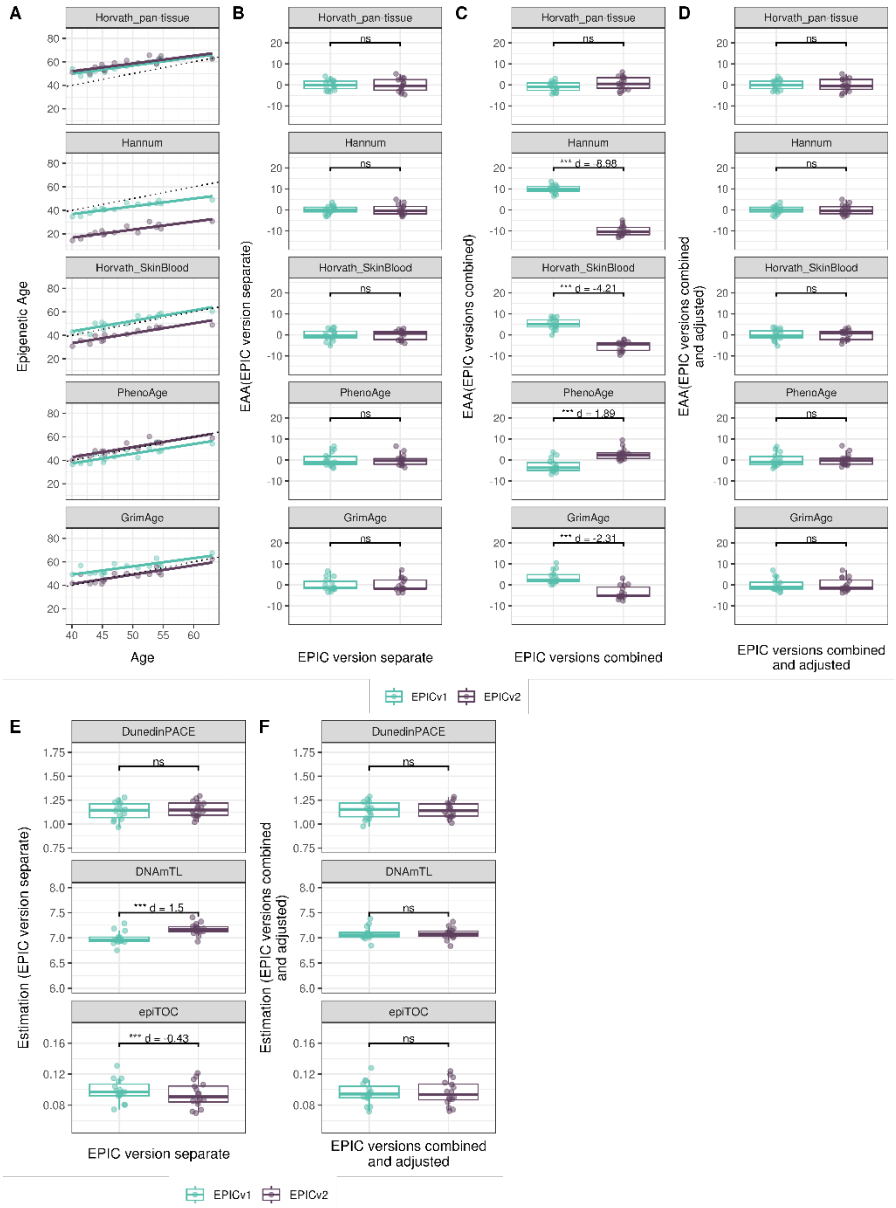
Supplementary Figure 3. Upset plot denoting overlap of probes selected when using the IDOL pre-selected probes and auto-selected probes in EPICv1 and EPICv2.



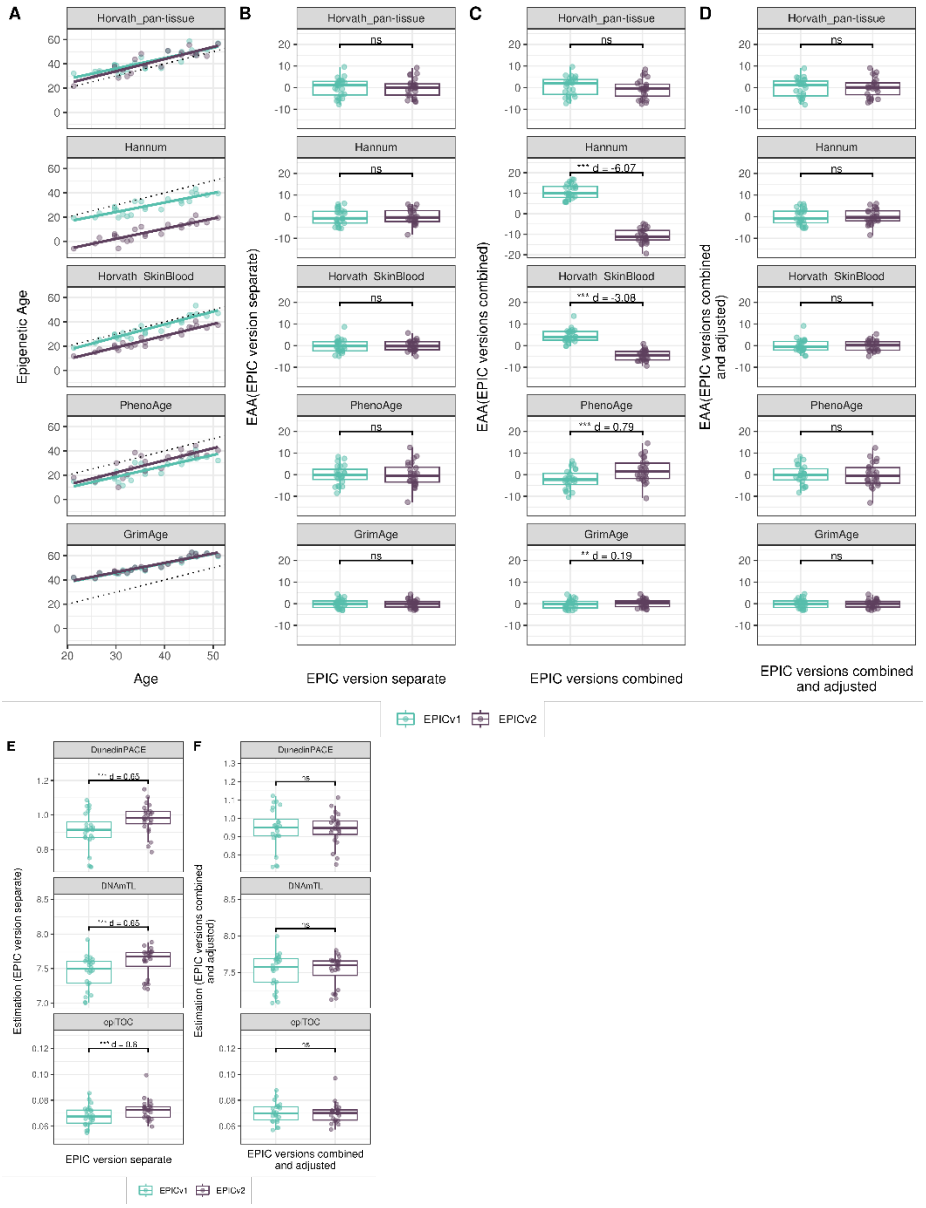
Supplementary Figure 4. Differences in DNA methylation-based immune cell type proportions estimated using the "auto" method on matched samples assessed on EPICv1 and EPICv2 in VHAS, CLHNS, and CALERIE. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher average estimated cell proportions in EPICv2 compared to EPICv1.



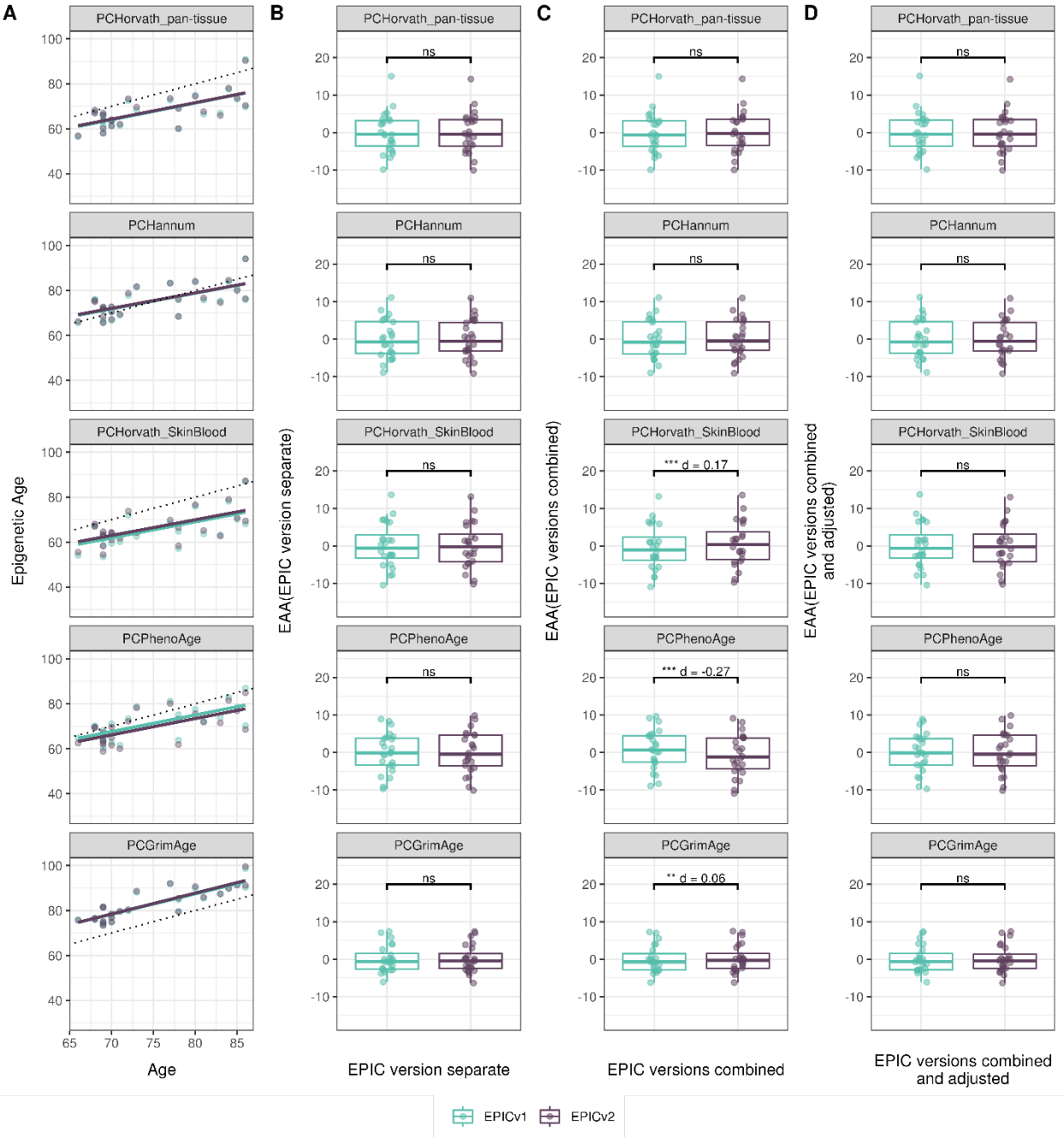
Supplementary Figure 5. Differences in epigenetic ages between EPICv1 and EPICv2 using the Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clocks in VHAS, CLHNS, and CALERIE when **(A)** using functional normalization and **(B)** normalizing EPICv1 and EPICv2 together using BMIQ normalization. **(C)**. functional normalization with batch-correction for EPIC version, chip and row. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates estimates in EPICv2 compared to EPICv1.



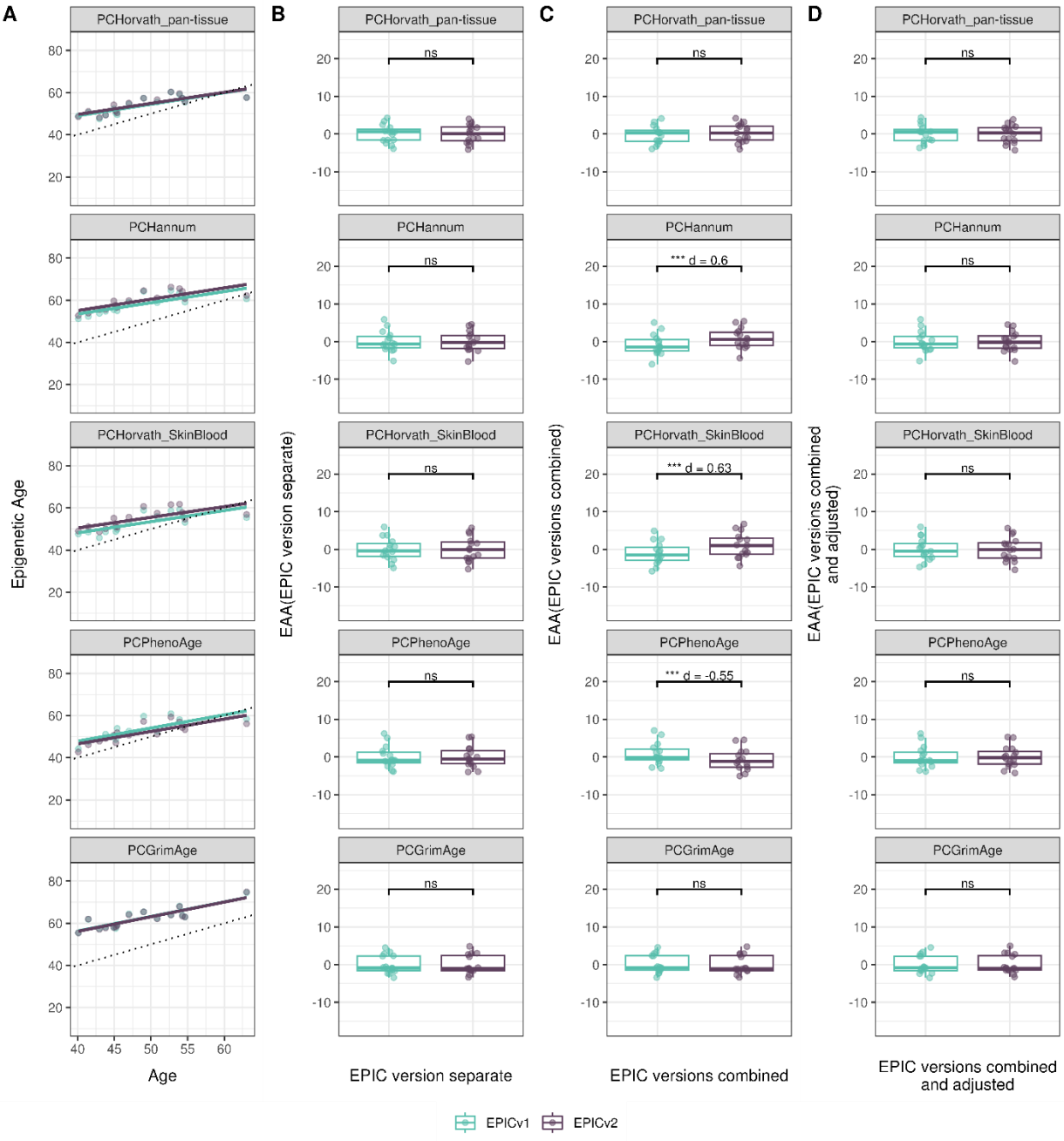
Supplementary Figure 6. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in CLHNS. **(A)** Scatter plot of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock estimates (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B-D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. **(E,F)** Boxplots comparing DunedinPACE, DNAmTL and epiTOC estimates calculated by considering EPIC versions separately (E) and combined and EPIC version adjusted (F), between EPICv1 and EPICv2. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates estimates in EPICv2 compared to EPICv1.



Supplementary Figure 7. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in CALERIE. **(A)** Scatter plot of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock estimates (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B-D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. **(E,F)** Boxplots comparing DunedinPACE, DNAmTL and epiTOC estimates calculated by considering EPIC versions separately (E) and combined and EPIC version adjusted (F), between EPICv1 and EPICv2. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates estimates in EPICv2 compared to EPICv1.



Supplementary Figure 8. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in VHAS. **(A)** Scatter plot of PC clocks of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock ages (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B, C and D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.



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121 **Supplementary Figure 9.** Epigenetic age estimated on matched samples assessed on EPICv1 and
122 EPICv2 in CLHNS. **(A)** Scatter plot of PC clocks of Horvath pan-tissue, Hannum, Horvath skin
123 and blood, PhenoAge, and GrimAge clock ages (Y axis) and chronological age (X axis) with dotted
124 line indicating x=y, coloured by EPIC version. **(B, C and D)** Boxplots comparing EPICv1 and
125 EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and
126 EPIC version adjusted, respectively. Statistical significance was defined as Bonferroni adjusted p-
127 value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not
128 significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates
129 higher estimates in EPICv2 compared to EPICv1.

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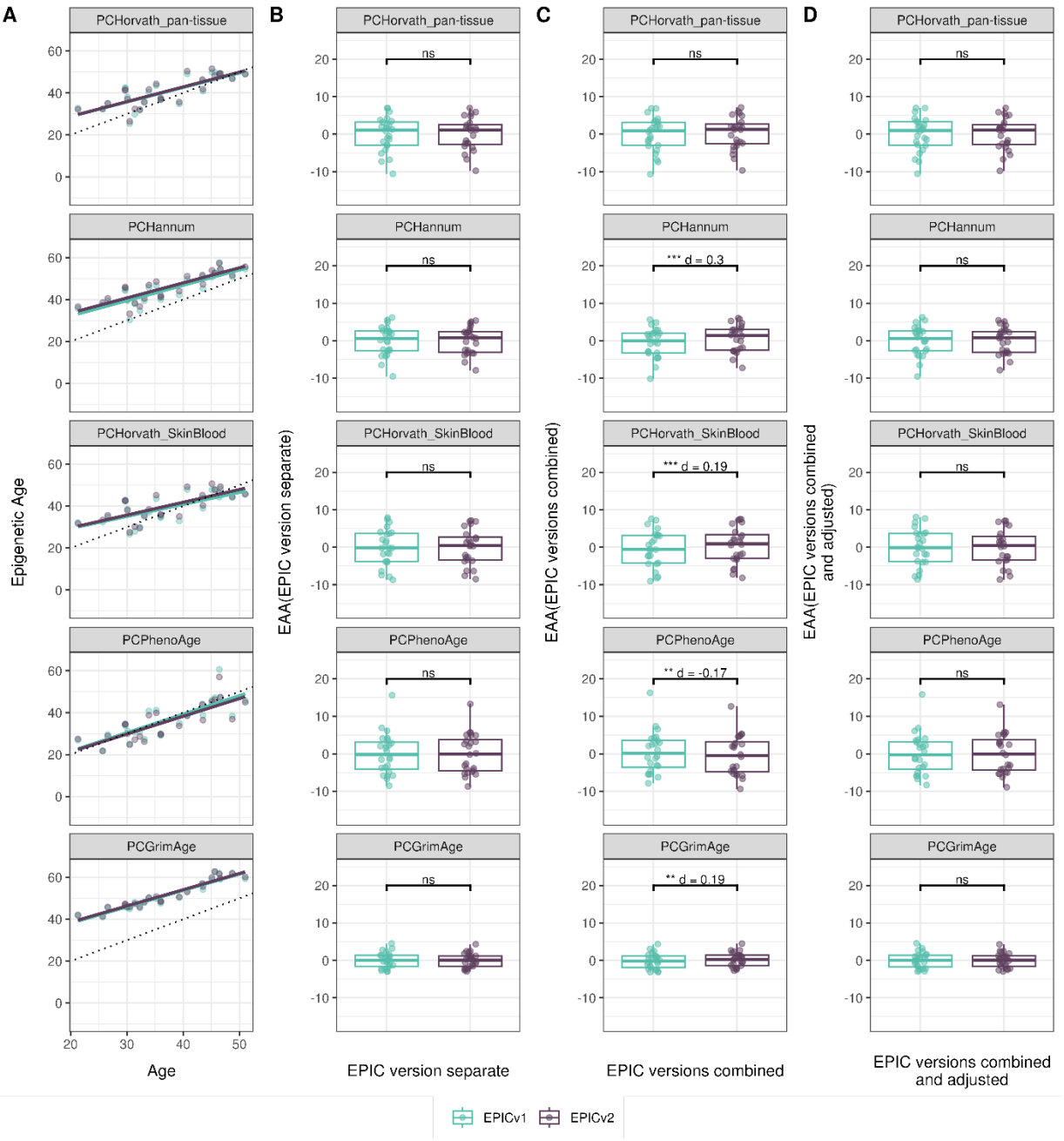
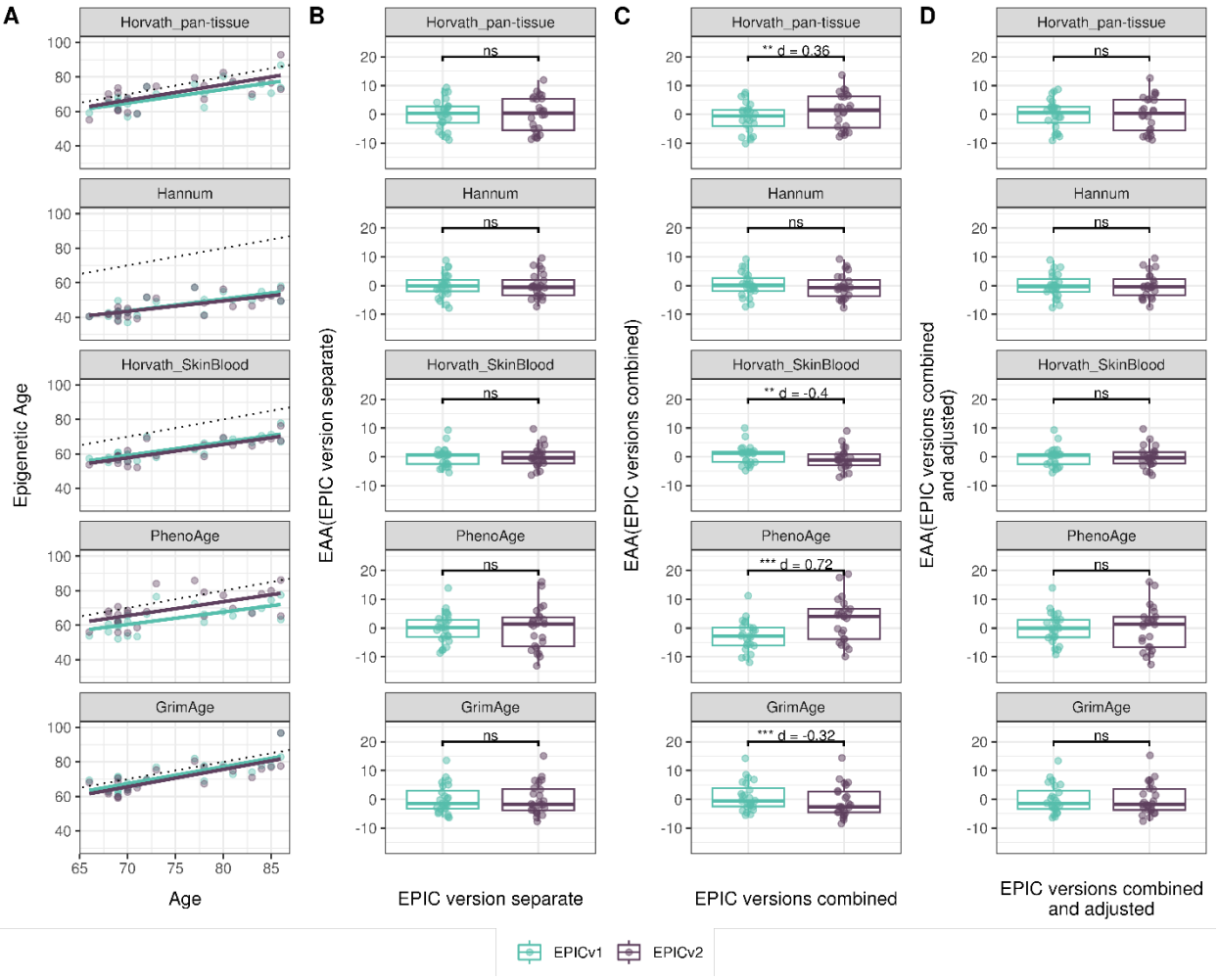


Supplementary Figure 10. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in CALERIE. **(A)** Scatter plot of PC clocks of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock ages (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B, C and D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.



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143 **Supplementary Figure 11.** Epigenetic clock estimates obtained after normalizing EPICv1 and
144 EPICv2 together using BMIQ in the VHAS cohort. (B-D) Boxplots comparing Horvath pan-tissue,
145 Hannum, Horvath skin and blood, PhenoAge, and GrimAge EAA, calculated on normalizing EPIC
146 versions together, between EPICv1 and EPICv2. Statistical significance was defined as Bonferroni
147 adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns"
148 denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive
149 Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.

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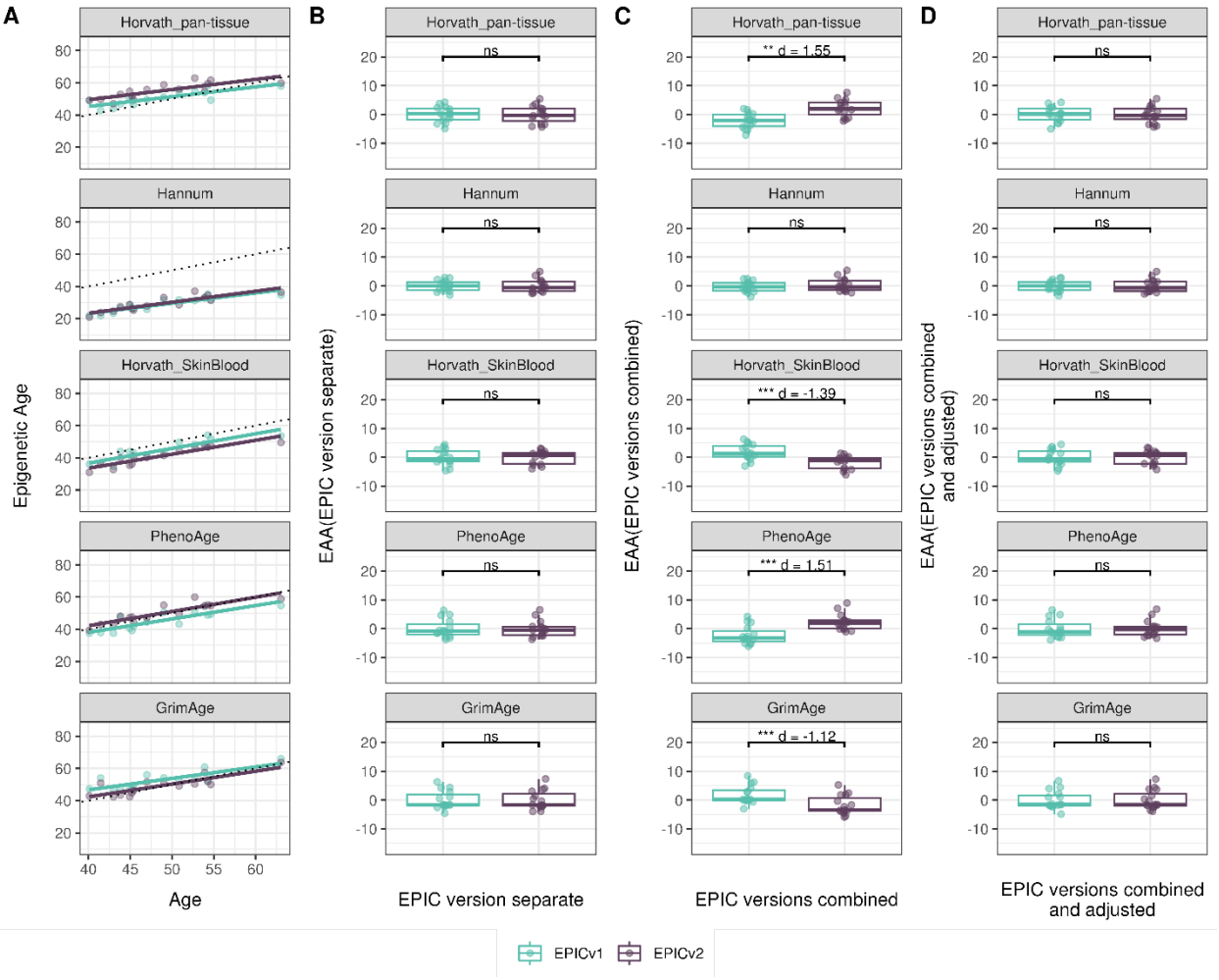

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153 **Supplementary Figure 12.** Epigenetic clock estimates obtained after normalizing EPICv1 and
154 EPICv2 together using BMIQ in the CLHNS cohort. **(B-D)** Boxplots comparing Horvath pan-
155 tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge EAA, calculated on
156 normalizing EPIC versions together, between EPICv1 and EPICv2. Statistical significance was
157 defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes
158 Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using
159 Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.

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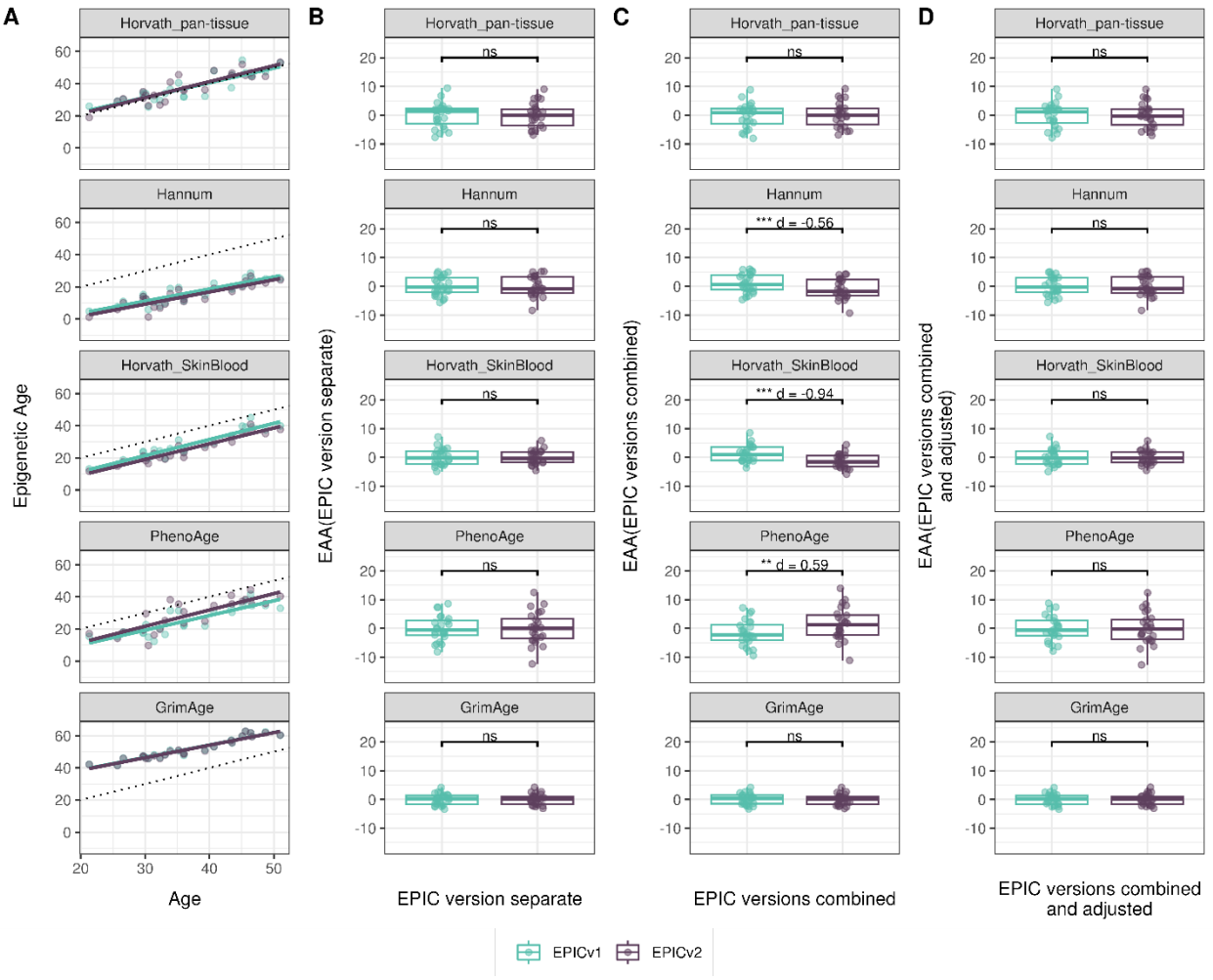
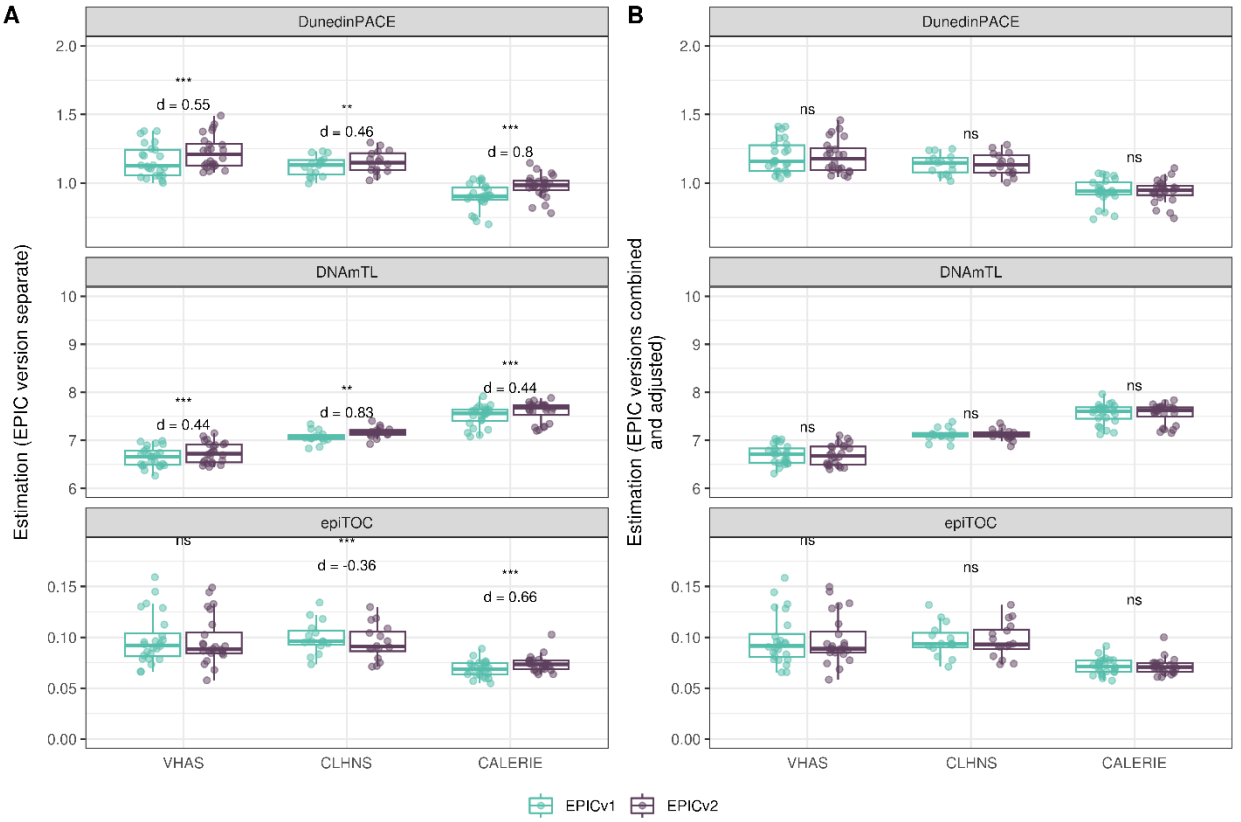
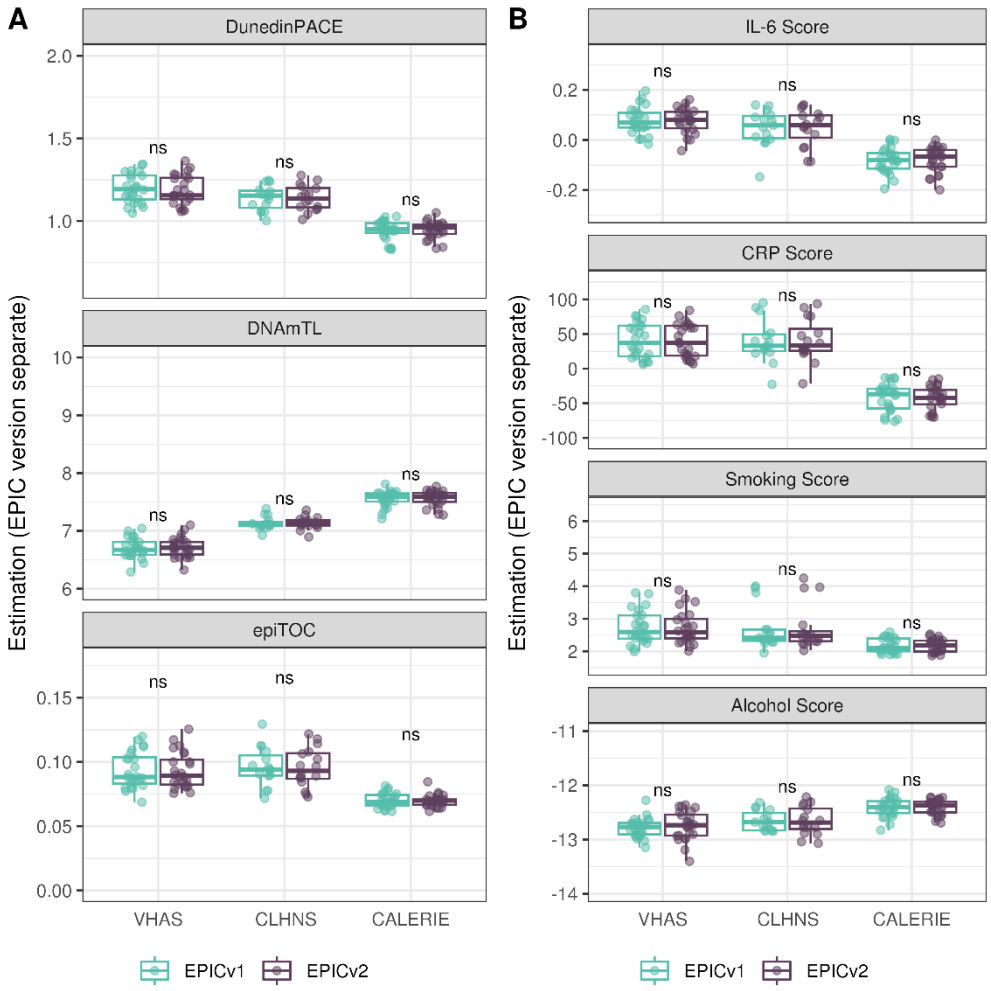


Supplementary Figure 13. Epigenetic clock estimates obtained after normalizing EPICv1 and EPICv2 together using BMIQ in the CALERIE cohort. **(B-D)** Boxplots comparing Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge EAA, calculated on normalizing EPIC versions together, between EPICv1 and EPICv2. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.



Supplementary Figure 14. Rate-based clock estimates obtained after normalizing EPICv1 and EPICv2 together using BMIQ in VHAS, CLHNS, and CALERIE. Boxplots comparing DunedinPACE, DNAmTL and epiTOC estimates calculated by considering EPIC versions separately **(A)** and combined and EPIC version adjusted **(B)**. Statistical significance was defined as Bonferroni adjusted *p*-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p <0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates estimates in EPICv2 compared to EPICv1.



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182 **Supplementary Figure 15.** Rate-based clock and DNA methylation-based predictor estimates
183 calculated by considering EPIC versions separately after functional normalization with batch-
184 correction for EPIC version, chip and row in VHAS, CLHNS, and CALERIE. **(A)** rate-based
185 clocks **(B)**. DNA methylation-based predictors. Statistical significance was defined as Bonferroni
186 adjusted *p*-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p <0.001, "ns"
187 denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive
188 Cohen's d indicates estimates in EPICv2 compared to EPICv1.

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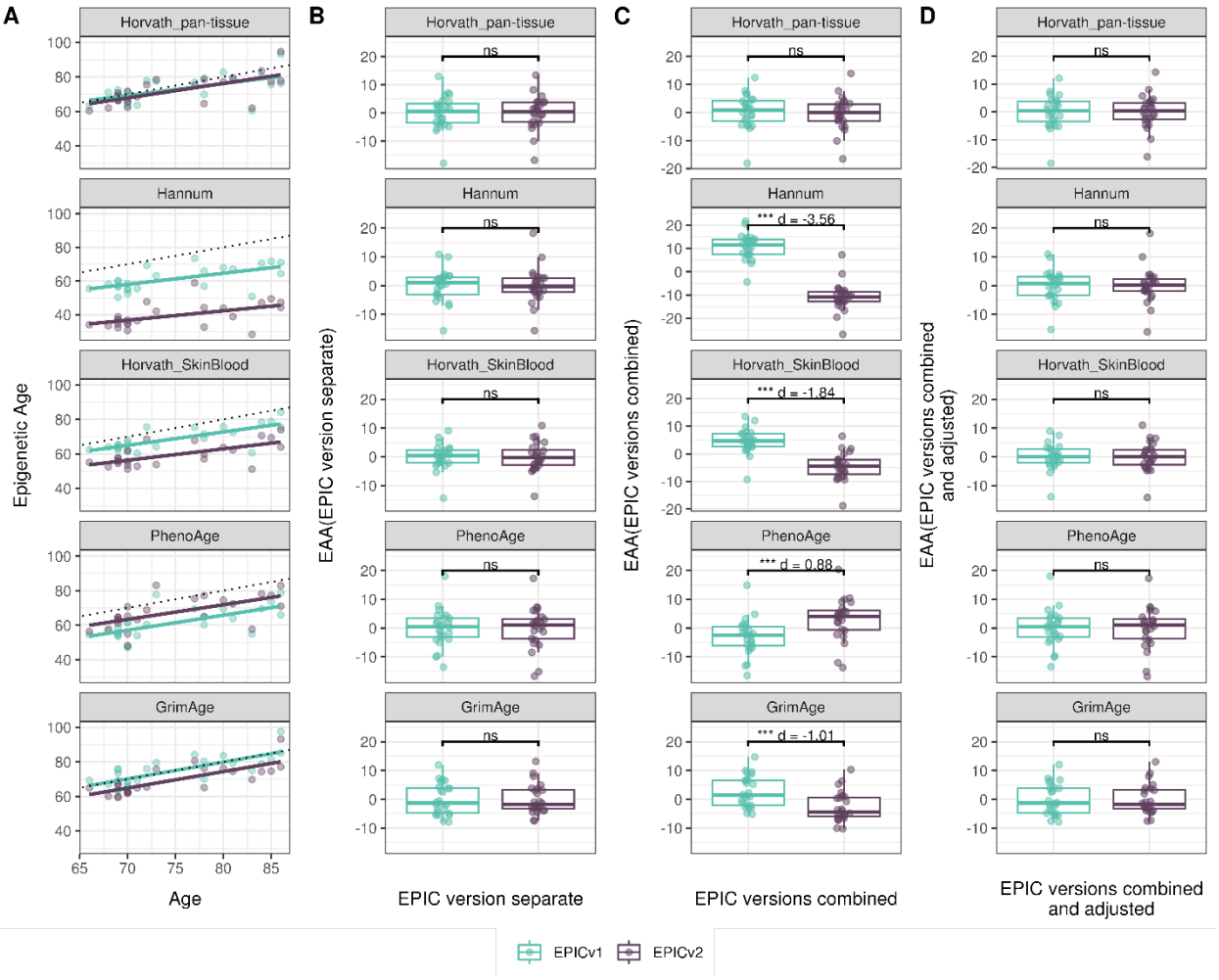
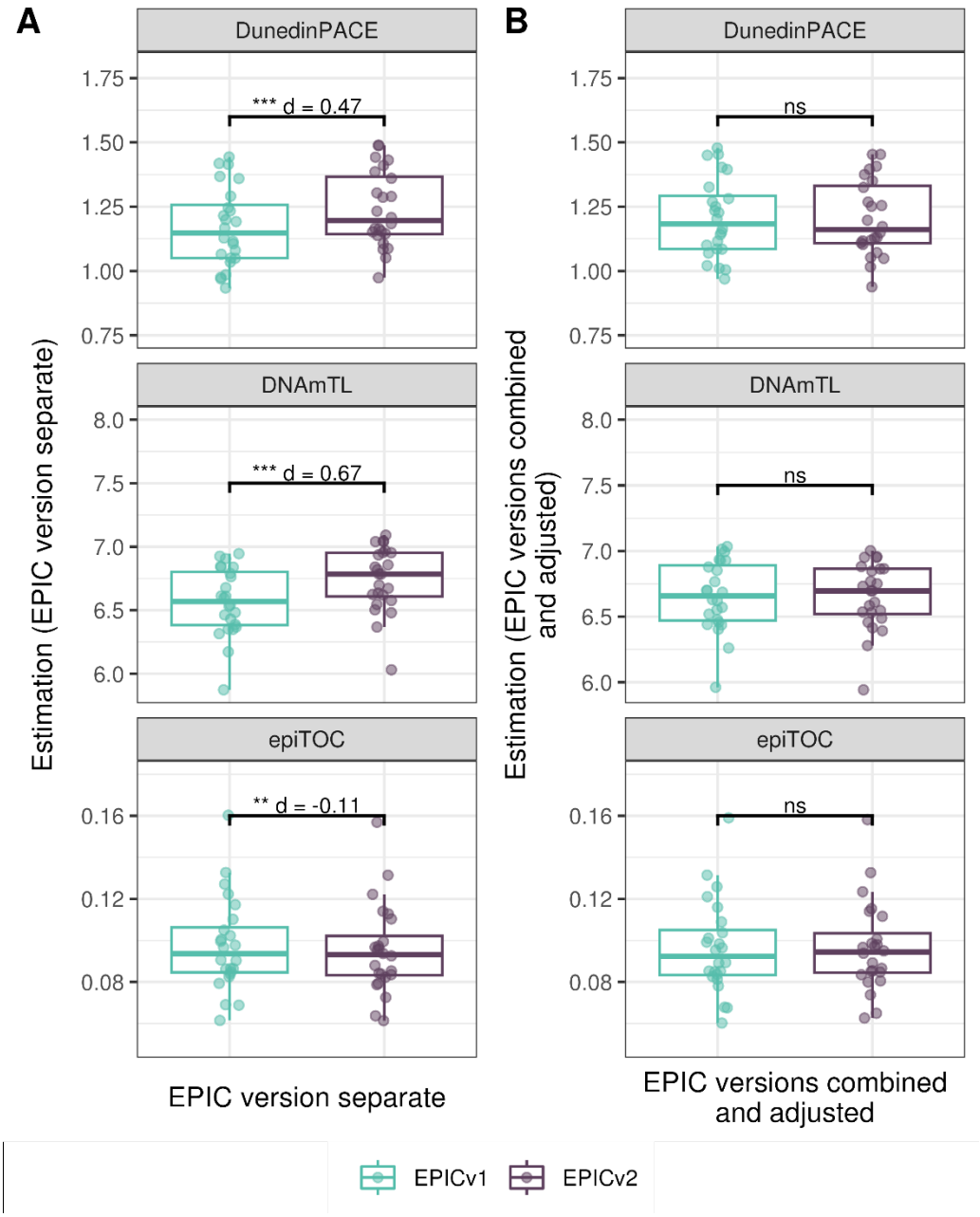
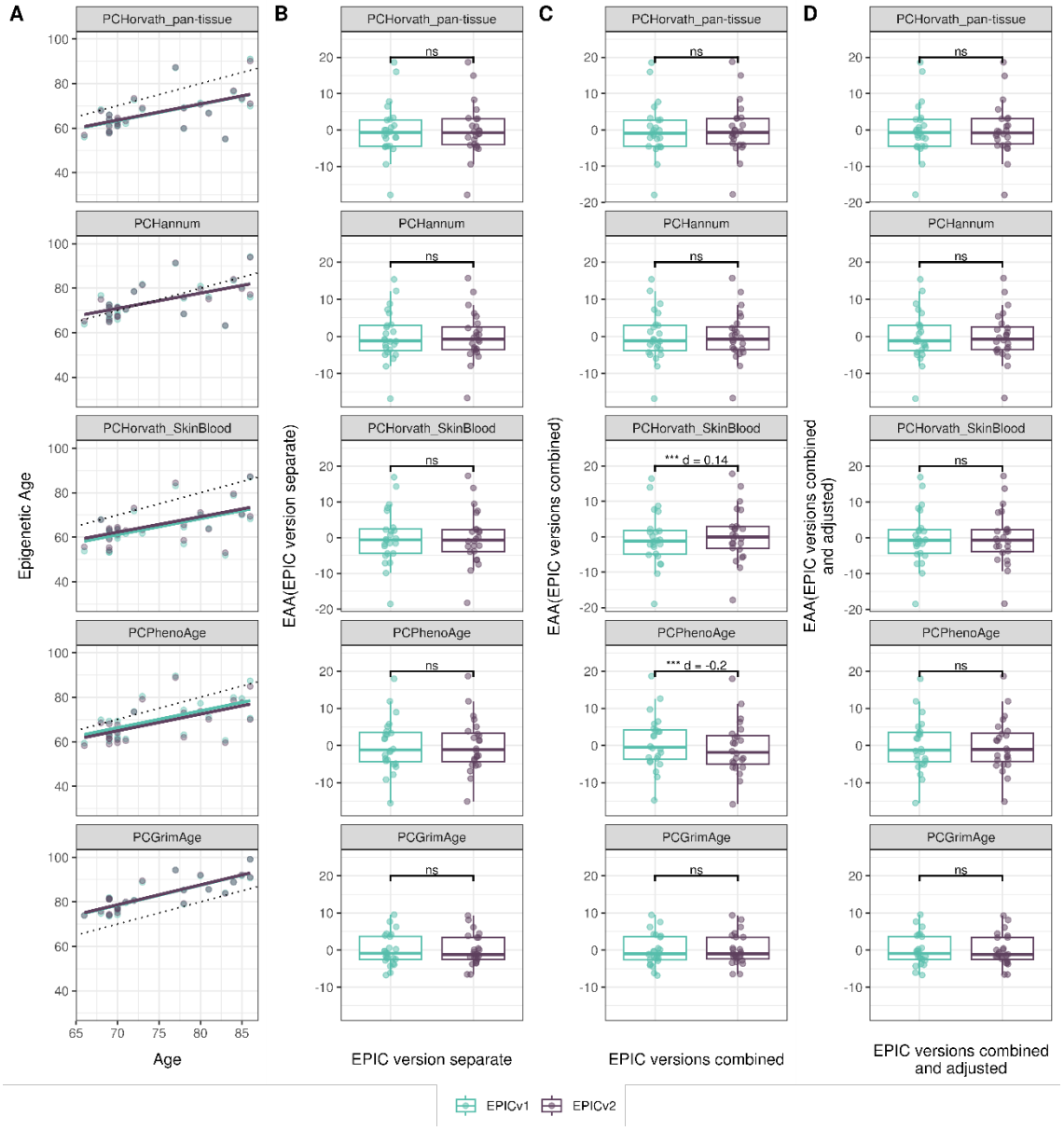


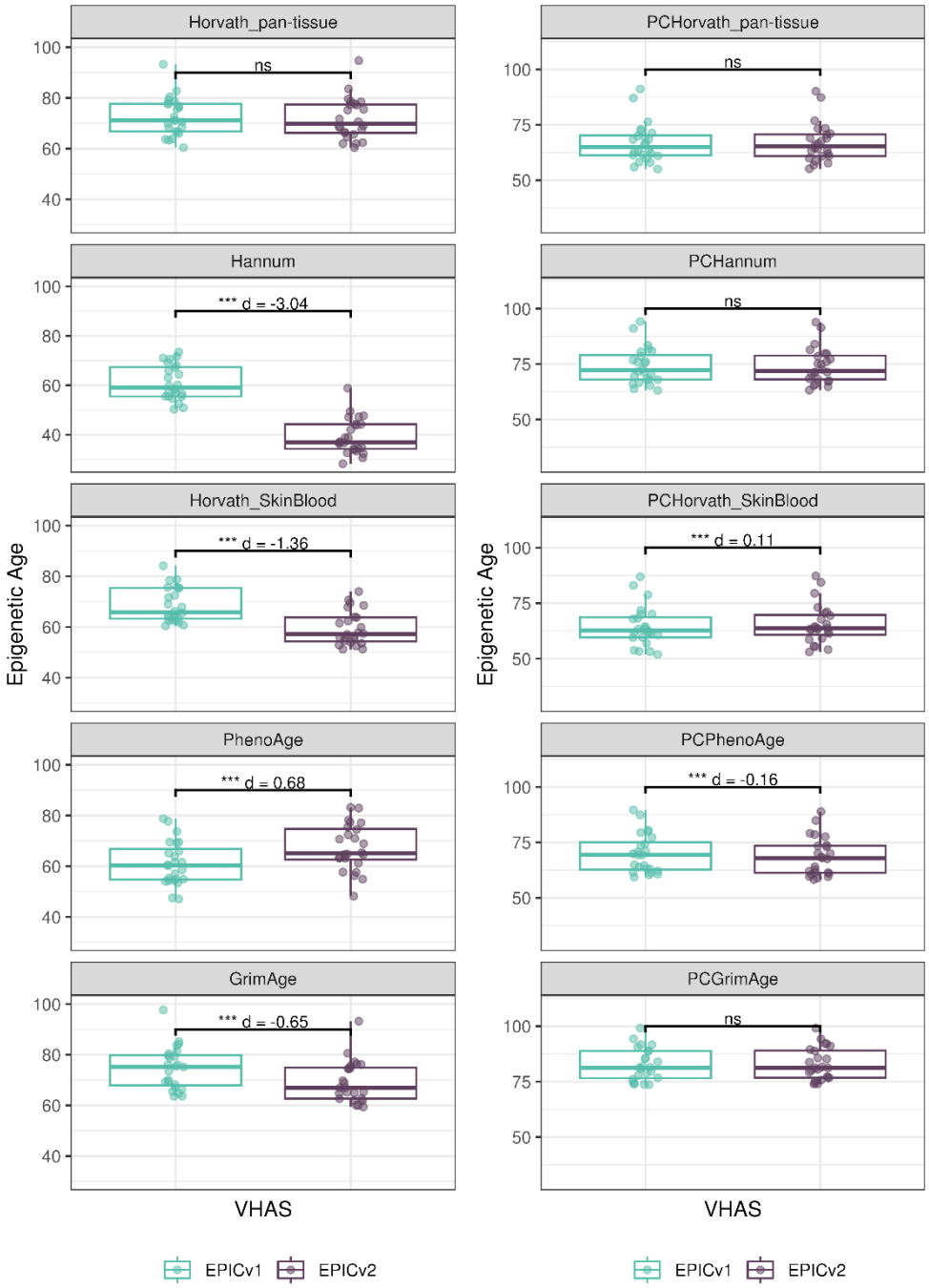
Supplementary Figure 16. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in VHAS capillary blood samples. **(A)** Scatter plot of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock ages (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B, C and D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.



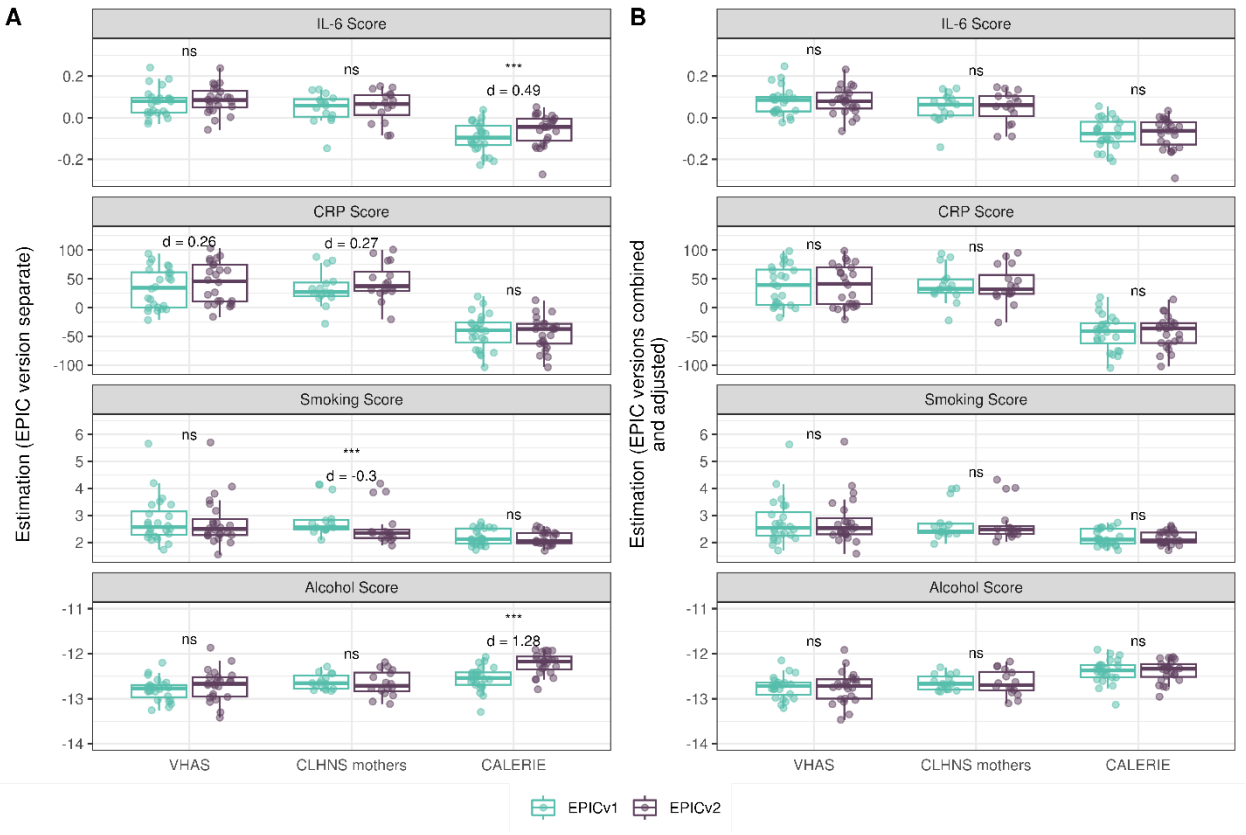
Supplementary Figure 17. Rate-based clock estimates on matched samples assessed on EPICv1 and EPICv2 in VHAS capillary blood samples. Boxplots comparing DunedinPACE, DNAmTL and epiTOC estimates calculated by considering EPIC versions separately **(A)** and combined and EPIC version adjusted **(B)**, between EPICv1 and EPICv2. Statistical significance was defined as Bonferroni adjusted *p*-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p <0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates estimates in EPICv2 compared to EPICv1.



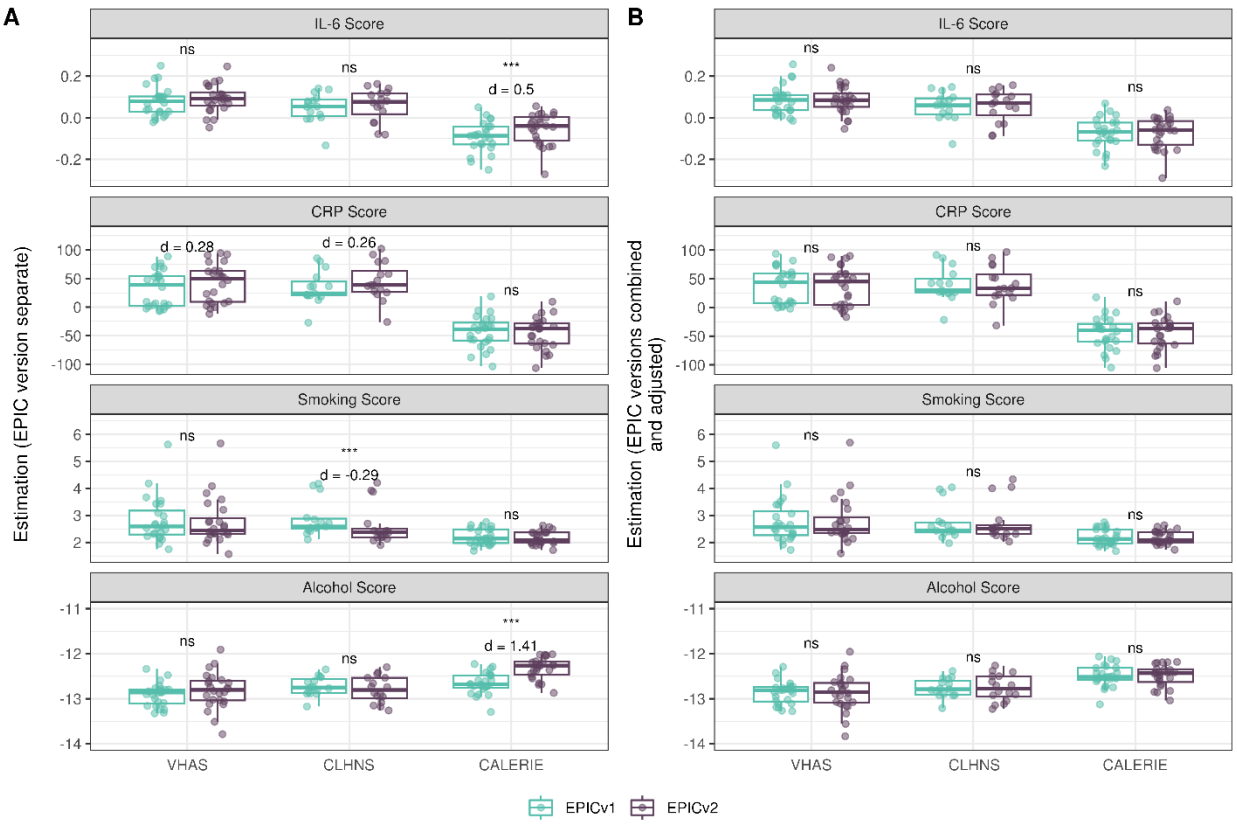
Supplementary Figure 18. Epigenetic age estimated on matched samples assessed on EPICv1 and EPICv2 in VHAS capillary blood samples. **(A)** Scatter plot of PC clocks of Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, and GrimAge clock ages (Y axis) and chronological age (X axis) with dotted line indicating x=y, coloured by EPIC version. **(B, C and D)** Boxplots comparing EPICv1 and EPICv2 EAAs calculated by considering EPIC versions separately, combined, and combined and EPIC version adjusted, respectively. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher estimates in EPICv2 compared to EPICv1.



Supplementary Figure 19. Differences in epigenetic ages between EPICv1 and EPICv2 using the Horvath pan-tissue, Hannum, Horvath skin and blood, PhenoAge, GrimAge clocks, and PC clocks in VHAS capillary blood samples.



Supplementary Figure 20. DNA methylation-based predictors estimated on matched samples assessed on EPICv1 and EPICv2 in VHAS, CLHNS, and CALERIE. Boxplots comparing EPICv1 and EPICv2 proxy IL-6, CRP, smoking, and alcohol scores calculated by considering **(A)** EPIC versions separately, and **(B)** combined and EPIC version adjusted. Statistical significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05, *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size measured using Cohen's d. A positive Cohen's d indicates higher average estimated cell proportions in EPICv2 compared to EPICv1.



243

244 **Supplementary Figure 21**. DNA methylation-based predictors estimated after normalizing
245 EPICv1 and EPICv2 together using BMIQ in VHAS, CLHNS, and CALERIE. Boxplots
246 comparing EPICv1 and EPICv2 proxy IL-6, CRP, smoking, and alcohol scores calculated by
247 considering EPIC versions separately **(A)**, and combined and EPIC version adjusted **(B)**. Statistical
248 significance was defined as Bonferroni adjusted p-value <0.05. ** denotes Bonferroni p <0.05,
249 *** denotes Bonferroni p<0.001, "ns" denotes "not significant", and "d" denotes effect size
250 measured using Cohen's d. A positive Cohen's d indicates higher average estimated cell
251 proportions in EPICv2 compared to EPICv1.

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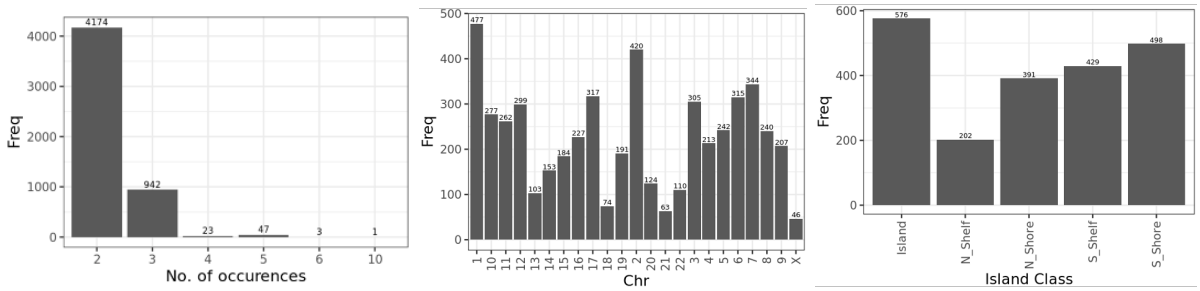
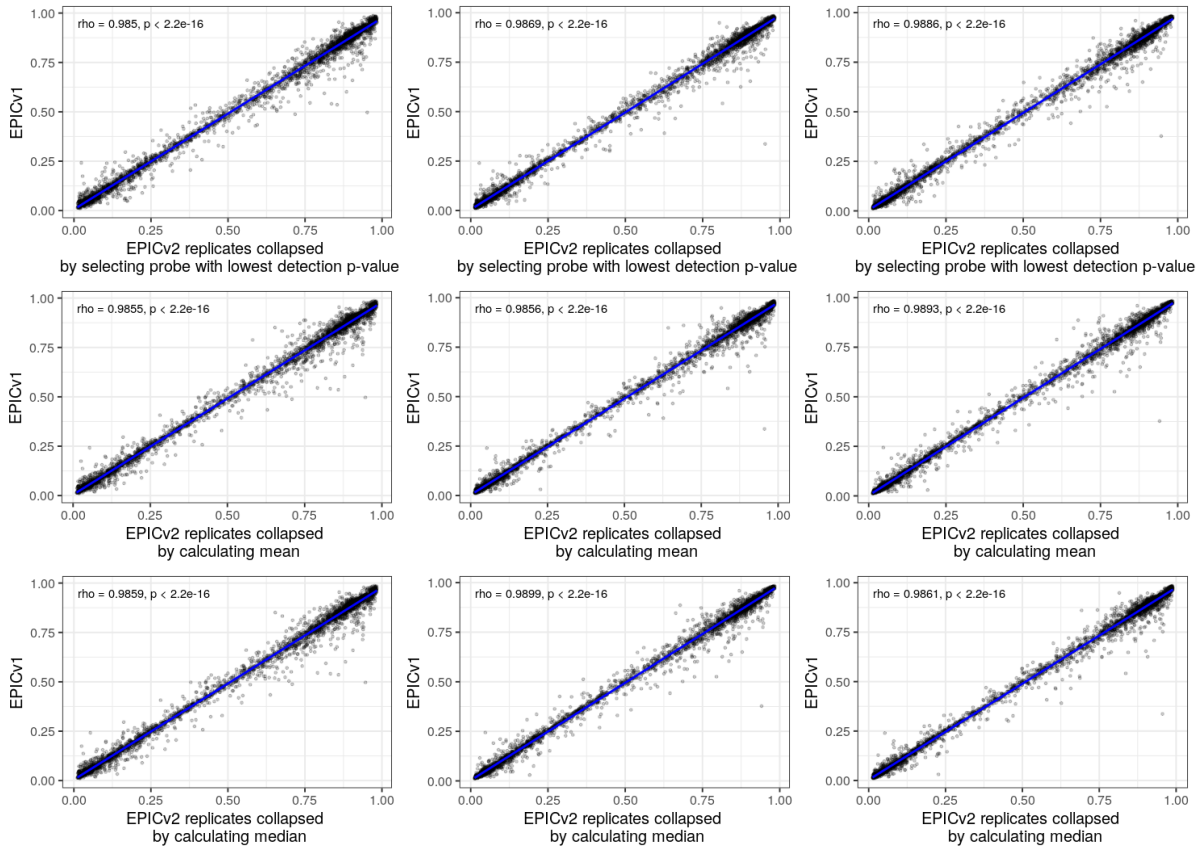


A B C

Supplementary Figure 22: Frequency of occurrence of replicate probes on EPIC v2 **(A)**, their distribution across chromosomes **(B)**, and CpG island classes **(C)**.

(A) VHAS **(B) CLHNS** **(C) CALERIE**



Supplementary Figure 23: Spearman correlation between β values of EPICv2 replicate probes and corresponding EPICv1 probes (n=3602). EPICv2 replicate probes were collapsed to obtain a single β value using detection *p*-value (row 1), mean (row 2), and median (row 3) based strategies for (A) VHAS, (B) CLHNS, and (C) CALERIE.