

Supplementary Figures

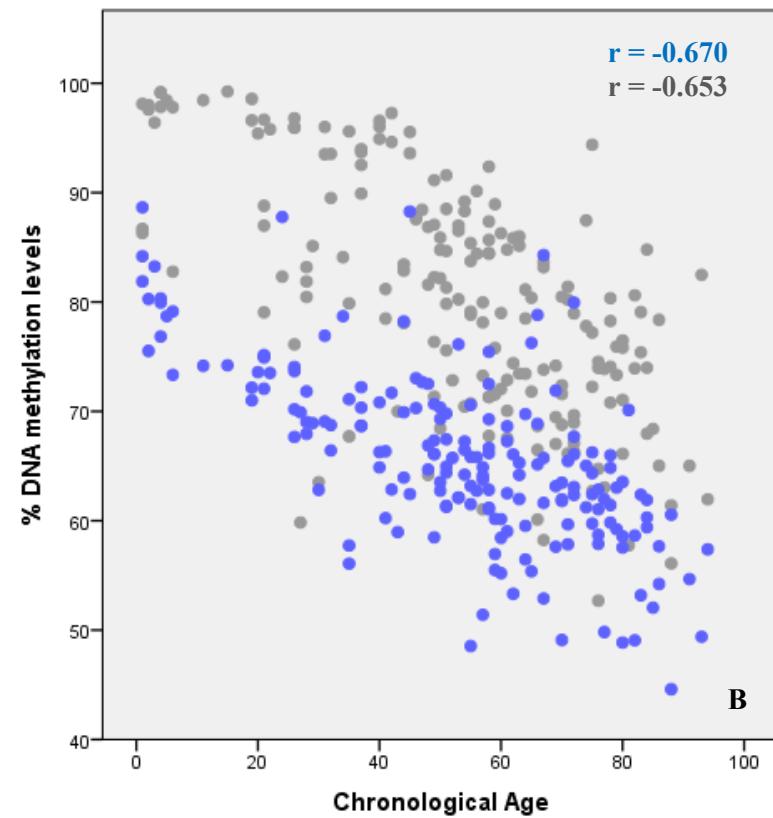
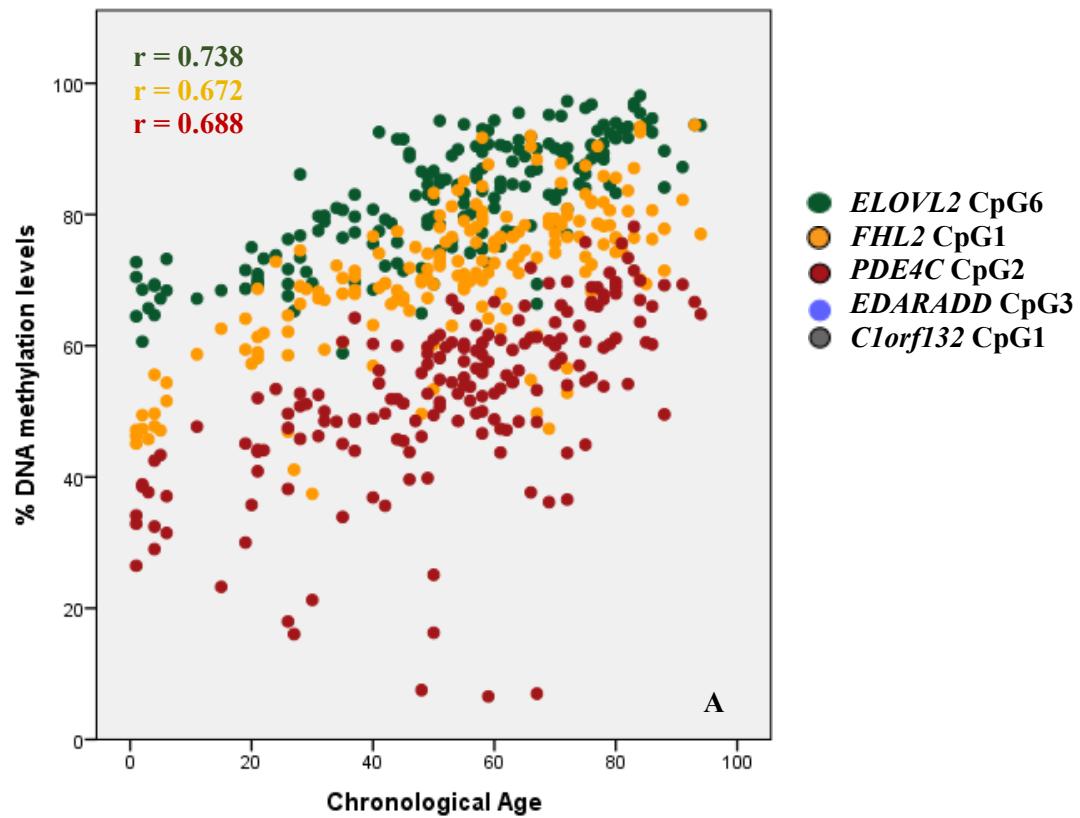


Figure S1: Correlations between DNAm levels and chronological age in 185 samples including blood samples from living and deceased individuals, bone samples collected from autopsies and teeth from living and deceased individuals, obtained through Sanger sequencing methodology. A) Positive correlation between methylation levels and chronological age for *ELOVL2* CpG6 (green), *PDE4C* CpG2 (dark red), *FHL2* CpG1 (yellow) markers; B) negative correlation between methylation levels and chronological age for *EDARADD* CpG3 (blue) and *Clorf132* CpG1 (gray) markers. The corresponding Spearman correlation coefficients (r) are depicted inside each plot.

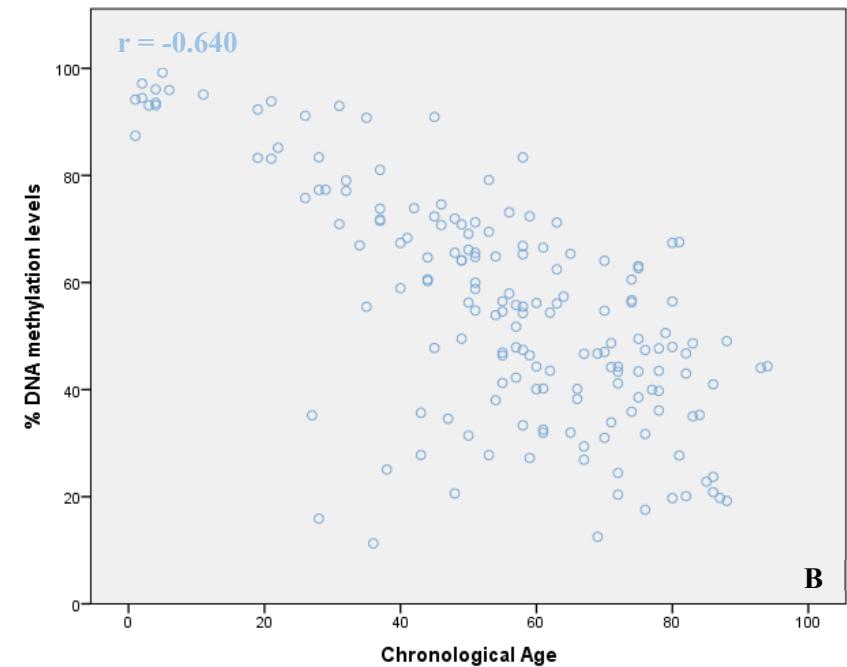
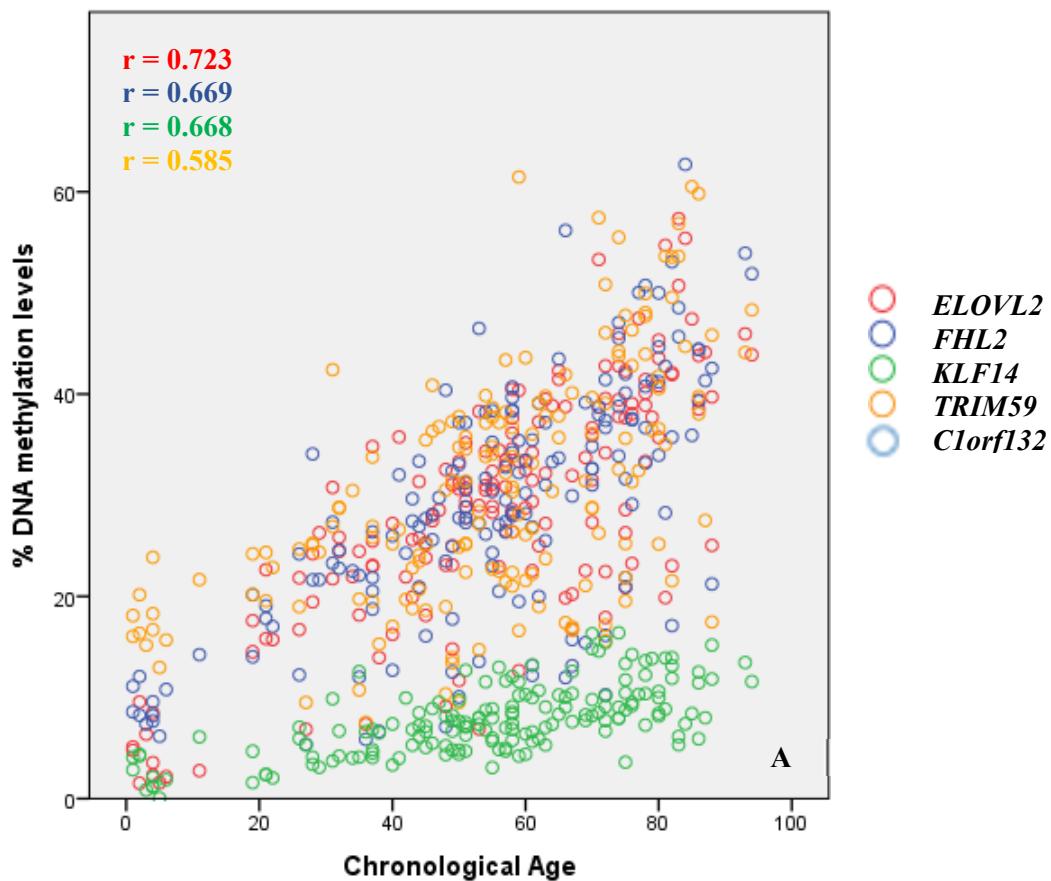


Figure S2: Correlations between DNAm levels and chronological age in 168 samples, including blood samples from living and deceased individuals, bone samples collected from autopsies and teeth from living and deceased individuals, obtained through SNaPshot methodology. A) Positive correlation between methylation levels and chronological age for CpG sites in *ELOVL2* (red), *FHL2* (blue), *KLF14* (green) and *TRIM59* (yellow) genes; B) negative correlation between methylation levels and chronological age for CpG site in *Clorf132* gene (light blue). The corresponding Spearman correlation coefficients (r) are depicted inside each plot.

Supplementary Tables

Table S1: Age distribution of the sample sets analyzed by Sanger sequencing and SNaPshot methodologies.

| Age range | Method | |
|-------------|-------------------|----------|
| | Sanger sequencing | SNaPshot |
| | N | N |
| 1-19 years | 16 | 13 |
| 20-29 years | 16 | 10 |
| 30-39 years | 13 | 13 |
| 40-49 years | 24 | 22 |
| 50-59 years | 39 | 37 |
| 60-69 years | 27 | 22 |
| 70-79 years | 30 | 30 |
| 80-89 years | 17 | 19 |
| 90-94 years | 3 | 2 |
| 1-94 years | 185 | 168 |

Table S2: Univariate linear regression analysis of the 43 CpG sites in *ELOVL2*, *FHL2*, *EDARADD*, *PDE4C* and *C1orf132* loci in 185 samples including blood from living and deceased individuals, teeth from living and deceased individuals and bone collected during autopsies.

| Gene | CpG site | Location | β | R | R^2 | Corrected R^2 | SE | P-value |
|------------------------------|-------------|----------------|-----------------|---------------|-------|-----------------|-------|--|
| <i>ELOVL2</i> | CpG1 | Chr6: 11044628 | 136.914 | 0.739 | 0.547 | 0.544 | 15.19 | 2.79×10^{-33} |
| | CpG2 | Chr6: 11044631 | 106.822 | 0.638 | 0.407 | 0.404 | 17.38 | 1.58×10^{-22} |
| | CpG3 | Chr6: 11044634 | 211.972 | 0.693 | 0.481 | 0.478 | 16.26 | 7.63×10^{-28} |
| | CpG4 | Chr6: 11044640 | 125.867 | 0.652 | 0.425 | 0.422 | 17.11 | 9.53×10^{-24} |
| | CpG5 | Chr6: 11044642 | 114.293 | 0.740 | 0.547 | 0.545 | 15.18 | 2.55×10^{-33} |
| | CpG6 | Chr6: 11044644 | 188.014 | 0.759 | 0.576 | 0.573 | 14.70 | 6.87×10^{-36} |
| | CpG7 | Chr6: 11044647 | 228.823 | 0.663 | 0.440 | 0.436 | 16.89 | 8.71×10^{-25} |
| | CpG8 | Chr6: 11044655 | 135.278 | 0.645 | 0.416 | 0.413 | 17.24 | 3.74×10^{-23} |
| | CpG9 | Chr6: 11044661 | 140.696 | 0.615 | 0.378 | 0.374 | 17.80 | 1.35×10^{-20} |
| <i>FHL2</i> | CpG1 | Chr2:105399282 | 139.052 | 0.692 | 0.479 | 0.476 | 16.29 | 1.11×10^{-27} |
| | CpG2 | Chr2:105399288 | 127.199 | 0.635 | 0.404 | 0.400 | 17.43 | 2.62×10^{-22} |
| | CpG3 | Chr2:105399291 | 133.508 | 0.640 | 0.410 | 0.407 | 17.33 | 9.95×10^{-23} |
| | CpG4 | Chr2:105399297 | 184.205 | 0.523 | 0.273 | 0.269 | 19.24 | 2.32×10^{-14} |
| | CpG5 | Chr2:105399300 | 158.887 | 0.647 | 0.419 | 0.416 | 17.02 | 2.43×10^{-23} |
| | CpG6 | Chr2:105399310 | 158.657 | 0.538 | 0.290 | 0.286 | 19.01 | 2.70×10^{-15} |
| | CpG7 | Chr2:105399314 | 96.186 | 0.308 | 0.095 | 0.090 | 21.47 | 0.000020 |
| | CpG8 | Chr2:105399316 | 112.689 | 0.389 | 0.151 | 0.147 | 20.79 | 4.43×10^{-8} |
| | CpG9 | Chr2:105399323 | 25.114 | 0.088 | 0.008 | 0.002 | 22.48 | 0.235207 |
| | CpG10 | Chr2:105399327 | 5.881 | 0.020 | 0.000 | -0.005 | 22.56 | 0.787338 |
| | CpG11 | Chr2:105399338 | -125.337 | -0.476 | 0.237 | 0.223 | 19.84 | 7.34×10^{-12} |
| | CpG12 | Chr2:105399340 | -35.520 | -0.140 | 0.020 | 0.014 | 22.34 | 0.056883 |
| <i>EDARADD</i> | CpG1 | Chr1:236394458 | 1.873 | 0.007 | 0.000 | -0.005 | 22.57 | 0.924703 |
| | CpG2 | Chr1:236394441 | -109.086 | -0.507 | 0.257 | 0.253 | 19.45 | 1.85×10^{-13} |
| | CpG3 | Chr1:236394382 | -189.185 | -0.682 | 0.465 | 0.462 | 16.51 | 1.21×10^{-26} |
| | CpG4 | Chr1:236394370 | -101.037 | -0.410 | 0.168 | 0.163 | 20.58 | 6.95×10^{-9} |
| <i>PDE4C</i> (N = 196) | CpG1 | Chr19:18233139 | 96.130 | 0.502 | 0.252 | 0.248 | 19.52 | 3.46×10^{-13} |
| | CpG2 | Chr19:18233133 | 103.448 | 0.613 | 0.376 | 0.372 | 17.83 | 1.79×10^{-20} |
| | CpG3 | Chr19:18233131 | 97.447 | 0.559 | 0.313 | 0.309 | 18.71 | 1.34×10^{-16} |
| | CpG4 | Chr19:18233127 | 45.141 | 0.238 | 0.057 | 0.051 | 21.92 | 0.001120 |
| | CpG5 | Chr19:18233105 | 100.544 | 0.462 | 0.213 | 0.209 | 20.02 | 3.71×10^{-11} |
| | CpG6 | Chr19:18233091 | -30.238 | -0.157 | 0.025 | 0.019 | 22.29 | 0.032991 |
| | CpG7 | Chr19:18233082 | 10.797 | 0.048 | 0.002 | -0.003 | 22.54 | 0.516987 |
| | CpG8 | Chr19:18233079 | -44.121 | -0.173 | 0.030 | 0.025 | 22.24 | 0.018301 |
| | CpG9 | Chr19:18233070 | -77.591 | -0.313 | 0.098 | 0.093 | 21.43 | 0.000014 |
| | CpG10 | Chr19:18233058 | -15.015 | -0.054 | 0.003 | -0.003 | 22.56 | 0.471827 |
| | CpG11 | Chr19:18233048 | -28.244 | -0.099 | 0.010 | 0.004 | 22.57 | 0.186649 |
| | CpG12 | Chr19:18233042 | -5.655 | -0.021 | 0.000 | -0.005 | 22.68 | 0.782908 |
| <i>C1orf132</i> (N = 126) | CpG1 | Chr1:207823681 | -135.517 | -0.654 | 0.428 | 0.425 | 17.07 | 5.67×10^{-24} |
| | CpG2 | Chr1:207823675 | -97.150 | -0.592 | 0.351 | 0.347 | 18.18 | 6.62×10^{-19} |
| | CpG3 | Chr1:207823672 | -88.567 | -0.572 | 0.327 | 0.324 | 18.51 | 1.79×10^{-17} |
| | CpG4 | Chr1:207823660 | -89.102 | -0.549 | 0.301 | 0.297 | 18.88 | 7.38×10^{-16} |
| | CpG5 | Chr1:207823657 | -96.852 | -0.570 | 0.325 | 0.321 | 18.60 | 4.49×10^{-17} |
| | CpG6 | Chr1:207823637 | -76.285 | -0.448 | 0.201 | 0.196 | 20.19 | 1.78×10^{-10} |

Abbreviations: N, number of samples; β , unstandardized coefficient; R, correlation coefficient; SE, standard error. Significant p-values are in bold. The strongest age-

associated CpG site is in bold and underlined. Genomic positions were based on the GRCh38/hg38 assembly.

Table S3: Statistical parameters obtained in a multiple regression model with the seven CpGs in genes *ELOVL2*, *FHL2*, *EDARADD*, *PDE4C* and *C1orf132* selected by stepwise regression approach, in blood, bone and tooth samples.

| Marker | Coefficient | P-value |
|----------------------|-------------|---------|
| (Intercept) | 26.852 | 0.009 |
| <i>EDARADD</i> CpG3 | -24.767 | 0.016 |
| <i>FHL2</i> CpG5 | 68.537 | 0.000 |
| <i>FHL2</i> CpG11 | -51.319 | 0.000 |
| <i>ELOVL2</i> CpG5 | 57.461 | 0.000 |
| <i>PDE4C</i> CpG5 | 41.449 | 0.000 |
| <i>PDE4C</i> CpG9 | -66.397 | 0.000 |
| <i>C1orf132</i> CpG3 | -27.418 | 0.000 |

Table S4: Statistical parameters obtained in a multiple regression model with the three CpGs in genes *ELOVL2*, *C1orf132* and *KLF14*, selected by stepwise regression approach, in blood, bone and tooth samples.

| Marker | Coefficient | P-value |
|-----------------|-------------|---------|
| (Intercept) | 29.220 | 0.000 |
| <i>C1orf132</i> | -33.437 | 0.000 |
| <i>ELOVL2</i> | 96.850 | 0.000 |
| <i>KLF14</i> | 208.747 | 0.000 |