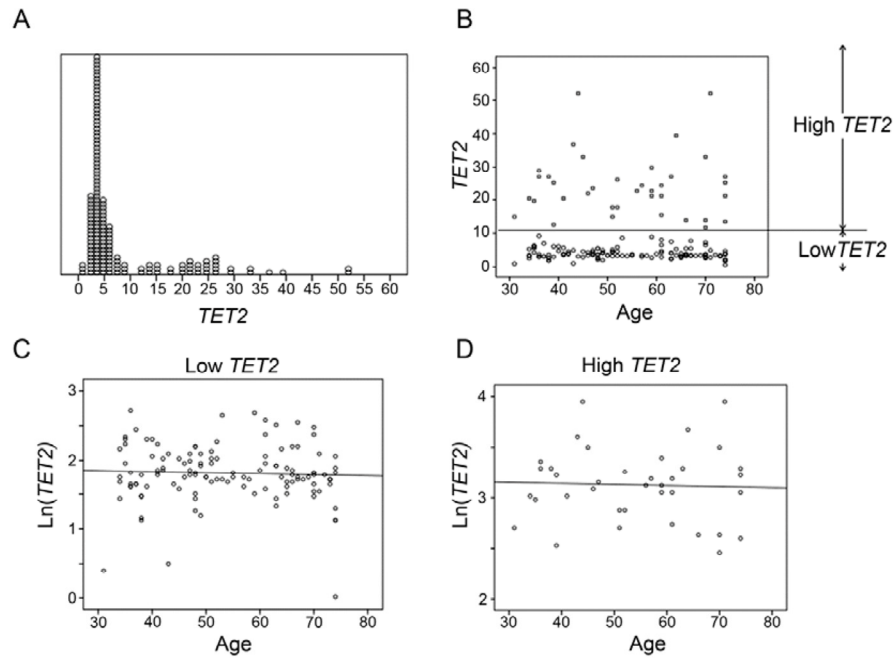


SUPPLEMENTARY MATERIAL

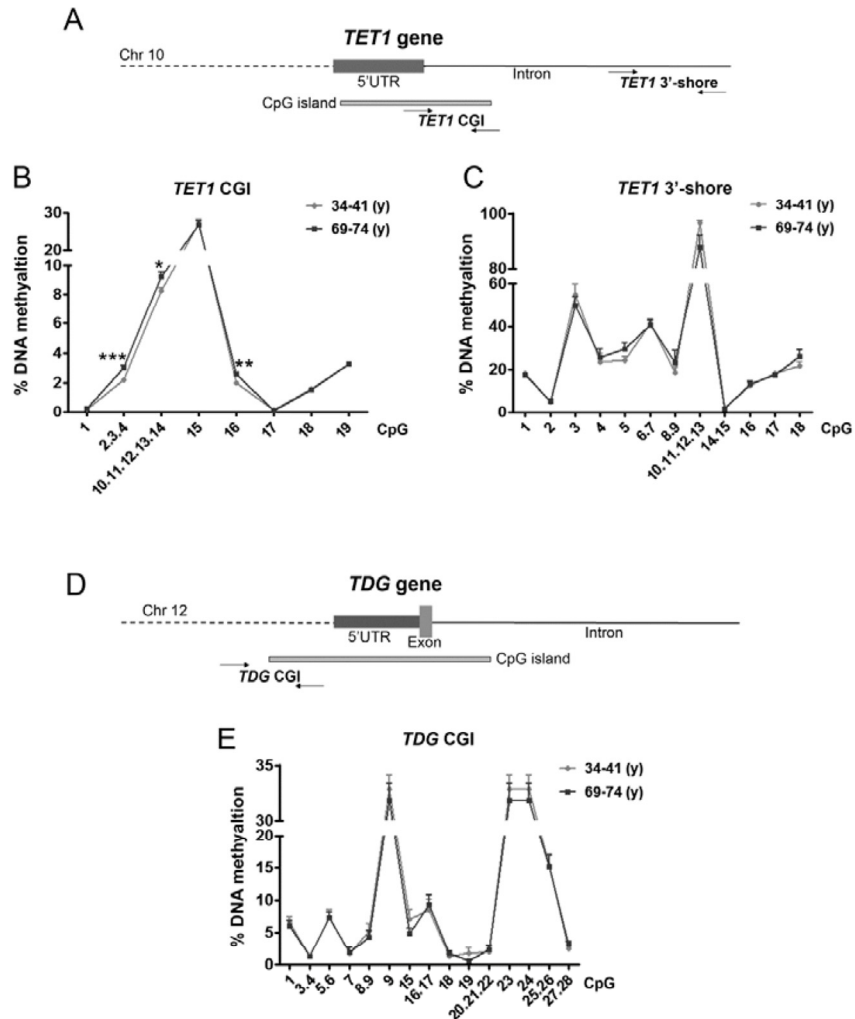


**Supplementary Figure 1. Bimodal distribution of *TET2* mRNA levels in PBMC.** (A) Cluster analysis was performed on *TET2* values data by two step cluster analysis implemented in SPSS package (based on log-likelihood distance measure) using Schwarz’s Bayesian as clustering criterion. The tool automatically identifies two clusters separated around the value of 10.0. The cut-off is slightly above the 75th percentile value (8.3). (B) *TET2* values showing a spread distribution across age. The subjects assigned to each cluster of *TET2* were named “low *TET2*” and “high *TET2*”. The panels below show the linear association with age of Ln(*TET2*) data with “low” (C) and “high” (D) levels of expression. Linear regression showed no significant changes with age for both classes.

**Supplementary Table 1. Regression analysis of *TET1* mRNA levels in PBMC†**

Model		Coefficients		Bootstrap for Coefficients		
		B ± SE	Beta	Bias	Sig	95% CI
<i>TET1</i>	(Constant)	0.266 ± 0.024		0.001	0.001	0.227;0.309
	Age (y)	-0.001±0.000	-0.239	< 0.001	0.001	-0.002;-0.001
Ln( <i>TET1</i> )	(Constant)	-1.316±0.113		0.005	0.001	-1.533;-1.112
	Age (y)	-0.008±0.002	-0.271	< 0.001	0.001	-0.011;-0.004
Ln( <i>TET1</i> ) B.C.	(Constant)	-1.349±0.098		< 0.001	0.001	-1.529;-1.148
	Age (y)	-0.007±0.002	-0.288	< 0.001	0.001	-0.010;-0.004
Ln( <i>TET1</i> ) B.C._age;gender	(Constant)	-1.273±0.097		0.000	0.001	-1.458;-1.089
	Age (y)	-0.008±0.002	-0.341	< 0.001	0.001	-0.012;-0.005

† Regression analysis was performed by using as dependent variable: *TET1* non-transformed data; Ln(*TET1*) data; Ln(*TET1*) data after batch correction (B.C); and Ln(*TET1*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Bootstrap results are based on 1000 stratified (by recruitment center and gender) bootstrap samples.



**Supplementary Figure 2. DNA methylation analysis of *TET1* and *TDG* regulatory regions. (A)** Schematic representation of *TET1* gene in which the localization of *TET1* CGI and *TET1* 3'-shore are shown. Age-related changes of DNA methylation in *TET1* CGI (**B**) and *TET1* 3'-shore (**C**) between the groups of young (< 41y) and old (>69 y) individuals are shown. (**D**) Schematic representation of *TDG* gene, in which the localization of CGI is shown. (**E**) Age-related changes of DNA methylation between the groups of young (< 41y) and the old (>69 y) individuals in the *TDG* CGI. Statistical significance was obtained using the Mann-Whitney test (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).  $n(34-41)=36$ ;  $n(69-74)=34$ . (y)= years.

**Supplementary Table 2.** Regression analysis of *TET3* mRNA levels in PBMC†

Model		Coefficients		Bootstrap for Coefficients		
		B ± SE	Beta	Bias	Sig	95% CI
<i>TET3</i>	(Constant)	3.684 ± 0.270		0.002	0.001	3.177;4.202
	Age (y)	-0.010±0.005	-0.157	< 0.001	0.025	-0.019;-0.001
Ln( <i>TET3</i> )	(Constant)	1.296±0.086		0.003	0.001	1.136;1.443
	Age (y)	-0.004±0.001	-0.172	< 0.001	0.012	-0.006;-0.001
Ln( <i>TET3</i> ) B.C.	(Constant)	1.308±0.070		-0.001	0.001	1.180;1.439
	Age (y)	-0.004±0.001	-0.218	< 0.001	0.005	-0.006;-0.001
Ln( <i>TET3</i> ) B.C._age;gender	(Constant)	1.361±0.071		0.002	0.001	1.233;1.493
	Age (y)	-0.005±0.001	-0.269	< 0.001	0.001	-0.007;-0.005

† Regression analysis was performed by using as dependent variable: *TET3* non-transformed data; Ln(*TET3*) data; Ln(*TET3*) data after batch correction (B.C); and Ln(*TET3*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Bootstrap results are based on 1000 stratified (by recruitment center and gender) bootstrap samples.

**Supplementary Table 3.** Influence of selected factors and covariates on age-related changes of *TET1* expression†

Test of Model Effects	<i>TET1</i>			Ln( <i>TET1</i> )		Ln( <i>TET1</i> ) B.C.		Ln( <i>TET1</i> ) B.C._age;gender	
	Type III	Type III	Type III	Type III	Type III	Type III	Type III		
Variables	df	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.
(Intercept)	1	84.628	<0.001	625.562	<0.001	856.957	<0.001	861.589	<0.001
Center	7	14.837	0.038	9.575	0.214	7.588	0.370	6.795	0.451
Gender	1	0.405	0.525	0.716	0.398	0.970	0.325	1.497	0.221
Age groups	2	5.544	0.063	5.884	0.053	6.138	0.046	9.690	0.008
Lympho/mono	1	6.152	0.013	9.594	0.002	14.020	<0.001	13.356	<0.001

†Analysis was performed by GLM using linear model with identity link-function considering as dependent variable: *TET1* non-transformed data; Ln(*TET1*) data; Ln(*TET1*) data after batch correction (B.C); and Ln(*TET1*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Model: (Intercept), center, gender, age groups, lympho/mono (included in the model as continuous variable).

**Supplementary Table 4.** Regression analysis of *TET1* mRNA levels with age considering lympho/mono ratio†

Model		Coefficients		Bootstrap for Coefficients		
		B ± SE	Beta	Bias	Sig	95% CI
<i>TET1</i>	(Constant)	0.210				
	Age (y)	-0.001 ± 0.0004 0.009 ± 0.003	-0.188 0.193	<0.001 <0.001 <0.001	0.001 0.001 0.005	0.154;0.270 -0.002;0.000 0.002;0.015
	Lympho/mono					
Ln( <i>TET1</i> )	(Constant)	-1.661 ± 0.152				
	Age (y)	-0.006 ± 0.002 0.054 ± 0.015	-0.203 0.241	<0.001 <0.001 <0.001	0.001 0.004 0.003	-1.926;-1.360 -0.009;-0.002 0.025;0.085
	Lympho/mono					
Ln( <i>TET1</i> ) B.C.	(Constant)	-1.673				
	Age (y)	-0.005 ± 0.002 0.053 ± 0.013	-0.224 0.275	-0.015 <0.001 0.001	0.001 0.001 0.001	-1.925;-1.439 -0.008;-0.002 0.031;0.082
	Lympho/mono					
Ln( <i>TET1</i> ) B.C._age;gender	(Constant)	-1.590				
	Age (y)	-0.007 ± 0.002 0.052 ± 0.012	-0.279 0.267	-0.004 <0.001 <0.001	0.001 0.001 0.001	-1.833;-1.327 -0.010;-0.004 0.027;0.078
	Lympho/mono					

† Regression analysis was performed by using as dependent variable: *TET1* not-transformed data; Ln(*TET1*) data; Ln(*TET1*) data after batch correction (B.C); and Ln(*TET1*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Bootstrap results are based on 1000 stratified (by recruitment center and gender) bootstrap samples.

**Supplementary Table 5.** Influence of selected factors and covariates on age-related changes of *TET2* expression†

Test of Model Effects	<i>TET2</i>		Ln( <i>TET2</i> )		Ln( <i>TET2</i> ) B.C.		Ln( <i>TET2</i> ) B.C._age;gender		
	Type III	Type III	Type III	Type III	Type III	Type III	Type III		
Variables	df	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.
(Intercept)	1	28.451	<0.001	118.959	<0.001	132.782	<0.001	132.671	<0.001
Center	7	5.158	0.641	3.342	0.852	6.244	0.512	5.507	0.598
Gender	1	3.306	0.069	3.933	0.047	2.523	0.112	3.441	0.064
Age groups	2	1.898	0.387	4.781	0.092	2.019	0.364	2.245	0.326
Lympho/mono	1	2.281	0.131	5.942	0.015	4.789	0.029	4.796	0.029

† Analysis was performed by GLM using linear model with identity link-function considering as dependent variable: *TET2* not-transformed data; Ln(*TET2*) data; Ln(*TET2*) data after batch correction (B.C); and Ln(*TET2*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Model: (Intercept), center, gender, age groups, lympho/mono (included in the model as continuous variable).

**Supplementary Table 6.** Influence of selected factors and covariates on age-related changes of *TET3* expression†

Test of Model Effects		<i>TET3</i>		Ln( <i>TET3</i> )		Ln( <i>TET3</i> ) B.C.		Ln( <i>TET3</i> ) B.C._age;gender	
Variables	df	Type III		Type III		Type III		Type III	
		Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.
(Intercept)	1	322.147	<0.001	416.554	<0.001	592.997	<0.001	624.541	<0.001
Center	7	19.893	0.006	17.491	0.014	15.306	0.032	13.952	0.052
Gender	1	6.964	0.008	8.302	0.004	4.650	0.031	5.990	0.014
Age groups	2	9.621	0.008	9.914	0.007	18.615	<0.001	30.389	<0.001
Lympho/mono	1	1.211	0.271	2.918	0.088	3.590	0.058	4.913	0.027

†Analysis was performed by GLM using linear model with identity link-function considering as dependent variable: *TET3* not-transformed data; Ln(*TET3*) data; Ln(*TET3*) data after batch correction (B.C); and Ln(*TET3*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Model: (Intercept), center, gender, age groups, lympho/mono (included in the model as continuous variable).

**Supplementary Table 7.** Regression analysis of *TDG* mRNA levels in PBMC†

Model		Coefficients		Bootstrap for Coefficients		
		B ± SE	Beta	Bias	Sig	95% CI
<i>TDG</i>	(Constant)	-0.246 ± 0.026		<0.001	0.001	0.195;0.303
	Age (y)	-0.001±0.000	-0.161	<0.001	0.050	-0.002;-3.349E <sup>-5</sup>
Ln( <i>TDG</i> )	(Constant)	1.436±0.109		0.003	0.001	-1.631;-1.234
	Age (y)	-0.005±0.002	-0.194	<0.001	0.005	-0.009;-0.001
Ln( <i>TDG</i> ) B.C.	(Constant)	-1.519±0.095		-0.001	0.001	-1.721;-1.325
	Age (y)	-0.004±0.002	-0.161	<0.001	0.034	-0.007;-1.777E <sup>-5</sup>
Ln( <i>TDG</i> ) B.C._age;gender	(Constant)	-1.485 ±0.094		0.003	0.001	-1.676;-1.292
	Age (y)	-0.004±0.002	-0.188	<0.001	0.020	-0.007;-0.005

† Regression analysis was performed by using as dependent variable: *TDG* not-transformed data; Ln(*TDG*) data; Ln(*TDG*) data after batch correction (B.C); and Ln(*TDG*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Bootstrap results are based on 1000 stratified (by recruitment center and gender) bootstrap samples.

**Supplementary Table 8.** Influence of selected factors and covariates on age-related changes of *TDG* expression†

Test of Model Effects	<i>TDG</i>		Ln( <i>TDG</i> )		Ln( <i>TDG</i> ) B.C.		Ln( <i>TDG</i> ) B.C._age;gender		
	Type III		Type III		Type III		Type III		
Variables	df	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.
<b>(Intercept)</b>	1	111.058	<0.001	542.769	<0.001	773.422	<0.001	764.387	<0.001
<b>Center</b>	7	40.802	<0.001	36.851	<0.001	32.153	<0.001	29.293	<0.001
<b>Gender</b>	1	1.082	0.297	0.821	0.365	0.931	0.334	1.129	0.288
<b>Age groups</b>	2	2.079	0.354	3.411	0.182	3.167	0.205	4.246	0.120
<b>Lympho/mono</b>	1	0.524	0.469	0.000	0.992	1.026	0.311	0.880	0.348

†Analysis was performed by GLM using linear model with identity link-function considering as dependent variable: *TDG* not-transformed data; Ln(*TDG*) data; Ln(*TDG*) data after batch correction (B.C); and Ln(*TDG*) data after batch correction retaining age and gender differences (B.C.\_age;gender). Model: (Intercept), center, gender, age groups, lympho/mono (included in the model as continuous variable).

**Supplementary Table 9.** Regression analysis of 5hm Clevels in PBMCT†

Model		Coefficients		Bootstrap for Coefficients		
		B ± SE	Beta	Bias	Sig	95% CI
<b>5hmC</b>	(Constant)	1.341 ± 0.106		0.001	0.001	1.165;1.527
	<b>Age (y)</b>	-0.004±0.002	-0.190	<0.001	0.008	-0.008;-0.001
<b>Ln(5hmC)</b>	(Constant)	0.298±0.102		-0.006	0.001	0.131;0.462
	<b>Age (y)</b>	-0.004±0.002	-0.199	<0.001	0.005	-0.007;-0.001
<b>Ln(5hmC) B.C.</b>	(Constant)	0.303±0.092		0.002	0.001	0.145;0.462
	<b>Age (y)</b>	-0.005±0.002	-0.227	<0.001	0.004	-0.007;-0.002
<b>Ln(5hmC) B.C._age;gender</b>	(Constant)	0.353±0.092		0.001	0.001	0.205;0.503
	<b>Age (y)</b>	-0.005±0.002	-0.266	<0.001	0.002	-0.008;-0.003

† Regression analysis was performed by using as dependent variable: 5hmC not-transformed data; Ln(5hmC) data; Ln(5hmC) data after batch correction (B.C); and Ln(5hmC) data after batch correction retaining age and gender differences (B.C.\_age;gender). Bootstrap results are based on 1000 stratified (by recruitment center and gender) bootstrap samples.

**Supplementary Table 10.** Influence of selected factors and covariates on age-related changes of 5hmC level<sup>†</sup>

Test of Model Effects		5hmC		Ln(5hmC)		Ln(5hmC) B.C.		Ln(5hmC) B.C._age;gender	
		Type III		Type III		Type III		Type III	
Variables	df	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.	Wald Chi-Square	Sig.
<b>(Intercept)</b>	1	220.480	<0.001	0.135	0.713	0.348	0.555	0.369	0.543
<b>Center</b>	7	13.213	0.067	15.579	0.029	16.095	0.024	15.522	0.030
<b>Gender</b>	1	0.916	0.339	1.755	0.185	3.273	0.070	4.089	0.043
<b>Age groups</b>	2	10.599	0.005	13.316	0.001	12.444	0.002	14.633	0.001
<b>Lympho/mono</b>	1	0.014	0.906	0.113	0.737	0.010	0.919	0.033	0.857

<sup>†</sup>Analysis was performed by GLM using linear model with identity link-function considering as dependent variable: 5hmCnot-transformed data; Ln(5hmC) data; Ln(5hmC) data after batch correction (B.C); and Ln(5hmC) data after batch correction retaining age and gender differences (B.C.\_age;gender). Model: (Intercept), center, gender, age groups, lympho/mono (included in the model as continuous variable).