

## Supplementary material

**Supplementary table 1:** List of biomarkers used to determine biological age as part of the MARK-AGE algorithm

<b>Biomarker</b>	<b>Unit of measure</b>	<b>Sample material</b>	<b>Males/Females</b>
Cumulative level of cytosine methylation at gene positions:			
ELOVL Fatty Acid Elongase 2 CpG 11,12,13,14	%	Blood cells	Both
ELOVL Fatty Acid Elongase 2 CpG 15,16,17	%	Blood cells	Both
FHL 2 CpG 11,12	%	Blood cells	Both
FHL 2 CpG 13,14,15	%	Blood cells	Both
FHL 2 CpG 16,17	%	Blood cells	Both
Dehydroepiandrosterone sulphate (DHEAS)	µmol/L	Plasma	Both
N-glycan peak 6	RFU	Plasma	Both
Alpha-2 macroglobulin (A2M)	mg/dL	Plasma	Only males
Lycopene	µmol/L	Plasma	Only males
Prostate specific antigen (PSA)	µg/L	Plasma	Only males
Ferritin	µg/L	Plasma	Only females
N-glycan log <sub>10</sub> (peak 1/peak 6)		Plasma	Only females
a-Tocopherol	µmol/L	Plasma	Only females

RFU: Relative fluorescence units

**Supplementary table 2:** Number and proportion of HIV-positive COBRA participants currently or ever receiving each ARV drug with median (IQR) cumulative exposure (months)

ARV drug	Currently receiving n (%)	Ever received n (%)	Cumulative exposure [months] Median (IQR)
Abacavir	18 (13.4%)	52 (38.8%)	50.4 (22.5, 79.2)
Didanosine		43 (32.1%)	43.2 (13.9, 73.0)
Lamivudine	31 (23.1%)	99 (73.9%)	97.0 (42.5, 140.4)
Stavudine		49 (36.6%)	44.8 (22.3, 75.5)
Tenofovir	97 (72.4%)	123 (91.8%)	61.1 (36.9, 91.2)
Zalcitabine		13 (9.7%)	18.0 (9.7, 24.0)
Zidovudine		86 (64.2%)	44.3 (19.7, 89.8)
Emtricitabine	85 (63.4%)	103 (76.9%)	52.6 (34.2, 68.5)
Any d-drug		60 (44.8%)	62.4 (38.1, 100.0)
<b>NRTI Total</b>	<b>123 (91.8%)</b>		<b>138.1 (77.4, 186.7)</b>
Nevirapine	31 (23.1%)	62 (46.3%)	67.2 (21.6, 141.7)
Efavirenz	30 (22.4%)	64 (47.8%)	44.2 (10.4, 106.9)
<b>NNRTI Total</b>	<b>70 (52.2%)</b>		<b>77.9 (25.4, 138.9)</b>
Darunavir	32 (23.9%)	43 (32.1%)	24.2 (4.4, 55.1)
Indinavir		34 (25.4%)	17.0 (11.4, 29.6)
Lopinavir		42 (31.3%)	37.1 (10.6, 98.0)
Boosting Ritonavir	66 (49.3%)	81 (60.5%)	65.2 (18.3, 104.4)
Saquinavir		37 (27.6%)	22.1 (13.3, 43.7)
Saquinavir + d-drug		26 (19.4%)	18.8 (13.8, 40.2)
Saquinavir + Ritonavir		26 (19.4%)	23.6 (13.7, 62.0)
Saquinavir without Ritonavir		27 (20.2%)	5.9 (0.1, 19.5)
Saquinavir + Other PI		5 (3.7%)	20.7 (12.1, 62.0)
Atazanavir	26 (19.4%)	47 (35.1%)	49.0 (11.7, 77.4)
Ritonavir		30 (22.4%)	16.9 (7.1, 30.4)
Nelfinavir		23 (17.2%)	26.0 (8.9, 34.2)
<b>PI Total</b>	<b>68 (50.8%)</b>		<b>65.7 (27.5, 120.2)</b>
Raltegravir	7 (5.2%)	15 (11.2%)	33.5 (16.5, 55.1)
<b>Integrase Inhibitors Total</b>	<b>7 (5.2%)</b>		<b>33.5 (16.5, 55.1)</b>

Note: d-drugs are Didanosine, Stavudine and Zalcitabine

**Supplementary table 3:** Distribution of the ten individual biomarkers of aging in COBRA participants and BD ( $p^1$ : HIV-positive vs HIV-negative COBRA participants;  $p^2$ : HIV-positive COBRA participants vs BD;  $p^3$ : HIV-negative COBRA participants vs BD – all  $p$ 's were obtained from Wilcoxon signed-rank tests)

Biomarker, median (IQR)	COBRA participants		$p^1$	BD (n=35)	$p^2$	$p^3$
	HIV-positive (n=134)	HIV-negative (n=79)				
Cumulative level of cytosine methylation at gene positions:						
ELOVL Fatty Acid Elongase 2 CpG 11,12,13,14 (%)	0.70 (0.67, 0.76)	0.67 (0.63, 0.71)	<0.001	0.62 (0.56, 0.64)	<0.001	<0.001
ELOVL Fatty Acid Elongase 2 CpG 15,16,17 (%)	0.63 (0.60, 0.68)	0.60 (0.57, 0.65)	<0.001	0.58 (0.52, 0.62)	<0.001	0.01
FHL 2 CpG 11,12 (%)	0.22 (0.19, 0.26)	0.21 (0.17, 0.24)	0.01	0.17 (0.15, 0.20)	<0.001	0.02
FHL 2 CpG 13,14,15 (%)	0.35 (0.30, 0.39)	0.32 (0.28, 0.37)	0.008	0.26 (0.24, 0.31)	<0.001	<0.001
FHL 2 CpG 16,17 (%)	0.54 (0.48, 0.59)	0.51 (0.46, 0.59)	0.26	0.42 (0.33, 0.50)	<0.001	<0.001
DHEAS ( $\mu\text{mol/L}$ )	2.54 (1.35, 3.88)	3.21 (2.07, 4.40)	0.03	2.70 (1.81, 3.80)	0.63	0.15
N-glycan peak 6 (RFU)	17.6 (16.0, 19.6)	18.0 (16.2, 20.2)	0.43	18.7 (17.5, 19.5)	0.04	0.18
A2M (mg/dL) $\sigma$	150.4 (123.0, 204.5)	134.6 (119.8, 154.5)	0.004	96.7 (78.0, 128.0)	<0.001	<0.001
Lycopene ( $\mu\text{mol/L}$ ) $\sigma$	0.62 (0.39, 1.00)	0.78 (0.52, 1.14)	0.09	0.89 (0.46, 1.10)	0.19	0.73
PSA ( $\mu\text{g/L}$ ) $\sigma$	0.91 (0.57, 1.36)	1.05 (0.63, 2.35)	0.04	0.80 (0.37, 1.50)	0.63	0.14
Ferritin ( $\mu\text{g/L}$ ) $\text{♀}$	31.7 (10.2, 46.7)	58.5 (25.0, 172.3)	0.33	52.3 (24.2, 79.2)	0.40	0.79
N-glycan $\log_{10}(\text{peak 1/peak 6})$ $\text{♀}$	-0.22 (-0.37, -0.10)	-0.27 (-0.47, -0.10)	0.60	-0.38 (-0.50, -0.23)	0.06	0.26
a-Tocopherol ( $\mu\text{mol/L}$ ) $\text{♀}$	37.1 (24.7, 38.9)	35.0 (28.2, 43.4)	0.95	34.2 (28.0, 39.1)	0.92	0.70

Note:  $\sigma$ : only in male individuals (n=124 in COBRA HIV-positive, n=73 in COBRA HIV-negative and n=18 in BD);  $\text{♀}$ : only in female individuals (n=9 in COBRA HIV-positive, n=6 in COBRA HIV-negative and n=17 in BD)

**Supplementary table 4:** Mean (95% CI) increase (if positive) or decrease (if negative) in age advancement among HIV-positive COBRA participants associated with prior exposure (yes vs no) and years of cumulative exposure to each ARV drug. Estimates were obtained with separate linear regression models (one for each factor considered); models assessing the association with cumulative exposure were based only on HIV-positive COBRA participants who ever received that ARV drug (numbers shown in Supplementary table 2)

Variable	Mean (95% CI) increase/decrease (in years)	p
Abacavir: prior exposure	0.43 (-2.96, 3.81)	0.80
cumulative exposure (per year)	-0.16 (-0.70, 0.37)	0.55
Didanosine: prior exposure	3.40 (-0.08, 6.89)	0.06
cumulative exposure (per year)	0.12 (-0.53, 0.78)	0.71
Lamivudine: prior exposure	2.75 (-0.98, 6.48)	0.15
cumulative exposure (per year)	-0.05 (-0.35, 0.25)	0.76
Stavudine: prior exposure	4.14 (0.79, 7.49)	0.02
cumulative exposure (per year)	0.74 (0.15, 1.34)	0.01
Tenofovir: prior exposure	2.33 (-3.66, 8.33)	0.44
cumulative exposure (per year)	0.40 (-0.12, 0.92)	0.13
Zalcitabine: prior exposure	6.12 (0.65, 11.60)	0.03
cumulative exposure (per year)	0.34 (-1.65, 2.34)	0.73
Zidovudine: prior exposure	1.39 (-2.04, 4.82)	0.42
cumulative exposure (per year)	-0.08 (-0.51, 0.35)	0.71
Emtricitabine: prior exposure	1.09 (-2.81, 5.00)	0.58
cumulative exposure (per year)	0.42 (-0.21, 1.06)	0.19
Any d-drug: prior exposure	3.57 (0.31, 6.83)	0.03
cumulative exposure (per year)	0.35 (-0.05, 0.76)	0.09
Nevirapine: prior exposure	-0.93 (-4.23, 2.38)	0.58
cumulative exposure (per year)	-0.16 (-0.49, 0.17)	0.35
Efavirenz: prior exposure	1.60 (-1.69, 4.89)	0.34
cumulative exposure (per year)	0.23 (-0.20, 0.66)	0.29
Darunavir: prior exposure	2.53 (-0.98, 6.03)	0.16
cumulative exposure (per year)	0.94 (0.04, 1.85)	0.04
Indinavir: prior exposure	1.41 (-2.37, 5.19)	0.46
cumulative exposure (per year)	-0.03 (-1.43, 1.38)	0.97
Lopinavir: prior exposure	1.68 (-1.87, 5.22)	0.35
cumulative exposure (per year)	0.39 (-0.15, 0.92)	0.15
Boosting Ritonavir: prior exposure	2.85 (-0.49, 6.18)	0.09
cumulative exposure (per year)	0.40 (0.01, 0.80)	0.05
Saquinavir: prior exposure	4.89 (1.29, 8.48)	0.008
cumulative exposure (per year)	1.39 (0.71, 2.07)	<0.001
Atazanavir: prior exposure	-2.40 (-5.83, 1.04)	0.17
cumulative exposure (per year)	-0.29 (-0.94, 0.36)	0.38
Ritonavir (non-boosting): prior exposure	1.35 (-2.60, 5.30)	0.50
cumulative exposure (per year)	0.72 (-0.06, 1.51)	0.07
Nelfinavir: prior exposure	1.71 (-2.65, 6.08)	0.44
cumulative exposure (per year)	1.52 (-0.09, 3.13)	0.06
Raltegravir: prior exposure	1.59 (-3.63, 6.82)	0.55
cumulative exposure (per year)	0.64 (-0.70, 1.97)	0.35

Note: d-drugs are Didanosine, Stavudine and Zalcitabine