SUPPLEMENTAL DIGITAL CONTENT

Material and methods

Measurement of telomere length (TL)

Total genomic DNA was extracted from either 6 DBS punches (3mm), or 0.1ml of peripheral or cord blood using QIAamp® DNA Mini Kit and a Qiacube (Qiagen). The relative average leukocyte TL was determined by qPCR as described [1,2] with the following modifications. The single copy nuclear gene coding for the accessory subunit of polymerase gamma (ASPG or POLG2) was used for nuclear DNA (S) copy number determination using primers ASPG3F (5'-GAGCTGTTGACGGAAAGGAG-3') and ASPG4R (5'-CAGAAGAGAATCCCGGCTAAG-3') at a final concentration of 1µM. The final primer concentrations for telomere (T) amplification were 0.3µM for tel 1b (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3') and 0.9µM for tel 2b (5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'). For both S and T PCR reactions, 8µL of LightCycler® 480 SYBR Green I master mix (ready-to-use hot-start reaction mix with $MgCl_2$, Roche) and 2µL of DNA extract were added to each well of a 96-well plate. Each sample was assayed in duplicate, in a random and blinded fashion. For both PCRs, thermal cycling began with a 10 min incubation at 95°C, followed by 45 cycles at 95°C/5s, 60°C/10s, 72°C/5s (for S), and 95°C/5s, 54°C/30s, and 72°C/1 min (for T). The ramping temperature rate for the annealing step was set at 2.2 °C/s for S PCR and 1.0 °C/s for T PCR. Standard curves were included in each run and prepared by serial dilutions (1:2) of pooled human blood genomic DNA, ranging from 30,000 to 469 copies of ASPG (S PCR) and 90 to 1.4 copies of telomere (T PCR) with DNA concentrations of 13.8ng/µL to 0.22ng/µL. LightCycler® 480 Software 1.5.0 SP4 was used to generate standard curves based on the maximum secondary derivative of each reaction and determine S and T copy numbers. The intra- and inter-assay coefficients of variation for S, T and T/S were 4%, 5%, and 7%, respectively. For a subset of 29 cord blood samples, lymphocyte median TL was independently determined by flow-fluorescence *in situ* hybridization (FISH) [3] and compared to the relative average leukocyte TL (TL) obtained by qPCR on the same frozen cord blood samples.

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Table S1: The study cohorts and their subjects, study sample collection schedule and ART exposure during the pregnancy

Cohort	**SJ**				**PR/CARMA**			
Infant blood	0-6 weeks				0-7 days			
Maternal blood	4 months before to 1 month after delivery				32-36 weeks of gestation			
Subjects	Infants		Mothers		Infants		Mothers	
Study sample	Heel prick DBS		Venous WB spotted into DBS		Heel prick WB		Venous WB	
HIV status	HEU		HIV^+		HEU	HIV-unexposed	HIV^+	HIV-
Pregnancy ART exposure	+	–	+	–	+	–	+	–
None	0	39	0	39	0	44	0	44
AZT only	45	0	45	0	0	0	0	0
AZT+3TC [a]	21	0	21	0	0	0	0	0
≥3 drugs ART	15	0	15	0	59	0	59	0

DBS, dried blood spot; WB, whole blood; HEU, HIV-exposed uninfected; ART, antiretroviral therapy. The number of subjects treated with each type of ART regimen is indicated at the bottom.

[a] One mother was treated with AZT + didanosine

Table S2. Correlations (Pearson's and Spearman's) between SJ maternal and infant peripheral blood (r (p value)).

	Pearson's R	**Spearman's R**
All subjects (n=120)	0.17 (0.057)	0.19 (0.040)
Unexposed (n=39)	0.15 (0.351)	0.11 (0.501)
Exposed (n=81)	0.18 (0.105)	0.22 (0.052)

Table S3. Correlations (Pearson's and Spearman's) between PR/CARMA maternal peripheral blood, infant peripheral blood, infant cord blood, and placenta (r (p value) (n)). Spearman's correlations are italicized on shaded background while Pearson's are on white background.

		Maternal	**Infant**	**Cord Blood**	**Placenta (maternal side)**	**Placenta (foetal side)**
All subjects	**Maternal**	---	0.44 (<0.0001) (n=99)	0.32 (0.004) (n=83)	0.32 (0.007) (n=73)	0.26 (0.027) (n=73)
	Infant	*0.46 (<0.0001) (n=99)*	---	0.47 (<0.0001) (n=83)	0.50 (<0.0001) (n=73)	0.47 (<0.0001) (n=73)
	Cord Blood	*0.32(0.003) (n=83)*	*0.54 (<0.0001) (n=83)*	---	0.31 (0.012) (n=66)	0.36 (0.003) (n=66)
	Placenta (maternal side)	*0.23 (0.052) (n=73)*	*0.55 (<0.0001) (n=73)*	*0.35 (0.004) (n=66)*	---	0.78 (<0.0001) (n=77)
	Placenta (foetal side)	*0.15 (0.201) (n=73)*	*0.49 (<0.0001) (n=73)*	*0.42 (0.0005) (n=66)*	*0.71 (<0.0001) (n=77)*	---
Unexposed	**Maternal**	**---**	0.47 (0.002) (n=43)	0.34 (0.051) (n=34)	0.25 (0.141) (n=35)	0.27 (0.116) (n=35)
	Infant	*0.44 (0.004) (n=43)*	---	0.41 (0.017) (n=34)	0.42 (0.011) (n=35)	0.43 (0.010) (n=35)
	Cord Blood	*0.42 (0.013) (n=34)*	*0.57 (0.001) (n=34)*	---	0.24 (0.202) (n=30)	0.31 (0.097) (n=30)
	Placenta (maternal side)	*0.06 (0.741) (n=35)*	*0.46 (0.005) (n=35)*	*0.29 (0.115) (n=30)*	---	0.82 (<0.0001) (n=36)
	Placenta (foetal side)	*0.17 (0.316) (n=35)*	*0.50 (0.002) (n=35)*	*0.42 (0.022) (n=30)*	*0.77 (<0.0001) (n=36)*	---
Exposed	**Maternal**	---	0.41 (0.002) (n=56)	0.27 (0.065) (n=49)	0.35 (0.029) (n=38)	0.23 (0.159) (n=38)
	Infant	*0.49 (0.0002) (n=56)*	---	0.49 (0.0004) (n=49)	0.60 (<0.0001) (n=38)	0.53 (0.001) (n=38)
	Cord Blood	*0.26 (0.077) (n=49)*	*0.53 (0.0001) (n=49)*	---	0.38 (0.021) (n=36)	0.46 (0.005) (n=36)
	Placenta (maternal side)	*0.37 (0.023) (n=38)*	*0.59 (0.0001) (n=38)*	*0.38 (0.024) (n=36)*	---	0.74 (<0.0001) (n=41)
	Placenta (foetal side)	*0.12 (0.478) (n=38)*	*0.42 (0.009) (n=38)*	*0.48 (0.003) (n=36)*	*0.64 (<0.0001) (n=41)*	---

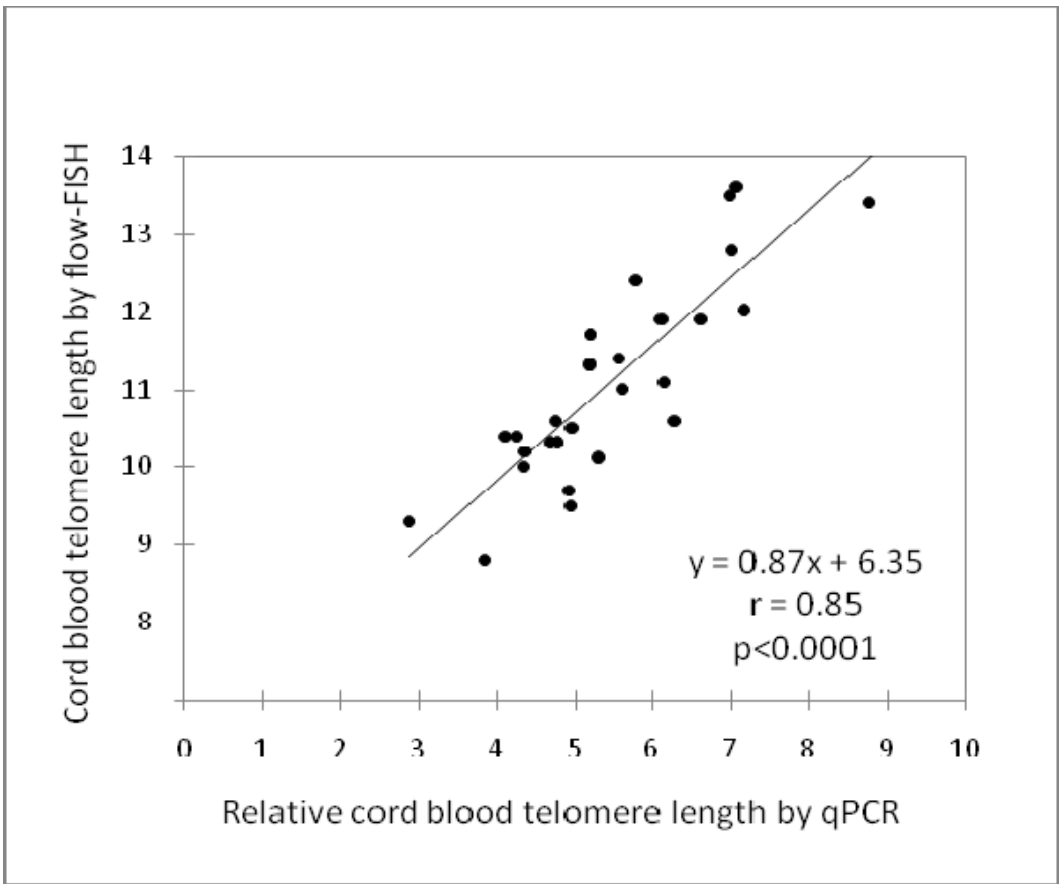

Figure S1. Pearson's correlation between PR/CARMA cohort infant umbilical cord blood median lymphocyte TL measured by flow-FISH and the relative average leukocyte TL measured by qPCR on the same sample (n=29).

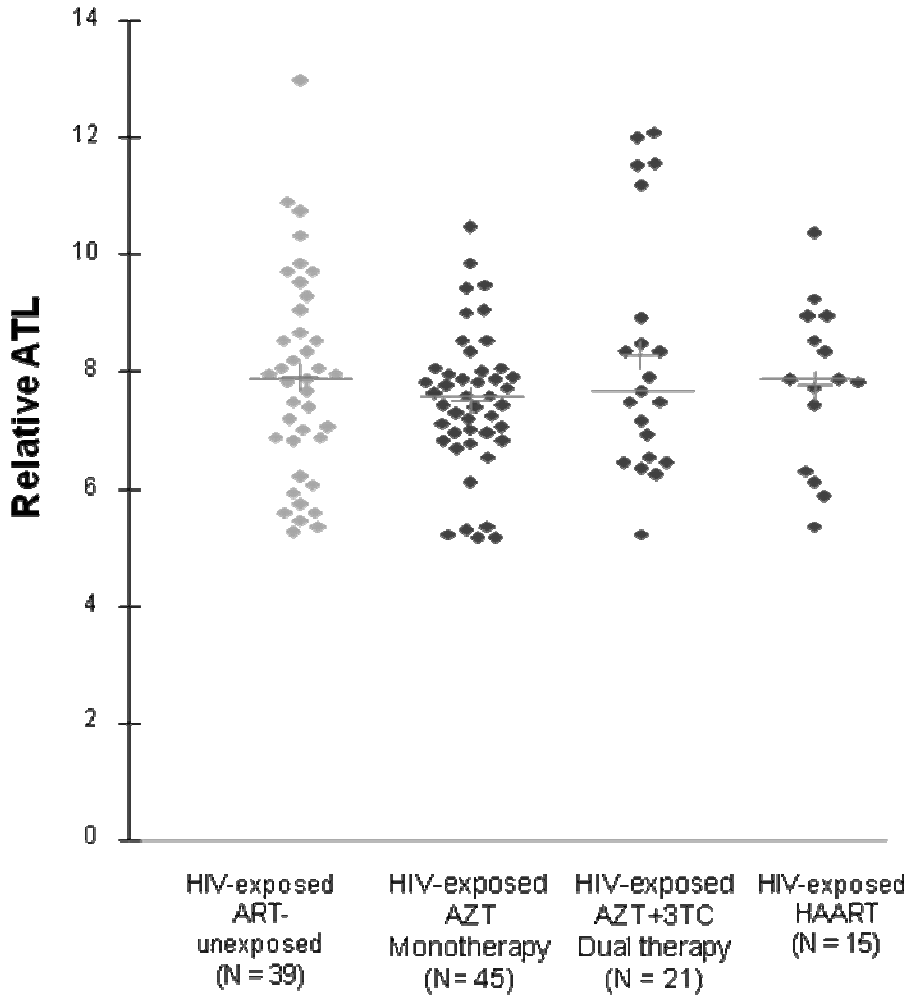

Figure S2. **Scatter plot of peripheral blood leukocyte telomere length in infants and mothers in the SJ cohort exposed to different maternal ART regimens in pregnancy.** The horizontal bars represent the median and the crosses the mean.

Supplemental References

1. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* May 15 2002;30(10):e47.

2. Gil ME, Coetzer TL. Real-time quantitative PCR of telomere length. *Mol Biotechnol.* Jun 2004;27(2):169-172.

3. Baerlocher GM, Vulto I, de Jong G, Lansdorp PM. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). *Nat Protoc.* 2006;1(5):2365-2376.