Supplementary Information

Mitochondrial aging is accelerated by anti-retroviral therapy through the clonal expansion of mtDNA mutations

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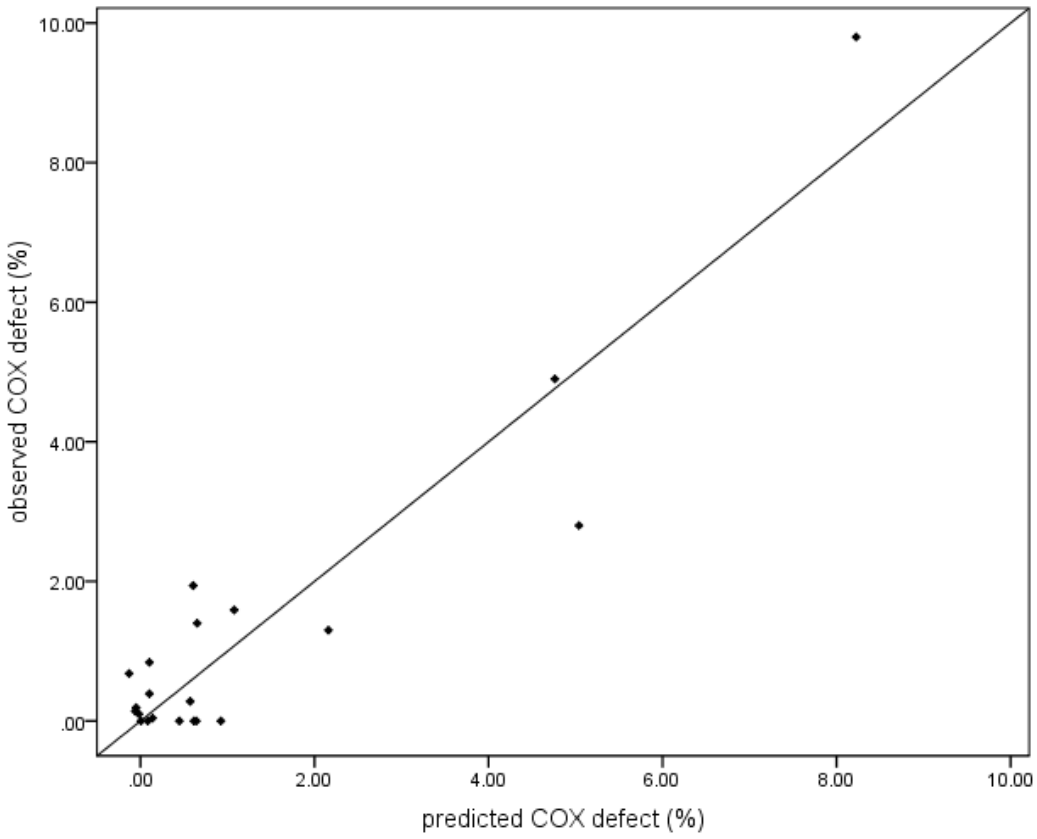
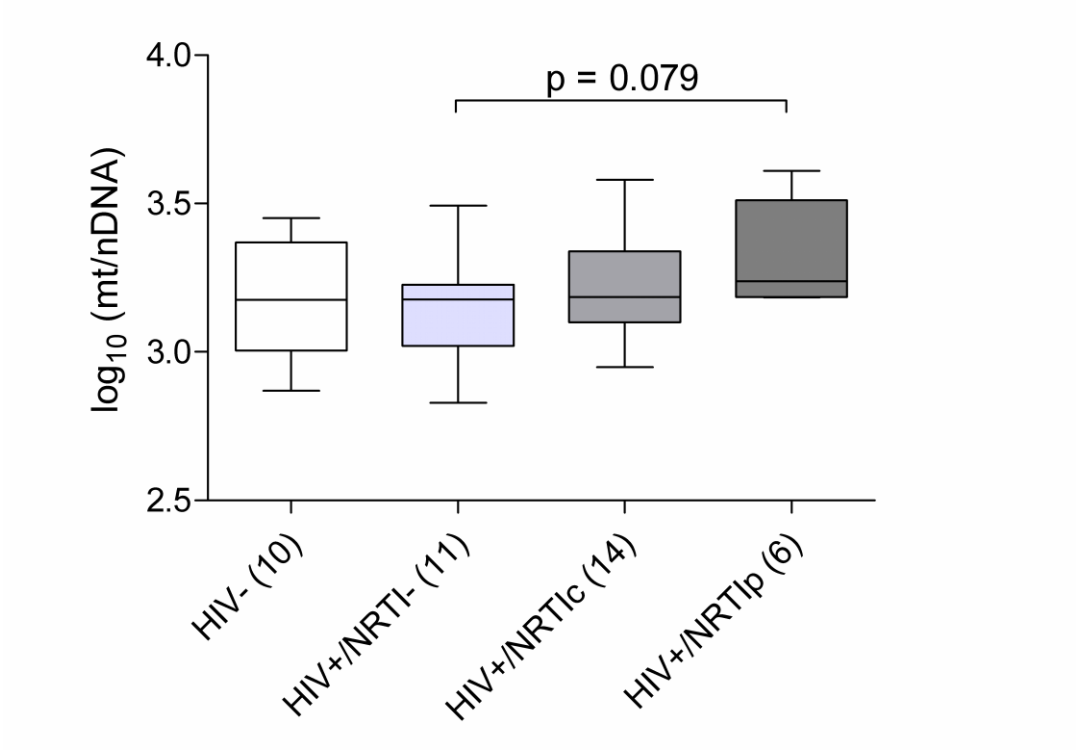
Supplementary Figure 1. Predicted COX defect according to cumulative NRTI exposure



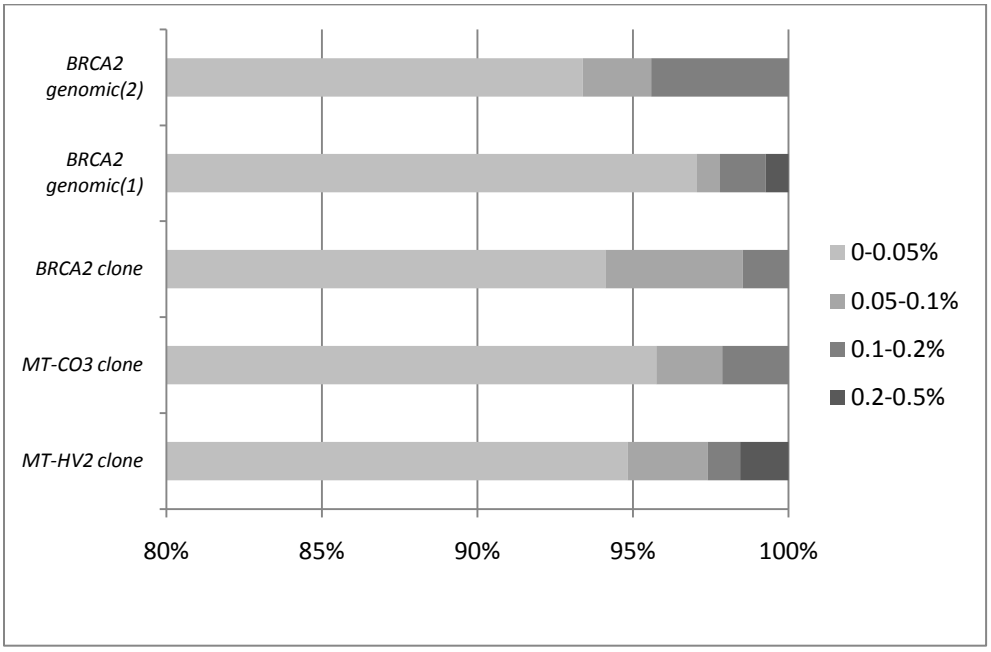
Supplementary Figure 1. Multivariate linear regression model of predicted percentage COX (cytochrome c oxidase) defects in skeletal muscle fibers from HIV-infected subjects, according to cumulative exposure to specific nucleoside analog anti-retroviral drugs (NRTIs). The inclusion in the model of cumulative (lifetime) exposure to those NRTIs implicated in perturbation of mtDNA replication (ddI, didanosine; ddC, zalcitabine; AZT, zidovudine; d4T, stavudine) was sufficient to explain 87% of the observed variation in COX defects (R = 0.93). Equation of regression line = $A + T_{ddI}\ B_{ddI} + T_{ddC}\ B_{ddC} + T_{AZT}\ B_{AZT} + T_{d4T}\ B_{d4T}$. T, duration of exposure (months). Coefficients: constant (A) = -0.459 ± 0.530; B_{ddI} = 0.107 ± 0.017; B_{ddC} = 0.093 ± 0.023; B_{AZT} = 0.011 ± 0.007; B_{d4T} = 0.017 ± 0.013. ddI ($p < 0.001$) and ddC ($p = 0.001$) were independently significantly associated with the proportion of COX deficient fibers. The p value for the model fit to the data is < 0.001.

Supplementary Figure 2. MtDNA content in homogenized skeletal muscle



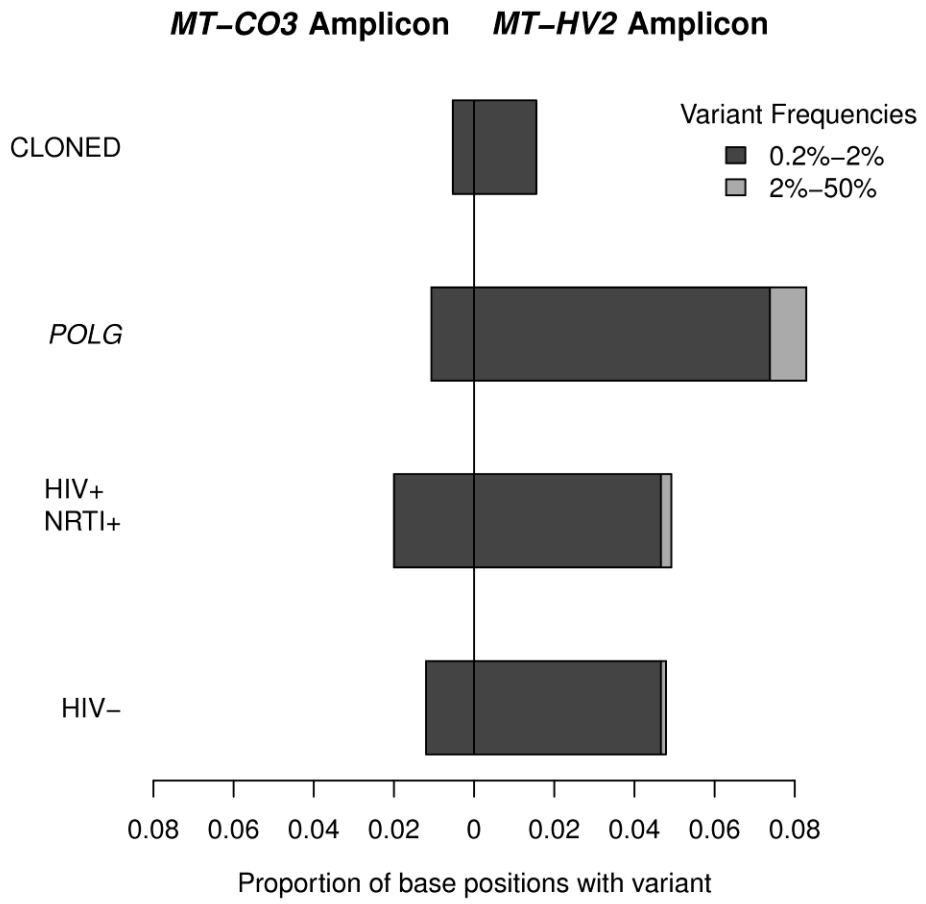
Supplementary Figure 2. Relative mitochondrial DNA (mean $\log_{10}$ (mt/nDNA) ±SEM) content in homogenized skeletal muscle. HIV-, uninfected controls (n=10; 3.17 ±0.06); HIV+/NRTI-, treatment-naïve (n=11; 3.15 ±0.05); HIV+/NRTIc, currently treated with relevant nucleoside analog drug (n=14, all with AZT (zidovudine) exposure only; 3.22 ±0.05); HIV+/NRTIp, *prior* (but not current) exposure to relevant NRTIs (n=6, with prior AZT, d4T (stavudine), ddI (didanosine) and / or ddC (zalcitabine) exposure; 3.31 ±0.07). There was therefore no evidence of persistent mtDNA depletion in HIV+/NRTIp subjects, in fact previously treated patients showed a trend towards higher mean mtDNA content compared with HIV+/NRTI- (p = 0.079).

Supplementary Figure 3. Determination of experimental noise in UDS assay



Supplementary Figure 3. Demonstration of low level of background noise in ultra-deep re-sequencing-by-synthesis (UDS, Roche 454 GS FLX) assay by study of autosomal genomic DNA (*BRCA2* amplicon) and cloned DNA (mtDNA hypervariable segment 2, *MT-HV2*; mtDNA COX subunit 3, *MT-CO3*; and *BRCA2*). Very few base positions (0.5% of total) demonstrate >0.2% variance frequency on UDS assay, confirming low intrinsic background noise, as well as no systematic difference in noise between autosomal and mtDNA amplicons.

Supplementary Figure 4. UDS replication experiment



Supplementary Figure 4. Ultra-deep re-sequencing-by-synthesis (UDS) replication experiment (Roche 454 GS FLX Titanium). Comparison of point mutation burden (>0.2% variant frequency) in skeletal muscle DNA extract from HIV-uninfected controls (HIV-), NRTI-treated HIV-infected (HIV+/NRTI+) subjects and patients with inherited defects of *POLG* (n=4 each) as well as cloned DNA. Two amplicons were located in mtDNA hypervariable segment 2 (*MT-HV2*) and mtDNA COX subunit 3 (*MT-CO3*). Subjects with inherited *POLG* defects show increased burden of low-level mutations compared with healthy controls in *MT-HV2* (OR 2.00, p =0.001), whereas HIV+/NRTI+ subjects do not.

Supplementary Table 1. Subject demographic and treatment details.

NRTI, history of nucleoside analogue reverse transcriptase inhibitor exposure; ART, anti-retroviral therapy; LDS, anti-retroviral-associated lipodystrophy syndrome. Polymerase γ inhibiting NRTIs: AZT, zidovudine; d4T, stavudine; ddI, didanosine; ddC, zalcitabine. Other ART: 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; TDF, tenofovir; NVP, nevirapine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir (therapeutic dosing); /r, ritonavir (pharmacokinetic boosting dosing); ATV, atazanavir; LPV, lopinavir; RAL, raltegravir. COX defect, proportion of cytochrome *c* oxidase deficient skeletal muscle fibers.

subject	HIV	NRTI	gender	age (years)	time since diagnosis (months)	current CD4 lymphocyte count (cells/μl)	nadir CD4 lymphocyte count (cells/μl)	current HIV viral load (copies/ml)	duration of ART (months)	poi y inhibitor NRTI (months)	current ART	lifetime ART	clinical LDS	clinical morbidity	COX defect
1	+	+	F	31	44	1048	218	<40	42	42	AZT 3TC NVP	AZT 3TC NVP	+		0.00%
2	+	+	F	46	38	566	8	<40	37	37	AZT 3TC EFV	AZT 3TC EFV	-		0.19%
3	+	+	M	38	138	682	150	<40	100	100	AZT 3TC NVP	AZT 3TC NVP	-		0.00%
4	+	+	F	31	49	630	168	<40	36	36	AZT 3TC EFV	AZT 3TC EFV	-		0.14%
5	+	+	M	32	95	553	197	<40	94	94	AZT 3TC EFV	AZT 3TC EFV	+		0.28%
6	+	+	F	30	51	532	83	<40	49	49	AZT 3TC EFV	AZT 3TC EFV	-		0.00%
7	+	+	M	44	52	619	259	<40	51	51	AZT 3TC EFV	AZT 3TC EFV	+		0.39%
8	+	+	F	48	32	253	17	<40	30	30	AZT 3TC EFV	AZT 3TC EFV	+		0.68%
9	+	+	M	35	83	585	4	<40	82	82	AZT 3TC EFV	AZT 3TC EFV	+		0.00%
10	+	+	F	33	54	397	114	<40	54	54	AZT 3TC NVP	AZT 3TC NVP	-		0.04%
11	+	+	M	49	98	588	107	<40	97	97	AZT 3TC EFV	AZT 3TC IDV EFV	+		1.94%
12	+	+	M	36	140	764	N/A	<40	140	140	AZT 3TC IDV	AZT 3TC IDV	-		1.6%
13	+	+	M	33	98	579	18	<40	98	98	AZT 3TC EFV	AZT 3TC EFV	-		0.0%
14	+	+	M	42	127	539	176	<40	126	126	AZT 3TC NVP	AZT 3TC IDV NVP	-		0.0%
15	+	+	M	43	119	1239	250	<40	118	101	TDF FTC EFV	AZT 3TC IDV EFV ABC TDF FTC	-		1.4%
16	+	+	M	45	165	592	305	<40	146	128	RAL ABC ATV/r	d4T 3TC NVP ddI IDV ABC ATV/r RAL	+		9.8%
17	+	+	M	48	158	872	10	<40	151	58	TDF ABC NVP	AZT ddI d4T 3TC RTV NVP IDV ddC ABC ATV/r TDF	-	fatigue	4.9%
18	+	+	M	40	54	309	152	<40	54	51	TDF FTC EFV	AZT 3TC EFV TDF FTC	-	LVF	0.8%
19	+	+	F	45	71	537	10	<40	70	40	TDF FTC NVP	AZT 3TC EFV TDF FTC NVP	-		0.1%
20	+	+	M	49	193	762	120	<40	193	122	TDF FTC ATV/r	AZT ddC ddI 3TC d4T SQV NVP IDV NFV ABC TDF LPV/r FTC ATV/r	+	LVF, CVD, fatigue	1.3%
21	+	+	M	50	140	669	0	<40	138	75	TDF FTC NVP	AZT d4T IDV NFV SQV 3TC NVP ddI TDF FTC	+	fatigue	2.8%
22	+	-	M	25	32	217	197	1250	-	-	-	-	-		0.0%
23	+	-	M	49	227	223	199	18900	-	-	-	-	-		0.3%
24	+	-	M	46	110	387	328	17000	-	-	-	-	-		0.5%
25	+	-	F	45	99	214	214	1050	-	-	-	-	-		0.2%
26	+	-	F	50	120	1358	541	20	-	-	-	-	-		0.0%
27	+	-	F	32	44	626	522	41600	-	-	-	-	-		0.1%
28	+	-	M	27	45	391	283	34300	-	-	-	-	-		0.1%
29	+	-	M	34	31	422	389	4700	-	-	-	-	-		0.1%
30	+	-	F	32	27	380	380	13900	-	-	-	-	-		0.0%
31	+	-	M	27	37	633	438	12700	-	-	-	-	-		0.0%
32	+	-	M	37	64	1165	1033	4300	-	-	-	-	-		0.0%
33	+	-	M	44	14	332	332	23600	-	-	-	-	-		0.0%
34	-	-	M	35	-	-	-	-	-	-	-	-	-		0.0%
35	-	-	F	31	-	-	-	-	-	-	-	-	-		0.0%
36	-	-	M	26	-	-	-	-	-	-	-	-	-		0.0%
37	-	-	M	47	-	-	-	-	-	-	-	-	-		0.3%
38	-	-	M	36	-	-	-	-	-	-	-	-	-		0.2%
39	-	-	F	42	-	-	-	-	-	-	-	-	-		0.2%
40	-	-	M	24	-	-	-	-	-	-	-	-	-		0.0%
41	-	-	M	21	-	-	-	-	-	-	-	-	-		0.0%
42	-	-	F	20	-	-	-	-	-	-	-	-	-		0.0%
43	-	-	M	52	-	-	-	-	-	-	-	-	-		0.2%

Supplementary Table 2. MtDNA deletion break-points in single muscle fibers

Sequence break-points for large scale mitochondrial DNA (mtDNA) deletions identified in individual COX (cytochrome c oxidase) deficient skeletal muscle fibers from nucleoside analog (NRTI) treated, HIV-infected subjects. Deletion shown as nucleotide positions (rCRS) and size of sequence overlap (where present). Bracketed nucleotides, sequence overlap (where repeat appears once only in the deleted molecule); bold italic nucleotides, partial mismatch in overlap sequences.

Subject	Deletion	Size	Flanking sequence
18	8145-8-14379	6243bp	AAACCACTTT(CA***C***CGCTA)-(CA***T***CGCTA)ACCCCACTAAAA
18	8246---14603	6358bp	AAAAATCTTTGA-AGGCTTAGAAGAAAA
18	9924-2-16070	6149bp	CGAAGCCGCCG(CC)-(CC)CATCAACAACCG
18	8718---14298	5581bp	TACACAACACTAA-TATTCAGCTTCCTA
18	8483-13-13446	4977bp	AAACTACCACCT(ACCTCCCTCACCA)-(ACCTCCCTCACCA)TTGGCAGCCTAGCA
18	9011-12-14931	5933bp	AGCCCTGGCCGT(ACGCCTAACCGC)-(ACGCCT***C***AACCGC)CTTTTCATCAATC
18	7376---13406	6031bp	AACCCTCCATAAA-AAAAATAGGAGGACT
20	7106-11-12082	4988bp	ATTCACTGATT(TCCCC***T***ATTCT)-(TCCCC***C***ATTCT)CCTCCTATCCC
20	7960-12-14481	6534bp	TTCAACTCCTA(CAT***AC***TTCCCCC)-(CAT***CA***TTCCCCC)TAAATAAATTAAAAAAA
15	7129-14-13991	6877bp	TCAGGCTACAC(CCTAGACC***A***AACCT)-(CCTAGACC***T***AACCT)GACTAGAAAA
15	8035-11-11422	3399bp	TACTCCCGATT(GAAGCCCCCAT)-(GAAGCCCCCAT)CGCTGGGTCAATA
12	8483-13-13446	4977bp	AAACTACCACCT(ACCTCCCTCACCA)-(ACCTCCCTCACCA)TTGGCAGCCTAGCA
12	6942---14816	7875bp	AGGATTCATCTTTC-CATCCAACATCTCC
12	6071---12499	6429bp	CCACATCTACAACGTT-TGTGCCTAGACCAAGAA
12	8936---16070	7135bp	CACCTACACCCC-CCCATCAACAACC

Supplementary Table 3. UDS (Roche 454 FLX GS) outputs (separate file).
Variance and read depth detected at individual base positions in mtDNA hypervariable segment 2 (*MT-HV2*), COX subunit 3 (*MT-CO3*) and autosomal (*BRCA2*) amplicons, for skeletal muscle DNA extracts from HIV-infected NRTI-treated subjects (HIV+/NRTI+, n=8), HIV-infected untreated subjects (HIV+/NRTI-, n=4), HIV-uninfected healthy controls (HIV-, n=4), subjects with *POLG* defects (*POLG*, n=4) and cloned DNA.

Raw 454 Flowgram output is available from the authors.

Supplementary Table 4. Primers.

All mitochondrial nucleotide positions refer to revised Cambridge Reference Sequence (rCRS, NC_012920).

Long-range PCR from single skeletal muscle fibers

Primary PCR: forward primer, nt5855-5875 (AGATTTACAGTCCAATGCTTC);
reverse primer, nt129-110 (AGATACTGCGACATAGGGTG).

Secondary PCR: forward primer, nt6358-6377 (TAGCAGGTGTCTCCTCTATC);
reverse primer, nt20-1 (AGGGTGATAGACCTGTGATC).

Real-time PCR from skeletal muscle homogenate and single fibers

B2M (nuclear)*:* forward primer, nt9145-9166 (CACTGAAAAAGATGAGTATGCC);
reverse primer, nt9375-9357 (AACATTCCCTGACAATCCC).

MT-ND1: forward primer, nt3458-3481 (ACGCCATAAAACTCTTCACCAAAG);
reverse primer, nt3569-3546 (GGGTTCATAGTAGAAGAGCGATGG).

MT-ND4: forward primer, nt11144-11165 (ACCTTGGCTATCATCACCCGAT);
reverse primer, nt11250-11230 (AGTGCGATGAGTAGGGGAAGG).

CD: forward primer, nt8393-8414 (CCCACCATAATTACCCCCATAC)
reverse primer, nt13509-13486 (GGAGTAGAAACCTGTGAGGAAAGG)

Whole mtDNA genome sequencing from single skeletal muscle fibers

Primary PCR	nt
AF GCTCACATCACCCCATAAAC	627-646
AR CTCGTCTTGCTGTGTTATGC	2721-2702
BF ACCAACAAGTCATTATTACCC	2395-2415
BR ATACTTGATGGCAGCTTCTG	4646-4627
CF GTCAGCTAAATAAGCTATCGG	4408-4428
CR GGACGGATCAGACGAAGAG	6468-6450
DF AATACCCATCATAATCGGAGG	6113-6133
DR GGTGATGAGGAATAGTGTAAG	8437-8417
EF TCAATGCTCTGAAATCTGTGG	8167-8187
ER TCGAAGCCGCACTCGTAAG	10183-10165
FF CTATTGATGAGGGTCTTACTC	9974-9994
FR GAGCTTTCTCGGTAAATAAGG	12216-12196
GF CTGTGCTAGTAACCACGTTC	11898-11917
GR GGTAGAATCCGAGTATGTTGG	13924-13904
HF TATTCGCAGGATTTCTCATTAC	13721-13742
HR GTGCTAATGGTGGAGTTAAAG	15989-15969
IF CCCATCCTCCATATATCCAAAC	15659-15680
IR TCACTGCTGTTTCCCGTGG	823 -805

Secondary PCR

Forward primers with M13 tag for cycle sequencing (TGTAAAACGACGGCCAGT)

1F TGTAAAACGACGGCCAGTTCACCCTCTAAATCACCACG	721-740
2F TGTAAAACGACGGCCAGTTTAAAACTCAAAGGACCTGGC	1157-1177
3F TGTAAAACGACGGCCAGTAACTTAACTTGACCGCTCTGAG	1650-1671
4F TGTAAAACGACGGCCAGTACTGTTAGTCCAAAGAGGAAC	2091-2111
5F TGTAAAACGACGGCCAGTCAGTGACACATGTTTAACGGC	2549-2569
6F TGTAAAACGACGGCCAGTCAGCCGCTATTAAAGGTTCG	3017-3036
7F TGTAAAACGACGGCCAGTACCATCACCCTCTACATCAC	3505-3524
8F TGTAAAACGACGGCCAGTTCGCCCTATTCTTCATAGCC	3965-3984
9F TGTAAAACGACGGCCAGTACACTCATCACAGCGCTAAG	4518-4537
10F TGTAAAACGACGGCCAGTCTCACTCTCTCAATCTTATCC	4932-4952
11F TGTAAAACGACGGCCAGTACCTCAATCACACTACTCCC	5367-5386
12F TGTAAAACGACGGCCAGTAGATTTACAGTCCAATGCTTC	5855-5875
13F TGTAAAACGACGGCCAGTTAGCAGGTGTCTCCTCTATC	6358-6377
14F TGTAAAACGACGGCCAGTATTTAGCTGACTCGCCACAC	6863-6882
15F TGTAAAACGACGGCCAGTGGCTCATTCATTTCTCTAACAG	7272-7293
16F TGTAAAACGACGGCCAGTTCCTAACACTCACAACAAAAC	7713-7723
17F TGTAAAACGACGGCCAGTACAGTTTCATGCCCATCGTC	8196-8215
18F TGTAAAACGACGGCCAGTACCACCCAACAATGACTAATC	8656-8676
19F TGTAAAACGACGGCCAGTATCCTAGAAATCGCTGTCGC	9127-9146
20F TGTAAAACGACGGCCAGTCATCCGTATTACTCGCATCAG	9607-9627
21F TGTAAAACGACGGCCAGTCAACACCCTCCTAGCCTTAC	10085-10104
22F TGTAAAACGACGGCCAGTATCGCTCACACCTCATATCC	10534-10553
23F TGTAAAACGACGGCCAGTTATCCAGTGAACCACTATCAC	11010-11030
24F TGTAAAACGACGGCCAGTTCCTTGTACTATCCCTATGAG	11541-11561
25F TGTAAAACGACGGCCAGTCTCCCTCTACATATTTACCAC	11977-11997
26F TGTAAAACGACGGCCAGTCTCTTCCCCACAACAATATTC	12478-12498
27F TGTAAAACGACGGCCAGTGCCCTTCTAAACGCTAATCC	12940-12959
28F TGTAAAACGACGGCCAGTCGGGTCCATCATCCACAAC	13365-13383
29F TGTAAAACGACGGCCAGTACCTAAAACTCACAGCCCTC	13790-13809
30F TGTAAAACGACGGCCAGTATTAAAGTTTACCACAACCACC	14317-14341
31F TGTAAAACGACGGCCAGTATTCATCGACCTCCCCACC	14797-14815
32F TGTAAAACGACGGCCAGTCATCTTGCCCTTCATTATTGC	15295-15315
D1F TGTAAAACGACGGCCAGTATCGGAGGACAACCAGTAAG	15758-15777
D2F TGTAAAACGACGGCCAGTCTCAACTATCACACATCAACTG	16223-16244
D3F TGTAAAACGACGGCCAGTCCTTAAATAAGACATCACGATG	16548-16569
D4F TGTAAAACGACGGCCAGTGCCACAGCACTTAAACACATC	323-343

Reverse primers with M13 tag (CAGGAAACAGCTATGACC)

1R CAGGAAACAGCTATGACCGATGGCGGTATATAGGCTGAG	1268-1248
2R CAGGAAACAGCTATGACCCTGGTAGTAAGGTGGAGTGGG	1709-1689
3R CAGGAAACAGCTATGACCATTGGTGGCTGCTTTTAGG	2193-2175
4R CAGGAAACAGCTATGACCTCGTGGAGCCATTCATACAG	2644-2625
5R CAGGAAACAGCTATGACCGATTACTCCGGTCTGAACTC	3087-3068
6R CAGGAAACAGCTATGACCGGAGGGGGGTTCATAGTAG	3374- 3356

7R CAGGAAACAGCTATGACCAGAGTGCGTCATATGTTGTTC 4057-4037
8R CAGGAAACAGCTATGACCGTTTATTTCTAGGCCTACTCAG 4577-4556
9R CAGGAAACAGCTATGACCGATTTTGCGTAGCTGGGTTTG 5003-4983
10R CAGGAAACAGCTATGACCTGTAGGAGTAGCGTGGTAAGG 5481-5462
11R CAGGAAACAGCTATGACCTAGTCAACGGTCGGCGAAC 5924-5906
12R CAGGAAACAGCTATGACCATGGCAGGGGGTTTTATATTG 6430-6410
13R CAGGAAACAGCTATGACCAAGAAAGATGAATCCTAGGGC 6944-6924
14R CAGGAAACAGCTATGACCCATCCATATAGTCACTCCAGG 7396-7376
15R CAGGAAACAGCTATGACCGGCAGGATAGTTCAGACGG 7791-7773
16R CAGGAAACAGCTATGACCTACAGTGGGCTCTAGAGGG 8301-8283
17R CAGGAAACAGCTATGACCGTATAAGAGATCAGGTTCGTC 8740-8720
18R CAGGAAACAGCTATGACCGTTGTCGTGCAGGTAGAGG 9201-9183
19R CAGGAAACAGCTATGACCATTAGACTATGGTGAGCTCAG 9661-9641
20R CAGGAAACAGCTATGACCTAGCCGTTGAGTTGTGGTAG 10147-10128
21R CAGGAAACAGCTATGACCAGGCACAATATTGGCTAAGAG 10649-10629
22R CAGGAAACAGCTATGACCATGATTAGTTCTGTGGCTGTG 11109-11089
23R CAGGAAACAGCTATGACCTAGGTCTGTTTGTCGTAGGC 11605-11586
24R CAGGAAACAGCTATGACCCGTGTGAATGAGGGTTTTATG 12054-12034
25R CAGGAAACAGCTATGACCGTGGCTCAGTGTCAGTTCG 12545-12527
26R CAGGAAACAGCTATGACCCTGATTTGCCTGCTGCTGC 13009-12991
27R CAGGAAACAGCTATGACCGGGAGGTTGAAGTGAGAGG 13453-13435
28R CAGGAAACAGCTATGACCGTTAGGTAGTTGAGGTCTAGG 13859-13839
29R CAGGAAACAGCTATGACCAGGATTGGTGCTGTGGGTG 14374-14356
30R CAGGAAACAGCTATGACCAAGGAGTGAGCCGAAGTTTC 14857-14838
31R CAGGAAACAGCTATGACCGGTTGTTTGATCCCGTTTCG 15368-15349
32R CAGGAAACAGCTATGACCTACAAGGACAGGCCCATTTG 15896-15877
D1R CAGGAAACAGCTATGACCAGGGTGATAGACCTGTGATC 19-1
D2R CAGGAAACAGCTATGACCAGATACTGCGACATAGGGTG 129-110
D3R CAGGAAACAGCTATGACCCTGGTTAGGCTGGTGTTAGG 389-370
D4R CAGGAAACAGCTATGACCTGCTGCGTGCTTGATGCTTG 771-752

Amplicon generation for ultra-deep sequencing-by-synthesis (454 GS FLX)

Primers comprised a sequence-specific segment, a barcode, and a fusion primer segment.

Sequence-specific segments

MT-HV2: forward, nt162-184 (CGCACCTACGTTCAATATTACAG)
reverse, nt455-434 (AAAATAATGTGTTAGTTGGGGG)

MT-CO3: forward, nt9307-9329 (GATTTCACTTCCACTCCATAACG)
reverse, nt9591-9572 (CTTCTAGGGGATTTAGCGGG)

Fusion primer segments

Forward: GCCTCCCTCGCGCCATCAG
Reverse: GCCTTGCCAGCCCGCTCAG

Barcode segments

1	AAGGAAGGT	15	TTACGTCCT
2	TTAAGGACT	16	ACTTAAGGT
3	TAAGGCCGT	17	TTACTTACT
4	TTAAGTAAT	18	TACTTCCGT
5	TAAGTACGT	19	TCCGGAAGT
6	AAGTCCGGT	20	CCGGACGGT
7	TAATTAAGT	21	TTCCGGCCT
8	AATTACGGT	22	CCGTAAGGT
9	TTAATTACT	23	TTCCGTACT
10	TTACGGAAT	24	TCCGTCCGT
11	TACGGACGT	25	TTCCTTAAT
12	ACGGCCGGT	26	TCCTTACGT
13	TACGTAAGT	27	CCTTCCGGT
14	ACGTACGGT		

Amplicon generation for ultra-deep sequencing-by-synthesis (454 GS FLX Titanium)

Primers comprised a sequence-specific segment, a barcode, and a fusion primer segment.

Sequence-specific segments

HVS2:	forward, nt.109-130 (GCACCCTATGTCGCAGTATCTG)
	reverse, nt.483-458 (GAGATTAGTAGTATGGGAGTGGGAGG)
CO3:	forward, nt.9304-9329 (TGTGATTTCACTTCCACTCCATAACG)
	reverse, nt.9653-9629 (ATGGTGAGCTCAGGTGATTGATACT)

Barcode segments

1	ACGAGTGCGT
2	ACGCTCGACA
3	AGACGCACTC
4	AGCACTGTAG

Fusion primer segments

Forward:	CGTATCGCCTCCCTCGCGCCATCAG
Reverse:	CTATGCGCCTTGCCAGCCCGCTCAG

Supplementary Note.

Clinical Data

All subjects were aged 50 years or below as no COX defect would be expected in skeletal muscle of healthy individuals in this age group [4]. All subjects gave informed consent to participation in the study. HIV-infected subjects were classified based on cumulative (lifetime) anti-retroviral drug exposure as HIV-infected, treatment naïve (HIV+/NRTI-) or as nucleoside analogue exposed (HIV+/NRTI+). We predicted that those NRTIs documented to disrupt mtDNA replication through pol γ inhibition may affect somatic mtDNA mutation [9,36]. Therefore all HIV+/NRTI+ subjects studied had history of exposure to at least one of the following NRTIs: zidovudine (AZT), stavudine (d4T), didanosine (ddI) and / or zalcitabine (ddC). Full subject treatment histories are presented in **Supplementary Table 1**. HIV-infected subjects were unselected with respect to the presence or absence of clinical complications of anti-retroviral therapy. Subjects with history of (non-HIV-related) neuromuscular disease, diabetes mellitus or chronic viral hepatitis were specifically excluded.

Lower limb skeletal muscle biopsies from HIV-infected subjects were obtained under local anesthesia. Open biopsies were obtained from HIV-uninfected control subjects (HIV-) at the time of elective orthopedic surgery. Samples were snap-frozen in the liquid phase of isopentane, cooled by liquid nitrogen, within 20 minutes of sampling.

Four patients with inherited defects of *POLG* were used as positive controls for UDS assay. Two of these patients (both 56 year old females) carried a compound heterozygous mutation, R627Q / W748S; one patient (17 year old male) carried compound heterozygous mutation, R627Q / R1096H; and one patient (45 year old female) carried homozygous A467T. All *POLG* patients showed minimal histochemical COX defects.

Supplementary references

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