Supplemental Digital Content 1. Primers and PCR conditions

cDNA synthesis:
Gene specific primers were used to generate cDNA from cell-associated and plasma HIV-1 RNA. For env we used the E115 reverse primer. For p24-RT we used the PCR 1 reverse primer. Both primers are listed in the table below. PCR conditions were as follows: 45 °C for 50 minutes, 85 °C for 10 minutes.

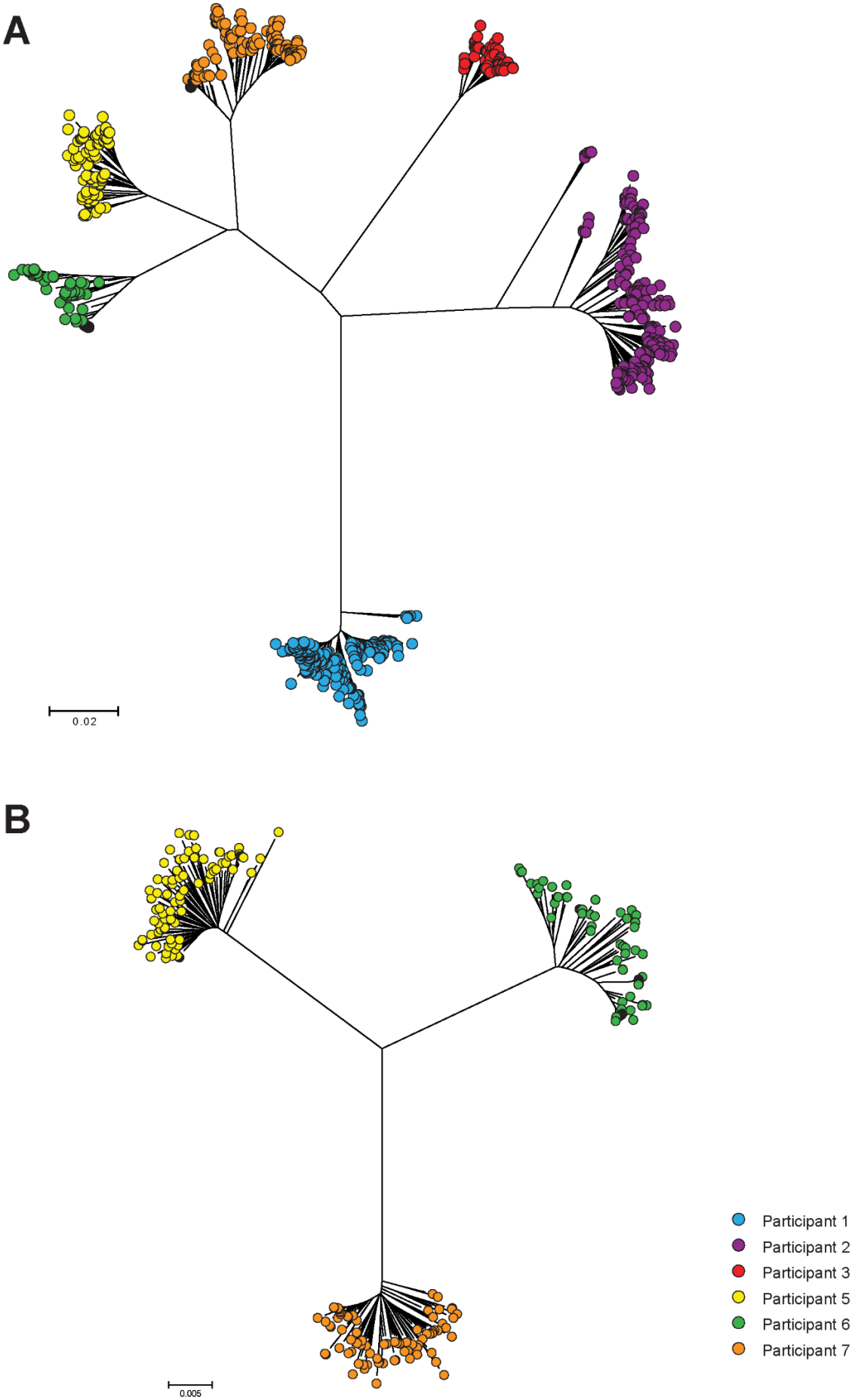
Env PCR Conditions:
The v1-v3 region of env was amplified using two rounds of nested PCR amplification. The primers listed in the table below were used for amplification: E20 Forward and E115 Reverse for PCR round one, and E30 Forward and E125 Reverse for PCR round 2. For participants 1,3,5,6,7 subtype B primers were used and for participant 2 subtype C primers were used. For round 1 of the PCR, the following thermocycler parameters were used: 94 °C for 2 minutes, 94 °C for 30 seconds, 52 °C for 30 seconds, 72 °C for 1 minute, 44 cycles of steps 2–4 and 72 °C for 3 minutes. For round 2 of the PCR, the following thermocycler parameters were used: 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 45 seconds, 41 cycles of steps 1–3 and 72 °C for 3 minutes. The PCR products (741-831 kb) representing single HIV-1 sequences were sequenced using Sanger sequencing (Australian Genome Research Facility, Sydney, Australia) with primers E30 Forward and E125 Reverse.

P24-RT PCR Conditions:
The p24-RT region was amplified using two rounds of nested PCR amplification. The primers listed in the table below were used for amplification: PCR1 Forward and PCR1 Reverse for PCR round one, and PCR2 Forward and PCR2 Reverse for PCR round 2. For round 1 of the PCR, the following thermocycler parameters were used: 94 °C for 2 minutes, 94 °C for 30 seconds, 53 °C for 30 seconds, 72 °C for 2 minutes and 30 seconds, 44 cycles of steps 2–4 and 72 °C for 3 minutes. For round 2 of the PCR, the following thermocycler parameters were used: 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 2 minutes and 30 seconds, 41 cycles of steps 1–3 and 72 °C for 3 minutes. The PCR products (2090-2099 kb) representing single HIV-1 sequences were sequenced using Sanger sequencing (Australian Genome Research Facility, Sydney, Australia) with sequencing primers F1-4 and R1-4 as listed in the table below.

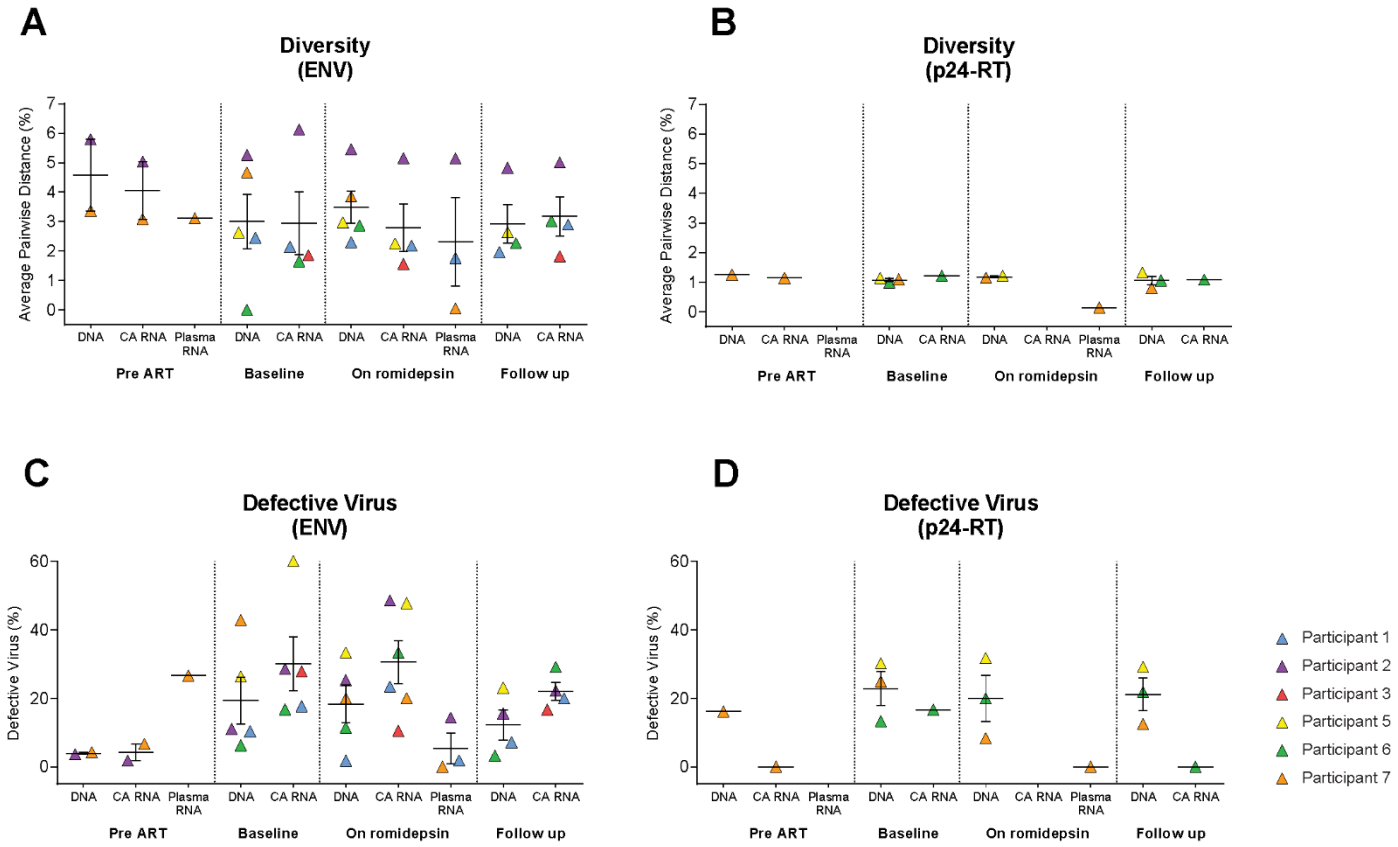
Primer Name	**Primer Sequence**
ENV: E20 Forward (Subtype B)	5’-GGGCCACACATGCCTGTGTACCCACAG-3’
ENV: E115 Reverse (Subtype B)	5’-AGAAAAATTCCCCTCCACAATTAA-3’
ENV: E30 Forward (Subtype B & C)	5’-GTGTACCCACAGACCCCAGCCCACAAG-3’
ENV: E125 Reverse (Subtype B)	5’-CAATTTCTGGGTCCCCTCCTGAGG-3’
ENV: E115 Reverse (Subtype C)	5’ AGAAAAATTCTCCTCTACAATTAA-3’
ENV: E20 Forward (Subtype C)	5’-GGGCTACACATGCCTGTGTACCCACAG-3’
ENV: E125 Reverse (Subtype C)	5’-TAATTTCTAGGTCCCCTCCTGAGG-3’
P24-RT: PCR1 Forward	5’-GCAAGCAGGGARCTAGAACGAT-3’
P24-RT: PCR 1 Reverse	5’-AGTGGTATTACTTCTGTTAGTGCTTT-3’
P24-RT: PCR2 Forward & Sequencing F1	5’-GTCAGCCAAAATTACCCTATAGT-3’
P24-RT: PCR2 Reverse	5’-TTGCCCAATTCAATTTTCCCACTAA-3’
P24-RT: Sequencing F2	5’-ATGACAGAAACCTTGTTGGTCCA-3’
P24-RT: Sequencing F3	5’-TGTTGGAAATGTGGAAAGGAAGGAC-3’
P24-RT: Sequencing F4	5’-ATGGCCCAAAAGTTAAACAATGGC-3’
P24-RT: Sequencing R1	5’-TGGACCAACAAGGTTTCTGTCAT-3’
P24-RT: Sequencing R2	5’-CTGAAGCTCTCTTCTGGTGG-3’
P24-RT: Sequencing R3	5’-TTCTTCTGTCAATGGCCATTGTTTAAC-3’
P24-RT: Sequencing R4	5’-GCTGTCYTTTTCTGGCAG-3’

Supplemental Digital Content 2. Number of intact env and p24-RT sequences obtained for each sample for each participant in the romidepsin trial. TP, time point; CRNA, cell-associated RNA; PRNA, plasma RNA; - indicates no sample available from this time point.

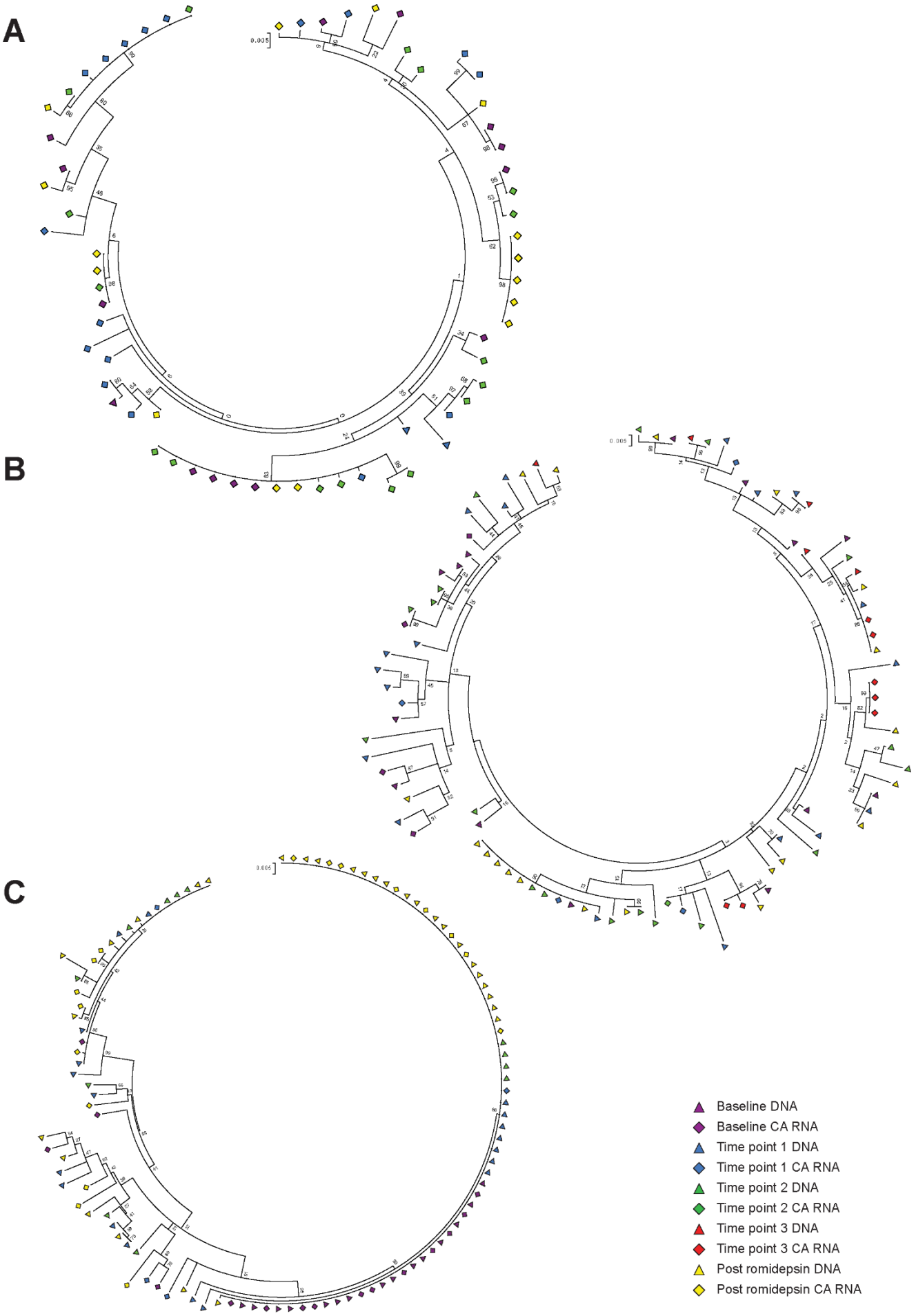
Participant	Pre-ART DNA	Pre-ART CRNA	Pre-ART PRNA	Baseline DNA	Baseline CRNA	TP1 DNA	TP1 CRNA	TP2 DNA	TP2 CRNA	TP3 DNA	TP3 CRNA	TP1 PRNA	TP2 PRNA	TP3 PRNA	Follow-up DNA	Follow-up CRNA
1 (env)	-	-	-	26	28	25	35	31	24	-	-	17	20	18	26	28
2 (env)	26	54	-	8	5	32	14	24	4	-	-	2	4		27	7
3 (env)	-	-	-	1	13	2	17	0	17	-	-	-	-	-	0	15
5 (env)	-	-	-	14	4	19	4	18	1	5	7	-	-	-	20	0
6 (env)	-	-	-	15	15	19	4	12	0	-	-	-	-	-	30	17
7 (env)	45	14	22	4	1	6	4	6	0	-	-	9	33	-	2	0
5 (p24-RT)	-	-	-	30	0	26	0	9	0	8	0	-	-	-	17	0
6 (p24-RT)	-	-	-	26	5	4	3	0	0	-	-	-	-	-	25	8
7 (p24-RT)	26	13	-	6	2	16	2	6	1	-	-	3	2	-	7	1



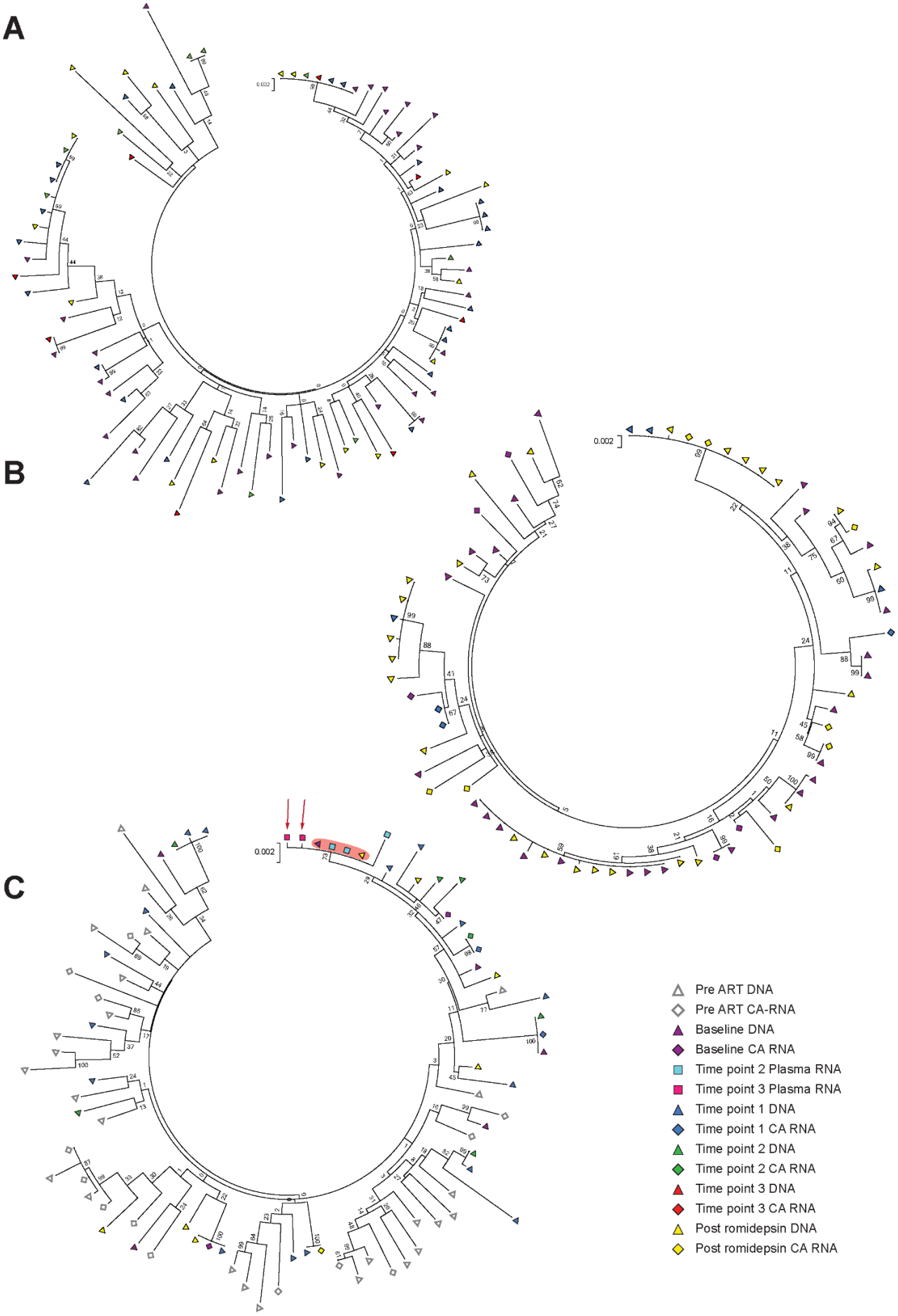
Supplemental Digital Content 3. Sequences from individual study participants segregate independently. Neighbor-joining trees of (A) env of HIV-1 sequences from all six participants in the romidepsin trial and (B) p24-RT of HIV-1 sequences from participants 5, 6, 7. Sequences that were classified as defective (containing stop codons or hypermutation) were not included. The phylogenetic analysis and tree construction were conducted using MEGA 6.0.



Supplemental Digital Content 4. Romidepsin nonselectively activates transcription from latent HIV-1 proviruses and romidepsin-induced plasma viremia contains low amounts of defective viruses. (A and B) Average pairwise distance of intact env (A) and p24-RT (B) sequences from HIV-1 DNA, cell-associated (CA) RNA and plasma RNA at study baseline, during romidepsin administration and at follow up. Error bars show mean ± standard error of the mean. On romidepsin data points represent the average pairwise distance of the pooled sequences for all time points during romidepsin treatment. (C and D) Defective sequences in percent of total number of sequences. Error bars show mean ± standard error of the mean. On romidepsin data points represent the percentage of defective sequences of the pooled sequences for all time points during romidepsin treatment.



Supplemental Digital Content 5. Maximum likelihood trees of env of HIV-1 for **(A)** participant 3, **(B)** participant 5 and **(C)** participant 6.



Supplemental Digital Content 6. Maximum likelihood trees of p24-RT of HIV-1 for **(A)** participant 5, **(B)** participant 6 and **(C)** participant 7. Highlighted in red are romidepsin-induced plasma sequences that are identical to DNA sequences. Red arrows point to plasma HIV RNA sequences that are >99.7% similar to HIV DNA.

Supplemental Digital Content 7. Viremic plasma samples do not contain HIV-1 DNA. Plasma HIV RNA levels during romidepsin treatment. SCA, single copy assay; RT, reverse transcriptase.

Participant ID	Time Point	Days after baseline	SCA (Copies/mL)	Plasma volume (mL)	No RT control	Internal viral control
1	1	1	69.6	2.0	Negative	Pass
1	2	10	33.1	2.0	Negative	Pass
1	3	17	39.3	2.0	Negative	Pass
2	1	3	2.5	2.0	Negative	Pass
2	2	10	10.8	1.0	Negative	Pass
7	2	10	21.0	2.0	Negative	Pass
7	3	17	47.0	2.0	Negative	Pass