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# Lorenzo–Redondo *et al.* reply

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In the accompanying Comment[1], Rosenbloom *et al.* present a model simulation that questions our report[2] of ongoing replication within drug sanctuaries in treated patients infected with HIV-1, which challenges the prevailing dogma that such replication cannot occur. They propose that cohorts of ever-older, latently infected cells revealed by differential decay of cell populations with varied half-lives after the start of antiretroviral therapy yield an illusion of viral evolution. We find this explanation untenable because Rosenbloom *et al.* make modelling assumptions that conflict with what is known about within-host virus evolution[3,4].

As Rosenbloom *et al.* acknowledge[1], they must apply very strong selective pressures in their simulations in order to match our observations (Supplementary Table 2). Selection must be both excessively strong (the selection coefficient $s = 0.2$ for each beneficial mutation) and pervasive (affecting about 3% of the genome) to reach the claimed 57% rate of false positives. However, even for escape mutations to cytotoxic T lymphocytes that target the virus, which are considered to be under exceptionally strong selection, the reported average strength of selection[5] is only $s = 0.03$, and the average selection coefficient across the entire HIV-1 genome[6,7] is $s = 0.005$. When $s$ is set to more plausible values, the simulations register nearly no forward evolution signal.

The model absolutely requires these biologically untenable selective forces, as revealed by the key difference between the simulated and observed within-host phylogenies. Internal branches in fig. 2c, d of ref. 1 comprise a single substitution that must be driven to fixation almost immediately by unrealistically large selective pressures. This level of divergence is three to five times lower than we report[2] (extended data fig. 3 of ref. 2), where multiple segregating (but mostly not adaptive) substitutions have accumulated between clades of haplotypes over the same period. Under any measure of phylogenetic support (for example, bootstrap), trees shown in fig. 2c, d of ref. 1 would collapse to rakes that bear no resemblance to experimental phylogenies[2].

The results like those in fig. 2 of ref. 1 are also likely to be influenced by the decision to sample a small number of sequences from simulated populations that generates limited information on within-host diversity; drawing only 50 sequences per time point (as done by Rosenbloom *et al.*[1]) will not reliably (in 80% or more of cases) capture variants with frequencies of less than 3%.

The model in ref. 1 predicts that, after treatment initiation, viral DNA will become more similar to viral RNA collected ever earlier in infection, giving a backwards evolution signal. Viral sequences in peripheral blood T cells sampled at successive time points from patients on suppressive therapy[8–10] do not support this prediction. The observation that DNA sequences in cells collected after the initiation of suppressive therapy retain close genetic similarity to the RNA sequences in plasma sampled in the late phase of HIV-1 infection before treatment[11] further undermines the model's predictions.

While differential decay of latently infected T cells may very well have a role in shaping sequence diversity, the process on its own fails to capture essential features of within-host sequence evolution and yields predictions not supported by available longitudinal data. Further multi-compartment deep sequencing studies of defined T cell subsets in larger cohorts of individuals on treatment are needed to establish the relative contributions of different mechanisms for viral persistence.

## References

1. Rosenbloom DIS, Hill AL, Laskey SB & Siliciano RF Re-evaluating evolution in the HIV reservoir. Nature 551, 10.1038/nature24634 (2017).
2. Lorenzo-Redondo R, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 530, 51–56 (2016). [PubMed: 26814962]

3. Rambaut A, Posada D, Crandall KA & Holmes EC The causes and consequences of HIV evolution. Nat. Rev. Genet. 5, 52–61 (2004). [PubMed: 14708016]

4. Lemey P, Rambaut A & Pybus OG HIV evolutionary dynamics within and among hosts. AIDS Rev. 8, 125–140 (2006). [PubMed: 17078483]

5. Liu Y et al. Selection on the human immunodeficiency virus type 1 proteome following primary infection. J. Virol. 80, 9519–9529 (2006). [PubMed: 16973556]

6. Neher RA & Leitner T Recombination rate and selection strength in HIV intra-patient evolution. PLOS Comput. Biol. 6, e1000660 (2010). [PubMed: 20126527]

7. Rouzine IM, Coffin JM & Weinberger LS Fifteen years later: hard and soft selection sweeps confirm a large population number for HIV *in vivo*. PLoS Genet. 10, e1004179 (2014). [PubMed: 24586204]

8. Finzi D et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278, 1295–1300 (1997). [PubMed: 9360927]

9. Wong JK et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278, 1291–1295 (1997). [PubMed: 9360926]

10. Hosmane NN et al. Proliferation of latently infected $CD4^+$ T cells carrying replication-competent HIV-1: Potential role in latent reservoir dynamics. J. Exp. Med. 214, 959–972 (2017). [PubMed: 28341641]

11. Brodin J et al. Establishment and stability of the latent HIV-1 DNA reservoir. eLife 5, e18889 (2016). doi:10.1038/nature24635 [PubMed: 27855060]