

## 1 **Viral and Host Mediators of Non-Suppressible HIV-1 Viremia**

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48 **Contributions**

49 AM, BE, XZ, YL, RS, DRK, and JZL conceived, designed, and supervised the project; AM, BE,  
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53 ZLB performed the statistical analysis; AM, BE, XZ, YL, GJB, RS, AK, MM, XL, RD, CK, PLA,  
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55 YL, RS, and JZL wrote the original draft of the paper; all of the authors contributed to the final  
56 review of the paper and the editing.

57 **Abstract**

58 Non-suppressible HIV-1 viremia (NSV) can occur in persons with HIV despite adherence to  
59 combination antiretroviral therapy (ART) and in the absence of significant drug resistance. Here,  
60 we show that plasma NSV sequences are comprised primarily of large clones without evidence  
61 of viral evolution over time. We defined proviruses that contribute to plasma viremia as  
62 “producer”, and those that did not as “non-producer”. Compared to ART-suppressed individuals,  
63 NSV participants had a significantly larger producer reservoir. Producer proviruses were  
64 enriched in chromosome 19 and in proximity to the activating H3K36me3 epigenetic mark. CD4<sup>+</sup>  
65 cells from NSV participants demonstrated upregulation of anti-apoptotic genes and  
66 downregulation of pro-apoptotic and type I/II interferon-related pathways. Furthermore, NSV  
67 participants showed no elevation in HIV-specific CD8<sup>+</sup> cell responses and producer proviruses  
68 were enriched for HLA escape mutations. We identified critical host and viral mediators of NSV  
69 that represent potential targets to disrupt HIV persistence and promote viral silencing.

70

71 **Key words:** HIV, non-suppressible viremia, persistent low-level viremia, producer provirus,  
72 integration, CD8 immune response

73 **Main**

74 For the majority of persons with HIV (PWH), antiretroviral therapy (ART) suppresses HIV RNA  
75 to below the level of commercial assay detection.<sup>1-5</sup> However, a subset of PWH demonstrate  
76 persistent (or non-suppressible) low-level viremia (NSV) while on ART.<sup>6,7</sup> NSV has historically  
77 been attributed to suboptimal ART adherence and/or accumulating HIV drug resistance.<sup>8,9</sup>  
78 Previous studies supporting the presence of active viral replication have reported that ART  
79 resistance mutations can accumulate when viremia persists in the low but detectable range<sup>10,11</sup>  
80 and that NSV can increase the risk of virologic failure.<sup>12</sup> While these factors can cause  
81 persistent NSV, other evidence has showed that persistent NSV can be maintained for long  
82 periods without leading to high-level virologic failure or the development of new resistance  
83 mutations.<sup>13-18</sup> While suboptimal ART adherence or emerging drug resistance may play a role in  
84 a subset of individuals with NSV, alternative mechanisms seem to underlie NSV in other PWH.

85 Clonal expansion of HIV-infected cells represents a key contributing factor for HIV  
86 persistence and recent studies have suggested this plays an important role in NSV as well.  
87 Halvas et al. reported that the majority of plasma variants were composed of clusters of identical  
88 sequences without signs of active viral replication.<sup>1</sup> While NSV was fueled by large populations  
89 of clonally-expanded HIV-infected cells, the mechanisms that lead to the establishment and  
90 maintenance of NSV, and the NSV-generating proviral reservoirs, remain understudied. In this  
91 study, we characterized a cohort of eight participants with NSV, and performed in-depth ART  
92 drug concentration testing, alongside viral and host cell genetics/genomics and immune profiling.  
93 We have identified features of host integration sites that differentiated proviruses fueling NSV  
94 from those that were not contributory. Transcriptomic and immunologic phenotyping studies  
95 highlighted host cell and cellular immune environments that distinguished PWH with and without  
96 NSV.

## 97 **Results**

### 98 **Participant characteristics and assessment of ARV drug levels**

99 We enrolled eight participants, 88% men, with a median age of 60 years and median ART  
100 duration of 10 years. The median duration of virologic suppression prior to the NSV and duration  
101 of NSV for all participants were 4 and 1.8 years, respectively. During the NSV episodes, the  
102 median viral load was 99 copies/ml and the median CD4 count was 798 cells/mm<sup>3</sup> (Table 1).  
103 Individual participant characteristics, ART regimens and genotypic susceptibility scores (GSS)<sup>19</sup>  
104 of plasma viruses sequenced during NSV are shown in Table S1. All participants were receiving  
105 at least 2 active antiretroviral drugs during the NSV episodes. Characteristics of the ART-  
106 suppressed comparator participants are shown in Table S2 and S3.

107 We assessed ART adherence by quantifying antiretroviral (and their anabolites) drug  
108 concentrations in plasma or through dried blood spot (DBS) testing. LV1 and LV2 had plasma  
109 dolutegravir (DTG) and darunavir (DRN) concentrations consistent with ongoing ART use (Table  
110 S4). LV3 and LV5-9 had DBS tests for tenofovir (TFV-DP, a measure of cumulative TDF/TAF  
111 adherence)<sup>20,21</sup> and emtricitabine (FTC-TP, a measure of recent FTC dosing)<sup>22</sup>. The median  
112 (range) FTC-TP levels was 5 (4.4-6.7) pmol/punches and TFV-DP levels was 3702 (2771-6684)  
113 fmol/punches.<sup>23</sup> These concentrations are consistent with the highest odds of suppression and  
114 lowest odds of future viremia,<sup>24,25</sup> suggesting that all study participants should have been virally  
115 suppressed on the basis of high adherence. Also, LV3 and LV5-9 had quantifiable FTC-TP,  
116 confirming dosing in the preceding 7 days before sampling.<sup>21</sup> These results demonstrate that  
117 our NSV participants had both high levels of short-term and cumulative ART adherence (Table  
118 S4).

119

120 **Plasma NSV sequences were comprised primarily of large clones without evidence of**  
121 **viral evolution**

122 HIV-1 integration targeting preferences are demarcated by various features of active chromatin,  
123 including transcription<sup>26</sup>, histone epigenetic marks<sup>27</sup>, and nuclear speckle proximity.<sup>28</sup> The  
124 provirus landscape morphs over time in response to ART and the host immune response to a  
125 quasi-homeostatic state marked by cell loss and clonal expansion.<sup>29-35</sup> A key goal of this study  
126 was to assess aspects of host proviruses that contributed to NSV. Longitudinal single-genome  
127 sequencing (SGS) of near-full length proviruses and plasma HIV *pol* and *env* RNA was  
128 performed.

129 A total of 1987 single-genome proviral sequences and 222 single-genome plasma sequences  
130 were generated for the 8 NSV participants. Longitudinal plasma HIV sequences were obtained  
131 for four participants with available sampling (LV1, LV7, LV8, and LV9), at a median 4.5 time  
132 points, an average of 9.7 months apart (Fig. 1a and S1).

133 Phylogenetic analysis confirmed that sequences from each participant partitioned into  
134 separate clusters (Fig. S2). Neighbor joining trees of proviral and plasma sequences for these  
135 eight participants showed that the plasma sequences were dominated by one or two clones,  
136 with no evidence of viral evolution from longitudinal samplings that would be consistent with  
137 active viral replication (Fig. 1b and Fig. 2a). For the initial analysis, proviral sequences were  
138 considered intact if they either did not harbor obvious defects or were linked to plasma  
139 sequences. At the time of study entry, the 2 largest plasma RNA clones comprised a median 71%  
140 (Q1-Q3: 27-83%) of all plasma sequences and were linked to a median 26% (Q1-Q3: 14-61%)  
141 of all intact proviral sequences (Fig. S3a). Overall, intact proviruses comprised a median 4.5%  
142 (Q1-Q3: 3.8-15%) of the proviral reservoir, with a high degree of variation evident from two  
143 participants (LV2 and LV9). LV2 and LV9 proviruses were dominated by several large clones of  
144 intact sequences that represented 76% and 34% of their total PBMC proviral reservoirs,  
145 respectively (Fig. S3b).

146 We categorized proviruses as producers if they matched a plasma sequence and as  
147 non-producers if they did not. There was a wide range of producer proviruses within the

148 reservoir. For LV2, the PBMC proviral reservoir was largely comprised of one large producer  
149 clone representing 98% of intact proviruses, which matched the large plasma NSV clone (Fig.  
150 2a). In contrast, LV3 had the smallest producer reservoir size, representing 3.5% of total intact  
151 sequences. These results demonstrate that while these individuals share a common NSV  
152 phenotype, their proviral landscape can be highly heterogenous (Fig. S3b).

153 We next compared the size of the intact and defective reservoir sizes between the NSV  
154 participants and a control group of 10 ART-suppressed participants (Table S2). NSV  
155 participants had a significantly larger total and intact PBMC proviral reservoir (NSV vs ART-  
156 suppressed: median total proviral genomes 34 vs 18 proviruses/million cells,  $P=0.08$  and  
157 median intact proviral genomes 4.3 vs 0.1 proviruses/million cells,  $P=0.001$ ). Specifically, the  
158 size of the producer proviral reservoir was significantly larger in the NSV participants than either  
159 the non-producer intact proviral reservoir in these participants or the intact proviral reservoir in  
160 the ART-suppressed participants (Fig. 2b). In addition, the NSV participants had a smaller  
161 number of proviruses with large deletions (median 2.6 vs 10.7 proviruses/million cells,  $P=0.006$ ).  
162 These results suggest that intact reservoir size could be a contributing factor to NSV.

163

### 164 **Integration site and epigenetic signatures of producer proviruses**

165 The location and chromatin landscape of HIV proviral integration sites can modulate the extent  
166 of proviral transcriptional activity.<sup>36,37</sup> We accordingly evaluated whether certain integration site  
167 features differentiated the producer, non-producer and defective proviruses. Using the Matched  
168 Integration Site and Proviral Sequencing (MIP-Seq) protocol, we identified host chromosomal  
169 integration sites for 11 producer, 21 intact non-producer and 44 defective proviruses across all  
170 NSV participants (we were unable to identify an integration site from one LV3 producer clone).  
171 Integration sites were identified across all autosomal and sex chromosomes with the exception  
172 of chromosome 21 (Fig. 3a). Compared to non-producer and defective proviruses, producer  
173 integration sites were enriched in chromosome 19. Twenty-seven percent (3/11) of producer



174 proviruses were located in chromosome 19 compared to none of the 21 non-producer and 44  
175 defective proviruses (producer vs non-producer  $P=0.03$  and producer vs defective  $P=0.006$ ).

176 Significant enrichment of producer proviruses for proximity to two activating epigenetic  
177 markers was also observed. Using ChIP-seq data from primary CD4<sup>+</sup> T cells published on the  
178 ROADMAP database,<sup>38</sup> we detected significantly elevated ChIP-seq reads for the H3K36me3  
179 and H3K9me3 histone marks in proximity to producer integration sites compared to either non-  
180 producer or defective integration sites (Fig. 3c). We calculated the level of plasma viral load  
181 contributed by the producer provirus, which we call the plasma clone viral load. We observed a  
182 significant positive correlation between the number of H3K36me3 ChIP-seq reads in proximity to  
183 the producer proviruses integration sites and the plasma clone viral load (Spearman  $r=0.83$ ,  
184  $p=0.001$ ) (Fig. 3d). Proximity to H3K36me3 has been linked to proviral gene expression<sup>36,37,39,40</sup>,  
185 suggesting that producer proviruses are enriched near transcriptionally active regions of the  
186 chromosome and that producers could potentially leverage cellular transcriptional machinery for  
187 proviral expression and virion production.<sup>36</sup> In addition, a higher number of proximal ChIP-seq  
188 peak numbers for two other activating histone marks (H3K27ac and H3K4me1) were linked to  
189 greater expression of host genes containing integrated proviruses (Fig. S4a), although these  
190 histone marks were not enriched near producer proviruses.

191 There were a number of chromosomal features that did not associate with the producer  
192 cell proviral phenotype. Distance to transcriptional start sites (TSSs) was statistically  
193 indistinguishable between producer, non-producer and defective proviruses, regardless of the  
194 orientation of the host gene and provirus (Fig. S4b-d). We also did not detect any significant  
195 differences between producer, non-producer and defective proviral classes and their distance to  
196 heterochromatic centromeres or the fraction of integration into transcriptionally active speckle-  
197 associated domains (Fig. S5a-b).

198 Finally, using NSV participant CD4 cellular RNA sequencing (RNA-Seq), we found no  
199 significant differences in host gene transcript levels between producer, non-producer and  
200 defective proviruses regardless of the integration orientation (Fig. S5c-d).

201  
202 **NSV association with upregulated cell survival signaling and downregulated interferon**  
203 **signaling**

204 Cell survival signaling has been linked to HIV persistence, especially in latently infected CD4<sup>+</sup> T  
205 cells.<sup>41,42</sup> To understand the association between cell signaling and NSV, we compared the  
206 CD4<sup>+</sup> T cell transcriptomic features between the NSV group (N=8) and a subgroup of the ART-  
207 suppressed individuals (N=5) using RNA-Seq. Compared to the ART-suppressed individuals,  
208 NSV participants had 481 upregulated genes and 558 downregulated genes (adjusted P value  
209 [Padj]<0.1) (Fig. 4a, red and blue dots). Among these differentially expressed genes (DEG),  
210 Gene Set Enrichment Analysis (GSEA) revealed enrichment of pathways related to HIV  
211 infection, HIV life cycle, and transcription in the NSV group (Fig. 4b). CD4<sup>+</sup> T cells from the NSV  
212 group exhibited enrichment in oxidative phosphorylation and apoptosis-related signals (Fig. 4b  
213 upper panel and Fig. S6a). Specifically, CD4<sup>+</sup> T cells from NSV participants appeared to be  
214 primed for survival via down-regulation of pro-apoptotic genes and upregulation of genes  
215 associated with anti-apoptotic pathways, including proteasome-related genes (e.g. PSMB1,  
216 PSMB2, PSMD14), ubiquitination-related genes, and oncogenes such as PIK3CA and  
217 PIK3R1 (Fig. 4c and 4d).<sup>43-46</sup>

218 Transcriptomic analysis also highlighted differences in the immune responses between  
219 NSV and ART-suppressed individuals. NSV participants demonstrated upregulation of  
220 immunosuppression-related genes, including CTLA4 and FOXP3 (Fig. 4a), pointing to an  
221 enrichment of the RUNX1-related pathway, which is associated with attenuation in antiviral and  
222 interferon (IFN) signaling through FOXP3 binding.<sup>47,48</sup> In fact, both IFN-alpha/beta and IFN-  
223 gamma signaling were enriched in ART-suppressed individuals (Fig. 4b lower panel, Fig. S6a-c).

224 IFN signaling plays a pivotal role in HIV pathogenesis by inducing viral restriction factors,  
225 causing depletion of CD4<sup>+</sup> T cells, and regulating systemic immune activation.<sup>49</sup> These results  
226 may point towards potential defects in immune-mediated control of a highly active HIV reservoir  
227 as a contributing factor for NSV. Finally, using the random forest algorithm, we identified genes  
228 that correlated with the proportion of intact and hypermutated sequences (Fig. S6d). String  
229 analysis revealed that the AKT1-centered signaling pathway gene set correlated with the size of  
230 the intact proviral reservoir (Fig. S6e).

231  
232 **Non-suppressible viremia does not increase HIV-specific CD8<sup>+</sup> T cell responses and is**  
233 **associated with HLA escape mutations**

234 Survival of CD4<sup>+</sup> T cells harboring producer proviruses not only relies on downregulation of  
235 apoptosis and IFN programs, but also resistance to killing.<sup>50</sup> HIV-specific CD8<sup>+</sup> T cells, which  
236 recognize viral peptides presented in complex with HLA Class-I (HLA-A, -B, and -C), are  
237 thought to be one of the most important mediators of viral control.<sup>51</sup> This appears to be the case  
238 even in the setting of ART, as CD8-depletion in the nonhuman primate model leads to loss of  
239 viral suppression<sup>52</sup>. Despite the higher antigen-exposure in NSV, we did not detect a more  
240 active effector HIV-specific T cell response as determined by IFN-gamma ELISOPT (Fig. 5a).  
241 We found no significant differences in HIV-specific CD8<sup>+</sup> T cell reactivity or proliferation between  
242 the NSV and ART-suppressed populations (Fig. 5a and 5b).

243 This relatively non-elevated CD8<sup>+</sup> T cell response in NSV was paired with high-levels of  
244 HLA-escape mutations in the producer proviruses. HLA class-I escape has long been  
245 recognized as a viral defense mechanism to evade host immune control.<sup>53</sup> We observed a  
246 modestly higher average number of HLA-adapted (i.e., escape) mutations in producer  
247 proviruses compared with non-producer (p=0.04) and a dramatically higher escape burden  
248 compared to defective proviruses adjusted for proviral length (p=0.001) (Fig. 5c). After  
249 normalizing for the size of each HIV gene, *nef* showed significantly higher numbers of adapted

250 and possible adapted mutations compared with other HIV genes (Fig. 5d, S7). Adapted and  
251 possible adapted mutations in HIV genomes in both producers and non-producers highly  
252 correlated with CD8<sup>+</sup> T cell IFN- $\gamma$  release but not with proliferation (Fig. 5e and 5f), suggesting a  
253 relationship between effector HIV-specific CD8<sup>+</sup> T cell responses and subsequent emergence of  
254 mutations within proviral clones. Among all genes, the number of *nef* adapted and possible  
255 adapted mutations in producer proviruses was strongly correlated with CD8<sup>+</sup> T cell IFN- $\gamma$   
256 release in NSV ( $r=0.94$ ,  $p=0.02$ ) (Fig. 5e and 5g). Adapted and possible adapted mutations in  
257 *pol* in producer proviruses also significantly correlated with total CD8<sup>+</sup> T cell activity ( $r=0.84$ ,  
258  $p=0.04$ ) (Fig. 5h). This seemingly represents immune-driven viral escape mutations that  
259 accumulated prior to ART initiation.

260

#### 261 **Replication incompetent producers with 5'-deletions in PSI ( $\Psi$ ) element**

262 Our sequencing revealed that NSV is largely comprised of one or two clonal populations that  
263 remain stable over time, which is consistent with high-level viral production from a large,  
264 clonally-expanded population of HIV-infected cells as the primary driver of NSV, rather than  
265 ongoing viral replication on ART. Thus, producer proviruses driving NSV need not be  
266 replication-competent, and we accordingly evaluated producer proviruses for potential  
267 replication defects, including deletions in the 5' PSI packaging element.<sup>54</sup> In 38% (3/8) of NSV  
268 participants, we observed that producer proviruses harbored deletions in the 5' end of HIV  
269 genome (Fig. S8, black boxes). These deletions, which encompassed 22, 15 and 41 nucleotides  
270 in participants LV4, LV7 and LV8, respectively, all occurred within SL1 and SL2 elements,  
271 ending at the same location within the splice donor site (Fig. S8). Plasma RNA sequencing of  
272 the 5' leader/*gag* region of HIV was performed to confirm the presence of these 5' defects within  
273 the plasma RNA sequences. To evaluate whether these proviruses were infectious, viral  
274 outgrowth assays (VOAs) were performed using a transwell system with participant CD4<sup>+</sup> T  
275 cells (LV4, LV7 and LV8; LV2, LV5 and LV9 served as controls) in the bottom chamber and

276 MOLT-4/CCR5 cells in the upper chamber. HIV DNA from the MOLT-4 cells was extracted and  
277 subjected to MIP-seq analysis. Producer provirus was isolated from the VOA for LV9 (Fig. 2a  
278 and Fig. S9). Non-producer proviruses were isolated from the VOA for LV2, LV5 and LV8 (Fig.  
279 2a).

280

## 281 **Discussion**

282 In this study, we have conducted a comprehensive assessment of NSV and have provided  
283 insight into ART-independent factors implicated in HIV suppression and persistence. Our results  
284 indicate that suboptimal ART adherence and drug resistance do not appear to be the drivers of  
285 non-suppressible viremia. In these participants, NSV is driven instead by the critical intersection  
286 of viral and host immune factors. Specifically, the NSV phenotype was highlighted by the  
287 presence of large, clonally-expanded reservoirs of proviruses frequently harboring immune  
288 escape mutations (and/or defects in the 5' leader region), integrated in transcriptionally-  
289 permissive chromosomal regions, within a CD4<sup>+</sup> T cell environment primed for survival, and  
290 without noticeable HIV-specific T cell responses (Fig. S10).

291 In one of the first in-depth reservoir studies of NSVs, Halvas et al. reported that NSV is  
292 comprised largely of identical populations of plasma viruses that arise from the expansion of  
293 HIV-infected CD4<sup>+</sup> T cell clones, which they termed repliclones.<sup>1</sup> However, most prior studies  
294 sequenced relatively short fragments of viral RNA, which can over-estimate the clonality of  
295 plasma sequences. Our plasma RNA sequencing assay combined an ultrasensitive RNA  
296 extraction process for 6.7 kb *pol-env* RNA sequencing (Fig. S9a-b). These results confirm that  
297 in our cohort, NSV is composed primarily of 1-2 large plasma viral clones that comprised >70%  
298 of plasma viruses. The role of these viral clones as the primary driver of NSV was confirmed  
299 across multiple longitudinal time points, which failed to reveal evidence of plasma viral  
300 sequence changes and evolution. For all of the NSV participants, we were able to identify exact  
301 proviral sequence matches for the large plasma clones. We found that the size of the producer

302 proviral reservoir was significantly larger than either the size of the nonproducer proviruses in  
303 NSV participants or intact proviruses in ART-suppressed participants, although admittedly with a  
304 broad distribution in size of the producer proviral reservoir. The large size of these producer  
305 proviral reservoir and their ability to maintain NSV over years highlight the relative stability of  
306 this reservoir. These results and the presence of NSV over many years suggests an intrinsic  
307 ability of these HIV infected cells to maintain prolonged survival and/or proliferate. Prior studies  
308 have reported that CD4<sup>+</sup> T cells modulating key pro- and anti-apoptotic pathways can maintain  
309 survival of HIV-infected cells, drive clonal expansion, and guard against CTLs.<sup>42,55</sup> Compared to  
310 ART-suppressed participants, CD4<sup>+</sup> T cells in NSV participants demonstrated transcriptional  
311 upregulation of anti-apoptotic pathways and down-regulation of pro-apoptotic pathways. While  
312 transcriptional analysis was not isolated to producer cells alone, these results suggest the CD4<sup>+</sup>  
313 T cell environment in NSV participants is primed for survival.

314         Using the MIP-Seq assay,<sup>39</sup> we were able to identify the location of HIV integration sites  
315 in host chromosomes for 11 producer, 21 intact non-producer and 44 defective proviruses  
316 across NSV participants (except producer proviruses from LV3). HIV integration is known to  
317 favor active chromatin, and proximity to activating epigenetic marks can modulate proviral gene  
318 expression and hence the fate of the resident provirus and infected cell.<sup>56,57</sup> We identified  
319 certain integration site features that were distinctive in producer proviruses, including  
320 enrichment in chromosome 19, which is distinctively enriched for gene density.<sup>58,59</sup> We also  
321 demonstrated that producer proviruses were located in regions enriched in certain epigenetic  
322 characteristics, including a greater number of H3K36me3 histone peaks, which are associated  
323 with a transcriptionally-permissive chromosomal regions and elevated proviral expression.<sup>37</sup> A  
324 higher number of H3K36me3 peaks surrounding the producer provirus was also strongly  
325 associated with higher plasma viral load comprised of that clone.

326         Given the transcriptionally-active nature of the producer proviruses, it is unclear why  
327 they are not rapidly targeted and cleared by the host immune response. We identified several

328 potential mechanisms that may lead to a more muted immune response to the producer  
329 proviruses, including down-regulation of interferon response genes, presence of HLA escape  
330 mutations, and lack of increase in activation of HIV-specific CD8<sup>+</sup> T cell responses despite  
331 prolonged elevated levels of HIV antigenemia in NSV. IFN plays a vital role in the innate host  
332 antiviral response and contributes to the suppression of HIV viremia.<sup>60</sup> Compared to ART-  
333 suppressed participants, NSV participants significantly down-regulated transcription of IFN  
334 genes and multiple genes involved in the IFN-response pathway (Fig. 4), including key  
335 regulators IRF3 and IRF7 (Fig. S6b).<sup>61</sup> Heightened HIV-specific CD8<sup>+</sup> T cell responses occur  
336 with HIV viremia, which is critical to suppress the HIV reservoir.<sup>62</sup> Thus, we were surprised to  
337 find a relatively muted CD8<sup>+</sup> T cell response, with no significant differences noted in HIV-specific  
338 CD8<sup>+</sup> T cell activity and proliferation between the NSV and ART-suppressed individuals,  
339 although there was a clear correlation between the magnitude of CD8<sup>+</sup> T cell response and  
340 mutational burden, suggesting a potential role for viral escape. Interestingly, the loss of SIV-  
341 specific CD8<sup>+</sup> T cell responses can lead to rebound viremia in non-human primates, even in the  
342 presence of ART.<sup>52</sup> Intact proviruses and producers in particular had high levels of  
343 adaptive HLA escape mutations associated with loss of HIV-specific CD8<sup>+</sup> T cell-mediated  
344 clearance of infected cells.<sup>63</sup> Of note, there was an enrichment of HLA-escape mutations in *nef*,  
345 which may be particularly immunogenic as previous studies have reported that the strength of  
346 the Nef-specific T cell activity is linked with the size of the HIV reservoir.<sup>64</sup> Additional studies of  
347 Nef function could assess its ability to downregulate HLA-A and B from the infected cell surface,  
348 thereby promoting immune escape.<sup>65</sup>

349 In 38% (3 of 8) of our NSV individuals, we detected deletions in the 5' leader sequence  
350 of the HIV genome. None of these sequences were detected using the VOA, suggesting these  
351 proviruses were replication-defective. The 5'-untranslated leader contains several structured  
352 motifs that are involved in multiple steps of HIV replication. The deletions are present in the PSI  
353 ( $\Psi$ ) element, which is a highly structured RNA sequence with four hairpin stem loops and a

354 strong affinity for the nucleocapsid (NC) domain of the viral Gag protein. Genome packaging  
355 during virus assembly and reverse transcription during the subsequent round of infection are  
356 some known functions of the 5' leader region.<sup>66,67</sup> A recent study by White et al. described 4  
357 NSV participants with apparent defects in the 5' leader sequence.<sup>68</sup> These defects generally  
358 spanned the major splice donor site (MSD) site and resulted in the creation of non-functional  
359 virions lacking the envelope glycoprotein. Interestingly, the 5' leader sequence deletions in our  
360 NSV participants spanned the same region and, in fact, LV4 shares the same 22 base deletion  
361 that was detected in three participants by White et al. The detection of 5' leader defects at high  
362 frequencies across multiple cohorts suggests a selective advantage of these proviruses in  
363 conferring the NSV phenotype, potentially by maintaining a plasma viral load in the absence of  
364 HIV replication and/or the ability of Env-deleted virions to escape from host immune  
365 surveillance.<sup>69</sup>

366 In addition to the previously noted limitations, we estimated proviral reservoir size by  
367 near-full length proviral sequencing. This could lead to some underestimation of the actual  
368 reservoir size, although other methods for reservoir quantification (e.g., IPDA) may over-  
369 estimate the size.<sup>70</sup> Future studies will need to investigate the size of producer proviruses within  
370 tissue reservoirs. We found that the peripheral blood reservoir of HIV-infected CD4<sup>+</sup> T cells  
371 contributing to NSV can differ dramatically between NSV participants. It's possible that NSV-  
372 generating CD4<sup>+</sup> T cells are also distributed within anatomical tissue compartments<sup>71,72</sup>,  
373 especially for those NSV participants with a relatively small producer proviral reservoir size in  
374 the peripheral blood. Prior studies have shown that *gag*- or CMV-specific antigens can drive the  
375 expansion of certain HIV-infected cellular clones.<sup>31</sup> Additional studies are needed to delineate  
376 which antigens might be playing a role in the expansion of the producer proviruses. Neutralizing  
377 antibody responses can also suppress viremia<sup>73</sup> and evaluation of the humoral immune  
378 responses are indicated, although prior studies suggest that some NSV is resistant to  
379 autologous neutralizing antibodies.<sup>68</sup>



380 In this study, we identified critical host and viral mediators of NSV that represent  
381 potential targets to disrupt HIV persistence and promote viral silencing. Importantly,  
382 ultrasensitive HIV viral load assays can detect residual low levels of HIV viremia in the vast  
383 majority of PWH, even on apparently suppressive ART.<sup>74</sup> Previous studies have reported that  
384 such residual viremia is largely comprised of drug-sensitive virus<sup>75</sup> and relatively homogeneous  
385 viral populations.<sup>18,76</sup> Thus, we believe it is likely that the mechanisms behind NSV that we  
386 describe here are present to some extent in most, if not all, of PWH. Achieving an in-depth  
387 understanding of the mechanisms behind NSV may provide insight on strategies for HIV  
388 reservoir eradication applicable to all PWH.

389

## 390 **Methods**

### 391 **Participants**

392 We enrolled 8 ART-treated participants with  $\geq 3$  HIV-1 RNA levels between 40-1000 copies/mL  
393 over 24 months and compared them to a group of ART-suppressed participants with similar  
394 demographic and HIV characteristics. A non-suppressible viremia participant enrolled in the HIV  
395 Eradication and Latency (HEAL) cohort, a biorepository of Brigham and Women's Hospital, was  
396 included. The NSV samples were taken from different time points enabling us to study these  
397 participants longitudinally. The ART-suppressed comparators included 11 participants from the  
398 AIDS Clinical Trials Group (ACTG) and 7 participants from the Ragon Institute of MGH, MIT and  
399 Harvard. Written informed consent was obtained from all participants.

400

### 401 **ARV drug level testing**

402 For plasma ARV testing, samples were sent to the infectious disease pharmacokinetics lab at  
403 the University of Florida. Testing was performed for darunavir and dolutegravir by liquid  
404 chromatography with tandem mass spectrometry. For dried blood spot (DBS) ARV testing, 25  
405 mL of whole blood were spotted five times onto Whatman 903 protein saver cards, as previously

406 described.<sup>21</sup> After spotting, cards were allowed to dry at room temperature for at least three  
407 hours (as long as overnight), after which they were stored at -80°C until analyzed. TFV-DP and  
408 FTC-TP were quantified from two 7-mm punches extracted with 2 mL of methanol:water to  
409 create a lysed cellular matrix using a previously validated method that was adapted and  
410 validated for TAF-containing regimens.<sup>77</sup> The assay was linear and ranged from 25 to 6,000  
411 fmol/sample for TFV-DP and from 0.1–200 pmol/sample for FTC-TP.<sup>21,77</sup>

412 **DNA isolation and HIV reservoir quantification**

413 DNA extractions were carried out from PBMCs using the QIAamp DNA Micro Kit (Catalog  
414 #56304), and the quantification of DNA was performed with Nanodrop (Applied Biosystems,  
415 ThermoFisher). To estimate the size of the reservoir, we employed NFL-seq, which is described  
416 below.

417

418 **Near-full length proviral sequencing, sequence alignments, quality control, and Neighbor**  
419 **joining analyses**

420 Extracted DNA was endpoint-diluted and subjected to NFL-seq, as previously described.<sup>78</sup> We  
421 classified our sequences into intact and different classes of defectives (e.g., 5'-defect, deletion,  
422 hypermutation, inversion) using a published proviral intactness pipeline.<sup>79</sup> Proviral sequences  
423 were categorized into intact and defective as previously described.<sup>79</sup> Briefly, after aligning to  
424 HXB2, we called our sequences to large deleterious deletions if they have <8000bp of the  
425 amplicon size, out-of-frame indels, premature/lethal stop codons, internal inversions, or  
426 packaging signal deletions ( $\geq 15$  bp). If a sequence that was almost full-length exhibited a  
427 mapped deletion at the 5' end, which eliminated the site where the primer binds, but did not  
428 display any fatal defects in its sequence, the absent 5' sequence was deduced to be present,  
429 and this sequence was regarded as an "inferred intact" HIV-1 sequence. The Los Alamos  
430 National Laboratory (LANL) HIV Sequence Database Hypermut 2. program was used to identify  
431 the existence or nonexistence of hypermutations linked to APOBEC-3G/3F. Sequences of the  
432 virus that did not have any of the mutations listed earlier were categorized as "genome-intact"  
433 sequences. Using MAFFT v7.2.0, we aligned the sequences and utilized MEGA 6 to deduce  
434 Neighbor joining trees. We called those intact proviruses with an exact match with plasma  
435 sequences as "producers" and other intact proviruses as "non-producers".

436

### 437 **Plasma RNA sequencing**

438 We sequenced plasma HIV RNA as previously described.<sup>80</sup> Extracted RNA was diluted to single  
439 viral genome levels to meet the criteria of single genome sequencing (SGS) of having no more  
440 than one template in each well, theoretically no more than 25% of wells being positive for HIV.  
441 Primers were designed to amplify *pol-env*, a 6.7 kb region. The amplification reaction was  
442 performed using 0.5  $\mu$ l primers (10  $\mu$ mol), 1  $\mu$ l (10 mmol) MgSO<sub>4</sub>, 1  $\mu$ l (10 mmol) dNTPs, and 1  
443 U Platinum Taq Polymerase (Invitrogen) in 25  $\mu$ L total volume. PCR conditions consisted of a  
444 denaturation step at 94°C for 2 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 56°C, 90  
445 sec at 68°C and 10 min at 68°C. Products were underwent Illumina barcoded library  
446 construction and MiSeq sequencing. Amplicons were assembled using the UltraCycler v1.0  
447 automated de novo sequence assembly to generate a continuous fragment. Plasma sequences  
448 that were within 1-2 nucleotides of the near-full length proviral sequence was considered part of  
449 the clonal cluster. We counted the total number of plasma sequences in each clone and divided  
450 them by all plasma sequences that we had generated. Then we multiplied the ratio with the  
451 plasma viral load determine the contribution of each clone for plasma viral load, which we  
452 termed the plasma clone viral load.

453 For each sequence, the genotypic susceptibility scores (GSS) versus the participants'  
454 ART regimen was calculated using the Stanford HIV database drug resistance scoring system.  
455 The Stanford HIV database provides a weighted penalty score for the effect of every resistance  
456 mutation and antiretroviral medication with 0 if there is no expected effect to 60 for high-level  
457 resistance. For each sequence, the estimated level of resistance for each antiretroviral  
458 medication (ARV) was determined by adding all of the penalty scores for each of the drug  
459 resistance mutations present. The GSS of each ARV was defined as the following: 1 (Stanford  
460 penalty score 0-9), 0.75 (Stanford penalty score 10-14), 0.5 (Stanford penalty score 15-29), 0.25

461 (Stanford penalty score 30-59), and 0 (Stanford penalty score  $\geq 60$ ). The GSS for the sequence  
462 was the sum of the GSS for each ARV as part of the participant's regimen.

### 463 **Total RNA transcripts sequencing (RNA-seq)**

464 CD4<sup>+</sup> T cells were selected from cryopreserved peripheral blood mononuclear cells (PBMC)  
465 using EasySep<sup>TM</sup> Human CD4<sup>+</sup> T Cell Enrichment Kit (STEMCELL Technologies Inc.). RNA was  
466 extracted from selected CD4<sup>+</sup> T cells with the AllPrep DNA/RNA kit (Qiagen) with subsequent  
467 ribosomal RNA depletion RNA reverse transcribed to cDNA library and sequenced by NovaSeq  
468 (Illumina). Sequencing results were processed with the VIPER pipeline for alignment, counting,  
469 and quality control.<sup>81</sup> Differentially expressed gene (DEG) analysis was performed with DESeq2  
470 package<sup>82</sup> and Gene Set Enrichment Analysis (GSEA) with fgsea package using the adaptive  
471 multilevel splitting Monte Carlo approach (n=10,000 for simple fgsea in preliminary estimation of  
472 P values).<sup>83</sup>

473

### 474 **Integration site (IS) identification and epigenetics**

475 We characterized single proviral genomes along with their matched genomic integration sites by  
476 (MIP-Seq).<sup>39</sup> Briefly, we initiated whole-genome amplification (WGA) by performing multiple  
477 displacement amplification (MDA) with phi29 polymerase using the QIAGEN REPLI-g Single  
478 Cell Kit, following the manufacturer's protocol. Afterward, we divided DNA from each sample  
479 and carried out proviral sequencing and integration site analysis. We utilized integration site  
480 loop amplification (ISLA), which has been previously described, to obtain the integration sites  
481 associated with each viral sequence.<sup>84</sup> One modification that we made to this assay is targeting  
482 both the 5' and 3' ends of HIV to assess the integration site on both ends and eliminate any  
483 potential bias that may arise from analyzing only one end of HIV. To determine the exact  
484 location of HIV in the host gene, we used an online tool for trimming integration sites  
485 (<https://indra.mullins.microbiol.washington.edu/integrationsites/>).<sup>85</sup> We analyzed our integration

486 sites for various histone marks by utilizing Chromatin Immunoprecipitation Sequencing (ChIP-  
487 Seq) datasets from primary CD4<sup>+</sup> T cells that were publicly available on the ROADMAP  
488 website.<sup>38</sup> The NIH Roadmap Epigenomics Mapping Consortium produces a public resource of  
489 human epigenomic data to catalyze basic biology and disease-oriented research  
490 (<http://www.roadmapepigenomics.org/>). We calculated the total number of peaks of histone  
491 marks in a 10kb window from the flanking sides of the integration site and regarded it as the  
492 total peak number. To determine the distance between the IS and the nearest transcriptional  
493 start site (TSS), we employed "nearestTSS: Find Nearest Transcriptional Start Site," which is a  
494 tool developed in R.<sup>86</sup>

495

#### 496 **Assessment of HIV-specific CD8<sup>+</sup> T cell Reactivity**

497 Peripheral blood mononuclear cells (PBMC) were resuspended at 1x10<sup>6</sup>/mL in RPMI  
498 supplemented with 10% FBS (R10) and plated 200  $\mu$ L per well in Immobilon-P 96-well microtiter  
499 plates (Millipore) pre-coated with 2  $\mu$ g/mL anti-IFN- $\gamma$  (clone DK1, Mabtech). Individual HLA-  
500 optimal HIV peptides matched to each subject's HLA genotype were added at 1  $\mu$ M and  
501 incubated at 37°C overnight. Negative control wells did not receive peptide and positive control  
502 wells were treated with 1  $\mu$ g/mL anti-CD3 (clone OKT3, Biolegend) and 1  $\mu$ g/mL anti-CD28  
503 (clone CD28.8, Biolegend) antibodies. ELISOPT assay was performed using manufacturer's  
504 protocol with anti-IFN- $\gamma$  (clone 1-DK1, Mabtech) capture, biotinylated anti-IFN- $\gamma$  (clone B6-1,  
505 Mabtech) detection, Streptavidin-ALP (Mabtech) and AP Conjugated Substrate (BioRad)  
506 followed by disinfection with 0.05% Tween-20 (Thermo Fisher) and analysis using S6 Macro  
507 Analyzer (CTL Analyzers). Responses greater than 10 spots per well and 3-fold above negative  
508 controls were scored as positive.<sup>87,88</sup>

509

#### 510 **Assessment of HIV-specific CD8<sup>+</sup> T cell Proliferation**

511 PBMCs were stained at 37°C for 20 minutes with 0.5 µM CellTrace CFSE (Thermo Fisher) as  
512 per manufacturer’s protocol at 1x10<sup>6</sup> cells/mL. Staining was quenched with FBS (Sigma), cells  
513 were washed twice with R10, resuspended at 1x10<sup>6</sup>/mL and plated 200 µL per well in 96-well  
514 round-bottom polystyrene plates (Corning). Individual HIV peptides corresponding to IFN-γ  
515 ELISPOT responses for each patient were added at 1 µM and incubated at 37°C for 6 days  
516 before flow cytometric assessment. Negative control wells did not receive peptide and positive  
517 control wells received 1 µg/mL anti-CD3 (clone OKT3, Biolegend) and anti-CD28 (clone CD28.8,  
518 Biolegend) antibodies. On day 6, cells were stained for viability using Live/Dead Violet (Thermo  
519 Fisher), AlexaFluor700-anti-CD3 (clone SK7, Biolegend), BUV395-anti-CD8 (clone RPA-T8, BD  
520 Biosciences), and APC-pHLA tetramer matching the peptide used for stimulation, then analyzed  
521 by flow cytometry (Fig. S11).

522

### 523 **HLA typing and HIV escape mutation data analysis**

524 HLA-A/B/C typing was performed using sequence-specific oligonucleotide probing (PCR-SSOP)  
525 and sequence-based typing as previously described.<sup>89</sup> We excised individual HIV genes from  
526 proviral sequences using Gene Cutter  
527 ([https://www.hiv.lanl.gov/content/sequence/GENE\\_CUTTER/cutter.html](https://www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html)). We then identified  
528 polymorphisms within these genes that are known to be associated with one or more host HLA  
529 alleles expressed, as defined using a published list of HLA-associated polymorphisms across  
530 the HIV subtype B proteome.<sup>90</sup> For the escape mutation analysis, each HLA-associated viral site  
531 was categorized into one of three groups. 1) “Nonadapted” viral sites showed the specific HIV-1  
532 residue predicted to be susceptible to the restricting HLA, 2) “adapted” sites showed the specific  
533 HIV-1 residue predicted to confer escape from the restricting HLA, and 3) “possibly-adapted”  
534 sites showed any residue other than the “nonadapted” form, supporting it as a possible escape  
535 variant.<sup>91</sup>

536

537 **Limiting dilution viral outgrowth assay (VOA)**

538 PBMC from participants and donors without HIV were stimulated with IL-2 (100 U/ml) and PHA  
539 (1µg/ml) for 72 hours in R20 culture media. Then, we continued the stimulation with only with IL-  
540 2 in R20 culture media. VOAs were performed by using CD4<sup>+</sup> T cells isolated from  
541 cryopreserved peripheral blood mononuclear cells (PBMC) using EasySep™ Human CD4<sup>+</sup> T  
542 Cell Enrichment Kit (STEMCELL Technologies Inc.) from participants and healthy donors. Then  
543 we used MOLT-4/CCR5 cell lines and co-cultured those for more than 30 days, as reported  
544 previously.<sup>92</sup> We started with  $0.1 \times 10^6$  MOLT-4/CCR5 cells and  $0.5 \times 10^6$  CD4<sup>+</sup> T cells from our  
545 participants and healthy donor and cultured them in each well of a 24-Transwell® plates  
546 (STEMCELL Technologies Inc.). We collected samples from supernatant and MOLT-4/CCR5  
547 every 3 days and refreshed the media with IL-2 (100 U/ml) in R20.

548

549 **Data analysis**

550 We analyzed our results by using Mann-Whitney U tests (2-tailed), Fisher's exact tests,  
551 Wilcoxon's tests as appropriate. Correlations were tested by the Spearman's rank test.  
552 Adjustment for multiple comparisons was made in the analysis of ChIP-seq histone marks and  
553 host gene transcription, RNA-seq, and numbers of HLA escape mutations per HIV gene. A P-  
554 value of less than 0.05 was deemed significant. We adjusted for multiple comparisons in the  
555 analysis of ChIP-seq histone marks and host gene transcription, RNA-seq, and the number of  
556 HLA escape mutations per HIV gene. However, we did not make any corrections for multiple  
557 comparisons in the other analyses, as it was an exploratory analysis. We performed the  
558 statistical analysis using Prism (GraphPad v.7) and the statistical packages in R (R Project for  
559 Statistical Computing, version 4.1.0).

560

561 **Study approval**



562 All study participants provided written informed consent. The study was approved by the Mass  
563 General Brigham Institutional Review Board.

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578

579 **Data availability**

580 All data and code are available by request. Sequence data were submitted to Genbank  
581 (Accession numbers *Pending*). ROADMAP epigenomic data are available at  
582 <http://www.roadmapepigenomics.org>.

583 **References:**

- 584 1 Halvas, E. K. *et al.* HIV-1 viremia not suppressible by antiretroviral therapy can originate  
585 from large T cell clones producing infectious virus. *J Clin Invest* **130**, 5847-5857,  
586 doi:10.1172/JCI138099 (2020).
- 587 2 Laprise, C., de Pokomandy, A., Baril, J. G., Dufresne, S. & Trottier, H. Virologic failure  
588 following persistent low-level viremia in a cohort of HIV-positive patients: results from 12  
589 years of observation. *Clin Infect Dis* **57**, 1489-1496, doi:10.1093/cid/cit529 (2013).
- 590 3 Ryscavage, P., Kelly, S., Li, J. Z., Harrigan, P. R. & Taiwo, B. Significance and clinical  
591 management of persistent low-level viremia and very-low-level viremia in HIV-1-infected  
592 patients. *Antimicrob Agents Chemother* **58**, 3585-3598, doi:10.1128/AAC.00076-14  
593 (2014).
- 594 4 Redd, A. D. *et al.* ART Adherence, Resistance, and Long-term HIV Viral Suppression in  
595 Postpartum Women. *Open Forum Infect Dis* **7**, ofaa346, doi:10.1093/ofid/ofaa346 (2020).
- 596 5 Dharan, N. J. & Cooper, D. A. Long-term durability of HIV viral load suppression. *Lancet*  
597 *HIV* **4**, e279-e280, doi:10.1016/s2352-3018(17)30063-2 (2017).
- 598 6 Fleming, J. *et al.* Low-level viremia and virologic failure in persons with HIV infection  
599 treated with antiretroviral therapy. *Aids* **33**, 2005-2012,  
600 doi:10.1097/qad.0000000000002306 (2019).
- 601 7 Prendergast, A. J. *et al.* The impact of viraemia on inflammatory biomarkers and CD4+  
602 cell subpopulations in HIV-infected children in sub-Saharan Africa. *Aids* **35**, 1537-1548,  
603 doi:10.1097/qad.0000000000002916 (2021).
- 604 8 Castillo-Mancilla, J. R. *et al.* Suboptimal Adherence to Combination Antiretroviral  
605 Therapy Is Associated With Higher Levels of Inflammation Despite HIV Suppression.  
606 *Clinical Infectious Diseases* **63**, 1661-1667, doi:10.1093/cid/ciw650 (2016).
- 607 9 Li, J. Z. *et al.* Prevalence and Significance of HIV-1 Drug Resistance Mutations among  
608 Patients on Antiretroviral Therapy with Detectable Low-Level Viremia. *Antimicrobial*  
609 *Agents and Chemotherapy* **56**, 5998-6000, doi:doi:10.1128/AAC.01217-12 (2012).
- 610 10 Gunthard, H. F. *et al.* Human immunodeficiency virus replication and genotypic  
611 resistance in blood and lymph nodes after a year of potent antiretroviral therapy. *J Virol*  
612 **72**, 2422-2428 (1998).
- 613 11 Martinez-Picado, J. *et al.* Antiretroviral resistance during successful therapy of HIV type  
614 1 infection. *Proceedings of the National Academy of Sciences* **97**, 10948-10953,  
615 doi:doi:10.1073/pnas.97.20.10948 (2000).
- 616 12 Elvstam, O. *et al.* Virologic Failure Following Low-level Viremia and Viral Blips During  
617 Antiretroviral Therapy: Results From a European Multicenter Cohort. *Clin Infect Dis* **76**,  
618 25-31, doi:10.1093/cid/ciac762 (2023).
- 619 13 Boillat-Blanco, N. *et al.* Virological outcome and management of persistent low-level  
620 viraemia in HIV-1-infected patients: 11 years of the Swiss HIV Cohort Study. *Antivir Ther*  
621 **20**, 165-175, doi:10.3851/IMP2815 (2015).
- 622 14 Vancoillie, L. *et al.* Longitudinal sequencing of HIV-1 infected patients with low-level  
623 viremia for years while on ART shows no indications for genetic evolution of the virus.  
624 *Virology* **510**, 185-193, doi:10.1016/j.virol.2017.07.010 (2017).
- 625 15 Podsadecki, T. J., Vrijens, B. C., Tousset, E. P., Rode, R. A. & Hanna, G. J. Decreased  
626 adherence to antiretroviral therapy observed prior to transient human immunodeficiency  
627 virus type 1 viremia. *J Infect Dis* **196**, 1773-1778, doi:10.1086/523704 (2007).
- 628 16 Hermankova, M. *et al.* HIV-1 Drug Resistance Profiles in Children and Adults With Viral  
629 Load of <math>\leq 50</math> Copies/mL Receiving Combination Therapy. *JAMA* **286**, 196-207,  
630 doi:10.1001/jama.286.2.196 (2001).
- 631 17 Havlir, D. V. *et al.* Prevalence and predictive value of intermittent viremia with  
632 combination hiv therapy. *JAMA* **286**, 171-179 (2001).

- 633 18 Bull, M. E. *et al.* Monotypic low-level HIV viremias during antiretroviral therapy are  
634 associated with disproportionate production of X4 virions and systemic immune  
635 activation. *AIDS* **32**, 1389-1401, doi:10.1097/QAD.0000000000001824 (2018).
- 636 19 Li, J. Z. *et al.* Impact of pre-existing drug resistance on risk of virological failure in South  
637 Africa. *J Antimicrob Chemother* **76**, 1558-1563, doi:10.1093/jac/dkab062 (2021).
- 638 20 Castillo-Mancilla, J. R. *et al.* Tenofovir, emtricitabine, and tenofovir diphosphate in dried  
639 blood spots for determining recent and cumulative drug exposure. *AIDS Res Hum*  
640 *Retroviruses* **29**, 384-390, doi:10.1089/aid.2012.0089 (2013).
- 641 21 Yager, J. *et al.* Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in  
642 Dried Blood Spots Following Tenofovir Alafenamide: The TAF-DBS Study. *J Acquir*  
643 *Immune Defic Syndr* **84**, 323-330, doi:10.1097/qai.0000000000002354 (2020).
- 644 22 Castillo-Mancilla, J. *et al.* Emtricitabine-Triphosphate in Dried Blood Spots as a Marker  
645 of Recent Dosing. *Antimicrob Agents Chemother* **60**, 6692-6697,  
646 doi:10.1128/aac.01017-16 (2016).
- 647 23 Frasca, K. *et al.* Emtricitabine triphosphate in dried blood spots is a predictor of viral  
648 suppression in HIV infection and reflects short-term adherence to antiretroviral therapy. *J*  
649 *Antimicrob Chemother* **74**, 1395-1401, doi:10.1093/jac/dky559 (2019).
- 650 24 Morrow, M. *et al.* Predictive Value of Tenofovir Diphosphate in Dried Blood Spots for  
651 Future Viremia in Persons Living With HIV. *J Infect Dis* **220**, 635-642,  
652 doi:10.1093/infdis/jiz144 (2019).
- 653 25 Morrow, M. *et al.* Emtricitabine triphosphate in dried blood spots predicts future viremia  
654 in persons with HIV and identifies mismatch with self-reported adherence. *Aids* **35**,  
655 1949-1956, doi:10.1097/qad.0000000000002981 (2021).
- 656 26 Schroder, A. R. *et al.* HIV-1 integration in the human genome favors active genes and  
657 local hotspots. *Cell* **110**, 521-529, doi:S0092867402008644 [pii] (2002).
- 658 27 Wang, G. P., Ciuffi, A., Leipzig, J., Berry, C. C. & Bushman, F. D. HIV integration site  
659 selection: analysis by massively parallel pyrosequencing reveals association with  
660 epigenetic modifications. *Genome Res* **17**, 1186-1194, doi:gr.6286907 [pii]  
661 10.1101/gr.6286907 (2007).
- 662 28 Francis, A. C. *et al.* HIV-1 replication complexes accumulate in nuclear speckles and  
663 integrate into speckle-associated genomic domains. *Nat Commun* **11**, 3505,  
664 doi:10.1038/s41467-020-17256-8 (2020).
- 665 29 Maldarelli, F. *et al.* HIV latency. Specific HIV integration sites are linked to clonal  
666 expansion and persistence of infected cells. *Science* **345**, 179-183, doi:science.1254194  
667 [pii]  
668 10.1126/science.1254194 (2014).
- 669 30 Wang, Z. *et al.* Expanded cellular clones carrying replication-competent HIV-1 persist,  
670 wax, and wane. *Proc Natl Acad Sci U S A* **115**, E2575-E2584,  
671 doi:10.1073/pnas.1720665115 (2018).
- 672 31 Simonetti, F. R. *et al.* Antigen-driven clonal selection shapes the persistence of HIV-1-  
673 infected CD4+ T cells in vivo. *J Clin Invest* **131**, doi:10.1172/JCI145254 (2021).
- 674 32 Coffin, J. M. *et al.* Integration in oncogenes plays only a minor role in determining the in  
675 vivo distribution of HIV integration sites before or during suppressive antiretroviral  
676 therapy. *PLoS Pathog* **17**, e1009141, doi:10.1371/journal.ppat.1009141 (2021).
- 677 33 Bedwell, G. J., Jang, S., Li, W., Singh, P. K. & Engelman, A. N. rigrag: high-resolution  
678 mapping of genic targeting preferences during HIV-1 integration in vitro and in vivo.  
679 *Nucleic Acids Res* **49**, 7330-7346, doi:10.1093/nar/gkab514 (2021).

- 680 34 Lian, X. *et al.* Progressive transformation of the HIV-1 reservoir cell profile over two  
681 decades of antiviral therapy. *Cell Host Microbe* **31**, 83-96.e85,  
682 doi:10.1016/j.chom.2022.12.002 (2023).
- 683 35 Sun, W. *et al.* Phenotypic signatures of immune selection in HIV-1 reservoir cells. *Nature*,  
684 doi:10.1038/s41586-022-05538-8 (2023).
- 685 36 Einkauf, K. B. *et al.* Parallel analysis of transcription, integration, and sequence of single  
686 HIV-1 proviruses. *Cell* **185**, 266-282.e215, doi:10.1016/j.cell.2021.12.011 (2022).
- 687 37 Vansant, G. *et al.* The chromatin landscape at the HIV-1 provirus integration site  
688 determines viral expression. *Nucleic Acids Research* **48**, 7801-7817,  
689 doi:10.1093/nar/gkaa536 (2020).
- 690 38 Kundaje, A. *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* **518**,  
691 317-330, doi:10.1038/nature14248 (2015).
- 692 39 Einkauf, K. B. *et al.* Intact HIV-1 proviruses accumulate at distinct chromosomal  
693 positions during prolonged antiretroviral therapy. *J Clin Invest* **129**, 988-998,  
694 doi:10.1172/JCI124291 (2019).
- 695 40 Sun, Z. *et al.* H3K36me3, message from chromatin to DNA damage repair. *Cell &*  
696 *Bioscience* **10**, 9, doi:10.1186/s13578-020-0374-z (2020).
- 697 41 Ren, Y. *et al.* Selective BCL-X(L) Antagonists Eliminate Infected Cells from a Primary-  
698 Cell Model of HIV Latency but Not from Ex Vivo Reservoirs. *Journal of virology* **95**,  
699 e0242520, doi:10.1128/jvi.02425-20 (2021).
- 700 42 Ren, Y. *et al.* BCL-2 antagonism sensitizes cytotoxic T cell-resistant HIV reservoirs to  
701 elimination ex vivo. *The Journal of clinical investigation* **130**, 2542-2559,  
702 doi:10.1172/jci132374 (2020).
- 703 43 Philp, A. J. *et al.* The phosphatidylinositol 3'-kinase p85alpha gene is an oncogene in  
704 human ovarian and colon tumors. *Cancer research* **61**, 7426-7429 (2001).
- 705 44 Samuels, Y. *et al.* Mutant PIK3CA promotes cell growth and invasion of human cancer  
706 cells. *Cancer cell* **7**, 561-573, doi:10.1016/j.ccr.2005.05.014 (2005).
- 707 45 Lata, S., Mishra, R. & Banerjee, A. C. Proteasomal Degradation Machinery: Favorite  
708 Target of HIV-1 Proteins. *Frontiers in microbiology* **9**, 2738,  
709 doi:10.3389/fmicb.2018.02738 (2018).
- 710 46 Satou, Y. *et al.* Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-  
711 cell leukemia cells both in vivo and in vitro. *Leukemia* **18**, 1357-1363,  
712 doi:10.1038/sj.leu.2403400 (2004).
- 713 47 Hu, Y. *et al.* RUNX1 inhibits the antiviral immune response against influenza A virus  
714 through attenuating type I interferon signaling. *Virology journal* **19**, 39,  
715 doi:10.1186/s12985-022-01764-8 (2022).
- 716 48 Ono, M. *et al.* Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1.  
717 *Nature* **446**, 685-689, doi:10.1038/nature05673 (2007).
- 718 49 Utay, N. S. & Douek, D. C. Interferons and HIV Infection: The Good, the Bad, and the  
719 Ugly. *Pathogens & immunity* **1**, 107-116, doi:10.20411/pai.v1i1.125 (2016).
- 720 50 Dash, P. K., Kevadiya, B. D., Su, H., Banoub, M. G. & Gendelman, H. E. Pathways  
721 towards human immunodeficiency virus elimination. *EBioMedicine* **53**, 102667,  
722 doi:10.1016/j.ebiom.2020.102667 (2020).
- 723 51 Kaseke, C. *et al.* HLA class-I-peptide stability mediates CD8(+) T cell immunodominance  
724 hierarchies and facilitates HLA-associated immune control of HIV. *Cell Rep* **36**, 109378,  
725 doi:10.1016/j.celrep.2021.109378 (2021).
- 726 52 Cartwright, E. K. *et al.* CD8(+) Lymphocytes Are Required for Maintaining Viral  
727 Suppression in SIV-Infected Macaques Treated with Short-Term Antiretroviral Therapy.  
728 *Immunity* **45**, 656-668, doi:10.1016/j.immuni.2016.08.018 (2016).

- 729 53 Goulder, P. J. R. *et al.* Late escape from an immunodominant cytotoxic T-lymphocyte  
730 response associated with progression to AIDS. *Nature Medicine* **3**, 212-217,  
731 doi:10.1038/nm0297-212 (1997).
- 732 54 White, J. A. *et al.* Clonally expanded HIV-1 proviruses with 5'-Leader defects can give  
733 rise to nonsuppressible residual viremia. *The Journal of Clinical Investigation*,  
734 doi:10.1172/JCI165245 (2023).
- 735 55 Kuo, H.-H. *et al.* Anti-apoptotic Protein BIRC5 Maintains Survival of HIV-1-Infected CD4+  
736 T Cells. *Immunity* **48**, 1183-1194.e1185,  
737 doi:<https://doi.org/10.1016/j.immuni.2018.04.004> (2018).
- 738 56 Janssens, J., De Wit, F., Parveen, N. & Debyser, Z. Single-Cell Imaging Shows That the  
739 Transcriptional State of the HIV-1 Provirus and Its Reactivation Potential Depend on the  
740 Integration Site. *mBio* **13**, e0000722, doi:10.1128/mbio.00007-22 (2022).
- 741 57 Vansant, G. *et al.* The chromatin landscape at the HIV-1 provirus integration site  
742 determines viral expression. *Nucleic Acids Res* **48**, 7801-7817, doi:10.1093/nar/gkaa536  
743 (2020).
- 744 58 Grimwood, J. *et al.* The DNA sequence and biology of human chromosome 19. *Nature*  
745 **428**, 529-535, doi:10.1038/nature02399 (2004).
- 746 59 Singh, P. K., Bedwell, G. J. & Engelman, A. N. Spatial and Genomic Correlates of HIV-1  
747 Integration Site Targeting. *Cells* **11**, doi:10.3390/cells11040655 (2022).
- 748 60 Rout, S. S., Di, Y., Dittmer, U., Sutter, K. & Lavender, K. J. Distinct effects of treatment  
749 with two different interferon-alpha subtypes on HIV-1-associated T-cell activation and  
750 dysfunction in humanized mice. *Aids* **36**, 325-336, doi:10.1097/qad.0000000000003111  
751 (2022).
- 752 61 Soper, A. *et al.* Type I Interferon Responses by HIV-1 Infection: Association with  
753 Disease Progression and Control. *Front Immunol* **8**, 1823,  
754 doi:10.3389/fimmu.2017.01823 (2017).
- 755 62 Chun, T. W. *et al.* Suppression of HIV replication in the resting CD4+ T cell reservoir by  
756 autologous CD8+ T cells: implications for the development of therapeutic strategies.  
757 *Proc Natl Acad Sci U S A* **98**, 253-258, doi:10.1073/pnas.98.1.253  
98/1/253 [pii] (2001).
- 758 63 Gulzar, N. & Copeland, K. F. CD8+ T-cells: function and response to HIV infection. *Curr*  
759 *HIV Res* **2**, 23-37, doi:10.2174/1570162043485077 (2004).
- 760 64 Thomas, A. S. *et al.* T-cell responses targeting HIV Nef uniquely correlate with infected  
761 cell frequencies after long-term antiretroviral therapy. *PLoS Pathog* **13**, e1006629,  
762 doi:10.1371/journal.ppat.1006629 (2017).
- 763 65 Sudderuddin, H. *et al.* Longitudinal within-host evolution of HIV Nef-mediated CD4, HLA  
764 and SERINC5 downregulation activity: a case study. *Retrovirology* **17**, 3,  
765 doi:10.1186/s12977-019-0510-1 (2020).
- 766 66 Lawrence, D. C., Stover, C. C., Noznitsky, J., Wu, Z. & Summers, M. F. Structure of the  
767 Intact Stem and Bulge of HIV-1  $\Psi$ -RNA Stem-Loop SL1. *Journal of Molecular Biology*  
768 **326**, 529-542, doi:[https://doi.org/10.1016/S0022-2836\(02\)01305-0](https://doi.org/10.1016/S0022-2836(02)01305-0) (2003).
- 769 67 Durand, S. *et al.* Quantitative analysis of the formation of nucleoprotein complexes  
770 between HIV-1 Gag protein and genomic RNA using transmission electron microscopy.  
771 *Journal of Biological Chemistry* **298**, 101500,  
772 doi:<https://doi.org/10.1016/j.jbc.2021.101500> (2022).
- 773 68 White, J. A. *et al.* Clonally expanded HIV-1 proviruses with 5'-Leader defects can give  
774 rise to nonsuppressible residual viremia. *J Clin Invest*, doi:10.1172/jci165245 (2023).
- 775 69 van Bel, N., Das, A. T., Cornelissen, M., Abbink, T. E. & Berkhout, B. A short sequence  
776 motif in the 5' leader of the HIV-1 genome modulates extended RNA dimer formation  
777

- 778 and virus replication. *J Biol Chem* **289**, 35061-35074, doi:10.1074/jbc.M114.621425  
779 (2014).
- 780 70 White, J. A. *et al.* Measuring the latent reservoir for HIV-1: Quantification bias in near  
781 full-length genome sequencing methods. *PLoS Pathog* **18**, e1010845,  
782 doi:10.1371/journal.ppat.1010845 (2022).
- 783 71 Bailey, J. R. *et al.* Residual Human Immunodeficiency Virus Type 1 Viremia in Some  
784 Patients on Antiretroviral Therapy Is Dominated by a Small Number of Invariant Clones  
785 Rarely Found in Circulating CD4<sup>+</sup> T Cells. *Journal of Virology* **80**, 6441-6457,  
786 doi:doi:10.1128/JVI.00591-06 (2006).
- 787 72 De Scheerder, M. A. *et al.* HIV Rebound Is Predominantly Fueled by Genetically  
788 Identical Viral Expansions from Diverse Reservoirs. *Cell Host Microbe* **26**, 347-358.e347,  
789 doi:10.1016/j.chom.2019.08.003 (2019).
- 790 73 Cizmeci, D. *et al.* Distinct clonal evolution of B-cells in HIV controllers with neutralizing  
791 antibody breadth. *Elife* **10**, doi:10.7554/eLife.62648 (2021).
- 792 74 Palmer, S. *et al.* Low-level viremia persists for at least 7 years in patients on suppressive  
793 antiretroviral therapy. *Proceedings of the National Academy of Sciences* **105**, 3879-3884,  
794 doi:doi:10.1073/pnas.0800050105 (2008).
- 795 75 Hermankova, M. *et al.* HIV-1 drug resistance profiles in children and adults with viral  
796 load of <50 copies/ml receiving combination therapy. *JAMA* **286**, 196-207, doi:jpc10019  
797 [pii] (2001).
- 798 76 Bailey, J. R. *et al.* Residual human immunodeficiency virus type 1 viremia in some  
799 patients on antiretroviral therapy is dominated by a small number of invariant clones  
800 rarely found in circulating CD4<sup>+</sup> T cells. *J Virol* **80**, 6441-6457, doi:80/13/6441 [pii]  
801 10.1128/JVI.00591-06 (2006).
- 802 77 Zheng, J. H. *et al.* Application of an intracellular assay for determination of tenofovir-  
803 diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. *J*  
804 *Pharm Biomed Anal* **122**, 16-20, doi:10.1016/j.jpba.2016.01.038 (2016).
- 805 78 Einkauf, K. B. *et al.* Intact HIV-1 proviruses accumulate at distinct chromosomal  
806 positions during prolonged antiretroviral therapy. *The Journal of Clinical Investigation*  
807 **129**, 988-998, doi:10.1172/JCI124291 (2019).
- 808 79 Jiang, C. *et al.* Distinct viral reservoirs in individuals with spontaneous control of HIV-1.  
809 *Nature* **585**, 261-267, doi:10.1038/s41586-020-2651-8 (2020).
- 810 80 Tosiano, M. A., Jacobs, J. L., Shutt, K. A., Cyktor, J. C. & Mellors, J. W. A Simpler and  
811 More Sensitive Single-Copy HIV-1 RNA Assay for Quantification of Persistent HIV-1  
812 Viremia in Individuals on Suppressive Antiretroviral Therapy. *J Clin Microbiol* **57**,  
813 doi:10.1128/JCM.01714-18 (2019).
- 814 81 Cornwell, M. *et al.* VIPER: Visualization Pipeline for RNA-seq, a Snakemake workflow  
815 for efficient and complete RNA-seq analysis. *BMC bioinformatics* **19**, 135,  
816 doi:10.1186/s12859-018-2139-9 (2018).
- 817 82 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion  
818 for RNA-seq data with DESeq2. *Genome biology* **15**, 550, doi:10.1186/s13059-014-  
819 0550-8 (2014).
- 820 83 Korotkevich, G. *et al.* Fast gene set enrichment analysis. *bioRxiv*, 060012,  
821 doi:10.1101/060012 (2021).
- 822 84 Wagner, T. A. *et al.* Proliferation of cells with HIV integrated into cancer genes  
823 contributes to persistent infection. *Science* **345**, 570-573,  
824 doi:doi:10.1126/science.1256304 (2014).
- 825 85 Mullins Lab, U. o. W. *Integration sites*,  
826 <<https://indra.mullins.microbiol.washington.edu/integrationsites/>> (2015).

827 86 Smyth, G. *nearestTSS: Find Nearest Transcriptional Start Site*,  
828 <<https://rdrr.io/bioc/edgeR/man/nearestTSS.html>> (2021).  
829 87 Gaiha, G. D. *et al.* Structural topology defines protective CD8(+) T cell epitopes in the  
830 HIV proteome. *Science* **364**, 480-484, doi:10.1126/science.aav5095 (2019).  
831 88 Garcia-Bates, T. M. *et al.* Dendritic cells focus CTL responses toward highly conserved  
832 and topologically important HIV-1 epitopes. *EBioMedicine* **63**, 103175,  
833 doi:<https://doi.org/10.1016/j.ebiom.2020.103175> (2021).  
834 89 Apps, R. *et al.* Influence of HLA-C expression level on HIV control. *Science* **340**, 87-91,  
835 doi:10.1126/science.1232685 (2013).  
836 90 Carlson, J. M. *et al.* Correlates of Protective Cellular Immunity Revealed by Analysis of  
837 Population-Level Immune Escape Pathways in HIV-1. *Journal of Virology* **86**, 13202-  
838 13216, doi:doi:10.1128/JVI.01998-12 (2012).  
839 91 Warren, J. A. *et al.* The HIV-1 latent reservoir is largely sensitive to circulating T cells.  
840 *Elife* **9**, doi:10.7554/eLife.57246 (2020).  
841 92 Laird, G. M. *et al.* Rapid quantification of the latent reservoir for HIV-1 using a viral  
842 outgrowth assay. *PLoS Pathog* **9**, e1003398, doi:10.1371/journal.ppat.1003398 (2013).  
843



## 844 Main Figures description

845 **Figure 1.** Example participant with non-suppressible viremia (LV1). (a) Viral loads and CD4<sup>+</sup> T  
846 cell count from the time of virologic suppression. Downward green and orange arrows indicate  
847 timing of drug resistance and plasma drug level testing, respectively. Sampling times for viral  
848 genetic analyses are in black arrows. Antiretroviral resistance mutations are shown in the insert.  
849 (b) Neighbor joining trees of proviral and plasma *pol-env* sequences in blue and red,  
850 respectively. Producers (green boxes) defined as proviruses with exact matches to plasma RNA  
851 sequences. Non-producers (purple boxes) are proviruses that do not match any plasma RNA  
852 sequences. Shape indicates sampling time point, corresponding to black arrows in part (a). RPV,  
853 rilpivirine; TDF, tenofovir; FTC, emtricitabine; ATV/r, atazanavir/ritonavir; TAF, tenofovir  
854 alafenamide; DTG, dolutegravir; DRV/r, darunavir/ritonavir.

855

856 **Figure 2.** Sequencing overview of the non-suppressible viremia cohort. (a) Pie charts represent  
857 percentage of intact and defective proviral sequences for each participant. Neighbor joining  
858 trees show intact proviral and plasma sequences from different timepoints. The host integration  
859 sites of the producer proviruses are labeled. (b) Comparison of reservoir size (number of  
860 proviral sequences per million cells) for intact and defective proviruses between NSV and ART-  
861 suppressed individuals. For intact proviruses, a comparison is made of producer proviral versus  
862 non-producer proviral reservoir size in NSV participants versus intact proviral reservoir size of  
863 ART-suppressed individuals. Wilcoxon matched-pairs signed rank test and Mann-Whitney U  
864 tests were used for comparisons. ns, not significant; \*P < 0.05, \*\*P < 0.01.

865

866 **Figure 3.** Integration sites and chromatin features of HIV-1 proviruses. (a) Circos plot showing  
867 the location of each integration site across human chromosomes. (b) Karyotyping heatmap  
868 showing the percentage of integration sites in each human chromosome for different classes of  
869 proviruses. Fisher's exact test was used. (c) Number of peaks for key histone marks in 10 kb  
870 regions flanking the proviral integration sites. Mann-Whitney U test was used. (d) Correlation  
871 between enrichment of H3K36me3 histone marks near producer proviral integration sites and  
872 plasma clone viral loads (viral load multiplied by fraction of plasma sequences matching the  
873 producer provirus). Host gene integration sites are labeled. Spearman correlation test was used.  
874 \*P < 0.05, \*\*P < 0.01.

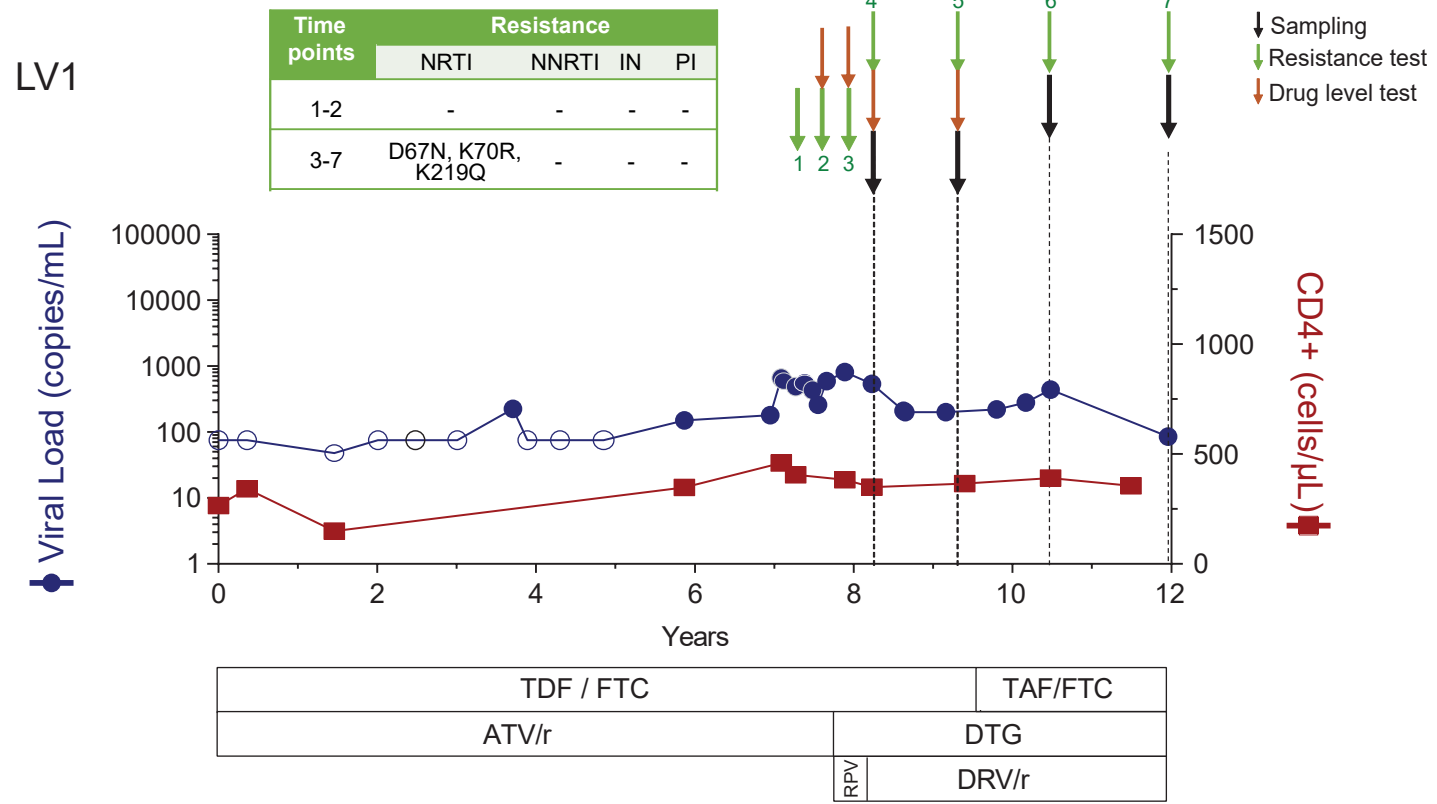
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876 **Figure 4.** Transcriptomic analysis of CD4<sup>+</sup> T cells from NSV participants. (a) Volcano plot shows  
877 differentially expressed genes in NSV versus ART-suppressed individuals. Red and blue colors  
878 highlight different extents of statistical significance. (b) Normalized enrichment score (NES)  
879 reflects the degree to which a set of genes is overrepresented among genes that are  
880 differentially expressed between NSV and ART-suppressed control participants. Bar-plot  
881 represents positively (red) and negatively (blue) correlated pathways. (c) Genes related to  
882 proteasome/ubiquitination in NSV participants. (d) Comparing anti-apoptotic and pro-apoptotic  
883 gene transcription levels between NSV and ART-suppressed control group. Mann-Whitney U  
884 tests were used for comparisons. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

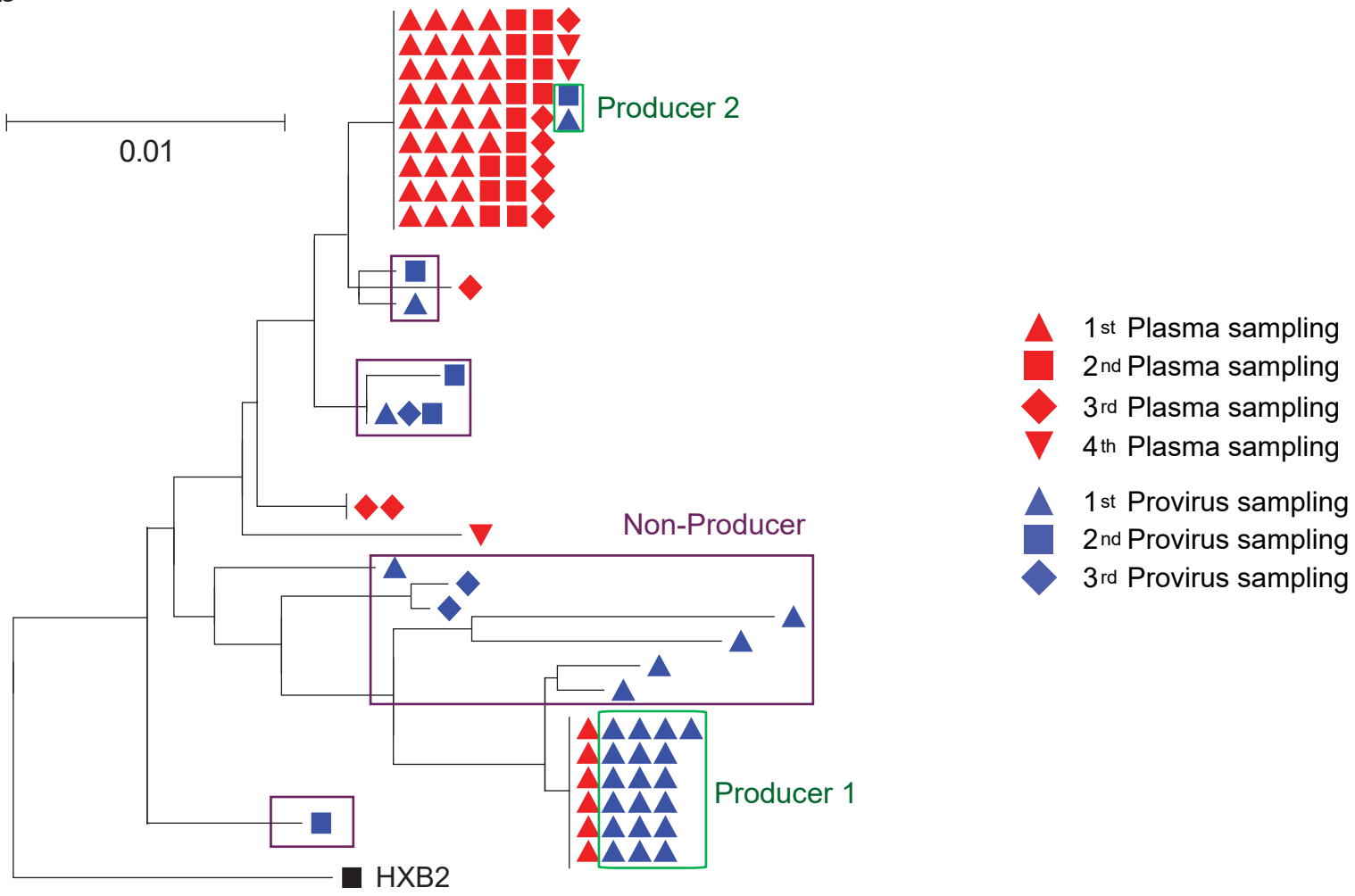
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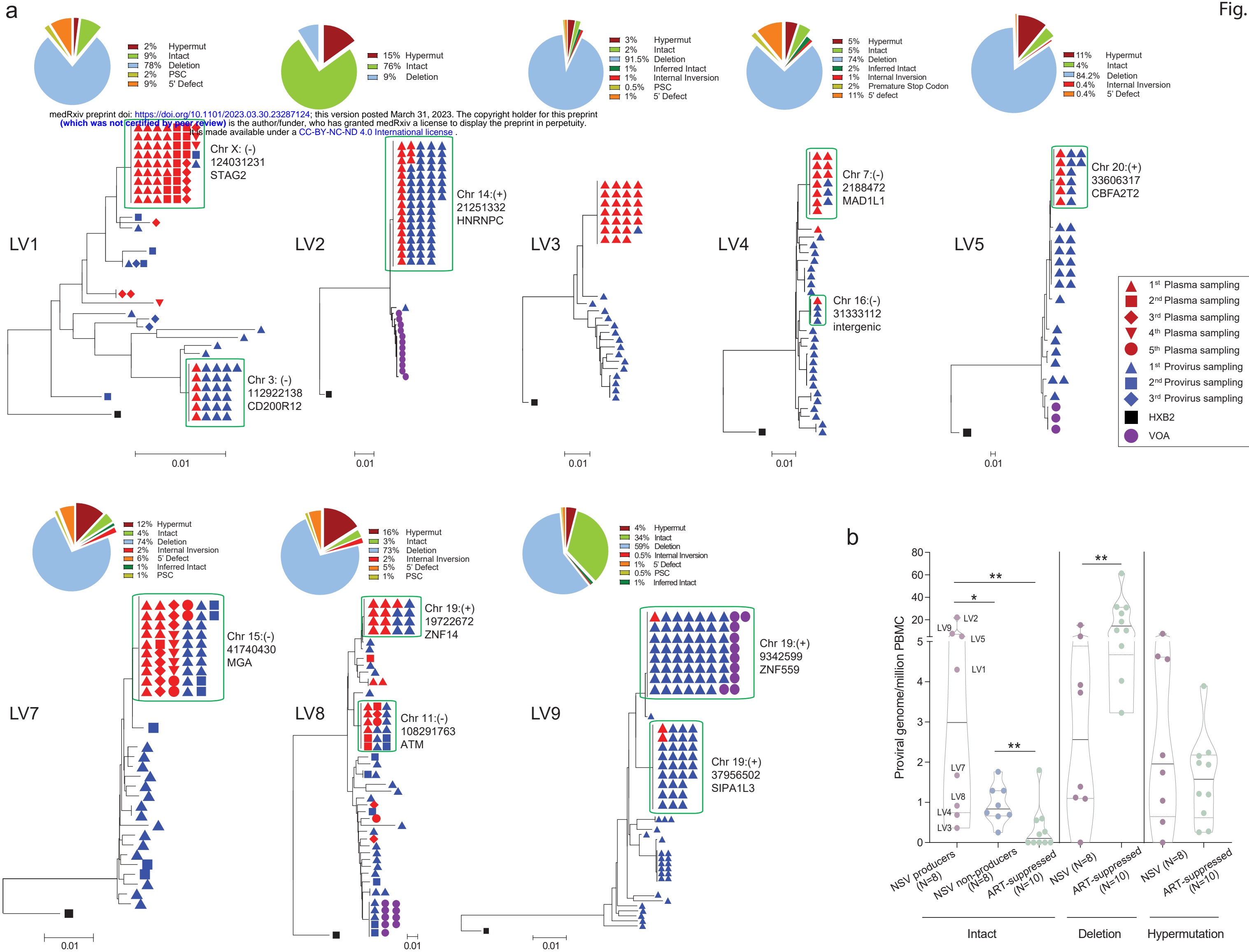
886 **Figure 5.** HIV-specific CD8<sup>+</sup> T cell response and HLA class I escape mutations. (a) HIV-specific  
887 CD8<sup>+</sup> T cell ELISPOT responses in NSV and control (ART-suppressed) cohorts. Mann-Whitney  
888 U test was used. (b) HIV-specific CD8<sup>+</sup> T cell proliferation responses. Mann-Whitney U test was  
889 used. (c) Average number of adapted and possible adapted HLA escape mutations across  
890 producer, non-producer, and defective proviral sequences. Wilcoxon matched-pairs signed rank  
891 testing was used. (d) Average number of mutations per base pair for each HIV gene in intact  
892 producer proviruses. Wilcoxon matched-pairs signed rank test was used. (e) Correlation  
893 between adapted and possible adapted mutations in different HIV genes in producer proviruses  
894 alongside CD8<sup>+</sup> T cell proliferation activity and percent intact provirus. Spearman correlation test  
895 was used. (f) Correlation between adapted and possible adapted mutations in three proviral  
896 classes and CD8<sup>+</sup> T cell activity (ELISPOT). Spearman correlation test was used. (g) and (h)  
897 Correlation between CD8<sup>+</sup> T cell activity (ELISPOT) versus average adapted and possible  
898 adapted mutations in *nef* and *pol* in producer proviruses (normalized for gene size). Spearman  
899 correlation test was used. ns, not significant; P > 0.05, \*P < 0.05, \*\*P < 0.01.

a

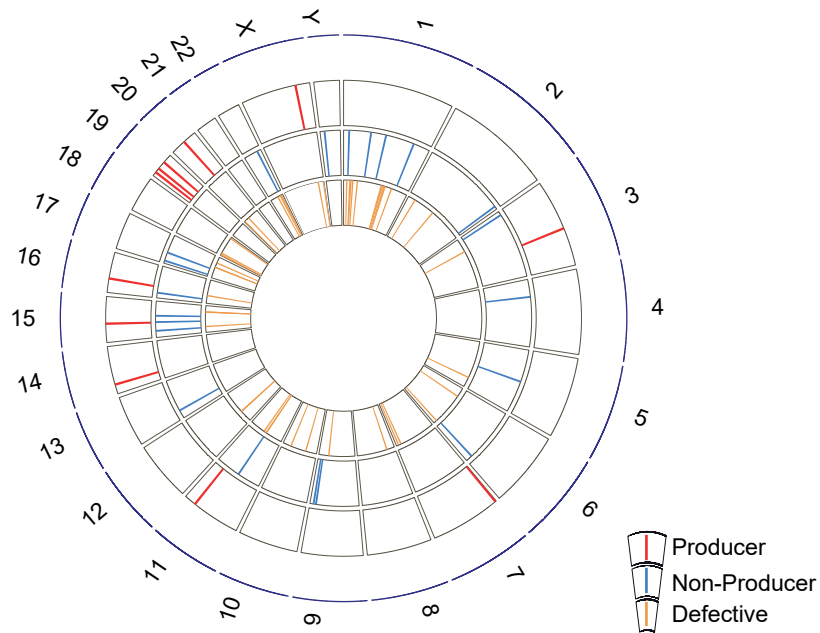


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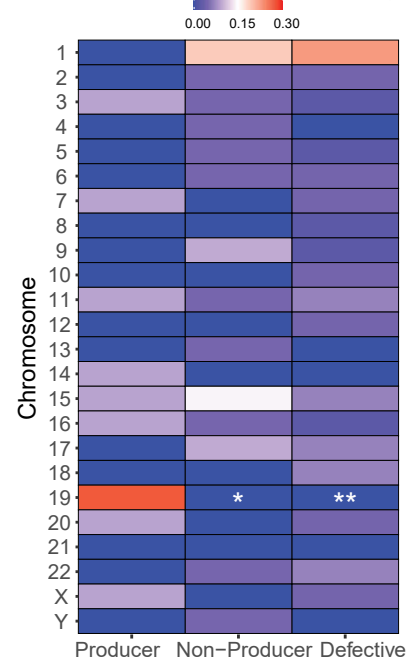




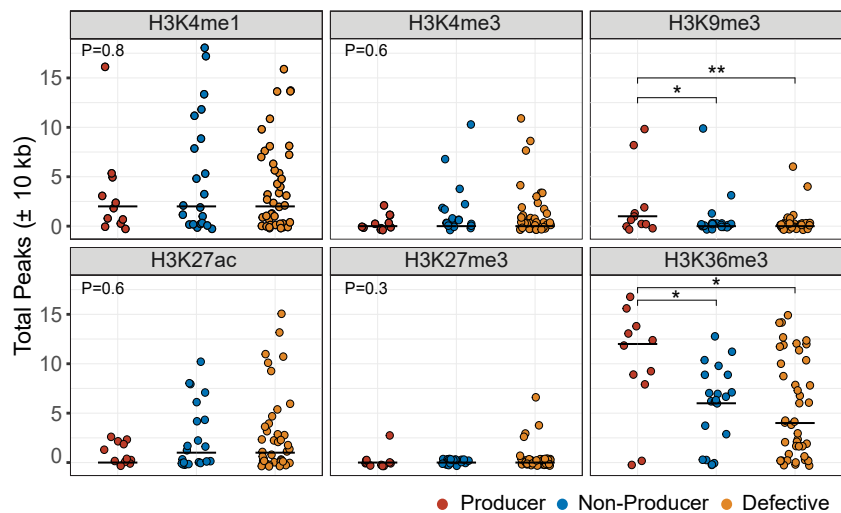
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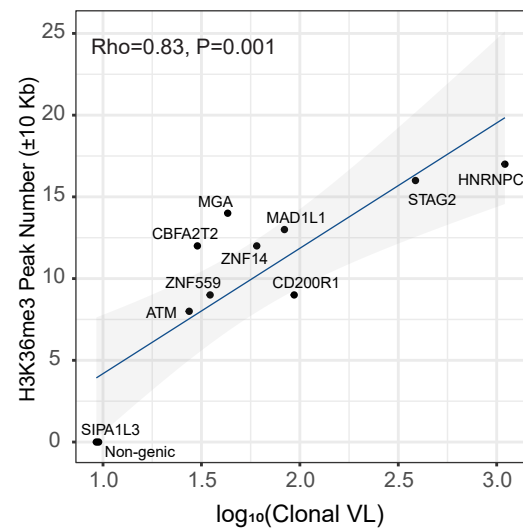


Fig. 4

