

## HIV

# Augmentation of HIV-specific T cell function by immediate treatment of hyperacute HIV-1 infection

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Sustained viremia after acute HIV infection is associated with profound CD4<sup>+</sup> T cell loss and exhaustion of HIV-specific CD8<sup>+</sup> T cell responses. To determine the impact of combination antiretroviral therapy (cART) on these processes, we examined the evolution of immune responses in acutely infected individuals initiating treatment before peak viremia. Immediate treatment of Fiebig stages I and II infection led to a rapid decline in viral load and diminished magnitude of HIV-specific (tetramer<sup>+</sup>) CD8<sup>+</sup> T cell responses compared to untreated donors. There was a strong positive correlation between cumulative viral antigen exposure before full cART-induced suppression and immune responses measured by MHC class I tetramers, IFN- $\gamma$  ELISPOT, and CD8<sup>+</sup> T cell activation. HIV-specific CD8<sup>+</sup> T responses of early treated individuals were characterized by increased CD127 and BCL-2 expression, greater in vitro IFN- $\gamma$  secretion, and enhanced differentiation into effector memory (T<sub>em</sub>) cells. Transcriptional analysis of tetramer<sup>+</sup> CD8<sup>+</sup> T cells from treated persons revealed reduced expression of genes associated with activation and apoptosis, with concurrent up-regulation of prosurvival genes including *BCL-2*, *AXL*, and *SRC*. Early treatment also resulted in robust HIV-specific CD4<sup>+</sup> T cell responses compared to untreated HIV-infected individuals. Our data show that limiting acute viremia results in enhanced functionality of HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, preserving key antiviral properties of these cells.

## INTRODUCTION

Despite considerable prevention efforts, continuing global HIV transmissions result in increasing numbers of lifelong infections; moreover, substantial scientific challenges remain in the quest for an effective vaccine or cure (1, 2). After transmission, there is considerable heterogeneity in the rate of disease progression, which is affected by the magnitude of the set point viral load and the initial CD4<sup>+</sup> T cell loss (3–5). Therefore, investigating factors that influence the quality of antiviral immune responses during the earliest stages of HIV infection may reveal specific responses that affect the clinical course of disease and inform vaccine development.

The emergence of HIV-specific CD8<sup>+</sup> T cell responses has consistently been associated with reduction in peak virus replication during primary infection (6–10). Rapid escape from HIV-specific CD8<sup>+</sup> T cell responses has been observed in acute infection, indicating antiviral function for at least a subset of these cells (11, 12). By twice-weekly screening of uninfected women at high risk of HIV infection in South Africa from the Females Rising Through Education Support and Health (FRESH) cohort (13, 14), we identified persons with hyperacute infection (defined as the period from onset of plasma viremia to peak viral load) and previously showed that

untreated (UnTx) infection is associated with massive HIV-specific CD8<sup>+</sup> T cell activation and that the magnitude and kinetics of the initial response affect set-point viral load (8); similar results have been reported in UnTx cohorts in Thailand and East Africa (15, 16). These studies identified defects in early responses that could contribute to lack of complete viral suppression, including increased T cell apoptosis and the inability to up-regulate prosurvival molecules, such as CD127 required for establishment of long-term immunologic memory (8). However, how these defects relate to overall antigen exposure and whether such defects could be abrogated by limiting antigen exposure in the earliest stages of infection remain unknown.

In contrast to UnTx acute infection, combination antiretroviral therapy (cART) initiation during and after peak viremia in primary HIV infection is associated with improved T cell functionality and reduction in the size of the viral reservoir and, in some cases, has been associated with prolonged remission after treatment discontinuation (17–20), suggesting that very early cART treatment in infection has a positive effect on antiviral immune responses. Moreover, numerous studies have shown that early therapy augments HIV-specific CD4<sup>+</sup> T cell responses [reviewed in (21)]. More recent studies demonstrate that initiation of cART before peak viremia limits antigen exposure and results in maintenance of CD4<sup>+</sup> T cell numbers (13, 22), but abrogates antibody responses and can result in nonreactive HIV serology (13, 23). However, the impact of immediate cART on the induction, evolution, and function of HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells has not been fully elucidated.

In this study, we conducted a detailed analysis of immune responses generated when cART is initiated in Fiebig stages I and II infection, before peak viremia, compared to treatment initiation at Fiebig stage III and later. Our findings have implications for the HIV vaccine and cure research and provide insights into the relationship between acute antigen exposure and T cell functionality.

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**RESULTS****A description of the HIV statuses and sample collection schedules for the three arms of the study**

We studied a total of 46 HIV-infected FRESH cohort participants classified into three groups (table S1). Group 1 consisted of 26 participants identified in Fiebig stages I and II who were initiated on cART within 24 to 48 hours of detection of plasma HIV RNA [treated (Tx) Fiebig stages I and II]. Group 2 consisted of 8 individuals identified in Fiebig stages III to V who were similarly immediately initiated on cART (Tx Fiebig stages III to V), and group 3 consisted of 12 participants identified in Fiebig stages I to V who did not initiate therapy during acute HIV infection (UnTx). A subset of the UnTx individuals was also sampled after subsequent ART initiation, which was based on the then-current South African guidelines of a CD4 count below 350 cells/ $\mu$ l. Group 1 samples were collected at a median of 28 days after enrollment and treatment initiation [interquartile range (IQR), 19.2 to 30 days], group 2 samples were collected at a median of 25 days (IQR, 16.5 to 29.5 days) after enrollment and treatment initiation, and UnTx samples were collected at a median of 28 days (IQR, 24 to 30.5 days) after enrollment. For groups 1 and 2, most samples were at a time when complete plasma viral suppression had already been achieved. Linear regression analysis showed that differences in treatment duration before sample collection among early Tx individuals did not significantly affect the measured immunological parameters reported in subsequent figures (table S2).

**Very early cART initiation results in induction of significantly lower magnitude HIV-specific CD8<sup>+</sup> T cell responses compared to UnTx HIV infection**

Very early cART initiation in hyperacute infection leads to lack of antibody seroconversion in the majority of infected individuals (table S3) (13, 23), but the impact of early therapy on the induction and magnitude of emerging CD8<sup>+</sup> T cell responses is not well characterized. To address this, we assessed HIV-specific CD8<sup>+</sup> T cell responses in individuals initiating therapy during Fiebig stages I and II or III to V and compared these responses to analogous time points during UnTx infection.

First, we used an interferon- $\gamma$  (IFN- $\gamma$ ) enzyme-linked immunospot (ELISPOT) assay with overlapping consensus clade C peptides spanning HIV Gag, Nef, and Env proteins to measure the breadth of HIV-specific T cell responses at the time of peak CD8<sup>+</sup> T cell activation in UnTx infection (8). Eighteen of 26 (69%) Tx Fiebig stages I and II individuals had detectable ELISPOT responses, whereas 5 of 6 (83%) Tx Fiebig stages III to V and 12 of 12 UnTx persons had detectable responses, differences that did not reach statistical significance ( $\chi^2 = 4.84$ ,  $df = 2$ ,  $P = 0.09$ ; Fig. 1A). However, UnTx individuals targeted significantly more peptides compared to Tx Fiebig stages I and II (Mann-Whitney test,  $P = 0.0001$ ) or Tx Fiebig stages III to V ( $P = 0.02$ ; Fig. 1B).

Next, we performed HIV-specific CD8<sup>+</sup> T cell proliferation assays after stimulation with Gag, Nef, and Env peptide pools. These were performed at similar time points to those used in the ELISPOT assays, but because of sample limitations, not all individuals could be tested with both assays. Eighteen of 20 Tx Fiebig stages I and II individuals tested (90%) had detectable proliferative responses, as did 6 of 7 (86%) Tx Fiebig stages III to V and 5 of 6 (83%) UnTx donors. There was no significant difference between groups in the number of donors with detectable responses ( $\chi^2 = 0.23$ ,  $df = 2$ ,  $P = 0.9$ ; Fig. 1C), nor in the overall breadth of responses [Mann-Whitney test,  $P =$  not significant (ns); Fig. 1D].

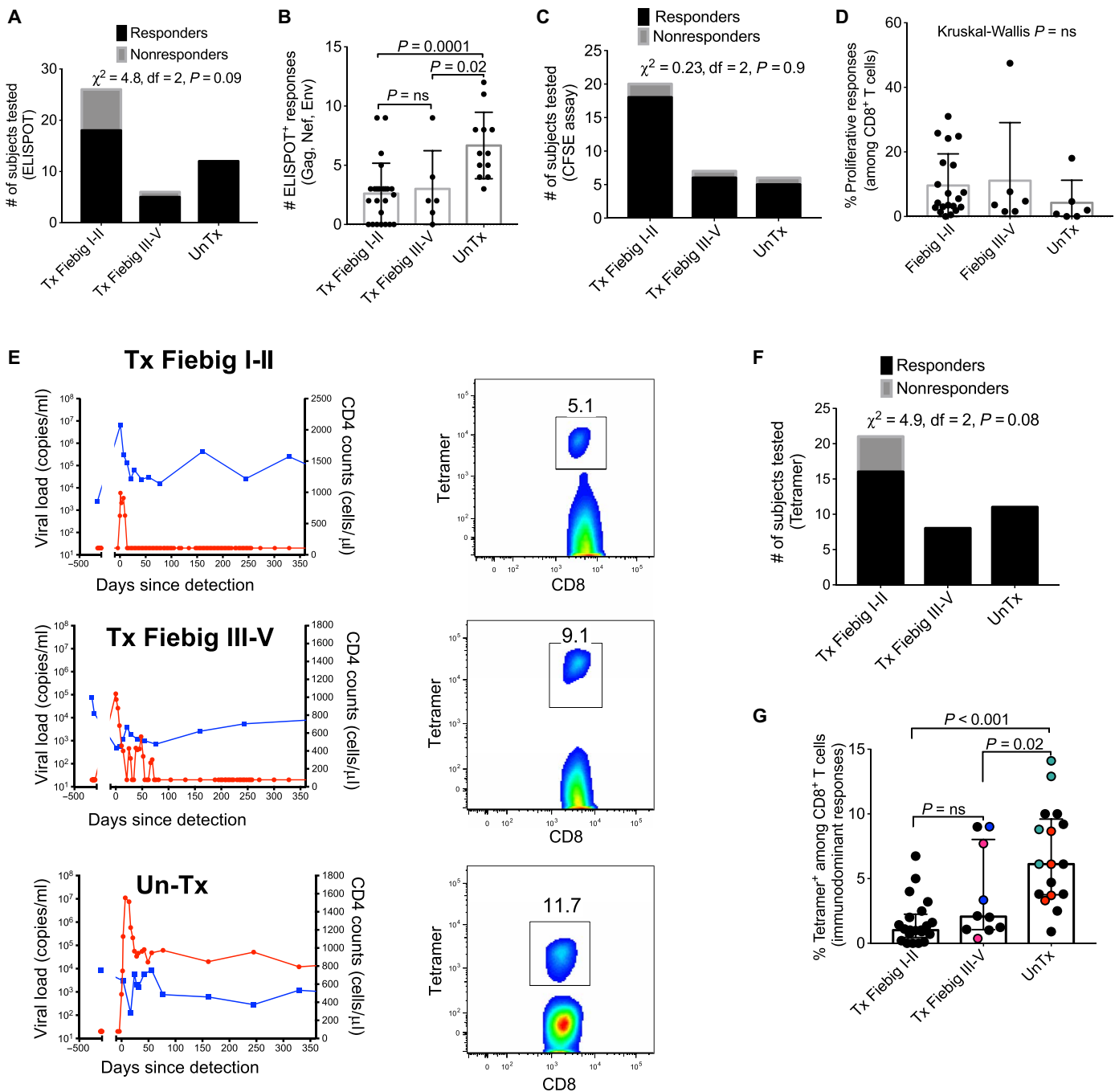
We then performed tetramer staining in samples from Tx Fiebig stages I and II individuals as a quantitative measure of individual HIV-specific T cell responses, independent of function, at similar time points as the ELISPOT and proliferation analyses. These studies were limited to those who expressed human leukocyte antigen (HLA) class I alleles for whom tetramers were available, with representative examples shown in Fig. 1E. All UnTx ( $n = 12$ ) and Tx Fiebig stages III to V ( $n = 8$ ) participants had detectable tetramer responses (Fig. 1F), and 80% of Tx Fiebig stages I and II participants had detectable responses, a difference that was not statistically significant ( $\chi^2 = 4.92$ ,  $df = 2$ ,  $P = 0.08$ ). Early Tx individuals had significantly lower magnitude of tetramer<sup>+</sup> cells compared to UnTx individuals (linear mixed-effects regression analysis: Tx Fiebig stages I and II versus UnTx,  $P = <0.001$ ; Tx Fiebig stages III to V versus UnTx,  $P = 0.02$ ; Fig. 1G).

Overall, 25 of 26 (96%) Tx Fiebig stages I and II individuals had detectable HIV-specific CD8<sup>+</sup> T cell responses by at least one of the assays used. Of note, seven of the Tx Fiebig stages I and II individuals with detectable CD8<sup>+</sup> T cell responses had no detectable HIV antibody responses by either Western blot or by enzyme immunoassay (table S3). Thus, in contrast to HIV-specific antibody responses, most of early Tx individuals develop detectable HIV-specific CD8<sup>+</sup> T cell responses, but with reduced magnitude and breadth compared to UnTx individuals.

**Cumulative HIV exposure before cART-mediated suppression correlates with the magnitude of HIV-specific CD8<sup>+</sup> T cell responses in individuals who initiate cART in Fiebig stage I or II**

We next investigated the impact of cumulative viral exposure on the generation of CD8<sup>+</sup> T cell responses by calculating the area under the plasma viral load curve over time. We termed this parameter “viremia copy-days” (VCD), similar to how cumulative HIV burden has been estimated in chronic HIV infection (24). VCD for Tx Fiebig stages I and II donors ranged over 2000-fold, from as little as 3.2 log<sub>10</sub> VCD to as much as 6.6 log<sub>10</sub> VCD (IQR, 4.1 to 5.7 log<sub>10</sub> VCD). We observed a positive correlation between VCD and the magnitude of tetramer<sup>+</sup> responses at peak time point in the 21 Tx Fiebig stages I and II participants for whom class I tetramer responses were detected (Spearman  $r = 0.6$ ,  $P = 0.007$ ; Fig. 2A). Similarly, there was a positive correlation between the breadth of responses (number of positive responses) measured by IFN- $\gamma$  ELISPOT and VCD in the 26 Tx Fiebig stages I and II participants with positive ELISPOT responses (Spearman  $r = 0.6$ ,  $P = 0.003$ ; Fig. 2B).

We and others have previously shown that during hyperacute HIV infection, most activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells are directed toward HIV antigens (8, 16). Therefore, we next investigated the relationship between the frequency of antigen-specific CD8<sup>+</sup> T cells measured by coexpression of activation markers (CD38<sup>+</sup>HLA-DR<sup>+</sup>) and VCD. Similarly, we found a strong positive correlation between the two parameters in the 20 Fiebig stages I and II participants tested (Spearman  $r = 0.8$ ,  $P = 0.001$ ; Fig. 2C). We then used the Youden index, typically used to determine the optimal cutoff for diagnostic tests (25), to identify the minimal VCD that would predict the development of a positive HIV-specific T cell ELISPOT response. We found the cutoff to be 3.8 log<sub>10</sub> VCD (Youden index, 0.541; sensitivity, 0.94; specificity, 0.60; area under receiver-operator curve at cutoff point, 0.77). Together, these data indicate that total antigen exposure in persons initiating ART during Fiebig stages I and II infection influences the detection, magnitude, and breadth of HIV-specific CD8<sup>+</sup> T cell responses.

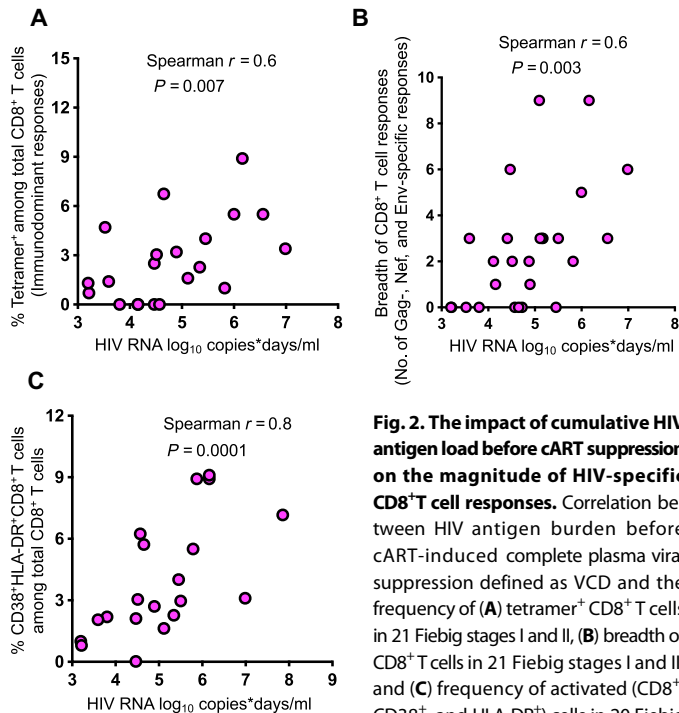


**Fig. 1. Effect of very early cART initiation on the induction of HIV-specific CD8<sup>+</sup>T cell responses.** (A) Number of study participants tested in each of the three donor groups using ELISPOT assay. Peripheral blood mononuclear cells (PBMCs) were stimulated with individual overlapping peptides spanning Gag, Nef, and Env proteins derived from HIV-1 clade C. (B) ELISPOT data denoting cumulative number of responses to clade C HIV Gag, Nef, and Env peptides in 24 Fiebig stages I and II, 6 Fiebig stages III to V, and 12 UnTx individuals. (C) Number of study participants tested in each group using the carboxyfluorescein succinimidyl ester (CFSE) proliferation assays. PBMCs were stimulated with Gag, Nef, and Env peptide pools derived from HIV-1 clade C. One peptide pool was used for each protein. (D) Cumulative percentage of proliferating CD8<sup>+</sup> T cells in response to Gag, Nef, and Env HIV-1 clade C peptide pools in 21 Fiebig stages I and II, 6 Fiebig stages III to V, and 6 UnTx individuals. (E) Left column shows longitudinal plasma HIV RNA (red; RNA copies per milliliter plasma) and absolute CD4<sup>+</sup> T cell counts (blue; CD4<sup>+</sup> T lymphocytes per microliter) before HIV infection and after onset of detectable plasma viremia in three representative individuals. The right column shows representative immunodominant tetramer<sup>+</sup> responses for each individual measured at the peak of the response (14 to 42 days after diagnosis). Data are arranged according to treatment initiation status. (F) Number of responding and nonresponding participants tested in each group using the MHC class I tetramers. (G) Frequencies of immunodominant tetramer<sup>+</sup> CD8<sup>+</sup> T cells in 21 Fiebig stages I and II, 8 Fiebig stages III to V, and 11 UnTx individuals. Statistical significance was calculated using multilevel linear mixed-effects regression analyses when comparing between groups to account for multiple measurements within some individuals. Black dots denote a single tetramer measurement per donor. Dots of the same color denote the sum of multiple tetramer measurements from a single donor. Horizontal lines represent median with IQR.

**Table 1. Baseline parameters and sample collection time points for the three arms of the study.**

Number in group	Group	Days from diagnosis to Tx*	Days to sample collection*	Viral load at sample collection*	CD4 counts at sample collection*
26	1: Tx Fiebig stages I and II	1.0 (1.0–2.0)	28.0 (20.3–30.0)	20 (20–20)	862.0 (669–977)
8	2: Tx Fiebig stages III to V	1.0 (1.0–23.0)	27.0 (21.0–28.0)	20 (20–20)	486 (423–599)
12	3: UnTx	-	28 (24.0–30.0)	665,000 (18,750–1,357,500)	547 (502–623)

\*Values reported as median (IQR).



**Fig. 2. The impact of cumulative HIV antigen load before cART suppression on the magnitude of HIV-specific CD8<sup>+</sup> T cell responses.** Correlation between HIV antigen burden before cART-induced complete plasma viral suppression defined as VCD and the frequency of (A) tetramer<sup>+</sup> CD8<sup>+</sup> T cells in 21 Fiebig stages I and II, (B) breadth of CD8<sup>+</sup> T cells in 21 Fiebig stages I and II, and (C) frequency of activated (CD8<sup>+</sup>, CD38<sup>+</sup>, and HLA-DR<sup>+</sup>) cells in 20 Fiebig

stages I and II individuals, respectively, at 14 to 42 days after detection of plasma viremia. Spearman's rank correlation test was used. Two-tailed *P* values are reported.

### Treatment initiation during hyperacute HIV infection results in greater IFN- $\gamma$ production and increased CD127 expression on HIV-specific CD8<sup>+</sup> T cells compared to UnTx HIV infection

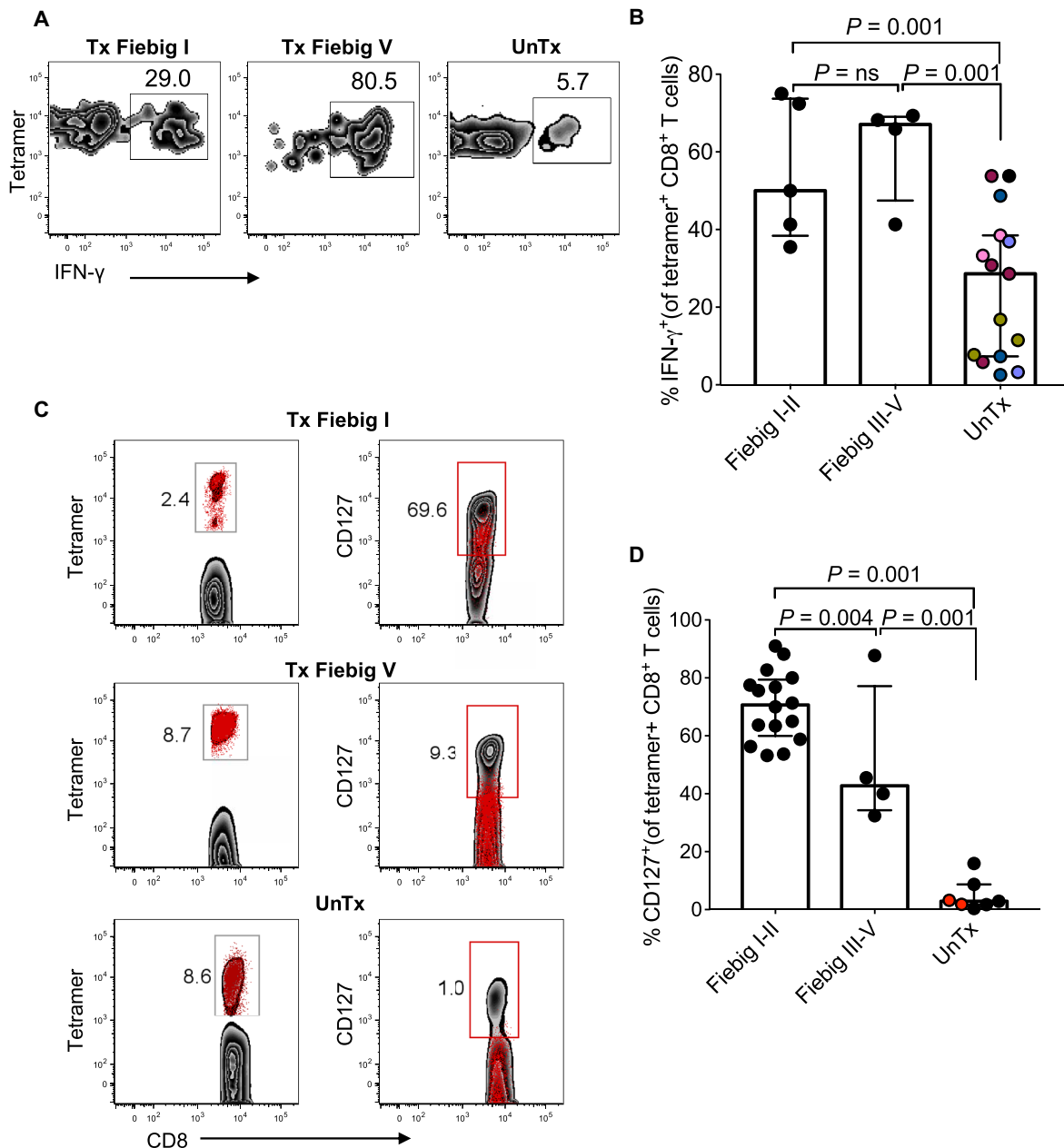
We previously demonstrated that UnTx hyperacute HIV infection is associated with high-magnitude HIV-specific CD8<sup>+</sup> T cell responses, exhibiting functional defects including decreased IFN- $\gamma$  secretion, down-regulation of the antiapoptotic marker B cell lymphoma 2 (BCL-2), and lower expression of the memory marker CD127, which is required for long-term survival (8). We next investigated whether prompt cART-mediated reduction in HIV replication alters the expression of these proteins. Tetramer staining, together with intracellular cytokine staining (ICS), demonstrated that early treatment improved IFN- $\gamma$  secretion capacity of HIV-specific responses after stimulation with Gag peptides compared to UnTx controls (Fig. 3A) and that this was significant for both Tx Fiebig stages I and II and Tx Fiebig stages III to V donors (linear mixed-effects regression analysis: Tx Fiebig stages I and II versus UnTx, *P* = 0.001; Tx Fiebig stages III to V versus UnTx, *P* < 0.001; Fig. 3B).

Another profound defect observed in HIV-specific CD8<sup>+</sup> T cell differentiation in UnTx infection is reduced ability to express the interleukin-7 receptor  $\alpha$  (CD127) (8, 16, 26, 27), which is required for long-term cell survival (28) and has been shown to inversely correlate with viral load set point (29). We next evaluated the impact of early treatment on the expression of this marker for long-lived memory cells. Representative results (Fig. 3C) and aggregate data (Fig. 3D) show that HIV-specific CD8<sup>+</sup> T cells associated with early treatment, whether in Fiebig stages I and II or III to V, resulted in higher frequencies of tetramer<sup>+</sup> CD127<sup>+</sup> cells among tetramer<sup>+</sup> cells compared to UnTx infection (Mann-Whitney test, *P* = <0.001), indicating that cumulative antigen exposure affects long-term survival of antigen-specific cells.

### cART-mediated reduction in antigen burden limits transcriptional activation of HIV-specific CD8<sup>+</sup> T cells in hyperacute infection

The observed impact of early treatment on cytokine secretion and CD127 expression by HIV-specific CD8<sup>+</sup> T cells led us to investigate the broader impact of treatment by performing longitudinal transcriptional analysis on this cell population. Tetramer<sup>+</sup> CD8<sup>+</sup> T cells were sorted at multiple time points after infection, using PBMC from four early Tx (Fiebig stages III to V) and four UnTx individuals, profiled by RNA sequencing (RNA-seq; Fig. 4A). We also sorted cytomegalovirus (CMV)-specific tetramer<sup>+</sup> CD8<sup>+</sup> T cells in one early Tx and two UnTx individuals and CD8<sup>+</sup> T cell populations depleted of tetramer<sup>+</sup> cells for all individuals. Across all time points sampled, the HIV-specific tetramer<sup>+</sup> CD8<sup>+</sup> T cells demonstrated the largest number of differentially expressed genes [false discovery ratio (FDR)-adjusted *P* value, *q* < 0.05] during the first year of infection (fig. S1A and tables S4 and S5). We observed very few transcriptional changes in the CMV-specific CD8<sup>+</sup> T cells or the remaining bulk CD8<sup>+</sup> T cells at all the time points measured compared to pre-HIV infection time point, consistent with our earlier report of a lack of bystander CD8<sup>+</sup> T cell activation in UnTx (8). Furthermore, direct comparison of CMV-specific and HIV-specific transcriptome data at the available time points revealed no significant differentially expressed genes between these infection-specific responses at late and long-term time points (table S6).

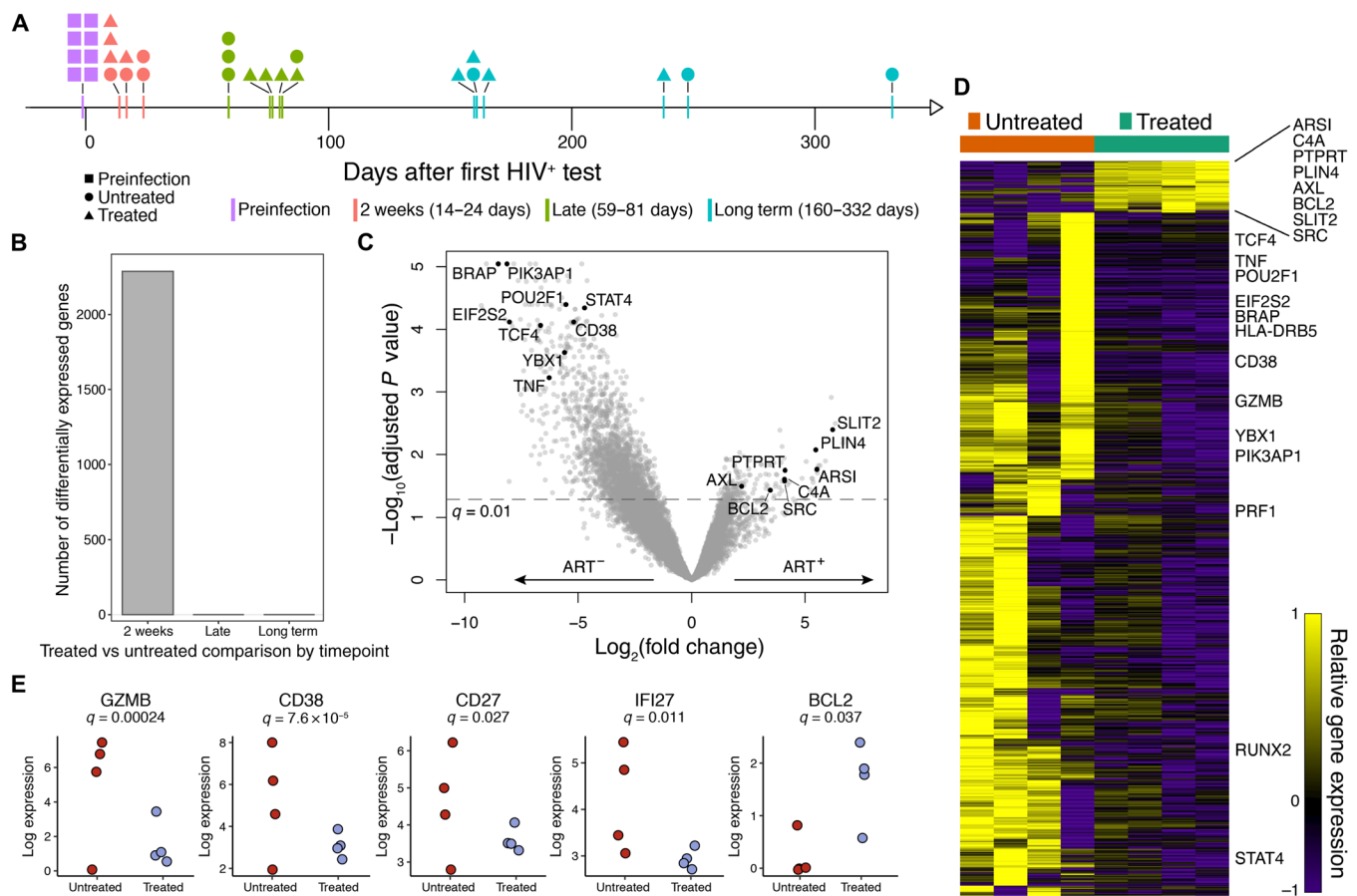
Comparing the transcriptomes of the HIV-specific tetramer<sup>+</sup> CD8<sup>+</sup> T cells between early Tx and UnTx individuals as a function of time since HIV detection, we observed substantial differential expression only at the time point closest to peak viremia ("2 weeks"; Fig. 4B and table S7). Early treatment mitigated the transcriptional response in HIV-specific CD8<sup>+</sup> T cells (Fig. 4, C and D). Specifically, genes associated not only with immune activation (e.g., *CD38*, *TNF*,



**Fig. 3. The impact of cART initiation on cytokine secretion potential and phenotypic characteristics of HIV-specific CD8<sup>+</sup>T cell responses.** HLA class I tetramer binding cells were tested by ICS for IFN- $\gamma$  production in response to HIV peptide stimulation. (A) Representative data for one Tx Fiebig stage I and II donor, one Tx Fiebig stages III to V individual, and one UnTx donor are shown. Flow panels are gated on IFN- $\gamma$ -secreting cells. (B) Aggregate data depicting IFN- $\gamma$ -secreting tetramer<sup>+</sup> CD8<sup>+</sup> T cells. Black dots denote a single measurement per donor in five Fiebig stages I and II, four Fiebig stages III to V, and six UnTx individuals. Dots of the same color denote multiple measurements from a single donor. (C) All flow plots gated on CD8<sup>+</sup> T cells. The left column shows flow plots gated on tetramer<sup>+</sup> cells (red dots). The right column shows tetramer<sup>+</sup> cells (red dots) overlaid over total CD8<sup>+</sup> T cells (gray background). (D) Aggregate for frequencies of CD127<sup>+</sup> tetramer<sup>+</sup> cells in 16 Fiebig stages I and II, 4 Fiebig stages III to V, and 6 UnTx individuals. Black dots denote a single measurement per donor. Dots of the same color denote multiple measurements from a single donor. Samples were tested between 21 and 28 days after diagnosis, as indicated in the figures. Statistical significance for aggregate data was calculated using multilevel linear mixed-effects regression analyses when comparing between groups to account for multiple measurements within some individuals. Horizontal lines represent median with IQR.

and *STAT4*) but also with cellular translation (e.g., *BRAP*, *POU2F1*, and *EIF2S2*) were down-regulated in tetramer<sup>+</sup> cells from Tx individuals compared to UnTx persons. Fewer genes were up-regulated in cells from Tx individuals compared to UnTx, suggesting a much tighter transcriptional response in the presence of cART and an antigen-limited environment. Some of the genes up-regulated in cells

from Tx individuals (e.g., *BCL-2*, *AXL*, and *SRC*) have known roles in cell survival with the potential to give rise to memory CD8<sup>+</sup> T cells (30–32), whereas many of the genes up-regulated in cells from UnTx individuals were significantly enriched for pathways associated with overactivation and cellular dysfunction (fig. S1B). In particular, the eukaryotic initiation factor 2 signaling pathway, involved in



**Fig. 4. Transcriptional signatures of HIV-specific CD8<sup>+</sup> T cell responses in Tx hyperacute and UnTx hyperacute HIV infection.** Transcriptional responses of CD8<sup>+</sup> T cells in UnTx ( $n = 4$ ) and early Tx ( $n = 4$ ) individuals along the course of acute infection. (A) Timeline of collected RNA samples with samples binned into time frames denoted by color. (B) Number of differentially expressed genes between HIV-specific CD8<sup>+</sup> T cells from UnTx and early Tx individuals at various time points. (C) Volcano plot depicting differentially expressed genes ( $FDR, q < 0.01$ , edgeR likelihood ratio test) in HIV-specific CD8<sup>+</sup> T cells at the 2-week time frame comparing Tx and UnTx individuals. Genes of interest are annotated by name. (D) Heatmap depicting differentially expressed genes from (C), row normalized expression. (E) Scaled log-normalized expression values of genes of interest. The significances reported were calculated as in (C).

protein synthesis, and the integrated stress response were strongly enriched in UnTx individuals ( $P = 7.9 \times 10^{-23}$ ), as well as several genes associated with apoptosis (fig. S1C).

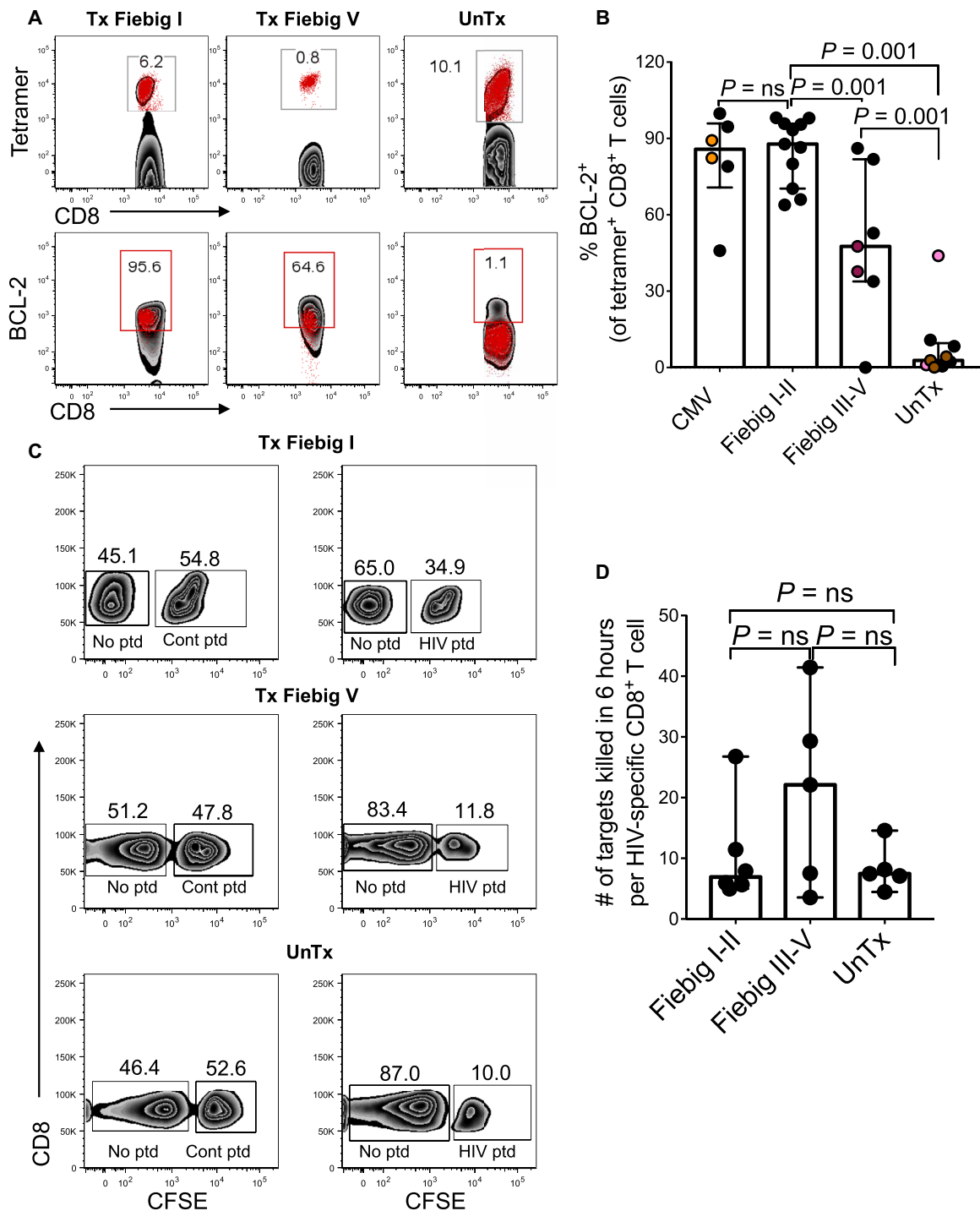
One of the few genes that was differentially up-regulated in cells from Tx donors compared to UnTx donors was *BCL-2* ( $q = 0.04$ ), an antiapoptotic molecule implicated in memory generation (31). We verified the transcriptional data (Fig 4E) by measuring the BCL-2 protein expression in tetramer-sorted HIV-specific CD8<sup>+</sup> T cells at peak viremia and found the highest expression in Tx Fiebig stages I and II compared to UnTx participants (linear mixed-effects regression analysis: Mann-Whitney test,  $P < 0.001$ ; Fig. 5, A and B). Early treatment led to BCL-2 expression comparable to CMV-specific CD8<sup>+</sup> T cells (Fig. 5B).

Transcriptional analysis also revealed that HIV-specific CD8<sup>+</sup> T cells from UnTx donors expressed significantly more granzyme B ( $FDR, q = 0.00024$ ) compared to early Tx donors (11). Thus, we investigated whether higher mRNA expression of cytolytic genes translated into superior killing of HIV-infected targets by measuring the intrinsic killing capacity of HIV-specific CD8<sup>+</sup> T cells using a 4-hour direct killing assay. Representative plots (Fig. 5C) and summary data (Fig. 5D) show CD8<sup>+</sup> T cells killing peptide-pulsed targets incu-

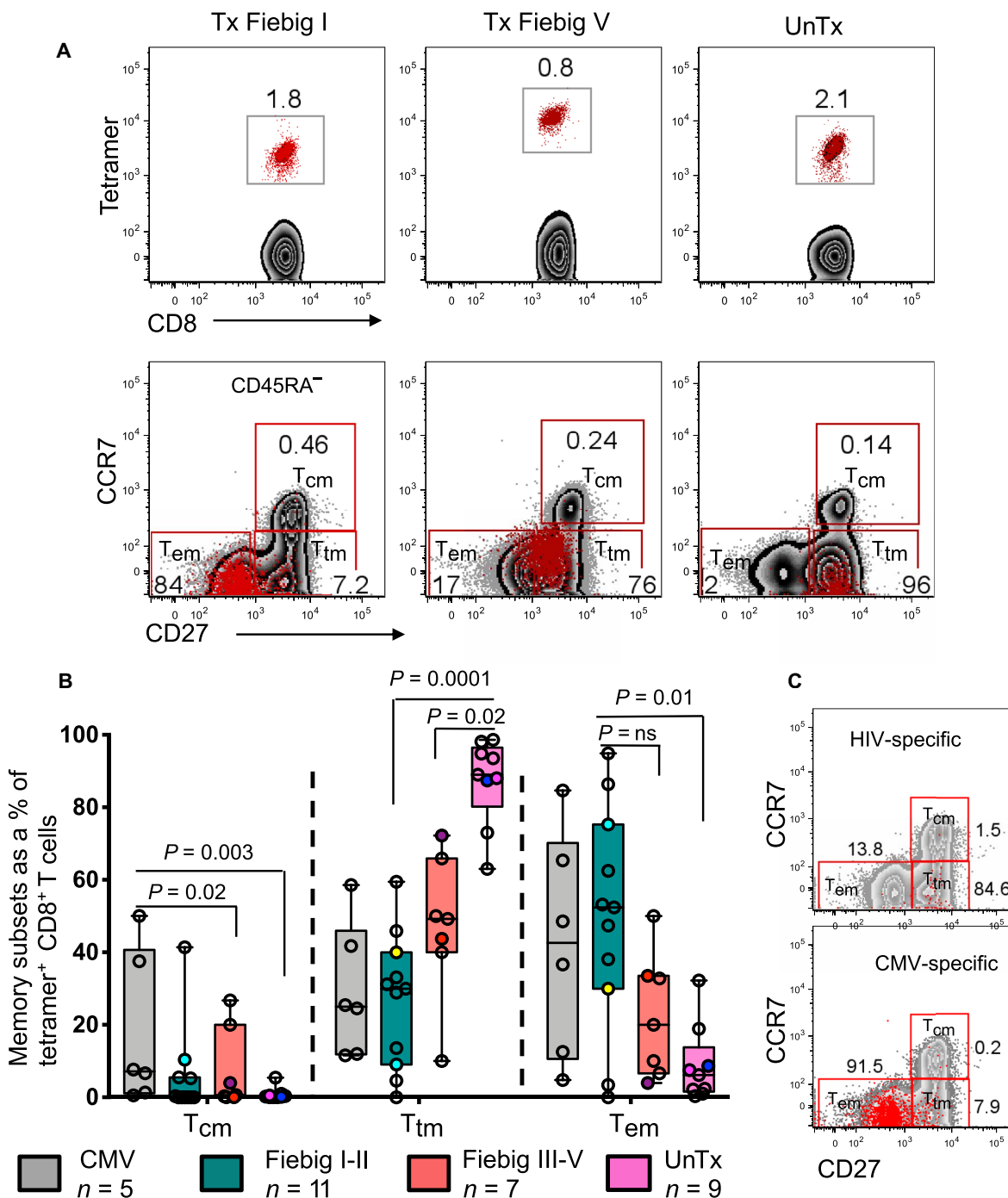
bated at a 1:1 effector target ratio. To account for differences in the frequencies of effector cells among the individuals studied, we measured frequency of tetramer<sup>+</sup> cells that could recognize peptide-loaded target cells to calculate per-cell CD8<sup>+</sup> T cell killing capacity. Despite the higher granzyme B expression in cells from UnTx infection, this analysis found no significant difference in the per-cell CD8<sup>+</sup> T cell killing activity between the groups. Together, these data demonstrate that at peak viremia, the HIV-specific CD8<sup>+</sup> T cell response is dominated by highly activated, short-lived effector cells, whereas early treatment skews CD8<sup>+</sup> T cells toward enhanced survival capacity and memory generation without impairing cytolytic function.

#### Limited cART-induced antigen exposure affects the differentiation status and durability of HIV-specific CD8<sup>+</sup> T cell responses

The functional attributes of antigen-specific T cell responses are influenced by the differentiation state of the cell (33). Chronic HIV infection has been shown to skew the maturation of HIV-specific CD8<sup>+</sup> T cells toward a transitional memory ( $T_{tm}$ ) phenotype, with suboptimal effector functions (34, 35). However, the effect of very early treatment on memory differentiation has not been explored. We



**Fig. 5. The effect of transient antigen exposure on the functional qualities of HIV-specific T cell responses.** (A) PBMCs isolated within 28 days of ART initiation were stained with a panel of MHC class I peptide tetramers specific for HIV epitopes and antibodies against BCL-2. All flow plots are gated on CD8<sup>+</sup> T cells. Top panels show flow plots gated on tetramer<sup>+</sup> CD8<sup>+</sup> T cells for each HIV tetramer tested. Bottom panels show tetramer<sup>+</sup> cells (red dots) overlaid on total CD8<sup>+</sup> T cells (black background). (B) Aggregate BCL-2 expression on tetramer<sup>+</sup> cells specific for CMV or HIV measured in 5 persons with CMV responses, and 11 Fiebig stages I and II, 6 Fiebig stages III to V, and 6 UnTx individuals with HIV-specific responses. Black dots denote single measurement per donor. Dots of the same color denote multiple measurements within a donor. (C) Representative results of direct killing activity of HIV-specific CD8<sup>+</sup> T cells measured in a 4-hour killing assay. Peptide-pulsed CFSE<sup>hi</sup> CD8-depleted cells designated as targets were mixed with CFSE<sup>lo</sup> unpulsed control cells in a 1:1 ratio and coincubated with autologous CD8<sup>+</sup> T cells. Reduction in the CFSE<sup>hi</sup> population was compared to target cells pulsed with an irrelevant peptide (ptd). (D) The killing capacity was calculated as percent reduction in CFSE<sup>hi</sup> HIV peptide-pulsed targets relative to control ovalbumin (SIINFEKL) peptide-pulsed condition. Six Tx Fiebig stages I and II, five Tx Fiebig stages III to V, and five UnTx individuals were used for these experiments. Statistical significance for aggregated data (B and D) was determined using linear mixed-effects regression analyses when comparing between groups to account for multiple measurements within some individuals. Horizontal lines represent median with IQR.



**Fig. 6. The effect of early cART initiation on memory differentiation of HIV-specific CD8<sup>+</sup> T cells.** HIV-specific (tetramer<sup>+</sup>) CD8<sup>+</sup> T cell memory subpopulations defined using CD45RA, CD27, and CCR7 at 14 to 36 days after viremia using flow cytometry. These markers were used to discriminate three distinct memory populations: T<sub>cm</sub> (CD45RA<sup>-</sup>CD27<sup>+</sup>CCR7<sup>+</sup>), T<sub>tm</sub> (CD45RA<sup>-</sup>CD27<sup>+</sup>CCR7<sup>-</sup>), and T<sub>em</sub> (CD45RA<sup>-</sup>CD27<sup>-</sup>CCR7<sup>-</sup>). **(A)** Representative flow plots for early Tx individuals and UnTx individuals are shown. **(B)** Aggregate data for the frequencies of the three memory subsets in 11 Tx Fiebig stages I and II, 7 Tx Fiebig stages III to V, and 9 UnTx individuals, as well as 6 CMV responses in early Tx individuals, are shown. Statistical significance was calculated using two-sided Mann-Whitney test. Horizontal lines represent median with IQR. Dots of the same color present data from the same donor. **(C)** Representative flow plots showing intradonor differences in the phenotype of CMV- and HIV-specific CD8<sup>+</sup> T cells in a Tx Fiebig stage V donor are shown.

assessed the differentiation state of tetramer<sup>+</sup> cells at 2 to 5 weeks after detection of plasma viremia using the well-defined phenotypic markers CD45RA, CCR7, and CD27 that are typically used to define central memory (T<sub>cm</sub>) (CD45RA<sup>-</sup>CD27<sup>+</sup>CCR7<sup>+</sup>), T<sub>tm</sub> (CD45RA<sup>-</sup>CD27<sup>+</sup>CCR7<sup>-</sup>),

and effector memory (T<sub>em</sub>) (CD45RA<sup>-</sup>CD27<sup>-</sup>CCR7<sup>-</sup>). As shown in the representative flow cytometry plot (Fig. 6A), the phenotype of UnTx responses was dominated by predifferentiated T<sub>tm</sub> cells (UnTx versus Tx Fiebig stages I and II: Mann-Whitney test, P = 0.0001; UnTx versus



Tx Fiebig stages III to V:  $P = 0.02$ ), analogous to what has been reported in chronic HIV infection (34). In contrast, responses in early Tx donors were predominantly  $T_{em}$  phenotype (Tx Fiebig stages I and II versus UnTx: Mann-Whitney test,  $P = 0.01$ ), comparable to CMV-specific responses in the same individuals (Fig. 6B). Moreover, representative comparative analysis of antigen-specific cells in a late Tx (Fiebig stage V) individual showed that the vast majority of HIV-specific  $CD8^+$  T cells were skewed toward  $T_{cm}$ , whereas the majority of CMV-specific responses were predominantly  $T_{em}$  (Fig. 6C). These data suggest that very early cART-mediated viral suppression affects phenotypic differentiation, leading to an increased frequency of more differentiated HIV-specific  $CD8^+$  T cells.

To understand the dynamics of the virus-specific  $CD8^+$  T cell response in more detail, we next examined the effect of early treatment on durability of T cell clonotypes. For two early Tx and two UnTx individuals, we sorted tetramer<sup>+</sup> populations reactive to the same epitope at an acute (28 or 35 days after diagnosis) and a chronic (336 days after diagnosis) time point. Repertoire diversity was assessed by sequencing T cell receptor  $\beta$  (TCR $\beta$ ) genes in each population. Consistent with the transcriptional profiles and differentiation status observed for  $CD8^+$  T cells in early Tx individuals, we found that the dominant TCR $\beta$  clones present at the acute time point in both individuals were maintained at the chronic time point (fig. S2 and table S8). In contrast, in both UnTx individuals, we observed that the dominant TCR $\beta$  clone present at the acute time point diminished in frequency and was replaced by a new dominant clone at the chronic time point. This was illustrated for an identical public B58-TW10-reactive TCR clone present in one early Tx and one UnTx participant, suggesting that the difference was due to variation in the  $CD8^+$  T cell response rather than intrinsic characteristics of individual TCR. Overall, the changes in TCR $\beta$  clonotype frequency (for all clones observed more than once) were greater in the UnTx individuals (Mann-Whitney test,  $P = 0.002$ ). These results indicate that early treatment results in better maintenance of dominant TCR clonotypes elicited during acute infection.

#### **cART-mediated prolonged viral suppression skews HIV-specific $CD8^+$ T cell differentiation toward $T_{cm}$ phenotype**

Next, we investigated the effect of prolonged HIV suppression on the phenotypic differentiation trajectories of HIV-specific  $CD8^+$  T cells. Tetramer<sup>+</sup>  $CD8^+$  T cells were phenotypically analyzed at 2 to 5 weeks and at a median of 336 days (IQR, 252 to 378 days) after onset of plasma viremia. Representative flow plots for a Tx Fiebig stage I donor and aggregate data for five Tx Fiebig stages I and II donors show a significant shift in the phenotype of HIV-specific  $CD8^+$  T cells from a predominantly  $T_{em}$  to a  $T_{cm}$  phenotype (change in  $T_{cm}$  frequencies between 14 and 42 days and >256 days: Mann-Whitney test,  $P = 0.01$ ; Fig. 7A). Similarly, five Fiebig stages III to V donors showed a significant increase in the  $T_{cm}$  phenotype with a concurrent reduction in the  $T_{em}$  phenotype [14 to 42 days versus >256 days: Mann-Whitney test,  $P = 0.001$ ; ( $T_{cm}$ ) and  $P = 0.04$  ( $T_{em}$ ); Fig. 7B]. There was no notable difference in the memory differentiation at the acute and late time points in four UnTx individuals despite plasma viral suppression by cART in some, at the chronic time point (Fig. 7C).

These data collectively demonstrate that immune responses from early Tx individuals had profiles consistent with establishing long-term persistence, so we investigated whether higher expression of survival genes translated into long-lived memory responses. Tetramer analysis for a representative UnTx donor who maintained high

plasma viremia of more than 4 log<sub>10</sub> RNA copies/ml had two persisting responses and two responses that diminished over time (fig. S3A), as has been previously observed (9, 36). Overall, most responses in UnTx donors diminished over time in spite of persistently high viral loads (fig. S3B). Of six UnTx individuals evaluated, three had responses that disappeared, whereas one had newly emerging responses that never reached the magnitude of the initial responses and two had responses that diminished over time. With prompt viral load suppression, most responses in early Tx individuals remained low in magnitude but persisted over time, as shown by tetramer analysis in representative Tx Fiebig stage III (fig. S3C) and Tx Fiebig stage I (fig. S3D) individuals and summarized in fig. S3E.

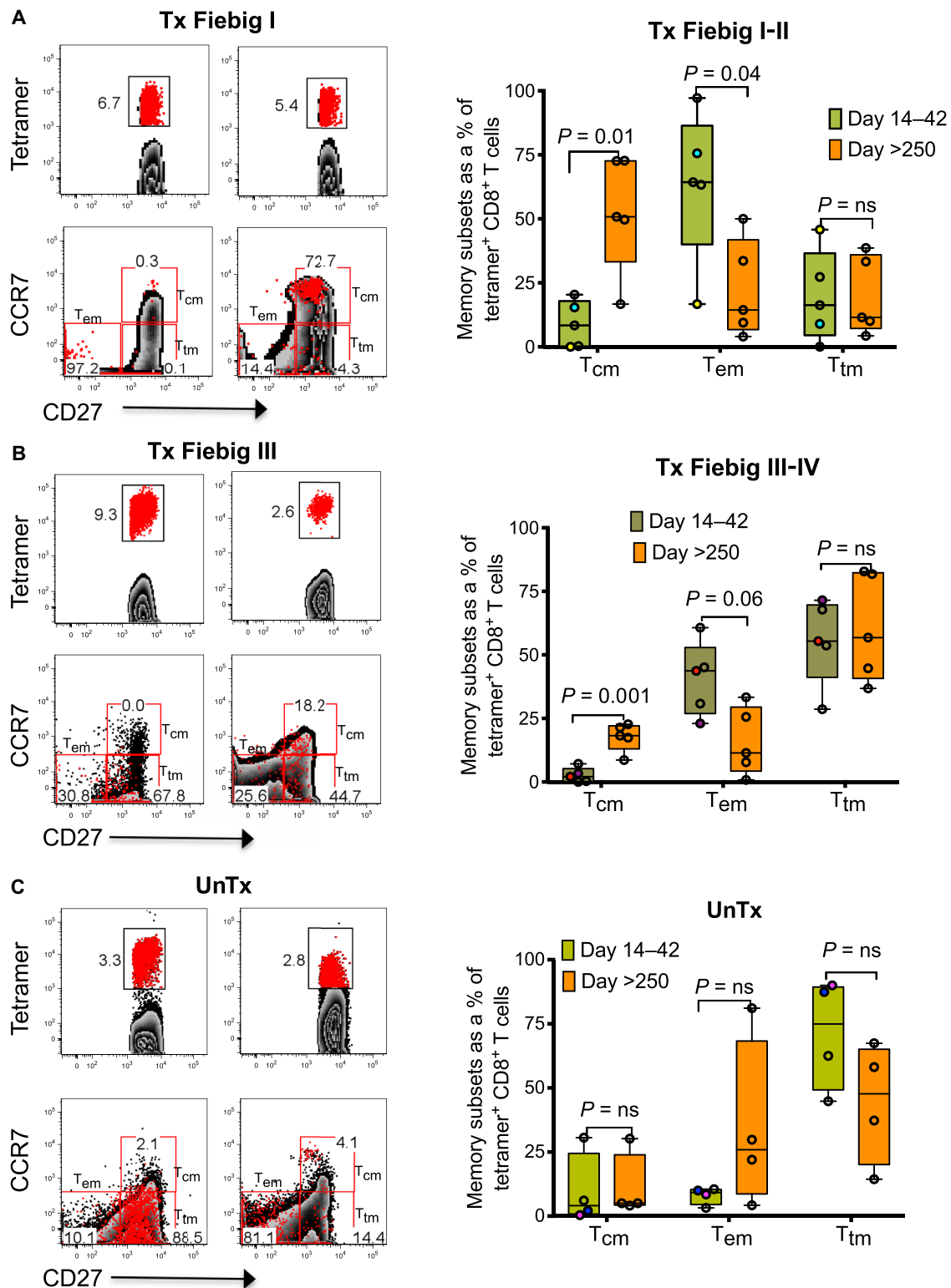
#### **Immediate cART in hyperacute infection preserves HIV-specific $CD4^+$ T helper cells and enhances HIV-specific $CD8^+$ T cell proliferation**

Acute HIV infection is associated with profound HIV-specific  $CD4^+$  T cell abnormalities due to selective depletion and severely impaired effector functions, whereas treatment at or just after peak viremia leads to robust antigen-specific responses (37–39). We next investigated the impact of treatment before peak viremia on induction of HIV-specific  $CD4^+$  T cell responses. ICS analysis between 21 and 28 days after diagnosis (Fig. 8, A and B) shows that early treatment was associated with significantly higher frequencies of IFN- $\gamma$ -secreting HIV-specific  $CD4^+$  T cells compared to UnTx individuals (linear mixed-effects regression analysis:  $P = 0.007$ ). Immediate cART was associated with strong HIV-specific  $CD4^+$  T cell proliferative responses that were otherwise diminished in UnTx infection (Fig. 8, C and D). Moreover, early Tx donors maintained robust  $CD4^+$  T cell proliferative responses for more than 250 days after diagnosis despite sustained viral suppression, whereas samples from UnTx donors had no notable improvement in  $CD4^+$  T cell proliferative capacity even when durable cART-mediated viral suppression was achieved later during chronic infection (Fig. 8, C and E). There was no detectable difference in  $CD8^+$  T cell proliferation in early Tx individuals at acute time points, possibly due to small sample size (Fig. 8, F and G).

Next, we used samples from Tx Fiebig stages I and II individuals sampled 14 to 35 days after diagnosis, which had large proliferative responses in previous experiments to investigate the effect of  $CD4^+$  T cell responses on  $CD8^+$  T cell proliferative responses.  $CD4^+$  T cell proliferation was positively correlated with  $CD8^+$  T cell proliferation after stimulation with HIV Gag antigens (Spearman  $r = 0.9$ ,  $P = 0.0009$ ; Fig. 8H), suggesting that proliferative responses of the two cell subsets could be interdependent. In addition, in vitro depletion of  $CD4^+$  T cells before stimulation with HIV peptides or with *Staphylococcus aureus*, enterotoxin type B severely blunted  $CD8^+$  T cell proliferation (Mann-Whitney test,  $P = 0.008$ ; Fig. 8, I and J). Together, these data indicate that proliferative  $CD4^+$  T cells sustain functional  $CD8^+$  T cell responses after immediate treatment of hyperacute infection.

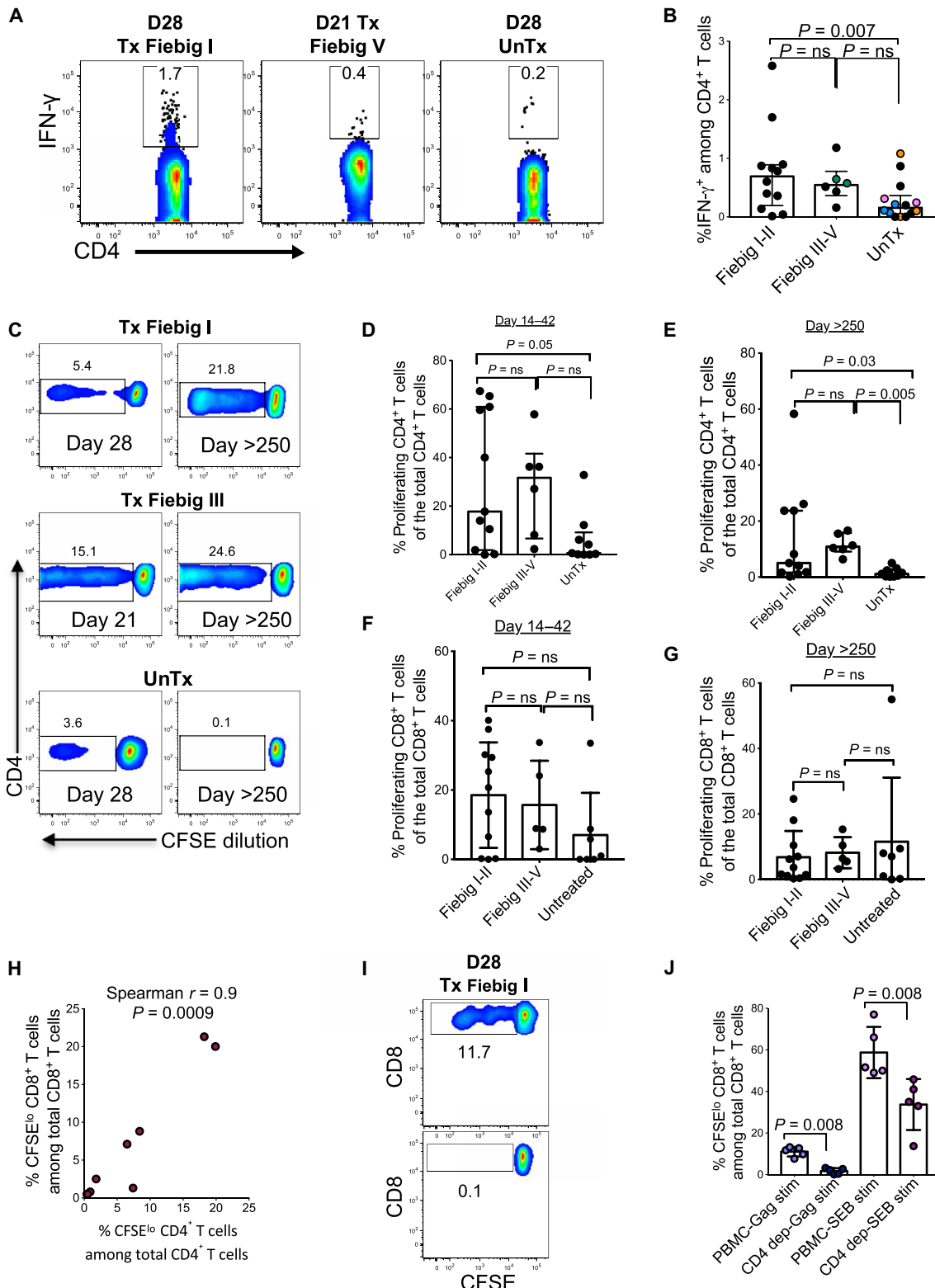
#### **DISCUSSION**

UnTx hyperacute HIV infection leads to robust induction of virus-specific  $CD8^+$  T cell responses, but these cells rapidly become poorly functional. Here, we conducted an analysis of the impact of treatment initiated before peak viremia on HIV-specific  $CD8^+$  T cell responses. This approach enabled us to examine the host response to HIV while limiting concurrent immune destruction, which typifies UnTx acute and chronic infection. These findings provide insight



**Fig. 7. Effect of prolonged viral suppression on the phenotype of persistent HIV-specific CD8<sup>+</sup> T cell responses.** HIV-specific (tetramer<sup>+</sup>) CD8<sup>+</sup> T cell memory subpopulations defined using CD45RA, CD27, and CCR7 during acute (14 to 36 days after diagnosis) and chronic (more than 250 days after diagnosis) infection. (A) Representative flow plots for a Tx Fiebig stage I individual and aggregate data for the frequencies of the three memory subsets in five Tx Fiebig stages I and II individuals. (B) Representative flow plots for a Tx Fiebig stage III individual and aggregate data for the frequencies of the three memory subsets in five Tx Fiebig stages III to V individuals. (C) Representative flow plots for one UnTx individual and aggregate data for the frequencies of the three memory subsets in four UnTx individuals. Dots of the same color represent data from the same donor. The whiskers represent minimum and maximum values. *P* values were adjusted using Bonferroni-Dunn method.

**Fig. 8. The effect of early cART initiation on the functional qualities of HIV-specific CD4<sup>+</sup> T cell responses.** HIV-specific CD4<sup>+</sup> T cell responses were measured by IFN- $\gamma$  ICS after overnight incubation in the presence of overlapping HIV-1 clade C peptide pools. **(A)** Representative flow plots gated on IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells. **(B)** Aggregate data for frequencies of IFN- $\gamma$ -producing CD4<sup>+</sup> T cells in response to Gag, Nef, and Env peptide pools in 12 Fiebig stages I and II, 5 Fiebig stages III to V, and 9 UnTx donors are shown. Black dots denote a single measurement per donor. Dots of the same color denote multiple measurements within a donor. Statistical significance was determined using multi-level linear mixed-effects regression analyses when comparing between groups to account for multiple measurements within some individuals. Horizontal lines represent median with IQR. **(C)** Flow cytometry of CFSE-labeled CD4<sup>+</sup> T cells for one representative donor from each group measured at 21 days and more than 250 days. **(D)** Aggregate CD4<sup>+</sup> T cell proliferative responses measured between 14 and 42 days after diagnosis. **(E)** Aggregate CD4<sup>+</sup> T cell proliferative responses measured after 250 days after diagnosis. **(F)** Aggregate CD8<sup>+</sup> T cell proliferative responses measured between 14 and 42 days after diagnosis. **(G)** Aggregate CD8<sup>+</sup> T cell proliferative responses measured 250 days after diagnosis. Data in (F) and (G) were generated from 11 Fiebig stages I and II, 5 Fiebig stages III to V, and 7 UnTx individuals. Statistical significance was determined using two-tailed Mann-Whitney test. **(H)** Correlation between CD4<sup>+</sup> and CD8<sup>+</sup> T cell proliferative responses in eight Tx Fiebig stages I and II donors. Spearman's rank correlation test was used. Two-tailed *P* values are reported. **(I)** Representative flow plot depicting CD8<sup>+</sup> T cell proliferation in unfractionated PBMC and in CD4-depleted PBMC. **(J)** Aggregate CD8<sup>+</sup> T cell proliferative responses to HIV antigens with and without CD4<sup>+</sup> T cells in five Tx Fiebig stages I and II donors. Statistical significance was determined using two-tailed Mann-Whitney test. Horizontal lines represent median with IQR.



Statistical significance was determined using two-tailed Mann-Whitney test. Horizontal lines represent median with IQR.

into features associated with natural priming of the immune system by HIV infection in the absence of CD4<sup>+</sup> T cell depletion. These studies are relevant to vaccine design because the phenotypes in early Tx donors such as improved CD8 effector function, robust CD4 proliferative responses and maintenance of long-lived T cell memory responses are key antiviral functions, which are desirable to induce with a vaccine.

Several studies of early treatment of HIV infection have examined the effect of treatment initiation after peak viremia (17, 40, 41). Because of the design of the FRESH cohort, we were able to identify infection during Fiebig stages I and II and initiate therapy within 1 to 2 days of detectable viremia in some cases, with peak viremia less than 1000 RNA copies/ml. Despite this limited exposure to detectable viremia, which has been shown to diminish HIV-specific B cell responses (13, 23), most of the individuals developed detectable HIV-specific CD8<sup>+</sup> T cell responses. However, the overall magnitude of responses was dependent on the relative exposure to virus, as measured by VCD analogous to viremia copy-years described in chronic HIV infection (42), and was shown to correlate with magnitude and breadth of HIV-specific CD8<sup>+</sup> T cell responses. This suggests that the degree of HIV antigen burden is a key driver of acute phase cytotoxic T lymphocyte responses. Whether some of the early Tx individuals lacked HIV-specific CD8<sup>+</sup> T cell responses altogether (43), or whether they were not cross-reactive with the reference strain of virus used, or simply below the limits of detection as shown previously in elite controllers (44), is not clear.

Recent studies have highlighted the role of metabolic state in shaping immune cell differentiation and function (45, 46). Through whole-genome RNA-seq analysis, we show that during UnTx acute HIV infection, HIV-specific CD8<sup>+</sup> T cells are metabolically hyperactive, functionally impaired, and have a skewed maturation phenotype. Early cART comparatively diminished these immune dysfunctions by relative coordinated down-regulation of the stress response genes, with a concurrent up-regulation of antiapoptotic and prosurvival genes, which are intricately involved in shaping the overall long-term survival of immune responses (31). Few transcriptional differences were found in bystander and CMV-specific CD8<sup>+</sup> T cells, highlighting the direct effect of treatment on only those cells targeting HIV-infected cells. This study shows that the key benefit of initiating cART very early is the generation of transcriptionally quiescent immune responses. It is plausible that the less stressed state of the responses in early Tx people could provide better immune priming amenable to rapid boosting with therapeutic vaccines. Moreover, our data provide insights to focus mechanistic studies into the biology of immune responses in early Tx people and identify key molecular targets such as the *BCL-2*, *AXL*, and *SRC* genes and cellular pathways that could be manipulated to enhance the generation of long-lived immune responses by future therapeutic interventions.

Furthermore, we observed divergent differentiation states of HIV-specific CD8<sup>+</sup> T cell responses both at the peak of the response and in the chronic phase of infection, an intermediate T<sub>cm</sub> population dominated UnTx responses at both the peak and chronic time points. These data suggest that the skewing of the response to this intermediate phenotype happens very early in infection and remains stable over time. In contrast, responses in Tx Fiebig stages I and II individuals were heavily skewed toward the T<sub>cm</sub> phenotype, classically superior at responding to immune stimuli (47). Sustained viral suppression in early Tx participants converted the responses to T<sub>cm</sub> phenotype.

These data further support the notion that it will be easier to boost preexisting T<sub>cm</sub> responses with a therapeutic vaccine in early Tx individuals due to the inherent ability of T<sub>cm</sub> to rapidly expand and acquire effector functions upon restimulation (33).

Last, we investigated whether loss of CD4<sup>+</sup> T cells contributed to the observed differences in CD8<sup>+</sup> T cell function and phenotype. UnTx HIV infection results in massive depletion of CD4<sup>+</sup> T cells, whereas early treatment preserves this population (13, 48, 49). In this study, we observed several CD4<sup>+</sup> T cell functional abnormalities in UnTx persons that were not apparent in early Tx donors. Consistent with previous reports (37), UnTx HIV infection was associated with a paucity of HIV-specific CD4<sup>+</sup> T cell responses. In addition, we observed diminished responses to HIV antigens and mitogens signifying HIV-induced generalized CD4<sup>+</sup> T cell functional impairment. In contrast, robust CD4<sup>+</sup> T cell proliferative responses were induced in early Tx donors and were maintained with prolonged cART-mediated viral suppression. Together, these results highlight the ability of CD4<sup>+</sup> T cell helper function to enhance CD8<sup>+</sup> T cell responses and underscore the need for more mechanistic work focused on defining the nature of CD4<sup>+</sup> T cell help to CD8<sup>+</sup> T cells.

Notable limitations of the study include variability in the number of days between diagnosis and sample collection, which was due to sample availability constraints. However, difference in number of days between diagnosis and sample collection did not substantially affect the immune parameters measured in the early Tx groups. Second, tetramer analysis was not performed for some of study participants due to a lack of appropriate tetramers. Nonetheless, the wide range of tetramers used in this study covered most of the immunodominant responses in our cohort. Third, despite the differences in the phenotypic composition of memory responses between experimental groups later in infection, RNA-seq only revealed significant differences at the 2-week time point, which is around the peak of activation in UnTx samples. The cells at later time points where more quiescent, which makes it difficult to detect transcriptional differences without previous ex vivo stimulation. Increasing sample size, read depth, and introducing ex vivo stimulation might overcome this limitation. Last, we have not investigated the impact of viral sequence diversity, which may affect antigen exposure in an epitope-specific manner that is not captured by VCD measurements. Early treatment will prevent viral evolution, but escape mutations have been observed in conjunction with the development of CD8<sup>+</sup> T cell responses during the first weeks of infection (50, 51). Therefore, additional analyses will be necessary to fully assess the contribution of viral sequence adaptation to differences in TCR clonotype frequencies and transcriptional profiles, particularly for UnTx individuals at later time points.

There is compelling evidence that early diagnosis and treatment of HIV infection have the benefits of reducing the HIV reservoir size and decreasing transmission of the virus to sexual partners (52). However, the impact of extremely early cART on induction of adaptive cellular immune responses with the potential to suppress HIV infection was unknown. In this study, we showed that treatment initiated during hyperacute infection induces HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses that differ in magnitude, phenotype, and function from responses generated in people who delay treatment. We show that preservation of CD4<sup>+</sup> T cells by early cART initiation may have a critical role in shaping CD8<sup>+</sup> T cell function. We also identified key molecules that could be potential targets for manipulation

to restore CD8<sup>+</sup> T cell function, such as BCL-2 and CD127. Overall, our results show that early cART leads to persistent functional T cell responses that are generated in most individuals with hyperacute infection. These data open up the possibility of harnessing immune responses generated under early treatment as a first critical step in the path toward immune-mediated HIV cure or remission strategies.

## MATERIALS AND METHODS

### Study design

Study participants were recruited from the FRESH cohort, a longitudinal study in which women aged 18 to 23 at enrollment are seen twice weekly for empowerment and life skills training and HIV prevention education. At each visit, they are tested for HIV RNA by finger-prick blood draw to detect acute infection and are offered immediate treatment when infection is detected (13). In this study, we included 12 HIV-infected individuals identified in Fiebig stages I and II who delayed treatment until they met the South African guidelines at the time (CD4 counts below 350 cells/mm<sup>3</sup>) and who we refer to as UnTx. Twenty-six participants were identified in Fiebig stages I and II and initiated on cART within 24 to 48 hours of detection of plasma HIV RNA, and eight individuals were identified in Fiebig stages III to V who were similarly immediately initiated on cART. Sample size for each experimental group and selection of sample time points were based on the availability of PBMC samples rather than a prespecified sample effect size. Class I and II HLA typing, longitudinal CD4<sup>+</sup> T cell counts, and viral loads were obtained, and longitudinal blood samples were collected starting before infection. Clinical characteristics of the study participants are shown in table S1. Written informed consent was obtained from each study participant in accordance with a protocol no. BF298/14 approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal and protocol no. 201P001018/PHS approved by the Institutional Review Board for Massachusetts General Hospital, Partners Human Research Committee. Following IRB approval in 2018 all women are offered pre-exposure prophylaxis (PrEP). Primary data are reported in data file S1.

### Statistical analyses

Comparisons of continuous variables between groups were performed using the nonparametric Mann-Whitney test, and the Dunn's test was used to adjust for multiple comparisons that were appropriate. Pearson's  $\chi^2$  test was used to analyze categorical data. Relationships between continuous variables were assessed using the Spearman's rank-order correlation. In instances where multiple measurements were available on a single individual, resulting in correlated responses, linear mixed-effects regression models (with random intercepts at the individual level) were used to compare outcomes between groups. Linear regression analyses and multilevel linear mixed-effects regression analyses were performed in the Tx groups (Fiebig stages I and II and Fiebig stages III to V) to assess the relationship between immune responses and the duration of treatment. Cumulative HIV exposure, here referred to as VCD, was determined by calculating the area under curve of viral load over time with a baseline of 20 RNA copies/ml (limit of detection for the quantification of plasma viral load).  $P < 0.05$  was considered significant. Analyses were performed on GraphPad Prism version 7 (GraphPad Software Inc.) and Stata version 15 (StataCorp).

RNA-seq data were analyzed as follows: Differential expression was performed using edgeR (53) with default dispersion calculation settings. Significance for differential expression was set at an FDR-adjusted  $P$  value,  $q < 0.05$ . Gene-set analysis was performed using all differentially expressed genes within each comparison in ingenuity pathway analysis (<https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/>). Upstream drivers and canonical pathways were extracted directly from IPA and reorganized for visual clarity.

## SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Transcriptional analysis reveals predicted dysfunctional responses in CD8<sup>+</sup> T cells from UnTx individuals versus early Tx individuals.

Fig. S2. TCR clonotype dynamics in early Tx and UnTx HIV.

Fig. S3. Longitudinal characterization of HIV-specific CD8<sup>+</sup> T cell responses in Tx and UnTx hyperacute HIV infection.

Table S1. Baseline characteristics of study participants.

Table S2. Effect of interval between treatment initiation and sample collection on immune parameters in Fiebig stages I and II and Fiebig stages III to V participants.

Table S3. Immunodominant responses measured by HLA class I tetramers in individuals Tx in Fiebig stages III to V and UnTx donors.

Table S4. Results from the RNA-seq differential expression tests in HIV-specific, CMV-specific, and total CD8<sup>+</sup> T cells (excluding the tetramer-specific cells) between the time points assayed in Tx individuals.

Table S5. Results from the RNA-seq differential expression tests in HIV-specific, CMV-specific, and total CD8<sup>+</sup> T cells (excluding the tetramer-specific cells) between the time points assayed in UnTx individuals.

Table S6. Results from the RNA-seq differential expression tests between CMV-specific and HIV-specific CD8<sup>+</sup> T cells at the late and long-term time points and the difference between those cells between the two time points.

Table S7. Results from the RNA-seq differential expression tests between HIV-specific CD8<sup>+</sup> T cells from Tx and UnTx individuals at each time point.

Table S8. TCR $\beta$  clonotype sequences.

Table S9. Table contains alignment statistics and metadata on the RNA-seq samples in this study.

Data file S1. Primary data.

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## Augmentation of HIV-specific T cell function by immediate treatment of hyperacute HIV-1 infection

Zaza M. Ndhlovu, Samuel W. Kazer, Thandeka Nkosi, Funsho Ogunshola, Daniel M. Muema, Gursev Anmole, Shayda A. Swann, Amber Moodley, Krista Dong, Tarylee Reddy, Mark A. Brockman, Alex K. Shalek, Thumbi Ndung'u and Bruce D. Walker

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### Keeping it FRESH in HIV

HIV infection affects the immune system beyond infected CD4<sup>+</sup> T cells and can cause systemic immune dysfunctions even when the virus is controlled by antiretroviral treatment. To determine how early treatment affects anti-HIV T cell responses, Ndhlovu *et al.* examined samples from the FRESH cohort, which enables analysis of hyperacute infection. They compared HIV-specific CD8<sup>+</sup> T cells from women who initiated treatment within 2 days of HIV diagnosis to those that started treatment during the acute or chronic phase of infection. CD8<sup>+</sup> T cells from individuals that started treatment immediately had preserved functionality, likely due to limited viremia. The antiviral capacity of these cells may be a useful goal for HIV vaccine design.

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