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Using mathematical modeling to define kinetic properties of HIV-specific CD8$^+$ T-cell responses

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1 **Abstract**

2 Multiple lines of evidence indicate that CD8$^+$ T cells are important in the control of HIV-1
3 (HIV) replication. However, CD8$^+$ T cells induced by natural infection cannot eliminate the virus
4 or reduce viral loads to acceptably low levels in most infected individuals. Understanding the
5 basic quantitative features of CD8$^+$ T-cell responses induced during the course of HIV infection
6 may therefore inform us about the limits that HIV vaccines, which aim to induce protective CD8$^+$
7 T-cell responses, must exceed. Using previously published experimental data from a cohort of HIV-
8 infected individuals with sampling times from acute to chronic infection we defined the quantitative
9 properties of CD8$^+$ T-cell responses to the whole HIV proteome. In contrast with a commonly
10 held view, we found that the relative number of HIV-specific CD8$^+$ T-cell responses (response
11 breadth) changed little over the course of infection (first 400 days post-infection), with moderate
12 but statistically significant changes occurring only during the first 35 symptomatic days. This
13 challenges the idea that a change in the T-cell response breadth over time is responsible for the slow
14 speed of viral escape from CD8$^+$ T cells in the chronic infection. The breadth of HIV-specific CD8$^+$
15 T-cell responses was not correlated with the average viral load for our small cohort of patients,
16 highlighting the possibility that statistically significant correlations previously found in other small
17 cohorts of patients arose by chance. Metrics of relative immunodominance of HIV-specific CD8$^+$ T-
18 cell responses such as Shannon entropy or the Evenness index were also not significantly correlated
19 with the average viral load. Our mathematical-model-driven analysis suggested extremely slow
20 expansion kinetics for the majority of HIV-specific CD8$^+$ T-cell responses and the presence of
21 intra- and interclonal competition between multiple CD8$^+$ T-cell responses; such competition
22 may limit the magnitude of CD8$^+$ T-cell responses, specific to different epitopes, and the overall
23 number of T-cell responses induced by vaccination. Together, our results suggest that vaccines
24 inducing T-cell responses with breadth and expansion kinetics similar to those induced by natural
25 HIV infection are unlikely to be highly efficacious, and we propose minimum quantitative features
26 of CD8$^+$ T-cell responses (breadth, expansion kinetics) that vaccines must induce to be deemed
27 acceptable for further testing.

28 **Keywords**: acute HIV infection, vaccines, CD8$^+$ T cells, immune response, multiple epitopes,
29 competition, mathematical model.

30 **Abbreviations**: CTL, cytotoxic T lymphocyte; HIV, human immunodeficiency virus, SE,
31 Shannon entropy, EI, Evenness index, PBMC, peripheral blood mononuclear cells, SFC, spot-
32 forming cells, IFN, interferon.

33 **Short running title**: T-cell response kinetics in HIV

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34 # 1 Introduction

35 HIV-1 (HIV) remains a major global infectious disease with more than 35 million infected in-
36 dividuals, and millions of deaths due to AIDS every year [1, 2]. Despite decades of research, a
37 highly effective vaccine against HIV/AIDS is not yet available; several vaccine candidates failed
38 in large phase II or III clinical trials [3–5]. One set of such failed trials investigated the efficacy
39 of a CD8$^+$ T-cell-based vaccine against HIV that had shown reasonable protection following the
40 infection of immunized monkeys with SIV [6, 7]. Although it is likely that multiple factors con-
41 tributed to the failure of this vaccine in humans, the limited breadth and small magnitude of the
42 vaccine-induced T-cell response might have been important [8, 9]. However, the magnitude and
43 breadth of HIV-specific CD8$^+$ T-cell response needed for a protective vaccine are not well defined
44 [9, 10]. Although most recent vaccine developments have shifted toward the induction of broadly
45 neutralizing antibodies [11–14], it is likely that the induction of both neutralizing antibodies and
46 memory CD8$^+$ T cells will be needed for adequate control of HIV [10, 15].

47 Multiple lines of evidence suggest that CD8$^+$ T cells play an important role in the control
48 of HIV replication; some evidence is based on correlational studies in humans and some on ex-
49 periments with SIV-infected monkeys [16–18]. In particular, 1) the appearance of CD8$^+$ T-cell
50 responses in the blood is correlated with a decline in viremia [16, 19–22]; 2) the rate of disease pro-
51 gression of HIV-infected individuals is strongly dependent on MHC-I locus combinations [23–25];
52 3) HIV escapes recognition from multiple CD8$^+$ T-cell responses during the infection [16, 26]. No
53 consensus has been reached on the relationship between magnitude of HIV-specific CD8$^+$ T-cell
54 responses and viral load [27–32]; several studies, but not all, have indicated a statistically signifi-
55 cant negative correlation between viral load and the number of Gag-specific CD8$^+$ T-cell responses
56 [32–36]. Important data also came from experiments on SIV-infected monkeys; depletion of CD8$^+$
57 T cells prior to or after infection leads to significantly higher viral loads [37–40]. Some vaccination
58 protocols in monkeys, in which high levels of SIV-specific CD8$^+$ T cells were induced, resulted in
59 a reduced viral load and, under certain conditions, apparent elimination of the virus [6, 7, 41–44].

60 Despite these promising experimental observations, following natural infection, CD8$^+$ T-cell
61 responses have not cleared HIV in any patient, or reduced viral loads to acceptably low levels
62 in many individuals [16, 45, 46]. While some HIV-infected individuals do not appear to progress
63 to AIDS and maintain high CD4$^+$ T-cell counts in their peripheral blood (so-called long-term
64 non-progressors or elite controllers, [46–48]), whether CD8$^+$ T cells are solely responsible for such
65 control remains undetermined [46, 49–53]. It is clear that if we are to pursue the development of
66 CD8$^+$ T-cell-based vaccines against HIV, such vaccines must induce more effective CD8$^+$ T-cell
67 responses than those induced during natural HIV infection. However, the definition of a "more
68 effective" response is not entirely clear. If induction of a broad (i.e., specific to multiple epitopes)
69 and high magnitude CD8$^+$ T-cell response is not feasible, it remains to be determined whether
70 vaccination strategies should focus on the induction of broad and low magnitude or narrow and
71 high magnitude CD8$^+$ T-cell responses. The basic quantitative aspects of HIV-specific CD8$^+$ T-
72 cell responses induced during natural infection may indicate which parameters of vaccine-induced
73 responses should be targeted for improvement so that the vaccine provides reasonable protection
74 in humans.

75 There are several studies documenting the kinetics of HIV-specific CD8$^+$ T-cell responses in
76 humans from acute to chronic infection [54–59]. In some cases, the data are restricted to a few well-
77 defined epitopes, often inducing immunodominant responses [59–61]. Similarly, only the kinetics
78 of immunodominant CD8$^+$ T cell responses to SIV in monkeys following vaccination have been
79 analyzed and well quantified [62, 63]. Many theoretical studies developed mathematical models of

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within-host HIV dynamics and their control by T-cell responses [64–69], but these models have not been well parametrized due to a lack of appropriate experimental data. Furthermore, these models involved different *a priori* assumptions on how CD8$^+$ T-cell responses to HIV are generated and maintained; the dynamics of these responses are often responsible for the observed changes in viral load and kinetics of viral escape from T cells [64, 68, 70]. Further refinements of such models and investigations of the robustness of their predictions will benefit greatly from the systematic analysis of the kinetics of HIV-specific CD8$^+$ T-cell responses. In particular, it remains unclear whether CD8$^+$ T-cell responses specific to different epitopes of HIV compete during infection as many mathematical models assume [64, 69, 71]. Studies on the competition between CD8$^+$ T cells specific to the same or different epitopes in mice are inconclusive, with some documenting competition and others a lack of competition [72–82]. A recent study using cross-sectional data suggested an absence of competition between CD8$^+$ T-cell responses, specific to different HIV epitopes [83]. The absence of such interclonal competition would also predict that it is possible for a vaccine to generate a very broad HIV-specific CD8$^+$ T-cell response.

In the present study, we performed mathematical-model-driven analysis of experimental data on viral load and HIV-specific CD8$^+$ T-cell dynamics from a study of 22 patients who had been followed from acute to chronic infection [55]. The useful features of these data include the high temporal resolution of CD8$^+$ T-cell responses and viral load measurement, with the detection of many viral epitopes recognized by CD8$^+$ T cells using the ELISPOT assay. In contrast with several previous studies (e.g., [60, 61, 83]), which focused on a subset of well-defined epitopes and epitope-specific CD8$^+$ T cells, we followed CD8$^+$ T cell responses to the whole viral proteome, which enabled detailed quantitative investigation of CD8$^+$ T-cell responses to HIV.

2 Material and methods

2.1 Experimental data

The data collection methods were as described in detail previously [55]. Briefly, individuals with acute HIV subtype B infection were recruited into the study, blood samples from the patients were taken at multiple, sequential time points over several months following symptomatic presentation. All measurements were timed in days since onset of symptoms. The time interval between infection and onset of symptoms is likely to vary somewhat between individuals [84]. Viral load was recorded for all patients. Protein regions targeted by patients' HIV-specific T-cell responses were mapped using either autologous or consensus HIV-1B viral sequences obtained within the first 6 months of infection by peptide-stimulated interferon (IFNγ ELISPOT assay or tetramer immunolabeling. Both assays show similar patterns of responses kinetics [55], but in our analyses we only used data obtained by ELISPOT. Note that in patients WEAU, SUMA, and BORI, T-cell responses were not mapped to the whole proteome; in these patients, responses measured in a previous study [85] were followed over time. In total, there were data for 22 patients (two additional patients only had tetramer immunolabeling measurements and were therefore not included in the analysis). Experimental data on the dynamics of HIV-specific CD8$^+$ T-cell responses and viral loads are shown in Figs. S1–S4.

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2.2 Mathematical model of CD8$^+$ T-cell response to a viral infection

To quantify the kinetics of HIV-specific CD8$^+$ T-cell responses, we used a simple $T_{\text{on}}/T_{\text{off}}$ mathematical model [86, Fig. 1]. The model assumes that the response starts at time $t = 0$ with frequency E_0 of epitope-specific CD8$^+$ T cells that become activated at time T_{on}. Activated T cells start proliferating at rate ρ and reach the peak at time T_{off}. Thereafter, epitope-specific CD8$^+$ T cells decline at rate α. The dynamics of the CD8$^+$ T-cell response $E(t)$ are therefore represented by the following differential equation:

$$\frac{dE}{dt} = \begin{cases} 0, & \text{if } t < T_{\text{on}}, \\ \rho E, & \text{if } T_{\text{on}} \leq t \leq T_{\text{off}}, \\ -\alpha E, & \text{if } t > T_{\text{off}} \end{cases} \tag{1}$$

with $E(0) = E_0$ as the predicted initial frequency of epitope-specific CD8$^+$ T cells at time $t = 0$ days since symptom onset.

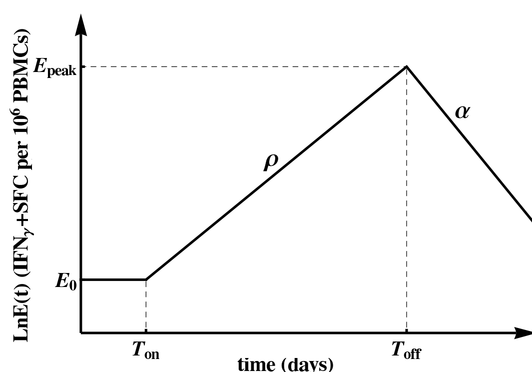

Figure 1: Schematic representation of the $T_{\text{on}}/T_{\text{off}}$ mathematical model fitted to the epitope-specific CD8$^+$ T-cell response kinetics data [86]. In this model, E_0 epitope-specific naive CD8$^+$ T cells become activated at time $t = T_{\text{on}}$ and start proliferating at rate ρ. At $t = T_{\text{off}}$, T cell response peaks and declines at rate α. We refer to E_0 as the predicted initial frequency of epitope-specific CD8$^+$ T cells [87]. Evidently, E_0 may over- or under-estimate the response precursor frequency depending on exactly when the T cells became activated and how adequate the mathematical model is for describing immune response data during the expansion phase.

Most immune responses (about 80%) had a detectable frequency at the first time point at which the response was measured, so we could not estimate when the response became activated (T_{on}). Therefore, when fitting the mathematical model (eqn. (1)) to such data, we set $T_{\text{on}} = 0$. This implies that we assumed each epitope-specific CD8$^+$ T cell response is triggered at $t = 0$ (onset of symptoms) with E_0 activated cells; this is clearly a simplification. In this way, the predicted initial frequency E_0 is a generalized recruitment parameter, which combines the true precursor frequency and the recruitment rate/time [86, 88]. For a minority of responses (about 20%) there were one or several consecutive measurements in the first few days since symptom onset that did not result in detectable T-cell responses. In those cases, we set T_{on} as the first day with detectable measurements or the last consecutive day with non-detectable measurements. We fitted the model (eqn. (1)) to the data on each measured epitope-specific CD8$^+$ T-cell response in all patients using `Mathematica 8` with nonlinear least squares by log-transforming the model predictions and data. For those responses that only expanded or only declined, we estimated only the expansion rate ρ or contraction rate α, respectively.

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142 ## 2.3 Statistics

143 Depending on the specific analysis, we used either parametric (e.g., Pearson correlation or linear
144 regression) or nonparametric (Spearman's rank correlation) methods. In most cases, significance
145 was not strongly dependent on the method used and in cases when normality of the data was
146 violated we used nonparametric tests. We used three metrics to estimate the strength of HIV-
147 specific, Gag-specific, or Env-specific CD8$^+$ T-cell response. Our focus on Gag and Env stems
148 from previous observations on the relative importance of T-cell responses specific to these proteins
149 in viral control [33, 34].

150 The first metric was immune response breadth, which is the number of responses specific to
151 either all HIV proteins, Gag, or Env at time t, $n(t)$. For this metric, we took into account all time
152 points at which CD8$^+$ T-cell responses were measured for each patient. In some patients, there
153 were missing measurements for some T-cell responses (marked "nd" for "not done"), so we tried
154 two methods: i) substituting "nd" with 1 (detection level), or ii) removing that time point from the
155 analysis. To estimate the breadth of the immune response it was important to exclude the data for
156 that specific time point from the analysis; inclusion of such data might lead to an overestimation
157 of the immune response breadth. There were subtle differences in estimated breadth using these
158 two methods, but these did not substantially influence our conclusions. A second metric for the
159 strength of the immune response was Shannon entropy (SE). While breadth only accounts for the
160 number of responses, SE takes into account the relative abundance of individual responses, and
161 reaches its maximum when all responses are of identical magnitude. SE at time t was calculated
162 as $SE(t) = \sum_{i=1}^{n(t)} f_i(t) \log_2(f_i(t))$ where $n(t)$ is the number of HIV-, Gag-, or Env-specific T-cell
163 responses at time t, and $f_i(t)$ is the frequency of the epitope-specific T-cell response in the total
164 response at time t. Importantly, measurements of SE do not depend on "nd" or below-level-of-
165 detection values; however, the number of detected responses $n(t)$ may have a large impact on
166 the actual value of SE. A third metric, Evenness index (EI) was calculated as the normalized
167 SE: $EI(t) = SE(t)/\log_2(n(t))$ where $\log_2(n(t))$ is the maximum value SE can reach for $n(t)$
168 immune responses. EI measures the degree of vertical immunodominance of HIV-specific T cell
169 responses [56] and varies between 0 and 1. Larger values indicate more "even" responses which,
170 based on our and others' previous work, should predict a longer time to viral escape from CD8$^+$
171 T cell responses and therefore better virus control [56, 89]. Both SE and EI are undefined for
172 $n = 0$. Furthermore, EI is ill-defined when only one immune response is measured per time point;
173 this is relevant when looking at Gag- and Env-specific T-cell responses as some patients had few
174 or none of those. We performed alternative analyses by i) removing data points where $n = 1$,
175 or ii) assigning $EI = 1$ or $EI = 0$ when $n = 1$. These modifications did not influence most of
176 our conclusions involving this metric. Because both viral load and breadth of T cell responses
177 changed within patients, in one set of analyses we calculated the mean breadth per time interval
178 by averaging several measurements of breadth.

179 In addition to SE and EI, other measures of immunodominance could also be used. For
180 example, Simpson's diversity index is used in ecology to estimate species richness [90]. In our
181 analyses, Simpson's diversity index led to predictions similar to SE (results not shown), so we
182 have reported only the results for SE and EI here.

183 As some of our correlations turned out to be statistically nonsignificant we performed several
184 power analyses to determine the numbers of patients needed to detect significance. We reanalyzed
185 previously published data from Geldmacher *et al.* [34] to determine whether the small sample
186 size in our cohort was responsible for the nonsignificant correlations. We performed these power
187 analyses using a bootstrap approach by resampling from the data with replacement using $10^3 - 10^4$

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188 simulations.

189 2.4 Ethics statement

190 This paper uses experimental data obtained previously [55] and no new observations requiring
191 patient consent or institutional review board approval have been performed.

192 3 Results

193 3.1 Moderate changes in the breadth of HIV-specific CD8$^+$ T-cell re-
194 sponse over the course of infection

195 While CD8$^+$ T-cell responses are thought to play an important role in control of HIV replication,
196 the kinetics of CD8$^+$ T-cell responses specific to most HIV proteins, especially during the acute
197 phase of infection, have not been quantified. Here, we reanalyzed data from a previous study that
198 included patients infected with HIV-1 subtype B [55].
199 First, we investigated how many responses there were in a given patient and how the breadth
200 of the HIV-specific CD8$^+$ T-cell response changed over the course of infection. For every patient,
201 we counted the maximum number of responses detected by ELISPOT assay to the whole viral
202 proteome and their specificity (Fig. 2). Similarly to several previous studies [16, 54, 56], we found
203 that most T-cell responses were directed against Gag and Env and this distribution changed little
204 after 100 days since symptom onset (Fig. 2A and results not shown). Interestingly, responses to
205 Nef, Integrase, or Reverse Transcriptase constituted a substantial fraction of all responses. We
206 found a median of eight epitope-specific CD8$^+$ T-cell responses per patient, with two patients
207 having over 15 responses and three patients having only three responses. Because of the potential
208 limit of detection associated with ELISPOT assays, the true breadth of HIV-specific CD8$^+$ T cell
209 response may be even higher [59]. The distribution of the number of responses in a given patient
210 did not change significantly over the course of infection, except in patients with many responses in
211 which some T-cell responses disappeared in chronic infection (Fig. 2B and Fig. S5 in Supplement).
212 There was no change in the average total HIV-specific T-cell response over time in this cohort of
213 patients (Fig. S6).
214 The breadth of the CD8$^+$ T-cell response, measured as the number of HIV-specific CD8$^+$ T-
215 cell responses (or breadth of protein-specific (such as Gag-specific) CD8$^+$ T-cell responses) has
216 been implicated in protection against disease progression [33, 34, 36, 91]. Some, but not all,
217 previous analyses suggested an increase in the breadth of HIV-specific CD8$^+$ T cell responses over
218 time [54, 55, 92, 93]. We found variable patterns for the change in breadth over time, i.e., there
219 were patients with increasing breadth (e.g., patients MM45, MM48, MM49), decreasing breadth
220 (e.g., MM43, MM55), or with non-monotonically changing breadth (e.g., MM23, MM42; Fig. S5).
221 Because there was no significant change in the average number of T-cell responses in all patients
222 (Fig. S5), we calculated the dynamics of normalized breadth for individual patients, dividing the
223 number of HIV-specific T-cell responses detected at a particular time point in a given patient by
224 the total number of responses in that patient (Fig. 3). Our analysis suggested that there was a
225 moderate but statistically significant increase in the average normalized breadth over time (from
226 85% to 95%), and this increase was limited to the first 35 days after symptom onset (results not
227 shown).

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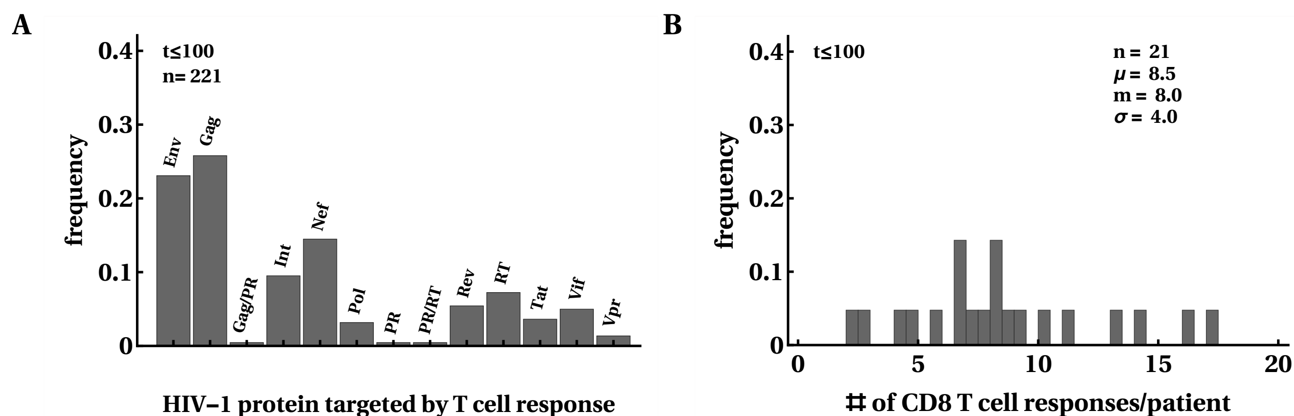

Figure 2: Most HIV proteins were recognized by CD8$^+$ T cell responses. We calculated the frequency at which HIV proteins were recognized by CD8$^+$ T cells; overall, 50% of responses were directed against Env or Gag (A). $m = 8$ CD8$^+$ T cell responses were detected in this cohort of 22 patients at any given time point after infection (B). In B (and other figures in the paper), μ denotes the average, m is the median, and σ is the standard deviation. The distributions are shown for the first 100 days after symptom onset but, overall, distributions changed little over the course of 400 days of infection (results not shown). Patient SUMA0874 was excluded from the analysis in B due to a lack of measurements of all T cell responses at all time points.

228 A relatively high breadth in the first month after infection, averaged over many patients ($\sim$ 229 85% of the maximum), may arise from the mixture of patients in the early and late stages of 230 acute infection; it may be expected that patients with early acute infection have few CD8 T-cell 231 responses, whereas patients with late acute infection have many CD8 T-cell responses. To address 232 this caveat we analyzed the dynamics of relative breadth in a subset of patients with a declining 233 viral load, which may be an indication of early acute HIV infection (patients MM25, MM28, 234 MM39, MM40, MM23, MM33, MM45, MM49, MM55, MM56). We found that similarly to the 235 previous analysis, there was a statistically significant increase in the average (or median) relative 236 breadth over time ($\rho = 0.36$, $p = 0.004$), and this increase was limited to the first 12 days after 237 symptom onset. The average normalized breadth increased from 73% to 96% between 12 and 400 238 days after symptom onset. Together, our results suggest a moderate increase in T-cell response 239 breadth by the first few weeks after symptom onset; however, there is a possibility that an increase 240 in breadth may be larger for patients progressing from very early acute to chronic infection. In 241 a recent paper [58] a moderate increase in CD8$^+$ T-cell response breadth within the first several 242 weeks of symptom onset and then relatively stable maintenance of breadth was observed in one 243 of two patients; the second patient showed a large increase in CD8$^+$ T-cell response breadth over 244 time.

245 Although the immune response breadth is considered to be a good measure of effective immune 246 response [10], there is no reason for this conjecture other than to simplify calculation. In fact, it is 247 possible that many HIV-specific T-cell responses with small magnitudes do not contribute to viral 248 control but would be counted when calculating immune response breadth. Studies in mice indicate 249 that the efficacy of effector and memory CD8$^+$ T cells in killing peptide-pulsed targets in the spleen 250 is directly proportional to the T cell frequency [94], meaning responses with a low frequency would 251 contribute little to the killing of targets. Other studies have suggested that equal magnitudes of 252 T-cell responses may be beneficial by limiting viral escape [56, 71]. Therefore, we introduced 253 two additional measures of HIV-specific T-cell response efficacy, allowing us to quantify T cell

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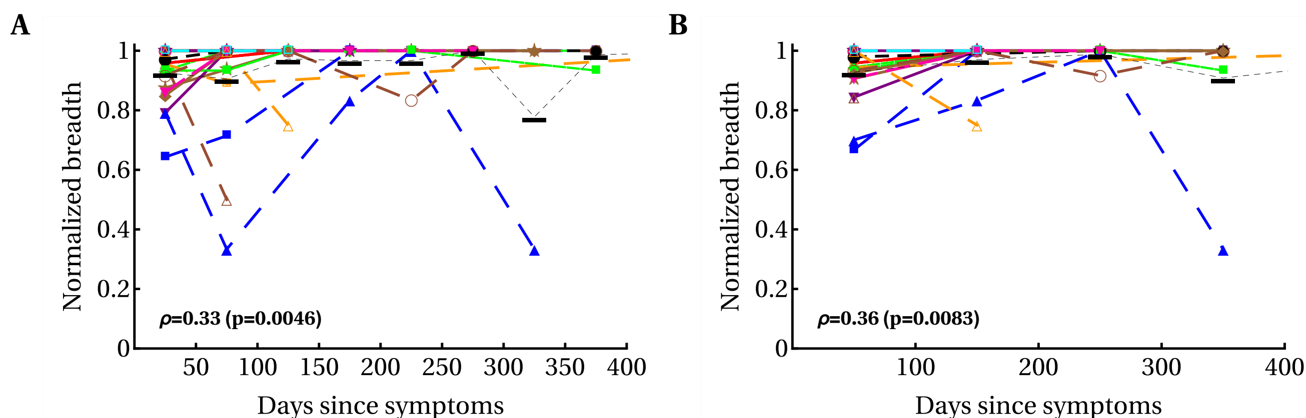

Figure 3: Modest yet statistically significant increase in the average normalized T-cell response breadth over the course of the first year of HIV infection. We divided the observations into different time bins (A, 50-day intervals; B, 100-day intervals) and calculated the relative breadth for the corresponding interval. The relative breadth was calculated as the number of HIV-specific CD8$^+$ T-cell responses detected in a given time period divided by the number of all responses measured for that patient in all time periods; data were averaged to simplify presentation. Averaging did not influence the statistical significance of conclusions (not shown). Colors and symbols represent the data from different patients as shown in Fig. S5 in Supplementary Material. Black horizontal bars denote the mean relative breadth for that time interval for all patients. There was a statistically significant increase in relative breadth (Spearman's rank correlation coefficient ρ and p values indicated on panels). There was no change in the average total immune response in all patients (Fig. S6). Detailed analysis of the relative number of CD8$^+$ T-cell responses in individual patients revealed variable patterns: constant breadth, increasing breadth, decreasing breadth, and breadth changing non-monotonically over time (Fig. S7). Also, no overall change in the average breadth (un-normalized) was observed (Fig. S5). We observed a similarly modest but significant increase in SE and EI of HIV-specific CD8$^+$ T-cell response with time (Fig. S8).

254 immunodominance (or richness): Shannon entropy (SE) and Evenness index (EI, see Materials
255 and Methods for details). While SE has been used to measure HIV genome variability in sequence
256 alignments, it has not previously been used to estimate immunodominance of immune responses.
257 Our analysis suggested that both SE and EI increased over the course of infection and that
258 this change was more significant for EI, in part because EI cannot exceed 1 by definition (Figs.
259 S8–S10). However, the statistically significant increases in these two metrics were also mainly
260 restricted to the first 40 days since symptom onset (not shown). Thus, the number and magnitude
261 of evenness for HIV-specific CD8$^+$ T cell responses both appear to increase very early in infection
262 and stabilize within 40 days of symptom onset.

263 3.2 Variable correlations between immune response breadth and viral 264 load

265 Correlates of protection against disease progression of HIV-infected individuals are incompletely
266 understood. It is well known that viral load is strongly correlated with risk of disease progression
267 in HIV-infected patients [95] and many other parameters have been measured to reveal potential
268 markers of protection. Among these, the breadth of HIV-specific CD8$^+$ T-cell response has been
269 widely emphasized as a potential predictor of viral control. Several studies found a statistically
270 significant negative correlation between the number of Gag-specific CD8$^+$ T-cell responses and

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271 viral load [33, 34, 36, 91, 96] whereas others did not [32]. In some of these studies, statistically
272 significant negative correlations were based on relatively small numbers of patients, e.g., $n = 18$
273 in Radebe *et al.* [91]. A negative correlation between viral load and breadth of Gag-specific CD8$^+$
274 T-cell responses was also found using bioinformatic predictions of potential T cell epitopes [35].
275 Negative correlations between viral load and CD8$^+$ T-cell response breadth have generally been
276 interpreted as an indication of protection even though it has been shown that viral load has an
277 impact on the change in the number of Gag-specific T-cell responses over time [97].

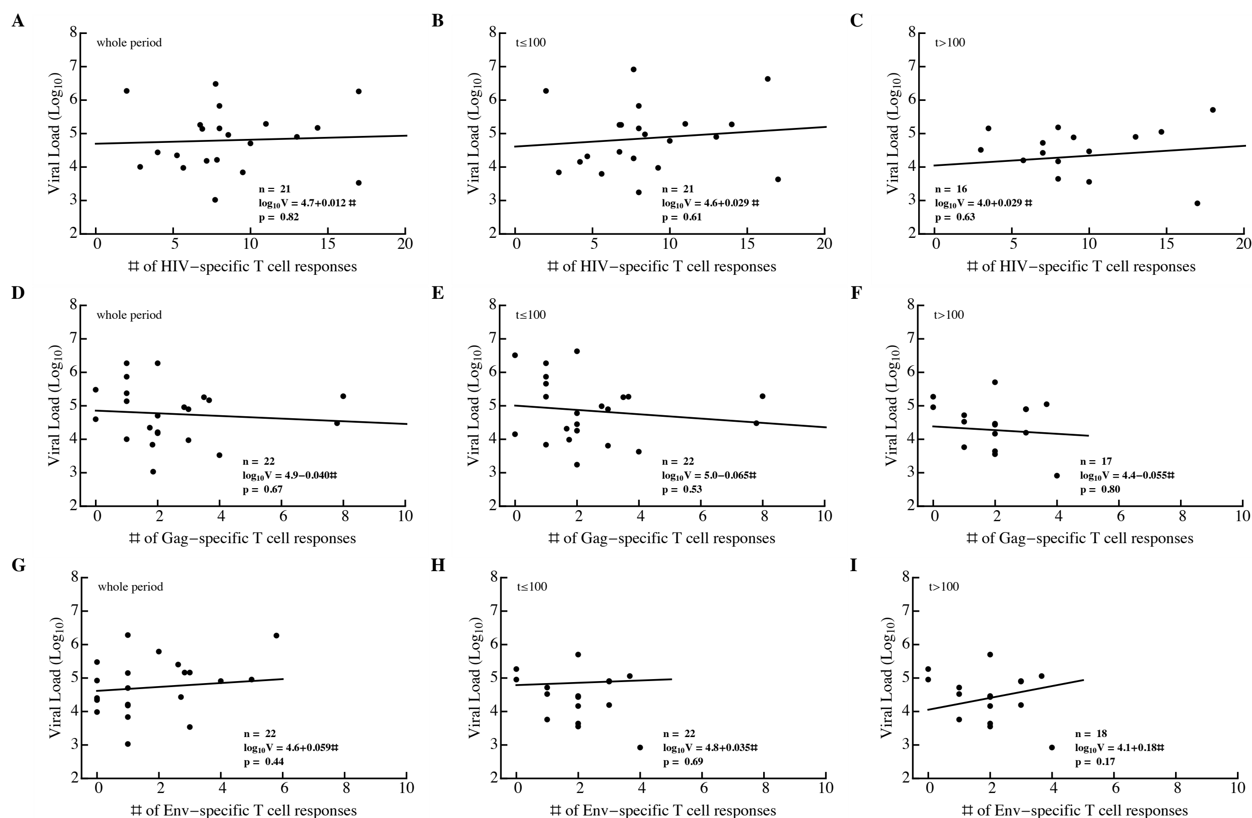

Figure 4: Breadth of HIV-specific CD8$^+$ T-cell response in a patient does not correlate significantly with average viral load. We calculated the average number of HIV-specific (A–C), Gag-specific (D–F), and Env-specific (G–I) CD8$^+$ T-cell responses over the whole observation period (A, D, G), during acute infection ($t \leq 100$ days since symptom onset; B, E, H), or during chronic infection ($t > 100$ days since symptom onset; C, F, I) and $\log_{10}$ average viral load in that time period. The average viral load during infection was not dependent on the breadth of the Gag-specific CD8$^+$ T-cell response during the infection (D–F). Patient SUMA0874 was excluded from the analysis in A–C due to insufficient measurements of all T-cell responses at all time points.

278 We investigated the relationship between three different metrics of T-cell response efficacy:
279 breadth, SE, and EI (see Material and Methods). For that, we calculated the average viral load
280 and average metric for the whole observation period in a patient, during the acute ($t \leq 100$ days
281 since symptoms) or chronic ($t > 100$ days) phase of infection. None of the correlations between
282 metric and viral load were significant, independent of the time period of infection or protein
283 specificity (Figs. 4 and S11).
284 We also investigated whether changes in the immune response breadth over time were nega-
285 tively correlated with viral load. Because there was a statistically significant increase in breadth
286 within the first month of symptom onset, a negative correlation between the change in breadth

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and viral load may indicate that a larger breadth is associated with viral control. However, both negative and positive correlations were found in similar proportions, indicating that a greater breadth did not necessarily drive reduction in viral load (or vice versa). To determine if individual epitope-specific CD8$^+$ T cells contribute to viral control, we calculated Spearman's rank correlation coefficients between the magnitude of epitope-specific T-cell response and viral load for all T-cell responses over time (Fig. 5). We found that there were disproportionally more negative than positive correlations, which suggested that increasing T-cell responses drive the decline in viral load (Fig. 5A). By dividing the data into correlations during the immune response expansion ($t \leq t_{\text{peak}}$, Fig. 5B) and contraction phases ($t > t_{\text{peak}}$, Fig. 5C) we found that most negative correlations are observed when T-cell responses expand (and the viral load declines). These analyses are consistent with the idea that expansion of HIV-specific CD8$^+$ T-cell responses is strongly associated with viral decline and that the contribution of T cells to viral control could be lower during chronic infection.

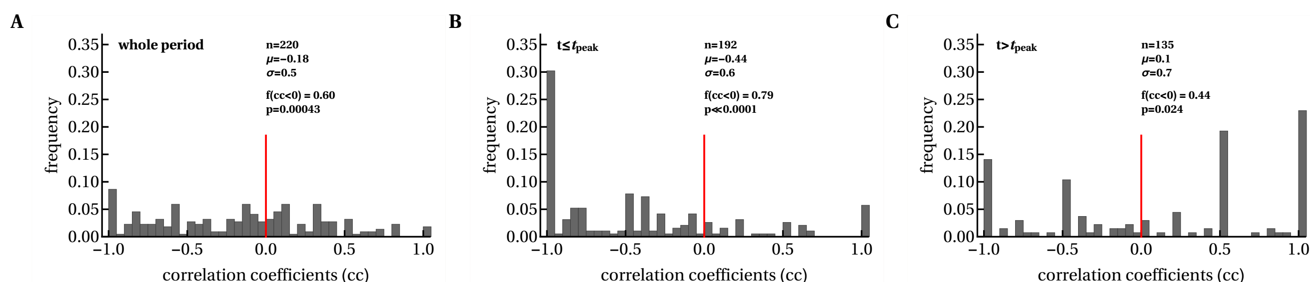

Figure 5: Expanding CD8$^+$ T-cell responses were negatively correlated with viral load before T cell numbers reached their peak values. We calculated Spearman's correlation coefficients between longitudinal changes in viral load and epitope-specific CD8$^+$ T-cell responses in each patient during the whole period (A), and before (B) and after (C) the peak of CD8$^+$ T-cell response. The $f(cc < 0)$ value denotes the fraction of negative correlation coefficients (cc), and p values are indicated for the binomial test of equal distribution of positive and negative correlations.

3.3 Most HIV-specific CD8$^+$ T-cell responses expand slowly and peak early

Several recent studies have quantified HIV dynamics during acute infection in patients either by using data from blood banks or by frequent sampling of individuals at high risk of HIV infection [84, 98]. However, as far as we know there are no accurate estimates of parameters characterizing the kinetics of HIV-specific CD8$^+$ T-cell response in acute infection. Therefore, we used a simple mathematical model (see eqn. (1) in Material and Methods) to characterize the kinetics of epitope-specific CD8$^+$ T-cell responses during acute HIV infection (Fig. S13). Since our mathematical model (eqn. (1)) describes T-cell responses specific to different viral epitopes in uncoupled form, all model parameters could be estimated for each T-cell response independently (Fig. 6).

The dynamics of HIV-specific CD8$^+$ T-cell responses were variable in individual patients. To further our analysis, we divided all HIV-specific T-cell responses into two subsets. In the first, a larger subset (about 80%) of T-cell responses were predicted to either expand or contract from the onset of symptoms ("early" responses, see Figs. 1 and 6). In a smaller subset, CD8$^+$ T-cell responses had a delay T_{on} in the expansion kinetics ("delayed" or "late" responses, see Figs. 1 and 6).

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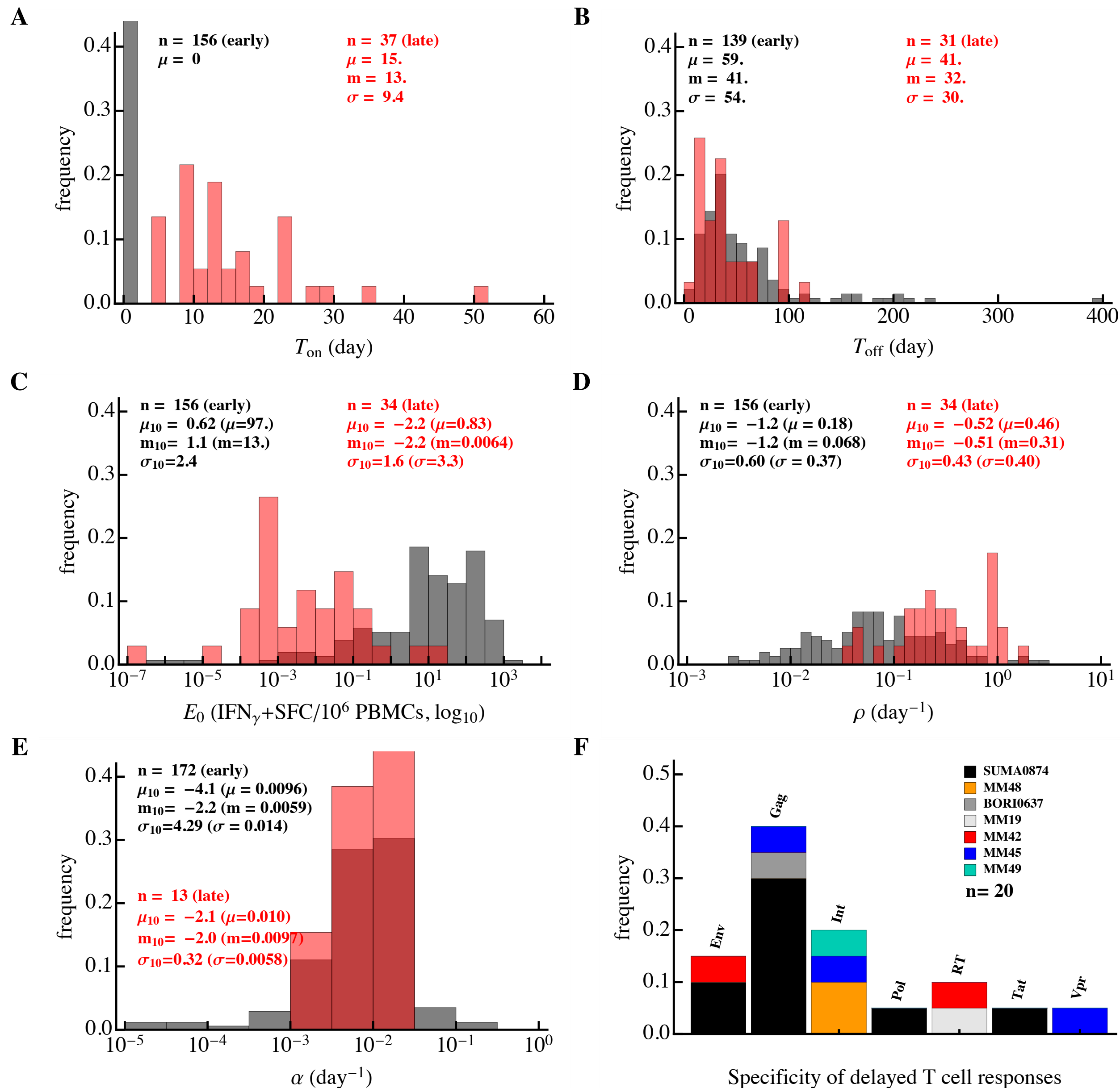

Figure 6: Differences in the kinetics of early and late HIV-specific CD8$^+$ T-cell responses. We fitted the $T_{\rm on}/T_{\rm off}$ model (eqn. (1)) to the data on the dynamics of epitope-specific CD8$^+$ T-cell response in each patient and plotted the distribution of the estimated parameters. The results are presented separately for T cell responses that started expanding (or contracting) from the first observation ("early" responses, about 80% of all responses; black) or delayed responses, which were undetectable at one or several initial time points ("late" responses; red). Panels show distributions for (A) time of expansion of T-cell response ($T_{\rm on}$), (B) time to peak of each T-cell response ($T_{\rm off}$), (C) initial predicted frequency of epitope-specific CD8$^+$ T cells (E_0), (D, E) expansion (ρ) and contraction (α) rates of T-cell responses, respectively, and (F) proteins recognized by late CD8$^+$ T cell responses. In A–E, n represents the number of fitted responses, and μ, m and σ represent mean, median and standard deviation, respectively (μ_{10}, m_{10}, and σ_{10} are mean, median and standard deviation for log$_{10}$-scaled parameters). Late responses were predicted to have a higher expansion rate ρ (Mann–Whitney, $p < 0.001$) and smaller frequency E_0 (Mann–Whitney, $p < 0.001$) than early responses.

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316 Several parameter estimates differed between the two response subsets. In general, early re-
317 sponses expanded slower, peaked later, and had a higher predicted frequency E_0 than late responses
318 (Fig. 6). The average delay T_{on} in the expansion kinetics of late responses was only 15 days since
319 symptom onset but some responses started expanding even later (Fig. 6A). There was a minor
320 difference in the timing of the T-cell response peak (Mann–Whitney, $p = 0.035$) and over 90% of
321 epitope-specific CD8$^+$ T-cell responses peaked before 100 days since symptom onset (Fig. 6B).
322 For the early responses, we found that there was an average of 97 antigen-specific CD8$^+$ T cells
323 per million peripheral blood mononuclear cells (PBMC) detected at the first time point (median,
324 13 IFN-γ^+ spot forming cells (SFC) per million PBMC, Figure 6C). Note that this is not very
325 different from the experimental estimates of the frequency of human naive CD8$^+$ T cells specific to
326 viral epitopes [87, 99]. To predict a theoretical frequency E_0 at which late responses would start
327 to expand exponentially from $t = 0$ days since symptom onset, we extended the fitted curve in the
328 negative time direction to estimate the intercept with the y-axis. Around 24% of epitope-specific
329 CD8$^+$ T-cell responses including many "delayed" responses were predicted to have a precursor
330 frequency $E_0 < 10^{-2}$ per million PBMC. Because this estimate is physiologically unreasonable
331 [87, 99], many of the "late" responses are likely to have started expanding after the onset of
332 symptoms (i.e., were "delayed").
333 Importantly, the majority (60%) of early epitope-specific CD8$^+$ T cells expanded extremely
334 slowly at a rate of < 0.1 day^{-1} (median, 0.068 day^{-1}, Fig. 6D). An expansion rate of 0.1 day^{-1}
335 corresponds to a doubling time of 7 days and this suggests that even in acute infection the majority
336 of HIV-specific T cell responses expanded very slowly. In contrast, delayed responses expanded
337 significantly faster, with a median rate $\rho = 0.31$/day, which was only slightly lower than the T
338 cell expansion rate in response to the yellow fever virus vaccine [61]. A small fraction of early
339 responses (6%) expanded at a fast rate of > 0.5 day^{-1}, but most responses contracted very slowly
340 at a rate of < 0.01 day^{-1} (Fig. 6E). This implies that HIV-specific T-cell responses were relatively
341 stable after their peak with a half-life of 70 days or longer. Thus, our analysis suggests that
342 most HIV-specific CD8$^+$ T cell responses expand slowly, peak early, and remain relatively stable
343 thereafter.
344 It was unclear why not all T-cell responses started expanding from symptom onset when viral
345 loads are relatively high (Fig. S4). For example, CD8$^+$ T-cell responses to multiple epitopes of
346 influenza virus or lymphocytic choriomeningitis virus (LCMV) in mice appear to start expanding
347 almost simultaneously [81, 88, 100–102]. One hypothesis is that late T cell responses are restricted
348 to proteins that are not expressed at high levels during the HIV life cycle. However, this hypothesis
349 was not supported by our data as delayed T-cell responses recognized multiple proteins, similarly
350 to all T-cell responses in the cohort (Fig. 2A and Fig. 6F). A second explanation is that these
351 delayed responses may be actively suppressed by the early responses. To investigate this, we
352 calculated the Pearson correlation coefficients between 20 delayed responses (with a predicted
353 frequency $E_0 < 0.01$, i.e., $T_{\text{on}} > 0$) and all other responses in these patients; most of these
354 delayed responses were specific to Gag and found predominantly in patient SUMA0874 (Fig. 2A
355 and Fig. 6F). Interestingly, only 20% of these correlations were negative, suggesting that other
356 early responses continued expanding as late responses appeared. The observation that most early
357 responses peaked after starting to expand further argues against an "active" suppression of delayed
358 responses by early responses. Third, it is possible that late responses simply start from a smaller
359 number of precursors [87]; this hypothesis could not be tested with our current data because
360 estimated frequencies E_0 are unlikely to be true precursor frequencies. Fourth and finally, delayed
361 expansion in the blood could simply be due to the retention of expanding T cell populations in
362 the lymphoid tissues. Testing this hypothesis would require measurements of HIV-specific T cell

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363 responses in the lymph nodes and/or spleen. Taking together, the reasons why some HIV-specific
364 CD8$^+$ T cell responses appear late in the blood of infected patients remain unclear.

365 3.4 Evidence of intraclonal competition of CD8$^+$ T cells

366 Magnitude of epitope-specific CD8$^+$ T-cell response is likely to be important in limiting virus
367 replication (Fig. 5). However, factors that influence the expansion kinetics of the CD8$^+$ T cell
368 response and response peak in humans remain poorly defined. Recent work suggested that viral
369 load in the blood of human volunteers during vaccination is the major determinant of the peak
370 T-cell response following yellow fever virus vaccination [103]. We found that the frequency E_0 had
371 a limited impact on the timing of the T-cell response peak (Fig. 7A) and the rate of T-cell response
372 expansion strongly affected the timing of the peak (Fig. 7B). The latter suggests that more rapidly
373 expanding responses peak early, which is markedly different from CD8$^+$ T-cell responses in mice
374 infected with LCMV where T-cell responses, specific to different viral epitopes, expand at different
375 rates but peak at the same time [86, 88, 100, 101].

376 Interestingly, we found that the expansion rate of epitope-specific T cell responses was strongly
377 dependent on the average viral load during the expansion phase (Fig. 7C) and on the estimated
378 frequency E_0 (Fig. 7D). The dependence of the expansion rate on viral load was nonlinear, in
379 contrast with the linear or "saturating" function used in mathematical models describing the
380 dependence of T cell proliferation rate on viral load [64, 66, 71, 86, 93, 104]. The observed decline
381 in expansion rate of T-cell responses with a higher frequency E_0 strongly indicates the presence of
382 intraclonal competition, suggesting that increasing precursor frequency of T cells by vaccination
383 (an expected result of vaccination) may dramatically reduce expansion kinetics of such responses
384 following exposure to HIV and this may limit T cell efficacy in controlling virus replication.
385 Similar intraclonal competition was also documented in some cases with T cell responses in mice
386 [105, 106]. In particular, increasing the number of chicken ovalbumin-specific naive CD8$^+$ T cells
387 in mice reduced the expansion rate of the ovalbumin-specific CD8$^+$ T cell population following
388 priming with ovalbumin [105].

389 Both the average viral load and predicted frequency E_0 had minimal impact on the peak
390 CD8$^+$ T-cell response (Fig. 7E&F); interestingly, no correlation between CD8$^+$ T cell precursor
391 frequency and peak T-cell response was found in mice [105]. The length of the expansion phase
392 ($T_{\text{off}} - T_{\text{on}}$) had little influence on the peak immune response (not shown). It is therefore possible
393 that the peak immune response was determined by virus-independent factors (e.g., cytokines);
394 further analyses are needed to better understand the mechanisms limiting the magnitude of T cell
395 responses to HIV.

396 It has been previously proposed that some viral infections such as HIV and hepatitis C virus
397 in humans and LCMV in mice induce a delayed CD8$^+$ T-cell response, and this delayed response
398 results in viral persistence [107, 108]. We sought to determine whether HIV-specific CD8$^+$ T-cell
399 responses appear late in infection compared, for example, to viruses causing only acute infections
400 in humans. It is clear that the expansion kinetics of virus-specific CD8$^+$ T cell responses are likely
401 to depend on viral load (e.g., Fig. 7C&D). Therefore, for an appropriate comparison of acute and
402 chronic viral infections we calculated the time intervals between the maximum observed viral load
403 and the time when epitope-specific CD8$^+$ T cells were predicted to reach their peak (T_{off}). About
404 40% of HIV-specific T cells peaked only 10 days after the maximum viremia (not shown). A 10-
405 day delay in CD8$^+$ T-cell response peak after the peak viremia is similar to that which has been
406 observed following yellow fever vaccination [60, 61]. Therefore, these results suggest that many
407 HIV-specific CD8$^+$ T-cell responses are generated with similar kinetics relative to viral load for

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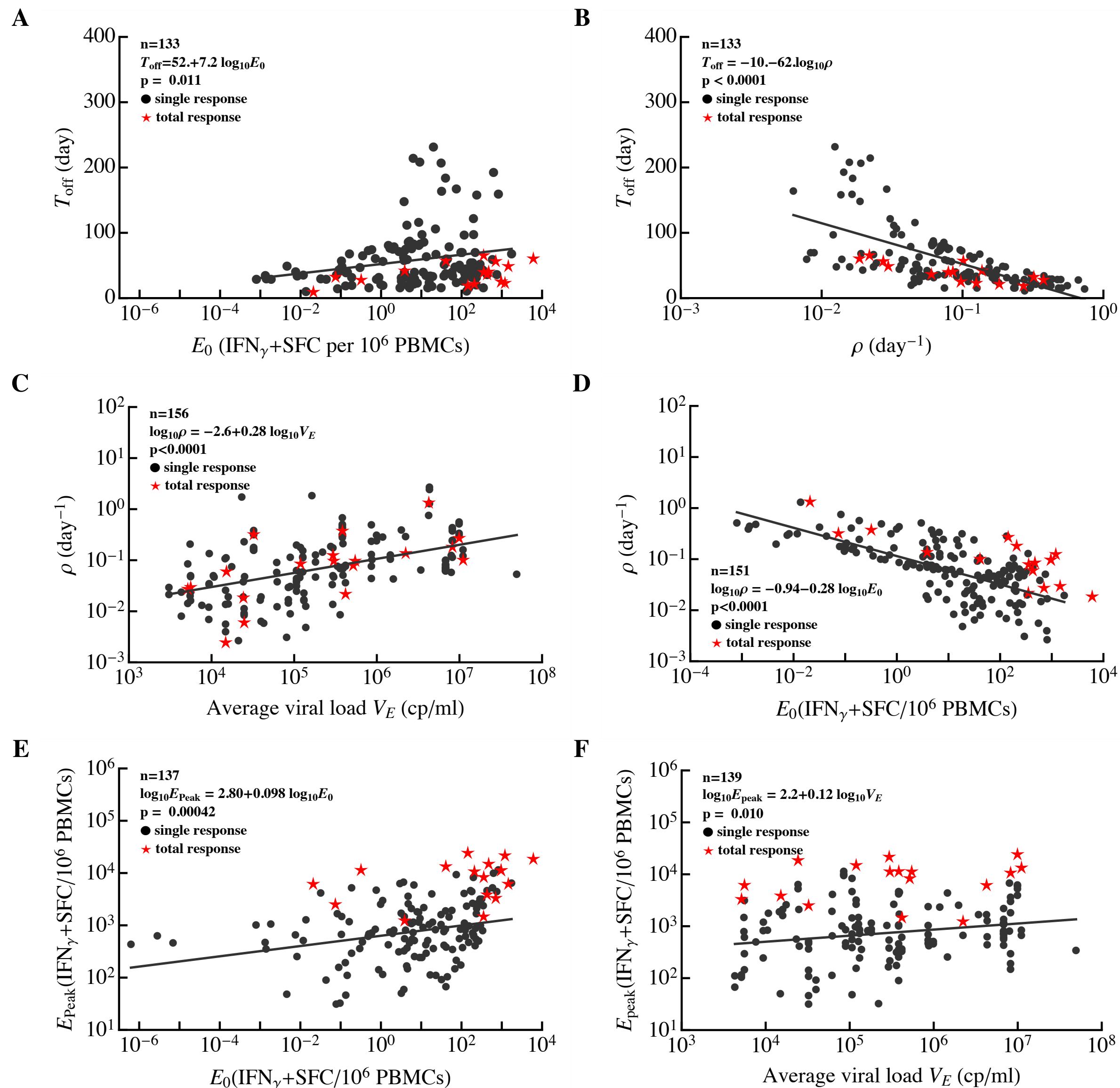

Figure 7: Correlations between major parameters determining dynamics of HIV-specific CD8$^+$ T-cell responses in acute infection. For all epitope-specific CD8$^+$ T-cell responses in all 22 patients (circles) or the total HIV-specific CD8$^+$ T-cell response per patient (stars), we estimated the initial frequency of epitope-specific CD8$^+$ T cells (E_0), rate of expansion of T cell populations (ρ), time of the peak of the response ($T_{\rm off}$), rate of contraction of the immune response after the peak (α), predicted peak values reached by the epitope-specific CD8$^+$ T-cell response ($E_{\rm peak} = E(T_{\rm off})$), and the average viral load (V_E). Solid lines denote regression lines; regression equations and p values are indicated on individual panels for all epitope-specific CD8$^+$ T-cell responses. The total HIV-specific CD8$^+$ T-cell response showed a similar trend to all epitope-specific CD8$^+$ T-cell responses (results not shown). Panels show correlations between the timing of the immune response peak $T_{\rm off}$ and predicted frequency E_0 (A), $T_{\rm off}$ and ρ (B), expansion rate ρ and average viral load V_E (C), ρ and E_0 (D), peak immune response $E_{\rm peak}$ and E_0 (E), and $E_{\rm peak}$ and V_E (F). For a given patient, we calculated the total HIV-specific CD8$^+$ T cell response as the sum of all epitope-specific CD8$^+$ T-cell responses at the same time point (i.e., by ignoring "nd"). For patient MM42, we could not fit the $T_{\rm on}/T_{\rm off}$ model to the dynamics of total CD8$^+$ T cell response data because of wide oscillations in the data. Identified relationships did not change if estimates for responses with unphysiological initial frequencies ($E_0 \leq 10^{-2}$) were excluded from the analysis (results not shown).

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408 both acute and chronic infections in humans and yet most of them expand significantly slower than
409 during an acute viral infection. This could, in part, be simply a consequence of HIV replication
410 being slower than yellow fever virus replication.

411 3.5 Evidence of interclonal competition of CD8$^+$ T cells

412 Many mathematical models of the CD8$^+$ T-cell response to HIV assume competition between
413 responses specific to different viral epitopes [64, 68, 71]. In fact, the presence of such competition
414 is important for explaining the kinetics and timing of viral escape from CD8$^+$ T-cell responses
415 [64, 71]. However, to our knowledge, there is no experimental evidence of competition between
416 different CD8$^+$ T-cell responses in HIV infection. Studies of CD8$^+$ T-cell responses to intracellular
417 pathogens in mice reached conflicting conclusions, with some reporting no evidence for competition
418 [73, 78, 100, 109] and others reporting some evidence for competition [77, 82, 110, 111]. A recent
419 analysis of data on the magnitude of CD8$^+$ T-cell responses specific to several HIV epitopes found
420 no evidence for such interclonal competition during the chronic phase of HIV infection [83].

421 This previous study suffered from two major limitations: only a few CD8$^+$ T-cell responses
422 were analyzed, and the analysis was restricted to a single time point [83]. Therefore, we sought
423 to determine if there is any evidence for competition between T-cell responses specific to different
424 viral epitopes in the data of Turnbull *et al.* [55]. If there is competition between two responses, we
425 expect that an increase in the magnitude of one response should lead to a decline in the magnitude
426 of another, i.e., there should be a negative correlation between longitudinal changes in magnitudes
427 of the two responses (Fig. S14). We therefore calculated correlations between magnitudes of all
428 pairs of epitope-specific CD8$^+$ T-cell responses over time for every patient (Fig. 8). The proportion
429 of negative correlations indicating potentially competing immune responses varied by patient and
430 was not strongly dependent on the time since infection (e.g., see Fig. S15). In some patients,
431 the proportion of positively and negatively correlated responses were similar (e.g., MM39, MM47,
432 MM51) but in most patients, negative correlations were significantly under-represented as judged
433 by the binomial test (Fig. 8). Overall, approximately 18% of correlation coefficients were negative,
434 suggesting that a small proportion of T-cell responses may be competing during the infection.
435 However, in contrast with the assumptions of many mathematical models, the vast majority of
436 responses do not appear to compete during the infection.

437 Previous analysis also suggested that in the presence of competition between epitope-specific
438 CD8$^+$ T cells, a larger number of responses should result in a smaller average size of epitope-
439 specific T cell response [83]. However, Fryer *et al.* [83] did not find a significant correlation
440 between the number of responses and average size of epitope-specific T-cell response, indicating
441 an absence of competition. One potential problem with this previous analysis was that it did not
442 take viral load into account in the correlation, and it is possible that viral load may affect the
443 strength of competition. For example, competition may be weak at high viral loads owing to an
444 abundance of the antigen, and may be strong at lower viral loads (or vice versa). Furthermore, we
445 showed that viral load influences the dynamics of HIV-specific CD8$^+$ T-cell responses (Fig. 7C)
446 and thus may confound the correlation. Therefore, we repeated the analysis of Fryer *et al.* [83] by
447 dividing the cohort data into three groups with different average viral loads (low, intermediate,
448 and high, Fig. 9). During the acute infection ($t \leq 100$ days after symptom onset) there was
449 a statistically significant negative correlation between the number of responses and the number
450 of T-cell responses (Fig. 9C) suggesting interclonal competition. However, significant negative
451 correlations were not observed for all time periods or all viral loads (e.g., $t > 100$ with low or
452 high viral load, Fig. S12); thus, overall, by correcting for multiple comparisons we must conclude

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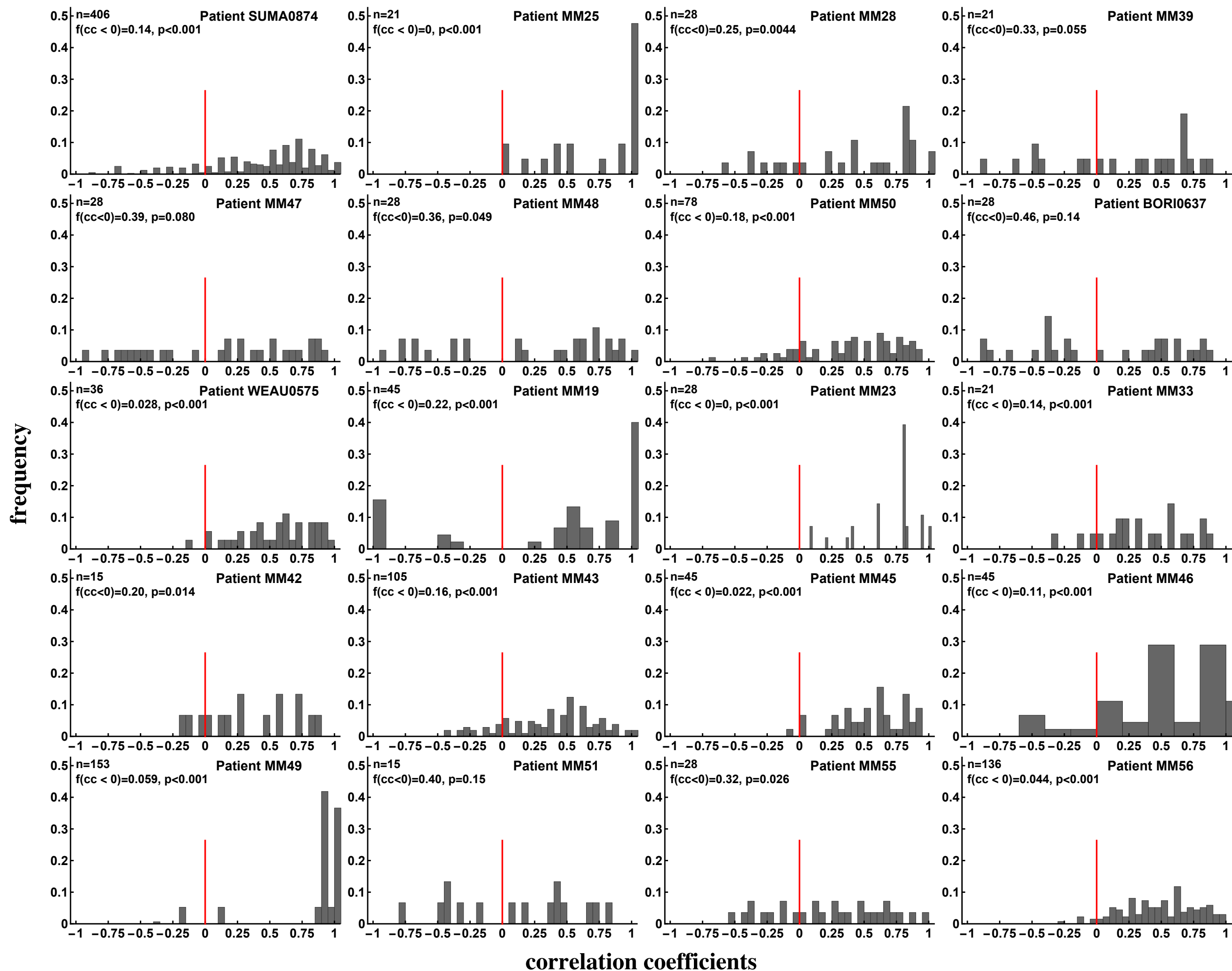

Figure 8: Evidence of interclonal competition between epitope-specific CD8$^+$ T cell responses. We calculated Spearman's rank correlation coefficients between longitudinal changes of pairs of epitope-specific CD8$^+$ T cell responses in a given patient (see individual panels) and plotted the distribution of these coefficients. Panels show the number of correlations (n), fraction of negative correlation coefficients ($f(cc) < 0$), and p values for the deviance of the distribution from uniform, found using the binomial test with null being the equal fraction of positive and negative correlations. We found that the majority of CD8$^+$ T cell populations expand and contract in unison and therefore do not appear to compete during the infection. Overall, discordant dynamics (negative correlation coefficients) were observed for 18% of all responses irrespective of the stage of infection (acute or chronic). Patients MM38 and MM40 were excluded from the analysis for having too few correlation pairs (two or three).

453 that there is no correlation between T-cell response breadth and average size of epitope-specific
454 T-cell response. The two types of analyses (longitudinal in Fig. 8 and cross-sectional in Fig. 9)
455 may thus have different power in detecting competition between immune responses. Our analysis
456 of longitudinal data suggests that a sizable proportion of HIV-specific T-cell responses may be
457 competing during infection.

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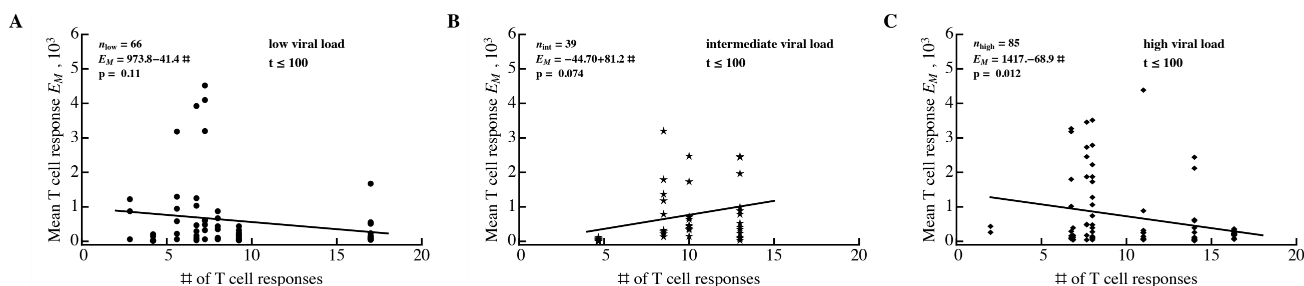

Figure 9: Average size of epitope-specific CD8$^+$ T-cell response is unrelated to the number of HIV-specific T-cell responses. For every patient, we calculated the average number of HIV-specific CD8$^+$ T-cell responses and the average density of epitope-specific T cells in a given observation period. To exclude the contribution of viral load to this relationship, we divided all 22 patients into three groups according to their mean viral load (low $\log_{10}$ viral load: 3.40–4.44; intermediate viral load: 4.60–5.03; high viral load: 5.25–6.83). Groups were estimated using the Manhattan Distance with the `FindClusters` function in `Mathematica`. Regression lines and corresponding p values are indicated on individual panels. Overall, results varied by time period and most correlations were not statistically significant (Fig. S12).

4 Discussion

It is generally accepted that CD8$^+$ T cells play an important role in controlling HIV replication. Features of HIV-specific CD8$^+$ T cell responses that are important in mediating this control remain incompletely understood. T cell specificity, polyfunctionality and ability to proliferate have been cited as important correlates of protection [17, 33, 47, 112]. Here, we analyzed the kinetics of the CD8$^+$ T cell response to the whole HIV proteome in patients controlling HIV poorly, and thus identified features associated with poor viral control.

In these patients, HIV infection induced a reasonably large number of CD8$^+$ T-cell responses, most of which were generated during the earliest stages of infection (first 35 days after symptom onset). On average, CD8$^+$ T cell response breadth increased moderately during the first month since symptom onset and remained relatively stable for the next year. However, breadth varied differently in individual patients. In some patients, breadth increased twofold over the course of 2 months after symptom onset, and in some patients, breadth remained constant or even declined. Importantly, a minimal change in CD8$^+$ T-cell response breadth from symptom onset to chronic phase was also observed in three patients from the Center of HIV Vaccine Immunology cohort [93]. However, our finding seems to contradict a conclusion reached by Turnbull *et al.* [55] who found that the median breadth of CD8$^+$ T-cell response increased from 2 to 6. The major difference between our analysis and the previous study is how we counted responses. Turnbull *et al.* [55] only counted responses that peaked within 2–3 weeks post symptoms, whereas we counted all detected responses.

Because of the high variability in the rate of exponential growth of CD8$^+$ T-cell responses (e.g., Fig. 7B) it is perhaps expected that only few rapidly expanding responses should be observed early in infection. Later in infection, immune response with slower expansion rates would be detected, creating the impression of T-cell response breadth increasing with time. This idealistic interpretation may be an artifact of a limited sensitivity of ELISPOT and difficulty tracking T cell response at the place of their generation, i.e., secondary lymphoid tissues. Better methods of T cell response detection in the blood and tissues are likely to provide a more complete picture of the dynamics of T cell response breadth.

Because T cell response breadth may not be stable over the course of infection in individual

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487 patients, interpreting relationships between the breadth and other parameters, e.g., viral load,
488 must be done with care. For example, it was observed that a change in the number of Gag-specific
489 $CD8^+$ T cell responses with time was dependent on a patient's viral load, suggesting that a larger
490 breadth in chronic HIV infection may be the consequence and not the cause of a lower viral load
491 [97].

492 We found no significant correlation between breadth, SE, or EI of HIV-, Gag-, or Env-specific
493 $CD8^+$ T-cell responses and viral load. This was in contrast with several (but not all) previous
494 studies that identified a statistically significant negative correlation between the number of Gag-
495 specific T cell responses and viral load [33, 34, 36, 91]; some of those studies included patient
496 cohorts of a similar size. This could be due to limited power in our study. Power analysis
497 indicated that for a sufficiently large number of patients, statistically significant correlations could
498 be found; however, such correlations were dependent on the measure of immune response efficiency.
499 Efficiency measured as the number of Gag-specific T cell responses was negatively correlated with
500 viral load, whereas EI for HIV- or Gag-specific T-cell responses was positively correlated with
501 viral load. The latter result, if confirmed in a larger cohort, is surprising, since T-cell responses of
502 a similar magnitude were predicted to limit viral escape from T cells [56, 93], and would therefore
503 be expected to lead to a lower viral load.

504 It is not clear whether the small number of patients in our cohort ($n = 22$) was responsible for
505 the absence of a statistically significant correlation. Two previous studies also involved a relatively
506 small number of patients and yet reached a statistically significant negative correlation between
507 the number of Gag-specific T-cell responses and viral load [34, 91]. Statistically significant results
508 may arise in underpowered studies by chance [113], and a small number of patients in the study
509 by Radebe *et al.* [91] may indicate an accidental statistically significant correlation. To investigate
510 the potential difference between our result and that from Geldmacher *et al.* [34], we reanalyzed
511 the data from the latter (data were provided by Chriss Geldmacher). The re-analysis revealed
512 several major differences between our study and theirs. First, we found that Geldmacher *et al.*
513 [34] detected more Gag-specific responses than Env-specific responses (slope of the Env vs. Gag
514 regression was 0.11 with $p \ll 10^{-3}$ when compared to slope = 1; t test). In Turnbull *et al.* [55] data,
515 the number of Gag and Env-specific T-cell responses were more similar (slope = 0.56, $p = 0.07$
516 for the comparison with slope = 1; t test). Second, the correlation strength between the number
517 of Gag-specific T-cell responses and viral load was previously overestimated; a non-parametric
518 Spearman's rank correlation test resulted in a higher, but still significant, p value ($p = 0.013$)
519 than that found previously (see Fig. 2 in [34]; the published value was $p = 0.0016$). Third and
520 finally, we found that the statistical significance of the negative correlation was driven exclusively
521 by four patients (out of 54) with a large number of Gag-specific responses (≥ 6); removing these
522 patients from the analysis made the correlation between viral load and number of Gag-specific
523 $CD8^+$ T-cell responses statistically nonsignificant ($p = 0.085$, results not shown). Resampling
524 data from 18–22 patients from the Geldmacher *et al.* [34] cohort with replacement demonstrated
525 low power in correlation between T-cell response breadth and viral load (power = 46%); however,
526 including the four outliers with high numbers of Gag-specific T-cell responses increased the power
527 to 63% (not shown). Together, these results suggest that the potential protection by Gag-specific
528 T-cell responses may not extend to all Gag-specific T-cell responses and may be a feature of only
529 some patients. This interpretation is consistent with previous analyses that only looked at T-cell
530 responses to defined Gag epitopes, and not to the whole gene [33, 96]. More studies are needed
531 to understand the protective nature of Gag- specific $CD8^+$ T-cell responses; for example, the
532 breadth of Gag-specific $CD8^+$ T-cell responses did not predict the control of HIV after cessation
533 of antiretroviral therapy in patients treated for acute HIV infection [114].

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534 An additional important part of our analysis is an illustration of other metrics that can be
535 used to evaluate the potential efficacy of CD8$^+$ T cell responses such as SE and EI. While it is
536 clear they can complement a commonly used measure of efficacy (response breadth), these metrics
537 have a strong limitation in that they ignore data from patients with no immune response, and
538 EI is ill-defined for cases when only one immune response is present. Furthermore, calculation of
539 these metrics requires measurement of the magnitude of epitope-specific T-cell responses.

540 By fitting a simple mathematical model to the longitudinal dynamics data for epitope-specific
541 CD8$^+$ T-cell responses, we estimated the parameters for T-cell responses in HIV infection. We
542 predict that the vast majority of HIV-specific T cell responses (80%) recognize HIV early and ex-
543 pand (or are already contracting) during the onset of symptoms. These T-cell responses expanded
544 extremely slowly, at a rate of < 0.1 day^{-1}, indicating that vaccines may need to induce responses
545 with significantly quicker expansion kinetics. A small proportion of responses (20%) had a delayed
546 expansion, and these late responses expanded at significantly higher rates than early responses.
547 All responses appeared to be relatively stable after reaching their peak (the contraction rate was
548 < 0.01 day^{-1} for most epitope-specific CD8$^+$ T cells).

549 Slow expansion of the early T-cell responses may be due to intraclonal competition for resources
550 such as antigens. Indeed, we found a strong negative correlation between the predicted initial
551 frequency of the response and the rate of response expansion, which is consistent with the presence
552 of intraclonal competition. Several previous reports documented the presence of such competition
553 under some, often unphysiological, circumstances (e.g., by artificially increasing the number of
554 naive CD8$^+$ T cells specific to an antigen) [105, 106, 115]. Slow expansion of T-cell responses
555 may also arise as an artifact of the measurement of T-cell response magnitude as frequency (i.e.,
556 number of spots per million PBMC); however, because most of our total responses reach only about
557 1% of PBMCs (e.g., Fig. 7E) and in general, about 10% of PBMCs are CD8$^+$ T cells (personal
558 communication from Seph Borrow), this alternative seems unlikely. The presence of intraclonal
559 competition may strongly limit the magnitude of epitope-specific T-cell responses induced by
560 vaccination.

561 A previous study found that the amount of yellow fever virus in the blood of volunteers greatly
562 affects the magnitude of CD8$^+$ T-cell response induced by vaccination [103]. In our analysis,
563 however, this correlation was not significant if we corrected for multiple comparisons (Fig. 7F).
564 More work is needed to understand the factors regulating the magnitude of the T cell response
565 following acute and chronic viral infections, as these may be different.

566 If broad HIV- or Gag-specific CD8$^+$ T-cell responses are protective (as several studies have
567 suggested; see above), induction of a broad T cell response may be difficult in the presence of in-
568 terclonal competition. One previous study suggested that interclonal competition between CD8$^+$
569 T-cell responses specific to different viral epitopes is absent in chronic HIV infection [83]. Inter-
570 estingly, we found that the vast majority of HIV-specific T-cell responses (about 82%) appeared
571 to have "synchronous" dynamics. Yet a substantial fraction of all responses did show evidence
572 of competition when an increase in the magnitude of one response was associated with a decline
573 in another (Fig. 8). The relative fraction of such potentially "competing" T-cell responses varied
574 by patient. Interestingly, using the method of Fryer *et al.* [83] to correlate the average size and
575 number of T-cell responses did not allow the detection of competition. This indicates that longi-
576 tudinal data may provide a higher power for detecting competition between epitope-specific CD8$^+$
577 T-cell responses. Our results thus suggest that interclonal competition may potentially limit the
578 breadth of vaccine-induced CD8$^+$ T-cell responses.

579 It should be emphasized, however, that correlation does not necessarily indicate causality
580 and negative associations between kinetics of individual T cell responses may arise for reasons

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581 unrelated to competition. Understanding why some responses are discordant while others increase
582 or decrease in unison is likely to shed more light on the degree of T cell competition during HIV
583 infection. Recent work suggests that competition between HIV-specific CD8$^+$ T cells for access to
584 infected cells may influence the rate of virus escape [71, 93]. Detecting competition in a biological
585 system is a complicated problem (e.g., [116]). Direct fitting of classical mathematical models
586 (Lotka–Volterra and predator–prey) revealed that these models can be consistent with some data
587 but in some cases failed to accurately describe the data (results not shown). Therefore, using
588 mathematical models alone does not allow discrimination between alternative mechanisms of T-
589 cell response competition, and further experiments are needed. One possible way of investigating
590 whether responses compete is to boost the magnitude of a given response (e.g., by therapeutic
591 vaccination) and see if this influences the magnitude of other T cell responses. Clinical evidence
592 suggests there is limited competition between humoral immune responses specific to different
593 infections [117].

594 A number of important caveats could not be addressed in this study. These include issues
595 with experimental data and mathematical model assumptions. First, CD8$^+$ T-cell responses were
596 mapped at 6 months after symptom onset, so some T-cell responses appearing earlier or later
597 than that time point could have been missed in the analysis. It is important to note, though,
598 that mapping of CD8$^+$ T-cell responses is often done at a single time point (e.g., [54, 56, 58]),
599 meaning such analyses suffer from a similar limitation. Second, the IFNγ ELISPOT may not be
600 sensitive enough to detect all the responses, and some evidence suggests that the sensitivity of
601 this method may vary during the infection [59]. This is likely to affect some parameters but not
602 others; for example, estimates of the rate of expansion of HIV-specific CD8$^+$ T cell responses are
603 likely to be dependent on ELISPOT sensitivity. Third, responses were measured only in the blood
604 whereas interactions between the virus and T cells are occur in lymphoid tissues. This problem is
605 unlikely to be resolved in human studies because it will be difficult to obtain longitudinal samples
606 of lymphoid tissues from patients. Fourth, the simple $T_{\text{on}}/T_{\text{off}}$ model may not fully describe T-cell
607 response kinetics, especially during early acute infection. However, this model has been successful
608 in describing the dynamics of the CD8$^+$ T-cell response to viral infections in both mice and humans
609 [61, 86, 88, 118]. Fifth, averaging of the viral load to infer correlations between parameters may
610 not be fully appropriate because in many patients there were large changes in viral load over
611 time (Fig. S4). However, explicit inclusion of viral load dynamics in some simple models proved
612 difficult (results not shown). Sixth, the data do not include the virus ramp-up phase, meaning
613 the earliest CD8$^+$ T-cell responses may be missed. Indeed, this might be an issue with many of
614 the recent analyses and, to date, the available data on CD8$^+$ T-cell response during the virus
615 expansion phase are limited. It should be noted that the data in which viral load in the blood
616 is measured soon after exposure (e.g., [84]) often comes from individuals who are at high risk of
617 acquiring HIV infection, and thus virus dynamics in such patients may not represent an "average"
618 patient. Finally, alignment of patient's data by the day since symptom onset may be misleading
619 as different patients are likely to experience symptoms at different times after infection. Methods
620 such as Fiebig staging or Poisson fitter may allow better alignment data in terms of days since
621 infection [119, 120] but the accuracy of these novel methods has not been well studied, in part,
622 because the exact date for HIV infection is rarely known.

623 In summary, our study provides basic information on the kinetics of CD8$^+$ T cell responses
624 specific to the whole HIV proteome given the limitations of current methods of measuring such
625 responses in humans. Understanding the complex underlying biology of interactions between
626 the virus and virus-specific CD8$^+$ T-cell response, and of the factors driving changes in T cells,
627 is instrumental in determining which T-cell-based vaccines induce a T-cell response exceeding

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628 that induced during natural HIV infection. We expect that such vaccines alone would induce
629 responses with a substantial impact on virus replication. Results of the present analysis will also
630 be helpful in developing better calibrated mathematical models of T-cell responses to HIV, which
631 will be valuable in predicting whether and how T-cell-based vaccines can provide protection upon
632 infection with the virus [10, 121].

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991 # List of Figures

992 1 Schematic representation of the $T_{\text{on}}/T_{\text{off}}$ mathematical model fitted to the epitope-
993 specific CD8+ T-cell response kinetics data [86]. In this model, E_0 epitope-specific
994 naive CD8+ T cells become activated at time $t = T_{\text{on}}$ and start proliferating at rate
995 ρ. At $t = T_{\text{off}}$, T cell response peaks and declines at rate α. We refer to E_0 as
996 the predicted initial frequency of epitope-specific CD8+ T cells [87]. Evidently, E_0
997 may over- or under-estimate the response precursor frequency depending on exactly
998 when the T cells became activated and how adequate the mathematical model is
999 for describing immune response data during the expansion phase. 3
1000 2 Most HIV proteins were recognized by CD8+ T cell responses. We calculated the
1001 frequency at which HIV proteins were recognized by CD8+ T cells; overall, 50%
1002 of responses were directed against Env or Gag (A). $m = 8$ CD8+ T cell responses
1003 were detected in this cohort of 22 patients at any given time point after infection
1004 (B). In B (and other figures in the paper), μ denotes the average, m is the median,
1005 and σ is the standard deviation. The distributions are shown for the first 100 days
1006 after symptom onset but, overall, distributions changed little over the course of 400
1007 days of infection (results not shown). Patient SUMA0874 was excluded from the
1008 analysis in B due to a lack of measurements of all T cell responses at all time points. 6
1009 3 Modest yet statistically significant increase in the average normalized T-cell re-
1010 sponse breadth over the course of the first year of HIV infection. We divided the
1011 observations into different time bins (A, 50-day intervals; B, 100-day intervals) and
1012 calculated the relative breadth for the corresponding interval. The relative breadth
1013 was calculated as the number of HIV-specific CD8+ T-cell responses detected in
1014 a given time period divided by the number of all responses measured for that pa-
1015 tient in all time periods; data were averaged to simplify presentation. Averaging
1016 did not influence the statistical significance of conclusions (not shown). Colors and
1017 symbols represent the data from different patients as shown in Fig. S5 in Supple-
1018 mentary Material. Black horizontal bars denote the mean relative breadth for that
1019 time interval for all patients. There was a statistically significant increase in relative
1020 breadth (Spearman's rank correlation coefficient ρ and p values indicated on pan-
1021 els). There was no change in the average total immune response in all patients (Fig.
1022 S6). Detailed analysis of the relative number of CD8+ T-cell responses in individual
1023 patients revealed variable patterns: constant breadth, increasing breadth, decreas-
1024 ing breadth, and breadth changing non-monotonically over time (Fig. S7). Also, no
1025 overall change in the average breadth (un-normalized) was observed (Fig. S5). We
1026 observed a similarly modest but significant increase in SE and EI of HIV-specific
1027 CD8+ T-cell response with time (Fig. S8). 7

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1028 4 Breadth of HIV-specific CD8$^+$ T-cell response in a patient does not correlate significantly with average viral load. We calculated the average number of HIV-specific (A–C), Gag-specific (D–F), and Env-specific (G–I) CD8$^+$ T-cell responses over the whole observation period (A, D, G), during acute infection ($t \leq 100$ days since symptom onset; B, E, H), or during chronic infection ($t > 100$ days since symptom onset; C, F, I) and $\log_{10}$ average viral load in that time period. The average viral load during infection was not dependent on the breadth of the Gag-specific CD8$^+$ T-cell response during the infection (D–F). Patient SUMA0874 was excluded from the analysis in A–C due to insufficient measurements of all T-cell responses at all time points. 8

1038 5 Expanding CD8$^+$ T-cell responses were negatively correlated with viral load before T cell numbers reached their peak values. We calculated Spearman's correlation coefficients between longitudinal changes in viral load and epitope-specific CD8$^+$ T-cell responses in each patient during the whole period (A), and before (B) and after (C) the peak of CD8$^+$ T-cell response. The $f(cc < 0)$ value denotes the fraction of negative correlation coefficients (cc), and p values are indicated for the binomial test of equal distribution of positive and negative correlations. 9

1045 6 Differences in the kinetics of early and late HIV-specific CD8$^+$ T-cell responses. We fitted the $T_{\text{on}}/T_{\text{off}}$ model (eqn. (1)) to the data on the dynamics of epitope-specific CD8$^+$ T-cell response in each patient and plotted the distribution of the estimated parameters. The results are presented separately for T cell responses that started expanding (or contracting) from the first observation ("early" responses, about 80% of all responses; black) or delayed responses, which were undetectable at one or several initial time points ("late" responses; red). Panels show distributions for (A) time of expansion of T-cell response (T_{on}), (B) time to peak of each T-cell response (T_{off}), (C) initial predicted frequency of epitope-specific CD8$^+$ T cells (E_0), (D, E) expansion (ρ) and contraction (α) rates of T-cell responses, respectively, and (F) proteins recognized by late CD8$^+$ T cell responses. In A–E, n represents the number of fitted responses, and μ, m and σ represent mean, median and standard deviation, respectively (μ_{10}, m_{10}, and σ_{10} are mean, median and standard deviation for $\log_{10}$-scaled parameters). Late responses were predicted to have a higher expansion rate ρ (Mann–Whitney, $p < 0.001$) and smaller frequency E_0 (Mann–Whitney, $p < 0.001$) than early responses. 10

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7 Correlations between major parameters determining dynamics of HIV-specific CD8$^+$ T-cell responses in acute infection. For all epitope-specific CD8$^+$ T-cell responses in all 22 patients (circles) or the total HIV-specific CD8$^+$ T-cell response per patient (stars), we estimated the initial frequency of epitope-specific CD8$^+$ T cells (E_0), rate of expansion of T cell populations (ρ), time of the peak of the response (T_{off}), rate of contraction of the immune response after the peak (α), predicted peak values reached by the epitope-specific CD8$^+$ T-cell response ($E_{\text{peak}} = E(T_{\text{off}})$), and the average viral load (V_E). Solid lines denote regression lines; regression equations and p values are indicated on individual panels for all epitope-specific CD8$^+$ T-cell responses. The total HIV-specific CD8$^+$ T-cell response showed a similar trend to all epitope-specific CD8$^+$ T-cell responses (results not shown). Panels show correlations between the timing of the immune response peak T_{off} and predicted frequency E_0 (A), T_{off} and ρ (B), expansion rate ρ and average viral load V_E (C), ρ and E_0 (D), peak immune response E_{peak} and E_0 (E), and E_{peak} and V_E (F). For a given patient, we calculated the total HIV-specific CD8$^+$ T cell response as the sum of all epitope-specific CD8$^+$ T-cell responses at the same time point (i.e., by ignoring "nd"). For patient MM42, we could not fit the $T_{\text{on}}/T_{\text{off}}$ model to the dynamics of total CD8$^+$ T cell response data because of wide oscillations in the data. Identified relationships did not change if estimates for responses with unphysiological initial frequencies ($E_0 \leq 10^{-2}$) were excluded from the analysis (results not shown). . . 13

8 Evidence of interclonal competition between epitope-specific CD8$^+$ T cell responses. We calculated Spearman's rank correlation coefficients between longitudinal changes of pairs of epitope-specific CD8$^+$ T cell responses in a given patient (see individual panels) and plotted the distribution of these coefficients. Panels show the number of correlations (n), fraction of negative correlation coefficients ($f(cc) < 0$), and p values for the deviance of the distribution from uniform, found using the binomial test with null being the equal fraction of positive and negative correlations. We found that the majority of CD8$^+$ T cell populations expand and contract in unison and therefore do not appear to compete during the infection. Overall, discordant dynamics (negative correlation coefficients) were observed for 18% of all responses irrespective of the stage of infection (acute or chronic). Patients MM38 and MM40 were excluded from the analysis for having too few correlation pairs (two or three). 15

9 Average size of epitope-specific CD8$^+$ T-cell response is unrelated to the number of HIV-specific T-cell responses. For every patient, we calculated the average number of HIV-specific CD8$^+$ T-cell responses and the average density of epitope-specific T cells in a given observation period. To exclude the contribution of viral load to this relationship, we divided all 22 patients into three groups according to their mean viral load (low $\log_{10}$ viral load: 3.40–4.44; intermediate viral load: 4.60–5.03; high viral load: 5.25–6.83). Groups were estimated using the Manhattan Distance with the `FindClusters` function in `Mathematica`. Regression lines and corresponding p values are indicated on individual panels. Overall, results varied by time period and most correlations were not statistically significant (Fig. S12). 16

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1103 S1 Kinetics of HIV-specific $CD8^+$ T-cell responses measured by IFN-γ ELISPOT assay
1104 in patients SUMA0874, MM49, MM56, MM43, MM50, and MM19. Measurements
1105 below the level of detection are plotted as having a value of 0.1. Patients are listed
1106 in descending order according to the total number of T-cell responses measured.
1107 We divided T cell responses into four groups according to their target protein (1:
1108 Env; 2: Gag, Gag/Protease, Integrase, RT; 3: Nef, Pol, Protease, Protease/Rt; 4:
1109 Rev, Tat, Vif, Vpr). S1
1110 S2 Kinetics of HIV-specific $CD8^+$ T-cell responses measured by IFN-γ ELISPOT assay
1111 in patients MM46, MM45, WEAU0575, MM55, MM23, BORI0637, MM48, and
1112 MM47. See Fig. S1 caption for more detail. S2
1113 S3 Kinetics of HIV-specific $CD8^+$ T-cell responses measured by IFN-γ ELISPOT assay
1114 in patients MM28, MM25, MM33, MM39, MM51, MM42, MM40, and MM38,
1115 respectively. See Fig. S1 caption for more detail. S3
1116 S4 Kinetics of total HIV-specific $CD8^+$ T-cell response measured by IFN-γ ELISPOT
1117 assay and viral load in 20 patients in the cohort. For each patient, total $CD8^+$
1118 T-cell response (squares) and viral load (circles) are plotted over time. Note that
1119 patient WEAU0575 was followed for longer than all other patients (772 days after
1120 symptom onset). Patients SUMA0874 and MM19 were excluded from this plot due
1121 to insufficient measurements of all T-cell responses at all time points. S4
1122 S5 Nonsignificant change in the number of HIV-specific $CD8^+$ T cell responses in all
1123 patients over the course of infection. We divided the whole observation period into
1124 different time bins (50-day intervals (A) or 100-day intervals (B)) and calculated
1125 the number of T-cell responses (breadth) for the corresponding group. Small hori-
1126 zontal bar denotes mean breadth for that time interval. Spearman's rank coefficient
1127 was used to determine the significance of breadth change over time (correlation co-
1128 efficient ρ and p values). Patient SUMA0874 was excluded from this plot due to
1129 insufficient measurements in all T-cell responses at all time points. S5
1130 S6 Nonsignificant changes of total $CD8^+$ T-cell response in all patients. We divided the
1131 whole observation period into different time bins (50-day intervals (A) or 100-day
1132 intervals (B)) and calculated the sum of all T-cell responses for a given patient.
1133 Small horizontal bar denotes average $CD8^+$ T cell response level for that time
1134 interval. For different time intervals (e.g., 15- or 30-day intervals), we found similar
1135 trends (results not shown). S5

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S7 Variable dynamics of T-cell response breadth in individual patients. Normalized immune response breadth was defined as the number of responses at a particular time divided by the total number of responses measured in that patient. The shaded bars (or vertical lines) denote times when T-cell response mapping was performed with pooled PBMCs in each patient; in patients MM33 and MM39, mapping was performed twice. Due to missing measurements ("nd") in some epitope-specific $CD8^+$ T cell responses, we estimated the breadth at certain time points for a particular patient in two ways: 1) ignoring the time point (red crosshair ×), or 2) replacing the "nd" with 0 (black dot •) when there was at least one missing measurement at this time point. We found that in some patients (e.g., MM45, MM48, MM49) the breadth expanded slightly to saturation level, and in others, contraction phases followed the saturation (e.g., MM43, MM55). Patient WEAU0575 was followed for 772 days after symptom onset, so the x-axis for this patient is longer. Patients SUMA0874 and MM19 were excluded from this plot due to insufficient measurements of all T-cell responses at all time points. S6

S8 SE and EI of T-cell responses moderately increased over time. SE and EI were calculated at different time points for all patients (see Material and Methods for more detail); we found a moderate but statistically significant positive trend (Spearman Rank Correlation: $\rho = 0.30$ ($p = 0.00074$) and $\rho = 0.49$ ($p \ll 0.0001$)). Major significant changes in both measures of breadth occurred within the first 40 days of symptom onset. Analyses included only the time points at which all $CD8^+$ T-cell responses were measured. Detailed SE and EI kinetics in each patient are shown in Figs. S9 and S10, respectively. S7

S9 Kinetics of SI (dashed line) and corresponding linear fitted curve (solid line) for all patients. No trends were statistically significant (p values from linear regressions are indicated on panels). S8

S10 Kinetics of EI (dashed line) and corresponding linear fitted curve (solid line) for all patients. Two out of 24 patients showed significant increase in EI over time while other trends were not significant (p values for linear regressions are indicated on the panels). S9

S11 Variable correlations between viral load (V) and SE (A–C) or EI (B–F) of Gag-specific $CD8^+$ T-cell responses. Note a positive (but nonsignificant) correlation between viral load and breadth measured by EI, and positive correlation between breadth measured as SE and V for chronic infection ($t > 100$ days after symptom onset). S10

S12 Correlation between number of T-cell responses and average size of T-cell response depends on viral load and time period since infection. Correlation between number of immune responses and average size of T-cell response is shown for chronic infection (top row) or for the whole time period (middle row) for different average viral loads. Bottom row shows correlation for all data at different time periods since infection. p values are from linear regressions; best fit equations are shown on individual panels. Some correlations are negative, indicating the presence of interclonal competition. Low, intermediate, and high viral loads were defined as described in Fig. 9. . . . . S11

S13 Examples of data on the kinetics of eptiope-specific $CD8^+$ T-cell responses and the predicted fits of the basic T_{on}-T_{off} model eqn. (1) to these data. In all three examples there were no initial zeroes recorded so we set $T_{\text{on}} = 0$ for simplicity. S11

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1183 S14 Examples of strongly negatively (A&C) and strongly positively(B&D) correlated
1184 viral load and epitope-specific $CD8^+$ T-cell responses or different epitope-specific
1185 $CD8^+$ T-cell responses. The correlation coefficients (ρ) were used to generate the
1186 histogram in Figures 5 or 8. . . . . S12
1187 S15 Detailed distributions of correlation coefficient (cc) between different epitope-specific
1188 $CD8^+$ T-cell responses (IRs) in different patients for $t \leq 100$ days after symp-
1189 tom onset (acute infection). Negatively correlated epitope-specific $CD8^+$ T cell
1190 responses were observed for nearly all patients, suggesting that interclonal compe-
1191 tition between T cell responses specific to different HIV epitopes may occur in all
1192 HIV-infected patients. S13

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Supplementary Information

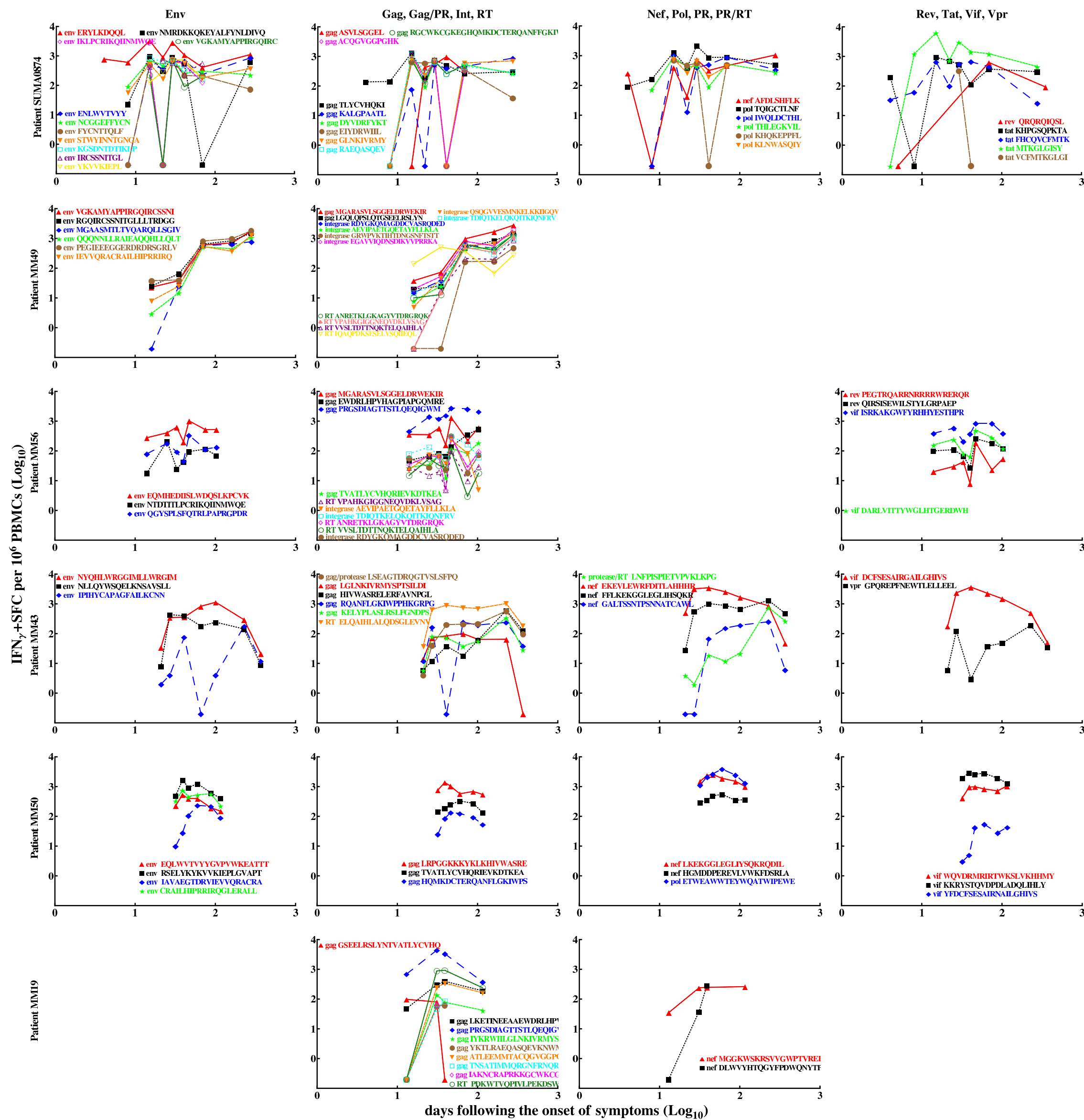

Figure S1: Kinetics of HIV-specific CD8$^+$ T-cell responses measured by IFN-γ ELISPOT assay in patients SUMA0874, MM49, MM56, MM43, MM50, and MM19. Measurements below the level of detection are plotted as having a value of 0.1. Patients are listed in descending order according to the total number of T-cell responses measured. We divided T cell responses into four groups according to their target protein (1: Env; 2: Gag, Gag/Protease, Integrase, RT; 3: Nef, Pol, Protease, Protease/Rt; 4: Rev, Tat, Vif, Vpr).

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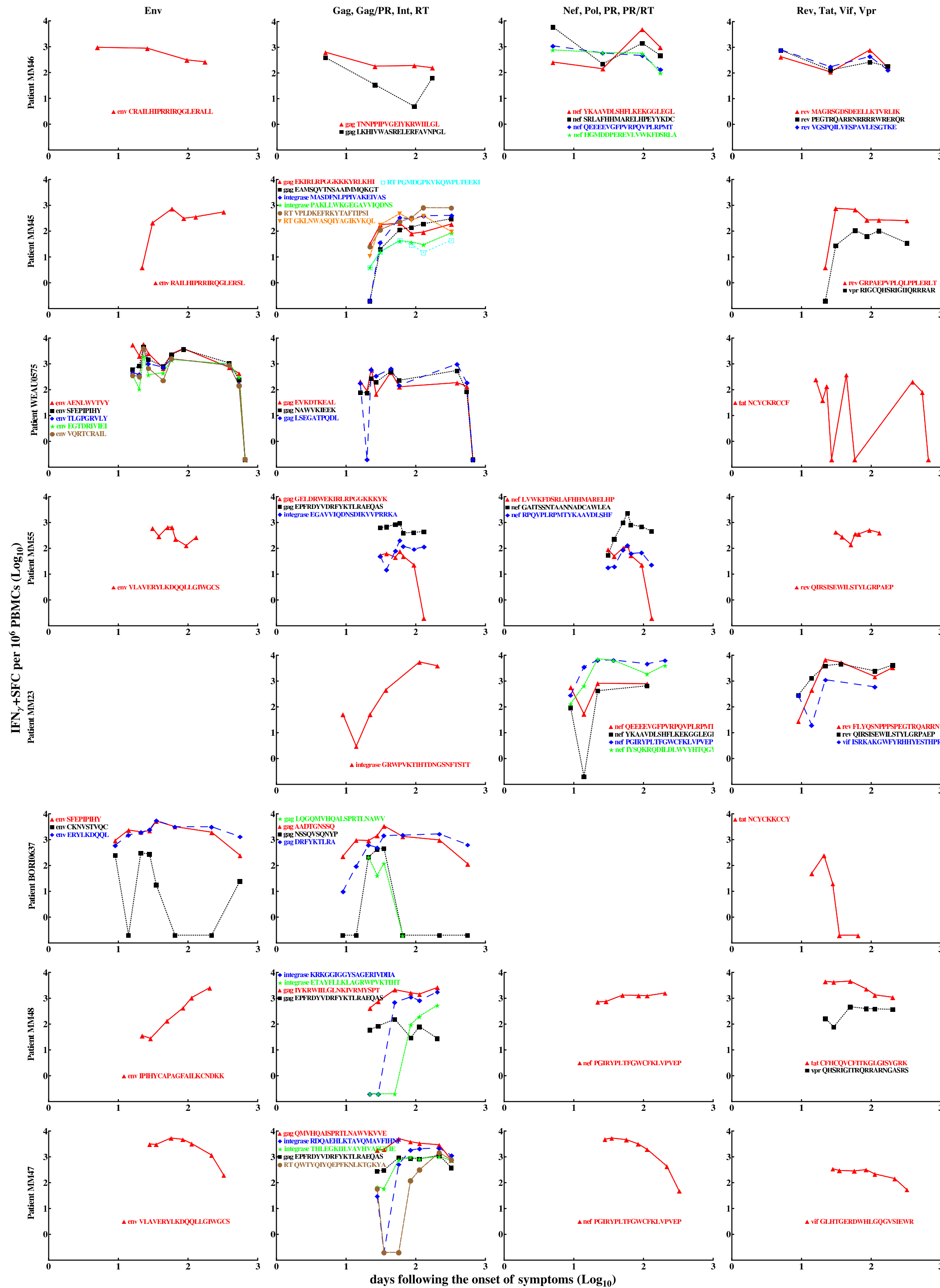

Figure S2: Kinetics of HIV-specific $CD8^+$ T-cell responses measured by IFN-γ ELISPOT assay in patients MM46, MM45, WEAU0575, MM55, MM23, BORI0637, MM48, and MM47. See Fig. S1 caption for more detail.

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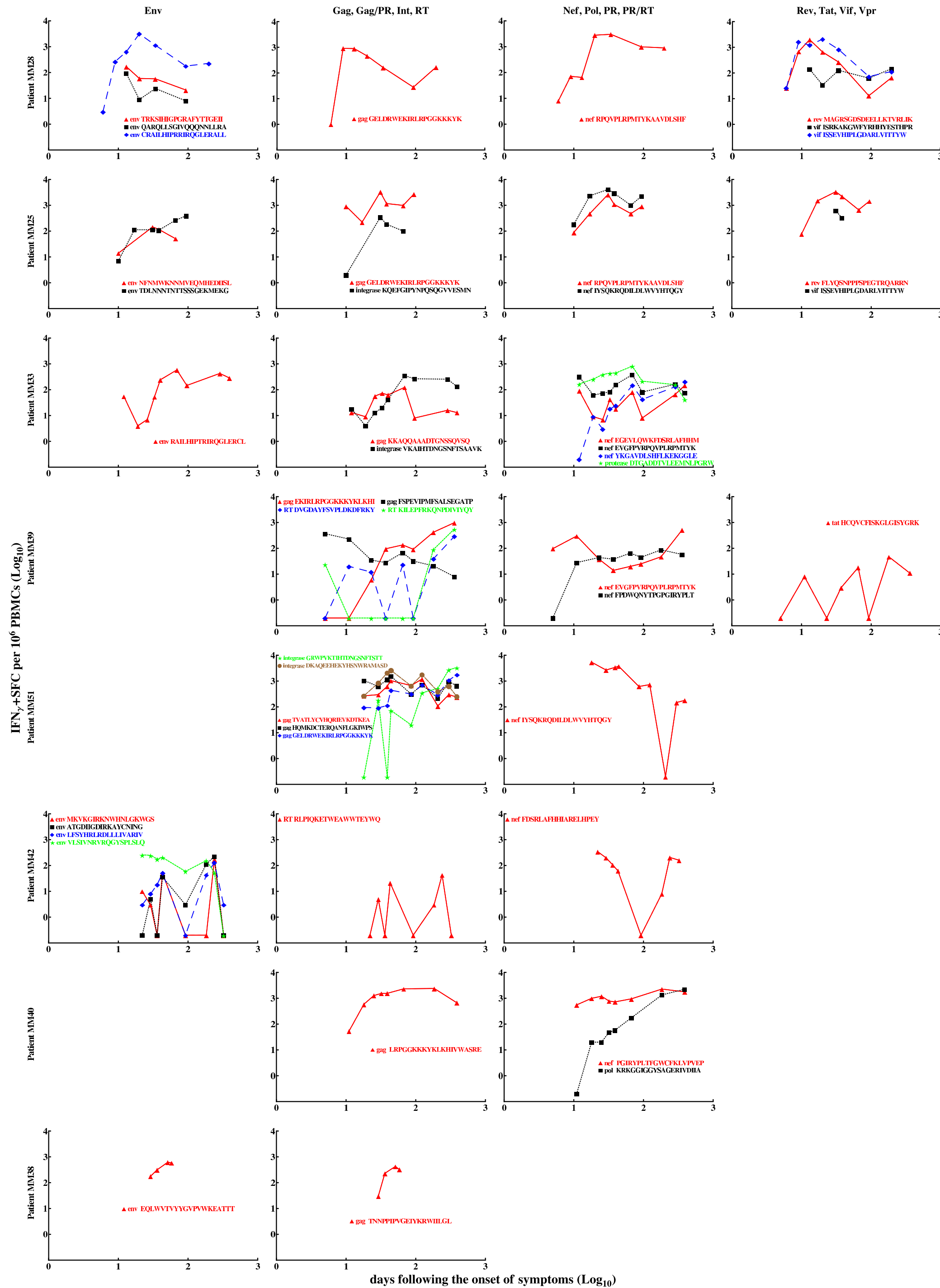

Figure S3: Kinetics of HIV-specific $CD8^+$ T-cell responses measured by IFN-γ ELISPOT assay in patients MM28, MM25, MM33, MM39, MM51, MM42, MM40, and MM38, respectively. See Fig. S1 caption for more detail.

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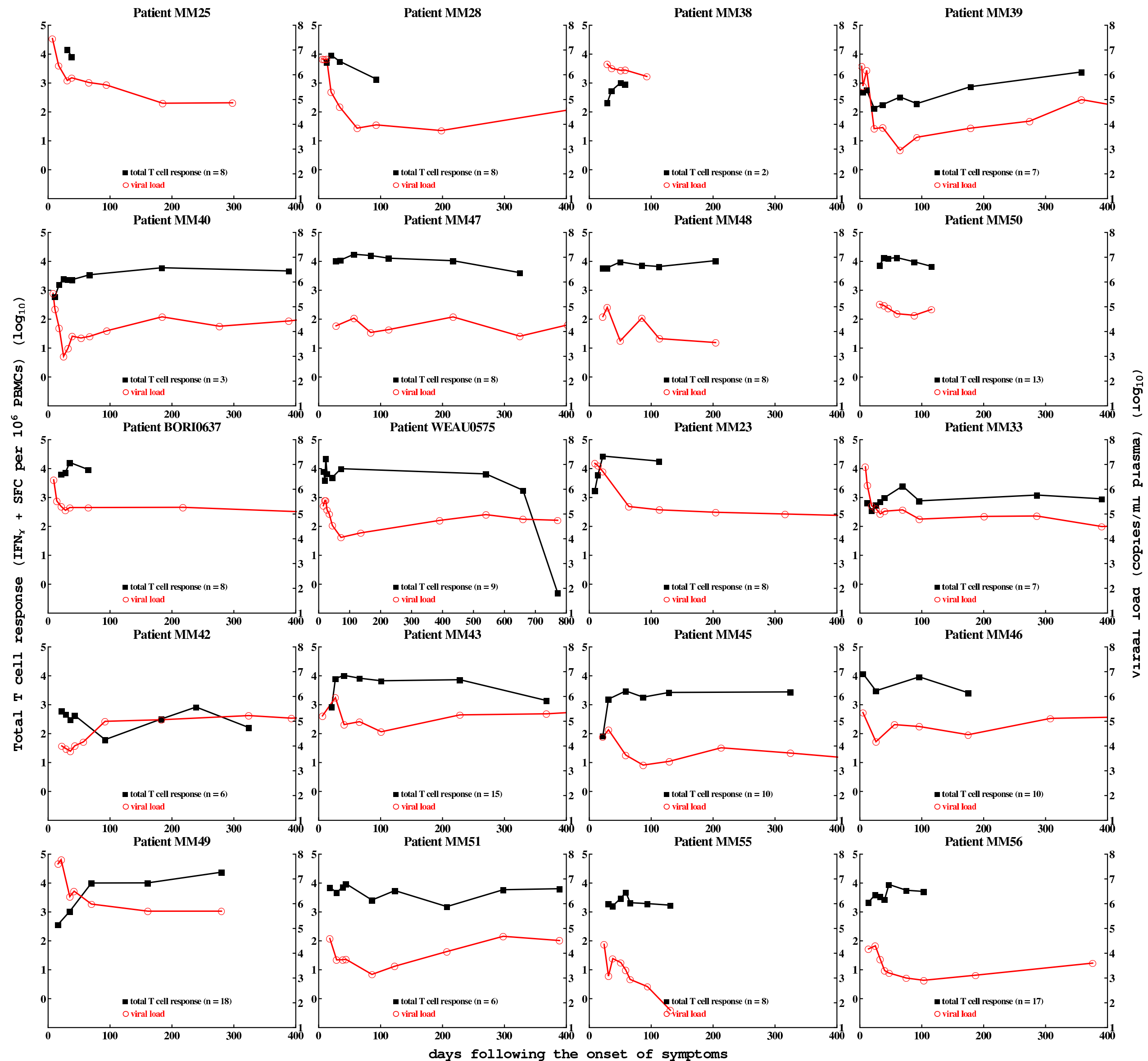

Figure S4: Kinetics of total HIV-specific CD8$^+$ T-cell response measured by IFN-γ ELISPOT assay and viral load in 20 patients in the cohort. For each patient, total CD8$^+$ T-cell response (squares) and viral load (circles) are plotted over time. Note that patient WEAU0575 was followed for longer than all other patients (772 days after symptom onset). Patients SUMA0874 and MM19 were excluded from this plot due to insufficient measurements of all T-cell responses at all time points.

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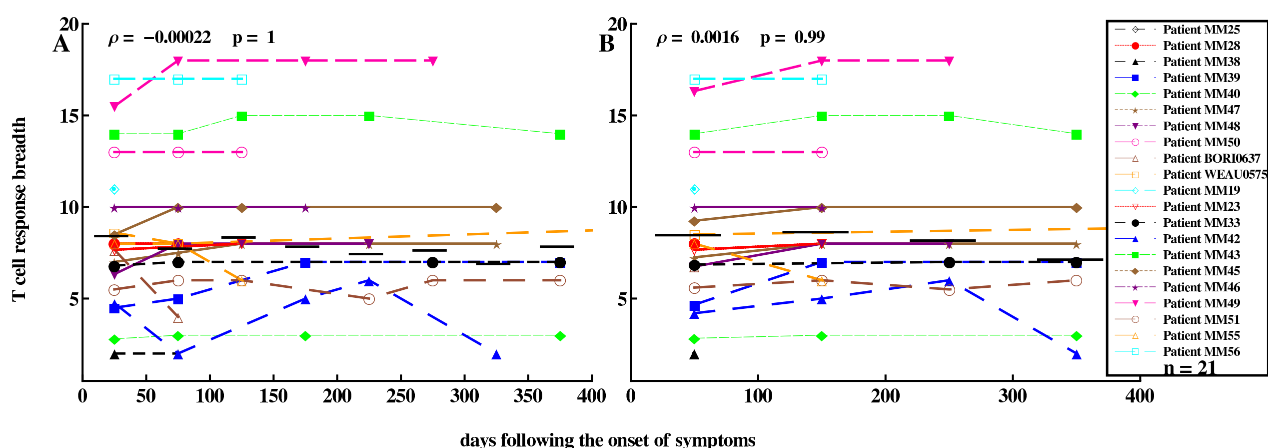

Figure S5: Nonsignificant change in the number of HIV-specific CD8$^+$ T cell responses in all patients over the course of infection. We divided the whole observation period into different time bins (50-day intervals (A) or 100-day intervals (B)) and calculated the number of T-cell responses (breadth) for the corresponding group. Small horizontal bar denotes mean breadth for that time interval. Spearman's rank coefficient was used to determine the significance of breadth change over time (correlation coefficient ρ and p values). Patient SUMA0874 was excluded from this plot due to insufficient measurements in all T-cell responses at all time points.

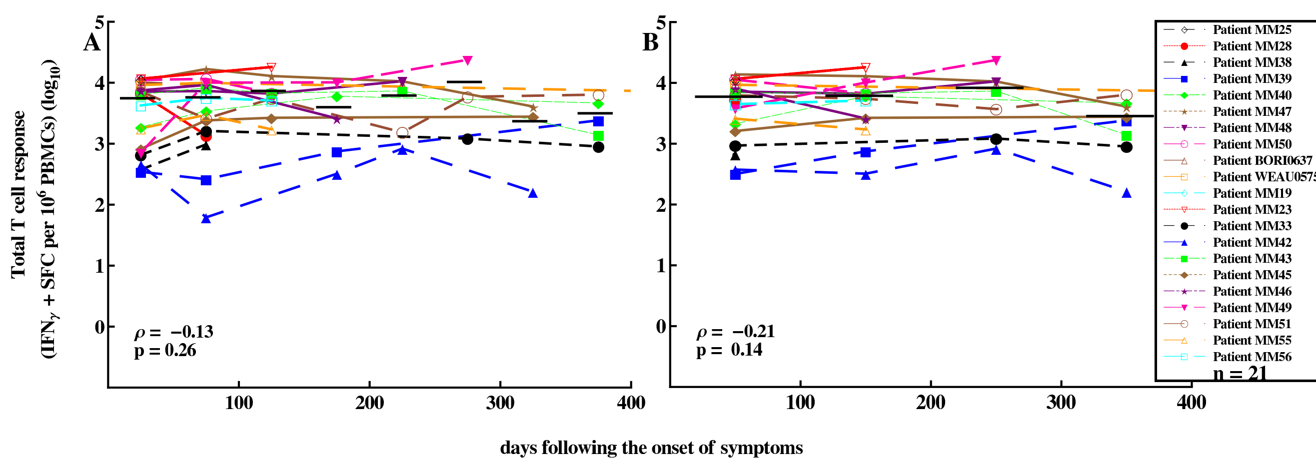

Figure S6: Nonsignificant changes of total CD8$^+$ T-cell response in all patients. We divided the whole observation period into different time bins (50-day intervals (A) or 100-day intervals (B)) and calculated the sum of all T-cell responses for a given patient. Small horizontal bar denotes average CD8$^+$ T cell response level for that time interval. For different time intervals (e.g., 15- or 30-day intervals), we found similar trends (results not shown).

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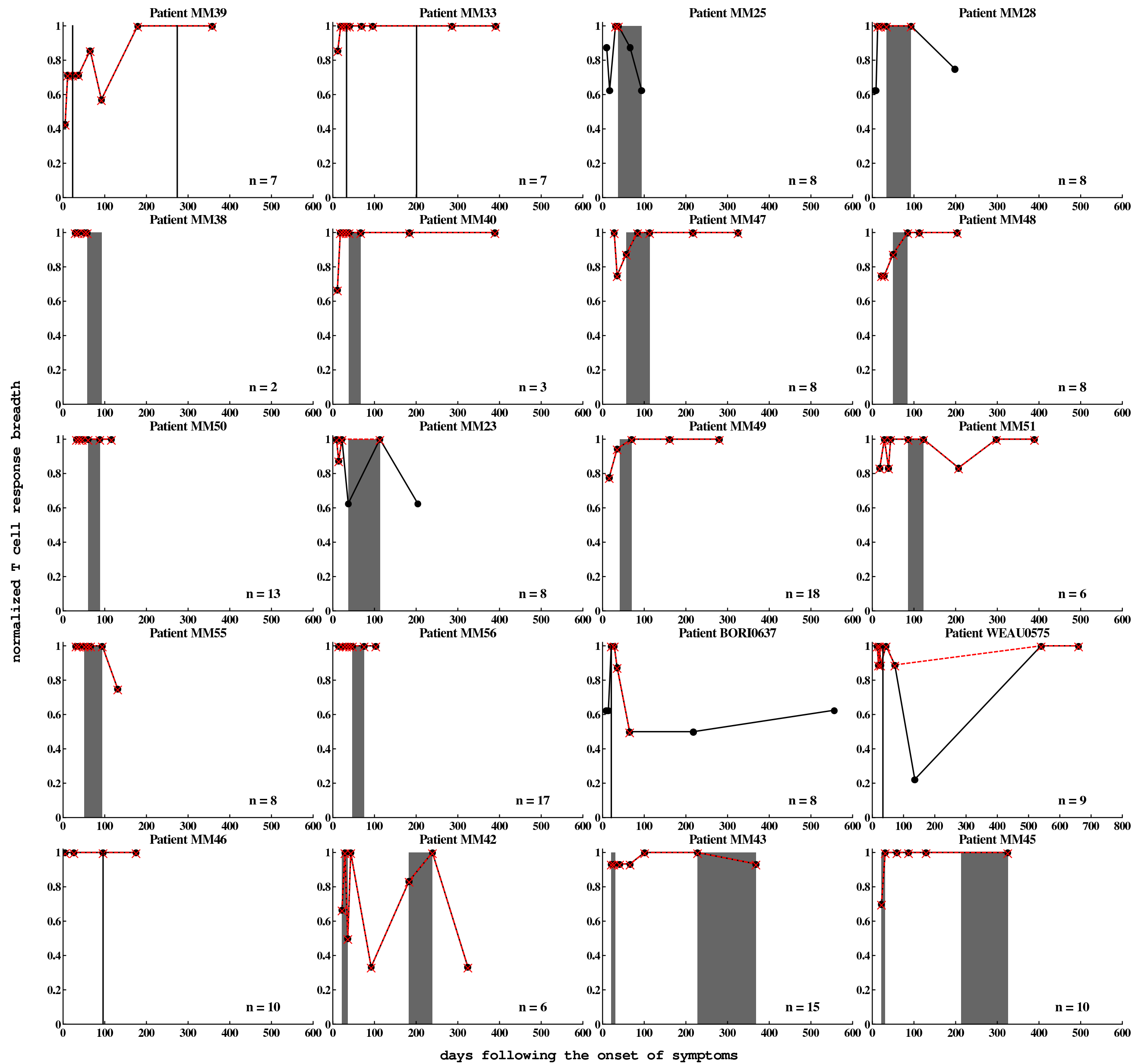

Figure S7: Variable dynamics of T-cell response breadth in individual patients. Normalized immune response breadth was defined as the number of responses at a particular time divided by the total number of responses measured in that patient. The shaded bars (or vertical lines) denote times when T-cell response mapping was performed with pooled PBMCs in each patient; in patients MM33 and MM39, mapping was performed twice. Due to missing measurements ("nd") in some epitope-specific CD8$^+$ T cell responses, we estimated the breadth at certain time points for a particular patient in two ways: 1) ignoring the time point (red crosshair ×), or 2) replacing the "nd" with 0 (black dot •) when there was at least one missing measurement at this time point. We found that in some patients (e.g., MM45, MM48, MM49) the breadth expanded slightly to saturation level, and in others, contraction phases followed the saturation (e.g., MM43, MM55). Patient WEAU0575 was followed for 772 days after symptom onset, so the x-axis for this patient is longer. Patients SUMA0874 and MM19 were excluded from this plot due to insufficient measurements of all T-cell responses at all time points.

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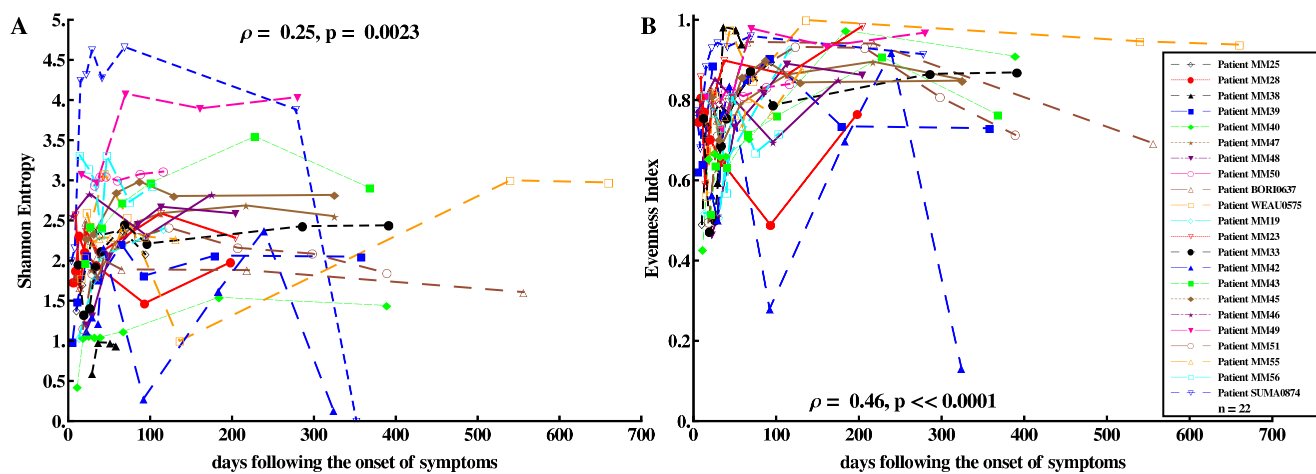

Figure S8: SE and EI of T-cell responses moderately increased over time. SE and EI were calculated at different time points for all patients (see Material and Methods for more detail); we found a moderate but statistically significant positive trend (Spearman Rank Correlation: $\rho = 0.30$ ($p = 0.00074$) and $\rho = 0.49$ ($p \ll 0.0001$)). Major significant changes in both measures of breadth occurred within the first 40 days of symptom onset. Analyses included only the time points at which all CD8$^+$ T-cell responses were measured. Detailed SE and EI kinetics in each patient are shown in Figs. S9 and S10, respectively.

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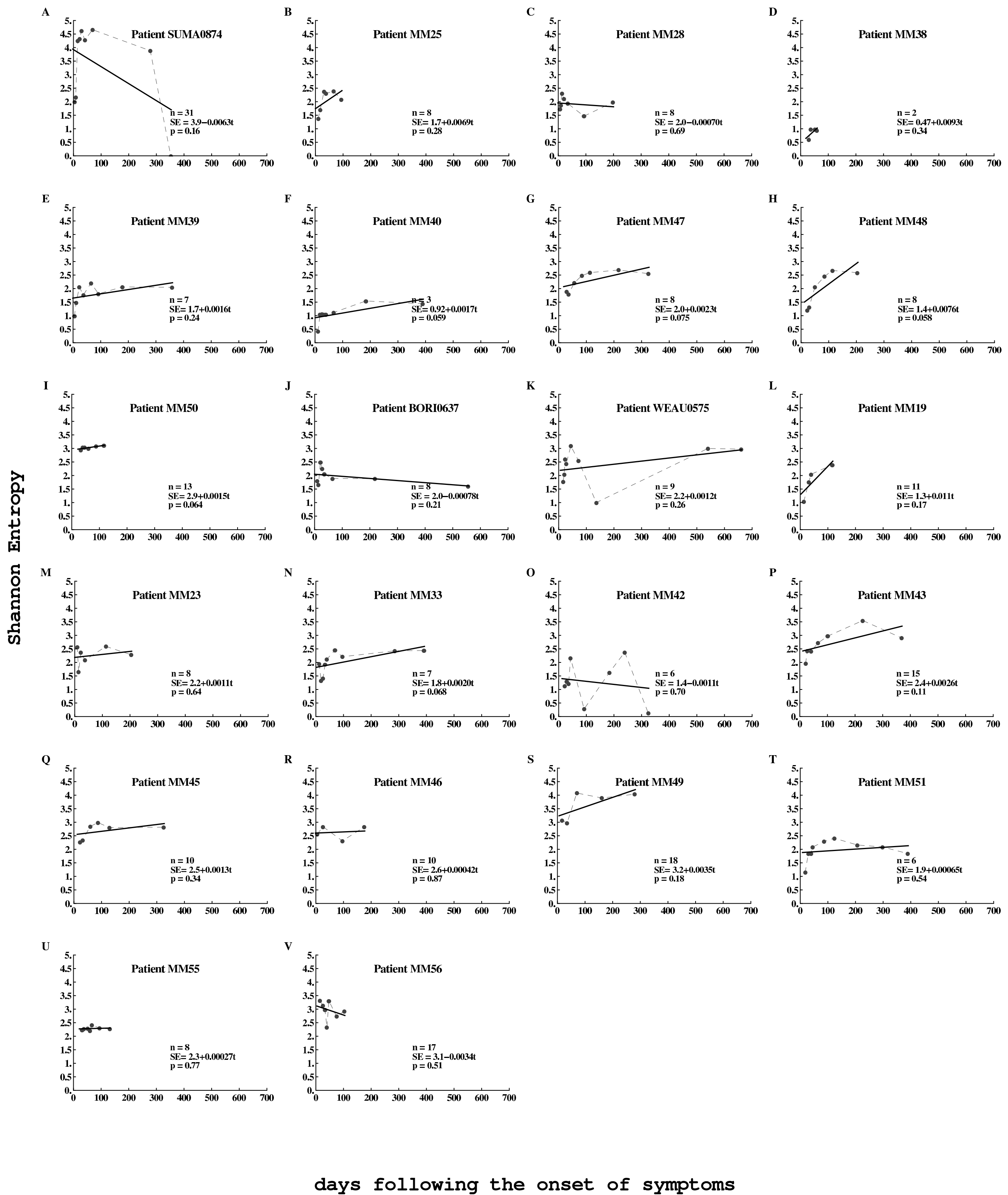

Figure S9: Kinetics of SI (dashed line) and corresponding linear fitted curve (solid line) for all patients. No trends were statistically significant (p values from linear regressions are indicated on panels).

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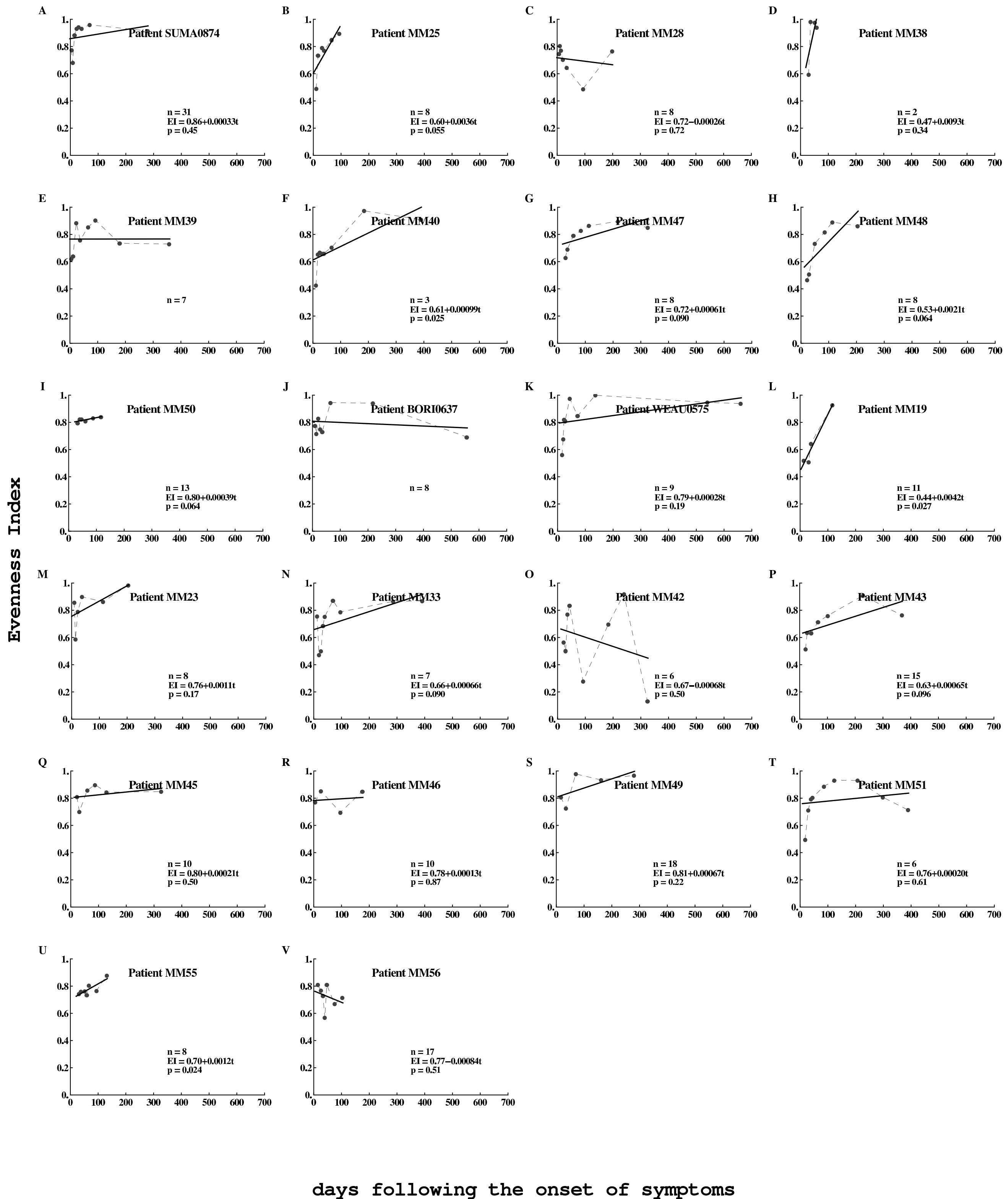

Figure S10: Kinetics of EI (dashed line) and corresponding linear fitted curve (solid line) for all patients. Two out of 24 patients showed significant increase in EI over time while other trends were not significant (p values for linear regressions are indicated on the panels).

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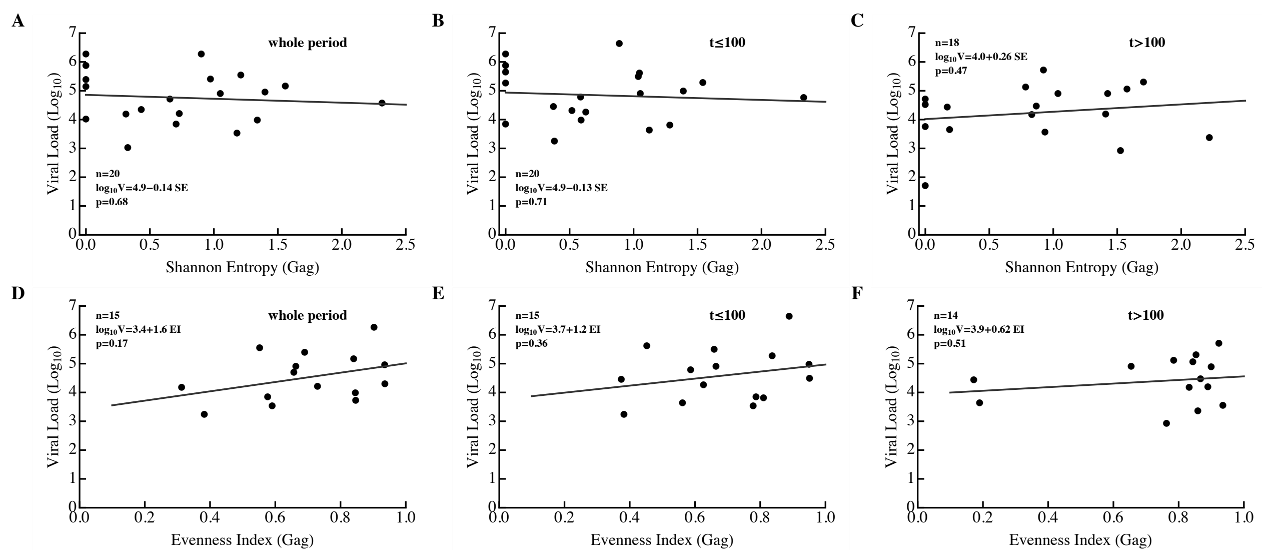

Figure S11: Variable correlations between viral load (V) and SE (A–C) or EI (B–F) of Gag-specific CD8$^+$ T-cell responses. Note a positive (but nonsignificant) correlation between viral load and breadth measured by EI, and positive correlation between breadth measured as SE and V for chronic infection ($t > 100$ days after symptom onset).

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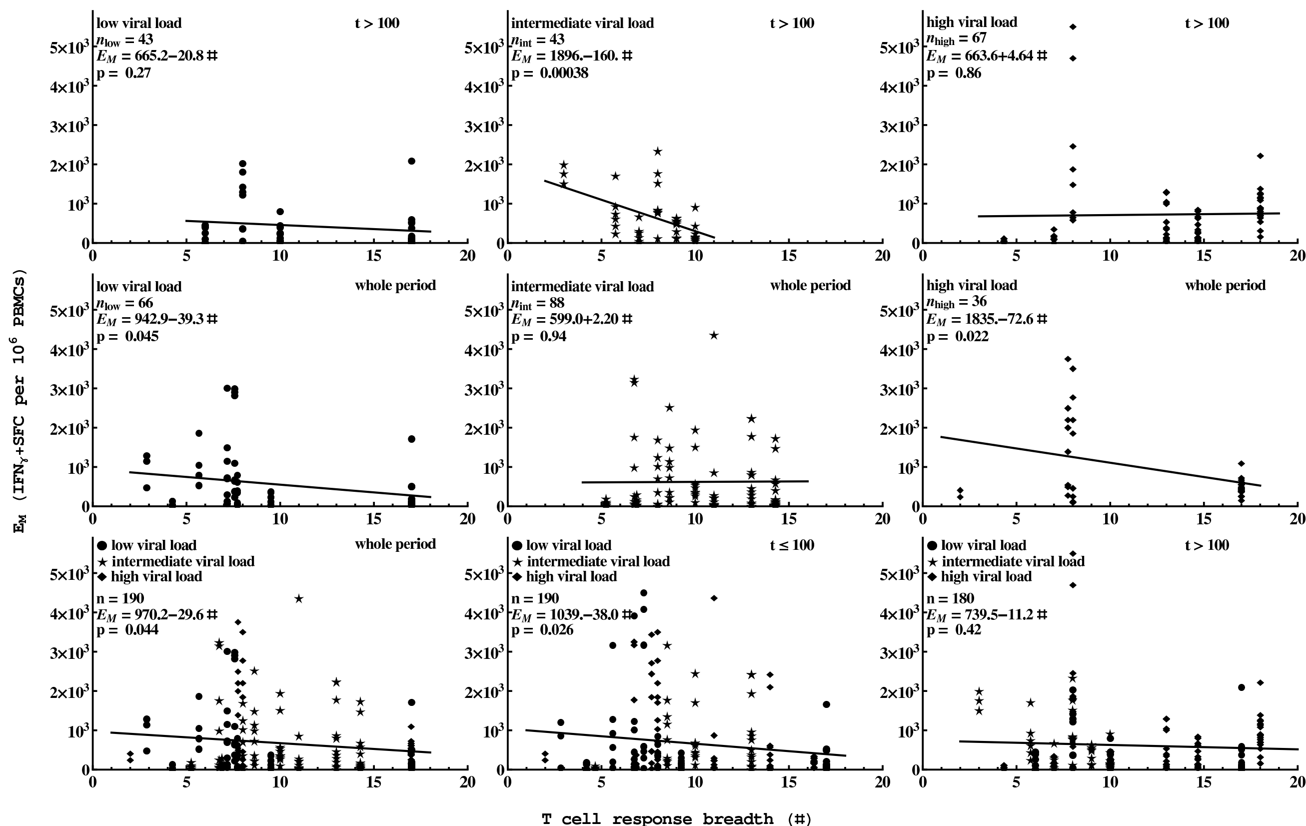

Figure S12: Correlation between number of T-cell responses and average size of T-cell response depends on viral load and time period since infection. Correlation between number of immune responses and average size of T-cell response is shown for chronic infection (top row) or for the whole time period (middle row) for different average viral loads. Bottom row shows correlation for all data at different time periods since infection. p values are from linear regressions; best fit equations are shown on individual panels. Some correlations are negative, indicating the presence of interclonal competition. Low, intermediate, and high viral loads were defined as described in Fig. 9.

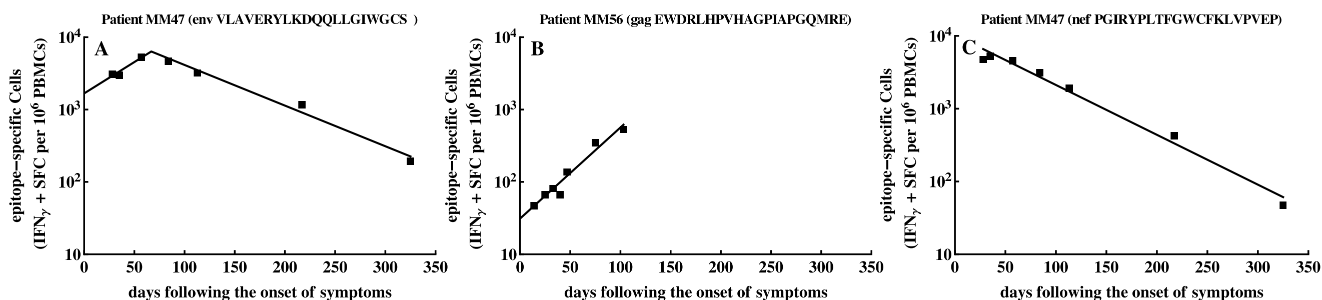

Figure S13: Examples of data on the kinetics of eptiope-specific CD8$^+$ T-cell responses and the predicted fits of the basic T_{on}-T_{off} model eqn. (1) to these data. In all three examples there were no initial zeroes recorded so we set $T_{\text{on}} = 0$ for simplicity.

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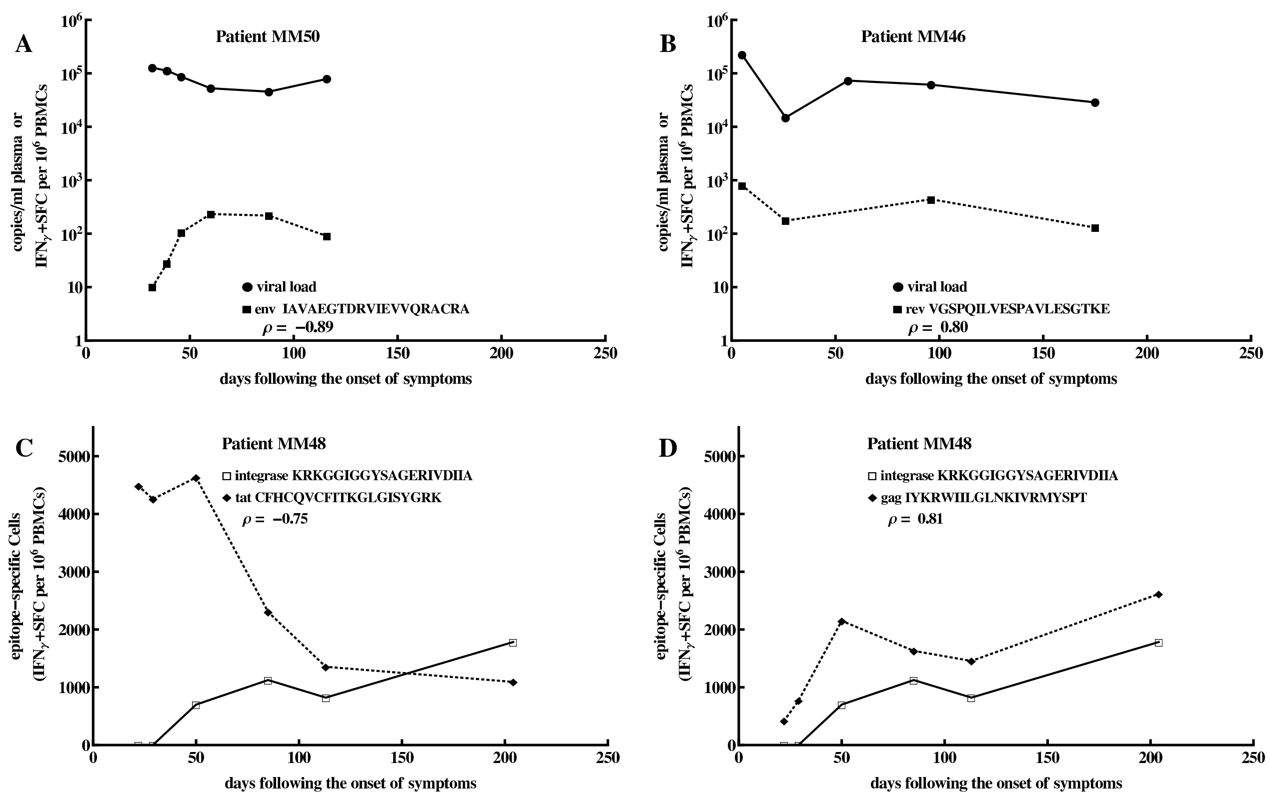

Figure S14: Examples of strongly negatively (A&C) and strongly positively(B&D) correlated viral load and epitope-specific $CD8^+$ T-cell responses or different epitope-specific $CD8^+$ T-cell responses. The correlation coefficients (ρ) were used to generate the histogram in Figures 5 or 8.

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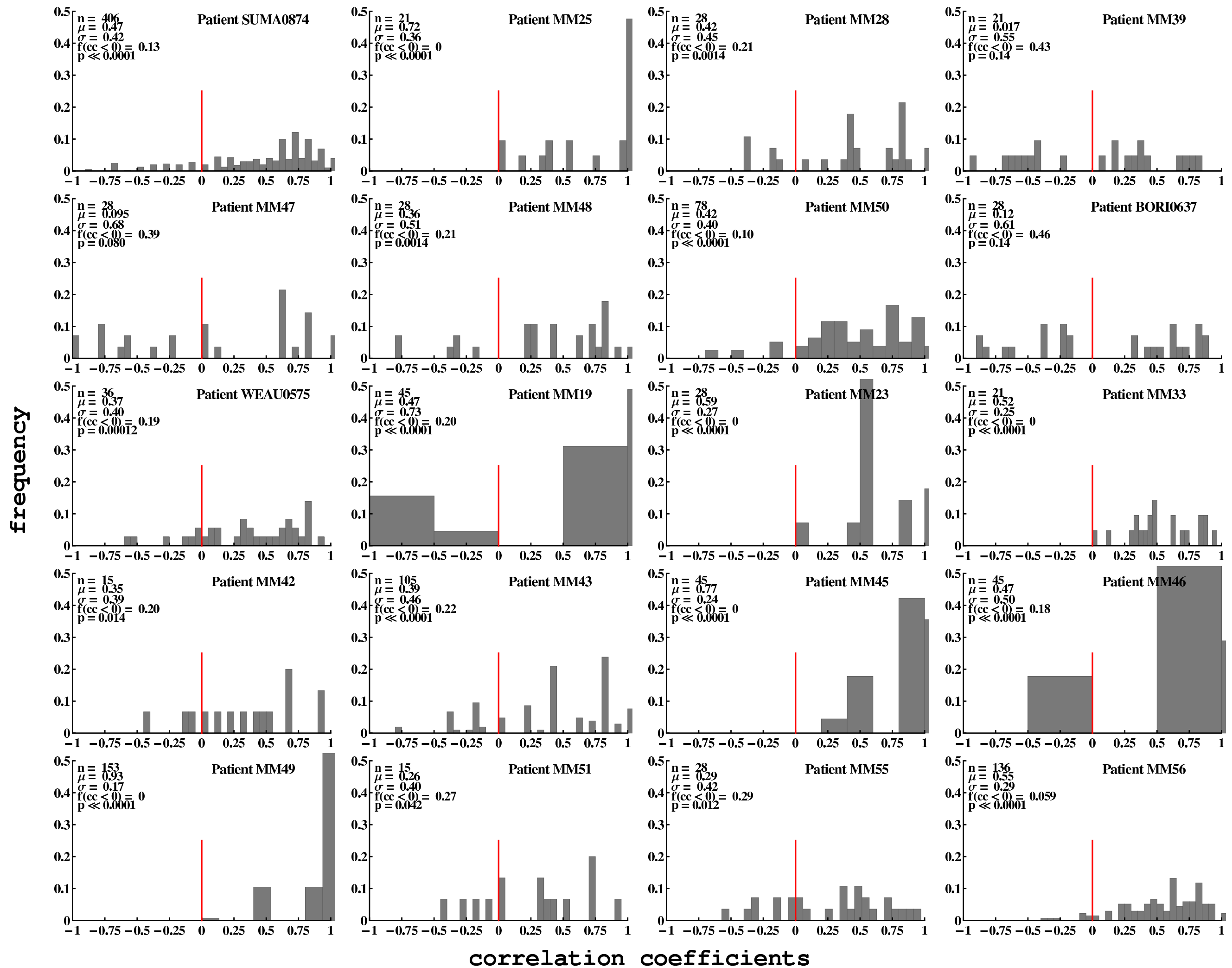

Figure S15: Detailed distributions of correlation coefficient (cc) between different epitope-specific CD8$^+$ T-cell responses (IRs) in different patients for $t \leq 100$ days after symptom onset (acute infection). Negatively correlated epitope-specific CD8$^+$ T cell responses were observed for nearly all patients, suggesting that interclonal competition between T cell responses specific to different HIV epitopes may occur in all HIV-infected patients.