The viral reservoir represents a critical challenge for human immunodeficiency virus type 1 (HIV-1) eradication strategies. However, it remains unclear when and where the viral reservoir is seeded during acute infection and the extent to which it is susceptible to early antiretroviral therapy (ART). Here we show that the viral reservoir is seeded rapidly after mucosal simian immunodeficiency virus (SIV) infection of rhesus monkeys and before systemic viraemia. We initiated suppressive ART in groups of monkeys on days 3, 7, 10 and 14 after intrarectal SIVMAC251 infection. Treatment with ART on day 3 blocked the emergence of viral RNA and proviral DNA in peripheral blood but also substantially reduced levels of proviral DNA in lymph nodes and gastrointestinal mucosa as compared with treatment at later time points. In addition, treatment on day 3 abrogated the induction of SIV-specific humoral and cellular immune responses. Nevertheless, after discontinuation of ART following 24 weeks of fully suppressive therapy, virus rebounded in all animals, although the monkeys that were treated on day 3 exhibited a delayed viral rebound as compared with those treated on days 7, 10 and 14. The time to viral rebound correlated with total viraemia during acute infection and with proviral DNA at the time of ART discontinuation. These data demonstrate that the viral reservoir is seeded rapidly after intrarectal SIV infection of rhesus monkeys, during the ‘eclipse’ phase, and before detectable viraemia. This strikingly early seeding of the refractory viral reservoir raises important new challenges for HIV-1 eradication strategies.

The viral reservoir in memory CD4+ T cells in HIV-1-infected individuals cannot be eliminated by current antiretroviral drugs or HIV-1-specific immune responses. This archive of replication-competent virus is the source of viral rebound in nearly all HIV-1-infected individuals who discontinue ART and represents a critical hurdle for HIV-1 eradication strategies. The temporal dynamics of seeding the viral reservoir have not been previously defined but have been presumed to occur during peak viraemia in acute HIV-1 infection. To evaluate the impact of early ART on the viral reservoir, we initiated suppressive ART at various time points after mucosal SIV infection of rhesus monkeys.

We inoculated 20 Indian-origin adult rhesus monkeys (Macaca mulatta) that did not express the protective major histocompatibility complex (MHC) class I alleles Mamu-A*01, Mamu-B*08 and Mamu-B*17 with 500 median tissue culture infective doses (TCID50) of SIVMAC251 (refs 8–10) by the intrarectal route. We initiated ART on days 3, 7, 10 and 14 after infection with a pre-formulated cocktail of tenofovir, emtricitabine and dolutegravir (see Methods), and a control group received no ART (n = 4 per group). ART was administered daily by subcutaneous injection for 24 weeks. Treatment on day 3 after infection resulted in no detectable viraemia (<50 RNA copies ml\(^{-1}\)) at any time point in four of four monkeys (Fig. 1a). In contrast, treatment on days 7, 10 and 14 abruptly interrupted the exponential growth of the virus and reduced plasma viral RNA to undetectable levels within 3–4 weeks. The mean levels of plasma viral RNA at the time of ART initiation in these groups of monkeys were 5.88 log copies ml\(^{-1}\) (day 7), 7.11 log copies ml\(^{-1}\) (day 10) and 7.50 log copies ml\(^{-1}\) (day 14), which were comparable with the levels of plasma viral RNA in untreated controls at these time points (Fig. 1b). Viral dynamics modelling revealed an initial exponential growth rate of 1.5 ± 0.5 per day, corresponding to a basic reproductive ratio of \(R_0 = 9.5 ± 5.1\) (see Methods; Extended Data Fig. 1 and Extended Data Table 1). An exponential decay rate of plasma viraemia after ART initiation of 0.60 ± 0.17 per day was observed in all the treated groups, corresponding to a 1.3 ± 0.4 day half-life of infected cells (Extended Data Fig. 1).

After initial control of viraemia, all animals treated with ART exhibited undetectable plasma viral loads (<50 RNA copies ml\(^{-1}\)) for the full duration of the experiment, whereas untreated control animals (basal viraemia of 5.88–7.11 log copies ml\(^{-1}\)) exhibited undetectable plasma viraemia at these time points (Fig. 1b). This was confirmed by quantitative real-time reverse transcription polymerase chain reaction analysis of the lymph nodes and gastrointestinal mucosa in the animals treated with ART. The results from analysis of the lymph nodes and gastrointestinal mucosa in the untreated control group were consistent with those from the plasma (Extended Data Fig. 2).

Figure 1 Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. James B. Whitney1,2, Alison L. Hill3, Srisowmya Sanisetty1, Pablo Penaloza-MacMaster1, Jinyan Liu1, Mayuri Shetty1, Lily Parenteau1, Crystal Cabral1, Jennifer Shields1, Stephen Blackmore1, Jeffrey Y. Smith1, Amanda L. Brinkman1, Lauren E. Peter1, Sheeba I. Mathew1, Kaitlin M. Smith3, Erica N. Borducchi1, Daniel I. S. Rosenbloom3, Mark G. Lewis4, Jillian Hattersley5, Bei Li5, Joseph Hesselgesser5, Romas Geleziunas5, Merlin L. Robb6, Jerome H. Kim6, Nelson L. Michael6 & Dan H. Barouch1,2

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24-week course of suppressive therapy with no detectable viral blips (Fig. 1a), demonstrating the potency and consistency of this ART regimen. Moreover, ultrasensitive plasma viral load assays at week 20 also proved negative (≤ 6 RNA copies ml⁻¹) in all animals (Extended Data Fig. 2). In addition, viral sequences from stimulated peripheral blood mononuclear cells (PBMCs) from ART-suppressed, SIV-infected monkeys using the same ART regimen revealed no viral sequence evolution over 6 months in a separate study (J.B.W., unpublished observations). Furthermore, treatment intensification studies in other SIV-infected rhesus monkeys in which the protease inhibitor darunavir was added to the current ART regimen did not lead to enhanced virological control (R.G., unpublished observations). Taken together, these data indicate that the ART regimen that was used in the present study was fully suppressive.

We next assessed the development of SIV-specific humoral and cellular immune responses in these animals. Monkeys treated on day 3 after infection developed no detectable SIV Env-specific antibody responses by enzyme-linked immunosorbent assay (ELISA) (Fig. 2a) and no detectable SIV Env-, Pol- or Gag-specific T lymphocyte responses by interferon (IFN)-γ ELISPOT assays (Fig. 2b) at weeks 4, 10, and 20 or 24 of infection. In contrast, monkeys treated on days 7, 10 and 14 developed detectable but lower SIV-specific humoral and cellular immune responses as compared with untreated controls, presumably as a result of reduced antigenic stimulus after ART initiation. Multiparameter intracellular cytokine staining (ICS) assays confirmed that SIV Gag-specific CD8⁺ and CD4⁺ T lymphocyte responses were undetectable in animals treated on day 3 and were lower in animals treated on days 7, 10 and 14 as compared with untreated controls (Extended Data Figs 3 and 4). Gag-specific CD8⁺ and CD4⁺ T lymphocytes in monkeys treated on days 7, 10 and 14 also exhibited reduced immune activation and proliferation as measured by Ki67 expression (Extended Data Fig. 4). These data demonstrate that initiation of ART on day 3 blocked the emergence of plasma viraemia and abrogated the induction of SIV-specific humoral and cellular immune responses.

We next determined the impact of early ART on levels of proviral DNA in PBMCs, lymph node mononuclear cells (LNMCs) and gastrointestinal mucosa mononuclear cells (GMMCs) over the course of 24 weeks of treatment with suppressive ART. In monkeys that initiated ART on day 3, there was a striking anatomical discordance with no proviral DNA detected in PBMCs at any time point (< 3 DNA copies per 10⁶ cells) (Fig. 3a). In contrast, clear but low levels of proviral DNA were detected in inguinal LNMCs and in colorectal GMMCs in these animals, although proviral DNA declined to undetectable or nearly undetectable levels in three of four of these animals by week 24. In monkeys treated with ART on days 7, 10 and 14, proviral DNA was readily detected in PBMCs as well as in LNMCs and GMMCs (Fig. 3b–d). Moreover, in animals treated with ART on days 10 and 14, proviral DNA in LNMCs appeared to stabilize by week 12 with minimal subsequent decline, consistent with a stable viral reservoir (Fig. 3c, d). In untreated animals, proviral DNA was markedly higher than in ART-treated animals with minimal decline over time (Fig. 3e). Analysis of sorted cell subpopulations demonstrated that proviral DNA was found primarily in central memory and transitional memory CD4⁺ T lymphocytes in lymph nodes on day 3 and in both PBMCs and lymph nodes on day 7 after SIV infection (Extended Data Fig. 5).
These data indicate that initiation of ART on day 3 reduced levels of proviral DNA at week 24 by at least 2.2 log in PBMCs ($P < 0.0001$; Fig. 3f) and 1.0 log in LNMCs ($P = 0.004$; Fig. 3g), and 0.9 log in GMMCs ($P = $ not significant; Fig. 3h) as compared with initiation of ART on day 14. The high variability in the GMMC samples probably reflected sampling variation in this anatomical compartment. Compared to untreated animals, initiation of ART on day 3 reduced levels of proviral DNA at week 24 by at least 3.3 log in PBMCs ($P < 0.0001$; Fig. 3f) and 3.1 log in LNMCs ($P < 0.0001$; Fig. 3g).

At week 24, ART was discontinued, and monkeys were monitored twice weekly for evidence of viral rebound. Viral rebound, defined as plasma viral RNA >50 copies ml$^{-1}$, occurred in all animals (Fig. 4a). In particular, viral rebound occurred in four of four animals that initiated ART on day 3, albeit with threefold delayed kinetics as compared with animals that initiated ART at later time points (median 21 days to viral rebound in the day 3 treated animals compared with 7 days in the day 14 treated animals; $P < 0.001$; Fig. 4b). The median log setpoint viral load after rebound, defined as viral loads on days 56–112 following ART discontinuation, was also 1.04 log RNA copies ml$^{-1}$ lower for all the ART-treated animals as compared with untreated controls (4.59 log RNA copies ml$^{-1}$ for all treatment groups combined versus 5.63 log RNA copies ml$^{-1}$ for untreated monkeys; $P = 0.01$; Fig. 4c), suggesting that there is a benefit to early ART, although no significant differences were observed among setpoint viral loads in the day 3, 7, 10 and 14 treatment groups. Setpoint viral loads in the untreated animals were comparable with historical controls. Taken together, these data show that the persistent viral reservoir was seeded by day 3 of infection and led to viral rebound in all monkeys after ART discontinuation.

To gain mechanistic insight into the kinetics of the viral rebound, we used viral dynamics modelling$^{1,5,16}$ (see Methods; Extended Data Fig. 6 and Extended Data Table 2). Initiation of ART on day 3 as compared with days 7, 10 and 14 resulted in lower modelled residual viral loads at the time of ART discontinuation ($P = 0.01$) and a trend towards a greater viral growth rate $R_0$ during viral rebound ($P = 0.06$), but no difference in post-rebound setpoint viral loads (Extended Data Fig. 7). Average $R_0$ during viral rebound was $4.2 \pm 1.8$ in the day 3 treated animals as compared with $2.3 \pm 0.6$ in the day 14 treated animals ($P = 0.05$; Extended Data Fig. 7), presumably reflecting the partially effective SIV-specific immune responses in the latter group (Fig. 2). Total plasma viraemia during acute infection was interpolated and calculated as the area under the curve for pre-ART viral loads (AUC VL) (Extended Data Fig. 8). Consistent with recent findings in acute HIV-1 infection in humans$^{17}$, the AUC VL during acute infection correlated with levels of proviral DNA in PBMCs ($P < 0.0001$; Fig. 5a), LNMCs ($P = 0.005$; Fig. 5b) and GMMCs ($P = 0.04$; data not shown) at the time of ART discontinuation. Moreover, both the AUC VL ($P < 0.0001$; Fig. 5c) and proviral DNA in PBMCs at the time of ART discontinuation ($P = 0.003$; Fig. 5d) correlated inversely with the interpolated time to viral rebound. These data suggest that total plasma viraemia during acute infection and proviral DNA immediately before ART discontinuation may predict the time to viral recrudescence.

We show that the viral reservoir can be seeded substantially earlier than previously recognized. After intrarectal SIV infection of rhesus monkeys, the viral reservoir was seeded during the first few days of infection, during the eclipse phase, and before detectable viraemia, probably in the mucosal and lymphoid tissues that represent the first sites of viral replication$^{17}$. Consistent with this finding, we observed proviral DNA in lymph nodes and in gastrointestinal mucosa but not in PBMCs in monkeys treated on day 3 after infection (Fig. 3a and Extended Data Fig. 5). The observation that the viral reservoir can be seeded before detectable viraemia suggests that substantial pathogenesis occurs in tissues in the first few days after mucosal virus exposure and prior to virus replication in peripheral blood, which has important implications for HIV-1 therapeutic and eradication strategies.

Our data are concordant with recent clinical studies that have demonstrated that early ART can reduce the size of the viral reservoir and delay or reduce viral rebound after ART discontinuation in humans$^{18–22}$. Our findings similarly show that early ART decreased proviral DNA in blood and tissues (Fig. 3f–h) and delayed and reduced viral rebound (Fig. 4b, c) after ART discontinuation in SIV-infected rhesus monkeys. Moreover,
our observations extend previous studies that have shown effective post-exposure prophylaxis with short courses of ART when initiated 24 h after SIV infection in monkeys.23,24 However, in the present study, initiation of suppressive ART even as early as day 3 after infection failed to eliminate the viral reservoir and did not prevent viral rebound subsequent to 24 weeks of fully suppressive ART. In addition, our data (Fig. 4a) are consistent with clinical studies that have shown that the vast majority of HIV-1-infected individuals who initiate ART during acute infection show viral rebound after discontinuation of ART25–27.

Our findings contrast with the sustained remission and potential cure of a viremic baby that was treated with ART at 30 h of life.28 It is possible that this baby was inoculated perinatally with maternal cells instead of mucosally with virus, resulting in rapid viremia without a previraemic eclipse phase of viral replication in mucosal and lymphoid tissues. The positive outcome in this baby therefore might have reflected the route of transmission, the lack of an eclipse phase, the very rapid initiation of ART, and/or the paucity of memory CD4+ T lymphocytes in the neonatal immune system.29

Clinical studies are required to confirm our observations, since important differences exist between SIV infection of rhesus monkeys and HIV-1 infection of humans. For example, the SIV dose used in the present study in monkeys was selected to limit the number of transmitted/founder viruses but also to infect all animals and was substantially higher than typical HIV-1 doses in humans. Nevertheless, the higher challenge dose has been shown to shorten the eclipse period and to lead to earlier plasma viraemia.30 Additional differences may also exist between SIV-infected rhesus monkeys and HIV-1-infected humans. The strikingly early seeding of the viral reservoir within the first few days of infection is sobering and presents new challenges to HIV-1 eradication strategies. If HIV-1 similarly seeds a persistent viral reservoir in infected rhesus monkeys and HIV-1-infected humans, our findings will help guide future HIV-1 eradication efforts. Since the acceptance of this paper, the ‘Mississippi baby’ that was treated with ART at 30 h of life and had a prolonged remission off therapy has now developed detectable levels of HIV-1 replication and has restarted therapy, demonstrating that early ART did not eradicate the viral reservoir in this child.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Animals. Twenty outbred, Indian-origin, young adult, male and female rhesus monkeys (Macaca mulatta) were genotyped and selected as negative for the protective MHC class I alleles Mamu-A^*01, Mamu-B^*08 and Mamu-B^*17. Animals expressing susceptible and resistant Trim5a alleles were distributed amongst the groups. Animals were otherwise randomly allocated to groups. All monkeys were housed at Bioquark, Rockville, Maryland. Animals were infected with 500 TCID50 of our SIVmac251 challenge stock by the intrarectal route. Monkeys were bled up to two times per week for viral load determinations. Assays were performed blinded. All animal studies were approved by the appropriate Institutional Animal Care and Use Committee (IACUC).

ART regimen. The pre-formulated antiretroviral therapy (ART) cocktail contained two reverse transcriptase inhibitors, 20 mg ml^-1 tenofovir (TFV) and 50 mg ml^-1 emtricitabine (FTC) plus 2.5 mg ml^-1 of the integrase inhibitor dolutegravir (DTG) in a solvent containing 25% (v/v) polyethylene glycol 400 (PEG-400), 15% (v/v) captisol and 0.075 N sodium hydroxide (NaOH) in water. This ART cocktail was administered once daily at 1 ml kg^-1 body weight via the subcutaneous route. The cocktail was prepared by mixing DTG stock solution (10 mg ml^-1 of DTG in PEG-400), TFV and FTC stock solution (80 mg ml^-1 of TFV and 200 mg ml^-1 of FTC in 0.3 N NaOH), and 30% (w/v) captisol solution at a 1:1:2 (v:v:v) ratio. The final solution was clear and at pH 6.5.

Cellular immune assays. SIV-specific cellular immune responses were assessed by IFN-γ ELISPOT assays and multiparameter ICS assays essentially as previously described. Twelve-colour ICS assays were performed with the Aqua green-fluorescent reactive dye (Invitrogen, L23101) and predetermined titres of monoclonal antibodies (Becton Dickinson) against CD3 (SP34; Alexa Fluor 700), CD4 (OKT4; BV711; Biolegend), CD8a (KJ126; phycoerythrin-cyanine (PE-Cy7)), CCR7 (3D12; Pacific Blue) and PD-1 (EH21.1; peridinin chlorophyll-a-cyanine (PERC-Pearl)) (ECD); Beckman Coulter), IFN-γ (B27; phycoerythrin-texas red reactive dye (Invitrogen, L23101) and predetermined titres of monoclonal antibodies (Becton Dickinson) against CD95 (DX2; allophycocyanin (APC)), CD69 (TP1.55.3; phycoerythrin-texas red reactive dye (Invitrogen, L23101) and predetermined titres of monoclonal antibodies (Becton Dickinson) against CD3 (SP34; Alexa Fluor 700), CD4 (OKT4; BV711; Biolegend), CD8a (KJ126; phycoerythrin-cyanine (PE-Cy7)), CCR7 (3D12; Pacific Blue) and PD-1 (EH21.1; peridinin chlorophyll-a- cyanine (PERC-Pearl)). IFN-γ backgrounds were consistently <0.01% in PBMCs.

Humoral immune assays. SIV Env-specific antibody responses were assessed by a direct ELISA essentially as previously described.

Viral RNA assays. Viral RNA was isolated from cell-free plasma using a viral RNA extraction kit (Qiagen) and was quantitated essentially as previously described. RNA was isolated by phenol-chloroform purification followed by ethanol precipitation. All purified RNA preparations were quantified by optical density. Quantitative RT–PCR was conducted in a two-step process. First, RNA was reverse transcribed in parallel with an SIV-gag RNA standard using the gene-specific primer sGag-R 5′-CAGCAGGCTTTCTCCTGACTATTTTG-3′. All samples were then treated with RNase H (Stratagene) for 20 min at 37 °C. Primer sequences were adapted from those described including the forward primer sGag-F 5′- GAGGCTTTCTCATAGGCATGAGACGCTATGCG-3′, linked to Fam and BQH (Invitrogen). Reactions were performed on a 7300 ABI Real-Time PCR system (Applied Biosystems) in triplicate according to the manufacturer’s protocols. Ultrasensitive viral load assays were performed essentially as described.

Proviral DNA assays. Lymph node and gastrointestinal mucosal biopsies were processed as single-cell suspensions, and levels of tissue-specific proviral DNA were quantitated as previously described. Total cellular DNA was isolated from 5 × 10^6 cells using a QIAamp DNA Blood Mini kit (Qiagen). The absolute quantification of viral DNA in each sample was determined by qPCR using primers specific to a conserved region of SIVmac239. All samples were directly compared to a linear virus standard and the simultaneous amplification of a fragment of the human GAPDH gene. The sensitivity of linear standards was compared against the 3D8 cell line as a reference standard as described. PCR assays were performed with 100–200 ng sample DNA.

Viral dynamics modelling. Plasma viral loads pre-treatment, during treatment, and after treatment interruption were fit to a viral dynamics model to determine key parameters. In order to derive individual level estimates with limited data points for each animal, we used a simplified version of the full system of differential equations describing the viral dynamics model, which had fewer parameters and could be solved analytically. All fits were evaluated using the nlinfit function in the MATLAB Statistical Toolbox. For acute infection in all animals treated on days 7, 10 or 14, and for rebound in all animals, data points below the limit of detection were treated as missing data (except for the last undetectable value after infection or interruption, which we took to equal the detection limit of 50 copies ml^-1). Treating undetectable viral load values as left-censored data using a maximum likelihood approach did not noticeably change the results.

Pre-treatment and during treatment: We fit the viral dynamics observed following infection and treatment up to week 24 using a three-parameter piecewise function:

$$v(t) = \begin{cases} v_0e^{d_1t}, & t < t_{\text{ART}} \\ v_0e^{d_2t}e^{(-t-d_1)}, & t \geq t_{\text{ART}} \end{cases}$$

(1)

where \(t_{\text{ART}}\), the time that treatment was started, was defined based on the cohort as 3, 7, 10 or 14 days. Here \(v_0\) is the effective infectious viral load from which the infection starts, \(d\) is the growth rate of viral load during acute infection, and \(d_1\) is the decay rate of viral load once treatment is started (if treatment is effective, the lifespan of infected cells is approximately \(1/d\)). In essence, the model allows viral load to increase exponentially until treatment begins, and then decrease exponentially until it is below the detection limit. This approximates the observed dynamics well because a stable setpoint was not reached before treatment, and insufficient data points above the detection limit existed during viral decay to observe biphasic behaviour.

The basic reproductive ratio of the infection, defined as the average number of new infected cells produced by a single infected cell during early infection, can be calculated from these parameters as \(R_0 = a(d+1).\) However, others have reported that due to the intracellular delay between infection and the production of virus, this formula underestimates \(R_0\). To correct for this effect, we used a modified expression, \(R_0 = a(d+1)(p+1)\), where \(p\) is the average length of the exponentially distributed delay time (which we take to be 1 day). The AUC VL was used as an estimate of the total number of cells infected prior to treatment, which is a reasonable approximation for acute SIV infection since CD4+ T-cell levels do not change consistently or significantly. We assumed that ART was fully effective so that there were no new infections after ART initiation, leading to the following formula:

$$\text{AUC} = \frac{t_{\text{ART}}}{a} \int_0^{t_{\text{ART}}} v(t) dt = \frac{v_0}{a} e^{d_1 t_{\text{ART}}} - 1$$

(2)

No fits were conducted for animals treated after 3 days, since all observed viral load values were below the limit of detection. To estimate the AUC VL for these, we used the average values of \(v_0\) and \(a\) from the remaining 12 animals in the above formula with \(t_{\text{ART}} = 3\).

Post-ART discontinuation: We fit the viral dynamics observed after ART discontinuation (starting at week 24) using a separate three-parameter function:

$$v(t) = \frac{v_0 v_{\text{max}} e^{r t}}{v_{\text{max}} + v_0 e^{r t} - 1}$$

(3)

Here \(t\) is the time since interruption. The parameter \(v_0\) is the infectious residual viral load from which the infection starts, \(r\) is the growth rate of viral load during rebound, and \(v_{\text{max}}\) is the viral setpoint reached after rebound. This logistic equation is an approximation to the full viral dynamics model that smooths out both high frequency fluctuations in viral load and lags in uninfected CD4+ T-cell dynamics (such as overshooting the setpoint). Using these fits, we can interpolate the time at which viral load crossed the threshold of 50 copies ml^-1 to define the rebound time. Similar to pre-treatment, we can calculate the post-rebound basic reproductive ratio as \(R_0 = (r/d + 1)/(p + 1)\), where \(d\) is the viral decay rate measured during treatment and \(p\) is the intracellular delay between infection and virion production (which we take to be 1 day).

Statistical analyses. All comparisons between cohorts were done using unpaired, equal variance, one-tailed t-tests. Correlation analysis was carried out in using the MATLAB corrcoef function (Pearson correlation). The standardized major axis method was employed for regression analysis (also known as ‘geometric mean regression’ or ‘reduced major axis regression’) using the gmrregress function for the MATLAB Statistical Toolbox (available at http://www.mathworks.com/matlabcentral/fileexchange/27918-gmregress).

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**Extended Data Figure 1**  | Viral dynamics modelling of initial viral growth and decay after ART initiation. Red lines indicate fitted values from the model in monkeys that initiated ART on days 3, 7, 10 and 14 of infection and were used for AUC VL calculations. Red asterisks indicate the time of treatment initiation. Values below the assay detection limit of 50 copies ml$^{-1}$ are not shown.
Extended Data Figure 2 | Ultrasensitive plasma viral loads in monkeys during ART. Log plasma viral RNA (copies ml\(^{-1}\)) at week 20 in rhesus monkeys infected with SIV\(_{\text{MAC251}}\) and after initiation of ART on days 3, 7, 10 and 14 of infection. Assay sensitivity is 6 RNA copies ml\(^{-1}\).
Extended Data Figure 3 | Intracellular cytokine staining raw data of Gag-specific CD8\(^+\) and CD4\(^+\) T cells. Representative data for the magnitude of Gag-specific IFN-\(\gamma\)^+ CD8\(^+\) and CD4\(^+\) T-cell responses at week 20 in monkeys that initiated ART on days 3, 7, 10 and 14 of infection or with no ART.
Extended Data Figure 4 | Intracellular cytokine staining of Gag-specific CD8\(^+\) and CD4\(^+\) T cells. Summary data for the magnitude of Gag-specific IFN-\(\gamma\) CD8\(^+\) and CD4\(^+\) T-cell responses and Ki67 expression at week 4 and week 20 in monkeys that initiated ART on days 3, 7, 10 and 14 of infection or with no ART (\(N = 4\) animals per group). Error bars show s.e.m.
Extended Data Figure 5 | Proviral DNA in CD4+ T-cell subpopulations during ART. Log proviral DNA (copies per 10^6 CD4+ T cells) in sorted naive (N), transitional effector memory (TM) and central memory (CM) CD4+ T-cell subpopulations from PBMCs and from genital, inguinal, iliac, para-aortic, axillary and/or mesenteric lymph nodes obtained from two animals necropsied on day 3 and two animals necropsied on day 7 after mucosal SIV_MAC251 infection. Error bars show s.e.m.
Extended Data Figure 6 | Viral dynamics modelling of viral rebound after ART discontinuation. Red lines indicate fitted values from the model in monkeys that initiated ART on days 3, 7, 10 and 14 after infection. Values below the assay detection limit of 50 copies ml$^{-1}$ are not shown.
**Extended Data Figure 7** | Viral kinetics and setpoint viral loads following ART discontinuation. Log initial viral loads, exponential viral growth rate and log setpoint viral loads after viral rebound derived from the model fits in groups of monkeys that initiated ART on days 3, 7, 10 and 14 of infection ($N = 4$ animals per group). Error bars show standard deviation.
Extended Data Figure 8 | Early ART impacts AUC VL and time to viral rebound. Log AUC VL and interpolated time of viral rebound derived from the model fits are shown in monkeys that initiated ART on days 3, 7, 10 and 14 of infection (N = 4 animals per group). Error bars show standard deviation.
## Extended Data Table 1 | Best-fit parameters for viral dynamics pre-ART and during ART

Fitted parameters include $v_0$, the effective infectious viral load from which the infection starts, $a$, the growth rate of viral load during acute infection, and $d$, the decay rate of viral load once treatment is started. See Methods, including equations (1) and (2), for detailed descriptions.

| Day of ART initiation | $\log_{10}(v_0)$ | $a$ | $d$ | Half-life $(\ln(2)/d)$ | $R_0$ | $\log_{10}(AUC)$ |
|-----------------------|------------------|-----|-----|------------------------|-------|------------------|
| 3                     | -                | -   | -   | -                      | -     | 2.6              |
| 3                     | -                | -   | -   | -                      | -     | 2.6              |
| 3                     | -                | -   | -   | -                      | -     | 2.6              |
| 3                     | -                | -   | -   | -                      | -     | 2.6              |
| 7                     | -1.16            | 2.19 | 0.50 | 1.38                   | 17.10 | 5.1              |
| 7                     | 1.44             | 1.45 | 0.29 | 2.37                   | 14.57 | 5.7              |
| 7                     | -1.77            | 2.66 | 0.58 | 1.19                   | 20.30 | 5.9              |
| 7                     | 1.34             | 1.49 | 0.56 | 1.24                   | 9.13  | 5.7              |
| 10                    | 1.27             | 1.38 | 0.63 | 1.11                   | 7.65  | 7.1              |
| 10                    | 2.16             | 1.25 | 0.91 | 0.76                   | 5.33  | 7.5              |
| 10                    | -0.25            | 1.55 | 0.51 | 1.36                   | 10.32 | 6.3              |
| 10                    | -0.24            | 1.57 | 0.60 | 1.15                   | 9.23  | 6.4              |
| 14                    | 1.76             | 0.94 | 0.62 | 1.12                   | 4.86  | 7.5              |
| 14                    | 1.79             | 1.05 | 0.49 | 1.42                   | 6.45  | 8.2              |
| 14                    | 2.30             | 0.94 | 0.53 | 1.30                   | 5.36  | 8.0              |
| 14                    | 1.54             | 1.11 | 0.91 | 0.76                   | 4.69  | 8.3              |
| Mean                  | 0.85             | 1.46 | 0.60 | 1.26                   | 9.58  | NA               |
| STD                   | 1.35             | 0.51 | 0.17 | 0.41                   | 5.16  | NA               |

Fitted parameters include $v_0$, the effective infectious viral load from which the infection starts, $a$, the growth rate of viral load during acute infection, and $d$, the decay rate of viral load once treatment is started. See Methods, including equations (1) and (2), for detailed descriptions.
Extended Data Table 2 | Best-fit parameters for viral dynamics after ART discontinuation

| Day of ART initiation | $\log_{10}(v_r)$ | $r$ | $\log_{10}(v_{max})$ | $R_0$ | Estimated days to VL=50 copies ml$^{-1}$ |
|-----------------------|------------------|-----|----------------------|-------|----------------------------------------|
| 3                     | -6.57            | 1.12| 4.22                 | 6.10  | 17.0                                   |
| 3                     | -0.27            | 0.27| 4.83                 | 1.84  | 16.8                                   |
| 3                     | -2.18            | 0.85| 4.88                 | 4.51  | 10.5                                   |
| 3                     | -4.34            | 0.80| 5.22                 | 4.20  | 17.5                                   |
| 7                     | 0.72             | 0.40| 4.70                 | 2.53  | 5.6                                    |
| 7                     | -1.23            | 0.94| 3.62                 | 8.12  | 7.2                                    |
| 7                     | 0.76             | 0.49| 3.79                 | 2.75  | 4.4                                    |
| 7                     | -0.60            | 0.69| 5.01                 | 3.78  | 7.7                                    |
| 10                    | -1.33            | 0.96| 5.26                 | 4.95  | 7.3                                    |
| 10                    | -1.17            | 0.89| 4.00                 | 3.74  | 7.4                                    |
| 10                    | 1.25             | 0.24| 5.71                 | 1.84  | 4.2                                    |
| 10                    | 0.12             | 0.38| 2.50                 | 2.24  | 10.1                                   |
| 14                    | 1.38             | 0.19| 3.39                 | 1.56  | 3.9                                    |
| 14                    | 1.23             | 0.36| 4.22                 | 2.34  | 3.0                                    |
| 14                    | 1.07             | 0.52| 4.41                 | 3.02  | 2.7                                    |
| 14                    | 0.24             | 0.51| 5.00                 | 2.37  | 6.6                                    |

Fitted parameters include $v_r$, the infectious residual viral load from which the infection starts, $r$, the growth rate of viral load during rebound, and $v_{max}$, the viral setpoint reached after rebound. See Methods, including equation (3), for detailed descriptions.