LETTER

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Early antibody therapy can induce long-lasting immunity to SHIV

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Highly potent and broadly neutralizing anti-HIV-1 antibodies (bNAbs) have been used to prevent and treat lentivirus infections in humanized mice, macaques, and humans[1–12]. In immunotherapy experiments, administration of bNAbs to chronically infected animals transiently suppresses virus replication, which invariably returns to pre-treatment levels and results in progression to clinical disease. Here we show that early administration of bNAbs in a macaque simian/human immunodeficiency virus (SHIV) model is associated with very low levels of persistent viraemia, which leads to the establishment of T-cell immunity and resultant long-term infection control. Animals challenged with $SHIV_{AD8\text{-}EO}$ by mucosal or intravenous routes received a single 2-week course of two potent passively transferred bNAbs (3BNC117 and 10-1074 (refs 13, 14)). Viraemia remained undetectable for 56–177 days, depending on bNAb half-life *in vivo*. Moreover, in the 13 treated monkeys, plasma virus loads subsequently declined to undetectable levels in 6 controller macaques. Four additional animals maintained their counts of T cells carrying the CD4 antigen ($CD4^+$) and very low levels of viraemia persisted for over 2 years. The frequency of cells carrying replication-competent virus was less than 1 per 10^6 circulating $CD4^+$ T cells in the six controller macaques. Infusion of a T-cell-depleting anti-CD8β monoclonal antibody to the controller animals led to a specific decline in levels of $CD8^+$ T cells and the rapid reappearance of plasma viraemia. In contrast, macaques treated for 15 weeks with combination anti-retroviral therapy, beginning on day 3 after infection, experienced sustained rebound plasma viraemia when treatment was interrupted. Our results show that passive immunotherapy during acute SHIV infection differs from combination anti-retroviral therapy in that it facilitates the emergence of potent $CD8^+$ T-cell immunity able to durably suppress virus replication.

After an initial and massive burst of HIV replication[15], viraemia is incompletely controlled by the emergence of a virus-specific $CD8^+$ T-cell response, leading to a chronic phase during which the infection is never cleared[16,17]. Virions that integrate their DNA into the host cell genome become a source of virus production, which persists despite anti-retroviral treatment over extended periods of time and gives rise to a recrudescent progressive infection when treatment is stopped[18]. This virus reservoir persists for the lifetime of the infected individual[19].

Nonetheless, initiation of combination anti-retroviral therapy (cART) within 6 months of HIV-1 infection may limit damage to the immune system as well as the size of the reservoir, as measured by preservation of anti-virus T-cell responses and lower levels of cell-associated viral DNA[20]. In a rare subpopulation of people with HIV-1 infection, cART initiated during the early phases of infection resulted in sustained control of viraemia to very low levels, after treatment interruption[21]. Durable control of plasma viraemia has also been reported in some SIVsmE660-infected rhesus macaques after anti-retroviral treatment, started 24 or 72 h after intravenous inoculation, was discontinued[22].

Because bNAbs suppress viraemia[3,23], accelerate the clearance of cell-free HIV-1 virions[24], enhance clearance of infected cells[6,25,26], and boost host humoral immunity[23] in humans, they have the potential to mitigate deleterious events occurring during the acute infection and possibly alter the long-term clinical course. Consistent with this hypothesis, experiments in humanized mice indicate that early intervention with bNAbs may be more effective in preventing establishment of the reservoir than cART[5]. However, humanized mice do not have intact immune systems, and fail to sustain infection beyond 3–4 months.

We previously reported that initiation of bNAb monotherapy, 12 weeks after $SHIV_{AD8\text{-}EO}$ inoculation, controls plasma viraemia in macaques for 1 or 2 weeks, after which resistant viral variants emerge[12]. Thus, it is problematical whether even early combination bNAb immunotherapy might durably control viraemia. To address this question, combination 10-1074 (ref. 13) and 3BNC117 (ref. 14) immunotherapy, which targets non-overlapping epitopes on the envelope spike, was administered to rhesus monkeys at the earliest possible time (that is, 3 days after infection) when we were certain that a $SHIV_{AD8\text{-}EO}$ infection had been established.

$SHIV_{AD8\text{-}EO}$ infection resembles HIV-1 infection in several important ways. It is R5 tropic, generates sustained levels of plasma viraemia in inoculated macaques, exhibits a Tier 2 neutralization sensitivity phenotype, produces resistant variants in bNAb- and ART-treated animals, and causes irreversible depletions of $CD4^+$ T cells in infected monkeys (refs 12 and 27–29, and Y.N. and M.A.M., unpublished observations). Infection leads to symptomatic immunodeficiency associated with opportunistic infections and a fatal clinical outcome in untreated monkeys.

In the initial experiment to assess the potency of combination bNAbs during acute infection, six macaques were inoculated intrarectally with 1,000 $TCID_{50}$ (50% tissue culture infective dose) of $SHIV_{AD8\text{-}EO}$, which is sufficient to infect all animals challenged by this route[11]. Beginning on day 3 after inoculation, each monkey received a single course of three weekly intravenous bNAb infusions (on days 3, 10, and 17 after infection) of 10-1074 (ref. 13) plus 3BNC117 (ref. 14). Compared with untreated animals, extremely low levels of plasma viraemia could be detected in two of the six bNAb recipients during the first 30 days of infection (Fig. 1a). Plasma viraemia in the other four macaques was not measurable (<100 SHIV RNA copies per millilitre) using standard PCR with reverse transcription (RT–PCR) assays.

All six monkeys infused with the bNAbs experienced sustained periods of virus suppression lasting 56–177 days, at which point

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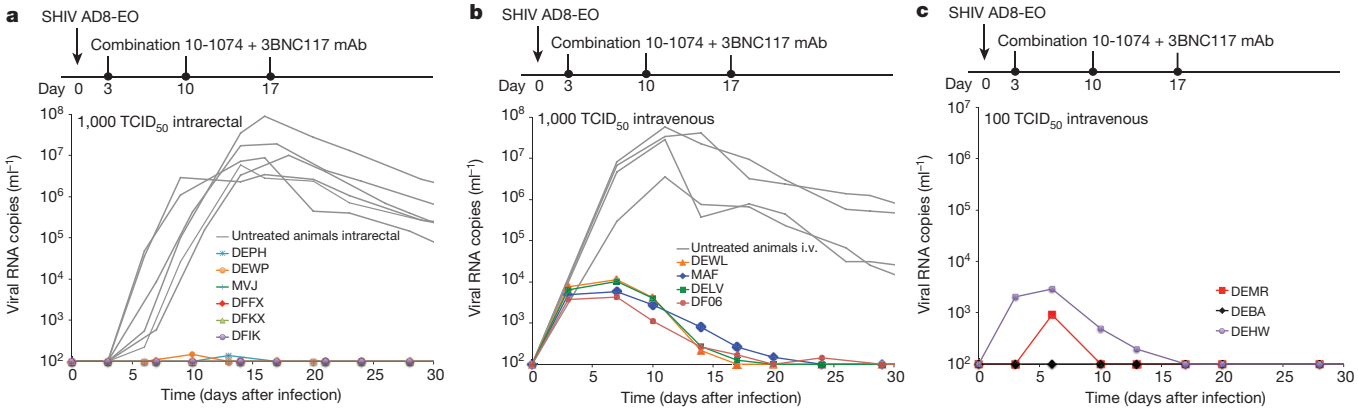

Figure 1 | Control of $SHIV_{AD8\text{-}EO}$ replication by a 2-week course of combination bNAb therapy. a, Six macaques were inoculated intrarectally with 1,000 $TCID_{50}$ of $SHIV_{AD8\text{-}EO}$ and were treated with 10-1074 plus 3BNC117 (10 mg kg^{-1} of each) mAbs on days 3, 10, and 17 after infection. Grey curves denote replication profiles of six similarly inoculated but untreated animals. **a**, **b**, Macaques inoculated intravenously (i.v.) with 1,000 $TCID_{50}$ ($n = 4$) (**b**) or 100 $TCID_{50}$ ($n = 3$) (**c**) of $SHIV_{AD8\text{-}EO}$ were treated with 10-1074 plus 3BNC117 mAbs as in **a**. Grey curves in **b** indicate the replication kinetics of four untreated monkeys inoculated intravenously with 5,000 or 500 $TCID_{50}$ of $SHIV_{AD8\text{-}EO}$. Plasma viral loads were measured by the standard RT–PCR assay (limit of detection, 100 SHIV RNA copies per millilitre) at the indicated times.

rebound viraemia occurred in five of the six treated macaques (Extended Data Fig. 1). Plasma viraemia remained below levels of detection in the sixth macaque (DFIK) during the first 150 days of infection/treatment (Extended Data Fig. 1f). As expected, the time to virus rebound was directly related to the concentration of bNAb in the plasma. For example, the $SHIV_{AD8\text{-}EO}$ rebound in macaque MVJ occurred on day 56 when the level of 3BNC117 decayed below 1 μg ml^{-1} in plasma, whereas monkeys DEPH and DEWP experienced prolonged suppression of viraemia before rebound was observed (Extended Data Fig. 1a, d, e). We conclude that a single 2-week course of combination antibody therapy with 3BNC117 and 10-1074, administered early after an intrarectal infection, can control $SHIV_{AD8\text{-}EO}$ viraemia for up to 177 days.

To determine whether low-level viral replication before virus rebound persisted despite apparent control of viraemia, we performed ultrasensitive nested RT–PCR (qRT–PCR) on plasma samples from animals with viral loads below levels of detection by standard methods (Extended Data Table 1). For example, in macaque DEPH, which did not generate a detectable virus rebound until day 177 (Extended Data Fig. 2d), the ultrasensitive assay measured fewer than two viral RNA copies per millilitre in plasma, between days 34 and 106 after infection, and ten RNA copies per millilitre on day 141 after infection, a month before rebound. We conclude that low levels of virus are continuously being produced systemically and released into the circulation in the infected and bNAb-treated macaques, even when plasma viraemia is not detected by standard assays.

Two patterns of 'post-rebound' plasma viraemia were observed in the six bNAb-treated monkeys. In the first (DFIK, MVJ, and DEWP; controller (Fig. 2a–c)), the rebound $SHIV_{AD8\text{-}EO}$ RNA loads in plasma returned to undetectable levels after a maximum of 20 weeks and remained so for an additional 20–30 weeks of observation. The second pattern occurred in monkeys DEPH, DFKX, and DFFX (non-controller

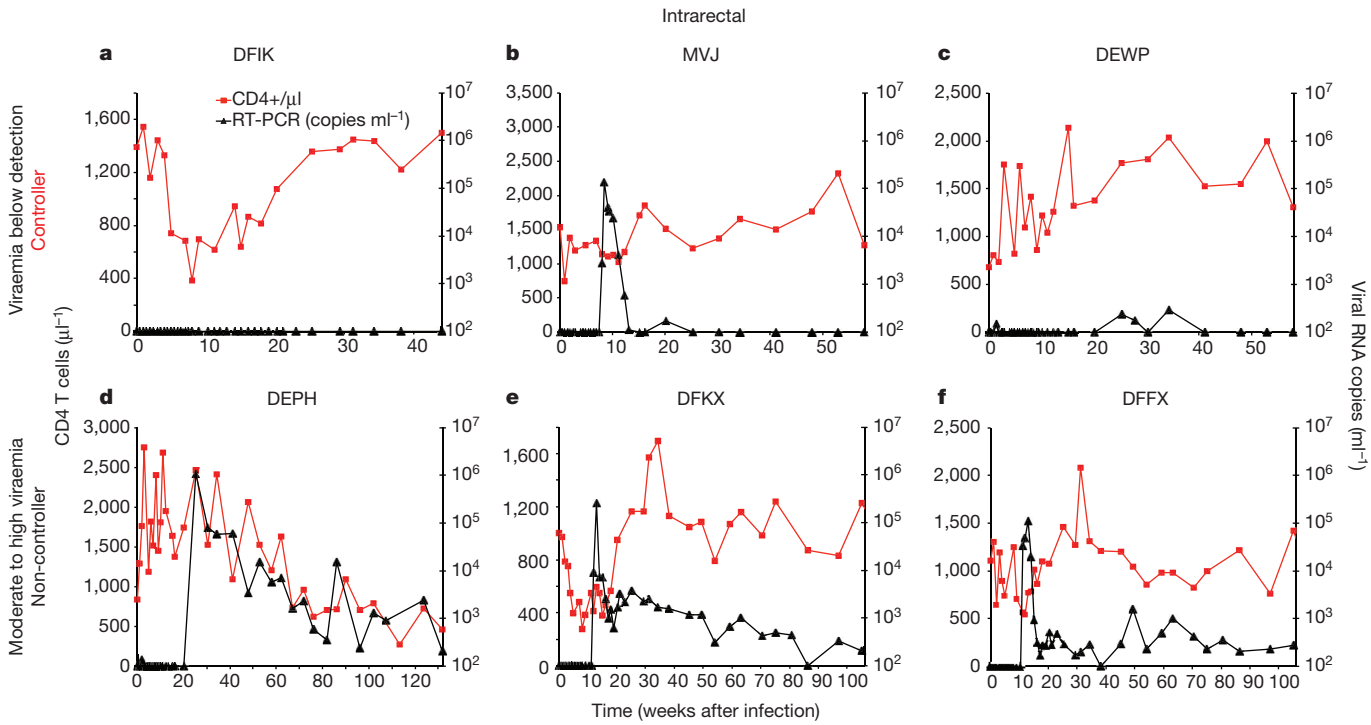

Figure 2 | Establishment of controller status in bNAb-treated animals inoculated with $SHIV_{AD8\text{-}EO}$ by the intrarectal route. a–f, Plasma virus loads (black) and $CD4^+$ T-cell levels (red) are shown in three controller (**a–c**) or three non-controller (**d–f**) macaques.

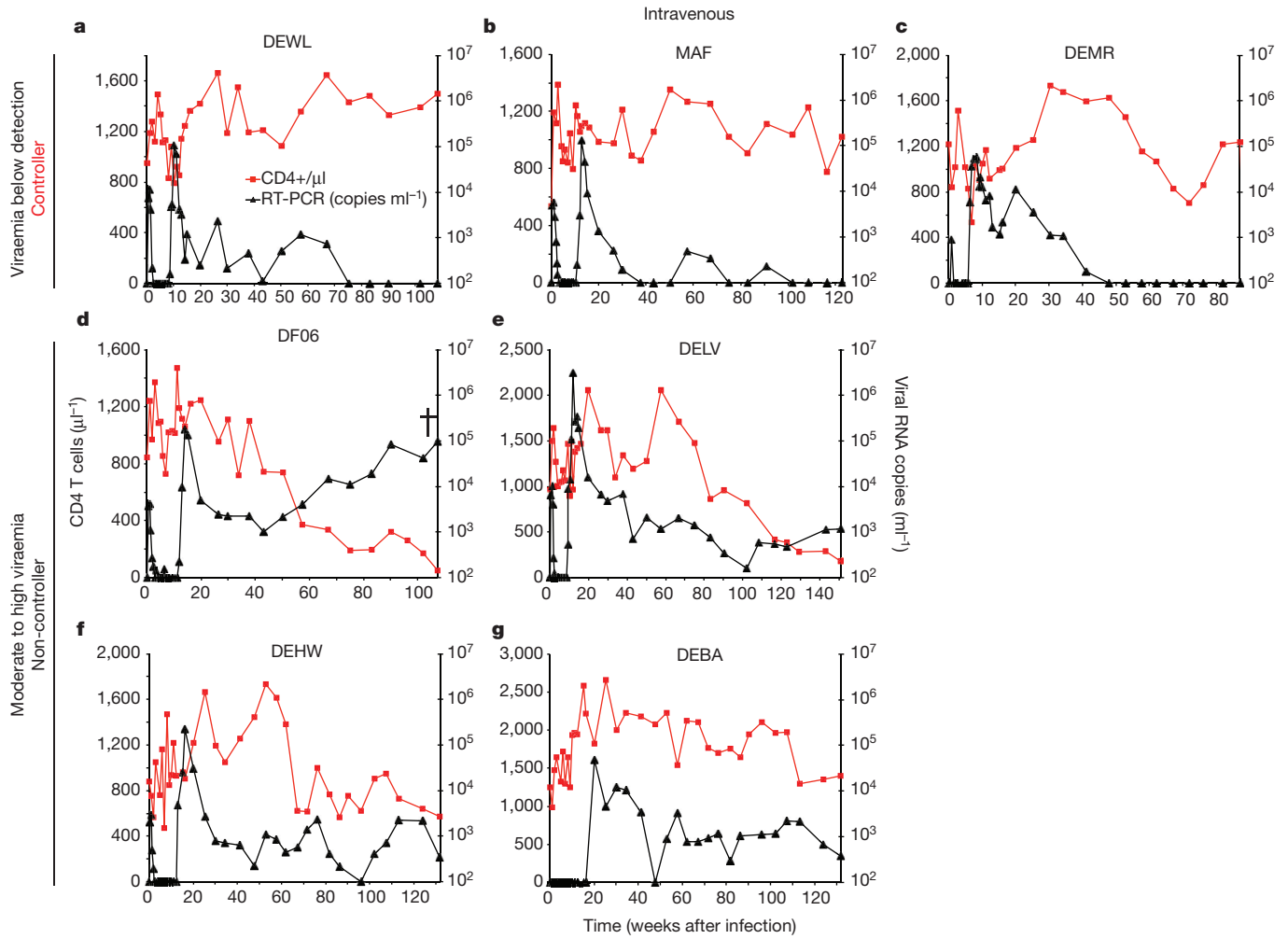

Figure 3 | Establishment of controller status in bNAb-treated animals inoculated with $SHIV_{AD8\text{-}EO}$ by the intravenous route. a–g, Plasma virus loads (black) and $CD4^+$ T-cell levels (red) are shown in three controller (**a–c**) or four non-controller (**d–g**) macaques.

(Fig. 2d–f)). These three macaques never fully controlled their viraemia after rebound and displayed viral loads of 1×10^2 to 10^3 RNA copies per millilitre between 110 and 130 weeks after virus inoculation.

Sustained control of plasma viraemia by early bNAb therapy after a mucosal $SHIV_{AD8\text{-}EO}$ challenge in three out of six monkeys suggested that the same intervention might also be effective against challenge by the intravenous route, for which a smaller virus inoculum is typically required to establish an infection. Macaques were therefore inoculated with either 100 or 1,000 $TCID_{50}$ of $SHIV_{AD8\text{-}EO}$ intravenously and then administered three weekly bNAb infusions starting on day 3 after infection. As shown in Fig. 1b, the four recipients of 1,000 $TCID_{50}$ of virus showed peak plasma virus loads ranging from 4.2×10^3 to 1.1×10^4 RNA copies per millilitre during the first 2 weeks of bNAb administration. Two of the three recipients (DEMR and DEHW) inoculated with 100 $TCID_{50}$ of virus intravenously had lower levels of plasma viraemia during the early treatment period, and in the third animal (DEBA), viral loads remained below the level of detection throughout this time (Fig. 1c). Viraemia declined to undetectable levels by 30 days after bNAb administration and remained suppressed for 48–110 days after infection in all seven combination bNAb-treated monkeys inoculated by the intravenous route (Extended Data, Fig. 2). As previously observed in the intrarectally challenged animals, virus rebound in the intravenously inoculated monkeys was directly associated with the decline of circulating bNAb levels.

To determine whether virus rebound was due to declining levels of bNAbs in the plasma or to the emergence of mutation(s) conferring resistance, we sequenced plasma viral RNA from the four monkeys (DELV, DF06, DEWL, and MAF), generating detectable viraemia at a time when the 3BNC117 monoclonal antibody (mAb) was not detectable, but 10-1074 mAb concentrations remained above threshold levels (see Extended Data Fig. 2). When samples collected at the peak of virus rebound from these 4 animals were analysed, no changes were observed in amplicons from macaques DELV and DEWL, but 9 out of 18 amplified sequences from macaque DF06 carried the N332S change and 12 out of 24 amplicons from monkey MAF had the S334N substitution, both of which eliminated the glycan at position 332 of gp120 (Extended Data Fig. 3), the epitope targeted by the 10-1074 mAb. This result indicated that resistance to the 10-1074 mAb probably occurred in these two animals during the time when 10-1074, but not 3BNC117, was detectable in the plasma.

Similar to the macaques challenged by the intrarectal route, passive immunotherapy after intravenous infection also led to sustained virus control after the resolution of rebound viraemia in some of the challenged monkeys. In three macaques (DEWL, MAF, and DEMR; controllers), rebound viraemia remained detectable for 42–90 weeks, and was then followed by long periods (25–56 additional weeks) during which virus replication was stably suppressed below the limits of detection (Fig. 3a–c). $CD4^+$ T-cell counts were maintained at a level of 800 cells per microlitre or higher in these three controller animals. In the second group of intravenously challenged animals (monkeys DF06, DELV, DEHW, and DEBA; non-controllers), virus replication was never completely suppressed after rebound (Fig. 3d–g). Failure to control viraemia was associated with $CD4^+$ T-cell loss and progression to AIDS in one (macaque DF06) of these four monkeys.

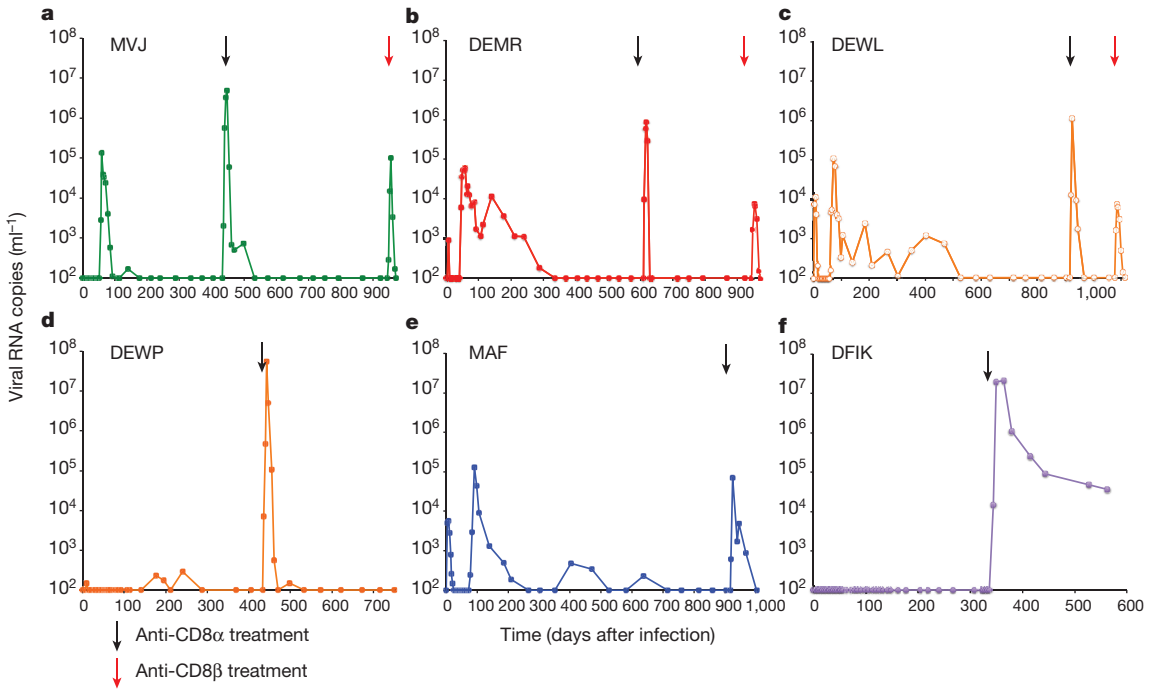

Figure 4 | Depletion of CD8$^+$ T cells in controller macaques results in the rapid induction of plasma viraemia. a–f, Plasma SHIV$_{AD8\text{-}EO}$ levels before and after administration of anti-CD8 depleting mAbs to six controller monkeys. Black arrows in each panel indicate the time of infusion of the anti-CD8α depleting mAb MT807R1. Red arrows in **a–c** indicate infusion of the anti-CD8β depleting mAb CD8b255R1 to controller macaques MVJ, DEMR, and DEWL.

Humoral and cellular immune responses were assessed before and after SHIV$_{AD8\text{-}EO}$ rebound to determine whether anti-viral immunity was associated with controller status. No measurable correlation was observed between virus control and antibody levels. The controllers generated very low levels of anti-gp120 binding antibodies that were barely detectable by enzyme-linked immunosorbent assay. Anti-viral CD8$^+$ T-cell responses were measured at four different times during the bNAb treatment, virus rebound, and post-rebound phases of their infections in both controllers and non-controllers. Although anti-SIVmac239 Gag CD8$^+$ T-cell responses were higher in animals challenged by the intravenous route, no major differences were observed between controllers and non-controllers (Extended Data Fig. 4a). Furthermore, the polyfunctionality of the CD8$^+$ T-cell responses and distribution of CD8$^+$ T-cell memory subsets in intravenously inoculated controllers and non-controllers at different times after infection, treatment, and rebound were comparable (Extended Data Fig. 4b, c).

To determine whether CD8$^+$ T cells might, in fact, be mediating the sustained suppression of virus replication, we initially administered the CD8 T-cell-depleting mAb MT807R1, which is specific for the CD8α chain, to all six controller macaques (MVJ (at day 434 after infection), DEWP (at day 434 after infection), DFIK (at day 336 after infection), DEMR (at day 609 after infection), MAF (at day 917 after infection), and DEWL (at day 917 after infection)) (Fig. 4a–f). All animals responded with a burst of plasma viraemia, reaching levels between 10^5 and 10^7 viral RNA copies per millilitre, which subsequently declined to baseline in all of the monkeys, except for DFIK.

Before the administration of the anti-CD8α mAb, quantitative virus outgrowth assays were performed on samples collected from the six controllers to measure the frequency of circulating CD4$^+$ T cells releasing replication-competent virions. As shown in Extended Data Table 2, fewer than 1 in 10^6 CD4$^+$ T cells carrying infectious virus was detected in all of the controller monkeys immediately before infusion of the depleting anti-CD8α mAb. Interestingly, in five of the six anti-CD8α mAb recipients in which plasma viraemia had returned to baseline levels after mAb infusion, the frequency of cells carrying replication-competent virus, determined by virus outgrowth assays, was also less than 1 in 10^6 CD4$^+$ T cells (Extended Data Table 2).

A major deficiency of using the anti-CD8α mAb to deplete CD8$^+$ T cells is that NK, NKT, and γδ T cells are also targeted for depletion, as documented by fluorescence-activated cell sorting (FACS) analyses for monkeys MVJ, DEWL, and DEMR (Extended Data Fig. 5a, b). We therefore administered the CD8b255R1 anti-CD8β mAb, which specifically targets macaque CD8$^+$ T cells, to deplete CD8$^+$ T cells in these same three controller monkeys. As shown in Fig. 4a–c (red arrows), infusion of the anti-CD8β mAb caused an immediate increase in plasma virus loads, a decline in levels of CD3$^+$CD8$^+$ T cells (Extended Data Fig. 5c), and no changes in circulating CD3$^-$CD8$^+$ cells (Extended Data Fig. 5d). We conclude that CD8$^+$ T cells are responsible for control of virus replication in controller macaques and depletion of this subset leads to recrudescence of viraemia.

Although the non-controller monkeys failed to suppress plasma viraemia to undetectable levels, four (DFKX, DFFX, DEHW, and DEBA) of seven of these macaques maintained low virus loads (105–385 RNA copies per millilitre) and did not experience significant changes in levels of circulating CD4$^+$ T cells for 2–3 years after SHIV$_{AD8\text{-}EO}$ infection (Figs 2 and 3). Taken together, this indicates that 10 (6 controllers and 4 non-controllers) of the 13 bNAb-treated monkeys benefited from early immunotherapy.

As a control for bNAb immunotherapy during the acute SHIV$_{AD8\text{-}EO}$ infection, three macaques were inoculated by the intrarectal route and treated daily with cART for 15 weeks, starting on day 3 after infection. This course of cART corresponded to the mean period during which 2 weeks of bNAb therapy suppressed virus replication before rebound. cART therapy controlled and maintained undetectable levels of viraemia for the entire 15-week treatment period (Extended Data Fig. 6). However, all three animals developed high sustained levels of plasma viraemia after cessation of cART and none became controllers.

We speculate that the continuous production of low levels of progeny virions, as measured by ultrasensitive RT–PCR (Extended Data Table 1) during the 50- to 140-day period before virus rebound in the antibody

treated macaques, could drive the formation of immune complexes. Antigen-presenting dendritic cells expressing activating Fc receptors can bind to these immune complexes, leading to their activation and efficient antigen processing for presentation and cross-presentation to $CD4^+$ and $CD8^+$ T cells[30]. In contrast, the near-complete inhibition of virus replication by the cART regimen used may limit the amount of viral antigen available to induce immunity.

As proof of concept, our results demonstrate that a SHIV infection, established during the acute phase, can, in fact, be controlled. They also suggest that a delicate balance may exist between (1) preservation of helper $CD4^+$ T cells, (2) the size and stability of the virus reservoir, and (3) the continuous production of sufficient quantities of antigen to generate a potent and sustained $CD8^+$ T-cell response. Although rhesus macaque infections with SHIV differ from HIV-1 infections in several important ways, immunotherapy should be explored as a way of controlling systemic dissemination of virus, of containing damage to the $CD4^+$ T-cell lineage, and of mobilizing a robust immune response that may be capable of controlling the infection in humans.

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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Supplementary Information is available in the online version of the paper.

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METHODS

Animal experiments. Eighteen 2- to 4-year-old male and female rhesus macaques (*M. mulatta*) of Indian genetic origin were housed and cared for in accordance with Guide for Care and Use of Laboratory Animals Report number NIH 82-53 (Department of Health and Human Services, Bethesda, Maryland, USA, 1985) in a biosafety level 2 National Institute of Allergy and Infectious Diseases (NIAID) facility. All animal procedures and experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the National Institute of Allergy and Infectious Diseases (NIH). No statistical methods were used to predetermine sample size. Animals and experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment. Phlebotomies, euthanasia, and sample collection were performed as previously described[31]. All of the macaques used in this study were negative for MHC class I *Mamu-A*01*, *Mamu-B*08*, and *Mamu-B*17* alleles. No animals were excluded from the analysis.

Antibodies. The 3BNC117 and 10-1074 anti-HIV-1 monoclonal NAbs were isolated and produced as described elsewhere[13,14]. MAb 10-1074 was produced by transient transfection of IgH and IgL expression plasmids into human embryonic kidney cells whereas 3BNC117 was produced from Chinese hamster ovary cells. All of the MAbs were IgG1. All of the MAbs were purified by chromatography and sterile filtration and were endotoxin free. A combination of single dose (10 mg kg^{-1}) of each MAb was administered intravenously to individual animals.

ART regimen. Three animals were treated with a three-drug ART regimen comprising two nucleoside reverse transcriptase (RT) inhibitors (tenofovir (PMPA) and emtricitabine (FTC)) and one integrase inhibitor (raltegravir (RAL)). PMPA and FTC were administered intramuscularly once a day at dosages of 20 mg kg^{-1} and 40 mg kg^{-1}, respectively. RAL was administered orally (mixed with food) at a dosage of 200 mg twice a day. The macaques received the ART regimen for 15 weeks, starting on day 3 after virus challenge.

Virus challenge. The origin and preparation of the tissue-culture-derived $SHIV_{AD8\text{-}EO}$ stock have been previously described[29]. Animals were challenged with $SHIV_{AD8\text{-}EO}$ intravenously (100 or 1,000 $TCID_{50}$) or intrarectally (1,000 $TCID_{50}$). For intrarectal inoculation, a paediatric nasal speculum was used to gently open the rectum and a 1 ml suspension of virus was slowly infused into rectal cavity using a plastic tuberculin syringe as previously described[11]. One thousand $TCID_{50}$ of $SHIV_{AD8\text{-}EO}$ administered intrarectally resulted in the establishment of infections in 30 of 30 rhesus monkeys (refs 11 and 29, and M.S., Y.N., R.G., and M.A.M., unpublished observations).

Quantification of plasma viral RNA. Viral RNA levels in plasma were determined by qRT–PCR (ABI Prism 7900HT sequence detection system; Applied Biosystems) as previously described[31]. Ultrasensitive measurement of plasma SIV gag RNA was performed as described previously[32].

Lymphocyte immunophenotyping and intracellular-cytokine assays. EDTA-treated blood samples were stained for flow cytometric analysis for lymphocyte immunophenotyping as previously described[28].

Intracellular cytokine staining. Cryopreserved macaque peripheral blood mononuclear cells (PBMCs) were thawed and rested overnight in a 37 °C/5% CO_2 incubator. The next morning, cells were stimulated with HIV Clade B envelope and SIV_{Mac239} gag peptide pools (final concentration of 2 μg ml^{-1}) in the presence of Brefeldin A, monensin, and CD107a-Cy5 PE (clone H4A3, BD Biosciences, San Jose, California, USA) for 6 h. Negative controls received an equal concentration of DMSO instead of peptides. Intracellular cytokine staining was performed as described[33] except the following mAbs were used: CD4-Cy5.5 PE (clone S3.5; Invitrogen, Carlsbad, California, USA), CD8-BV570 (clone RPA-T8; BioLegend), CD45RA-Cy5 PE (clone 5H9, BD Biosciences), PD-1-BV785 (clone EH12.2H7, BioLegend), CCR7-BV650 (clone G043H7, BioLegend), CD39-PerCpeF710 (clone eBioA1, e Bioscience), MIP-1B-PE (clone D21-1351, BD Biosciences), Granzyme B-APC (clone GB12, Invitrogen), CD69-ECD (clone TP1.55.3; Beckman Coulter), CD3-Cy7APC (clone SP34.2; BD Biosciences), IFNγ-Alexa700 (clone B27; BioLegend), IL-2-BV605 (clone MQ1-17H12; BD Biosciences), IL-10-BV421 (clone JES3-9D7, BD Biosciences), and TNF-FITC (clone Mab11; BD Biosciences). Aqua LIVE/DEAD kit (Invitrogen) was used to exclude dead cells. All antibodies were previously titrated to determine the optimal concentration. Samples were acquired on an LSR II flow cytometer and analysed using FlowJo version 9.6.3 (Treestar, Ashland, Oregon, USA).

Measurement of anti-gp120 antibodies. An enzyme-linked immunosorbent assay to detect antibodies generated against the HIV-1 gp120 envelope protein was performed as previously described[27].

MAb concentrations in plasma. Plasma concentrations of 10-1074 and 3BNC117 NAbs were separately determined against HIV-1 virus strains that were sensitive to one but not the other mAb as previously described[12].

Quantitative virus outgrowth assay. Macaque PBMCs were purified by Ficoll-Hypaque density gradient centrifugation. $CD4^+$ T cells were isolated from the PBMCs using a cell enrichment cocktail (StemCell Technologies) and enumerated using an automatic cell counter (Muse Cell Analyzer, EMD Millipore). The frequency of circulating $CD4^+$ T cells carrying replication-competent virus was determined using quantitative co-culture assays as previously described[34]. Briefly, serially diluted $CD4^+$ T cells (fivefold dilutions from 1×10^6 to 320 cells per well in duplicate) as well as replicates of 1×10^6 cells were stimulated with irradiated PBMCs from healthy HIV-negative donors in the presence of 1 μg ml^{-1} of anti-CD3 antibody (BD Pharmingen) for 24 h. To further propagate the virus produced by the SHIV-infected $CD4^+$ T cells, anti-CD3-stimulated, CD8-depleted PBMCs from healthy HIV-negative donors were added to the culture on days 1 and 7 followed by periodic removal of cell suspensions and replenishment of culture medium. After up to 21 days of incubation periods, wells positive for SHIV-AD8 were identified by SIV p27 Antigen Capture Assay (Advanced Bioscience Laboratories) and infectious units per million cells were determined by a maximum likelihood method[35].

$CD8^+$ T lymphocyte depletion *in vivo*. Six controller animals were injected subcutaneously with anti-CD8 mAbα M-T807R1 (National Institutes of Health Nonhuman Primate Reagent Resource Program) (10 mg kg^{-1}) on day 0, and intravenously (5 mg kg^{-1}) on days 3, 7, and 10 (ref. 36). Three of the six controller animals were also injected intravenously with anti-CD8β mAb CD8b255R1 (National Institutes of Health Nonhuman Primate Reagent Resource Program) (50 mg kg^{-1}).

Data availability statement. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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32. Hansen, S. G. *et al.* Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* **473,** 523–527 (2011).
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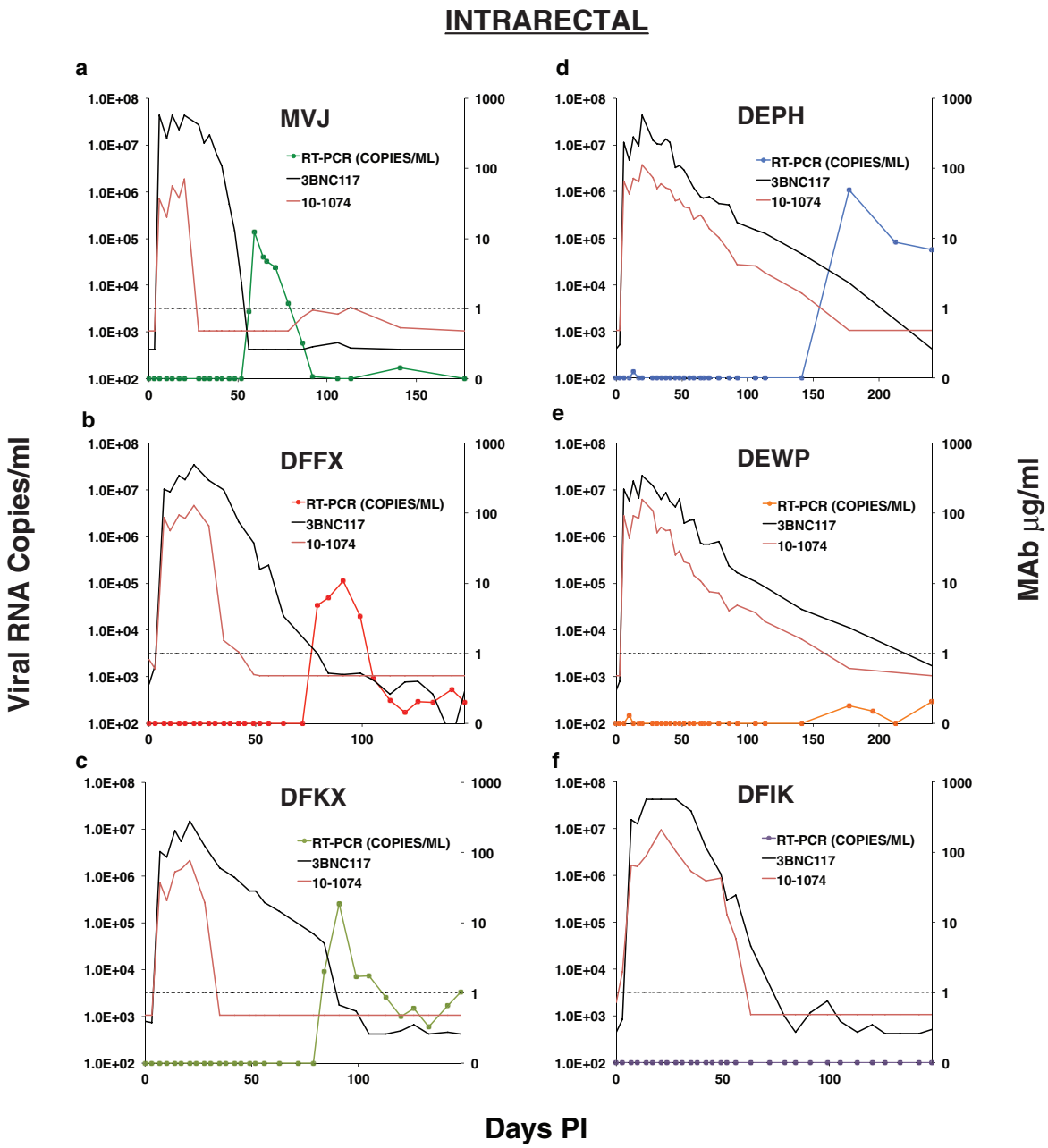
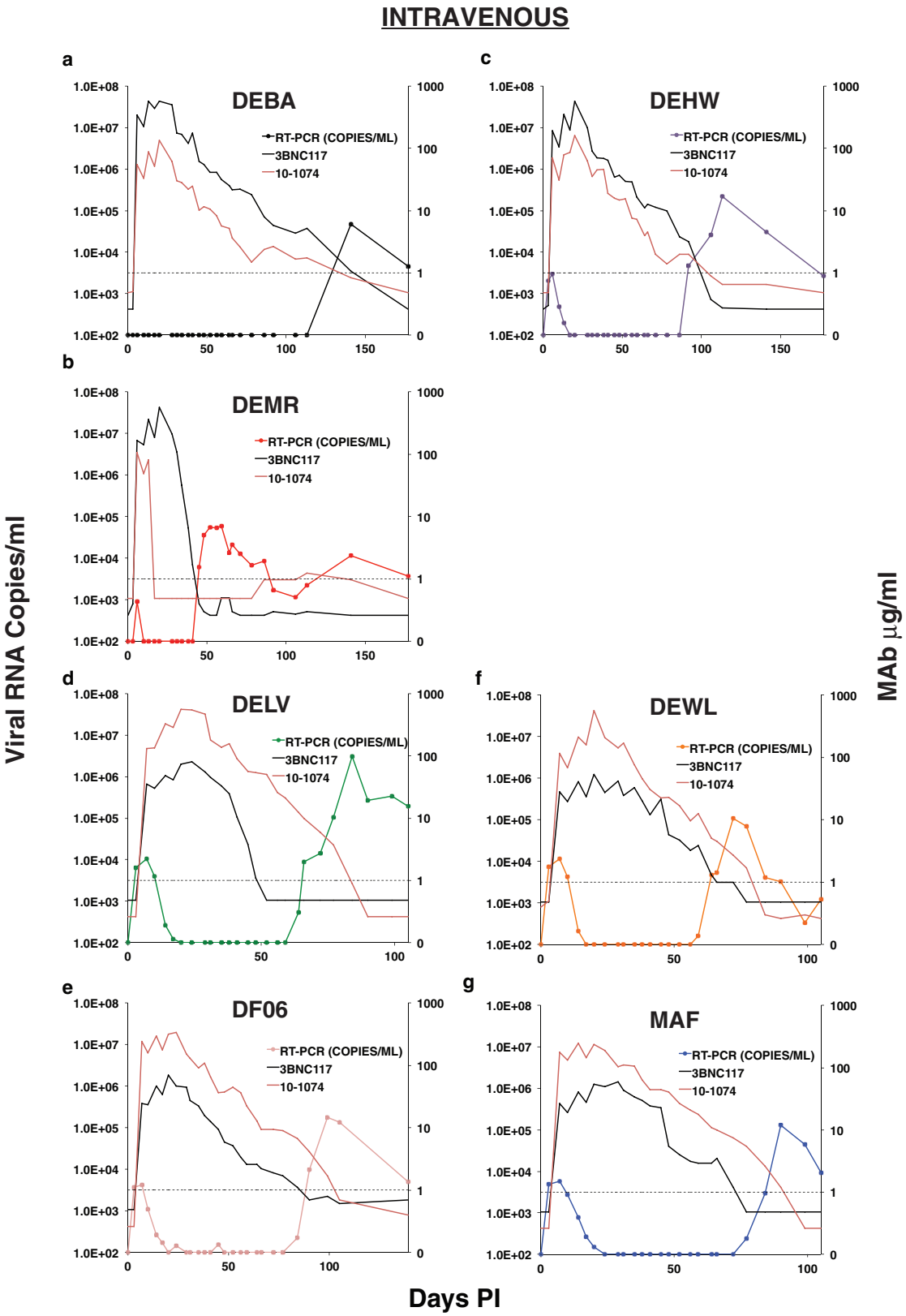
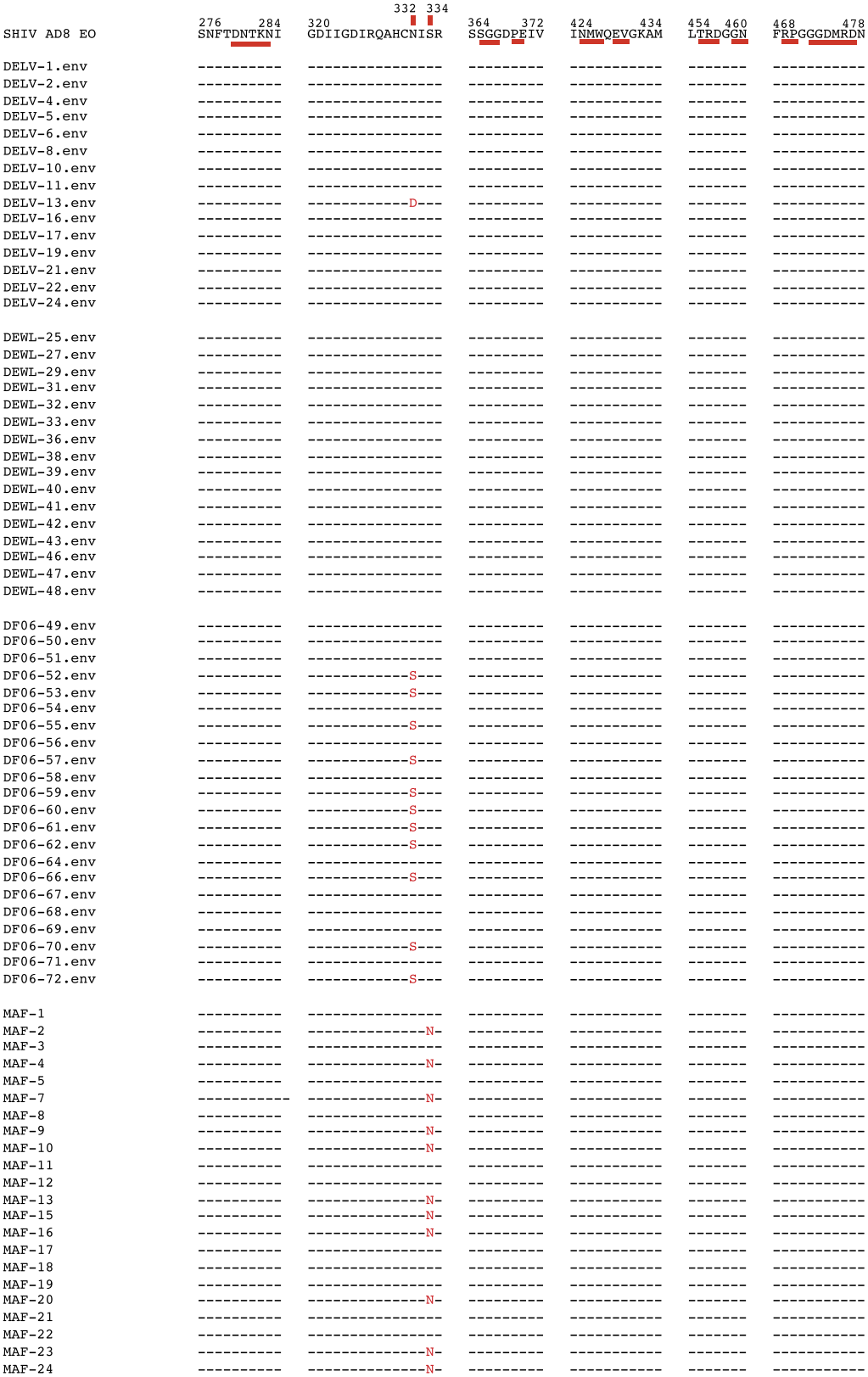


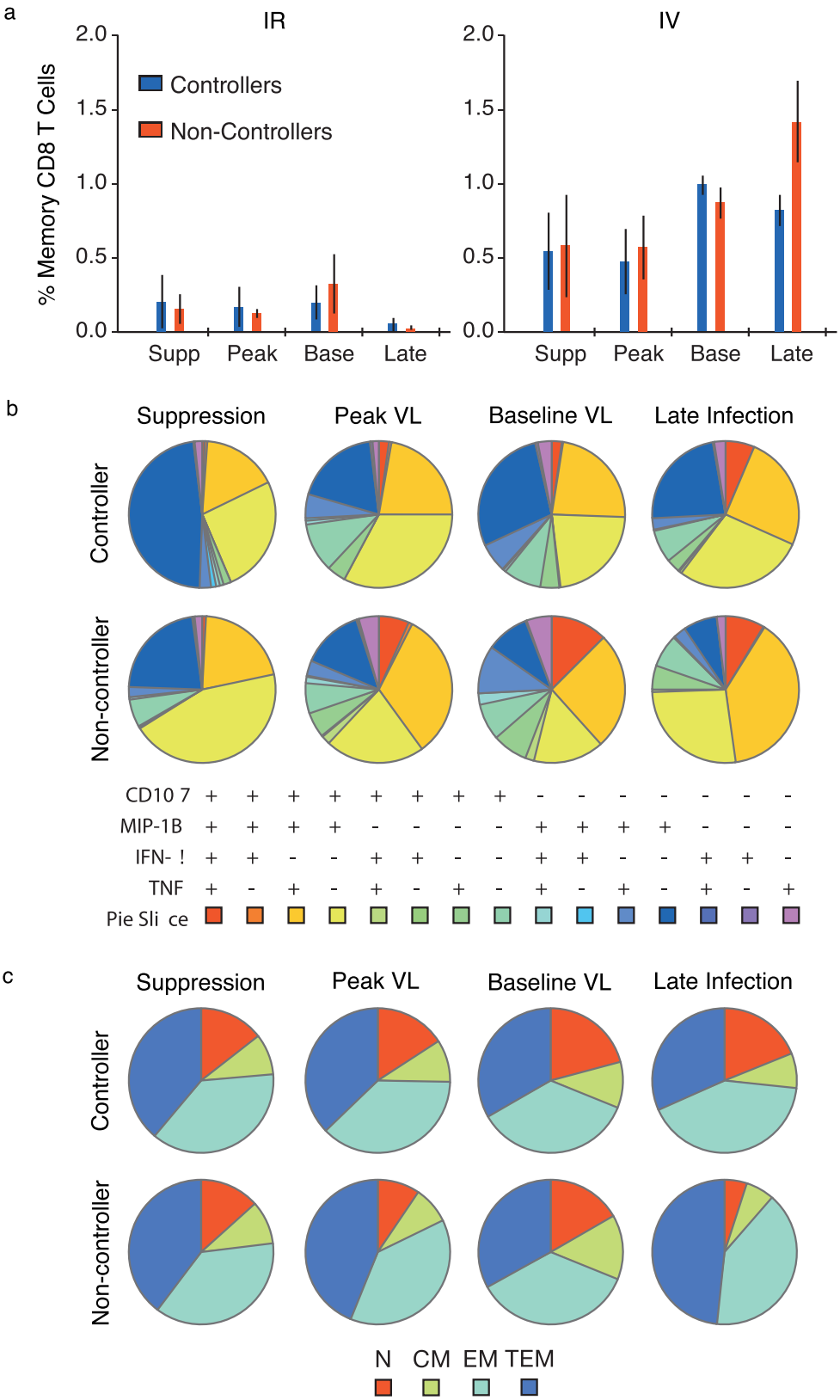
Extended Data Figure 1 | Sustained suppression of virus replication by a 2-week course of combination bNAb treatment after intrarectal $SHIV_{AD8\text{-}EO}$ challenge. Plasma viral RNA levels and 10-1074 or 3BNC117 mAb concentrations after bNAb therapy beginning on day 3 after intrarectal challenge are shown ($n = 6$).



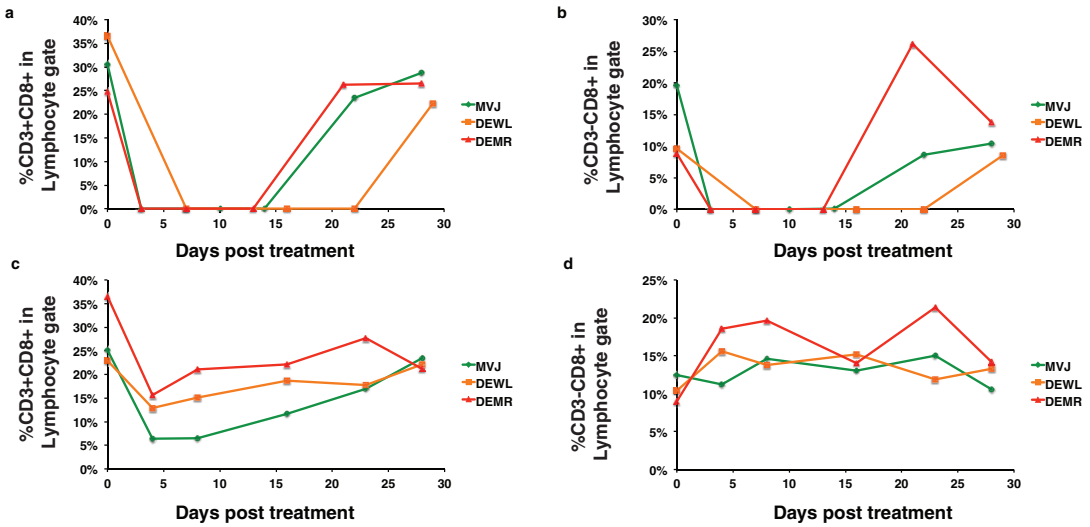
Extended Data Figure 2 | Sustained suppression of virus replication by a 2-week course of combination bNAb treatment after intravenous $SHIV_{AD8\text{-}EO}$ challenge. Plasma viral RNA levels and 10-1074 or 3BNC117 mAb concentrations after bNAb therapy beginning on day 3 after intravenous challenge are shown ($n = 7$).



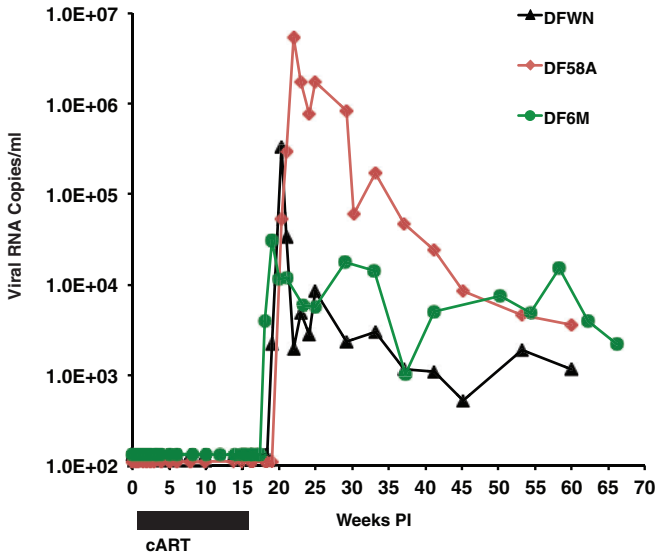
Extended Data Figure 3 | Analyses of selected SHIV$_{AD8}$ gp120 sequences known to confer resistance to 10-1074 or 3BNC117 mAbs present in virus rebounding after immunotherapy. Nucleotide sequences present in amplicons, obtained from animals DELV (day 84 after infection), DEWL (day 72 after infection), DF06 (day 99 after infection), or MAF (day 90 after infection) during virus rebound (shown in Extended Data Fig. 2d–g), were evaluated. SHIV$_{AD8\text{-}EO}$ gp120 sequences are shown at the top. Mutations conferring resistance to 10-1074 (vertical bars) and 3BNC117 (horizontal bars) are highlighted.



Extended Data Figure 4 | CD8$^+$ T-cell responses and memory phenotype. a, Memory CD8$^+$ T-cell responses to SIVmac239 Gag measured by intracellular cytokine staining as a sum of IFN-γ, IL-2, TNF, CD107a, MIP-1B, and IL-10. Supp, during bNAb-mediated viral suppression; Peak, during peak of virus rebound; Base, after resolution of rebound; Late, during late stage of virus infection; coloured bars, mean; whiskers, s.e.m. **b**, Polyfunctionality of the CD8$^+$ T-cell responses in macaques inoculated by the intravenous route. **c**, CD8$^+$ T-cell memory subsets in macaques inoculated by the intravenous route. N, naive; CM, central memory; EM, effector memory; TEM, terminal effector memory.



Extended Data Figure 5 | FACS analyses of CD8$^+$ cells in controller animals after administration of depleting anti-CD8 mAbs. CD8$^+$ lymphocytes were collected from controller monkeys MVJ, DEWL, and DEMR after infusion of the MT807R1anti-CD8α mAb (**a**, **b**) or the CD8b255R1 anti-CD8β mAb (**c**, **d**) and the CD3$^+$CD8$^+$ and CD3$^-$CD8$^+$ fractions determined.



Extended Data Figure 6 | Administration of cART during the acute $SHIV_{AD8\text{-}EO}$ infection of macaques does not result in controller status. cART (tenofovir, emtricitabine, and raltagravir) was administered daily to three animals for 15 weeks beginning on day 3 after intrarectal challenge with 1,000 $TCID_{50}$ of $SHIV_{AD8\text{-}EO}$.

Extended Data Table 1 | Plasma viral RNA levels in macaques by ultrasensitive RT–PCR assay

Animal	Days post infection	Plasma viral RNA (copies/ml)
DEPH	10	10
	13	5
	34	<2
	48	<2
	106	<2
	141	10
DEWP	10	5
	13	3
	34	<2
	48	<2
	106	<2
	113	<2
	141	10
	177	260
	212	50
MVJ	10	213
	13	20
	34	<2
	48	<2
	106	30
	113	160
	141	30
	177	10
DFFX	7	<2
	14	<2
DFKX	7	<2
	14	<2
DFIK	7	<2
	14	<2

Extended Data Table 2 | Circulating $CD4^+$ T-cell frequency in controller monkeys capable of releasing replication-competent $SHIV_{AD8\text{-}EO}$ by virus outgrowth assay

Animal ID	Route of Virus Inoculation	Infectious SHIV (per 10^6 $CD4^+$T cells)		
		Pre-CD8 depletion	Post-CD8 depletion	
			Viremic Phase	Controlled Phase
DEWP	IR	0.167	747	<0.100
MVJ	IR	0.566	2503	<0.100
DFIK	IR	<0.112	ND	ND
DEMR	IV	<0.100	ND	0.519
DEWL	IV	<0.100	ND	<0.065
MAF	IV	0.424	ND	0.2245