LETTER

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# Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia

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**Neutralizing antibodies can confer immunity to primate lentiviruses by blocking infection in macaque models of AIDS[1–4]. However, earlier studies of anti-human immunodeficiency virus type 1 (HIV-1) neutralizing antibodies administered to infected individuals or humanized mice reported poor control of virus replication and the rapid emergence of resistant variants[5–7]. A new generation of anti-HIV-1 monoclonal antibodies, possessing extraordinary potency and breadth of neutralizing activity, has recently been isolated from infected individuals[8]. These neutralizing antibodies target different regions of the HIV-1 envelope glycoprotein including the CD4-binding site, glycans located in the V1/V2, V3 and V4 regions, and the membrane proximal external region of gp41 (refs 9–14). Here we have examined two of the new antibodies, directed to the CD4-binding site and the V3 region (3BNC117 and 10-1074, respectively), for their ability to block infection and suppress viraemia in macaques infected with the R5 tropic simian–human immunodeficiency virus (SHIV)-AD8, which emulates many of the pathogenic and immunogenic properties of HIV-1 during infections of rhesus macaques[15,16]. Either antibody alone can potently block virus acquisition. When administered individually to recently infected macaques, the 10-1074 antibody caused a rapid decline in virus load to undetectable levels for 4–7 days, followed by virus rebound during which neutralization-resistant variants became detectable. When administered together, a single treatment rapidly suppressed plasma viraemia for 3–5 weeks in some long-term chronically SHIV-infected animals with low $CD4^+$ T-cell levels. A second cycle of anti-HIV-1 monoclonal antibody therapy, administered to two previously treated animals, successfully controlled virus rebound. These results indicate that immunotherapy or a combination of immunotherapy plus conventional antiretroviral drugs might be useful as a treatment for chronically HIV-1-infected individuals experiencing immune dysfunction.**

SHIV-AD8 was selected as challenge virus for this study because several clinical features observed during infections of macaques were similar to those reported in HIV-1-infected individuals. SHIV-AD8 consistently establishes sustained set-point viraemia in macaques inoculated by the intravenous or intrarectal routes and causes unrelenting depletion of $CD4^+$ T lymphocytes[15,16]. During the acute infection, SHIV-AD8 targets memory $CD4^+$ T cells in blood and at effector sites in tissues. Their gradual depletion is subsequently followed by the loss of the naive $CD4^+$ T lymphocyte subset. The latter heralds the onset of symptomatic immunodeficiency in macaques characterized by the development of opportunistic infections (*Mycobacterium*, *Pneumocystis*, *Cryptosporidium* species), lymphomas, marked weight loss, and death within 2–4 years of virus inoculation. In addition, SHIV-AD8-infected macaques generate cross-reactive antibodies, capable of neutralizing tier 1 and tier 2 HIV-1 isolates, including one 'elite neutralizer' macaque producing potent cross-clade neutralizing activity[8,17,18]. As is frequently the case for HIV-1 elite neutralizers, resistant variants emerged in this SHIV-AD8-infected animal, which succumbed to AIDS at week 117 after infection[19].

The neutralization sensitivities of SHIV-AD8EO (ref. 17)—a molecularly cloned derivative of SHIV-AD8 (ref. 16)—to the 10-1074 (ref. 20), 3BNC117 (ref. 21) and VRC01 (ref. 22) monoclonal antibodies were measured in the TZM-bl cell assay (Fig. 1a). The half-maximum inhibitory concentration ($IC_{50}$) values determined for 10-1074, 3BNC117 and VRC01 against SHIV-AD8EO were 0.20 $\mu g\,ml^{-1}$, 0.14 $\mu g\,ml^{-1}$ and 0.63 $\mu g\,ml^{-1}$, respectively, indicating that the 10-1074 and 3BNC117 monoclonal antibodies had similar activities and either one was more potent than VRC01 against SHIV-AD8EO *in vitro*.

A more critical test of neutralization efficacy is the prevention of virus acquisition *in vivo* after passive transfer of neutralizing antibodies and subsequent virus challenge. This was examined by administering the 10-1074 or 3BNC117 monoclonal antibodies 24 h before an intrarectal challenge of Indian origin rhesus macaques with 1,000 tissue infectious $dose_{50}$ ($TCID_{50}$) (approximately 3 animal infectious $doses_{50}$ ($AID_{50}$)[15]), an inoculum size we have previously determined is sufficient to establish SHIV-AD8EO infections *in vivo* after a single inoculation by this route in 10 of 10 macaques. As shown in Fig. 1b, the transfer of the 10-1074 monoclonal antibody at a dose of 20 $mg\,kg^{-1}$ or 5 $mg\,kg^{-1}$ to macaques prevented virus acquisition in 2 of 2 and 2 of 2 macaques, respectively. The administration of 1 $mg\,kg^{-1}$ of 10-1074, however, failed to protect either of two animals. For the 3BNC117 passive transfer, the monoclonal antibody *in vivo* titration was initiated at a dose of 5 $mg\,kg^{-1}$, which blocked virus acquisition in 2 of 2 macaques (Fig. 1c). In contrast, 2 of 2 macaques became infected when the dose of 3BNC117 was reduced to 1 $mg\,kg^{-1}$. The plasma concentrations at the time of challenge for both monoclonal antibodies were comparable (approximately100 $\mu g\,ml^{-1}$) in the four macaques treated with a dose of 5 $mg\,kg^{-1}$.

Recent studies have reported that combinations of three or more of the newly cloned broad and potent antibodies effectively suppressed HIV-1 viraemia in humanized mice[10,23,24]. However, humanized mice carry a lower viral load, and do not have an intact adaptive or innate immune system. We have also previously reported that in the presence of neutralizing antibody, the clearance of circulating HIV-1 *in vivo* is accelerated from approximately 20 min to 3 or 4 min[25]. The potential therapeutic benefit of anti-HIV-1 neutralizing antibodies controlling virus replication in infected macaques was evaluated by administering either 10-1074 or 3BN117 alone (monotherapy) to two animals, 12 weeks after virus inoculation, when post-peak set-point viraemia levels had been established. On the basis of the pre-exposure prevention experiments

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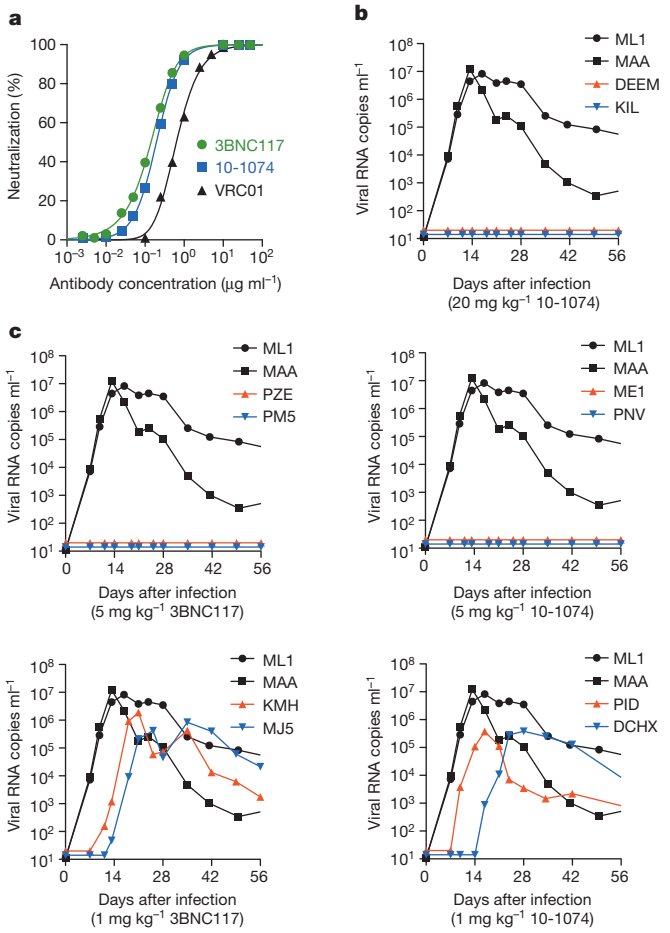


**Figure 1 | HIV monoclonal antibodies block SHIV acquisition.** **a**, Neutralization of SHIV-AD8EO by three anti-HIV-1 monoclonal antibodies assayed in TZM-bl cells. **b**, Pre-exposure passive transfer of 10-1074 monoclonal antibody to macaques followed by SHIV-AD8EO intrarectal challenge (20 mg kg$^{-1}$ (top), 5 mg kg$^{-1}$ (middle) and 1 mg kg$^{-1}$ (bottom)). **c**, Pre-exposure passive transfer of 3BNC117 monoclonal antibody to macaques followed by SHIV-AD8EO intrarectal challenge. Macaques ML1 and MAA received 20 mg kg$^{-1}$ of control anti-dengue virus NS1 IgG1 monoclonal antibody.

described above, infected macaques were treated with either antibody at a dose of 10 mg kg$^{-1}$ (Table 1). Both recipients of the 3BNC117 monoclonal antibody experienced rapid declines of plasma viraemia to background levels at day 10 after the start of treatment (Fig. 2a). Virus rebound became detectable on day 20 in these two macaques. The 10-1074-treated animal MCN sustained an unexpectedly rapid and a greater than $10^3$ reduction of plasma viral loads to undetectable levels by day 6 of treatment initiation. However, the effects of 10-1074 monoclonal antibody administration were more variable and of short duration (4 days) (Fig. 2a and Extended Data Fig. 1). Furthermore, single genome amplification (SGA) analysis of virus in the four monotherapy recipients revealed that the rebound virus present in both of the 10-1074-treated macaques had sustained changes that eliminated the gp120 Asn 332 glycan, rendering the virus resistant to this antibody (Extended Data Fig. 2)[10].

The known failure of antiretroviral drug monotherapy to durably control HIV-1 and SIV replication *in vivo* prompted us to co-administer 3BNC117 and 10-1074 monoclonal antibodies, each at a dose of 10 mg kg$^{-1}$, to chronically SHIV-AD8EO-infected macaques. In addition, the capacity of the molecularly cloned SHIV-AD8EO to cause unrelenting CD4$^+$ T-cell depletion and symptomatic immunodeficiency provided the opportunity to assess the potential therapeutic effects of combination anti-HIV-1 neutralizing monoclonal antibodies in two groups of infected macaques experiencing the pathogenic effects of SHIV-AD8EO infection. The first group consisted of two clinically asymptomatic animals (DBZ3 and DC99A) that had been infected for 159 weeks and had sustained similar and significant declines of circulating and bronchoalveolar lavage (BAL) CD4$^+$ T cells (Table 1 and Extended Data Figs 3 and 4). At the time of monoclonal antibody administration, the plasma viral loads in macaques DBZ3 and DC99A were $1.08 \times 10^4$ and $7.6 \times 10^3$ RNA copies ml$^{-1}$, respectively. Both macaques responded to combination anti-HIV-1 monoclonal antibody treatment with immediate and rapid reductions of plasma viraemia to undetectable levels within 7–10 days (Fig. 2b, c). Suppression of measurable SHIV-AD8EO in the plasma of macaques DBZ3 and DC99A, after a single administration of the two monoclonal antibodies, lasted 18 and 36 days, respectively, exceeding the 4–7 day window of virus suppression observed during monotherapy. In each case, plasma viraemia rebounded to pre-treatment levels. A second cycle of combination immunotherapy was administered to macaques DBZ3 and DC99A to determine whether the re-emergence of detectable plasma viraemia simply reflected insufficient levels of circulating antibodies in these animals. As shown in the right panels of Figs 2b, c, the viral loads in each animal became undetectable by day 7 of the second treatment cycle. Viraemia was suppressed for 4 days in macaque DBZ3 and 28 days in macaque DC99A. SGA analysis demonstrated that the rebound virus that emerged in macaque DC99A, after the second round of immunotherapy, carried a gp120 Asn332Asp mutation, indicating that resistance to the 10-1074 component of the antibody combination had occurred (Extended Data Fig. 5b).

Combination monoclonal antibody therapy was also evaluated in a second group of three chronically infected animals (DBX3, DCF1 and DCM8), which had also been infected for more than 3 years but were clinically symptomatic, experiencing intermittent diarrhoea and or anorexia (Table 1). At the time of monoclonal antibody administration, the level of circulating CD4$^+$ T cells in one of these macaques (DCM8) was only 43 cells μl$^{-1}$ and was higher in animals DCF1 (105 cells μl$^{-1}$) and DBXE (158 cells μl$^{-1}$). Plasma viral loads exceeded $10^5$ RNA copies ml$^{-1}$ in animals DBXE and DCF1 and were significantly lower ($1.59 \times 10^3$ RNA copies ml$^{-1}$) in macaque DCM8. As shown in Fig. 2d–f and Extended Data Fig. 3, the administration of

**Table 1 | SHIV-AD8-infected macaques treated with anti-HIV monoclonal antibodies**

| Animal | Pre-infection (CD4$^+$ T cells (cells μl$^{-1}$)) | Treatment initiation (weeks after infection) | Pre-monoclonal antibody treatment | | Clinical status | Monoclonal antibody administered | |
|---|---|---|---|---|---|---|---|
| | | | CD4$^+$ T cells (cells μl$^{-1}$) | Viral load (RNA copies ml$^{-1}$) | | 3BNC117 (mg kg$^{-1}$) | 10-1074 (mg kg$^{-1}$) |
| KZ6 | 881 | 12 | 1,447 | $5.22 \times 10^3$ | Post-acute set point | 10 | 0 |
| MB6 | 545 | 12 | 1,598 | $7.43 \times 10^3$ | Post-acute set point | 10 | 0 |
| MB8 | 1,512 | 12 | 2,625 | $1.14 \times 10^4$ | Post-acute set point | 0 | 10 |
| MCN | 210 | 12 | 599 | $2.14 \times 10^4$ | Post-acute set point | 0 | 10 |
| DBZ3 | 650 | 159 | 118 | $1.08 \times 10^4$ | Asymptomatic | 10 | 10 |
| DC99A | 623 | 159 | 165 | $7.60 \times 10^3$ | Asymptomatic | 10 | 10 |
| DBXE | 1,585 | 163 | 158 | $1.96 \times 10^5$ | Intermittent diarrhoea | 10 | 10 |
| DCF1 | 1,203 | 157 | 105 | $1.44 \times 10^5$ | Intermittent diarrhoea | 10 | 10 |
| DCM8 | 608 | 163 | 43 | $1.59 \times 10^3$ | Intermittent diarrhoea | 10 | 10 |

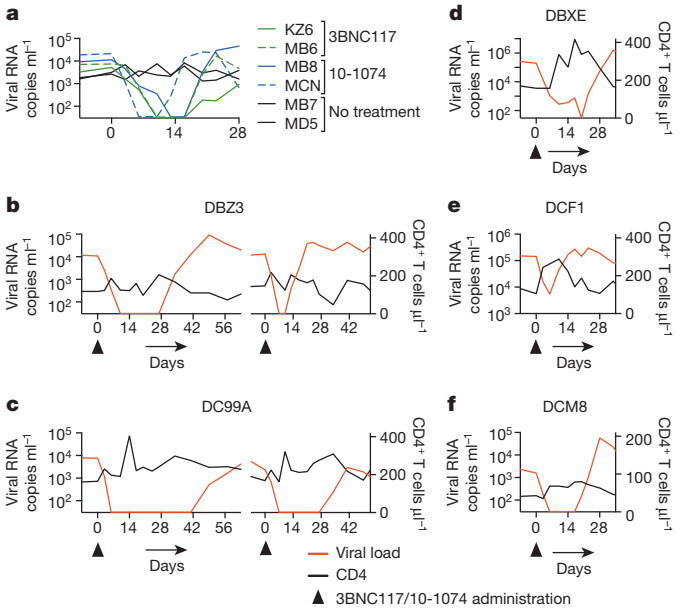


**Figure 2 | Suppression of plasma viraemia after monotherapy or combination anti-HIV-1 neutralizing antibody treatment.** **a**, Plasma viral loads in post-acute set-point SHIV-infected rhesus macaques with/without single monoclonal antibody treatment. **b**–**f**, Plasma viral loads and total $CD4^+$ T-cell numbers in chronically SHIV-infected rhesus macaques (DBZ3 (**b**), DC99A (**c**), DBXE (**d**), DCF1 (**e**) and DCM8 (**f**)) after combination monoclonal antibody treatment.

the two monoclonal antibodies to macaque DBXE resulted in reduction of viraemia from $2.0 \times 10^5$ RNA copies at day 0 to undetectable levels in plasma at day 20. This was followed, within a few days, by a resurgence of high levels of circulating virus in DBXE. Macaque DCM8, with more modest plasma virus loads and very low numbers of circulating $CD4^+$ T cells, experienced a rapid decline of viraemia to undetectable levels between days 6 and 17 after the initiation of monoclonal antibody treatment. Finally, animal DCF1, previously reported to have generated broadly reacting anti-HIV-1 neutralizing antibodies[17], exhibited a transient and a comparatively modest 27-fold reduction of plasma viraemia by day 6 in response to combination monoclonal antibody therapy, before the viral loads returned to high pre-treatment levels.

SGA analysis of emerging virus populations in the five long-term chronically infected macaques revealed no changes affecting sensitivity to either 10-1074 or 3BNC117 except for the rebounding virus after the second treatment cycle of macaque DC99A noted above (Extended Data Fig. 5).

Because some of the recipients of combination antibody treatment had prolonged control of detectable plasma viraemia, peripheral blood mononuclear cell (PBMC)-associated viral RNA and DNA levels were evaluated before and after monoclonal antibody administration. As shown in Extended Data Table 1, monoclonal antibody treatment resulted in reduced levels of cell-associated viral RNA in each animal contemporaneous with the suppression of its plasma viraemia. No consistent change in cell-associated viral DNA levels occurred as a result of antibody treatment.

Co-administration of neutralizing monoclonal antibodies to chronically SHIV-AD8EO-infected macaques resulted in a transient elevation of circulating $CD4^+$ T-cell levels, particularly in late-stage animals with very high viral loads. The $CD4^+$ T-cell numbers in macaques DBXE and DCF1 increased 2–3-fold during the period of monoclonal-antibody-mediated virus suppression, but gradually declined to pre-treatment levels as viraemia again became detectable (Extended Data Fig. 6). The corresponding elevations and declines of both the naive and memory $CD4^+$ T-cell subsets in animals DBXE and DCF1 would be consistent with an antibody-mediated tissue redistribution mechanism, given that the R5 tropic SHIV-AD8EO exclusively targets memory, not naïve, $CD4^+$ T lymphocytes. The levels of memory $CD4^+$ T cells at an effector site (from BAL) did not change appreciably during immunotherapy (Extended Data Fig. 4).

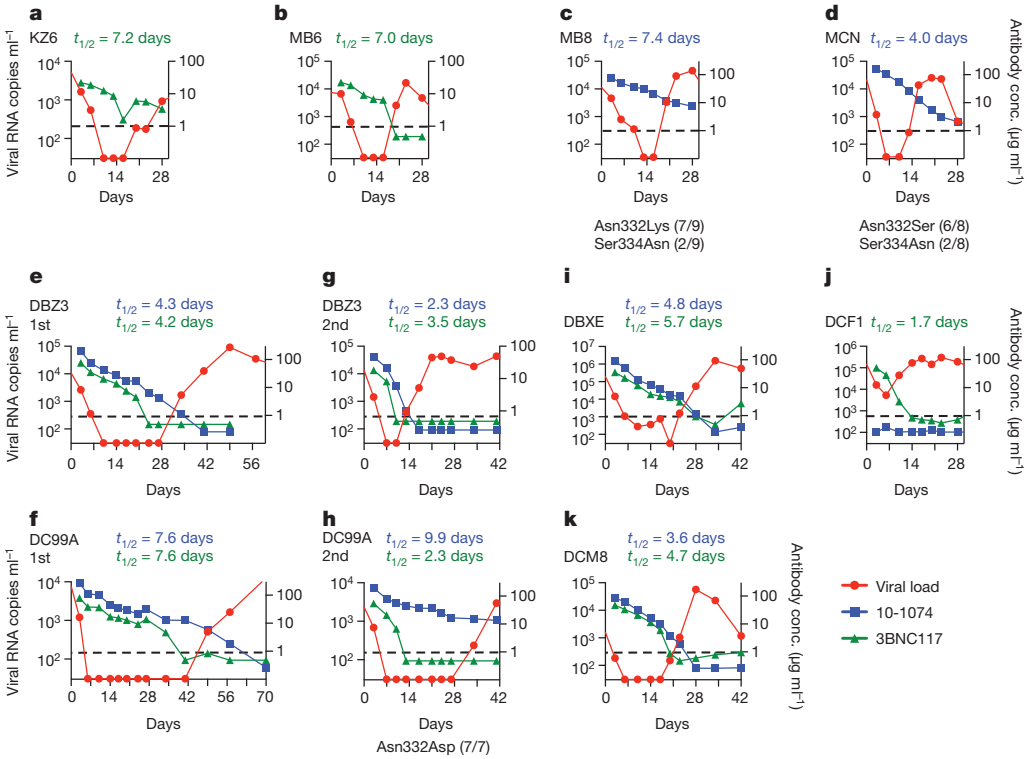


**Figure 3 | Plasma viraemia rebounds in SHIV-infected macaques when neutralizing antibody levels decline.** **a**–**k**, Viral RNA levels and concentrations of 10-1074 or 3BNC117 monoclonal antibodies in plasma at various times after initiation of single or combination antibody treatment. In panels **c**, **d**, **h**, the gp120 changes present in the rebound virus populations and their frequencies are indicated at the bottom.

Plasma concentrations of each monoclonal antibody were determined by measuring the neutralizing activity in plasma against selected HIV-1 pseudovirus strains sensitive to one or the other, but not to both, antibodies (Extended Data Fig. 7). In treated animals, suppression of SHIV-AD8EO viraemia was maintained until a threshold plasma monoclonal antibody concentration of approximately 5 $\mu g\,ml^{-1}$ was reached, except when escape variants emerged in animals MB8, MCN and the second cycle of treatment in macaque DC99A (Fig. 3 and Extended Data Fig. 8). This was even the case for macaque DCF1, for which only a modest and transient reduction of plasma viral RNA levels was observed after combination therapy. Notably, the monoclonal antibodies administered to this clinically symptomatic macaque had a shortened half-life or were undetectable (Fig. 3j). In this regard, macaque DCF1 had to be euthanized on day 56 after treatment initiation and a necropsy revealed severe enteropathy, characterized by disseminated gastrointestinal cryptosporidiosis, pancreatitis and cholangitis, perhaps explaining the rapid loss of neutralizing antibodies in this animal.

The decay rate constants and the corresponding half-lives of plasma viral RNA after monotherapy or combination neutralizing antibody treatments were calculated from the initial slopes of the HIV-1 plasma RNA decline (Extended Data Table 2). Furthermore, unlike pharmacological antiretroviral therapy (ART) in which two phases of viral decay lasting several weeks is commonly seen, treatment with neutralizing antibodies induced a single phase of exponential decline ($t_{1/2} = 1.13$ days). For the macaques (DC99A and DBZ3) that received two cycles of treatment, the decay rate constant for the second treatment was higher than that for the first treatment for one of the animals and lower for the other (Extended Data Table 2).

Our findings demonstrate that combination anti-HIV neutralizing monoclonal antibody treatment is superior to monotherapy and can rapidly and potently suppress plasma viraema in chronically SHIV-infected macaques with low $CD4^+$ T-cell levels and symptomatic disease. Animals administered a single neutralizing monoclonal antibody experienced a relatively short period of plasma viral RNA suppression, and neutralization-resistant progeny virus emerged in some macaques, attesting to the robustness of SHIV-AD8EO *in vivo*. Our results further indicate that administration of monoclonal antibodies, alone or in combination with currently available antiretroviral drug regimens[24], could be useful therapeutically, particularly for chronically infected individuals with compromised immune systems.

## METHODS SUMMARY

The neutralization activities of the broadly acting 3BNC117 (ref. 21) CD4-binding site and 10-1074 (ref. 20) gp120 Asn-332-dependent neutralizing monoclonal antibodies against SHIV-AD8EO were initially determined in the TZM-bl cell system against SHIV-AD8EO as previously described[15,17]. Their capacities to block virus acquisition or to control plasma viraemia in chronically infected animals challenged with the R5-tropic SHIV-AD8EO were assessed by monitoring plasma viral loads and cell-associated viral nucleic acids; levels of $CD4^+$ T-cell subsets were measured by flow cytometry as previously reported[16]. SGA analyses of circulating viral variants and the determination of antibody levels in plasma have been previously described[26]. Plasma concentration of neutralizing antibodies was determined by measuring neutralizing activity against HIV-1 pseudovirus preparations only susceptible to either 10-1074 or 3BNC117.

**Online Content** Any additional Methods, 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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**Author Contributions** M.S., Y.N., M.C.N. and M.A.M. designed the experiments; M.S., Y.N., F.K., H.M., O.K.D., R.P., A.B.-W. and M.P. performed the experiments; M.S., Y.N., F.K., M.P., J.D.L., D.D., M.C.N. and M.A.M. analysed the data; and M.S., Y.N., M.C.N. and M.A.M. wrote the manuscript. M.S. and Y.N. contributed equally to the work.

**Author Information** The SHIV-AD8 gp120 sequences known to confer resistance to the 10-1074 or 3BNC117 monoclonal antibodies have been deposited in GenBank/EMBL/DDBJ under accession numbers KF738375 to KF738446. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.C.N. (nussen@mail.rockefeller.edu) or M.A.M. (malm@nih.gov).

## METHODS

**Virus.** The origin and preparation of the tissue-culture-derived SHIV-AD8EO stock have been previously described[17].

**Animal experiments.** Twenty-three male and female rhesus macaques (*Macaca mulatta*) of Indian genetic origin ranging from 2 to 10 years of age were maintained in accordance with Guide for the Care and Use of Laboratory Animals Report no. NIH 85-23 (Department of Health and Human Services, Bethesda, Maryland, 1985) and were housed in a biosafety level 2 NIAID facility. Phlebotomies, euthanasia and sample collection were performed as previously described[27]. All animals were negative for the MHC class I *Mamu-A*01*, *Mamu-B*08* and *Mamu-B*17* allele.

**Antibodies.** Two monoclonal antibodies 3BNC117 (ref. 21) and 10-1074 (ref. 20) were isolated and produced as described elsewhere. DEN3, a dengue virus NS1-specific human IgG1 antibody[2], was used as the negative control antibodies in this study. All antibodies were administered intravenously.

**Quantification of viral nucleic acids.** Viral RNA levels in plasma were determined by real-time reverse transcription-PCR (ABI Prism 7900HT sequence detection system; Applied Biosystems) as previously reported[27]. Ultrasensitive measurement of cell-associated SIV RNA and DNA were determined by a nested, hybrid real-time/digital PCR assay[28].

**Neutralization assays.** The *in vitro* potency of each monoclonal antibody was assessed by TZM-bl entry assay with pseudotyped SHIV-AD8EO as previously reported[15,17].

**Lymphocyte immunophenotyping.** EDTA-treated blood samples were stained for flow cytometric analysis as previously described[16].

**Antibody concentrations and $t_{1/2}$ estimations in plasma.** Plasma concentrations of 10-1074 and 3BNC117 neutralization activity were separately determined against HIV-1 virus strains that are sensitive to one but not the other monoclonal antibody as well as not, or very weakly, sensitive to autologous antibodies in macaque plasma. All samples were heat-inactivated for 1 h at 56 °C and neutralizing activity was measured by using a TZM-bl assay as previously described[29]. The half-maximum inhibitory dose ($ID_{50}$) titres were determined by serial plasma dilutions starting at 1:20; antibody concentrations were calculated by multiplying each $ID_{50}$ value. $t_{1/2}$ of plasma monoclonal antibodies was estimated by $\ln(2)/k$.

**Single genomic analysis.** Single-genome amplification of full-length HIV-1 *env* genes was performed as reported[26].

**Determination of plasma viral RNA decay.** The kinetics of plasma HIV-1 RNA after the start of therapy was analysed as previously described[30] by a simple one-exponential model: $\ln(V) = c - kt$, where $V$ is the HIV-1 RNA concentration, and $k$ is virus decay rate constant (note that here $k$ corresponds to $k1$ (ref. 30) and $c$ = a constant close to the ln of the baseline virus concentration). The half-life $t_{1/2}$ was calculated as $0.693/k$. The data were fitted using the program Excel and the goodness of fit was estimated by the correlation coefficients, which were in the range from 0.91 to 1.0. All time points in the exponential phase of decline were used for calculation.


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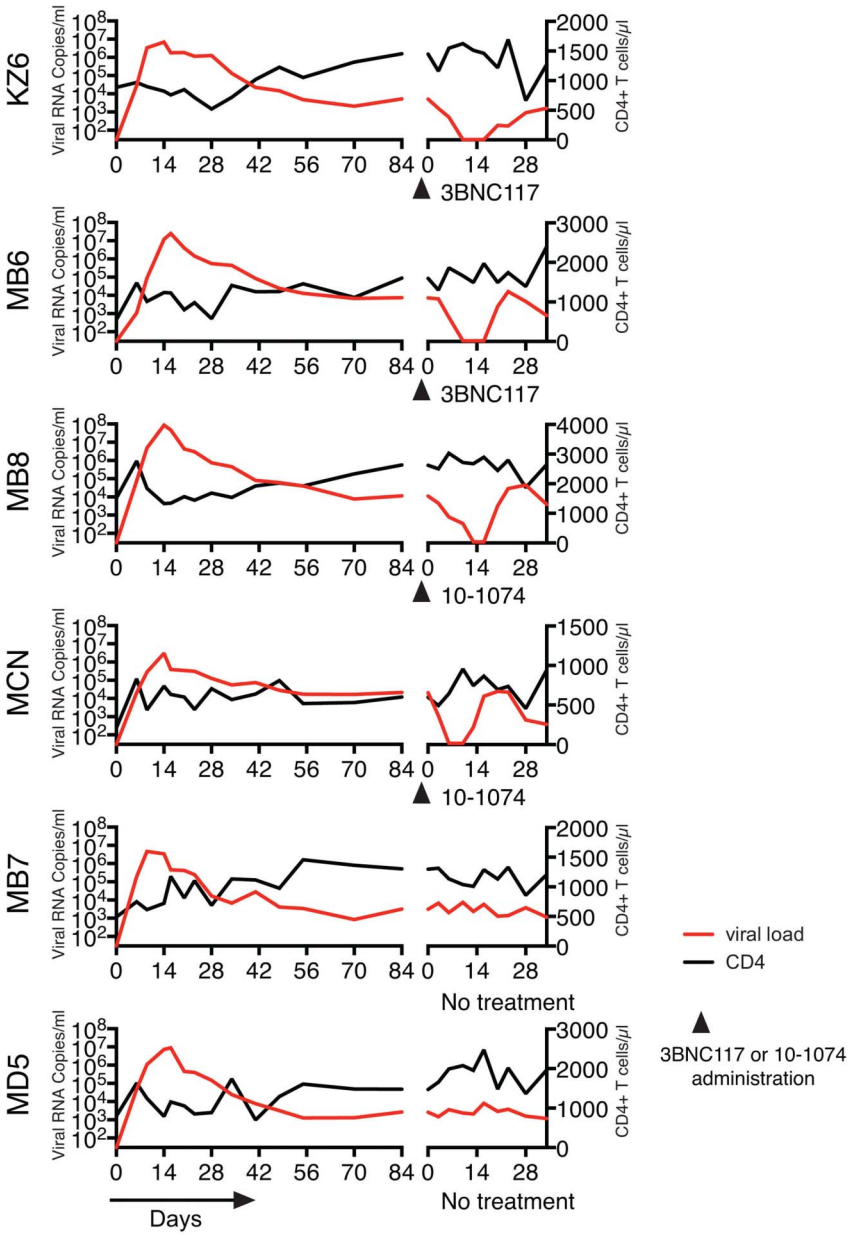
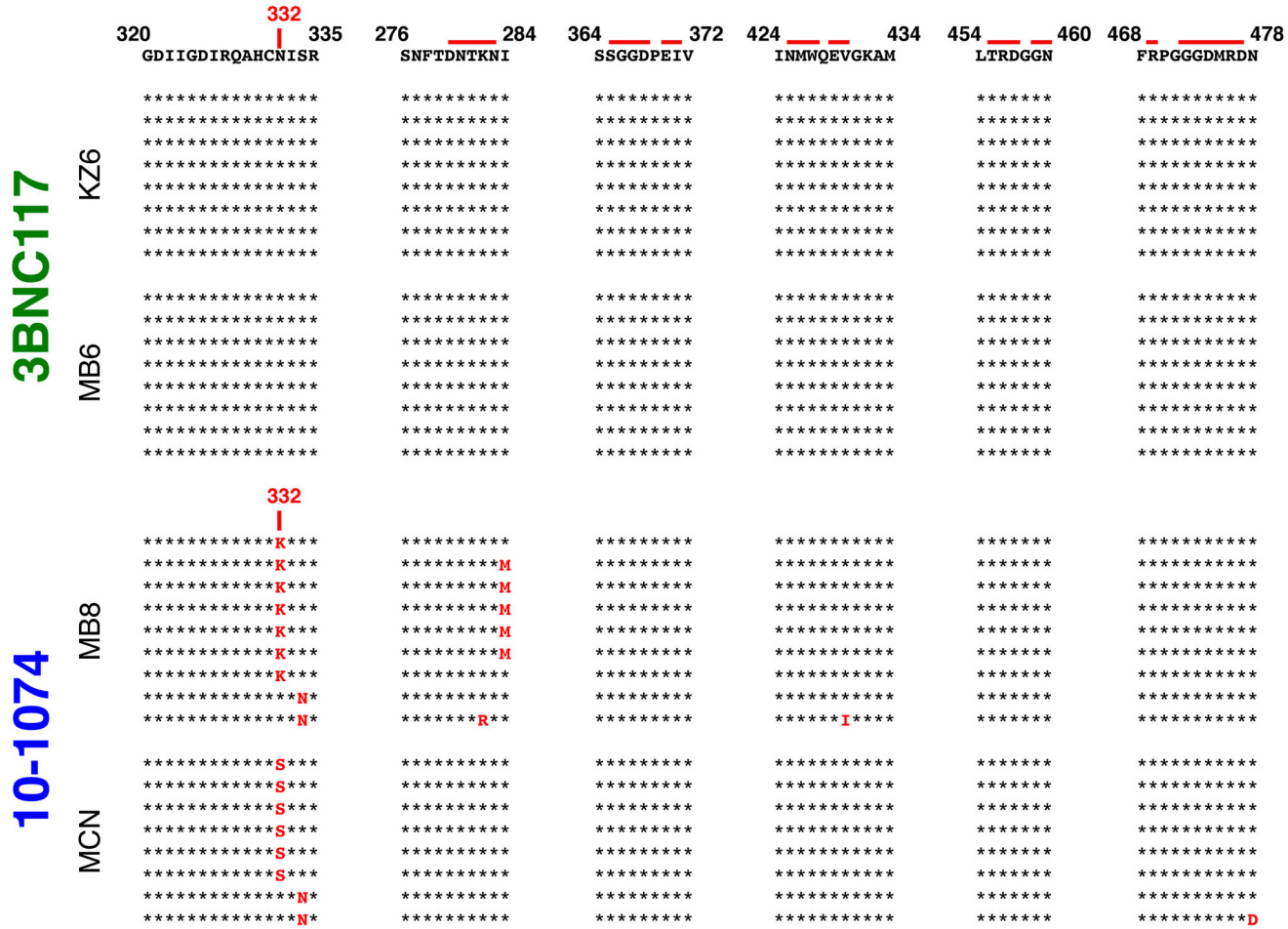
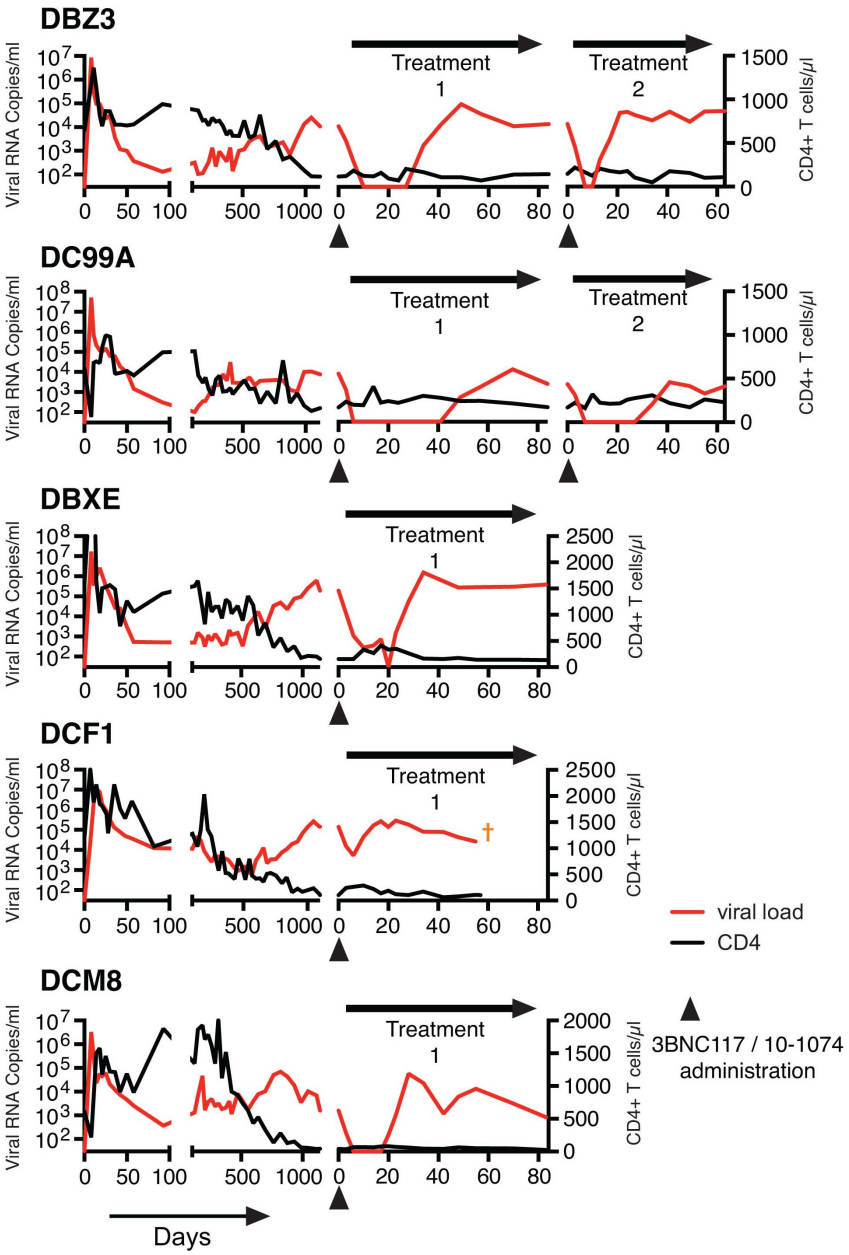
30. Polis, M. A. *et al.* Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy. *Lancet* **358,** 1760–1765 (2001).



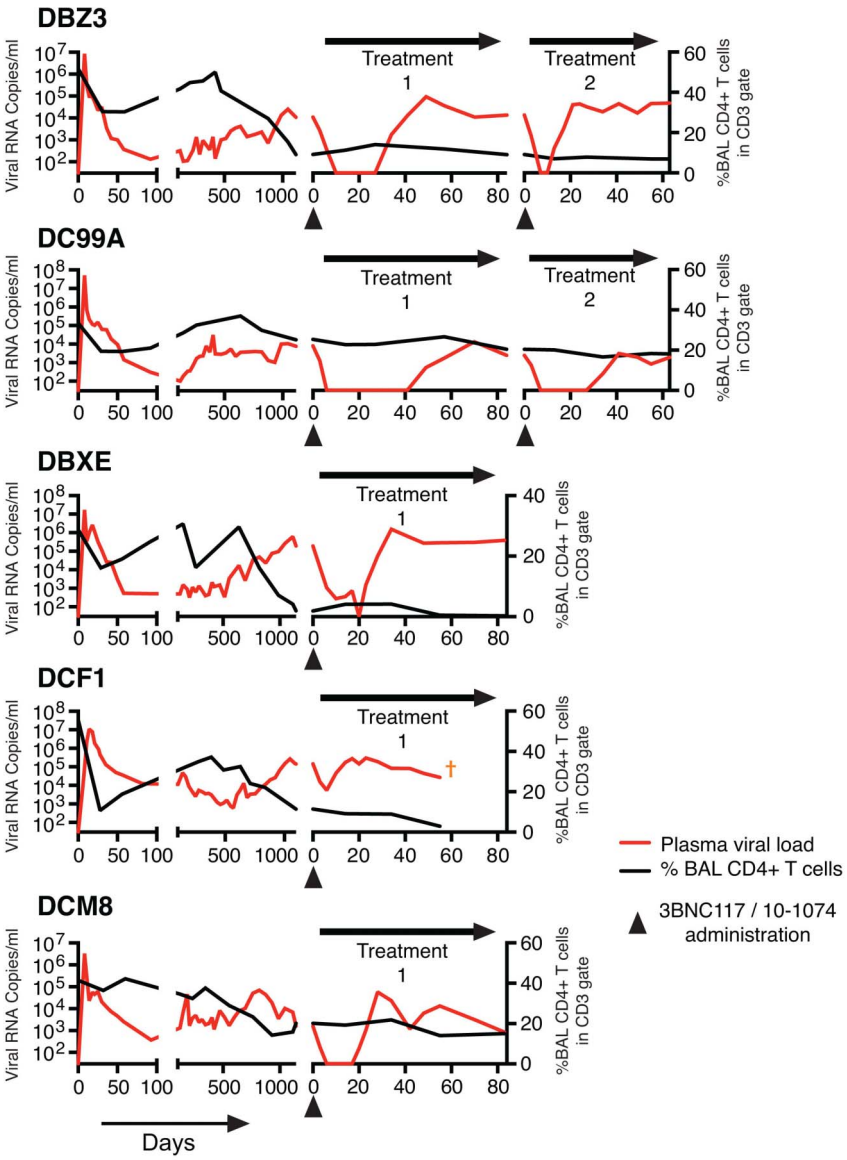

**Extended Data Figure 1 | Treatment of SHIV-infected macaques with single anti-HIV-1 neutralizing monoclonal antibodies.** Plasma viral loads and total $CD4^+$ T cell numbers before (the initial 84 days of the SHIV-AD8EO infection) and during single monoclonal antibody treatment are shown. KZ6 and MB6 received the 3BNC117 monoclonal antibody and MB8 and MCN were administered the 10-1074 monoclonal antibody. Macaques MB7 and MD5 were not treated.



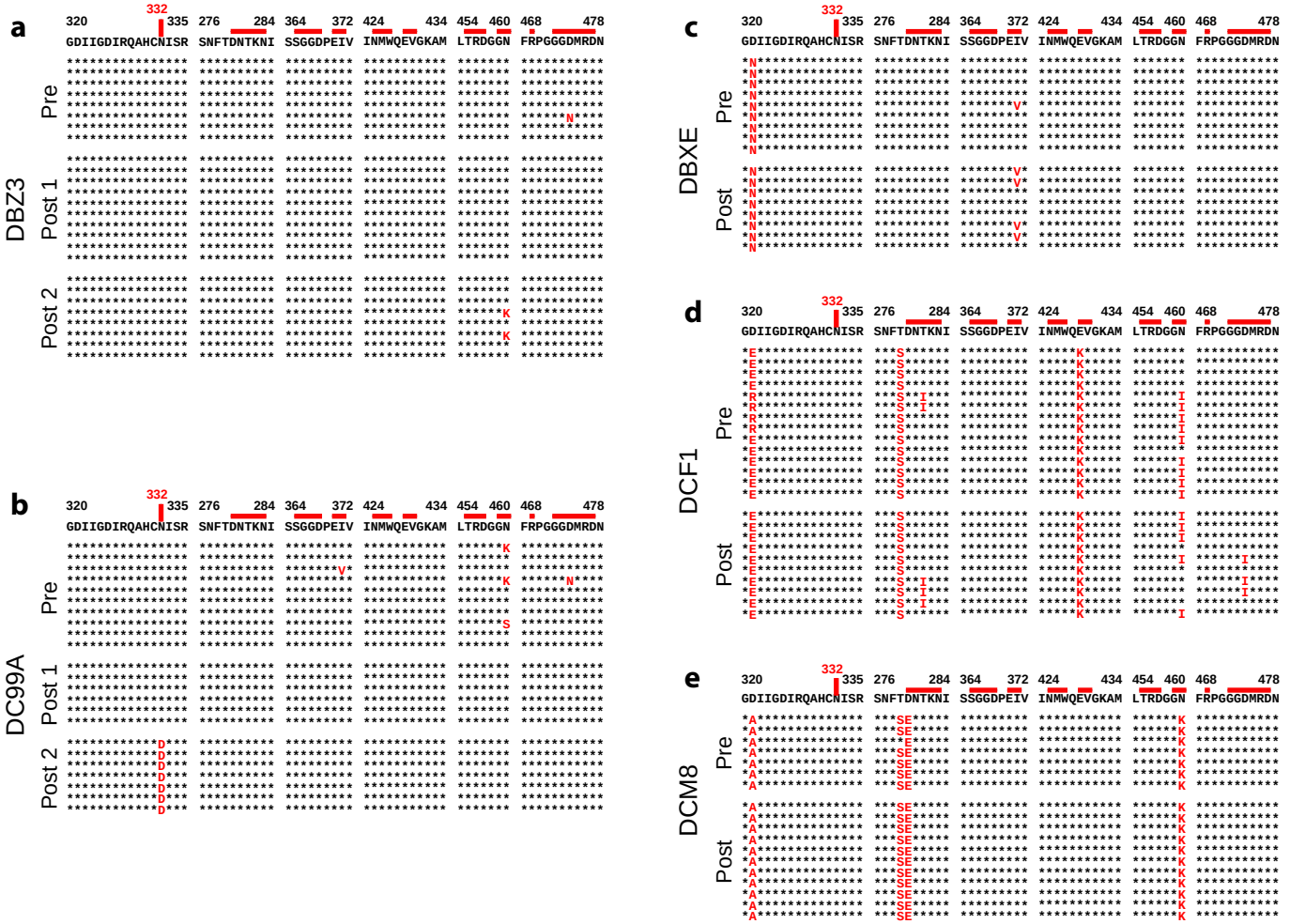

**Extended Data Figure 2 | SGA analysis of selected SHIV-AD8EO gp120 sequences, present in rebound virus after single monoclonal antibody immunotherapy, and known to confer resistance to 10-1074 or 3BNC117 monoclonal antibody.** SGA was used to amplify plasma viral RNA after monoclonal antibody treatment from the plasma of animals KZ6 (day 28 ($n = 8$)), MB6 (day 23 ($n = 8$)), MB8 (day 23 ($n = 9$)) and MCN (day 23 ($n = 8$)). The gp120 sequences at the top are present in the SHIV-AD8EO molecular clone inoculum. Mutations conferring resistance are highlighted in red.



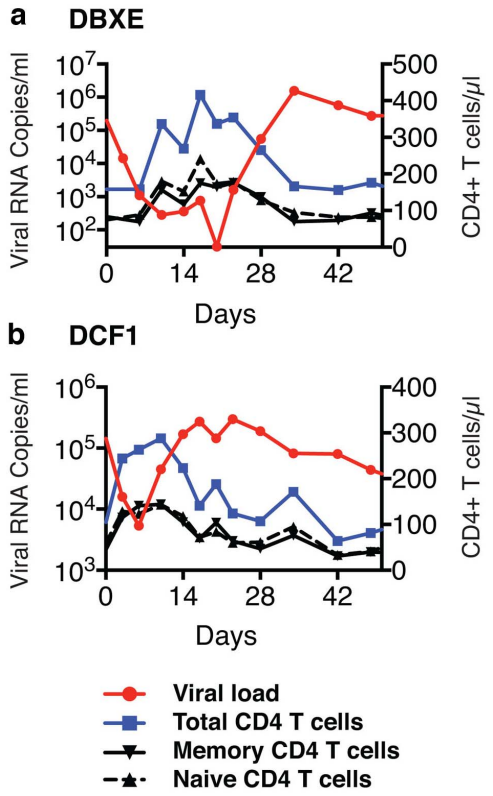

**Extended Data Figure 3 | Circulating CD4$^+$ T cells in five chronically SHIV-infected macaques treated with two anti-HIV-1 neutralizing monoclonal antibodies.** Plasma viral loads and total CD4$^+$ T-cell numbers before (first 1,100 to 1,140 days) and during the first or second cycle of combination monoclonal antibody treatment are shown.




**Extended Data Figure 4 | BAL CD4$^+$ T cells in five chronically SHIV-infected macaques treated with two anti-HIV-1 neutralizing monoclonal antibodies.** Plasma viral loads and the percentage of CD4$^+$ T cells in CD3$^+$ gated BAL specimens, before (first 1,100 to 1,140 days) and during the first or second cycle of combination monoclonal antibody treatment, are shown.




**Extended Data Figure 5 | SGA analysis of selected SHIV-AD8EO gp120 sequences known to confer resistance to 10-1074 or 3BNC117 monoclonal antibody, before and after combination immunotherapy. a–e**, Plasmas from animals DBZ3 (pre ($n = 8$); day 49 post first treatment ($n = 10$); day 24 post second treatment ($n = 8$)) (**a**), DC99A (pre ($n = 10$); day 57 post first treatment ($n = 6$); day 41 post second treatment ($n = 7$)) (**b**), DBXE (pre ($n = 9$), day 28 ($n = 8$)) (**c**), DCF1 (pre ($n = 14$), day 28 ($n = 10$)) (**d**) and DCM8 (pre ($n = 7$), day 28 ($n = 11$)) (**e**) were evaluated. The gp120 sequences at the top are present in the SHIV-AD8EO molecular clone inoculum. Mutations conferring resistance are highlighted in red.




**Extended Data Figure 6 | $CD4^+$ T-cell numbers increase during combination monoclonal antibody treatment of SHIV-AD8EO-infected macaques. a**, **b**, Levels of viral RNA and total $CD4^+$ T-cell/$CD4^+$ T-cell subsets in symptomatic chronically infected macaques DBXE (**a**) and DCF1 (**b**).

**a**

| | ID$_{50}$ (μg/ml) | |
|---|---|---|
| | 10-1074 | 3BNC117 |
| X2088_9 | 0.013 | >100 |
| Q769_d22 | >100 | 0.024 |

**b**

| | Plasma ID$_{50}$ Titer | | Plasma ID$_{50}$ converted to respected antibody concentration of 10-1074 and 3BNC117 (μg/ml) | |
|---|---|---|---|---|
| Plasma + mAb | X2088_9 | Q769_d22 | X2088_9 | Q769_d22 |
| preP_0.01_3BNC117 | <20 | <20 | <0.26 | <0.48 |
| preP_0.1_3BNC117 | <20 | <20 | <0.26 | <0.48 |
| preP_1_3BNC117 | <20 | 27 | <0.26 | 0.65 |
| preP_10_3BNC117 | <20 | 340 | <0.26 | 8.16 |
| preP_100_3BNC117 | <20 | 5,369 | <0.26 | 128.86 |
| preP_0.01_10-1074 | <20 | <20 | <0.26 | <0.48 |
| preP_0.1_10-1074 | <20 | <20 | <0.26 | <0.48 |
| preP_1_10-1074 | 54 | <20 | 0.70 | <0.48 |
| preP_10_10-1074 | 516 | <20 | 6.71 | <0.48 |
| preP_100_10-1074 | 11,419 | <20 | 148.45 | <0.48 |

preT - pre-treatment plasma of differerent NHPs (DBZ3, DC99A, DCM8, DCF1, DBXE)

| ID$_{50}$ Titer | μg/ml |
|---|---|
| >5000 | >75 |
| >1000 - 5000 | >15 - 75 |
| >200 -1000 | >5 -15 |
| 40 - 200 | 1 - 5 |

**Extended Data Figure 7 | Assays to identify 10-1074- or 3BNC117-specific neutralizing activities in the plasma of monoclonal-antibody-treated macaques.** **a**, ID$_{50}$ values measured in the TZM-bl neutralization assay of 10-1074 and 3BNC117 against HIV-1 strains that are sensitive to one but not the other broadly neutralizing antibody (that is, HIV-1 strain X2088_9 (10-1074 sensitive); HIV-1 strain Q769_d22 (3BNC117 sensitive)). **b**, Neutralizing activities in plasma before antibody administration (preP), but spiked with 0.01, 0.1, 1, 10 and 100 μg ml$^{-1}$ of antibodies 10-1074 (blue) or 3BNC117 (green). Neutralizing activities are reported as plasma ID$_{50}$ titres (left columns) and converted to antibody concentrations (right columns) based on measured ID$_{50}$ values in **a**.

**a**

| NHP | days post injection | Plasma $ID_{50}$ Titer X2088_9 | Plasma $ID_{50}$ Titer Q769_d22 | Plasma $ID_{50}$ converted to respected antibody concentration of 10-1074 and 3BNC117 (μg/ml) 10-1074 | 3BNC117 |
|---|---|---|---|---|---|
| KZ6 | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | d3 | <20 | 916 | <0.26 | 21.98 |
| | d6 | 21 | 780 | 0.27 | 18.72 |
| | d10 | <20 | 519 | <0.26 | 12.46 |
| | d13 | <20 | 357 | <0.26 | 8.57 |
| | d16 | <20 | 65 | <0.26 | 1.56 |
| | d20 | <20 | 250 | <0.26 | 6.00 |
| | d23 | <20 | 237 | <0.26 | 5.69 |
| | d28 | <20 | 143 | <0.26 | 3.43 |
| MB6 | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | d3 | <20 | 930 | <0.26 | 22.32 |
| | d6 | <20 | 766 | <0.26 | 18.38 |
| | d10 | <20 | 385 | <0.26 | 9.24 |
| | d13 | <20 | 286 | <0.26 | 6.86 |
| | d16 | <20 | 271 | <0.26 | 6.50 |
| | d20 | <20 | <20 | <0.26 | <0.48 |
| | d23 | <20 | <20 | <0.26 | <0.48 |
| | d28 | <20 | <20 | <0.26 | <0.48 |
| MB8 | Prebleed | 54 | 52 | <0.26 | 1.25 |
| | d3 | 5957 | 52 | 77.44 | 1.25 |
| | d6 | 3950 | <20 | 51.35 | <0.48 |
| | d10 | 2706 | 42 | 35.18 | 1.01 |
| | d13 | 2454 | 34 | 31.90 | 0.82 |
| | d16 | 1617 | 24 | 21.02 | 0.58 |
| | d20 | 912 | <20 | 11.86 | <0.48 |
| | d23 | 773 | 37 | 10.05 | 0.89 |
| | d28 | 568 | 38 | 7.38 | 0.91 |
| MCN | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | d3 | 12652 | <20 | 164.48 | <0.48 |
| | d6 | 7908 | <20 | 102.80 | <0.48 |
| | d10 | 4149 | <20 | 53.94 | <0.48 |
| | d13 | 1983 | <20 | 25.78 | <0.48 |
| | d16 | 988 | <20 | 12.84 | <0.48 |
| | d20 | 417 | <20 | 5.42 | <0.48 |
| | d23 | 237 | 22 | 3.08 | 0.53 |
| | d28 | 159 | 21 | 2.07 | 0.50 |

**b**

| NHP | days post injection | Plasma $ID_{50}$ Titer X2088_9 | Plasma $ID_{50}$ Titer Q769_d22 | Plasma $ID_{50}$ converted to respected antibody concentration of 10-1074 and 3BNC117 (μg/ml) 10-1074 | 3BNC117 |
|---|---|---|---|---|---|
| DBZ3 | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | 3 | 15385 | 3178 | 200.01 | 76.27 |
| | 6 | 5784 | 1460 | 75.19 | 35.04 |
| | 10 | 3150 | 848 | 40.95 | 20.35 |
| | 14 | 2114 | 567 | 27.48 | 13.61 |
| | 17 | 1331 | 317 | 17.30 | 7.61 |
| | 20 | 1300 | 188 | 16.90 | 4.51 |
| | 24 | 485 | <20 | 6.31 | <0.48 |
| | 27 | 333 | <20 | 4.33 | <0.48 |
| | 34 | 85 | <20 | 1.11 | <0.48 |
| | 41 | <20 | <20 | <0.26 | <0.48 |
| | 49 | <20 | <20 | <0.26 | <0.48 |
| Retreatment | | | | | |
| DBZ3 | R0 | <20 | <20 | <0.26 | <0.48 |
| | R3 | 3651 | 750 | 47.46 | 18.00 |
| | R7 | 1640 | 342 | 21.32 | 8.21 |
| | R10 | 438 | <20 | 5.69 | <0.48 |
| | R13 | 75 | <20 | 0.98 | <0.48 |
| | R17 | <20 | <20 | <0.26 | <0.48 |
| | R21 | <20 | <20 | <0.26 | <0.48 |
| | R24 | <20 | <20 | <0.26 | <0.48 |
| | R27 | <20 | <20 | <0.26 | <0.48 |
| | R34 | <20 | <20 | <0.26 | <0.48 |
| | R41 | <20 | <20 | <0.26 | <0.48 |
| DC99A | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | 3 | 20244 | 3306 | 263.17 | 79.34 |
| | 6 | 8614 | 1595 | 111.98 | 38.28 |
| | 10 | 7756 | 1532 | 100.83 | 36.77 |
| | 14 | 3429 | 717 | 44.58 | 17.21 |
| | 17 | 2655 | 647 | 34.52 | 15.53 |
| | 20 | 2242 | 540 | 29.15 | 12.96 |
| | 24 | 1638 | 384 | 21.29 | 9.22 |
| | 27 | 2439 | 576 | 31.71 | 13.82 |
| | 34 | 1001 | 195 | 13.01 | 4.68 |
| | 41 | 968 | <20 | 12.58 | <0.48 |
| | 49 | 461 | 35 | 5.99 | 0.84 |
| | 57 | 144 | <20 | 1.87 | <0.48 |
| | 70 | <20 | <20 | <0.26 | <0.48 |
| Retreatment | | | | | |
| DC99A | R0 | <20 | <20 | <0.26 | <0.48 |
| | R3 | 14993 | 2274 | 194.91 | 54.58 |
| | R7 | 6079 | 875 | 79.03 | 21.00 |
| | R10 | 4426 | 280 | 57.54 | 6.72 |
| | R13 | 3458 | <20 | 44.95 | <0.48 |
| | R17 | 2844 | <20 | 36.97 | <0.48 |
| | R21 | 2773 | <20 | 36.05 | <0.48 |
| | R24 | 1935 | <20 | 25.16 | <0.48 |
| | R27 | 1279 | <20 | 16.63 | <0.48 |
| | R34 | 1186 | <20 | 15.42 | <0.48 |
| | R41 | 1037 | <20 | 13.48 | <0.48 |

**c**

| NHP | days post injection | Plasma $ID_{50}$ Titer X2088_9 | Plasma $ID_{50}$ Titer Q769_d22 | Plasma $ID_{50}$ converted to respected antibody concentration of 10-1074 and 3BNC117 (μg/ml) 10-1074 | 3BNC117 |
|---|---|---|---|---|---|
| DCM8 | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | 3 | 6649 | 1977 | 86.44 | 47.45 |
| | 6 | 4799 | 1388 | 62.39 | 33.31 |
| | 10 | 2432 | 877 | 31.62 | 21.05 |
| | 14 | 1222 | 477 | 15.89 | 11.45 |
| | 17 | 797 | 249 | 10.36 | 5.98 |
| | 20 | 296 | 37 | 3.85 | 0.89 |
| | 23 | 148 | <20 | 1.92 | <0.48 |
| | 28 | <20 | 25 | <0.26 | 0.60 |
| | 34 | <20 | 33 | <0.26 | 0.79 |
| | 42 | 21 | 40 | 0.27 | 0.96 |
| | 48 | <20 | 43 | <0.26 | 1.03 |
| DBXE | Prebleed | <20 | 24 | <0.26 | 0.58 |
| | 3 | 6962 | 1497 | 90.51 | 35.93 |
| | 6 | 3827 | 927 | 49.75 | 22.25 |
| | 10 | 1446 | 504 | 18.80 | 12.10 |
| | 14 | 957 | 242 | 12.44 | 5.81 |
| | 17 | 696 | 208 | 9.05 | 4.99 |
| | 20 | 415 | 190 | 5.40 | 4.56 |
| | 23 | 378 | 131 | 4.91 | 3.14 |
| | 28 | 85 | 39 | 1.11 | 0.94 |
| | 34 | <20 | <20 | <0.26 | <0.48 |
| | 42 | 29 | 115 | 0.38 | 2.76 |
| | 48 | <20 | <20 | <0.26 | <0.48 |
| DCF1 | Prebleed | <20 | <20 | <0.26 | <0.48 |
| | 3 | <20 | 2138 | <0.26 | 51.31 |
| | 6 | 29 | 1172 | 0.38 | 28.13 |
| | 10 | <20 | 133 | <0.26 | 3.19 |
| | 14 | 21 | 35 | 0.27 | 0.84 |
| | 17 | <20 | 30 | <0.26 | 0.72 |
| | 20 | 23 | 28 | 0.30 | 0.67 |
| | 23 | <20 | 23 | <0.26 | 0.55 |
| | 28 | <20 | 30 | <0.26 | 0.72 |
| | 34 | <20 | 65 | <0.26 | 1.56 |
| | 42 | <20 | 74 | <0.26 | 1.78 |
| | 48 | 21 | 83 | 0.27 | 1.99 |

| $ID_{50}$ Titer | μg/ml |
|---|---|
| >5000 | >75 |
| >1000 - 5000 | >15 - 75 |
| >200 - 1000 | >5 - 15 |
| 40 - 200 | 1 - 5 |

**Extended Data Figure 8 | Monoclonal antibody levels in the plasmas of monotherapy and combination monoclonal antibody macaque recipients.** **a**–**c**, Macaques treated with one neutralizing monoclonal antibody (**a**); macaques receiving two cycles of combination monoclonal antibody treatment (**b**); macaques receiving a single cycle of combination monoclonal antibody treatment (**c**). $ID_{50}$ titres (left columns) and monoclonal antibody concentrations (right columns) were measured in the indicated macaque plasma samples before (Prebleed) and after (Day) monoclonal antibody administration.

**Extended Data Table 1 | Cell-associated viral RNA/DNA in rhesus macaques receiving combination monoclonal antibody therapy**

| Animal | Treatment Time (Days) | Plasma Viral RNA (copies/ml) | SIV Gag RNA Copies per $10^8$ Cell Eq | SIV Gag DNA Copies per $10^8$ Cell Eq |
|---|---|---|---|---|
| DBZ3 1st | 0 | 1.08E+04 | 9,000 | 6,700 |
| | 10 | < 100 | 360 | 7,500 |
| | 20 | < 100 | 2,600 | 14,000 |
| | 24 | < 100 | 1,600 | 6,400 |
| | 27 | < 100 | 670 | 5,700 |
| DBZ3 2nd | 0 | 1.32E+04 | 52,000 | 15,000 |
| | 10 | < 100 | 380 | 4,700 |
| | 13 | 4.09E+02 | 1,000 | 11,000 |
| DC99A 1st | 0 | 7.60E+03 | 31,000 | 1,400 |
| | 14 | < 100 | 18,000 | 5,600 |
| | 20 | < 100 | 8,100 | 2,700 |
| | 27 | < 100 | 400 | 790 |
| | 34 | < 100 | 550 | 1,100 |
| | 41 | < 100 | 7,200 | 780 |
| DC99A 2nd | 0 | 2.35E+03 | 23,000 | 2,100 |
| | 21 | < 100 | 570 | 1,100 |
| | 27 | < 100 | 1,100 | 2,100 |
| DBXE | 0 | 1.96E+05 | 470,000 | 71,000 |
| | 14 | 3.59E+02 | 17,000 | 33,000 |
| | 17 | 7.55E+02 | 11,000 | 22,000 |
| | 20 | < 100 | 11,000 | 33,000 |
| | 23 | 1.60E+03 | 17,000 | 27,000 |
| DCM8 | 0 | 1.59E+03 | 110,000 | 8,600 |
| | 14 | < 100 | 1,700 | 1,600 |
| | 17 | < 100 | 880 | 5,000 |
| | 20 | 1.51E+02 | 22,000 | 6,600 |
| DCF1 | 0 | 1.44E+05 | 240,000 | 15,000 |
| | 6 | 5.38E+03 | 34,000 | 5,500 |
| | 14 | 1.69E+05 | 190,000 | 11,000 |
| | 20 | 1.45E+05 | 1,100,000 | 14,000 |

**Extended Data Table 2 | Decay rate constants of SHIV-AD8 RNA in plasma after monoclonal antibody treatment**

| Animal | k ($days^{-1}$) | $T_{1/2}$ (days) |
|---|---|---|
| DC99A 1st | 0.72 | 1.0 |
| DC99A 2nd | 0.45 | 1.5 |
| DBZ3 1st | 0.47 | 1.4 |
| DBZ3 2nd | 0.70 | 1.0 |
| DBXE | 0.66 | 1.0 |
| DCF1 | 0.55 | 1.3 |
| DCM8 | 0.46 | 1.5 |
| KZ6 | 0.40 | 1.8 |
| MB6 | 0.43 | 1.5 |
| MB8 | 0.36 | 1.9 |
| MCN | 0.89 | 0.8 |

Rate constants of initial viral RNA decline in plasma (decay rate constants *k*) and corresponding half-lives ($t_{1/2}$) were determined as previously described (ref. 26). The decay rate constants *k* were calculated by fitting the data with an exponential function. The fit was very good with correlation coefficients in the range from 0.91 to 1.0.