SUPPLEMENTAL FILE 2

SUPPLEMENTAL TABLES

TABLE S1. VHH selected for further characterisation[1].

VHH	Llama	Immunogen	Selection
A12	L44	CN54 gp120	IIIB, 92UG037, 92BR025 gp120[2]
D7	L44	CN54 gp120	IIIB gp120
C8	L44	CN54 gp120	IIIB gp120

[1]VHH phage display libraries derived from llamas immunized with recombinant gp120 were panned on gp120 from various HIV-1 isolates followed by a competitive elution with sCD4. Individual VHH clones were isolated and screened for HIV-1 neutralization activity and binding to recombinant gp120. A number of similar VHH sequences were identified and representative clones were chosen for further characterization.

[2]A12 was isolated upon panning of the VHH phage libraries on gp120 derived from HIV-1 of subtype A, B and C, varying the antigen in different combinations, in two subsequent rounds of selection.

SUPPLEMENTAL FIGURES

FIGURE S1

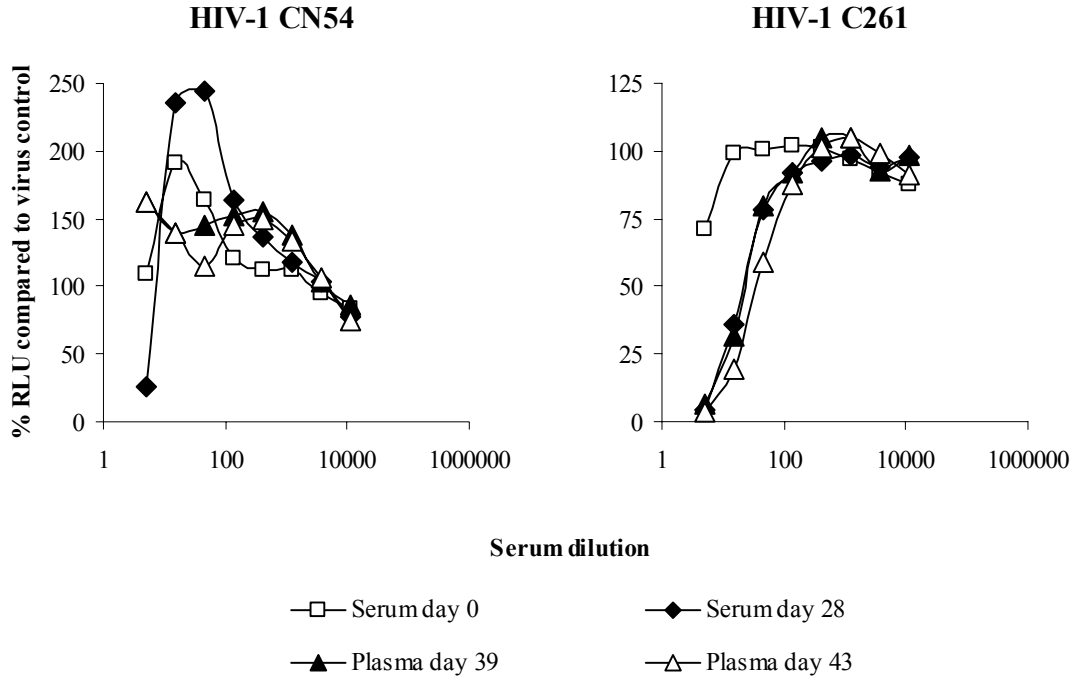

FIGURE S1. Neutralization activity of sera and plasma from llama L44, immunized with HIV-1 CN54 gp120. The neutralization activity of serum samples from day 0 (pre-immunization) and 28 (post-immunization) and plasma samples from day 39 and 43 (post-immunization) was evaluated against HIV-1 CN54 and C261 in TZM-bl cells. Shown is the percentage of relative light units (RLU) obtained in samples wells, compared to the RLU obtained in wells containing virus and cells only. Data points represent the mean of duplicate readings. Some reduction of infectivity was observed in serum sample from day 28 against HIV-1 CN54 at a 1:5 dilution. Against HIV-1 C261, the IC_{50} was 1:21, 1:22, and 1:37 dilution for the serum sample from day 28 and the plasma samples from day 39 and 43, respectively. HIV-1 CN54 is relatively insensitive to neutralization by antibodies to the CD4bs, whereas HIV-1 C261 is sensitive to neutralization by

VHH A12 and D7. The neutralization activity in the serum and plasma samples may be mediated by conventional as well as heavy-chain antibodies, as the two antibody types were not separated for this experiment.

FIGURE S2

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D7          AVQLVESGGGLAQAGGSLRLSCTVSGRTSSSHDMGWFRQAPGKEREFVAAISWSGGTTNY 60
A12         AVQLVESGGGLVQAGGSLRLSCTASGRISSSYDMGWFRQAPGKEREFVAAISWSGGTTDY 60
C8          AVQLVDSGGGLVQAGGSLRLSCVVSGSIFSINAMGWYRQAPGKQRDLVARISGDS-STYY 59
            *****:*****.**********..**    *   ***:******:*::** ** .. :* *

D7          ADSVKGRFAISKDNAKNAVSLQMNSLKPEDTAVYYCAAKWRPLRYSDNPSNSDYNYWGQG 120
A12         ADSVKGRFAISKDNAKNAVSLQMNSLKPEDTAVYYCAAKWRPLRYSDYPSNSDYYDWGQG 120
C8          IDSVKGRFTISRDNAANTVYLQMNSLKPEDTAVYYCAARRLPIG--------DYTDWGQG 111
             *******:**:*** *:* *****************:  *:         **  ****

D7          TQVTVSS 127
A12         TQVTVSS 127
C8          TQVTVSS 118
            *******
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FIGURE S2. Multiple amino acid sequence alignment of VHH A12, D7 and C8. The CDR 1, 2 and 3 are shaded in grey. The alignment was made using the CLUSTAL W (http://www.ebi.ac.uk/Tools/clustalw2) multiple sequence alignment software.

FIGURE S3

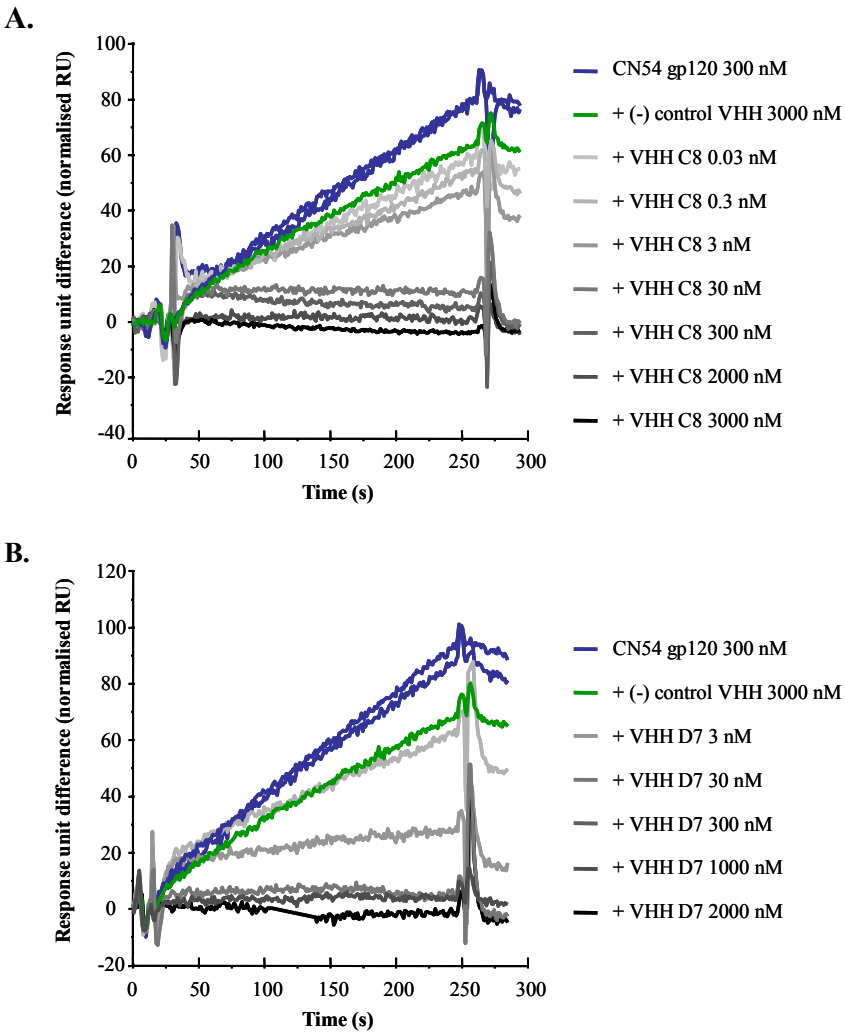

FIGURE S3. Dose-dependent inhibition of sCD4 binding to CN54 gp120 by VHH C8 and D7 as assayed by surface plasmon resonance. Soluble CD4 was cross-linked to an anti-CD4 Ab coupled to the chip. Serial dilutions of VHH were pre-incubated with HIV-1 CN54 gp120 and subsequently injected onto the chip. Data were analyzed using the BIAevaluation software. Binding of HIV-1 CN54 gp120 to sCD4 is represented as the normalized difference between the response units (RU) observed in the cell containing the chip with sCD4 and the negative control flow cell, plotted versus time.