(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 January 2013 (10.01.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/006688 A2

(51) **International Patent Classification**: Not classified

(21) **International Application Number**:
PCT/US2012/045530

(22) **International Filing Date**:
5 July 2012 (05.07.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/457,906 5 July 2011 (05.07.2011) US
61/529,137 30 August 2011 (30.08.2011) US

(71) **Applicants** *(for all designated States except US)*: **DUKE UNIVERSITY** [US/US]; 2812 Erwin Road, Durham, NC 27705 (US). **CHILDREN'S MEDICAL CENTER CORPORATION** [US/US]; 55 Shattuck Street, Boston, MA 02115 (US). **THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY OF THE ARMY, ON BEHALF OF WALTER REED ARMY INSTITUTE OF RESEARCH** [US/US]; 504 Scott Street, Fort Detrick, MD 21702-5012 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **KIM, Jerome** [US/US]; C/o The Government Of The U.s, As Represented By, The Secretary Of The Army, On Behalf Of Walter, Reed Army Institute Of Research, 504 Scott Street, Fort Detrick, MD 21702-5012 (US). **HARRISON, Stephen** [US/US]; 19r Sparhawk Street, Boston, MA 02135 (US). **HAYNES, Barton, F.** [US/US]; C/o Duke University, 2812 Erwin Road, Durham, NC 27705 (US). **TOMARAS, Georgia, D.** [US/US]; C/o Duke University, 2812 Erwin Road, Durham, NC 27705 (US). **MICHAEL, Nelson** [US/US]; c/o The Government Of The U.s, As Represented By, The Secretary Of The Army, On Behalf Of Walter, Reed Army Institute Of Research, 504 Scott Street, Fort Detrick, MD 21702-5012 (US).

(74) **Agent**: **WILSON, Mary, J.**; Nixon & Vanderhye P.c., 901 North Glebe Road, 11th Floor, Arlington, VA 22203-1808 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2013/006688 A2

(54) **Title**: N-TERMINAL DELETED GP120 IMMUNOGENS

(57) **Abstract**: The present invention relates, in general, to human immunodeficiency virus (HIV), and in particular to a vaccine for HIV-1 and to methods of making and using same.

N-TERMINAL DELETED GP120 IMMUNOGENS

This application claims priority from U.S. Provisional Application No. 61/457,906, filed July 5, 2011 and U.S. Provisional Application No. 61/529,137, filed August 30, 2011, the entire contents of both applications are incorporated herein by reference.

This invention was made with government support under Grant No. A1067854 awarded by the National Institutes of Health, Bethesda, Maryland. The government has certain rights in this invention.

TECHNICAL FIELD

The present invention relates, in general, to human immunodeficiency virus (HIV), and, in particular, to a vaccine for HIV-1 and to methods of making and using same.

BACKGROUND

Development of a safe, practical and effective HIV-1 vaccine is one of the highest priorities of the global scientific community (Klausner et al, Science 5628:2036-2039 (2003); Esparza et al, Science Strategic Plan, DOI: 10.1371/journal.pmed.0020025, Policy Forum Vol. 2, February 2005)). While anti-retroviral treatment (ART) has dramatically prolonged the lives of HIV-1 infected patients, anti-retroviral therapy is not yet routinely available in developing countries, and the global rate of spread of HIV-1 continues unabated.

There are multiple components for successful HIV vaccine development. First is the production of HIV envelope constructs that express neutralizing epitopes reflective of the native envelope (Env) to ensure that the regions and epitopes to which it is desired to induce protective antibodies are indeed present on the Env immunogen (i.e., envelope antigenicity). Second, for scalablility of

Env protein production, it is important to be able to make monomeric Envs that are not disulfide linked. The Sodroski laboratory has previously shown that when gp120 Envs are produced in 293T mammalian cells, there is nearly always a major component of the Env that is disulfide linked (Finzi A, Pacheco B, Zeng,X, Young DK, Kwong, PD, Sodroski, J, J. Virol. Methods 168: 155-161, 2010). This disulfide linked Env has many of the desired epitopes occluded and not available for antibody binding (Finzi A, Pacheco B, Zeng,X, Young DK, Kwong, PD, Sodroski, J, J. Virol. Methods 168: 155-161, 2010). Third, many of the regions of the HIV Env are poorly immunogenic or the responses to these epitopes are down regulated by tolerance mechanisms or represent rare maturation pathways (i.e., are subdominant in nature) (McElrath J, Haynes, BF, Immunity 33:542-54. 2010; Verkoczy L, Kelsoe, G, Moody, MA, Haynes, BF, Current Opinion in Immunology 23:383-390, 2011).

The first and second components described above can be dealt with by immunogen design. The third component is dealt with by taking an optimally antigenic Env and formulating it with appropriate adjuvants to drive an otherwise subdominant antibody response in an immunodominant manner by design of immunogens that can optimally trigger naïve B cell receptors of clonal lineages that can make protective antibodies (Ma, BJ, Alam, SM, Go, EP, Lu, X, Desaire, H, Tomaras, GD, Bowman, C, Sutherland, LL, Scearce, RM, Santra, S, Letvin, NL, Kepler, TB, Liao, HX, Haynes, BF, PLoS Pathogens, in press, 2011).

The present invention relates, at least in part, to a gp120 Env design strategy that addresses the first two components of the HIV-1 vaccine problem referenced above: optimal antigenicity and stable gp120 monomer production for scalability of vaccine production.

SUMMARY OF THE INVENTION

The present invention relates generally to HIV. More specifically, the invention relates to a vaccine for HIV-1 and to methods of making and using same.

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Dissociation constants (Kd) of CH01, PG9, A32 and 697D mAbs binding to RV144 Env.

Figure 2. D11((Δ)11) A244 gp120 design expressed in 293T cells. Green box (more shaded) highlights the effect of gD on Kd of binding to A244 Env.

Figure 3. Black-and-white ribbon diagram of gp120, based on Peter Kwong structure deposited in NCBI database as 3JWD.pdb.

Figures 4A and 4B. Reactivity of the neutralizing V2 mab 697D (Fig. 4A), and the V2, V3 neutralizing Ab CH03 Fab (Fig. 4B).

Figure 5. Reactivity of the A32 mAb.

Figure 6. Fast protein liquid chromatography (FPLC) size exclusion profile of unpurified A244 gp120 gD+ (with 11 amino acid (aa) deletion).

Figure 7. FPLC size exclusion profile of purified A244 gp120 gD+ (with 11 aa deletion)

Figure 8. FPLC size exclusion profile of unpurified A244 gp120 gD negative with the N-terminal 11 aa.

Figure 9. FPLC size exclusion profile of purified A244 gp120 gD negative with the N-terminal 11 aa.

Figure 10. FPLC size exclusion profile of unpurified gD- A244 gp120 Delta11.

Figure 11. FPLC size exclusion profile of purified gD- A244 gp120 Delta11.

Figures 12A and 12B. Surface plasmon reasonance (SPR) sensorgrams of the antigenic make-up of the A244 D(delta) negative 11 Env (batch 110608) showing that it binds A32 (C1 conformational), T8 (C1 mabs and sCD4 recombinant Env) (Fig. 12A). Fig. 12B shows the SPR sensorgrams of CCR5 receptor gp120 binding site mAb 17b binding to either A32 triggered, sCD4 triggered or T8 triggered Env D11 gp120.

Figures 13A-13C. Antibody titrations and Kd determinations for mAb 697D (V2 neutralizing); A244Δ11 (Fig. 13A), A244gDneg (Fig. 13B), A244gD/293T (Fig. 13C).

Figures 14A-14C. Antibody titrations and Kd determinations for mAb PG9 (V2,V3 conformational); A244Δ11 (Fig. 14A), A244gDneg/293T (Fig. 14B), A244gD/293T (Fig. 14C).

Figures 15A-15C. Antibody titrations and Kd determinations for mAb CH01 (V2,V3 conformational); A244Δ11/293T (Fig. 15A), A244gDneg/293T (Fig. 15B), A244gD/293T (Fig. 15C).

Figures 16A-16B. Antibody titrations and Kd determinations for mAb CH03 (V2,V3 conformational); A244Δ11 (Fig. 16A), A244gDneg (Fig. 16B).

Figures 17A and 17B. Binding to CH01-04_RUA1; A244Δ11 (Fig. 17A), A244gDneg (Fig. 17B).

Figures 18A and 18B. Binding to CH01-04_RUA2; A244Δ11 (Fig. 18A), A244gDneg/293T (Fig. 18B).

Figure 19. Full length nucleotide sequence and amino acid sequence of the Delta 11 gp120 A244 Env protein.

Figure 20. Full length nucleotide sequences and amino acid sequences of the Delta11 gp120s of 63521.B, 6240.B, 089.C and the group M consensus Env CON-S gp120, and the Delta7 gp120 of 1086.C.

Figure 21. Full nucleotide sequences and amino acid sequences of the full length gp140s of 63521.B, 6240.B, 1086.C, 089.C and the group M consensus Env CON-S gp140.

Figure 22. Binding responses of plasma IgG from RV144 vaccinees in surface plasmon reasonance assays. RV144 vaccinees see the same gp120 epitopes on the Delta 11 env as are seen on the gD+ A244 Envs, but not on the gD negative A244 Env.

Figure 23. Deleted N-terminal gp120 sequences on Delta 11 Envs.

Figure 24. Differential CD4 and MAb A32 binding to the SIVcpz gp140 Env versus the A244 gp120 Delta 11 Env. Whereas the A244 gp120 binds both sCD4 and the C1 mAb A32 well, the SIVcpz gp140 Env only binds CD4 and not A32. Since the dominant response in the RV144 trial is to C1 region , particuarly with regard to the IgA response, and the IgA binding response correlated directly with infection in the RV144 case control immune correlates analysis, then an additional strategy to preventing induction of C1 antibodies is to induce antibodies with an envelope like US1 that doesnt bind A32 but does bind CD4.

Figure 25. Tables.

Figure 26. HIV-1 Env sequences with delta 11 and C1 deletion as HIV-1 vaccine design as examples.

Figure 27. Diagram of HIV-1 gp120 proteins constructs with modifications. Designs for each of A244, MN and 92TH023 gp120 proteins with either no modifications (gp120), with both the HSV gD tag (first 27 aa of mature HSV gD protein) and N-terminal 11 aa deletion (gDΔ11 gp120), or with only 11 aa deletion (Δ11 gp120) are outlined. The gDΔ11 gp120 constructs for all three proteins are similar with respect to modifications to the design used in the RV144 vaccine trial for A244-rgp120 and MN-rgp120. The Env gp120 proteins were expressed and purified as described in Example 3.

Figure 28. Binding of C1, V2 and V3 antibodies to RV144 immunogen gp120 proteins. RV144 Env proteins MN-rgp120 and A244-rgp120 binding at varying concentrations (0.5 – 5 μg/mL for A32; 5.0 -100 μg/mL for 697D; 0.5 – 5 μg/mL for 19b) to A32, 697D and 19b are shown. The calculated K_d values and with fitted curves (black line) for each of the binding interactions are shown. Each mAb was captured on anti-Fc antibody immobilized surfaces and gp120 monomeric proteins were in injected as analyte and as described in Example 3. The data is representative of three measurements.

Figures 29A-29D: Relative proportion of monomer and dimer in A244 gp120 proteins. Fig. 29A) SDS PAGE analysis (under reduced and non-reduced condition) of A244, A244gDΔ11, and A244Δ11 gp120 proteins showing the presence of disulfide linked dimers in varying proportions. Each of the gp120 preparations were analyzed by size exclusion chromatography (SEC) which showed relatively larger proportion of monomer in A244 gp120 (Fig. 29B) than in either A244 Δ11 (Fig. 29C) or A244 gDΔ11 (Fig. 29D). Peak volume analysis of the monomer and dimer fractions gave the following proportion of monomer and dimer in Fig. 29B) and Fig. 29C) and Fig. 29D) respectively.

Figures 30A-30E: Enhanced binding of C1, V2 and V1V2 antibodies to E.A244gp120 proteins with Δ11 deletion. Each of the analyte gp120 proteins (left panel: A244 gp120; middle panel: A244gDΔ11; right panel: A244Δ11 gp120) were injected over the listed antibodies captured on an anti-Fc immobilized surface. Each gp120 protein was titrated at 0.5 – 5.0 μg/mL for 19b and A32; A244 gp120 at 10 – 50 μg/mL for 697D, CH01 and PG9; A244gDD11 at 10-100 μg/mL for 697-D and CH01 and at 10-40 μg/mL for PG9; A244Δ11 at 2-50 μg/mL for 697-D, 10-100μg/mL for CH01 and PG9 mAb captured surfaces. Data is

representative of at least 3 measurements made on individual flow cells with equivalent amounts of captured antibodies. (Fig. 30A-19b, Fig. 30B-A32, Fig. 30C-697D, Fig. 30D-CH01, and Fig. 30E-PG9.)

Figure 31. Higher affinity binding of CH01_RUA1 and CH01_RUA2 to A244Δ11 gp120. Each of the CH01 RUAs was captured as described in Figure 30. Each of the three forms of A244 gp120 was flowed at concentration ranging from 10 -100μg/mL. Kinetic rate constants and K_d were derived as described in Example 3. When compared to A244 gp120, both CH01 RUAs bound with higher affinities to A244 with either gDΔ11 (middle panel) or Δ11 (right panel). Data is representative of at least 3 measurements made on individual flow cells with equivalent amounts of captured antibodies.

Figures 32A and 32D. RV144 vaccinee sera antibody responses. Fig. 32A) RV144 vaccinee IgG binding to A244 gp120 proteins show higher avidity binding to A244 gp120 with Δ11. RV144 visit 8 (week 26, two weeks following the final immunization) IgG (n=97) binding avidity was measured against A244 gp120, A244gDΔ11 and A244 Δ11 gp120 proteins. Binding responses and dissociation rate constants for Avidity score measurements were calculated as described Example 3. The mean Avidity of binding to A244 gp120, A244gDΔ11 and A244 Δ11 gp120 were 1.0±1.5, 10.0±0.5, and 5.7±0.7 RU.s (x 10^5) respectively. The differences in binding avidity was significant for A244 gp120 vs A244gDΔ11 (Student t-test, $p<0.001$) and A244 gp120 vs A244Δ11 g120 (Student t-test, $p<0.001$). No binding was observed for IgG samples from Vaccinee plasma IgG from visit 0 or from placebo group. Fig. 32B) RV144 vaccinee plasma IgG binding to A244 gp120 proteins show higher relative binding to A244 gp120 with Δ11. RV144 visit 8 (week 26, two weeks following the final immunization) plasma

antibody was measured against A244 gp120, A244gDΔ11 and A244 Δ11 gp120 proteins. The differences in binding responses was significant for A244 gp120 vs A244Δ11 g120 (Student t-test, p<0.001). Fig. 32C) Blocking of RV144 induced IgG binding to A244gDΔ11 gp120 by conformational C1 (A32), V2 (697D, 830A), V3 (19b) and V2/V3 (CH01) antibodies. RV144 IgG samples (n=109) with high and low level (>80 Response Unit measured at 100ug/mL) of binding to A244D11 gp120 were selected for antibody blocking studies. A control group (n=19) showing no binding to A244 Δ11 gp120 was included to assess non-specific signal in IgG samples. Fig. 32D) ELISA assay showing high level of A32 blocking (mean =39.6% ±19.2) by RV144 IgG and low level of CD4 blocking antibodies (mean = 13% ±8.9). Blocking of IgG from visit 1 were 6.7%±4.2 and 8.9%±7.6 for A32 and CD4 respectively.

Figures 33A-33B. RV144 mAbs CH51 and CH54 show higher affinity binding to A244gp120 with Δ11 modification. A244, A244gDΔ11 and A244Δ11 gp120 were each injected at concentrations ranging from 5 -25 μg/mL over either CH51 (Fig. 33A) or CH54 (Fig. 33B) captured on anti-Fc immobilized surfaces. Binding of both CH51 and CH54 was an order of magnitude higher for both A244 gp120 with the Δ11 modification than A244 gp120 (left panel) with no modification.

DETAILED DESCRIPTION OF THE INVENTION

The efficacy seen in the RV144 ALVAC prime gp120 B/E boost Thai clinical trial demonstrated that a protective HIV-1 vaccine could be made (Rerks-Ngarm, S et al NEJM 361: 2209-30, 2009). However, the efficacy was modest at 31% and the duration of protection short, demonstrating the need for

improvement in level of protection. To improve on the efficacy of RV144 results, it is critical to understand the nature of the immunogens in RV144 and to understand why the trial worked, and to define any immune correlates of protection in the trial.

The gD HSV tag positioned, in the immunogens in RV144, N terminal to the gp120, and in place of the first 11 amino acids of the gp120 expressed coding region, has been implicated as being responsible for effecting the enhanced antigenicity of the A244 gp120 molecule used in the Thai trial. The present invention results, at least in part, from the realization that it was not the 27 amino acid gD tag (Lasky et al, Science 233:209-212 (1986)) that upregulated the gp120 epitopes but, rather, it was the truncation and deletion of the first 11 amino acids of the N-terminus of the gp120 (Fig. 23). Thus, the envelope design in accordance with the present invention involves deletion of residues (e.g., about 11 amino acids) at the N-terminus of the gp120. The result of this deletion on, for example, the A244 gp120 AE envelope is stabilization of the V1V2 conformational neutralizing epitopes, stabilization and enhanced expression of V2,V3 confirmation (so-called quaternary neutralizing epitopes) and enhanced expression of the C1 A32-like ADCC epitope. Moreover, the general strategy of deletion of N-terminal amino acids (e.g., about 11) of gp120 results in gp120s expressed in mammalian cells that are primarily monomeric, as opposed to dimeric, and, therefore, solves the production and scalability problem of commercial gp120 Env vaccine production.

The present invention relates generally to an immunogen, gp120 or gp140, without an N-terminal Herpes Simplex gD tag substituted for amino acids of the N-terminus of gp120, with an HIV leader sequence (or other leader sequence), and without the original about 4 to about 25, preferably about 11, amino acids of the N-terminus of gp120. While the invention includes deletions of various lengths and combinations of amino acids, deletions of between 5-11 amino acids are preferred. The 1086.C gp120 Env has an N-terminal set of amino acids only 7 amino acids long and

manifests ~ 50% dimer formation when expressed in 293T or CHO cells. Thus, it is likely that deletions of greater than 5 amino acids are required for prevention of dimer formation. In one embodiment, 11 residues are deleted; in another embodiment, between 2 and 10 residues are deleted, either consecutive amino acids or selected amino acids; in yet another embodiment, a short peptide sequence chosen for stability is substituted for one or more of the 11 residues.

Advantageously, the Env gp120 or gp140 is a transmitted founder virus Env such as 1086.C, 089.C, 63521.B, 6240.B, 040.B or A1C recombinant transmitted founder Env 707-01-069-2 (see sequences, for example, in U.S. Provisional Application No. 61/344,622 and PCT/US2011/000352). In addition, the 0219.A signature Env of broad Nabs can also be advantageously used, as can transmitted/founder Env sequences derived from a subject with broadly neutralizing antibodies. In addition, N-terminus truncated consensus sequences such as the group M consensus CON-S gp140 or gp120 sequence (Liao et al, Virology 353(2):268 (2006), PCT/US04/30397, U.S. Application Nos. 10/572,638 and 11/896,934) or the gp140 or the gp120 of the mosaic Env sequences (PCT/US2009/004664, U.S. Application Nos. 11/990,222 and 12/192,015) can be used.

The N-terminal gp120 truncation is preferably 11 amino acids in length, however, as noted above, truncations of about 4 to about 25 amino acids can be used. Other combinations of amino acid substitutions can also be used.

The N-terminus truncated gp120 or gp140 envelopes can be formulated as DNAs (Santra S. et al. Nature Med. 16: 324-8, 2010) and as inserts in vectors including rAdenovirus (Barouch DH, et al. Nature Med. 16: 319-23, 2010), recombinant mycobacteria (i.e., BCG or M smegmatis) (Yu, JS et al. Clinical Vaccine Immunol. 14: 886-093, 2007; ibid 13: 1204-11, 2006), and recombinant vaccinia type of vectors (Santra S. Nature Med. 16: 324-8, 2010). The truncated envelopes can also be administered as a protein boost in combination with a variety of vectored Env primes (i.e., HIV-1 Envs expressed in

non-HIV viral or bacterial vectors) (Barefoot B et al. Vaccine 26: 6108-18, 2008), or as protein alone (Liao HC et al Virology 353: 268-82, 2006). The protein can be administered with an adjuvant such as MF59, AS01B, polyI, polyC or alum and administered, for example, subcutaneously or intramuscularly. Alternatively, the protein or vectored Env can be administered mucosally such as via intranasal immunization or by other mucosal route (Torrieri DL et al Mol. Ther. Oct. 19 2010, E put ahead of print).

Immunogens of the invention are suitable for use in generating an immune response in a patient (e.g., a human patient) to HIV-1. The mode of administration of the HIV-1 protein/polypeptide/peptide, or encoding sequence, can vary with the immunogen, the patient and the effect sought, similarly, the dose administered. As noted above, typically, the administration route will be intramuscular or subcutaneous injection (intravenous and intraperitoneal can also be used). Additionally, the formulations can be administered via the intranasal route, or intrarectally or vaginally as a suppository-like vehicle. Optimum dosing regimens can be readily determined by one skilled in the art. The immunogens are preferred for use prophylactically, however, their administration to infected individuals may reduce viral load.

Certain aspects of the invention are described in greater detail in the non-limiting Examples that follow. (U.S. Provisional Application No. 61/457,906 is incorporated herein by reference.)

EXAMPLE 1

Figure 1 shows that putting the gD herpes simplex 27 aa tag N-terminal to gp120 AE.92Th023, MN gp120 and A244 gp120 (gp120s used in the Thai HIV vaccine efficacy trial (Lasky et al, Science 233:209-212 (1986); Rerks-Ngarm et al, N. Eng. J. Med. 361:2209-2220 (2009)) and MN and A244 used as gD+,

gp120 proteins (Rerks-Ngarm et al, N. Eng. J. Med. 361:2209-2220 (2009)), particularly with A244 gp120, results in upregulation of V2,V3 conformational neutralizing epitopes (CH01, PG9 binding), C1 ADCC A32 mAb binding, and V2 conformational neutralizing Ab binding (mAb 697D). The gD HSV tag is placed N terminal to the gp120 in place of the first 11 amino acids of gp120 expressed coding region. This tag has been implicated as being responsible for effecting the enhanced antigenicity of the A244 gp120 molecule. It has now been shown that the presence of the 27 aa gD tag (Lasky et al, Science 233:209-212 (1986)) has no effect itself on the upregulation of gp120 epitopes. Rather, what is required is truncation of the N-terminus of the gp120 for upregulation of the gp120 epitopes and for production of primarily monomeric gp120 species.

Figure 2 shows the D11 A244 gp120 design that has been expressed in 293T cells. The N-terminus of the D11 gp120 starts at the VPV... aa gp120 sequence.

Figure 3 shows the black-and-white ribbon diagram of gp120, based on structure from Peter Kwong deposited in the public NCBI database as 3JWD.pdb. The region in this figure that is 31-42 is the region that is deleted in the Delta 11 gp120 Env construct. It includes the full N- and C-terminal segments of gp120 (but not V1-V2 or V3). Residues 31-41 stick out into space, as do residues 494-511. Residues 31-41, are the residues deleted in D11 A244 gp120 which are relatively hydrophobic and which would, in gp160, interact with gp41, likely lead to some misfolding or aggregation of gp120 in the endoplamic reticulum, so that disulfides form between two gp120s. The C-terminal extension, which would also interact with gp41 (it leads to the cleavage point), is very polar (indeed, charged), so it may help solubilize the expressed protein. The Delta 11 substitution apparently eliminated (or at least partly eliminated) the aggregation aberrant disulfide linkage, and/or misfolding tendency of the whole Env during folding.

Figures 4 and 5 show the reactivity of the neutralizing V2 mab 697D, the V2, V3 neutralizing Ab CH03 Fab (Fig. 4), and the A32 mAb (Fig. 5) binding to A244 gD+ gp120, A244 gD negative (with no delta 11 deletion) and A244 gD negative D11 (Delta 11 deletion present with no gD tag present). Data show that each of the three Abs bind better to Delta11 Env gp120 A244 than to Envs with either no gD tag but with the 11 aa N terminus of gp120 (gDneg gp210) or to an Env with the gD tag that has gD substituted for the 11 proximal N-terminal amino acids.

Figures 6-11 show the FPLC profiles of unpurified and purified A244 gp120 gD+ (with 11 aa deletion) (Figs. 6,7), A244 GP120 gD negative with the N-terminal 11 aa (Figs. 8,9) and gD- A244 gp120 Delta11 (Figs. 10 and 11). Figs. 6, 8 and 10 are unpurfied post lectin column Envs and Figs. 7, 9, and 11 are post FPLC purification monomers of each Env. The figures show that after lectin purification columns, the Delta 11 gpD- Env gp120 is the most monomer after lectin column purification and before FPLC and the gD- gp120 without the delta 11 truncation has the most dimers and least monomers of the three Envs. Thus, the Delta11 Env gp120 solves the gp120 production problem by deletion of the 11 N-terminal aa of gp120, thus prohibiting C-C mediated gp120 dimerization.

Figure 12 shows surface plasmon reasonance (SPR) sensorgrams of the antigenic make-up of the A244 D(delta) negative 11 Env (batch 110608) showing that it binds A32 (C1 conformational), T8 (C1 mabs and sCD4 recombinant Env (Fig. 12A). Fig. 12B shows the SPR sensorgrams of CCR5 receptor gp120 binding site mAb 17b binding to either A32 triggered, sCD4 triggered or T8 triggered env D11 gp120. Figs. 12A and 12B demonstrate that the Delta 11 Env gp120 expresses the A32 epitope, binds CD4 and as is the case with a functional Env, both A32 and sCD4 upregulate the CCR5 binding site as measured by 17b mAb binding.

Figures 13-16 show antibody titrations and Kd determinations for mAbs 697D (V2 neutralizing) (Fig. 13), PG9 (V2,V3 conformational) (Fig. 14), CH01

(V2,V3 conformational) (Fig. 15), CH03 (V2,V3 conformational) (Fig. 16). In all cases the delta (Δ))11 A244 gp120 Env bound the antibodies in question better than did the gD-A244 (with no delta11 deletion) and even bound better than the gD+ A244 with both the gD and the delta 11 deletion.

It was of great interest to know how a particular antigen/immunogen binds to the naïve B cell receptor of some of these antibodies. The way this has been accomplished is to revert the mutated CH01-CH04 antibodies back to two candidate reverted unmutated ancestor antibodies (RUAs) that are the putative B cell receptors of naïve B cells (Termed CH01-04_RUA1 and CH01-04_RUA2) and determine the binding of delta11 A244 vs gD- A244 without the delta 11 deletion. It was found that the delta 11 A244 Env bound better to both of the CH01-CH04 clonal lineage RUAs than did the gD- A244 Env without the delta 11 deletion. (See Figs. 17 and 18.)

Figure 19 shows the full nucleotide sequence and aa sequence of the Delta (Δ))11 gp120 A244 Envelope protein.

Figure 20 shows the full nucleotide sequence and aa sequence of the Delta(Δ))11 gp120s of 63521.B, 6240.B, 089.C and the group M consensus Env CON-S gp120, and the Delta (Δ))7 gp120 of 1086.C

Figure 21 shows the full nucleotide sequence and aa sequence of the full gp140s of of 63521.B, 6240.B, 1086.C, 089.C and the group M consensus Env CON-S gp140.

Figure 22 shows the binding responses of plasma IgG from RV144 vaccinees in surface plasmon reasonance assays. The median binding response of vaccinee IgG was the same to both the vaccine immunogen gp120 (A244gD CHO GSID) and the delta (Δ)) 11 gp120 (A244gDneg)11 293T) but were 5-fold higher than the Env gp120 without the deletion (A244gDneg 293T). The response to delta (Δ)) 11 gp120 was also similar to the same Env gp120 with the HSV gD peptide tag (A244gD+ gp120 293T), both of which have the same Δ 11 deletion.

These data shows that the RV144 vaccine induced IgGs that bind with higher avidity to delta (Δ)) 11 gp120.

Figure 23 shows the N-terminal amino acids that are deleted in various Delta 11 gp120 designs. Bold amino acids are hydrophobic. Deletions can be made in any length and in any combination of amino acids to have the effect of the Delta 11 deletion. However, optimal deletions are of between 5-11 amino acids. The 1086.C gp120 Env has an N-terminal set of amino acids only 7 aa long and manifests ~ 50% dimer formation when expressed in 293T or CHO cells. Thus, it is likely that at least greater than 5 amino acid deletions are needed for prevention of dimer formation. In one embodiment of the invention, 11 residues are deleted; in another embodiment, between 2 and 10 residues are deleted, either consecutive amino acids or selected amino acids. In yet another embodiment, a short peptide sequence chosen for stability can be substituted for one or more of the 11 residues.

Thus, what these data clearly show is that the delta 11 gp120 Env design is a novel immunogen design that both conveys upon the A244, and likely other Envs, the ability to: 1) be produced as primarily a monomer - thus solving the problem of gp120 Env monomer production for appropriate yield of monomer for scaleup of vaccine protein production, and 2) this delta (Δ))11 mutation has conferred on the remaining gp120 the ability to bind better to C1 and V2 mAbs.

The delta 11 Env design is expected to be applicable to multiple Envs (for examples but not exclusive examples see Figures 20 and 21). Moreover, truncations of any length from about 4 aa to about 25 aa are expected to have the same effect. The delta11 gp120s or other truncated versions of gp120 can be administered as proteins, DNAs, and as inserts in a myriad of vectors including rAd5, other rAdenoviruses, recombinant mycobacteria, and a myriad of poxvirus vectors including MVA, NYVAC, and WT vaccinia. In addition attenuated polio virus and the bacterial vector listeria can be used.

The delta 11 and related truncations can as well be made to recombinant gp140 and gp160 constructs that also can be expressed and used in the same manner as Delta 11 gp120s.

Delta 11 Env mutated Env proteins and vectored inserts can thus be used as optimally antigenic Envs for formulation with the most potent adjuvants such as AS01B, MF-59 and polyI, polyC. The plasmids of these Delta 11 Env mutated Env proteins can also be randomly mutated for screens for higher levels of binding to various Env antibody RUAs and intermediate clonal lineage antibodies to improve Env immunogenicity.

EXAMPLE 2

Epitope specificity in RV144 vaccine recipients were determined using peptide microarray and binding antibody multiplex assays. The vaccine elicited IgG responses were against 4 predominant regions in HIV-1 gp120 Env (C1, V2, V3, and C5). In 20 subjects measured, 100% had IgG responses against the C1 region. Of interest, functional antibody responses (ADCC) were also elicited by RV144 and these responses were epitope mapped to the C1 region (Ferrari, Haynes 2011). RV144 also elicited IgA antibody responses (~60%) and in ~1/3 of these vaccines, the response targets the C1 (CRF1) epitope (as identified by IgG HIV1 Env microarray). The plasma IgA response (Env magnitude and breadth) was a primary variable in the RV144 case control study, along with 5 other primary variables. The anti-Env IgA response showed a statistically significant direct correlation with infection. In further analyses of the secondary variables, the IgA response to several Env (most significantly A1.Congp140) and to the C1 CRF-1 peptide were more strongly correlated with infection. In further analysis of the primary variables, the IgA response was shown to interact (as a variable) with 4 of the other primary variables to directly increase the relative risk of infection. Thus, this work indicates that the anti-IgA Env C1 epitope may

either directly contribute to virus replication or may serve to inhibit the protective effects of functional antibody responses (ADCC, neutralization). The C1 epitope chosen for analysis in the case control study was based on the predominance of the IgG response to the C1 region. Therefore, IgA antibodies targeting C1 could directly block functional IgG binding to this region.

A strategy going forward would be to delete the C1 epitope that both IgG and IgA are targeting, while maintaining potentially important epitopes for other functional antibodies (ie ADCC directed against V2 targets rather than C1). Another strategy would be to block the C1 region by mutations in the C1 region so that the epitope does not induce antibodes to that region.

EXAMPLE 3

The RV144 vaccine trial in Thailand demonstrated an estimated vaccine efficacy of 31.2% in preventing HIV-1 acquisition in a heterosexual population (37). A previous trial in high risk intravenous drug users (IVDU) using AIDSVAX B/E® (4, 5, 16, 27) did not show protection (15, 36). The RV144 vaccine is comprised of a canarypox ALVAC prime with the E.92TH023 gp120 membrane anchored insert and AIDSVAX B/E® gp120 boost. This vaccine regimen induced Env antibody responses in 98.6% and CD4 T cell responses in 90.1% of vaccinated subjects (15), and induced Tier 1 virus-, but not Tier 2, neutralizing antibodies (37). The majority (89%) of breakthrough infections in RV144 vaccinees were subtype CRF01_AE (15) suggesting that the immune responses elicited against the clade E gp120 A244 Env protein were involved in lowering infection risk of HIV-1 acquisition.

The target of potentially protective or neutralizing antibodies is the trimeric Env spike, which is sparsely present on HIV-1 virions (30, 47). Neutralizing epitopes presented on gp120 may be masked by glycans, may be

exposed only transiently following receptor/co-receptor engagement, or may depend strongly on intact quaternary structures (19, 22, 26). A major hurdle in HIV-1 Env protein vaccine design is the preservation of the structural properties in soluble versions of Env proteins that mimic those on intact viruses (20), particularly when the Env gp120 proteins are expressed as monomers. Furthermore, the gp120 inner domains and the co-receptor binding epitopes can be occluded in dimeric (and probably misfolded) forms of recombinant gp120, which are often produced by mammalian cells together with gp120 monomers (13). Thus, optimal presentation of neutralizing epitopes on gp120 depends critically on its conformational state.

A number of gp120 V2 antibodies have been described that bind well to conformational epitopes on the scaffolded murine leukemia viruses gp70-HIV-1 V1V2 and to other recently described V1V2 scaffold proteins (18, 24, 32, 34, 35). A clonal lineage of V2V3 conformational gp120 broadly neutralizing antibodies (bnAbs) CH01-CH04, that show blocking by the prototype V2V3 conformational gp120 mAb, PG9 and PG16, bind only to a subset of gp120 monomers including clade E A244 gp120 (7). Although previously described as quaternary structure specific mAbs, with preferential binding to membrane anchored trimeric HIV Env (45), PG9 and PG16 bnAbs can bind to both monomeric and trimeric gp140 (9), and as well to monomeric gp120 (7). The PG9 bnAb has been crystallized bound to a V1V2 scaffold protein and shown to bind primarily to the V1V2 C–β strand and to adjacent glycans (32). Thus the V2V3 conformational bnAbs of which PG9 is a prototype, bind to a conformational peptidoglycan epitope of gp120 V1V2 (32). The RV144 Env, A244-rgp120 (7), a component of AIDSVAX B/E® (4, 27) is among the rare monomeric gp120s to which the CH01-CH04 and PG9 antibodies bind. The unmutated ancestor antibodies of the CH01-CH04 clonal lineage also bind A244 gp120 monomers, with an affinity within the range appropriate for B-

cell receptor triggering (7). One unique feature of the RV144 protein gp120 design was that the proteins were constructed with a HSV gD peptide tag and an 11-amino acid (aa) deletion at the gp120 N-terminus (4, 27). Could features of the A244-rgp120 design have contributed to enhanced exposure of V1V2 conformational epitopes on the vaccine proteins? If so, induction of antibodies with specificity for the more prominently exposed epitopes might be observed in RV144 vaccinees. A recently conducted analysis of the RV144 case-control study showed that antibody responses were to the C1, V2, V3 and C5 gp120 regions and that high levels of IgG antibodies to a V1V2 scaffold protein correlated inversely with HIV-1 infection rate in vaccinees (21). Thus, one hypothesis is that addition of the gD tag and/or the Δ11 mutation provided enhanced presentation of certain gp120 epitopes and contributed to the induction of V1V2 antibody responses in RV144 vaccinated subjects.

The studies described below demonstrate that the RV144 gp120 protein immunogen, A244-rgp120, was associated with enhanced antigenicity for C1, V2 and V2V3 conformational epitopes, and that the gp120 N-terminal deletion (Δ11), without the inclusion of HSV gD tag, was sufficient for both the enhanced antigenicity and immunogenicity in humans.

EXPERIMENTAL DETAILS

Proteins and Antibodies

RV144 vaccine immunogen proteins (Table 1 below) A244-rgp120 and MN-rgp120 were produced originally by Genentech, Inc., further developed by VaxGen Inc., and supplied for this study by GSID (Global Solutions for Infectious Diseases, South San Francisco, CA). A244 gp120, A244 gDΔ11, A244 Δ11, A244 gD N160K, MN gDΔ11 and MN gp120 were expressed in 293T cells (Table 1, Figure 27) and lectin-affinity purified (28) followed by size exclusion

chromatography on a Superdex 200 FPLC (GE Healthcare) to homogeneity for monomeric gp120. Expression of additional gp120 proteins with N-terminal deletion included the subtype B (63521 and 6240) and subtype C (C.089C, C.1086) Env proteins described earlier (7, 25, 42). N-terminal deletion for all Env gp120 involved 11 aa, except for C.1086 in which the corresponding shorter N-terminal segment (7aa) of the mature Env protein was deleted. Synagis (MedImmune, Gaithersburg, MD), anti-RSV mAb, was used as a negative control. The C1 mAb A32 and the V3 mAb 19b were supplied by James Robinson (Tulane University, LA). CH01 mAb as previously described was isolated, and its unmutated ancestor antibodies inferred, from IgG+ memory B cells of a broad neutralizer subject (7). V2 mAb 697D, 830A, 2158, and 697D Fab were provided by S. Zolla-Pazner (New York University, NY) and described previously (17, 18). V2V3 conformational/quaternary mAbs PG9 and PG16 were provided by Dennis Burton (IAVI, and Scripps Research Institute, La Jolla, CA) and Peter Kwong (NIH, Bethesda, CA).

Surface plasmon resonance (SPR) kinetics and Dissociation constant (K_d) measurements.

Env gp120 binding K_d and rate constant measurements were carried out on BIAcore 3000 instruments as described earlier (1-3). Anti-human IgG Fc antibody (Sigma Chemicals) was immobilized on a CM5 sensor chip to about 15000 Response Unit (RU), and each antibody was captured to about 50-100 RU on three individual flow cells for replicate analysis, in addition to one flow cell with the control Synagis mAb on the same sensor chip. Non-specific binding of Env gp120 to the control surface and/or blank buffer flow was subtracted for each mAb-gp120 binding interactions. Antibody capture level, which ranged from 50-100 RU, on the sensor surface was optimized for each mAb to minimize

rebinding and any associated secondary effects. 697D Fab was directly coupled via amine coupling chemistry to the sensor surfaces, and Env gp120 was flowed and data collected as above. All curve fitting analyses were performed using global fit of multiple titrations to the 1:1 Langmuir model. Mean and standard deviation (s.d.) of rate constants and K_d were calculated from at least three measurements on individual sensor surfaces with equivalent amounts of captured antibody. All data analysis was performed using the BIAevaluation 4.1 analysis software (GE Healthcare).

Isolation and Purification of IgG from Plasma.

Total IgG was isolated from individual RV144 vaccine recipient plasma samples using Protein G resin pre-packed into 96-well depletion plates (GE Healthcare) as previously described (31). Plasma was diluted 2-fold with TBS, pH 7.5, and 200 µl of the diluted sample was added per well. The plates were incubated at room temperature, with shaking, for one hour. The unbound fractions were removed by centrifugation at 700 x g for 3 minutes. Wells were then washed 3 times with 200 µl of TBS to remove loosely bound material. The IgG bound to the resin was eluted with 200 µl of 2.5% glacial acetic acid, pH 2.53, and immediately neutralized with 120 µl of 1M Tris-HCL pH 9.0. The eluted IgG fractions were concentrated using Amicon Ultra centrifugal filters (Millipore) with a 30kDa cut-off. The sample volume was reduced to 50 µl by centrifugation at 14,000 x g in a microcentrifuge pre-cooled to 4 °C. A buffer exchange was then performed using 2.5 volumes of PBS, pH 7.5. The concentrated IgG was diluted to the desired volume with PBS and assayed for protein concentration using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific) using the IgG reference setting.

Binding Antibody Multiplex Assays for anti-Env IgG were performed as previously described (41). Briefly, antibody measurements from vaccine plasma (1:200 dilution) were acquired on a Bio-Plex instrument (Bio-Rad) and the readout was expressed as mean fluorescent intensity (MFI) and concentration (µg/ml) based on a HIVIG standard curve. Positive and negative controls were included in each assay to ensure specificity and for maintaining consistency and reproducibility between assays. The preset assay criteria for sample reporting were: coefficient of variation (CV) per duplicate values for each sample were ≤15% and >100 beads counted per sample. To control for Env protein performance, the positive control titer (HIVIG) included on each assay had to be within +/- 3 standard deviations of the mean for each antigen (tracked with a Levy-Jennings plot with preset acceptance of titer (calculated with a four-parameter logistic equation, SigmaPlot, Systat Software).

Surface Plasmon Resonance (SPR) measurements of Plasma IgG Avidity.

RV144 vaccine recipient IgG avidity was measured on a BIAcore 4000 instrument (BIAcore/GE Healthcare) using the multiplex array format (1x16) in which each IgG sample was flowed over duplicate spots of 8 different Env gp120 antigen surfaces. Using a Series S CM5 sensor chip (BIAcore/GE Healthcare) gp120 proteins were amine coupled in duplicate on 16 different spots on four flow channels of the chip. The negative control mAb Synagis was flowed over each surface, and the signal was used to subtract out non-specific interactions with each individual spot. Each of the above listed gp120 Env proteins, including the vaccine immunogens A244-rgp120 and MN-rg120, were immobilized to about 6000-8000 RU using amine coupling chemistry as described earlier (1-3) . Antigen surface activity was monitored using the C1 mAb A32 as positive control and an irrelevant anti-RSV (Synagis) mAb as negative control. V1V2 mAb CH01,

which is sensitive to N160K substitution, was used as a negative control for antigen spots with A244gD/N160K gp120. An anti-gD Fab was used to monitor binding to the gD peptide tag in Env gp120 with gD and to select IgG samples with low gD reactivity for mAb blocking studies. The IgG samples (n=97) from vaccinee plasma at week 26 (two weeks following final immunization) and week 0 were diluted in PBS to 200μg/mL and injected over each of the flow cells with replicate spots (2x) at 10μL/min for an association time of 120 s and a dissociation time of 600s. A random selection of IgG samples collected at visit 0 from 20 vaccinees was also included. Following each binding cycle, surfaces were regenerated with a short injection (20s) of glycine, pH2.5. Each surface activity was monitored by including A32 mAb (20μg/mL) injection every 20 cycles of IgG samples and surface decay of A32 binding over the entire experimental run was used to normalize binding signal of plasma IgG samples. Non-specific binding of the negative control mAb was subtracted from each IgG sample binding data. Data analyses were performed with BIAevaluation 4000 and BIAevaluation 4.1 software (BIAcore/GE Healthcare) as described earlier for Biacore 3000 (2) and Biacore A100 (38) data analysis respectively. Kinetic binding responses were measured by averaging post-injection response unit (RU) over a 20s window, and dissociation rate constant, k_d (s-1) was measured during the post-injection/buffer wash phase (after the first 20s to allow stabilization of signal) following curve fitting to a Langmuir dissociation equation. The majority of IgG bound with a relatively slow dissociation rate ($<10^{-3}$ s^{-1}), and the previously described method for BIAcore A100 ranking of dissociation rates in complex or polyclonal samples as a ratio of response units measured as binding late and stability late (23, 38) was modified to include binding response and dissociation rate constant measurements and as described earlier (14, 21). A relative avidity binding score was calculated for each IgG sample as follows,

Avidity score (RU.s) = Binding Response (RU)/k_d, s-1, with higher binding responses and slower k_d as an indicator of higher affinity interaction (14).

Antibody blocking assay.

Antibody blocking using an ELISA assay was carried out as described earlier (3, 21). 384 well ELISA plates (Costar #3700) were coated with 30ng/well Env overnight at 4 °C and blocked with assay diluent (PBS containing 4% (w/v) whey protein/ 15% Normal Goat Serum/ 0.5% Tween20/ 0.05% Sodium Azide) for 1 hour at room temperature. All assay steps, were conducted in assay diluent (except substrate step) and incubated for 1 hour at room temperature followed by washing with PBS/0.1% Tween-20. Sera were diluted 1:50 and incubated in quadruplicate wells. For CD4 (binding site) blocking assays, 10µl of a saturating concentration soluble CD4 (Progenics Pharm Inc.) was added following serum incubation step. 10µl of biotinylated target mAb was added at the EC50 (determined by a direct binding of biotinylated-mAb to JRFL gp140). Biotin-mAb binding was detected with streptavidin-alkaline phosphatase at 1:1000 (Promega V5591) followed by substrate (CBC buffer + 2mM $MgCl_2$ +1mg/ml p-npp [4-Nitrophenyl phosphate di(2-amino-2-ethyl-1,3-propanediol) salt]), and plates were read at 405nm at 45 minutes. Quadruplicate wells were background subtracted and averaged. Percent inhibition was calculated as follows: 100-(sera triplicate mean/no inhibition control mean) x100.

SPR antibody blocking using RV144 vaccinee IgG samples was measured on BIAcore 3000 instruments. Env immunogen A244 Δ11 gp120 was immobilized on all flow cells of a CM5 sensor chip to about 5,000-6,000 RU using standard amine coupling chemistry. Blocking antibodies were sequentially injected at predetermined concentration to capture near saturation. A zero baseline was set, and RV144 patient IgGs were injected at 10µL/min for an

association time of 180s and a dissociation time of 600s. Kinetic binding responses were measured 15s after the end of the injection. The IgG samples (n=119) with high and mid-level binding (>80 Response Units) to A244 Δ11 gp120 were selected from a panel of week 26 (two weeks following final immunization) plasma samples that included infected vaccinee (n=41) and uninfected vaccinee (n=205) groups. Randomly selected Visit 0 vaccinee IgG samples (n=19) with no binding to A244Δ11 gp120 were included to assess non-specific interactions. Anti-gp41 mAb 7B2 was used as a negative control blocking antibody. Test antibodies included A32 (C1 region), PG9 and CH01 (V2V3 Conformational/Quaternary), 2158, 697-30D, and 830A (V2), and 19b (V3) mAbs. Following each binding cycle, surfaces were regenerated with a short injection (10-15s) of either Glycine-HCl pH2.0 or 100 mM phosphoric acid. Blocking percentages were calculated from the ratio of binding response after negative control 7B2 mAb block to the binding response after test antibody block [% block = (1 – (RU after 7B2 block/RU after test mAb block))*100].

Isolation of antibodies from RV144 vaccinee plasma memory B cell.

Monoclonal antibodies CH51 and CH54 were isolated from circulating IgG+ memory B cells obtained from a vaccine recipient (subject 210884) as previously described ((7). Briefly, CD2(neg), CD14(neg), CD16(neg), CD235a(neg), IgD(neg) and IgG(pos) cells were isolated from frozen PBMCs using magnetic activated cell sorting (Miltenyi Biotec, Auburn, CA) and resuspended in complete medium containing 2.5 μg/ml oCpG ODN2006 (tlrl-2006, InvivoGen, San Diego, CA), 5μM CHK2 kinase inhibitor (Calbiochem/EMD Chemicals, Gibbstown, NJ) and EBV (200 μl supernatant of B95-8 cells/10^4 memory B cells). After overnight incubation in bulk, cells were distributed into 96-well round-bottom tissue culture plates at a cell density of 8 cells/well in presence of

ODN2006, CHK2 kinase inhibitor and irradiated (7500 cGy) CD40 ligand-expressing L cells (5000 cells/ well). Cells were re-fed at day 7 and harvested at day 14. Cultures were screened for binding to HIV-1 gp120 envelope glycoproteins contained in the vaccine formulation (Bonsignori et al. manuscript in preparation). Cells from positive cultures were single-cell sorted and PCR was performed as previously described (29, 46). Briefly, reverse transcription (RT) was performed using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) and human constant region primers for IgG, IgA1, IgA2, IgM, IgD, Igκ, Igλ; separate reactions amplified individual VH, Vκ, and Vλ families from the cDNA template using two rounds of PCR. Products were analyzed with agarose gels (1.2%) and purified with PCR purification kits (QIAGEN, Valencia, CA). Products were sequenced in forward and reverse directions using a BigDye® sequencing kit using an ABI 3700 (Applied Biosystems, Foster City, CA). Sequence base calling was performed using Phred (10, 11); forward and reverse strands were assembled using an assembly algorithm based on the quality scores at each position (33). The estimated PCR artifact rate was 0.28 or approximately one PCR artifact per five genes amplified. Ig isotype was determined by local alignment with genes of known isotype (40); V, D, and J region genes, CDR3 loop lengths, and mutation rates were identified using SoDA (44) and data were annotated so that matching subject data and sort information was linked to the cDNA sequence and analysis results. Isolated Ig V(D)J gene pairs were assembled by PCR into linear full-length Ig heavy- and light-chain gene expression cassettes (29) and optimized as previously described for binding to the Fcγ-Receptors (39). Human embryonic kidney cell line 293T (ATCC, Manassas, VA) was grown to near confluence in 6-well tissue culture plates (Becton Dickson, Franklin Lakes, NJ) and transfected with 2 μg per well of purified PCR-produced IgH and IgL linear Ig gene expression cassettes using Effectene (Qiagen). The supernatants were

harvested from the transfected 293T cells after three days of incubation at 37°C in 5% CO_2 and the monoclonal antibodies were purified as previously described (29).

RESULTS

Expression of gp120 variants of the RV144 trial protein immunogens

To address the effect of the modifications on the RV144 clinical trial protein Env gp120 antigenicity, the immunogen proteins A244-rgp120 and MN-rgp120 were expressed in 293T cells, with no modifications (A244 gp120 and MN gp120); with only the 11 aa N-terminal deletion (A244Δ11 gp120); or, as in the RV144 Env immunogens, with both the HSV gD peptide tag and the N-terminal deletion (A244 gDΔ11 gp120, MN gDΔ11 gp120) (Figure 27, Table 1). The E clade 92TH023 gp120 was also expressed either with no modification (92TH023 gp120) or with both Δ11 deletion and gD tag (92TH023 gDΔ11 gp 120, Figure 27). These Env proteins were compared for gp120 monomer expression and for their binding to mAbs that recognize conformational epitopes on gp120.

Presentation of gp120 conformational epitopes on RV144 vaccine Env gp120 proteins with the gD tag and Δ11 deletion

It has been reported previously that one component of the RV144 Env immunogen, A244-rgp120, binds to mAbs with specificity for the gp120 conformational V1V2 epitopes (7, 32). The CH01-CH04 lineage V2V3 bnAbs and PG9 bnAb bound to A244 gp120 with $_{D}$s ranging from 100-300nM (7). Since PG9/PG16 mAbs bind preferentially to native trimers (45) and only to rare gp120 monomers (7), the binding of PG9 and CH01 mAbs suggests that the RV144 Env gp120 might show enhanced expression of conformational epitopes in the V1V2 loops.

It was found that other conformational epitopes were also presented on both RV144 Env A244- rgp120 and MN-rgp120; the V2 mAb 697-D that does not bind to linear V2 peptides (18) bound to MN- and A244-rgp120 with K_ds of 477nM and 318nM respectively (Figure 28) The gp120 C1 mAb A32, which binds to the surface of transmitted/founder infected CD4 T cells and mediates ADCC (12), also bound strongly to the two RV144 Env gp120 proteins, with a relatively higher affinity for MN-rgp120 (Figure 28). The K_d of the V3 mAb 19b for MN-rgp120 was about 5-fold lower than that of A244-rgp120 but was within the range reported for other V3 mAb binding to Env gp120 proteins (43). Thus, both RV144 vaccine Envelope gp120 immunogens expressed conformational epitopes within the C1, V2 and V2V3 regions of gp120. The presentation of gp120 variable loop conformational epitopes and the recent association of conformational V1V2 antibodies with a lower rate of HIV-1 infection in RV144 (21) raised the question whether the two RV144 vaccine Env modifications - inclusion of the HSV gD peptide tag and/or the N-terminal Δ11 deletion might have led to the enhanced exposure of conformational epitopes within the C1 and V1V2 regions.

The N-terminal 11 aa deletion (Δ11) in A244 gp120 reduces dimer formation

Expression of recombinant gp120 produces a substantial amount of disulfide-linked gp120 dimer, in which gp120 inner domain epitopes and the co-receptor binding surface are occluded (13). To determine the effect the two modifications on A244 gp120 might have on protein expression, a comparison was first made of the oligomerization states of the three different A244 gp120 proteins. In reducing SDS-PAGE gel, A244 gp120 proteins migrated as single bands of the expected size; in non-reducing conditions, they gave a mixture of bands that corresponded to monomers and dimers of gp120 (Figure 29A). Size exclusion chromatography showed that A244 gp120 (Figure 29B) had more

dimer (58 ±1%) than monomer (38 ± 1%). In contrast, the monomer fraction of Env A244 Δ11 gp120 was enriched by almost two-fold (66 ± 1%) and the dimer fraction was correspondingly reduced (30 ± 1%; monomer to dimer ratio= 2.2:1 (Student t test, $p < 0.001$ for monomer fractions in A244 gp120 and A244Δ11 gp120 ; Figure 29C). The inclusion of the HSV gD peptide, in addition to the Δ11 modification, in A244 gDΔ11 (Figure 29D) did not further improve monomer enrichment and gave a similar ratio of monomer to dimer (63 ± 1 and 33 ± 1% respectively) as did A244 Δ11 (Student t test, $p=0.11$ for monomer fractions in A244gDΔ11 and A244Δ11 gp120). The amount of higher order oligomers or aggregates was the same for all three expressed proteins (about 3-4%) (Figure 29). A similar profile was observed for the 92TH023 gp120 proteins, with a higher proportion of monomers (~65%) in 92TH023 gp120s with Δ11 and gD tag than without any modifications (~38%). MN gp120 expressed with Δ11 and gD (MN gD gp120) or with no modifications (MN gp120) gave similar proportions of dimers (34% and 31% respectively). Additional gp120 constructs derived from different clades including clade B (625321 and 6240) and clade C (C.089C) were designed to contain their original signal peptide and deletion of the first 11 or 7 (C.1086) amino acid residues, and produced in 293 cells by transient transfection. For each of the above proteins it was found that inclusion of Δ11 alone yielded predominantly monomers, as detected by SDS-PAGE under non-reducing conditions (data not shown) and greater than 90% monomers in gel filtration chromatography analysis. Thus, the N-terminal Δ11 modification alone resulted in markedly lower amounts of gp120 dimer formation when Env proteins were expressed in mammalian cells.

Enhanced binding of conformational V2 antibodies and V2V3 bnAbs to A244 Δ11 gp120 monomers

Monomers of each of the A244 gp120 proteins (A224, A244 Δ11, A244 gDΔ11) were purified to homogeneity following removal of dimeric and aggregate fractions by size exclusion chromatography (SEC). Following SEC fractionation, the monomeric gp120 proteins were stable and did not redistribute into dimer or aggregate fractions. Each of the three purified gp120 monomers bound to CD4 and showed CD4 induced (CD4i) epitope upregulation as assessed by 17b mAb binding (data not shown). A comparison of the binding of the size-fractionated monomeric and dimeric A244 gp120 showed that the binding of the C1 mAb A32 was markedly reduced and the binding of the V2 mAb 697D was near completely lost upon dimer formation. This is consistent with the previously reported results (13) that the V1V2 loop and the N- and C-termini are involved in gp120 dimer formation, and that the epitopes on the Env inner domain are occluded in gp120 dimers.

Using a panel of antibodies with specificities that included conformational C1, V2 and V2V3 epitopes, a comparison was made of mAb binding K_d and rate constants for each of the monomeric clade E A244 gp120 proteins, to assess whether the Δ11 and/or gD tag had any effect on Env antigenicity (Figure 30, Table 2). It was observed that inclusion of Δ11 had no effect on exposure of the V3 loop, since the V3 mAb 19b bound with similar K_d and kinetic rate constants to each of A244 gp120 proteins (Table 2, Figure 30A). The ADCC mediating C1 mAb A32 (12), however, bound with a 9-fold and 6-fold higher affinity to A244 Δ11 and A244 gDΔ11 respectively than to A244 gp120 (Table 2, Figure 30B). Similarly, the conformational V2 mAb 697-D (18) bound to A244 gDΔ11 and to A244 Δ11 with nearly an order of magnitude higher affinity (Figure 30D, Table 2) than to A244 gp120 (K_d = 218, 157 and 1465nM,

respectively). These differences were also observed using the Fab fragment of the V2 conformational mAb 697-D, which bound to A244 gDΔ11 with 8-fold higher affinity than to unmodified A244 gp120 (K_d = 690 and 5700nM respectively). While the dissociation rate constants were similar, the ka (on-rate) was nearly 10-fold higher for binding of 697-D to A244gDΔ11 than to A244 gp120. Two other conformation-dependent V2 mAbs, 2158 and 830A, had higher affinities for A244 gp120 than did 697-D, but both also had higher affinities for A244 with gD and Δ11 modifications (Table 2). In particular, V2 mAb 2158 bound to both A244gDΔ11 and A44Δ11 gp120 with K_d ~ 3.7 nM and had approximately 3-fold lower affinity for unmodified A244 gp120 (K_d =11.2nM) (Table 2). Likewise, V2 mAb 830A bound to A244 Δ11gD and to A244Δ11 gp120 about 5-fold more avidly than it did to unmodified A244 gp120 (Table 2). Finally, the affinities of A244Δ11 (K_d=278nM) and A244 gDΔ11 (K_d = 317nM) gp120 for V2V3 bNab CH01 were about 5-fold higher than for unmodified A244 gp120 (K_d = 1638nM: Figure 30D, Table 2) and modified Env affinities for PG9, about 3-fold higher (K_d = 48nM, 53nM, and 183nM for A244Δ11, A244 gDΔ11, and unmodified A244, respectively; Figure 30E, Table 2).

These results suggest that the conformational V2 and V2V3 epitopes recognized by mAbs 697-D, and CH01 and PG9 are better exposed or conformationally more stable on A244 gp120 proteins with the Δ11 modification and without the inclusion of HSV gD. In most cases, the differences in K_d are due to differences in the association rates, k_a, with roughly 10-fold faster rates of both 697D Fab and CH01 mAb for association with gp120 with a Δ11 modification (Table 2). The contribution of ka to the differences in K_d support the notion that exposure of particular conformational epitopes is a critical factor in the enhanced antigenicity.

RV144 A244 Δ11 Env is antigenically reactive with CH01-CH04 clonal lineage unmutated antibodies

Two unmutated ancestor antibodies (UAs) of the mAb CH01-VH04 clonal lineage, CH01_RUA1 and CH01_RUA2, have recently been shown to bind to the RV144 vaccine trial immunogen A244-rgp120 (8). Binding of the CH01-04 UAs to A244Δ11 gp120 was compared with their binding to unmodified A244 gp120. As observed with the mature CH01 mAb, CH01_RUA1 and CH01_RUA2 bound to A244 Δ11 with about 4 to 5-fold higher affinity than to A244 gp120 (Figure 31); the two CH01 UAs had roughly equivalent K_ds for the A244 gp120Δ11 proteins. As observed for the mAbs, the higher affinity of CH01 UAs for A244Δ11 was largely due to relatively faster association rates; the dissociation rates were similar (Figure 31). This result was interpreted to imply that the Δ11 modification in A244 gp120 results in enhanced exposure of the V2V3 conformational epitopes to the UAs, just as it does for the CH01 lineage mature antibodies.

HSV gD and Δ11 modifications have small effects on the antigenicity of MN gp120 and 92TH023 gp120

The results showing the effect of Δ11 modifications on antigenicity of A244 gp120 raises the question whether similar modification of either MN gp120 or the 92TH023 gp120 would influence their antigenicity. The antigenicities of MN and TH023 gp120 Δ11gD were compared with those of the unmodified proteins. The modifications enhanced binding of the C1 mAb A32 and of the V2 mAb 697-D to MN or 92TH023 gp120 by 2-3fold. PG9 binding to MN and 92TH023 gp120 was unaffected by the presence of gD or Δ11 modification. Thus, in contrast to A244 gp120, the gD and Δ11 modifications had either no effect

(conformational V2V3) or a much weaker effect (conformational V2, C1) on the antigenicity of MN or 92TH023 gp120s.

An assessment was made of the effect of an N-terminal Δ11 deletion and gD tag on a clade C Env gp120 (1086). 1086 gp120 monomers with a 7aa deletion of the same region as in A244 gp120 (1086 Δ7 gp120) had the same affinity for A32 (C1), VRC01 (CD4bs) and 19b (V3) mAbs as had the unmodified protein. 1086 gp120 did not bind to PG9 or CH01, as previously reported (7). Binding of the V2 mAbs 697-D and 830A was likewise unaffected by modification. Thus, neither the gD addition nor the N-terminal deletion had any effect on the antigenicity of this clade C gp120 for the antibodies tested. That is, the effects of the Δ11 deletion in the RV144 immunogen apparently depended on the particular gp120 tested.

Plasma IgG from RV144 vaccinees bind with higher avidity to A244gD Δ11 gp120

The effect of Δ11 modification in enhancing the antigenicity of gp120 conformational epitopes to C1, V2 and V2V3 raises the question, whether antibodies induced by the RV144 immunogen (A244gDΔ11) also bind more avidly to A244 Δ11 than to A244 gp120. IgG was purified from RV144 vaccinee plasma taken two weeks after the final ALVAC/AIDSVAX B/E immunization (week 26) and their relative avidity scores were measured for each of the A244 gp120 proteins in a SPR binding assay. Compared to placebo and pre-vaccination visit 1 IgG samples (no binding), IgG samples from the week-26 vaccinee group bound A244gDΔ11 gp120 with avidity scores that ranged over 2-orders of magnitude (Figure 32A). The mean avidity scores for both A244 Δ11 and A244 gDΔ11 were, however, significantly higher ($p<0.001$) than that for A244 gp120 (Figure 32A). In the HIV-1 binding antibody multiplex assay, a significant difference was found; RV144 plasma IgG showed tighter binding to A244 Δ11 gp120 than did A244

gp120 ($p<0.001$; Figure 32B). Thus the RV144 vaccine gave rise to antibodies with higher magnitude and avidity for A244 gp120 with the Δ11 modification than for unmodified A244 gp120.

Conformational antibodies to C1, V2 and V2V3 block RV144 induced IgG binding to A244 Δ11 gp120

To assess the specificity of the antibodies induced by RV144 vaccine gp120 immunogens, a measurement was made of the relative level of blocking of vaccinee IgG binding by a panel of mAbs, including those that showed higher affinity for A244 Δ11 gp120. As shown in Figure 32C, the binding of vaccinee IgG was blocked by each of C1 (A32), V2 (697-D) and the V2V3 (CH01) bnAb, with the strongest blocking observed with A32 (66%). For the V2 epitope, V2 mAbs 2158 and 830A were also used, which show varying levels of overlap with each other; 830A strongly blocks all other V2 mAbs (data not shown). Among these V2 mAbs, blocking of RV144 IgG was strongest with 697-D and 830A, both of which have enhanced binding to A244 with Δ11 modifications (Table 2).

Among the two conformational V2V3 gp120 bnAbs, we found no blocking of RV144 IgG binding by PG9, but detectable blocking (24.6%) by CH01 (Figure 32C). We also determined blocking of plasma antibodies in ELISA assays for antibodies that inhibit binding of biotinylated mAb A32 and of soluble CD4. In these assays, the mean blocking of A32 and sCD4 binding was 39.6% and 13% respectively (Figure 32D).

These results suggest that the RV144 vaccine induced a relatively larger proportion of antibodies directed against the conformational C1 (A32) epitope than against epitopes in or around the conformational V2 and V1V2 epitopes recognized by the mAbs 697-D, 830A and the bnAb CH01.

Monoclonal antibodies from RV144 vaccinees recognize epitopes enhanced on A244gDΔ11 gp120 monomers

Using previously described methodologies for isolating antibodies from memory B cells (7), two IgG antibodies, CH51 and CH54, were isolated from circulating IgG+ memory B cells of an RV144 vaccine recipient (subject 210884). A32 blocked binding of both CH51 and CH54, suggesting that RV144 derived mAbs bound to epitopes that overlap with the C1 conformational epitope of A32. The overall K_ds of CH51 and CH54 mAbs for binding to A244gDΔ11 were higher than that of the C1 mAb A32 (Figure 33), but both of these RV144 mAbs bound to A244gDΔ11 and A244 Δ11 with an order of magnitude lower K_d than they did to A244 gp120 (Figure 33A, 33B). Thus, the RV144 vaccinee-derived antibodies (CH51 and CH54) with A32-like specificity showed tighter binding to A244gp120 with the N-terminal deletion and mirrored the overall enhanced avidity detected in RV144 plasma samples with A244gDΔ11.

Summarizing, the RV144 trial showed estimated vaccine efficacy to be 31.2%. Future HIV-1 vaccine efficacy trials will therefore require an improved immunogen design, and analysis of the RV144 immunogens is an important first step. In the work done above, a study has been made of the effects of gp120 design on antigenicity and immunogenicity of the immunogens used in the RV144 HIV-1 vaccine efficacy trial. It has been demonstrated that deletion of the N-terminal amino-acid residues of the A244 gp120 and, to a lesser degree, of the MN gp120, both of which were used as boosts in the trial, enhanced the antigenicity of gp120 conformational epitopes to C1 and V1V2 regions. The enhanced epitopes were immunogenic in the human vaccinees, and they appear to have induced immune responses with higher avidity for these conformational epitopes than for the same epitopes on the unmodified immunogen A244 gp120. It has also been shown that a gD tag, introduced into the AIDSVAX B/E rgp120 as

part of early expression and purification strategies (27), does not contribute to the enhancement, as long as the N-terminal deletion is retained. Antigenic enhancement by the N-terminal deletion, Δ11, was more pronounced with A244 (clade E) than with MN (clade B), 92TH023 (clade E), or 1086C (clade C) gp120 proteins, suggesting that these effects may depend strongly on the particular gp120 vaccine immunogen.

The higher proportion of disulfide-linked dimers in the preparations of unmodified A244 rgp120 than in those with a Δ11 deletion (with or without the gD-tag replacement) suggests that the principal effect of removing the N-terminal residues has been to enhance the reliability of folding in the ER. Correctly folded gp120 has no unapired cysteines, and any inter-chain disulfides must form at the expense of correct intra-chain pairings. That is, at least part of the protein must be misfolded for disulfide-linked dimers to form at all. Even the monomeric protein in any preparation may be conformationally heterogeneous; the proportion of dimer will tend to reflect the degree of misfolding within the monomer population. Because the consequences of the deletion depend on the rest of the gp120 sequence -- it had a less marked effect on rgp120 from other isolates -- its influence on folding is probably non-specific. The N-terminal 11 residues, which immediately follow the signal-peptide cleavage site in the polypeptide chain, presumably interact with gp41 near the membrane-proximal part of the molecule, and they are unlikely to influence the conformation of most neutralizing epitopes directly, but rather through their effect on the yield of correctly folded protein.

Two observations show that the upregulation of C1, V2, and V1V2 epitopes on A244 gp120 was relevant to the antibody responses induced in the RV144 trial. First, RV144 vaccinee antibodies have been identified that recognize these epitopes in SPR and ELISA blocking assays, and human mAbs have been

isolated from RV144 vaccinees that are blocked in their binding to A244 gp120 by the conformational C1 antibody, A32 (6, 21, 48). Moreover, mAb 697-D, which binds to A244 gp120 and the gp70V1V2 Case A2 clade B scaffolded protein, binds to A244 Δ11 gp120 nearly ten-fold more tightly than it does to A244 gp120 with no modifications. Second, the RV144 induced plasma antibody response had a higher avidity for A244 Envs with gD Δ11 or with Δ11 alone than for their unmodified counterparts (Figure 32). The conformational V2 and V1V2 epitope specificities induced by the vaccine included those that could be blocked by mAbs CH01 and 697D, but not by mAb PG9 (Figure 32B). Although it has not yet been possible to rescue a V2 mAb against the conformational V2 or V1V2 epitopes selectively recognized by 697-D or CH01, the presence of plasma antibodies with specificities capable of blocking the binding of these mAbs to A244 gp120 has been demonstrated (Figure 32B). Furthermore, both the A244 gp120 mAbs isolated from RV144 vaccinees (CH51, CH54) bound A244 and MN gp120s, their binding was blocked by A32 and both mediated antibody dependent cellular cytotoxicity (ADCC) to HIV-1 AE_01 infected CD4 T cell targets. Binding of both of these A32-like mAbs (CH51 and CH54) to A244 gp120 was enhanced when the Δ11 deletion was introduced. These data strongly suggest that the observed Δ11-enhanced gp120 antigenicity of RV144 gp120 immunogens played a role in the induction of certain antibody types (C1, V2 and V1V2) in the RV144 vaccinees.

In a RV144 immune correlates analysis, plasma IgA Env antibodies correlated directly with infection rate while V1V2 antibodies correlated inversely with infection rate (21). While ADCC alone did not correlate with infection rate in this analysis, ADCC in the presence of low levels of IgA antibodies did correlate weakly with a lower infection rate, demonstrating an interaction between ADCC-mediating antibodies and high IgA levels for mitigation of any potential

protective ADCC response (21). The underlying mechanism of protection in the RV144 trial has yet to be elucidated—the immune correlates study has so far only identified antibody responses that correlate directly (plasma HIV-1 Env IgA) or inversely (plasma Abs binding to gp70-V1V2) with infection risk (21). Subsequent studies are required to determine if either of these antibody types are causal correlates or are surrogate markers of other factors.

Thus, it has been shown that the Δ11 N-terminal deletion on the gp120 Envs used in the AIDSVAX B/E boost of the RV144 HIV-1 vaccine trial enhanced gp120 epitope expression and augmented both antigencity and immunogenicity for the C1, V2 and V1V2 gp120 regions. The Δ11 deletion (with or without gD) leads to expression of a higher proportion of correctly folded recombinant protein, and the stability and conformational homogeneity of the immunogen is likely to have contributed substantially to its properties. The data suggest that careful attention to Env conformations and antigenicity will be critical when designing immunogens in future trials.

Table 1. Env gp120 protein constructs used in the study.

Env protein	gD peptide	N-terminal deletion
A244-rgp120*	+	11 aa
MN-rgp120*	+	11aa
A244 gp120	-	-
A244gDΔ11 gp120	+	11 aa
A244 Δ11 gp120	+	11aa
MN gp120	-	-
MN gDΔ11 gp120	+	11aa
92TH023 gp120	-	-
92TH023 gDΔ11 gp120	+	11aa
63521 Δ11 gp120	-	11aa
6240 Δ11 gp120	-	11aa
O89C Δ11 gp120	-	11aa
1086 Δ7 gp120**	-	7aa

* RV144 vaccine immunogen proteins A244-rgp120 and MN-rgp120 were produced by Genentech Inc., developed by VaxGen Inc and supplied by GSID.

** 1086 Env, in which the corresponding N-terminal segment (7aa) is shorter, was designed with Δ7 deletion.

Table 2. Dissociation and kinetic rate constants of antibody binding to E.A244 gp120 proteins.

Antibody/Protein	Rate Constants/K_d	E.A244 gp120	E.A244gDΔ11 gp120	E.A244Δ11 gp120
A32 (C1)	k_a ($x10^3$ $M^{-1}s^{-1}$)	76.8±11.4	134±14.0	222.6±20.4
	k_d ($x10^{-3}$ s^{-1})	0.47±0.05	0.133±0.017	0.15±0.03
	K_d (nM)	6.25±1.4	1.0±0.22	0.67±0.13
19b (V3)	k_a ($x10^3$ $M^{-1}s^{-1}$)	130.3±10.5	170.3±8.5	239.3±19.8
	k_d ($x10^{-3}$ s^{-1})	1.54±0.095	1.4±0.08	1.56±0.08
	K_d (nM)	11.8±0.21	8.24±0.23	6.54±0.38
697D (V2)	k_a ($x10^3$ $M^{-1}s^{-1}$)	4.9±1.1	24.9±5.6	26.75±0.71
	k_d ($x10^{-3}$ s^{-1})	7.0±1.98	5.24±0.54	5.18±0.6
	K_d (nM)	1465.3±317	217.6±45.7	156.7±34.0
830A (V2)	k_a ($x10^3$ $M^{-1}s^{-1}$)	21.8±3.1	41.1±1.9	59.9±4.6
	k_d ($x10^{-3}$ s^{-1})	0.22±0.06	0.07±0.003	0.088±0.01
	Kd (nM)	10.2±3.6	1.7±0.16	1.56±0.09
2158 (V2)	k_a ($x10^3$ $M^{-1}s^{-1}$)	16.4±0.98	28.7±1.0	36.5±1.8
	k_d ($x10^{-3}$ s^{-1})	0.19±0.04	0.10±0.03	0.13±0.04
	K_d (nM)	11.2±1.6	3.7±0.9	3.68±1.1

CH01 (V2V3)	k_a ($x10^3$ $M^{-1}s^{-1}$)	3.73±1.6	37.2±15.1	49.0±5.4
	k_d ($x10^{-3}$ s^{-1})	4.38±0.52	9.9±2.8	15.6±1.5
	K_d (nM)	1639±601	277.8±42	317±31.9
PG9 (V2V3)	k_a ($x10^3$ $M^{-1}s^{-1}$)	5.0±3.5	11.5±0.6	10.9±0.9
	k_d ($x10^{-3}$ s^{-1})	1.1±0.4	0.55±0.03	0.57±0.06
	K_d (nM)	183±44.0	48.1±0.15	52.6±2.9
VRC01 (CD4 bs)	k_a ($x10^3$ $M^{-1}s^{-1}$)	17.6±0.52	13.3±0.57	9.7±0.43
	k_d ($x10^{-3}$ s^{-1})	0.28±0.02	0.21±0.06	0.39±0.03
	K_d (nM)	15.7±1.7	15.8±3.9	36.7±1.4

Each of the rate constants and K_d values were derived from at least three measurements on individual flow cells of the same sensor chip or from binding data collected on a different sensor chips. The mean and s.d. of rate constants (k_a, k_d) and K_d values are reported for each antibody binding to the three different forms of monomeric E.A244 gp120 proteins.

REFERENCES CITED IN EXAMPLE 3

1. **Alam, S. M., M. McAdams, D. Boren, M. Rak, R. M. Scearce, F. Gao, Z. T. Camacho, D. Gewirth, G. Kelsoe, P. Chen, and B. F. Haynes.** 2007. The role of antibody polyspecificity and lipid reactivity in binding of broadly neutralizing anti-HIV-1 envelope human monoclonal antibodies 2F5 and 4E10 to glycoprotein 41 membrane proximal envelope epitopes. Journal of Immunology **178:**4424-35.
2. **Alam, S. M., M. Morelli, S. M. Dennison, H. X. Liao, R. Zhang, S. M. Xia, S. Rits-Volloch, L. Sun, S. C. Harrison, B. F. Haynes, and B. Chen.** 2009. Role of HIV membrane in neutralization by two broadly neutralizing antibodies. Proceedings of the National Academy of Sciences of the United States of America **106:**20234-9.
3. **Alam, S. M., R. M. Scearce, R. J. Parks, K. Plonk, S. G. Plonk, L. L. Sutherland, M. K. Gorny, S. Zolla-Pazner, S. Vanleeuwen, M. A. Moody, S. M. Xia, D. C. Montefiori, G. D. Tomaras, K. J. Weinhold, S. A. Karim, C. B. Hicks, H. X. Liao, J. Robinson, G. M. Shaw, and B. F. Haynes.** 2008. Human immunodeficiency virus type 1 gp41 antibodies that mask membrane proximal region epitopes: antibody binding kinetics, induction, and potential for regulation in acute infection. J Virol **82:**115-25.
4. **Berman, P. W.** 1998. Development of bivalent rgp120 vaccines to prevent HIV Type 1 infection. . AIDS Res Hum Retroviruses **14:**S277-S289.
5. **Berman, P. W., Huang, W., Riddle, L. et al.** 1999. Development of bivalent (B/E) vaccines able to neutralize CCR5-dependent viruses from the United States and Thailand. Virology **265:**1-9.

6. **Billings, E. A., KArasavvas, N., de Souza, M.S., Currier, J., Pitisuttithum, P. et al.** 2011. Surface Plasmon Resonance Analysis of anti-gp120 V2-specific IgG antibodies generated in the RV144 Thai Trial. AIDS Res Hum Retroviruses **27**:21.

7. **Bonsignori, M., Hwang, K.K., Chen, X., Tsao, C.Y., Morris, L., Gray, E. et al.** 2011. Analysis of a clonal lineage of HIV-1 Envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J. Virol. **85**:9998-10009.

8. **Bonsignori, M., X. Wu, M. A. Moody, H. Liao, K. Hwang, J. A. Crump, S. H. Capiga, N. E. Sam, G. D. Tomaras, X. Chen, C. Tsao, S. M. Alam, G. J. Nabel, P. D. Kwong, L. Morris, D. Montefiori, J. R. Mascola, and B. F. Haynes.** 2011. Isolation of CD4-Binding Site and V2/V3 Conformational (Quaternary) Broadly Neutralizing Antibodies from the Same HIV-1 Infected African Subject. AIDS Research and Human Retroviruses **27**:A120-A120.

9. **Davenport, T., Friend D, Ellingson K, Xu H, Caldwell Z, Sellhorn G, Kraft Z, Strong RK, Stamatatos L.** 2011. Binding interactions between soluble HIV envelope glycoproteins and quaternary-structure-specific monoclonal antibodies PG9 and PG16. J. Virol. **85**:7095-7107.

10. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res **8**:186-94.

11. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res **8**:175-85.

12. **Ferrari, G., Pollara J, Kozink D, Harms T, Drinker M, Freel S, Moody MA, Alam SM, Tomaras GD, Ochsenbauer C, Kappes JC, Shaw GM, Hoxie JA, Robinson JE, Haynes BF.** 2011. An HIV-1 gp120 envelope human

monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. J. Virol. **85**:7029-7036.

13. **Finzi, A., Pacheco, B., Zeng, X., Do Kwon, Y., Kwong, P.D., Sodroski, J.** 2010. Conformatinal characterization of aberrant disulfide-linked HIV-1 gp120 dimers secreted from overexpressing cells. J. Virol. Methods **168**:155-161.

14. **Flynn, B. J., Kastenmuller,K., Willie-Reece, U., Tomaras, G.D., Alam, S.M., Lindsay, R.W. et al.** 2011. Immunization with HIV Gag targeted to dendritic cells followed by recombinant New York Vaccinia virus induces ribust T-cell immunity in nonhuman primates. Proc Natl Acad Sci U S A **108**:7131-7136.

15. **Flynn, N. M., Fortahl, D.N., Harro, C.D., Judson, F.N., Mayer, K.H., Para, M.F., rgp120 HIV Vaccine Study Group.** 2005. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J. Infect. Dis. **191**:654-665.

16. **Francis, D. P., Gregory, T., McElarath, M.J. et al.** 1998. Advancing AIDSVAX to phase 3: safety, immunogenicity, and plans for phase 3. AIDS Res Hum Retroviruses **14**:325-331.

17. **Gorny, M. K., C. Williams, X. Wang, B. Volsky, T. O'Neal, L. Li, M. S. Seaman, and S. Zolla-Pazner. .** 2011. Functional and immunochemical cross-reactivity of V2-specific monoclonal antibodies from human immunodeficiency virus type 1-infected individuals. Virology **in press**.

18. **Gorny, M. K., J. P. Moore, A. J. Conley, S. Karwowska, J. Sodroski, C. Williams, S. Burda, L. J. Boots, and S. Zolla-Pazner.** 1994. Human anti-V2

monoclonal antibody that neutralizes primary but not laboratory isolates of human immunodeficiency virus type 1. J Virol **68:**8312-20.

19. **Gorny, M. K., L. Stamatatos, B. Volsky, K. Revesz, C. Williams, X. H. Wang, S. Cohen, R. Staudinger, and S. Zolla-Pazner.** 2005. Identification of a new quaternary neutralizing epitope on human immunodeficiency virus type 1 virus particles. J Virol **79:**5232-7.

20. **Harris, A., Borgnia, M. J., Shi, D., et al.** 2011. Trimeric HIV-1 glycoprotein gp140 immunogens and native HIV-1 envelope glysoproteins display the same and open quaternary molecular architectures. Proc Natl Acad Sci U S A **108:**11440-11445.

21. **Haynes, B. F.** 2011. Case control study of the RV144 trial for immune correlates: the analysis and way forward AIDS Vaccine Conference 2011, Bangkok, Thailand.

22. **Honnen, W. J., C. Krachmarov, S. C. Kayman, M. K. Gorny, S. Zolla-Pazner, and A. Pinter.** 2007. Type-specific epitopes targeted by monoclonal antibodies with exceptionally potent neutralizing activities for selected strains of human immunodeficiency virus type 1 map to a common region of the V2 domain of gp120 and differ only at single positions from the clade B consensus sequence. J Virol **81:**1424-32.

23. **Kasturi, S. P., Skountozi, I., Albrecht, R.A., Koutsonanos, D. et al.** 2011. Programming the magnitude and persistence of antibody responses with innate immunity. Nature **470** 543-547.

24. **Kayman, S. C., Wu, Z., Revesz, K., Chen, H., Kopelman, R., Pinter, A.** 1994. Presentation of native epitopes in the V1V2 and V3 regions of human immunodeficiency virus type I gp120 by fusion glycoprotsins containing isolated gp120 domains. J. Virol. **68:**400-410.

25. **Keele, B. F., E. E. Giorgi, J. F. Salazar-Gonzalez, J. M. Decker, K. T. Pham, M. G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J. L. Kirchherr, F. Gao, J. A. Anderson, L. H. Ping, R. Swanstrom, G. D. Tomaras, W. A. Blattner, et al.** 2008. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. . Proc. Natl. Acad. Sci **105**.

26. **Kwong, P. D., ML Doyle , DJ Casper , C. Cicala, S.A. Leavitt S. Majeed, T.D. Steenbeke, M. Venturi, I. Chaiken, M. Fung, H. Katinge, P.W. Parren, J. Robinson, D. Van Ryk, L. Wang, D.R. Burton, E. Freire, R. Wyatt, J. Sodroski W.A. Hendrickson, J. Arthos.** 2002. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature **420:**678-682.

27. **Lasky, L. A., Groopman, J.E., Fennie, C.W. et al.** 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **233:**209-212.

28. **Liao, H.-X., L.L. Sutherland, S-M Xia, M.E. Brock, R.M. Scearce, S. Vanleeuwen, M-S. Alam, M. McAdams, E.A. Weaver, Z.T. Camacho, B-J. Ma, Y. Li, J.M. Decker, G.J. Nabel, D.C. Montefiori, B.H. Hahn, B.T. Korber, F. Gao, B.F. Haynes.** 2006. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. Virology **353:**268-282.

29. **Liao, H. X., M. C. Levesque, A. Nagel, A. Dixon, R. Zhang, E. Walter, R. Parks, J. Whitesides, D. J. Marshall, K. K. Hwang, Y. Yang, X. Chen, F. Gao, S. Munshaw, T. B. Kepler, T. Denny, M. A. Moody, and B. F. Haynes.** 2009. High-throughput isolation of immunoglobulin genes from single human B cells and expression as monoclonal antibodies. J Virol Methods **158:**171-9.

30. **Liu, J., Bartesaghi, A., Borgnia, M.J.., Sapiro, G., Subramniam, S.** 2008. Molecular architecture of native HIV-1 gp120 trimers. Nature **455:**109-113.

31. **Liu, P., Overman, R.G., Yates, N.L., Alam, S.M., Vandergrift, N., Chen, Y., Graw, F., Freel, S.A., Kappes, J.C., Ochsenbauer, C., Montefiori, D.C., Gao, F., Perelson, A.S., Cohen, M.S., Haynes, B.F., Tomaras, G.D.** 2011. Dynamic antibody specificities and virion concentrations in circulating immune complexes in acute to chronic HIV-1 infection. J. Virol. **85:**11196-11207.

32. **McLellan, J., Pancera M, Carrico C, Gorman J, Julien JP, Khayat R, Louder R, Pejchal R, Sastry M, Dai K, O'Dell S, Patel N, Shahzad-ul-Hussan S, Yang Y, Zhang B, Zhou T, Zhu J, Boyington JC, Chuang GY, Diwanji D, Georgiev I, Kwon YD, Lee D, Louder MK, Moquin S, Schmidt SD, Yang ZY, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Burton DR, Koff WC, Walker LM, Phogat S, Wyatt R, Orwenyo J, Wang LX, Arthos J, Bewley CA, Mascola JR, Nabel GJ, Schief WR, Ward AB, Wilson IA, Kwong PD.** 2011. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature **480:**336-343.

33. **Munshaw, S., and T. B. Kepler.** 2010. SoDA2: a Hidden Markov Model approach for identification of immunoglobulin rearrangements. Bioinformatics **26:**867-72.

34. **Pinter, A., W. J. Honnen, Y. He, M. K. Gorny, S. Zolla-Pazner, and S. C. Kayman.** 2004. The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary human immunodeficiency virus type 1 isolates to neutralization by antibodies commonly induced upon infection. J Virol **78:**5205-15.

35. **Pinter, A., Honnen, W.J., Kayman, S.C., Trochev, O. Wu, Z.** 1998. Potent neutralization of primary HIV-1 isolates by antibodies directed against epitopes present in the V1/V2 domain of HIV-1 gp120. Vaccine **16**:1803-1811.

36. **Pitisuttithum, P.** 2008. HIV vaccine research in Thailand: lessons learned. Expert Rev. Vaccines **7**:311-317.

37. **Rerks-Ngam, S., Pitisuttithum, P., Nitayaphan, S. et al.** 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N. Eng. J. Med. **361**:2209-2220.

38. **Safsten, P., Klakamp, S.L., Drake, A.W., Karlsson, R., Myszka, D.G.** 2006. Screening antibody-antigen interactions in parallel using BIAcore A100. Anal. Biochem. **353**:181-190.

39. **Shields, R. L., A. K. Namenuk, K. Hong, Y. G. Meng, J. Rae, J. Briggs, D. Xie, J. Lai, A. Stadlen, B. Li, J. A. Fox, and L. G. Presta.** 2001. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem **276**:6591-604.

40. **Smith, T. F., and M. S. Waterman.** 1981. Identification of common molecular subsequences. J Mol Biol **147**:195-7.

41. **Tomaras, G. D., N. L. Yates, P. Liu, L. Qin, G. G. Fouda, L. L. Chavez, A. C. Decamp, R. J. Parks, V. C. Ashley, J. T. Lucas, M. Cohen, J. Eron, C. B. Hicks, H. X. Liao, S. G. Self, G. Landucci, D. N. Forthal, K. J. Weinhold, B. F. Keele, B. H. Hahn, M. L. Greenberg, L. Morris, S. S. Karim, W. A. Blattner, D. C. Montefiori, G. M. Shaw, A. S. Perelson, and B. F. Haynes.** 2008. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies

followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J Virol **82:**12449-63.

42. **Tsao, C., et al.** 2010. Antigenicity and immuogenicity of transmitted/founder HIV envelope oligomers compared to chronic HIV envelopes. AIDS Res. Hum. Retroviruses **10.01:**A27.

43. **VanCott, T. C., Bethke, F.R., Polonis, V.R., Gorny, M.K., Zolla-Pazner, S., Redfield R.R., Birx, D.L.** 1994. Dissociation rate of antibody-gp120 binding interactions is predictive of V3-mediated neutralization of HIV-1. J. Immunol. **153:**449.

44. **Volpe, J. M., L. G. Cowell, and T. B. Kepler.** 2006. SoDA: implementation of a 3D alignment algorithm for inference of antigen receptor recombinations. Bioinformatics **22:**438-44.

45. **Walker, L. M., S. K. Phogat, P. Y. Chan-Hui, D. Wagner, P. Phung, J. L. Goss, T. Wrin, M. D. Simek, S. Fling, J. L. Mitcham, J. K. Lehrman, F. H. Priddy, O. A. Olsen, S. M. Frey, P. W. Hammond, S. Kaminsky, T. Zamb, M. Moyle, W. C. Koff, P. Poignard, and D. R. Burton.** 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science **326:**285-9.

46. **Wrammert, J., K. Smith, J. Miller, W. A. Langley, K. Kokko, C. Larsen, N. Y. Zheng, I. Mays, L. Garman, C. Helms, J. James, G. M. Air, J. D. Capra, R. Ahmed, and P. C. Wilson.** 2008. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature **453:**667-71.

47. **Zhu, P., J. Liu, J. Bess, E. Chertova, J. D. Lifson, H. Grise, G. A. Ofek, K. A. Taylor, and K. H. Roux.** 2006. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature **441:**847-852.

48. **Zolla-Pazner, S., Cardozo, T., Decamp, A., Haynes, B., Kim, J., Kong, X., Michael, N., Rerks-Ngam, S., Williams, C.** 2011. V2-reactive Antibodies in

RV144 Vaccinees' Plasma. AIDS Res Hum Retroviruses (Abstracts from AIDS Vaccine 2011) **27**:A21.

* * *

All documents and other information sources cited above are hereby incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An immunogen comprising an HIV-1 Env gp120 or gp140, wherein the original about 4 to about 25 amino acids of the N-terminus of gp120 are deleted and wherein an N-terminal Herpes Simplex gD tag is not substituted for amino acids of the N-terminus of gp120.

2. The immunogen according to claim 1 wherein about 5 to 11 amino acids of the N-terminus of gp120 are deleted.

3. The immunogen according to claim 1 wherein said about 4 to about 25 amino acids are consecutive amino acids.

4. The immunogen according to claim 1 wherein said Env gp120 or gp140 is a transmitted founder virus Env.

5. The immunogen according to claim 4 wherein said transmitted founder virus Env is 1086.C, 089.C, 63521.B, 6240.B, 040.B or A1C recombinant transmitted founder Env 707-01-069-2.

6. A nucleic acid comprising a nucleotide sequence encoding the immunogen according to claim 1.

7. A vector comprising the nucleic acid according to claim 6.

8. The vector according to claim 7 wherein said vector is a rAdenovirus, recombinant mycobacteria or recombinant vaccinia type vector.

9. A composition comprising said immunogen according to claim 1 or said nucleic acid according to claim 6 and a carrier.

10. A method of inducing an immune response in a subject comprising administering to said subject said immunogen according to claim 1 or said nucleic acid according to claim 6 in an amount sufficient to effect said induction..

Figure 1

Dissociation Constants (Kd) of CH01, PG9, A32 and 697D mAbs binding to RV144 Env

	CH01 nM	CH01 nM	PG9 nM	PG9 nM	A32 nM	A32 nM	697D nM	697D nM
ENV	gD+	gD-	gD+	gD-	gD+	gD-	gD+	gD-
AE.92TH023 gp120	-	-	212	-	1.35	4.6	734	1313
B.MN gp120	288	457	542	347	4.15	0.8	488	1073
AE.A244 gp120	200	2100	128	1100	0.13	2.6	180	1000

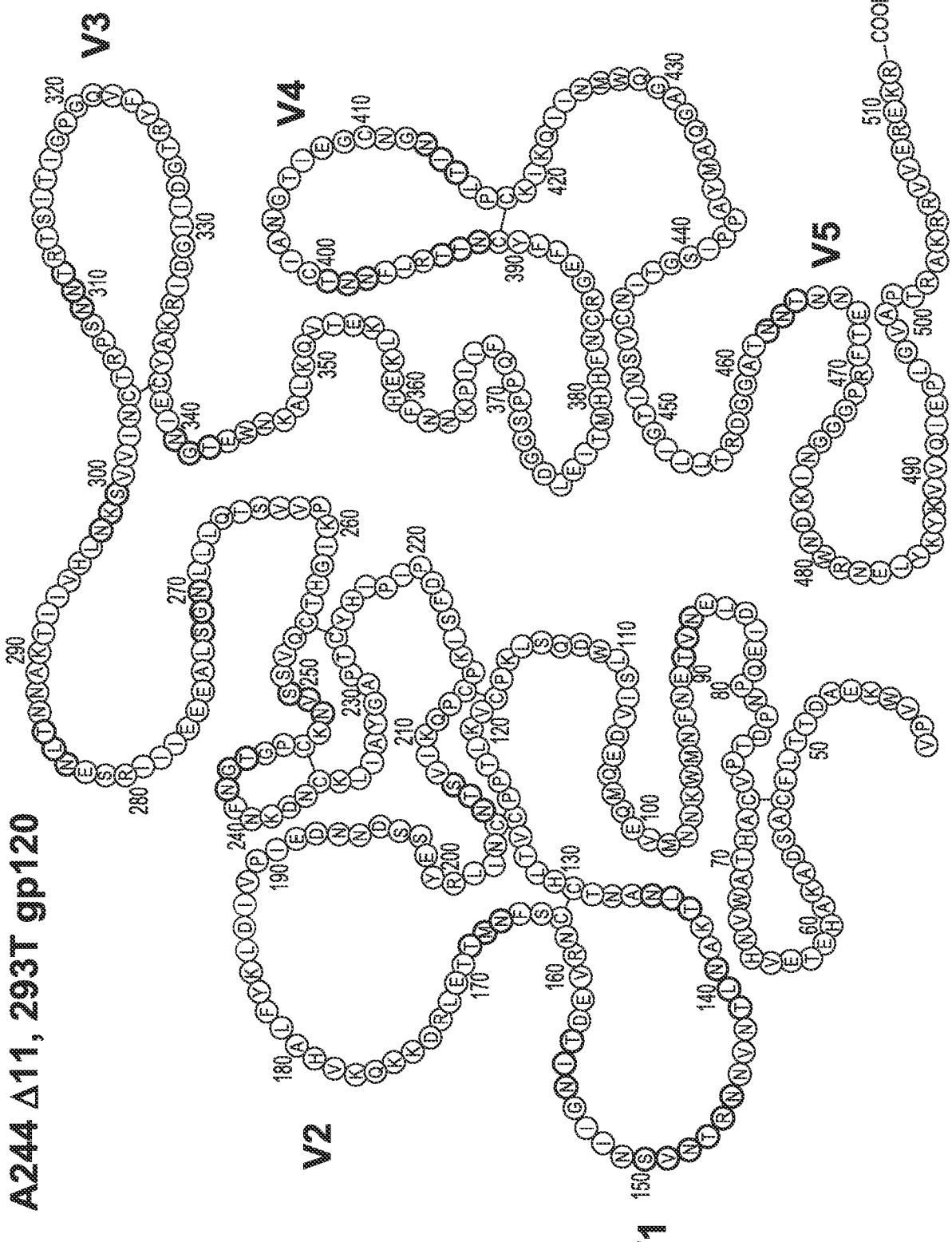
CH01, PG9 = V2,V3 conformational (quaternary) Abs

A32 = C1 epitope Ab ; mediates ADCC

697D = conformational V2 Ab

Larry Liao, Munir Alam

Figure 2



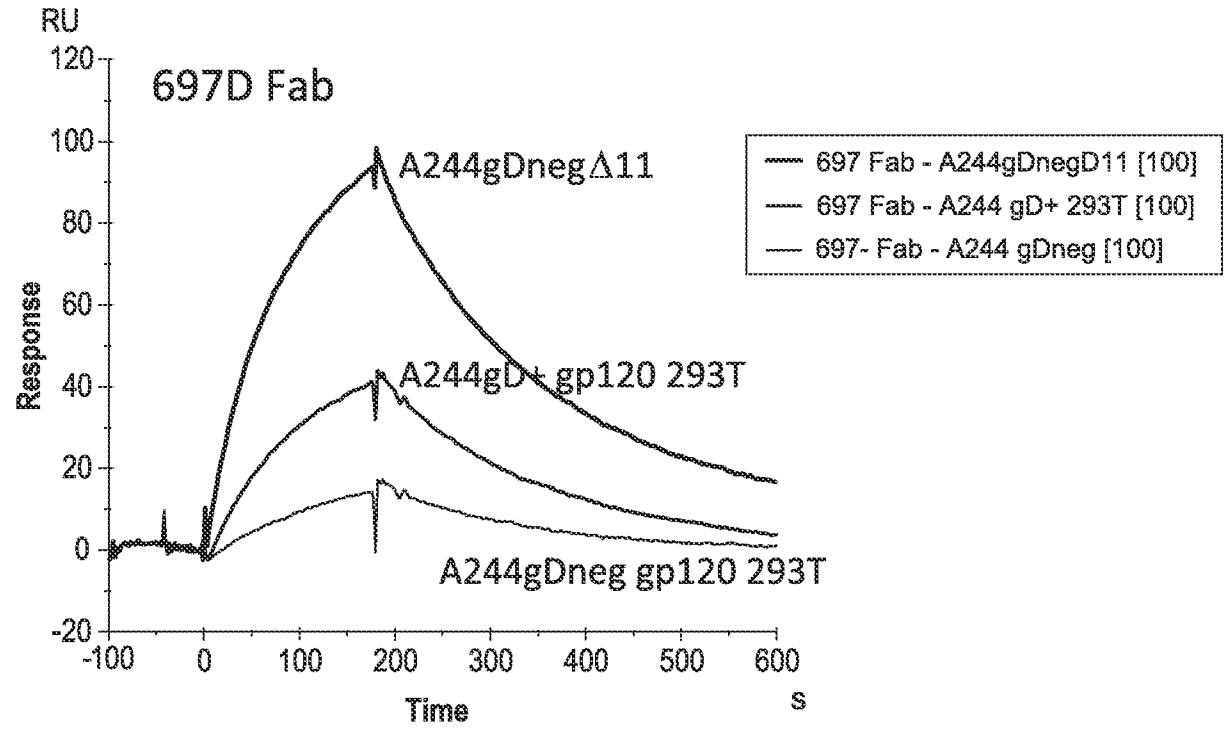
Amino acid sequence inside circles represents A244 gp120 gD(-). (adapted from Leonard, et al.,J.Biol. Chem. 265, 10373, 1990).

Figure 3

black-and-white ribbon diagram of gp120, based on Peter Kwong structure deposited in NCBI database as 3JWD.pdb. It included the full N- and C-terminal segments of gp120 (but NOT V1-V2 or V3). Residues 31-41 stick out into space, as do residues 494-511. Residues 31-41, are the residues deleted in Δ11 A244 gp120 which are relatively hydrophobic and which would, in gp160, interact with gp41, likely lead to some misfolding or aggregation of gp120 in the endoplamic reticulum, so that disulfides form between two gp120s. The C-terminal extension, which would also interact with gp41 (it leads to the cleavage point), is very polar (indeed, charged), so it may even help solubilize the expressed protein. The Δ11 substitution apparently eliminated (or at least partly eliminated) the aggregation and/or misfolding tendency and the addition of the HSV gD tag in gD+ A244 gp120 did not cause disulfide formation to the extent that the whole gp120 does.

Figure 4

A


RU
697D Fab
A244gDnegΔ11
A244gD+ gp120 293T
A244gDneg gp120 293T
697 Fab - A244gDnegD11 [100]
697 Fab - A244 gD+ 293T [100]
697- Fab - A244 gDneg [100]
Response
Time
s

B

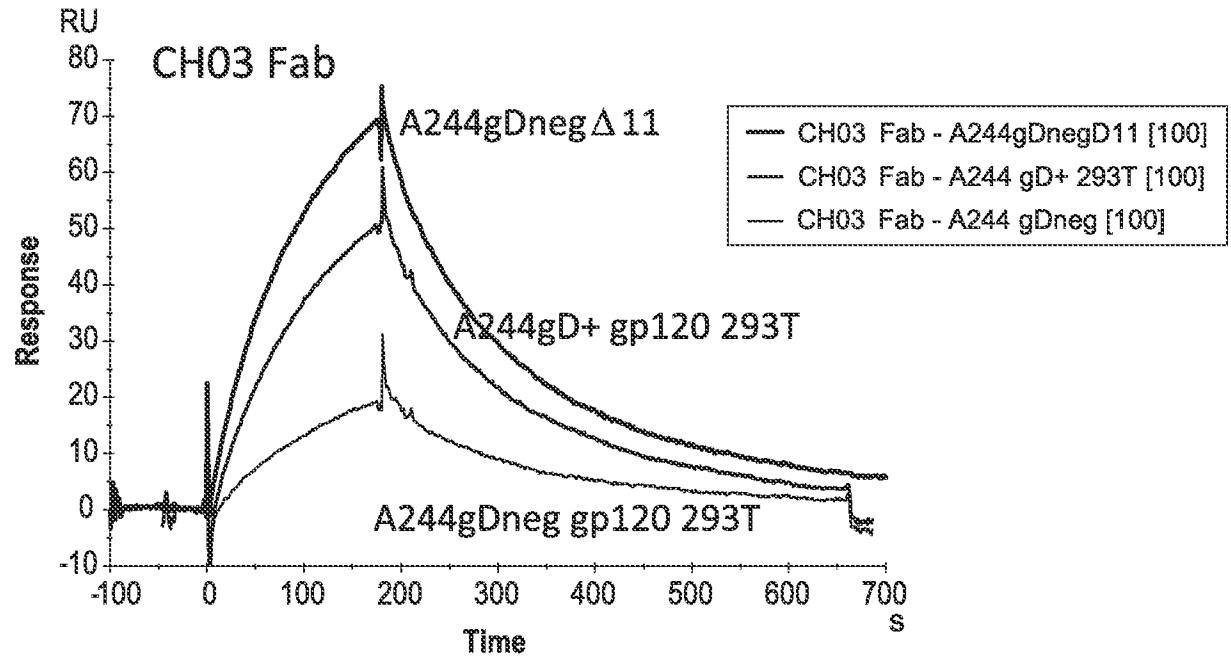

RU
CH03 Fab
A244gDnegΔ11
A244gD+ gp120 293T
A244gDneg gp120 293T
CH03 Fab - A244gDnegD11 [100]
CH03 Fab - A244 gD+ 293T [100]
CH03 Fab - A244 gDneg [100]
Response
Time
s

Figure 5

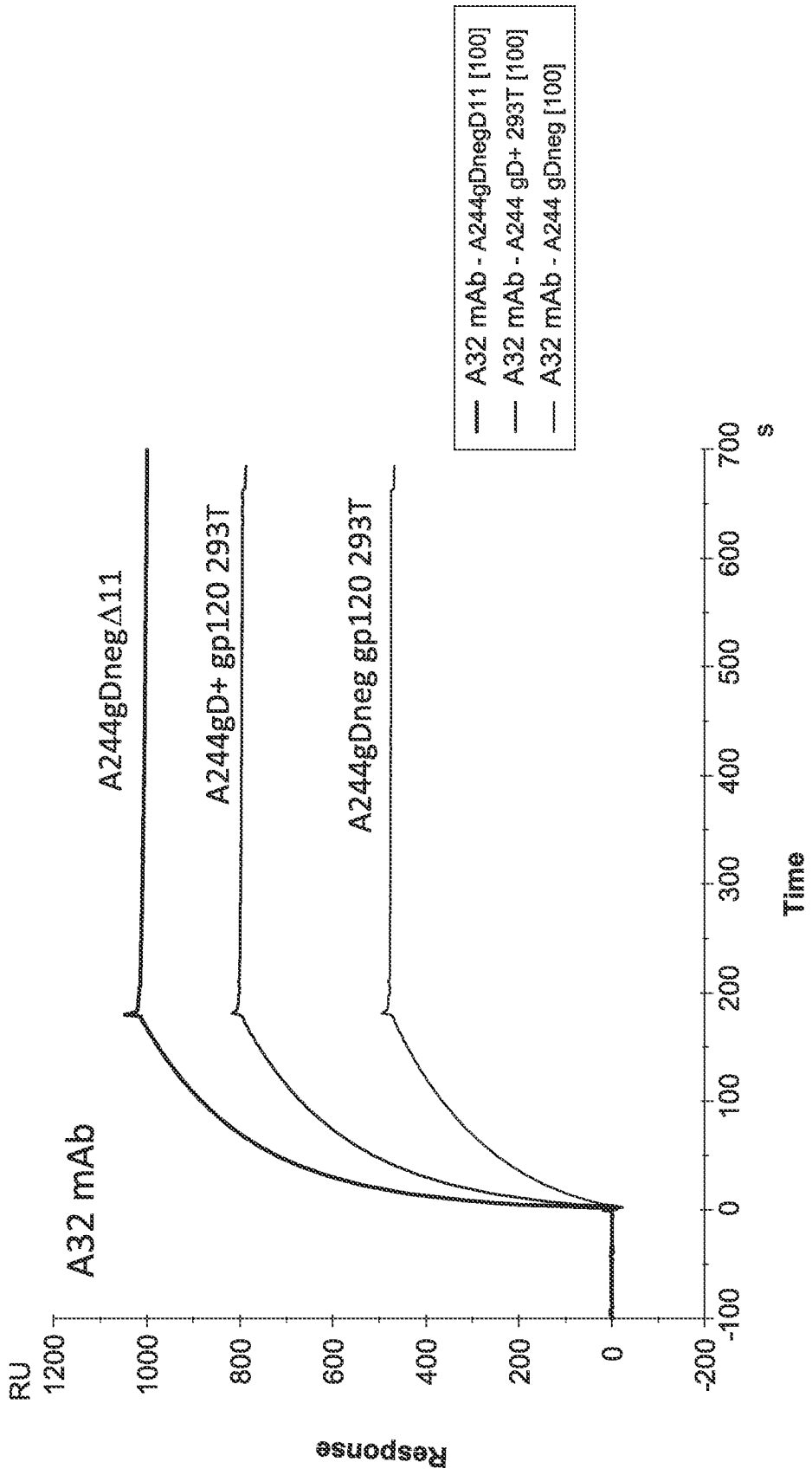
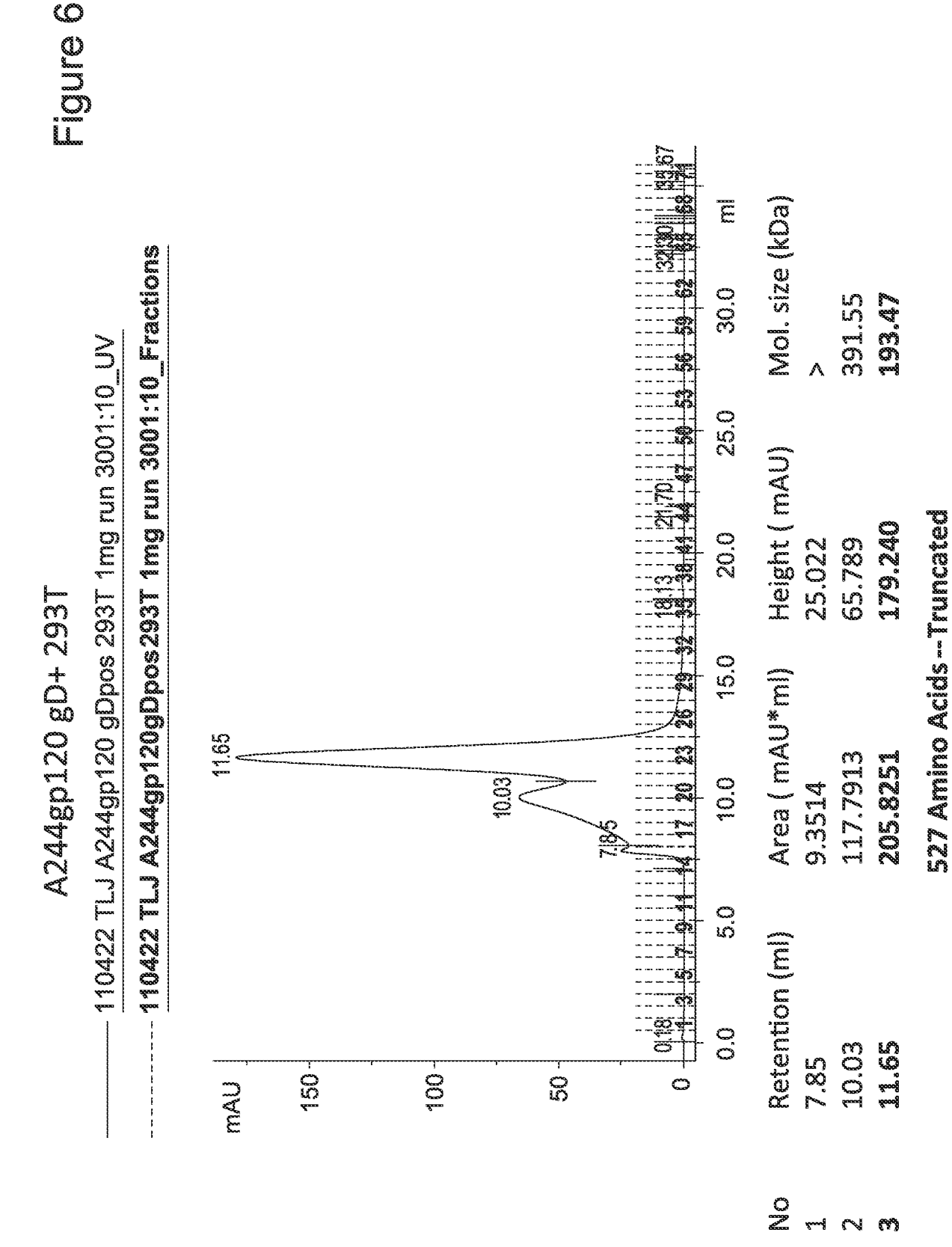

A32 mAb - A244gDnegD11 [100]
A32 mAb - A244 gD+ 293T [100]
A32 mAb - A244 gDneg [100]
A244gDnegΔ11
A244gD+ gp120 293T
A244gDneg gp120 293T
A32 mAb
RU
1200
1000
800
600
400
200
0
-200
Response
-100
0
100
200
300
400
500
600
700
s
Time

Figure 6

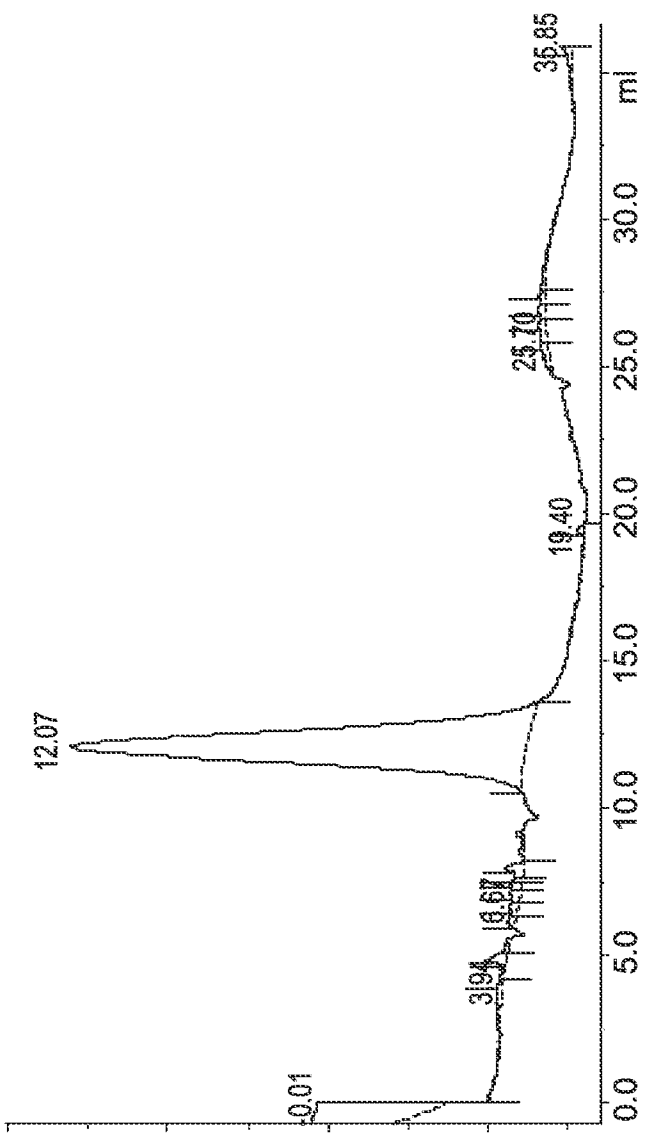


No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	7.85	9.3514	25.022	>
2	10.03	117.7913	65.789	391.55
3	11.65	205.8251	179.240	193.47

527 Amino Acids--Truncated

Figure 7

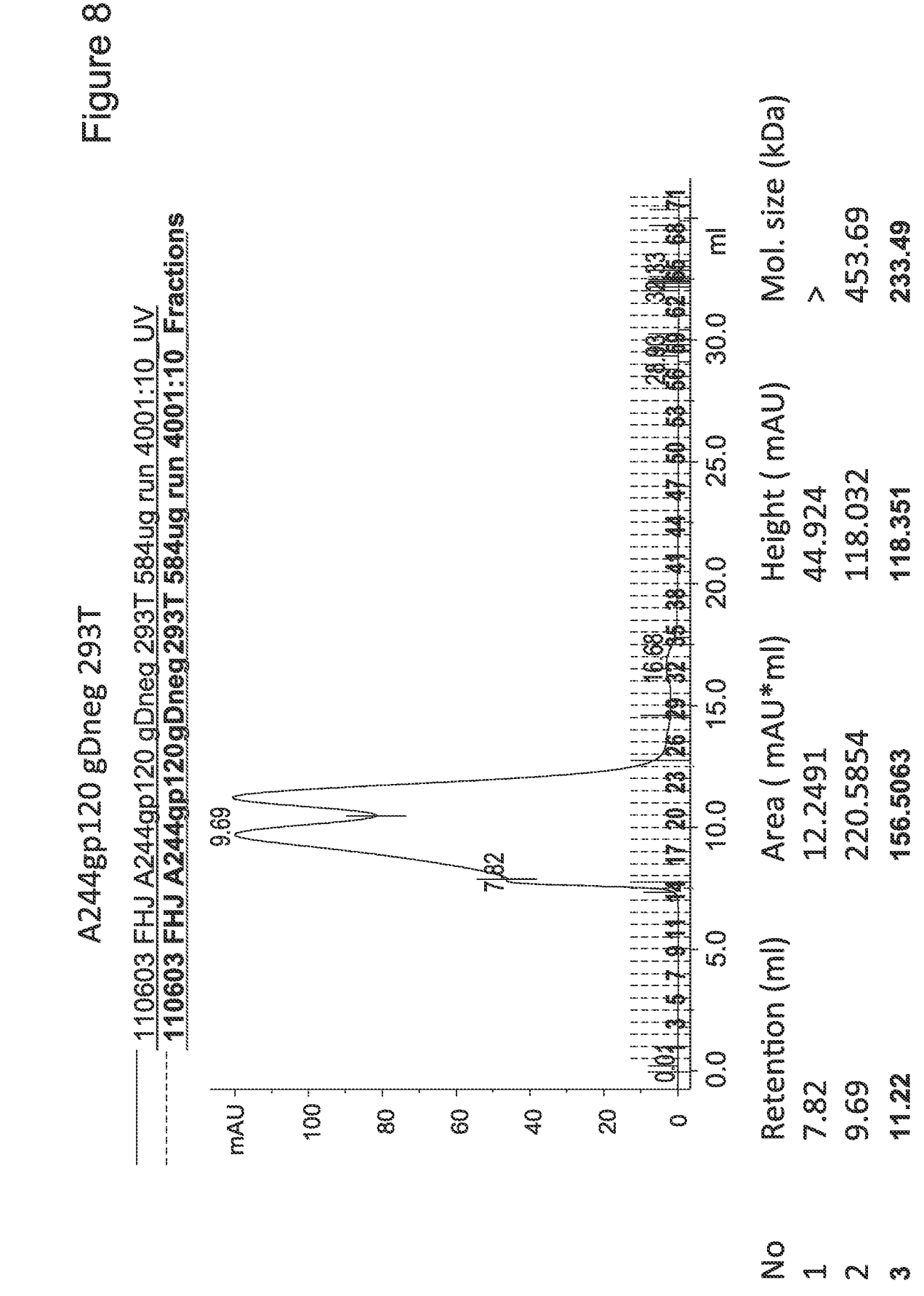
A244gp120 gDpos 293T MON b#110422



No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	12.07	3.4638	2.838	184.56

R.T. Difference due to different instrument being run for QC

Figure 8

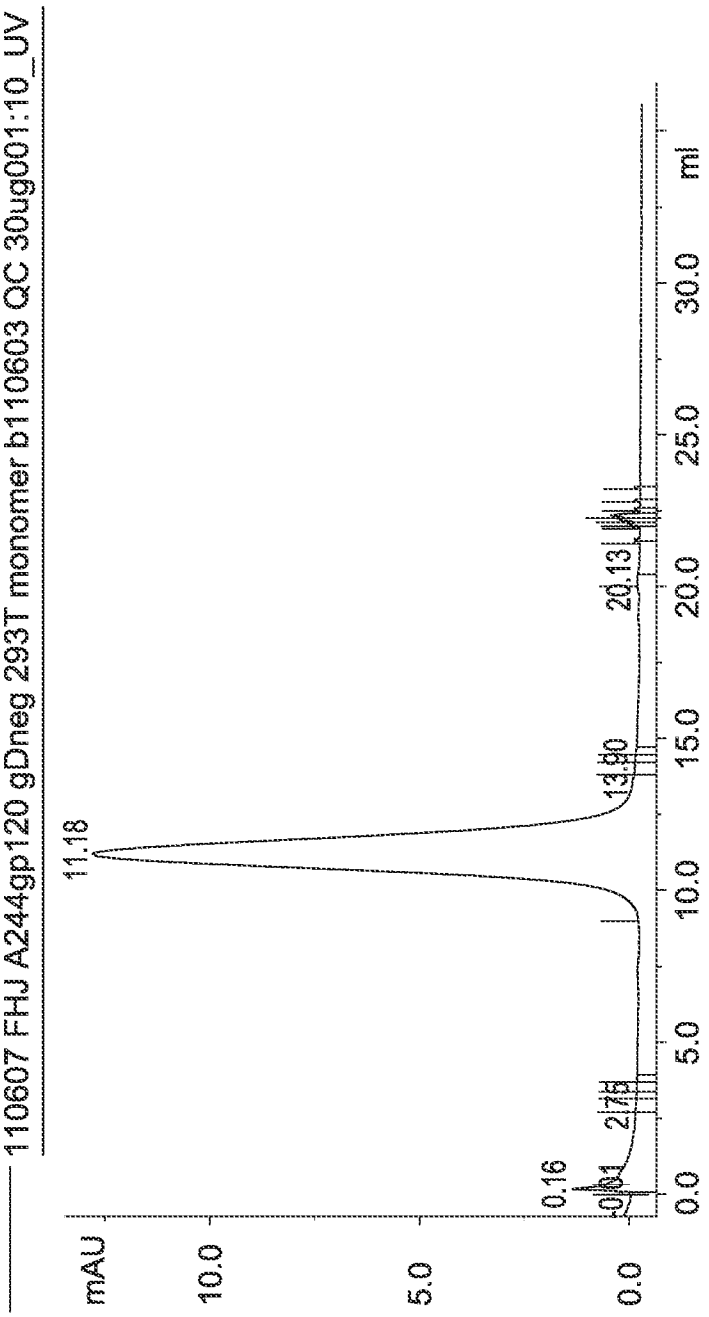


No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	7.82	12.2491	44.924	>
2	9.69	220.5854	118.032	453.69
3	11.22	156.5063	118.351	233.49

512 Amino Acids

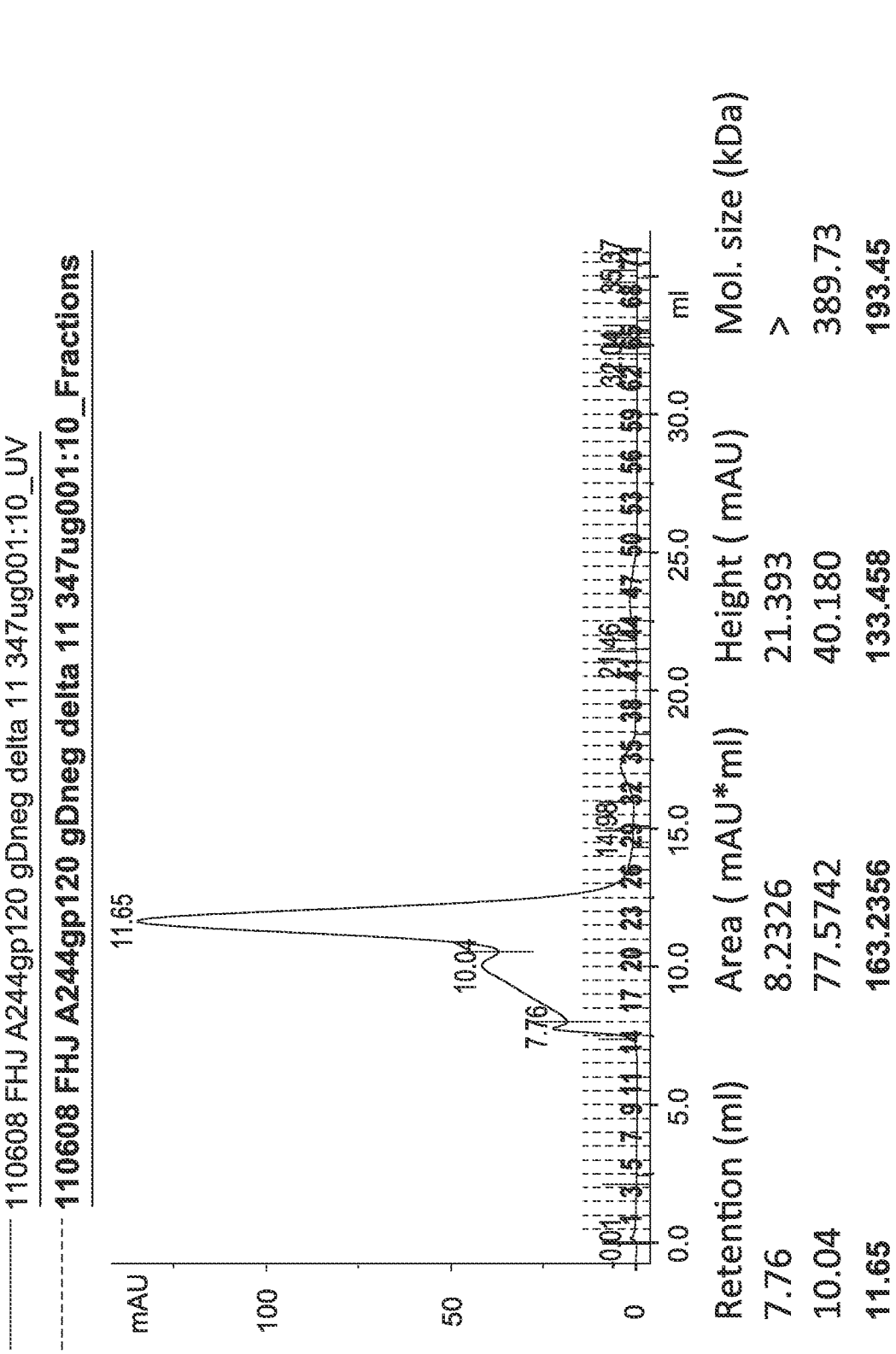
Figure 9

A244 gp120 gDneg 293T Monomer b110603



No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	11.18	16.4260	13.008	237.28

Figure 10

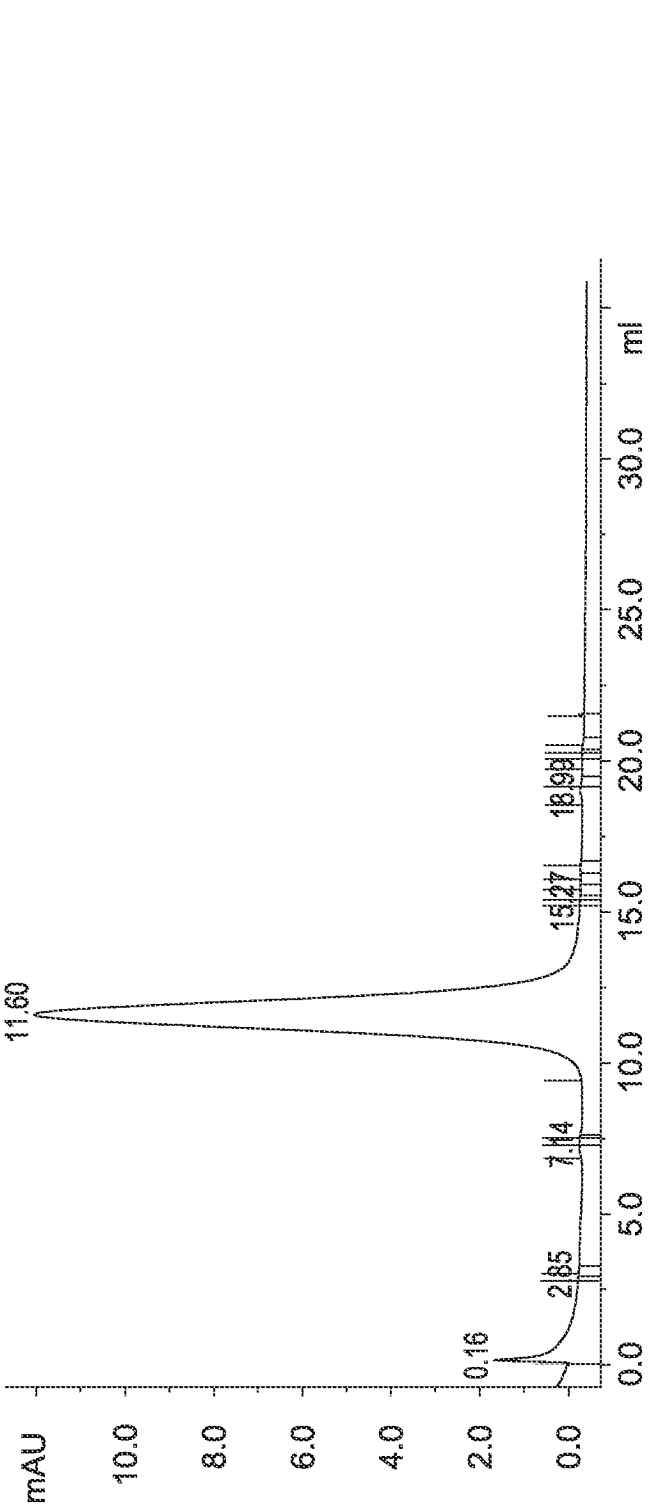


No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	7.76	8.2326	21.393	>
2	10.04	77.5742	40.180	389.73
3	11.65	163.2356	133.458	193.45

501 Amino Acids – Truncated

Figure 11

A244gp120 gDneg DELTA 11 293T monomer batch#110608



No	Retention (ml)	Area (mAU*ml)	Height (mAU)	Mol. size (kDa)
1	11.60	15.0307	12.338	197.69

Figure 12

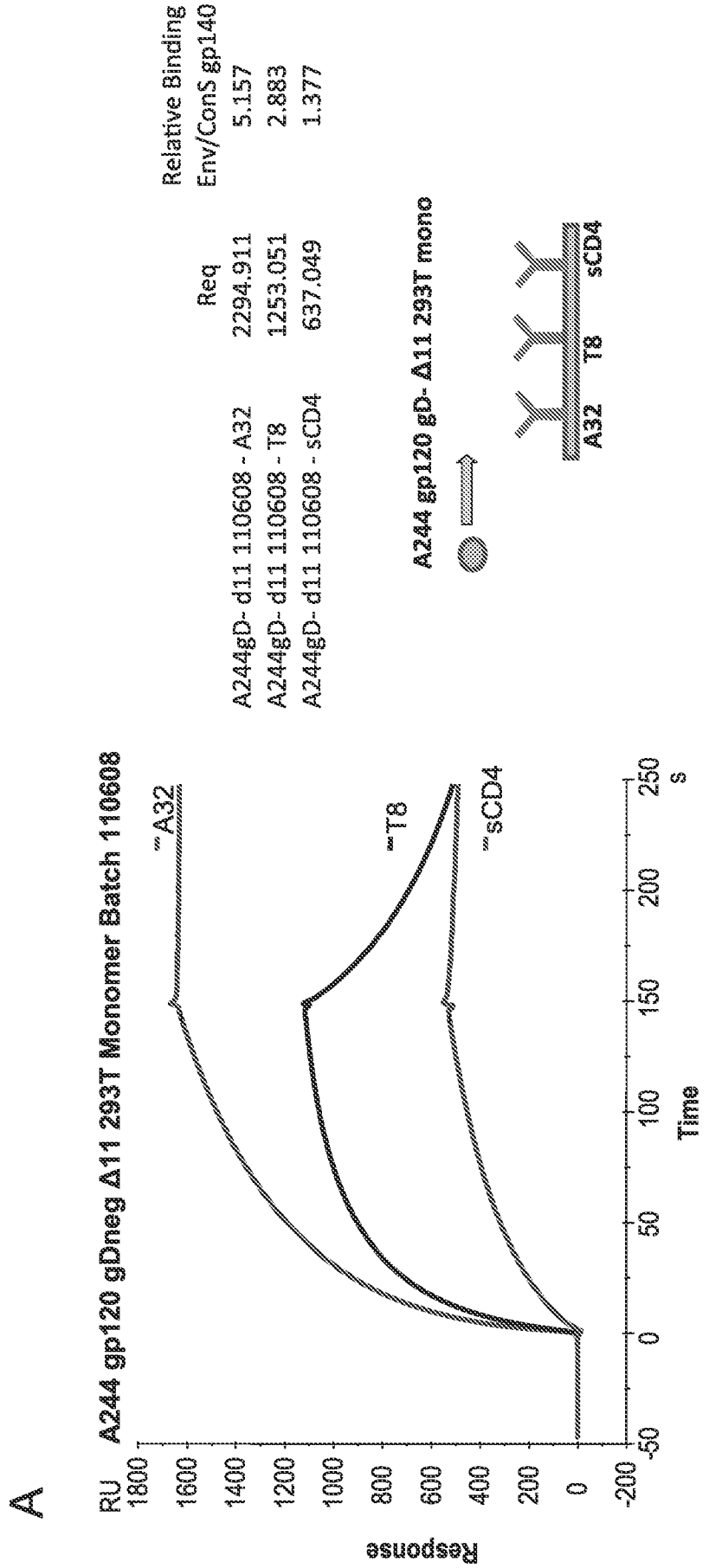

A
A244 gp120 gDneg Δ11 293T Monomer Batch 110608
RU
Response
Time
s
A32
T8
sCD4
A244gD- d11 110608 - A32
A244gD- d11 110608 - T8
A244gD- d11 110608 - sCD4
Req
2294.911
1253.051
637.049
Relative Binding Env/ConS gp140
5.157
2.883
1.377
A244 gp120 gD- Δ11 293T mono
A32
T8
sCD4

Figure 12

B

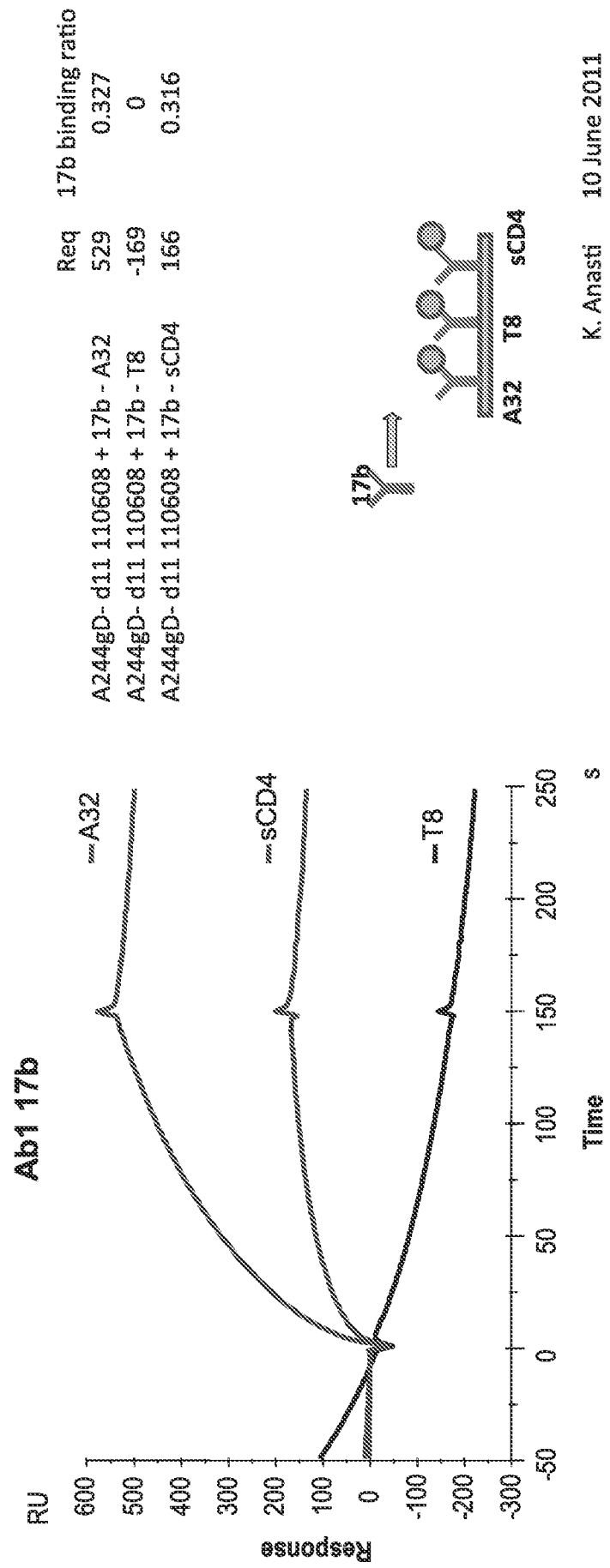

Ab1 17b
RU
Response
600
500
400
300
200
100
0
-100
-200
-300
-50
0
50
100
150
200
250
Time
s
A32
sCD4
T8
A244gD- d11 110608 + 17b - A32
A244gD- d11 110608 + 17b - T8
A244gD- d11 110608 + 17b - sCD4
Req
529
-169
166
17b binding ratio
0.327
0
0.316
17b
A32
T8
sCD4
K. Anasti
10 June 2011

Figure 13

Binding to 697D mAb

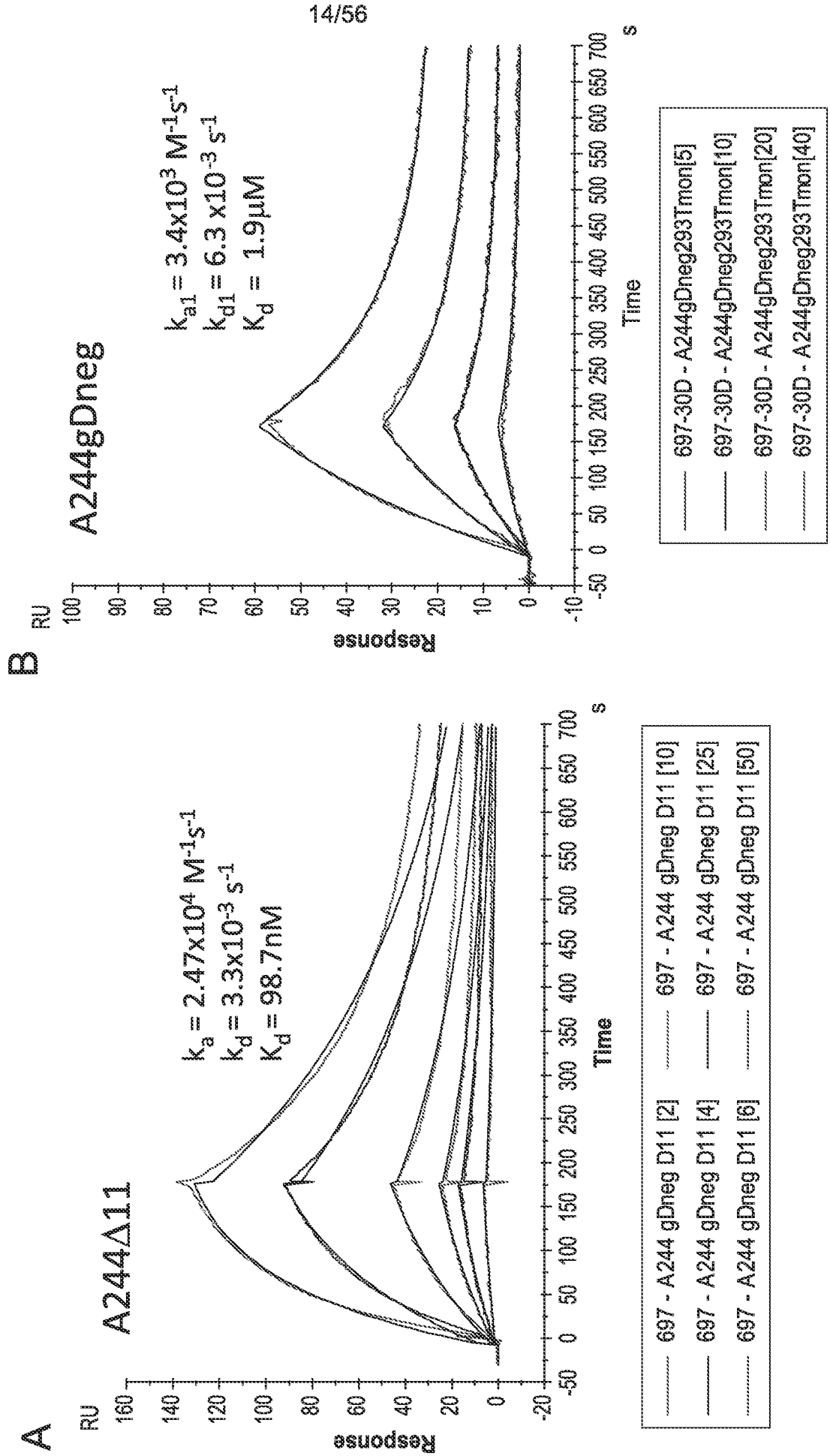


Figure 13

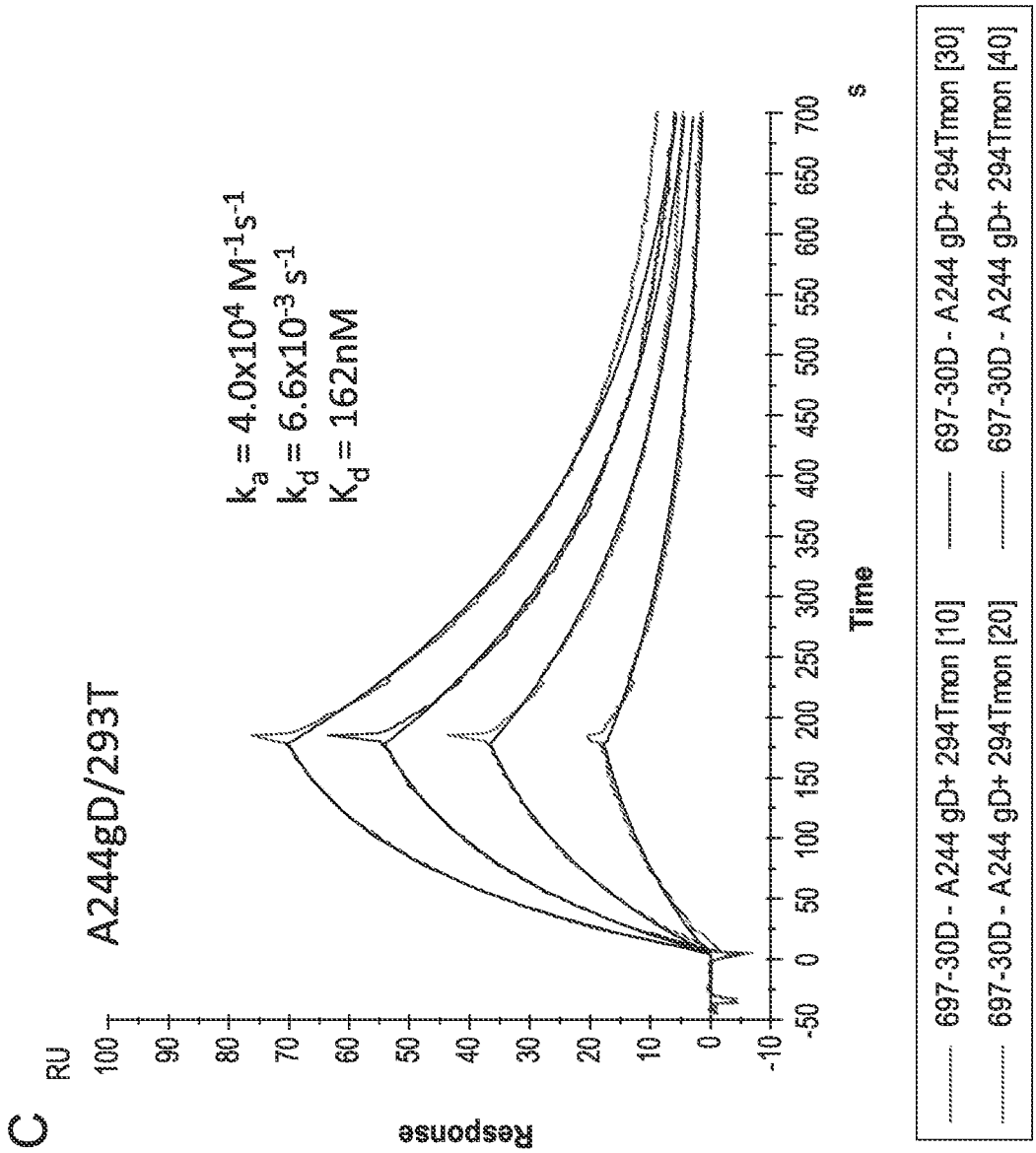

C
Binding to 697D mAb
A244gD/293T
$k_a = 4.0x10^4\ M^{-1}s^{-1}$
$k_d = 6.6x10^{-3}\ s^{-1}$
$K_d = 162nM$
RU
Response
Time
s
697-30D - A244 gD+ 294Tmon [10]
697-30D - A244 gD+ 294Tmon [20]
697-30D - A244 gD+ 294Tmon [30]
697-30D - A244 gD+ 294Tmon [40]

Figure 14

Binding to PG9 mAb

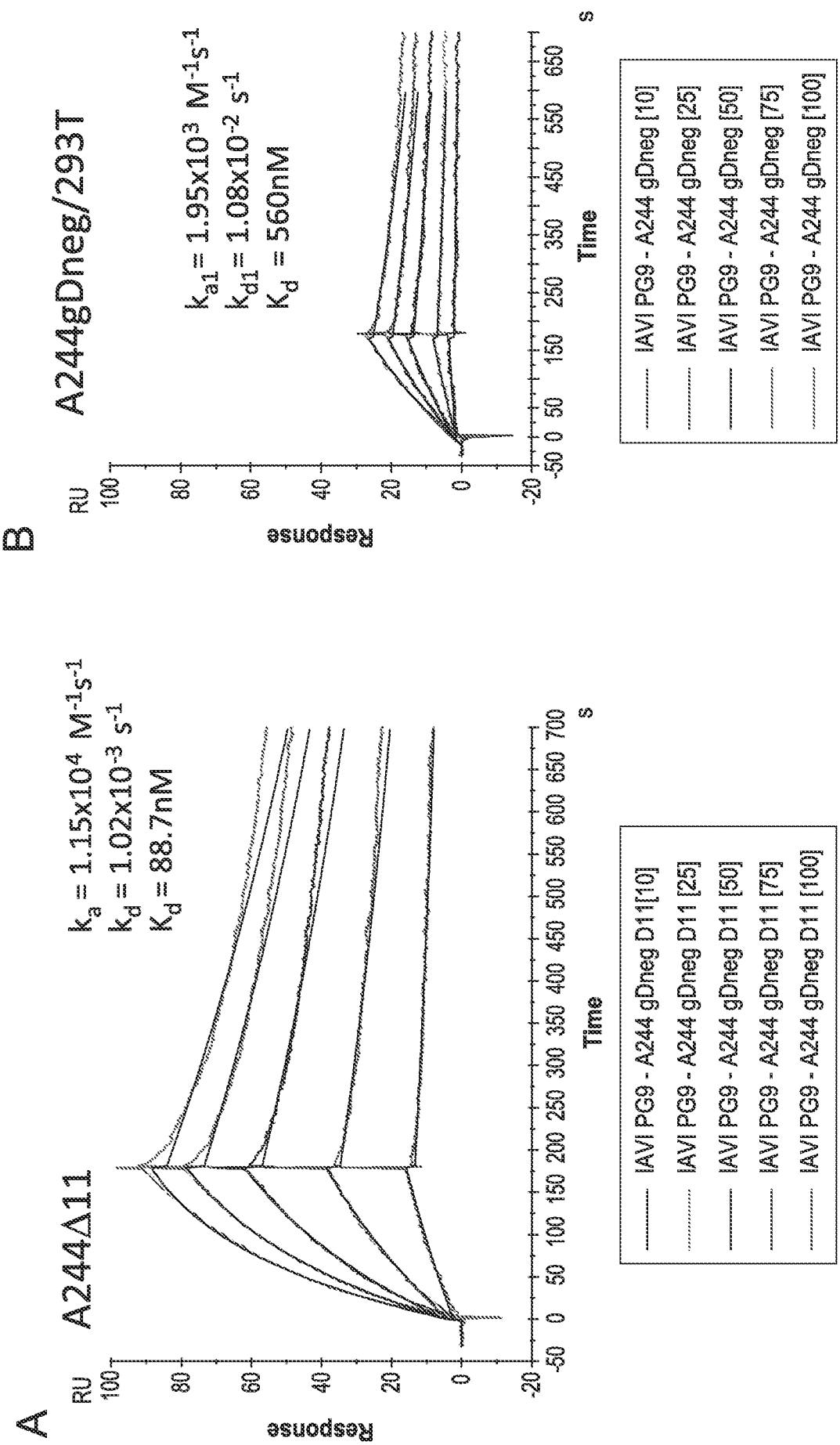


Figure 14

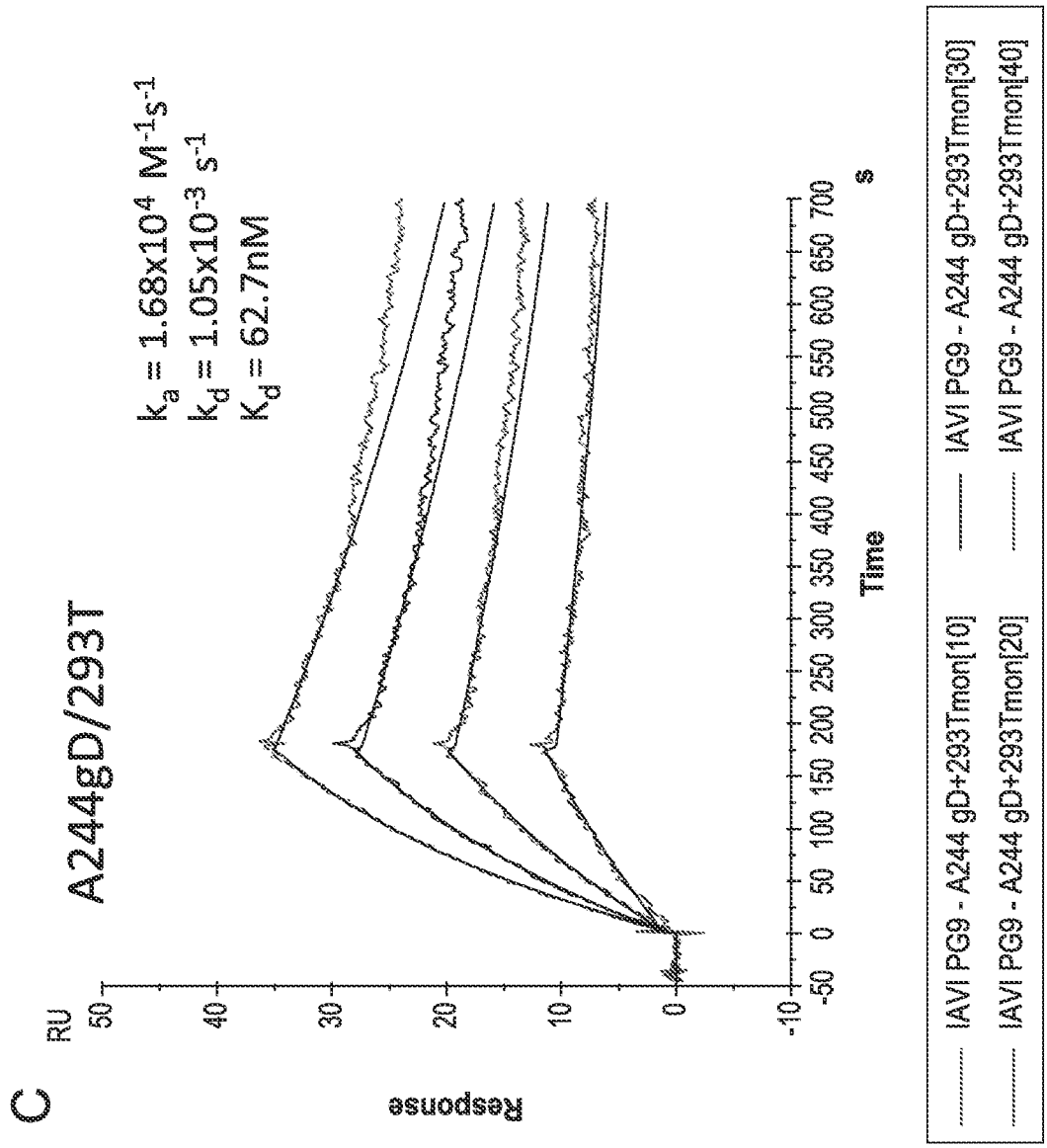

Binding to PG9 mAb
C
A244gD/293T
k_a = 1.68x10^4 M^{-1}s^{-1}
k_d = 1.05x10^{-3} s^{-1}
K_d = 62.7nM
RU
50
40
30
20
10
0
-10
Response
-50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700
Time
s
IAVI PG9 - A244 gD+293Tmon[10]
IAVI PG9 - A244 gD+293Tmon[20]
IAVI PG9 - A244 gD+293Tmon[30]
IAVI PG9 - A244 gD+293Tmon[40]

Figure 15

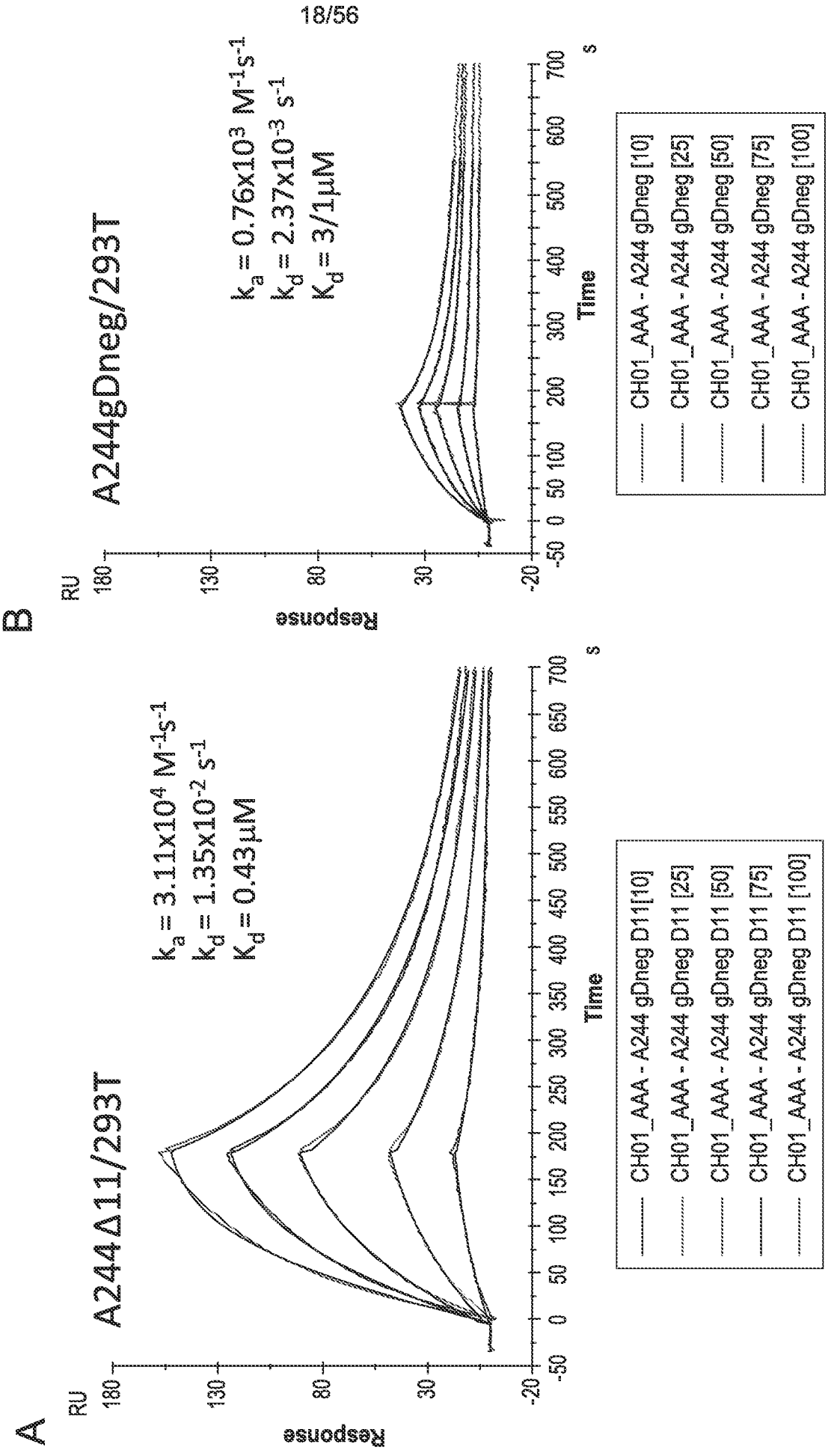

Binding to CH01 mAb
A
A244Δ11/293T
RU
Response
Time
s
k_a = 3.11x10^4 M^{-1}s^{-1}
k_d = 1.35x10^{-2} s^{-1}
K_d = 0.43µM
CH01_AAA - A244 gDneg D11[10]
CH01_AAA - A244 gDneg D11 [25]
CH01_AAA - A244 gDneg D11 [50]
CH01_AAA - A244 gDneg D11 [75]
CH01_AAA - A244 gDneg D11 [100]
B
A244gDneg/293T
k_a = 0.76x10^3 M^{-1}s^{-1}
k_d = 2.37x10^{-3} s^{-1}
K_d = 3/1µM
CH01_AAA - A244 gDneg [10]
CH01_AAA - A244 gDneg [25]
CH01_AAA - A244 gDneg [50]
CH01_AAA - A244 gDneg [75]
CH01_AAA - A244 gDneg [100]

Figure 15

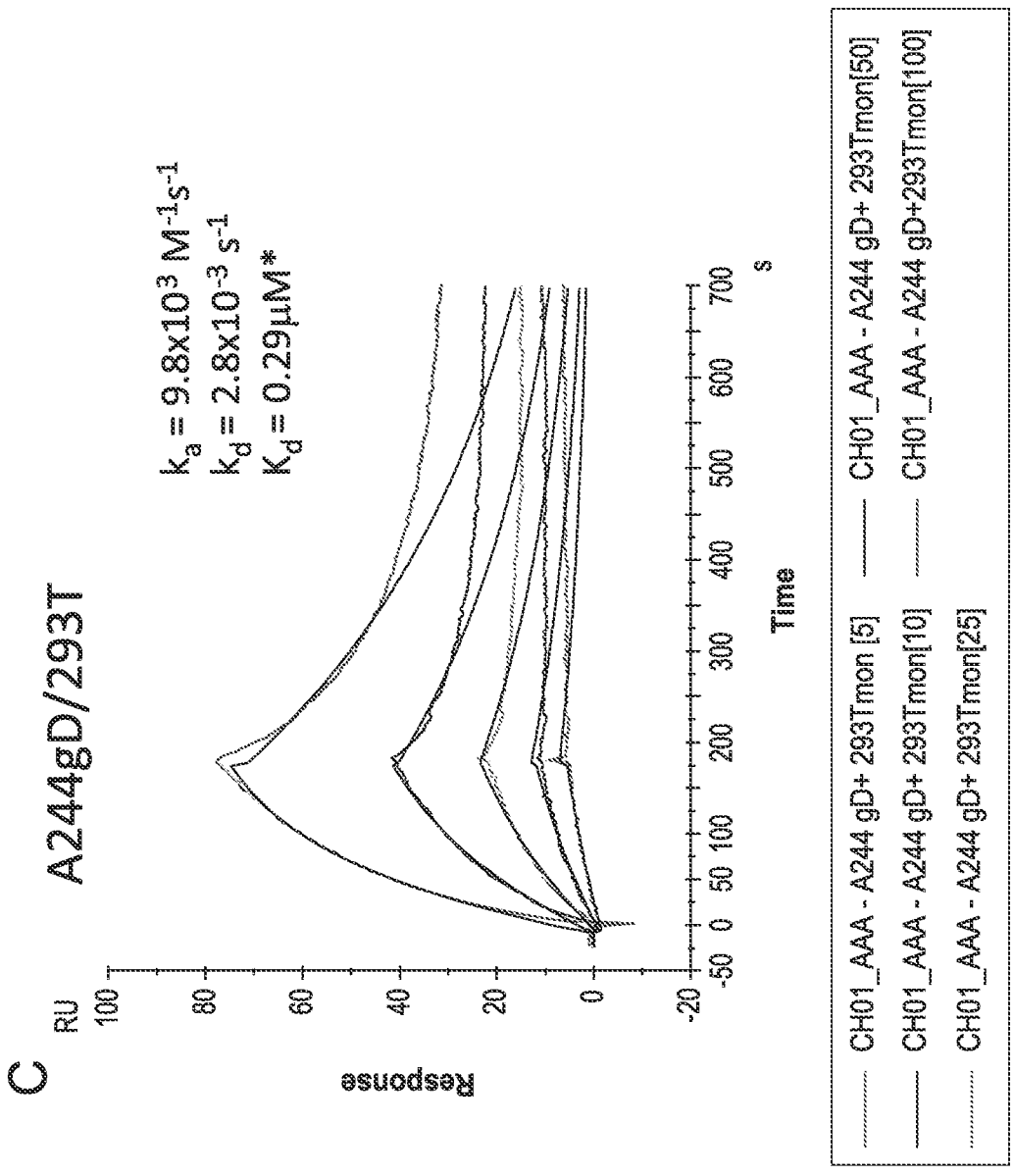

Binding to CH01 mAb
C
A244gD/293T
RU
100
80
60
40
20
0
-20
Response
$k_a = 9.8x10^3\ M^{-1}s^{-1}$
$k_d = 2.8x10^{-3}\ s^{-1}$
$K_d = 0.29\mu M^*$
-50 0 50 100 200 300 400 500 600 700
Time
s
CH01_AAA - A244 gD+ 293Tmon [5]
CH01_AAA - A244 gD+ 293Tmon[10]
CH01_AAA - A244 gD+ 293Tmon[25]
CH01_AAA - A244 gD+ 293Tmon[50]
CH01_AAA - A244 gD+293Tmon[100]
* Fit deviation, need to lower Ag density

Figure 16

A

Binding to CH03 mAb

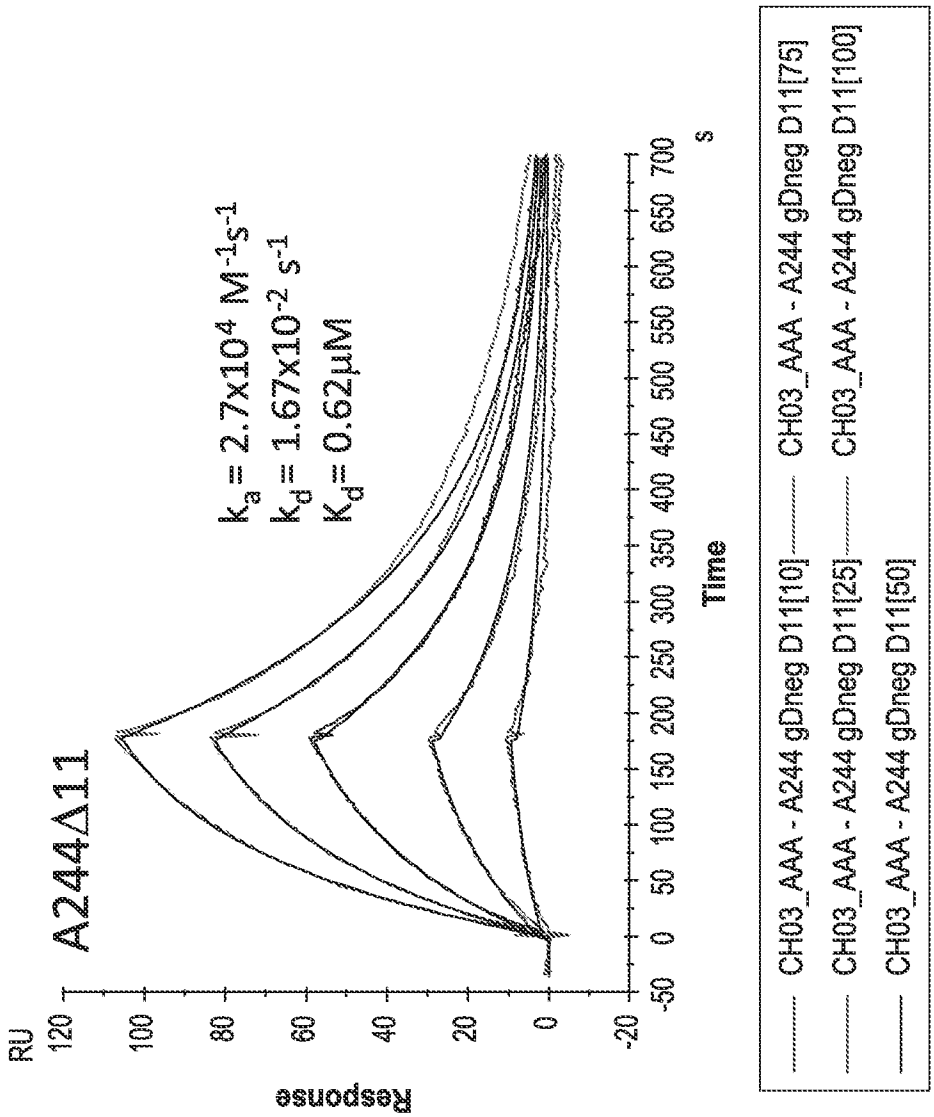


Figure 16

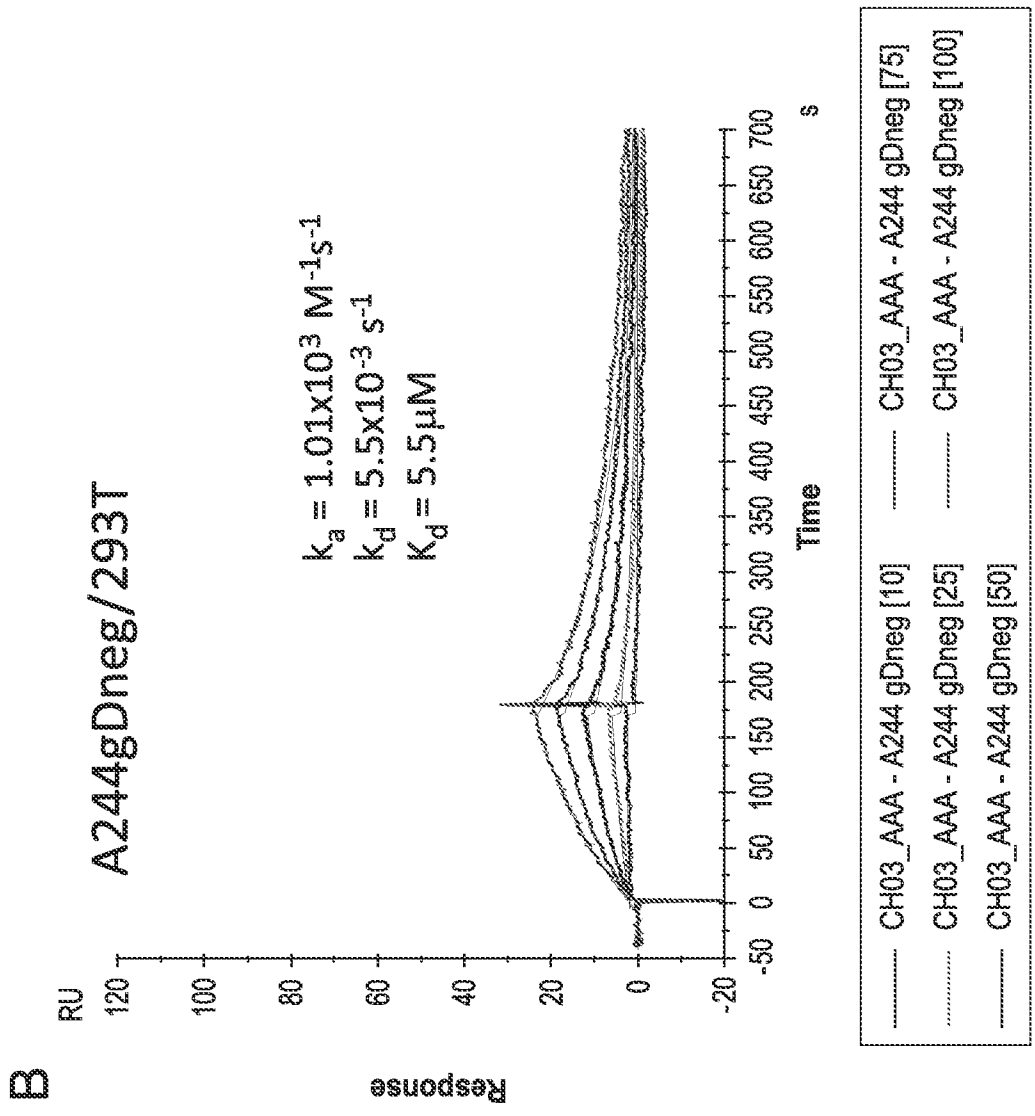

B
Binding to CH03 mAb
A244gDneg/293T
k_a = 1.01x10^3 M^{-1}s^{-1}
k_d = 5.5x10^{-3} s^{-1}
K_d = 5.5µM
Response
RU
120
100
80
60
40
20
0
-20
-50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700
Time
s
CH03_AAA - A244 gDneg [10]
CH03_AAA - A244 gDneg [25]
CH03_AAA - A244 gDneg [50]
CH03_AAA - A244 gDneg [75]
CH03_AAA - A244 gDneg [100]

Figure 17

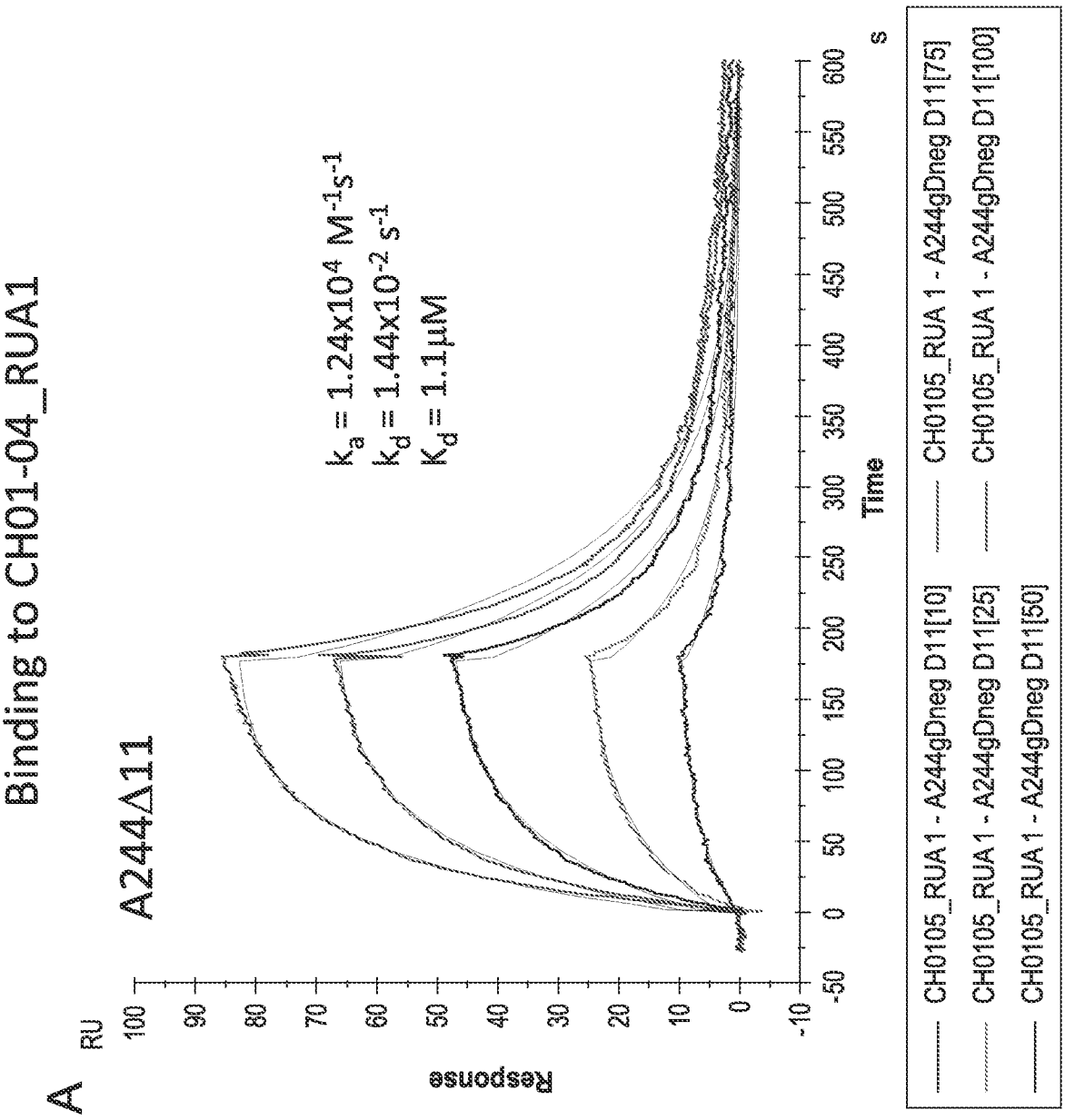

A
Binding to CH01-04_RUA1
A244Δ11
RU
Response
Time
s
k_a = 1.24x10^4 M^{-1}s^{-1}
k_d = 1.44x10^{-2} s^{-1}
K_d = 1.1µM
CH0105_RUA 1 - A244gDneg D11[10]
CH0105_RUA 1 - A244gDneg D11[25]
CH0105_RUA 1 - A244gDneg D11[50]
CH0105_RUA 1 - A244gDneg D11[75]
CH0105_RUA 1 - A244gDneg D11[100]

Figure 17

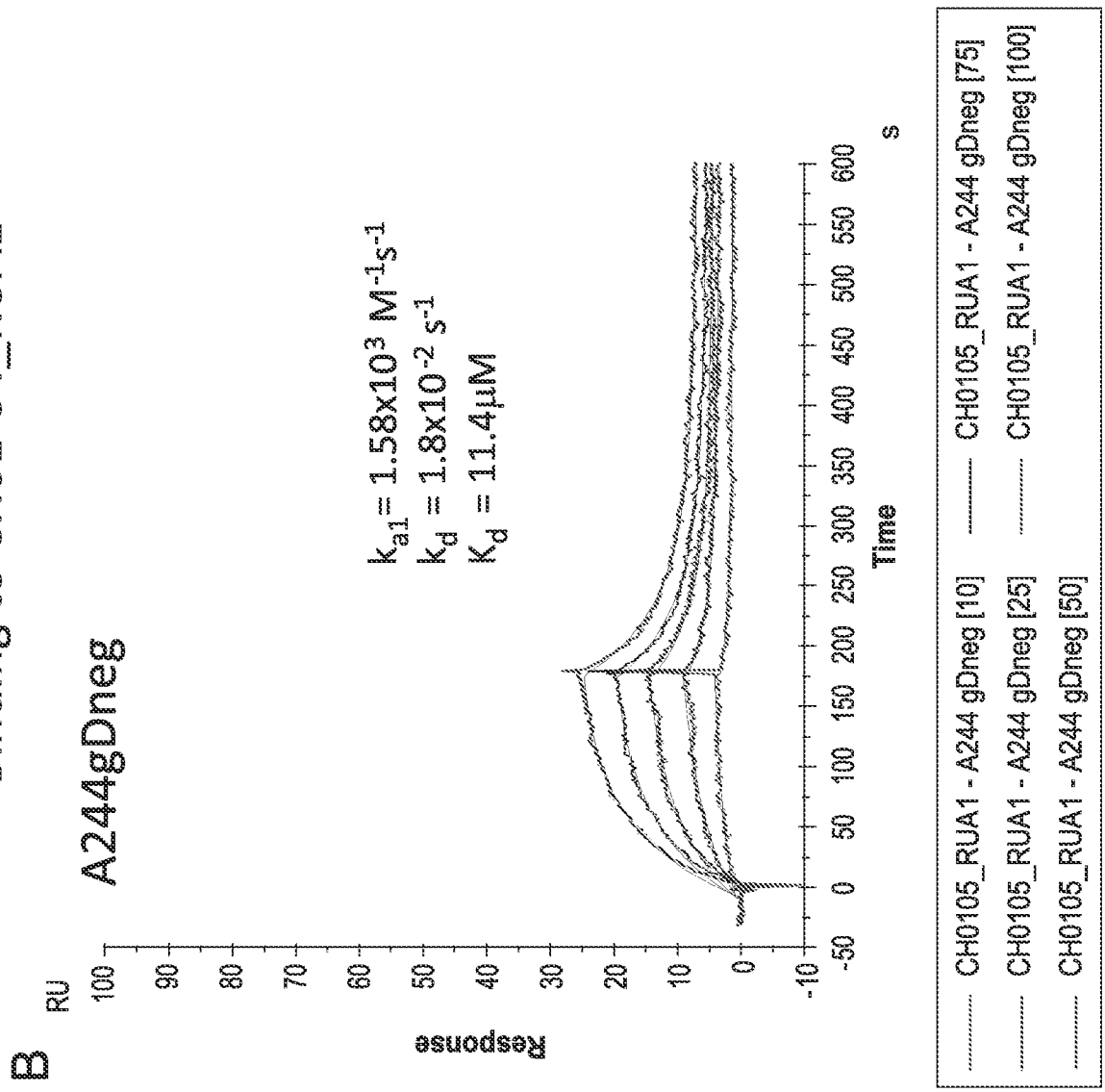

B
Binding to CH01-04_RUA1
A244gDneg
RU
Response
Time
s
k_{a1}= 1.58x10^3 M^{-1}s^{-1}
k_d = 1.8x10^{-2} s^{-1}
K_d = 11.4µM
CH0105_RUA1 - A244 gDneg [10]
CH0105_RUA1 - A244 gDneg [25]
CH0105_RUA1 - A244 gDneg [50]
CH0105_RUA1 - A244 gDneg [75]
CH0105_RUA1 - A244 gDneg [100]

Figure 18

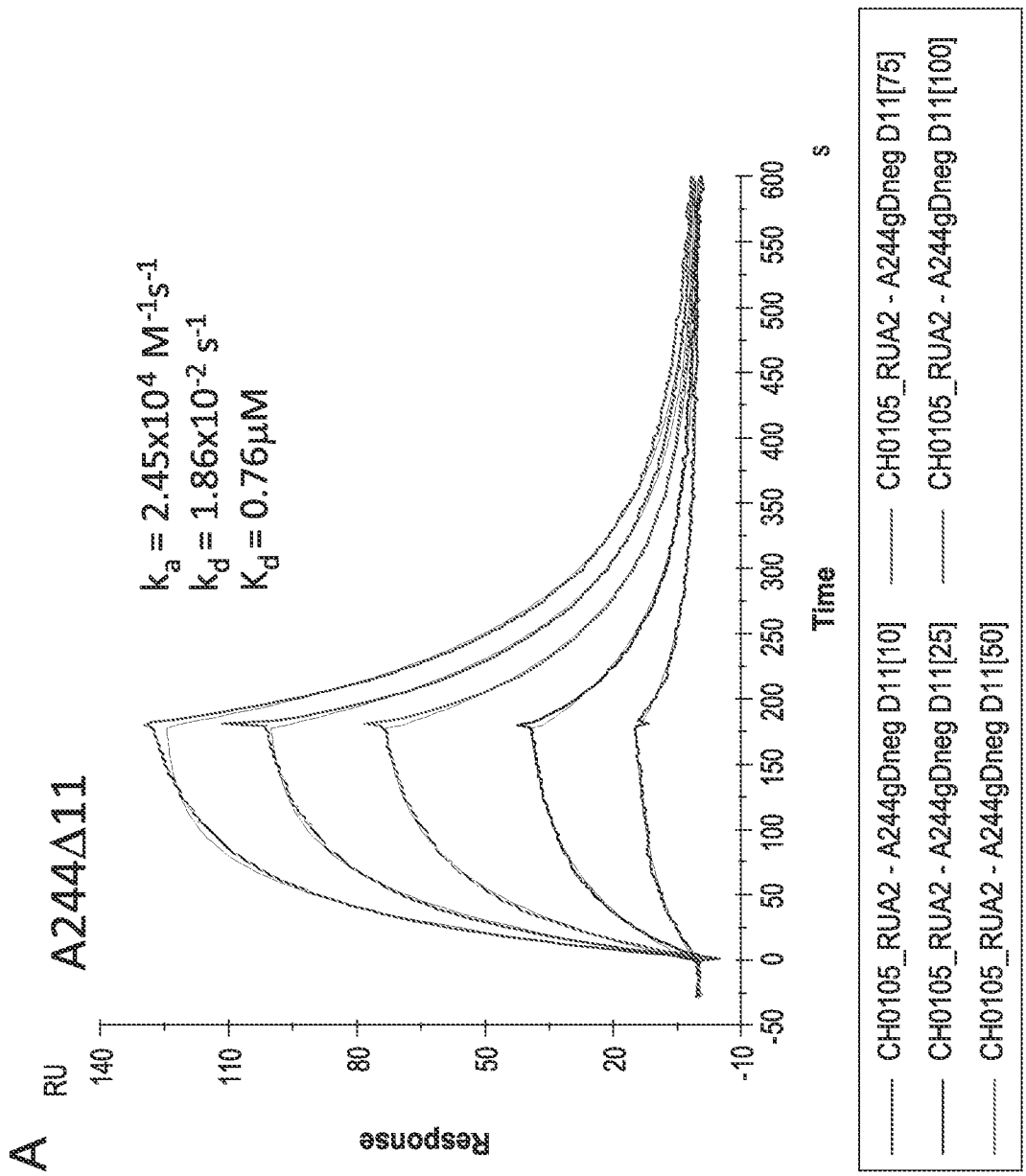

A
Binding to CH01-04_RUA2
A244Δ11
RU
140
110
80
50
20
-10
Response
k_a = 2.45x10^4 M^{-1}s^{-1}
k_d = 1.86x10^{-2} s^{-1}
K_d = 0.76µM
-50
0
50
100
150
200
250
300
350
400
450
500
550
600
Time
s
CH0105_RUA2 - A244gDneg D11[10]
CH0105_RUA2 - A244gDneg D11[25]
CH0105_RUA2 - A244gDneg D11[50]
CH0105_RUA2 - A244gDneg D11[75]
CH0105_RUA2 - A244gDneg D11[100]

Figure 18

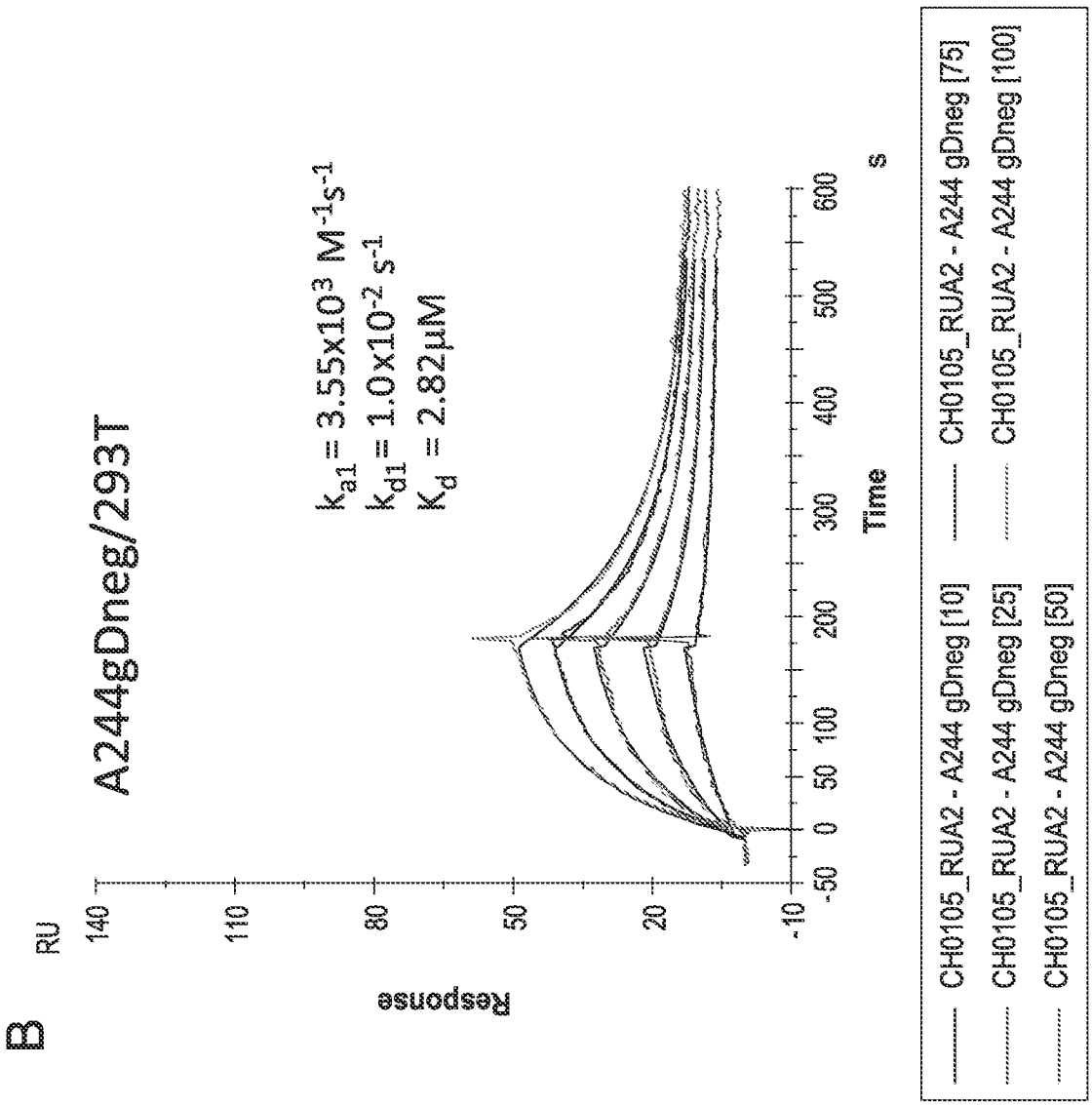

B
Binding to CH01-04_RUA2
A244gDneg/293T
RU
Response
Time
s
k_{a1}= 3.55x10^3 M^{-1}s^{-1}
k_{d1}= 1.0x10^{-2} s^{-1}
K_d = 2.82µM
CH0105_RUA2 - A244 gDneg [10]
CH0105_RUA2 - A244 gDneg [25]
CH0105_RUA2 - A244 gDneg [50]
CH0105_RUA2 - A244 gDneg [75]
CH0105_RUA2 - A244 gDneg [100]

Figure 19

Amino acid and nucleotide sequences the A244 gp120Δ11 envelope protein.

>A244gp120gDneg-Δ11

MRVKETQMNWPNLWKWGTLILGLVIICSAVPVWKEADTTLFCASDAKAHETEVHNVWATHACVPTDPNPQEIDLENVTENFN
MWKNNMVEQMEDVISLWDQSLKPCVKLTPPCVTLHCTNANLTNVNNRTNVSNIIGNITDEVRNCSFNMTTELRDKK
QKVHALFYKLDIVPIEDNNDSSEYRLINCNTSVIKQPCPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPCKNVSSVQCTH
GIKPVVSTQLLLNGSLAEEEIIIRSENLTNNAKTIIVHLNKSVVINCTRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYCE
INGTEWNKALKQVTEKLKEHFNNKPIIFQPPSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCIANGTIEGCNGNITLPCKIK
QIINMWQGAGQAMYAPPISGTINCVSNITGILLTRDGGATNNTNNETFRPGGGNIKDNWRNELYKYKVVQIEPLGVAPTRAK
RRVVEREKR*

>A244gp120gDneg-Δ11.opt

aagctt**gtcgacacc**ATGCGCGTGAAGGAGACCCAGATGAACTGGCCCAACCTGTGGAAGTGGGGCACCCTGATCCTGGGCC
TGGTGATCATCTGCTCCGCCGTGCCCGTGTGGAAGGAGGCCGACACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCCACGA
GACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGATCGACCTGGAGAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCAGGAGGACGTGATCTCCCTGTGGGACCAGTCCCTGA
AGCCCTGCGTGAAGCTGACCCCCCTGCGTGACCCTGCACTGCACCAACGCCACCTGACCAACGCCAAGGCCACCTGACCAACGT
GAACAACCGCACCAACGTGTCCAACATCATCACCGACGAGGTGCGCAACTGCTCCTTCAACATGACCACCGAG
CTGCGCGACAAGAAGCAGAAGGTGCACGCCCTGTTCTACAAGCTGGACATCGTGCCCATCGAGGACAACAACGACTCCTCCG
AGTACCGCCTGATCAACTGCAACACCTCCGTGATCAAGCAGCCCTGCCCCAAGATCTCCTTCGACCCCATCCCCATCCACTA
CTGCACCCCCGCCGGCTACGCCATCCTGAAGTGCAACGACAAGAACTTCAACGGCACCGGCCCCTGCAAGAACGTGTCCTCC
GTGCAGTGCACCCACGGCATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCCTGGCCGAGGAGGAGATCATCA
TCCGCTCCGAGAACCTGACCAACAACGCCATCATCGTGCACCTGAACAAGTCCGTGGTGATCAACTGCACCCGCCC
CTCCAACACACCCGCACCTCCATCACCATCGGCCCCGGCCAGGTGTTCTACCGCACCGGCGACATCATCGGCGACATCCGC
AAGGCCTACTGCGAGATCAACGGCACCGAGTGGAACAAGGCCCTGAAGCAGGTGACCGAGAAGCTGAAGGAGCACTTCAACA
ACAAGCCCATCATCTTCCAGCCCCCCTCCGGCGGCGACCTGGAGATCACCATGCACCACTTCAACTGCCGCGGCGAGTTCTT
CTACTGCAACACCACCCGCCTGTTCAACAACACCTGCATCGCCAACGGCACCATCGAGGGCTGCAACGGCAACATCACCCTG
CCCTGCAAGATCAAGCAGATCATCAACATGTGGCAGGGCGCCGGCCAGGCCATGTACGCCCCCCCCATCTCCGGCACCATCA
ACTGCGTGTCCAACATCACCGGCATCCTGCTGACCCGCGACGGCGGCGCCACCAACACCAACGAGACCTTCCGCCCC
CGGCGGCGGCAACATCAAGGACAACTGGCGCAACGAGCTGTACAAGTACAAGGTGGTGCAGATCGAGCCCCTGGGCGTGGCC
CCCACCCGCGCCAAGCGCCGTGGTGGAGCGCGAGAAGCGCT*taggatccatctaga*

Figure 20

Amino acid and nucleotide sequences of B63521, B.6240, M.Con-S, C.1086C and C.089C gp120 envelope proteins.

>B.63521_gp120Δ11
MRVKGIRKNYQHLWRWGTMLLGILMICSAVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQELVLANVTENFN
MWNNTMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDVTNATNINNSSGGVESGEIKNCSFNITTSVRDKVQKEY
ALFYKLDIVPITNESSKYRLISCNTSVLTQACPKVSFEPIPIHYCAPAGFAILKCNNETFNGKGPCINVSTVQCTHGIRPVV
STQLLLNGSLAEKEVIIRSDNFSDNAKNIIVQLKEYVKINCTRPNNNTRKSIHIGPGRAFYATGEIIGNIRQAHCNISRSKW
NDTLKQIAAKLGEQFRNKTIVFNPSSGGDLEIVTHSFNCGGEFFYCNTTKLFNSTWIREGNNGTWNGTIGLNDTAGNDTIIL
PCKIKQIINMWQEVGKAMYAPPIRGQIRCSSNITGLILTRDGGKDDSNGSEILEIFRPGGGDMRDNWRSELYKYKVVRIEPL
GVAPTRARERVVQKEKE

>B.63521_gp120Δ11
aagcttgtcgacaccATGCGCGTGAAGGGCATCCGCAAGAACTACCAGCACCTGTGGCGCTGGGGCACCATGCTGCTGGGCA
TCCTGATGATCTGCTCCGCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGA
CACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGCTGGTGCTGGCCAACGTG
ACCGAGAACTTCAACATGTGGAACAACACCATGGTGGAGCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTGA
AGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCGACGTGACCAACGCCACCACCAACATCAACGCCACCAA
CATCAACAACTCCTCCGGCGCGTGGAGTCCGGCGAGATCAAGAACTGCTCCTTCAACATCACCACCTCCGTGCGCGACAAG
GTGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGACATCGTGCCCATCACCAACGAGTCCTCCAAGTACCGCCTGATCTCCT
GCAACACCTCCGTGCTGACCCAGGCCTGCCCCAAGGTGTCCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTT
CGCCATCCTGAAGTGCAACGACAAGACCTTCAACGGCACAGGGCCCCCTGCATCAACGTGTCCACCGTGCAGTGCACCCACGGC
ATCCGCCCCGTGGTGTCCACCCAGCTGCTGAACGGCTCCCTGGCCGAGAAGGAGGTGATCATCCGCTCCGACAACTTCT
CCGACAACGCCAAGAACATCATCGTGCAGCTGAAGGAGTACGTGAAGATCAACTGCACCCGCCCCAACAACACCCGCAAA
GTCCATCCACATCGGCCCCGGCCGCTTCTACGCCACCGGCGAGATCATCGGCAACATCCGCCAGGCCCACTGCAACATC
TCCCGCTCCAAGTGGAACGACACCCTGAAGCAGATCGCCGCCAAGCTGGGCGAGCAGTTCCGCAACAAGACCATCGTGTTCA
ACCCCTCCTCCGGCGGCGACCTGGAGATCGTGACCCACTCCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCACCCAA
GCTGTTCAACTCCACCTGGATTCGCGAGGGCAACAACGGCACCATCGGCCTGAACGACACCGCCGGCAAC
GACACCATCATCCTGCCCCTGCAAGATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCA
TCCGCGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTGATCCTGACCCGCGACGGCGGCAAGGACGACTCCAACGGCTC
CGAGATCCTGGAGATCTTCCGCCCCGGCGGCGACATGCGCGACAACTGGCGCTCCGAGCTGTACAAGTACAAGGTGGTG
CGCATCGAGCCCCTGGGCGTGGCCCCCACCCGCGCCCGAGCGCGTGGTGCAGAAGGAGGAGGAGGAGTAGggatcctctaga_

Figure 20 Cont'd-1

>B.6240_gp120Δ11
MRVKGIRKNYQHLWRWGIMLLGTLMICSAVPVWKEATTTLFCASDAKAYSPEKHNIWATHACVPTDPNPQELVLGNV
TEDFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLKNSATDTNGTSGTNNRTVEQGMETEIKNCSFNITTGI
GNKMQKEYALFYKLDVVPIDSNNNSDNTSYRLISCNTSVVTQACPKTSFEPIPIHYCAPAGFAILKCNNKTFSGKGPCKNVS
TVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENFTNNAKTIIVQLNESVIINCTRPNNNTRKGIHIGLGRALYATGDIIGDI
RQAHCNLSSKSWNKTLQQVVRKLREQFGNKTIAFNQSSGGDQEIVKHSFNCGGEFFYCDTTQLFNSTWSSNDTWNSTGVQDN
NITLPCRIKQIINMWQEVGKAMYAPPIQGLISCSSNITGLLLTRDGGTNNTNATEIFRPGGGDMRDNWRSELYKYKVVKIEP
LGIAPTKAKRRVVQREKR

>B.6240_gp120Δ11.□□□
aagcttgtcgaccaccATGCGCGTGAAGGGCATCCGCAAGAACTACCAGCACCTGTGGCGCTGGGGCATCTGGCGCTGGGGCA
TCATGCTGCTGGGCACCCTGATGATCTGCTCCGCCGTGTGGAAGGAGGCCACCACCCTGTTCTGCGCCTCCGA
CGCCAAGGCCTACTCCCCCGAGAAGCACAACATCTGGGCCACCCGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGCTG
GTGCTGGGGCAACGTGACCGAGGACTTCAACATGTGGAAGAACAACATGGTGGAGGCAGATGCACGAGGACATCATCTCCCTGT
GGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCGACCTGAAGAACTCCGCCAC
CGACACCAACGGCACCTCCGGCACCAACACCGCACCGTGGAGCAGGGCATGGAGACCGAGATCAAGAACTGCTCCTTCAAC
ATCACCACCGGCATCGGCAACAAGATGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACTCCAACA
ACAACTCCGACACAACACCTCCTACCGCCTGATCTCCTGCAACACCTCCGTGGTGACCCAGGCCTGCCCCAAGACCTCCTTCGA
GCCCATCCCCATCCACTACTGCGCCCCCGGCTTCGCCATCCTGAAGTGCAACAACAAGACCTTCTCCGGCAAGGGCCCC
TGCAAGAACGTGTCCACCGTGCAGTGCACCCACGGCATCCGCCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCCTGG
CCGAGGAGGAGATCGTGATCCGCTCCGAGAACTTCACCAACAACGCCAAGAACCATCATCGTGCAGCTGAACGAGTCCGTGAT
CATCAACTGCACCCGCCCCAACAACACCCGCAAGGGCATCCACATCGGCCCTGGGCCGCGCCCTGTACGCCACCGGCGAC
ATCATCGGCGACATCCGCCAGGCCCACTGCAACCTGTCCTCCAAGTCCTGGAACAAGACCCTGCAGCAGGTGGTGCGCAAGC
TGCGCGAGCAGTTCGGCAACAAGACCATCGCCTTCAACCAGTCCTCCGGCGGCGACCCAGGAGATCGTGAAGCACTCCTTCAA
CTGCGGCGGCGAGTTCTTCTACTGCGACACCACCCAGCTGTTCAACTCCACCTGGTCCTCCAACGACACCTGGAACTCCACC
GGCGTGCAGGACAACACATCACCCTGCCCTGCCGCATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGT
ACGCCCCCCCCATCCAGGGCCTGATCTCCTGCTCCTCCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAACCAACAA
CACCAACGCCACCGAGATCTTCCGCCCCGGCGGCGACATGCGCGACAACTGGCGCTCCGAGCTGTACAAGTACAAGGTG
GTGAAGATCGAGCCCCTGGGCATCGCCCCCACCAAGGCCAAGCGCCGTGGTGCAGCGCGAAGAAGCGCTAGggatcctcta
ga_

>M.CON-S_gp120Δ11
MRVRGIQRNCQHLWRWGTLILGMLMICSAVPVWKEANTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIVLENVTENFN

Figure 20 Cont'd-2

MWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVNVTNTTNNTEEKGEIKNCSFNITTEIRDKKQKVYALFYRLDV
VPIDDNNNNSSNYRLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLL
LNGSLAEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHCNISGTKWNKTLQ
QVAKKLREHFNNKTIIFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTWIGNGTKNNNNTNDTITLPCRIKQIINMWQGV
GQAMYAPPIEGKITCKSNITGLLLTRDGGNNNTNETEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVEREKR

>M.CON-S_gp120Δ11.□□□
aagcttgtcgacaccATGCGCGTGCGCGGCATCCAGCGCAACTGCCAGCACCTGTGGCGCTGGGGCACCCTGATCCTGGGCA
TGCTGATGATCTGCTCCGCCGTGCCCGTGTGGAAGGAGGCCAACACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGA
CACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGATCGTGCTGGAGAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTGA
AGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCAACGTGAACGTGACCAACACCACCAACACCGA
GGAGAAGGGCGAGATCAAGAACTGCTCCTTCAACATCACCACCGAGATCCGCGACAAGAAGCAGAAGGTGTACGCCCTGTTC
TACCGCCTGGACGTGGTGCCCATCGACGACAACAACACCAACTACCGCCTGATCAACTGCAACACCTCCGCCA
TCACCCAGGCCTGCCCCAAGGTGTCCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTG
CAACGACAAGAAGTTCAACGGCACCGGCCCCTGCAAGAACGTGTCCACCGTGCAGTGCACCCACGGCATCAAGCCCGTGGTG
TCCACCCAGCTGCTGCTGAACGGCTCCCTGGCCGAGGAGGAGATCATCATCCGCTCCGAGAACATCACCAACAACGCCAAGA
CCATCATCGTGCAGCTGAACGAGTCCGTGGAGATCAACTGCACCCGCCCCAACAACACCCGCAAGTCCATCCGCATCGG
CCCCGGCCAGGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCTCCGGCACCAAGTGG
AACAAGACCCTGCAGCAGGTGGCCAAGAAGCTGCGCGAGCACTTCAACAACAAGACCATCATCTTCAAGCCCTCCTCCGGCG
GCGACCTGGAGATCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTTCTACTGCAACACCTCCGGCCTGTTCAACTCCAC
CTGGATCGGCAACGGCACCAAGAACACAACACCAACGACACCATCACCCTGCCCTGCCGCATCAAGCAGATCATCAACAAC
ATGTGGCAGGGCGTGGGCCAGGCCATGTACGCCCCCCCCATCGAGGGCAAGATCACCTGCAACATCACCGGCCTGC
TGCTGACCCGCGACGGCGGCAACAACACCAACGAGACCGAGATCTTCCGCCCCGGCGGCGACATGCGCGACAACTG
GCGCTCCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCTGGGCGTGGCCCCCACCAAGGCCAAGCGCCGCGTGGTG
GAGCGCGAGAAGCGCTAGggatcctctaga_

>C.1086C_gp120Δ7
MRVRGIWKNWPQWLIWSILGFWIGNMEGSVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEMVLANVTENFN
MWKNDMVEQMHEDIISLWDESLKPCVKLTPLCVTLNCTNVKGNESDTSEVMKNCSFKATTELKDKKHKVHALFYKLDVVPLN
GNSSSSGEYRLINCNTSAITQACPKVSFDPIPLHYCAPAGFAILKCNNKTFNGTGPCRNVSTVQCTHGIKPVVSTQLLLNGS

Figure 20 Cont'd-3

LAEEEIIIRSENLTNNAKTIIVHLNESVNIVCTRPNNNTRKSIRIGPGQTFYATGDIIGNIRQAHCNINESKWNNTLQKVGE
ELAKHFPSKTIKFEPSSGGDLEITTHSFNCRGEFFYCNTSDLFNGTYRNGTYNHTGRSSNGTITLQCKIKQIINMWQEVGRA
IYAPPIEGEITCNSNITGLLLLRDGGQSNETNDTETFRPGGGDMRDNWRSELYKYKVVEIKPLGVAPTEAKRRVVEREKR

>C.1086C_gp120Δ7.opt
aagcttgtcgaccaccATGCGCGTGCGCGGCATCTGGAAGAACTGGCCCCAGTGGCTGATCTGGTCCATCCTGGGCTTCTGGA
TCGGCAACATGGAGGGCTCCGTGCCCGTGTGGAAGGAGGCCAAGACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGA
GAAGGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCCCCAACCCCCAGGAGATGGTGCTGGCCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACGACATGGTGGAGCAGATGCACGAGGACATCATCTCCCTGTGGGACGAGTCCCTGA
AGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCAACGTGAAGGGCAACGAGTCCGACACCTCCGAGGT
GATGAAGAACTGCTCCTTCAAGGCCACCACCGAGCTGAAGGACAAGAAGCACAAGGTGCACGCCCTGTTCTACAAGCTGGAC
GTGGTGCCCCTGAACGGCAACTCCTCCTACCGGCGAGTACCGCCTGATCAACTGCAACACCTCCGCCATCACCCAGGCCT
GCCCCAAGGTGTCCTTCGACCCCATCCCCATCCACTACTGCGCCCCCGGCTTCGCCATCCTGAAGTGCAACAACAAGAC
CTTCAACGGCACCGGCCCCTGCCGCAACGTGTCCACCGTGCAGTGCACCCACGGCATCAAGCCCGTGGTGTCCACCCAGCTG
CTGCTGAACGGCTCCCTGGCCGAGGAGGAGATCATCATCCGCTCCGAGAACCTGACCAACAACGCCAAGACCATCATCGTGC
ACCTGAACGAGTCCGTGAACATCGTGTGCACCCCGCCCCAACACACCCGCAAGTCCATCCGCATCGGCCCCGGCCAGAC
CTTCTACGCCACCGGCGACATCATCGGCAACATCCGCCACTGCAACATCAACGAGTCCAAGTGGAACAACACCCTG
CAGAAGGTGGGCGAGGAGCTGGCCAAGCACTTCCCCTCCAAGACCATCAAGTTCGAGCCCCTCCTCCGGCGCGACCTGGAGA
TCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTTCTACTGCAACACCTCCGACCTGTTCAACGGCACCTACCGCAACGG
CACCTACAACCACACCGGCCGCTCCTCCAACGGCACCATCACCCTGCAGTGCAAGATCATCAACATGTGGCAG
GAGGTGGGCCGCGCCATCTACGCCCCCCCCATCGAGGGCGAGATCACCTGCAACTCCAACATCACCGGCCTGCTGCTGC
GCGACGGCGGCCAGTCCAACGAGACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCGACATGCGCGACAACTGGCGCTC
CGAGCTGTACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCGTGGCCCCCACCGAGGCCAAGCGCCGCGTGGTGGAGCGC
GAGAAGCGCTAGggatcctctaga

>C.089C_gp120Δ11
MRVRGMLRNCQQWWIWGILGFWMLMICSVVPVWKEAKTTLFCASDARAYEREVHNVWATHACVPTDPNPQEMVLVNVTENFN
MWKNDMVDQMHEDIISLWDQSLKPCVKLTPLCVILECNNANGTTNNGSVIVVNENSTMYGEIQNCSFKVNSEIKGKKQDVYA
LFNSLDIVKLYNNGTSQYRLINCNTSTLTQACPKVSFDPIPIHYCAPAGYAILKCNNKTFNGTGPCNNVSTVQCTHGIKPVV
STQLLLNGSLAEGEIIIRSKNLTDNTKTIIVHLNESIKINCIRPNNNTRSIRIGPGQAFYAANGIVGNIRQAHCNISEGEW
NKTLYRVSRKLAEHFPGKEIKFKPHSGGDLEITTHSFNCRGEFFYCNTSKLFNGTYNGTYTNNDTNSTIILPCRIKQIINMW

Figure 20 Cont'd-4

QEVGQAMYAPPIEGIIACNSTITGLLLTRDGGDKNGSKPEIFRPGGGDMRDNWRSELYKYKVVEIKPLGIAPTKAKRRVVEK
EKTIQKR

>C.089C_gp120Δ11.□□□

aagcttgtcgacaccATGCGCGTGCGCGGCATGCTGCGCAACTGCCAGCAGTGGTGGATCTGGGGCATCCTGGGCTTCTGGA
TGCTGATGATCTGCTCCGTGGTGCCCGTGTGGAAGGAGGCCAAGACCACCCTGTTCTGCGCCTCCGACGCCCCGCGCCTACGA
GCGCGAGGTGCACACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCCAGGAGATGGTGCTGGTGAACGTG
ACCGAGAACTTCAACATGTGGAAGAACGACATGGTGGACCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTGA
AGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGATCCTGGAGTGCAACAACGCCAACGGCACCACCAACACGGCTCCGTGAT
CGTGGTGAACGAGAACTCCACCATGTACGGCGAGATCCAGAACTGCTCCTTCAAGGTGAACTCCGAGATCAAGGGCAAGAAG
CAGGACGTGTACGCCCTGTTCAACTCCCTGGACATCGTGAAGCTGTACAACAACGGCACCTCCCAGTACCGCCTGATCAACT
GCAACACCTCCACCCTGACCCAGGCCTGCCCCAAGGTGTCCTTCGACCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTA
CGCCATCCTGAAGTGCAACAACAAGACCTTCAACGGCACCGGCCCCTGCAACACGTGTCCACCGTGCAGTGGCACCCACGGC
ATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCCTGGCCGAGGGCGAGATCATCATCCGCTCCAAGAACCTGA
CCGACAACACCAAGACCATCATCGTGCACCTGAACGAGTCCATCAAGATCAACTGCACATCCGCCCCAACAACACCCGCCG
CTCCATCCGCATCGGCCCCGGCCAGGCCTTCTACGCCGCCAACGGCATCGTGGGCAACATCCGCCAGGCCCACTGCAACATC
TCCGAGGGCGAGTGGAACAAGACCCTGTACCGCGTGTCCCGCAAGCTGGCCGAGCACTTCCCCGGCAAGGAGATCAAGTTCA
AGCCCCACTCCGGCGGCGACCTGGAGATCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTTCTACTGCAACACCTCCCAA
GCTGTTCAACGGCACCTACAACGGCACCTACACCAACGACACCAACTCCACCATCATCCTGCCCTGCCGCATCAAGCAG
ATCATCAACATGTGGCAGGAGGTGGGCCAGGCCATGTACGCCCCCCCCATCGAGGGCATCATCGCCTGCAACTCCACCATCA
CCGGCCTGCTGCTGACCCGCGACGGCGGCAAGAACGGCTCCAAGCCCGAGATCTTCCGCCCCGGCGGCGACATGCG
CGACAACTGGCGCTCCGAGCTGTACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCATCGCCCCCACCAAGGCCAAGCGC
CGCGTGGTGGAGAAGGAGAAGAGACCATCCAGAAGCGCTAGggatcctctaga_

Figure 21

Figure 21. Nucleotide and amino acid sequences of B63521, B.6240, M.Con-S, C.1086C and C.089C gp140C envelope proteins.

>B.63521_gp140C

MRVKGIRKNYQHLWRWGTMLLGILMICSAAAQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQE
LVLANVTENFNMWNNTMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDVTNATNINNSSGGVESGEIKNCSFNIT
TSVRDKVQKEYALFYKLDIVPITNESSKYRLISCNTSVLTQACPKVSFEPIPIHYCAPAGFAILKCNNETFNGKGPCINVST
VQCTHGIRPVVSTQLLLNGSLAEKEVIIRSDNFSDNAKNIIVQLKEYVKINCTRPNNNTRKSIHIGPGRAFYATGEIIGNIR
QAHCNISRSKWNDTLKQIAAKLGEQFRNKTIVFNPSSGGDLEIVTHSFNCGGEFFYCNTTKLFNSTWIREGNNGTWNGTIGL
NDTAGNDTIILPCKIKQIINMWQEVGKAMYAPPIRGQIRCSSNITGLILTRDGGKDDSNGSEILEIFRPGGGDMRDNWRSEL
YKYKVVRIEPLGVAPTRARERVVQKEKEAVGLGAMFLGFLGAAGSAMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHM
LQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTDVPWDTSWSNKTLDDIWGSNMTWMEWEREIDNYTSTIYTLL
EEAQYQQEKNEKELLELDKWASLWNWFDITNWLWYIR

>B.63521_gp140C.opt

ATGCGCGTGAAGGGCATCCGCAAGAACTACCAGCACCTGTGGCGCTGGGGCACCATGCTGCTGGGCATCCTGATGATCTGCT
CCGCCGCCGCCCAGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCCTGTTCTGCGCCTC
CGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAG
CTGGTGCTGGCCAACGTGACCGAGAACTTCAACATGTGGAACAACACCATGGTGGAGCAGATGCACGAGGACATCATCTCCC
TGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCGACGTGACCAACGCCAC
CAACATCAACGCCACCAACATCAACAACTCCTCCGGCGGCGTGGAGTCCGGCGAGATCAAGAACTGCTCCTTCAACATCACC
ACCTCCGTGCGCGACAAGGTGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGACATCGTGCCCATCACCAACGAGTCCTCCA
AGTACCGCCTGATCTCCTGCAACACCTCCGTGCTGACCCAGGCCTGCCCCAAGGTGTCCTTCGAGCCCATCCCCATCCACTA
CTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAACAACGAGACCTTCAACGGCAAGGGCCCCTGCATCAACGTGTCCACC
GTGCAGTGCACCCACGGCATCCGCCCCGTGGTGTCCACCCAGCTGCTGCTCCCTGGCCGAGAAGGAGGTGATCA
TCCGCTCCGACAACTTCTCCGACAACGCCAAGAACATCATCGTGCAGCTGAAGGAGTACGTGAAGATCAACTGCACCCGCCC
CAACAACACACCCGCAAGTCCATCCACATCGGCCCCGGCCGCTTCTACGCCACCGGCGAGATCATCGGCAACATCCGC
CAGGCCCACTGCAACATCTCCCGCTCCAAGTGGAACGACACCCTGAAGCAGATCGCCGCCAAGCTGGGCGAGCAGTTCCGCA
ACAAGACCATCGTGTTCAACCCCTCCTCCGGCGGCGACCCTGAGATCGTGACCCACTCCTTCAACTGCGGCGAGTTCTT
CTACTGCAACACCACCCAAGCTGTTCAACTCCACCTGGATCCGCGAGGGCAACAACGGCACCCTGGAACGGCACCATCGGCCTG
AACGACACCGCCGGCAACGACACCATCATCCTGCCCTGCAAGATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGCAAGG
CCATGTACGCCCCCCCCCCATCCGGCGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTGATCCTGACCCGCGACGGCGGCAA

Figure 21 Cont'd-1

GGACGACTCCAACGGCTCCGAGATCCTGGAGATCTTCCGCCCCGGCGGCGGCGACATGCGCGACAACTGGCGCTCCGAGCTG
TACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGGCCCCCACCCGCGCCCCGCGAGCGCGTGGTGTGCAGAAGGAGGAG
AGGCCGTGGGCCTGGGCGCCATGTTCCTGGGCGCCGGCTCCGCCATGGGCGCCGCCTCCATGACCCTGAC
CGTGCAGGCCCGCCAGCTGCTGTCCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCCAGCAGCACATG
CTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCGTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGC
TGGGCATCTGGGGCTGCTCCGGCAAGCTGATCTGCACCACCGACGTGCCCTGGGACACCTCCTGGTCCAACAAGACCCTGGA
CGACATCTGGGGCCTCCAACATGACCTGGATGGGAGTGGGGACGCGCGAGATCGACAACTACACCTCCACCATCTACACCCTGCTG
GAGGAGGCCCAGTACCAGCAGGAGAAGAACGAGAAGGAGCTGCTGGAGCTGGACAAGTGGGCCTCCCTGTGGAACTGGTTCG
ACATCACCAACTGGCTGTGGTACATCCGCTAGGGATCC

>B.6240_140C

MRVKGIRKNYQHLWRWGIWRWGIMLLGTLMICSATEKLWVTVYYGVPVWKEATTTLFCASDAKAYSPEKHNIWATHACVPTD
PNPQELVLGNVTEDFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLKNSATDTNGTSGTNNRTVEQGMETEI
KNCSFNITTGIGNKMQKEYALFYKLDVVPIDSNNNSDNTSYRLISCNTSVVTQACPKTSFEPIPIHYCAPAGFAILKCNNKT
FSGKGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENFTNNAKTIIVQLNESVIINCTRPNNNTRKGIHIGLGRA
LYATGDIIGDIRQAHCNLSSKSWNKTLQQVVRKLREQFGNKTIAFNQSSGGDQEIVKHSFNCGGEFFYCDTTQLFNSTWSSN
DTWNSTGVQDNNITLPCRIKQIINMWQEVGKAMYAPPIQGLISCSSNITGLLLTRDGGTNNTNATEIFRPGGGDMRDNWRSE
LYKYKVVKIEPLGIAPTKAKERVVQREKEAVGLGAVFIGFLGAAGSTMGAASVTLTVQARQLLSGIVQQQNNLLRAIEAQQH
MLQLTVWGIKQLQARILAVERYLKDQQILGIWGCSGKLICPTAVPWNASWSNKSLTAIWNNMTWMEWEREIDNYTGLIYSLI
EESQIQQEQNEKELLELDKWASLWNWFDITKWLWYIK

>B.6240_140C.opt

ATGCGCGTGAAGGGCATCCGCAACTACCAGCACCTGTGGCGCTGGGGCATCATGCTGCTGGGCA
CCCTGATGATCTGCTCCGCCACCGAGAAGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCAC
CCTGTTCTGCGCCTCCGACGCCAAGGCCTACTCCCCCGAGAAGCACACAACATCTGGGCCACCCACGCCTGCGTGCCCACCGAC
CCCAACCCCCAGGAGCTGGTGCTGGGCAACGTGACCGAGGACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCACG
AGGACATCATCTCCCTGTGGGACCAGTCCCTGAAGCCTGACCCCCCCTGTGCGTGACCCTGAACTGCACCGA
CCTGAAGAACTCCGCCACCGACACCAACGGCACCTCCGGCACCAACAACCGCACCGTGGAGGCAGGGCATGGAGACCGAGATC
AAGAACTGCTCCTTCAACATCACCACCGGCATCGGCAACAAGATGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGG
TGCCCATCGACTCCAACAACAACTCCGACAACACCTCCTACCGCCTGATCTCCTGCAACACCTCCGTGGTGACCCAGGCCCTG
CCCCAAGACCTCCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGGCTTCGCCATCCTGAAGTGCAACAACAAGACC

Figure 21 Cont'd-2

TTCTCCGGCAAGGGCCCCTGCAAGAACGTGTCCACCGTGCAGTGCACCCACGGCATCCGCCCCGTGGTGTCCACCCAGCTGC
TGCTGAACGGCTCCCTGGCCGAGGAGATCGTGATCCGCTCCGAGAACTTCACCAACAACGCCAAGACCATCATCGTGCA
GCTGAACGAGTCCGTGATCATCAACTGCACCCGCCCCAACAACACCCGCAAGGGCATCCACATCGGCCTGGGCCGCGCC
CTGTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACCTGTCCTCCAAGTCCTGGAACAAGACCCTGC
AGCAGGTGGTGCGCAAGCTGCGCGAGCAGTTCGGCAACAAGACCATCGCCTTCAACCAGTCCTCCGGCGGCGACCAGGAGAT
CGTGAAGCACTCCTTCAACTGCGGCGGCGAGTTCTTCTACTGCGACACCACCCAGCTGTTCAACTCCACCTGGTCCTCCAAC
GACACCTGGAACTCCACCGGCGTGCAGGACAACAACATCACCCTGCCCTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
AGGTGGGCAAGGCCATGTACGCCCCCCCCATCCAGGGCCTGATCTCCTGCTCCTCCAACATCACCGGCCTGCTGCTGACCCG
CGACGGCGGCACCAACAACACCGAGATCTTCCGCCCCGGCGGCGACATGCGCGACAACTGGCGCTCCGAG
CTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCTGGGCATCGCCCCCACCAAGGCCAAGGAGCGCGTGGTGCAGCGCGAGA
AGGAGGCCGTGGGCCTGGGCGCCGTGTTCATCGGCTTCCTGGGCGCCGGCTCCACCATGGGCGCCGCCTCCGTGACCCT
GACCGTGCAGGCCCGCCAGCTGCTGTCCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCCAGCAC
ATGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGA
TCCTGGGCATCTGGGGCTGCTCCGGCAAGCTGATCTGCCCCACCGCCGTGCCCTGGAACGCCTCCTGGTCCAACAAGTCCCT
GACCGCCATCTGGAACACAACATGACCTGGATGGGAGTGGGAGCGCGAGATCGACAACTACACCGGCCTGATCTACTCCCTGATC
GAGGAGTCCCAGATCCAGCAGGAGCAGAACGAGAAGGAGCTGCTGGAGCTGGACAAGTGGGCCTCCCTGTGGAACTGGTTCG
ACATCACCAAGTGGCTGTGGTACATCAAGTAG

>M.CON-S_140C
MRVRGIQRNCQHLWRWGTLILGMLMICSAAENLWVTVYYGVPVWKEANTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQE
IVLENVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVNVTNTTNNTEEKGEIKNCSFNITTEIRDKKQ
KVYALFYRLDVVPIDDNNNNSSNYRLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTH
GIKPVVSTQLLLNGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHCN
ISGTKWNKTLQQVAKKLREHFNNKTIIFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTWIGNGTKNNNNTNDTITLPCR
IKQIINMWQGVGQAMYAPPIEGKITCKSNITGLLLTRDGGNNNTNETEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTK
AKERVVEREKEAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLRAIEAQQHLLQLTVWGIKQLQARVLA
VERYLKDQQLLGIWGCSGKLICTTTVPWNSSWSNKSQDEIWDNMTWMEWEREINNYTDIIYSLIEESQNQQEKNEQELLALD
KWASLWNWFDITNWLWYIK

Figure 21 Cont'd-3

>M.CON-S_140C.opt

ATGAGGGTCCGGGGAATCCAGCGCAACTGCCAGCACCTCTGGAGGTGGGGCACGCTGATCCTGGGGATGCTGATCTGCA
GCGCGGCTGAGAACCTGTGGGTGACAGTGTACTACGGCGTGCCTGTGTGGAAGGAGGCCAACACCACCCTGTTCTGCGCCTC
GGACGCCAAGGCCTACGACACGGAGGTCCACAACGTGTGGGCTACCCACGCCTGCCGTGCCCACCGACCCCAATCCTCAGGAG
ATCGTCCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCACGAGGACATCATCAGCCT
TGTGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCTGTGCGTGACCCTGAACTGCACGAACGTGAACGTGACCAA
CACCACGAACACAACGGAGGAGAAGGGGGAGATCAAGAACTGCAGCTTCAACATCACCACCGAGATCCGGAAGAAGCAG
AAGGTGTACGCCCTGTTCTACCGGCTGGACGTCGTGCCGATCGACGACAACAACACAGAACTCCAGCAACTACAGGCTGATCA
ACTGCAACACCCAGCGCGATCACCCAGGCCTGCCCTAAGGTGTCGTTCGAGCCCATCCCCATCCACTACTGCGCGCCCTGCCGG
CTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCACCGGCCCCTGCAAGAACGTCAGCACCGTCCAGTGCACCCAC
GGCATCAAGCCTGTGGTGTCCACCCAGCTGCTCCTGAACGGCAGCCTGGCCGAGGAGGAGATCATCATCAGGAGCGAGAACA
TCACCAACAACGCCAAGACGATCATCGTGCAGCTGAACGAGTCGGTGGAGATCAACTGCACCCGGCCCAACAACACGCG
GAAGAGCATCCGGATCGGCCCTGGACAGGCGTTCTACGCCACGGGCGACATCATCGGCGACATCAGGCAGGCCCACTGCAAC
ATCTCGGGGACGAAGTGGAACAAGACCCTGCAGCAGGTCGCGAAGAAGCTGAGGGAGCACTTCAACAACAAGACCATCATCT
TCAAGCCCGAGGCAGCGGCGGAGACCTGGAGATCACCACGCACTCGTTCAACTGCCGGGGCGAGTTCTTCTACTGTAACACGTC
GGGCCTGTTCAACAGCACCCTGGATCGGCACCGGAAGAACAACAACACTAACGACACCATCACCCTGCCCTGCCGG
ATCAAGCAGATCATCAACATGTGGCAGGGCGTGGGCCAGGCTATGTACGCCCCTCCCATCGAGGGCAAGATCACGTGCAAGA
GCAACATCACCGGCCTGCTGACCAGGGACGGCGGAACACAACACGAACGAGACCGAGATCTTCAGACCTGGCGGCGG
AGACATGAGACAACTGGCGGAGCGAGCTGTACAAGTACAAGGTCGTGAAGATCGAGCCCCTGGGCGTCGCCACCCACCAAG
GCCAAGGAGAGGGTGGTGGAGCGGGAGAAGGAGGCGGTCGGCATCGGCGCCGTGTTCCTGGGAGCAGCCGGCA
GCACCATGGGAGCCGCCTCGATCACCCTGACCGTGCAGGCGAGGCAGCTGCTGTCCGGCATCGTGCAGCAGCAGTCGAACCT
GCTGAGGGCCATCGAGGCCCAGCACCTGCTCCAGCTGACCGTGTGGGGCATCAAGCAGCTCCAGGCCAGGGTGCTGGCC
GTCGAGCGCTACCTGAAGGACCAGCAGCTGCTCGGCATCTGGGGCTGCAAGCTGATCTGCACCACCGTGCCCT
GGAACAGCAGCTGGAGCAACAAGAGCCAGGACGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGGGAGATCAACAA
CTACACCGACATCATCTACAGCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGCGCTGGAC
AAGTGGGCGTCGCTGTGGAACTGGTTCGACATCACCAACTGGCTGTGGTACATCAAGTGAGGATCCTCTAGA

>C.1086C _140C

MRVRGIWKNWPQWLIWSILGFWIGNMEGSWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEMVLA
NVTENFNMWKNDMVEQMHEDIISLWDESLKPCVKLTPLCVTLNCTNVKGNESDTSEVMKNCSFKATTELKDKKHKVHALFYK
LDVVPLNGNSSSGEYRLINCNTSAITQACPKVSFDPIPLHYCAPAGFAILKCNNKTFNGTGPCRNVSTVQCTHGIKPVVST

Figure 21 Cont'd-4

QLLLNGSLAEEEIIIRSENLTNNAKTIIVHLNESVNIVCTRPNNNTRKSIRIGPGQTFYATGDIIGNIRQAHCNINESKWNN
TLQKVGEELAKHFPSKTIKFEPSSGGDLEITTHSFNCRGEEFFYCNTSDLFNGTYRNGTYNHTGRSSNGTITLQCKIKQIINM
WQEVGRAIYAPPIEGEITCNSNITGLLLTRDGGQSNETNDTETFRPGGGDMRDNWRSELYKYKVVEIKPLGVAPTEAKERVV
EREKEAVGIGAVFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQSNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYLK
DQQLLGMWGCSGKLICTTAVPWNSSWSNKSQNEIWGNMTWMQWDREINNYTNTIYRLLEDSQNQQEKNEKDLLALDSWKNLW
NWFDISKWLWYIK

>C.1086C_140C.opt

ATGCGCGTGCGCGGCATCTGGAAGAACTGGCCCCAGTGGCTGATCTGGTCCATCCTGGGCTTCTGGATCGGCAACATGGAGG
GCTCCTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCAAGACCACCCTGTTCTGCGCCTCCGACGCCAAGGC
CTACGAGAAGGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGATGGTGCTGGCC
AACGTGACCGAGAACTTCAACATGTGGAAGAACGACATGGTGGAGCAGATGCACGAGGACATCATCTCCCTGTGGGACGAGT
CCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCAACGTGAAGGGCAACGAGTCCGACACCTC
CGAGGTGATGAAGAACTGCTCCTTCAAGGCCACCACCGAGCTGAAGGACAAGAAGCACAAGGTGCACGCCCTGTTCTACAAG
CTGGACGTGGTGCCCCTGAACGGCAACTCCTACCGCCTGATCAACTGCAACACCTCCGCCATCACCC
AGGCCTGCCCCAAGGTGTCCTTCGACCCCATCCCCATCCACTACTGCGCCCCCGGCTTCGCCATCCTGAAGTGCAACAA
CAAGACCTTCAACGGCACCGGCCCCTGCCGCAACGTGTCCACCGTGCAGTGCACCCACGGCATCAAGCCCGTGGTGTCCACC
CAGCTGCTGCTGAACGGCTCCCTGGCCGAGGAGGAGATCATCATCCGCTCCGAGAACCTGACCAACAACGCCAAGACCATCA
TCGTGCACCTGAACGAGTCCGTGAACATCGTGTGCACCCCGCCCCAACAACACCCGCAAGTCCATCCGCATCGGCCCCGG
CCAGACCTTCTACGCCACCGGCGACATCATCGGCAACATCCGCCAGGCCCACTGCAACATCAACGAGTCCAAGTGGAACAAC
ACCCTGCAGAAGGTGGGCGAGGAGCTGGCCAAGCACTTCCCCTCCAAGACCATCAAGTTCGAGCCCTCCTCCGGCGGCGACC
TGGAGATCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTTCTACTGCAACACCTCCGACCTGTTCAACGGCACCTACCG
CAACGGCACCTACAACCACACCGGCCGCTCCTCCAACGGCACCATCACCCTGCAGTGCAAGATCAAGCAGATCATCAACATG
TGGCAGGAGGTGGGCCGCGCCATCTACGCCCCCCCCATCGAGGGCGAGATCACCTGCAACTCCAACATCACCGGCCTGCTGC
TGCTGCGCGACGGCGGCCAGTCCAACGAGACCAACGACACCGAGACCTTCCGCCCCGGCGGCGACATGCGCGACAACTG
GCGCTCCGAGCTGTACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCGTGGCCCCCACCGAGGCCAAGGAGCGCGTGGTG
GAGCGCGAGAAGGAGGCCGTGGGCATCGGCGCCGTGTTCCTGGGCTTCCTGGGCGCCGCGCTCCACCATGGGCGCCGCCT
CCATGACCCTGACCGTGCAGGCCCGCCAGCTGCTGTCCGGCATCGTGCAGCAGCAGTCCAACCTGCTGCGCGCCATCGAGGC
CCAGCAGCACATGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCGTGCTGGCCATCGAGCGCTACCTGAAG
GACCAGCAGCTGCTGGGCATGTGGGGCTGCTCCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGAACTCCTCCTGGTCCA

Figure 21 Cont'd-5

ACAAGTCCCAGAACGAGATCTGGGGCAACATGACCTGGATGCAGTGGGACCGCGAGATCAACAACTACACCAACACCATCTA
CCGCCTGCTGGAGGACTCCCAGAACCAGCAGGAGAAGGACCTGCTGGCCCTGGACTCCTGGAAGAACCTGTGG
AACTGGTTCGACATCTCCAAGTGGCTGTGGTACATCAAGTAGGGATCCTCTAGA_

>C.089C_140C

MRVRGMLRNCQQWWIWGILGFWMLMICSVVGNLWVTVYYGVPVWKEAKTTLFCASDARAYEREVHNVWATHACVPTDPNPQE
MVLVNVTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTPLCVILECNNANGTTNNGSVIVVNENSTMYGEIQNCSFKVNS
EIKGKKQDVYALFNSLDIVKLYNNGTSQYRLINCNTSTLTQACPKVSFDPIPIHYCAPAGYAILKCNNKTFNGTGPCNNVST
VQCTHGIKPVVSTQLLLNGSLAEGEIIIRSKNLTDNTKTIIVHLNESIKINCIRPNNNTRRSIRIGPGQAFYAANGIVGNIR
QAHCNISEGEWNKTLYRVSRKLAEHFPGKEIKFKPHSGGDLEITTHSFNCRGEFFYCNTSKLFNGTYTNGTYTNNDTNSTIIL
PCRIKQIINMWQEVGQAMYAPPIEGIIACNSTITGLLLTRDGGDKNGSKPEIFRPGGGDMRDNWRSELYKYKVVEIKPLGIA
PTKAKERVVEKEKTIQKEAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLRAIEAQQHMLQLTVWGIKQ
LQARVLAMERYLQDQQLLGIWGCSGKLICTTAVPWNSSWSNKTLEYIWGNMTWMQWDREIDNYTGIIYDLLEDSQIQQEKNE
KDLLALDSWKNLWSWFSITNWLWYIK

>C.089C_140C.opt

ATGCGCGTGCGCGGCATGCTGCGCAACTGCCAGCAGTGGTGGATCTGGGGCATCCTGGGCTTCTGGATGATGCTGATCTGCT
CCGTGGTGGGCAACCTGTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCCTGTTCTGCGCCTC
CGACGCCCCGCGCCTACGAGCGCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCGACCCCAACCCCCAGGAG
ATGGTGCTGGTGAACGTGACCGAGAACTTCAACATGTGGAAGAACGACATGGTGGACCAGATGCACGAGGACATCATCTCCC
TGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGATCCTGGAGTGCAACAACGCCAACGGCACCAC
CAACAACGGCTCCGTGATCGTGGTGAACGAGAACTCCACCATGTACGGCGAGATCCAGAACTGCTCCTTCAAGGTGAACTCC
GAGATCAAGGGCAAGAAGCAGGACGTGTACGCCCTGTTCAACTCCCTGGACATCGTGAAGCTGTACAACAACGGCACCTCCC
AGTACCGCCTGATCAACTGCAACACCTCCACCCTGACCCAGGCCTGCCCCAAGGTGTCCTTCGACCCCATCCCCATCCACTA
CTGCGCCCCCGCCGGCTACGCCATCCTGAAGTGCAACAACAAGACCTTCAACGGCACCGGCCCCTGCAACAACGTGTCCACC
GTGCAGTGCACCCACGGCATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCCTGGCCGAGGGCGAGATCATCA
TCCGCTCCAAGAACCTGACCGACAACACCATCATCGTGCACCTGAACGAGTCCATCAAGATCAACTGCATCCGCCCC
CAACAACACACCCGCCGCTCCATCCGCATCGGCCCCGGCCAGGCCTTCTACGCCGCCAACGGCATCGTGGGCAACATCCGC
CAGGCCCACTGCAACATCTCCGAGGGCGAGTGGAACAAGACCCTGTACCGCGTGTCCCGCAAGCTGGCCGAGCACTTCCCCG
GCAAGGAGATCAAGTTCAAGCCCCACTCCGGCGGCGACCTGGAGATCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTT

Figure 21 Cont'd-6

CTACTGCAACACCTCCAAGCTGTTCAACGGCACCTACACCAACACGACACCAACTCCACCATCCTG
CCCTGCCGCATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGCCAGGCCATGTACGCCCCCCCCATCGAGGGCATCATCG
CCTGCAACTCCACCATCACCGGCCTGCTGACCCGCGACGGCGGCGACAAGAACGGCTCCAAGCCCGAGATCTTCCGCCCC
CGGCGGCGGCGACATGCGCGACAACTGGCGCTCCGAGCTGTACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCATCGCC
CCCACCAAGGCCAAGGAGCGCGTGGTGGAGAAGGAAGACCATCCAGAAGGAGGCCGTGGGCATCGGCGCCGTGTTCCTGG
GCTTCCTGGGCGCCGCCGGCTCCACCATGGGCGCCGCCTCACCCTGACCGTGCAGGCCCCGCCAGCTGCTGTCCGGCAT
CGTGCAGCAGCAGTCCAACCTGCTGCGCGCCATCGAGGCCCAGCACATGCTGCAGCTGACCGTGTGGGGCATCAAGCAG
CTGCAGGCCCGCGTGCTGGCCATGGAGCGCTACCTGCAGGACCAGCTGCTGGGCATCTGGGGCTGCTCCGGCAAGCTGA
TCTGCACCACCGCCGTGCCCTGGAACTCCTCCTGGTCCAACAAGACCCTGGAGTACATCTGGGGCAACATGACCTGGATGCA
GTGGGACCGCGAGATCGACAACTACACCGGCATCATCTACGACCTGCTGGAGGACTCCCAGATCCAGCAGGAGAAGAACGAG
AAGGACCTGCTGGCCCTGGACTCCTGGAAGAACCTGTGGTTCTCCATCACCAACTGGCTGTGGTACATCAAG_

Figure 22

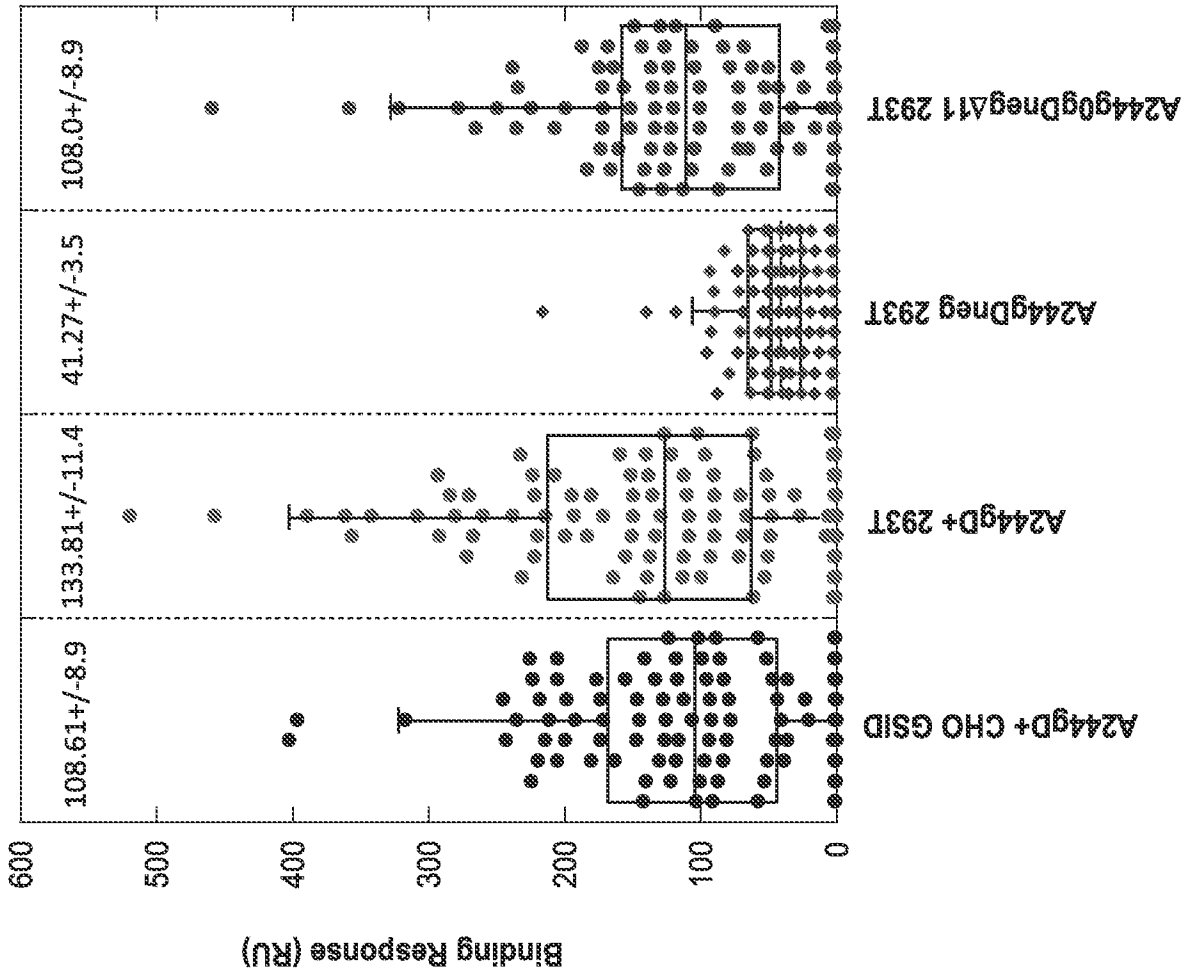

Binding Response (RU)
600
500
400
300
200
100
0
108.61+/-8.9
133.81+/-11.4
41.27+/-3.5
108.0+/-8.9
A244gD+ CHO GSID
A244gD+ 293T
A244gDneg 293T
A244gDnegA11 293T

Figure 23

N-terminal sequences of HIV Envs

CON-S. Mcon **A A** E N **L W V** T **V Y Y** G

A244.AE **A** S D N **L W V** T **V Y Y** G

63521.B **A A A** Q **L W V** T **V Y Y** G

6240.B **A** T E K **L W V** T **V Y Y** G

1086.C - - - - **W V** T **V Y Y** G

089.C **V V** G N **L W V** T **V Y Y** G

Bold amino acids are hydrophobic. Deletions can
Be made in any length and in any combination of
amino acids to have the effect of the
Delta 11 deletion. However, optimal deletions will be of
Between 5-11 amino acids. The 1086.C gp120 Env has
an N-terminal set of amino acids only 7 aa long and
manifests ~ 50% dimer formation when expressed in 293T or
CHO cells. Thus it is likely that at least more than 5 aa
deletions are needed for prevention of dimer formation. As one instance
of the design, 11 residues will be deleted; in another instance,
between 2 and 10 residues will be deleted, either in a consecutive
strength or selectively; "in yet another instance, a short peptide
sequence chosen for stability will be substituted for one or more of
the 11 residues.

Figure 24

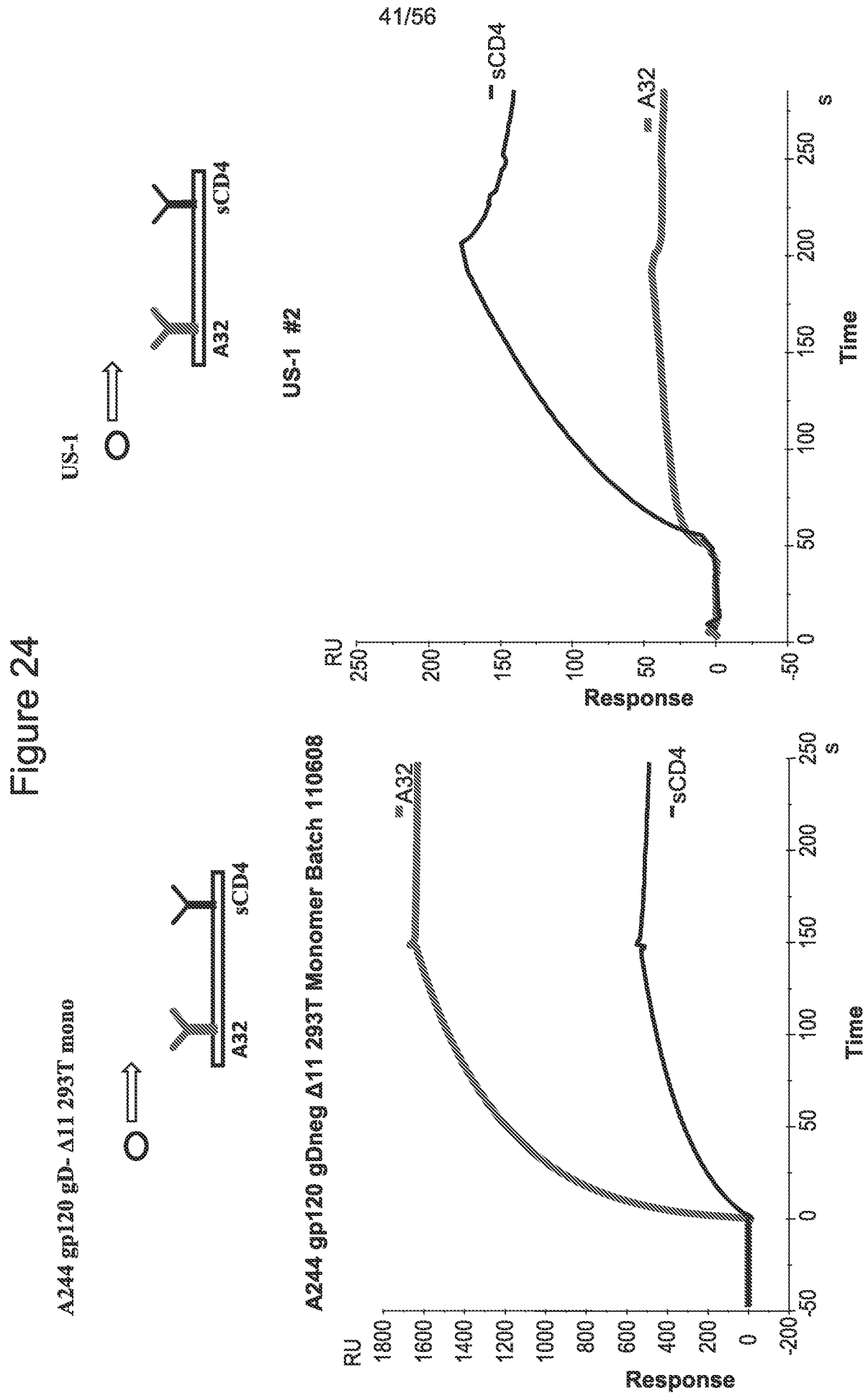

A244 gp120 gD- Δ11 293T mono
A32
sCD4
A244 gp120 gDneg Δ11 293T Monomer Batch 110608
sCD4
A32
RU
Response
Time
s
US-1
A32
sCD4
US-1 #2
sCD4
A32
RU
Response
Time
s

Figure 25

Variables/High Risk	Est. HR Quantitative/Categorical/Hazards Quantitative Hazards Categorical	P-value Quantitative/Categorical/Hazards Hazards Quantitative Hazards Categorical
IgA Main Breadth	1.43/1.51/1.52/1.66	0.0275/ *ns* /0.012/ *ns*
IgA OOMSA	1.38/2.63/1.39/2.83	0.0315/0.0159/0.0273/0.0102
IgA A1.con.env03	3.71/1.57/1.62/4.16	0.0019/0.0012/0.0011/0.0007
IgA DRCBL gp140	1.34/2.02/1.36/2.07	0.0709/0.0499/0.0584/0.0412
IgA C1 CRF1	1.69/3.15/1.75/3.26	0.0004/0.003/0.0003/0.0022
A32(A244d11)	1.11/2.33/1.33/2.67	ns/ns/ns/0.0468
Variables/ Lower Risk	Est. HR Quantitative	
IgG A244gD-293T	0.71	0.0292
IgG A244 K178C	0.74	0.0543
ADCC CM243 AUC	1.07/.96/1.1	Ns/0.0232/ns

Figure 26

Sequences highlighted in red (underlined) above are deleted to make HIV-1 Env gp120_Delta111 mutants, sequences highlighted in blue (double underlined) are C1 region and also deleted.

HIV-1 Env gp120_Delta111 and deletion of C1 mutants:

>AE.A244_gp120
MRVKETQMNWPNLWKWGTLILGLVIICSA**SDNLWVTVYYG**VPVWKEADTTLFCASDAKAHETEVHNVWATHACVPTDP
NPQEIDLENVTENFNMWKNNMVEQMQEDVISLWDQSLKPCVKLTPPCVTLHCTNANLTKANLTNVNNRTNVSNIIGNI
TDEVRNCSFNMTTELRDKKQKVHALFYKLDIVPIEDNNDSSEYRLINCNTSVIKQPCPKISFDPIPIHYCTPAGYAIL
KCNDKNFNGTGPCKNVSSVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTNNAKTIIVHLNKSVVINCTRPSNNTR
TSITIGPGQVFYRTGDIIGDIRKAYCEINGTEWNKALKQVTEKLKEHFNNKPIIFQPPSGGDLEITMHHFNCRGEFFY
CNTTRLFNNTCIANGTIEGCNGNITLPCKIKQIINMWQGAGQAMYAPPISGTINCVSNITGILLTRDGGATNNTNNET
FRPGGGNIKDNWRNELYKYKVVQIEPLGVAPTRAKRRVVEREKR
>B.63521_gp120
MRVKGIRKNYQHLWRWGTMLLGILMICSA**AAQLWVTVYYG**VPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDP
NPQELVLANVTENFNMWNNTMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDVTNATNINNSSGGVESGEI
KNCSFNITTSVRDKVQKEYALFYKLDIVPITNESSKYRLISCNTSVLTQACPKVSFEPIPIHYCAPAGFAILKCNNET
FNGKGPCINVSTVQCTHGIRPVVSTQLLLNGSLAEKEVIIRSDNFSDNAKNIIVQLKEYVKINCTRPNNNTRKSIHIG
PGRAFYATGEIIGNIRQAHCNISRSKWNDTLKQIAAKLGEQFRNKTIVFNPSSGGDLEIVTHSFNCGGEFFYCNTTKL
FNSTWIREGNNGTWNGTIGLNDTAGNDTIILPCKIKQIINMWQEVGKAMYAPPIRGQIRCSSNITGLILTRDGGKDDS
NGSEILEIFRPGGGDMRDNWRSELYKYKVVRIEPLGVAPTRARERVVQKEKE
>B.62400_gp120
MRVKGIRKNYQHLWRWGIMLLGTLMICSA**TEKLWVTVYYG**VPVWKEATTTLFCASDAKAYSPEKHNIWATHAC
VPTDPNPQELVLGNVTEDFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLKNSATDTNGTSGTNNRTV
EQGMETEIKNCSFNITTGIGNKMQKEYALFYKLDVVPIDSNNNSDNTSYRLISCNTSVVTQACPKTSFEPIPIHYCAP
AGFAILKCNNKTFSGKGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENFTNNAKTIIVQLNESVIINCTR
PNNNTRKGIHIGLGRALYATGDIIGDIRQAHCNLSSKSWNKTLQQVVRKLREQFGNKTIAFNQSSGGDQEIVKHSFNC
GGEFFYCDTTQLFNSTWSSNDTWNSTGVQDNNITLPCRIKQIINMWQEVGKAMYAPPIQGLISCSSNITGLLLTRDGG
TNNTNATEIFRPGGGDMRDNWRSELYKYKVVKIEPLGIAPTKAKRRVVQREKR

Figure 26 Cont'd-1

>M.CON-S_gp120
MRVRGIQRNCQHLWRWGTLILGMLMICSA**AENLWVTVYYG**VPVWKEANTTLFCASDAKAYDTEVHNVWATHACVPTDP
NPQEIVLENVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVNVTNTTNNTEEKGEIKNCSFNIT
TEIRDKKQKVYALFYRLDVVPIDDNNNNSSNYRLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTG
PCKNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRIGPGQAF
YATGDIIGDIRQAHCNISGTKWNKTLQQVAKKLREHFNNKTIIFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTW
IGNGTKNNNNTNDTITLPCRIKQIINMWQGVGQAMYAPPIEGKITCKSNITGLLLTRDGGNNNTNETEIFRPGGGDMR
DNWRSELYKYKVVKIEPLGVAPTKAKRRVVEREKR
>C.1086C_gp120
MRVRGIWKNWPQWLIWSILGFWIGNMEGS**WVTVYYG**VPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQE
MVLANVTENFNMWKNDMVEQMHEDIISLWDESLKPCVKLTPLCVTLNCTNVKGNESDTSEVMKNCSFKATTELKDKKH
KVHALFYKLDVVPLNGNSSSSGEYRLINCNTSAITQACPKVSFDPIPLHYCAPAGFAILKCNNKTFNGTGPCRNVSTV
QCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTNNAKTIIVHLNESVNIVCTRPNNNTRKSIRIGPGQTFYATGDIIG
NIRQAHCNINESKWNNTLQKVGEELAKHFPSKTIKFEPSSGGDLEITTHSFNCRGEFFYCNTSDLFNGTYRNGTYNHT
GRSSNGTITLQCKIKQIINMWQEVGRAIYAPPIEGEITCNSNITGLLLLRDGGQSNETNDTETFRPGGGDMRDNWRSE
LYKYKVVEIKPLGVAPTEAKRRVVEREKR_
>C.089C_gp120
MRVRGMLRNCQQWWIWGILGFWMLMICSV**VGNLWVTVYYG**VPVWKEAKTTLFCASDARAYEREVHNVWATHACVPTDP
NPQEMVLVNVTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTPLCVILECNNANGTTNNGSVIVVNENSTMYGEIQ
NCSFKVNSEIKGKKQDVYALFNSLDIVKLYNNGTSQYRLINCNTSTLTQACPKVSFDPIPIHYCAPAGYAILKCNNKT
FNGTGPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEGEIIIRSKNLTDNTKTIIVHLNESIKINCIRPNNNTRRSIRIG
PGQAFYAANGIVGNIRQAHCNISEGEWNKTLYRVVSRKLAEHFPGKEIKFKPHSGGDLEITTHSFNCRGEFFYCNTSKL
FNGTYNGTYTNNDTNSTITLPCRIKQIINMWQEVGQAMYAPPIEGIIACNSTITGLLLTRDGGDKNGSKPEIFRPGGG
DMRDNWRSELYKYKVVEIKPLGIAPTKAKRRVVEKEKTIQKR
Sequences with deltal1 and C1 deletion:

>AE.A244_gp120deltal1_C1
MRVKETQMNWPNLWKWGTLILGLVIICSAVPVWKEADTTLFCASDAKAHETEVHNVWATHACVPTDPNPQEIDLENVT
ENFNMWKNNMVEQTLHCTNANLTKANLTNVNNRTNVSNIIGNITDEVRNCSFNMTTELRDKKQKVHALFYKLDIVPIE
DNNDSSEYRLINCNTSVIKQPCPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPCKNVSSVQCTHGIKPVVSTQLLL
NGSLAEEEIIIRSENLTNNAKTIIVHLNKSVVINCTRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYCEINGTEWNK

Figure 26 Cont'd-2

ALKQVTEKLKEHFNNKPIIFQPPSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCIANGTIEGCNGNITLPCKIKQIIN
MWQGAGQAMYAPPISGTINCVSNITGILLTRDGGATNNTNNETFRPGGGNIKDNWRNELYKYKVVQIEPLGVAPTRAK
RRVVEREKR_
>B.63521_gp120delta11_C1
MRVKGIRKNYQHLWRWGTMLLGILMICSAVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQELVLANVT
ENFNMWNNTMVEQTLNCTDVTNATNINATNINNSSGGVESGEIKNCSFNITTSVRDKVQKEYALFYKLDIVPITNESS
KYRLISCNTSVLTQACPKVSFEPIPIHYCAPAGFAILKCNNETFNGKGPCINVSTVQCTHGIRPVVSTQLLLNGSLAE
KEVIIRSDNFSDNAKNIIVQLKEYVKINCTRPNNNTRKSIHIGPGRAFYATGEIIGNIRQAHCNISRSKWNDTLKQIA
AKLGEQFRNKTIVFNPSSGGDLEIVTHSFNCGGEFFYCNTTKLFNSTWIREGNNGTWNGTIGLNDTAGNDTIILPCKI
KQIINMWQEVGKAMYAPPIRGQIRCSSNITGLILTRDGGKDDSNGSEILEIFRPGGGDMRDNWRSELYKYKVVRIEPL
GVAPTRARERVVQKEKE
>B.6240_gp120delta11_C1
MRVKGIRKNYQHLWRWGIMLLGTLMICSAVPVWKEATTTLFCASDAKAYSPEKHNIWATHACVPTDPNPQELV
LGNVTEDFNMWKNNMVEQTLNCTDLKNSATDTNGTSGTNNRTVEQGMETEIKNCSFNITTGIGNKMQKEYALFYKLDV
VPIDSNNNSDNTSYRLISCNTSVVTQACPKTSFEPIPIHYCAPAGFAILKCNNKTFSGKGPCKNVSTVQCTHGIRPVV
STQLLLNGSLAEEEIVIRSENFTNNAKTIIVQLNESVIINCTRPNNNTRKGIHIGLGRALYATGDIIGDIRQAHCNLS
SKSWNKTLQQVVRKLREQFGNKTIAFNQSSGGDQEIVKHSFNCGGEFFYCDTTQLFNSTWSSNDTWNSTGVQDNNITL
PCRIKQIINMWQEVGKAMYAPPIQGLISCSSNITGLLLTRDGGTNNTNATEIFRPGGGDMRDNWRSELYKYKVVKIEP
LGIAPTKAKRRVVQREKR

>M.CON-S_gp120delta11_C1
MRVRGIQRNCQHLWRWGTLILGMLMICSAVPVWKEANTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIVLENVT
ENFNMWKNNMVEQTLNCTNVNVTNTTNNTEEKGEIKNCSFNITTEIRDKKQKVYALFYRLDVVPIDDNNNNSSNYRLI
NCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIII
RSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHCNISGTKWNKTLQQVAKKLRE
HFNNKTIIFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTWIGNGTKNNNNTNDTITLPCRIKQIINMWQGVGQAM
YAPPIEGKITCKSNITGLLLTRDGGNNNTNETEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVEREKR

Figure 26 Cont'd-3

>C.1086C_gp120delta7_C1
MRVRGIWKNWPQWLIWSILGFWIGNMEGSVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEMVLANVT
ENFNMWKNDMVEQTLNCTNVKGNESDTSEVMKNCSFKATTELKDKKHKVHALFYKLDVVPLNGNSSSGEYRLINCNT
SAITQACPKVSFDPIPLHYCAPAGFAILKCNNKTFNGTGPCRNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSEN
LTNNAKTIIVHLNESVNIVCTRPNNNTRKSIRIGPGQTFYATGDIIGNIRQAHCNINESKWNNTLQKVGEELAKHFPS
KTIKFEPSSGGDLEITTHSFNCRGEFFYCNTSDLFNGTYRNGTYNHTGRSSNGTITLQCKIKQIINMWQEVGRAIYAP
PIEGEITCNSNITGLLLTRDGGQSNETNDTETFRPGGGDMRDNWRSELYKYKVVEIKPLGVAPTEAKRRVVEREKR
>C.089C_gp120delta11_C1
MRVRGMLRNCQQWWIWGILGFWMLMICSVVPVWKEAKTTLFCASDARAYEREVHNVWATHACVPTDPNPQEMVLVNVT
ENFNMWKNDMVDQILECNNANGTTNNGSVTVVNENSTMYGEIQNCSFKVNSEIKGKKQDVYALFNSLDIVKLYNNGTS
QYRLINCNTSTLTQACPKVSFDPIPIHYCAPAGYAILKCNNKTFNGTGPCNNVSTVQCTHGIKPVVSTQLLLNGSLAE
GEIIIRSKNLTDNTKTIIVHLNESIKINCIRPNNNTRRSIRIGPGQAFYAANGIVGNIRQAHCNISEGEWNKTLYRVS
RKLAEHFPGKEIKFKPHSGGDLEITTHSFNCRGEFFYCNTSKLFNGTYTNNDTNSTIILPCRIKQIINMWQEVG
QAMYAPPIEGIIACNSTITGLLLTRDGGDKNGSKPEIFRPGGGDMRDNWRSELYKYKVVEIKPLGIAPTKAKRRVVEK
EKTIQKR

Figure 27

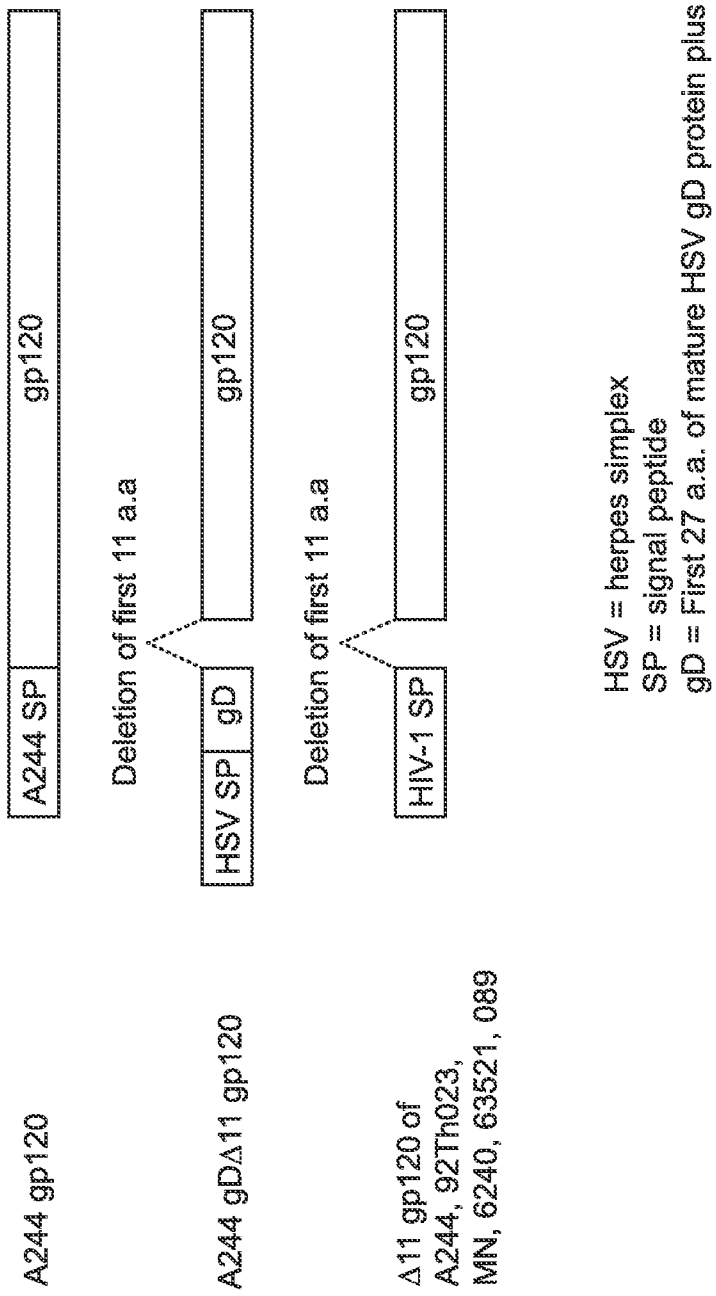

A244 SP
gp120
A244 gp120
Deletion of first 11 a.a
HSV SP
gD
gp120
A244 gDΔ11 gp120
RV144 clinical trial design (A244-rgp120)
Deletion of first 11 a.a
HIV-1 SP
gp120
Δ11 gp120 of A244, 92Th023, MN, 6240, 63521, 089
HSV = herpes simplex
SP = signal peptide
gD = First 27 a.a. of mature HSV gD protein plus 3 a.a (LLE) linker

Figure 28

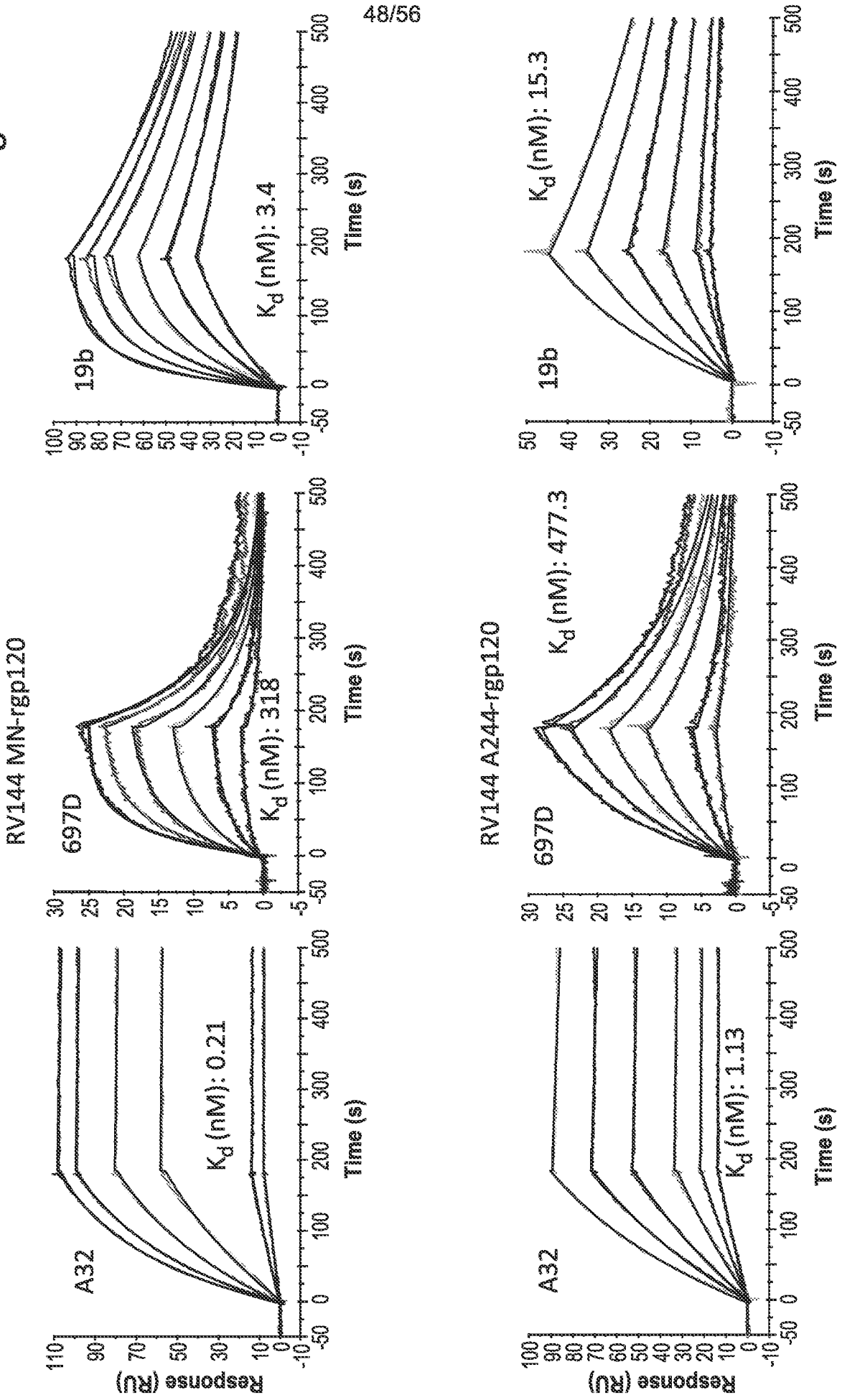

RV144 MN-rgp120
RV144 A244-rgp120
A32
697D
19b
Response (RU)
Time (s)
K_d (nM): 0.21
K_d (nM): 318
K_d (nM): 3.4
K_d (nM): 1.13
K_d (nM): 477.3
K_d (nM): 15.3

Figure 29

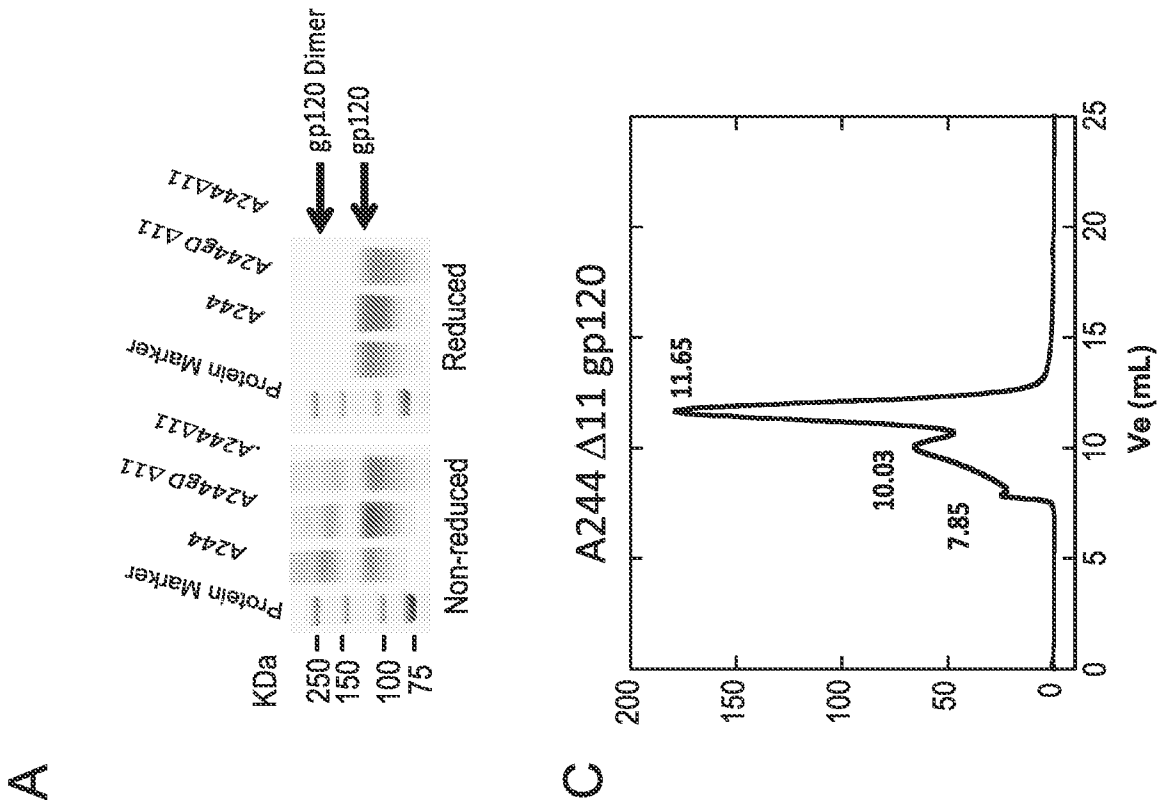

A
KDa
250
150
100
75
Protein Marker
A244
A244gD Δ11
A244Δ11
Protein Marker
A244
A244gD Δ11
A244Δ11
Non-reduced
Reduced
gp120 Dimer
gp120
B
A244 gp120
mAu
Ve (mL)
9.69
11.2
7.82
C
A244 Δ11 gp120
11.65
10.03
7.85
D
A244 gD Δ11 gp120
11.67
10.07
7.76

Figure 30

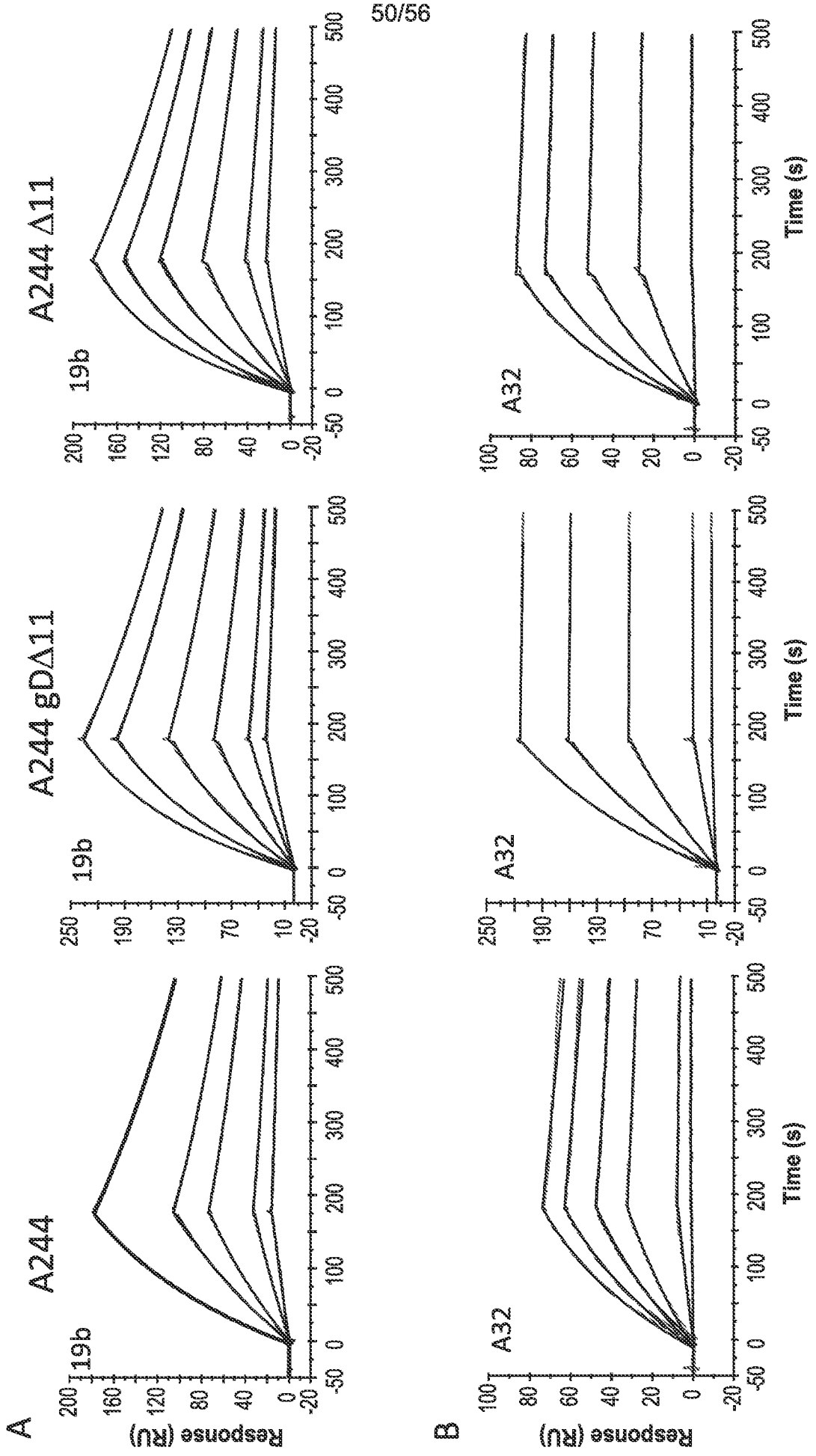

A
A244
19b
A244 gDΔ11
19b
A244 Δ11
19b
B
A32
A32
A32
Response (RU)
Time (s)

Figure 30

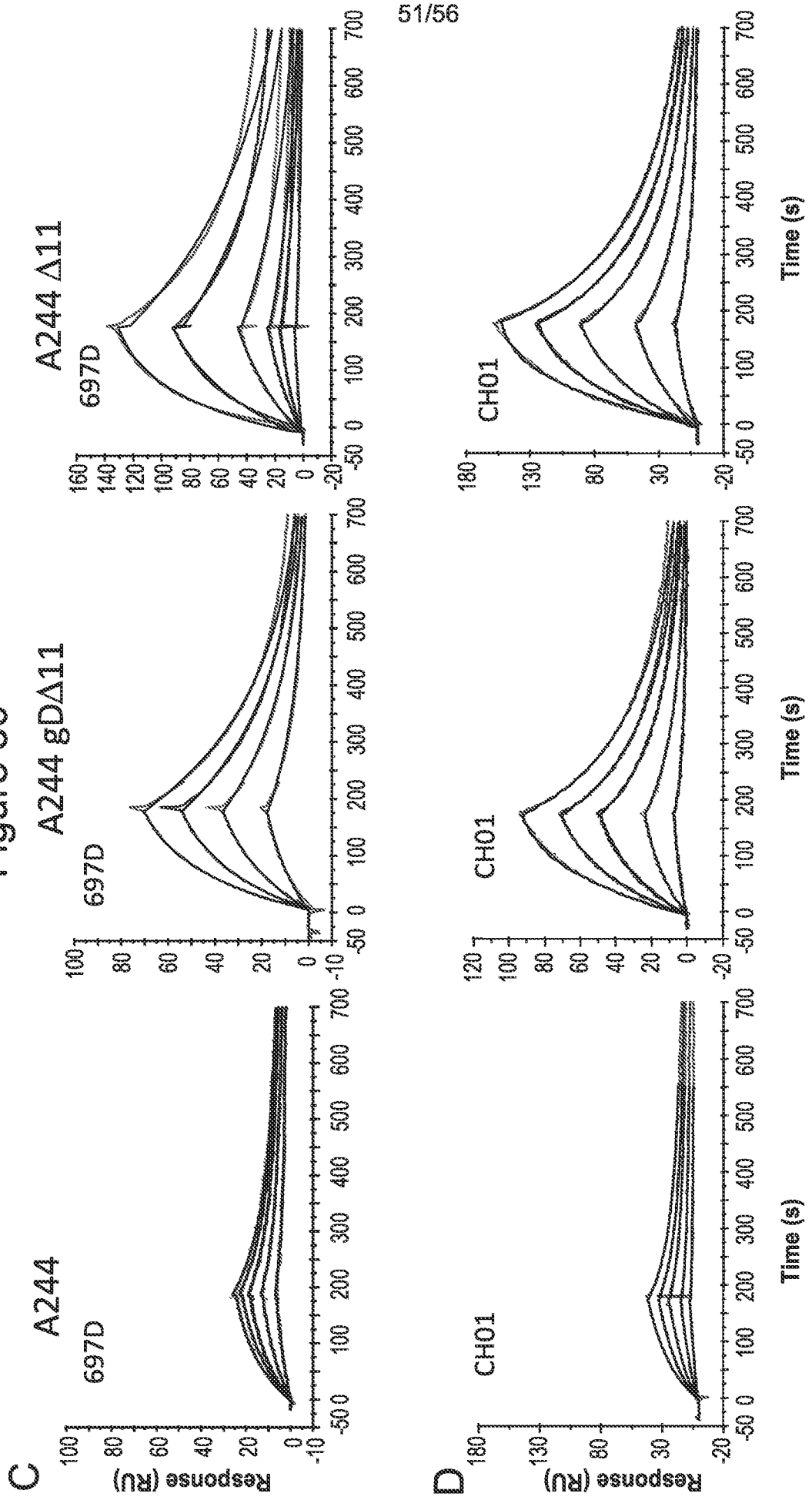


Figure 30

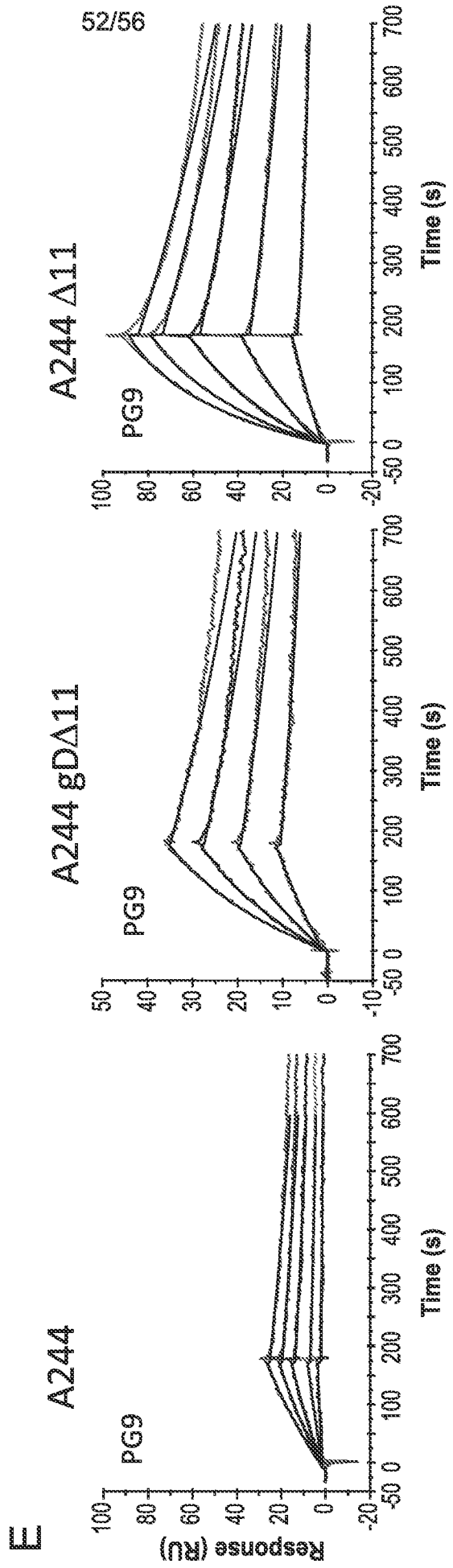

E
A244
PG9
Response (RU)
Time (s)
A244 gDΔ11
PG9
Time (s)
A244 Δ11
PG9
Time (s)
52/56

Figure 31

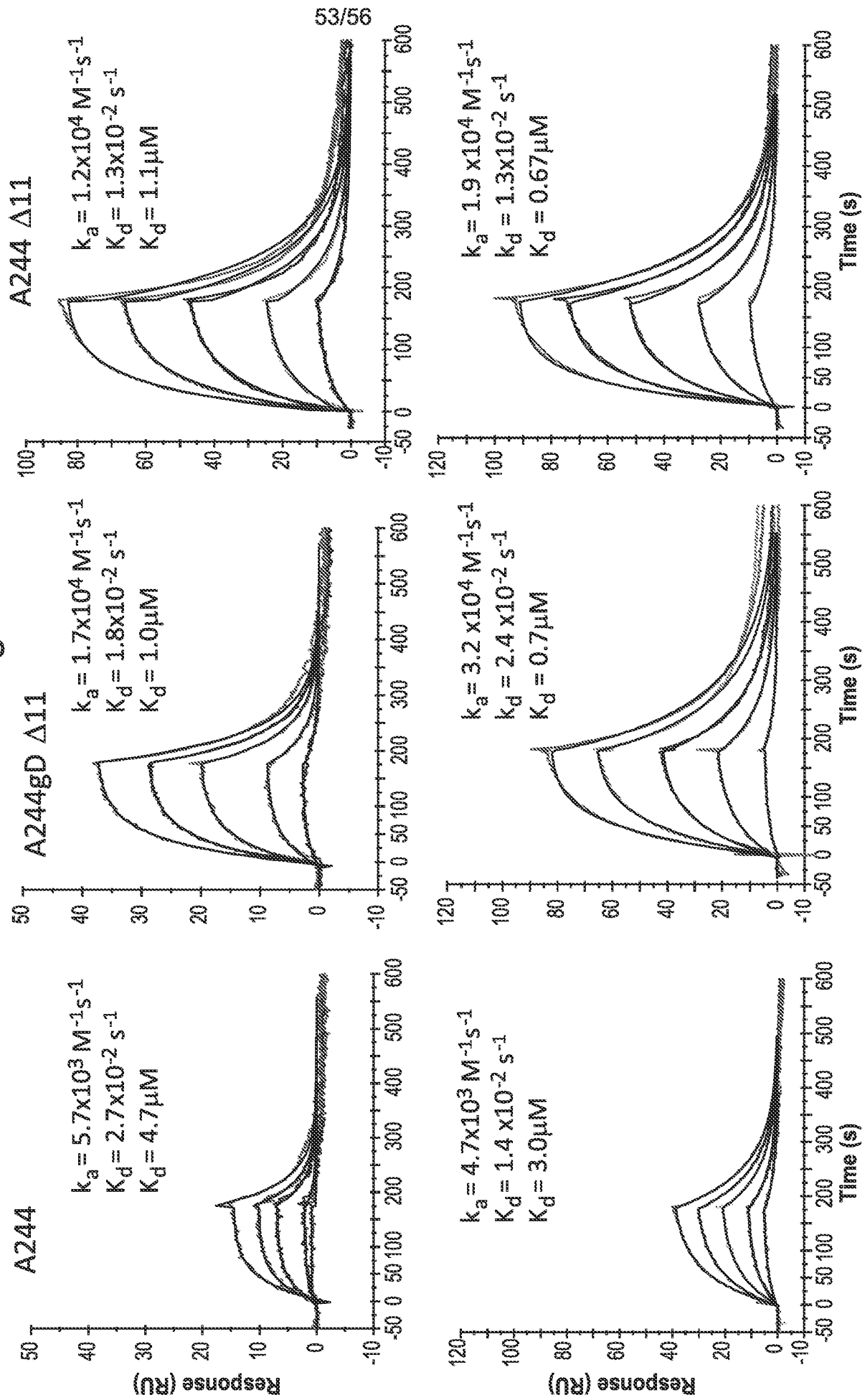

A244
k_a = 5.7x10^3 M^{-1}s^{-1}
K_d = 2.7x10^{-2} s^{-1}
K_d = 4.7µM
A244gD Δ11
k_a = 1.7x10^4 M^{-1}s^{-1}
K_d = 1.8x10^{-2} s^{-1}
K_d = 1.0µM
A244 Δ11
k_a = 1.2x10^4 M^{-1}s^{-1}
K_d = 1.3x10^{-2} s^{-1}
K_d = 1.1µM
k_a = 4.7x10^3 M^{-1}s^{-1}
K_d = 1.4 x10^{-2} s^{-1}
K_d = 3.0µM
k_a = 3.2 x10^4 M^{-1}s^{-1}
K_d = 2.4 x10^{-2} s^{-1}
K_d = 0.7µM
k_a = 1.9 x10^4 M^{-1}s^{-1}
K_d = 1.3x10^{-2} s^{-1}
K_d = 0.67µM
Response (RU)
Time (s)

Figure 32

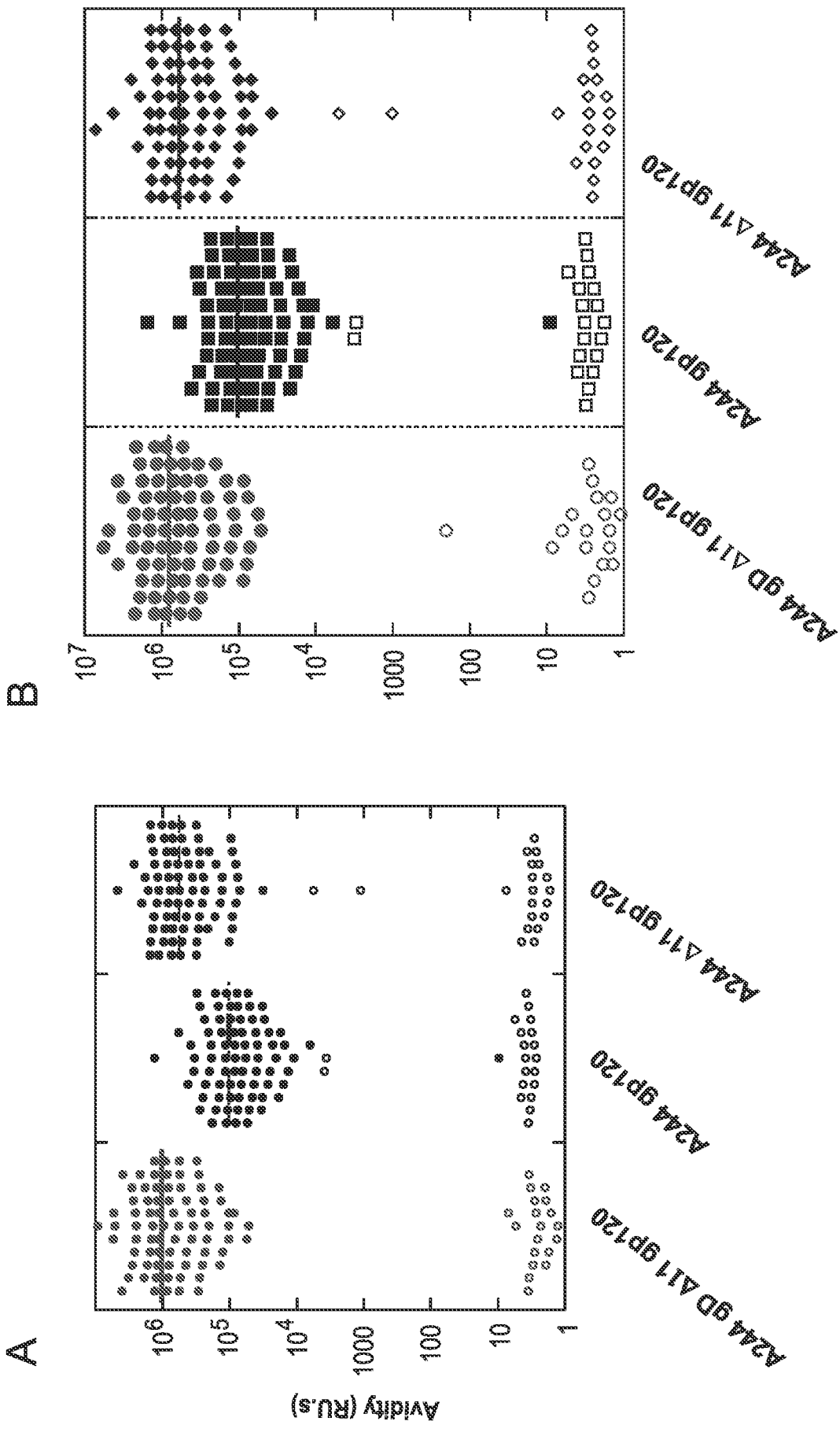

A
B
Avidity (RU.s)
10^6
10^5
10^4
1000
100
10
1
10^7
A244 GD Δ11 GP120
A244 GP120
A244 Δ11 GP120

Figure 32

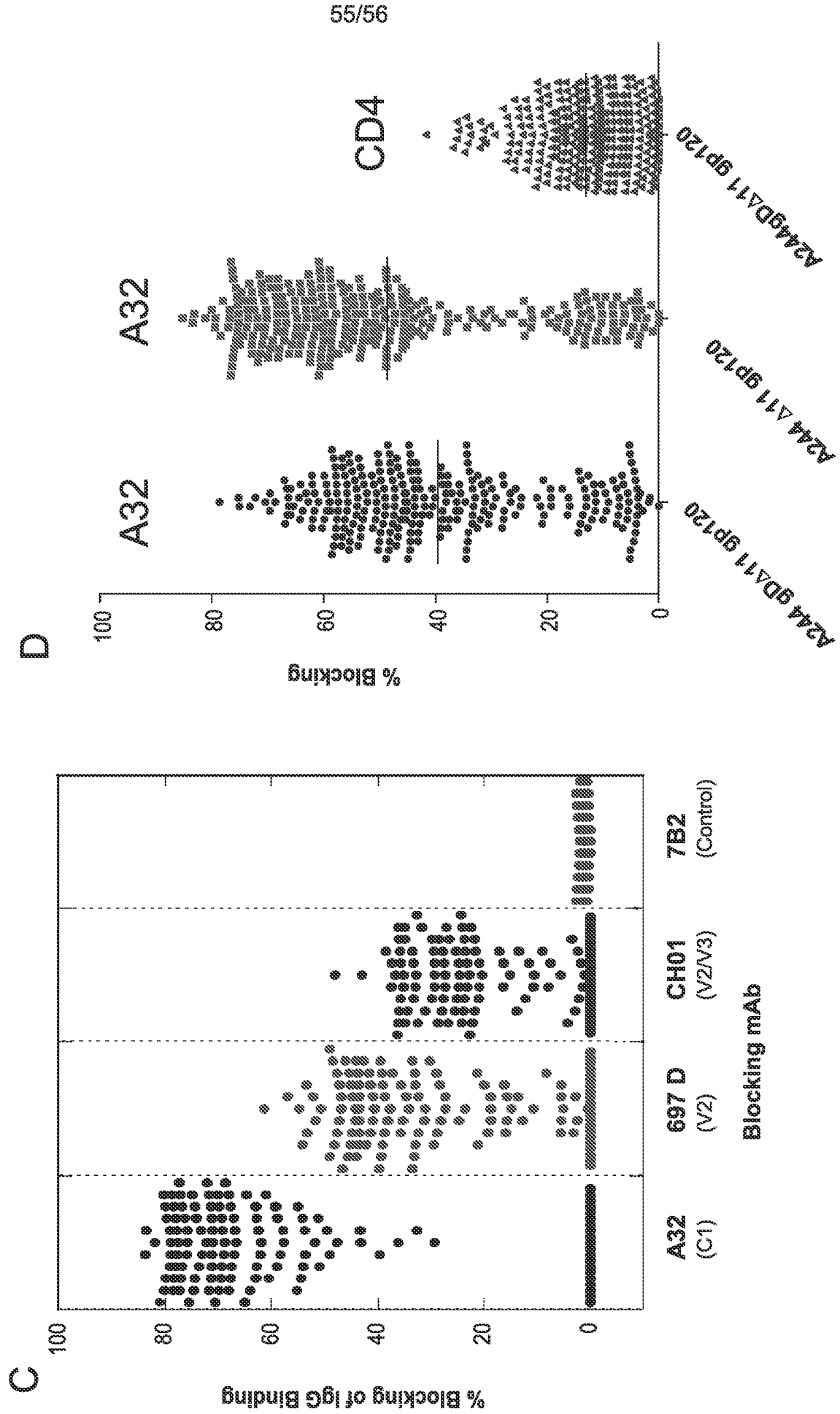
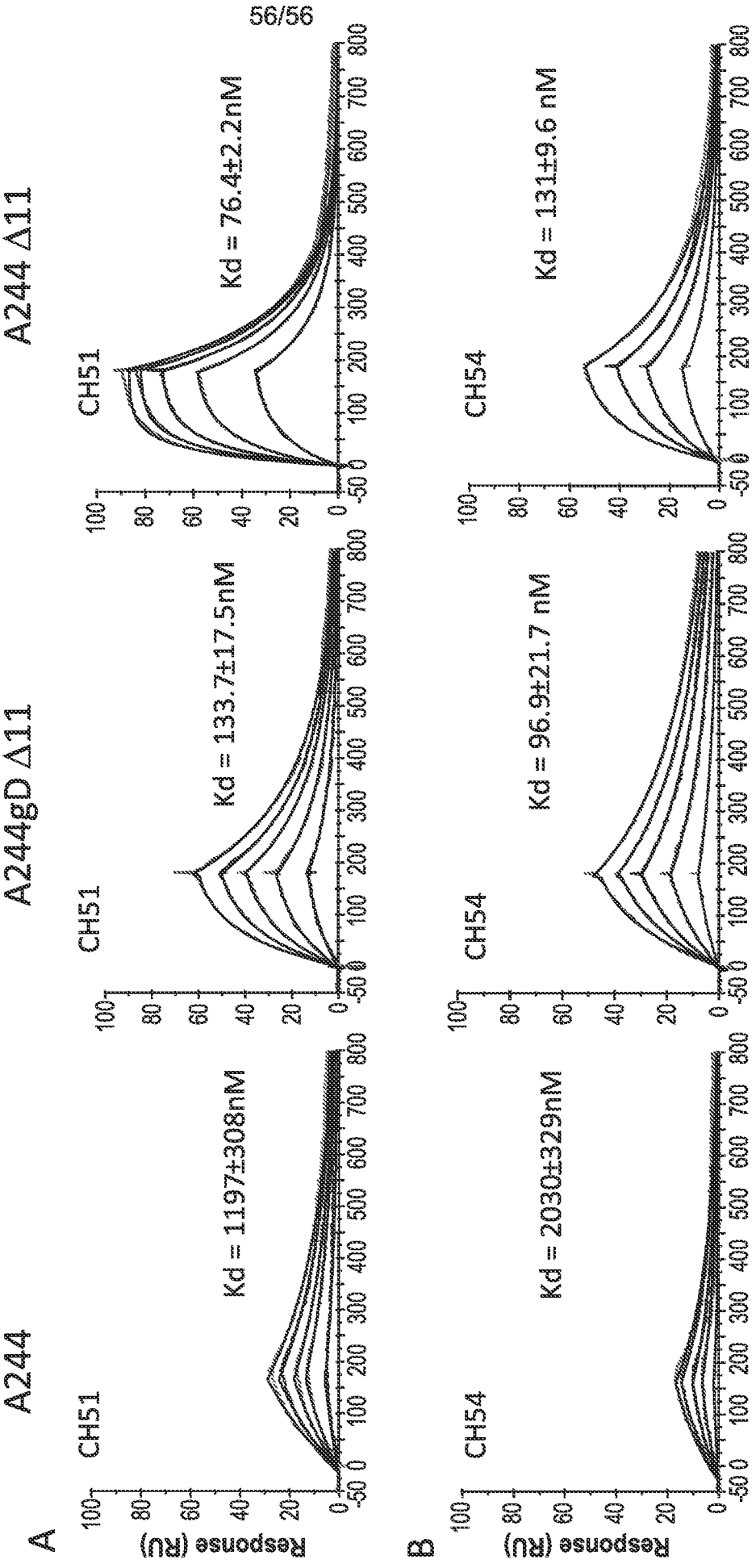

C
% Blocking of IgG Binding
100
80
60
40
20
0
A32
(C1)
697 D
(V2)
CH01
(V2/V3)
7B2
(Control)
Blocking mAb
D
% Blocking
A32
A32
CD4
A244 GDΔ11 gp120
A244 Δ11 gp120
A244GDΔ11 gp120

Figure 33


A
A244
CH51
Kd = 1197±308nM
A244gD Δ11
CH51
Kd = 133.7±17.5nM
A244 Δ11
CH51
Kd = 76.4±2.2nM
B
CH54
Kd = 2030±329nM
CH54
Kd = 96.9±21.7 nM
CH54
Kd = 131±9.6 nM
Response (RU)