



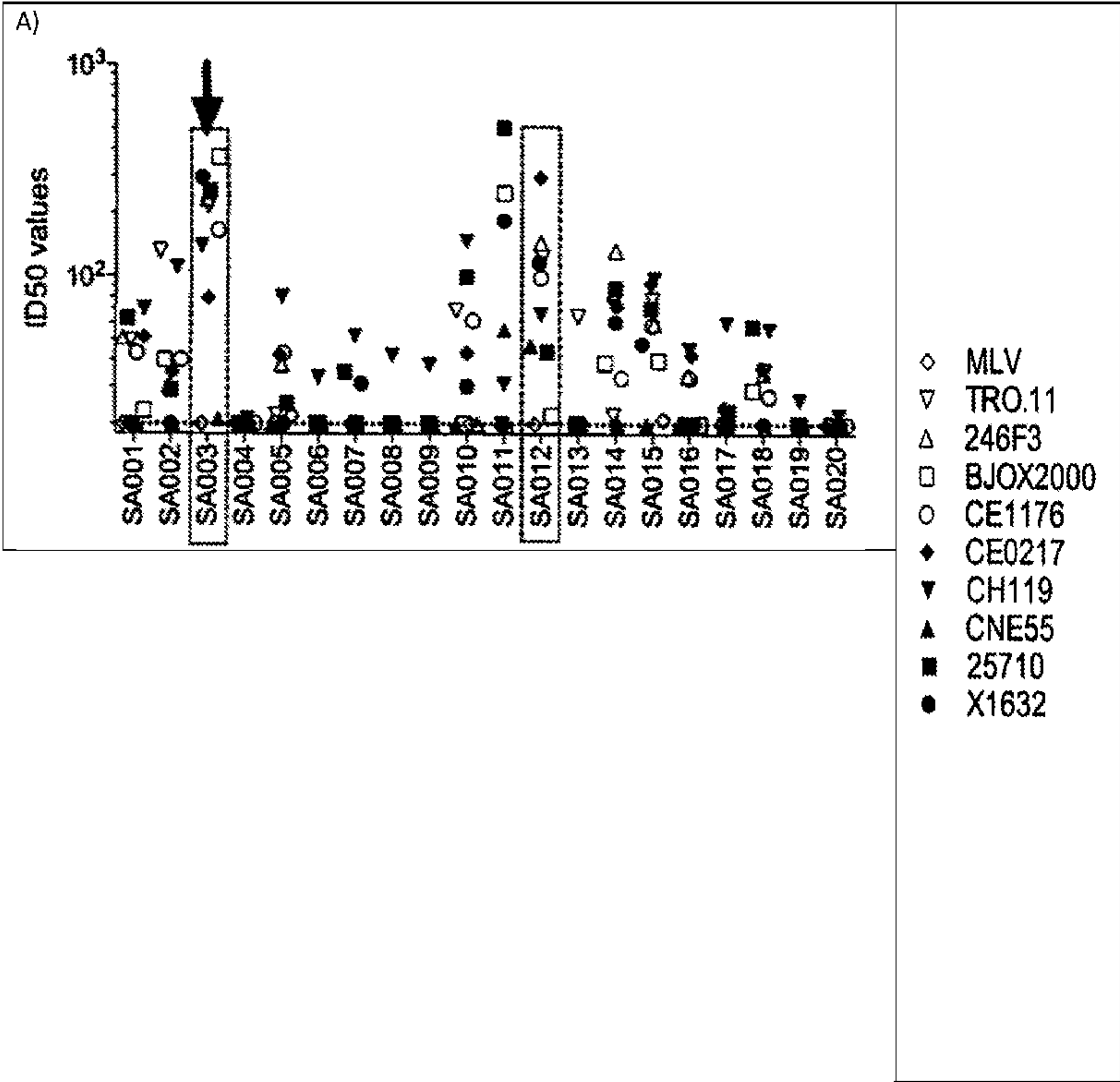
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(71) Demandeurs/Applicants:
CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS,
CH;
INSTITUTE FOR RESEARCH IN BIOMEDICINE, CH
(72) Inventeurs/Inventors:
PANTALEO, GIUSEPPE, CH;
LANZAVECCHIA, ANTONIO, CH
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : AGENTS DE LIAISON AU VIH
(54) Title: HIV BINDING AGENTS

FIGURE 1A



(57) **Abrégé/Abstract:**
This disclosure relates to binding agents with specificity for HIV and to methods for using the same to treat, prevent and / or ameliorate HIV infection and/or AIDS.

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(71) Applicants: **CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS** [CH/CH]; Rue du Bugnon 21, BH10-513, 1011 Lausanne (CH). **INSTITUTE FOR RESEARCH IN BIOMEDICINE** [CH/CH]; Via Vincenzo Vela 6, 6500 Bellinzona (CH).

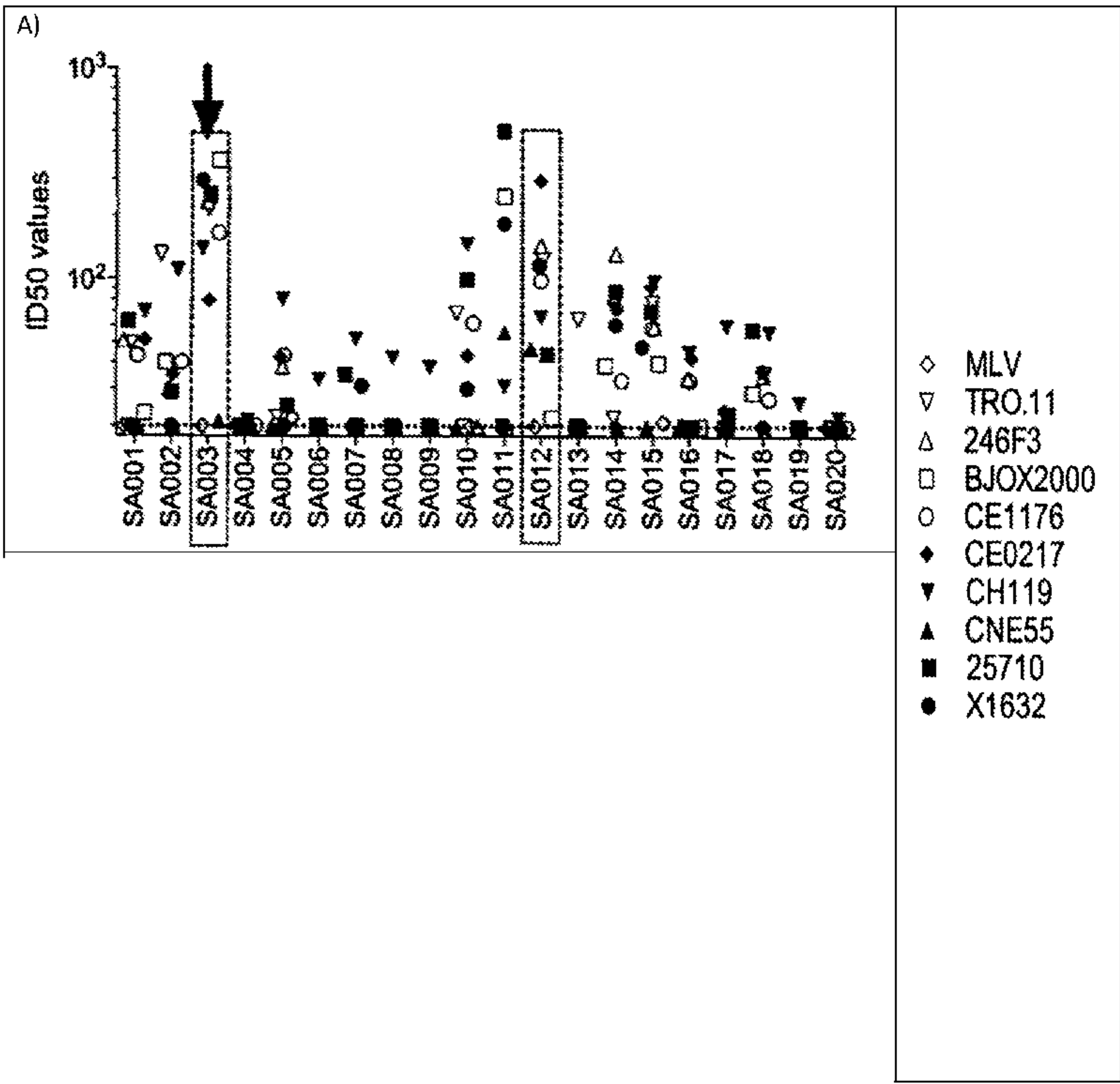
(72) Inventors: **PANTALEO, Giuseppe**; c/o Centre Hospitalier Universitaire Vaudois, Rue du Bugnon 21, 1011 Lausanne (CH). **LANZAVECCHIA, Antonio**; c/o Institute for Research in Biomedicine, Via Vincenzo Vela 6, 6500 Bellinzona (CH).

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(54) Title: HIV BINDING AGENTS

FIGURE 1A



[Continued on next page]

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(57) Abstract: This disclosure relates to binding agents with specificity for HIV and to methods for using the same to treat, prevent and / or ameliorate HIV infection and/or AIDS.

HIV BINDING AGENTS

Related Applications

[001] This application claims priority to U.S. Ser. No. 62/263,618 filed on December 5, 2015.

Field of the Disclosure

[002] This disclosure relates to binding agents with specificity for human immunodeficiency virus (HIV), methods for making the same, and to methods for using the same to treat and / or prevent HIV infection.

Background of the Disclosure

[003] As we enter the fourth decade of the HIV epidemic, significant advances have been made in the understanding of HIV pathogenesis and in the development of potent and safe antiviral drugs. More than 30 antiviral drugs have been registered and the impact of combination antiretroviral therapy (ART) on both morbidity and mortality has been remarkable. However, despite the long-term suppression of HIV replication achieved in patients with optimal adherence to ART, HIV invariably rebounds after interruption of therapy. Furthermore, successful therapy does not induce or allow restoration/development of virus-specific immune responses capable of controlling HIV replication in the absence of ART. Thus, life-long ART is needed to control HIV replication and associated disease in the large majority of HIV infected subjects.

[004] A number of immunological interventions have been investigated in the past and currently being further developed with the goal to achieve HIV functional cure, wherein viral replication is suppressed without sustained antiviral therapy. Therapeutic vaccine strategies have been the primary intervention strategy investigated but the results have shown modest efficacy in experimental animal models and patients with the exception of a CMV-based vector HIV vaccine (50% efficacy in the NHP model). Recent studies have generated interesting results on the possibility of using anti-envelope broad neutralizing antibodies (bNabs) as therapeutic agents in HIV infection.

[005] There is a need in the art for additional reagents for targeting HIV, especially neutralizing antibodies, and methods for using the same. This disclosure addresses those

needs by providing reagents and methods that may be used to target HIV and cells and / or tissues infected by and /or harboring the same.

Brief Description of the Drawings

[006] In the following a brief description of the appended figures will be given. The figures are intended to illustrate the present invention in more detail. However, they are not intended to limit the subject matter of the invention in any way.

[007] Figures 1A-D show the results of neutralization of a panel of nine (9) HIV-1 pseudoviruses from the Global Panel of HIV-1 reference strains by 70 plasma samples from chronically infected patients naïve to antiretroviral therapy. MLV pseudovirus is used as a negative control. Boxed are the seven donors selected for the collection of lymph nodes to isolate potent broadly neutralizing antibodies. Highlighted with the arrow is donor SA003 who was selected for the isolation of the broadly neutralizing antibody described in the present invention. ID50 values indicate the dilution of plasma capable of neutralizing 50% of viral infection.

[008] Figure 2 shows the gating and sorting strategy used to purify memory and germinal center IgG B cells from lymph node samples. B cells were selected for the expression of the surface marker CD19 and IgG B cells were negatively selected for the lack of IgA and IgM B cell receptor (BCR) expression. Germinal center B cells were further selected for the expression of the CD38 marker (that is absent on memory B cells).

[009] Figures 3A-B show the results of neutralization of a panel of nine (9) HIV-1 pseudoviruses (and MLV as negative control) from the Global Panel of HIV-1 reference strains by different concentration (in µg/ml) of the monoclonal antibody LN01. IC50 values indicate the concentration of monoclonal antibody capable of neutralizing 50% of viral infection. Error bars indicate the standard deviation of duplicates.

[0010] Figure 4 shows the results of neutralization of a multi-clade panel of 118 HIV-1 pseudoviruses by the monoclonal antibody LN01. IC50 values indicate the concentration of monoclonal antibody capable of neutralizing 50% of viral infection.

[0011] Figures 5A-J show the distribution of IC50 values on the whole panel of 118 viruses described in **Figure 4** and on individual clades or circulating recombinant forms.

[0012] Figure 6 shows the results of neutralization of a panel of nine (9) HIV-1 pseudoviruses from the Global Panel of HIV-1 reference strains by the monoclonal antibody LN01 when using TZM-bl or TZM-bl expressing Fc gamma Receptor I as target cells.

[0013] Figure 7 shows the results of neutralization of a panel of seven (7) HIV-2/HIV-1 chimeric pseudoviruses where the HIV-2 (strain 7312A) MPER region is mutagenized by introducing corresponding residues from the MPER consensus sequence of clade B or clade C (in the case of the variant 7312A.C1C) HIV-1 strains.

[0014] Figures 8A-D show the binding of LN01 or 7B2 monoclonal antibodies to an array of 1423 15-mer peptides, overlapping by 12 amino acids, that cover the full length of the consensus HIV-1 Env gp160 sequences for clades A, B, C, D, group M, CRF01_AE and CRF02_AG. Signals below 2.0E+4 are scored as negative. As expected 7B2 reacts with the gp41 immunodominant region (gp41 ID).

[0015] Figures 9A-D show the binding, as assessed by surface plasmon resonance, of LN01, PGT145, PGT151 and 17B monoclonal antibodies to the cleaved and soluble HIV-1 Env trimer BG505 SOSIP.664 gp140 that expresses multiple epitopes for broadly neutralizing in the presence or absence of soluble CD4 (sCD4). As expected PGT145 (V1-V2 glycan specific) and PGT151 (binding to a site at the interface between gp120 and gp41) bound with high affinity to BG505 SOSIP.664 gp140 in the absence and presence of sCD4, while 17b (binding to a CD4 binding induced site) bound only in the presence of sCD4.

[0016] Figures 10A-B show the binding, as measured by ELISA, of LN01 and 10E8 (MPER-specific broadly neutralizing antibody) antibodies to a set of HIV-1 Env antigens and a negative control (Ctr) antigen. 10E8 antibody reacted to the recombinant ecto-domain of gp41 that contains the MPER region.

[0017] Figures 11A-B shows the binding, as measured by ELISA, of LN01 and 10E8 monoclonal antibodies to a fusion intermediate gp41 (gp41int) or uncoated plates as a negative control (PBS). 10E8 antibody reacted to gp41 that contains the MPER region.

[0018] **Figure 12** shows the binding of mAb 10E8 to the gp41 peptide RRR-NEQELLELDKWASLWNWFDITNWLWYIRRRR (SEQ ID NO. 89).

[0019] **Figures 13A-C** show the potency of LN01 antibody point mutation variants. A. LN01 VH and VL variants. B. IC_{50} (μ g/ml) of LN01 variants. C. IC_{50} ratio (IC_{50} LN01 wt / IC_{50} LN01 variants ("var.")).

[0020] **Figure 14A-K** show testing of LN01 variants 7, 8 and 38 in parallel with the parental LN01 antibody against a multiclade panel of eight viruses. A-I. Percent (%) neutralization. J. IC_{50} (μ g/ml). K. IC_{50} ratio (IC_{50} LN01 wt / IC_{50} LN01 variants ("var.")).

[0021] **Figures 15A-B** show testing of LN01 variants 41, 42, 43, 44, 48 and 50 against a multiclade panel of seven viruses. A. IC_{50} (μ g/ml). B. IC_{50} ratio (IC_{50} LN01 wt / IC_{50} LN01 variants ("var.")).

[0022] **Figures 16A-B** show testing of LN01 variant 49 against a panel of seven viruses. A. IC_{50} (μ g/ml). B. IC_{50} ratio (IC_{50} LN01 wt / IC_{50} LN01 variants ("var.")).

[0023] **Figure 17** shows testing of LN01 variant 82 (IC_{50} (μ g/ml)).

Summary of the Disclosure

[0024] This disclosure relates to binding agents with specificity for human immunodeficiency virus (HIV), methods for producing such binding agents, as well as methods for using such binding agents to treat, prevent and / or ameliorate HIV infection.

Detailed Description

[0025] This disclosure relates to binding agents having binding affinity for human immunodeficiency virus (HIV). In some embodiments, the binding agent can bind HIV antigens on viral particles per se or on the surface of cells *in vitro* and / or *in vivo*. The binding agents may also bind isolated HIV antigens and / or fragments and / or derivatives thereof, typically *in vitro*. Also provided are methods for using such binding agents to diagnose, treat, prevent and / or ameliorate one or more diseases associated with HIV. For instance, the binding agents may be antibodies (e.g., monoclonal antibodies) that may react

with and / or bind to the epitopes of HIV or polypeptides thereof. The binding agents may be useful for treating disease caused by HIV, such as Acquired Immune Deficiency Syndrome (AIDS). In some embodiments, the binding agents described herein may selectively target and/or eliminate HIV and/or HIV-infected cells containing HIV (e.g., replication competent HIV) and/or expressing proteins thereof. In some embodiments, such cells may be reservoirs for replication competent HIV. In some embodiments, binding agents having, for instance, different specificities (e.g., recognizing different epitopes) may be combined to HIV activity such as infection, replication and/or spread to other cells. In some embodiments, the binding agents described herein may also provide for the selective elimination and / or suppression of HIV or HIV-expressing cells. In some embodiments, the binding agents described herein may be used to suppress and / or eliminate HIV and/or HIV-expressing cells to treat, for instance, HIV infection and/or AIDS. Other embodiments, uses and the like are described below.

[0026] The binding agents may be antibodies such as monoclonal antibodies. As shown in the examples herein, the techniques discussed below have been used to identify a fully human mAb termed "LN01", having particular characteristics that are described herein and shown in the examples. The LN01 antibody was isolated and the amino acid sequences of variable heavy (V_H) and light (V_L) chain domains of said antibody determined. A binding agent such as LN01 may be identified by referencing the amino acid and/or nucleic acid sequences corresponding to the variability and / or complementarity determining regions ("CDRs") thereof. A CDR comprises amino acid residues within the variable region identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts described by MacCallum et al., 1996, J. Mol. Biol., 262:732-745. In the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding (Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166). Still other CDR boundary definitions may not strictly follow one of the above approaches, but may nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including

combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

[0027] The amino acid sequences of the heavy chain CDRs (CDRH1, CDRH2, CDRH3), light chain CDRs (CDRL1, CDRL2 (and CDRL2 long), CDRL3), V_H and V_L domains of LN01 and certain exemplary variants thereof are shown in **Table 1** below.

Table 1

LN01 region	SEQ ID NO.	Amino Acid Sequence (one letter code)
LN01 CDRH1	1	GDSVSNDNYY
LN01 CDRH2	2	IYYSGTT
LN01 CDRH3	3	VRMP SHGFWSTSFSYWYFDL
LN01 CDRL1	4	QSVTKY
LN01 CDRL2	-	GTY
LN01 CDRL2 (long)	5	LIYGTYTLL
LN01 CDRL3	6	QQAHS TPWT
LN01 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	7	EVQLVESGPGLVQPWGTL SLTCRVSG <u>GDSVSNDN</u> <u>YYWAWIRQTPGRELQVIGTIYYSGTTYYNP</u> SLRN RVTISLDKSVNVVSLRLG SVSAADTAQYYC <u>VRMP</u> <u>SHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	8	DIQMTQSPSSLSASVGD KVTITCRAS <u>QSVTKYLN</u> <u>WYQFKTGQAPRIL IYGTYTLLSGVSPR</u> FSGAGSG SLYTLTITNIQPEDFATYYC <u>QQAHS TPWTFGQGT</u> HVAAN
LN01 variant 7 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	9	EVQLVESGPGLVQPWGTL SLTCRVSG <u>GDSVSNDN</u> <u>WYYWAWIRQTPGRELQVIGTIYYSGTTYYNP</u> SLR NRVTISLDKSVNVVSLRLG SVSAADTAQYYC <u>VRM</u> <u>PSHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 7 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	10	DIQMTQSPSSLSASVGD KVTITCRAS <u>QSVTKYLN</u> <u>WYQFKTGQAPRIL IYGTYTLLSGVSPR</u> FSGAGSG SLYTLTITNIQPEDFATYYC <u>QQAHS TPWTFGQGT</u> HVAAN
LN01 variant 8 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	11	EVQLVESGPGLVQPWGTL SLTCRVSG <u>GDSVSND</u> <u>WYYWAWIRQTPGRELQVIGTIYYSGTTYYNP</u> SLR NRVTISLDKSVNVVSLRLG SVSAADTAQYYC <u>VRM</u> <u>PSHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 8 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	12	DIQMTQSPSSLSASVGD KVTITCRAS <u>QSVTKYLN</u> <u>WYQFKTGQAPRIL IYGTYTLLSGVSPR</u> FSGAGSG SLYTLTITNIQPEDFATYYC <u>QQAHS TPWTFGQGT</u> HVAAN
LN01 variant 38 Variable Heavy (V _H)	13	EVQLVESGPGLVQPWGTL SLTCRVSG <u>GDSVSNDN</u> <u>YYWAWIRQTPGRELQVIGTIYYSGTTYYNP</u> SLRN

(CDRH1, CDRH2 and CDRH3 underlined)		RVTISLDKSVNVVSLRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 38 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	14	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGWG SLYTLTITNIQPEDFATYYCQQAHSPTWTFGQGT HVAAN
LN01 variant 41 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	15	EVQLVESGPGLVQPWGTLSTLCRVSGDSVSNFN YYWAWIRQTPGRELQVIGTIYYSGTTYYNP SLRN RVTISLDKSVNVVSLRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 41 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	16	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGSG SLYTLTITNIQPEDFATYYCQQAHSPTWTFGQGT HVAAN
LN01 variant 42 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	17	EVQLVESGPGLVQPWGTLSTLCRVSGDSVSNYN YYWAWIRQTPGRELQVIGTIYYSGTTYYNP SLRN RVTISLDKSVNVVSLRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 42 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	18	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGSG SLYTLTITNIQPEDFATYYCQQAHSPTWTFGQGT HVAAN
LN01 variant 48 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	19	QLQLQESGPGLVKPSETLSLTCTVSGDSVSNWN YYWAWIRQTPGRELQVIGTIYYSGTTYYNP SLRN RVTISLDKSVNVVSLRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGRGTLVTVSS</u>
LN01 variant 48 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	20	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGSG SLYTLTITNIQPEDFATYYCQQAHSPTWTFGQGT HVAAN
LN01 variant 49 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	21	EVQLVESGPGLVQPWGTLSTLCRVSGDSVSNW <u>WYYWAWIRQTPGRELQVIGTIYYSGTTYYNP</u> SLR NRVTISLDKSVNVVSLRLGSVSAADTAQYYCVRM PSHGFWSTSFSYWYFDLWGRGHFVAVSW
LN01 variant 49 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	22	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGSG SLYTLTITNIQPEDFATYYCQQAHSPTWTFGQGT HVAAN
LN01 variant 82 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	23	QVQLEESGPGLVQPWGTLSTLCRVSGGSISSSS YYWAWIRQTPGRELQVIGTIYYSGTTYYNP SLRN RVTISLDKSVNVVSLRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGRGHFVAVSW</u>
LN01 variant 82 Variable Light (V _L)	24	DIQMTQSPSSLSASVGDKVTITCRASQSVTKYLN WYQFKTGQAPRILIYGTYTLLSGVSPRFSGAGSG

(CDRL1, CDRL2 and CDRL3 underlined)		SLYTLTITNIQPEDFATYYC <u>QQAHSTPWTF</u> GQGT HVAAN
LN01 variants 7 and 48 Variable Heavy (V _H) CDRH1	25	GDSVSNWNY
LN01 variant 8 Variable Heavy (V _H) CDRH1	26	GDSVSNWNY
LN01 variant 41 Variable Heavy (V _H) CDRH1	27	GDSVSNFNY
LN01 variant 42 Variable Heavy (V _H) CDRH1	28	GDSVSNYNY
LN01 variant 43 Variable Heavy (V _H) CDRH1	29	GDSVSNLNY
LN01 variant 44 Variable Heavy (V _H) CDRH1	30	GDSVSNINY
LN01 variant 49 Variable Heavy (V _H) CDRH1	31	GDSVSNWNY
LN01 variant 82 Variable Heavy (V _H) CDRH1	32	GSISSSSY

[0028] A binding agent of this disclosure may comprise, for example, any one or more of the amino acid sequences shown in **Table 1** (i.e., any one or more of SEQ ID NOS. 1-32 or GTY (LN01 CDRL2)). Fragments and/or derivatives (e.g., comprising substituted amino acids, such as conservative substitutions) thereof are also disclosed. An exemplary derivative of the LN01 antibody (an IgG3 antibody), for instance, is termed “IgG1 LN01” in which the LN01 variable regions were cloned into an IgG1 backbone. In some embodiments, then, a binding agent of this disclosure may comprise one or more (i.e., one, two, three, four, five, six or seven) of SEQ ID NOS. 1-32. In some embodiments, it is preferred that the binding agent comprise each of SEQ ID NOS. 1-6 and or GTY (LN01 CDRL2). In some embodiments, such a binding agent may comprise SEQ ID NO. 7 and / or SEQ ID NO. 8; SEQ NOS. 9 and/or 10; SEQ NOS. 11 and/or 12; SEQ NOS. 13 and/or 14; SEQ NOS. 15 and/or 16; SEQ NOS. 17 and/or 18; SEQ NOS. 19 and/or 20; SEQ NOS. 21 and/or 22; or SEQ NOS. 23 and/or 24; or a conservatively substituted variant thereof. In preferred embodiments, the binding agent comprises SEQ ID NO. 7 and SEQ ID NO. 8; SEQ NOS. 9 and 10; SEQ NOS. 11 and 12; SEQ NOS. 13 and 14; SEQ NOS. 15 and 16; SEQ NOS. 17 and 18; SEQ NOS. 19 and 20; SEQ NOS. 21 and 22; or SEQ NOS. 23 and 24; or a conservatively substituted variant thereof. In some embodiments,

the binding agent may comprise any of SEQ ID NOS. 1-32 comprising one or more amino acid substitutions, in particular conservative substitutions (see, e.g., **Table 2**). Exemplary variants of SEQ ID NO.:1 (LN01 CDR H1) include, for instance, any of SEQ ID NOS. 25-32. In some embodiments, the binding agent may be a monoclonal antibody or a fragment or derivative thereof. In some embodiments, the binding agent may be an HIV-binding fragment of such a monoclonal antibody. Such embodiments would typically include at least one or more of SEQ ID NOS. 1-32, and preferably include each of SEQ ID NOS. 1-6 (or GTY (LN01 CDRL2) such as SEQ ID NOS. 7-8; SEQ ID NO. 25 (LN01 variants 7 and 48 CDR H1); SEQ ID NO. 26 (LN01 variant 8 CDR H1); SEQ ID NO. 27 (LN01 variant 41 CDR H1); SEQ ID NO. 28 (LN01 variant 42 CDR H1); SEQ ID NO. 29 (LN01 variant 43 CDR H1); SEQ ID NO. 30 (LN01 variant 44 CDR H1); SEQ ID NO. 31 (LN01 variant 49, a combination of LN01 variant 7 CDR H1 and LN01 variant 8 CDR H1); or SEQ ID NO. 32 (LN01 variant 82 CDR H1). In some embodiments, it may be beneficial to avoid variants comprising a variable heavy chain region comprising SEQ ID NO. 70 or 71 (LN01 variants 13 and 14, respectively); tryptophan (W) at an amino acid corresponding to amino acid 22 of SEQ ID NO. 70 (LN01 variant 13); tryptophan (W) at an amino acid corresponding to amino acid 23 of SEQ ID NO. 71 (LN01 variant 14); a CDRH3 amino acid sequence of any of SEQ ID NOS. 72-78 (LN01 variants 18-24, respectively); alanine (A) at an amino acid corresponding to amino acid 8 of SEQ ID NO. 72 (LN01 variant 18); alanine (A) at an amino acid corresponding to amino acid 9 of SEQ ID NO. 73 (LN01 variant 19); tryptophan (W) at an amino acid corresponding to amino acid 10 of SEQ ID NO. 74 (LN01 variant 20); tryptophan (W) at an amino acid corresponding to amino acid 11 of SEQ ID NO. 75 (LN01 variant 21); tryptophan (W) at an amino acid corresponding to amino acid 12 of SEQ ID NO. 76 (LN01 variant 22); tryptophan (W) at an amino acid corresponding to amino acid 14 of SEQ ID NO. 77 (LN01 variant 23); tryptophan (W) at an amino acid corresponding to amino acid 15 of SEQ ID NO. 78 (LN01 variant 24); a variable light chain region comprising SEQ ID NO. 79 or 80 (LN01 variants 32 and 33, respectively); tryptophan (W) at an amino acid corresponding to amino acid 5 of SEQ ID NO. 79 (LN01 variant 32); and/or, tryptophan (W) at an amino acid corresponding to amino acid 6 of SEQ ID NO. 80 (LN01 variant 33), as these may not provide suitable HIV neutralizing activity. Thus, in some embodiments, the binding agent may comprise any of SEQ ID NOS. 1-32 but not those of any one or more of SEQ ID NOS. 70-80. Other suitable embodiments may be derived by those of ordinary skill in the art from this disclosure.

[0029] It is preferred that the binding agent (e.g., antibody, or the antigen binding fragment thereof), comprises one or more amino acid sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to at least one of SEQ ID NOS. 1-32 (i.e., the CDR sequences, the VH sequence and/or the VL sequence shown in **Table 1**). In some embodiments, that percent identity is with respect to an amino acid sequence of at least three amino acids (e.g., as in GTY of LN01 CDRL2), six amino acids (e.g., as in LN01 CDRL1 (SEQ ID NO.:4)), seven amino acids (e.g., as in LN01 CDRH2 (SEQ ID NO.:2)), nine amino acids (e.g., as in LN01 CDRL2 (long), (SEQ ID NO.:5), LN01 CDRL3 (SEQ ID NO.:6), LN01 variant 82 CDRH1 (SEQ ID NO. 32)), ten amino acids (e.g., as in LN01 CDRH1 (SEQ ID NO.:1) or any of SEQ ID NOS. 25-31), or twenty amino acids (e.g., as in LN01 CDRH3 (SEQ ID NO.:3)). As discussed below, identities of less than 100% may result from the natural or synthetic substitution of one or more amino acids with another amino acid(s), as in a conservative substitution (see, e.g., **Table 2**). Various combinations of SEQ ID NOS. 1-32 may be useful as may be ascertained by one of ordinary skill in the art using the techniques described herein or as may be otherwise available to those of ordinary skill in the art. In preferred embodiments, the binding agent binds HIV and/or cells infected by HIV and/or expressing HIV proteins. In some especially preferred embodiments, the binding agent neutralizes HIV as described herein. In preferred embodiments, the binding agent both binds HIV and/or cells infected by HIV and/or expressing HIV proteins, and neutralizes HIV.

[0030] The variable region and/or CDR sequences (e.g., of **Table 1**) may be used in combination with one or more other variable region / CDR amino acid sequences available to those of ordinary skill in the art. Such variable region / CDR amino acid sequences may alternatively and / or also be adjoined to one or more types of constant region polypeptides of an antibody molecule. For instance, the CDR amino acid sequences shown in **Table 1** may be adjoined to or associated with the constant regions of any antibody molecule of the same or a different species (e.g., human, goat, rat, sheep, chicken) and / or antibody subtype of that from which the CDR amino acid sequence was derived. For instance, an exemplary binding agent may be, or may be derived from, one having about the same neutralizing activity and/or binding the same or similar epitopes and/or exhibiting about the same affinity as another binding agent comprising one or more of the amino acid sequences shown in **Table 1** (e.g., LN01 and IgG1 LN01). The binding agent may comprise an antibody heavy and / or a light chain that each comprises one or more constant and / or variable regions.

Any of the amino acid sequences described herein (e.g., as in **Table 1**), and / or any fragments and / or derivatives thereof, may also be combined with any other variable region and / or CDR in any order and / or combination to form new binding agents, e.g., hybrid and / or fusion binding agents, and / or inserted into other heavy and / or light chain variable regions using standard techniques.

[0031] This disclosure also provides for the use of such binding agents to isolate, identify, and / or target HIV and / or cells harboring and/or infected by HIV and / or expressing HIV antigens. In certain embodiments, such binding agents may be reactive against HIV antigens such as proteins expressed on the surface of cells. In some embodiments, the binding agent(s) is an antibody (antibodies). The term "antibody" or "antibodies" may refer to whole or fragmented antibodies in unpurified or partially purified form (e.g., hybridoma supernatant, ascites, polyclonal antisera) or in purified form. The antibodies may be of any suitable origin or form including, for example, murine (e.g., produced by murine hybridoma cells), or expressed as humanized antibodies, chimeric antibodies, human antibodies, and the like. For instance, antibodies may be wholly or partially derived from human (e.g., IgG (IgG1, IgG2, IgG2a, Ig2b, IgG3, IgG4), IgM, IgA (IgA1 and IgA2), IgD, and IgE), canine (e.g., IgGA, IgGB, IgGC, IgGD), chicken (e.g., IgA, IgD, IgE, IgG, IgM, IgY), goat (e.g., IgG), mouse (e.g., IgG, IgD, IgE, IgG, IgM), pig (e.g., IgG, IgD, IgE, IgG, IgM), and / or rat (e.g., IgG, IgD, IgE, IgG, IgM) antibodies, for instance. Methods of preparing, utilizing and storing various types of antibodies are well-known to those of skill in the art and would be suitable in practicing the present invention (see, for example, Harlow, et al. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; Harlow, et al. *Using Antibodies: A Laboratory Manual, Portable Protocol No. 1*, 1998; Kohler and Milstein, *Nature*, 256:495 (1975)); Jones et al. *Nature*, 321:522-525 (1986); Riechmann et al. *Nature*, 332:323-329 (1988); Presta (*Curr. Op. Struct. Biol.*, 2:593-596 (1992); Verhoeyen et al. (*Science*, 239:1534-1536 (1988); Hoogenboom et al., *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991); Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991); Marks et al., *Bio/Technology* 10, 779-783 (1992); Lonberg et al., *Nature* 368 856-859 (1994); Morrison, *Nature* 368 812-13 (1994); Fishwild et al., *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995); as well as U.S. Pat. Nos. 4,816,567; 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and, 5,661,016). In certain applications, the antibodies may be contained within hybridoma supernatant or

ascites and utilized either directly as such or following concentration using standard techniques. In other applications, the antibodies may be further purified using, for example, salt fractionation and ion exchange chromatography, or affinity chromatography using Protein A, Protein G, Protein A/G, and / or Protein L ligands covalently coupled to a solid support such as agarose beads, or combinations of these techniques. The antibodies may be stored in any suitable format, including as a frozen preparation (e.g., -20°C or -70°C), in lyophilized form, or under normal refrigeration conditions (e.g., 4°C). When stored in liquid form, for instance, it is preferred that a suitable buffer such as Tris-buffered saline (TBS) or phosphate buffered saline (PBS) is utilized. In some embodiments, the binding agent may be prepared as an injectable preparation, such as in suspension in a non-toxic parenterally acceptable diluent or solvent. Suitable vehicles and solvents that may be utilized include water, Ringer's solution, and isotonic sodium chloride solution, TBS and / or PBS, among others. Such preparations may be suitable for use in vitro or in vivo may be prepared as is known in the art and the exact preparation may depend on the particular application.

[0032] The binding agents described herein are not, however, in any way limited to antibodies (i.e., whole antibodies). For example, the binding agent may be any compound exhibiting similar binding properties as another (e.g., a mimetic). For example, an exemplary binding agent may be one that binds HIV and/or can compete with another binding agent having specificity therefor (e.g., a monoclonal antibody such as LN01). In some embodiments, the mimetic may exhibit substantially the same affinity in binding assays as the binding agent (e.g., monoclonal antibody) to which it is being compared. The affinity a particular binding agent may be measured by any suitable assay including but not limited to FACS staining of cell surface HIV antigens (e.g., polypeptides). One binding agent may be said to have “substantially the same affinity” as another where the measurements (e.g., nm) are within about any of 1-20, 1-5, 5-10, 10-15, or 15-20 percent of one another. Exemplary mimetics may include, for example, organic compounds that specifically bind HIV, or an affibody (Nygren, et al. FEBS J. 275 (11): 2668–76 (2008)), affilin (Ebersbach, et al. J. Mol. Biol. 372 (1): 172–85 (2007)), affitin (Krehenbrink, et al. J. Mol. Biol. 383 (5): 1058–68 (2008)), anticalin (Skerra, A. FEBS J. 275 (11): 2677–83 (2008)), avimer (Silverman, et al. Nat. Biotechnol. 23 (12): 1556–61 (2005)), DARPin (Stumpp, et al. Drug Discov. Today 13 (15–16): 695–701 (2008)), Fynomer (Grabulovski, et al. J. Biol. Chem. 282 (5): 3196–3204 (2007)), Kunitz domain peptide (Nixon, et al. Curr. Opin. Drug Discov. Devel. 9 (2): 261–8 (2006)), and / or a monobody (Koide, et al. Methods Mol. Biol. 352: 95–109 (2007)). Other

mimetics may include, for example, a derivative of an antibody such as, for example, an F_{ab}, F_{ab2}, Fab' single chain antibody, F_v, single domain antibody, mono-specific antibody, bi-specific antibody, tri-specific antibody, multi-valent antibody, chimeric antibody, canine-human chimeric antibody, canine-mouse chimeric antibody, antibody comprising a canine Fc, humanized antibody, human antibody, caninized, CDR-grafted antibody (i.e., comprising any of SEQ ID NOS. 1-32 shown in **Table 1**), shark antibody, nanobody, canelid antibody, microbody, and / or intrabody, or derivative thereof. Other binding agents are also provided herein as would be understood by one of ordinary skill in the art.

[0033] Any method known to those of ordinary skill in the art may be used to generate binding agents having specificity for (e.g., binding to) HIV. For instance, to generate and isolate monoclonal antibodies an animal such as a mouse may be administered (e.g., immunized) with one or more HIV proteins. Animals exhibiting serum reactivity to HIV expressed on activated human T lymphocytes (as determined by, for instance, flow cytometry and / or microscopy) may then be selected for generation of anti-HIV hybridoma cell lines. This may be repeated for multiple rounds. Screening may also include, for instance, affinity binding and / or functional characterization to identify the binding agent as an being specific for HIV. In some embodiments, such as in the Examples herein, human beings may be screened for the expression of antibodies against HIV. In some embodiments, plasma samples of human beings infected by HIV may be screened to identify persons expressing anti-HIV antibodies, and in particular, neutralizing antibodies. Neutralizing antibody-producing cells of such persons may then be isolated, followed by the isolation and characterization of the antibodies produced thereby (e.g., as in the examples herein). A neutralizing antibody may be one that exhibits the ability to neutralize, or inhibit, infection of cells by HIV. In general, a neutralization assay typically measures the loss of infectivity of the virus through reaction of the virus with specific antibodies. Typically, a loss of infectivity is caused by interference by the bound antibody with any of the virus replication steps including but not limited to binding to target cells, entry, and/or viral release. The presence of unneutralized virus is detected after a predetermined amount of time, e.g., one, two, three, four, five, six, seven, eight, nine, 10, 12 or 14 days, by measuring the infection of target cells using any of the systems available to those of ordinary skill in the art (e.g., a luciferase-based system). A non-limiting example of a neutralization assay may include combining a given amount of a virus or pseudovirus (see below) and different concentrations of the test or control (typically positive and negative controls assayed separately) antibody or antibodies

are mixed under appropriate conditions (e.g., one (1) hour at room temperature) and then inoculated into an appropriate target cell culture (e.g., TZM-bl cells). For instance, binding agent-producing cells (e.g., B cells producing antibodies) may be assayed for the production of HIV-1 neutralizing antibodies by seeding such cells in separate plates as single cell microcultures on human feeder cells in the presence of Epstein-Barr Virus (EBV) (which also stimulate polyclonally memory B cells), a cocktail of growth factors (e.g., TLR9 agonist CpG-2006, IL-2 (1000 IU/ml), IL-6 (10 ng/ml), IL-21 (10 ng/ml), and anti-B cell receptor (BCR) goat antibodies (which trigger BCRs). After an appropriate time (e.g., 14 days), supernatants of such cultures may be tested in a primary luciferase-based screening system using two or more representative HIV-1 viruses or pseudoviruses that productively infect such cells. The pseudoviruses may be incubated with B cell culture supernatants for an appropriate time and temperature (e.g., one (1) h at 37°C (5% CO₂)) before the addition of host cells (e.g., 3000 TZM-bl cells). Incubation for an appropriate time (e.g., 72 hours) may then follow, after which the supernatant may be removed and Steadylite reagent (Perkin Elmer) added (e.g., 15 µl). Luciferase activity may then be determined (e.g., five minutes later) on a Synergy microplate luminometer (BioTek). Decreased luciferase activity relative to a negative control typically indicates virus neutralization. Neutralization assays such as these, suitable for analyzing binding agents of this disclosure, are known in the art (see, e.g., Montefiori, D.C. *Curr. Protocol. Immunol.* Chapter 12, Unit 12.11 (2005); Edmonds, et al. *Virology*, 408(1): 1-13 (2010); Seaman, et al. *J. Virol.* 84(3): 1439-1452 (2010); Pace, et al. *PNAS USA*, 110(33): 13540-13545 (2013)). In some embodiments, test samples may be screened for the presence of antibodies able to neutralize a panel of HIV pseudoviruses (e.g., nine (9) HIV-1 pseudoviruses from the Global Panel of HIV-1 reference strains as conducted in the examples herein (those pseudoviruses being BJOX (CRF007_BC), CE1176 (C), TRO.11 (B), X1632 (G), CH119 (CRF07_BC), CNE55 (CRF01_AE), 25710 (C), 246F3 (AC), CE0217 (C)); DeCamp, A. et al. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 88, 2489–2507 (2014)). Neutralization of a larger panel of pseudoviruses may also be tested; for instance, de Camp et al. describe a group of 12 pseudoviruses (also known as HIV-1 Env Reference Strains): 398F1, 25710, CNE8, TRO11, X2278, BJOX2000, X1632, CE1176, 246F3, CH119, CE0217, and CNE55. In some embodiments, a panel of ten HIV isolates may be tested and a BNA may be identified as one that neutralizes six, seven, eight, nine members of a panel of nine pseudoviruses; or six, seven, eight, nine, 10, 11 or 12 members of a panel of 12 pseudoviruses. Screening of larger panels of such pseudoviruses (e.g., a panel of 118

pseudoviruses as in the examples herein) may also be carried out. An exemplary panel of 118 pseudoviruses used in the examples against which test samples may be tested for neutralizing antibodies may include, for instance, those shown in **Fig. 4** (including clade A, clade B, clade C, clade D, clade G, circulating recombinant forms CRF10_CD, CRF01_AE, CRF02_AG and CRF07_BC, as well as non-circulating recombinants AC and ACD strains). In some embodiments, neutralization may be determined as a measure of the concentration (e.g., $\mu\text{g/ml}$) of monoclonal antibody capable of neutralizing any of about 50%, 60%, 70%, 80%, 90%, 95%, or 99% of viral infection (an “IC₅₀” value). In some embodiments, a binding agent may be considered neutralizing if it is able to neutralize 50% of viral infection at a concentration of, for instance, about any of 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , 10^0 , 10^1 , 10^2 , or 10^3 $\mu\text{g/ml}$ (an IC₅₀ value as shown in in Fig. 3). In some embodiments, as in the Examples herein, it is preferred that this IC₅₀ value be below 25 $\mu\text{g/ml}$, and even more preferably below about any of 15, 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05, or 0.01 $\mu\text{g/ml}$. In preferred embodiments, as for the LN01 antibody described herein, the IC₅₀ value may be less than 0.011 (e.g., Fig. 7). In some embodiments, the ability of a neutralizing antibody to neutralize viral infection may also be expressed as a percent neutralization (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% (e.g., as in **Fig. 3**)). Other measures of neutralization may also be suitable as may be determined by those of ordinary skill in the art.

[0034] In some embodiments, the binding agents described herein may be broadly neutralizing antibodies (BNAs) identified in biological samples (e.g., plasma) obtained from HIV-infected persons. As mentioned above and shown in the examples herein, such BNAs may be identified by testing plasma samples of patients chronically infected by HIV (preferably those naïve to antiretroviral therapy) for the ability to neutralize multi-clade HIV isolates (e.g., initially using a nine or 12-member panel and then a larger panel (e.g., 118 members) of pseudoviruses)). In some embodiments, the samples may be derived from patients known to be “Elite Controllers” with viremia <50 HIV RNA copies per ml of plasma. Screening procedures such as these may result in the identification of patients that may serve as lymph node donors for the subsequent isolation and characterization of B cells producing BNAs. In carrying out such screening assays, neutralizing activity is typically compared to a negative control such as murine leukemia virus (MLV) pseudovirus.

[0035] In some embodiments, germinal center and memory IgG B cells of patients expressing neutralizing binding agents (e.g., antibodies) may be isolated and further studied.

In some embodiments, the cells may be sorted separately according to IgG (e.g., IgA and IgM negative cells), CD19, and CD38 expression (germinal center B cells are CD38 positive) (see, e.g., **Figure 2**) and interrogated for the production of HIV-1 neutralizing antibodies. For instance, highly pure IgG memory B cells and IgG germinal cells may be seeded in separate plates as single cell micro-cultures on human feeder cells in the presence of Epstein-Barr Virus (EBV) (which also stimulate polyclonally memory B cells) and a cocktail of growth factors and the like (e.g., composed TLR9 agonist CpG-2006, IL-2 (1000 IU/ml), IL-6 (10 ng/ml), IL-21 (10 ng/ml), and anti-BCR goat antibodies (B cell receptor (BCR) triggering)). Supernatants of such cultures (e.g., from day 14 cultures) may then be tested in a primary screening (e.g., using a 384-well based HIV-1 pseudoviruses neutralization assay using in parallel two strains, CE1176 and BJOX2000, representative of clade C and CRF07, as shown in the examples herein). Neutralization assays may be carried out using any suitable host cells (e.g., TZM-bl cells (Seaman, et al. J. Virol. 84(3): 1439-52 (2010); NIH AIDS Reagent Program Catalog Number 8129)). HIV-1 pseudoviruses resulting in a significant output relative light units (RLU) (e.g., of $50-100 \times 10^4$ RLU) (i.e., indicating productive infection of cells) may then incubated with B cell culture supernatants for an appropriate time and temperature (e.g., one (1) h at 37% (5% CO₂)) before the addition of host cells (e.g., 3000 TZM-bl cells). Incubation for an appropriate time (e.g., 72 hours) typically follows, after which the supernatant may be removed and Steadylite reagent (Perkin Elmer) added (e.g., 15 µl). Luciferase activity may then detected (e.g., five minutes later) on a Synergy microplate luminometer (BioTek). Decreased luciferase activity indicates a lesser amount of virus being released by the cells and virus neutralization. For instance, if the base RLU for a particular pseudovirus is $50-100 \times 10^4$ RLU, a neutralizing antibody may be determined to decrease the RLU for that pseudovirus to $25-50 \times 10^4$ RLU (i.e., a 50% decrease), or less. Using such systems, supernatants capable of cross-neutralizing strains may be identified, further harvested, and tested for their ability to neutralize other pseudoviruses.

[0036] The antibodies derived from such neutralizing antibody-containing cultures may then be further characterized by determining the amino acid and nucleotide sequences of the antibody variable and complementarity determining regions (CDRs) regions. Using these techniques, the HIV-neutralizing binding agent termed "LN01" was identified as an IgG3-type fully human monoclonal antibody having the CDR, VH and VL sequences shown in **Table 1** (SEQ ID NOS. 1-7 and/or GTY). LN01 was determined to be derived from the IGHV4-39*07 and IGKV1-39*01 germline genes and highly somatically mutated in variable genes of both

heavy chain (28%) and kappa light chain (27%) compared to germ line. LN01 was also found to possess a long heavy-chain complementarity-determining 3 region ("CDR H3") loop composed of 20 amino acids. In some embodiments, the variable heavy chain (V_H) and variable light chain (V_L) genes of a binding agent may then be cloned into an IgG expression vector of the same or a different isotype. As shown in the examples, for instance, nucleic acids encoding LN01 CDRs (**Table 3**) were cloned into IgG1 backbone, and the recombinant IgG1-based antibody (IgG1 LN01) was produced by transfecting appropriate host cells (e.g., Expi293F cells). The antibody full-length IgG1-based antibody may then be purified using standard techniques (e.g., a full-length IgG1-based antibody may be purified using a recombinant protein-A column (GE-Healthcare)). The recombinantly-produced IgG1 antibody may then be tested against any of a panel of pseudoviruses such as any of those described herein (e.g., the Global Panel of nine (9) HIV-1 reference pseudoviruses used in the examples) on an appropriate host cell (e.g., TZM-bl cells). In preferred embodiments, the binding agent will exhibit the ability to neutralize a majority (i.e., at least about 50% or greater) of the pseudovirus panel members (e.g., comprising nine, 12 or 118 members) without neutralizing a negative control virus (e.g., MLV pseudovirus). It is preferred that the binding agent exhibit the ability to neutralize a majority of such viruses (e.g., neutralization of greater than about 50%, such as any of about 60%, 70%, 80%, 90%, 95%, 99%, or 100%) with IC_{50} values considered neutralizing (see below). For example, in some embodiments, a binding agent of this disclosure may exhibit neutralization of HIV-1 pseudoviruses BJOX (CRF07_BC), CE1176, TRO.11 (B), X1632 (G), CH119 (CRF07_BC), CNE55 (CRF01_AE), 25710 (C), CD0217(C) but not of the control virus SVA-MLV at about 10^0 μ g/ml or less (**Figure 3**). In some embodiments, neutralization of the HIV-1 pseudoviruses viruses may be observed where the antibody concentration is from 10^2 - 10^0 μ g/ml, or between 10^0 - 10^1 μ g/ml (**Figure 3**). In some such embodiments, the percent neutralization by the binding agent is at least about 50% (**Figure 3**). In some embodiments, infection of one HIV-1 isolate is considered neutralized by a binding agent (e.g., antibody) at an IC_{50} of less than 25 μ g/ml, if infection of at least one isolate of this isolate is neutralized with an IC_{50} of less than 25 μ g/ml. In some embodiments, the binding agent may be considered neutralizing where a majority of the 118 HIV-1 pseudoviruses listed in **Figure 4** are considered neutralized at an IC_{50} of less than 25 μ g/ml, such as about 10 μ g/ml, 9 μ g/ml, 8 μ g/ml, 7 μ g/ml, 6 μ g/ml, 5 μ g/ml, 4 μ g/ml, 3 μ g/ml, 2 μ g/ml, 1 μ g/ml, 0.9 μ g/ml, 0.8 μ g/ml, 0.7 μ g/ml, 0.6 μ g/ml, 0.5 μ g/ml, 0.4 μ g/ml, 0.3 μ g/ml, 0.2 μ g/ml, 0.1 μ g/ml, 0.09 μ g/ml, 0.08 μ g/ml, 0.07 μ g/ml, 0.06 μ g/ml, 0.05 μ g/ml, 0.04 μ g/ml, 0.03 μ g/ml, 0.02 μ g/ml, or 0.01 μ g/ml. In preferred embodiments, the binding

agent may neutralize HIV-1 pseudovirus strains of ID 6535.3, QH0692.42, SC422661.8, PVO.4, TRO.11, PEJO4541.67, WITO4160.33, 1006_11_C3_1601, 1054_07_TC4_1499, 1056_TA11_1826, 1012_11_TC21_3257, 6244_13_B5_4576, SC05_8C11_2344, Du156.12, Du172.17, Du422.1, ZM197M.PB7, ZM214M.PL15, ZM233M.PB6, ZM249M.PL1, ZM109F.PB4, ZM135M.PL10a, HIV-0013095-2.11, HIV-16055-2.3, HIV-16845-2.22, Ce1086_B2, Ce1176_A3, Ce0682_E4, Ce1172_H1, ZM247v1(Rev-), 3016.v5.c45, A07412M1.vrc12, 231966.c02, CNE20, CNE21, CNE17, CNE30, CNE53, CNE58, 9004SS_A3_4, 928-28, 263-8, T255-34, 211-9, 235-47, CNE8, C1080.c03, R2184.c04, R1166.c01, C2101.c01, C3347.c11, BJOX015000.11.5, BJOX010000.06.2, BJOX025000.01.1, BJOX028000.10.3, X1193_c1, X2131_C1_B5, P1981_C5_3, 6952.v1.c20, and 0815.v3.c3 at an IC_{50} of less than or about 1 μ g/ml (**Fig. 4**). In some preferred embodiments, the binding agent may neutralize HIV-1 pseudovirus strains of ID QH0692.42, SC422661.8, PVO.4, TRO.11, PEJO4541.67, WITO4160.33, 1006_11_C3_1601, 1054_07_TC4_1499, 1056_TA11_1826, 6244_13_B5_4576, SC05_8C11_2344, Du156.12, Du172.17, ZM197M.PB7, HIV-0013095-2.11, HIV-16055-2.3, HIV-16845-2.22, Ce1086_B2, Ce1172_H1, ZM247v1(Rev-), 3016.v5.c45, A07412M1.vrc12, CNE20, CNE21, CNE53, CNE58, 9004SS_A3_4, 928-28, 263-8, T255-34, CNE8, C1080.c03, BJOX015000.11.5, BJOX010000.06.2, BJOX025000.01.1, BJOX028000.10.3, X2131_C1_B5, P1981_C5_3, 6952.v1.c20, and 0815.v3.c3 at an IC_{50} of less than or about 0.5 μ g/ml (**Fig. 4**). In some preferred embodiments, the binding agent may neutralize HIV-1 pseudovirus strains of ID 1054_07_TC4_1499, 1056_TA11_1826, 6244_13_B5_4576, Du172.17, HIV-0013095-2.11, HIV-16845-2.22, Ce1172_H1, CNE20, CNE21, 928-28, CNE8, P1981_C5_3 at an IC_{50} of less than or about 0.1 μ g/ml (**Fig. 4**). It is further preferred that the binding agent not exhibit clade-dependency. For instance, in some embodiments, the binding agent may exhibit the ability to neutralize pseudoviruses of HIV-1 Clades B, B (T/F), C, C (T/F), D, D (T/F), BC, A, A (T/F), CRF02_AG, CRF01_Ae, CRF01_AE (T/F), G, CD, AC and ACD (**Fig. 4**). In some preferred embodiments, the binding agent may neutralize at least one pseudovirus in each of clades B, B (T/F), C, C (T/F), D, BC, A, A (T/F), CRF02_AG, CRF01_Ae, CRF01_AE (T/F), G, CD, and ACD at an IC_{50} of less than or about 1 μ g/ml (**Fig. 4**). In some preferred embodiments, the binding agent may neutralize at least one pseudovirus in each of clades B, B (T/F), C, C (T/F), D, BC, A (T/F), CRF02_AG, CRF01_Ae, CRF01_AE (T/F), G, CD, and ACD at an IC_{50} of less than or about 0.5 μ g/ml (**Fig. 4**). In some preferred embodiments, the binding agent may neutralize at least one pseudovirus in each of clades B (T/F), C, C (T/F), BC, A (T/F), CRF02_AG, CRF01_AE, CRF01_AE (T/F),

G, and CD at at an IC_{50} of less than or about 0.1 $\mu\text{g/ml}$ (**Fig. 4**). In some embodiments, the binding agent comprises any one or more of these properties and one or more of SEQ ID NOS. 1-32, preferably each of SEQ ID NOS. 1-7 and/or SEQ ID NOS. 8-9 and/or fragments and/or derivatives thereof. These are characteristics of LN01-type binding agents, such as IgG1 LN01, as shown in **Fig. 4**.

[0037] In some embodiments, the binding agents may be tested for neutralization capacity against HIV reference pseudoviruses (e.g., the above-described Global Panel of nine (9) HIV-1 reference pseudoviruses) using cells expressing or not expressing one or more types of Fc receptors (e.g., parental TZM-bl cells and TZM-bl cells expressing Fc-gamma receptor I (CD64) as in the examples; see e.g. Perez, et al. Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. J Virol 83, 7397–7410 (2009); NIH AIDS Reagent Program Catalog No. 11798). Enhanced neutralizing activity in cells expressing Fc receptors may provide antibodies a kinetic advantage for virus inhibition. This kinetic advantage could be unique to antibodies, whose epitopes are thought to be difficult to access or exposed for only a short time on intermediate conformations of the Env protein during an early stage of fusion. Fc-gamma receptors could also potentially facilitate HIV-1 neutralization is phagocytosis, thereby increasing neutralization capacity of the antibodies. To this point, HeLa cells, from which the TZM-bl cell line was constructed, are known to exhibit properties of nonprofessional phagocytes. Thus, it is possible that TZM-bl cells were converted to professional phagocytic cells by introducing Fc-gamma receptor on their surface. Any Fc-gamma-receptor-mediated antiviral effects on HIV-1 neutralizing antibodies, whether by entry inhibition or phagocytosis, might be beneficial in HIV treatment and vaccine regimens. Fc-gamma receptors are rarely expressed on CD4+ lymphocytes, but several other HIV-1-susceptible cell types express multiple Fc-gamma receptors and are involved in sexual transmission and the early establishment of long-lived viral reservoirs. In particular, macrophages are among the first infection-susceptible cells that the virus encounters after mucosal exposure, and are thought to serve as a long-lived virus reservoir in chronic infection. Macrophages, as well as certain subsets of monocytes and dendritic cells, are known to express multiple Fc-gamma receptors. It is also important to mention that Fc-gamma receptors play a role in regulating adaptive immunity and peripheral tolerance, by facilitating antigen uptake, antigen presentation, cell activation and B cell tolerance. Thus, in some embodiments, the binding agents described herein may be used in conjunction with

agents that induce and/or enhance Fc receptor expression, including the introduction of nucleic acids encoding one or more Fc receptors with or in conjunction with treatment by the binding agents described herein.

[0038] The specificity of the binding agents described herein may be determined using any of the many techniques available to those of ordinary skill in the art. For instance, as shown in the examples herein, the specificity of a binding agent (e.g., IgG1 LN01 antibody), with respect to particular epitopes, may be ascertained using a panel of chimeric pseudoviruses (e.g., HIV-2/HIV-1 chimeras containing various segments of the HIV-1 MPER into the parental HIV-2/7312A) to test for neutralization capacity. For instance, the examples herein demonstrate that IgG1 LN01 antibody does not neutralize the parental HIV-2 7312A strain. However, IgG1 LN01 antibody was found to potently neutralize the chimeric virus 7312A.C4 in which six (6) residues of HIV-2 MPER (LASWVKYIQ (SEQ ID NO. 65)) were replaced by those of HIV-1 MPER region (ITKWLWYIK (SEQ ID NO. 66)). IgG1 LN01 antibodies was also found not to neutralize the chimeric virus 7312A.C6 in which only three (3) residues in the same region were replaced (ITSWIKYIQ (SEQ ID NO. 67)) (see, e.g., **Fig. 7**). A similar finding was obtained with the chimeric virus 7312A.C1C where the same six (6) changes to 7312A.C4 were combined with an additional seven (7) mutations in the N-terminal region. These results indicate that amino acid residues L679, W680 and K683 in the C-terminal region of gp41 MPER are involved in IgG1 LN01 antibody binding. Thus, in some embodiments, the binding agent exhibits the capacity to bind to and/or neutralize HIV expressing gp41 comprising the amino acid residues L679, W680 and K683 as found in the HIV-1 envelope amino acid sequence of GenBank Accession No. K03455 (NCBI GenPept Accession No. AAB50262), such as, for instance, SEQ ID NO. 68 (L679, W680 and K683 underlined):

1	MRVKEKYQHL	WRWGWRWGTM	LLGMLMICSA	TEKLWVTVYY	GVPVWKEATT	TLFCASDAKA
61	YDTEVHNVWA	THACVPTDPN	PQEVVLVNVT	ENFNMWKNDM	VEQMHEDIIS	LWDQSLKPCV
121	KLTPLCVSLK	CTDLKNDTNT	NSSSGRMIME	KGEIKNCSFN	ISTSIRGKVQ	KEYAFFYKLD
181	IIPIDNDTTS	YKLTSCNTSV	ITQACPKVSF	EPIPIHYCAP	AGFAILKCNN	KTFNGTGPCT
241	NVSTVQCTHG	IRPVVSTQLL	LNGSLAEEEV	VIRSVNFTDN	AKTIIVQLNT	SVEINCTRPN
301	NNTRKRIRIQ	RGPGRAFVTI	GKIGNMRQAH	CNISRAKWNN	TLKQIASKLR	EQFGNNKTII
361	FKQSSGGDPE	IVTHSFNCGG	EFFYCNSTQL	FNSTWFNSTW	STEGSNNTG	SDTITLPCRI
421	KQIINMWQKV	GKAMYAPPIS	GQIRCSSNIT	GLLLTRDGGN	SNNESEIFRP	GGGDMRDNWR
481	SELYKYKVVK	IEPLGVAPTK	AKRRVVQREK	RAVGIGALFL	GFLGAAGSTM	GAASMTLTVQ
541	ARQLLSGIVQ	QQNNLLRAIE	AQQHLLQLTV	WGIKQLQARI	LAVERYLKDQ	QLLGIWGCSG
601	KLICTTAVPW	NASWSNKSLE	QIWNHTTWME	WDREINNYTS	LIHSLIEESQ	NQQEKNEQEL
661	LELDKASLW	NWFNITNWLW	YIKLFIMIVG	GLVGLRIVFA	VLSIVNRVRQ	GYSPLSFQTH
721	LPTPRGPDRP	EGIEEEGGER	DRDRSIRLVN	GSLALIWDDL	RSLCLFSYHR	LRDLLLLIVTR

781 IVELLGRRGW EALKYWWNLL QYWSQELKNS AVSLLNATAI AVAEGTDRVI EVVQGACRAI
 841 RHIPRRIRQG LERILL (SEQ ID NO. 68),

or equivalents thereof as would be understood by those of ordinary skill in the art. An exemplary gp41 polypeptide is shown below:

AVGIGALFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTV
 WGIKQLQARILAVERYLKDQQLLGIWGCSGKLICTTAVPWNASWSNKSLEQIWNMTWM
 EWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITNWLWYIKLFI
 VGGLVGLRIVFAVLSIVNRVRQG (SEQ ID NO. 69).

Another exemplary gp41 polypeptide has GenBank Accession No. 1103299B (Meusing, et al. Nature 313 (6002), 450-458 (1985)). Exemplary equivalents to amino acid residues L679, W680 and K683 are the underlined amino acid residues L168, W169 and K172 of SEQ ID NO. 14 (as well as GenBank Accession No. 1103299B). In some embodiments, the binding agent exhibits the capacity to bind to and/or neutralize HIV expressing amino acid sequence ITKWLWYIK (SEQ ID NO. 66). In some embodiments, the binding agent exhibits the capacity to bind to and/or neutralize HIV expressing amino acid sequence ITKWLWYIK (SEQ ID NO. 66) but not LASWVKYIQ (SEQ ID NO. 65) and/or ITKWIKYIQ (SEQ ID NO. 67). In some embodiments, the epitope(s) to which the binding agent binds (i.e., has specificity) includes the amino acid residues L679, W680 and K683, or equivalents thereof of, HIV env (e.g., SEQ ID NO. 68). With respect gp41, in preferred embodiments, the epitope(s) to which the binding agent binds (i.e., has specificity) includes the amino acid residues L168, W169 and K172 of SEQ ID NO. 69, or equivalents thereof. In some embodiments, a binding agent of this disclosure may comprise these binding specificities along with the neutralization characteristics described above (i.e., neutralization of HIV-1 pseudoviruses BJOX (CRF07_BC), CE1176, TRO.11 (B), X1632 (G), CH119 (CRF07_BC), CNE55 (CRF01_AE), 25710 (C), CD0217(C) but not of the control virus SVA-MLV at a concentration is from 10^2 - 10^0 ug/ml, or between 10^0 - 10^1 ug/ml, to at least about 50% (**Fig. 3**), as well as the neutralization a majority of the 118 HIV-1 pseudoviruses listed in **Figure 4** at an IC_{50} of less than 25 μ g/ml).

[0039] Peptide microarrays may alternatively and/or also be used to determine the binding specificity of the binding agents described herein. One or more peptide microarrays may be designed such that overlapping peptides encompassing the entire amino acid sequence of a HIV polypeptide. For instance, as shown in the examples herein, a peptide microarray formed by 1423 overlapping (by 12 amino acids) 15-mer peptides covering the consensus HIV-1 Env gp160 sequences for clades A, B, C, D, group M, CRF01_AE and CRF02_AG

was utilized to test the specificity of binding agent IgG1 LN01. In the examples herein, the peptides were printed onto 3D-Epoxy glass slides and were analyzed with a GenePix 4000B scanner (Tomaras, G. D. et al. Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1-infected individuals. *J Virol* 85, 11502–11519 (2011)) but any suitable system available to those of ordinary skill in the art may be utilized. The binding agent may be tested along with a control binding agent (e.g., in the examples herein IgG01 LN01 was tested at 20 µg/ml in parallel with a control antibody called 7B2 having specificity for the immunodominant region of gp41). The binding of the binding agent to the peptides in the microarray may be detected by any suitable process, including by incubation with DyLight 649-labeled goat anti-human IgG as in the examples herein. Fluorescence intensity may be measured by any suitable system, such as a GenePix 4000B scanner/GenePix software as in the examples herein (see, e.g., **Fig. 8**). As shown in the examples herein, IgG1 LN01 antibody did not clearly react with any of the peptides in that library, while the control antibody (7B2) strongly reacted with 190-195 peptides that spanned the gp41 immunodominant region, indicating that the IgG1 LN01 antibody does not recognize a linear epitope in HIV-1 Env. Similar tests may also be performed on any of the binding agents contemplated herein.

[0040] The specificity of a binding agent may also be tested for binding to soluble trimers representing HIV proteins (e.g., soluble, cleaved SOSIP.664 gp140 trimers based on the subtype A transmitted/founder strain, BG505 as used in the examples herein). Preferred trimers (such as those used in the examples herein) are those being highly stable, homogenous and closely resembling native virus spikes when visualized by negative stain electron microscopy (EM) (Sanders, R. W. et al. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog.* 9, e1003618 (2013)). Typically, broadly neutralizing antibodies against multiple neutralizing epitopes on HIV-1 Env will be highly reactive with such trimers (e.g., the BG505 SOSIP.664 gp140 trimers, including quaternary epitopes antibodies (CH01, PG9, PG16 and PGT145)). Conversely, non-neutralizing antibodies (NAbs) to the CD4-binding site, CD4-induced epitopes or gp41 ectodomain would not (and did not in the example) react with the trimers, even when their epitopes were present on simpler forms of Env (e.g., gp120 monomers or dissociated gp41 subunits). The examples also included a test, which may be used in testing any of binding agents described herein, in which the MPER was also deleted to improve trimer solubility and reduce

aggregate formation. The binding agents may also be tested for binding to such trimers in the presence or absence of soluble CD4 (sCD4). The examples herein describe the testing of the IgG1 LN01, PGT145 (V1-V2 glycan specific), PGT151 (binding to a site at the interface between gp120 and gp41) and 17b (binding to a CD4 binding induced site) antibodies for binding to BG505 SOSIP.664 gp140 trimers in the presence or absence of sCD4 (measured by surface plasmon resonance (SPR)). As shown therein, the PGT145 and PGT151 antibodies reacted strongly to BG505 SOSIP.664 gp140 trimers in the presence and absence of sCD4; 17b reacted to BG505 SOSIP.664 gp140 trimers only in the presence of sCD4; and IgG1 LN01 did not react with BG505 SOSIP.664 gp140 trimers neither in the presence nor in the absence of sCD4 (see, e.g., **Fig. 9**). Similar tests may also be performed on any of the binding agents contemplated herein.

[0041] Other assay systems such as ELISA may also be used to test the binding agents contemplated herein. For instance, as shown in the examples, the IgG1 LN01 antibody, in parallel with MPER-specific 10E8 antibody (Huang, J. et al. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature* 491, 406–412 (2012)), was tested by ELISA against a panel of HIV-1 antigens (ConsB, consensus clade B gp140, 426c, clade C gp140, 426c-NLGS, 426c gp140 where the N-linked glycosylation sites were removed, 426c core, gp140 where the V loops were removed, UG37 gp140, clade A and gp41, recombinant ecto-domain of gp41, amino acids 541-682 from HxB2 strain, Vybion). None of the tested antigens was recognized by IgG1 LN01 antibody by ELISA (see, e.g., **Fig. 10**). Conversely, 10E8 antibody reacted to the recombinant ecto-domain of gp41. The results presented in the examples therefore indicate that IgG1 LN01 antibody might recognize an epitope in the MPER of gp41 different from 10E8. The examples herein also demonstrate the testing of IgG1 LN01 antibody against a fusion intermediate gp41 and uncoated plates (PBS), called gp41int (Lai, R. P. J. et al. A fusion intermediate gp41 immunogen elicits neutralizing antibodies to HIV-1. *J Biol Chem* 289, 29912–29926 (2014)), using ELISA. The gp41int antigen is recognized with high affinity by MPER antibodies 4E10, 2F5 and 10E8. While 10E8 reacted to gp41int by ELISA, IgG1 LN01 antibody did not (see, e.g., **Fig. 11**). These results indicate that IgG1 LN01 antibody recognizes a conserved epitope, possibly in the MPER region, that is not readily displayed in any of the antigens tested. Similar tests may be performed on any of the binding agents contemplated herein.

[0042] The term “binding affinity” and/or K_D refers to the dissociation rate of a particular

antibody-antigen interaction. The K_D is the ratio of the rate of dissociation ("off-rate (k_d)") to the association rate ("on-rate (k_a)"). K_D therefore equals k_d/k_a and is expressed as a molar concentration (M). Thus, the smaller the K_D , the stronger the affinity of binding. For example, a K_D of 1 mM indicates weak binding as compared to a K_D of 1 nM. K_D values for antibodies can be determined using methods well established in the art such as by using a Biacore[®] system. In some embodiments, the binding agents described herein may be compared with another binding agent with reference to the respective K_D values of each. These properties may be combined with other characteristics such as neutralization capacity and/or epitope specificity in order to compare binding agents to one another. Accordingly, binding agents having a similar K_D to those described herein, perhaps also sharing the neutralization capacity and epitope specificity described herein (e.g., as exhibited by LN01), are also contemplated as part of this disclosure.

[0043] Any of the amino acid sequences of **Tables 1** (and / or any one or more fragments and / or derivatives thereof) may be also substituted by any other amino acid as desired by one of ordinary skill in the art. For example, one of skill in the art may make conservative substitutions by replacing particular amino acids with others as shown in **Table 2** below. The specific amino acid substitution selected may depend on the location of the site selected. An amino acid substitution may be said to "correspond to" where one of ordinary skill in the art could ascertain a significant amount of similarity between the amino acid sequences surrounding the amino acid being substituted. For instance, a particular amino acid sequence may correspond to another where two, three, four or more N-terminal and C-terminal amino acids surrounding the amino acid being substituted are the same or similar (e.g., as described in **Table 2**) in the polypeptides being compared. Conservative amino acid substitutions may involve a substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge, hydrophobicity, or hydrophilicity of the amino acid residue at that position and, in particular, does not result in, e.g., decreased HIV neutralization capacity and/or different epitope specificity.

Table 2

Original Amino Acid Residue	Exemplary Conservative Substitutions of the Original Amino Acid Residue	Preferred Conservative Substitution of the Original Amino Acid Residue
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0044] In certain embodiments, a nucleic acid molecule encoding one or more binding agents described herein may be inserted into one or more expression vectors, as discussed below in greater detail. In such embodiments, the binding agent may be encoded by nucleotides corresponding to the amino acid sequence. The particular combinations of nucleotides (codons) that encode the various amino acids (AA) are well known in the art, as described in various references used by those skilled in the art (e.g., Lewin, B. *Genes V*, Oxford University Press, 1994). The nucleotide sequences encoding the amino acids of said binding agents may be ascertained with reference to **Table 3**, for example. Nucleic acid variants may use any combination of nucleotides that encode the binding agent.

Table 3
Codons Encoding Amino Acids (AA)

AA	Codon	AA	Codons	AA	Codons	AA	Codons
Phe (F)	TTT	Ser (S)	TCT	Tyr (Y)	TAT	Cys (C)	TGT
	TTC		TCC		TAC		TGC
Leu (L)	TTA		TCA	TERM	TAA	TERM	TGA
	TTG	Pro (P)	TCG		TAG	Trp (W)	TGG
	CTT		CCT	His (H)	CAT	Arg (R)	CGT
	CTC		CCC		CAC		CGC
	CTA		CCA	Gln (Q)	CAA		CGA
	CTG		CCG		CAG		CGG
Ile (I)	ATT	Thr (T)	ACT	Asn (N)	AAT	Ser (S)	AGT
	ATC		ACC		AAC		AGC
	ATA		ACA	Lys (K)	AAA	Arg (R)	AGA
Met (M)	ATG		ACG		AAG		AGG
Val (V)	GTT	Ala (A)	GCT	Asp (D)	GAT	Gly (G)	GGT
	GTC		GCC		GAC		GGC
	GTA		GCA	Glu (E)	GAA		GGA
	GTG		GCG		GAG		GGG

Those of ordinary skill in the art understand that the nucleotide sequence encoding a particular amino acid sequence may be easily derived from the amino acid sequence of any of SEQ ID NOS. 1-7 and the information presented in **Table 4**. For instance, it may be deduced from the amino acid sequence GDSVSNDNYY (SEQ ID NO.: 1) and the information presented in **Table 4** that the amino acid sequence may be encoded by the nucleotide sequence GGTGACTCAGTCAGTAATGATAATTATTAT (SEQ ID NO.: 33). Those of ordinary skill in the art would understand that nucleotide sequences encoding SEQ ID NOS. 2-32 and 34-36 may be deduced in the same way, and such nucleotide sequences are contemplated herein. **Table 4** provides exemplary nucleic acid sequences encoding the amino acid sequences shown in **Table 1** (SEQ ID NOS. 1-32):

TABLE 4

LN01 region	Amino Sequence	Exemplary Nucleotide Sequence
LN01 CDRH1	GDSVSNDNYY (SEQ ID NO. 1)	GGTGACTCAGTCAGTAATGATAATTATTAT (SEQ ID NO. 33)
LN01 CDRH2	IYYSGTT (SEQ ID NO. 2)	ATCTATTACAGCGGCACAACC (SEQ ID NO. 34)
LN01 CDRH3	VRMPSHGFWSTSFYWYFDL (SEQ ID NO. 3)	GTTCGCATGCCCAGTCACGGATTTTGGAGTACTTCTTTCTCTTAC TGGTATTTTCGATCTC (SEQ ID NO. 35)
LN01 CDRL1	QSVTKY (SEQ ID NO. 4)	CAGAGTGTCAACCAAATAT (SEQ ID NO. 36)

LN01 CDRL2	GTY	GGGACTTAT
LN01 CDRL2 (long)	LIYGTYTLL (SEQ ID NO. 5)	CTCATCTATGGGACTTATACTT TACTC (SEQ ID NO. 37)
LN01 CDR3	QQAHSPTWT (SEQ ID NO. 6)	CAACAGGCTCACAGTACTCCC TGGACC (SEQ ID NO. 38)
LN01 Variable Heavy (VH)	EVQLVESGPGLVQPWGTLST TCRVSGDSVSNDNYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWYFDLWGR GHFVAVSW (SEQ ID NO. 7)	GAGGTGCAGCTGGTGGAGTCG GGCCCAGGACTGGTGCAGCCC TGGGGGACCCTGTCCCTCACCT GTCGTGTCTCTGGTGA CTAGT CAGTAATGATAATTATTATTGGG CCTGGATTCGCCAGACCCCCGG GAGGGA ACTGCAGGTCATCGGA ACTATCTATTACAGCGGCACAAC CTACTACAATCCGTGCTCAGG AATCGAGTCACGATCTCATTGG ACAAGTCCGTCAATGTGGTCTC CCTGAGATTGGGGTCTGTGAGT GCCGCGGACACGGCCCAATATT ATTGCGTTTCGCATGCCCAGTCA CGGATTTTGGAGTACTTCTTTCT CTTACTGGTATTTTCGATCTCTGG GGCCGTGGTCATTTTCGTGCTG TCTCCTGG (SEQ ID NO. 39)
LN01 Variable Light (VL)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRILIYGTYTLLSGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQAHSPTWTFGQG THVAAN (SEQ ID NO. 8)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO. 40)
LN01 variant 7 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTLST TCRVSGDSVSNDNYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWYFDLWG RGHFVAVSW (SEQ ID NO.: 9)	GAAGTGCAGCTGGTGGAACTCTG GCCCTGGCCTGGTGCAGCCTTG GGGCACACTGAGCCTGACCTGT AGAGTGTCCGGCGACAGCGTGT CCA ACTGGA ACTACTACTGGGC CTGGATCCGGCAGACCCCCGG CAGAGAACTGCAAGTGATCGGC ACCATCTACTACAGCGGCACAA CCTACTACAACCCAGCCTGCG GAACAGAGTGACCATCAGCCTG GACAAGAGCGTGAACGTGGTGT CCCTGAGACTGGGCTCTGTGTC TGCCGCGGATACCGCCCAGTAC

		TACTGCGTGCGGATGCCCAGCC ACGGCTTCTGGTCTACCAGCTT CAGCTACTGGTACTTCGACCTG TGGGGCAGAGGCCACTTCGTG GCCGTGTCTTGG (SEQ ID NO. 41)
LN01 variant 7 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LS</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQA <u>HSTPWTF</u> GGQ THVAAN (SEQ ID NO.: 10)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO. 42)
LN01 variant 8 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTL SLTCRVSGDSVSNDWYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWYFDLWGR GHFVAVSW (SEQ ID NO.: 11)	GAAGTGCAGCTGGTGGAACTCTG GCCCTGGCCTGGTGCAGCCTTG GGGCACACTGAGCCTGACCTGT AGAGTGTCCGGCGACAGCGTGT CCAACGACTGGTACTACTGGGC CTGGATCCGGCAGACCCCCGG CAGAGAACTGCAAGTGATCGGC ACCATCTACTACAGCGGCACAA CCTACTACAACCCAGCCTGCG GAACAGAGTGACCATCAGCCTG GACAAGAGCGTGAACGTGGTGT CCCTGAGACTGGGCTCTGTGTC TGCCGCCGATAACCGCCAGTAC TACTGCGTGCGGATGCCCAGCC ACGGCTTCTGGTCTACCAGCTT CAGCTACTGGTACTTCGACCTG TGGGGCAGAGGCCACTTCGTG GCCGTGTCTTGG (SEQ ID NO. 43)
LN01 variant 8 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LS</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQA <u>HSTPWTF</u> GGQ THVAAN (SEQ ID NO.:12)	GACATCCAGATGACCCAGAGCC CCAGCAGCCTGTCTGCCAGCGT GGGCGACAAAGTGACCATCACC TGTCGGGCCAGCCAGAGCGTG ACCAAGTACCTGAACTGGTATC AGTTTAAGACCGGCCAGGCCCC CAGAATCCTGATCTACGGCACC TACACCCTGCTGAGCGGCGTGT CCCCTAGATTCTCTGGCGCCGG AAGCGGCAGCCTGTACACCCTG ACAATCACCAACATCCAGCCCG

		AGGACTTCGCCACCTACTACTG CCAGCAGGCCCACAGCACCCCT TGGACATTTGGCCAGGGAACAC ACGTGGCCGCCAAC (SEQ ID NO. 44)
LN01 variant 38 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTL SLTCRVSGDSVSNDNYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWYFDLWGR GHFVAVSW (SEQ ID NO.: 13)	AAGTGCAGCTGGTGGGAATCTGG CCCTGGCCTGGTGCAGCCTTGG GGCACACTGAGCCTGACCTGTA GAGTGTCCGGCGACAGCGTGTC CAACGACAACACTACTACTGGGCC TGGATCCGGCAGACCCCCGGC AGAGAACTGCAAGTGATCGGCA CCATCTACTACAGCGGCACAAC CTACTACAACCCCAGCCTGCGG AACAGAGTGACCATCAGCCTGG ACAAGAGCGTGAACGTGGTGTC CCTGAGACTGGGCTCTGTGTCT GCCGCCGATACCGCCCAGTACT ACTGCGTGCGGATGCCAGCCA CGGCTTCTGGTCTACCAGCTTC AGCTACTGGTACTTCGACCTGT GGGGCAGAGGCCACTTCGTGG CCGTGTCTTGG (SEQ ID NO. 45)
LN01 variant 38 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRILIYGTYTLLSGVSPR FSGAGWGSlyTLTITNIQPED FATYYCQQAHSPTWTFGQG THVAAN (SEQ ID NO.: 14)	ACATCCAGATGACCCAGAGCCC CAGCAGCCTGTCTGCCAGCGTG GGCGACAAAGTGACCATCACCT GTCGGGCCAGCCAGAGCGTGA CCAAGTACCTGAACTGGTATCA GTTTAAGACCGGCCAGGCCCCC AGAATCCTGATCTACGGCACCT ACACCCTGCTGAGCGGCGTGTC CCCTAGATTCTCTGGCGCCGGA TGGGGCAGCCTGTACACCCTGA CAATCACCAACATCCAGCCCGA GGACTTCGCCACCTACTACTGC CAGCAGGCCCACAGCACCCCTT GGACATTTGGCCAGGGAACACA CGTGGCCGCCAAC (SEQ ID NO. 46)
LN01 variant 41 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTL SLTCRVSGDSVSNFNYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWYFDLWGR GHFVAVSW (SEQ ID NO.: 15)	GAGGTGCAGCTGGTGGGAATCTG GACCTGGACTGGTGCAGCCTTG GGGCACTCTGTCTCTGACATGC CGGGTGAGCGGGGACAGCGTC TCCAACTTTAATTACTATTGGGC TTGGATCAGGCAGACACCAGGG CGCGAGCTGCAGGTCATCGGG ACTATCTACTATTCCGGAACCAC ATACTATAACCCCTCTCTGCGGA ATAGAGTGACCATTCTCTGGAC AAGAGTGTCAACGTGGTCAGTC TGCGACTGGGATCTGTGAGTGC

		CGCTGATACCGCACAGTACTAT TGCGTGCGGATGCCCTCTCACG GCTTCTGGTCAACAAGCTTTTCC TACTGGTATTTTCGATCTGTGGG GACGGGGGCCATTTTCGTGGCCGT CTCCTGG (SEQ ID NO. 47)
LN01 variant 41 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LL</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYC <u>QQA</u> HSTPWTFGQG THVAAN (SEQ ID NO.: 16)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO. 48)
LN01 variant 42 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTL SLTCRVSGDSVSNYNYWAWI RQTPGRELQVIGTIY <u>YS</u> GTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWF</u> DLWGR GHFVAVSW (SEQ ID NO.: 17)	GAGGTGCAGCTGGTGGAACTCG GACCTGGACTGGTGCAGCCTTG GGGCACTCTGTCTCTGACATGC CGGGTGAGCGGGGACAGCGTC TCCAATAACAATTACTATTGGGC TTGGATCAGGCAGACACCAGGG CGCGAGCTGCAGGTCATCGGG ACTATCTACTATTCCGGAACCAC ATACTATAACCCCTCTCTGCGGA ATAGAGTGACCATTCTCTGGAC AAGAGTGTCAACGTGGTCAGTC TGCGACTGGGATCTGTGAGTGC CGCTGATACCGCACAGTACTAT TGCGTGCGGATGCCCTCTCACG GCTTCTGGTCAACAAGCTTTTCC TACTGGTATTTTCGATCTGTGGG GACGGGGGCCATTTTGTGGCCGT CTCCTGG (SEQ ID NO. 49)
LN01 variant 42 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LL</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYC <u>QQA</u> HSTPWTFGQG THVAAN (SEQ ID NO.: 18)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC

		AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO. 50)
LN01 variant 48 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	QLQLQESGPGLVKPSETLSL TCTVSGDSVSN <u>WNYYWAWI</u> RQTPGRELQVIGTI <u>YYSGTTY</u> YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGR</u> GTLVTVSS (SEQ ID NO.:19)	CAGCTGCAGCTGCAGGAGAGTG GACCTGGACTGGTGAAGCCTTC AGAAACACTGAGCCTGACTTGC ACCGTGTCCGGCGACTCTGTCA GTAAGTGGAAATTACTATTGGGCA TGGATTAGACAGACACCAGGAA GAGAGCTGCAGGTCATCGGGAC AATCTACTATAGTGGAACCACAT ACTATAACCCCTCACTGCGGAA TAGAGTGACCATTTCCTGGAC AAATCTGTCAACGTGGTCTCTCT GCGACTGGGCTCAGTGAGCGC CGCTGATACTGCCCAGTACTATT GCGTGCGGATGCCAGCCACG GCTTCTGGTCCACCTCTTTTAGT TACTGGTATTTTCGATCTGTGGG GACGGGGCACACTGGTGACTGT CAGCTCC (SEQ ID NO. 51)
LN01 variant 48 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRILIYGTYTLLSGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQAHSPTWTFGQG THVAAN (SEQ ID NO.: 20)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO. 52)
LN01 variant 49 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	EVQLVESGPGLVQPWGTL TCRVSGDSVSN <u>WYWWYWAWI</u> RQTPGRELQVIGTI <u>YYSGTTY</u> YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP <u>SHGFWSTSFSYWYFDLWGR</u> GHFVAVSW (SEQ ID NO.:21)	GAGGTGCAGCTGGTGGAAATCTG GACCTGGACTGGTGCAGCCTTG GGGCACTCTGTCTCTGACATGC CGGGTGAGCGGGGACAGCGTC TCCAAGTGGTGGTACTATTGGG CTTGGATCAGGCAGACACCAGG GCGCGAGCTGCAGGTCATCGG GACTATCTACTATTCCGGAACCA CATACTATAACCCCTCTCTGCG GAATAGAGTGACCATTTCCTCTG GACAAGAGTGTCAATGTGGTCA GTCTGCGACTGGGATCTGTGAG TGCCGCTGATACCGCACAGTAC TATTGCGTGCGGATGCCCTCTC ACGGCTTCTGGTCAACAAGCTT

		TTCCTACTGGTATTTTCGATCTGT GGGGACGGGGCCATTTTGTGG CCGTCTCCTGG (SEQ ID NO.:53)
LN01 variant 49 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LS</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQAHSPTWTFGQG THVAAN (SEQ ID NO.:22)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO.: 54)
LN01 variant 82 Variable Heavy (V _H) (CDRH1, CDRH2 and CDRH3 underlined)	QVQLEESGPGLVQPWGTL LTCRVSGGSISSSSYYWAWI RQTPGRELQVIGTIYYSGTTY YNPSLRNRVTISLDKSVNVVS LRLGSVSAADTAQYYCVRMP SHGFWSTSFSYWF ~~DL~~ WGR GHFVAVSW (SEQ ID NO. 23)	CAGGTGCAGCTGGAGGAATCTG GACCTGGACTGGTCCAGCCTTG GGGGACTCTGAGCCTGACCTGC CGGGTGTCAGGCGGGAGCATC AGCTCCTCTAGTTACTATTGGGC TTGGATTAGGCAGACACCAGGC CGCGAGCTGCAGGTCATCGGCA CTATCTACTATAGTGGGACCACA TACTATAACCCCTCACTGCGGA ATAGAGTGACCATCTCCCTGGA CAAGTCTGTCAACGTGGTCTCT CTGCGACTGGGATCAGTGAGCG CCGCTGATACCGCACAGTACTA TTGCGTGCGGATGCCAGCCAC GGCTTCTGGTCCACATCTTTTAG TACTGGTATTTTCGACCTGTGG GGGCGGGGACATTTTGTGGCC GTCAGTTGG (SEQ ID NO.:55)
LN01 variant 82 Variable Light (V _L) (CDRL1, CDRL2 and CDRL3 underlined)	DIQMTQSPSSLSASVGDKVTI TCRASQSVTKYLNWYQFKT GQAPRIL <u>IYGT</u> YTL <u>LS</u> SGVSPR FSGAGSGSLYTLTITNIQPED FATYYCQQAHSPTWTFGQG THVAAN (SEQ ID NO.:24)	GACATCCAGATGACCCAGTCTC CGTCCTCCCTGTCTGCCTCTGT TGGAGACAAAGTCACCATCACC TGCCGGGGCCAGTCAGAGTGTCA CCAAATATTTAAATTGGTATCAG TTTAAGACCGGCCAAGCCCCAA GAATCCTCATCTATGGGACTTAT ACTTTACTCAGTGGCGTCTCGC CTCGGTTCAGTGGCGCCGGATC TGGTTCACTCTACACTCTGACCA TCACCAATATACAGCCTGAAGA CTTCGCCACCTATTATTGTCAAC AGGCTCACAGTACTCCCTGGAC CTTCGGCCAAGGAACCCACGTG GCGGCCAAC (SEQ ID NO.:56)

LN01 variants 7 and 48 Variable Heavy (V _H) CDRH1	GDSVSNWNY (SEQ ID NO.:25)	GGCGACAGCGTGTCCAAGTGA ACTACTAC (SEQ ID NO. 57)
LN01 variant 8 Variable Heavy (V _H) CDRH1	GDSVSNWY (SEQ ID NO.:26)	GGCGACAGCGTGTCCAAGTGA GGTACTAC (SEQ ID NO. 58)
LN01 variant 41 Variable Heavy (V _H) CDRH1	GDSVSNFN (SEQ ID NO.:27)	GGGGACAGCGTCTCCAAGTTA ATTACTAT (SEQ ID NO. 59)
LN01 variant 42 Variable Heavy (V _H) CDRH1	GDSVSNYNY (SEQ ID NO.:28)	GGGGACAGCGTCTCCAAGTACA ATTACTAT (SEQ ID NO. 60)
LN01 variant 43 Variable Heavy (V _H) CDRH1	GDSVSNLN (SEQ ID NO.:29)	GGGGACAGCGTCTCCAAGTTAA ATTACTAT (SEQ ID NO. 61)
LN01 variant 44 Variable Heavy (V _H) CDRH1	GDSVSNIN (SEQ ID NO.:30)	GGGGACAGCGTCTCCAAGATTA ATTACTAT (SEQ ID NO. 62)
LN01 variant 49 Variable Heavy (V _H) CDRH1	GDSVSNWY (SEQ ID NO.:31)	GGGGACAGCGTCTCCAAGTGGT GGTACTAT (SEQ ID NO. 63)
LN01 variant 82 Variable Heavy (V _H) CDRH1 (germline sequence)	GSISSSSY (SEQ ID NO.:32)	GGGAGCATCAGCTCCTCTAGTT ACTAT (SEQ ID NO. 64)

Thus, a nucleic acid encoding a binding agent of this disclosure may comprise one or more SEQ ID NOS. 33-64 or GGGACTTAT, or a derivative thereof that encodes any of SEQ ID NOS. 1-32 or GTY (LN01 CDRL2) and/or a derivative thereof (e.g., any of SEQ ID NOS. 1-32 conservatively substituted as described in **Table 2**, encoded by a nucleotide sequence determined using standard techniques, e.g., as described in **Table 3**, including but not limited to those described in **Table 4**). Expression vectors comprising such nucleic acid sequences are also contemplated by this disclosure. Where the binding agents are antibodies, nucleotide sequences encoding the variable regions thereof may also be isolated from the phage and / or hybridoma cells expressing the same cloned into expression vectors. Methods for producing such preparations are well-known in the art.

[0045] Nucleic acid molecules encoding one or more HIV binding agents may be contained within a viral and / or a non-viral vector. In one embodiment, a DNA vector is utilized to deliver nucleic acids encoding one or more HIV binding agents to the patient. In doing so, various strategies may be utilized to improve the efficiency of such mechanisms including, for example, the use of self-replicating viral replicons (Caley, et al. 1999. *Vaccine*, 17: 3124-

2135; Dubensky, et al. 2000. *Mol. Med.* 6: 723-732; Leitner, et al. 2000. *Cancer Res.* 60: 51-55), codon optimization (Liu, et al. 2000. *Mol. Ther.*, 1: 497-500; Dubensky, *supra*; Huang, et al. 2001. *J. Virol.* 75: 4947-4951), *in vivo* electroporation (Widera, et al. 2000. *J. Immunol.* 164: 4635-3640), incorporation of nucleic acids encoding co-stimulatory molecules, cytokines and / or chemokines (Xiang, et al. 1995. *Immunity*, 2: 129-135; Kim, et al. 1998. *Eur. J. Immunol.*, 28: 1089-1103; Iwasaki, et al. 1997. *J. Immunol.* 158: 4591-3201; Sheerlinck, et al. 2001. *Vaccine*, 19: 2647-2656), incorporation of stimulatory motifs such as CpG (Gurunathan, *supra*; Leitner, *supra*), sequences for targeting of the endocytic or ubiquitin-processing pathways (Thomson, et al. 1998. *J. Virol.* 72: 2246-2252; Velders, et al. 2001. *J. Immunol.* 166: 5366-5373), prime-boost regimens (Gurunathan, *supra*; Sullivan, et al. 2000. *Nature*, 408: 605-609; Hanke, et al. 1998. *Vaccine*, 16: 439-445; Amara, et al. 2001. *Science*, 292: 69-74), proteasome-sensitive cleavage sites, and the use of mucosal delivery vectors such as *Salmonella* (Darji, et al. 1997. *Cell*, 91: 765-775; Woo, et al. 2001. *Vaccine*, 19: 2945-2954). Other methods are known in the art, some of which are described below. Various viral vectors that have been successfully utilized for introducing a nucleic acid to a host include retrovirus, adenovirus, adeno-associated virus (AAV), herpes virus, and poxvirus, among others. The vectors may be constructed using standard recombinant techniques widely available to one skilled in the art. Such techniques may be found in common molecular biology references such as *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), and *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, ca). “Non-viral” plasmid vectors may also be suitable in certain embodiments. Preferred plasmid vectors are compatible with bacterial, insect, and / or mammalian host cells. Such vectors include, for example, PCR-ii, PCR3, and pcDNA3.1 (Invitrogen, San Diego, CA), pBSii (Stratagene, La Jolla, CA), pet15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFp-n2 (Clontech, Palo Alto, CA), pETI (Bluebacii, Invitrogen), pDSR-alpha (PCT pub. No. WO 90/14363) and pFASTBACdual (Gibco-BRL, Grand island, NY) as well as Bluescript[®] plasmid derivatives (a high copy number COLe1-based phagemid, Stratagene Cloning Systems, La Jolla, CA), PCR cloning plasmids designed for cloning TAQ-amplified PCR products (e.g., TOPO[™] TA cloning[®] kit, PCR2.1[®] plasmid derivatives, Invitrogen, Carlsbad, CA). Bacterial vectors may also be used. These vectors include, for example, *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, *Bacille Calmette Guérin* (BCG), and *Streptococcus* (see for example, WO 88/6626; WO 90/0594; WO 91/13157; WO 92/1796; and WO

92/21376). Many other non-viral plasmid expression vectors and systems are known in the art and may be used. Other delivery techniques may also suffice including, for example, DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, CaPO_4 precipitation, gene gun techniques, electroporation, and colloidal dispersion systems. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system is a liposome, which are artificial membrane vesicles useful as delivery vehicles *in vitro* and *in vivo*. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, R., *et al.*, 1981, *Trends Biochem. Sci.*, 6: 77). The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

[0046] A cultured cell comprising the vector is also provided. The cultured cell may be a cultured cell transfected with the vector or a progeny of the cell, wherein the cell expresses the immunogenic polypeptide. Suitable cell lines are known to those of skill in the art and are commercially available, for example, through the American Type Culture Collection (ATCC). The transfected cells can be used in a method of producing an immunogenic polypeptide. The method comprises culturing a cell comprising the vector under conditions that allow expression of the immunogenic polypeptide, optionally under the control of an expression sequence. The immunogenic polypeptide can be isolated from the cell or the culture medium using standard protein purification methods. In some embodiments, the binding agents described herein may be conjugated to active agents to target and inhibit the function of and/or eliminate cell populations expressing HIV polypeptides and/or harboring HIV (and/or another antigen in the case of binding agents with multiple specificities). For instance, CD4^+ T-cell populations containing replication competent HIV may be targeted and eliminated

using binding agent / drug conjugates (e.g., antibody-drug conjugates (ADC)). Mono- and/or bi-specific candidate binding agents may be conjugated with one or more types of drugs (e.g., drugs damaging DNA, targeting microtubules). The binding agents described herein and/ or derivatives thereof may also be adjoined to and / or conjugated to functional agents for *in vitro* and / or *in vivo* use. For instance, the binding agent may be adjoined to and / or conjugated to functional moieties such as cytotoxic drugs or toxins, and / or active fragments thereof such as diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin, among others. Suitable functional moieties may also include radiochemicals. Binding agents, such as antibodies, may be adjoined to and / or conjugated to the one or more functional agents using standard techniques in the art.

[0047] In some embodiments, this disclosure provides binding agents with multiple specificities such that epitopes bound by LN01 (e.g., ITKWLWYIK (SEQ ID NO. 72) but not LASWVKYIQ (SEQ ID NO. 71) and/or ITKWIKYIQ (SEQ ID NO. 73)) and at least one other secondary antigen (e.g., a cell surface protein) may be bound by a single binding agent. In some embodiments, the secondary antigen may be one expressed by cells infected by an infectious agent. For instance, an exemplary secondary antigen may be HIV Env antigen other than gp41. Such binding agents may bind the secondary antigen and / or may serve to neutralize the infectious agent as may be determined using the assays described herein. Combinations of binding agents, such as one or more described herein with another available to those of ordinary skill in the art, are also contemplated herein. For instance, in some embodiments, the combinations may be identified to provide statistically significant differences from results (e.g., neutralization assays) obtained using only one or more of the binding agents and not others. In some embodiments, combinations exhibit synergistic neutralization of HIV, for example. In some embodiments, the combination may comprise a first binding agent having the characteristics of LN01 (e.g., such as IgG1 LN01) and/or comprising any one or more of SEQ ID NOS. 1-32 (and/or as described in **Table 1**), and/or derivatives thereof, and any one or more of the antibodies described in any one or more of U.S. Pat. No. 5,087,557; U.S. Pat. No. 5,298,419; U.S. Pat. No. 5,459,060; U.S. Pat. No. 5,693,752; U.S. Pat. No. 5,731,189; U.S. Pat. No. 5,753,503; U.S. Pat. No. 5,756,674; U.S. Pat. No. 5,777,074; U.S. Pat. No. 5,804,440; U.S. Pat. No. 5,831,034; U.S. Pat. No. 6,008,044; U.S. Pat. No. 7,774,887B2; U.S. Pat. Publications 2003/0118985A1, 2007/0292390A1, or 2014/0205612A1 (e.g., binding the gp41 epitopes ELDKWA, ELEKWA, ELNKWA, ELDEWA); EP0335134B1 US 176077 (e.g, a humanized

version of the mouse mAbs described therein); DE3932461A1 (mAb against the epitope Arg-Ile-Leu-Ala-Val-Glu-Arg-Leu-Lys-Try-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser); Evans, et al. J. Immunol. 140(3): 941-3 (1988); Gorney, et al. Proc. Natl. Acad. Sci. USA, 86: 1624-28 (1989); Teeuwsen, et al. (1990) *AIDS Res. Hum. Retroviruses* 6, 381-392; Earl, et al. J. Virol. 71(4): 2674-2684 (1997); Jiang, et al. J. Virol. 72(12): 10213-17 (1998); Zwick, et al. J. Virol. 75(22): 10892-10905 (2001); Eckert et al. PNAS USA, 98(20): 11187-11192 (2001); Louis, et al. J. Biol. Chem. 278(22): 20278-20285 (2003); and/or Pietzsch, et al. J. Virol. 84(10): 5032-42 (2010); all of which are incorporated herein in their entirety. For instance, any of the binding agents described herein may be combined with (i.e., as a single composition, and/or used in conjunction with) one or more the antibodies commonly known as 2F5, 4E10 and/or Z13e1, and/or derivatives thereof, among others. The binding agents of such compositions may be different entities such as two or more different monoclonal antibodies or derivatives thereof, or may be found on the same entity such as a bi-functional antibody (a single antibody or derivative thereof comprising multiple binding specificities). Such combinations as described herein may also be combined with one or more other agents that may effect immune cell function such as antibodies against CTLA-4, and the like. One of ordinary skill in the art would recognize that many such combinations may be suitable for use as described herein.

[0048] As mentioned above, the HIV binding agents described herein may be used to treat and / or prevent and / or ameliorate the symptoms of infection by HIV. As is well-known in the art, HIV isolates are now classified into discrete genetic subtypes. HIV-1 is known to comprise at least ten subtypes (A1, A2, A3, A4, B, C, D, E, F1, F2, G, H, J and K) (Taylor et al, NEJM, 359(18):1965-1966 (2008)). HIV-2 is known to include at least five subtypes (A, B, C, D, and E). Subtype B has been associated with the HIV epidemic in homosexual men and intravenous drug users worldwide. Most HIV-1 immunogens, laboratory adapted isolates, reagents and mapped epitopes belong to subtype B. In sub-Saharan Africa, India and China, areas where the incidence of new HIV infections is high, HIV-1 subtype B accounts for only a small minority of infections, and subtype HIV-1 C appears to be the most common infecting subtype. Any of these types of isolates may be addressed using the binding agents described herein. One or more binding agents may also be administered with or in conjunction with one or more agents used to prevent, treat and / or ameliorate HIV such as for example, a protease inhibitor, an HIV entry inhibitor, a reverse transcriptase inhibitor, and / or an anti- retroviral nucleoside analog. Suitable compounds include, for example,

Agenerase (amprenavir), Combivir (Retrovir / Epivir), Crixivan (indinavir), Emtriva (emtricitabine), Epivir (3tc / lamivudine), Epzicom, Fortovase / Invirase (saquinavir), Fuzeon (enfuvirtide), Hivid (ddc / zalcitabine), Kaletra (lopinavir), Lexiva (Fosamprenavir), Norvir (ritonavir), Rescriptor (delavirdine), Retrovir / AZT (zidovudine), Reyatax (atazanavir, BMS-232632), Sustiva (efavirenz), Trizivir (abacavir / zidovudine / lamivudine), Truvada (Emtricitabine / Tenofovir DF), Videx (ddI / didanosine), Videx EC (ddI, didanosine), Viracept (nevirapine), Viread (tenofovir disoproxil fumarate), Zerit (d4T / stavudine), and Ziagen (abacavir) may be utilized. Other suitable agents are known to those of skill in the art and may be suitable for use as described herein. Such agents may either be used prior to, during, or after administration of the binding agents and / or use of the methods described herein.

[0049] The skilled artisan has many suitable techniques for using the binding agents (e.g., antibodies) described herein to identify biological samples containing proteins that bind thereto. For instance, antibodies may be utilized to isolate HIV or cells containing HIV and / or expressing HIV antigens using, for example, immunoprecipitation or other capture-type assay. This well-known technique is performed by attaching the antibody to a solid support or chromatographic material (e.g., a bead coated with Protein A, Protein G and / or Protein L). The bound antibody is then introduced into a solution either containing or believed to contain HIV antigens (e.g., an HIV-infected cell). The HIV antigen(s) may then bind to the antibody and non-binding materials are washed away under conditions in which the HIV antigen(s) remains bound to the antibody. The bound protein may then be separated from the antibody and analyzed as desired. Similar methods for isolating a protein using an antibody are well-known in the art. The binding agents (e.g., antibodies) may also be utilized to detect HIV or HIV antigens within a biological sample. For instance, the antibodies may be used in assays such as, for example, flow cytometric analysis, ELISA, immunoblotting (e.g., western blot), *in situ* detection, immunocytochemistry, and / or immunohistochemistry. Methods of carrying out such assays are well-known in the art. In some embodiments, the binding agents may be adjoined to and / or conjugated to one or more detectable labels. For instance, suitable detectable labels may include, for instance, fluorosceins (e.g., DyLight, Cy3, Cy5, FITC, HiLyte Fluor 555, HiLyte Fluor 647; 5-carboxy-2,7-dichlorofluorescein; 5-Carboxyfluorescein (5-FAM); 5-HAT (Hydroxy Tryptamine); 5-Hydroxy Tryptamine (HAT); 6-JOE; 6-carboxyfluorescein (6-FAM); FITC; 6-carboxy-1,4-dichloro-2',7'-dichlorofluorescein (TET); 6-carboxy-1,4-dichloro-2',4', 5', 7'-tetrachlorofluorescein (HEX); 6-carboxy-4',5'-

dichloro-2', 7'-dimethoxyfluorescein (JOE); Alexa fluors (e.g., 350, 405, 430, 488, 500, 514, 532, 546, 555, 568, 594, 610, 633, 635, 647, 660, 680, 700, 750); BODIPY fluorophores (e.g., 492/515, 493/503, 500/510, 505/515, 530/550, 542/563, 558/568, 564/570, 576/589, 581/591, 630/650-X, 650/665-X, 665/676, FL, FL ATP, FI-Ceramide, R6G SE, TMR, TMR-X conjugate, TMR-X, SE, TR, TR ATP, TR-X SE)), rhodamines (e.g., 110, 123, B, B 200, BB, BG, B extra, 5-carboxytetramethylrhodamine (5-TAMRA), 5 GLD, 6-Carboxyrhodamine 6G, Lissamine, Lissamine Rhodamine B, Phalloidine, Phalloidine, Red, Rhod-2, ROX (6-carboxy-X-rhodamine), 5-ROX (carboxy-X-rhodamine), Sulphorhodamine B can C, Sulphorhodamine G Extra, TAMRA (6-carboxytetramethylrhodamine), Tetramethylrhodamine (TRITC), WT), Texas Red, and / or Texas Red-X. Other detectable labels known in the art may also be suitable for use. Binding agents, such as antibodies, may be adjoined to and / or conjugated to the one or more detectable labels using standard techniques in the art.

[0050] The binding agents described herein may be also be used to determine the presence of a disease state in a patient, to predict prognosis, or to determine the effectiveness of a chemotherapeutic or other treatment regimen. Expression profile assays, performed as described herein or as is otherwise known in the art, may be used to determine the relative level of expression of HIV in a cell, for instance. The level of expression may then be correlated with base (e.g., control) levels to determine whether a particular disease is present within the patient, the patient's prognosis, or whether a particular treatment regimen is effective. For example, if the patient is being treated with a particular anti-infective regimen, an increased or decreased level of expression of HIV in the patient's tissues (e.g., in plasma) may indicate the regimen is worsening or improving the load of HIV in that host. The increase or decrease in expression may indicate the regimen is having or not having the desired effect and another therapeutic modality may therefore be selected.

[0051] It is also possible to use the binding agents described herein as reagents in drug screening assays to test, for example, new drug candidates. The reagents may be used to ascertain the effect of a drug candidate on the expression of the immunogenic target in a cell line, or a cell or tissue of a patient. The expression profiling technique may be combined with high throughput screening techniques to allow rapid identification of useful compounds and monitor the effectiveness of treatment with a drug candidate (see, for example, Zlokarnik, et al., Science 279, 84-8 (1998)). Drug candidates may be chemical compounds, nucleic acids, proteins, antibodies, or derivatives therefrom, whether naturally occurring or synthetically

derived. Drug candidates thus identified may be utilized, among other uses, as pharmaceutical compositions for administration to patients or for use in further screening assays.

[0052] In some embodiments, the binding agents are in purified form. A “purified” binding agent (e.g., antibody) may be one that is separated from at least about 50% of the proteins and / or other components with which it is initially found (e.g., as part of a hybridoma supernatant or ascites preparation in the case of a monoclonal antibody). A purified binding agent (e.g., antibody) may be one that is separated from at least about 50%, 60%, 75%, 90%, or 95% of the proteins and / or other components with which it is initially found.

[0053] The polypeptides and nucleic acids described herein may also be combined with one or more pharmaceutically acceptable carriers prior to administration to a host. A pharmaceutically acceptable carrier is a material that is not biologically or otherwise undesirable, e.g., the material may be administered to a subject, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. Suitable pharmaceutical carriers and their formulations are described in, for example, *Remington's: The Science and Practice of Pharmacy, 21st Edition*, David B. Troy, ed., Lippicott Williams & Wilkins (2005). Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carriers include, but are not limited to, sterile water, saline, buffered solutions like Ringer's solution, and dextrose solution. The pH of the solution is generally from about 5 to about 8 or from about 7 to about 7.5. Other carriers include sustained-release preparations such as semipermeable matrices of solid hydrophobic polymers containing polypeptides or fragments thereof. Matrices may be in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered. Carriers are those suitable for administration of polypeptides and / or fragments thereof to humans or other subjects. Pharmaceutical compositions may also include carriers, thickeners, diluents, buffers, preservatives, surface active agents, adjuvants, immunostimulants, in addition to the

immunogenic polypeptide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents and anesthetics. The pharmaceutical composition may be administered orally, parentally, by inhalation spray, rectally, intranodally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term “pharmaceutically acceptable carrier” or “physiologically acceptable carrier” as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of a nucleic acid, polypeptide, or peptide as a pharmaceutical composition. A “pharmaceutical composition” is a composition comprising a therapeutically effective amount of a nucleic acid or polypeptide. The terms “effective amount” and “therapeutically effective amount” each refer to the amount of a binding agent, nucleic acid or the like used to observe the desired therapeutic effect (e.g., eliminating HIV).

[0054] Methods for treating one or more disease conditions (e.g., HIV or cancer) in a mammalian host comprising administering to the mammal at least one or more effective doses of one or more binding agents (and / or derivative(s) thereof) described herein are also provided. In some embodiments, the binding agent is a monoclonal antibody or fragment or derivative thereof comprising one or more of SEQ ID NOS. 1-32 and / or shown in **Table 1**. The one or more binding agents may be administered in a dosage amount of about 1 to about 50 mg / kg, about 1 to about 30 mg / kg, or about 5 to about 30 mg / kg (e.g., about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, or 40 mg / kg). In certain embodiments, the one or more binding agents may be administered to the mammal (e.g., intradermally, intravenously, orally, rectally) at about 10 mg / kg one or more times. When multiple doses are administered, the doses may comprise about the same or different amount of binding agent in each dose. The doses may also be separated in time from one another by the same or different intervals. For instance, the doses may be separated by about any of 6, 12, 24, 36, 48, 60, 72, 84, or 96 hours, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, 10 months, 11 months, 12 months, 1.5 years, 2 years, 3 years, 4 years, 5 years, or any time period before, after, and / or between any of these time periods. In some embodiments, the binding agents may be administered in conjunction with other agents (e.g., anti-infective agents and/or chemotherapeutic agent). Such other agents may be administered about simultaneously with the binding agents, or at a different time and / or frequency. Other embodiments of such methods may also be

appropriate as could be readily determined by one of ordinary skill in the art.

[0055] To assist the skilled artisan in using the binding agents such as antibodies described herein, the same may be provided in kit format. A kit including one or more of such binding agents and optionally other components necessary for using the same to detect cells expressing HIV is also provided. The binding agents of the kit may be provided in any suitable form, including frozen, lyophilized, or in a pharmaceutically acceptable buffer such as TBS or PBS. The kit may also include other reagents required for utilization of the binding agents *in vitro* or *in vivo* such as buffers (e.g., TBS, PBS), blocking agents (solutions including nonfat dry milk, normal sera, Tween-20 Detergent, BSA, or casein), and / or detection reagents (e.g., goat anti-mouse IgG biotin, streptavidin-HRP conjugates, allophycocyanin, B-phycoerythrin, R-phycoerythrin, peroxidase, detectable labels, and other labels and / or staining kits (e.g., ABC Staining Kit, Pierce)). The kits may also include other reagents and / or instructions for using the antibodies in commonly utilized assays described above such as, for example, flow cytometric analysis, ELISA, immunoblotting (e.g., western blot), *in situ* detection, immunocytochemistry, immunohistochemistry. In one embodiment, the kit provides a binding agent in purified form. In another embodiment, the binding agent may be provided in biotinylated form either alone or along with an avidin-conjugated detection reagent (e.g., antibody). In another embodiment, the kit includes a binding agents comprising one or more detectable labels that may be used to directly detect HIV. Buffers and the like required for using any of these systems are well-known in the art and / or may be prepared by the end-user or provided as a component of the kit. The kit may also include a solid support containing positive- and negative-control protein and / or tissue samples. For example, kits for performing spotting or western blot-type assays may include control cell or tissue lysates for use in SDS-PAGE or nylon or other membranes containing pre-fixed control samples with additional space for experimental samples. Kits for visualization of HIV in cells on slides may include pre-formatted slides containing control cell or tissue samples with additional space for experimental samples. Other embodiments of kits are also contemplated herein as would be understood by those of ordinary skill in the art.

[0056] Thus, this disclosure provides binding agents such as the LN01 antibody with specificity for HIV. In some embodiments, the binding agent is a polypeptide comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOS. 1-32 and / or shown in **Table 1**. In some embodiments, the binding agent is a polypeptide comprising

one or more combinations of SEQ ID NOS. 1-32. In some embodiments, the binding agent is an antibody. In some embodiments, the binding agent is a polypeptide such as an antibody comprising a heavy chain CDR amino acid sequence selected from the group consisting of SEQ ID NOS. 1-3. In some embodiments, the binding agent is a polypeptide such as an antibody comprising a light chain CDR amino acid sequence selected from the group consisting of SEQ ID NOS. 4-7. In some embodiments, the binding agent is a polypeptide such as an antibody comprising a V_H amino acid sequence of SEQ ID NO. 7 (LN01), SEQ ID NO. 9 (LN01 variant 7), SEQ ID NO. 11 (LN01 variant 8), SEQ ID NO. 13 (LN01 variant 38), SEQ ID NO. 15 (LN01 variant 41), SEQ ID NO. 17 (LN01 variant 42), SEQ ID NO. 19 (LN01 variant 48), SEQ ID NO. 21 (LN01 variant 49), or SEQ ID NO. 23 (LN01 variant 82). In some embodiments, the binding agent is a polypeptide such as an antibody comprising a V_L amino acid sequence of SEQ ID NO. 8 (LN01), SEQ ID NO. 10 (LN01 variant 7), SEQ ID NO. 12 (LN01 variant 8), SEQ ID NO. 14 (LN01 variant 38), SEQ ID NO. 16 (LN01 variant 41), SEQ ID NO. 18 (LN01 variant 42), SEQ ID NO. 20 (LN01 variant 48), SEQ ID NO. 22 (LN01 variant 49), or SEQ ID NO. 28 (LN01 variant 24). In some embodiments, the binding agent comprises the combinations of CDRs (SEQ ID NOS. 1-6 or GNT (LN01 CDRL1)) and/or variable regions (SEQ ID NOS. 7 and 8; SEQ NOS. 9 and 10; SEQ NOS. 11 and 12; SEQ NOS. 13 and 14; SEQ NOS. 15 and 16; SEQ NOS. 17 and 18; SEQ NOS. 19 and 20; SEQ NOS. 21 and 22; or, SEQ ID NOS. 23 and 24; or a conservatively substituted variant thereof) shown in **Table 1**.

[0057] In some embodiments, the binding agents have specificity for an epitope comprising amino acid residues L679, W680 and K683 of SEQ ID NO. 68 and/or amino acid residues L168, W169 and K172 of SEQ ID NO. 69. In some embodiments, the binding agent exhibits the capacity to bind to and/or neutralize HIV expressing amino acid sequence ITKWLWYIK (SEQ ID NO. 66). In some embodiments, the binding agent exhibits the capacity to bind to and/or neutralize HIV expressing amino acid sequence ITKWLWYIK (SEQ ID NO. 66) but not LASWVKYIQ (SEQ ID NO. 65) and/or ITKWIKYIQ (SEQ ID NO. 67). In some embodiments, a binding agent of this disclosure may comprise any one or more of these binding specificities along with the neutralization characteristics described above (i.e., neutralization of HIV-1 pseudoviruses BJOX (CRF07_BC), CE1176, TRO.11 (B), X1632 (G), CH119 (CRF07_BC), CNE55 (CRF01_AE), 25710 (C), CD0217(C) but not of the control virus SVA-MLV at a concentration is from 10²-10⁰ ug/ml, or between 10⁰-10¹ ug/ml, to at least about 50%, and/or the ability to the neutralize a majority of the 118 HIV-1 pseudoviruses listed in **Figure 4** at an

IC₅₀ of less than 25). In some embodiments, nucleic acids encoding such binding agents are also provided as in **Table 4**.

[0058] In some embodiments, the binding agent is derived from or related to (e.g., by sequence or derivation) a human antibody, human IgG, human IgG1, human IgG2, human IgG2a, human IgG2b, human IgG3, human IgG4, human IgM, human IgA, human IgA1, human IgA2, human IgD, human IgE, canine antibody, canine IgGA, canine IgGB, canine IgGC, canine IgGD, chicken antibody, chicken IgA, chicken IgD, chicken IgE, chicken IgG, chicken IgM, chicken IgY, goat antibody, goat IgG, mouse antibody, mouse IgG, pig antibody, and / or rat antibody, and / or a derivative thereof. In some embodiments, the derivative may be selected from the group consisting of an F_{ab}, F_{ab2}, Fab' single chain antibody, F_v, single chain, mono-specific antibody, bispecific antibody, trimeric antibody, multi-specific antibody, multivalent antibody, chimeric antibody, canine-human chimeric antibody, canine-mouse chimeric antibody, antibody comprising a canine Fc, humanized antibody, human antibody, caninized antibody, CDR-grafted antibody, shark antibody, nanobody, and / or canelid antibody. In some embodiments, the binding agent comprises at least a first and second specificity, the first being against HIV gp41 and the second being against a different antigen (e.g., an antigen of an infectious agent such as HIV (e.g., env) and / or a tumor antigen). In some embodiments, the binding agent and / or derivative thereof may comprise a detectable label fixably attached thereto. In some embodiments, the binding agent of any one and / or derivative thereof comprises an effector moiety (e.g., a cytotoxic drug, toxin, diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin, and radiochemical) fixably attached thereto. In some embodiments, polynucleotides encoding one or more binding agents are also provided (e.g., as an expression vector). Host cells comprising and / or expressing the polypeptide products of such polynucleotides are also provided. In some embodiments, compositions comprising at least one binding agent or derivative; at least one isolated polynucleotide; at least one expression vector; and / or, at least one host cell; or a combination thereof; and, a pharmaceutically acceptable carrier are also provided.

[0059] This disclosure also provides methods for detecting HIV on a cell, the method comprising contacting a test biological sample with a binding agent or derivative described herein and detecting the binding agent bound to the biological sample or components thereof. Such methods may be an *in vivo* method or an *in vitro* method. In some embodiments, the

method may comprise comparing the amount of binding to the test biological sample or components thereof to the amount of binding to a control biological sample or components thereof, wherein increased binding to the test biological sample or components thereof relative to the control biological sample or components thereof indicates the presence of a cell expressing HIV polypeptides in the test biological sample (e.g., mammalian blood). In some embodiments, a kit for detecting the expression of HIV in or on a cell, the kit comprising a binding agent or derivative thereof and instructions for use. In some embodiments, the binding agent and / or derivative thereof is in lyophilized form. In some embodiments, this disclosure provides methods for treating, preventing and / or ameliorating an infectious disease, cancer and / or autoimmunity in a mammal comprising administering to the mammal at least one effective dose of a pharmaceutical composition comprising a binding agent or derivative thereof. In some embodiments, the infectious disease is human immunodeficiency virus (HIV). In some embodiments, multiple doses are administered to the animal. In some embodiments, the binding agent and / or derivative thereof may be administered in a dosage amount of about 1 to 50 mg / kg.

[0060] The terms “about”, “approximately”, and the like, when preceding a list of numerical values or range, refer to each individual value in the list or range independently as if each individual value in the list or range was immediately preceded by that term. The terms mean that the values to which the same refer are exactly, close to, or similar thereto.

[0061] As used herein, a subject or a host is meant to be an individual. The subject can include domesticated animals, such as cats and dogs, livestock (e.g., cattle, horses, pigs, sheep, and goats), laboratory animals (e.g., mice, rabbits, rats, guinea pigs) and birds. In one aspect, the subject is a mammal such as a primate or a human.

[0062] Optional or optionally means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase optionally the composition can comprise a combination means that the composition may comprise a combination of different molecules or may not include a combination such that the description includes both the combination and the absence of the combination (i.e., individual members of the combination).

[0063] Ranges may be expressed herein as from about one particular value, and/or to about another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent about or approximately, it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. Ranges (e.g., 90-100%) are meant to include the range *per se* as well as each independent value within the range as if each value was individually listed.

[0064] The term “combined” or “in combination” or “in conjunction” may refer to a physical combination of agents that are administered together or the use of two or more agents in a regimen (e.g., administered separately, physically and / or in time) for treating, preventing and / or ameliorating a particular disease.

[0065] When the terms treat, prevent, and / or ameliorate or derivatives thereof are used herein in connection with a given treatment for a given condition (e.g., preventing cancer infection by HIV), it is meant to convey that the treated patient either does not develop a clinically observable level of the condition at all, or develops it more slowly and/or to a lesser degree than he/she would have absent the treatment. These terms are not limited solely to a situation in which the patient experiences no aspect of the condition whatsoever. For example, a treatment will be said to have prevented the condition if it is given during exposure of a patient to a stimulus that would have been expected to produce a given manifestation of the condition, and results in the patient's experiencing fewer and/or milder symptoms of the condition than otherwise expected. For instance, a treatment can “prevent” infection by resulting in the patient's displaying only mild overt symptoms of the infection; it does not imply that there must have been no penetration of any cell by the infecting microorganism.

[0066] Similarly, reduce, reducing, and reduction as used herein in connection with prevention, treatment and / or amelioration of a given condition by a particular treatment typically refers to a subject developing an infection more slowly or to a lesser degree as compared to a control or basal level of developing an infection in the absence of a treatment (e.g., administration of one or more HIV binding agents). A reduction in the risk of infection

may result in the patient's displaying only mild overt symptoms of the infection or delayed symptoms of infection; it does not imply that there must have been no penetration of any cell by the infecting microorganism.

[0067] All references cited within this disclosure are hereby incorporated by reference in their entirety. Certain embodiments are further described in the following examples. These embodiments are provided as examples only and are not intended to limit the scope of the claims in any way.

EXAMPLES

Example 1

Lymph Node Donors

[0068] Selection of HIV-1 lymph node donors for the isolation of broadly neutralizing antibodies. In order to isolate broadly neutralizing antibodies capable to broadly neutralize multi-clade HIV-1 isolates 70 plasma samples from chronically infected patients naïve to antiretroviral therapy were screened for the presence of high titers of antibodies able to neutralize a panel of nine (9) HIV-1 pseudoviruses from the Global Panel of HIV-1 reference strains (DeCamp, A. et al. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol 88, 2489–2507 (2014)). This analysis resulted in the identification of seven (7) patients (**Figure 1**) as lymph node donors for the subsequent isolation and characterization of potent broadly neutralizing antibodies. In particular, donor SA003 was selected for the presence high level of antibodies found to neutralize eight (8) out of nine (9) isolates tested (and for the lack of background activity against the negative control MLV pseudovirus). Of note, SA003 donor is an Elite Controller with viremia <50 HIV RNA copies per ml of plasma (infected with clade B HIV-1).

Example 2

Isolation and characterization of a potent HIV-1 broadly neutralizing antibody

[0069] Germinal center and Memory IgG B cells from donor SA003 were sorted separately according to IgG (i.e. IgA and IgM negative cells), CD19 and CD38 expression (germinal center B cells are CD38 positive) (**Figure 2**) and interrogated for the production of HIV-1 neutralizing antibodies. In particular, highly pure IgG memory B cells and IgG germinal cells were seeded in separate plates as single cell micro-cultures on human feeder cells in the presence of Epstein-Barr Virus (EBV) (which also stimulate polyclonally memory B cells) and

a cocktail composed TLR9 agonist CpG-2006, IL-2 (1000 IU/ml), IL-6 (10 ng/ml), IL-21 (10 ng/ml), and anti-BCR goat antibodies (BCR triggering). Supernatants from day 14 cultures were then tested in a primary screening using a 384-well based HIV-1 pseudoviruses neutralization assay (using in parallel two strains, CE1176 and BJOX2000, representative of clade C and CRF07). Neutralization assays were undertaken on TZM-bl cells. In a 384-well plate, HIV-1 pseudoviruses that resulted in an output of 50-100 x 10⁴ relative light units (RLU) were incubated with B cell culture supernatants for 1 h at 37% (5% CO₂) before the addition of 3000 TZM-bl cells. These were incubated for a further 72 h, after which supernatant was removed and 15 µl Steadylite reagent (Perkin Elmer) was added. Luciferase activity was detected 5 min later by reading the plates on a Synergy microplate luminometer (BioTek). The supernatants derived from two cultures of germinal center B cells were found able to cross-neutralize both CE1176 and BJOX2000 strains. The supernatants from these two cultures were further harvested and tested for their ability to neutralize four (4) pseudoviruses (CE1176, BJOX2000, X1632 and 25710). Of note, one of the two supernatants neutralized all four pseudoviruses. The antibody derived from the neutralizing culture was characterized by determining the amino acid and nucleotide sequences of its variable regions (**Tables 1 and 5**) and the complementarity determining regions (CDRs) therein and termed "LN01". Accordingly, the binding agent termed "LN01" is an IgG3-type fully human monoclonal antibody having the CDR, VH and VL sequences as shown above in **Tables 1 and 2**. This antibody was derived fromIGHV4-39*07 and IGKV1-39*01 germline genes, and was highly somatically mutated in variable genes of both heavy chain (28%) and kappa light chain (27%) compared to germ line. The LN01 antibody also possessed a long heavy-chain complementarity-determining 3 region (CDR H3) loop composed of 20 amino acids. The LN01 VH and VL genes were cloned into IgG1 expression vectors, and the recombinant IgG1 LN01 antibody was produced by transfecting Expi293F cells. The full-length IgG1 LN01 antibody was then purified using a recombinant protein-A column (GE-Healthcare).

[0070] The recombinantly produced IgG1 LN01 antibody was then tested against the Global Panel of nine (9) HIV-1 reference pseudoviruses on TZM-bl cells. Strikingly, IgG1 LN01 antibody neutralized eight (8) out of nine (9) HIV-1 pseudoviruses with IC₅₀ values ranging from 0.03 to 1.6 µg/ml (**Figure 3**) and did not neutralize the negative control MLV pseudovirus. IgG1 LN01 antibody was subsequently tested on an extended panel of 118 HIV-1 pseudoviruses including clade A, clade B, clade C, clade D, clade G, circulating recombinant forms CRF10_CD, CRF01_AE, CRF02_AG and CRF07_BC and non-circulating

recombinants AC and ACD strains. IgG1 LN01 antibody broadly neutralized 109 viruses out of 118 with IC₅₀ below 25 µg/ml, i.e. the 92% of the tested viruses, with a median IC₅₀ of 1.1 µg/ml (**Figures 4 and 5**). The analysis of the viruses neutralized indicated that LN01 neutralizing activity is not clade-dependent.

Example 3

Effect of Fc Receptors on LN01 Neutralizing Activity

[0071] IgG1 LN01 antibody was tested against the Global Panel of nine (9) HIV-1 reference pseudoviruses on parental TZM-bl cells and TZM-bl cells expressing Fc-gamma receptor I (CD64) (Perez, L. G., Costa, M. R., Todd, C. A., Haynes, B. F. & Montefiori, D. C. Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. J Virol 83, 7397–7410 (2009)). Of note, the neutralizing activity of IgG1 LN01 antibody was enhanced 100-fold in TZM-bl cells expressing Fc-gamma receptor I (**Figure 6**). In addition, IgG1 LN01 antibody showed potent neutralization against the strain CE0217 using TZM-bl cells expressing Fc-gamma receptor I that was not neutralized by IgG1 LN01 antibody on TZM-bl cells (IC₅₀>25 µg/ml)). These results suggest that by prepositioning of IgG1 LN01 antibody at the cell surface, Fc-gamma receptors might give Abs a kinetic advantage for virus inhibition. This kinetic advantage could be unique to antibodies, whose epitopes are thought to be difficult to access or exposed for only a short time on intermediate conformations of the Env protein during an early stage of fusion. Another mechanism by which Fc-gamma receptors could potentially facilitate HIV-1 neutralization is phagocytosis. HeLa cells, from which the TZM-bl cell line was constructed, are known to exhibit properties of nonprofessional phagocytes. Thus, it is possible that TZM-bl cells were converted to professional phagocytic cells by introducing Fc-gamma receptor on their surface. Any Fc-gamma-receptor-mediated antiviral effects on HIV-1 neutralizing antibodies, whether by entry inhibition or phagocytosis, might be beneficial to several cell types in vaccine setting. Fc-gamma receptors are rarely expressed on CD4⁺ lymphocytes, several additional HIV-1-susceptible cell types express multiple Fc-gamma receptors and are involved in sexual transmission and the early establishment of long-lived viral reservoirs. In particular, macrophages are among the first infection-susceptible cells that the virus encounters after mucosal exposure, and they are thought to serve as a long-lived virus reservoir in chronic infection. Macrophages are well known to express multiple Fc-gamma receptors as well as certain subsets of monocytes and dendritic cells. It is also important to mention that Fc-gamma receptors play a role in regulating

adaptive immunity and peripheral tolerance, by facilitating antigen uptake, antigen presentation, cell activation and B cell tolerance.

Example 4

LN01 Specificity

[0072] In order to better define the specificity of IgG1 LN01 antibody we used a panel of HIV-2/HIV-1 chimeric pseudoviruses containing various segments of the HIV-1 MPER into the parental HIV-2/7312A. IgG1 LN01 antibody did not neutralize the parental HIV-2 7312A strain. Of note, IgG1 LN01 antibody was found to potently neutralize the chimeric virus 7312A.C4 in which only 6 residues from HIV-1 were replaced in the HIV-2 MPER region (LASWVKYIQ (SEQ ID NO. 65) was replaced into ITKWLWYIK (SEQ ID NO. 66)), but not the chimeric virus 7312A.C6 in which only 3 residues in the same region were replaced (LASWVKYIQ (SEQ ID NO. 65) was replaced into ITKWIKYIQ (SEQ ID NO. 67)) (**Figure 7**). A similar finding was obtained with the chimeric virus 7312A.C1C where the same 6 mutations of 7312A.C4 were combined with additional 7 mutations in the N-terminal region. These results indicate that residues in the C terminal region of the gp41 MPER (L679, W680 and K683) are involved in IgG1 LN01 antibody binding.

[0073] In order to better define the specificity of LN01 we used a peptide microarray formed by 1423 15-mer peptides, overlapping by 12 amino acids, that cover the full length of the consensus HIV-1 Env gp160 sequences for clades A, B, C, D, group M, CRF01_AE and CRF02_AG. The peptides were printed onto 3D-Epoxy glass slides and were analyzed with a GenePix 4000B scanner (Tomaras, G. D. et al. Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1-infected individuals. *J Virol* 85, 11502–11519 (2011)). LN01 was tested at 20 µg/ml in parallel with a control antibody called 7B2 (that is specific for the immunodominant region of gp41) for binding to the peptide microarray. The binding of LN01 and 7B2 was detected by incubation with DyLight 649-labeled goat anti-human IgG. Fluorescence intensity was measured using a GenePix 4000B scanner and was analyzed with GenePix software (**Figure 8**). Of note, IgG1 LN01 antibody did not clearly react with any of the peptides in this library, while 7B2 strongly reacted with 190-195 peptides that spanned the gp41 immunodominant region. These results indicate that IgG1 LN01 antibody does not recognize a linear epitope in HIV-1 Env.

[0074] IgG1 LN01 antibody was also tested for binding to soluble, cleaved SOSIP.664 gp140

trimers based on the subtype A transmitted/founder strain, BG505. These trimers are highly stable, homogenous and closely resemble native virus spikes when visualized by negative stain electron microscopy (EM) (Sanders, R. W. et al. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog. 9, e1003618 (2013)). All broadly neutralizing antibodies against multiple neutralizing epitopes on HIV-1 Env were highly reactive with the BG505 SOSIP.664 gp140 trimers, including quaternary epitopes antibodies (CH01, PG9, PG16 and PGT145). Conversely, non-NAbs to the CD4-binding site, CD4-induced epitopes or gp41 ectodomain did not react with the trimers, even when their epitopes were present on simpler forms of Env (e.g. gp120 monomers or dissociated gp41 subunits). The MPER was also deleted to improve trimer solubility and reduce aggregate formation. IgG1 LN01 antibody, PGT145 (V1-V2 glycan specific), PGT151 (binding to a site at the interface between gp120 and gp41) and 17b (binding to a CD4 binding induced site) antibodies were tested for binding to BG505 SOSIP.664 gp140 trimers in the presence or absence of soluble CD4 (sCD4) by surface plasmon resonance (SPR). PGT145 and PGT151 antibodies reacted strongly to BG505 SOSIP.664 gp140 trimers in the presence and absence of sCD4; 17b reacted to BG505 SOSIP.664 gp140 trimers only in the presence of sCD4. LN01 did not react with BG505 SOSIP.664 gp140 trimers neither in the presence nor in the absence of sCD4 (**Figure 9**).

[0075] IgG1 LN01 antibody, in parallel with MPER-specific 10E8 antibody (Huang, J. et al. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature 491, 406–412 (2012)), was also tested by ELISA against a panel of HIV-1 antigens (ConsB, consensus clade B gp140, 426c, clade C gp140, 426c-NLGS, 426c gp140 where the N-linked glycosylation sites were removed, 426c core, gp140 where the V loops were removed, UG37 gp140, clade A and gp41, recombinant ecto-domain of gp41, amino acids 541-682 from HxB2 strain, Vybion). None of the tested antigens was recognized by IgG1 LN01 antibody by ELISA (**Figure 10**). Conversely, 10E8 antibody reacted to the recombinant ecto-domain of gp41. These results indicate that IgG1 LN01 antibody may recognize an epitope in the MPER of gp41 different from 10E8.

[0076] Finally, IgG1 LN01 antibody was tested against a fusion intermediate gp41 and uncoated plates (PBS), called gp41int (Lai, R. P. J. et al. A fusion intermediate gp41 immunogen elicits neutralizing antibodies to HIV-1. J Biol Chem 289, 29912–29926 (2014)).

The gp41int antigen is recognized with high affinity by MPER antibodies 4E10, 2F5 and 10E8. While 10E8 reacted to gp41int by ELISA, IgG1 LN01 antibody did not react (**Figure 11**). These results indicate that IgG1 LN01 antibody recognizes a conserved epitope, possibly in the MPER region, that is not readily displayed in any of the antigens tested. We can hypothesize that IgG1 LN01 antibody recognizes its cognate conformational epitope in the C-terminal region of the MPER region when displayed in the prefusion native Env conformation.

Example 5

LN01 lack of high affinity binding to a MPE peptide

[0077] In order to better define the specificity of IgG1 LN01 antibody we tested its binding by ELISA to a 28 amino acids long peptide that was used to co-crystallize the MPER-specific antibody 10E8 (Huang et al. Nature 2012). This peptide encompasses the entire 28-residue gp41 MPER (residues 656-683) (sequence RRR-NEQELLELDKWASLWNWFDITNWLWYIRRRR (SEQ ID NO.:81)). While 10E8 reacted to this MPER peptide avidly, IgG1 LN01 antibody reacted to it very poorly (**Figure 12**). These results indicate that IgG1 LN01 antibody recognizes a conserved epitope, possibly in the MPER region, that is not readily displayed in a linear peptide encompassing the entire MPER gp41 region.

Example 6

LN01 variants with improved neutralizing activity

[0078] In order to improve the potency of LN01 antibody we produced a set of variants where we introduced point mutations in the LN01 VH (25 variants) or in VL (15 variants) (**Figure 13A**). These substitutions were selected based on a predicted surface exposure by replacing the original residues by W or A. Forty (40) LN01 variants were produced recombinantly by combining the mutated VH or VL with the parental VL or VH, respectively. These variants were tested against an initial panel of three HIV-1 strains (CH119, X1632; BJOX and a control virus SVA-MLV). Several LN01 variants lost neutralizing activity partially or completely (variants 13, 14, 21, 18, 19, 20, 22, 23, 24, 32 and 33), indicating an important role for the original residues in antigen recognition (in particular residues in the FR3 of the VH, in the CDR3 of the VH and in residues of the CDR1 of the VL). Of note, three variants showed a more than three-fold (3X) increase in neutralizing potency: variant 7 (D32W, mutation in the CDR1 of the VH), variant 8 (N33W in the CDR1 of the VH) and variant 38

(S67W in the FR3 of the VL) (**Figures 13B and 13C**). Remarkably, LN01 variant 7 showed 39-fold increase in potency as compared to the parental LN01 antibody. These results were confirmed when LN01 variants 7, 8 and 38 were tested in parallel with the parental LN01 antibody against a multiclade panel of 8 viruses (**Figures 14A-K**). In this second test the increased potency observed for LN01 variants 7, 8 and 38 was on average 47, 2.3 and 2.7 fold, respectively.

[0079] Based on these results additional LN01 variants (variants 41, 42, 43, 44, 48 and 50) were synthesized and tested against a panel of seven HIV-1 strains (**Figures 15A-B**). In variants (v) 41, 42, 43 and 44 the D32 residue of the CDR1 of the VH was mutated to either F (v41), Y (v42), L (v43) or I (v44). Of interest, none of these 4 mutations conferred the same increased in potency observed with the introduction of W in variant 7. In addition, only the introduction of aromatic residues (i.e., F or Y) improved LN01 activity, with Y (V42) conferring the most significant improvement of on average 3.7-fold (**Figure 15B**). The introduction of hydrophobic residues in the same position did not confer any benefit in terms of neutralizing activity. Two variants in which the somatic mutations in FR1 and FR4 were reverted to the germline configuration in the presence (variant 48) or absence (variant 50) of the D32W mutation used in variant 7 were also tested. The removal of somatic mutations in framework (FR) 1 and FR4 of the VH did not alter LN01 neutralizing activity significantly. The introduction of the D32W on the backbone of variant 50 conferred a more potent neutralizing activity (on average 4.7 fold better than the parental LN01 antibody). It is worth noting, however, that D32W in the context of germlined FRs in the VH did not achieve the same level of potency improvement observed with the same mutation on the backbone of the fully mutated parental LN01 antibody (i.e., variant 7).

[0080] The combination of the mutations introduced in variants 7 and 8 (D32W and N33W) was also tested in a new variant called 49 that showed an average 81-fold higher potency as compared to the parental LN01 antibody (**Figures 16A-B**) on a panel of 7 viruses. This result indicate that LN01 variant 49 is comparable or superior to the highly potent LN01 variant 7 antibody.

[0081] Finally, we tested another LN01 variant in which the five CDR1 somatic mutations were reverted back to the germline configuration. The mutated CDR1 sequence DSVSNDNYY (SEQ ID NO.:82; including the underlined five CDR1 somatic mutations) was

reverted to GSISSSSYY (SEQ ID NO.:32; germline CDR1) to generate the LN01 variant 82. Surprisingly, LN01 variant 82 showed a potency 2.9-fold higher than that of the parental LN01 antibody (**Figure 17**). This result indicates that VH CDR1 plays an important role in LN01 neutralizing activity.

[0082] While certain embodiments have been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the following claims.

AMENDED CLAIMS
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1. A binding agent that neutralizes HIV in an in vitro HIV neutralization assay and/or in vivo, the binding agent comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOS. 1-32 and / or shown in Table 1.
2. The binding agent of claim 1 comprising at least one amino acid sequence selected from the group consisting of SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28, SEQ ID NO. 29, SEQ ID NO. 30, SEQ ID NO. 31, and SEQ ID NO. 32.
3. The binding agent of claim 1 comprising:
 - at least one of SEQ ID NOS. 1-6 or GNT, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 1-8, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 9 and 10, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 11 and 12, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 13 and 14, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 15 and 16, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 17 and 18, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 19 and 20, or a conservatively substituted variant thereof;
 - SEQ ID NOS. 21 and 22, or a conservatively substituted variant thereof; and,
 - SEQ ID NOS. 23 and 24, or a conservatively substituted variant thereof.
4. The binding agent of any one of claims 1-3 that does not comprise:
 - a variable heavy chain region comprising SEQ ID NO. 70 or 71;
 - tryptophan (W) at an amino acid corresponding to amino acid 22 of SEQ ID NO. 70;
 - tryptophan (W) at an amino acid corresponding to amino acid 23 of SEQ ID NO. 71;
 - a CDRH3 amino acid sequence of any of SEQ ID NOS. 72-78;
 - alanine (A) at an amino acid corresponding to amino acid 8 of SEQ ID NO. 72;
 - alanine (A) at an amino acid corresponding to amino acid 9 of SEQ ID NO. 73;
 - tryptophan (W) at an amino acid corresponding to amino acid 10 of SEQ ID NO. 74;

tryptophan (W) at an amino acid corresponding to amino acid 11 of SEQ ID NO. 75;

tryptophan (W) at an amino acid corresponding to amino acid 12 of SEQ ID NO. 76;

tryptophan (W) at an amino acid corresponding to amino acid 14 of SEQ ID NO. 77;

tryptophan (W) at an amino acid corresponding to amino acid 15 of SEQ ID NO. 78;

a variable light chain region comprising SEQ ID NO. 79 or 80;

tryptophan (W) at an amino acid corresponding to amino acid 5 of SEQ ID NO. 79; and/or,

tryptophan (W) at an amino acid corresponding to amino acid 6 of SEQ ID NO. 80.

5. The binding agent of any one of claims 1-4 wherein the binding agent exhibits binding specificity for amino acid residues L679, W680 and K683 of SEQ ID NO. 68, and/or amino acid residues L168, W169 and K172 of SEQ ID NO. 69.
6. The binding agent of any one of claims 1-4 wherein the binding agent exhibits binding specificity for ITKWLWYIK (SEQ ID NO. 66).
7. The binding agent of any one of claims 1-4 wherein the binding agent exhibits binding specificity for ITKWLWYIK (SEQ ID NO. 66) but not LASWVKYIQ (SEQ ID NO. 65) and/or ITKWIKYIQ (SEQ ID NO. 67).
8. The binding agent of any one of claims 1-4 wherein the binding agent exhibits neutralization of HIV-1 pseudoviruses BJOX (CRF07_BC), CE1176, TRO.11 (B), X1632 (G), CH119 (CRF07_BC), CNE55 (CRF01_AE), 25710 (C), CD0217(C) but not of the control virus SVA-MLV at a concentration is from 10^2 - 10^0 ug/ml, or between 10^0 - 10^1 μ g/ml.
9. The binding agent of claim 8 wherein the percent neutralization is at least about 50%.
10. The binding agent of any one of claims 1-9 wherein the binding agent neutralizes a majority of the 118 HIV-1 pseudoviruses listed in **Figure 4** at an IC_{50} of less than 25 μ g/ml.
11. The binding agent of any one of claims 1-10 that is an isolated monoclonal antibody.
12. The binding agent of claim 11 wherein the monoclonal antibody is a human monoclonal antibody.
13. The binding agent of claim 11 or 12 wherein the antibody isotype is IgG1 or IgG3.

14. The binding agent of claim 1 comprising at least one heavy chain CDR amino acid sequence selected from the group consisting of SEQ ID NOS. 1-3.
15. The binding agent of claim 1 comprising at least one light chain CDR amino acid sequence selected from the group consisting of SEQ ID NOS. 4-6 or GNT.
16. The binding agent of claim 1 comprising at least one heavy chain variable amino acid sequence selected from the group consisting of SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, and SEQ ID NO. 23.
17. The binding agent of claim 1 comprising at least one light chain variable amino acid sequence selected from the group consisting of SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, and SEQ ID NO. 24.
18. The binding agent of any one of claims 1-17 comprising a conservative substitution of an amino acid residue of any one or more of SEQ ID NOS. 1-32.
19. The binding agent of any one of claims 1-18 derived from a human antibody, human IgG, human IgG1, human IgG2, human IgG2a, human IgG2b, human IgG3, human IgG4, human IgM, human IgA, human IgA1, human IgA2, human IgD, human IgE, canine antibody, canine IgGA, canine IgGB, canine IgGC, canine IgGD, chicken antibody, chicken IgA, chicken IgD, chicken IgE, chicken IgG, chicken IgM, chicken IgY, goat antibody, goat IgG, mouse antibody, mouse IgG, pig antibody, and rat antibody.
20. A derivative of a binding agent of any one of claims 1-19.
21. The derivative of claim 21 selected from the group consisting of an F_{ab}, F_{ab2}, Fab' single chain antibody, F_v, single chain, mono-specific antibody, bispecific antibody, trimeric antibody, multi-specific antibody, multivalent antibody, chimeric antibody, canine-human chimeric antibody, canine-mouse chimeric antibody, antibody comprising a canine Fc, humanized antibody, human antibody, caninized antibody, CDR-grafted antibody, shark antibody, nanobody, and canelid antibody.
22. The binding agent or derivative of any one of claims 1-21 comprising at least a first and second specificity, the first being against gp41 and the second being against a different antigen.
23. The binding agent of any one of claims 1-22 or derivative thereof comprising a detectable label fixably attached thereto.

24. The binding agent of claim 23 wherein the detectable label is selected from the group consisting of fluorescein, DyLight, Cy3, Cy5, FITC, HiLyte Fluor 555, HiLyte Fluor 647, 5-carboxy-2,7-dichlorofluorescein, 5-carboxyfluorescein, 5-FAM, hydroxy tryptamine, 5-hydroxy tryptamine (5-HAT), 6-carboxyfluorescein (6-FAM), FITC, 6-carboxy-1,4-dichloro-2',7'-dichlorofluorescein (TET), 6-carboxy-1,4-dichloro-2',4',5',7'-tetra-chlorofluorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (6-JOE), an Alexa fluor, Alexa fluor 350, Alexa fluor 405, Alexa fluor 430, Alexa fluor 488, Alexa fluor 500, Alexa fluor 514, Alexa fluor 532, Alexa fluor 546, Alexa fluor 555, Alexa fluor 568, Alexa fluor 594, Alexa fluor 610, Alexa fluor 633, Alexa fluor 635, Alexa fluor 647, Alexa fluor 660, Alexa fluor 680, Alexa fluor 700, Alexa fluor 750, a BODIPY fluorophores, BODIPY 492/515, BODIPY 493/503, BODIPY 500/510, BODIPY 505/515, BODIPY 530/550, BODIPY 542/563, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650-X, BODIPY 650/665-X, BODIPY 665/676, FL, FL ATP, FI-Ceramide, R6G SE, TMR, TMR-X conjugate, TMR-X, SE, TR, TR ATP, TR-X SE, a rhodamine, rhodamine 110, rhodamine 123, rhodamine B, rhodamine B 200, rhodamine BB, rhodamine BG, rhodamine B extra, 5-carboxytetramethylrhodamine (5-TAMRA), 5 GLD, 6-carboxyrhodamine 6G, Lissamine, Lissamine Rhodamine B, Phalloidine, Phalloidine, rhodamine red, Rhod-2, 6-carboxy-X-rhodamine (ROX), carboxy-X-rhodamine (5-ROX), Sulphorhodamine B can C, Sulphorhodamine G Extra, 6-carboxytetramethylrhodamine (TAMRA), tetramethylrhodamine (TRITC), rhodamine WT, Texas Red, and Texas Red-X.
25. The binding agent of any one of claims 1-22 or derivative thereof comprising an effector moiety fixably attached thereto.
26. The binding agent or derivative of claim 25 wherein the effector moiety is selected from the group consisting of a cytotoxic drug, toxin, diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin, and radiochemical.
27. An isolated polynucleotide encoding a binding agent of any one of claims 1-22.
28. The isolate polynucleotide of claim 24 comprising a nucleic acid sequence of at least one of SEQ ID NOS. 37-72.
29. An expression vector comprising one or more polynucleotides of claim 27 or 28.
30. A host cell comprising the isolated polynucleotide of claim 27 or 28 and / or the expression vector of claim 30.
31. A composition comprising at least one binding agent or derivative of any one of claims 1-26; at least one isolated polynucleotide of claim 27 or 28; or at least one expression

vector of claim 29; and / or, at least one host cell of claim 30; or a combination thereof; and, a pharmaceutically acceptable carrier.

32. A method for detecting HIV on a cell, the method comprising contacting a test biological sample with a binding agent or derivative of any one of claims 1-26 and detecting the binding agent bound to the biological sample or components thereof.
33. The method of claim 32, further comprising comparing the amount of binding to the test biological sample or components thereof to the amount of binding to a control biological sample or components thereof, wherein increased binding to the test biological sample or components thereof relative to the control biological sample or components thereof indicates the presence of a cell expressing HIV in the test biological sample.
34. The method of claim 32 or 33 wherein the test biological sample is mammalian blood.
35. The method of any one of claims 32-34 wherein the method is an *in vivo* method.
36. The method of any one of claims 32-34 wherein the method is an *in vitro* method.
37. A method for treating, preventing and / or ameliorating HIV infection and/or AIDS in a mammal comprising administering to the mammal at least one effective dose of a pharmaceutical composition comprising a binding agent or derivative of any one of claims 1-26.
38. The method of claim 37 wherein multiple doses are administered to the animal.
39. The method of claim 37 or 38 wherein the binding agent is administered in a dosage amount of about 1 to 50 mg / kg.
40. A kit for detecting the expression of HIV in or on a cell, the kit comprising a binding agent or derivative of any one of claims 1-26 and instructions for use.
41. The kit of claim 40 wherein the binding agent, antibody, or derivative is in lyophilized form.

FIGURE 1A-B

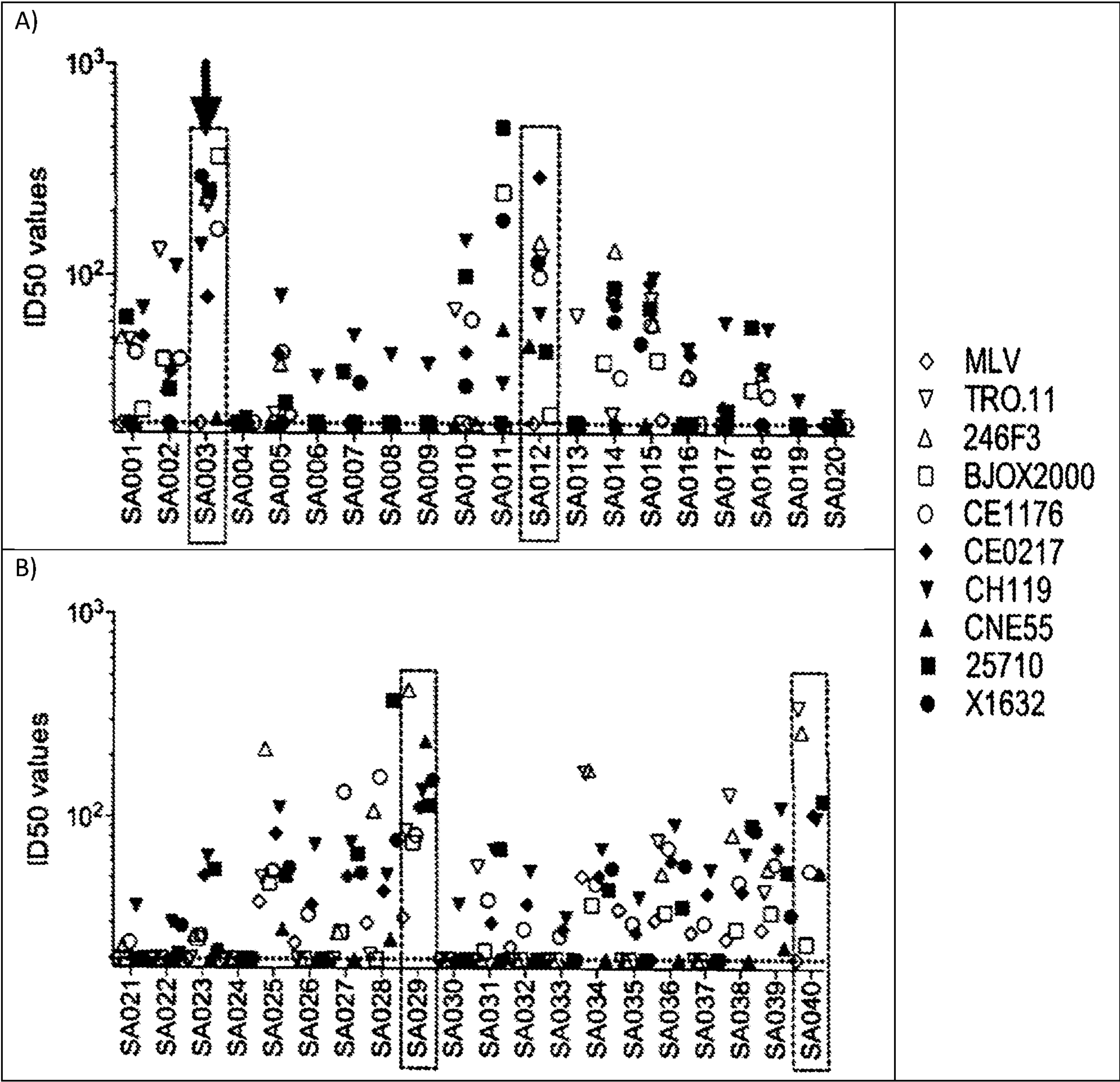


FIGURE 1C-D

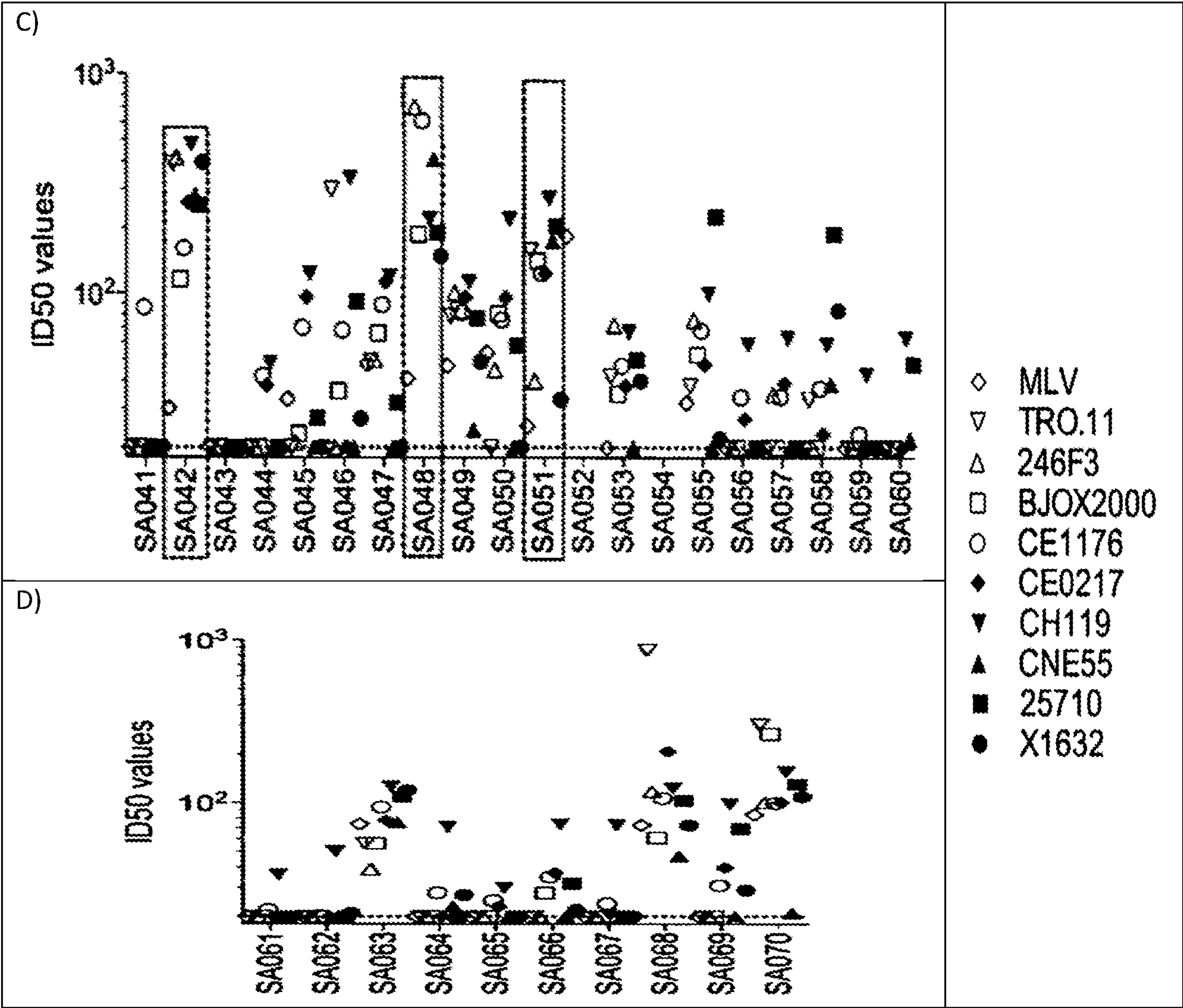


FIGURE 2

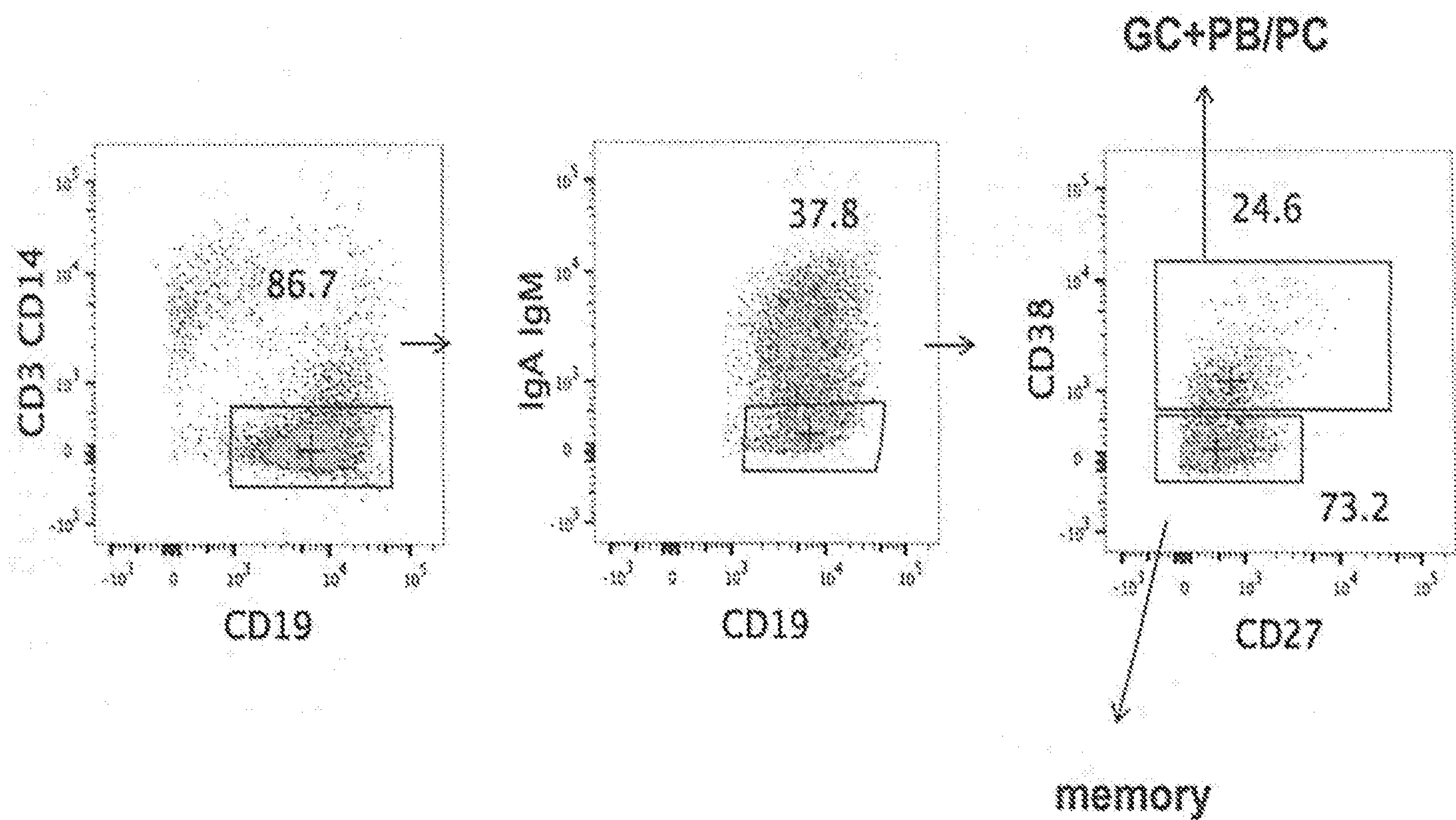


FIGURE 3

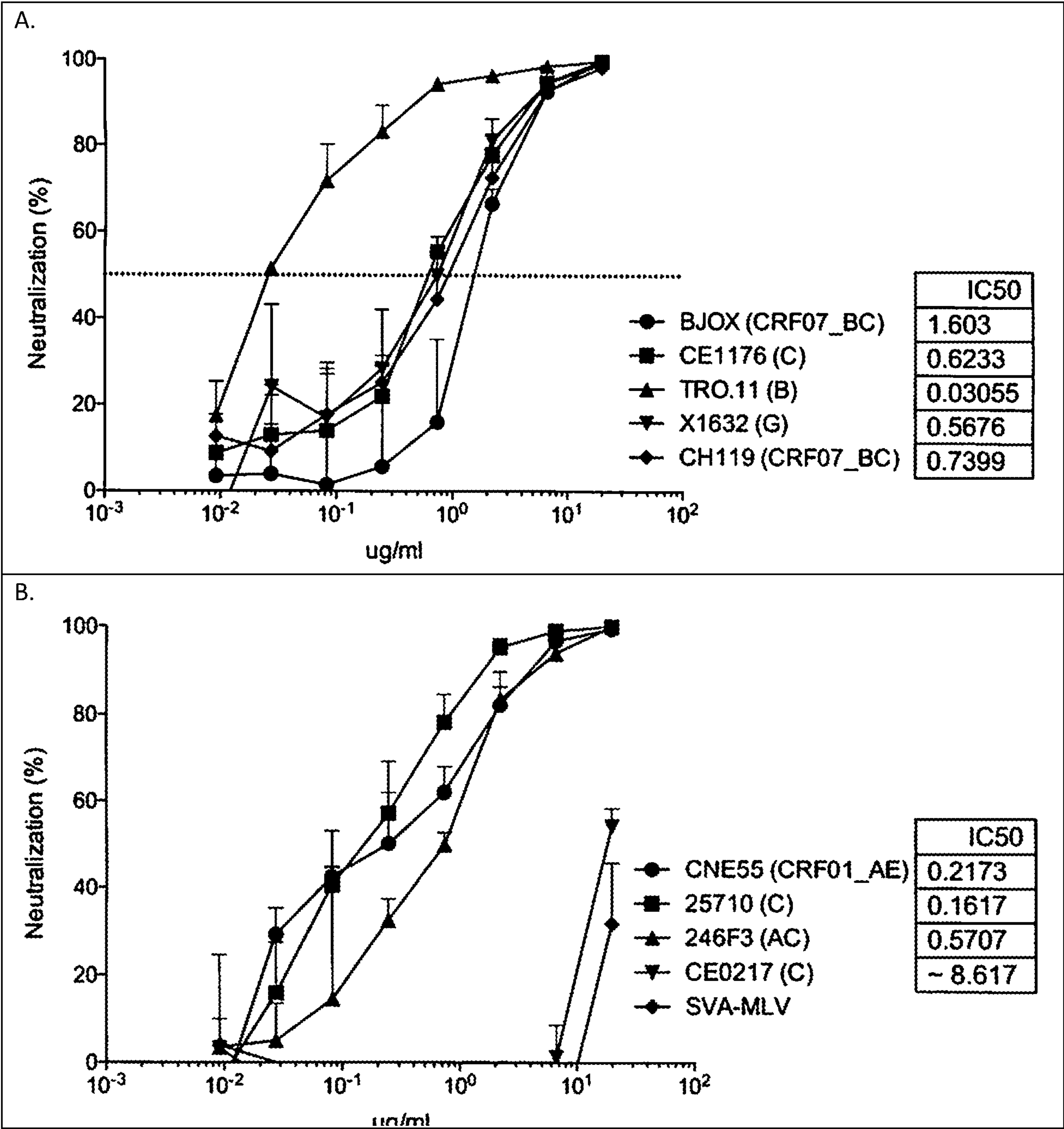


FIGURE 4

Virus ID	Clade*	IC50
B535.3	B	1.031
QH0892.42	B	0.515
SC422661.8	B	0.292
PVO.4	B	0.403
TRO.11	B	0.185
AC10.029	B	1.894
RHPA4259.7	B	10.308
THRO4156.18	B	9.831
REJO4541.87	B	0.281
TRJO4551.58	B	8.416
WITO4160.33	B	0.224
CAAN5342.A2	B	2.242
WEAU.415.410.5017	B (TF)	2.471
1006.11.C3.1601	B (TF)	0.548
1054.07.TC4.1498	B (TF)	0.092
1056.10.TA11.1826	B (TF)	0.060
1012.11.TC21.3257	B (TF)	0.641
6240.08.TA5.4622	B (TF)	1.978
6244.13.B5.4576	B (TF)	0.088
62357.14.D3.4589	B (TF)	5.043
SC05.AC11.2344	B (TF)	0.710
Du156.12	C	0.146
Du172.17	C	0.059
Du422.1	C	0.929
ZM197MPB7	C	0.127
ZM214MPL.15	C	0.840
ZM233MPB6	C	0.673
ZM249MPL.1	C	0.944
ZM53MPB12	C	11.114
ZM109FPB4	C	0.618
ZM135MPL.10a	C	0.710
CAP45.2.00.G3	C	1.878
CAP210.2.00.E8	C	>25
HN-001428-2.42	C	24.889
HN-0013085-2.11	C	0.028
HN-16055-2.3	C	0.174
HN-16845-2.22	C	0.040
Ce1086.B2	C (TF)	0.328
Ce0393.C3	C (TF)	>25
Ce1176.A3	C (TF)	1.092
Ce2010.F5	C (TF)	11.611
Ce0682.F4	C (TF)	0.628
Ce1172.H1	C (TF)	0.070
Ce2080.G9	C (TF)	4.050
Ce703010054.2A2	C (TF)	1.703
BE1268.431a	C (TF)	>25
246F.C1G	C (TF)	4.894
249M.B10	C (TF)	2.769
ZM247v1 (Rev.)	C (TF)	0.501
7030102001E5 (Rev.)	C (TF)	4.385
1394C9G1 (Rev.)	C (TF)	5.864
Ce704809221.1B3	C (TF)	3.078
3016.v5.c45	D	0.250
A07412M1.vrc12	D	0.326
231965.c01	D	8.503
231966.c02	D	0.948
191821.E6.1	D (TF)	1.239

Virus ID	Clade*	IC50
CNE19	BC	1.208
CNE20	BC	0.018
CNE21	BC	0.053
CNE17	BC	1.112
CNE30	BC	0.597
CNE52	BC	>25
CNE53	BC	0.321
CNE58	BC	0.297
MS208A1	A	>25
Q23.17	A	5.513
Q461.a2	A	2.829
Q769.d22	A	6.420
Q259.d2.17	A	8.498
Q842.d12	A	7.934
Q330.v4.c3	A	8.243
Q260.v5.c36	A	>25
191955.A11	A (TF)	1.668
191084.B7-19	A (TF)	2.614
9004SS.A3.4	A (TF)	0.409
T257-31	CRF02_AG	21.964
928-28	CRF02_AG	0.086
263-8	CRF02_AG	0.355
T250-4	CRF02_AG	2.202
T251-18	CRF02_AG	>25
T278-50	CRF02_AG	5.102
T255-34	CRF02_AG	0.277
211-9	CRF02_AG	0.583
235-47	CRF02_AG	0.674
620345.c01	CRF01_AE	1.547
CNE8	CRF01_AE	0.028
C1080.c03	CRF01_AE	0.124
R2184.c04	CRF01_AE	0.890
R1166.c01	CRF01_AE	0.803
R3265.c06	CRF01_AE	3.139
C2101.c01	CRF01_AE	0.739
C3347.c11	CRF01_AE	0.901
C4118.c09	CRF01_AE	2.022
CNE5	CRF01_AE	4.345
BJOX009000.024	CRF01_AE	1.212
BJOX015000.115	CRF01_AE (TF)	0.529
BJOX010000.062	CRF01_AE (TF)	0.353
BJOX025000.01.1	CRF01_AE (TF)	0.186
BJOX028000.10.3	CRF01_AE (TF)	1.097
X1193.c1	G	0.851
F0402.c2.11	G	3.836
X1254.c3	G	3.005
X2088.c9	G	>25
X2131.C1.B5	G	0.229
P1981.C5.3	G	0.115
X1632.S2.B10	G	1.846
9817.v2.c59	CD	14.639
6480.v4.c25	CD	6.271
6952.v1.c20	CD	0.168
6811.v7.c18	CD	5.002
89-F1.2.25	CD	3.043
3301.v1.c24	AC	7.543
6041.v3.c23	AC	1.720
6540.v4.c1	AC	3.885
6545.v4.c1	AC	2.977
0815.v3.c3	ACD	0.384
3103.v3.c10	ACD	>25

FIGURE 5A-F

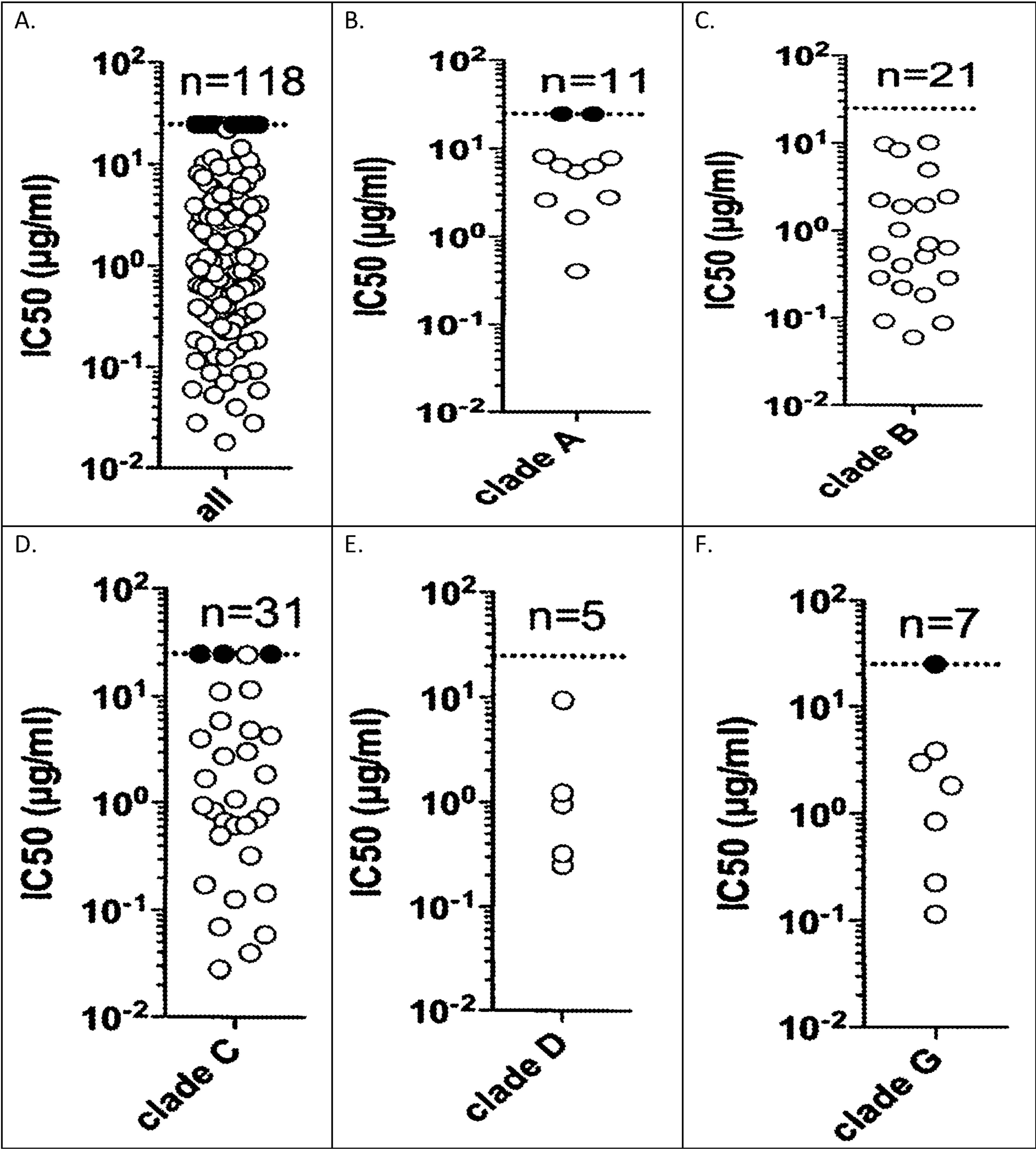


FIGURE 5G-J

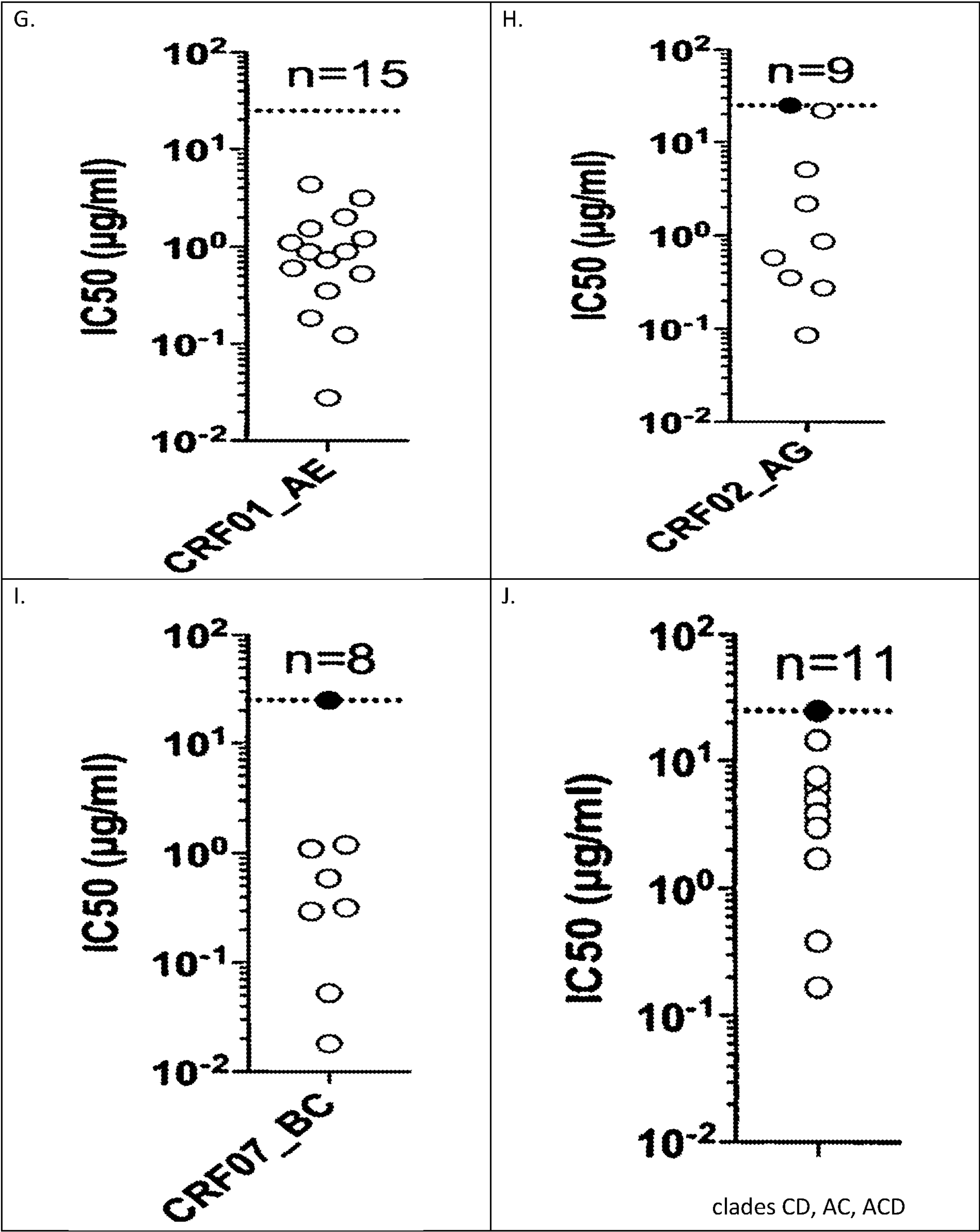


FIGURE 6

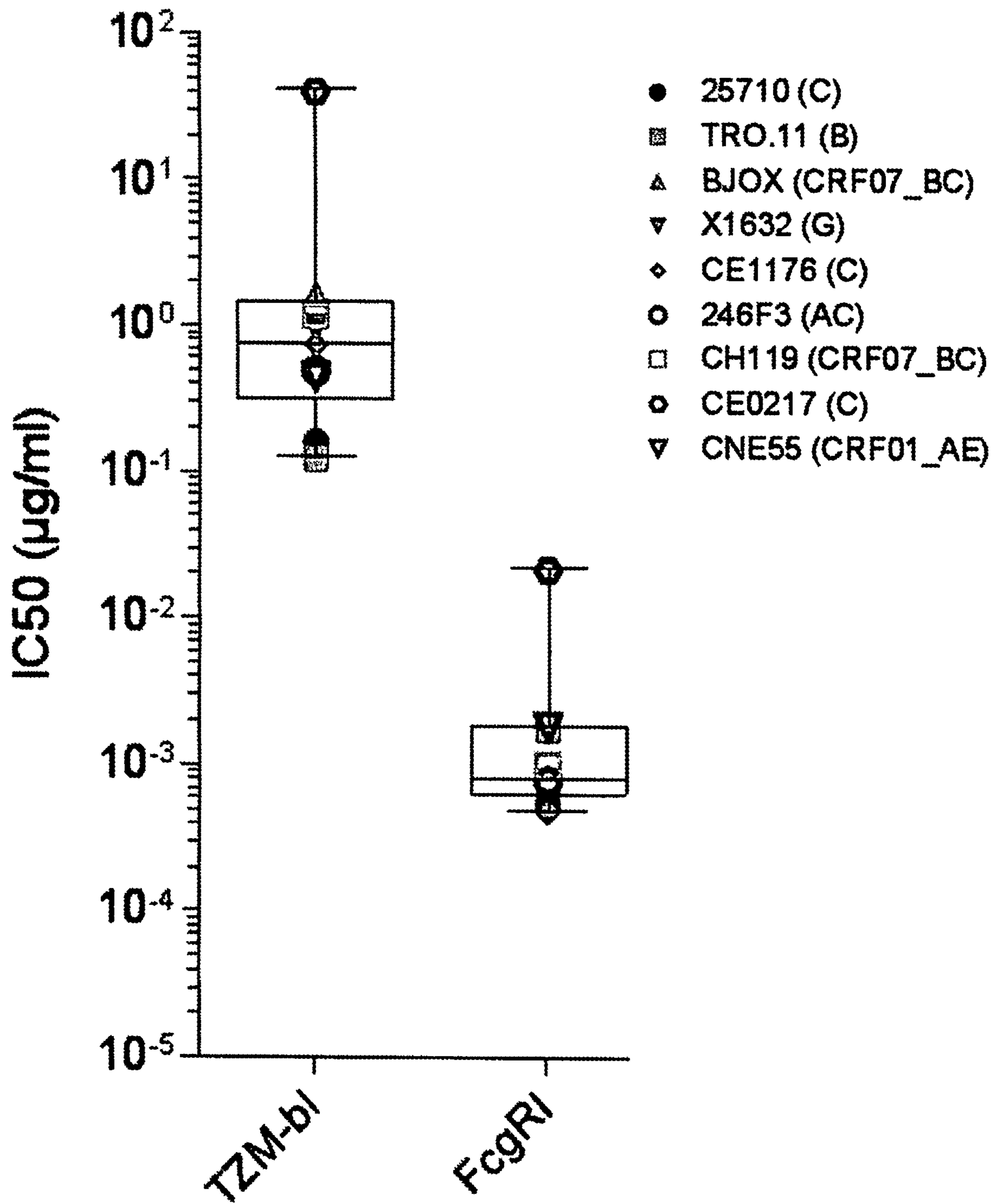


FIGURE 7

IC50 (ug/ml) in TZM-bl cells		656-	661-	666-	671-	676-	681-	686-
Virus	LN01							
Clade B HIV-1		NEQELLALDKWASLWNWFDITKWLWYIKIFIMIV						
HIV-2/7312A	>25	NMYELQKLNSWDVFGNWFDLASWVKYIQYGVYIV						
HIV-2/7312A.C1C	<0.011	-----LA-D--KNLW-----ITK-LW--K-----						
HIV-2/7312A.C3	>25	-----LA-DK-ASLW-----						
HIV-2/7312A.C4	<0.011	-----ITK-LW--K-----						
HIV-2/7312A.C6	>25	-----IT--I-----						
HIV-2/7312A.C7	>25	-----A-DKWA-----						
HIV-2/7312A.C8	<0.011	-----SLW-----ITK-LW--K-----						

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FIGURE 8A-B

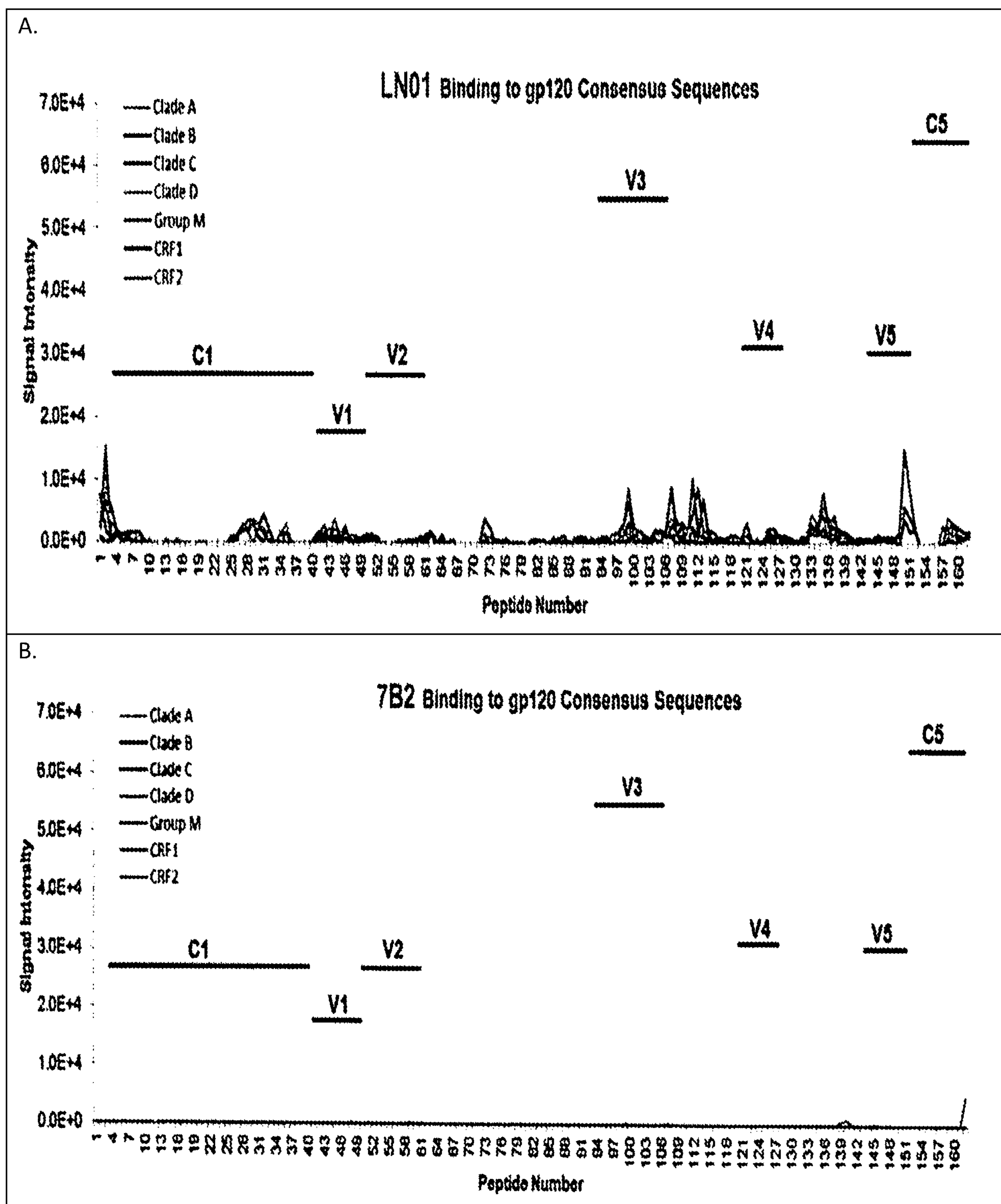


FIGURE 8C-D

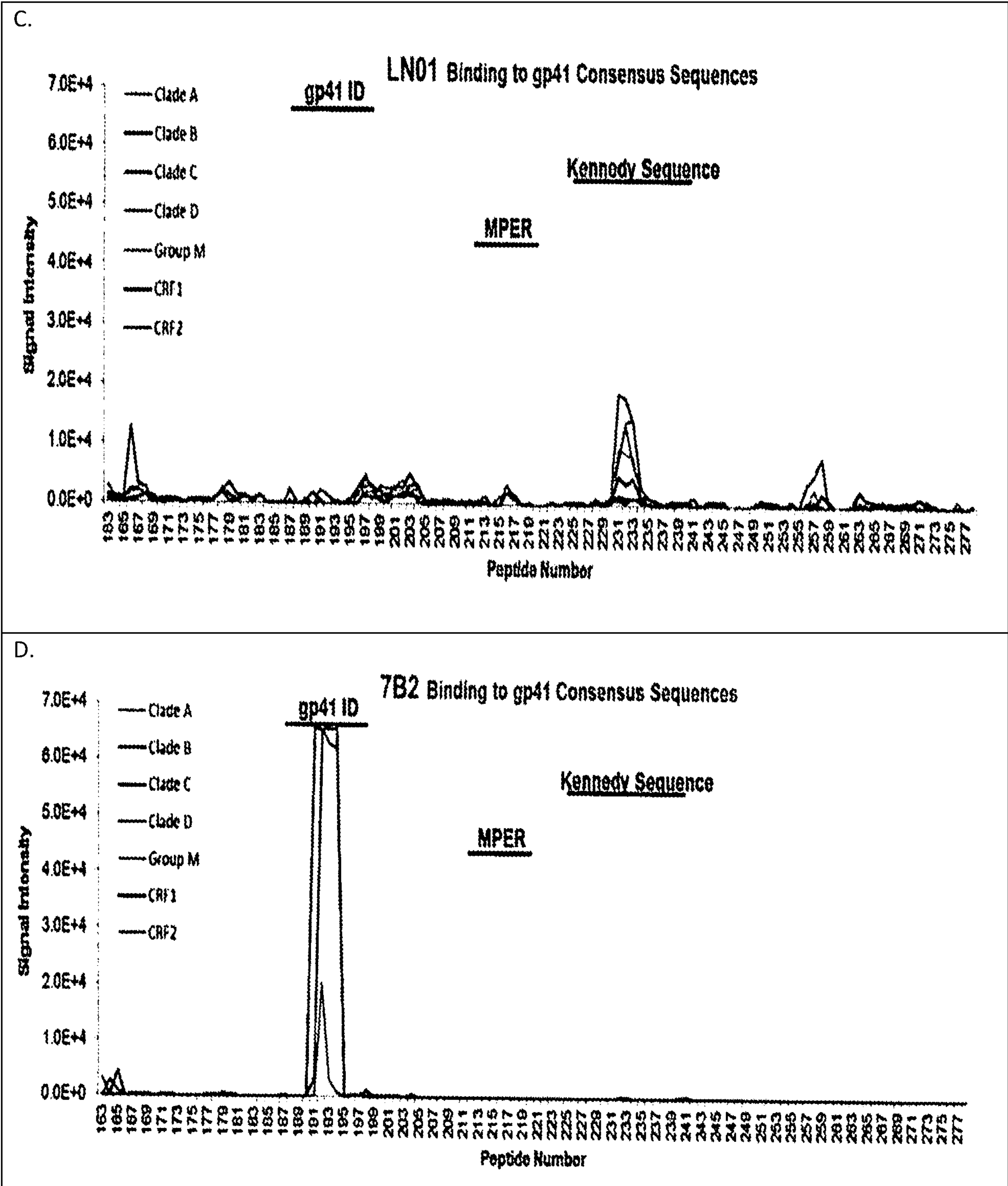


FIGURE 9A-B

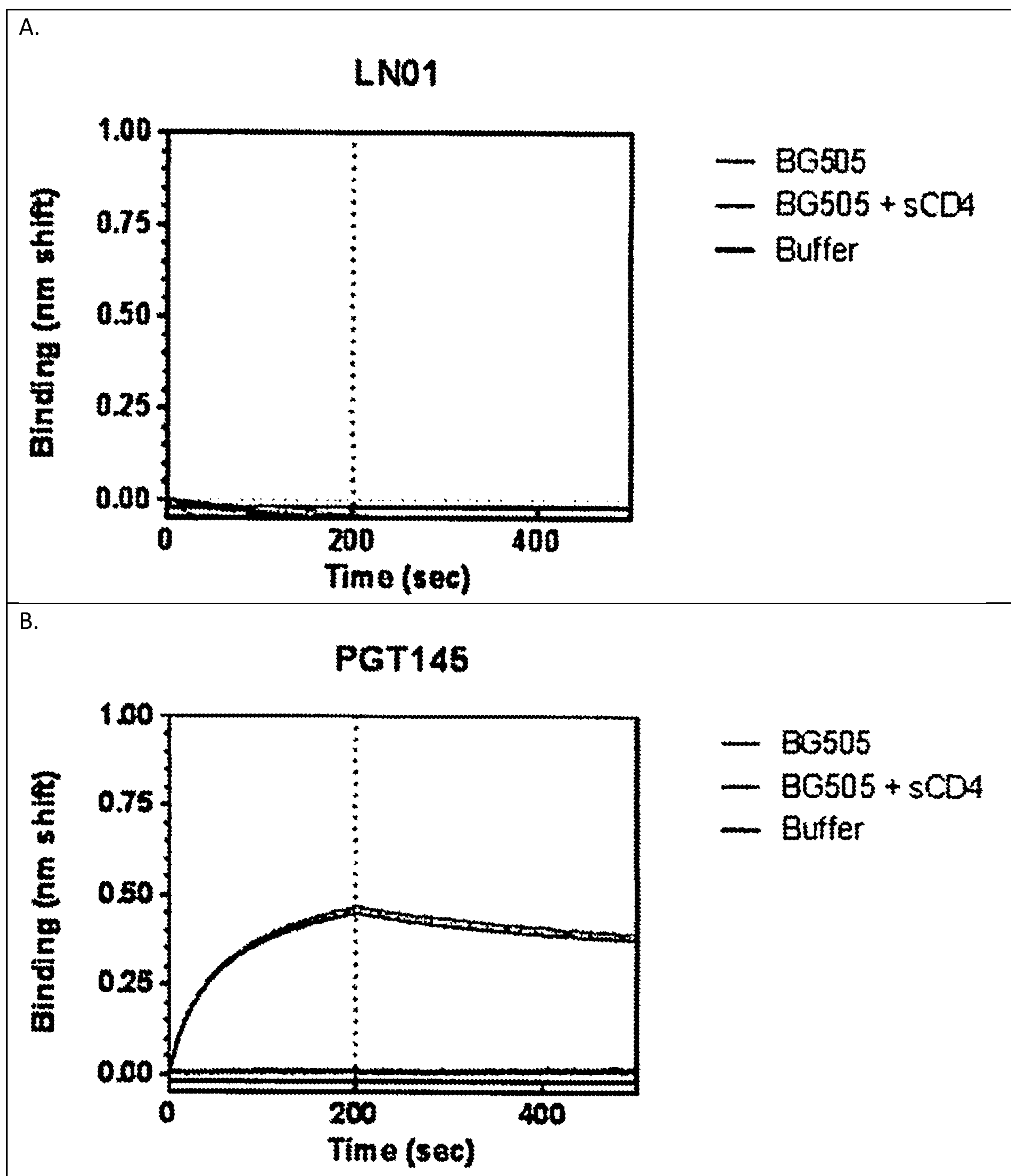


FIGURE 9C-D

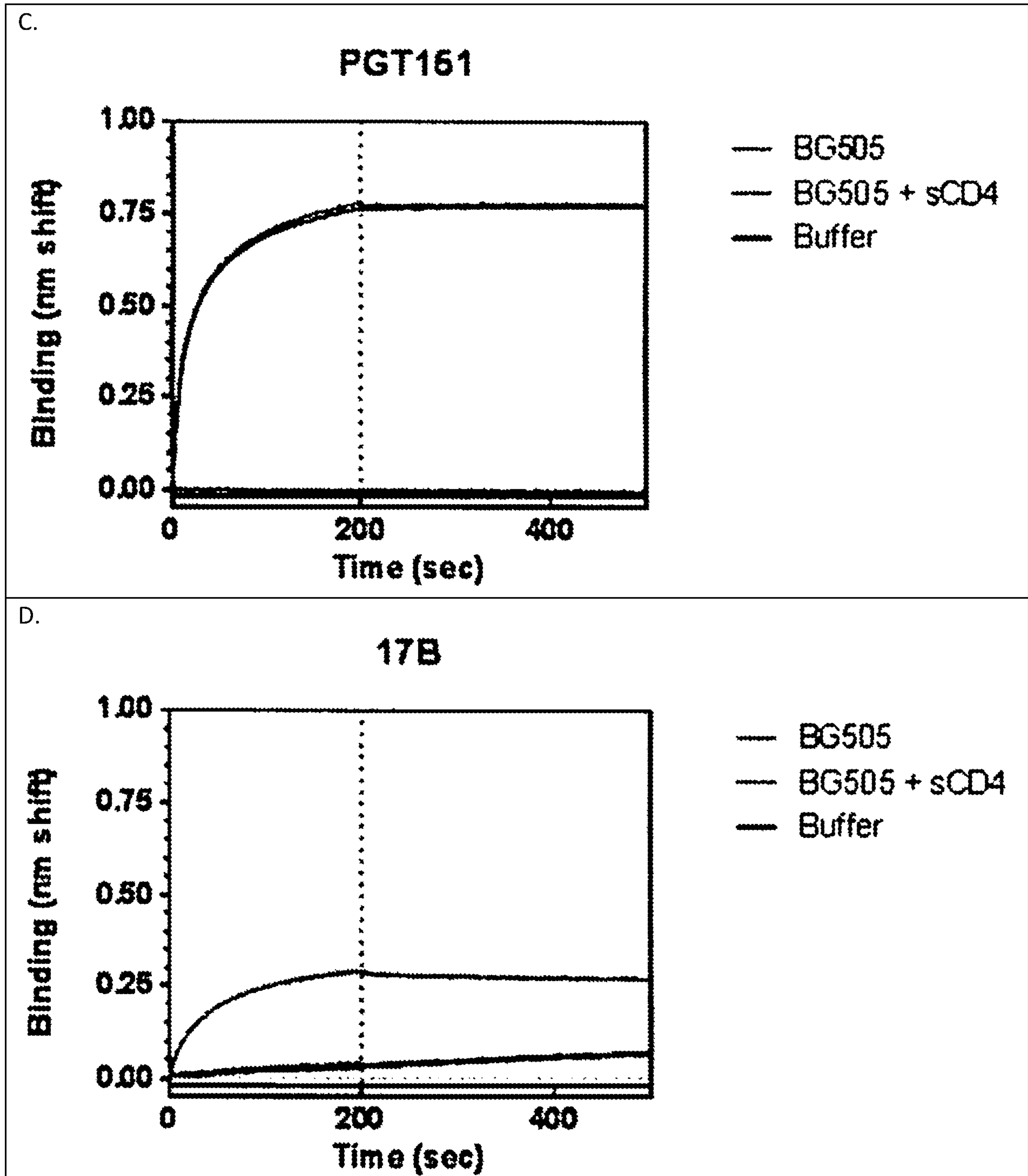


FIGURE 10

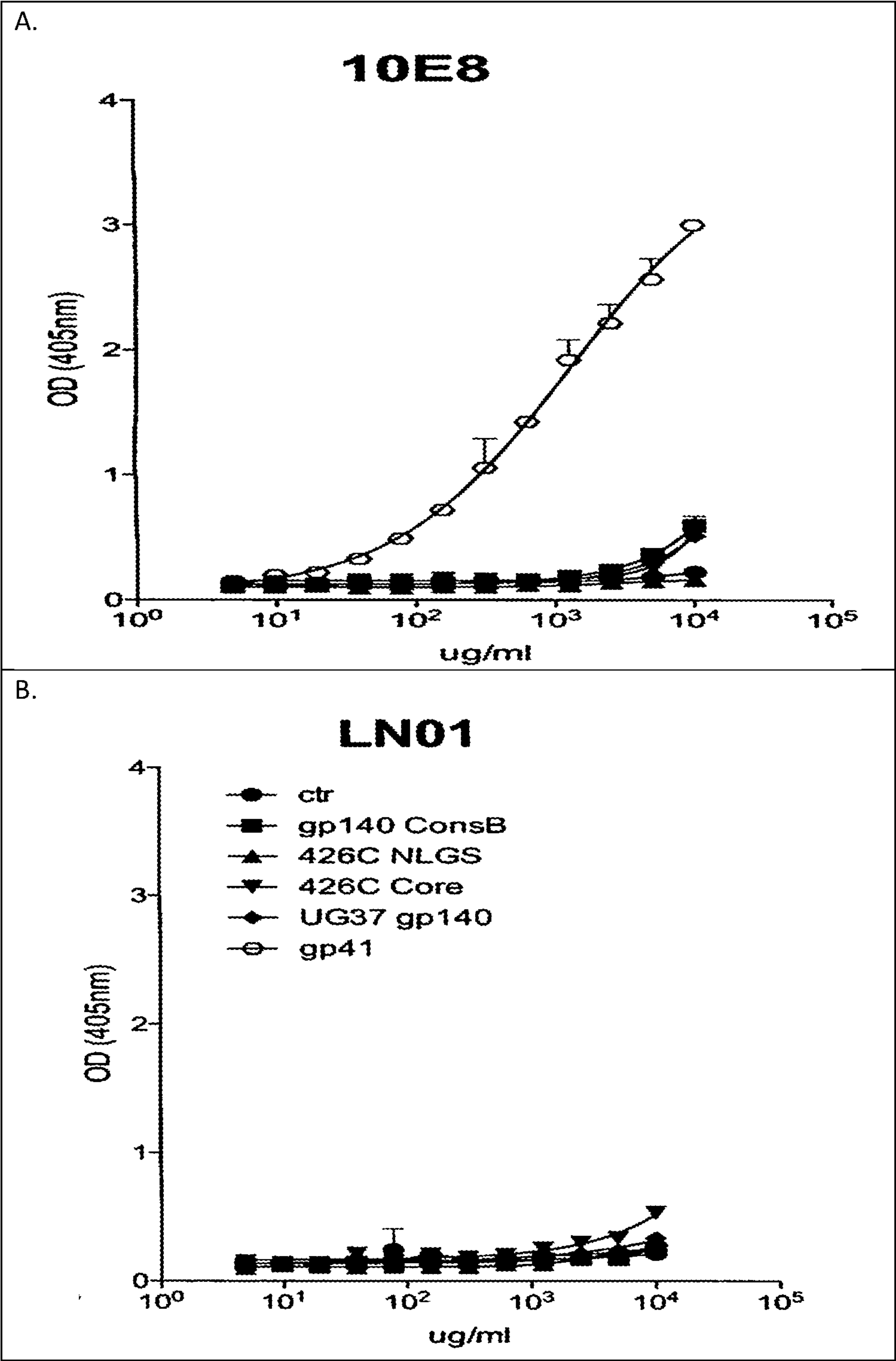


FIGURE 11

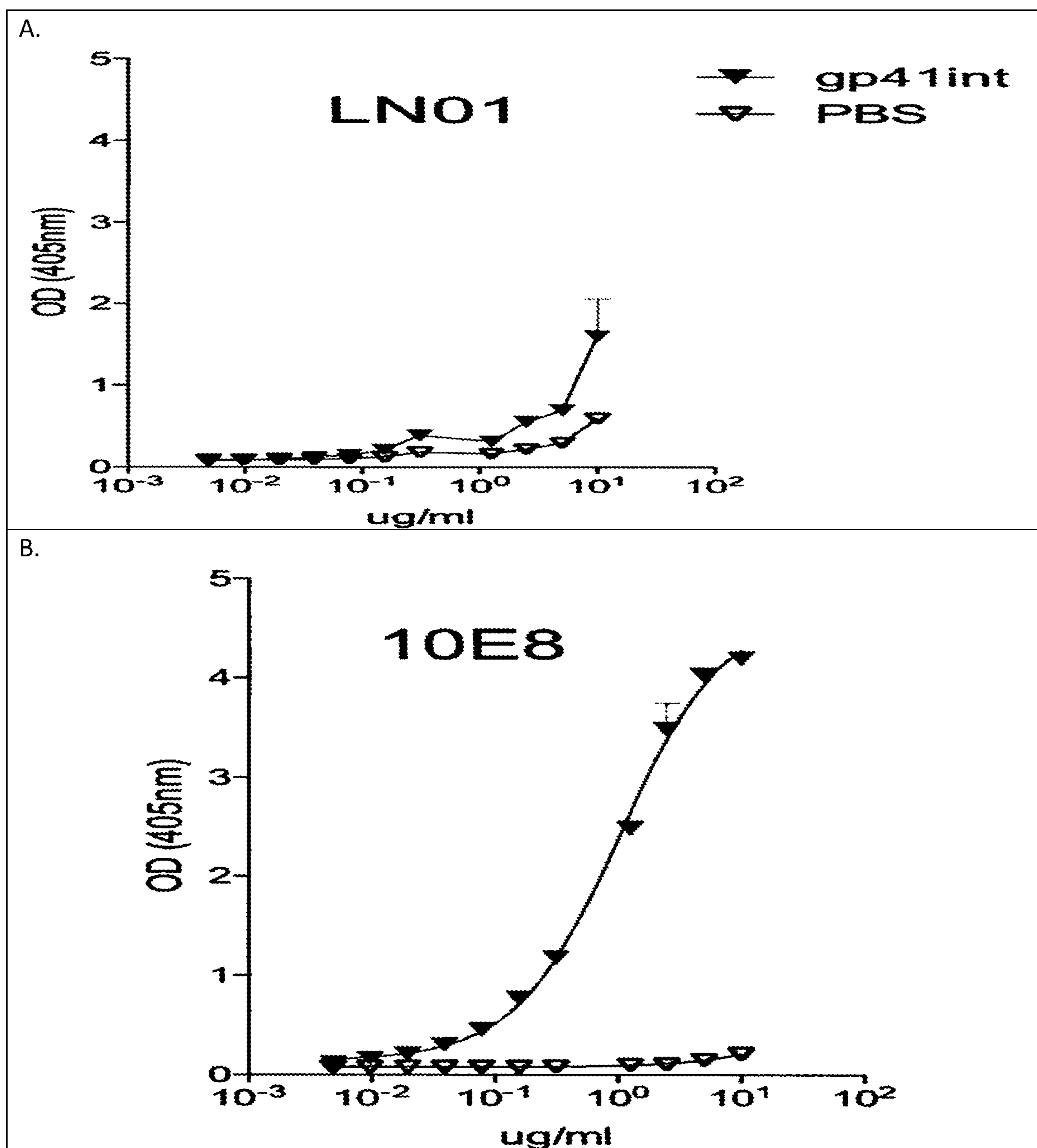


FIGURE 12

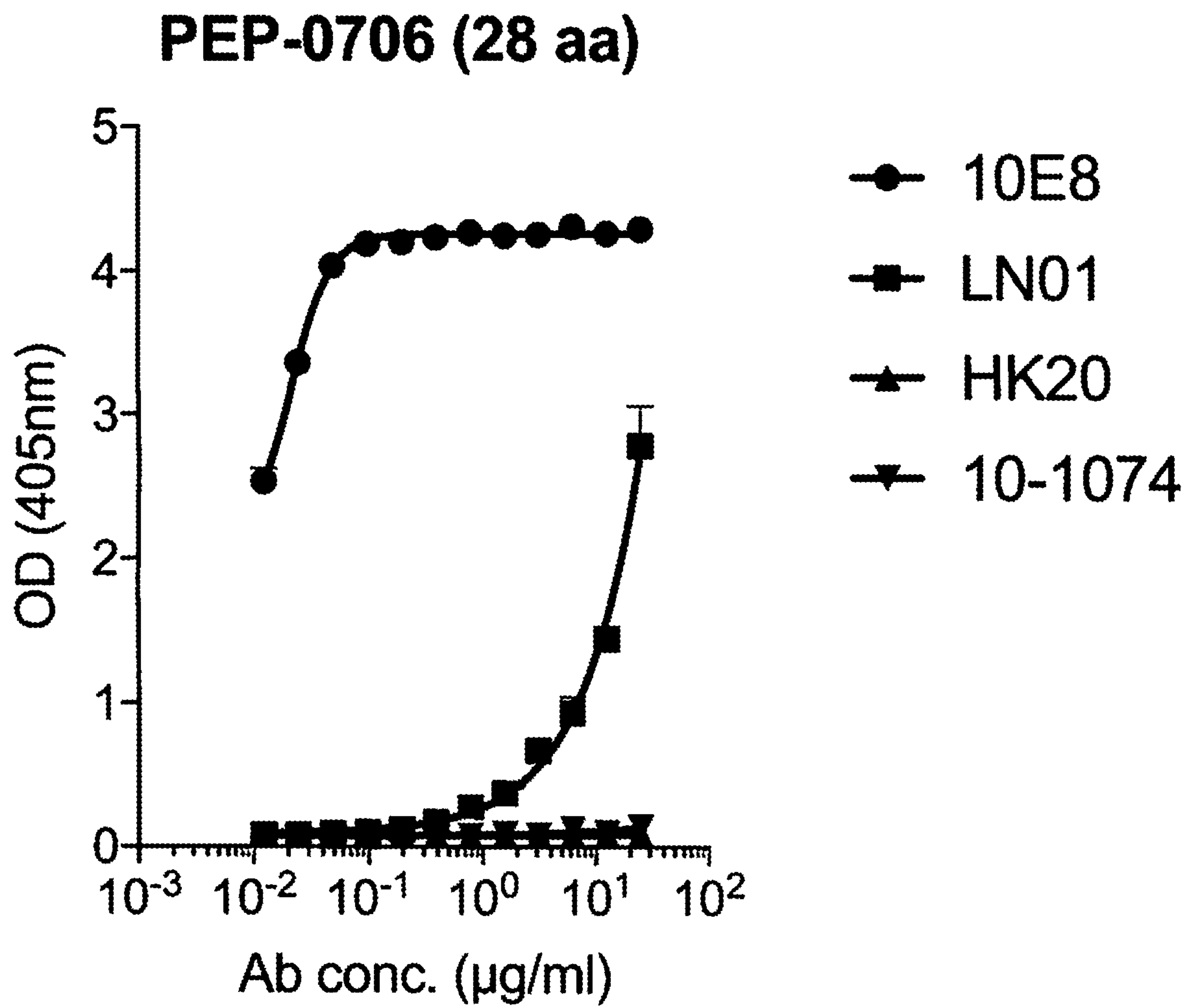


FIGURE 13A

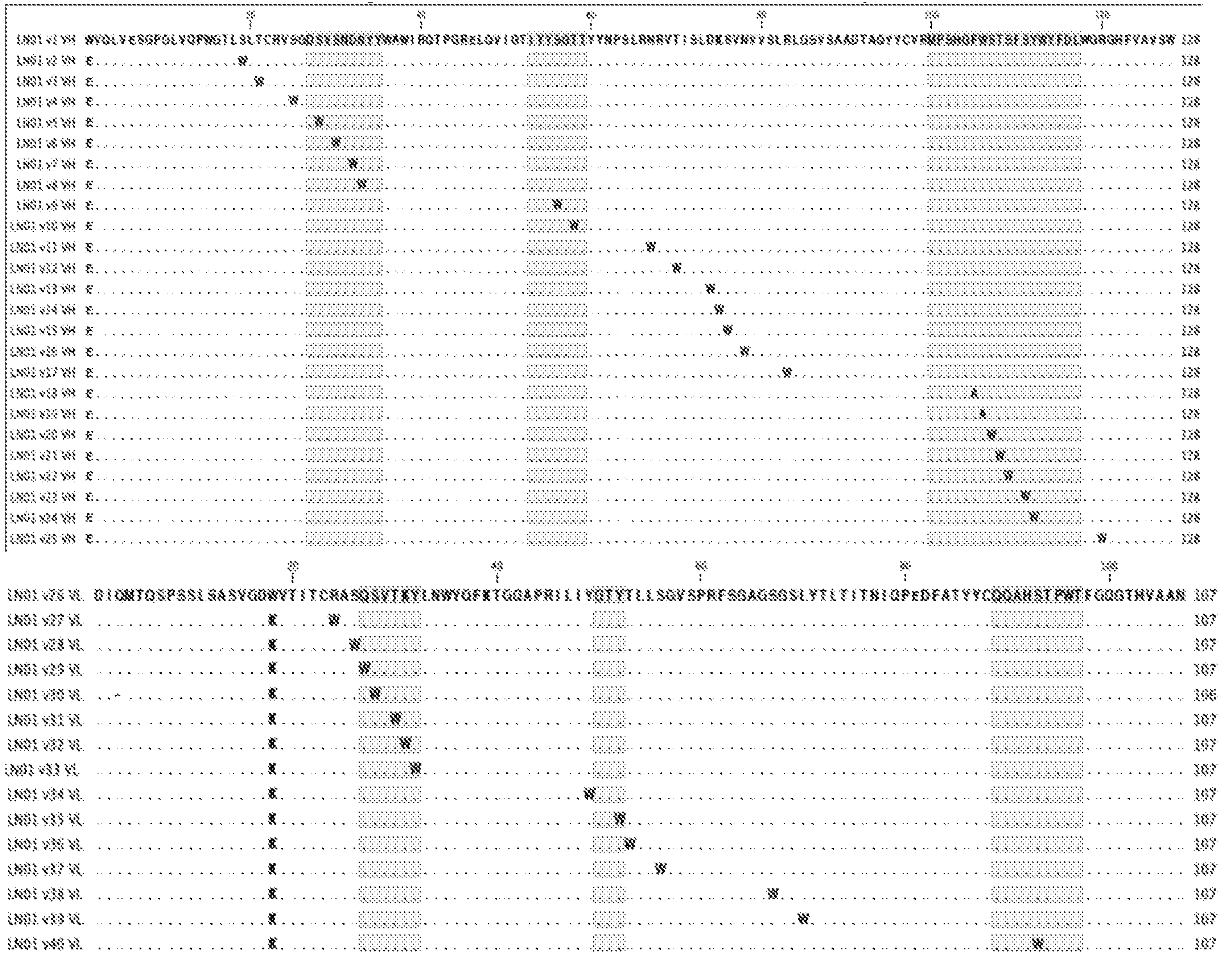


FIGURE 13B-C

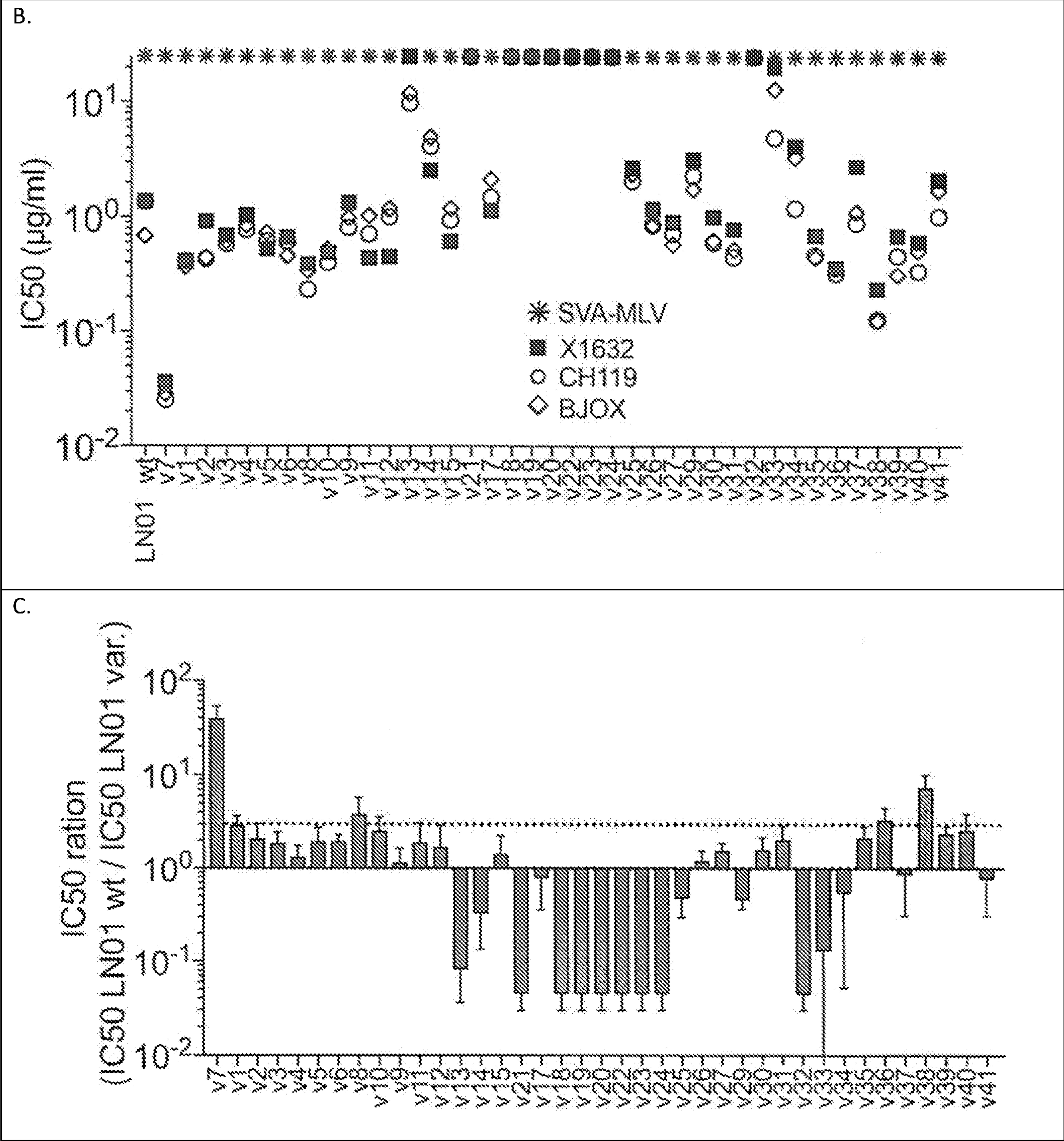
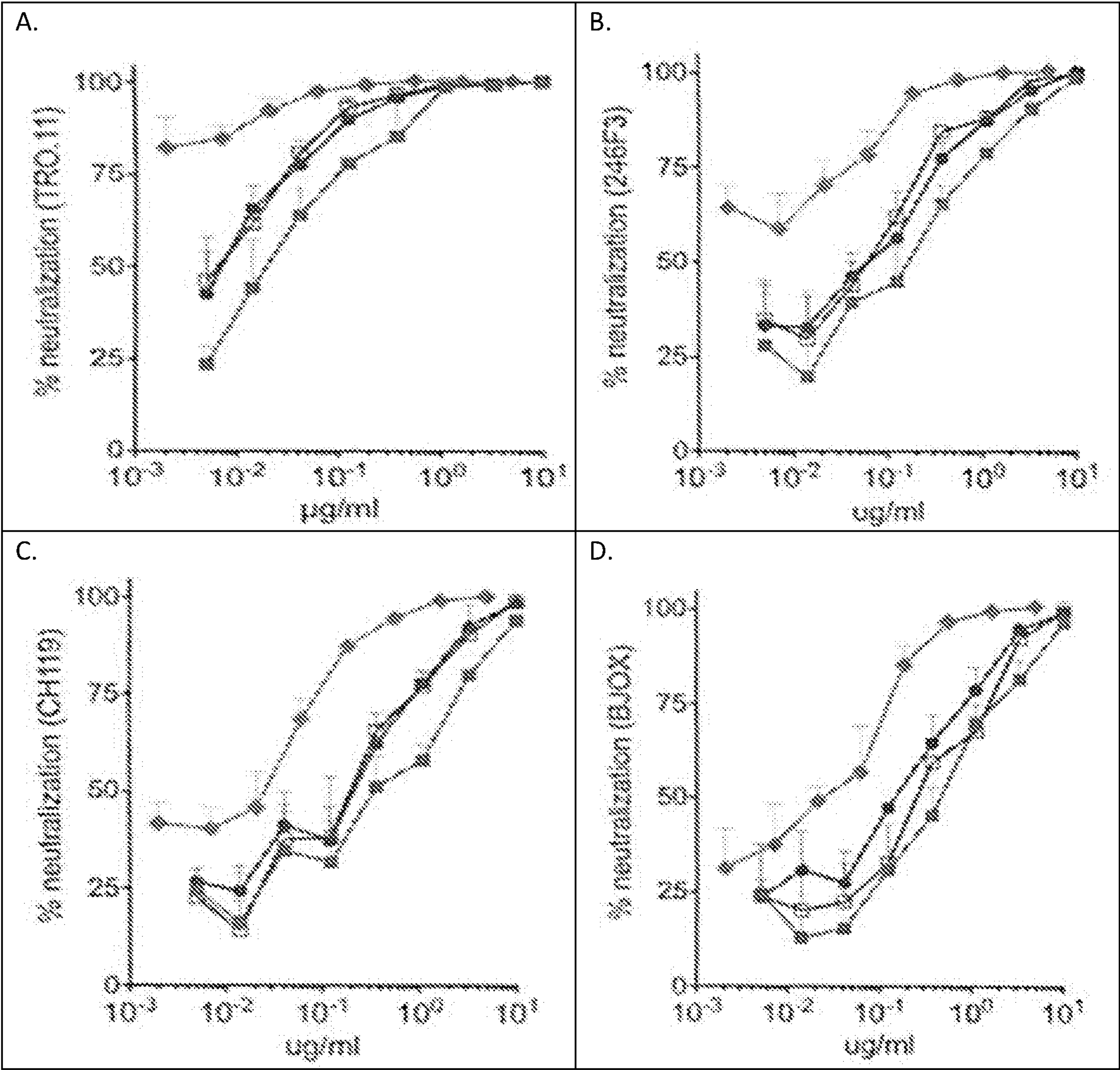
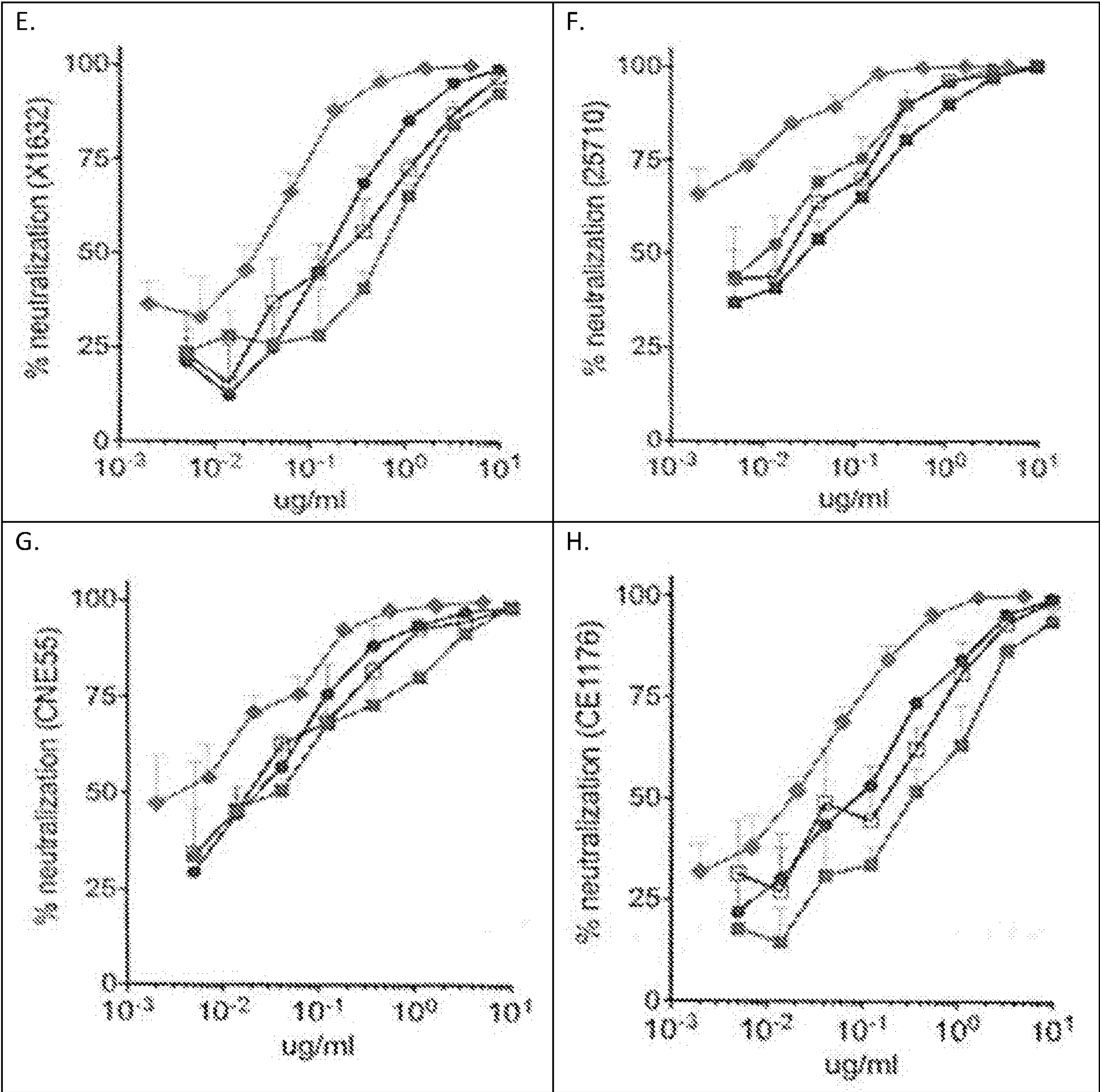


FIGURE 14A-D



■ L1401 #1
◆ V7
▲ V8
● V39

FIGURE 14E-H



UNO161
V7
V8
V29

FIGURE 14I-K

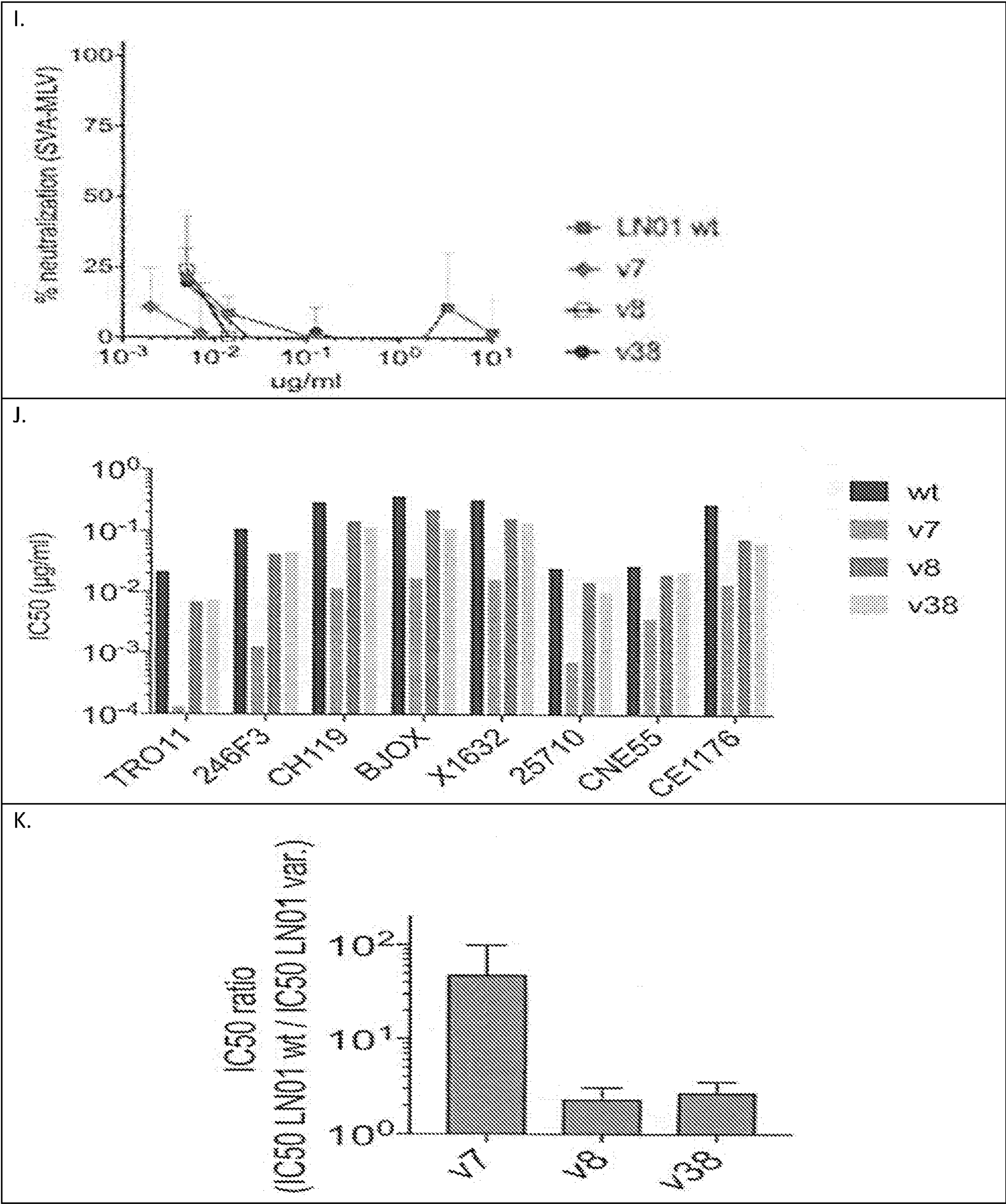


FIGURE 15A-B

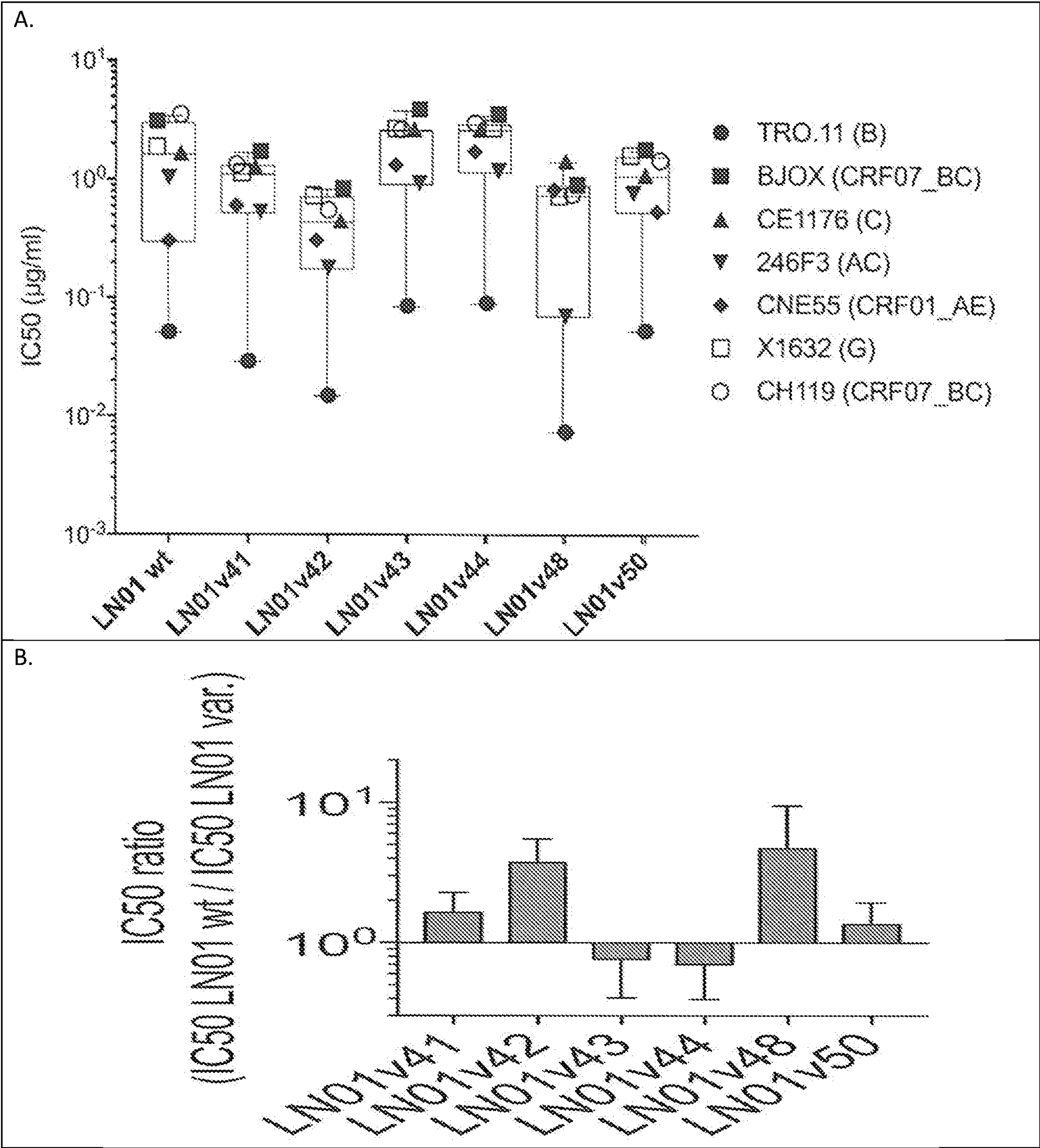


FIGURE 16A-B

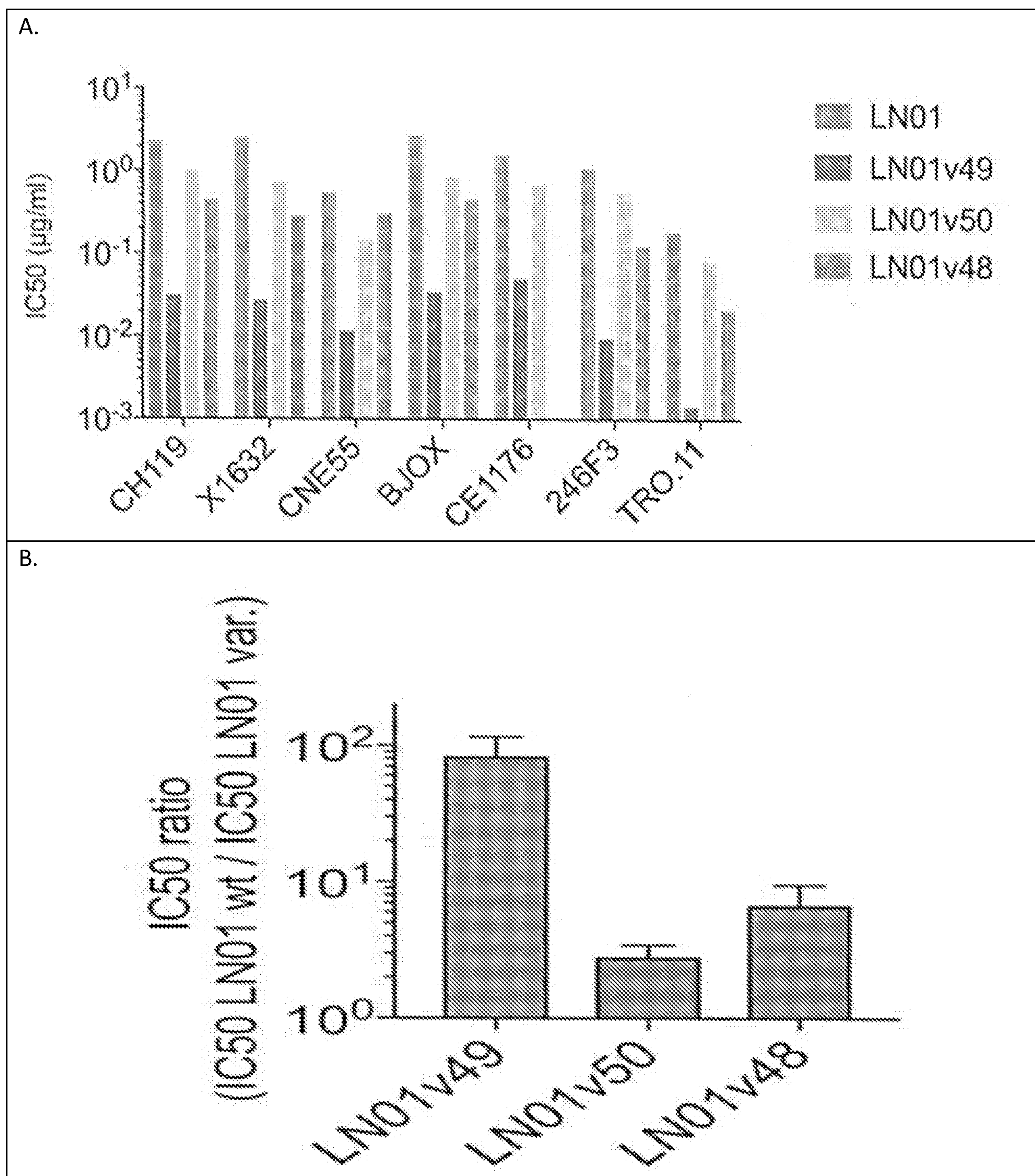


FIGURE 17

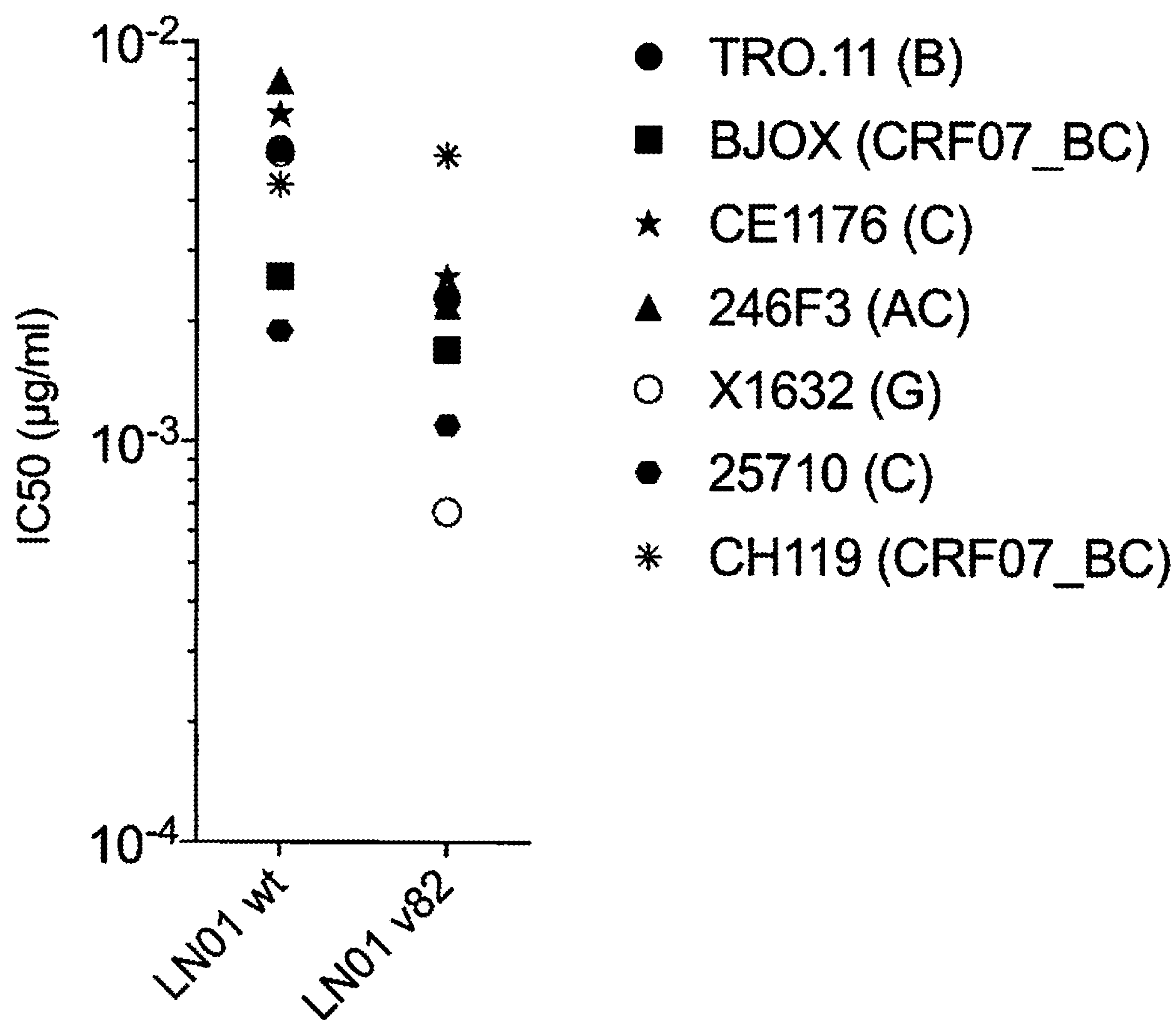


FIGURE 1A

