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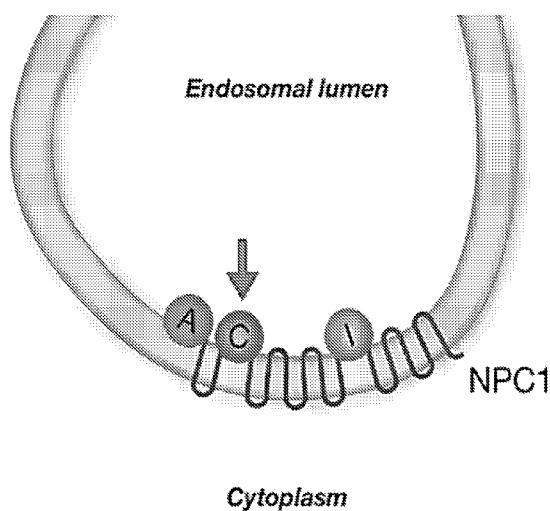


FIG. 1A

(57) Abstract: Disclosed are bispecific antibodies and bispecific fusion constructs that bind to Niemann-Pick C1 (NPC1) receptor for treating or preventing filovirus infections, pharmaceutical compositions comprising the bispecific antibodies, and therapeutic methods using the bispecific antibodies.



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ANTIBODY THERAPEUTICS AGAINST FILOVIRUS INFECTIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application number 62/157,104, filed on May 5, 2015, the contents of which are herein incorporated by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant numbers AI101436, AI109762 and AI090249 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Throughout this application various publications are referred to in parentheses. Full citations for these references may be found at the end of the specification. The disclosures of these publications are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0004] Multiple members of the family *Filoviridae* of enveloped negative-strand RNA viruses (filoviruses) cause a highly lethal hemorrhagic fever for which no approved treatments are available. While Ebola virus (EBOV) is responsible for the ongoing unprecedented epidemic in West Africa, the antigenically-distinct Sudan virus (SUDV), Bundibugyo virus (BDBV), Marburg virus (MARV), and Ravn virus (RAVV) have also caused outbreaks with high case fatality rates (30–90%). Moreover, novel filoviruses with zoonotic potential likely await discovery in the filovirus-endemic zone of equatorial Africa and elsewhere. As a case in point, the founding member of a distinct filovirus clade, Lloviu virus (LLOV), was recently isolated in southern Spain and Portugal, where it is suspected to have caused mass die-offs of insectivorous cave bats (Negredo, *PLoS Pathog*, 2011).

[0005] The current outbreak in West Africa has seen ZMapp (Mapp Biopharmaceuticals), a mixture of three humanized mouse monoclonal antibodies (mAbs) against the EBOV spike glycoprotein (GP), emerge as a promising treatment for Ebola virus disease. More generally, it has provided a powerful proof-of-concept for mAb-based prophylactics and therapeutics against filoviruses. However, a major limitation of ZMapp

and other planned mAb cocktails is their narrow spectrum of action (against EBOV only), dictated by the high antigenic diversity of filovirus GP proteins. Because the development, stockpiling, and deployment of separate mAb cocktails against each virulent filovirus is impractical, broad-spectrum mAb-based treatments that target multiple filoviruses are highly desirable.

[0006] The endo/lysosomal cholesterol transporter Niemann-Pick C1 (NPC1) is a universal intracellular receptor for entry and infection by filoviruses, and is required for *in vivo* pathogenesis by both EBOV and MARV (and almost certainly by BDBV, SUDV, and RAVV as well) (Carette, *Nature*, 2011; Ng, *Virology*, 2014). NPC1 protein has a cytoplasmic C-terminus, 13 transmembrane domains, and 3 large loops in the lumen of the endosome (Davies et al., 2000). One complication in targeting NPC1 with monoclonal antibodies (mAbs) is its exclusive localization to late endosomal compartments, where it is protected from extracellular antibodies.

[0007] The present invention uses bispecific antibodies (bsAbs) with potent anti-filovirus activity to address the need for methods for treating subjects infected with filoviruses or who are at risk for infection with filoviruses.

SUMMARY OF THE INVENTION

[0008] The invention provides methods of treating or preventing or reducing or inhibiting or reducing the risk or incidence of a filovirus infection in a subject comprising administering to the subject a bispecific antibody or a bispecific fusion construct that binds to Niemann-Pick C1 (NPC1) receptor in an amount effective to treat or prevent or reduce or inhibit or reduce the risk or incidence of a filovirus infection in a subject.

[0009] The invention further provides bispecific antibodies and bispecific fusion constructs that bind to Niemann-Pick C1 (NPC1) receptor for treating or preventing or reducing or inhibiting or reducing the risk or incidence of a filovirus infection in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Fig. 1A. NPC1 is required for Ebola virus infection. Niemann-Pick C1 (NPC1), a ubiquitous multi-pass membrane protein localized to late endosomes is required for cytoplasmic entry and infection by all filoviruses. The filovirus spike glycoprotein, GP, must engage NPC1's second luminal domain (domain C; arrow) to drive viral membrane fusion and cytoplasmic escape.

[0011] Fig. 1B. NPC1 is required for Ebola *in vivo* pathogenesis. NPC1-knockout mice (NPC1^{-/-}), but not their WT littermates (NPC1^{+/+}), are completely resistant to infection and killing by EBOV and MARV.

[0012] Fig. 2A-2D. A illustrates the X-ray crystal structure of a cleaved EBOV GP (GPCL) trimer bound to three copies of a soluble human NPC1 domain C protein. In B, side bottom views of NPC1 domain C are shown, with residues contacting GPCL (left panel) and mAb 548 (right panel) highlighted. C-D demonstrate the kinetics of binding of 548 to human NPC1 domain C by biolayer interferometry (BLI). D shows BLI-derived kinetic binding constants for the interaction of 548 with human and non-human primate (NHP; *Colobus guereza*) NPC1 domain C. 548 recognizes these proteins with picomolar affinity, at both neutral and acid pH.

[0013] Fig 3. mAbs specific to human NPC1 domain C block GP–NPC1 binding *in vitro*. Capacity of three anti-NPC1 mAbs to block GP–NPC1 binding in an ELISA. A soluble, flag-tagged form of NPC1 domain C was pre-incubated with the indicated concentrations of each mAb, and the protein-mAb mixtures were then added to plates coated with vesicular stomatitis virus (VSV) particles bearing EBOV GP. Bound domain C was detected with an anti-flag antibody. Irrelevant isotype-matched antibodies had no effect on GP-NPC1 domain C binding (not shown).

[0014] Fig. 4. Anti-NPC1 domain C mAbs neutralize VSV-EBOV GP infection weakly or not at all. VSV-GP particles were exposed to U2OS human osteosarcoma cells in the presence of increasing concentrations of NPC1-specific mAbs or a control GP-specific neutralizing mAb (KZ52). The number of infected cells was determined at 16 h post-infection, and normalized to that obtained in the absence of antibody (set to 100%).

[0015] Fig. 5A. Engineering antibodies with dual binding specificities for GP and NPC1. Constructs encoding bsAbs were generated by fusing NPC1-specific or control IgG sequences to a single-chain variable fragment (scFv) sequence derived from the EBOV GP-specific mAb KZ52. Fusions were done in four configurations: to the N- or C-terminus of the IgG heavy chain [HC] or light chain [LC].

[0016] Fig. 5B. Engineering antibodies with dual binding specificities for GP and NPC1. bsAbs and IgGs were expressed in 293-Freestyle cells by co-transfection of HC and LC expression vectors, and purified by protein A affinity chromatography.

[0017] Fig. 5C. bsAbs, but not NPC1-specific IgGs, can simultaneously bind to GP and NPC1 domain C. bsAbs and IgGs were captured onto GP-coated ELISA plates and then

incubated with NPC1 domain C-flag. Bound NPC1 domain C was detected with an anti-flag antibody.

[0018] Fig. 6. GP-NPC1 bsAbs neutralize VSV-EBOV GP infection with enhanced potency. Left panel, GP-NPC1 bsAbs show enhanced potency at viral neutralization relative to control GP-only bsAbs. VSV-EBOV GP particles were pre-incubated with the indicated concentrations of each bsAb for 1 h at room temp and then allowed to infect U2OS cells. Infected cells were quantified at 16 h post-infection and normalized to a no-antibody control (100%). Right panel, the neutralization curves were fit to a logistic equation to extract the concentration of half-maximal neutralization ($IC_{50} \pm 95\%$ confidence intervals).

[0019] Fig. 7. The enhanced neutralization potency of GP-NPC1 bsAbs can be attributed to their receptor blocking activity. Left panel, VSV-EBOV GP particles were pre-incubated with the indicated concentrations of each bsAb for 1 h at room temp and then allowed to infect matched U2OS cell lines expressing endogenous levels of NPC1 or over-expressing NPC1 (NPC1^{hi}). Infected cells were quantified at 16 h post-infection and normalized to a no-antibody control (100%). Right panel, the neutralization curves were fit to a logistic equation to extract the concentration of half-maximal neutralization ($IC_{50} \pm 95\%$ confidence intervals). The ratio of IC_{50} s for each bsAb in NPC1^{hi} vs. WT U2OS cells is shown.

[0020] Fig. 8. Fusion of a receptor-blocking IgG to a late endosome-localizing protein facilitates viral neutralization. bsAbs comprising an NPC1-specific IgG (401 or 548) fused to NPC2, a soluble late endosomal/lysosomal protein, were expressed and purified, and examined for their capacity to neutralize VSV-EBOV GP infection, as described in Figs. 6–7.

[0021] Fig. 9A-9D. A illustrates a bispecific antibody strategy for delivery of an NPC1-binding antibody to NPC1-positive endosomal/lysosomal compartments. In this strategy, the heavy and light chains of an NPC1-binding antibody like 548 are genetically fused to the variable VH and VL domains of a delivery antibody like FVM09, to generate a dual-variable domain Ig (DVD-IgTM). FVM09 broadly recognizes ebolavirus GP in extracellular viral particles or on the cell surface. However, the delivery antibody could also target a cell-surface marker, instead of viral GP. In B, the binding of the FVM09~548 DVD-Ig to each of its antigens (ebolavirus GP, human NPC1-C) is measured by BLI. In C, two-phase BLI binding curves show that FVM09~548 can simultaneously bind to both of its antigens. D shows BLI-derived kinetic binding constants for the interaction of the

FVM09~548 DVD-Ig with each of its antigens, and compares these constants to those obtained with the respective parent IgGs.

[0022] Fig. 10A-10B. A shows an SDS-polyacrylamide gel of purified FVM09~548 resolved under non-reducing (NR) and reducing (R) conditions and stained with Commassie Brilliant Blue. In B, FVM09~548 was subjected to size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS). The SEC-MALS trace indicates that the majority species of FVM09~548 (main peak; retention time = 5.10 min) is an IgG-like monomer with an apparent molecular weight of ~175 kDa. A small (typical) amount of soluble aggregates (retention time = 4.46 min) is also present.

[0023] Fig. 11A-11C measures the capacity of the FVM09~548 bispecific antibody to neutralize ebolavirus GP-dependent entry and infection in human cells. In A, vesicular stomatitis viruses bearing EBOV GP (VSV-EBOV GP) were incubated with increasing concentrations of FVM09~548, its parent IgGs FVM09, 548, or an equimolar mixture of the parent IgGs (FVM09+548), and then exposed to cells. Only FVM09~548 showed potent, dose-dependent neutralization of VSV-EBOV GP infection. B shows that FVM09~548 can broadly neutralize infection mediated by multiple ebolavirus GPs. C shows that authentic EBOV, BDBV, and SUDV can be potentially neutralized by FVM09~548.

[0024] Fig. 12A-12C examines the mechanism of action of the FVM09~548 bispecific antibody. In A, wild-type (WT) VSV-EBOV GP particles, or mutant particles containing two point mutations (E288D/W292R) in the FVM09 epitope ((FVM09mut) were incubated with increasing concentrations of the FVM09~548 antibody, and then exposed to cells. Loss of FVM09-GP binding is associated with a 99% reduction in the potency of antiviral neutralization, providing evidence that engagement of GP in extracellular virus particles by FVM09~548 is necessary for its neutralizing activity. In B, VSV-EBOV GP particles were incubated with FVM09~548 and then exposed to isogenic cell lines expressing physiological levels of human NPC1 (WT), or expressing high levels of human NPC1 (NPC1-overexp). The neutralization potency of FVM09~548 was abolished by NPC1 overexpression, likely because of the titration of available antibody by excess NPC1. This experiment provides evidence that engagement of NPC1 domain C by 548 in endosomes is required for the neutralizing activity of FVM09~548. In C, the delivery of FVM09~548 to NPC1-containing endosomes was directly examined. VSV-EBOV GP particles labeled with the fluorescent protein mNeongreen (mNG) were incubated with antibodies labeled with the fluorophore pHrodo-Red™, which only fluoresces at acid pH (left panel). The virus-

antibody mixture was exposed to cells at 37°C for 30 min, after which time, cells were examined for both virus- and antibody-associated fluorescence by two-color flow cytometry. The flow cytometric analysis is shown in the right panel. Cells were gated into virus-negative and virus-positive populations based on mNeongreen fluorescence, and each subpopulation was further examined for pHrodo-Red™ fluorescence. Strong pHrodo-Red™ fluorescence was seen with FVM09 in the virus-positive sample only, indicating that binding of this antibody to GP on extracellular virus particles affords its delivery to acidic endosomes. Little or no pHrodo-Red™ fluorescence was observed with 548 in the absence or presence of virus, indicating that this antibody cannot access endosomal compartments on its. By contrast FVM09~548 gave a strong pHrodo-Red™ signal in virus-positive cells. Therefore, combining FVM09 and 548 into a single bispecific antibody promotes efficient delivery of 548 to endosomes through the action of FVM09.

[0025] Fig. 13A-13C. The capacity of the human NPC1 domain C binding antibody 548 to bind to a non-human primate (*Colobus guereza*) NPC1 domain C protein and block EBOV GP-NPC1 binding is shown. In A, BLI is employed to detect and measure 548 binding to *C. guereza* NPC domain C at the acidic pH of late endosomes. B shows the capacity of 548 to block GP-*C. guereza* NPC1 domain C binding in an ELISA. A soluble, flag-tagged form of *C. guereza* NPC1 domain C was pre-incubated with the indicated concentrations of 548, and the protein-mAb mixtures were then added to plates coated with vesicular stomatitis virus (VSV) particles bearing EBOV GP. Bound domain C was detected with an anti-flag antibody. Because the sequences of *C. guereza* NPC1 that contact GP are identical to those of NPC1 proteins from rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) (Fig. 13C), 548 can be evaluated for antiviral protection in these two established non-human primate (NHP) models of filovirus challenge. In C, sequences corresponding to NPC1 residues 485–514 (human NPC1 numbering) are shown. The 548 epitope is indicated—it is identical in humans and the three indicated NHP species. *Homo sapiens* - SEQ ID NO:24, *Macaca mulatta* - SEQ ID NO:25, *Colobus guereza* - SEQ ID NO:26, *Macaca fascicularis* - SEQ ID NO:27.

DETAILED DESCRIPTION OF THE INVENTION

[0026] The present invention provides a method for treating or preventing or reducing or inhibiting or reducing the risk or incidence of a filovirus infection in a subject comprising administering to the subject a bispecific antibody or a bispecific fusion construct that binds

to Niemann-Pick C1 (NPC1) receptor in an amount effective to treat or prevent or reduce or inhibit or reduce the risk or incidence of a filovirus infection in a subject.

[0027] The invention also provides a bispecific antibody or a bispecific fusion construct that binds to Niemann-Pick C1 (NPC1) receptor for treating or preventing or reducing or inhibiting or reducing the risk or incidence of a filovirus infection in a subject.

[0028] The bispecific antibody or bispecific fusion construct can combine both antiviral and anti-NPC1 specificities in the same molecule. For example, the bispecific antibody or bispecific fusion construct can bind both to NPC1 and to filovirus glycoprotein (GP).

[0029] The bispecific antibody can be generated by fusing a NPC1-specific sequence to a single-chain variable fragment (scFv) sequence derived from EBOV GP-specific monoclonal antibody KZ52, where the fusion can be to a N- or C-terminus of an IgG heavy chain or light chain.

[0030] The bispecific antibody or bispecific fusion construct can combine an anti-NPC1 specificity with an endosomal targeting functionality. This can include an antibody specific for a cellular molecule that affords endosomal delivery (e.g., NPC2) or a peptide or protein that would do the same (not necessarily just an antibody). Thus, the bispecific antibody or bispecific fusion construct can bind both to NPC1 and to Niemann-Pick C2 (NPC2).

[0031] The bispecific antibody or bispecific fusion construct can be generated by fusing heavy and light chains of a NPC1-specific sequence to variable VH and VL domains of a delivery antibody to generate a dual-variable domain Ig. The dual variable domain-Ig (DVD-Ig) bispecific antibody format was developed by AbbVie (Wu *et al.*, 2007). The delivery antibody can target, for example, a filovirus glycoprotein (GP) or a cell-surface marker. The delivery antibody can be, for example, FVM09 (Keck *et al.* 2015). FVM09 broadly recognizes ebolavirus GP in extracellular viral particles or on the cell surface.

[0032] The anti-NPC1 amino acid sequence of a variable region of a light chain of the bispecific antibody or bispecific fusion construct can comprise the amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. The anti-NPC1 amino acid sequence of a variable region of a heavy chain of the bispecific antibody or bispecific fusion construct can comprise the amino acid sequence set forth in SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8. The anti-NPC1 amino acid sequence of a variable region of a light chain can comprise the amino acid sequence set forth in SEQ ID NO:3 and a variable region of a heavy chain can comprise the amino acid sequence set forth in SEQ ID NO:4. The anti-NPC1 amino acid sequence of a variable region of a light chain can comprise the amino

acid sequence set forth in SEQ ID NO:5 and a variable region of a heavy chain can comprise the amino acid sequence set forth in SEQ ID NO:6. The anti-NPC1 amino acid sequence of a variable region of a light chain can comprise the amino acid sequence set forth in SEQ ID NO:7 and a variable region of a heavy chain can comprise the amino acid sequence set forth in SEQ ID NO:8.

[0033] The bispecific antibody can comprise the amino acid sequence set forth in any one of SEQ ID NOs:9-20. The bispecific fusion construct can comprise the amino acid sequence set forth in SEQ ID NO:21 or SEQ ID NO:22.

[0034] In different uses, the subject can be infected with a filovirus. Alternatively, the subject can be at risk for infection with a filovirus. Subjects who are at risk for infection with filoviruses include subjects who have been exposed to filovirus or are at risk of exposure to filovirus. In addition to the natural occurrence of filoviruses, there is the potential for exposure to these pathogens if they are used as agents of bioterrorism or biological warfare. For example, the subject can be a family member or healthcare worker in an area of an outbreak of a filovirus infection. The subject can be a medical personnel, first responder or military personnel potentially exposed or exposed to a filovirus as the result of bioterrorism or biological warfare. The subject can be a biosafety level 3/4 laboratory personnel or animal worker potentially exposed or exposed to a filovirus.

[0035] The family *Filoviridae* is a family of viruses including genera *Ebolavirus* and *Marburgvirus*. The Ebola virus species can be *Zaire ebolavirus* or *Sudan ebolavirus*. Additional examples of filovirus include a Bundibugyo virus, a Sudan virus, a Ravn virus and a Lloviu virus.

[0036] The subject can be a mammal, such as, for example, livestock, a bat, a primate or a human.

[0037] To treat a subject with a filovirus infection means to reduce or stop the spread of filovirus in the subject, or to eliminate the filovirus from the subject, or to reduce or eliminate a sign or symptom of filovirus infection in the subject. Filovirus infection is characterized by hemorrhagic fever, including abnormalities in blood coagulation. As used herein, “preventing” a filovirus infection means reducing the development of, or reducing the extent of, one or more symptoms of the condition, as compared to the development or extent the condition takes in the absence of preventative treatment. In an embodiment, “preventing” as used herein does not mean an absolute prevention, but a lessened extent of

the condition brought about prophylactically, or to reduce the risk or incidence of a filovirus infection.

[0038] Human NPC1 receptor protein has the amino acid sequence (SEQ ID NO:1) (NCBI Reference Sequence: NM_000271.4):

MTARGIALGL	LLLLLCPAQV	FSQSCVWYGE	CGIAYGDKRY	NCEYSGPPKP	LPKDGVDLVQ	60
ELCPGFFFGN	VSLCCDVRQL	QTLKDNLQLP	LQFLSRCPSC	FYNLLNLFCE	LTCSPRQSQF	120
LNVTATEDYV	DPVTNQTKTN	VKELQYYVGQ	SFANAMYNAC	RDVEAPSSND	KALGLLCGKD	180
ADACNATNWI	EYMFNKDNGQ	APFTITPVFS	DFPVHGMPEM	NNATKGCDES	VDEVTA PCSC	240
QDCSIVCGPK	PQPPPPAPW	TILGLDAMYV	IMWITYMAFL	LVFFGAFFAV	WCYRKRYFVS	300
EYTPIDSNIA	FSVNASDKGE	ASCCDPVSAA	FEGCLRRLEF	RWGSFCVRNP	GCVIFFSLVF	360
ITACSSGLVF	VRVTNPNVDL	WSAPSSQARL	EKEYFDQHFG	PPFRTEQLII	RAPLTDKHIY	420
QPYPSGADVP	FGPPLDIQIL	HQVLDLQIAI	ENITASYDNE	TVTLQDICLA	PLSPYNTNCT	480
ILSVLNIFYQN	SHSLDHRKKG	DDFFVYADYH	THFLYCVRAP	ASLNDTSLLH	DPCLGTFGGP	540
VFPWLVLGGY	DDQNYNNATA	LVITFPVNNY	YNDTEKLQRA	QAWKEKEFINF	VKNYKNPNLT	600
ISFTAERSIE	DELNRESDS	VFTVVISYAI	MFLYISLALG	HMKSCRLLV	DSKVS LGIAG	660
ILIVLSSVAC	SLGVFSYIGL	PLTLIVIEVI	PFLVLAVGVD	NIFILVQAYQ	RDERLQGETL	720
DQQLGRVLGE	VAPSMFLSSF	SETVAFFLGA	LSVMPAVHTF	SLFAGLAVFI	DFLLQITCFV	780
SLLGLDIKRQ	EKNRLDIFCC	VRGAEDGTSV	QASESCLFRF	FKNSYSPLLL	KDWMRPVIA	840
IFVGVLFSFI	AVLNKVDIGL	DQSLSMPDDS	YMDYFYSIS	QYLHAGPPVY	FVLEE GHDT	900
SSKGQNMVCG	GMGCNDSL	QQIFNAAQLD	NYTRIGFAPS	SWIDDYFDWV	KPQSSCCRVD	960
NITDQFCNAS	VDPACVRCR	PLTPEGKQRP	QGGDFMRFLP	MFLSDNPNPK	CGKGHAAAYS	1020
SAVNILLGHG	TRVGATYFMT	YHTVLQTSAD	FIDALKKARL	IASNVTETMG	INGSAYRVFP	1080
YSVFYVFYEQ	YLTIIDDTIF	NLGVSLGAIF	LVTMVLGCE	LWSAVIMCAT	IAMVLVNMFG	1140
VMWLWGISLN	AVSLVNLVMS	CGISVEFCSH	ITRAFTVSMK	GSRVERAEEA	LAHMGSSVFS	1200
GITLTKFGGI	VVLAFKSKI	FQIFYFRMYL	AMVLLGATHG	LIFLPVLLSY	IGPSV NKA	1260
CATEERYKGT	ERERLLNF					1278

[0039] Nucleic acid (mRNA) encoding human NPC1 receptor protein has the nucleotide sequence (SEQ ID NO:2) (NCBI Reference Sequence: NM_000271.4):

1	gaagggcaac	acggggacct	tgaagcgggg	tcgcggcgcc	gccccagccc	gggccaggga
61	gtccccggcag	cggcacctcc	cagaaagggc	ggagccgacg	acgccttctt	ccttcctgac
121	cggcgcgcgc	agcctgctgc	cgcggtcagc	gcctgctcct	gctcctccgc	tcctcctgcg
181	cggggtgctg	aaacagcccg	gggaagtaga	gccgcctccg	gggagcccaa	ccagccgaac
241	gccgcccggc	tcagcagcct	tgccggcgca	cagcatgacc	gctcgcggcc	tgcccttg
301	cctcctcctg	ctgctactgt	gtccagcgca	ggtgttttca	cagtcctgtg	tttggtatgg
361	agagtgtgga	attgcatatg	gggacaagag	gtacaattgc	gaatattctg	gccccacaaa
421	accattgcca	aaggatggat	atgacttagt	gcaggaactc	tgtccaggat	tcttcttttg
481	caatgtcagt	ctctgttgtg	atgttcggca	gcttcagaca	ctaaaagaca	acctgcagct
541	gcctctacag	tttctgtcca	gatgtccatc	ctgtttttat	aacctactga	acctgttttg
601	tgagctgaca	tgtagccctc	gacagagtca	gtttttgaat	gttacagcta	ctgaagatta
661	tgttgatcct	gttacaacc	agacgaaaac	aaatgtgaaa	gagttacaat	actacgtcgg
721	acagagtttt	gccaatgcaa	tgtacaatgc	ctgccgggat	gtggaggccc	cctcaagtaa
781	tgacaaggcc	ctgggactcc	tgtgtgggaa	ggacgctgac	gcctgtaatg	ccaccaactg
841	gattgaatac	atgttcaata	aggacaatgg	acaggcacct	tttaccatca	ctcctgtgtt
901	ttcagatttt	ccagtcctatg	ggatggagcc	catgaacaat	gccaccaaag	gctgtgacga
961	gtctgtggat	gaggtcacag	caccatgtag	ctgccaaagc	tgctctattg	tctgtggccc
1021	caagccccag	ccccacctc	ctcctgctcc	ctggacgatc	cttggttg	acgccatgta
1081	tgtcatcatg	tggatcacct	acatggcgtt	tttgcttgtg	ttttttggag	cattttttgc
1141	agtggtggtg	tacagaaaac	ggtattttgt	ctccaggtac	actcccatcg	atagaatat
1201	agctttttct	gttaatgcaa	gtgacaaagg	agaggcgctc	tgctgtgacg	ctgtcagcgc
1261	agcatttgag	ggctgcttga	ggcggctgtt	cacacgctgg	gggtctttct	gcgtccgaaa
1321	ccctggctgt	gtcattttct	tctcgtgtgt	cttcattact	gcgtgttctg	caggcctgg
1381	gtttgtccgg	gtcacaacca	atccagttga	cctctggtca	gccccagca	gccaggctcg
1441	cctggaaaaa	gagtactttg	accagcactt	tgggcctttc	ttccggacgg	agcagctcat
1501	catccggggc	cctctcactg	acaaacacat	ttaccagcca	tacccttcgg	gagctgatgt

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1561 acccttttggga cctccgcttg acatacagat actgcaccag gttcttgact tacaaatagc
1621 catcgaaaaac attactgcct cttatgacaa tgagactgtg acacttcaag acatctgctt
1681 ggccctctt tcaccgtata acacgaactg caccattttg agtgtgttaa attacttcca
1741 gaacagccat tccgtgctgg accacaagaa aggggacgac ttctttgtgt atgccgatta
1801 ccacacgcac tttctgtact gcgtacgggc tcctgcctct ctgaatgata caagtttgct
1861 ccatgaccct tgtctgggta cgtttggtgg accagtgttc ccgtggcttg tgttgggagg
1921 ctatgatgat caaaactaca ataacgccac tgcccttggtg attaccttcc ctgtcaataa
1981 ttactataat gatacagaga agctccagag ggcccaggcc tgggaaaaag agtttattaa
2041 ttttgtgaaa aactacaaga atcccaatct gaccatttcc ttcactgctg aacgaagtat
2101 tgaagatgaa ctaaatcgtg aaagtgcag tgatgtcttc accgttgtaa ttagctatgc
2161 catcatgttt ctatatattt ccctagcctt ggggcacatg aaaagctgtc gcaggcttct
2221 ggtggattcg aaggtctcac taggcacgcg gggcatcttg atcgtgctga gctcggtggc
2281 ttgctccttg ggtgtcttca gctacattgg gttgcccttg accctcattg tgattgaagt
2341 catcccgttc ctggtgctgg ctggttgagt ggacaacatc ttcattctgg tgcaggccta
2401 ccagagagat gaacgtcttc aaggggaaac cctggatcag cagctgggga gggctcagg
2461 agaagtggct ccagtatgt tcctgtcatc cttttctgag actgtagcat ttttcttagg
2521 agcattgtcc gtgatgccag ccgtgcacac cttctctctc tttgcgggat tggcagtctt
2581 cattgacttt cttctgcaga ttacctgttt cgtgagtctc ttggggtag acattaaacg
2641 tcaagagaaa aatcggctag acatcttttg ctgtgtcaga ggtgctgaag atggaacaag
2701 cgtccaggcc tcagagagct gtttgtttcg cttcttcaaa aactcctatt ctccacttct
2761 gctaaaggac tggatgagac caattgtgat agcaatattt gtgggtgttc tgtcattcag
2821 catcgcagtc ctgaacaaag tagatatgg attggatcag tctctttcga tgcagatga
2881 ctctacatg gtgattatt tcaaatccat cagtcagtac ctgcatgcg gtcgcgtgt
2941 gtactttgtc ctggagggaag ggcacgacta cacttcttcc aaggggcaga acatgggtgtg
3001 cggcggcatg ggctgcaaca atgattccct ggtgcagcag atatttaacg cggcgcagct
3061 ggacaactat acccgaatag gcttcgcccc ctctcctgg atcgacgatt atttcgactg
3121 ggtgaagcca cagtctctt gctgtcagat ggacaatatc actgaccagt tctgcaatgc
3181 ttcagtgggt gaccctgcct gcgttcgctg caggcctctg actccggaag gcaaacagag
3241 gcctcagggg ggagacttca tgagattcct gcccatgttc ctttcggata accctaacc
3301 caagtgtggc aaagggggac atgctgccta tagttctgca gttaacatcc tccttgcca
3361 tggcaccagg gtcggagcca cgtacttcat gacctaccac accgtgctgc agacctctgc
3421 tgactttatt gacgtcttga agaaagccg acttatagcc agtaatgtca ccgaaacct
3481 gggcattaac ggcagtgcct accgagtatt tccttacagt gtgttttatg tcttctacga
3541 acagtacctg accatcattg acgacactat cttcaacctc ggtgtgtccc tgggcgcgat
3601 atttctgggt accatgggtc tcctgggctg tgagctctgg tctgcagtca tcatgtgtgc
3661 caccatcgcc atggtcttgg tcaacatgtt tggagttagg tggctctggg gcatcagtct
3721 gaacgtgtga tccttgggtc acctggtgat gagctgtggc atctccgtgg agttctgcag
3781 ccacataacc agagcgttca cggtgagcat gaaaggcagc cgcgtggagc gcgcggaaga
3841 ggcacttgcc cacatgggca gctccgtgtt cagtggaaac acacttaca aatttgagg
3901 gattgtgggt ttggcttttg ccaaactca aattttccag atattctact tcaggatgta
3961 tttggccatg gtcttactgg gagccactca cggattaata tttctcctg tcttactcag
4021 ttacataggg ccatcagtaa ataaagccaa aagttgtgcc actgaagagc gatacaaagg
4081 aacagagcgc gaacggcttc taaatttcta gccctctcgc agggcatcct gactgaactg
4141 tgtctaaggg tcggtcgggt taccactgga cgggtgctgc atcggaagg ccaagttgaa
4201 caccgatgg tgccaaccat cgggtgtttg gcagcagctt tgaacgtagc gcctgtgaac
4261 tcaggaatgc acagttgact tgggaagcag tattactaga tctggaggca accacaggac
4321 actaaacttc tcccagcctc ttcaggaaag aaacctcatt ctttggcaag caggaggtga
4381 cactagatgg ctgtgaatgt gatccgctca ctgacactct gtaaaggcca atcaatgcac
4441 tgtctgtctc tccttttagg agtaagccat ccacaagtt ctataccata tttttagtga
4501 cagttgaggt tgtagataca ctttataaca ttttatagtt taaagagctt tattaatgca
4561 ataaattaac tttgtacaca tttttatata aaaaaacagc aagtgtttc agaagtgtgt
4621 aggcctcatt agagcttggc ctccaaaaat ctgtttgaaa aaagcaacat gttcttcaca
4681 gtgttcccct agaaaggaag agatttaatt gccagttaga tgtggcatga aatgagggac
4741 aaagaaagca tctcgtaggt gtgtctactg ggttttaact tatttttctt taataaaata
4801 cattgttttc ctaaaaaaa aaaaaaa

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[0040] The invention also provides monoclonal antibodies to NPC1.

[0041] Examples of anti-NPC1 antibody amino acid sequences of variable regions for light and heavy chains include:

401-variable light chain (SEQ ID NO:3) (mouse)

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQKSPQLLVYNAKTLVEAVPSR
FSGSGSGTQFSLKINSLQPEDFGTYCYCQHHYGSPWTFGGGTKLEIK,

401-variable heavy chain (SEQ ID NO:4) (mouse)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEQGLEWIGRIDPANGNTEYD
TKFQGKATITADTSSNTAYLQLSSLTSEDTAIVYYCSRGIYWGRGTTTLTVSS,

548-variable light chain (SEQ ID NO:5) (mouse)

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQKSPQLLVYNAKTLAEGVPSR
FSGSGSGTQFSLKINSLQPEDFGIYYCQHHYGSPWAFGGGTKLEIK,

548-variable heavy chain (SEQ ID NO:6) (mouse)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEEGLEWIGRIDPADGNTEYV
PKFQGKATITADTFSTNTVYLQLSGLTSEDTAIVYYCSRGIYWGGTTTLTVSS,

952-variable light chain (SEQ ID NO:7) (mouse)

DIVMTQSHKFMSTSVGDRVSICTKASQDVNTAVVWYQQKPGQSPKLLIYWASTRHTGVPDR
FTGSGSGTDFTLTISVQAEDLALYYCQQHYTSPWTFGGGTKLEIK, and

952-variable heavy chain (SEQ ID NO:8) (mouse)

DVQLQESGPDLVKPKQSLSLTCTVTGYSITSGYSWHWIRQFPGNRLEWMDYIHYSGSINYN
PSLKSRIISITRDTSKNQFFLQLNSVTTEDTATYYCARWGATGFDYWGQGTTLTVSS.

[0042] Examples of bispecific Ab design include the following. The underlined region designates glycine-rich linker polypeptide. The bold region designates fusion linker polypeptide. The italicized region corresponds to the constant sequences of the pMAZ-encoded heavy or light constant region sequences for human IgG1 (heavy) and kappa constant domain (light).

401-scFv-KZ52-HCN (heavy chain N-terminal fusion) (in all of the following scFv-KZ52 bispecifics: scFv is human, IgG HC and LC constant domains are human IgG1, IgG VH and VL domains are mouse) (SEQ ID NO:9)

EVQLLESGGGLVKPGGSLRLSCAASGFTLINYRMNWRQAPGKGLEWVSSISSSSSYIHYA
DSVKGRFTISRDNALNSLYLQMNSLRAEDTAIVYYCVREGPRATGYSMADVFDIWGQGMVT
VSSGGGGSGGGSGGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ

QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSAPLTF
 GGGTKVEIK**GGGSAGSAGSAGSGGS**EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVW
 KQRPEQGLEWIGRIDPANGNTEYDTKFQGKATITADTSSNTAYLQLSSLTSEDVAVYYCSR
 GYYWGRGTTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
 CPPCPAPELLGRPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
 KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK,

401-scFv-KZ52-LCN (light chain N-terminal fusion) (SEQ ID NO:10)

EVQLLES GGGLVKPGGSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSYIHYA
 DSVKGRFTISRDN AENSLYLQMNSLR AEDTAVYYCVREGPRATGYSMADVFDIWGQGMVT
 VSSGGGGSGGGSGGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ
 QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSAPLTF
 GGGTKVEIK**GGGSAGSAGSAGSGGS**DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQ
 QKQKSPQLLVYNAKTLVEAVPSRFSGSGSGTQFSLKINSLQPEDFGTYQCQHHYGSPTWF
 GGGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
 QESVTEQDSKDSTYSLSSLTTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC,

401-scFv-KZ52-HCC (heavy chain C-terminal fusion) (SEQ ID NO:11)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVWKQRPEQGLEWIGRIDPANGNTEYD
 TKFQGKATITADTSSNTAYLQLSSLTSEDVAVYYCSRGYWGRGTTTLTVSSASTKGPSVFP
 LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGRPSVFLFPPKPKD
 TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
 HNHYTQKSLSLSPGK**GGGSAGSAGSAGSGGS**EVQLLES GGGLVKPGGSLRLSCAASGFTLIN
 YRMNWVRQAPGKGLEWVSSISSSSYIH YADSVKGRFTISRDN AENSLYLQMNSLR AEDTA
 VYYCVREGPRATGYSMADVFDIWGQGMVTVSSGGGGSGGGSGGGGSELVMTQSPDSLAV
 SLGERATINCKSSQSVLYSSNNKSYLAWYQKPGQPPKLLIYWASTRESGVPDRFSGSGSG
 TDFTLTISSLQAEDVAVYYCQQYYSAPLTFGGGTKVEIK,

401-scFv-KZ52-LCC (light chain C-terminal fusion) (SEQ ID NO:12)

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQGKSPQLLVYNAKTLVEAVPSR
 FSGSGSGTQFSLKINSLOPEDFGTYCYQHGYGSPWTFGGGKLEIKRTVAAPSVFIFPPSD
 EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK
 ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**GGSAGSAGSAGSGGSE**VQLLESGGGLVKPG
 GSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSSYIHYADSVKGRFTISRDAE
 NSLYLQMNSLRAEDTAVYYCVREGPRATGYSMADVFDIWQGTMVTVSSGGGGSGGGSGG
GGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQQKPGQPPKLLIYWAS
 TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYSAPLTFGGGKVEIK,

548-scFv-KZ52-HCN (heavy chain N-terminal fusion) (SEQ ID NO:13)

EVQLLESGGGLVKPGGSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSSYIHYA
 DSVKGRFTISRDAENSLYLQMNSLRAEDTAVYYCVREGPRATGYSMADVFDIWQGTMVT
 VSSGGGGSGGGSGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ
 QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYSAPLTF
 GGGKVEIK**GGSAGSAGSAGSGGSE**VQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHV
 KQRPEEGLEWIGRIDPADGNTEYVPKFQGKATITADTFSTNTVYLQSLGTS EDTAVYYCSR
 GYYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
 CPPCPAPELLGRPSVFLEPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
 KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK,

548-scFv-KZ52-LCN (light chain N-terminal fusion) (SEQ ID NO:14)

EVQLLESGGGLVKPGGSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSSYIHYA
 DSVKGRFTISRDAENSLYLQMNSLRAEDTAVYYCVREGPRATGYSMADVFDIWQGTMVT
 VSSGGGGSGGGSGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ
 QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYSAPLTF
 GGGKVEIK**GGSAGSAGSAGSGGS**DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQ
 QKQKSPQLLVYNAKTLAEGVPSRFSGSGSGTQFSLKINSLOPEDFGIYYCQHGYGSPWAF
 GGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
 QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC,

548-scFv-KZ52-HCC (heavy chain C-terminal fusion) (SEQ ID NO:15)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVWKQRPEEGLEWIGRIDPADGNTEYV

PKFQ GKATITADTF SNTVYLQLSGLT SEDTAVYYCSRGIYWGGTTTLTVSSASTKGPSVFP
 LAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGRPSVFLFPPKPKD
 TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL
 HNHYTQKSLSLSPGK**GGSAGSAGSAGSGGSE**VQLLES GGGGLVKPGGSLRLSCAASGFTLIN
 YRMNWVRQAPGKGLEWVSSISSSSSSYIHYADSVKGRFTISRDN AENSLYLQMNSLRAEDTA
 VYYCVREGPRATGYSMADVFDIWGGQTMVTVSSGGGGSGGGSGGGGSELVMTQSPDSLAV
 SLGERATINCKSSQSVLYSSNNKSYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSG
 TDFTLTISSLQAEDVAVYYCQQYYSAPLTFGGGTKEIK,

548-scFv-KZ52-LCC (light chain C-terminal fusion) (SEQ ID NO:16)

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQKSPQLLVYNAKTLAEGVPSR
 FSGSGSGTQFSLKINS LQPEDFGIYYCQH HYGSPWAFGGGTKEIKRTVAAPSVFIFPPSD
 EQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK
 ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**GGSAGSAGSAGSGGSE**VQLLES GGGGLVKPG
 GSLRLSCAASGFTLIN YRMNWVRQAPGKGLEWVSSISSSSSSYIHYADSVKGRFTISRDN A
 NSLYLQMNSLRAEDTAVYYCVREGPRATGYSMADVFDIWGGQTMVTVSSGGGGSGGGSGG
GGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQQKPGQPPKLLIYWAS
 TRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSAPLTFGGGTKEIK,

952-scFv-KZ52-HCN (heavy chain N-terminal fusion) (SEQ ID NO:17)

EVQLLES GGGGLVKPGGSLRLSCAASGFTLIN YRMNWVRQAPGKGLEWVSSISSSSSSYIHYA
 DSVKGRFTISRDN AENSLYLQMNSLRAEDTAVYYCVREGPRATGYSMADVFDIWGGQTMV
 TVSSGGGGSGGGSGGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ
 QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSAPLTF
 GGGTKEIK**GGSAGSAGSAGSGGS**DVQLQESGPD LVPKXQSLSLTCTVTGYSITSGYSWHW
 IRQFPGNRLEWMDYIHYSGSINYNPSLKSRI SITRDTSKNQFFLQLNSVTTEDTATYYCAR
 WGATGFDYWGQTTTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVT VSWN
 SGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSC
 DKTHTCPPCPAPELLGRPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGS
 FFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK,

952-scFv-KZ52-LCN (light chain N-terminal fusion) (SEQ ID NO:18)

EVQLLES GGGGLVKPGGSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSSSYIHYA
 DSVKGRFTISRDN AENSLYLQMNSLR AEDTAVYYCVREGPRATGYSMADVFDIWGQGMVT
 VSSGGGGSGGGSGGGGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ
 QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTIS SLQAEDVAVYYCQQYYSA PLTF
 GGGTKVEIK **GGGSAGSAGSAGSGGS** DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVVWYQ
 QKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTIS SVQAEDLALYYCQQHYTSPWTF
 GGGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC,

952-scFv-KZ52-HCC (heavy chain C-terminal fusion) (SEQ ID NO:19)

DVQLQESGPDLVKPXQSLSLTCTVTGYSITSGYSWHWIRQFPGNRLEWMDYIHYSGSINYN
 PSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARWGATGFDYWGQGTTLTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGRPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV
MHEALHNHYTQKSLSLSPGK **GGGSAGSAGSAGSGGSE** EVQLLES GGGGLVKPGGSLRLSCAASG
 FTLINYRMNWVRQAPGKGLEWVSSISSSSSSYIH YADSVKGRFTISRDN AENSLYLQMNSLR
 AEDTAVYYCVREGPRATGYSMADVFDIWGQGMVT VSSGGGGSGGGSGGGGSELVMTQSP
 DSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ QKPGQPPKLLIYWASTRESGVPDRFS
 GSGSGTDFTLTIS SLQAEDVAVYYCQQYYSA PLTFGGGTKVEIK, and

952-scFv-KZ52-LCC (light chain C-terminal fusion) (SEQ ID NO:20)

DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVVWYQ QKPGQSPKLLIYWASTRHTGVPDR
 FTGSGSGTDFTLTIS SVQAEDLALYYCQQHYTSPWTFGGGTKLEIKRTVAAPS VFIFPPSD
EQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC **GGGSAGSAGSAGSGGSE** EVQLLES GGGGLVKPG
 GSLRLSCAASGFTLINYRMNWVRQAPGKGLEWVSSISSSSSSYIH YADSVKGRFTISRDN A
 NSLYLQMNSLR AEDTAVYYCVREGPRATGYSMADVFDIWGQGMVT VSSGGGGSGGGSGG
GGSELVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKSYLAWYQ QKPGQPPKLLIY WAS
 TRESGVPDRFSGSGSGTDFTLTIS SLQAEDVAVYYCQQYYSA PLTFGGGTKVEIK.

[0043] Additional examples include the following. The italicized region corresponds to the constant sequences of the pMAZ-encoded heavy or light constant region sequences for

human IgG1. The underlined region designates fusion linker polypeptide. The bold region designates the sequence of human NPC2.

401-NPC2-HCC (NPC2 C-terminal fusion to heavy chain of 401 IgG1) (in all of the following bispecifics: NPC2 is human, IgG HC and LC constant domains are human IgG1, IgG VH and VL domains are mouse) (SEQ ID NO:21)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEQGLEWIGRIDPANGNTEYD
TKFQ GKATITADTSSNTAYLQLSSLTSED TAVYYCSR GYYWGRGTTLT VSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGRPSVFLFPPKPKD
TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL
HNHYTQKSLSLSPGKGGSAGSAGSAGSGGSE**EPVQFKDCGSVDGVIKEVNVSPCPTQPCQLS**
KGQSYSVNVTFTSNIQSKSSKAVVHGILMGVPVPFPPIPEPDGCKSGINCPIQDKTYSYLN
KLPVKSEYPSIKLVVEWQLQDDKNQSLFCWEIPVQIVSHL, and

548-NPC2-HCC (NPC2 C-terminal fusion to heavy chain of 548 IgG1) (SEQ ID NO:22)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEEGLEWIGRIDPADGNTEYV
PKFQ GKATITADTFSTNTVYLQLSGLTSED TAVYYCSR GYYWQGTTLT VSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGRPSVFLFPPKPKD
TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL
HNHYTQKSLSLSPGKGGSAGSAGSAGSGGSE**EPVQFKDCGSVDGVIKEVNVSPCPTQPCQLS**
KGQSYSVNVTFTSNIQSKSSKAVVHGILMGVPVPFPPIPEPDGCKSGINCPIQDKTYSYLN
KLPVKSEYPSIKLVVEWQLQDDKNQSLFCWEIPVQIVSHL.

[0044] Preferably, 548 has the following epitope, DFFVYADYHT (SEQ ID NO: 23), which corresponds to residues 502–511 of human NPC1.

[0045] Preferably, the monoclonal antibody or bispecific antibody is a human antibody or humanized antibody. “Humanized” forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin

(recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin variable domain are replaced by corresponding non-human residues. These modifications may be made to further refine antibody performance. Furthermore, in a specific embodiment, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. In an embodiment, the humanized antibodies do not comprise residues that are not found in the recipient antibody or in the donor antibody. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, e.g., 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); Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409, the contents of each of which references and patents are hereby incorporated by reference in their entirety. In one embodiment where the humanized antibodies do comprise residues that are not found in the recipient antibody or in the donor antibody, the Fc regions of the antibodies are modified as described in WO 99/58572, the content of which is hereby incorporated by reference in its entirety.

[0046] Techniques to humanize a monoclonal antibody are described in U.S. Pat. Nos. 4,816,567; 5,807,715; 5,866,692; 6,331,415; 5,530,101; 5,693,761; 5,693,762; 5,585,089; and 6,180,370, the content of each of which is hereby incorporated by reference in its entirety.

[0047] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains. See, for example, Winter et al. *Nature* 349: 293-299 (1991), Lobuglio et al. *Proc. Nat. Acad. Sci. USA* 86: 4220-4224 (1989), Shaw et al. *J. Immunol.* 138: 4534-4538 (1987), and Brown et al. *Cancer Res.* 47:

3577-3583 (1987), the content of each of which is hereby incorporated by reference in its entirety. Other references describe rodent hypervariable regions or CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain. See, for example, Riechmann et al. *Nature* 332: 323-327 (1988), Verhoeyen et al. *Science* 239: 1534-1536 (1988), and Jones et al. *Nature* 321: 522-525 (1986), the content of each of which is hereby incorporated by reference in its entirety. Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions - European Patent Publication No. 0519596 (incorporated by reference in its entirety). These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. The antibody constant region can be engineered such that it is immunologically inert (e.g., does not trigger complement lysis). See, e.g. PCT Publication No. WO99/58572; UK Patent Application No. 9809951.8. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty et al., *Nucl. Acids Res.* 19: 2471-2476 (1991) and in U.S. Pat. Nos. 6,180,377; 6,054,297; 5,997,867; 5,866,692; 6,210,671; and 6,350,861; and in PCT Publication No. WO 01/27160 (each incorporated by reference in their entirety).

[0048] Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[0049] The invention also provides pharmaceutical compositions for treating or preventing or reducing or inhibiting a filovirus infection in a subject comprising any of the bispecific antibodies or bispecific fusion constructs disclosed herein and a pharmaceutically acceptable carrier. Examples of acceptable pharmaceutical carriers include, but are not limited to, additive solution-3 (AS-3), saline, phosphate buffered saline, Ringer's solution, lactated Ringer's solution, Locke-Ringer's solution, Krebs Ringer's solution, Hartmann's balanced saline solution, and heparinized sodium citrate acid dextrose solution. The bispecific antibody or bispecific fusion construct can be administered to the subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier used can depend on the route of administration. The pharmaceutical composition can be formulated for administration by any method known in

the art, including but not limited to, oral administration, parenteral administration, intravenous administration and administration through an osmotic mini-pump.

[0050] The invention further provides an isolated nucleic acid encoding any of the bispecific antibodies or bispecific fusion constructs disclosed herein. The isolated nucleic acid can be, or comprise, a cDNA.

[0051] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

[0052] Introduction and Overview: Ebola virus (EBOV) and related filoviruses are associated with sporadic outbreaks of highly lethal hemorrhagic fever in Middle and West Africa. The ongoing regional EBOV epidemic in West Africa has underscored the urgent need for antiviral treatments and demonstrated the potential of passive immunotherapy to reverse advanced filovirus disease. However, existing monoclonal antibody (mAb) cocktails such as ZMapp are limited by a narrow spectrum of antiviral action, which stems from viral strain-specific neutralization of the highly variable entry glycoprotein, GP, by most mAbs. Accordingly, set forth herein is an immunotherapeutic strategy targeting the broadly required and highly conserved filovirus entry receptor Niemann-Pick C1 (NPC1) instead of GP. Unfortunately, anti-NPC1 mAbs that efficiently blocked GP-NPC1 interaction *in vitro* failed to neutralize viral infection in cells, presumably because of an unusual feature of filovirus receptor recognition—the GP-NPC1 interaction can occur only in cellular endosomes where both virus and receptor are likely protected from extracellular antibodies. To overcome this limitation, bispecific Abs (bsAbs) were generated that combine both antiviral and anti-receptor specificities in the same molecule. These bsAbs potently neutralized EBOV infection in a manner that required their engagement of both GP and NPC1, indicating a two-step mechanism of action in which they exploit extracellular virus particles to gain access to NPC1-containing endosomes. bsAbs that combine broadly-reactive (but non-neutralizing) anti-GP Abs with anti-NPC1 Abs are expected to afford broad-spectrum protection against filovirus infection *in vivo*.

[0053] NPC1 is required for Ebola virus infection and *in vivo* pathogenesis. Niemann-Pick C1 (NPC1), a ubiquitous multi-pass membrane protein localized to late endosomes is

required for cytoplasmic entry and infection by all filoviruses. The filovirus spike glycoprotein, GP, must engage NPC1's second luminal domain (Fig. 1A, domain C) to drive viral membrane fusion and cytoplasmic escape (Miller, *EMBO J*, 2012). As shown in Fig. 1B, NPC1-knockout mice (NPC1^{-/-}), but not their WT littermates (NPC1^{+/+}), are completely resistant to infection and killing by EBOV and MARV (Carette, *Nature* 2011; Herbert, submitted).

[0054] Mouse hybridomas were raised that express mAbs specific for human NPC1 domain C (data not shown). Screens identified two mAbs (548 and 952) that bound with high affinity to NPC1 and potently blocked its interaction with the EBOV glycoprotein GP *in vitro*. A third mAb (401) had much more modest receptor-blocking activity. Fig. 3 shows the capacity of three anti-NPC1 mAbs to block GP-NPC1 binding in an ELISA. A soluble, flag-tagged form of NPC1 domain C was pre-incubated with the indicated concentrations of each mAb, and the protein-mAb mixtures were then added to plates coated with vesicular stomatitis virus (VSV) particles bearing EBOV GP. Bound domain C was detected with an anti-flag antibody. Irrelevant isotype-matched antibodies had no effect on GP-NPC1 domain C binding (not shown).

[0055] As exemplified in Fig. 4, anti-NPC1 domain C mAbs neutralize VSV-EBOV GP infection weakly or not at all. VSV-GP particles were exposed to U2OS human osteosarcoma cells in the presence of increasing concentrations of NPC1-specific mAbs or a control GP-specific neutralizing mAb (KZ52). The number of infected cells was determined at 16 h post-infection, and normalized to that obtained in the absence of antibody (set to 100%). The NPC1-specific mAbs 548 and 401 neutralized VSV-EBOV GP infection weakly or not at all, respectively. By contrast, the previously described GP-specific neutralizing mAb KZ52 was highly effective at neutralization.

[0056] Fig. 5 illustrates engineering antibodies with dual binding specificities for GP and NPC1. In Fig. 5A, constructs encoding bsAbs were generated by fusing NPC1-specific or control IgG sequences to a single-chain variable fragment (scFv) sequence derived from the EBOV GP-specific mAb KZ52. Fusions were done in four configurations: to the N- or C-terminus of the IgG heavy chain [HC] or light chain [LC]. In Fig. 5B, bsAbs and IgGs were expressed in 293-Freestyle cells by co-transfection of HC and LC expression vectors, and purified by protein A affinity chromatography. In Fig. 5C, it is shown that bsAbs, but not NPC1-specific IgGs, can simultaneously bind to GP and NPC1 domain C. bsAbs and

IgGs were captured onto GP-coated ELISA plates and then incubated with NPC1 domain C-flag. Bound NPC1 domain C was detected with an anti-flag antibody.

[0057] It was postulated that the NPC1-specific mAbs do not neutralize EBOV entry because they cannot efficiently access NPC1-containing late endosomes. To overcome this obstacle, an exploratory panel of bispecific antibodies (bsAbs) containing both NPC1-binding (401 or 548) and GP-binding (KZ52) specificities was generated. It was reasoned that one or more of these bsAbs might be able to 'ride' virus particles into late endosomes and then bind to NPC1, thereby inhibiting viral entry by both GP-blocking and receptor-blocking mechanisms. It was found that all four purified bsAbs could bind simultaneously to EBOV GP and NPC1 (e.g., see Fig. 5C), setting the stage for infection neutralization studies.

[0058] As shown in Fig. 6, GP-NPC1 bsAbs neutralize VSV-EBOV GP infection with enhanced potency. In the left panel, GP-NPC1 bsAbs show enhanced potency at viral neutralization relative to control GP-only bsAbs. VSV-EBOV GP particles were pre-incubated with the indicated concentrations of each bsAb for 1 hour at room temperature and then allowed to infect U2OS cells. Infected cells were quantified at 16 hours post-infection and normalized to a no-antibody control (100%). In the right panel, the neutralization curves were fit to a logistic equation to extract the concentration of half-maximal neutralization ($IC_{50} \pm 95\%$ confidence intervals). The GP-NPC1 bsAbs were 10–60 fold more potent at neutralizing EBOV entry than control bsAbs in which an irrelevant (non-NPC1 binding) IgG was fused to the same scFv, derived from the GP-specific mAb, KZ52. These results indicate that the bsAbs possess an enhanced capacity to block GP-NPC1 binding within endosomes, relative to the NPC1-specific IgGs.

[0059] As shown in Fig. 6, the enhanced neutralization potency of GP-NPC1 bsAbs can be attributed to their receptor blocking activity. In the left panel, VSV-EBOV GP particles were pre-incubated with the indicated concentrations of each bsAb for 1 hour at room temperature and then allowed to infect matched U2OS cell lines expressing endogenous levels of NPC1 or over-expressing NPC1 (NPC1^{hi}). Infected cells were quantified at 16 hours post-infection and normalized to a no-antibody control (100%). In the right panel, the neutralization curves were fit to a logistic equation to extract the concentration of half-maximal neutralization ($IC_{50} \pm 95\%$ confidence intervals). The ratio of IC_{50} s for each bsAb in NPC1^{hi} vs. WT U2OS cells is shown. It was reasoned that the neutralization potency of GP-NPC1 bsAbs should be reduced in cells engineered to overexpress NPC1, because the

excess NPC1 should titrate the available NPC1-binding sites in the bsAbs and thereby increase the probability of productive GP-NPC1 binding. This is precisely what was observed. The bsAb 548-HCN' suffered a 24-fold loss in potency in U2OS-NPC1^{hi} cells, relative to WT U2OS cells. By contrast, the potency of the Ctrl-HCN' bsAb, which cannot bind to NPC1, was reduced much more modestly (~2-fold). These findings provide strong evidence that the bsAbs neutralize viral infection by deploying both GP- and NPC1-binding specificities within cells.

[0060] An additional strategy to deliver NPC1-specific IgGs to NPC1-positive late endosomes was also developed. The IgGs were fused to NPC2, a late endosomal/lysosomal host protein that interacts with NPC1 and that can autonomously traffic to late endosomes when added to cells (Naureckiene, *Science* 2000). As shown in Fig. 8, fusion of a receptor-blocking IgG to a late endosome-localizing protein facilitates viral neutralization. bsAbs comprising an NPC1-specific IgG (401 or 548) fused to NPC2, a soluble late endosomal/lysosomal protein, were expressed and purified, and examined for their capacity to neutralize VSV-EBOV GP infection, as described in Figs. 6–7. Fusion of the potent receptor-blocking IgG 548 to NPC2 afforded neutralization of infection, whereas fusion of the weak receptor-blocking IgG 401 to NPC2 did not. Therefore, direct, cellular protein-mediated delivery of NPC1-specific IgGs to NPC1+ endosomes is also a feasible strategy for targeting NPC1 and preventing NPC1-mediated filovirus entry into cells.

[0061] The NPC1-targeting bispecific antibody approaches described herein provide an unprecedented opportunity for anti-filovirus therapeutics with increased antiviral breadth.

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What is claimed is:

1. A method of treating or preventing or reducing or inhibiting a filovirus infection in a subject comprising administering to the subject a bispecific antibody or a bispecific fusion construct that binds to Niemann-Pick C1 (NPC1) receptor in an amount effective to treat or prevent or reduce or inhibit a filovirus infection in a subject.
2. The method of claim 1, wherein the bispecific antibody or bispecific fusion construct combines both antiviral and anti-NPC1 specificities in the same molecule.
3. The method of claim 1, wherein the bispecific antibody or bispecific fusion construct binds to NPC1 and to filovirus glycoprotein (GP).
4. The method of claim 1, wherein the bispecific antibody is generated by fusing a NPC1-specific sequence to a single-chain variable fragment (scFv) sequence derived from EBOV GP-specific monoclonal antibody KZ52.
5. The method of claim 4, wherein fusion is to a N- or C-terminus of an IgG heavy chain or light chain.
6. The method of claim 1, wherein the bispecific antibody or bispecific fusion construct combines an anti-NPC1 specificity with an endosomal targeting functionality.
7. The method of claim 1, wherein the bispecific antibody or bispecific fusion construct binds to NPC1 and to Niemann-Pick C2 (NPC2).
8. The method of claim 1, wherein the bispecific antibody is generated by fusing heavy and light chains of a NPC1-specific sequence to variable VH and VL domains of a delivery antibody to generate a dual-variable domain Ig.
9. The method of claim 8, wherein the delivery antibody targets a filovirus glycoprotein (GP) or a cell-surface marker.

10. The method of any of claims 1-9, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.
11. The method of any of claims 1-9, wherein the anti-NPC1 amino acid sequence of a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8.
12. The method of any of claims 1-9, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:3 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:4.
13. The method of any of claims 1-9, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:5 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:6.
14. The method of any of claims 1-9, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:7 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:8.
15. The method of any of claims 1-5, wherein the bispecific antibody comprises the amino acid sequence set forth in any one of SEQ ID NOs:9-20.
16. The method of claim 1, 6 or 7, wherein the bispecific fusion construct comprises the amino acid sequence set forth in SEQ ID NO:21 or SEQ ID NO:22.
17. The method of any of claims 1-16, wherein the subject is infected with a filovirus.
18. The method of any of claims 1-16, wherein the subject is at risk for infection with a filovirus.

19. The method of claim 18, wherein the subject is a family member or healthcare worker in an area of an outbreak of a filovirus infection.
20. The method of claim 18, wherein the subject is a medical personnel, first responder or military personnel potentially exposed or exposed to a filovirus as the result of bioterrorism or biological warfare.
21. The method of claim 18, wherein the subject is a biosafety level 3/4 laboratory personnel or animal worker potentially exposed or exposed to a filovirus.
22. The method of any of claims 1-21, wherein the filovirus is an Ebola virus.
23. The method of claim 22, wherein the Ebola virus species is *Zaire ebolavirus* or *Sudan ebolavirus*.
24. The method of any of claims 1-21, wherein the filovirus is a Marburg virus, a Bundibugyo virus, a Sudan virus, a Ravn virus or a Lloviu virus.
25. The method of any of claims 1-24, wherein the subject is a mammal.
26. The method of any of claims 1-25, wherein the subject is a human.
27. A bispecific antibody or a bispecific fusion construct that binds to Niemann-Pick C1 (NPC1) receptor for treating or preventing or reducing or inhibiting a filovirus infection in a subject.
28. The bispecific antibody or bispecific fusion construct of claim 27, wherein the bispecific antibody or bispecific fusion construct combines both antiviral and anti-NPC1 specificities in the same molecule.
29. The bispecific antibody of claim 27, wherein the bispecific antibody or bispecific fusion construct binds to NPC1 and to filovirus glycoprotein (GP).

30. The bispecific antibody of claim 27, wherein the bispecific antibody is generated by fusing a NPC1-specific sequence to a single-chain variable fragment (scFv) sequence derived from EBOV GP-specific monoclonal antibody KZ52.

31. The bispecific antibody of claim 30, wherein fusion is to a N- or C-terminus of an IgG heavy chain or light chain.

32. The bispecific antibody or bispecific fusion construct of claim 27, wherein the bispecific antibody or bispecific fusion construct combines an anti-NPC1 specificity with an endosomal targeting functionality.

33. The bispecific antibody or bispecific fusion construct of claim 27, wherein the bispecific antibody binds to NPC1 and to Niemann-Pick C2 (NPC2).

34. The bispecific antibody or bispecific fusion construct of claim 27, wherein the bispecific antibody or bispecific fusion construct is generated by fusing heavy and light chains of a NPC1-specific sequence to variable VH and VL domains of a delivery antibody to generate a dual-variable domain Ig.

35. The bispecific antibody or bispecific fusion construct of claim 34, wherein the delivery antibody targets a filovirus glycoprotein (GP) or a cell-surface marker.

36. The bispecific antibody or bispecific fusion construct of any of claims 27-35, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

37. The bispecific antibody or bispecific fusion construct of any of claims 27-35, wherein the anti-NPC1 amino acid sequence of a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8.

38. The bispecific antibody or bispecific fusion construct of any of claims 27-35, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises

the amino acid sequence set forth in SEQ ID NO:3 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:4.

39. The bispecific antibody or bispecific fusion construct of any of claims 27-35, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:5 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:6.

40. The bispecific antibody or bispecific fusion construct of any of claims 27-35, wherein the anti-NPC1 amino acid sequence of a variable region of a light chain comprises the amino acid sequence set forth in SEQ ID NO:7 and a variable region of a heavy chain comprises the amino acid sequence set forth in SEQ ID NO:8.

41. The bispecific antibody of any of claims 27-31, wherein the bispecific antibody comprises the amino acid sequence set forth in any one of SEQ ID NOs:9-20.

42. The bispecific fusion construct of claim 27, 32 or 33, wherein the bispecific fusion construct comprises the amino acid sequence set forth in SEQ ID NO:21 or SEQ ID NO:22.

43. The bispecific antibody or bispecific fusion construct of any of claims 27-42, wherein the filovirus is an Ebola virus.

44. The bispecific antibody or bispecific fusion construct of claim 43, wherein the Ebola virus species is *Zaire ebolavirus* or *Sudan ebolavirus*.

45. The bispecific antibody or bispecific fusion construct of any of claims 27-42, wherein the filovirus is a Marburg virus, a Bundibugyo virus, a Sudan virus, a Ravn virus or a Lloviu virus.

46. The bispecific antibody or bispecific fusion construct of any of claims 27-45, wherein the subject is a mammal.

47. The bispecific antibody or bispecific fusion construct of any of claims 27-46, wherein the subject is a human.

48. A pharmaceutical composition for treating or preventing or reducing or inhibiting a filovirus infection in a subject comprising the bispecific antibody or bispecific fusion construct of any of claims 27-47 and a pharmaceutically acceptable carrier.

49. An isolated nucleic acid encoding the bispecific antibody or bispecific fusion construct of any of claims 27-47.

50. The isolated nucleic acid of claim 49, wherein the nucleic acid is, or comprises, a cDNA.

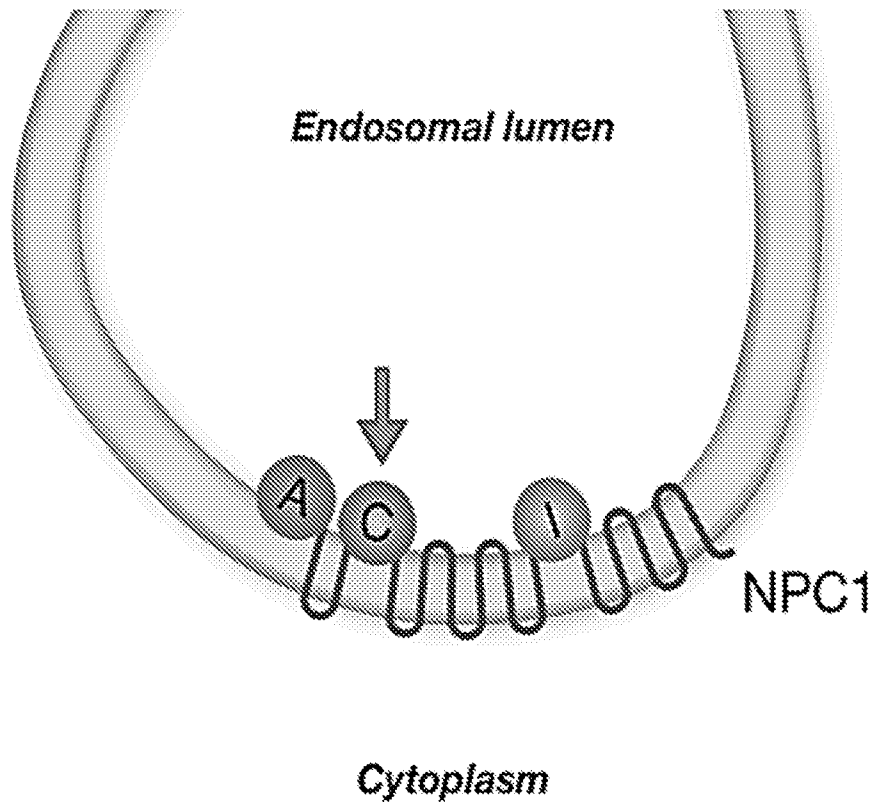


FIG. 1A

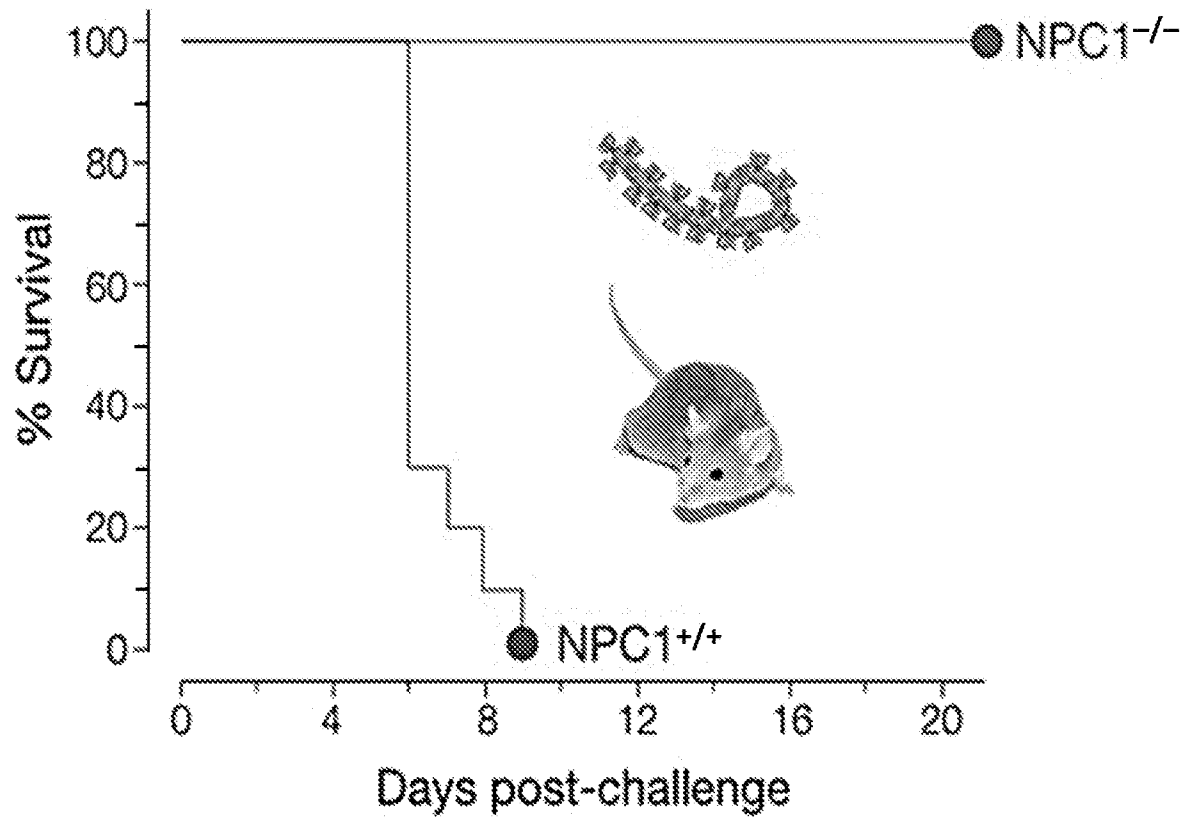


FIG. 1B

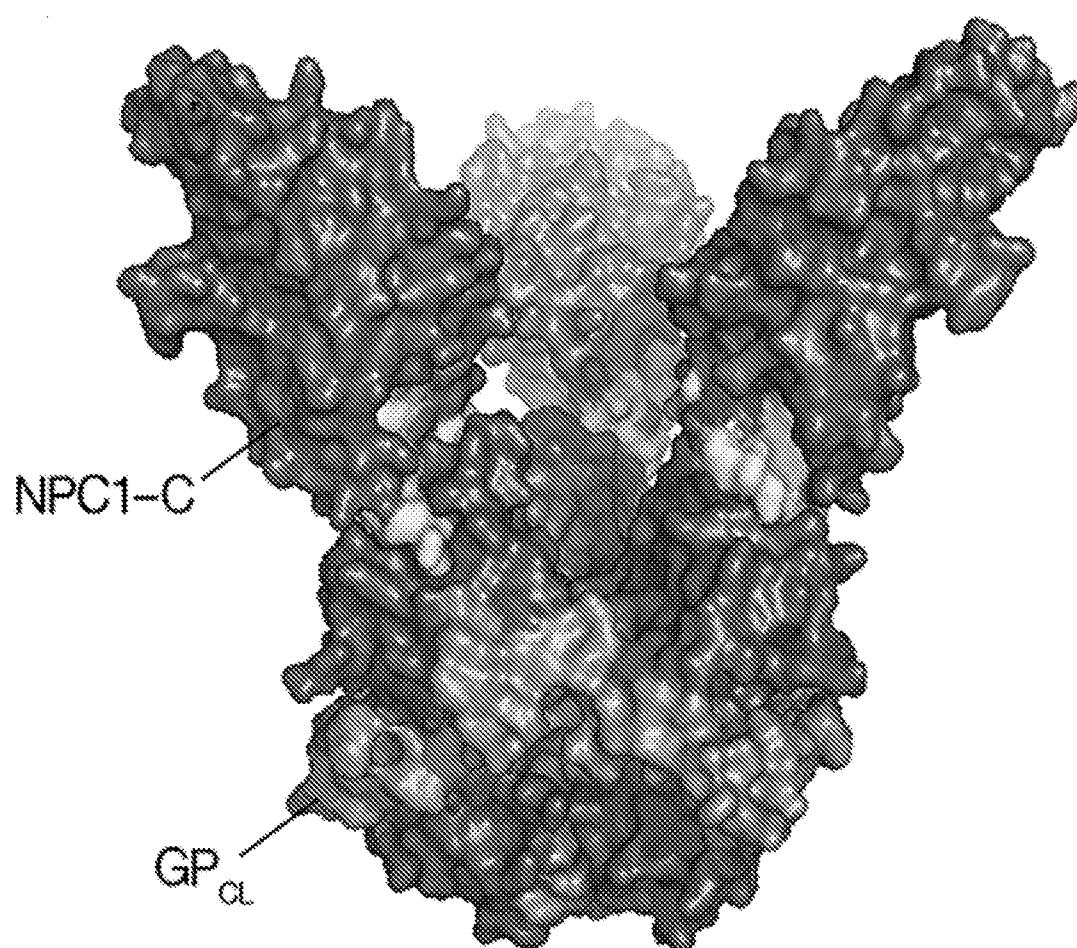


FIG. 2A

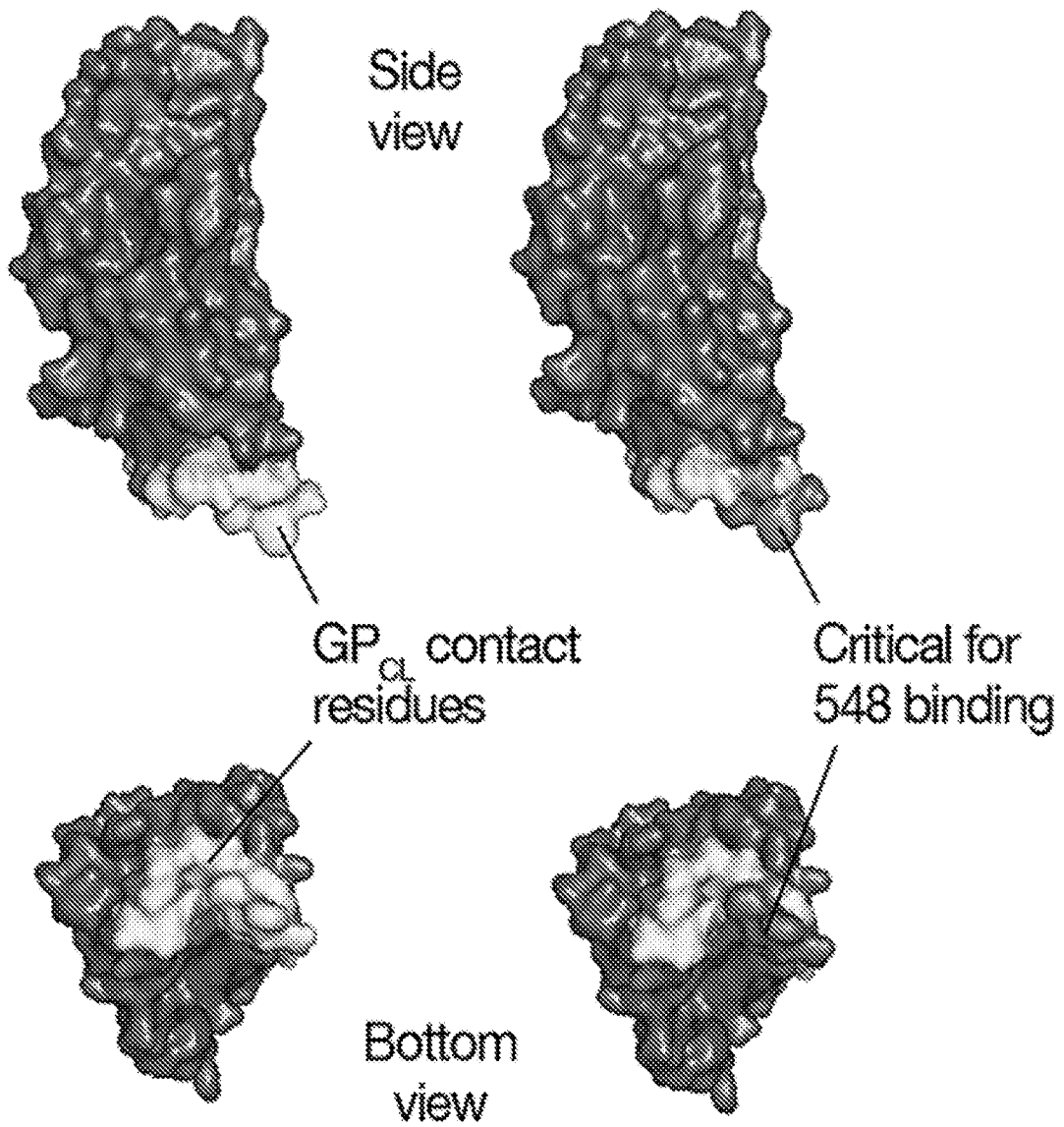


FIG. 2B

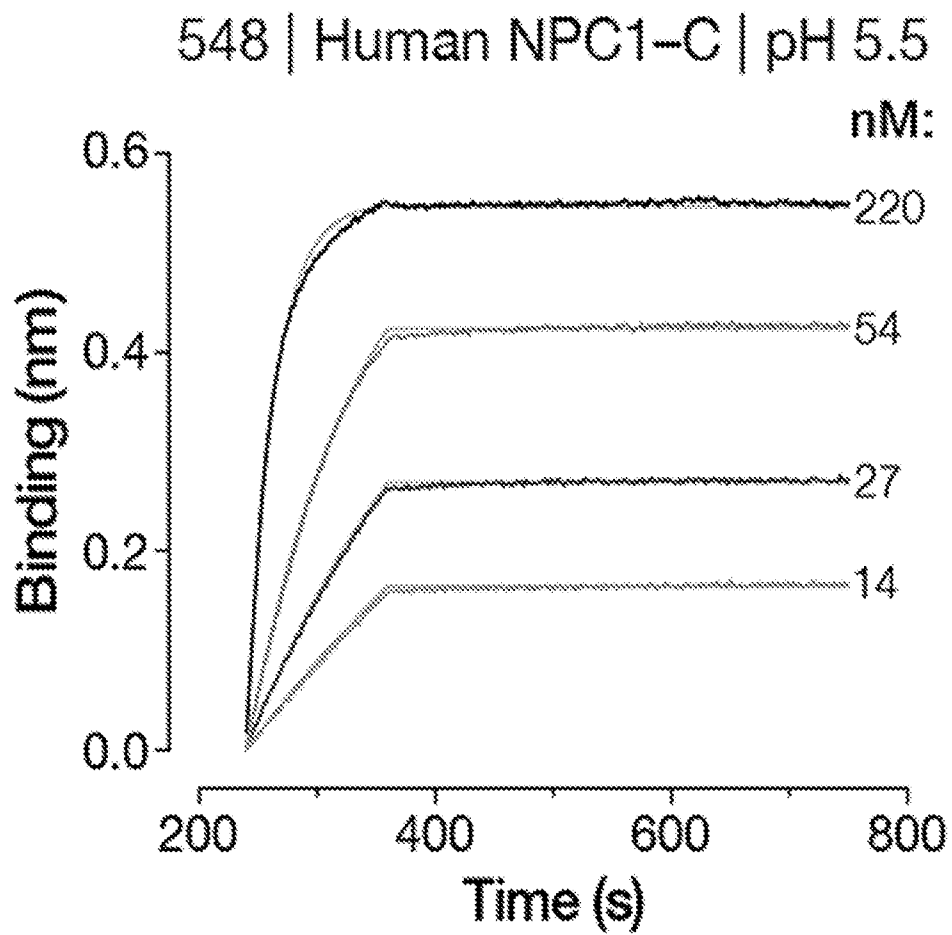


FIG. 2C

Antibody	Antigen	pH	$k_a(\text{M}^{-1} \text{s}^{-1})^a$	$k_d(\text{s}^{-1})^b$	$K_D(\text{nM})^o$
548	Human NPC1-C	7.5	$(1.870 \pm 0.004) \times 10^5$	$< 1.0 \times 10^{-7}$	< 0.001
548	Human NPC1-C	5.5	$(2.020 \pm 0.005) \times 10^5$	$< 1.0 \times 10^{-7}$	< 0.001
548	NHP NPC1-C	5.5	$(2.24 \pm 0.01) \times 10^5$	$< 1.0 \times 10^{-7}$	< 0.001
548	Mouse NPC1-C	5.5	NA ^d	NA	NA

FIG. 2D

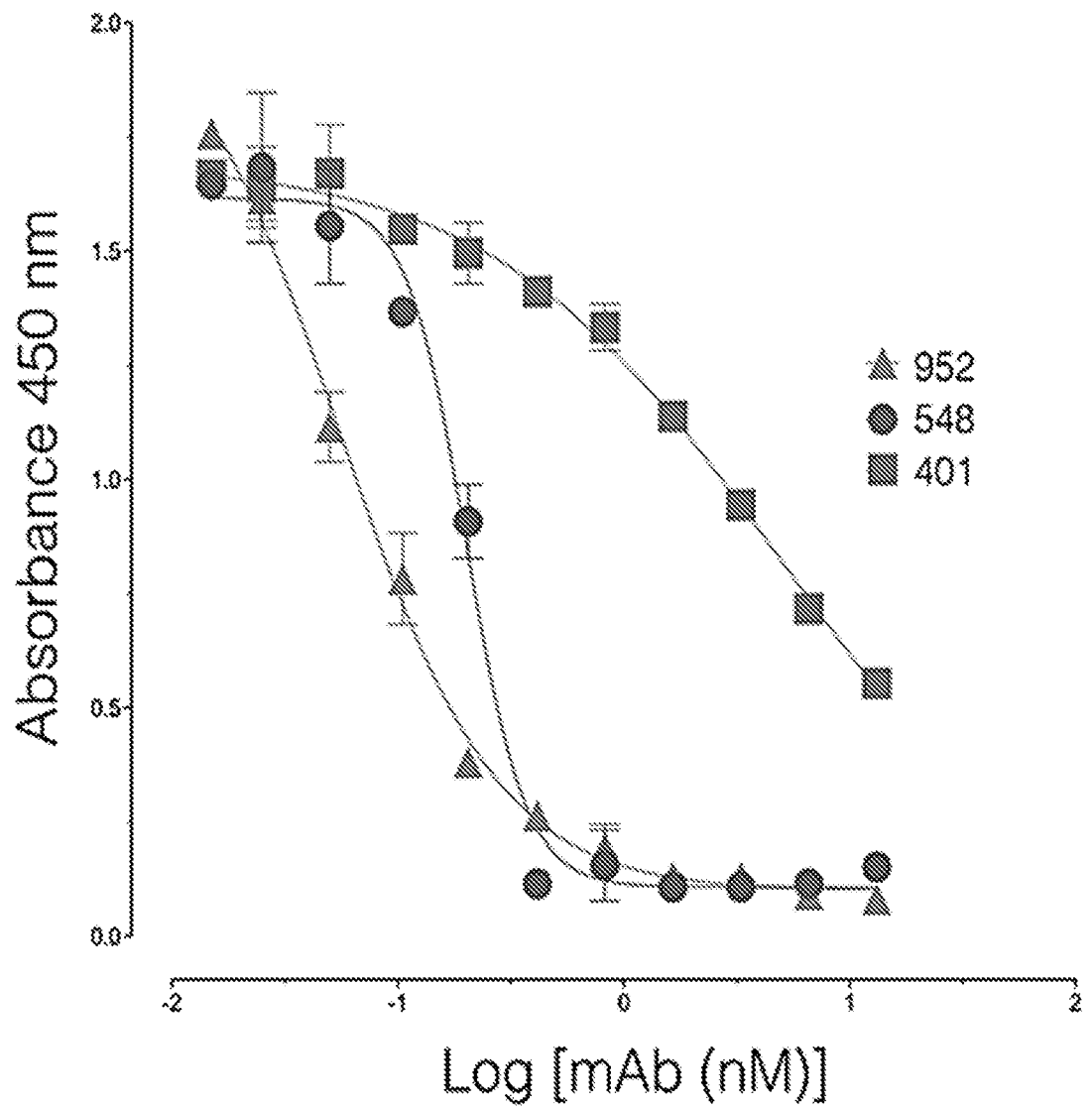


FIG. 3

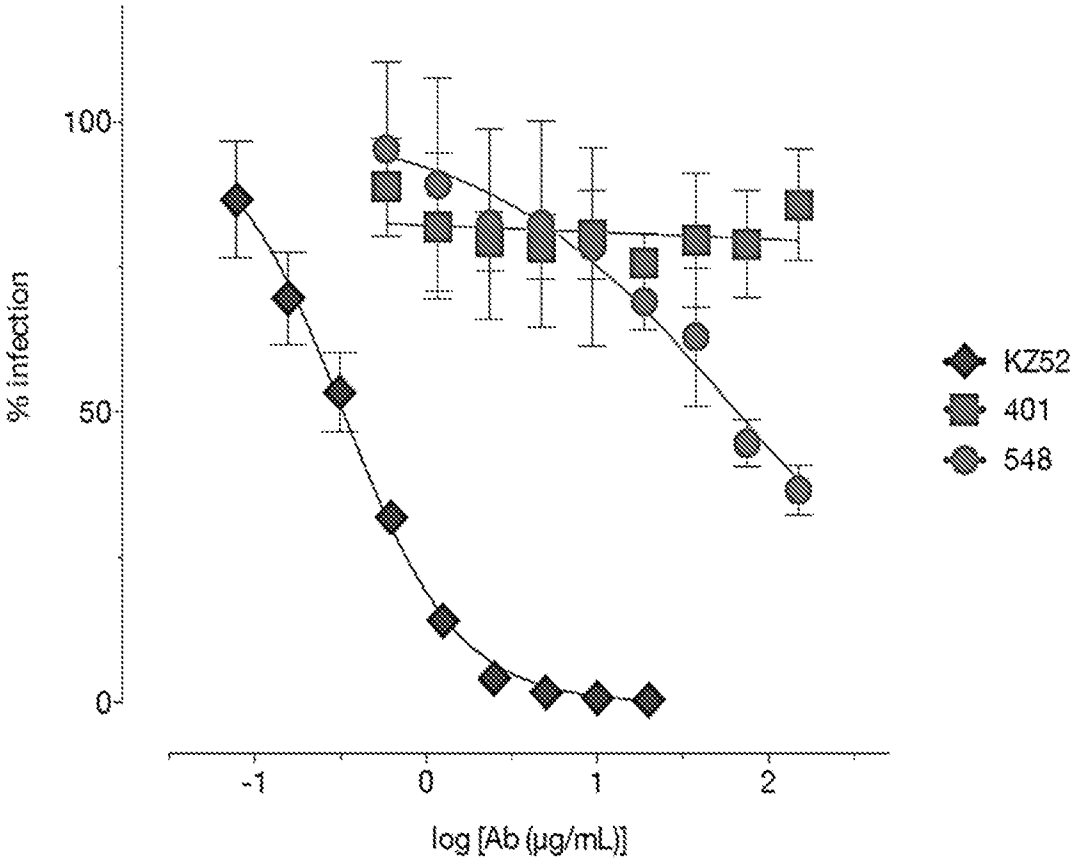


FIG. 4

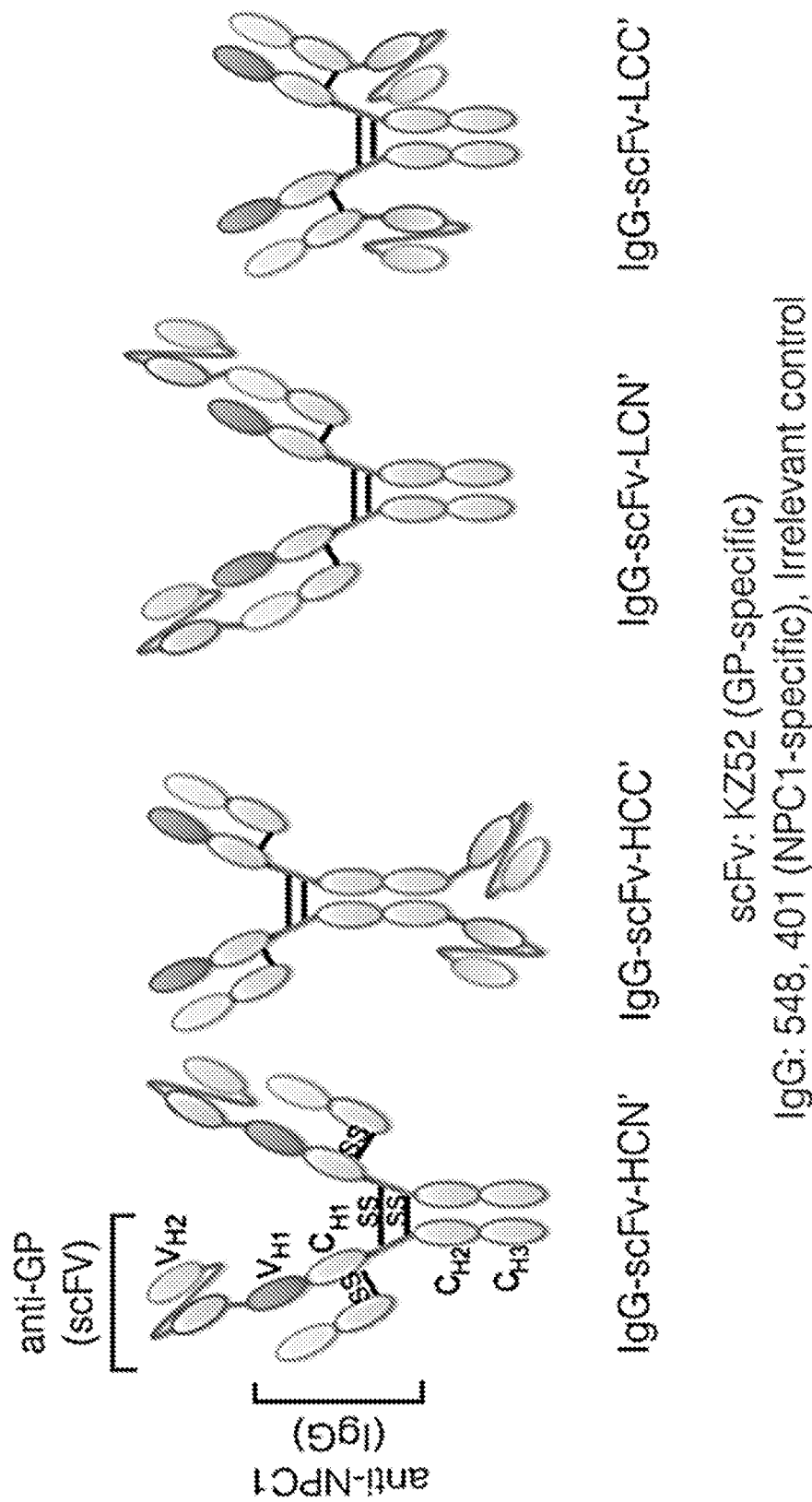


FIG. 5A

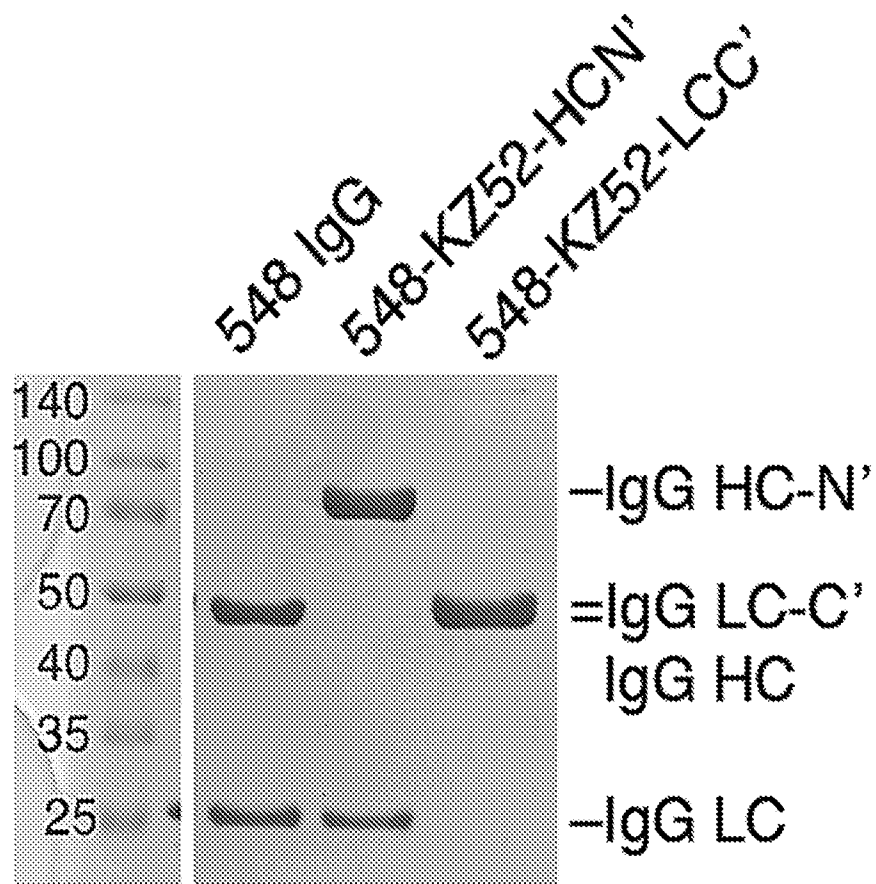


FIG. 5B

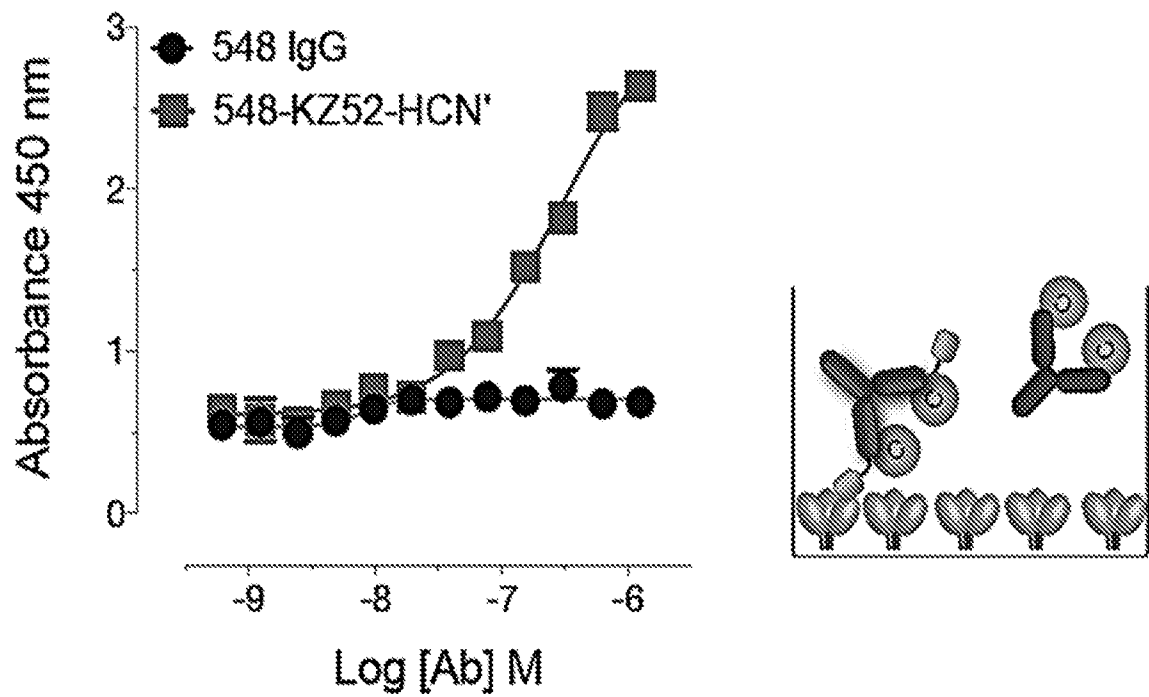


FIG. 5C

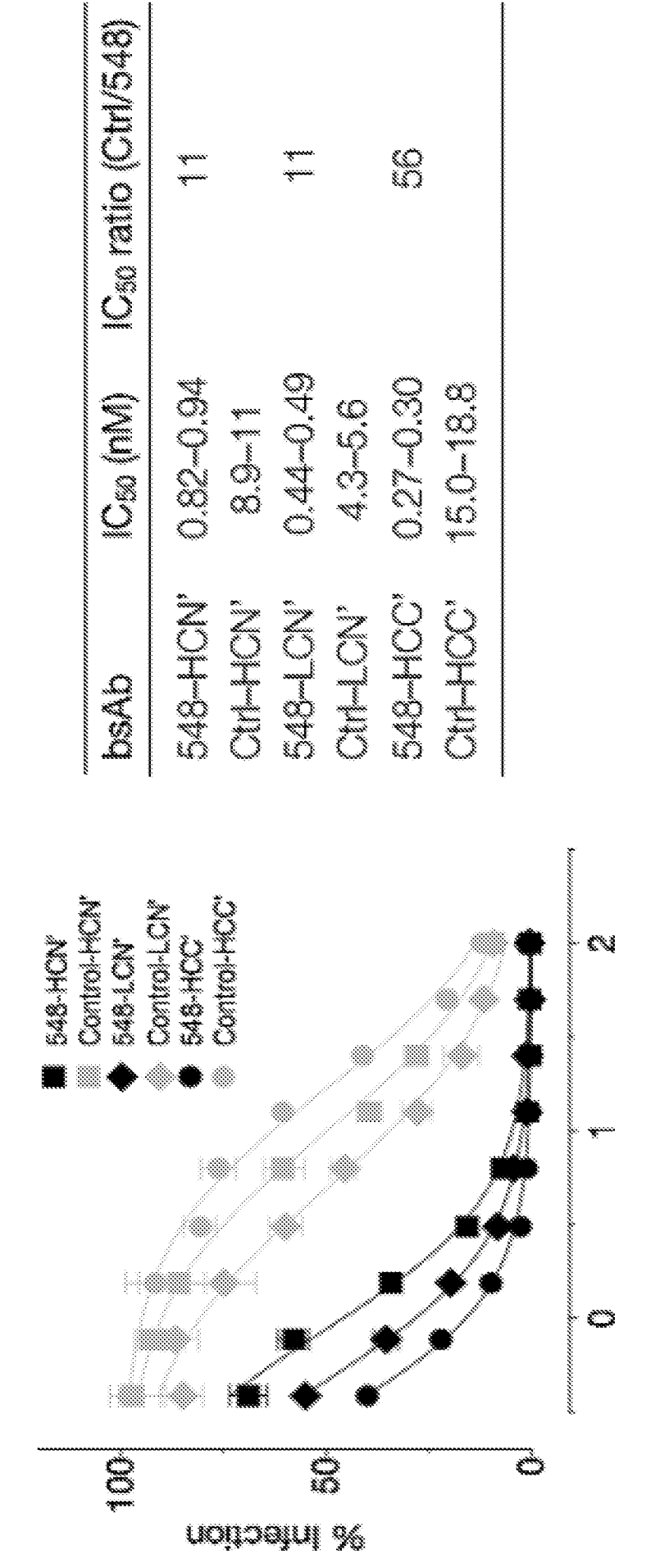
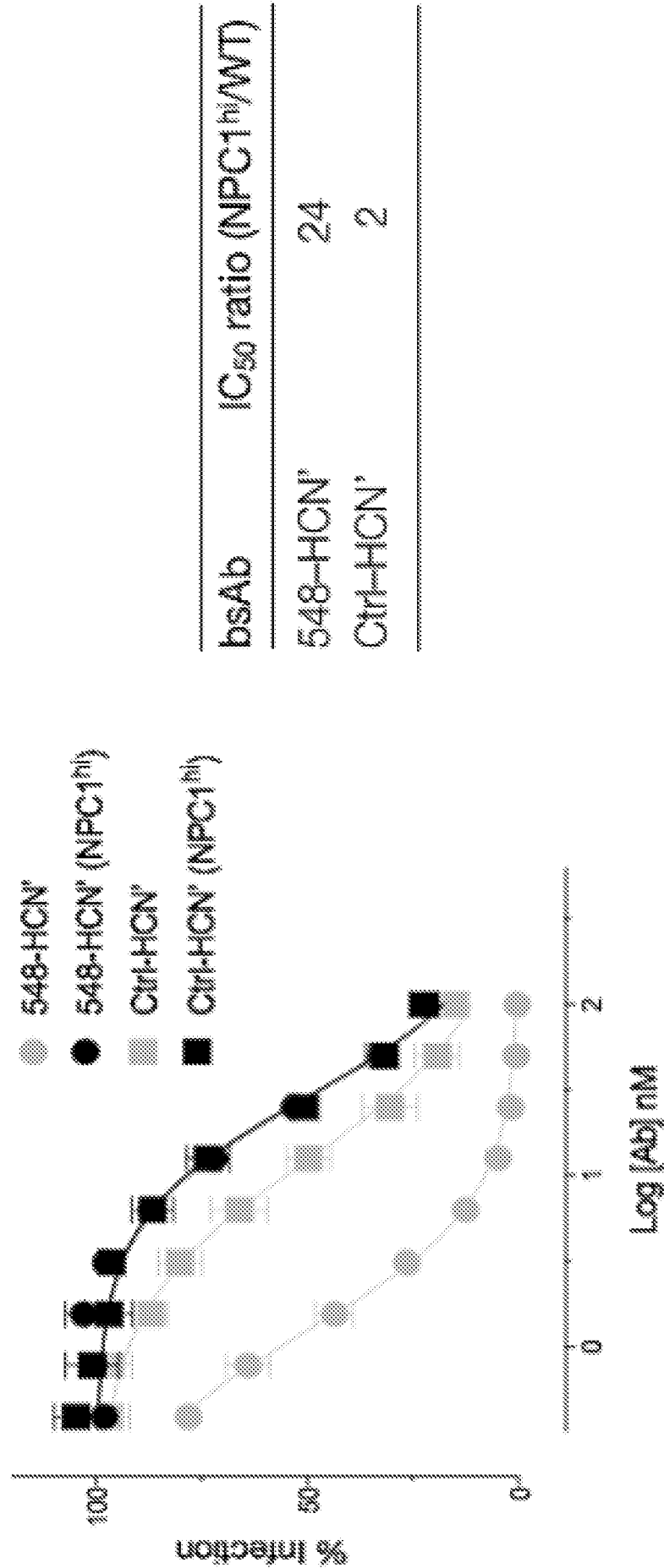


FIG. 6



bsAb	IC ₅₀ ratio (NPC1 ^{hi} /WT)
548-HCN*	24
Ctrl-HCN*	2

FIG. 7

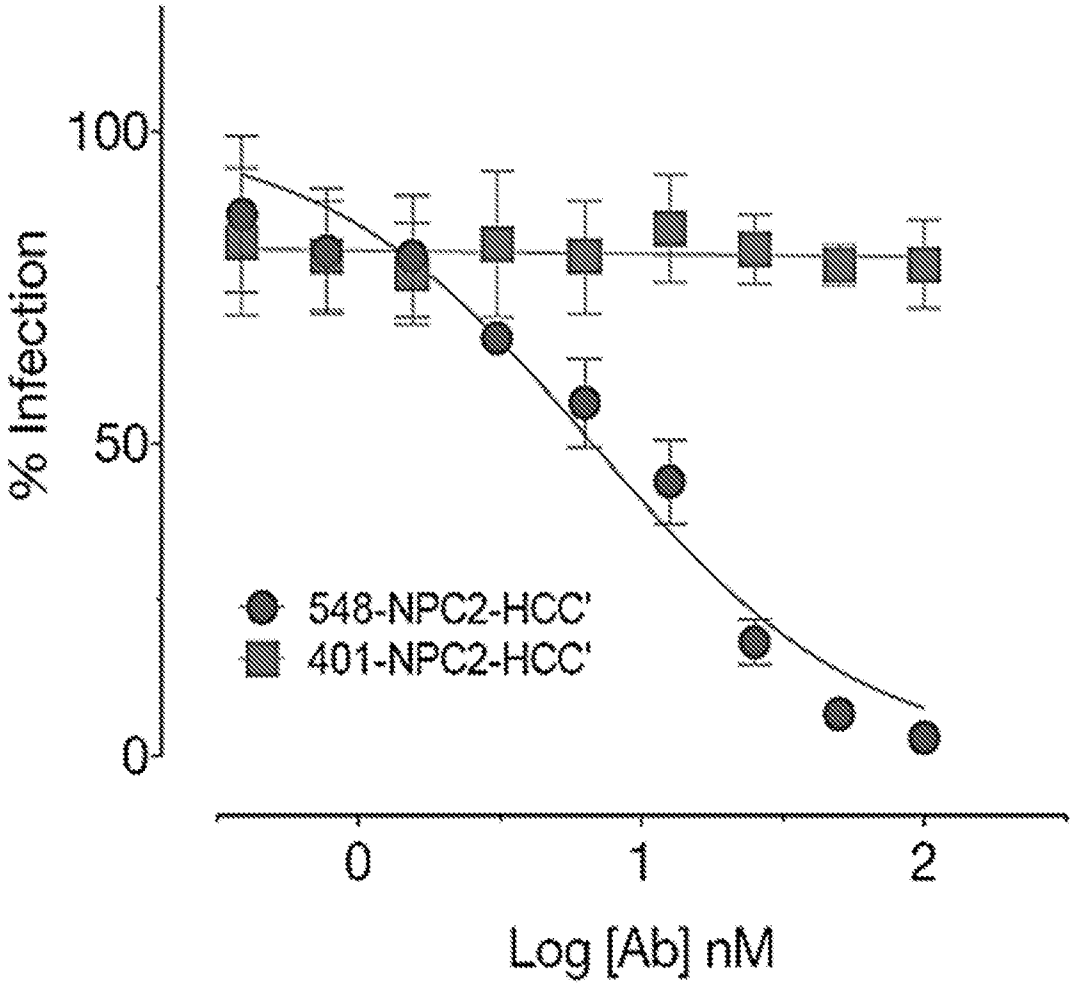


FIG. 8

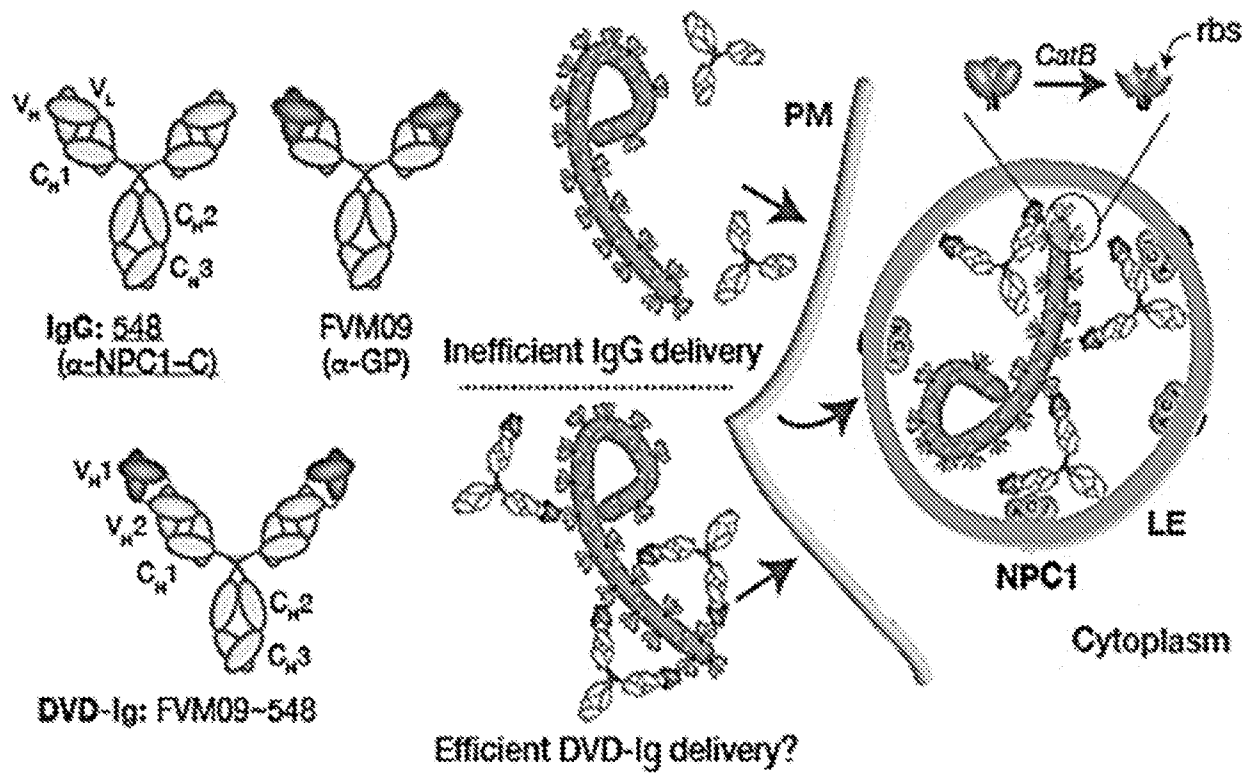


FIG. 9A

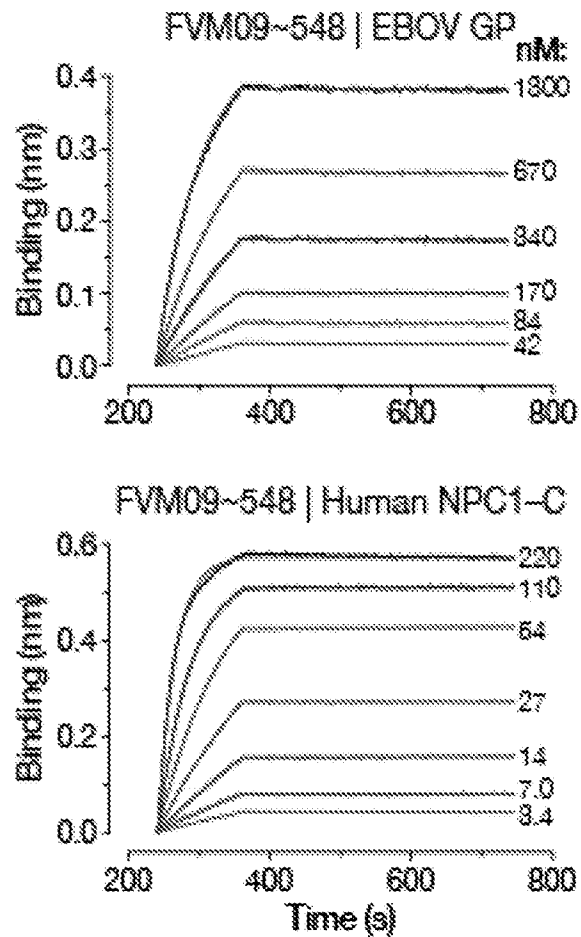


FIG. 9B

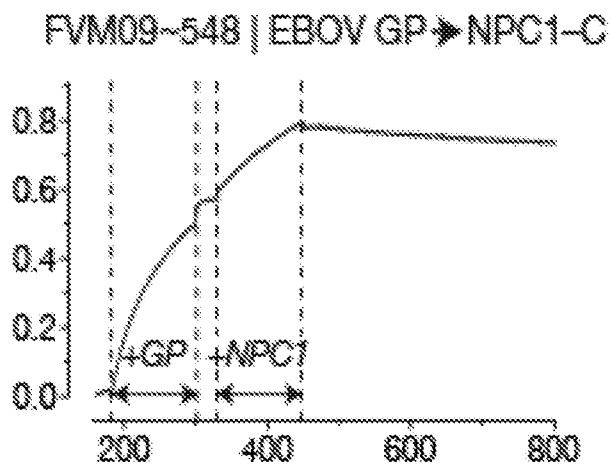


FIG. 9C

15/24

Antibody	Antigen	pH	$k_a(M^{-1} S^{-1})^a$	$k_d(S^{-1})^b$	$K_D(nM)^o$
FVM09	EBOV GP	7.5	$(1.200 \pm 0.004) \times 10^4$	$(5.5 \pm 0.1) \times 10^{-5}$	4.50 ± 0.09
548	Human NPC1-C	7.5	$(1.870 \pm 0.004) \times 10^5$	$< 1.0 \times 10^{-7}$	< 0.001
FVM09~548	EBOV GP	7.5	$(1.220 \pm 0.004) \times 10^4$	$(2.2 \pm 0.2) \times 10^{-5}$	1.79 ± 0.07
FVM09~548	Mouse NPC1-C	7.5	$(8.58 \pm 0.08) \times 10^4$	$< 1.0 \times 10^{-7}$	< 0.001

FIG. 9D

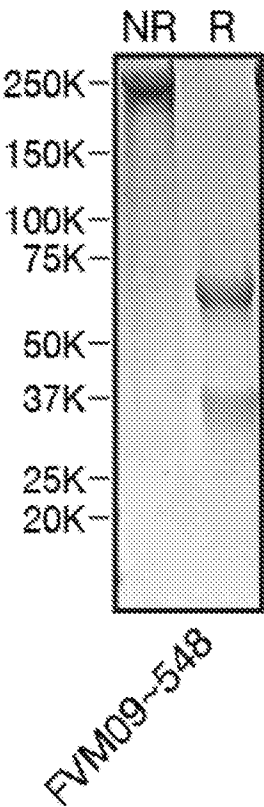


FIG. 10A

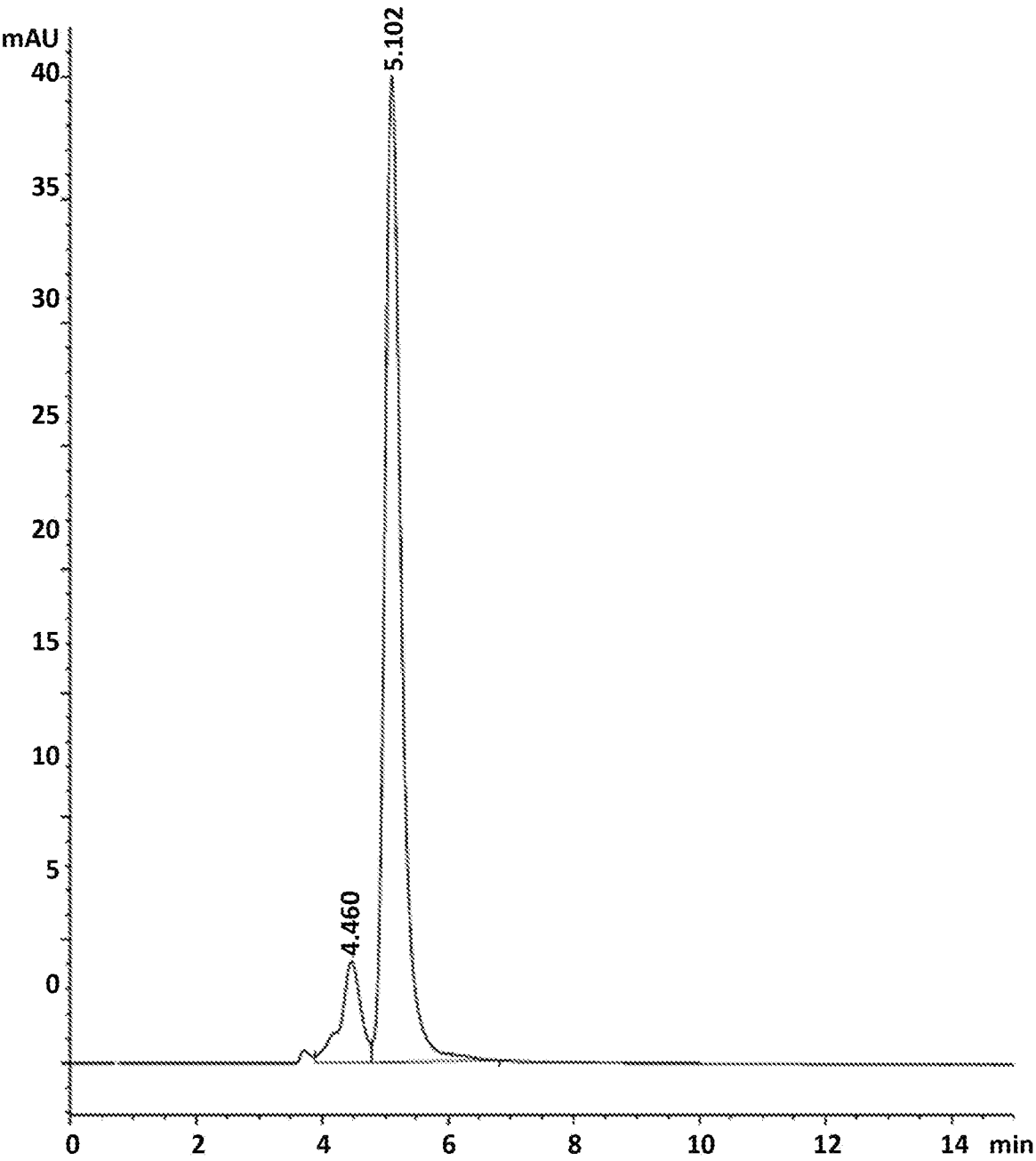


FIG. 10B

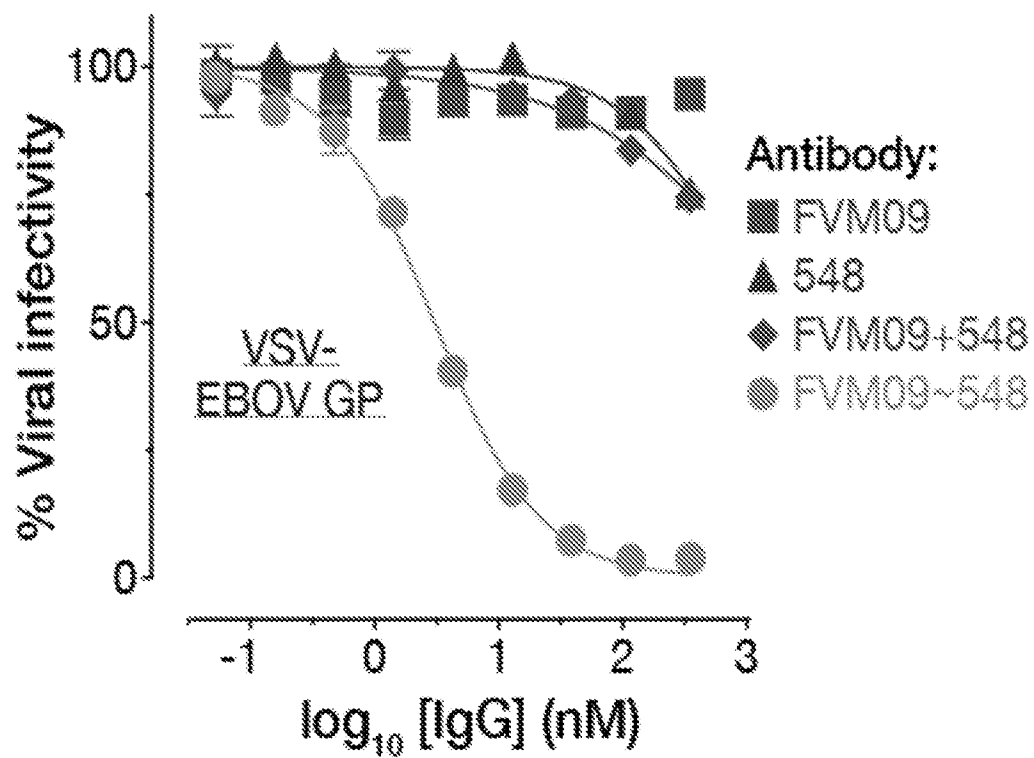


FIG. 11A

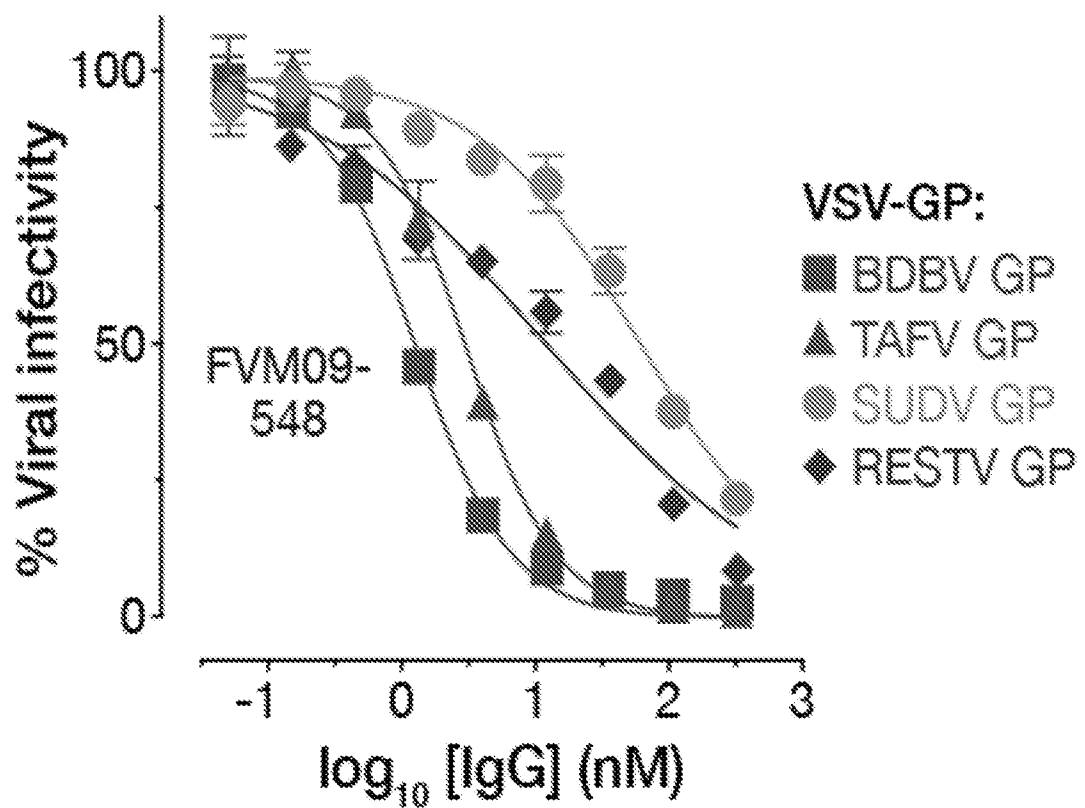


FIG. 11B

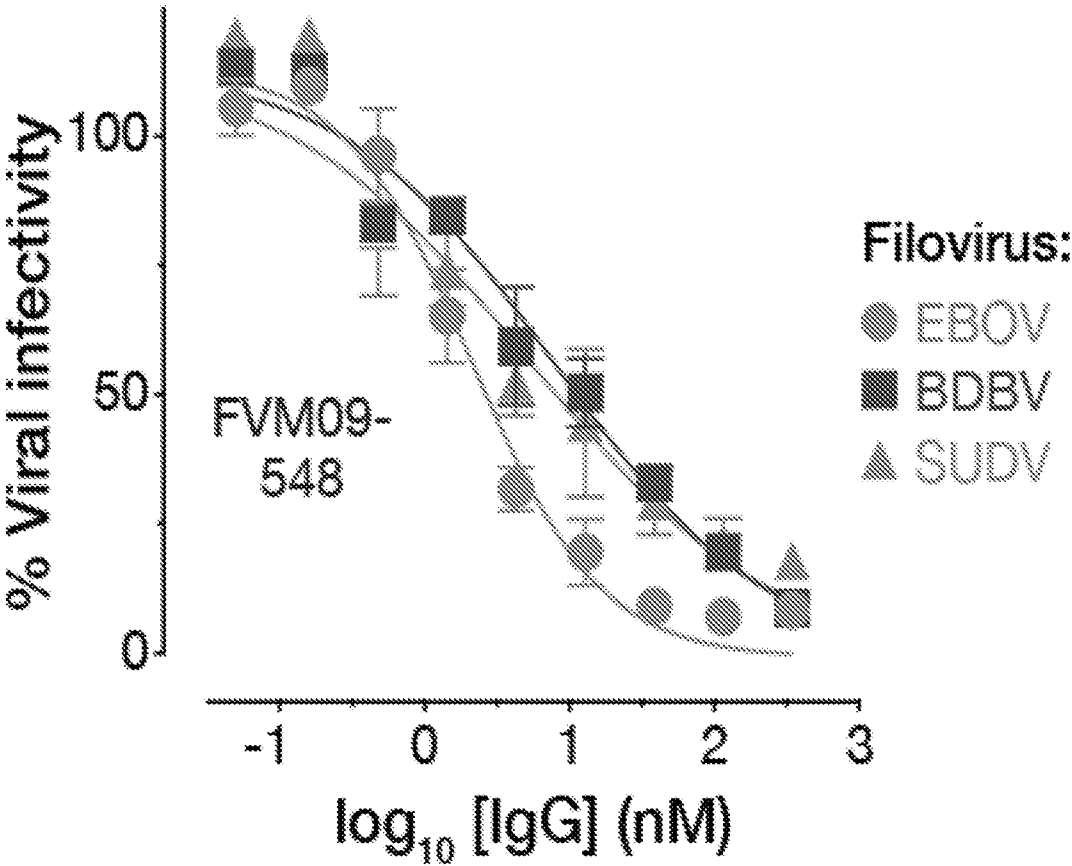


FIG. 11C

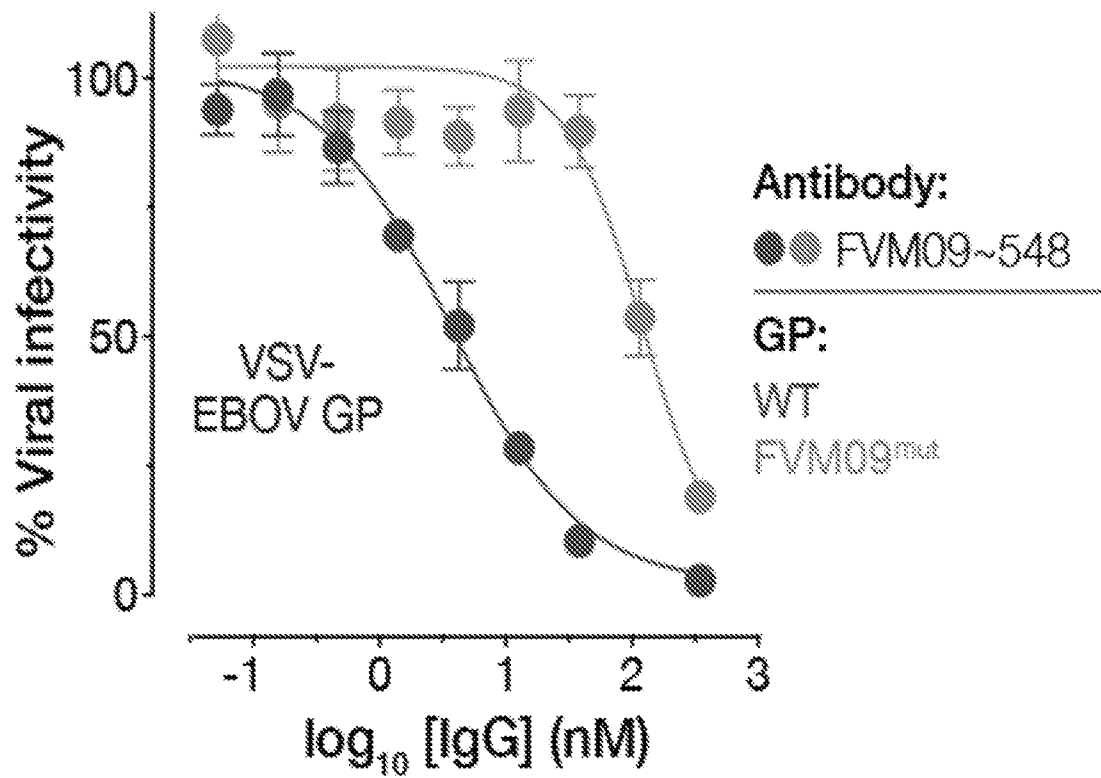


FIG. 12A

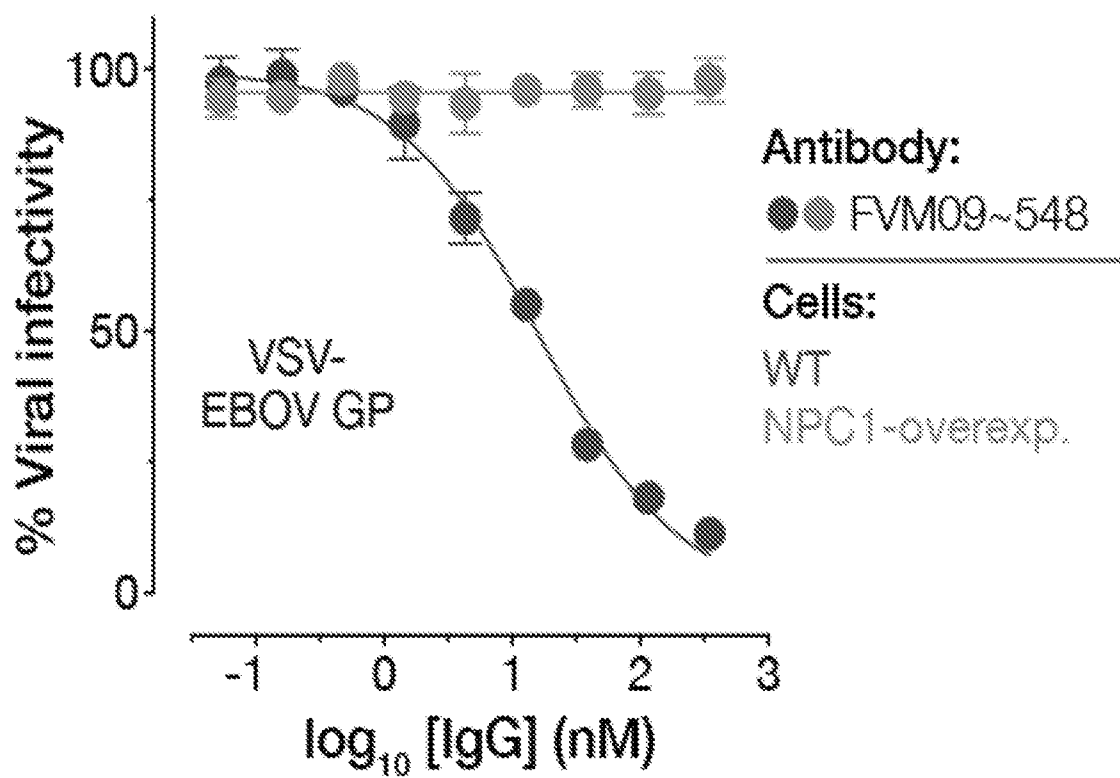


FIG. 12B

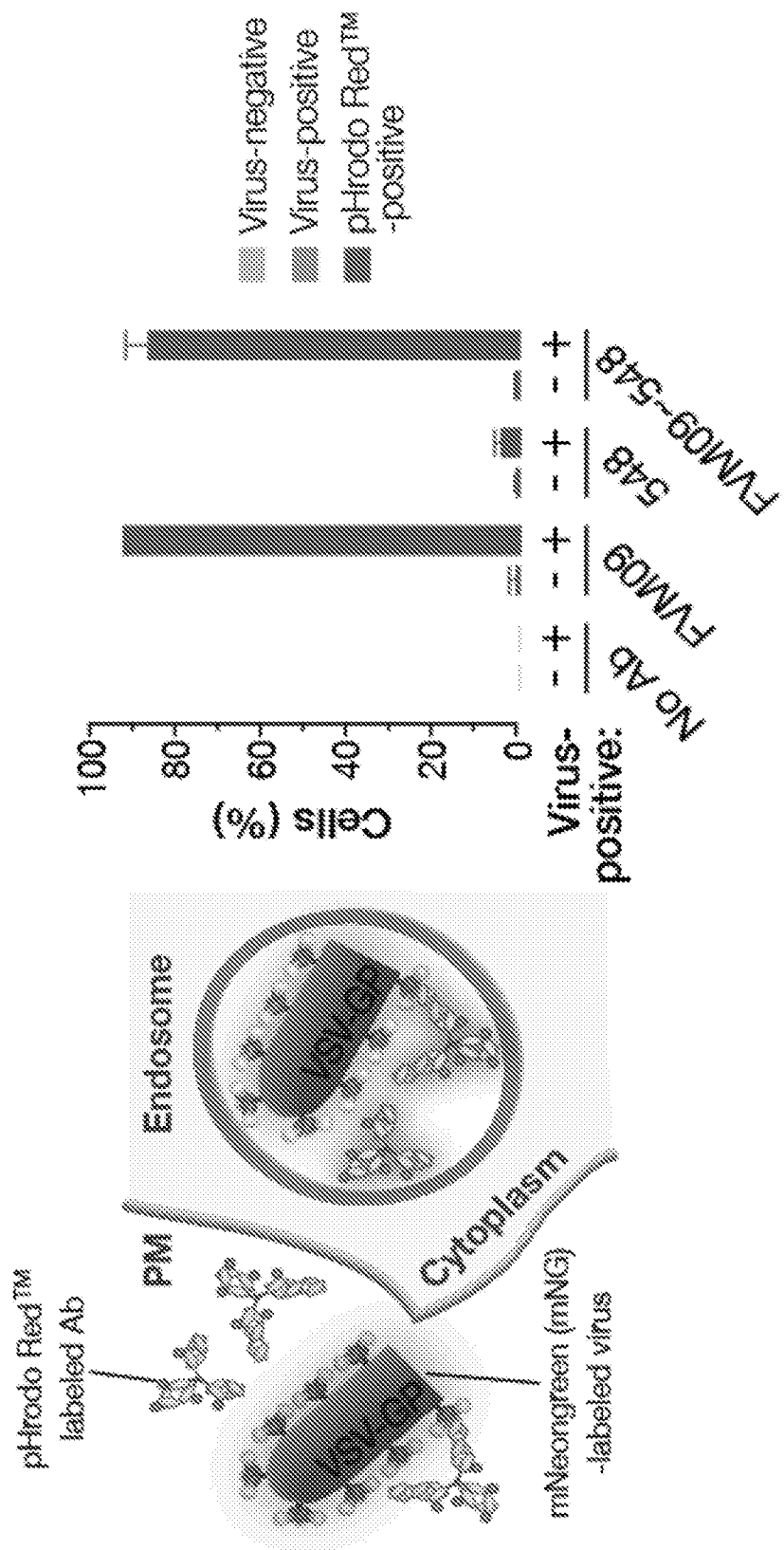


FIG. 12C

548 | *C. guereza* NPC1-C | pH 5.5

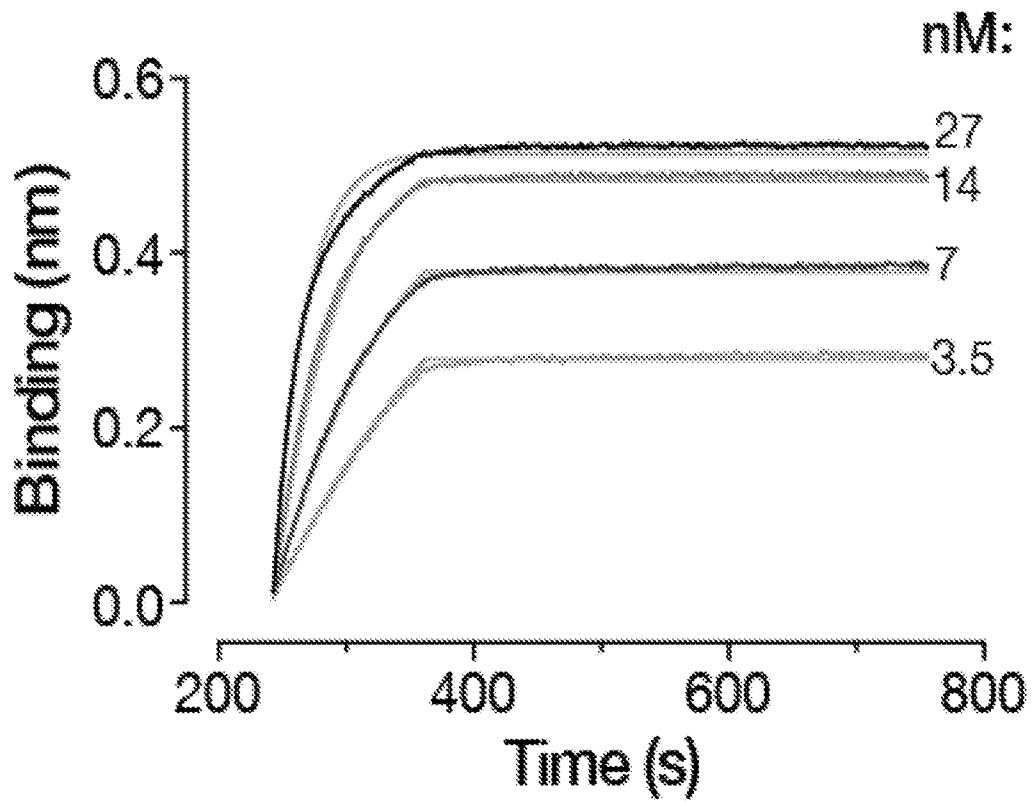


FIG. 13A

Binding competition:
C. guereza NPC1-C vs. 548 | GP_{CL}

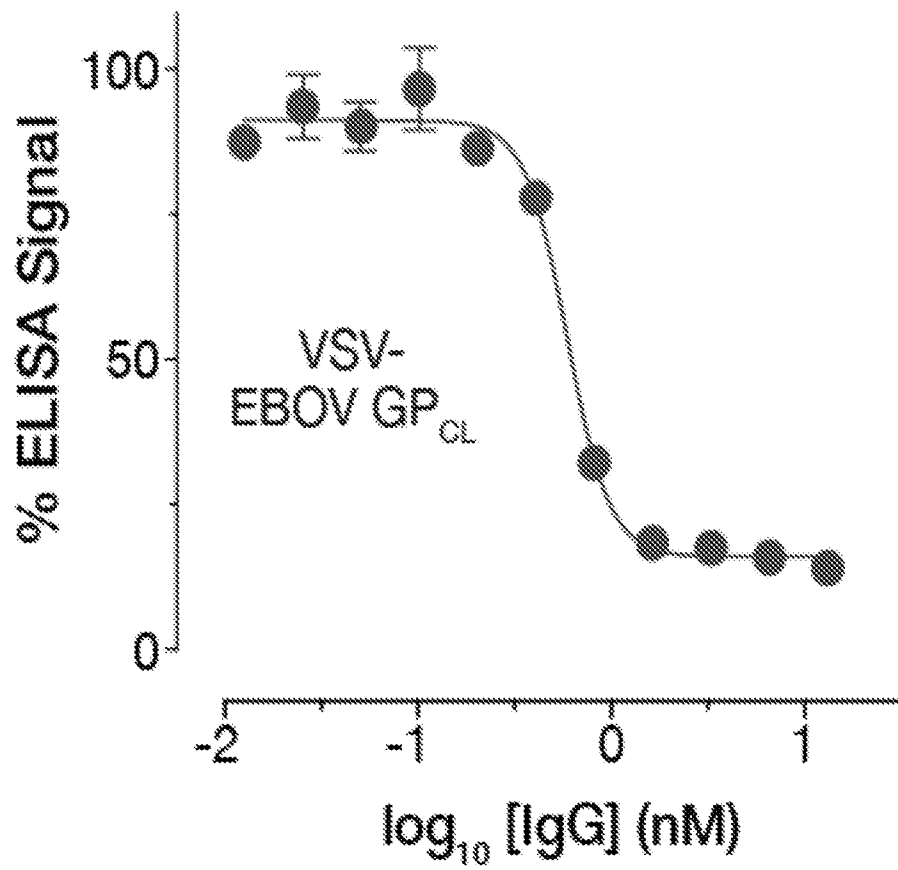


FIG. 13B

	485		514
<i>Homo sapiens</i>	LN	YFQNSHSV LDHKKGDDEF	VYADYHTHFL
<i>Macaca mulatta</i>	LN	YFQNSHSV LDHKKGDDEF	VYADYHTHFL
<i>Colobus guereza</i>	LN	YFQNSHSV LDHKKGDDEF	VYADYHTHFL
<i>Macaca fascicularis</i>	LN	YFQNSHSV LDHKKGDDEF	VYADYHTHFL

548 binding region

FIG. 13C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/030652

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; A61K 39/395; A61K 39/42; C07K 16/10; C07K 16/46 (2016.01)

CPC - A61K 2039/505; C07K 16/10; C07K 16/468; C07K 2317/31; C07K 2317/51; C07K 2317/515 (2016.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC - A61K 39/00; A61K 39/395; A61K 39/42; C07K 16/10; C07K 16/46

CPC - A61K 2039/505; A61K 39/00; A61K 39/42; C07K 16/10; C07K 16/468; C07K 2317/30; C07K 2317/31; C07K 2317/51;

C07K 2317/515; C07K 2317/52; C07K 2317/52; C07K 2317/622; C07K 2317/622; C07K 2317/76

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/130.1; 424/131.1; 424/139.1; 424/141.1; 424/159.1; 424/184.1 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar, Google, PubMed

Search terms used: ((Niemann Pick type C1) OR NPC1) W3 (antibod* OR (binding protien%)) ebolavirus OR ebola OR ebov OR (ebola virus)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0009461 A1 (GARCIA-CALVO) 14 January 2010 (14.01.2010) entire document	1-16, 27-42
A	US 2014/0329834 A1 (CUNNINGHAM et al) 06 November 2014 (06.11.2014) entire document	1-16, 27-42
A	US 2003/0092038 A1 (CARSTEADT et al) 15 May 2003 (15.05.2003) entire document	1-16, 27-42
A	MILLER et al. "Ebola virus entry requires the host-programmed recognition of an intracellular receptor," The EMBO Journal, 06 March 2012 (06.03.2012), Vol. 31, Pgs. 1947-1960. entire document	1-16, 27-42
P, A	BORNHOLDT et al. "Host-Primed Ebola Virus GP Exposes a Hydrophobic NPC1 Receptor-Binding Pocket, Revealing a Target for Broadly Neutralizing Antibodies," mBio, 23 February 2016 (23.02.2016), Vol. 7, No. 1, Pgs. 1-11. entire document	1-16, 27-42

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 August 2016

Date of mailing of the international search report

09 SEP 2016

Name and mailing address of the ISA/

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Blaine R. Copenheaver

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/030652

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-27 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/030652

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 17-26, 43-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.