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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO ENTEROVIRUS D68

(57) Abstract: The present disclosure is directed to antibodies binding to enterovirus D68 (EV-D68) and methods for use thereof.

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DESCRIPTION

HUMAN MONOCLONAL ANTIBODIES TO ENTEROVIRUS D68

PRIORITY CLAIM

This application claims benefit of priority to U.S. Provisional Application Serial Nos. 62/878,955, 62/899,503 and 63/047,330, filed July 26, 2019, September 12, 2019 and July 2, 2020, respectively, the entire contents of each application being incorporated by reference herein.

BACKGROUND

1. Field of the Disclosure

The present disclosure relates generally to the fields of medicine, infectious disease, and immunology. More particular, the disclosure relates to human antibodies binding to enterovirus D68 and methods of using such antibodies to diagnose and treat enterovirus D68 infections.

2. Background

Enterovirus-D68 (EV-D68) is a positive-sense, single-stranded RNA virus of the *Enterovirus* genus of the *Picornaviridae* family. Other common human pathogens within this genus include poliovirus, echovirus, coxsackievirus, rhinovirus, and other numbered enteroviruses such as enterovirus-A71 (Zell *et al.*, 2017). While structurally and genetically similar, these viruses cause a wide variety of childhood diseases including neonatal sepsis, myocarditis, poliomyelitis, meningitis, respiratory tract infections, and hand, foot, and mouth disease. Much like the rhinoviruses, EV-D68 is primarily a respiratory pathogen that is acid-labile and replicates best at 33 °C (Oberste *et al.*, 2004). These similarities are such that a strain of EV-D68 was even initially reported as rhinovirus 87 (Blomqvist *et al.*, 2002).

From its initial identification in a California child with pneumonia in 1962 (Schieble *et al.*, 1967) through the turn of the century, EV-D68 was detected only rarely (Khetsuriani *et al.*, 2006). Since then, EV-D68 has been recognized increasingly as a pathogen of emerging importance due to its worldwide detection in outbreaks of primarily respiratory illness in children (Xiang and Wang, 2016). The largest ever known outbreak occurred in 2014 in the United States with 1,152 confirmed cases spanning all states except for Alaska (Oermann *et al.*, 2015). This number likely grossly underestimates the actual number of cases of EV-D68 in 2014 because mild upper respiratory tract infection would not likely result in the specialized testing needed to detect this

virus. Children with asthma experienced especially severe infections (Biggs *et al.*, 2017), although a study of one institution found that EV-D68 was no more severe than non-EV-D68 enterovirus/rhinovirus infections in children with asthma (Overdahl *et al.*, 2016). Over half of the hospitalized patients were admitted to intensive care units, with 80% receiving supplemental oxygen, 23% requiring non-invasive ventilation, 8% requiring intubation and mechanical ventilation, and 1% died (Midgley *et al.*, 2015).

Concurrent with the 2014 outbreak, a small cluster of pediatric patients with acute onset flaccid paralysis and cranial nerve dysfunction was noted in Colorado (Messacar *et al.*, 2015). This syndrome has been designated acute flaccid myelitis (AFM), defined as a poliomyelitis-like illness with typically asymmetric, flaccid limb weakness and myelitis of primarily gray matter seen on spinal cord imaging (Div. of Viral Diseases, CDC, 2018). Since the 2014 outbreak, many more cases and clusters of AFM have been associated with EV-D68. To date, 74 AFM cases with positive EV-D68 testing from any patient source have been identified across 6 continents (Messacar *et al.*, 2018). While the Centers for Disease Control and Prevention (CDC) does not officially recognize EV-D68 as a proven cause of AFM (Nat'l Center for Immun. Resp. Disease), many experts find the preponderance of evidence compelling enough to consider the relationship between the two to be causal (Messacar *et al.* 2018). Because of continued worldwide outbreaks, the World Health Organization Research and Development Blueprint now lists EV-D68 as a major public health risk (Ann. Rev. of Diseases, 2018).

As EV-D68 has emerged only recently as a priority pathogen, most initial studies focused on defining the epidemiology of the virus rather than characterizing the immune response. Therefore, the study of humoral immunity to EV-D68 is nascent. The role of serum antibodies in protection from other viruses of the *Enterovirus* genus is varied. For example, three doses of inactivated poliovirus vaccine approaches 100% induction of serum neutralizing antibodies and is 80-96% effective at preventing paralytic poliomyelitis; however, vaccination does not fully prevent enteric or nasopharyngeal poliovirus shedding (Vidor *et al.*, 2018) due to its inability to induce nasal or duodenal IgA (Sutter *et al.*, 2018). Studies of rhinovirus infection show that humoral immunity to specific serotypes of virus fails to protect reliably against homotypic virus reinfection within months (Howard *et al.*, 2016). These differences in extent of protection associated with antibody responses likely are due to the differing sites of pathology for these viruses: secondary neuronal spread after initial enteral infection for polioviruses versus localized respiratory tract infection for rhinoviruses. EV-D68 infection can cause disease in the respiratory tract and is associated with disease in the central nervous system, so the role of antibodies in

protection and disease pathogenesis is likely to be complex. A better understanding this role would aid in the development of vaccines and therapies for EV-D68 infections.

SUMMARY

Thus, in accordance with the present disclosure, there is provided a method of detecting enterovirus D68 (EV-D68) infection in a subject comprising (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (b) detecting EV-D68 in said sample by binding of said antibody or antibody fragment to a EV-D68 antigen in said sample. The sample may be a body fluid, such as blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, tissue scrapings, respiratory droplets or aerosol, feces, *etc.* Detection may comprise, for example, ELISA, RIA, lateral flow assay, Western blot, and the like. The method may further comprise performing steps (a) and (b) a second time and determining a change in EV-D68 antigen levels as compared to the first assay.

The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

In another aspect, the present disclosure provides a method of treating a subject infected with enterovirus D68 (EV-D68) or reducing the likelihood of infection of a subject at risk of contracting EV-D68, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain

variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be any isotype, including without limitation IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody.

The antibody or antibody fragment may be administered prior to infection or after infection, *e.g.*, such as at or less than about 7 days, about 5 days, about 3 days, about 2 days, or about 1 day following infection. Treating may treat lung infection and/or neurologic infection, or wherein treating reduces lung infection and/or neurologic infection. Delivering may comprise antibody or antibody fragment administration systemically, by aerosol delivery, *etc.*, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment. The method may further comprise treating said subject with a second therapy, such as an anti-viral therapy or an anti-inflammatory therapy.

In yet another aspect, there is provided a monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be any isotype, including without limitation IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as

enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

5 A hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The hybridoma or engineered cell may encode an antibody or antibody fragment encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired
10 variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The hybridoma or engineered cell may contain an antibody or antibody fragment comprising light and heavy chain variable sequences according to clone-paired sequences from Table 2, comprising light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or
15 comprising light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy,
20 such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.

25 Also provided is a vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy
30 chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may

comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.

Still another embodiment comprises a vaccine formulation comprising one or more expression vectors encoding an antibody or antibody fragment characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The expression vector(s) is/are Sindbis virus or VEE vector(s). The vaccine formulation may be formulated for delivery by needle injection, jet injection, or electroporation. The vaccine formulation may further comprise one or more expression vectors encoding for a second antibody or antibody fragment, such as a distinct antibody or antibody fragment characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or

antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may
5 comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

Still a further embodiment comprises a method of protecting the health of a placenta and/or
10 fetus of a pregnant subject infected with or at risk of infection with enterovirus D68, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set
15 forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having
20 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS
25 or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.

30 The antibody or antibody fragment may be administered prior to infection or after infection, *e.g.*, such as at or less than about 7 days, about 5 days, about 3 days, about 2 days, or about 1 day following infection. The subject may be a pregnant female, a sexually active female, or a female undergoing fertility treatments. Delivering may comprise antibody or antibody fragment

administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment. The antibody or antibody fragment may increase the size of the placenta as compared to an untreated control. The antibody or antibody fragment may reduce viral load and/or pathology of the fetus as compared to an untreated control.

5 In still yet another embodiment, there is provided a method of determining the antigenic integrity, correct conformation and/or correct sequence of an enterovirus D68 antigen comprising (a) contacting a sample comprising said antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (b) determining antigenic integrity, correct conformation and/or correct sequence of said antigen by
10 detectable binding of said first antibody or antibody fragment to said antigen. The sample may comprise recombinantly produced antigen or may comprise a vaccine formulation or vaccine production batch. Detection may comprise ELISA, RIA, western blot, a biosensor using surface plasmon resonance or biolayer interferometry, or flow cytometric staining.

The first antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The first antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences
15 having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The first antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The method may further comprise performing steps (a) and (b) a second time to determine the antigenic stability of the antigen over time.

25 The method may further comprise (c) contacting a sample comprising said antigen with a second antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (d) determining antigenic integrity of said antigen by detectable binding of said second antibody or antibody fragment to said antigen. The second antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1, by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The second antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired
30

sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The second antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab
5 fragment, F(ab')₂ fragment, or Fv fragment. The method may comprise performing steps (c) and (d) a second time to determine the antigenic stability of the antigen over time.

Also provided is a human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody binds to EV-D68 across at least two viral clades.

10 An additional embodiment comprises a human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody binds to EV-D68 VP1, VP2 and VP3. The antibody may bind to EV-D68 VP1 DE loop, and/or to EV-D68 VP2 EE loop, and/or to EV-D68 VP3 N-terminal loop.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in
15 the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The word “about” means plus or minus 5% of the stated number.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and
20 advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

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BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific
5 embodiments presented herein.

FIG. 1. Antibody isolation. Sixty-four human monoclonal antibodies were isolated and screened using an indirect ELISA in which live virus isolates from the 2014 EV-D68 outbreak are directly coated onto an ELISA plate. Any hits are then fused with myeloma cells to make a
10 hybridoma, which will theoretically continue dividing forever. After purifying antibodies, a number of *in vitro* characterization steps are performed to define their phenotypes.

FIG. 2. Antibody neutralization *in vitro*. *In vitro* neutralization is performed using the cell culture 50% infectious dose, or CCID₅₀ assay. Some antibodies are quite potent, some still very potent, and others weak, with about 1/3 of the mAbs showing some detectable neutralizing
15 activity

FIG. 3. Cross-reactive binding among clades. To compare across all mAbs, a heat map was generated using the half maximal effective concentration, or EC₅₀, from ELISAs. Some bind very well to all clades, while some have notable dropout, typically with the distant A2 clade.

FIG. 4. Competition reveals at least four main surface epitopes. Using Pearson
20 correlation, the inventors generated relatedness values as the colored readout to cluster. This relatedness helps to sort the antibodies, but not actual interference percentage. The inventors have now described three to four phenotypes, which can be related to each other. This permits identification of candidates for clinical development.

FIG. 5. Clinical candidates. In addition to neutralization, further characterization
25 revealed additional clinical candidates. mAb 159 does not cross-react in ELISA or CCID₅₀, even though it is incredibly potent and does not bind to a mock preparation of virus. mAb 105 is still quite potent, but it cross-reacts quite well.

FIGS. 6A-C. Expt. NIA-1849. Lung virus titers of EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with EV-D68-228 significantly reduced lung virus titers on day 1 (FIG. 6A), day 3 (FIG. 6B), and day 5 (FIG. 6C) post-infection. No lung virus titers were
30 detected in mice treated with 10, 3, or 1 mg/kg of EV-D68-228 at days 1, 3, or 5 post-infection. Treatment with IVIg was able to reduce lung virus titers only on day 1 post-infection. Guanidine significantly reduced lung virus titers on days 1 and 3 but not day 5 post-infection. ** $P < 0.01$, **** $P < 0.0001$ compared to placebo-treated mice.

FIGS. 7A-C. Expt. NIA-1849. Blood virus titers of EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with the EV-D68-228 mAb reduced blood virus titers at day 1 (FIG. 7A), day 3 (FIG. 7B), and day 5 (FIG. 7C) post-infection. Treatment with IVIg significantly reduced blood virus titers at days 1, 3, and 5 post-infection. Guanidine reduced blood virus titers on days 1 and 5 but not day 3 post-infection. $**P<0.01$, $***P<0.001$, $****P<0.0001$ compared to placebo-treated mice.

FIG. 8. Expt. NIA-1849. Lung concentrations of IL-1 α and IL-1 β from EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with EV-D68-228 significantly reduced concentrations of IL-1 α and IL-1 β on day 3 post-infection compared to placebo-treated mice. Treatment with IVIg or guanidine did not significantly reduce lung concentrations of IL-1 α or IL-1 β . $****P<0.0001$ compared to placebo-treated mice. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 9. Expt. NIA-1849. Lung concentrations of IL-6 and MCP-1 from EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with EV-D68-228 significantly reduced concentrations of IL-6 and MCP-1 on day 3 post-infection compared to placebo-treated mice. Treatment with IVIg or guanidine significantly reduced lung concentrations of MCP-1 on day 3 post-infection. $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$ compared to placebo-treated mice. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 10. Expt. NIA-1849. Lung concentrations of RANTES from EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with EV-D68-228 significantly reduced concentrations of RANTES on days 3 and 5 post-infection compared to placebo-treated mice. Treatment with IVIg or guanidine did not significantly reduce lung concentrations of RANTES at any day post-infection. $**P<0.01$, $***P<0.001$, $****P<0.0001$ compared to placebo-treated mice. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 11. Expt. NIA-1849. Lung concentrations of IL-2, IL-3, and IL-4 from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of IL-2, IL-3, or IL-4 were observed post-infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 12. Expt. NIA-1849. Lung concentrations of IL-5, IL-10, and IL-12p70 from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of IL-5, IL-10, or IL-12p70 were observed post-infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 13. Expt. NIA-1849. Lung concentrations of IL-17, IFN γ , and TNF α from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of IL-17, IFN γ , or TNF α were observed post-infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 14. Expt. NIA-1849. Lung concentrations of MIP-1 α and GM-CSF from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of MIP-1 α or GM-CSF were observed post-infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 15. Expt. NIA-1850. Survival of 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228. (n=6 mice/group). Treatment with a dose of 10, 3 or 1 mg/kg of EV-D68-228 completely protected mice from mortality. A dose of 10 mg/kg of IVIg protected four of six mice from mortality. Guanidine at a dose of 100 mg/kg/day completely protected mice from mortality. All six of the placebo-treated mice succumbed to the infection * P <0.05, ** P <0.01 compared to placebo-treated mice.

FIG. 16. Expt. NIA-1850. Mean body weights of EV-D68-infected 10-day-old AG129 mice treated with EV-D68-228. Treatment with doses of 10, 3, or 1 mg/kg protected mice from infection-associated weight loss. Treatment with IVIg at a dose of 10 mg/kg also protected mice from weight loss. Mice treated with guanidine at a dose of 100 mg/kg/day were also protected from weight loss. * P <0.05, *** P <0.001, **** P <0.0001 compared to placebo-treated mice.

FIGS. 17A-C. Expt. NIA-1850. Blood virus titers of 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228. No blood virus titers were detected in mice treated with doses of 10, 3, or 1 mg/kg of EV-D68-228 day 1 (FIG. 17A), day 3 (FIG. 17B), or day 5 (FIG. 17C) post-infection. Treatment with a dose of 10 mg/kg of IVIg significantly reduced blood virus titers on days 1, 3, and 5 post-infection. Blood virus titers were also significantly reduced on days 1, 3, and 5 post-infection by treatment with guanidine at a dose of 100 mg/kg/day. * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001 compared to placebo-treated mice.

FIG. 18. Expt. NIA-1850. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228. Treatment with EV-D68-228 at doses of 10, 3, or 1 mg/kg prevented clinical signs of paralysis as measured by neurological scores. Treatment with a dose of 10 mg/kg of IVIg reduced neurological scores on days 3, 4, and 5 post-infection. No neurological scores were observed in the mice treated with 100 mg/kg/day of guanidine. ** P <0.01, *** P <0.001, **** P <0.0001 compared to placebo-treated mice. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIGS. 19A-B. Expt. NIA-1869. Lung virus titers of EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Treatment with EV-D68-228 significantly reduced lung virus titers on day 3 (FIG. 19A) and day 5 (FIG. 19B) post-infection. No lung virus titers were detected in mice treated with 10 mg/kg of EV-D68-228 at within 24 hours post-infection. Treatment with EV-D68-228 at doses of 10 and 1 mg/kg at 48 hours post-infection significantly reduced lung virus titers at days 3 and 5 post-infection. Treatment with IVIg did not reduce lung virus titers when treated 24 hours post-infection. (**** $P < 0.0001$ compared to placebo-treated mice).

FIGS. 20A-B. Expt. NIA-1869. Blood virus titers of EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Treatment with the EV-D68-228 mAb reduced blood virus titers at day 3 (FIG. 20A) and day 5 (FIG. 20B) post-infection. Treatment with EV-D68-228 up to 48 hours post-infection reduced blood virus titers to below the limit of detection at both days 3 and 5 post-infection. Treatment with IVIg significantly reduced blood virus titers at days 3 and 5 post-infection. (* $P < 0.05$, **** $P < 0.0001$ compared to placebo-treated mice).

FIG. 21. Expt. NIA-1869. Lung concentrations of IL-1 α and IL-1 β from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Treatment with EV-D68-228 significantly reduced concentrations of IL-1 α and IL-1 β on day 3 and day 5 post-infection compared to placebo-treated mice. Treatment with IVIg only significantly reduced lung concentrations of IL-1 α or IL-1 β on day 3 and day 5 post-infection. (* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 22. Expt. NIA-1869. Lung concentrations of IL-5 and IL-6 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Treatment with EV-D68-228 up to 48 hours post-infection significantly reduced concentrations of IL-5 and IL-6 on day 3 post-infection compared to placebo-treated mice. Treatment with IVIg 24 hours post-infection significantly reduced lung concentrations of IL-5 and IL-6 on day 3 post-infection. (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 23. Expt. NIA-1849. Lung concentrations of MCP-1 and RANTES from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Treatment with EV-D68-228 within 48 hours post-infection significantly reduced concentrations of MCP-1 and RANTES on days 3 and 5 post-infection compared to placebo-treated mice. Treatment with IVIg 24 hours post-infection significantly reduced lung concentrations of MCP-1 at day 3 post-infection and reduced concentrations of RANTES at days 3 and 5 post-infection. (** $P < 0.01$, *** $P < 0.001$,

**** $P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 24. Expt. NIA-1869. Lung concentrations of IL-2, IL-3, and IL-4 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of IL-2, IL-3, or IL-4 were observed after infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 25. Expt. NIA-1869. Lung concentrations of IL-10, IL-12p70, and IL-17 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of IL-5, IL-10, or IL-12p70 were observed after infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 26. Expt. NIA-1869. Lung concentrations of IFN γ and TNF α from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of IFN γ or TNF α were observed after infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 27. Expt. NIA-1869. Lung concentrations of MIP-1 α and GM-CSF from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of MIP-1 α or GM-CSF were observed after infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 28. Expt. NIA-1870. Survival of 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228. (n=6 mice/group, n=7 mice/group treated 120 hours post-infection). Treatment with a dose of 10 mg/kg of EV-D68-228 completely protected mice from mortality at 24 hours post-infection. Treatment with EV-D68-228 at 48 hours post-infection protected four of six mice from mortality. Only one of six mice treated 72 hours post-infection with EV-D68-228 survived the infection. Despite none of the seven mice surviving in the group treated 120 hours post-infection with EV-D68-228, the survival curve was different than placebo-treated mice due to a delay in the day of death. A dose of 10 mg/kg of IVIg protected all six mice from mortality when treated 24 hours post-infection. All six of the placebo-treated mice succumbed to the infection (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to placebo-treated mice).

FIG. 29. Expt. NIA-1870. Mean body weights of EV-D68-infected 10-day-old AG129 mice treated post-infection with EV-D68-228. Treatment with doses of 10 mg/kg of EV-D68-228 at 24 or 48 hours post-infection protected mice from infection-associated weight loss. Treatment with IVIg at a dose of 10 mg/kg 24 hours post-infection did not protect mice from weight loss. (* $P < 0.05$, ** $P < 0.01$ compared to placebo-treated mice).

FIGS. 30A-C. Expt. NIA-1870. Blood virus titers of 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228. Blood virus titers are shown at day 1 (FIG. 30A), day 3 (FIG. 30B), or day 5 (FIG. 30C) post-infection. Treatment with EV-D68-228 at a dose of 10 mg/kg significantly reduced blood virus titers on days 3 and 5 post-infection when administered 24, 48, or 72 hours after infection. IVIg at a dose of 10 mg/kg administered 24 hours post-infection also reduced blood virus titers at days 3 and 5 post-infection. (* $P<0.05$, *** $P<0.001$, **** $P<0.0001$ compared to placebo-treated mice).

FIGS. 31A-B. Expt. NIA-1870. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228. Neurological scores are shown at days 2 and 3 post-infection (FIG. 31A) and days 4 and 5 post-infection (FIG. 31B). Treatment with EV-D68-228 at a dose of 10 mg/kg at 24 hours post-infection prevented clinical signs of paralysis on days 3, 4, and 5 as measured by neurological scores. Treatment with a dose of 10 mg/kg of IVIg 24 hours post-infection reduced neurological scores on days 3, 4, and 5 post-infection. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 32. Competition binding groups of mAbs from EV-D68-immune human subjects. Relatedness scores were generated from competition-binding ELISAs with a B1 clade EV-D68 isolate and used to cluster mAbs into four competition-binding groups designated 1 to 4. Clone numbers listed in red or blue are potentially neutralizing mAbs, with blue clone names indicating the two mAbs studied in detail in later figures.

FIGS. 33A-C. Neutralization potency and binding capacity of human mAbs. (FIG. 33A) MAbs were ranked within competition-binding group (Comp. group, group 5 indicates the residual collection of singletons) by IC_{50} value in a CCID₅₀ neutralization assay using a B1 clade isolate. The inventors also tested neutralization of a D clade and Fermon (Fer.) isolate for the 21 most potently neutralizing mAbs. “>” denotes neutralization was not detected when tested in concentrations up to 50 μ g/mL. Blank cells indicate not tested. Clone numbers listed in red or blue are potentially neutralizing mAbs, with blue clone names indicating the two mAbs studied in detail in later figures. (FIG. 33B) Binding strength to live virus isolates or a mock virus preparation is denoted using EC_{50} values generated by using (FIG. 33C) indirect ELISA with purified mAb dilutions to fit sigmoidal dose response curves. “>” indicates EC_{50} value exceeds the maximum concentration tested of 100 μ g/mL, suggesting poor or no binding.

FIGS. 34A-C. Structural feature comparison between two immune complexes. (FIG. 34A) Radially colored cryo-EM maps of EV-D68:Fab EV68-159 (left) or EV-D68:Fab EV68-228 (right). Each map is projected down a two-fold axis of symmetry. The five-, three-, and two-fold

axes of each asymmetric unit are depicted using a triangle outline labeled with one pentagon, two small triangles, and one oval, respectively. (FIG. 34B) Binding position comparison on an asymmetric unit. Viral proteins are colored in blue (VP1), green (VP2) and red (VP3). Fab molecules are colored in grey (EV68-159) or purple (EV68-228), and the heavy or light chains are shown in the same colors with dark or light intensities, respectively. (FIG. 34C) Footprints of EV68-159 Fab (left) or EV68-228 Fab (right). Radially colored 2D projections of the viral surface were created with RIVEM. Virus surface residues facing any atoms from the Fab molecules within a distance of 4 Å are outlined in light blue (VP1), light green (VP2) and light red (VP3). The canyon region is outlined in yellow. Scale bars in (FIG. 34A) and (FIG. 34C) indicate radial distance measured in Å.

FIG. 35. Close-up view of the binding interfaces of EV68-159 and EV68-228. The viral capsid is shown as surface and the Fab is shown in a cartoon representation. VP1, VP2 and VP3 are colored in white, dark grey and silver, respectively. Heavy or light chains are colored in orange or yellow, respectively. Viral residues making interactions are colored based on the heavy and light chains, and the color intensities vary based on which of the VPs. The heavy and light chain complementarity-determining regions (HCDR and LCDR, respectively) involved in the binding interfaces are shown with arrows.

FIGS. 36A-D. Molecular detail of virion-Fab interactions. Representative interactions at the binding interface of EV-D68:Fab EV68-159 (FIGS. 36A-C) and EV-D68:Fab EV68-228 (FIG. 36D). Hydrogen bonds are colored in cyan and salt bridges are colored in magenta.

FIGS. 37A-D. MAb EV68-228 protects mice from EV-D68-induced respiratory disease, when used as either prophylaxis or therapy. Four-week-old AG129 strain mice (n = 4 per time point) were inoculated with mouse-adapted B1 clade EV-D68 intranasally; antibody was administered intraperitoneally; and viral titers for indicated tissue were measured by a CCID₅₀ assay. (FIGS. 37A-B) Mice were inoculated with virus 24 hours after indicated dose of antibody, then viral titers were measured at indicated time points. (FIGS. 37C-D) Mice were inoculated with virus followed by 10 mg/kg (except where indicated) of antibody 4, 24, or 48 hours later, then viral titers were measured.

FIGS. 38A-F. MAb EV68-228 decreases lung inflammation in EV-D68 infected mice. Four-week-old AG129 mice (n = 4 per time point) (FIGS. 38A-C) were inoculated with virus intranasally 24 hours after indicated dose of antibody or (FIGS. 38D-F) were inoculated with virus intranasally followed by 10 mg/kg (except where indicated) of antibody 4, 24, or 48 hours later, then cytokines were measured at indicated time points. Cytokines were quantified from lung homogenates using an ELISA. Values from the treatment groups were compared to the placebo

group for each time point using a one-way ANOVA with Dunnett's T3 multiple comparisons test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). IL - interleukin; MCP - monocyte chemoattractant protein. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIGS. 39A-F. MAb EV68-228 protects mice from EV-D68-induced neurologic disease, when used as either prophylaxis or therapy. Ten-day-old mice were inoculated with mouse-adapted B1 clade EV-D68 intraperitoneally; antibody was administered intraperitoneally; and viral titers for indicated tissue were measured by a CCID₅₀ assay. (FIGS. 39A-C) Mice were inoculated with virus 24 hours after indicated dose of antibody, then (FIG. 39A) viral titers were measured ($n = 3$ per time point), (FIG. 39B) survival was monitored, and (FIG. 39C) neurologic scores ($n = 6$ per time point) were recorded at indicated time points. Higher scores indicate more severe motor impairment. (FIGS. 39D-F) Mice were inoculated with virus followed by a 10 mg/kg dose of antibody, then (FIG. 39D) viral titers were measured ($n = 3$ per time point), (FIG. 39E) survival was monitored, and (FIG. 39F) neurologic scores ($n = 6$ per time point, except $n = 9$ for 120 hr post) were recorded. Colored vertical arrows indicate time of treatment.

FIG. 40. Western blot data. All mAbs were tested for the ability to stain B1 clade virus by western blot. All positive results are shown.

FIG. 41. Detailed characteristics of human mAbs. MAbs were ranked within competition-binding group by IC₅₀ value in a CCID₅₀ neutralization assay using a B1 clade isolate. The inventors also tested neutralization of a D clade and Fermon (Fer.) isolate for the 21 most potentially neutralizing mAbs. ">" denotes neutralization was not detected when tested at concentrations up to 50 $\mu\text{g/mL}$. Blank cells indicate not tested. Clone numbers listed with red or blue font are potentially neutralizing mAbs, with blue clone names indicating the two mAbs studied in detail in later figures. Final IC₅₀ values for each mAb are the average of the logarithm of the EC₅₀ values from three separate experiments performed in duplicate. Binding strength to live virus isolates are denoted based on EC₅₀ values generated using indirect ELISA with purified mAb dilutions to fit sigmoidal dose response curves. ">" indicates the predicted EC₅₀ value exceeds the maximum concentration tested of 100 $\mu\text{g/mL}$, suggesting poor or no binding. Final EC₅₀ values for each mAb are the average of the logarithm of the EC₅₀ values from three separate experiments performed in duplicate. IgG subtypes were determined phenotypically. Light chain types are kappa (K) or lambda (L) and were determined genetically. WB indicates western blot reactivity, and all mAbs were tested, with blank rows indicating no reactivity.

FIG. 42. Indirect ELISA data for all mAbs. Binding strength to live virus isolates is denoted by EC₅₀ values generated by using indirect ELISA with purified mAb dilutions to fit

sigmoidal dose response curves. Shown are representative data from one of three experiments, with error bars indicating the standard deviation of duplicate technical replicates. Final EC₅₀ values for each mAb are the average of the logarithm of the EC₅₀ values from all three experiments. The mock preparation of virus was from an RD cell monolayer that was not inoculated with virus but was prepared in the same manner as virus-infected cells.

FIG. 43. Representative densities from the EV-D68: Fab EV68-228 electron density map. Viral proteins are colored in blue (VP1), green (VP2), red (VP3) and magenta (VP4).

FIG. 44. Estimates of immune complex map resolutions. Map resolutions are estimated based on the gold-standard Fourier shell correlation (FSC) cutoff of 0.143. The final resolutions for EV68-159 (red curve) or EV68-228 (blue curve) complexes are 2.9Å or 3.1Å, respectively.

FIG. 45. Comparison of The Fab binding sites. EV68-159 and EV68-228 are colored in gold and blue, respectively, and the viral surface is colored in cyan. The left panel shows both variable domains and constant domains, whereas the right panel shows only the variable domains, demonstrating that the footprints of the two Fabs do not overlap.

FIG. 46. Roadmaps showing an enlarged view of the Fab footprints. The radially colored 2D projection of the viral surface was created with RIVEM. Virus surface residues facing any atoms from the Fab molecules within a distance of 4Å are outlined in light blue (VP1), light green (VP2) and light red (VP3). The canyon region is outlined in yellow.

FIG. 47. Bulky side chains of the EV68-228 Fab heavy chain. This view shows an example of bulky side chains forming a hydrophobic interaction network to stabilize the EV68-228 Fab, which also is seen in the structure of the EV68-159 Fab.

FIGS 48A-C. Expt. NIA-1930. Lung virus titers of EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Lung virus titers are shown on day 1 (FIG. 48A), day 3 (FIG. 48B), and day 5 (FIG. 48C) post-infection. No lung virus titers were detected in mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO at days 1, 3, or 5 post-infection. Treatment with 10 mg/kg of hIVIg only significantly reduced lung virus titers on day 5 post-infection. (** $P < 0.01$, **** $P < 0.0001$ compared to placebo-treated mice.)

FIGS. 49A-C. Expt. NIA-1930. Blood virus titers of EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown at day 1 (FIG. 49A), day 3 (FIG. 49B), and day 5 (FIG. 49C) post-infection. No virus was detected in the blood of mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO on days 1, 3, or 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced blood virus titers at days 1, 3, and 5 post-infection. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared to placebo-treated mice.)

FIG. 50. Expt. NIA-1930. Lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-1 α on days 1, 3, and 5 post-infection. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO also reduced concentrations of IL-1 β on day 3 post-infection. Treatment with hIVIg only significantly reduced lung concentrations of IL-1 β on day 3 post-infection. No significant changes were observed in concentrations of IL-2 or IL-3 following infection. (* P <0.05, *** P <0.001, **** P <0.0001 compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 51. Expt. NIA-1930. Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-5 on day 3 post-infection and IL-6 on days 3 and 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced lung concentrations of IL-5 and IL-6 on day 3 post-infection. No significant changes were observed in concentrations of IL-4 or IL-10 following infection. (* P <0.05, *** P <0.001, **** P <0.0001 compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 52. Expt. NIA-1930. Lung concentrations of IL-12p70, IL-17, MCP-1, and IFN- γ from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of MCP-1 on days 3 and 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced lung concentrations of MCP-1 at day 3 and 5 post-infection. No significant changes were observed in concentrations of IL-12p70, IL-17, or IFN- γ following infection. (** P <0.01, *** P <0.001, **** P <0.0001 compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 53. Expt. NIA-1930. Lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced lung concentrations of MIP-1 α on days 1 and 3 post-infection and reduced concentrations of RANTES on days 3 and 5 post-infection. A 10 mg/kg dose of hIVIg significantly reduced concentrations of MIP-1 α on days 1 and 3 post-infection and also reduced concentrations of RANTES on day 3 post-infection. No significant changes in concentrations of TNF α or GM-CSF were observed after infection with EV-D68. (** P <0.01, *** P <0.001,

$P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIGS. 54A-B. Expt. NIA-1930. Lung virus titers of EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Lung virus titers are shown on day 3 (FIG. 54A) and day 5 (FIG. 54B) post-infection. No lung virus titers were detected in mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO at days 3 or 5 post-infection. Treatment with 10 mg/kg of hIVIg only significantly reduced lung virus titers on day 3 post-infection. ($**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ compared to placebo-treated mice.)

FIGS. 55A-B. Expt. NIA-1930. Blood virus titers of EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown at day 3 (FIG. 55A) and day 5 (FIG. 55B) post-infection. No virus was detected in the blood of mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO on days 3 or 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced blood virus titers at days 3 and 5 post-infection. ($*P < 0.05$, $***P < 0.001$, $****P < 0.0001$ compared to placebo-treated mice.)

FIG. 56. Expt. NIA-1930. Lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-1 α and IL-1 β on day 3 post-infection. Concentrations of IL-3 were significantly reduced in mice treated with EV68-228-TP as well as mice treated with hIVIg on day 3 post-infection. No significant changes were observed in concentrations of IL-2 following infection. ($*P < 0.05$, $**P < 0.01$, $****P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 57. Expt. NIA-1930. Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-5 on day 3 post-infection and IL-6 on days 3 and 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced lung concentrations of IL-5 on day 3 post-infection. No significant changes were observed in concentrations of IL-4 or IL-10 following infection. ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ compared to placebo-treated mice).

Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 58. Expt. NIA-1930. Lung concentrations of IL-12p70, IL-17, MCP-1, and IFN- γ from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly

reduced concentrations of MCP-1 on days 3 and 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced lung concentrations of MCP-1 at day 3 and 5 post-infection. No significant changes were observed in concentrations of IL-12p70, IL-17, or IFN- γ following infection. (**** $P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 59. Expt. NIA-1930. Lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced lung concentrations of MIP-1 α on day 3 post-infection and reduced concentrations of RANTES on days 3 and 5 post-infection. No significant changes in concentrations of TNF α or GM-CSF were observed after infection with EV-D68. (**** $P < 0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIGS. 60A-B. Expt. NIA-1930. Lung virus titers of EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Lung virus titers are shown on day 3 (FIG. 60A) and day 5 (FIG. 60B) post-infection. Only EV68-228-CHO significantly reduced lung virus titers on days 3 or 5 post-infection. (**** $P < 0.0001$ compared to placebo-treated mice).

FIGS. 61A-B. Expt. NIA-1930. Blood virus titers of EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown at day 3 (FIG. 61A) and day 5 (FIG. 61B) post-infection. No virus was detected in the blood of mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO on days 3 or 5 post-infection. Treatment with 10 mg/kg of hIVIg significantly reduced blood virus titers at days 3 and 5 post-infection. (**** $P < 0.0001$ compared to placebo-treated mice).

FIG. 62. Expt. NIA-1930. Lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Only treatment with 10 mg/kg of EV68-228-CHO significantly reduced concentrations of IL-1 α and IL-1 β on day 3 post-infection. No significant changes were observed in concentrations of IL-2 or IL-3 following infection. (** $P < 0.01$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 63. Expt. NIA-1930. Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or

EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-6 on day 3 post-infection. No significant changes were observed in concentrations of IL-4, IL-5, or IL-10 following infection. (** $P < 0.01$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 64. Expt. NIA-1930. Lung concentrations of IL-12p70, IL-17, MCP-1, and IFN- γ from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. No significant changes were observed in concentrations of IL-12p70, IL-17, MCP-1, or IFN- γ following infection. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 65. Expt. NIA-1930. Lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. No significant changes in concentrations of TNF α , MIP-1 α , GM-CSF, or RANTES were observed after infection with EV-D68. Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 66. Expt. NIA-1931. Survival of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. ($n=10$ mice/group). Treatment with a dose of 10 mg/kg of EV68-228-TP or completely protected mice from mortality. All ten mice treated with 10 mg/kg of EV68-228-CHO were protected from mortality. A dose of 10 mg/kg of hIVIg protected eight of ten mice from mortality. Nine of the ten of the placebo-treated mice succumbed to the infection (*** $P < 0.001$, **** $P < 0.0001$ compared to placebo-treated mice).

FIG. 67. Expt. NIA-1931. Percentages of initial body weight of EV-D68-infected 10-day-old AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. No significant differences in weight loss were observed in mice treated 24 hours pre-infection with 10 mg/kg doses EV68-228-TP or EV68-228-CHO compared to placebo-treated mice. Treatment with a 10 mg/kg dose of hIVIg did not protect mice from weight loss.

FIGS. 68A-B. Expt. NIA-1931. Blood virus titers on days 1 and 3 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 1 (FIG. 68A) and day 3 (FIG. 68B) post-infection. Treatment 24 hours pre-infection with 10 mg/kg of EV68-228-TP significantly reduced virus titers on days 1 and 3 post-infection. Treatment 24 hours pre-infection with 10 mg/kg of EV68-228-CHO significantly reduced virus titers on days 1 and 3

post-infection. Treatment 24 hours pre-infection with a dose of 10 mg/kg of IVIg significantly reduced blood virus titers on day 3 post-infection. (* $P<0.05$, ** $P<0.01$, **** $P<0.0001$ compared to placebo-treated mice).

FIGS. 69A-B. Expt. NIA-1931. Blood virus titers on days 5 and 7 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 5 (FIG. 69A) and day 7 (FIG. 69B) post-infection. Treatment 24 hours pre-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO reduced virus titers on day 5 post-infection. No virus was detected at day 7 post-infection.

FIGS. 70A-C. Expt. NIA-1931. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO on days 2-10 post-infection. Neurological scores are shown for days 2-4 (FIG 70A), days 5-7 (FIG. 70B), and days 8-10 (FIG. 70C) post-infection. Treatment 24 hours pre-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO completely prevented clinical signs of paralysis as measured by neurological scores. No neurological scores were observed in mice treated with a dose of 10 mg/kg of IVIg 24 hours pre-infection. (**** $P<0.0001$ compared to placebo-treated mice).

FIG. 71. Expt. NIA-1931. Survival of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. (n=10 mice/group). Treatment with a dose of 10 mg/kg of EV68-228-TP or completely protected mice from mortality. All ten mice treated with 10 mg/kg of EV68-228-CHO were protected from mortality. A dose of 10 mg/kg of IVIg protected ten of ten mice from mortality. Eight of the ten of the placebo-treated mice succumbed to the infection (*** $P<0.001$ compared to placebo-treated mice).

FIG. 72. Expt. NIA-1931. Percentages of initial body weight of EV-D68-infected 10-day-old AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. No significant differences in weight loss were observed in mice treated 24 hours post-infection with 10 mg/kg doses EV68-228-TP or EV68-228-CHO compared to placebo-treated mice. Treatment with a 10 mg/kg dose of hIVIg did not protect mice from weight loss.

FIGS. 73A-B. Expt. NIA-1931. Blood virus titers on days 1 and 3 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 1 (FIG. 73A) and day 3 (FIG. 73B) post-infection. Treatment 24 hours post-infection with 10 mg/kg of EV68-228-TP significantly reduced virus titers on day 3 post-infection. Treatment 24 hours post-infection

with 10 mg/kg of EV68-228-CHO significantly reduced virus titers on day 3 post-infection. Treatment 24 hours post-infection with a dose of 10 mg/kg of IVIg significantly reduced blood virus titers on day 3 post-infection. (* $P<0.05$, ** $P<0.01$, **** $P<0.0001$ compared to placebo-treated mice).

FIGS. 74A-B. Expt. NIA-1931. Blood virus titers on days 5 and 7 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 5 (FIG. 74A) and day 7 (FIG. 74B) post-infection. No virus was detected after treatment 24 hours post-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO on day 5 post-infection. No virus was detected in any mice at day 7 post-infection.

FIGS. 75A-B. Expt. NIA-1931. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO on days 2-9 post-infection. Neurological scores are shown for days 2-5 (FIG. 75A) and days 6-9 (FIG. 75B) post-infection. Treatment 24 hours post-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced signs of paralysis as measured by neurological scores on days 2-9 post-infection. Treatment with hIVIg at a dose of 10 mg/kg 24 hours after infection only reduced neurological scores on days 2 and 3 post-infection. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIGS. 76A-B. Expt. NIA-1931. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO on days 10-17 post-infection. Neurological scores are shown for days 10-13 (FIG. 76A) and days 14-17 (FIG. 76B) post-infection. Treatment 24 hours post-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced signs of paralysis as measured by neurological scores on days 10-16 post-infection. Treatment with hIVIg at a dose of 10 mg/kg 24 hours after infection did not significantly reduce neurological scores. (* $P<0.05$, ** $P<0.01$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

FIG. 77. Expt. NIA-1931. Survival of 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. (n=10 mice/group). Treatment with a dose of 10 mg/kg of EV68-228-TP protected nine of ten mice from mortality. Eight of ten mice treated with 10 mg/kg of EV68-228-CHO were protected from mortality. A dose of 10 mg/kg of IVIg protected six of ten mice from mortality. Nine of

the ten of the placebo-treated mice succumbed to the infection (** $P<0.01$, *** $P<0.001$ compared to placebo-treated mice).

FIG. 78. Expt. NIA-1931. Percentages of initial body weight of EV-D68-infected 10-day-old AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. No significant differences in weight loss were observed in mice treated 24 hours post-infection with 10 mg/kg doses EV68-228-TP or EV68-228-CHO compared to placebo-treated mice. Treatment with a 10 mg/kg dose of hIVIg did not protect mice from weight loss.

FIGS. 79A-B. Expt. NIA-1931. Blood virus titers on days 1 and 3 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 1 (FIG. 79A) and day 3 (FIG. 79B) post-infection. Treatment 48 hours post-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO did not significantly reduce blood virus titers. Treatment 48 hours post-infection with a dose of 10 mg/kg of IVIg did not significantly reduce blood virus titers.

FIGS. 80A-B. Expt. NIA-1931. Blood virus titers on days 5 and 7 post-infection of 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Blood virus titers are shown on day 5 (FIG. 80A) and day 7 (FIG. 80B) post-infection. No virus was detected after treatment 24 hours post-infection with 10 mg/kg of EV68-228-TP or EV68-228-CHO on day 5 post-infection. No virus was detected in any mice at day 7 post-infection.

FIGS. 81A-B. Expt. NIA-1931. Neurological scores of 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO on days 3-10 post-infection. Neurological scores are shown for days 3-6 (FIG. 81A) and days 7-10 (FIG. 81B) post-infection. Treatment 48 hours post-infection with 10 mg/kg of EV68-228-TP significantly reduced neurological scores on days 3-5 post-infection. A single administration of a 10 mg/kg dose of EV68-228-CHO 48 hours after infection significantly reduced signs of paralysis as measured by neurological scores on days 3-6 post-infection. Treatment with hIVIg at a dose of 10 mg/kg 48 hours after infection did not significantly reduce neurological scores post-infection. (* $P<0.05$, *** $P<0.001$, **** $P<0.0001$ compared to placebo-treated mice). Designation of samples in graph corresponds left-to-right with vertical legend top-to-bottom.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

As discussed above, enterovirus D68 (EV-D68) can cause outbreaks of respiratory illness across the world, mostly in children. Clusters of acute flaccid myelitis, a poliomyelitis-like neuromuscular weakness syndrome, often occur concurrently with EV-D68 respiratory outbreaks. Seroepidemiologic studies have found that the serum of nearly everyone older than 2 to 5 years contains anti-EV-D68 neutralizing antibodies, suggesting that EV-D68 is a ubiquitous pathogen of childhood. However, knowledge of the viral epitopes against which the humoral immune response is directed is only inferred from previous studies of related viruses. Here, the inventors have generated 64 human monoclonal antibodies against EV-D68, some of which are potently neutralizing. These are believed to be the first human mAbs against this pathogen and the inventors propose their use in detecting, treating and preventing EV-D68 infections. These and other aspects of the disclosure are described in detail below.

I. Enterovirus D68

Enterovirus 68 (EV68, EV-D68, HEV68) is a member of the *Picornaviridae* family, an enterovirus. First isolated in California in 1962 and once considered rare, it has been on a worldwide upswing in the 21st century. It is suspected of causing a polio-like disorder called acute flaccid myelitis.

EV68 is one of the more than one hundred types of enteroviruses, a group of ssRNA viruses containing the polioviruses, coxsackieviruses, and echoviruses, and it is unenveloped. Unlike all other enteroviruses, EV68 displays acid lability and a lower optimum growth temperature, both characteristic features of the human rhinoviruses. It was previously called human rhinovirus 87 by some researchers. Children less than 5 years old and children with asthma appear to be most at risk for the illness, although illness in adults with asthma and immunosuppression have also been reported.

Since its discovery, EV68 had been described mostly sporadically in isolated cases. Six clusters (equal to or more than 10 cases) or outbreaks between 2005 and 2011 have been reported from the Philippines, Japan, the Netherlands, and the states of Georgia, Pennsylvania and Arizona in the United States. EV68 was found in 2 of 5 children during a 2012/13 cluster of polio-like disease in California. In August 2014, the virus caused clusters of respiratory disease in the United States. By mid-October 691 people in 46 states and the District of Columbia had come down with a respiratory illness caused by EV-D68 and five children died.

In 2016, 29 cases were reported in Europe (5 in France and Scotland. 3 each in Sweden, Norway and Spain).

Since the year 2000, the original virus strains diversified and evolved a genetically distinct outbreak strain, clade B1. It is Clade B1, but not older strains, which has been associated with AFM and is neuropathic in animal models. Cases have been described to occur late in the enterovirus season (roughly the period of time between the spring equinox and autumn equinox), which is typically during August and September in the Northern Hemisphere.

EV68 almost exclusively causes respiratory illness, which varies from mild to severe, but can cause a range of symptoms, from none at all, to subtle flu-like symptoms, to debilitating respiratory illness and a suspected rare involvement in a syndrome with polio-like symptoms. Like all enteroviruses, it can cause variable rashes, abdominal pain and soft stools. Initial symptoms are similar to those for the common cold, including a runny nose, sore throat, cough, and fever. As the disease progresses, more serious symptoms may occur, including difficulty breathing as in pneumonia, reduced alertness, a reduction in urine production, and dehydration, and may lead to respiratory failure.

The degree of severity of symptoms experienced seems to depend on the demographic population in question. Experts estimate that the majority of the population has, in fact, been exposed to the enterovirus, but that no symptoms are exhibited in healthy adults. In contrast, EV-D68 is disproportionately debilitating in very young children, as well as the very weak. While several hundred people (472), mostly youth, have been exposed to the disease, less than a hundred of those patients have been diagnosed with severe symptoms (such as paralysis).

The virus has been suspected as one cause of acute flaccid myelitis a rare muscle weakness, usually due to polio, since two California children who tested positive for the virus had paralysis of one or more limbs reaching peak severity within 48 hours of onset. Recovery of motor function was poor at 6-month follow-up. As of October 2014, the CDC was investigating 10 cases of paralysis and/or cranial dysfunction in Colorado and other reports around the country, coinciding with the increase in enterovirus D68 activity. As of October 2014, it was believed that the actual number of cases might be 100 or more. As of 2018 the link of EV-D68 and the paralysis is strong, meeting six Bradford Hill criteria fully and two partially. The CDC issued a statement on October 17, 2018 claiming “Right now, we know that poliovirus is not the cause of these AFM cases. CDC has tested every stool specimen from the AFM patients, none of the specimens have tested positive for the poliovirus.” In 2014, a real-time PCR test to speed up detection was developed by CDC.

There is no specific treatment and no vaccine, so the illness has to run its course; treatment is directed against symptoms (symptomatic treatment). Most people recover completely; however, some need to be hospitalized, and some have died as a result of the virus. Five EV68 paralysis cases were unsuccessfully treated with steroids, intravenous
5 immunoglobulin and/or plasma exchange. The treatment had no apparent benefit as no recovery of motor function was seen. A 2015 study suggested the antiviral drug pleconaril may be useful for the treatment of EV-D68.

Since the virus is spread through saliva and phlegm as well as stool, washing hands is important. Sick people can attempt to decrease spreading the virus by basic sanitary measures,
10 such as covering the nose and mouth when sneezing or coughing. Other measures including cleaning surfaces and toys. For hospitalized patients with EV-D68 infection, the CDC recommends transmission-based precautions, *i.e.*, standard precautions, contact precautions, as is recommended for all enteroviruses, and to consider droplet precautions. According to the CDC in 2003, surfaces in healthcare settings should be cleaned with a hospital-grade
15 disinfectant with an EPA label claim for any of several non-enveloped viruses (*e.g.*, norovirus, poliovirus, rhinovirus).

A. Viral Epitopes

Measurements of serum antibody virus neutralization capacity reflect the activity of an
20 entire polyclonal antibody repertoire. However, to fully understand the humoral response to a virus it is necessary to define the specific viral epitopes to which individual antibodies bind and determine whether antibody binding to specific epitopes protects against disease. Historically, four neutralizing immunoepitopes (Nim) for viruses of the *Enterovirus* genus were identified through studies of murine monoclonal antibodies raised against rhinovirus-B14
25 (Rossmann *et al.*, 1985). Studies of EV-D68 specific monoclonal antibody epitopes have not been performed to date. Determination of the crystal structure of EV-D68, however, suggested the likely location of the four Nims on the EV-D68 virion by observations of structural homology with rhinoviruses (Liu *et al.*, 2015a). Studies comparing the amino acid sequences of the surface proteins of recent human isolates of EV-D68 from the U.S. (Zhang *et al.*, 2015)
30 or Japan (Imamura *et al.*, 2014) to that of the Fermon reference virus strain suggest that mutations in the Nims and nearby flanking residues have occurred, especially in the BC and DE loops of capsid protein VP1 that are disordered in the crystal structure (Liu *et al.*, 2015a). It is possible that these VP1 polymorphisms contribute to increased pathogenicity of the virus by eluding pre-existing humoral immunity. Murine monoclonal antibodies are available

commercially (sources include GeneTex, ThermoFisher, and Sigma), but aside from listing the specific viral surface protein that was used as an immunogen, information is not available on the epitopes for these antibodies. Notably, some of these commercial antibodies were generated from immunogens of other *Enterovirus* genus viruses, suggesting the ability of heterotypic antibodies to bind to EV-D68. Further supporting this possibility, two murine mAbs cross-reacted to 40 different enterovirus species, although curiously EV-D68 was the only virus tested that they did not react to (Miao *et al.*, 2009). Heterotypic molecular recognition of EV-D68 by antibodies induced by other types of enteroviruses might contribute to the observed universal EV-D68 seroprevalence early in life. Understanding the fine specificity of human antibody epitopes on EV-D68 and the type specificity or breadth of such antibodies will require studies with cloned human monoclonal antibodies induced by natural infection.

During the 2014 EV-D68 outbreak in the U.S., nearly all viral isolates were of the newly emergent B1 clade, with fewer detections of virus from the closely related B2 or distantly related D clades (Tan *et al.*, 2016). All but one of the subjects for this study were infected with B1 clade isolates (Table E). Since 2014, B3 clade viruses have dominated, and B1 clade viruses are no longer circulating (Dyrdak *et al.*, 2019); in 2018 all EV-D68 isolates sequenced by the U.S. Centers for Disease Control and Prevention were from the B3 clade (Kujawski *et al.*, 2019). The inventors first measured the *in vitro* neutralization capability of each mAb in a 50% cell culture infectious dose (CCID₅₀) assay using a B1 clade EV-D68 isolate (FIG. 33A). Twenty-eight mAbs demonstrated neutralization with a half maximal inhibitory concentration (IC₅₀) below 50 µg/mL, with mAb EV68-159 exhibiting the strongest neutralization at an IC₅₀ value of 0.32 ng/mL (FIG. 41). The inventors further tested the 21 most potently neutralizing mAbs against a D clade isolate and found that 11 mAbs neutralized that virus, with 7 of those exhibiting at least a ten-fold decrease in potency by IC₅₀ value for the heterologous virus. The Fermon strain is an isolate from 1962 and is so distantly related to modern EV-D68 isolates that it does not fit into the clade classification scheme (Tan *et al.*, 2016). Nine mAbs neutralized the Fermon laboratory reference strain, but less potently than they inhibited the contemporary B1 clade virus.

Recognizing that neutralization assays may underestimate cross-reactivity, the inventors used the same indirect ELISA approach described above to generate half-maximal effective concentrations (EC₅₀) of purified mAb for binding to representative EV-D68 isolates from the B1, B2, or D clades (FIGS. 33B-C, and FIG. 42). Of the mAbs with EC₅₀ values for binding of ≤ 1 µg/mL to B1 clade isolates, all bound to a B2 clade isolate, whereas about half

also bound to a D clade isolate (FIG. 33B and FIG. 41). An additional class of mAbs was observed that bound weakly in general but cross-reacted to viruses from all clades tested.

To date, structural studies of antibody-EV-D68 interactions have been limited to murine mAbs (Zheng *et al.*, 2019). The inventors selected two potentially neutralizing human mAbs, the clade-specific mAb EV68-159 and the highly cross-reactive mAb EV68-228, to make immune complexes with antigen binding fragments (Fabs) and a B1 clade EV-D68 isolate for cryo-electron microscopy (cryo-EM) studies. The final density maps attained a resolution of 2.9 Å (EV68-159) or 3.1 Å (EV68-228) (FIG. 34A, FIG. 43, FIG. 44, and Table G). The structures revealed two distinct binding sites: EV68-159 attached around the three-fold axes of symmetry, whereas EV68-228 bound around the five-fold axes between depressions that form the canyon regions (FIGS. 34A-C, FIG. 45). Thus, for each Fab, a total of 60 copies bound to the virus particle. The Fab variable domains, which interacted with the viral surface, displayed strong densities similar to the viral capsid proteins, and an atomic model of each Fab was built together with the four viral capsid proteins. In contrast, the Fab constant domains, which are located further from the viral surface, displayed weaker densities and were excluded from atomic model building. The backbone of the polypeptide chains and the majority of amino acid side chains are well-ordered in the density maps, demonstrating the critical features of the binding interface between virus particle and Fab molecule.

B. Animal Models of Neurologic Disease

Measuring neutralization of viruses by antibodies *in vitro* is typically the most rapid and reproducible method to characterize the antiviral function of antibodies. However, this mode of testing does not measure effector functions mediated by the Fc portion of antibodies that operate only *in vivo*, such as complement activation or engagement with cell-surface Fc receptors. Consequently, the magnitude of *in vitro* neutralization capacity may not correlate perfectly with the levels of inhibition of viral replication or protection against disease observed *in vivo* (reviewed in (Lu *et al.*, 2018)). Many of the Fc-mediated effector functions of antibodies require Fc receptor bearing cells of the innate or adaptive immune systems to mediate full protective effects. Small animal models of infection are needed for assessment of efficacy of humoral immunity in preclinical studies.

Investigators in Colorado (Hixon *et al.*, 2017a; 2017b), Utah (Morrey *et al.*, 2018), and China (Zhang *et al.*, 2018) have reported murine models of EV-D68 since early 2017. In all three reported models, mice of no greater than 10 days old are inoculated with virus. Across these models, EV-D68 has been shown capable of causing flaccid limb paralysis and death

when administered by intraperitoneal (Hixon *et al.*, 2017a; 2017b; Morrey *et al.*, 2018; Zhang *et al.*, 2018), intramuscular (Hixon *et al.* 2017a; 2017b), or intracerebral routes (Hixon *et al.*, 2017b). Notably, intranasal inoculation led to paralysis in only two of 73 outbred NIH Swiss Webster mice tested, with virus detected in the spinal cord (Hixon *et al.*, 2017b), whereas
5 intranasal inoculation of AG129 mice, which lack interferon type-I and –II receptors, caused paralysis in two of four mice with virus detected in muscle but not spinal cord (Morrey *et al.*, 2018). However, all studies in which humoral immunity was evaluated in mice used non-physiologic routes of virus inoculation.

Viral antigen has been visualized in tissues by immunostaining in skeletal muscle
10 (Morrey *et al.*, 2018; Zhang *et al.*, 2018) and spinal cord (Hixon *et al.*, 2017b; Morrey *et al.*, 2018). Involvement of both muscle and spinal cord suggests that EV-D68 may induce paralysis by two mechanisms: 1) direct pathologic effect on skeletal muscles and 2) loss of central motor neurons. Motor neurons in the spinal cord were infected, which is analogous to the pathogenesis of poliomyelitis. This pattern of murine neuron involvement in immunostaining
15 studies also correlates with findings of gray matter change on spinal cord imaging in patients with AFM (Hixon *et al.*, 2017b; Morrey *et al.*, 2018). More sensitive real-time quantitative RT-PCR tests detected EV-D68 RNA chiefly in muscle and spinal cord, but also in brain, heart, lung, intestine, liver, spleen, kidney, and blood (Zhang *et al.*, 2018). However, other than in spinal cord and muscle, the amounts of RNA detected were of questionable significance and
20 may well reflect viral genome copies present in blood within these tissues rather than true tropism for these tissues.

Studies in these models of paralysis and lethality facilitated preliminary studies of the ability of antibodies to protect from neurologic disease. Heat-inactivated serum from immune mice passively transferred to naïve mice prevented neurologic disease, even when EV-D68 was
25 inoculated directly by the intracerebral route (Hixon *et al.*, 2017b). Also, therapeutic administration of human IVIG reduced motor impairment even up to 6 days after infection of mice (Hixon *et al.*, 2017a), consistent with findings of high titers of EV-D68 specific antibodies in IVIG (Zhang *et al.*, 2015). This observation provides hope that vaccines or human monoclonal antibodies could mediate a therapeutic effect after an individual patient is
30 identified to have features of AFM temporally associated with EV-D68. Further, targeted vaccination or immunoprophylaxis of populations such as infants and toddlers with waning maternal humoral immunity during a known outbreak of EV-D68 could prevent cases of AFM.

C. Animal Models of Respiratory Disease

The major limitations of current murine studies of EV-D68 infection are the use of non-physiologic routes of inoculation to induce paralysis and death and the lack of respiratory disease, which is the chief manifestation of EV-D68 infection in humans. For both cotton rats (*Sigmodon hispidus*) (Patel *et al.*, 2016) and ferrets (*Mustela putorius furo*) (Zheng *et al.*, 2017a) a single study of each has demonstrated that intranasal inoculation with EV-D68 results in replication of virus in the nose and lungs and induces increases in pro-inflammatory innate immune molecule transcripts in the lungs. Clinical symptoms consistent with upper respiratory tract infection (cough, nasal discharge, and dry nose) also developed in ~25% of inoculated ferrets. Neither study assessed for the presence of EV-D68 in muscle or central nervous system tissues nor noted apparent limb weakness or paralysis. Vaccination of cotton rats indicated that some levels of pre-existing humoral immunity did not protect fully against respiratory infection and may in fact be harmful (Patel *et al.*, 2016), in findings that are described further below.

The theoretical advantage of cotton rats and ferrets as models for human respiratory viral infections is that their respiratory epithelia may more closely mimic that of humans, especially in terms of sialylation of glycans on epithelial surfaces, which are heavily α 2,6-linked in humans but not in mice (Gagneux *et al.*, 2003). EV-D68 bound preferentially to α 2,6-linked sialic acids over α 2,3-linked sialic acids in an *in vitro* glycan array (Imamura *et al.*, 2014). Staining of the respiratory tract with fluorescently labeled lectins indicated that cotton rats have only α 2,6-linked sialic acids in their lower respiratory tracts with a mix of α 2,6- and α 2,3-linked sialic acids in the upper airways (Blanco *et al.*, 2018). Using similar lectin-based methods, ferrets were shown to have α 2,6-linked sialic acids on epithelial glycans of the upper respiratory tract (Leigh *et al.*, 1995), with a mix of α 2,6- and α 2,3-linked sialic acids in the lower airways (Zheng *et al.*, 2017a). However, more recent and sophisticated glycomic analyses of human (Walther *et al.*, 2013), mouse (Bern *et al.*, 2013), and ferret (Jia *et al.*, 2014) respiratory tissues indicated that a more complex array of glycan modifications than simply density of α 2,6-linked sialylation likely determines the tropism of respiratory viruses for different animal species. Nonetheless, the success of initial studies in rats and ferrets at mimicking human EV-D68 respiratory disease are encouraging for their further development as models of human pathogenesis.

D. Vaccines

Experimental vaccine candidates for EV-D68 have not yet entered clinical development, however initial studies inoculating mice with either virus-like particle (VLP) vaccines made in yeast (Zhang *et al.*, 2018a) or insect cells (Dai *et al.*, 2018) or beta-propiolactone-inactivated EV-D68 (Zhang *et al.*, 2018) have shown promise. The VLP vaccines induced antibody responses that protected mice from subsequent lethal intraperitoneal challenge. With each of these vaccine candidates, passive transfer of vaccine immune serum to naïve mice was sufficient for protection from lethal intraperitoneal challenge. A possible drawback of VLP based vaccines is the uncertainty of whether these synthetic constructs fully recapitulate all conformationally sensitive structures on the surface of the particles. Many potent human virus neutralizing antibodies for other viruses recognize complex quaternary antigenic sites with strict conformational constraints (Crowe, JE, 2017). The integrity of human antibody epitopes on VLPs cannot be assessed currently since these epitopes are unknown. Further tempering the promise of these murine vaccine studies are findings in cotton rats inoculated intramuscularly with either live or UV-inactivated EV-D68 and subsequently challenged intranasally with a homologous live virus, in which enhanced inflammation was seen in the lung compared to infection of naïve animals (Patel *et al.*, 2016). Specifically, UV-inactivated virus vaccination did not limit viral replication in the lung or nose and skewed the resultant cytokine signature toward a Th2 phenotype, rather than a balanced Th1 and Th2 phenotype seen with immunity from live virus vaccination. Enhanced inflammation and disease have been noted with inactivated virus vaccine candidates for respiratory syncytial virus (Karron, R, 2018) and measles (Strebel *et al.*, 2018). Further careful study of the immune correlates of protection or enhancement caused by EV-D68 vaccine candidates is necessary given these mixed findings.

II. Monoclonal Antibodies and Production Thereof

An "isolated antibody" is one that has been separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In particular embodiments, the antibody is purified: (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most particularly more than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the

antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The basic four-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 basic heterotetramer units along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable region (V_H) followed by three constant domains (C_H) for each of the alpha and gamma chains and four C_H domains for mu and isotypes. Each L chain has at the N-terminus, a variable region (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable regions. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, *e.g.*, Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, Conn., 1994, page 71, and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda based on the amino acid sequences of their constant domains (C_L). Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha, delta, epsilon, gamma and mu, respectively. They gamma and alpha classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

The term "variable" refers to the fact that certain segments of the V domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino

acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable regions of native heavy and light chains each comprise four FRs, largely adopting a beta-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), and antibody-dependent complement deposition (ADCD).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the V_H when numbered in accordance with the Kabat numbering system; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)); and/or those residues from a "hypervariable loop" (*e.g.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and 26-32 (H1), 52-56 (H2) and 95-101 (H3) in the V_H when numbered in accordance with the Chothia numbering system; Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)); and/or those residues from a "hypervariable loop"/CDR (*e.g.*, residues 27-38 (L1), 56-65 (L2) and 105-120 (L3) in the V_L, and 27-38 (H1), 56-65 (H2) and 105-120 (H3) in the V_H when numbered in accordance with the IMGT numbering system; Lefranc, M. P. *et al.* Nucl. Acids Res. 27:209-212 (1999), Ruiz, M. *et al.* Nucl. Acids Res. 28:219-221 (2000)). Optionally the antibody has symmetrical insertions at one or more of the following points 28, 36 (L1), 63, 74-75 (L2) and 123 (L3) in the V_L, and 28, 36 (H1), 63, 74-75 (H2) and 123 (H3) in the V_{subH} when numbered in accordance with AHO; Honneger, A. and Plunkthun, A. J. Mol. Biol. 309:657-670 (2001)).

By "germline nucleic acid residue" is meant the nucleic acid residue that naturally occurs in a germline gene encoding a constant or variable region. "Germline gene" is the DNA found in a germ cell (*i.e.*, a cell destined to become an egg or in the sperm). A "germline

mutation" refers to a heritable change in a particular DNA that has occurred in a germ cell or the zygote at the single-cell stage, and when transmitted to offspring, such a mutation is incorporated in every cell of the body. A germline mutation is in contrast to a somatic mutation which is acquired in a single body cell. In some cases, nucleotides in a germline DNA sequence encoding for a variable region are mutated (*i.e.*, a somatic mutation) and replaced with a different nucleotide.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations that include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present disclosure may be prepared by the hybridoma methodology first described by Kohler *et al.*, Nature, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, *e.g.*, U.S. Patent 4,816,567) after single cell sorting of an antigen specific B cell, an antigen specific plasmablast responding to an infection or immunization, or capture of linked heavy and light chains from single cells in a bulk sorted antigen specific collection. The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature, 352:624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222:581-597 (1991), for example.

A. General Methods

It will be understood that monoclonal antibodies binding to EV-D68 will have several applications. These include the production of diagnostic kits for use in detecting and diagnosing EV-D68 infection, as well as for treating the same. In these contexts, one may link such antibodies to diagnostic or therapeutic agents, use them as capture agents or competitors in competitive assays, or use them individually without additional agents being attached thereto. The antibodies may be mutated or modified, as discussed further below. Methods for preparing and characterizing antibodies are well known in the art (see, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; U.S. Patent 4,196,265).

The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. The first step for both these methods is immunization of an appropriate host or identification of subjects who are immune due to prior natural infection or vaccination with a licensed or experimental vaccine. As is well known in the art, a given composition for immunization may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine. As also is well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants in animals include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant and in humans include alum, CpG, MFP59 and combinations of immunostimulatory molecules ("Adjuvant Systems", such as AS01 or AS03). Additional experimental forms of inoculation to induce EV-D68-specific B cells is possible, including nanoparticle vaccines, or gene-encoded antigens delivered as DNA or RNA genes in a physical delivery system (such as lipid nanoparticle or on a gold biolistic bead), and delivered with needle, gene gun, transcutaneous electroporation device. The antigen gene also can be carried as encoded by a replication competent or defective viral vector such as adenovirus, adeno-associated virus, poxvirus, herpesvirus, or alphavirus replicon, or alternatively a virus like particle.

In the case of human antibodies against natural pathogens, a suitable approach is to identify subjects that have been exposed to the pathogens, such as those who have been diagnosed as having contracted the disease, or those who have been vaccinated to generate protective immunity against the pathogen or to test the safety or efficacy of an experimental vaccine. Circulating anti-pathogen antibodies can be detected, and antibody encoding or producing B cells from the antibody-positive subject may then be obtained.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored

by sampling blood of the immunized animal at various points following immunization. A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens, lymph nodes, tonsils or adenoids, bone marrow aspirates or biopsies, tissue biopsies from mucosal organs like lung or GI tract, or from circulating blood. The antibody-producing B lymphocytes from the immunized animal or immune human are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized or human or human/mouse chimeric cells. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984). HMM2.5 cells or MFP-2 cells are particularly useful examples of such cells.

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. In some cases, transformation of human B cells with Epstein Barr virus (EBV) as an initial step increases the size of the B cells, enhancing fusion with the relatively large-sized myeloma cells. Transformation efficiency by EBV is enhanced by using CpG and a Chk2 inhibitor drug in the transforming medium. Alternatively, human B cells can be activated by co-culture with transfected cell lines expressing CD40 Ligand (CD154) in medium containing additional soluble factors, such as IL-21 and human B cell Activating Factor (BAFF), a Type II member of the TNF superfamily. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Geftter *et al.* (1977). The use of electrically induced fusion methods also is appropriate (Goding, pp. 71-74, 1986) and there are processes for better efficiency (Yu *et al.*, 2008). Fusion procedures usually produce viable hybrids at low frequencies, about 1×10^{-6} to 1×10^{-8} , but with optimized procedures one can achieve fusion efficiencies close to 1 in 200 (Yu *et al.*,

2008). However, relatively low efficiency of fusion does not pose a problem, as the viable, fused hybrids are differentiated from the parental, infused cells (particularly the infused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture medium. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the medium is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the medium is supplemented with hypoxanthine. Ouabain is added if the B cell source is an EBV-transformed human B cell line, in order to eliminate EBV-transformed lines that have not fused to the myeloma.

The preferred selection medium is HAT or HAT with ouabain. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells. When the source of B cells used for fusion is a line of EBV-transformed B cells, as here, ouabain may also be used for drug selection of hybrids as EBV-transformed B cells are susceptible to drug killing, whereas the myeloma partner used is chosen to be ouabain resistant.

Culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays dot immunobinding assays, and the like. The selected hybridomas are then serially diluted or single-cell sorted by flow cytometric sorting and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into an animal (*e.g.*, a mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. When human hybridomas are used in this way, it is optimal to inject immunocompromised mice, such as SCID mice, to prevent tumor rejection. The injected

animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily
5 obtained in high concentrations. Alternatively, human hybridoma cells lines can be used *in vitro* to produce immunoglobulins in cell supernatant. The cell lines can be adapted for growth in serum-free medium to optimize the ability to recover human monoclonal immunoglobulins of high purity.

MAbs produced by either means may be further purified, if desired, using filtration,
10 centrifugation and various chromatographic methods such as FPLC or affinity chromatography. Fragments of the monoclonal antibodies of the disclosure can be obtained from the purified monoclonal antibodies by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present disclosure can be
15 synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonal antibodies. Single B cells labelled with the antigen of interest can be sorted physically using paramagnetic bead selection or flow cytometric sorting, then RNA can be isolated from the single cells and antibody genes amplified by RT-PCR. Alternatively, antigen-
20 specific bulk sorted populations of cells can be segregated into microvesicles and the matched heavy and light chain variable genes recovered from single cells using physical linkage of heavy and light chain amplicons, or common barcoding of heavy and light chain genes from a vesicle. Matched heavy and light chain genes from single cells also can be obtained from populations of antigen specific B cells by treating cells with cell-penetrating nanoparticles
25 bearing RT-PCR primers and barcodes for marking transcripts with one barcode per cell. The antibody variable genes also can be isolated by RNA extraction of a hybridoma line and the antibody genes obtained by RT-PCR and cloned into an immunoglobulin expression vector. Alternatively, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the cell lines and phagemids expressing appropriate antibodies are selected by
30 panning using viral antigens. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present disclosure include U.S. Patent 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Patent 4,816,567 which describes recombinant immunoglobulin preparations; and U.S. Patent 4,867,973 which describes antibody-therapeutic agent conjugates.

B. Antibodies of the Present Disclosure

Antibodies according to the present disclosure may be defined, in the first instance, by their binding specificity. Those of skill in the art, by assessing the binding specificity/affinity of a given antibody using techniques well known to those of skill in the art, can determine whether such antibodies fall within the scope of the instant claims. For example, the epitope to which a given antibody bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) amino acids located within the antigen molecule (*e.g.*, a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within the antigen molecule (*e.g.*, a conformational epitope).

Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody “interacts with one or more amino acids” within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Cross-blocking can be measured in various binding assays such as ELISA, biolayer interferometry, or surface plasmon resonance. Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) *Methods Mol. Biol.* 248: 443-63), peptide cleavage analysis, high-resolution electron microscopy techniques using single particle reconstruction, cryoEM, or tomography, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) *Prot. Sci.* 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino

acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. See, *e.g.*, Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A. When the antibody neutralizes EV-D68, antibody escape mutant variant organisms can be isolated by propagating EV-D68 *in vitro* or in animal models in the presence of high concentrations of the antibody. Sequence analysis of the EV-D68 gene encoding the antigen targeted by the antibody reveals the mutation(s) conferring antibody escape, indicating residues in the epitope or that affect the structure of the epitope allosterically.

The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the disclosure into groups of antibodies binding different epitopes.

The present disclosure includes antibodies that may bind to the same epitope, or a portion of the epitope. Likewise, the present disclosure also includes antibodies that compete for binding to a target or a fragment thereof with any of the specific exemplary antibodies described herein. One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference, the

reference antibody is allowed to bind to target under saturating conditions. Next, the ability of a test antibody to bind to the target molecule is assessed. If the test antibody is able to bind to the target molecule following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the reference antibody. On the other hand, if the test antibody is not able to bind to the target molecule following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference antibody.

To determine if an antibody competes for binding with a reference anti- EV-D68 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to the EV-D68 antigen under saturating conditions followed by assessment of binding of the test antibody to the EV-D68 antigen. In a second orientation, the test antibody is allowed to bind to the EV-D68 antigen molecule under saturating conditions followed by assessment of binding of the reference antibody to the EV-D68 antigen. If, in both orientations, only the first (saturating) antibody is capable of binding to EV-D68, then it is concluded that the test antibody and the reference antibody compete for binding to EV-D68. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. Structural studies with

EM or crystallography also can demonstrate whether or not two antibodies that compete for binding recognize the same epitope.

In another aspect, there are provided monoclonal antibodies having clone-paired CDRs from the heavy and light chains as illustrated in Tables 3 and 4, respectively. Such antibodies
5 may be produced by the clones discussed below in the Examples section using methods described herein.

In another aspect, the antibodies may be defined by their variable sequence, which include additional "framework" regions. These are provided in Tables 1 and 2 that encode or represent full variable regions. Furthermore, the antibodies sequences may vary from these
10 sequences, optionally using methods discussed in greater detail below. For example, nucleic acid sequences may vary from those set out above in that (a) the variable regions may be segregated away from the constant domains of the light and heavy chains, (b) the nucleic acids may vary from those set out above while not affecting the residues encoded thereby, (c) the nucleic acids may vary from those set out above by a given percentage, *e.g.*, 70%, 75%, 80%,
15 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, (d) the nucleic acids may vary from those set out above by virtue of the ability to hybridize under high stringency conditions, as exemplified by low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C, (e) the amino acids may vary from those set out above by a given percentage, *e.g.*, 80%, 85%,
20 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, or (f) the amino acids may vary from those set out above by permitting conservative substitutions (discussed below). Each of the foregoing applies to the nucleic acid sequences set forth as Table 1 and the amino acid sequences of Table 2.

When comparing polynucleotide and polypeptide sequences, two sequences are said to
25 be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40
30 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, Wis.), using default parameters. This program embodies several alignment schemes described in the

following references: Dayhoff, M. O. (1978) A model of evolutionary change in proteins--
Matrices for detecting distant relationships. In Dayhoff, M. O. (ed.) Atlas of Protein Sequence
and Structure, National Biomedical Research Foundation, Washington D.C. Vol. 5, Suppl. 3,
pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogeny pp. 626-645
5 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, Calif.; Higgins, D. G. and
Sharp, P. M. (1989) CABIOS 5:151-153; Myers, E. W. and Muller W. (1988) CABIOS 4:11-
17; Robinson, E. D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) Mol. Biol. Evol.
4:406-425; Sneath, P. H. A. and Sokal, R. R. (1973) Numerical Taxonomy--the Principles and
Practice of Numerical Taxonomy, Freeman Press, San Francisco, Calif.; Wilbur, W. J. and
10 Lipman, D. J. (1983) Proc. Natl. Acad., Sci. USA 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the
local identity algorithm of Smith and Waterman (1981) Add. APL. Math 2:482, by the identity
alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for
similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. USA 85: 2444, by
15 computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and
TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575
Science Dr., Madison, Wis.), or by inspection.

One particular example of algorithms that are suitable for determining percent sequence
identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are
20 described in Altschul *et al.* (1977) Nucl. Acids Res. 25:3389-3402 and Altschul *et al.* (1990) J.
Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with
the parameters described herein, to determine percent sequence identity for the polynucleotides
and polypeptides of the disclosure. Software for performing BLAST analyses is publicly
available through the National Center for Biotechnology Information. The rearranged nature
25 of an antibody sequence and the variable length of each gene requires multiple rounds of
BLAST searches for a single antibody sequence. Also, manual assembly of different genes is
difficult and error-prone. The sequence analysis tool IgBLAST (world-wide-web at
ncbi.nlm.nih.gov/igblast/) identifies matches to the germline V, D and J genes, details at
rearrangement junctions, the delineation of Ig V domain framework regions and
30 complementarity determining regions. IgBLAST can analyze nucleotide or protein sequences
and can process sequences in batches and allows searches against the germline gene databases
and other sequence databases simultaneously to minimize the chance of missing possibly the
best matching germline V gene.

In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

In one approach, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Yet another way of defining an antibody is as a "derivative" of any of the below-described antibodies and their antigen-binding fragments. The term "derivative" refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to an antigen but which comprises, one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to a "parental" (or wild-type) molecule. Such amino acid substitutions or additions may introduce naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. The term "derivative" encompasses, for example, as

variants having altered CH1, hinge, CH2, CH3 or CH4 regions, so as to form, for example antibodies, *etc.*, having variant Fc regions that exhibit enhanced or impaired effector or binding characteristics. The term “derivative” additionally encompasses non-amino acid modifications, for example, amino acids that may be glycosylated (*e.g.*, have altered mannose, 2-N-acetylglucosamine, galactose, fucose, glucose, sialic acid, 5-N-acetylneuraminic acid, 5-glycolneuraminic acid, *etc.* content), acetylated, pegylated, phosphorylated, amidated, derivatized by known protecting/blocking groups, proteolytic cleavage, linked to a cellular ligand or other protein, *etc.* In some embodiments, the altered carbohydrate modifications modulate one or more of the following: solubilization of the antibody, facilitation of subcellular transport and secretion of the antibody, promotion of antibody assembly, conformational integrity, and antibody-mediated effector function. In a specific embodiment, the altered carbohydrate modifications enhance antibody mediated effector function relative to the antibody lacking the carbohydrate modification. Carbohydrate modifications that lead to altered antibody mediated effector function are well known in the art (for example, see Shields, R. L. *et al.* (2002) “*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcγ₃ RIII And Antibody-Dependent Cellular Toxicity*,” J. Biol. Chem. 277(30): 26733-26740; Davies J. *et al.* (2001) “*Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC Gamma RIII*,” Biotechnology & Bioengineering 74(4): 288-294). Methods of altering carbohydrate contents are known to those skilled in the art, see, *e.g.*, Wallick, S. C. *et al.* (1988) “*Glycosylation Of A VH Residue Of A Monoclonal Antibody Against Alpha (1---6) Dextran Increases Its Affinity For Antigen*,” J. Exp. Med. 168(3): 1099-1109; Tao, M. H. *et al.* (1989) “*Studies Of Aglycosylated Chimeric Mouse-Human IgG. Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region*,” J. Immunol. 143(8): 2595-2601; Routledge, E. G. *et al.* (1995) “*The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody*,” Transplantation 60(8):847-53; Elliott, S. *et al.* (2003) “*Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering*,” Nature Biotechnol. 21:414-21; Shields, R. L. *et al.* (2002) “*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcγ₃ RIII And Antibody-Dependent Cellular Toxicity*,” J. Biol. Chem. 277(30): 26733-26740).

A derivative antibody or antibody fragment can be generated with an engineered sequence or glycosylation state to confer preferred levels of activity in antibody dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-

dependent neutrophil phagocytosis (ADNP), or antibody-dependent complement deposition (ADCD) functions as measured by bead-based or cell-based assays or *in vivo* studies in animal models.

A derivative antibody or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* In one embodiment, an antibody derivative will possess a similar or identical function as the parental antibody. In another embodiment, an antibody derivative will exhibit an altered activity relative to the parental antibody. For example, a derivative antibody (or fragment thereof) can bind to its epitope more tightly or be more resistant to proteolysis than the parental antibody.

C. Engineering of Antibody Sequences

In various embodiments, one may choose to engineer sequences of the identified antibodies for a variety of reasons, such as improved expression, improved cross-reactivity or diminished off-target binding. Modified antibodies may be made by any technique known to those of skill in the art, including expression through standard molecular biological techniques, or the chemical synthesis of polypeptides. Methods for recombinant expression are addressed elsewhere in this document. The following is a general discussion of relevant goals techniques for antibody engineering.

Hybridomas may be cultured, then cells lysed, and total RNA extracted. Random hexamers may be used with RT to generate cDNA copies of RNA, and then PCR performed using a multiplex mixture of PCR primers expected to amplify all human variable gene sequences. PCR product can be cloned into pGEM-T Easy vector, then sequenced by automated DNA sequencing using standard vector primers. Assay of binding and neutralization may be performed using antibodies collected from hybridoma supernatants and purified by FPLC, using Protein G columns.

Recombinant full-length IgG antibodies can be generated by subcloning heavy and light chain Fv DNAs from the cloning vector into an IgG plasmid vector, transfected into 293 (*e.g.*, Freestyle) cells or CHO cells, and antibodies can be collected and purified from the 293 or CHO cell supernatant. Other appropriate host cells systems include bacteria, such as *E. coli*, insect cells (S2, Sf9, Sf29, High Five), plant cells (*e.g.*, tobacco, with or without engineering for human-like glycans), algae, or in a variety of non-human transgenic contexts, such as mice, rats, goats or cows.

Expression of nucleic acids encoding antibodies, both for the purpose of subsequent antibody purification, and for immunization of a host, is also contemplated. Antibody coding sequences can be RNA, such as native RNA or modified RNA. Modified RNA contemplates certain chemical modifications that confer increased stability and low immunogenicity to mRNAs, thereby facilitating expression of therapeutically important proteins. For instance, N1-methyl-pseudouridine (N1m Ψ) outperforms several other nucleoside modifications and their combinations in terms of translation capacity. In addition to turning off the immune/eIF2 α phosphorylation-dependent inhibition of translation, incorporated N1m Ψ nucleotides dramatically alter the dynamics of the translation process by increasing ribosome pausing and density on the mRNA. Increased ribosome loading of modified mRNAs renders them more permissive for initiation by favoring either ribosome recycling on the same mRNA or de novo ribosome recruitment. Such modifications could be used to enhance antibody expression *in vivo* following inoculation with RNA. The RNA, whether native or modified, may be delivered as naked RNA or in a delivery vehicle, such as a lipid nanoparticle.

Alternatively, DNA encoding the antibody may be employed for the same purposes. The DNA is included in an expression cassette comprising a promoter active in the host cell for which it is designed. The expression cassette is advantageously included in a replicable vector, such as a conventional plasmid or minivector. Vectors include viral vectors, such as poxviruses, adenoviruses, herpesviruses, adeno-associated viruses, and lentiviruses are contemplated. Replicons encoding antibody genes such as alphavirus replicons based on VEE virus or Sindbis virus are also contemplated. Delivery of such vectors can be performed by needle through intramuscular, subcutaneous, or intradermal routes, or by transcutaneous electroporation when *in vivo* expression is desired.

The rapid availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce the duration of process development programs. Lonza has developed a generic method using pooled transfectants grown in CDACF medium, for the rapid production of small quantities (up to 50 g) of antibodies in CHO cells. Although slightly slower than a true transient system, the advantages include a higher product concentration and use of the same host and process as the production cell line. Example of growth and productivity of GS-CHO pools, expressing a model antibody, in a disposable bioreactor: in a disposable bag bioreactor culture (5 L working volume) operated in fed-batch mode, a harvest antibody concentration of 2 g/L was achieved within 9 weeks of transfection.

Antibody molecules will comprise fragments (such as F(ab'), F(ab')₂) that are produced, for example, by the proteolytic cleavage of the mAbs, or single-chain immunoglobulins producible, for example, via recombinant means. F(ab') antibody derivatives are monovalent, while F(ab')₂ antibody derivatives are bivalent. In one embodiment, such fragments can be combined with one another, or with other antibody fragments or receptor ligands to form “chimeric” binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule.

In related embodiments, the antibody is a derivative of the disclosed antibodies, *e.g.*, an antibody comprising the CDR sequences identical to those in the disclosed antibodies (*e.g.*, a chimeric, or CDR-grafted antibody). Alternatively, one may wish to make modifications, such as introducing conservative changes into an antibody molecule. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: basic amino acids: arginine (+3.0), lysine (+3.0), and histidine (-0.5); acidic amino acids: aspartate (+3.0 ± 1), glutamate (+3.0 ± 1), asparagine (+0.2), and glutamine (+0.2); hydrophilic, nonionic amino acids: serine (+0.3), asparagine (+0.2), glutamine (+0.2), and threonine (-0.4), sulfur containing amino acids: cysteine (-1.0) and methionine (-1.3); hydrophobic, nonaromatic amino acids: valine (-1.5), leucine (-1.8), isoleucine (-1.8), proline (-0.5 ± 1), alanine (-0.5), and glycine (0); hydrophobic, aromatic amino acids: tryptophan (-3.4), phenylalanine (-2.5), and tyrosine (-2.3).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity and produce a biologically or immunologically modified protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred,

those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The present disclosure also contemplates isotype modification. By modifying the Fc region to have a different isotype, different functionalities can be achieved. For example, changing to IgG₁ can increase antibody dependent cell cytotoxicity, switching to class A can improve tissue distribution, and switching to class M can improve valency.

Alternatively or additionally, it may be useful to combine amino acid modifications with one or more further amino acid modifications that alter C1q binding and/or the complement dependent cytotoxicity (CDC) function of the Fc region of an IL-23p19 binding molecule. The binding polypeptide of particular interest may be one that binds to C1q and displays complement dependent cytotoxicity. Polypeptides with pre-existing C1q binding activity, optionally further having the ability to mediate CDC may be modified such that one or both of these activities are enhanced. Amino acid modifications that alter C1q and/or modify its complement dependent cytotoxicity function are described, for example, in WO/0042072, which is hereby incorporated by reference.

One can design an Fc region of an antibody with altered effector function, *e.g.*, by modifying C1q binding and/or Fc γ R binding and thereby changing CDC activity and/or ADCC activity. "Effector functions" are responsible for activating or diminishing a biological activity (*e.g.*, in a subject). Examples of effector functions include, but are not limited to: C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), *etc.* Such effector functions may require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain) and can be assessed using various assays (*e.g.*, Fc binding assays, ADCC assays, CDC assays, *etc.*).

For example, one can generate a variant Fc region of an antibody with improved C1q binding and improved Fc γ RIII binding (*e.g.*, having both improved ADCC activity and improved CDC activity). Alternatively, if it is desired that effector function be reduced or ablated, a variant Fc region can be engineered with reduced CDC activity and/or reduced

ADCC activity. In other embodiments, only one of these activities may be increased, and, optionally, also the other activity reduced (*e.g.*, to generate an Fc region variant with improved ADCC activity, but reduced CDC activity and *vice versa*).

FcRn binding. Fc mutations can also be introduced and engineered to alter their interaction with the neonatal Fc receptor (FcRn) and improve their pharmacokinetic properties. A collection of human Fc variants with improved binding to the FcRn have been described (Shields *et al.*, (2001). High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR, (J. Biol. Chem. 276:6591-6604). A number of methods are known that can result in increased half-life (Kuo and Aveson, (2011)), including amino acid modifications may be generated through techniques including alanine scanning mutagenesis, random mutagenesis and screening to assess the binding to the neonatal Fc receptor (FcRn) and/or the *in vivo* behavior. Computational strategies followed by mutagenesis may also be used to select one of amino acid mutations to mutate.

The present disclosure therefore provides a variant of an antigen binding protein with optimized binding to FcRn. In a particular embodiment, the said variant of an antigen binding protein comprises at least one amino acid modification in the Fc region of said antigen binding protein, wherein said modification is selected from the group consisting of 226, 227, 228, 230, 231, 233, 234, 239, 241, 243, 246, 250, 252, 256, 259, 264, 265, 267, 269, 270, 276, 284, 285, 288, 289, 290, 291, 292, 294, 297, 298, 299, 301, 302, 303, 305, 307, 308, 309, 311, 315, 317, 320, 322, 325, 327, 330, 332, 334, 335, 338, 340, 342, 343, 345, 347, 350, 352, 354, 355, 356, 359, 360, 361, 362, 369, 370, 371, 375, 378, 380, 382, 384, 385, 386, 387, 389, 390, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 403, 404, 408, 411, 412, 414, 415, 416, 418, 419, 420, 421, 422, 424, 426, 428, 433, 434, 438, 439, 440, 443, 444, 445, 446 and 447 of the Fc region as compared to said parent polypeptide, wherein the numbering of the amino acids in the Fc region is that of the EU index in Kabat. In a further aspect of the disclosure the modifications are M252Y/S254T/T256E.

Additionally, various publications describe methods for obtaining physiologically active molecules whose half-lives are modified, see for example Kontermann (2009) either by introducing an FcRn-binding polypeptide into the molecules or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved but affinities for other Fc receptors have been greatly reduced or fusing with FcRn binding domains of antibodies.

Derivatized antibodies may be used to alter the half-lives (*e.g.*, serum half-lives) of parental antibodies in a mammal, particularly a human. Such alterations may result in a half-

life of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the antibodies of the present disclosure or fragments thereof in a mammal, preferably a human, results in a higher serum titer of said antibodies or antibody fragments in the mammal, and thus reduces the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or fragments thereof with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor.

Beltramello *et al.* (2010) previously reported the modification of neutralizing mAbs, due to their tendency to enhance dengue virus infection, by generating in which leucine residues at positions 1.3 and 1.2 of CH2 domain (according to the IMGT unique numbering for C-domain) were substituted with alanine residues. This modification, also known as “LALA” mutation, abolishes antibody binding to FcγRI, FcγRII and FcγRIIIa, as described by Hessel *et al.* (2007). The variant and unmodified recombinant mAbs were compared for their capacity to neutralize and enhance infection by the four dengue virus serotypes. LALA variants retained the same neutralizing activity as unmodified mAb but were completely devoid of enhancing activity. LALA mutations of this nature are therefore contemplated in the context of the presently disclosed antibodies.

Altered Glycosylation. A particular embodiment of the present disclosure is an isolated monoclonal antibody, or antigen binding fragment thereof, containing a substantially homogeneous glycan without sialic acid, galactose, or fucose. The monoclonal antibody comprises a heavy chain variable region and a light chain variable region, both of which may be attached to heavy chain or light chain constant regions respectively. The aforementioned substantially homogeneous glycan may be covalently attached to the heavy chain constant region.

Another embodiment of the present disclosure comprises a mAb with a novel Fc glycosylation pattern. The isolated monoclonal antibody, or antigen binding fragment thereof, is present in a substantially homogenous composition represented by the GNGN or G1/G2 glycoform. Fc glycosylation plays a significant role in anti-viral and anti-cancer properties of therapeutic mAbs. The disclosure is in line with a recent study that shows increased anti-

lentivirus cell-mediated viral inhibition of a fucose free anti-HIV mAb *in vitro*. This embodiment of the present disclosure with homogenous glycans lacking a core fucose, showed increased protection against specific viruses by a factor greater than two-fold. Elimination of core fucose dramatically improves the ADCC activity of mAbs mediated by natural killer (NK) cells but appears to have the opposite effect on the ADCC activity of polymorphonuclear cells (PMNs).

The isolated monoclonal antibody, or antigen binding fragment thereof, comprising a substantially homogenous composition represented by the GNGN or G1/G2 glycoform exhibits increased binding affinity for Fc gamma RI and Fc gamma RIII compared to the same antibody without the substantially homogeneous GNGN glycoform and with G0, G1F, G2F, GNF, GNGNF or GNGNFX containing glycoforms. In one embodiment of the present disclosure, the antibody dissociates from Fc gamma RI with a K_d of 1×10^{-8} M or less and from Fc gamma RIII with a K_d of 1×10^{-7} M or less.

Glycosylation of an Fc region is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. The recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain peptide sequences are asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. Thus, the presence of either of these peptide sequences in a polypeptide creates a potential glycosylation site.

The glycosylation pattern may be altered, for example, by deleting one or more glycosylation site(s) found in the polypeptide, and/or adding one or more glycosylation site(s) that are not present in the polypeptide. Addition of glycosylation sites to the Fc region of an antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). An exemplary glycosylation variant has an amino acid substitution of residue Asn 297 of the heavy chain. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original polypeptide (for O-linked glycosylation sites). Additionally, a change of Asn 297 to Ala can remove one of the glycosylation sites.

In certain embodiments, the antibody is expressed in cells that express beta (1,4)-N-acetylglucosaminyltransferase III (GnT III), such that GnT III adds GlcNAc to the IL-23p19 antibody. Methods for producing antibodies in such a fashion are provided in WO/9954342,

WO/03011878, patent publication 20030003097A1, and Umana *et al.*, *Nature Biotechnology*, 17:176-180, February 1999. Cell lines can be altered to enhance or reduce or eliminate certain post-translational modifications, such as glycosylation, using genome editing technology such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). For example, CRISPR technology can be used to eliminate genes encoding glycosylating enzymes in 293 or CHO cells used to express recombinant monoclonal antibodies.

Elimination of monoclonal antibody protein sequence liabilities. It is possible to engineer the antibody variable gene sequences obtained from human B cells to enhance their manufacturability and safety. Potential protein sequence liabilities can be identified by searching for sequence motifs associated with sites containing:

- 1) Unpaired Cys residues,
- 2) N-linked glycosylation,
- 3) Asn deamidation,
- 4) Asp isomerization,
- 5) SYE truncation,
- 6) Met oxidation,
- 7) Trp oxidation,
- 8) N-terminal glutamate,
- 9) Integrin binding,
- 10) CD11c/CD18 binding, or
- 11) Fragmentation

Such motifs can be eliminated by altering the synthetic gene for the cDNA encoding recombinant antibodies.

Protein engineering efforts in the field of development of therapeutic antibodies clearly reveal that certain sequences or residues are associated with solubility differences (Fernandez-Escamilla *et al.*, *Nature Biotech.*, 22 (10), 1302-1306, 2004; Chennamsetty *et al.*, *PNAS*, 106 (29), 11937-11942, 2009; Voynov *et al.*, *Biocon. Chem.*, 21 (2), 385-392, 2010) Evidence from solubility-altering mutations in the literature indicate that some hydrophilic residues such as aspartic acid, glutamic acid, and serine contribute significantly more favorably to protein solubility than other hydrophilic residues, such as asparagine, glutamine, threonine, lysine, and arginine.

Stability. Antibodies can be engineered for enhanced biophysical properties. One can use elevated temperature to unfold antibodies to determine relative stability, using average apparent melting temperatures. Differential Scanning Calorimetry (DSC) measures the heat

capacity, C_p , of a molecule (the heat required to warm it, per degree) as a function of temperature. One can use DSC to study the thermal stability of antibodies. DSC data for mAbs is particularly interesting because it sometimes resolves the unfolding of individual domains within the mAb structure, producing up to three peaks in the thermogram (from unfolding of the Fab, C_H2 , and C_H3 domains). Typically unfolding of the Fab domain produces the strongest peak. The DSC profiles and relative stability of the Fc portion show characteristic differences for the human IgG₁, IgG₂, IgG₃, and IgG₄ subclasses (Garber and Demarest, *Biochem. Biophys. Res. Commun.* 355, 751-757, 2007). One also can determine average apparent melting temperature using circular dichroism (CD), performed with a CD spectrometer. Far-UV CD spectra will be measured for antibodies in the range of 200 to 260 nm at increments of 0.5 nm. The final spectra can be determined as averages of 20 accumulations. Residue ellipticity values can be calculated after background subtraction. Thermal unfolding of antibodies (0.1 mg/mL) can be monitored at 235 nm from 25-95 °C and a heating rate of 1 °C/min. One can use dynamic light scattering (DLS) to assess for propensity for aggregation. DLS is used to characterize size of various particles including proteins. If the system is not disperse in size, the mean effective diameter of the particles can be determined. This measurement depends on the size of the particle core, the size of surface structures, and particle concentration. Since DLS essentially measures fluctuations in scattered light intensity due to particles, the diffusion coefficient of the particles can be determined. DLS software in commercial DLA instruments displays the particle population at different diameters. Stability studies can be done conveniently using DLS. DLS measurements of a sample can show whether the particles aggregate over time or with temperature variation by determining whether the hydrodynamic radius of the particle increases. If particles aggregate, one can see a larger population of particles with a larger radius. Stability depending on temperature can be analyzed by controlling the temperature *in situ*. Capillary electrophoresis (CE) techniques include proven methodologies for determining features of antibody stability. One can use an iCE approach to resolve antibody protein charge variants due to deamidation, C-terminal lysines, sialylation, oxidation, glycosylation, and any other change to the protein that can result in a change in pI of the protein. Each of the expressed antibody proteins can be evaluated by high throughput, free solution isoelectric focusing (IEF) in a capillary column (cIEF), using a Protein Simple Maurice instrument. Whole-column UV absorption detection can be performed every 30 seconds for real time monitoring of molecules focusing at the isoelectric points (pIs). This approach combines the high resolution of traditional gel IEF with the advantages of quantitation and automation found in column-based separations while eliminating the need for a mobilization step. The technique yields

reproducible, quantitative analysis of identity, purity, and heterogeneity profiles for the expressed antibodies. The results identify charge heterogeneity and molecular sizing on the antibodies, with both absorbance and native fluorescence detection modes and with sensitivity of detection down to 0.7 µg/mL.

5 **Solubility.** One can determine the intrinsic solubility score of antibody sequences. The intrinsic solubility scores can be calculated using CamSol Intrinsic (Sormanni *et al.*, *J Mol Biol* 427, 478-490, 2015). The amino acid sequences for residues 95-102 (Kabat numbering) in HCDR3 of each antibody fragment such as a scFv can be evaluated via the online program to calculate the solubility scores. One also can determine solubility using laboratory techniques.
10 Various techniques exist, including addition of lyophilized protein to a solution until the solution becomes saturated and the solubility limit is reached, or concentration by ultrafiltration in a microconcentrator with a suitable molecular weight cut-off. The most straightforward method is induction of amorphous precipitation, which measures protein solubility using a method involving protein precipitation using ammonium sulfate (Trevino *et al.*, *J Mol*
15 *Biol*, 366: 449-460, 2007). Ammonium sulfate precipitation gives quick and accurate information on relative solubility values. Ammonium sulfate precipitation produces precipitated solutions with well-defined aqueous and solid phases and requires relatively small amounts of protein. Solubility measurements performed using induction of amorphous precipitation by ammonium sulfate also can be done easily at different pH values. Protein
20 solubility is highly pH dependent, and pH is considered the most important extrinsic factor that affects solubility.

Autoreactivity. Generally, it is thought that autoreactive clones should be eliminated during ontogeny by negative selection, however it has become clear that many human naturally occurring antibodies with autoreactive properties persist in adult mature repertoires, and the
25 autoreactivity may enhance the antiviral function of many antibodies to pathogens. It has been noted that HCDR3 loops in antibodies during early B cell development are often rich in positive charge and exhibit autoreactive patterns (Wardemann *et al.*, *Science* 301, 1374-1377, 2003). One can test a given antibody for autoreactivity by assessing the level of binding to human origin cells in microscopy (using adherent HeLa or HEp-2 epithelial cells) and flow cytometric
30 cell surface staining (using suspension Jurkat T cells and 293S human embryonic kidney cells). Autoreactivity also can be surveyed using assessment of binding to tissues in tissue arrays.

Preferred residues (“Human Likeness”). B cell repertoire deep sequencing of human B cells from blood donors is being performed on a wide scale in many recent studies. Sequence information about a significant portion of the human antibody repertoire facilitates statistical

assessment of antibody sequence features common in healthy humans. With knowledge about the antibody sequence features in a human recombined antibody variable gene reference database, the position specific degree of “Human Likeness” (HL) of an antibody sequence can be estimated. HL has been shown to be useful for the development of antibodies in clinical use, like therapeutic antibodies or antibodies as vaccines. The goal is to increase the human likeness of antibodies to reduce potential adverse effects and anti-antibody immune responses that will lead to significantly decreased efficacy of the antibody drug or can induce serious health implications. One can assess antibody characteristics of the combined antibody repertoire of three healthy human blood donors of about 400 million sequences in total and created a novel “relative Human Likeness” (rHL) score that focuses on the hypervariable region of the antibody. The rHL score allows one to easily distinguish between human (positive score) and non-human sequences (negative score). Antibodies can be engineered to eliminate residues that are not common in human repertoires.

D. Single Chain Antibodies

A single chain variable fragment (scFv) is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short (usually serine, glycine) linker. This chimeric molecule retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide. This modification usually leaves the specificity unaltered. These molecules were created historically to facilitate phage display where it is highly convenient to express the antigen binding domain as a single peptide. Alternatively, scFv can be created directly from subcloned heavy and light chains derived from a hybridoma or B cell. Single chain variable fragments lack the constant Fc region found in complete antibody molecules, and thus, the common binding sites (*e.g.*, protein A/G) used to purify antibodies. These fragments can often be purified/immobilized using Protein L since Protein L interacts with the variable region of kappa light chains.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alanine, serine and glycine. However, other residues can function as well. Tang *et al.* (1996) used phage display as a means of rapidly selecting tailored linkers for single-chain antibodies (scFvs) from protein linker libraries. A random linker library was constructed in which the genes for the heavy and light chain variable domains were linked by a segment encoding an 18-amino acid polypeptide of variable composition. The scFv repertoire (approx. 5×10^6 different members) was displayed on filamentous phage and subjected to affinity selection with hapten. The population of selected variants exhibited significant increases in

binding activity but retained considerable sequence diversity. Screening 1054 individual variants subsequently yielded a catalytically active scFv that was produced efficiently in soluble form. Sequence analysis revealed a conserved proline in the linker two residues after the V_H C terminus and an abundance of arginines and prolines at other positions as the only
5 common features of the selected tethers.

The recombinant antibodies of the present disclosure may also involve sequences or moieties that permit dimerization or multimerization of the receptors. Such sequences include those derived from IgA, which permit formation of multimers in conjunction with the J-chain. Another multimerization domain is the Gal4 dimerization domain. In other embodiments, the
10 chains may be modified with agents such as biotin/avidin, which permit the combination of two antibodies.

In a separate embodiment, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus
15 of the light chain via an appropriate chemical bridge).

Cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules, *e.g.*, a stabilizing and coagulating agent. However, it is contemplated that dimers or multimers of the same analog or heteromeric complexes comprised of different
20 analogs can be created. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (*e.g.*, N-hydroxy succinimide) and the other reacting with a thiol group (*e.g.*, pyridyl disulfide, maleimides, halogens, *etc.*). Through the primary amine
25 reactive group, the cross-linker may react with the lysine residue(s) of one protein (*e.g.*, the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (*e.g.*, the selective agent).

It is preferred that a cross-linker having reasonable stability in blood will be employed.
30 Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*, preventing release of the targeting peptide prior to reaching the site of action. These linkers are thus one group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is “sterically hindered” by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (*e.g.*, the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent. Particular uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

E. Multispecific Antibodies

In certain embodiments, antibodies of the present disclosure are bispecific or multispecific. Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of a single antigen. Other such antibodies may combine a first antigen binding site with a binding site for a second antigen. Alternatively, an anti-pathogen arm may be combined with an arm that binds to a triggering molecule on a leukocyte, such as a T-cell receptor molecule (*e.g.*, CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and Fc gamma RIII (CD16), so as to focus and localize cellular defense mechanisms to the infected cell.

Bispecific antibodies may also be used to localize cytotoxic agents to infected cells. These antibodies possess a pathogen-binding arm and an arm that binds the cytotoxic agent (*e.g.*, saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies). WO 96/16673 describes a bispecific anti-ErbB2/anti-Fc gamma RIII antibody and U.S. Patent 5,837,234 discloses a bispecific anti-ErbB2/anti-Fc gamma RI antibody. A bispecific anti-ErbB2/Fc alpha antibody is shown in WO98/02463. U.S. Patent 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, EMBO J., 10:3655-3659 (1991).

According to a different approach, antibody variable regions with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_{H2}, and C_{H3} regions. It is preferred to have the first heavy-chain constant region (C_{H1}) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-

transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three
5 polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant effect on the yield of the desired chain combination.

In a particular embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid
10 immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating
15 bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 121:210 (1986).

According to another approach described in U.S. Patent 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_{H3} domain. In this method, one or more small amino acid side
20 chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such
25 as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent 4,676,980), and for treatment of HIV infection (WO 91/00360,
30 WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using

chemical linkage. Brennan *et al.*, Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Techniques exist that facilitate the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, J. Exp. Med., 175: 217-225 (1992) describe the production of a humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described (Merchant *et al.*, *Nat. Biotechnol.* 16, 677-681 (1998) doi:10.1038/nbt0798-677pmid:9661204). For example, bispecific antibodies have been produced using leucine zippers (Kostelny *et al.*, J. Immunol., 148(5):1547-1553, 1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a V_H connected to a V_L by a linker that is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, J. Immunol., 152:5368 (1994).

In a particular embodiment, a bispecific or multispecific antibody may be formed as a DOCK-AND-LOCK™ (DNL™) complex (see, e.g., U.S. Patents 7,521,056; 7,527,787;

7,534,866; 7,550,143 and 7,666,400, the Examples section of each of which is incorporated herein by reference.) Generally, the technique takes advantage of the specific and high-affinity binding interactions that occur between a dimerization and docking domain (DDD) sequence of the regulatory (R) subunits of cAMP-dependent protein kinase (PKA) and an anchor domain (AD) sequence derived from any of a variety of AKAP proteins (Baillie et al., FEBS Letters. 2005; 579: 3264; Wong and Scott, Nat. Rev. Mol. Cell Biol. 2004; 5: 959). The DDD and AD peptides may be attached to any protein, peptide or other molecule. Because the DDD sequences spontaneously dimerize and bind to the AD sequence, the technique allows the formation of complexes between any selected molecules that may be attached to DDD or AD sequences.

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared (Tutt *et al.*, J. Immunol. 147: 60, 1991; Xu *et al.*, *Science*, 358(6359):85-90, 2017). A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind.

The antibodies of the present disclosure can be multivalent antibodies with three or more antigen binding sites (*e.g.*, tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable regions. For instance, the polypeptide chain(s) may comprise VD1-(X1)_n-VD2-(X2)_n-Fc, wherein VD1 is a first variable region, VD2 is a second variable region, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable region polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable region

polypeptides. The light chain variable region polypeptides contemplated here comprise a light chain variable region and, optionally, further comprise a C_L domain.

Charge modifications are particularly useful in the context of a multispecific antibody, where amino acid substitutions in Fab molecules result in reducing the mispairing of light chains with non-matching heavy chains (Bence-Jones-type side products), which can occur in the production of Fab-based bi-/multispecific antigen binding molecules with a V_H/V_L exchange in one (or more, in case of molecules comprising more than two antigen-binding Fab molecules) of their binding arms (see also PCT publication no. WO 2015/150447, particularly the examples therein, incorporated herein by reference in its entirety).

Accordingly, in particular embodiments, an antibody comprised in the therapeutic agent comprises

(a) a first Fab molecule which specifically binds to a first antigen

(b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains V_L and V_H of the Fab light chain and the Fab heavy chain are replaced by each other,

wherein the first antigen is an activating T cell antigen and the second antigen is a target cell antigen, or the first antigen is a target cell antigen and the second antigen is an activating T cell antigen; and

wherein

i) in the constant domain C_L of the first Fab molecule under a) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain C_{H1} of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index); or

ii) in the constant domain C_L of the second Fab molecule under b) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain C_{H1} of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index).

The antibody may not comprise both modifications mentioned under i) and ii). The constant domains C_L and C_{H1} of the second Fab molecule are not replaced by each other (*i.e.*, remain unexchanged).

In another embodiment of the antibody, in the constant domain C_L of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K),

arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In an even more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

F. Chimeric Antigen Receptors

Artificial T cell receptors (also known as chimeric T cell receptors, chimeric immunoreceptors, chimeric antigen receptors (CARs)) are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell, with transfer of their coding sequence facilitated by retroviral vectors. In this way, a large number of target-specific T cells can be generated for adoptive cell transfer. Phase I clinical studies of this approach show efficacy.

The most common form of these molecules are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, fused to CD3-zeta transmembrane and endodomain. Such molecules result in the transmission of a zeta signal in response to recognition by the scFv of its target. An example of such a construct is 14g2a-Zeta, which is a fusion of a scFv derived from hybridoma 14g2a (which recognizes disialoganglioside GD2). When T cells express this molecule (usually achieved by oncoretroviral vector transduction), they recognize and kill target cells that express GD2 (*e.g.*, neuroblastoma cells). To target malignant B cells, investigators have redirected the specificity of T cells using a chimeric immunoreceptor specific for the B-lineage molecule, CD19.

The variable portions of an immunoglobulin heavy and light chain are fused by a flexible linker to form a scFv. This scFv is preceded by a signal peptide to direct the nascent protein to the endoplasmic reticulum and subsequent surface expression (this is cleaved). A flexible spacer allows to the scFv to orient in different directions to enable antigen binding. The transmembrane domain is a typical hydrophobic alpha helix usually derived from the original molecule of the signaling endodomain which protrudes into the cell and transmits the desired signal.

Type I proteins are in fact two protein domains linked by a transmembrane alpha helix in between. The cell membrane lipid bilayer, through which the transmembrane domain passes, acts to isolate the inside portion (endodomain) from the external portion (ectodomain). It is not so surprising that attaching an ectodomain from one protein to an endodomain of another protein results in a molecule that combines the recognition of the former to the signal of the latter.

Ectodomain. A signal peptide directs the nascent protein into the endoplasmic reticulum. This is essential if the receptor is to be glycosylated and anchored in the cell membrane. Any eukaryotic signal peptide sequence usually works fine. Generally, the signal

peptide natively attached to the amino-terminal most component is used (*e.g.*, in a scFv with orientation light chain - linker - heavy chain, the native signal of the light-chain is used

The antigen recognition domain is usually an scFv. There are however many alternatives. An antigen recognition domain from native T-cell receptor (TCR) alpha and beta single chains have been described, as have simple ectodomains (*e.g.*, CD4 ectodomain to recognize HIV infected cells) and more exotic recognition components such as a linked cytokine (which leads to recognition of cells bearing the cytokine receptor). In fact, almost anything that binds a given target with high affinity can be used as an antigen recognition region.

A spacer region links the antigen binding domain to the transmembrane domain. It should be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. The simplest form is the hinge region from IgG1. Alternatives include the CH₂CH₃ region of immunoglobulin and portions of CD3. For most scFv based constructs, the IgG1 hinge suffices. However, the best spacer often has to be determined empirically.

Transmembrane domain. The transmembrane domain is a hydrophobic alpha helix that spans the membrane. Generally, the transmembrane domain from the most membrane proximal component of the endodomain is used. Interestingly, using the CD3-zeta transmembrane domain may result in incorporation of the artificial TCR into the native TCR a factor that is dependent on the presence of the native CD3-zeta transmembrane charged aspartic acid residue. Different transmembrane domains result in different receptor stability. The CD28 transmembrane domain results in a brightly expressed, stable receptor.

Endodomain. This is the "business-end" of the receptor. After antigen recognition, receptors cluster and a signal is transmitted to the cell. The most commonly used endodomain component is CD3-zeta which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling is needed.

"First-generation" CARs typically had the intracellular domain from the CD3 ζ - chain, which is the primary transmitter of signals from endogenous TCRs. "Second-generation" CARs add intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 41BB, ICOS) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. Preclinical studies have indicated that the second generation of CAR designs improves the antitumor activity of T cells. More recent, "third-generation" CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, to further augment potency.

G. ADCs

Antibody Drug Conjugates or ADCs are a new class of highly potent biopharmaceutical drugs designed as a targeted therapy for the treatment of people with infectious disease. ADCs are complex molecules composed of an antibody (a whole mAb or an antibody fragment such as a single-chain variable fragment, or scFv) linked, via a stable chemical linker with labile bonds, to a biological active cytotoxic/anti-viral payload or drug. Antibody Drug Conjugates are examples of bioconjugates and immunoconjugates.

By combining the unique targeting capabilities of monoclonal antibodies with the cancer-killing ability of cytotoxic drugs, antibody-drug conjugates allow sensitive discrimination between healthy and diseased tissue. This means that, in contrast to traditional systemic approaches, antibody-drug conjugates target and attack the infected cell so that healthy cells are less severely affected.

In the development ADC-based anti-tumor therapies, an anticancer drug (*e.g.*, a cell toxin or cytotoxin) is coupled to an antibody that specifically targets a certain cell marker (*e.g.*, a protein that, ideally, is only to be found in or on infected cells). Antibodies track these proteins down in the body and attach themselves to the surface of cancer cells. The biochemical reaction between the antibody and the target protein (antigen) triggers a signal in the tumor cell, which then absorbs or internalizes the antibody together with the cytotoxin. After the ADC is internalized, the cytotoxic drug is released and kills the cell or impairs viral replication. Due to this targeting, ideally the drug has lower side effects and gives a wider therapeutic window than other agents.

A stable link between the antibody and cytotoxic/anti-viral agent is a crucial aspect of an ADC. Linkers are based on chemical motifs including disulfides, hydrazones or peptides (cleavable), or thioethers (non-cleavable) and control the distribution and delivery of the cytotoxic agent to the target cell. Cleavable and non-cleavable types of linkers have been proven to be safe in preclinical and clinical trials. Brentuximab vedotin includes an enzyme-sensitive cleavable linker that delivers the potent and highly toxic anti-microtubule agent Monomethyl auristatin E or MMAE, a synthetic antineoplastic agent, to human specific CD30-positive malignant cells. Because of its high toxicity MMAE, which inhibits cell division by blocking the polymerization of tubulin, cannot be used as a single-agent chemotherapeutic drug. However, the combination of MMAE linked to an anti-CD30 monoclonal antibody (cAC10, a cell membrane protein of the tumor necrosis factor or TNF receptor) proved to be stable in extracellular fluid, cleavable by cathepsin and safe for therapy. Trastuzumab emtansine, the other approved ADC, is a combination of the microtubule-formation inhibitor mertansine (DM-

1), a derivative of the Maytansine, and antibody trastuzumab (Herceptin®/Genentech/Roche) attached by a stable, non-cleavable linker.

The availability of better and more stable linkers has changed the function of the chemical bond. The type of linker, cleavable or non-cleavable, lends specific properties to the cytotoxic (anti-cancer) drug. For example, a non-cleavable linker keeps the drug within the cell. As a result, the entire antibody, linker and cytotoxic agent enter the targeted cancer cell where the antibody is degraded to the level of an amino acid. The resulting complex – amino acid, linker and cytotoxic agent – now becomes the active drug. In contrast, cleavable linkers are catalyzed by enzymes in the host cell where it releases the cytotoxic agent.

Another type of cleavable linker, currently in development, adds an extra molecule between the cytotoxic/anti-viral drug and the cleavage site. This linker technology allows researchers to create ADCs with more flexibility without worrying about changing cleavage kinetics. Researchers are also developing a new method of peptide cleavage based on Edman degradation, a method of sequencing amino acids in a peptide. Future direction in the development of ADCs also include the development of site-specific conjugation (TDCs) to further improve stability and therapeutic index and α emitting immunoconjugates and antibody-conjugated nanoparticles.

H. BiTES

Bi-specific T-cell engagers (BiTEs) are a class of artificial bispecific monoclonal antibodies that are investigated for the use as anti-cancer drugs. They direct a host's immune system, more specifically the T cells' cytotoxic activity, against infected cells. *BiTE* is a registered trademark of Micromet AG.

BiTEs are fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies, or amino acid sequences from four different genes, on a single peptide chain of about 55 kilodaltons. One of the scFvs binds to T cells via the CD3 receptor, and the other to an infected cell via a specific molecule.

Like other bispecific antibodies, and unlike ordinary monoclonal antibodies, BiTEs form a link between T cells and target cells. This causes T cells to exert cytotoxic/anti-viral activity on infected cells by producing proteins like perforin and granzymes, independently of the presence of MHC I or co-stimulatory molecules. These proteins enter infected cells and initiate the cell's apoptosis. This action mimics physiological processes observed during T cell attacks against infected cells.

I. Intrabodies

In a particular embodiment, the antibody is a recombinant antibody that is suitable for action inside of a cell – such antibodies are known as “intrabodies.” These antibodies may interfere with target function by a variety of mechanism, such as by altering intracellular protein trafficking, interfering with enzymatic function, and blocking protein-protein or protein-DNA interactions. In many ways, their structures mimic or parallel those of single chain and single domain antibodies, discussed above. Indeed, single-transcript/single-chain is an important feature that permits intracellular expression in a target cell, and also makes protein transit across cell membranes more feasible. However, additional features are required.

The two major issues impacting the implementation of intrabody therapeutic are delivery, including cell/tissue targeting, and stability. With respect to delivery, a variety of approaches have been employed, such as tissue-directed delivery, use of cell-type specific promoters, viral-based delivery and use of cell-permeability/membrane translocating peptides. With respect to the stability, the approach is generally to either screen by brute force, including methods that involve phage display and may include sequence maturation or development of consensus sequences, or more directed modifications such as insertion stabilizing sequences (*e.g.*, Fc regions, chaperone protein sequences, leucine zippers) and disulfide replacement/modification.

An additional feature that intrabodies may require is a signal for intracellular targeting. Vectors that can target intrabodies (or other proteins) to subcellular regions such as the cytoplasm, nucleus, mitochondria and ER have been designed and are commercially available (Invitrogen Corp.; Persic *et al.*, 1997).

By virtue of their ability to enter cells, intrabodies have additional uses that other types of antibodies may not achieve. In the case of the present antibodies, the ability to interact with the MUC1 cytoplasmic domain in a living cell may interfere with functions associated with the MUC1 CD, such as signaling functions (binding to other molecules) or oligomer formation. In particular, it is contemplated that such antibodies can be used to inhibit MUC1 dimer formation.

J. Purification

In certain embodiments, the antibodies of the present disclosure may be purified. The term “purified,” as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein is purified to any degree relative to its naturally-obtainable state. A purified protein therefore also refers to a protein, free from the environment in which it may naturally occur. Where the term “substantially purified” is used, this designation will

refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Other methods for protein purification include, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; gel filtration, reverse phase, hydroxylapatite and affinity chromatography; and combinations of such and other techniques.

In purifying an antibody of the present disclosure, it may be desirable to express the polypeptide in a prokaryotic or eukaryotic expression system and extract the protein using denaturing conditions. The polypeptide may be purified from other cellular components using an affinity column, which binds to a tagged portion of the polypeptide. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Commonly, complete antibodies are fractionated utilizing agents (*i.e.*, protein A) that bind the Fc portion of the antibody. Alternatively, antigens may be used to simultaneously purify and select appropriate antibodies. Such methods often utilize the selection agent bound to a support, such as a column, filter or bead. The antibodies are bound to a support, contaminants removed (*e.g.*, washed away), and the antibodies released by applying conditions (salt, heat, *etc.*).

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. Another method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity. The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to

follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

III. Active/Passive Immunization and Treatment/Prevention of Enterovirus D68 Infection

A. Formulation and Administration

The present disclosure provides pharmaceutical compositions comprising anti-EV-D68 antibodies and antigens for generating the same. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody or a fragment thereof, or a peptide immunogen, and a pharmaceutically acceptable carrier. In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a particular carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Other suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical agents are described in “Remington's Pharmaceutical Sciences.” Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of

carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration, which can be oral, intravenous, intraarterial, intrabuccal, intranasal, nebulized, bronchial inhalation, intra-rectal, vaginal, topical or delivered by mechanical ventilation.

5 Active vaccines are also envisioned where antibodies like those disclosed are produced *in vivo* in a subject at risk of EV-D68 infection. Such vaccines can be formulated for parenteral administration, *e.g.*, formulated for injection *via* the intradermal, intravenous, intramuscular, subcutaneous, aerosol or even intraperitoneal routes. Administration by intradermal and intramuscular routes are contemplated. The vaccine could alternatively be administered by a
10 topical route directly to the mucosa, for example by nasal drops, inhalation, by nebulizer, or via intrarectal or vaginal delivery. Pharmaceutically acceptable salts include the acid salts and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example,
15 sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Passive transfer of antibodies, known as artificially acquired passive immunity, generally will involve the use of intravenous or intramuscular injections. The forms of antibody can be human or animal blood plasma or serum, as pooled human immunoglobulin for
20 intravenous (IVIG) or intramuscular (IG) use, as high-titer human IVIG or IG from immunized or from donors recovering from disease, and as monoclonal antibodies (MAb). Such immunity generally lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. However, passive immunity provides immediate protection. The antibodies will be
25 formulated in a carrier suitable for injection, *i.e.*, sterile and syringeable.

Generally, the ingredients of compositions of the disclosure are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion,
30 it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from

hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

2. ADCC

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or fragments thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. By “antibody having increased/reduced antibody dependent cell-mediated cytotoxicity (ADCC)” is meant an antibody having increased/reduced ADCC as determined by any suitable method known to those of ordinary skill in the art.

As used herein, the term “increased/reduced ADCC” is defined as either an increase/reduction in the number of target cells that are lysed in a given time, at a given concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or a reduction/increase in the concentration of antibody, in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The increase/reduction in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard production, purification, formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example the increase in ADCC mediated by an antibody produced by host cells engineered to have an altered pattern of glycosylation (*e.g.*, to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein, is relative to the ADCC mediated by the same antibody produced by the same type of non-engineered host cells.

3. CDC

Complement-dependent cytotoxicity (CDC) is a function of the complement system. It is the processes in the immune system that kill pathogens by damaging their membranes without the involvement of antibodies or cells of the immune system. There are three main processes. All three insert one or more membrane attack complexes (MAC) into the pathogen which cause lethal colloid-osmotic swelling, *i.e.*, CDC. It is one of the mechanisms by which antibodies or antibody fragments have an anti-viral effect.

IV. Antibody Conjugates

Antibodies of the present disclosure may be linked to at least one agent to form an antibody conjugate. In order to increase the efficacy of antibody molecules as diagnostic or therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one effector or reporter molecule. Effector molecules comprise molecules having a desired activity, *e.g.*, cytotoxic activity. Non-limiting examples of effector molecules which have been attached to antibodies include toxins, anti-tumor agents, therapeutic enzymes, radionuclides, antiviral agents, chelating agents, cytokines, growth factors, and oligo- or polynucleotides. By contrast, a reporter molecule is defined as any moiety which may be detected using an assay. Non-limiting examples of reporter molecules which have been conjugated to antibodies include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, photoaffinity molecules, colored particles or ligands, such as biotin.

Antibody conjugates are generally preferred for use as diagnostic agents. Antibody diagnostics generally fall within two classes, those for use in *in vitro* diagnostics, such as in a variety of immunoassays, and those for use *in vivo* diagnostic protocols, generally known as "antibody-directed imaging." Many appropriate imaging agents are known in the art, as are methods for their attachment to antibodies (see, for *e.g.*, U.S. Patents 5,021,236, 4,938,948, and 4,472,509). The imaging moieties used can be paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one might mention astatine²¹¹, ¹⁴carbon, ⁵¹chromium, ³⁶chlorine, ⁵⁷cobalt, ⁵⁸cobalt, copper⁶⁷, ¹⁵²Eu, gallium⁶⁷, ³hydrogen, iodine¹²³, iodine¹²⁵, iodine¹³¹, indium¹¹¹, ⁵⁹iron, ³²phosphorus, rhenium¹⁸⁶, rhenium¹⁸⁸, ⁷⁵selenium, ³⁵sulphur, technetium^{99m} and/or yttrium⁹⁰. ¹²⁵I is often being preferred for use in certain embodiments, and technetium^{99m} and/or indium¹¹¹ are also often preferred due to their low energy and suitability for long range detection. Radioactively labeled monoclonal antibodies of the present disclosure may be produced according to well-known

methods in the art. For instance, monoclonal antibodies can be iodinated by contact with sodium and/or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Monoclonal antibodies according to the disclosure may be labeled with technetium^{99m} by ligand exchange process, for example, by
5 reducing pertechnate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column. Alternatively, direct labeling techniques may be used, *e.g.*, by incubating pertechnate, a reducing agent such as SnCl_2 , a buffer solution such as sodium-potassium phthalate solution, and the antibody. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to antibody are
10 diethylenetriaminepentaacetic acid (DTPA) or ethylene diaminetetracetic acid (EDTA).

Among the fluorescent labels contemplated for use as conjugates include Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG,
15 Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

Additional types of antibodies contemplated in the present disclosure are those intended primarily for use *in vitro*, where the antibody is linked to a secondary binding ligand and/or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a
20 chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase or glucose oxidase. Preferred secondary binding ligands are biotin and avidin and streptavidin compounds. The use of such labels is well known to those of skill in the art and are described, for example, in U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241.

Yet another known method of site-specific attachment of molecules to antibodies comprises the reaction of antibodies with hapten-based affinity labels. Essentially, hapten-based affinity labels react with amino acids in the antigen binding site, thereby destroying this site and blocking specific antigen reaction. However, this may not be advantageous since it results in loss of antigen binding by the antibody conjugate.
25

Molecules containing azido groups may also be used to form covalent bonds to proteins through reactive nitrene intermediates that are generated by low intensity ultraviolet light (Potter and Haley, 1983). In particular, 2- and 8-azido analogues of purine nucleotides have been used as site-directed photoprobes to identify nucleotide binding proteins in crude cell extracts (Owens & Haley, 1987; Atherton *et al.*, 1985). The 2- and 8-azido nucleotides have
30

also been used to map nucleotide binding domains of purified proteins (Khatoon *et al.*, 1989; King *et al.*, 1989; Dholakia *et al.*, 1989) and may be used as antibody binding agents.

Several methods are known in the art for the attachment or conjugation of an antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3 α -6 α -diphenylglycouril-3 attached to the antibody (U.S. Patents 4,472,509 and 4,938,948). Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate. In U.S. Patent 4,938,948, imaging of breast tumors is achieved using monoclonal antibodies and the detectable imaging moieties are bound to the antibody using linkers such as methyl-p-hydroxybenzimidate or N-succinimidyl-3-(4-hydroxyphenyl)propionate.

In other embodiments, derivatization of immunoglobulins by selectively introducing sulfhydryl groups in the Fc region of an immunoglobulin, using reaction conditions that do not alter the antibody combining site are contemplated. Antibody conjugates produced according to this methodology are disclosed to exhibit improved longevity, specificity and sensitivity (U.S. Patent 5,196,066, incorporated herein by reference). Site-specific attachment of effector or reporter molecules, wherein the reporter or effector molecule is conjugated to a carbohydrate residue in the Fc region have also been disclosed in the literature (O'Shannessy *et al.*, 1987). This approach has been reported to produce diagnostically and therapeutically promising antibodies which are currently in clinical evaluation.

V. Immunodetection Methods

In still further embodiments, the present disclosure concerns immunodetection methods for binding, purifying, removing, quantifying and otherwise generally detecting EV-D68 and its associated antigens. While such methods can be applied in a traditional sense, another use will be in quality control and monitoring of vaccine and other virus stocks, where antibodies according to the present disclosure can be used to assess the amount or integrity (*i.e.*, long term stability) of antigens in viruses. Alternatively, the methods may be used to screen various antibodies for appropriate/desired reactivity profiles.

Other immunodetection methods include specific assays for determining the presence of EV-D68 in a subject. A wide variety of assay formats are contemplated, but specifically

those that would be used to detect EV-D68 in a fluid obtained from a subject, such as saliva, blood, plasma, sputum, semen, urine, respiratory droplets or aerosol. In particular, semen has been demonstrated as a viable sample for detecting EV-D68 (Purpura *et al.*, 2016; Mansuy *et al.*, 2016; Barzon *et al.*, 2016; Gornet *et al.*, 2016; Duffy *et al.*, 2009; CDC, 2016; Halfon *et al.*, 2010; Elder *et al.* 2005). The assays may be advantageously formatted for non-healthcare (home) use, including lateral flow assays (see below) analogous to home pregnancy tests. These assays may be packaged in the form of a kit with appropriate reagents and instructions to permit use by the subject of a family member.

Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. In particular, a competitive assay for the detection and quantitation of EV-D68 antibodies directed to specific parasite epitopes in samples also is provided. The steps of various useful immunodetection methods have been described in the scientific literature, such as, *e.g.*, Doolittle and Ben-Zeev (1999), Gulbis and Galand (1993), De Jager *et al.* (1993), and Nakamura *et al.* (1987). In general, the immunobinding methods include obtaining a sample suspected of containing EV-D68, and contacting the sample with a first antibody in accordance with the present disclosure, as the case may be, under conditions effective to allow the formation of immunocomplexes.

These methods include methods for purifying EV-D68 or related antigens from a sample. The antibody will preferably be linked to a solid support, such as in the form of a column matrix, and the sample suspected of containing the EV-D68 or antigenic component will be applied to the immobilized antibody. The unwanted components will be washed from the column, leaving the EV-D68 antigen immunocomplexed to the immobilized antibody, which is then collected by removing the organism or antigen from the column.

The immunobinding methods also include methods for detecting and quantifying the amount of EV-D68 or related components in a sample and the detection and quantification of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing EV-D68 or its antigens and contact the sample with an antibody that binds EV-D68 or components thereof, followed by detecting and quantifying the amount of immune complexes formed under the specific conditions. In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing EV-D68 or EV-D68 antigen, such as a tissue section or specimen, a homogenized tissue extract, a biological fluid, including blood and serum, or a secretion, such as feces or urine.

Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the antibody composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form
5 immune complexes with, *i.e.*, to bind to EV-D68 or antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may
10 be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. Patents concerning the use of such labels include U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241. Of course, one may find additional advantages through the use of a secondary binding ligand such as a
15 second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second
20 binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an antibody, which may thus be termed a “secondary” antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The
25 secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

Further methods include the detection of primary immune complexes by a two-step approach. A second binding ligand, such as an antibody that has binding affinity for the
30 antibody, is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary

immune complexes thus formed. This system may provide for signal amplification if this is desired.

One method of immunodetection uses two different antibodies. A first biotinylated antibody is used to detect the target antigen, and a second antibody is then used to detect the biotin attached to the complexed biotin. In that method, the sample to be tested is first incubated in a solution containing the first step antibody. If the target antigen is present, some of the antibody binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin), biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution containing the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced which is macroscopically visible.

Another known method of immunodetection takes advantage of the immuno-PCR (Polymerase Chain Reaction) methodology. The PCR method is similar to the Cantor method up to the incubation with biotinylated DNA, however, instead of using multiple rounds of streptavidin and biotinylated DNA incubation, the DNA/biotin/streptavidin/antibody complex is washed out with a low pH or high salt buffer that releases the antibody. The resulting wash solution is then used to carry out a PCR reaction with suitable primers with appropriate controls. At least in theory, the enormous amplification capability and specificity of PCR can be utilized to detect a single antigen molecule.

A. ELISAs

Immunoassays, in their most simple and direct sense, are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. However, it will be readily appreciated that detection is not limited to such techniques, and western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the antibodies of the disclosure are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the EV-D68 or EV-D68 antigen is added to

the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection may be achieved by the addition of another anti-EV-D68 antibody that is linked to a detectable label. This type of ELISA is a simple “sandwich ELISA.” Detection may also be achieved by the addition of a second anti-EV-D68 antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing the EV-D68 or EV-D68 antigen are immobilized onto the well surface and then contacted with the anti-EV-D68 antibodies of the disclosure. After binding and washing to remove non-specifically bound immune complexes, the bound anti-EV-D68 antibodies are detected. Where the initial anti-EV-D68 antibodies are linked to a detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has binding affinity for the first anti-EV-D68 antibody, with the second antibody being linked to a detectable label.

Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating and binding, washing to remove non-specifically bound species, and detecting the bound immune complexes. These are described below.

In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period of hours. The wells of the plate will then be washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then “coated” with a nonspecific protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein or solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

In ELISAs, it is probably more customary to use a secondary or tertiary detection means rather than a direct procedure. Thus, after binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the biological sample to be tested under conditions effective to allow immune complex (antigen/antibody) formation. Detection of the immune complex then requires a labeled secondary binding ligand or antibody, and a secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or a third binding ligand.

“Under conditions effective to allow immune complex (antigen/antibody) formation” means that the conditions preferably include diluting the antigens and/or antibodies with

solutions such as BSA, bovine gamma globulin (BGG) or phosphate buffered saline (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background.

The “suitable” conditions also mean that the incubation is at a temperature or for a period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours or so, at temperatures preferably on the order of 25°C to 27°C, or may be overnight at about 4°C or so.

Following all incubation steps in an ELISA, the contacted surface is washed so as to remove non-complexed material. A preferred washing procedure includes washing with a solution such as PBS/Tween, or borate buffer. Following the formation of specific immune complexes between the test sample and the originally bound material, and subsequent washing, the occurrence of even minute amounts of immune complexes may be determined.

To provide a detecting means, the second or third antibody will have an associated label to allow detection. Preferably, this will be an enzyme that will generate color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact or incubate the first and second immune complex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immune complex formation (*e.g.*, incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, *e.g.*, by incubation with a chromogenic substrate such as urea, or bromocresol purple, or 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS), or H₂O₂, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generated, *e.g.*, using a visible spectra spectrophotometer.

In another embodiment, the present disclosure contemplates the use of competitive formats. This is particularly useful in the detection of EV-D68 antibodies in sample. In competition-based assays, an unknown amount of analyte or antibody is determined by its ability to displace a known amount of labeled antibody or analyte. Thus, the quantifiable loss of a signal is an indication of the amount of unknown antibody or analyte in a sample.

Here, the inventor proposes the use of labeled EV-D68 monoclonal antibodies to determine the amount of EV-D68 antibodies in a sample. The basic format would include contacting a known amount of EV-D68 monoclonal antibody (linked to a detectable label) with EV-D68 antigen or particle. The EV-D68 antigen or organism is preferably attached to a

support. After binding of the labeled monoclonal antibody to the support, the sample is added and incubated under conditions permitting any unlabeled antibody in the sample to compete with, and hence displace, the labeled monoclonal antibody. By measuring either the lost label or the label remaining (and subtracting that from the original amount of bound label), one can
5 determine how much non-labeled antibody is bound to the support, and thus how much antibody was present in the sample.

B. Western Blot

The Western blot (alternatively, protein immunoblot) is an analytical technique used to
10 detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.

15 Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication. Cells may also be broken open by one of the above mechanical methods. However, it should be noted that bacteria, virus or environmental samples can be the source of protein and thus Western blotting is not restricted
20 to cellular studies only. Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturing.

The proteins of the sample are separated using gel electrophoresis. Separation of
25 proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to determine a protein. It is also possible to use a two-dimensional (2-D) gel which spreads the proteins from a single sample out in two dimensions. Proteins are separated according to isoelectric point (pH at which they have neutral
30 net charge) in the first dimension, and according to their molecular weight in the second dimension.

In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or polyvinylidene difluoride (PVDF). The membrane is placed on top of the gel, and a stack of filter papers placed on top of that. The

entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it. Another method for transferring the proteins is called electroblotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization they had within the gel. As a result of this blotting process, the proteins are exposed on a thin surface layer for detection (see below). Both varieties of membrane are chosen for their non-specific protein binding properties (*i.e.*, binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF but are far more fragile and do not stand up well to repeated probings. The uniformity and overall effectiveness of transfer of protein from the gel to the membrane can be checked by staining the membrane with Coomassie Brilliant Blue or Ponceau S dyes. Once transferred, proteins are detected using labeled primary antibodies, or unlabeled primary antibodies followed by indirect detection using labeled protein A or secondary labeled antibodies binding to the Fc region of the primary antibodies.

C. Lateral Flow Assays

Lateral flow assays, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence (or absence) of a target analyte in sample (matrix) without the need for specialized and costly equipment, though many laboratory-based applications exist that are supported by reading equipment. Typically, these tests are used as low resources medical diagnostics, either for home testing, point of care testing, or laboratory use. A widely spread and well-known application is the home pregnancy test.

The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid (*e.g.*, urine) spontaneously. The first element (the sample pad) acts as a sponge and holds an excess of sample fluid. Once soaked, the fluid migrates to the second element (conjugate pad) in which the manufacturer has stored the so-called conjugate, a dried format of bio-active particles (see below) in a salt-sugar matrix that contains everything to guarantee an optimized chemical reaction between the target molecule (*e.g.*, an antigen) and its chemical partner (*e.g.*, antibody) that has been immobilized on the particle's surface. While the sample fluid dissolves the salt-sugar matrix, it also dissolves the particles and in one combined transport action the sample and conjugate mix while flowing through the porous structure. In this way, the analyte binds to the particles while migrating further through the third capillary bed. This material has one

or more areas (often called stripes) where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third 'capture' molecule binds the complex. After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes color. Typically there are at least two stripes: one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine, the second contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized. After passing these reaction zones, the fluid enters the final porous material – the wick – that simply acts as a waste container. Lateral Flow Tests can operate as either competitive or sandwich assays. Lateral flow assays are disclosed in U.S. Patent 6,485,982.

D. Immunohistochemistry

The antibodies of the present disclosure may also be used in conjunction with both fresh-frozen and/or formalin-fixed, paraffin-embedded tissue blocks prepared for study by immunohistochemistry (IHC). The method of preparing tissue blocks from these particulate specimens has been successfully used in previous IHC studies of various prognostic factors and is well known to those of skill in the art (Brown *et al.*, 1990; Abbondanzo *et al.*, 1990; Allred *et al.*, 1990).

Briefly, frozen-sections may be prepared by rehydrating 50 ng of frozen “pulverized” tissue at room temperature in phosphate buffered saline (PBS) in small plastic capsules; pelleting the particles by centrifugation; resuspending them in a viscous embedding medium (OCT); inverting the capsule and/or pelleting again by centrifugation; snap-freezing in -70°C isopentane; cutting the plastic capsule and/or removing the frozen cylinder of tissue; securing the tissue cylinder on a cryostat microtome chuck; and/or cutting 25-50 serial sections from the capsule. Alternatively, whole frozen tissue samples may be used for serial section cuttings.

Permanent-sections may be prepared by a similar method involving rehydration of the 50 mg sample in a plastic microfuge tube; pelleting; resuspending in 10% formalin for 4 hours fixation; washing/pelleting; resuspending in warm 2.5% agar; pelleting; cooling in ice water to harden the agar; removing the tissue/agar block from the tube; infiltrating and/or embedding the block in paraffin; and/or cutting up to 50 serial permanent sections. Again, whole tissue samples may be substituted.

E. Immunodetection Kits

In still further embodiments, the present disclosure concerns immunodetection kits for use with the immunodetection methods described above. As the antibodies may be used to detect EV-D68 or EV-D68 antigens, the antibodies may be included in the kit. The immunodetection kits will thus comprise, in suitable container means, a first antibody that
5 binds to EV-D68 or EV-D68 antigen, and optionally an immunodetection reagent.

In certain embodiments, the EV-D68 antibody may be pre-bound to a solid support, such as a column matrix and/or well of a microtiter plate. The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated
10 with or linked to the given antibody. Detectable labels that are associated with or attached to a secondary binding ligand are also contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody.

Further suitable immunodetection reagents for use in the present kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first
15 antibody, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label. As noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present disclosure.

The kits may further comprise a suitably aliquoted composition of the EV-D68 or EV-D68 antigens, whether labeled or unlabeled, as may be used to prepare a standard curve for a
20 detection assay. The kits may contain antibody-label conjugates either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit. The components of the kits may be packaged either in aqueous media or in lyophilized form.

The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the antibody may be placed, or preferably,
25 suitably aliquoted. The kits of the present disclosure will also typically include a means for containing the antibody, antigen, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

F. Vaccine and Antigen Quality Control Assays

The present disclosure also contemplates the use of antibodies and antibody fragments as described herein for use in assessing the antigenic integrity of a viral antigen in a sample. Biological medicinal products like vaccines differ from chemical drugs in that they cannot
30 normally be characterized molecularly; antibodies are large molecules of significant

complexity and have the capacity to vary widely from preparation to preparation. They are also administered to healthy individuals, including children at the start of their lives, and thus a strong emphasis must be placed on their quality to ensure, to the greatest extent possible, that they are efficacious in preventing or treating life-threatening disease, without themselves causing harm.

The increasing globalization in the production and distribution of vaccines has opened new possibilities to better manage public health concerns but has also raised questions about the equivalence and interchangeability of vaccines procured across a variety of sources. International standardization of starting materials, of production and quality control testing, and the setting of high expectations for regulatory oversight on the way these products are manufactured and used, have thus been the cornerstone for continued success. But it remains a field in constant change, and continuous technical advances in the field offer a promise of developing potent new weapons against the oldest public health threats, as well as new ones - malaria, pandemic influenza, and HIV, to name a few - but also put a great pressure on manufacturers, regulatory authorities, and the wider medical community to ensure that products continue to meet the highest standards of quality attainable.

Thus, one may obtain an antigen or vaccine from any source or at any point during a manufacturing process. The quality control processes may therefore begin with preparing a sample for an immunoassay that identifies binding of an antibody or fragment disclosed herein to a viral antigen. Such immunoassays are disclosed elsewhere in this document, and any of these may be used to assess the structural/antigenic integrity of the antigen. Standards for finding the sample to contain acceptable amounts of antigenically correct and intact antigen may be established by regulatory agencies.

Another important embodiment where antigen integrity is assessed is in determining shelf-life and storage stability. Most medicines, including vaccines, can deteriorate over time. Therefore, it is critical to determine whether, over time, the degree to which an antigen, such as in a vaccine, degrades or destabilizes such that it is no longer antigenic and/or capable of generating an immune response when administered to a subject. Again, standards for finding the sample to contain acceptable amounts of antigenically intact antigen may be established by regulatory agencies.

In certain embodiments, viral antigens may contain more than one protective epitope. In these cases, it may prove useful to employ assays that look at the binding of more than one antibody, such as 2, 3, 4, 5 or even more antibodies. These antibodies bind to closely related epitopes, such that they are adjacent or even overlap each other. On the other hand, they may

represent distinct epitopes from disparate parts of the antigen. By examining the integrity of multiple epitopes, a more complete picture of the antigen's overall integrity, and hence ability to generate a protective immune response, may be determined.

Antibodies and fragments thereof as described in the present disclosure may also be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective EV-D68 antibodies. Antibodies, antibody fragment, or variants and derivatives thereof, as described in the present disclosure may also be used in a kit for monitoring vaccine manufacture with the desired immunogenicity.

10 VI. Examples

The following examples are included to demonstrate preferred embodiments. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of embodiments, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 - Materials and Methods

20 **Animals.** Four-week-old male and female AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University.

25 **Antibodies and Compound.** The monoclonal antibody (mAb) EV-D68-228 was provided by James Crowe at Vanderbilt University Medical Center. EV-D68-228 was provided in solution at a concentration of 1.134 mg/ml and was diluted in sterile saline to doses of 10, 3, and 1 mg/kg for treatment. rRSV-90 was provided in solution at a concentration of 5 mg/ml and was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (IVIg, Carimune, CSL Behring, King of Prussia, PA) was purchased from a local pharmacy and was used as a comparator to the EV-D68-228 mAb. Guanidine HCl (guanidine) was obtained from Sigma-Aldrich (St. Louis, MO) and served as a positive control.

30 **Virus.** Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of

4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus stock was amplified twice in RD cells to create a working stock. The virus used for infection was designated EV-D68 MP30 PP.

5 **Experiment design.** A total of 78 mice were randomized into 6 groups of 12 mice each with a group of 6 mice used for normal controls as shown in Table I. Mice were treated via intraperitoneal (IP) administration of EV-D68-228 mAb, IVIg, or placebo mAb 24 hours prior to infection. Mice were infected via intranasal (IN) instillation of $1 \times 10^{4.5}$ CCID₅₀ of EV-D68 MP30 PP in a 90 µl volume of MEM. Treatment with guanidine started 4 hours post-infection
10 and continued twice daily for 5 days. Mice were weighed prior to treatment and daily thereafter. Four mice from each treatment group were euthanized on days 1, 3, and 5 post-infection for evaluation of lung virus titers, blood virus titers, and lung cytokine concentrations.

Lung Cytokine/Chemokine Evaluations. Each sample of lung homogenate was tested for cytokines and chemokines using a chemiluminescent ELISA-based assay according to the
15 manufacturer's instructions (Quansys Biosciences Q-Plex™ Array, Logan, UT). The Quansys multiplex ELISA is a quantitative test in which 16 distinct capture antibodies have been applied to each well of a 96-well plate in a defined array. Each sample supernatant was tested at for the following: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, MCP-1, IFN-γ, TNFα, MIP-1α, GM-CSF, and RANTES. Definitions of abbreviations are: IL - interleukin;
20 MCP - monocyte chemoattractant protein; IFN - interferon; TNF - tumor necrosis factor, MIP – macrophage inflammatory protein; GM-CSF - granulocyte/ macrophage colony stimulating factor; and RANTES - regulated upon activation, normal T cell expressed and secreted.

Statistical analysis. All figures and statistical analyses were completed using Prism 8.0.2. (GraphPad Software Inc.). For each day post-infection, lung and blood virus titers from
25 treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). For each cytokine/chemokine, the concentrations from treated mice were compared to placebo-treated mice using a two-way ANOVA.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University
30 dated March 2, 2019 (expires March 1, 2022). The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U. S. Government (National Institutes of Health) approval was renewed March 9, 2018 (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Table I - Experimental Design to Test Efficacy of EV-D68-228 for treatment of an EV-D68 respiratory infection in mice

Number /Cage	Group No.	Infected	Compound	Dosage	Route	Treatment Schedule	Observations
12	1	Yes	rRSV-90 (Placebo)	10 mg/kg	IP	Once, 24 hours pre-infection	4 mice per group euthanized at days 1, 3, and 5 post-infection for lung virus titers, blood virus titers, and lung cytokines.
12	3	Yes	EV-D68-228	10 mg/kg			
12	5	Yes	EV-D68-228	3 mg/kg			
12	7	Yes	EV-D68-228	1 mg/kg			
12	9	Yes	IVIg (Carimune NF)	10 mg/kg			
12	11	Yes	Guanidine	100 mg/kg/day		b.i.d. x 5 beginning 4 hours post-infection	
6	2	No	Normal Controls	-	-	-	2 mice per group euthanized at days 1, 3, and 5 post-infection for lung cytokines.

5

Example 2 – Results

The objective of this study was to determine the efficacy of treatment with EV-D68-228 for an Enterovirus D68 (EV-D68) respiratory infection in four-week-old AG129 mice. This study determined the efficacy of an EV-D68-228 mAb for treatment of an EV-D68 respiratory infection in four-week-old AG129 mice.

FIGS. 6A-C show lung virus titers for EV-D68-infected AG129 mice treated with EV-D68-228. No lung virus titers were detected at days 1, 3, or 5 post-infection in mice treated with doses of 10, 3, or 1 mg/kg of EV-D68-228. Treatment with a dose of 10 mg/kg of IVIg significantly reduced lung virus titers on day 1 post-infection, but not on days 3 or 5 post-infection. Guanidine treatment at a dose of 100 mg/kg/day significantly reduced lung virus titers on days 1 and 3 post-infection, but not on day 5 post-infection.

FIGS. 7A-C show blood virus titers for EV-D68-infected AG129 mice treated with EV-D68-228. No blood virus titers were detected at days 1, 3, or 5 post-infection in mice treated with doses of 10, 3, or 1 mg/kg of EV-D68-228. Treatment with IVIg at a dose of 10 mg/kg also reduced blood virus titers at days 1, 3, and 5 post-infection. Guanidine significantly reduced blood virus titers on days 1 and 5 but not day 3 post-infection.

FIG. 8 shows lung concentrations of IL-1 α and IL-1 β from EV-D68-infected AG129 mice treated with EV-D68-228. Treatment with EV-D68-228 significantly reduced

concentrations of IL-1 α and IL-1 β on day 3 post-infection. Treatment with IVIg or guanidine did not significantly reduce lung concentrations of IL-1 α or IL-1 β .

Lung concentrations of IL-6 and MCP-1 from EV-D68-infected AG129 mice treated with EV-D68-228 are shown in FIG. 9. Treatment with EV-D68-228 significantly reduced concentrations of IL-6 and MCP-1 on day 3 post-infection. Treatment with IVIg or guanidine significantly reduced lung concentrations of MCP-1 but not IL-6 on day 3 post-infection.

Lung concentrations of RANTES from EV-D68-infected AG129 mice treated with EV-D68-228 are shown in FIG. 10. Treatment with EV-D68-228 significantly reduced concentrations of RANTES on days 3 and 5 post-infection compared to placebo-treated mice. Treatment with IVIg or guanidine did not significantly reduce lung concentrations of RANTES at days 1, 3, or 5 post-infection.

FIG. 11 shows lung concentrations of IL-2, IL-3, and IL-4 from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of IL-2, IL-3, or IL-4 were observed post-infection with EV-D68.

FIG. 12 shows lung concentrations of IL-5, IL-10, and IL-12p70 from EV-D68-infected AG129 mice treated with EV-D68-228. No significant changes in concentrations of IL-5, IL-10, or IL-12p70 were observed post-infection with EV-D68.

Lung concentrations of IL-17, IFN γ , and TNF α from EV-D68-infected AG129 mice treated with EV-D68-228 are shown in FIG. 13. No significant changes in concentrations of IL-17, IFN γ , or TNF α were observed post-infection with EV-D68.

Lung concentrations of MIP-1 α and GM-CSF from EV-D68-infected AG129 mice treated with EV-D68-228 are shown in FIG. 14. No significant changes in concentrations of MIP-1 α or GM-CSF were observed post-infection with EV-D68.

Example 3 - Discussion

This study determined the efficacy of EV-D68-228, a mAb against EV-D68, for treatment of a respiratory infection caused by EV-D68 in four-week-old AG129 mice.

Doses of 10, 3, and 1 mg/kg of EV-D68-228 were highly effective at reducing lung virus titers, blood virus titers, and pro-inflammatory cytokines caused by EV-D68 infection. Lung concentrations of IL-1 α , IL-1 β , IL-6, MCP-1, and RANTES were significantly reduced by treatment with EV-D68-228.

EV-D68-228 was highly effective at preventing infection with EV-D68

Example 4 – Materials and Methods

Animals. Ten-day-old AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University.

Antibodies and Compound. The monoclonal antibody (mAb) EV-D68-228 was provided by James Crowe at Vanderbilt University Medical Center. EV-D68-228 was provided in solution at a concentration of 1.134 mg/ml and was diluted in sterile saline to doses of 10, 3, and 1 mg/kg for treatment. rRSV-90 was provided in solution at a concentration of 5 mg/ml and was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (IVIg, Carimune, CSL Behring, King of Prussia, PA) was purchased from a local pharmacy and was used as a comparator to the EV-D68-228 mAb. Guanidine HCl (guanidine) was obtained from Sigma-Aldrich (St. Louis, MO) and served as a positive control.

Virus. Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of 4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus stock was amplified twice in RD cells to create a working stock. The virus used for infection was designated EV-D68 MP30 PP.

Experiment design. A total of 42 mice were randomized into 7 groups of 6 mice each as shown in Table II. Mice were treated via intraperitoneal (IP) administration of EV-D68-228 mAb, IVIg, or placebo mAb 24 hours prior to infection. Mice were infected via intraperitoneal (IP) administration of $1 \times 10^{6.5}$ CCID₅₀ of EV-D68 MP30 PP in a 100 µl volume of MEM. Treatment with guanidine started 4 hours post-infection and continued twice daily for 5 days. Mice were weighed prior to treatment and daily thereafter. All mice were observed daily for morbidity, mortality, and neurological scores through day 21. Neurological scores (NS) were recorded as follows: NS0 – no observable paralysis, NS1 – abnormal splay of hindlimb but normal or slightly slower gait, NS2 – hindlimb partially collapsed and foot drags during use for forward motion, NS3 – rigid paralysis of hindlimb and hindlimb is not used for forward motion, NS4 – rigid paralysis in hindlimbs and no forward motion. Any animals observed with a score of NS4 were humanely euthanized.

Statistical analysis. Kaplan-Meier survival curves were generated Prism 8.0.2. (GraphPad Software Inc.). Survival curves were compared using the Log-rank (Mantel-Cox)

test followed by a Gehan-Breslow-Wilcoxon test. For each day post-infection, blood virus titers from treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). Mean body weights were compared using a one-way ANOVA. Neurological scores were compared using a Kruskal-Wallis test followed by a

5 Dunn's multiple comparisons test.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University dated March 2, 2019 (expires March 1, 2022). The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U. S. Government (National

10 Institutes of Health) approval was renewed March 9, 2018 (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Table II – Experimental Design to Test Efficacy of EV-D68-228 for treatment of an EV-D68 neurological infection in mice

Number /Cage	Group No.	Infected	Compound	Dosage	Route	Treatment Schedule	Observations
6	1	Yes	rRSV-90 (Placebo)	10 mg/kg	IP	Once, 24 hours pre-infection	Mice observed daily for survival, body weights, and neurological scores. Blood collected from 3 mice per group on days 1, 3, and 5 post-infection for blood virus titers.
6	3	Yes	EV-D68-228	10 mg/kg			
6	5	Yes	EV-D68-228	3 mg/kg			
6	7	Yes	EV-D68-228	1 mg/kg			
6	9	Yes	IVlg (Carimune NF)	10 mg/kg			
6	11	Yes	Guanidine	100 mg/kg/day		b.i.d. x 5 beginning 4 hours post-infection	
6	2	No	Normal Controls	-	-	-	Observed for normal weight gain.

Example 5 – Results

The objective of this study was to determine the efficacy of treatment with EV-D68-228 for an Enterovirus D68 (EV-D68) neurological infection in 10-day-old AG129 mice. This study determined the efficacy of an EV-D68-228 mAb for treatment of an EV-D68 neurological infection in 10-day-old AG129 mice.

FIG. 15 shows Kaplan-Meier survival curves for 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228, IVIg, or guanidine. Doses of 10, 3, or 1 mg/kg of EV-D68-228 provided 100% protection from mortality in each group of six mice infected with EV-D68. Two of six mice that were treated with IVIg at a dose of 10 mg/kg did not survive the infection. Treatment with guanidine at a dose of 100 mg/kg/day protected all six mice from mortality.

FIG. 16 shows mean body weights for 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228, IVIg, or guanidine. Doses of 10, 3, or 1 mg/kg protected mice from infection-associated weight loss. A dose of 10 mg/kg of IVIg also protected mice from weight loss. In addition, guanidine at a dose of 100 mg/kg/day protected mice from weight loss.

FIGS. 17A-C show blood virus titers for EV-D68-infected AG129 mice treated with EV-D68-228. No blood virus titers were detected at days 1, 3, or 5 post-infection in mice treated with doses of 10, 3, or 1 mg/kg of EV-D68-228. Treatment with IVIg at a dose of 10 mg/kg also reduced blood virus titers at days 1, 3, and 5 post-infection. Guanidine treatment at a dose of 100 mg/kg/day significantly reduced blood virus titers on days 1, 3, and 5 post-infection.

Neurological scores in 10-day-old AG129 mice infected with EV-D68 and treated with EV-D68-228, IVIg, or guanidine are shown in FIG. 18. No neurological scores were observed in mice treated with doses of 10, 3, or 1 mg/kg of the EV-D68-228 Ab. Neurological scores were observed in mice treated with IVIg at a dose of 10 mg/kg on days 4, 5, and 6 post-infection. However, neurological scores in mice treated with IVIg were significantly reduced compared to placebo-treated mice. No neurological scores were observed in the mice treated with guanidine at a dose of 100 mg/kg/day.

Example 6 – Discussion

This study determined the efficacy of EV-D68-228, a mAb against EV-D68, for treatment of a neurological infection caused by EV-D68 in 10-day-old AG129 mice.

Doses of 10, 3, and 1 mg/kg of EV-D68-228 provided protection from mortality and weight loss in EV-D68-infected mice. In addition, no blood virus titers were detected at days 1, 3, or 5 post-infection in mice treated with EV-D68-228. No paralysis, as measured by neurological scores, was observed in mice treated with doses of 10, 3, or 1 mg/kg of EV-D68-228.

EV-D68-228 appeared to provide protection from EV-D68 infection compared to commercial IVIg as indicated by survival, blood virus titers, and neurological scores observed in mice treated with IVIg at a dose of 10 mg/kg.

EV-D68-228 provided equivalent protection from mortality and paralysis when compared to treatment with the positive control, guanidine. However, while blood virus titers were still detected in guanidine-treated mice on day 3 post-infection, no virus was detected in the blood of mice treated with EV-D68-228 at doses of 10, 3, or 1 mg/kg at days 1, 3, or 5 post-infection.

Example 7 – Efficacy of EV-D68-228 on Respiratory Infection in Mice

Materials and Methods

Animals. Four-week-old male and female AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University.

Antibodies and Compound. The monoclonal antibody (mAb) EV-D68-228 was provided by James Crowe at Vanderbilt University Medical Center. EV-D68-228 was provided in solution at a concentration of 1.134 mg/ml and was diluted in sterile saline to doses of 10 or 1 mg/kg for treatment. rRSV-90 was provided in solution at a concentration of 5 mg/ml and was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (IVIg, Carimune, CSL Behring, King of Prussia, PA) was purchased from a local pharmacy and was used as a comparator to the EV-D68-228 mAb.

Virus. Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of 4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus stock was amplified twice in RD cells to create a working stock. The virus used for infection was designated EV-D68 MP30 PP.

Experiment design. A total of 52 mice were randomized into 6 groups of 8 mice each with a group of 4 mice used for normal controls as shown in Table C. Mice were treated via intraperitoneal (IP) administration of EV-D68-228 mAb, IVIg, or placebo mAb at 4, 24, or 48 hours post-infection. Mice were infected via intranasal (IN) instillation of $1 \times 10^{4.5}$ CCID₅₀ of EV-D68 MP30 PP in a 90 μ l volume of MEM. Mice were weighed prior to treatment and

daily thereafter. Four mice from each treatment group were euthanized on days 3 and 5 post-infection for evaluation of lung virus titers, blood virus titers, and lung cytokine concentrations.

Lung Cytokine/Chemokine Evaluations. Each sample of lung homogenate was tested for cytokines and chemokines using a chemiluminescent ELISA-based assay according to the manufacturer's instructions (Quansys Biosciences Q-Plex™ Array, Logan, UT). The Quansys multiplex ELISA is a quantitative test in which 16 distinct capture antibodies have been applied to each well of a 96-well plate in a defined array. Each sample supernatant was tested at for the following: IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, MCP-1, IFN- γ , TNF α , MIP-1 α , GM-CSF, and RANTES. Definition of abbreviations are: IL - interleukin; MCP - monocyte chemoattractant protein; IFN - interferon; TNF - tumor necrosis factor, MIP – macrophage inflammatory 3 protein; GM-CSF - granulocyte/ macrophage colony stimulating factor; and RANTES - regulated upon activation, normal T cell expressed and secreted.

Statistical analysis. All figures and statistical analyses were completed using Prism 8.2.0. (GraphPad Software Inc.). For each day post-infection, lung and blood virus titers from treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). For each cytokine/chemokine, the concentrations from treated mice were compared to placebo-treated mice using a two-way ANOVA.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University. The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U.S. Government (National Institutes of Health) approval was renewed (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Table C. Expt. NIA-1869. Experimental Design – Therapeutic Efficacy of EV-D68-228 for treatment of an EV-D68 respiratory infection in mice.

Number /Cage	Group No.	Infected	Compound	Dosage	Route	Treatment Schedule	Observations
12	1	Yes	rRSV-90 (Placebo)	10 mg/kg	IP	Once, 24 hours post-infection	4 mice per group euthanized at days 3 and 5 post-infection for lung virus titers, blood virus titers, and lung cytokines.
12	3	Yes	EV-D68-228	10 mg/kg		Once, 4 hours post-infection	
12	5	Yes	EV-D68-228	10 mg/kg		Once, 24 hours post-infection	
12	7	Yes	EV-D68-228	10 mg/kg		Once, 48 hours post-infection	
12	9	Yes	EV-D68-228	1 mg/kg			
12	11	Yes	IVIg (Carimune NF)	10 mg/kg		Once, 24 hours post-infection	
4	2	No	Normal Controls	-	-	-	2 mice per group euthanized at days 3 and 5 post-infection for lung cytokines.

5

Results and Discussion

This study determined the therapeutic efficacy of an EV-D68-228 mAb for treatment of an EV-D68 respiratory infection in four-week-old AG129 mice.

FIG. 19 shows lung virus titers for EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No lung virus titers were detected at days 3 or 5 post-infection in mice treated with doses of 10 mg/kg of EV-D68-228 when treatment started at 4 or 24 hours after infection. In mice treated 48 hours post-infection, a dose of 10 mg/kg of EV-D68-228 significantly reduced lung virus titers on days 3 and 5 post-infection. Even a dose of 1 mg/kg given 48 hours post-infection significantly reduced lung virus titers on days 3 and 5 post-infection. Treatment with a dose of 10 mg/kg of IVIg given 24 hours post-infection did not significantly reduce lung virus titers on days 3 or 5 post-infection.

FIG. 20 shows blood virus titers for EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No blood virus titers were detected at days 3 or 5 post-infection in mice treated with doses of 10 mg/kg of EV-D68-228 at 4, 24, or 48 hours after infection. A lower dose of 1 mg/kg of EV-D68-228 given 48 hours post-infection reduced blood virus titers below the limit of detection. Treatment with IVIg at a dose of 10 mg/kg also reduced blood virus titers at days 3, and 5 post-infection.

FIG. 21 shows lung concentrations of IL-1 α and IL-1 β from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. Therapeutic treatment with EV-D68-228 significantly reduced concentrations of IL-1 α and IL-1 β on days 3 and 5 post-infection. Treatment with IVIg significantly reduced lung concentrations of IL-1 α or IL-1 β , but only on day 3 post-infection.

Lung concentrations of IL-5 and IL-6 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228 are shown in FIG. 22. Therapeutic treatment with EV-D68-228 significantly reduced concentrations of IL-5 and IL-6 on day 3 post-infection. Treatment with IVIg 24 hours post-infection significantly reduced lung concentrations of IL-5 and IL-6 on day 3 post-infection.

Lung concentrations of MCP-1 and RANTES from EV-D68-infected AG129 mice treated post-infection with EV-D68-228 are shown in FIG. 23. Therapeutic treatment with EV-D68-228 significantly reduced lung concentrations of MCP-1 and RANTES on days 3 and 5 post-infection compared to placebo-treated mice. Treatment with IVIg significantly reduced lung concentrations of MCP-1 on day 3 post-infection and reduced concentrations of RANTES on days 3 and 5 post-infection.

FIG. 24 shows lung concentrations of IL-2, IL-3, and IL-4 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of IL-2, IL-3, or IL-4 were observed post-infection with EV-D68.

FIG. 25 shows lung concentrations of IL-10, IL-12p70, and IL-17 from EV-D68-infected AG129 mice treated post-infection with EV-D68-228. No significant changes in concentrations of IL-10, IL-12p70, or IL-17 were observed after infection with EV-D68.

Lung concentrations of IFN γ and TNF α from EV-D68-infected AG129 mice treated post-infection with EV-D68-228 are shown in FIG. 26. No significant changes in concentrations of IFN γ or TNF α were observed after infection with EV-D68.

Lung concentrations of MIP-1 α and GM-CSF from EV-D68-infected AG129 mice treated post-infection with EV-D68-228 are shown in FIG. 27. No significant changes in concentrations of MIP-1 α or GM-CSF were observed following infection with EV-D68.

Thus, this study determined the therapeutic efficacy of EV-D68-228, a mAb against EV-D68, for treatment of a respiratory infection caused by EV-D68 in four-week-old AG129 mice. Doses of 10 and 1 mg/kg of EV-D68-228 were highly effective at reducing lung virus titers, blood virus titers, and pro-inflammatory cytokines caused by EV-D68 infection when administered within 48 hours post-infection. Lung concentrations of IL-1 α , IL-1 β , IL-5, IL-6, MCP-1, and RANTES were significantly reduced by treatment with EV-D68-228. As EV-D68-

228 was highly effective as a therapeutic treatment for an EV-D68 respiratory infection in AG129 mice, plethysmography could be used in future studies to determine the impact of EV-D68-228 treatment on lung function.

5 **Example 8 – Efficacy of EV-D68-228 on Respiratory Infection in Mice**

Materials and Methods

The objective of this study was to determine the therapeutic efficacy of treatment with EV-D68-228 for an Enterovirus D68 (EV-D68) neurological infection in 10-day-old AG129 mice.

10 **Animals.** Ten-day-old AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University.

Antibodies and Compound. The monoclonal antibody (mAb) EV-D68-228 was
15 provided by James Crowe at Vanderbilt University Medical Center. EV-D68-228 was provided in solution at a concentration of 1.134 mg/ml and was diluted in sterile saline to doses of 10 mg/kg for treatment. rRSV-90 was provided in solution at a concentration of 5 mg/ml and was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (IVIg, Carimune, CSL Behring, King of Prussia, PA) was purchased from a local pharmacy and was
20 used as a comparator to the EV-D68-228 mAb.

Virus. Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of 4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus
25 stock was amplified twice in RD cells to create a working stock. The virus used for infection was designated EV-D68 MP30 PP.

Experiment design. A total of 42 mice were randomized into 6 groups of 6 treated-mice each with one group of 9 mice to account for deaths prior to treatment each as shown in Table D. One group of 3 mice was used as normal controls. Mice were infected via
30 intraperitoneal (IP) administration of $1 \times 10^{6.5}$ CCID₅₀ of EV-D68 MP30 PP in a 100 µl volume of MEM. Mice were treated via intraperitoneal (IP) administration of EV-D68-228 mAb at 24, 48, 72, or 120 hours post-infection. Treatment with IVIg or placebo Ab was given once 24 hours post-infection. Mice were weighed prior to treatment and daily thereafter. All mice were

observed daily for morbidity, mortality, and neurological scores through day 21. Neurological scores (NS) were recorded as follows: NS0 – no observable paralysis, NS1 – abnormal splay of hindlimb but normal or slightly slower gait, NS2 – hindlimb partially collapsed and foot drags during use for forward motion, NS3 – rigid paralysis of hindlimb and hindlimb is not used for forward motion, NS4 – rigid paralysis in hindlimbs and no forward motion. Any animals observed with a score of NS4 were humanely euthanized.

Statistical analysis. Kaplan-Meier survival curves were generated Prism 8.2.0. (GraphPad Software Inc.). Survival curves were compared using the Log-rank (Mantel-Cox) test followed by a Gehan-Breslow-Wilcoxon test. For each day post-infection, blood virus titers from treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). Mean body weights were compared using a one-way ANOVA. Neurological scores were compared using a Kruskal-Wallis test followed by a Dunn's multiple comparisons test.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University. The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U. S. Government (National Institutes of Health) approval was renewed (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Table D. Expt. NIA-1870. Experimental Design – Therapeutic efficacy of EV-D68-228 for treatment of an EV-D68 neurological infection in mice.

Number /Cage	Group No.	Infected	Compound	Dosage	Route	Treatment Schedule	Observations
6	1	Yes	rRSV-90 (Placebo)	10 mg/kg	IP	Once, 24 hours post-infection	Mice observed daily for survival, body weights, and neurological scores. Blood collected from 3 mice per group on days 1, 3, 5, and 7 post-infection for blood virus titers.
6	3	Yes	EV-D68-228	10 mg/kg		Once, 24 hours post-infection	
6	5	Yes	EV-D68-228			Once, 48 hours post-infection	
6	7	Yes	EV-D68-228			Once, 72 hours post-infection	
9	9	Yes	EV-D68-228}			Once, 120 hours post-infection	
6	11	Yes	IVIg (Canimmune NF)	10 mg/kg		Once, 24 hours post-infection	
3	2	No	Normal Controls	-	-	-	Observed for normal weight gain.

Results and Discussion

This study determined the efficacy of an EV-D68-228 mAb for therapeutic treatment of an EV-D68 neurological infection in 10-day-old AG129 mice.

FIG. 28 shows Kaplan-Meier survival curves for 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228. With a single administration of EV-D68-228 at a dose of 10 mg/kg, a significant survival benefit was observed in mice treated at 24, 48, and 120 hours post-infection. The difference in survival benefit in the mice treated 120 hours post-infection was due to an increase in the day of death as all seven mice that were treated still succumbed to the infection. A dose of 10 mg/kg of IVIg completely protected mice from mortality when administered 24 hours post-infection.

FIG. 29 shows mean body weights for 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228. A single dose of 10 mg/kg of EV-D68-228 protected mice from infection-associated weight loss when administered 24 or 48 hours after infection. A single administration of IVIg administered 24 hours post-infection did not protect mice from weight loss.

FIGS. 30A-C shows blood virus titers for EV-D68-infected AG129 mice treated post-infection with EV-D68-228. A single administration of 10 mg/kg of EV-D68-228 at 24, 48, or 72 hours post-infection significantly reduced blood virus titers at days 3 and 5 post-infection. Treatment with IVIg at a dose of 10 mg/kg 24 hours after infection reduced blood virus titers at days 3 and 5 post-infection. No virus titers were detected in any of the surviving mice at day seven post-infection.

Neurological scores in 10-day-old AG129 mice infected with EV-D68 and treated post-infection with EV-D68-228 are shown in FIGS. 31A-B. Neurological scores on days 3, 4, and 5 were significantly reduced in mice treated 24 hours post-infection with EV-D68-228 at a dose of 10 mg/kg. IVIg at a dose of 10 mg/kg given 24 hours post-infection reduced neurological scores at days 3, 4, and 5 post-infection. No comparisons in neurological score were completed after day 5 post-infection due to the death of all the placebo-treated mice.

Thus, this study determined the therapeutic efficacy of EV-D68-228, a mAb against EV-D68, for treatment of a neurological infection caused by EV-D68 in 10-day-old AG129 mice. When administered within 48 hours post-infection, a single dose of 10 mg/kg of EV-D68-228 protected mice from mortality and weight loss. A significant reduction in blood virus titers was observed in mice treated within 72 hours post-infection. A reduction in paralysis as measured by neurological scores was observed in mice treated with 10 mg/kg of EV-D68 at 24

hours post-infection. As EV-D68-228 could be used as a prophylactic therapy during an outbreak of EV-D68, determining the efficacy of EV-D68-228 when administered at various time points prior to infection would be beneficial.

Example 9 – Materials and Methods

Study design. The inventors designed this study to try to identify any antibodies that humans can make in response to EV-D68 infection. Therefore, they used live virus isolates in an indirect ELISA screen to identify B cells secreting EV-D68-binding antibodies, and then electrofused those B cells with myeloma cells to create monoclonal antibody secreting hybridomas. The inventors then characterized the neutralization and binding properties of these individual mAbs *in vitro* using CCID₅₀, ELISA, and cryo-EM based techniques. They pursued *in vivo* experiments to generate pre-clinical data supporting the development of mAb EV68-228 as a prophylactic and/or therapeutic agent in humans. For this purpose, they studied the effectiveness of mAb EV68-228 at protecting mice from EV-D68 infection as compared to human IVIG, which is widely used to treat humans with AFM based on theoretical benefit, but this IVIG treatment so far has not been proven to be effective. An advantage of the AG129 murine model of infection is that the inventors could measure the effect of antibody treatment in both respiratory and neurologic models of infection.

Cell lines. RD cells (human, female origin) were obtained from the American Type Culture Collection (ATCC CCL-136). RD cells were cultured in 5% CO₂ at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) (ThermoFisher Scientific) supplemented with 10% heat inactivated fetal bovine serum (HI-FBS; HyClone), 1 mM sodium pyruvate and 1% penicillin-streptomycin-amphotericin B (ThermoFisher Scientific). For structural studies, RD cells were cultured in DMEM (Sigma-Aldrich) supplemented with 10% HI-FBS (Sigma-Aldrich) and nonessential amino acids (NEAA, Life Technologies). The ExpiCHO (hamster, female origin) cell line was purchased from ThermoFisher Scientific and cultured according to the manufacturer's protocol. The HMMA2.5 line is a non-secreting mouse-human heteromyeloma cell line (sex information is not available) that was generated by fusing a murine myeloma cell line with a human myeloma cell line (Posner *et al.*, 1987). This cell line was cultured as described previously (Yu *et al.*, 2008). All cell lines were tested on a monthly basis for *Mycoplasma* and found to be negative in all cases.

Viruses. See Table K for a list of the virus isolates used in this study. EV-D68 isolates were propagated for two generations in RD cell monolayer cultures for use in enzyme linked

immunosorbent assays (ELISAs) described below. RD cell monolayers were inoculated with a given virus isolate and monitored until 70 to 90% cell death was observed. This cell culture flask then was frozen to -80°C, thawed, and the contents scraped and collected into a 50 mL conical tube. This preparation was sonicated three times for 20 s in an inverted cup sonicator at maximum power settings (Fisherbrand), vortexed for 30 s, and then sonicated two more times for 20 s. Cell debris was pelleted, and the virus-containing supernatant was spun over a 30% sucrose in PBS (w/v) cushion at 10°C for four hrs at 100,000 x g. Supernatant was discarded, and the pellet allowed to soak in 0.01% (w/v) bovine serum albumin (BSA) in NTE buffer (20 mM Tris, 120 mM NaCl, 1 mM EDTA pH 8.0) overnight at 4 °C. The resuspended pellet then was clarified further by centrifugation at 10,000 x g for 10 min, before storage of virus aliquots at -80 °C until ready for use.

For structural studies the US/MO/14-18947 isolate was used. Virus was passaged in RD cells and stored at -80°C before large scale propagation. RD cells were grown to 80% confluency and were infected with EV-D68 at a multiplicity of infection of 0.01. Two days post-infection, the cells were collected together with the supernatant and spun down. The cell pellets were collected and after multiple freeze/thaw cycles spun down to remove cell debris. All supernatants were combined and pelleted at 210,000 x g for 2 hours. The pellets were incubated and resuspended in 250 mM HEPES (pH = 7.5), 250 mM NaCl buffer, then supplemented with final concentration 5 mM MgCl₂, 0.01 mg/mL DNase (Sigma-Aldrich), 0.8 mg/mL trypsin, 15 mM EDTA and 1% (w/v) n-lauryl-sarcosine. The sample was then pelleted at 210,000 x g for 2 hours, resuspended, and loaded onto a potassium tartrate gradient (10 to 40%, w/v) for the last round of ultracentrifugation at 160,000 x g for 2 hours. The purified virus sample, which was observed as a blue band in the middle of the tube, was extracted and buffered exchanged into 20 mM Tris, 120 mM NaCl, 1 mM EDTA (pH=8.0) buffer to remove potassium tartrate.

Detection of virus load by CCID₅₀ assay. Titration of virus stocks or virus in murine blood or lung samples was performed by CCID₅₀ assay in RD cell culture monolayers. Briefly, increasing 10-fold dilutions of the samples were applied to RD cell monolayers in triplicate wells (50 µL) of a 96 well plate, incubated for five days in 5% CO₂ at 33 °C, and then fixed with 1% paraformaldehyde and stained with crystal violet. Wells with any cytopathic effect were scored as positive for virus, and titers were determined using a formula based on the Spearman-Kaerber equation (Ramakrishnan, 2016); the limit of detection was 136 CCID₅₀/mL.

Virus neutralization assay. Virus neutralization assays were performed in a CCID₅₀ format using the indicated viruses, essentially as described previously for poliovirus (Weldon

et al., 2016). Virus was incubated with increasing concentrations of mAb in duplicate for one hr at 33 °C, then each suspension was added to a monolayer of RD cells in technical quadruplicate wells (50 µL) of a 96-well plate. After five days incubation in 5% CO₂ at 33 °C, cells were fixed with 1% paraformaldehyde and stained with crystal violet. Wells with any cytopathic effect were scored as positive for virus, and half maximal inhibitory concentrations (IC₅₀) were determined using a formula based on the Spearman-Kaerber equation (Ramakrishnan, 2016); the limits of detection were 57 µg/mL to 4.8 pg/mL.

Mouse models. Ten-day old (neurologic model) or four-week old (respiratory model) male and female AG129 mice (deficient in receptors for interferon α/β and γ) were obtained from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University dated March 2, 2019 (expires March 1, 2022). The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U. S. Government (National Institutes of Health) approval was renewed March 9, 2018 (PHS Assurance No. D16-00468[A3801-01]) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Antibody and control treatments were diluted in PBS and administered by intraperitoneal injection at indicated time points before or after EV-D68 inoculation. Guanidine HCl (Sigma-Aldrich) served as a positive control for treatment (Hurst *et al.*, 2019), started 4 hours post-infection and continued twice daily for 5 days. A suspension of mouse adapted EV-D68 was administered by intraperitoneal injection (neurologic model; 10^{6.5} CCID₅₀ in a 100 µL volume of MEM) or intranasal instillation (respiratory model; 10^{4.5} CCID₅₀ in a 90 µL volume of MEM). Mice were weighed prior to treatment and daily thereafter. Mice were euthanized humanely at indicated time points post-infection for measurement of lung virus titers, blood virus titers, or lung cytokine concentrations, as indicated. For the neurologic model, all mice were observed daily for morbidity, mortality, and neurological scores through day 21. Neurological scores (NS) were recorded as follows: NS0 – no observable paralysis, NS1 – abnormal splay of hindlimb but normal or slightly slower gait, NS2 – hindlimb partially collapsed and foot drags during use for forward motion, NS3 – rigid paralysis of hindlimb and hindlimb is not used for forward motion, NS4 – rigid paralysis in hindlimbs and no forward motion. Any animals observed with a score of NS4 were euthanized humanely.

Lung cytokine/chemokine evaluations. Each sample of lung homogenate was tested for cytokines and chemokines using quantitative chemiluminescent ELISA-based assays according to the manufacturer's instructions (Quansys Biosciences Q-Plex™ Array, Logan, UT). The Quansys multiplex ELISA is a quantitative test in which 16 distinct capture antibodies are applied to each well of a 96-well plate in a defined array.

Generation of human monoclonal antibodies (mAbs). Subjects were identified from the Childhood Onset of Asthma (COAST) birth cohort (Lemanske, 2002) who had laboratory documented EV-D68 upper respiratory tract infections (Bochkov *et al.*, 2014). After written informed consent was obtained, peripheral blood was collected and stored at room temperature until peripheral blood mononuclear cells (PBMCs) could be purified using SepMate tubes (Stemcell Technologies), per the manufacturer's protocol, and then cryopreserved in 10% (v/v) dimethyl sulfoxide (DMSO) in fetal bovine serum (FBS) and stored in the vapor phase of liquid nitrogen. Lymphoblastoid cell lines (LCLs) were generated as described previously (Smith and Crowe, 2015) from memory B cells within the PBMCs by mixing with Epstein-Barr virus, cell cycle checkpoint kinase 2 (chk2) inhibitor (Sigma-Aldrich), CpG (Sigma-Aldrich), and cyclosporin A (Sigma-Aldrich) in Medium A (STEMCELL Technologies). One week later, LCLs were counted and then expanded on a feeder layer of γ -irradiated, human PBMCs from an unrelated donor. In one more week, LCL supernatants were screened for the presence of EV-D68-reactive IgG by indirect ELISA using live EV-D68 virus as the antigen, comprising cell culture grown EV-D68 virus generated from a 2014 clinical isolate. LCLs from wells containing virus-reactive antibodies were fused to HMMMA2.5 myeloma cells by electrofusion, as previously described (Yu *et al.*, 2008). After the fusion reaction, hybridoma lines were cultured in a selection medium containing HAT media supplements (Sigma-Aldrich) and ouabain (Sigma-Aldrich) in 384-well plates before screening of supernatants for antibody production. Two weeks later, supernatants from the resulting hybridoma cell lines were screened by indirect ELISA with live virus as antigen, and cell lines from wells with EV-D68-reactive antibodies were expanded in culture and then cloned by single-cell flow cytometric sorting into 384-well cell culture plates. These cloned cells were expanded in Medium E until about 50% confluent in 12-well tissue culture treated plates (Corning) and their supernatants screened for virus binding by ELISA. Wells with the highest signal in ELISA were selected as the mAb-producing hybridoma cell lines for further use.

mAb isotype and gene sequence analysis. The isotype and subclass of secreted antibodies were determined using mouse anti-human IgG1, IgG2, IgG3 or IgG4 antibodies conjugated with horseradish peroxidase (Southern Biotech). Antibody heavy- and light-chain

variable region genes were sequenced from RNA obtained from hybridoma lines that had been cloned biologically by flow cytometric sorting. Total RNA was extracted using the RNeasy Mini kit (Qiagen). A modified 5' RACE (Rapid Amplification of cDNA Ends) approach was used (Turchaninova *et al.*, 2016). Briefly, 5 µL total RNA was mixed with cDNA synthesis primer mix (10 µM each) and incubated for 2 min at 70 °C and then decrease the incubation temperature to 42 °C to anneal the synthesis primers (1 to 3 min). After incubation, a mixture containing 5× first-strand buffer (Clontech), DTT (20 mM), 5' template switch oligo (10 µM), dNTP solution (10 mM each) and 10× SMARTScribe Reverse Transcriptase (Clontech) was added to the primer-annealed total RNA reaction and incubated for 60 min at 42 °C. The first-strand synthesis reaction was purified using the Ampure Size Select Magnetic Bead Kit at a ratio of 1.8× (Beckman Coulter). Following, a single PCR amplification reaction containing 5 µL first-strand cDNA, 2× Q5 High Fidelity Mastermix (NEB), dNTP (10 mM each), forward universal primer (10 µM) and reverse primer mix (0.2 µM each in heavy-chain mix, 0.2 µM each in light-chain mix) were subjected to thermal cycling with the following conditions: initial denaturation for 90 s followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 40 s, followed by a final extension step at 72 °C for 4 min. All primer sequences used in this protocol were described previously ((Turchaninova *et al.*, 2016). The first PCR reaction was purified using the AMPure Size Select Magnetic Bead Kit at a ratio of 0.6× (Beckman Coulter). Amplicon libraries were then prepared according to the Multiplex SMRT Sequencing protocol (Pacific Biosciences) and sequenced on a Sequel platform instrument (Pacific Biosciences). Raw sequencing data was demultiplexed and circular consensus sequences (CCS) were determined using the SMRT Analysis tool suite (Pacific Biosciences). The identities of gene segments, complementarity-determining regions (CDRs), and mutations from germline genes were determined by alignment using the ImMunoGeneTics database (Giudicelli and Lefranc, 2011).

Antibody production and purification. For hybridoma-derived mAb, hybridoma cells were grown to exhaustion in Hybridoma SFM (1X) serum free medium (Gibco). For recombinant mAb production, cDNA encoding the genes of heavy and light chains were synthesized and cloned into a DNA plasmid expression vector encoding a full-length IgG1 protein (McLean *et al.*, 2000) and transformed into *E. coli* cells. MAb proteins were produced after transient transfection of ExpiCHO cells following the manufacturer's protocol. The resulting secreted IgGs were purified from filtered culture supernatants by fast protein liquid chromatography (FPLC) on an ÄKTA instrument using a Protein G column (GE Healthcare

Life Sciences). Purified mAbs were buffer exchanged into PBS, filtered using sterile 0.45- μ m pore size filter devices (Millipore), concentrated, and stored in aliquots at -80 °C until use. An aliquot of each mAb also was biotinylated directly in 96-well format using the EZ-Link NHS-PEG₄-biotin kit (ThermoFisher Scientific) with a 20-fold molar excess of biotin to mAb, followed by buffer exchange back to PBS using a desalting plate (Zeba, 7 kDa cutoff). Hybridoma-derived mAbs were used in *in vitro* experiments, and recombinant mAbs were used in *in vivo* experiments. Pooled human immunoglobulin was purchased as intravenous immunoglobulin (IVIG, Carimune, CSL Behring, King of Prussia, PA). RSV90 is a recombinant human IgG1 mAb produced in the inventors' laboratory that was used as a negative control, placebo mAb in mouse experiments. Polyclonal anti-VP1, -VP2, and -VP3 antibodies used in western blot were purchased from Genetex.

Fab fragment production. Fab fragments were generated and purified via Pierce Fab Preparation Kit (ThermoFisher Scientific). The Immobilized Papain vial spin column and Zeba Spin Desalting Column were equilibrated with digestion buffer (35 mg cysteine·HCl per 10 mL of supplied Fab Digestion Buffer, pH ~7.0) before use. The NAb Protein A Plus Spin Column was equilibrated with PBS buffer before use. The original IgG samples were passed through the Zeba Spin Desalting Column, and 0.5 mL of the prepared IgG samples were applied on the Immobilized Papain vial and incubated at 37 °C for 5 hours Fab digestion. Then the final Fab fragments were buffer exchanged to PBS and stored at 4 °C.

EV-D68-specific ELISA. Wells of medium binding, black fluorescent immunoassay microtiter plates (Greiner Bio-One) were coated with virus stocks diluted in 100 mM bicarbonate/carbonate buffer, pH 9.6 and incubated at 4°C overnight. Plates were blocked with 2% BSA in Dulbecco's phosphate-buffered saline (DPBS) containing 0.05% Tween-20 (DPBS-T) for 1 hr. For mAb screening assays, hybridoma culture supernatants were added to the wells and incubated for 2 hr at ambient temperature. The bound antibodies were detected using Fc-specific goat anti-human IgG conjugated with HRP (Southern Biotech) and QuantaBlu fluorogenic peroxidase substrate (ThermoFisher Scientific). After 20 min, 100 mM glycine (pH 10.5) was added to quench the reaction, and the emission was measured at 420 nm after excitement at 325 nm using a Synergy H1 microplate reader (Biotek). For dose-response and cross-reactivity assays, serial dilutions of purified mAbs were applied to the wells in duplicate technical replicates and mAb binding was detected as above; the experiments were performed at least three times. For the competition ELISA, microtiter plates were first coated with virus, and then a purified mAb was added at 100 μ g/mL and allowed to incubate at 33 °C for 3 hr. Then, a biotinylated mAb was spiked into this mixture at a final concentration of 5

µg/mL and allowed to incubate at ambient temperature for 1.5 hr. After a wash and 30 min of incubation with avidin-peroxidase (ThermoFisher Scientific), mAb binding was detected as above.

Western blot. B1 clade virus preparation was mixed with denaturing and reducing loading buffer, boiled at 100 °C for 5 min, and then run on an SDS-PAGE gel along with Novex sharp pre-stained protein standard (ThermoFisher Scientific). Protein was transferred to a membrane, blocked in blocking buffer (Li-Cor), and then cut into strips so that individual lanes could be stained with purified mAb in blocking buffer. An IRDye 800CW-conjugated goat anti-human secondary antibody (Li-Cor) was used to detect mAb binding. Strips were reassembled to visualize molecular weight and imaged on an Odyssey CLx near infrared imager (Li-Cor).

Cryo-EM sample preparation and data collection. For both EV-D68:Fab EV68-159 and EV-D68:Fab EV68-228 complexes, purified EV-D68 viruses and Fabs were mixed at a molar ratio of 1:200. After incubating at room temperature for 45 to 60 min, 3.5 µL of virus-Fab mixture sample were added to a glow-discharged 400 mesh lacey carbon film copper grid (Ted Pella Inc.). Grids were plunge frozen (Cryoplunge 3 system, Gatan) in liquid ethane after being blotted for 3.5s in 75 to 80% humidity. Cryo-EM datasets were collected on a 300 kV Titan Krios Microscope (Thermo Fisher Scientific). For the EV-D68:Fab EV68-228 dataset, movies were collected using the program Leginon (Suloway *et al.*, 2005) with a K3 Direct Detection Camera (Gatan) at a magnification of 64,000X, resulting in a super resolution pixel size of 0.662 Å, with a defocus range from 0.7 to 2 µm. A total electron dose of 44.2 electrons/Å² over 2.6 seconds of exposure was recorded over 50 frames. The EV-D68:Fab EV68-159 dataset was acquired with a K2 Summit direct electron detector (Gatan) at a nominal magnification of 81,000X, resulting in a super resolution pixel size of 0.874 Å, a defocus range from 0.7 to 3.5 µm. A total electron dose of 31.4 electrons/Å² over 12 seconds of exposure was split into 60 frames. Overall, 462 movies and 732 movies were acquired for the EV-D68:Fab EV68-228 and EV-D68:Fab EV68-159 datasets, respectively.

Image processing. For both datasets, motion correction was performed on the raw movie frames via MotionCor2 (Zheng *et al.*, 2017b) as implemented in Appion (Lander *et al.*, 2009) during data collection. The contrast transfer function (CTF) was estimated on the aligned frames with CTFFIND4 (Rohou and Grigorieff, 2015). Particle picking templates were generated using the Appion Manual Picker (Lander *et al.*, 2009) and templates for auto picking were obtained through 2-dimensional (2D) classification in XMIPP (Sorzano *et al.*, 2004). These templates were then used for auto-picking in FindEM (Roseman, 2004) and particles

were extracted using RELION. These particles were then subjected to multiple rounds of 2D and 3D classifications in RELION (Scheres, 2012). This resulted in 20,194 and 30,554 particles for the EV-D68:Fab EV68-228 and EV-D68:Fab EV68-159 datasets which were selected for final 3D icosahedral reconstructions using the program JSPR following the gold-standard refinement method (Guo and Jiang, 2014). The final resolutions for both maps were estimated based on a gold-standard Fourier shell correlation cutoff of 0.143 (Scheres and Chen, 2012). Map sharpening was done in RELION (Scheres, 2012) post-processing. Data collection parameters and related items are summarized in Table G.

Model building, refinement and analysis. The same methods were used for the atomic structures of both EV-D68:Fab EV68-159 and EV-D68:Fab EV68-228. The X-ray crystallography model of the EV-D68 Fermon strain (PDB code: 4WM8) was selected as a starting reference for model building and was manually fitted into the density maps using the program Chimera (Pettersen *et al.*, 2004). Using the initial fitting as a basis, the models were rebuilt in Coot (Emsley *et al.*, 2010) and refined using real-space-refinement in PHENIX (Adams *et al.*, 2010) to correct for outliers and poorly fitted rotamers. Chimera (Pettersen *et al.*, 2004), Coot (Emsley *et al.*, 2010) and CCP4i2-PISA (Potterton *et al.*, 2018) were used to determine the binding interface residues. The final atomic models were validated in MolProbity (Chen *et al.*, 2012). Refinement statistics are described in Table G.

Selection of neutralization escape mutant virus. A clade B1 EV-D68 isolate was passaged under selection with increasing amounts of purified mAb in RD cells. After incubating mAb and virus for 1 hr at 33 °C, this mixture was added to a cell monolayer for 2 hr at 33 °C. The monolayer then was rinsed thrice, and mAb containing medium was added back. This culture was incubated at 33 °C until at least 70% cytopathic effect was observed (cells lifted off of plate), at which point the cells and supernatant together were collected and frozen to -80 °C. This sample was thawed and sonicated in the same microfuge tube in an inverted cup sonicator at maximum power 3 x 20 s, vortexed for 30 s at maximum power, and sonicated again 2 x 20 s. Cellular debris was clarified for 10 min at 10,000 x g. Then the virus-containing supernatant was mixed 1:1 with fresh medium containing mAb at higher concentration. Over three passages, mAb concentration was increased from 5 to 50 to 500 ng/mL. Viral RNA was harvested using TRI Reagent and Direct-zol RNA MiniPrep kit (Zymo Research). In triplicate, the inventors generated cDNA templates, from which a 3,080 bp amplicon covering the P1 region of the viral genome was generated with the PrimeScript One Step RT-PCR Kit Ver. 2 (Takara) and primers 5'- CCTCCGGCCCCTGAAT (Fwd; SEQ ID NO: 641) and 5'- CCATTGAATCCCTGGGCCTT (Rev; SEQ ID NO: 642). They used a

Pacific Biosciences (PacBio) next generation sequencing platform to generate sequences of each of the three replicates. 2,000 reads of each sequencing run were used to quantitate the percentage of reads in which each mutation was observed. Mutations were determined as compared to a wild-type consensus sequence of all of the reads from the negative control selection mAb selection.

Quantification and statistical analysis. Technical and biological replicates are indicated in the methods and figure legends. Statistical analyses were performed using Prism v8 (GraphPad).

Competition-binding assay. ELISA fluorescence values were normalized to a percentage of maximal binding determined from a control well without an irrelevant prior competing mAb added. The Pearson correlation of each biotinylated mAb to each other biotinylated mAb was calculated using the median inhibition percentage from three different experiments using the *corr* method of the *Pandas* Python package (McKinney, 2010). Hierarchical clustering was then performed on these Pearson correlations using the *clustermap* method of the *Seaborn* Python package. The clustering information was exported in newick format and imported into Interactive Tree of Life v4 (Letunic and Bork, 2019), which was used to display the hierarchically-clustered heatmap before importation into Excel (Microsoft) for final display.

Antibody ELISA binding experiments. EC₅₀ values for mAb binding were determined after log transformation of antibody concentration using four-parameter sigmoidal dose-response nonlinear regression analysis constrained to a bottom value of zero and top value less than the maximal fluorescent value of the mAb with the highest saturation fluorescence value.

Virus assays. MAb IC₅₀ values were calculated using a formula based on the Spearman-Kaerber equation (Ramkrishnan, 2016). Viral titers in murine plasma and lungs were compared using a one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test, with a single pooled variance. A value of $p < 0.05$ was considered significant.

Lung cytokine/chemokine evaluations. For each cytokine/chemokine, the concentrations from treated mice were compared to placebo-treated mice using a Brown-Forsythe one-way ANOVA test and Dunnett's T3 multiple comparisons test, with individual variances computed for each comparison. This analysis was chosen because the inventors did not assume equal standard deviations for each measurement.

***In vivo* protection studies.** Survival curves were generated using the Kaplan-Meier method and curves compared using the log rank test (Mantel-Cox). Neurologic scores were compared using a chi-square test.

5

Example 10 – Results

After obtaining written informed consent, twelve subjects who had previous documented EV-D68 respiratory tract infections during the 2014 outbreak in the U.S. donated blood, from which the inventors isolated peripheral blood mononuclear cells (PBMCs). The subjects were 12 to 15 years old when infected and 16 to 18 years old at time of blood collection. Each subject had a history of EV-D68-associated respiratory disease, and none had symptoms of AFM (Table E). The collected PBMCs were transformed *in vitro* by inoculation with Epstein-Barr virus to generate memory B cell derived lymphoblastoid cell lines (LCLs), which secrete antibodies. LCL culture supernatants were used in an indirect ELISA to screen for the presence of EV-D68-reactive IgGs. The inventors selected cultures with antibodies that bound to laboratory-grown live virus preparations of EV-D68 generated from 2014 clinical isolates but that did not bind to a similarly prepared uninfected cell supernatant. After electrofusion of LCLs secreting EV-D68-specific antibodies with a non-secreting myeloma cell line, the resulting hybridoma cells were single-cell sorted to generate clonal hybridomas secreting fully human mAbs (Table F).

The inventors sought to determine how many major antigenic sites on the virus surface are bound by human mAbs made in response to natural infection. To identify groups of antibodies that recognized similar epitopes, they determined whether the mAbs could compete with the binding of each of the other mAbs to live virus in an indirect ELISA. For competition-binding experiments, virus was coated directly onto an ELISA plate and then incubated with high concentrations of one unlabeled mAb. Next, mAbs labeled by biotinylation were added at a lower concentration, and the ability of the second mAb to bind the virus in the presence of the first mAb was determined. The inventors then used a Pearson correlation with the inhibition data to determine the relatedness of the antibody binding patterns to each other and identified four main competition-binding groups (FIG. 32), which they termed groups 1 to 4. They used each mAb to stain a western blot of EV-D68 preparations and found that nearly all mAbs in competition-binding groups 2 and 3 bound to linear epitopes in the VP1 protein, whereas only a single other mAb bound to any protein in the virus preparation (FIG. 40).

During the 2014 EV-D68 outbreak in the U.S., nearly all viral isolates were of the newly emergent B1 clade, with fewer detections of virus from the closely related B2 or distantly

related D clades (Tan *et al.*, 2016). All but one of the subjects for this study were infected with B1 clade isolates (Table E). Since 2014, B3 clade viruses have dominated, and B1 clade viruses are no longer circulating (Dyrdak *et al.*, 2019); in 2018 all EV-D68 isolates sequenced by the U.S. Centers for Disease Control and Prevention were from the B3 clade (Kujawski *et al.*, 2019). The inventors first measured the *in vitro* neutralization capability of each mAb in a 50% cell culture infectious dose (CCID₅₀) assay using a B1 clade EV-D68 isolate (FIG. 33A). Twenty-eight mAbs demonstrated neutralization with a half maximal inhibitory concentration (IC₅₀) below 50 µg/mL, with mAb EV68-159 exhibiting the strongest neutralization at an IC₅₀ value of 0.32 ng/mL (FIG. 41). The inventors further tested the 21 most potently neutralizing mAbs against a D clade isolate and found that 11 mAbs neutralized that virus, with 7 of those exhibiting at least a ten-fold decrease in potency by IC₅₀ value for the heterologous virus. The Fermon strain is an isolate from 1962 and is so distantly related to modern EV-D68 isolates that it does not fit into the clade classification scheme (Tan *et al.*, 2016). Nine mAbs neutralized the Fermon laboratory reference strain, but less potently than they inhibited the contemporary B1 clade virus.

Recognizing that neutralization assays may underestimate cross-reactivity, the inventors used the same indirect ELISA approach described above to generate half-maximal effective concentrations (EC₅₀) of purified mAb for binding to representative EV-D68 isolates from the B1, B2, or D clades (FIGS. 33B-C, and FIG. 42). Of the mAbs with EC₅₀ values for binding of ≤ 1 µg/mL to B1 clade isolates, all bound to a B2 clade isolate, whereas about half also bound to a D clade isolate (FIG. 33B and FIG. 41). An additional class of mAbs was observed that bound weakly in general but cross-reacted to viruses from all clades tested.

To date, structural studies of antibody-EV-D68 interactions have been limited to murine mAbs (Zheng *et al.*, 2019). The inventors selected two potently neutralizing human mAbs, the clade-specific mAb EV68-159 and the highly cross-reactive mAb EV68-228, to make immune complexes with antigen binding fragments (Fabs) and a B1 clade EV-D68 isolate for cryo-electron microscopy (cryo-EM) studies. The final density maps attained a resolution of 2.9 Å (EV68-159) or 3.1 Å (EV68-228) (FIG. 34A, FIG. 43, FIG. 44, and Table G). The structures revealed two distinct binding sites: EV68-159 attached around the three-fold axes of symmetry, whereas EV68-228 bound around the five-fold axes between depressions that form the canyon regions (FIGS. 34A-C, FIG. 45). Thus, for each Fab, a total of 60 copies bound to the virus particle. The Fab variable domains, which interacted with the viral surface, displayed strong densities similar to the viral capsid proteins, and an atomic model of each Fab was built together with the four viral capsid proteins. In contrast, the Fab constant domains, which are located

further from the viral surface, displayed weaker densities and were excluded from atomic model building. The backbone of the polypeptide chains and the majority of amino acid side chains are well-ordered in the density maps, demonstrating the critical features of the binding interface between virus particle and Fab molecule.

5 For both models, the viral surface residues that were facing and within a 4 Å distance from the Fab were identified as the footprint (FIG. 34C, FIG. 46, and Table H). The footprints show that both Fab molecules sit within one protomer. In the EV-D68:Fab EV68-159 complex, each Fab masked a viral surface area around 990 Å². At the binding interface (FIG. 35), essential interactions were found between the EV68-159 light chain and three residues on the
10 C-terminus of VP1: Glu271 and Arg272 (FIG. 36A) and Asp285 (FIG. 36B). Residues Glu271 and Arg272 formed hydrogen bonds with CDR3 and CDR1. Arg272 and Asp285 formed salt bridges with CDR3 and CDR2 residues, respectively. The heavy chain of EV68-159 contributed 77% of the masked surface areas. A series of hydrogen bonds was found between the heavy chain complementarity-determining region 2 (CDR2) and CDR3 and the VP3 N-
15 terminal loop before the B-β strand (βB) (FIG. 36C).

In the EV-D68:Fab EV68-228 complex, each Fab masked approximately 1,170 Å² of the viral capsid surface. Similar to EV68-159, the heavy chain of the EV68-228 Fab dominated the interaction with the viral capsid by masking around 84% of the surface area. The binding interface (FIG. 35) was stabilized mainly by hydrogen bonds formed between the heavy chain
20 CDRs and the VP1 βB as well as the VP3 C-terminus (FIG. 36D). The light chain CDR1 interacted with the VP2 EF loop. In addition, hydrogen bonds formed between the heavy chain framework region (FR) 3 and the VP1 DE loop. Furthermore, a salt bridge formed between the light chain CDR3 and the VP1 C-terminus. Overall, the EV68-228 Fab bound the viral surface around the five-fold axes and recognized the classical picornavirus neutralizing immunogenic
25 sites (NIm)s NIm-IB (VP1 DE loop) and NIm-II (VP2 EF loop) (Rossmann *et al.*, 1985).

Bulky side chains were found at the interface for both Fabs and act to stabilize the structures through hydrophobic interaction networks (FIG. 47). Furthermore, disulfide bonds also were detected around CDR1 and CDR3 in heavy and light chains. Another pair of cysteines, Cys101 and Cys106, were found within the CDR3 of the EV68-228 heavy chain and were at
30 the correct distance and orientation to form a disulfide bond (FIG. 36D). Specifically, when the contour levels were reduced, the densities of the two cysteine side chains connected. As described above for the EV-D68:Fab EV68-228 complex, hydrogen bonds were observed between the heavy chain CDR3 and the VP3 C-terminal residues adjacent to the canyon

involving Cys101, forming both a hydrogen bond and a disulfide bond. These cysteine residues play critical roles in stabilizing both Fab structure and the virus-Fab binding interface.

The inventors next sought to determine if the potentially neutralizing and highly cross-reactive human mAb EV68-228 could prevent or treat infection and disease in small animal models of EV-D68 infection. They tested for this antiviral activity *in vivo* using two different established models of infection causing either respiratory or AFM-like neurologic disease in AG129 strain mice that are deficient in receptors for interferon α/β and γ (Evans *et al.*, 2019; Hurst *et al.*, 2019). First, the inventors tested whether antibodies could reduce viremia and lung virus replication in the respiratory model of infection. MAb EV68-228 administered systemically as prophylaxis a day before virus inoculation provided sterilizing immunity in the blood (FIG. 37A) and lungs (FIG. 37B) at each of the concentrations tested, whereas human IVIG only sterilized the blood. Induction of pro-inflammatory cytokine secretion was inhibited in the lungs of EV68-228 treated mice (FIGS. 38A-C). When used as treatment given at increasing times after virus inoculation, again all treatments were highly effective at sterilizing the blood (FIG. 37C), but only EV68-228 had efficacy in the lungs (FIG. 37D). The inventors similarly observed reduced pro-inflammatory cytokine levels in the lungs of EV68-228-treated mice (FIGS. 38D-F).

Next the inventors assessed the effect of passive transfer of antibodies in a neurologic model of infection that mimics AFM disease. EV68-228 prophylaxis provided sterilizing immunity of the blood (FIG. 39A) and complete protection from death (FIG. 39B) or development of any neurologic disease (FIG. 39C), whereas IVIG immunity protected only partially. Given therapeutically, EV68-228 treatment sterilized the blood within 24 hours of administration (FIG. 39D) at each of the time points given. EV68-228 improved survival (FIG. 39E) and neurologic disease when given as late as 48 hours after infection; when given at 72 hours post-infection, the mouse that survived improved clinically (FIG. 39F and Table I).

Table E – Characteristics of subjects who provided peripheral blood mononuclear cells

Subject	Age at donation ¹	Gender ²	Ethnicity ³	Race ⁴	GenBank accession numbers for virus isolate	Virus Clade	Illness date	Donation date	Symptoms ⁵	Severity ⁶
1	18	F	Unk.	W	KX255352 KX255415	B1 B1	8/11/14	12/27/17	R, H	Mod.
2	17	F	NHL	B & W	KX255378	B1	9/5/14	8/7/17	NR	NR
3	17	M	NHL	W	KX255387	B1	9/11/14	8/1/17	NR	Mild
4	17	M	NHL	W	KX255385 KX433183	B1	9/11/14	7/19/17	C, R, H, SP	Mild
5	18	M	NHL	W	KX255382	B1	8/3/14	7/12/17	NR	NR
6	17	F	NHL	W	KX255364 KX255373	B1 B1	8/13/14	7/5/17	C, R	Mod.
7	17	M	NHL	W	KX433162	A1	8/7/12	6/21/17	C, R, H, W	Severe
8	17	M	NHL	W	KX255407	B1	9/23/14	6/19/17	C, R, H, W	Mild
9	17	M	NHL	W	KX255379	B1	9/26/14	6/8/17	C, R	Mild
10	18	F	NHL	W	KX255411	B1	9/4/14	6/6/17	C	Mild
11	18	F	NHL	W	KX255390 KX255362 KX255403	B1 B1 B1	7/18/14	5/30/17	C, H, R, W	Mod.
12	18	M	NHL	W	KX255389	B1	9/26/14	1/22/18	C, R, W	Mod.

¹Age in years; ²F: female, M: male; ³Unk.: unknown, NHL: not Hispanic or Latino; ⁴B: black, W: white; ⁵C: cough, H: hoarse, R: rhinitis, SP: sinus pain, W: wheeze, NR: not recorded;

5 ⁶Mod.: moderate, NR: not recorded.

Table F – Sequence characteristics of human mAbs

Clone	Heavy chain					Light chain		
	V gene	J gene	D gene	CDR3		V gene	J gene	CDR3
EV68-37	V3-30*04	J4*02	D6-13*01	ARPTLPYNNWYAPEY		LV3-10*01	LJ2*01	SSTDSSGNFVL
EV68-40	V3-23*04	J4*02	D6-6*01	AKVVRIAAVLYYFDY		KV3-11*01	KJ5*01	QQRSSWPII
EV68-41	V3-23*04	J3*02	D1-4*01	ARVKESTIGTITALVFDI		LV3-61*01	LJ3*02	GLYMGSGIWI
EV68-43	V3-30*03	J6*02	D4-17*01	AKDKKHGDFDYGVVDV		KV1-12*01	KJ1*01	QQADSFPRT
EV68-46	V3-30*03	J4*02	D1-26*01	ARRRPGSFPGLCDY		KV1-5*03	KJ2*01	QQHNSYSYT
EV68-48	V1-69*06	J3*01	D3-12*01	ARMYSGHDDGVVDV		KV3-11*01	KJ1*01	QQRSTWPPGM
EV68-71	V4-39*01	J3*02	D4-17*01	ARHLTHLYGDYVTFDALDI		KV3-20*01	KJ1*01	QQYSNSRLT
EV68-72	V3-48*02	J5*02	D3-3*01	ARAHGRIVTSGVVTNRDP		LV3-44*01	LJ2*01	SSYTTSTNLVV
EV68-74	V1-2*02	J4*02	D2-2*01	ARMGCRSDRCYSTNYNFDQ		LV3-21*02	LJ2*01	QVWDSGIDVV
EV68-75	V3-21*01	J4*02	D6-6*01	ARERGHSTSSSYFDS		LV3-21*01	LJ3*02	RVWDSDTIDHRV
EV68-76	V3-15*01	J4*02	D3-22*01	STGPPYYDTSGYFPQFDY		LV3-1*01	LJ2*01	QAWDSSTVV
EV68-79	V3-21*01	J6*02	D3-9*01	ARDRPMMEGEGIDELTGYYVYQYYAMDV		ND		
EV68-84	V1-2*02	J4*02	D5-24*01	ARAGRNGYDY		LV3-25*03	LJ2*01	QSGDSSGTYLV
EV68-85	V3-23*01	J4*02	D4-11*01	TVPWGNYNNDYVSDY		KV3-11*01	KJ5*01	HQHSTWFRGT
EV68-88	V3-30-3*01	J4*02	D6-13*01	ARHFLPYSSSWYQGFNY		LV6-57*01	LJ2*01	QSYDNSNRAVV
EV68-89	V3-33*01	J4*02	D4-17*01	ARGVPYGDITLGLVY		LV6-57*01	LJ3*02	QSYDNSDRV
EV68-95	V2-36*01	J4*01	D1-15*01	ARLLVAGTFLPSHYFDY		LV3-21*01	LJ2*01	QVWDSSENHPV
EV68-97	V3-48*01	J4*02	D1-15*01	TRQVGADFSGRGFDY		LV3-1*01	LJ3*02	QAWDSSTAV
EV68-98	V3-48*01	J3*02	D1-14*01	ATARHITNDGFDI		KV1-9*01	KJ3*01	QQLNSHPRMFT
EV68-105	V4-38-2*01	J4*02	D1-21*02	ARGPGHCYGDDECYAVYFDQ		KV1-12*01	KJ3*01	QQANSFPFT
EV68-110	V1-69*06	J4*02	D1-8*01	ARSLPYCTNDVCSNQNFYDY		LV3-25*03	LJ2*01	QSADSSGTYVV
EV68-114	V3-23*01	J4*02	D4-17*01	VRRFPMITVTSFDS		LV2-41*01	LJ2*01	GAYAGFNAL
EV68-116	V3-7*03	J4*02	D2-2*01	VREQVRRVVVRSTGYPDF		LV3-16*01	LJ3*02	YSTDSSGYQRA
EV68-138	V4-31*03	J5*02	D1-21*02	ARHVVIASQWFDP		KV3-11*01	KJ2*01	QQRSRWPPPYT
EV68-132	V3-30-3*01	J6*02	D3-22*01	ARVTADYTESSGKVF		KV1-5*03	KJ1*01	QQYQTF8WT

Table F, cont'd

EV68-153	ND					LV1-47*01	LJ2*01	AAWDDRLSGVV
EV68-156	V4-59*01	J1*01	D2-8*02	ARGSMPHI		LV1-47*01	LJ1*01	AAWDDSLKAPV
EV68-158	V3-23*01	J3*02	D3-22*01	AKDSHSMIVVDHAFDI		LV3-21*02	LJ1*01	QVWDSYNVHYV
EV68-159	V3-21*01	J4*02	D1-14*01	AREEGFRAYNLY		LV1-47*01	LJ2*01	AAWDDHLSGVV
EV68-160	V3-30*04	J4*02	D2-8*01	ARDWDRLVRSVGV		LV2-11*01	LJ3*02	QSYAGTYTWV
EV68-161	V4-61*01	J6*02	D3-3*01	ERRLRILSIERNYYAMDV		KV3-20*01	KJ1*01	QQYGSPWT
EV68-162	V3-30-3*01	J6*02	D2-15*01	ARDHVPPKDCSDGNCHSDYGM DV		KV1-5*03	KJ2*01	QQHNSYSYT
EV68-164	V3-48*02	J6*02	D3-10*01	ARVYTMELRGASMDV		KV3-11*01	KJ3*01	QLRITWPPIFT
EV68-165	V1-2*02	J4*02	D2-2*01	ARVKKSSANCYGNFDY		LV2-8*01	LJ2*01	SSYAGSNLNV
EV68-166	V5-51*01	J5*01	D5-12*01	ARQTTQNSGYDRWFD		LV1-44*01	LJ3*02	AAWDDSLNGWV
EV68-178	ND					KV1-5*03	KJ1*01	QQYNSYPLT
EV68-181	V3-23*01	J4*02	D3-3*01	AISVPLLRFLWFQHPDF		KV3-15*01	KJ1*01	HQYENWPPWT
EV68-183	V3-31*01	J4*02	D4-11*01	VRFTMTVTNFD		LV3-11*01	LJ3*02	CSHAGSHTWV
EV68-185	V3-30*03	J3*02	D3-22*01	PKVIPHPYTDSSGDDAFDI		KV3-15*01	KJ5*01	QQYSKLPIT
EV68-208	V1-69*01	J4*03	D3-10*01	ARFISTASYVPGTFEDV		KV3-11*01	KJ2*02	QQRSDWPPGI
EV68-210	V3-21*01	J4*02	D4-17*01	ARMVBNVTIAFDY		LV2-23*02	LJ3*02	QSYGGSNSWM
EV68-219	V1-24*01	J4*02	D2-21*01	ATWQVEVVNGRDRYFDS		LV3-25*03	LJ2*01	Q8ADNTRITV
EV68-220	V3-48*03	J3*01	D3-22*01	ARDVRDCSALTCPRGDADF		KV2-30*01	KJ3*01	MQGTHWPRT
EV68-221	V3-21*01	J4*02	D2-15*01	VKVGGSKHQYTFDY		LV1-44*01	LJ2*01	AAWDDSLNGVV
EV68-224	V1-18*01	J4*02	D2-2*02	ARERCSTSTCYSRVADY		KV1-39*01	KJ1*01	QQSYRSPRT
EV68-225	V3-42*03	J3*02	D3-9*01	MREGLTYVDSTI		LV4-69*01	LJ3*02	QIWGTGFRV
EV68-227	V3-9*01	J3*02	D3-16*01	AKDDYEGAGFDI		KV3-11*01	KJ5*01	QQRSNWPPIT
EV68-228	V4-38-2*02	J4*02	D2-15*01	VRHEGSCNDGSCYGSFVDN		KV1-12*01	KJ4*01	QQADSFIT
EV68-229	ND					KV3-11*01	KJ4*01	QQRSNWPPIT
EV68-231	V3-23*01	J2*01	D2-21*02	ARGGTFHNWYFDL		KV3-15*01	KJ1*01	QQYKNWPPRT
EV68-234	V3-23*01	J6*03	D5-12*01	AKGHTYYSYTYMAV		KV3-20*01	KJ4*01	QQYGTST
EV68-235	V1-24*01	J4*02	D2-21*01	ATWQIEVVNGRDEFFDS		LV3-25*03	LJ2*01	Q8ADNTRITV
EV68-236	V1-24*01	J4*02	D2-21*01	ATWQVAVVSGRRDYFDS		LV3-25*03	LJ2*01	QTADIKYTV
EV68-241	V4-30-4*01	J5*02	D3-3*01	ARAYAYEFWEGYPNWDF		KV1-39*01	KJ1*01	QQSYSPWWT
EV68-242	ND					KV1-5*03	KJ1*01	QQYNSLPWT
EV68-247	V3-30*01	J4*02	D2-2*01	ARGLYGCSGTGGSCIPFEY		KV1-39*01	KJ4*01	QQSDSAPPT
EV68-254	V4-34*02	J5*02	D2-2*01	VRVPRRGFEFGFCDDTACRYGHTWDF		KV1-39*01	KJ1*01	QQSYLTPTT
EV68-266	V3-11*01	J2*01	D2-8*02	AGSKVGYITGRNWFYDL		LV2-8*01	LJ2*01	SSYAGNNLNV
EV68-269	V1-46*01	J3*02	D2-2*02	ARDLVVVVPVEMSRRAFDI		LV3-21*02	LJ3*01	QVWDSITDHGV
EV68-271	V1-2*06	J4*02	D3-16*02	ARDYRDDYMWGSRPLDY		KV3-11*01	KJ4*01	QQRSLGLT

- 5 Sequence characteristics as identified by the International ImMunoGeneTics (IMGT) Information System, world-wide-web at imgt.org. When IMGT could not rule out multiple genes, the first call is listed. ND: not determined.

Table G – Cryo-EM data acquisition parameters and refinement statistics

	EV-D68:Fab EV68-159	EV-D68:Fab EV68-228
Cryo-EM data acquisition and processing		
Magnification	81,000X	64,000X
Camera	K2 Summit direct electron detector (Gatan)	K3 Direct Detection Camera (Gatan)
Voltage (kV)	300	300
Pixel size (Å)	0.874	0.662
Defocus range (µm)	0.7-3.5	0.7-2.0
Total electron dose (electrons/Å ²)	31.4	44.2
Particles picked	42,078	27,390
Particles used	30,554	20,194
Map resolution (FSC threshold =0.143)	2.9	3.1
Model building and refinement		
Model building reference (PDB code)	4WM8	4WM8
MolProbity score	1.93	1.96
Clash score	9.05	8.7
Rotamer outliers (%)	0.46	0.00
R.m.s. deviations		
Bond length (Å)	0.005	0.005
Bond angles (°)	0.691	0.689
Ramachandran plot (%)		
Favored	93.08	91.70
Allowed	6.92	8.20
Outliers	0.00	0.10

Table H – Structural contact amino acid residues of EV-D68 and respective Fabs

Viral amino acid	Fab amino acid	Potential interactions
EV68-159		
VP1: GLU271	L: ILE95	Hydrogen bond
VP1: ARG272	L: SER26	Hydrogen bond
	L: SER27	Hydrogen bond
	L: ASP94	Salt bridge
VP1: ASP285	L: LYS51	Salt bridge
VP3: GLU59	H: ARG102	Hydrogen bond
VP3: SER60	H: GLY100	Hydrogen bond
VP3: MET64	H: TYR56	Hydrogen bond
VP3: GLU65	H: SER51	Hydrogen bond
	H: THR52	Hydrogen bond
	H: SER53	Hydrogen bond
EV68-228		
VP1: LYS71	H: SER30	Hydrogen bond
VP1: ARG72	H: TYR27	Hydrogen bond
VP1: SER73	H: ASN31	Hydrogen bond
VP1: GLY129	H: SER73	Hydrogen bond
VP1: LYS268	L: ASP92	Salt bridge
VP2: ASN136	L: SER30	Hydrogen bond
VP3: GLY 234	H: ASN102	Hydrogen bond
VP3: LEU236	H: ASN102	Hydrogen bond
VP3: ASP237	H: TYR34	Hydrogen bond
	H: SER100	Hydrogen bond
	H: CYS101	Hydrogen bond
	H: ASN102	Hydrogen bond
VP3: HIS238	H: TYR53	Hydrogen bond
	H: TYR54	Hydrogen bond
VP3: GLU243	H: TYR33	Hydrogen bond

Heavy and light chains are labeled as H and L, respectively. Notably, only direct interactions that are clearly observed in the electron density maps at high resolution are listed. This differs from contact residues highlighted in the roadmaps (FIG. 34C and FIG. 46), which use a 4Å cutoff for displaying the overall footprint at maximum hydrogen bonding distance.

5

Table I – Neurologic scores of individual mice treated with antibody after EV-68 inoculation

Day	Placebo – 24 hrs post						EV68-228 - 24 hrs post					
2	1	2	1	1	2	0	0	1	1	0	0	0
3	2	2	2	1	3	1	1	1	1	1	1	1
4	3	3	4	2	4	2	1	1	1	1	1	1
5	4	4		3		3	1	1	1	1	1	1
6				4		4	1	1	1	1	1	1
7							1	1	0	1	1	1
8							1	1	0	1	1	1
9							1	1	1	1	1	1
10							1	1	1	1	1	1

Day	EV68-228 - 48 hrs post						EV68-228 - 72 hrs post					
2	1	2	1	1	2	2	1	2	2	1	2	2
3	2	2	2	2	2	2	2	3	2	2	2	2
4	2	2	2	2	2	4	4	0	3	3	4	4
5	2	2	2	2	4			0	3	3		
6	2	2	2	2				0	4	4		
7	1	2	1	1				0				
8	1	2	1	1				0				
9	1	2	1	1				0				
10	1	1	1	1				0				

Day	EV68-228 - 120 hrs post							
2	1	0	1	0	1	1	1	0
3	1	1	1	2	1	1	1	1
4	1	1	2	3	2	3	2	3
5	2	2	3	3	3	4	3	4
6	3	4	4	3	4		4	
7	4			4				
8								
9								
10								

Day	IVIg - 24 hrs post					
2	0	1	0	0	1	0
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	2	2	1	1	2	2
7	1	2	2	1	1	1
8	1	2	2	1	2	1
9	0	2	2	0	1	0
10	0	1	1	0	0	1

5

Each column is the data from an individual mouse within the indicated treatment group corresponding to the graph in FIG. 39F. Day represents the number of days after EV-D68 inoculation the score was determined. Mice were sacrificed or already dead upon a score of 4, so no further scores are listed in days after a 4 was recorded. Highlighted cells indicate instances of a mouse improving clinically.

10

Table J – Neutralization escape amino acid mutants

Amino acid			Average percent of reads ¹			Contact residue ²	
Position	WT ³	Mutant	RSV-90	EV68-159	EV68-228	EV68-159	EV68-228
VP2 135	HIS	TYR	0	13	0		Adjacent
VP2 139	ALA	THR	20	2	3		Adjacent
VP3 42	SER	ASN	7	15	3		
VP1 71	LYS	MET	12	11	33		Contact
VP1 126	ALA	VAL	1	3	0		
VP1 157	LYS	GLU	6	17	4		
VP1 270	ARG	LYS	1	0	1	Adjacent	

¹Each of the mAbs listed was used as selection for three passages of EV-D68 in RD cell culture, with RSV-90 being the same negative control mAb used as placebo treatment for *in vivo* experiments. For each treatment, the structural genes were sequenced using next generation sequencing in 3 technical replicates with 2000 sequences analyzed per replicate. Numbers listed are the average of these replicates. ²Contact residues as determined by cryo-EM structures from this manuscript, which are virus amino acids located within 4Å of Fab amino acids. Adjacent refers to amino acids that are immediately adjacent to contact residues.

³WT: wild-type. Represents the consensus sequence of the RSV-90 selected virus sequences.

Table K – EV-68 isolates used

Strain name	Abbreviation	Clade	Source	Source ID	GenBank Accession	Pubmed ID
US/MO/14-18947	MO/47	B1	BEI	NR-49129	KM851225	25414503
US/MO/14-18949	MO/49	B1	BEI	NR-49130	KM851227	25414503
US/IL/14-18956	IL/56	B1	BEI	NR-49133	MK268345	25414503
US/IL/14-18952	IL/52	B2	BEI	NR-49131	KM851230	25414503
US/KY/14-18953	KY/53	D	BEI	NR-49132	KM851231	25414503
Fernon	Fer.	1962 reference strain	ATCC	VR-1826	AY426531	15302951
Mouse adapted US/MO/14-18949		B1	Hurst and Tarbet, USU	NA	MH708882	30521834

ATCC: American Type Culture Collection; BEI: Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH; USU: Utah State University; NA: not applicable.

Example 11 - Discussion

These studies reveal diverse features of the human B cell response to EV-D68 infection. The inventors attempted to be unbiased in their approach to isolating these mAbs, using live virus isolates as the screening antigen. The diversity in antibody phenotypes that they recovered may be a result of this strategy, as the inventors observed a broad range of cross-reactivity among clades of EV-D68, both with binding and neutralization. Interestingly, strong binding to live virus particles did not necessarily predict high neutralizing potency. Of the competition-binding groups observed, only groups 2 and 3 exhibited uniformity in phenotype. MABs in both groups were cross-reactive, but group 2 mAbs neutralized virus whereas group 3 mAbs did not. Nearly all of the group 2 and 3 mAbs bound to VP1 in western blot (FIGS. 40 and 41), suggesting that they bind to linear epitopes. Notably, the competition-binding studies used full-length IgG molecules, so the competition seen is functional as would occur in human tissues and does not necessarily indicate that there are only four structurally distinct epitopes on the viral surface.

The lack of western blot reactivity of EV68-159 and EV68-228 correlates with the findings in the structural studies that show both epitopes span all three major viral surface proteins. The conformation-dependent nature of the epitopes of these two potentially neutralizing mAbs is notable because recent diagnostic advances using peptide microarray (Mishra *et al.*, 2019) and phage library (Schubert *et al.*, 2019) technologies scanned for antibodies in the cerebrospinal fluid of patients with AFM that recognize linear epitopes. Detection of antibodies recognizing linear epitopes currently can be used in valuable diagnostic tools; however, these studies reveal these tests are at best only partially informative about the quality of antibody response these patients make in response to enterovirus infection. The structures also suggest the molecular basis for antibody-mediated neutralization. By contacting all three structural proteins within a protomer, both mAbs appear capable of inhibiting dynamic structural transitions necessary for infection, which are poorly understood.

The disulfide bond in the CDR3 of EV68-228 heavy chain is a structural moiety the inventors have now observed in broadly neutralizing antiviral human mAbs for a number of viruses, including both hepatitis C (Flyak *et al.*, 2018) and influenza A virus (Bangaru *et al.*, 2019). The intervening four to five amino acids between cysteines forms a smaller structured loop at the most distal tip of the full CDR3 loop, stabilizing the CDR3 in a preconfigured state optimal for binding the viral antigen. For EV68-228 specifically, the Cys101 also directly

interacts with VP3 via a hydrogen bond, so the cysteine participates in both CDR3 loop stabilization and interaction with target.

The three VP1 residues that interact with EV68-159 light chain (Glu271, Arg272 and Asp285) and Glu59 on the N-terminal loop of VP3, which interacts with EV68-159 heavy chain, are adjacent to the sialic acid receptor binding site (Liu *et al.*, 2015), suggesting that the EV68-159 Fab may block virus from binding sialic acid receptors. In particular, these three VP1 residues are located on a 22-amino acid VP1 C-terminal peptide that is bound by antibodies found in the cerebrospinal fluid of patients with AFM (Mishra *et al.*, 2019; Schubert *et al.*, 2019). Furthermore, the interaction of the EV68-159 Fab heavy chain with the VP3 N-terminal loop may prevent the virus from uncoating, since the N-termini of the four VPs contribute to capsid stability (Filman *et al.*, 1989). EV68-228 may prevent the virus from uncoating by binding VP1 β B, inhibiting the externalization of the N-terminus of VP1 that is required for entry. In addition, the antibody footprint includes residues on the C-terminus of VP3, which is not part of a classical NIm. These residues are adjacent to the canyon receptor binding site, suggesting that mAb EV68-228 also may block virus binding to receptors.

Finally, at a time when poliovirus types 2 and 3 have been eradicated, AFM is on the rise, and the role of EV-D68 in causing epidemics of this paralytic disease is increasingly evident. Given how well prophylaxis with human mAb EV68-228 works *in vivo*, these data suggest that an effective EV-D68 vaccine might prevent AFM disease. Indeed, recent studies indicate that virus-like particle (Zhang *et al.*, 2018a; Dai *et al.*, 2018) and inactivated EV-D68 (Patel *et al.*, 2016) vaccine candidates are immunogenic and protective against infection in mice. However, a study of cotton rats vaccinated with inactivated EV-D68 suggested that they may have suffered worse respiratory disease upon subsequent EV-D68 infection (Messacar *et al.*, 2016). While this finding could suggest the possibility of antibody-dependent enhancement (ADE) of EV-D68 infection, in mice the inventors did not observe ADE caused by polyclonal or monoclonal antibodies, within the range of antibody concentrations they tested. Also, the prospect of using mAb EV68-228 as a therapy early during EV-D68 infection is appealing, especially since this antibody potently neutralizes a diverse set of viral isolates without obvious autoreactive binding to human cell materials (FIG. 33C). Even though IVIG protected mice from AFM-like disease due to EV-D68 in prior *in vivo* studies (Hixon *et al.*, 2017a), so far IVIG has not been shown to confer benefit for humans with AFM (Messacar *et al.*, 2016). However, IVIG is a complex mixture of polyclonal antibodies with only a small fraction that recognize EV-D68. MAb prophylaxis or therapy for EV-D68 associated AFM is more promising than IVIG because of the high specificity, high potency, and lower antibody dose

that can be used. It is possible, however, that a cocktail of mAbs directed at multiple epitopes may be more protective than mAb monotherapy. A mAb cocktail theoretically would provide a higher barrier to emergence of mAb resistant virus, but the inventors did not observe resistance *in vivo* (FIGS. 37A-D and FIGS. 39A-F). Even under conditions optimized for
5 selecting EV68-228 resistant viruses *in vitro*, the inventors could only identify virus genomes with mutations of unclear significance (Table J). In the absence of a reverse genetics system for making recombinant viruses with these mutations, they were unable to verify specifically if these mutations caused escape from neutralization. Therefore, they find emergence of resistance during potential therapeutic use unlikely. These experiments also provide hope for
10 therapeutic efficacy in patients with severe respiratory disease due to EV-D68, which is the clinical syndrome that brought the 2014 EV-D68 outbreak to the attention of public health authorities prior to recognition of the association with AFM (Midgley *et al.*, 2014). Overall, the studies presented here show that natural EV-D68 infection of humans induces B cells encoding broad and potently neutralizing antibodies that can prevent or treat infection and
15 disease in both the respiratory tract and the nervous system.

Example 12

The objective of this study was to determine the efficacy of treatment with tobacco-produced EV68-228 for an Enterovirus D68 (EV-D68) respiratory infection in four-week-old
20 AG129 mice.

Materials and Methods

Animals. Four-week-old male and female AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah
25 State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water.

Antibodies and Compound. The monoclonal antibody (mAb) EV68-228 produced in tobacco plants (EV68-228-TP) as well as Chinese hamster ovary (CHO) cells (EV68-228-CHO) was provided by the inventors. Both the tobacco-produced and CHO antibodies were provided
30 as solutions and were dosed at a concentration of 10 mg/kg. A tobacco-produced anti-HIV (Anti-HIV-TP) antibody was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (HIVIg, Carimune, CSL Behring, King of Prussia, PA) was purchased from a local pharmacy and was used as a positive control.

Virus. Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of 4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus stock was amplified twice in RD cells to create a working stock. The virus used for infection was designated EV-D68 MP30 PP.

Experiment design. A total of 118 mice were randomized into 4 groups of 12 mice, 8 groups of 8, and an additional group of 6 mice used for normal controls as shown in Table 1. Mice were treated via intraperitoneal (IP) administration of EV68-228 mAb, HIVIg, or placebo mAb at 24 hours pre-infection or, 24- or 48-hours post-infection. Mice were infected via intranasal (IN) instillation of $1 \times 10^{4.5}$ CCID₅₀ of EV-D68 MP30 PP in a 90 μ l volume of MEM. Mice were weighed prior to treatment and daily thereafter. Four mice from each treatment group were euthanized on days 1, 3 and 5 post-infection for evaluation of lung virus titers, blood virus titers, and lung cytokine concentrations. For the mice treated 24- and 48-hours post-infection, samples were only collected on days 3 and 5 post-infection.

Lung Cytokine/Chemokine Evaluations. Each sample of lung homogenate was tested for cytokines and chemokines using a chemiluminescent ELISA-based assay according to the manufacturer's instructions (Quansys Biosciences Q-Plex™ Array, Logan, UT). The Quansys multiplex ELISA is a quantitative test in which 16 distinct capture antibodies have been applied to each well of a 96-well plate in a defined array. Each sample supernatant was tested at for the following: IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, MCP-1, IFN- γ , TNF α , MIP-1 α , GM-CSF, and RANTES. Definition of abbreviations are: IL - interleukin; MCP - monocyte chemoattractant protein; IFN - interferon; TNF - tumor necrosis factor, MIP - macrophage inflammatory protein; GM-CSF - granulocyte/ macrophage colony stimulating factor; and RANTES - regulated upon activation, normal T cell expressed and secreted.

Statistical analysis. All figures and statistical analyses were completed using Prism 8.4.2. (GraphPad Software Inc.). For each day post-infection, lung and blood virus titers from treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). For each cytokine/chemokine, the concentrations from treated mice were compared to placebo-treated mice using a two-way ANOVA.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University dated March 2, 2019 (expires March 1, 2022). The work performed done in the AAALAC-

accredited Laboratory Animal Research Center of Utah State University. The U.S. Government (National Institutes of Health) approval was renewed March 9, 2018 (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

5

Table L - Expt. NIA-1930/Experimental Design: Efficacy of EV68-228-TP for treatment of an EV-D68 respiratory infection in mice

No. /Cage	Group No.	Infected	Treatment	Dose	Treatment Schedule	Route	Observations	
12	1	Yes	Anti-HIV-TP*	10 mg/kg	Once, 24 hours pre-infection	IP	4 mice/group sacrificed at days 1, 3, and 5 post-infection for: lung virus titers, and lung cytokines.	
12	2	Yes	EV68-228-TP					
12	3	Yes	EV68-228-CHO					
12	4	Yes	hIVIg					
8	5	Yes	Anti-HIV-TP*	10 mg/kg	Once, 24 hours post-infection	IP	4 mice/group sacrificed at days 3 and 5 post-infection for: lung virus titers, and lung cytokines.	
8	6	Yes	EV68-228-TP					
8	7	Yes	EV68-228-CHO					
8	8	Yes	hIVIg					
8	9	Yes	Anti-HIV-TP*	10 mg/kg	Once, 48 hours post-infection	IP		4 mice/group sacrificed at days 3 and 5 post-infection for: lung virus titers, and lung cytokines.
8	10	Yes	EV68-228-TP					
8	11	Yes	EV68-228-CHO					
8	12	Yes	hIVIg					
6	13	No	Normal Controls	-	-	-	2 mice/group sacrificed at days 1, 3, and 5 for normal cytokine controls	

10

Results and Discussion

This study determined the efficacy of a tobacco-produced EV68-228 mAb for treatment of an EV-D68 respiratory infection in four-week-old AG129 mice.

FIGS. 48A-C show lung virus titers for EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. No lung virus titers were detected at days 1, 3, or 5 post-infection in mice treated with doses of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with a dose of 10 mg/kg of hIVIg only significantly reduced lung virus titers on day 5 post-infection.

FIGS. 49A-C show blood virus titers for EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at days 1, 3, or 5 post-infection in mice treated with doses of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with hIVIg at a dose of 10 mg/kg also reduced blood virus titers at days 1, 3, and 5 post-infection.

FIG. 50 shows lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Significant reductions in lung concentration of IL-1 α were observed on days 1 and 3 post-infection in mice treated with EV68-228-TP as well as mice treated with EV68-228-CHO. Both the tobacco-produced and the CHO-produced treatments reduced concentrations of IL-1 β at day 3 post-infection. Treatment with hIVIg did not significantly affect lung cytokine concentrations of IL-1 α or IL-1 β . No significant changes in lung concentrations of IL-2 or IL-3 were observed post-infection with EV-D68.

Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO are shown in FIG. 51. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-5 on day 3 post-infection and reduced concentrations of IL-6 on days 3 and 5 post-infection. Treatment with hIVIg significantly reduced lung concentrations of IL-5 and IL-6 on day 3 post-infection. No significant changes in lung concentrations of IL-4 or IL-10 were observed after infection with EV-D68.

FIG. 52 shows lung concentrations of IL-12p70, IL-17, MCP-1, and IFN γ from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced lung concentrations of MCP-1 days 3 and 5 post-infection. Treatment with hIVIg significantly reduced lung concentrations of MCP-1 on days 3 and 5 post-infection. No significant changes in concentrations of IL-12p70, IL-17, or IFN γ were observed after infection with EV-D68.

FIG. 53 shows lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO reduced lung virus concentrations of MIP-1 α on days 1 and 3 post-infection and reduced concentrations of RANTES on days 3 and 5 post-infection. A 10 mg/kg dose of hIVIg reduced lung concentrations of MIP-1 α on days 1 and 3 post-infection and reduced concentrations of

RANTES on day 3 post-infection. No significant changes in concentrations of TNF α or GM-CSF were observed after infection with EV-D68.

FIGS. 54A-B show lung virus titers for EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. No lung virus titers were detected at days 3 or 5 post-infection in mice treated with doses of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with a dose of 10 mg/kg of hIVIg only significantly reduced lung virus titers on day 3 post-infection.

FIGS. 55A-B show blood virus titers for EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at days 3 or 5 post-infection in mice treated with doses of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with hIVIg at a dose of 10 mg/kg also reduced blood virus titers at days 3 and 5 post-infection.

FIG. 56 shows lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Significant reductions in lung concentrations of IL-1 α and IL-1 β were observed on day 3 post-infection in mice treated with EV68-228-TP as well as mice treated with EV68-228-CHO. Treatment with hIVIg did not significantly affect lung cytokine concentrations of IL-1 α or IL-1 β . Concentrations of IL-3 were significantly reduced in mice treated with EV68-228-TP as well as mice treated with hIVIg on day 3 post-infection. No significant changes in lung concentrations of IL-2 were observed post-infection with EV-D68.

Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO are shown in FIG. 57. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced concentrations of IL-5 on day 3 post-infection and reduced concentrations of IL-6 on days 3 and 5 post-infection. Treatment with hIVIg significantly reduced lung concentrations of IL-5 on day 3 post-infection. No significant changes in concentrations of IL-4 or IL-10 in the lung tissue were observed after infection with EV-D68.

FIG. 58 shows lung concentrations of IL-12p70, IL-17, MCP-1, and IFN γ from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO significantly reduced lung concentrations of MCP-1 days 3 and 5 post-infection. Treatment with hIVIg significantly reduced lung concentrations of MCP-1 on days 3 and 5 post-infection. No significant changes in concentrations of IL-12p70, IL-17, or IFN γ were observed after infection with EV-D68.

FIG. 59 shows lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with 10 mg/kg of EV68-228-TP or EV68-228-CHO reduced lung virus concentrations of MIP-1 α on day 3 post-infection and reduced concentrations of RANTES on days 3 and 5 post-infection. A 10 mg/kg dose of hIVIg did not significantly reduce lung concentrations of MIP-1 α or RANTES post-infection. No significant changes in concentrations of TNF α or GM-CSF were observed after infection with EV-D68.

FIGS. 60A-B show lung virus titers for EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Only treatment with the 10 mg/kg dose of EV68-228-CHO reduced lung virus titers when treatment was given 48 hours after infection. Neither treatment with 10 mg/kg of EV68-228-TP or hIVIg significantly reduced lung virus titers when treatment was administered 48 hours after infection.

FIGS. 61A-B show blood virus titers for EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at days 3 or 5 post-infection in mice treated with doses of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with hIVIg at a dose of 10 mg/kg also reduced blood virus titers at days 3 and 5 post-infection.

FIG. 62 shows lung concentrations of IL-1 α , IL-1 β , IL-2, and IL-3 from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Significant reductions in lung concentrations of IL-1 α and IL-1 β were observed on day 3 post-infection in mice treated with EV68-228-CHO but not in the mice treated with EV68-228-TP. Treatment with hIVIg did not significantly affect lung cytokine concentrations of IL-1 α or IL-1 β . No significant changes in lung concentrations of IL-2 or IL-3 were observed post-infection with EV-D68.

Lung concentrations of IL-4, IL-5, IL-6, and IL-10 from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO are shown in FIG. 63. Treatment with 10 mg/kg of EV68-228-CHO significantly reduced concentrations of IL-6 on day 3 post-infection. Treatment with EV68-228-TP or hIVIg did not significantly reduce lung concentrations of IL-6. No significant changes in concentrations of IL-4, IL-5, or IL-10 in the lung tissue were observed after infection with EV-D68.

FIG. 64 shows lung concentrations of IL-12p70, IL-17, MCP-1, and IFN γ from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. When treatment was administered 48 hours after infection, no significant changes were

observed in lung concentrations of IL-12p70, IL-17, MCP-1, or IFN γ in mice infected with EV-D68.

FIG. 65 shows lung concentrations of TNF α , MIP-1 α , GM-CSF, and RANTES from EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. When treatment was administered 48 hours after infection, no significant changes were observed in lung concentrations of TNF α , MIP-1 α , GM-CSF, or RANTES in mice infected with EV-D68.

Conclusions

This study determined the efficacy of a tobacco-produced EV68-228 mAb against EV-D68 for treatment of a respiratory infection caused by EV-D68 in four-week-old AG129 mice. The CHO-produced EV68-228 Ab was used as a comparator.

When treatment was administered either 24 hours pre-infection or 24 hours post-infection, the tobacco-produced Ab produced similar reductions in lung virus titers, blood virus titers, and lung cytokine concentrations when compared to the CHO-produced Ab.

When administered 48 hours post-infection, slight differences in the efficacy of the Abs were observed. The CHO-produced Ab was able to significantly reduce lung virus titers on days 3 and 5 post-infection while the tobacco-produced Ab was not able to reduce lung virus titers at either day 3 or 5 post-infection. Both the tobacco-produced and CHO-produced Abs were able to reduce blood virus titers on days 3 and 5 post-infection. In addition, the CHO-produced Ab significantly reduced lung concentrations of IL-1 α , IL-1 β , and IL-6 while the tobacco-produced Ab did not produce similar reductions in cytokine concentrations.

These differences in lung virus titers and lung cytokine concentrations were only observed when treatment was administered 48 hours after infection and the variability may be due to the limits of Ab treatment post-infection. However, in the previous study (NIA-1869) evaluating post-treatment of an EV-D68 respiratory infection with the CHO-produced antibody, a dose of 1 mg/kg was able to reduce lung concentrations of IL-1 α , IL-1 β , IL-5, MCP-1 and RANTES. Additional studies would be valuable to determine if there are significant differences between the tobacco-produced and the CHO-produced antibody.

Example 13

The objective of this study was to determine the efficacy of treatment with tobacco-produced EV68-228 antibody for an Enterovirus D68 (EV-D68) neurological infection in 10-

day-old AG129 mice. Antibody produced in Chinese hamster ovary cells was used as a comparator for efficacy.

Materials and Methods

5 **Animals.** Ten-day-old AG129 mice from a specific-pathogen-free colony maintained at the Utah Science Technology and Research (USTAR) building at Utah State University. The mice were bred and maintained on irradiated Teklad Rodent Diet (Harlan Teklad) and autoclaved tap water at the USTAR building of Utah State University.

10 **Antibodies and Compound.** The monoclonal antibody (mAb) EV68-228 produced in tobacco plants (EV68-228-TP) as well as Chinese hamster ovary (CHO) cells (EV68-228-CHO) was provided by the inventors at Vanderbilt University Medical Center. Both the tobacco-produced and CHO antibodies were provided as solutions and were dosed at a concentration of 10 mg/kg. A tobacco-produced anti-HIV (Anti-HIV-TP) antibody was used as a negative control antibody at a dose of 10 mg/kg. Intravenous immunoglobulin (IVIg, Carimune, CSL
15 Behring, King of Prussia, PA) was purchased from a local pharmacy and was used as a positive control.

20 **Virus.** Enterovirus D68 was obtained from BEI Resources, NIAID, NIH: Enterovirus D68, US/MO/14-18949, NR-49130. The virus was serially passaged 30 times in the lungs of 4-week-old AG129 mice and then plaque-purified three times in Rhabdomyosarcoma (RD) cells obtained from the American Type Culture Collection (Manassas, VA). The resulting virus stock was amplified twice in RD cells to create a working stock. The virus used for
infection was designated EV-D68 MP30 PP.

25 **Experiment design.** A total of 125 mice were randomized into 12 groups of 10 mice each as shown in Table 1 with an additional 5 mice used as normal controls for weight gain. Mice were treated via intraperitoneal (IP) administration of EV68-228-TP or EV68-228-CHO, IVIg, or placebo 24 hours before, 24- or 48-hours post-infection. Mice were infected via intraperitoneal (IP) administration of $1 \times 10^{6.5}$ CCID₅₀ of EV-D68 MP30 PP in a 0.1 mL volume of MEM. Mice were weighed prior to treatment and daily thereafter. Blood was collected from groups of 5 mice on days 1, 3, 5, and 7 post-infection for evaluation of blood virus titers.
30 All mice were observed daily for morbidity, mortality, and neurological scores through day 21. Neurological scores (NS) were recorded as follows: NS0 – no observable paralysis, NS1 – abnormal splay of hindlimb but normal or slightly slower gait, NS2 – hindlimb partially collapsed and foot drags during use for forward motion, NS3 – rigid paralysis of hindlimb and

hindlimb is not used for forward motion, NS4 – rigid paralysis in hindlimbs and no forward motion. Any animals observed with a score of NS4 were humanely euthanized.

Statistical analysis. Kaplan-Meier survival curves were generated Prism 8.4.2. (GraphPad Software Inc.). Survival curves were compared using the Log-rank (Mantel-Cox) test followed by a Gehan-Breslow-Wilcoxon test. For each day post-infection, blood virus titers from treated groups were compared to lung and blood titers from placebo-treated mice using a one-way analysis of variance (ANOVA). Mean body weights were compared using a one-way ANOVA. Neurological scores were compared using a Kruskal-Wallis test followed by a Dunn's multiple comparisons test.

Ethics regulation of laboratory animals. This study was conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University dated March 2, 2019 (expires March 1, 2022). The work was done in the AAALAC-accredited Laboratory Animal Research Center of Utah State University. The U. S. Government (National Institutes of Health) approval was renewed March 9, 2018 (PHS Assurance No. D16-00468[A3801-01]) in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revision; 2011).

Table M - Expt. NIA-1931/Experimental Design: Efficacy of EV68-228-TP for treatment of an EV-D68 neurological infection in mice

No. /Cage	Group No.	Infected	Compound	Dosage	Route	Treatment Schedule	Observations
10	1	Yes	Anti-HIV-TP (Placebo)	10 mg/kg	IP	Once, 24 hours pre-infection	Mice observed daily for survival, body weights, and neurological scores.
10	2	Yes	EV68-228-TP				
10	3	Yes	EV68-228-CHO				
10	4	Yes	hIVlg				
10	5	Yes	Anti-HIV-TP (Placebo)	10 mg/kg	IP	Once, 24 hours post-infection	Blood collected from 5 mice per group on days 1, 3, 5, and 7 post-infection for blood virus titers.
10	6	Yes	EV68-228-TP				
10	7	Yes	EV68-228-CHO				
10	8	Yes	hIVlg				

10	9	Yes	Anti-HIV-TP (Placebo)	10 mg/kg	IP	Once, 48 hours post- infection	
10	10	Yes	EV68-228-TP				
10	11	Yes	EV68-228- CHO				
10	12	Yes	hIVIg				
5	13	No	Normal Controls	-	-	-	Observed for normal weight gain

Results and Discussion

This study determined the efficacy of a tobacco-produced EV68-228 mAb for treatment of an EV-D68 neurological infection in 10-day-old AG129 mice.

5 FIG. 66 shows Kaplan-Meier survival curves for 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. Both the EV68-228-TP or EV68-228-CHO treatment 24 hours prior to infection provided complete protection from mortality. A single 10 mg/kg dose of hIVIg protected eight of ten mice (80%) from mortality. Nine of the ten placebo-treated mice succumbed to the infection.

10 FIG. 67 shows percentages of initial body weights for 10-day-old AG129 mice infected with EV-D68 and treated 24 hours prior to infection with EV68-228-TP or EV68-228-CHO. No significant protection from weight loss was observed in mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO since the one surviving mouse in the placebo-treated group gained weight after recovering from infection.

15 FIG. 68A-B show blood virus titers on days 1 and 3 post-infection for EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at days 1 or 3 post-infection in mice treated with a dose of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with IVIg at a dose of 10 mg/kg also reduced blood virus titers at day 3 post-infection.

20 FIGS. 69A-B show blood virus titers on days 5 and 7 post-infection for EV-D68-infected AG129 mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at day 5 post-infection in mice treated with a dose of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with IVIg at a dose of 10 mg/kg did not significantly reduce blood virus titers at days 5 post-infection. No virus was detected in
25 the blood of any of the treatment groups at day 7 post-infection.

Neurological scores on days 2-10 post-infection in 10-day-old AG129 mice infected with EV-D68 and treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO are shown in FIGS. 70A-B. No neurological scores were observed in mice treated with a dose of 10 mg/kg of EV68-228-TP or EV68-228-CHO at any day post-infection. In addition, no neurological scores were observed in mice treated with IVIg at a dose of 10 mg/kg at any time post-infection. No significant reductions in neurological scores were observed after day 7 post-infection because the only surviving placebo-treated mouse did not display any signs of paralysis.

Kaplan-Meier survival curves of 10-day-old AG129 mice infected with EV-D68 and treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO are shown in FIG. 71. Treatment with a 10 mg/kg dose of EV68-228-TP or EV68-228-CHO 24 hours after infection completely protected mice from mortality. A single dose of hIVIg given 24 hours after infection also protected all ten mice (100%) from mortality. Eight of the ten placebo-treated mice succumbed to the infection.

FIG. 72 shows percentages of initial body weights for 10-day-old AG129 mice infected with EV-D68 and treated 24 hours after infection with EV68-228-TP or EV68-228-CHO. No significant protection from weight loss was observed in mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO since two surviving mice in the placebo-treated group gained weight infection.

Blood virus titers on days 1 and 3 post-infection from 10-day-old mice infected with EV-D68 and treated 24 hours after infection with EV68-228-TP or EV68-228-CHO are shown in FIGS. 73A-B. No significant reduction in blood virus titers were observed on day 1 post-infection since the treatment was administered 24 hours after infection. On day 3 post-infection, treatment with EV68-228-TP and EV68-228-CHO significantly reduced blood virus titers. Treatment with hIVIg 24 hours after infection significantly reduced blood virus titers on day 3 post-infection.

Blood virus titers on days 5 and 7 post-infection from 10-day-old mice infected with EV-D68 and treated 24 hours after infection with EV68-228-TP or EV68-228-CHO are shown in FIGS. 74A-B. No virus was detected in the blood of mice treated 24 hours after infection with EV68-228-TP or EV68-228-CHO, however, only two placebo-treated mice were still alive for collection of blood and virus was not detected in the blood of either animal. Due to the lack of virus titers in placebo-treated animals, no statistical significance could be determined for any of the treatment groups. No virus was detected in the blood of any of the groups at day 7 post-infection.

FIGS. 75A-B show neurological scores on days 2-9 post-infection in 10-day-old AG129 mice treated 24 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with EV68-228-TP or EV68-228-CHO 24 hours after infection significantly reduced signs of paralysis as indicated by reduced neurological scores at days 2-9 post-infection. A single 10 mg/kg dose of hIVIg 24 hours after infection reduced neurological scores at days 2 and 3 post-infection but did not improve the outcome for the remainder of the study.

Neurological scores on days 10-17 post-infection in 10-day-old AG129 mice treated 24 hours after infection with EV68-228-TP or EV68-228-CHO are shown in FIGS. 76A-B. Both EV68-228-TP and EV68-228-CHO significantly reduced neurological scores on days 10-15 post-infection. The administration of hIVIg did not significantly reduce neurological scores at days 10-17 post-infection.

FIG. 77 shows Kaplan-Meier survival curves for 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. Treatment with EV68-228-TP at a dose of 10 mg/kg protected nine of ten mice (90%) from mortality. Eight of ten mice (80%) survived the infection with treated 48 hours after infection with EV68-228-CHO. A single 10 mg/kg dose of hIVIg protected six of ten mice (60%) from mortality. One of the ten placebo-treated mice survived the infection.

FIG. 78 shows percentages of initial body weights for 10-day-old AG129 mice infected with EV-D68 and treated 48 hours after infection with EV68-228-TP or EV68-228-CHO. No significant protection from weight loss was observed in mice treated 24 hours pre-infection with EV68-228-TP or EV68-228-CHO as the one surviving mouse in the placebo-treated group gained weight following infection.

FIGS. 79A-B show blood virus titers on days 1 and 3 post-infection for EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. On day 1 post-infection, none of the treatment groups were significantly different since no treatments had occurred at that point. At day 3 post-infection, blood virus titers were lower in mice treated with 10 mg/kg of EV68-228-TP or EV68-228-CHO although the differences were not statistically significant. This is likely due to treatment being administered just 24 hours prior to the blood collection on day 3. Treatment with IVIg at a dose of 10 mg/kg did not significantly reduce blood virus titers at day 1 or day 3 post-infection.

FIGS. 80A-B show blood virus titers on days 5 and 7 post-infection for EV-D68-infected AG129 mice treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO. No blood virus titers were detected at day 5 post-infection in mice treated with a dose of 10 mg/kg of EV68-228-TP or EV68-228-CHO. Treatment with IVIg at a dose of 10 mg/kg did

not significantly reduce blood virus titers at days 5 post-infection. No virus was detected in the blood of any of the treatment groups at day 7 post-infection.

Neurological scores on days 3-10 post-infection in 10-day-old AG129 mice infected with EV-D68 and treated 48 hours post-infection with EV68-228-TP or EV68-228-CHO are shown in FIGS. 81A-B. Treatment with 10 mg/kg of EV68-228-TP significantly reduced neurological scores at days 3-5 post-infection. Treatment with 10 mg/kg of EV68-228-CHO significantly reduced neurological scores on days 3-6 post-infection. Neurological scores were significantly reduced in mice treated with 10 mg/kg of hVIG on days 3-5 post-infection. No significant reduction in neurological scores were observed in any of the treatment groups on days 7-10 post-infection.

Conclusions

This study determined the efficacy of a tobacco-produced EV68-228 mAb against EV-D68 neurological infection 10-day-old AG129 mice.

The tobacco-produced mAb provided similar protection from mortality compared to the CHO-produced mAb when treatment was administered 24 hours prior to infection as well as 24 or 48 hours after infection.

In addition, similar reductions in blood virus titers were observed in mice treated with both EV68-228-TP and EV68-228-CHO mAbs in 10-day-old AG129 mice infected with EV-D68.

Similar protection from paralysis as indicated by reductions in neurological scores was observed in mice treated with EV68-228-TP as well as EV68-228-CHO.

In summary, the tobacco-produced EV68-228 provided similar protection from mortality, reductions in blood virus titers, and protection from paralysis when compared to the CHO-produced EV68-228.

TABLE 1 – NUCLEOTIDE SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	SEQ ID NO:	Chain	Variable Sequence Region
EV-D68-37	1	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCATGTGCAGCCTCTGGAT TCATCTTCAGTCGCTACGCTCTGCACTGGGTCCGCCAGGC TCCAGGCAAGGGGCTGGACTGGGTGGCAGTTATATCATAT GATGCAAGAAATTCATATTACACAGACTCCGTGAAGGGCC GATTCACCATCTCCAGAGACAATTCCAAAAACACGCTGTTT CTGCAGATGAACAGTCTGAGAGCTGACGACACGGCTGTCT ATTACTGTGCGAGACCGACTTTGCCCTACAGCAACAACCTG GTACGCGCCTGAATACTGGGGCCAGGGAACCCTGGTCAC CGTCTCCTCA
	2	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTCAGTGTCCC CAGGACAAACGGCCAGGATCACCTGCTCTGGAGATGCATT ACCAAAAAAATATGCTTCTTGGTACCAGCAGAAGTCAGGCC AGGCCCTGTGCTGGTCATCTATGAAGACACCAAACGACC CTCCGGGATCCCTGAGAGATTCTCTGGCTCCAGCTCAGGG ACAATGGCCACCTTGACTATCAGTGGGGCCAGGTGGAAG ATGAAAGTGACTACTACTGTTCCCTCGACAGACAGCAGTGGT AATCCTGTGCTATTCGGCGGAGGGACCAAGTTGACCGTCC TA
EV-D68-40	3	heavy	GAGGTGCAGCTGGTAGAGTCTGGGGGAGACTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCTCCTTTAGCAGCTATGCCATGGCCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGCAGTGGGTCTCATCTATTAGTGGT AACGGTAATGGGAGATCCTATGCAGATTCTCTGAAGGGCC GGTTCACCACCTCCAAAGACCTTTCCAAGTATACCCTGTAT CTGCAAATGAACAATCTGAGACCCGAGGACACGGCCATAT ATTACTGTGCGAAAGTTGTCCGTATAGCAGCTGTTTTGTAT TACTTTGACTATTGGGGCCCGGGAACCCAGGTTACCGTCT CCTCA
	4	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTAGCACCTACTTAGCCTGGTACCAACAAAAGCCTG GCCAGGCTCCAGGCTCCTCATCTATGAAGCATCCACCAG GGCCACTGGCATCCCAGCCAGGTTCAAGTGGCAGTGGGTCT GGGACAGACTTCACTCTCATCATCAGCAGCCTAGAGCCTG AAGATTTTGCAGTTTATCACTGTCAGCAGCGCAGCTCCTGG CCGATCACCTTCGGCCAAGGGACACGACTGGAGATTGAA
EV-D68-41	5	heavy	GACGTGCAGCTGGTGGAGTCTGGGGGGGGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCTGCCTCTGGAT TCACTTTTAGCAACTATGCCATGACCTGGGTCCGCCAGGCT CTAGGGAAGGGGCTGGAGTGGGTCTCTTCTATTAGTGGTA GTGGTGGCCTCACATATTTGCGACACTCCGTGAAGGGCCG GCTCACCATCTCCAGAGACAACCTCCAAGAATACCCTCTATC TGCAAATGAGCAGCCTGAGAGCCGAAGACACGGCCGTATA TTACTGTGCGAGAGTGAAAAGTACAACCTGGAACGACGGCG TTAGTTTTTGTATCTGGGGCCAAGGGACAATGGTCACCGT CTCTTCG
	6	light	CAGACTGTGGTGACCCAGGAGCCATCGTTCTCAGTGTCCC CTGGAGGGACAGTCACACTCACTTGTGGCTTGAGTTCTGG

			CTCAGTCTCTAGTAGTTACTACCCAGTTGGTACCAGCAGACCCCAGGCCAGGCTCCACGCACGCTCATCTACAGTATAAACAGACGTTCTTCTGGGGTCCCTGATCGCTTCTCTGGCTTCA TCCTTGGGAACAAAGCTGCCCTCACCATCAGGGGGGCCCA GGCAGATGATGAATCTGATTATTACTGTGGGCTGTATATGG GTAGTGGCATTGATCTTCGGCGGAGGGACCAAGCTGAC CGTCCTA
EV-D68-43	7	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCATTAAGTATGGCATGCACTGGGTCCGCCAGGCT CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCAAAATG ATGGAAGTTATAACTACGATGCAGACTCCGTGAAGGGCCG ATTACCATCTCCAGAGACAATTCCAAGAACAAGGTTTATC TACAAATGAACAGCCTGAGACCTGAGGACACGGCTGTGTA TTTCTGTGCGAAAGACAAACACGGTGACTTCGACTACTACG GTGTGGACGTCTGGGGGCCAAGGGACCACGGTCACCGTCT CCCC
	8	light	GACGTCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATC TATAGGAGACAGAGTCAACATCACTTGTGCGGGCGGGTCAG GGAATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAACCAG GGAAAGCCCCCTAAGCTCCTGATCTATGCTGCATCCAATTTG CAAAGTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTG GGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGA AGATTTTGGAACTTACTATTGTCAACAGGCTGACAGTTTCC CTCGGACGTTTCGGGCCAAGGGACCAAGGTGGAAATCAAA
EV-D68-46	9	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAGTTATGGCATACTGGGTCCGCCAGGC TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCCTAT GATGGAAGTGATAACACCTATGCACCTTTTGTGAACGGCC GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT CTGCAAATGAACAGCCTGAGAGCTGACGACACGGCTGTGT ATTACTGTGCGAGGCGTCGGCCTGGGAGCTTCCCAGGACT TTGCGACTACTGGGGCCAGGGAGCCCTGGTCACCGTCTCC TCA
	10	light	GACATCCAGATGACCCAGTCGCCTTCCACTATGTCTGCTTC TGTAGGAGACAGAGTCAACATCACTTGCCGGGCCAGTCAG AGTATTAGTAAGTGGTTGGCCTGGTATCAGCAGAAGCCAG GGAAAGCCCCCTAACTCCTGATCTATAAGGCGTCTACCTTA CAACTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTG GGACAGAATTCACCTCTCACCATCAACAGCCTGCAGCCTGAT GATTTTGCAACTTATTACTGCCAACAGCATAATAGTTATTCTG TACACTTTTGGCCAGGGGACCAAGGTGGAGATCAAG
EV-D68-48	11	heavy	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAG GCAGCTTCAGCAGACTTACTATCATCTGGGTGCGGCAGGC CCCTGGACAAGGGCTTGAGTGGATGGGAGGGCACATCCC TATCTTTGGAACAACAACTACGCACTGAAGTTCCAGGGCA GAGTCACGATTACCGCGGACAAAACACGAGCACAGCCTA CATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCATA TATTACTGTGCGAGAATGTATAGTGGCCATGACGGCGTTGA TGTCTGGGGCCAAGGGACACTGGTCACCGTCTCTTCA
	12	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA

			GAGTGTTAGGAGCTACTTAGCCTGGTACCAACACAAACCT GGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACA GGGCCAAGGGCATCCCAGCCAGGTTCACTGGCAGTGGGT CTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCC TGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCACCT GGCCTCCGGGAATGTTTCGGCCAAGGGACCAGGGTGGAAA TCAAA
EV- D68- 71	13	heavy	CAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CATTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCATCAGCAGTGGTTTTTACTACTGGGGCTGGATCCG CCAGCCCCCAGGGAAGGGGCTAGAGTGGATTGGGACTAT CTATGATAGTGGGAGAACCTATGACAACCCGTCCCTCAAG AGTCGAGTCACCATATCCGCAGACACGTCCAAGAAGCAGT TCTCACTGACACTGAGGTCTGCGACCGCCGCGGACACGG CTGTGTATTTCTGTGCGAGACACCTTACCCACCTCTACGGT GACTACGTCACCCCTGATGCTTTAGATATCTGGGGCCAAG GGACAATGGTCACCGTCTCTTCA
	14	light	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCCTTGTC TCCAGGGGAAAGAGCCACCCTCTCTTGACAGGGCCAGTCAG AGCGTTAGTAGCAGCTTCTTAGCCTGGTACCAGCAGAAAC CTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCGTCCAG CAGGGCCACCGGCATCCCAGACAGGTTTCAAGAGGACAGTGG GTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG CCTGAAGATTTTGCAGTATATTACTGTCAGCAGTATAGTAA CTCACGTCTGACGTTTCGGCCAAGGGACCAAGGTGGAAATC AAA
EV- D68- 72	15	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCACTACCTATAGCATGAACTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGATTTTATACATTAGTAGTG GTAGTAGTAACATATACTACGCAGACTCTGTGAAGGGCCG ATTACCATCTCCAGAGACAATGCCAAGAACTCACTGAATC TGCAAATGAGCAGCCTGAGAGACGAGGACACGGCTGTGTA TTACTGTGCGAGAGCCCACGGACGTATAGTGAATTCTGGA GTGGTTATTAGTAGGTTTCGACCCCTGGGGCCAGGGAATCC TGGTCACCGTCTCTCTCA
	16	light	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTC CTGGACAGTCGATCACCATCTCCTGCACTGGAACCAGCAG TGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACTGC ACCCAGGCAAAGCCCCCAAACCTCATGATTTTTGAGGTCACT TATCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAA GTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAG GCTGAGGACGAGGCTGATTATTTCTGCAGCTCCTATACAAC CAGCAACACTCTCGTGGTGTTCGGCGGAGGGACCAAGCTG ACCGTCCTA
EV- D68- 74	17	heavy	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGGCCTCAGTGAAGGTCTCCTGTAGGACTTCTGGAT ACACCTTACGGCCTACTATATGCACTGGGTGCGACAGGC CCCCGACAAGGGCCTGAGTGGATGGGAAGGATCAACCC GAGCAGTGGTGGCGCACAGTATGCACAGAAGTTTCAGGGC AGGGTCACCATGACCAGGGACACGTCCATCAGCACAACT ACATGACCCTGAGCGGGCTGACATCTGACGACACGGCCGT GTTTTACTGTGCGAGAATGGGTTGTCGTAGTGACCGGTGC

			TATTCGACCAACTACAACCTTTGACCAGTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCA
	18	light	TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCC CAGGACAGACGGCCAGGATTCCCTGTGGGGGAAACAACAT TGGAATAAAAAGTGTTCACTGGTACCAGCAGAAGCCAGGC CAGGCCCCCTGTGCTGGTTCGTCTCTAATGACAGCGACCGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGG GAACACGGGCCACCCTGACCATCAGCAGGGTCTGAAGCCGG GGATGAGGCCGACTATTATTGTCAGGTGTGGGATAGTGGT ATTGATGTCGTTTTTCGGCGGAGGGACCAAGCTGACCGTCC TA
EV- D68- 75	19	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACTATCAGTCCCTATGGCATGAACTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATTATTAGTAGT AGTAGTCGTTACACATATTACGCAGACTCAGTGAAGGGCC GTTTCACCATCTCCAGAGACAACGCCAAGAATTCAGTGTCT CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGT ATTACTGTGCGAGAGAGAGGGGGCCATAGCACCTCGTCCTC ATACTTTGACTCCTGGGGCCAGGGAACCCTGGTCACCGTC TCCTCA
	20	light	TCCTATGTGCTGACTCAGCCACCCTCAGTGTCTATTGGCCC CAGGAAAGACGGCCAGGATTACCTGTGGGGGAAACAACAT TGGAATAAAAAGTGAGCTGGTACCAGCAGAAGCCAGGC CAGGCCCCCTGTGCTGGTCATGTATTATGATAGTGACCGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGG GAACACGGGCCACCCTGACCATCAACAGGGTCTGAGGCCGG GGATGAGGCCGACTATTACTGTCTGGGTGTGGGATAGTGAT ACTGATCATCGAGTGTTCTGGCGGGGGGACCAAGCTGACC GTCCTA
EV- D68- 76	21	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAG CCTGGGGGGTCCCTTAGACTCTCCTGTGCAGCCTCTGGAT TCACTTTTCAGTAACGCCTGGATTAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTTGGCCGTATTCAAACC AAAAGTATGGAGGGACAACAGACTACGCTGCACCCGTGA AAGGCAGATTCACCATCTCAAGAGATGATTCAAAAAACACG TTGTATCTGCAAATGAACAGCCTGAAAACCGAGGACACAG CCTTGTATTATTGTAGCACAGGACCGTATTACTATGATACTA GTGGTTACCCCCAACCCCTTTGACTACTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCA
	22	light	TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCC CAGGACAGACAGCCAGCATCACCTGCTCTGGAGATAAATT GGGAGATAAATATGCTTGCTGGTATCAGCAGAAGCCAGGC CAGTCCCCTGTGCTGGTCATCTATCAAGATAACCAAGCGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGG GAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTATG GATGAGGCTGACTATTACTGTGAGGCGTGGGACAGCAGCA CTGTGGTATTTCGGCGGAGGGACCAAGCTGACCGTCCTA
EV- D68- 80	23	heavy	GAGGTACAGATGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCGGGGGGGTCCCTCAGACTCTCCTGTTCACTCTCTGGAT TCGACTTCAGTAGATAACCATGAACTGGTTCCGCCAGGCT CCAGGGGAGGGGCTGAAGTGGGTCTCGTCCATTAGTAGTA CTAGTCTTTACACATTCTATGCGGACTCAGTGAAGGGCCGA TTCTCCATCTCCAGAGACAACGCCAGGGTTCCCTGTCTCT

			ACAAATGAGCAGCCTGAGACCCGAAGATACGGCTGTCTAT TATTGTGCGAGAGTCGTTGGTCCCGCCGAGTTAGATTACT GGGGCCAGGGAGTGCTGGTCACCGTCTCCTCA
	24	light	GTCACCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCTTC TGTCGGGGACAGAGTCACCATCACTTGCCGGGCAAGTCAG GACATTGGAGTTGACTTAGGTTGGTTTCAGCAGAGACCTG GGAAAGCCCCTAAACTCCTGATCTATGGTGCCTCCAGGTT GCAGAGTGGGGTCCCATCACGCTTCAGCGGGCGTGATC TGGCACATTTTTCACTCTCACCATCAGCAGCCTGCAGCCTG AAGATTTTACAACCTTACTTCTGTCTTCAAGATTATAATTACC CTTGGACGCTCGGGCCAGGGGACCACGGTGGGAGTCAAA
EV- D68- 84	25	heavy	CAGGTGCACCTGGTGCAGTCTGGGTCTGCGGTGAGGAAG CCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGAT ACACCTTCACCGACTACTATATTCACTGGGTGCGACAGGC CCCTGGACAAGGCCTTGAGTGGATGGGATGGATCAACCCT AAAAGTGGTGGCTCAAATTATACACAGAGGTTTCAGGCCAG GGTCACCATGACCTGGGACACGTCCATCAGTACAGCCTAC ATGGAGTTGAGCAGGCTGAGATCTGACGACACGGCCGTGT ATTATTGTGCGAGGGCGGGCAGAAATGGCTACGACTACTG GGGCCAGGGAACCCTGGTCACCGTCTCCTCA
	26	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTGAGTGTCCC CAGGACAGACGGCCAGGATCACCTGCTCTGCAGATGCATT GCCAAAGCAATATGCTTATTGGTACCAACAGAAGCCAGGC CAGGCCCTGTGTTGATGATATATCAAGACACTGAGAGGC CCTCAGGGATCCCTGAGCGATTCTCTGGGTCCAGCTCAGG GACAACAGTCACGTTGACCATCAGTGGAGTCCAGGCAGAA GACGAGGCTGACTATTACTGTCAATCAGGAGACAGCAGTG GTACTTATCTAGTTTTTCGGCGGAGGGACCAAGCTGACCGT CCTA
EV- D68- 85	27	heavy	GAGGTACAGCTCTTGGAGTCTGGGGGAGGCTTGGTACAGC CTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATT CAAATTTAGAACTATGCCATGACCTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTCTCAACTATTACTAGTG GTGGTAGTACAGAGTACGCAGACGCCGTGAAGGGCCGGT TCATCATCTCCAGAGACAATTCCAAGAACACGTTATATTTG CAAATGAACAGCCTGAGAGCCGACGACACGGCCGTATATT ACTGTACAGTGCCGTGGGGTAACTACAATGACTACGTGTCT GACTACTGGGGCCAGGGAACCCTGGTCCCCGTCTCCTCA
	28	light	GAAGTTGTATTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGCAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAG CGTGTTGGCAACTCCTTAGCCTGGTACCAACAAAAACCTG GCCAGGCTCCCAGCCTCCTCATCTATGATGCTTCCAAGAG GGCCACTGGCATCCCAGCCAGGTTCAAGTGGCAGTGGGTCT GGGACAGACTTCACTCTCACCATCATCAGCCTAGAGTCTGA AGATTTTGCAGTTTATTACTGTCACCAACATAGCACCTGGC CTCGGGGGACCTTCGGCCAAGGGACACGACTGGAGATTAA A
EV- D68- 88	29	heavy	CAGGTGCACCTGGTTGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCATCTTCAGTAGATATCCTATGCACTGGGTCCGCCAGGCT CCAGGCAAGGGTCTGGAGTGGGTGGCACTTATATCATATG ATGGAACAATAAATACTACGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTC TCCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTCTA

			TTACTGTGCGAGACATTTTCTCCCATATAGCAGTAGTTGGT ACCAGGGCTTTAACTACTGGGGCCAGGGAATCCTGGTCAC CGTCTCCTCA
	30	light	AATTTTATGCTGACTCAGCCCCACTCTGTGTCTGGAGTCTCC GGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTGG CAGCATTGCCACCACTATGTGCAGTGGTACCAGCAGCGC CCGGGCAGTTCCCCCACCCTATAATCTTTGAAGATAGTCA AAGACCGTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATC GACAGCTCCTCCAATTCTGCCTCCCTCACCATCTCTGGACT GAGGACTGACGACGAGGCTGACTACTACTGTCAGTCTTAT GATAACAGCAATCGGGCTGTTGTATTCTGGCGGAGGGACCA AGCTGACCGTCCTA
EV- D68- 89	31	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGAAGCGTCTGGAT TCCTCTTCAGTCGCTATGGCATGCACTGGGTCCGCCAGGC TCCAGGCAAGGGGCTGGACTGGGTGGCAGTTATATCGTAT GATGGAAATAAGAAATATTATGCAGACTCTGTGAAGGGCCG ATTCACCATCTCCAGAGACAATTCAAAGAACACGTTGTATC TGCAAGTGAACAGCCTGAGAGTCGAGGACACGGCTGTTTA TTACTGTGCGAGAGGTGTCCCGTACGGTGACACCCCTTACA GGGCTTGTCTACTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA
	32	light	AATTTTATGCTGACTCAGCCGCACTCTGTGTCTGGAGTCTCC GGGGAAGACGGTAACCATCTCCTGCACCCGCAGCAGTGG CACCATTGCCAGCACTATGTGCAGTGGTACCAGCAGCGC CCGGGCAGTGCCCCCACCCTGTAATCTATGAGGATAACC AAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCAT CGACAGCTCCTCCAATCTGCCTCCCTCACCATCTCTGGA CTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTT ATGATAACAGCGATCGGGTGTTCGGCGGAGGGACCAAGCT GACCGTCCTT
EV- D68- 95	33	heavy	CAGGTCACCTTGAAGGAGTCTGGTCCTGTGCTGGTGAAC CCACAGAGACCCTCACGCTGACCTGCACCGTCTCTGGGTT CTCACTCAGGAACGCTAGAATGGGAGTGAGCTGGATCCGT CAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGACACATTT TTTCGAATGACGAAAAATCCTACAACACATCTCTGAAGAGC AGACTCTCCATCTCCAAGGACACCTCCAAAAGCCAGGTGG TCCTTACCATGACCAGCATGGACCCTTTGGACACAGCCAC ATATTTCTGTGCACGGCTACTGGTGGCTGGTACTTTCTCTCC CCTCTCACTACTTTGACTACTGGGGCCAAGGAATCCTGGTC ACCGTCTCCTCA
	34	light	TCCTATGTGCTGACTCAGCCACCCTCAGTGTCACTGACCC CAGGAAAGACGGCCAGGATTACCTGTGGGGGAAACAACAT TGGACTTAAAAGTGTCTTCTGGTACCAGGAGAGGCCAGAC CAGGCCCTGTGGTGGTCATCTATTATGATAGCGCCCGGC CCTCAGGGATCCCTGAGCGAATCTCTGGCTCCAAGTCTGG GAACACGGCCACCCTGACCATCACCAGGGTCTGAAGCCGG GGATGAGGCCGACTATTTTGTGAGGTGTGGGATAGTAGT CGTAATCATCCGGTCTTCGGCGGAGGGACCAAACCTGACCG TCCTC
EV- D68- 97	35	heavy	GAGCTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTACCTATAGCATGAATTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTA

			GTAGTAGTACCATACAGTACGCAGACTCTGTGAAGGGCCG ATTACCATCTCCAGAGACAATGCCAAGAACTCACTGTATC TGCAAATGAATAGCCTGAGAGCCGAAGACACGGCCGTGTA TTATTGTACGAGACAGGTCGGGGCGGATTTTCAGTGGCCGC GGCTTTGACTACTGGGGCCAGGGAACCCTGCTCACCGTCT CCTCA
	36	light	TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCC CAGGACAGACAGCCACCATCACCTGCTTTGGAGATAAATT GGGGGATAAATATGCTTGCTGGTATCAGCAGAAGCCAGGC CAGTCCCCTGTGTTGGTCATCTACCAAGATAGCAAGCGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGG GAACACAGCCACTCTGACCATCAGCGGGACCCAGGCAATG GATGAGGCTGACTATTACT-GT- CAGGCGTGGGACAGCAGCACTGCAGTGTTCGGCGGAGGG ACCAAGCTGACCGTCCTC
EV- D68- 98	37	heavy	GAGGTACAACTAGTGGAGTCAGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCAAGTTTTCCGTCTATGCCTTGAGTTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGATTTTCATATATTAGTAGTA GTGGTTCTACCATATATTATTCAGACTCTGTGAAGGGCCGA TTCACCATCTCCAGAGACAATGTCGGGAACTCACTGTTTGT GCAAATGAACAGCCTGAGAGCCGAGGACACGGGTATTTAT TACTGTGCGACAGCCCGCCACATCACCATGATGGTTTTGA TATTTGGGGCCAAGGGACAATGGTCATCGTCTCTTCA
	38	light	GACATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCAG GGCATTAGTAGGTTTTAGCCTGGTATCAGCAGAAACCAGG GAAAGCCCCTAAGCTCCTGATCTATTCTGCGTCCACTTTAC AAAGGGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATTTGG GACAGATTTCACTCTCACAATCAGTAGCCTTCAGCCTGAAG ATTTTGCAACTTATTACTGTCAACAACCTTAATAGTCACCCCC GAATGTTCACTTTCCGGCCCTGGGACCACAGTGGATATCAA G
EV- D68- 105	39	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCGCTGTGTCTGGTT ACTTAATCAGCAATGGTTACTACTGGGGCTGGATCCGGCA GCCCCCGGGAAGGGGCTGGAGTGGATTGGGAGTATCTA TCATACTAGAAGCACCTACTACAACCCGTCCCTCAAGAGTC GAGTCAGCATCTCAGTAGACACGTCCAAGAACCGGTTCTC CCTGAGGCTGAGGTCTGTGACCGCCGCAGACACGGCCTTT TATTACTGTGCGAGAGGCCAGGCCACTGTTATGGTGATG ACGACTGCTACGCGTACTACTTTGACCAGTGGGGCCAGGG AACCCCGGTACCGTCTCCCCA
	40	light	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTTCATC TGTAGGAGACAGAGTCACCATCACTTGTCGGGCGAGTCAG GGTATTAGCAACTGGTTAGCCTGGTATCAGCAGAAACCAG GGAAAGCTCCTAACTCCTGATCTATGATGCCTCCAGTTTG CAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTG GGACAGATTTCACTCTCACCATCAACAGCCTGCAGCCTGAA GATTTTGCAACTTACTATTGTCAACAGGCTAACAGTTTCCCT TTCATTTCCGGCCCTGGGACCAAAGTGGATATCAAA
EV- D68- 110	41	heavy	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAG GCACCTTCAGCAGGTTTGCTATCAGCTGGGTGCGACAGGC

			CCCTGGACAAGGGCTTCAGTGGATGGGAGGGATCCTCCCT ATCTTTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCA GAGTCACGATTACCGCGGACACATCCACGAGCACAGCCTA CATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGT GTATTACTGTGCGAGATCCCTCCCCTATTGTACTAATGATG TATGCTCAAACCAGAACACATTTGACTACTGGGGCCAGGG AACCTGGTCACCGTCTCCTCA
	42	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTCAGTGTCCC CAGGACAGGCGGCCAGGATCACCTGCTCTGGAGATGCATT GCCTAAGCAATATGCTTATTGGTACCAGCAGAAGCCAGGC CAGGCCCCCTGTGTTGGTGATATATGAAGACAATAAGAGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAGCTCAGG GACAACAGTCACGTTGACCATCAGTGGAGTCCAGGCAGAA GACGAGGCTGACTATTACTGTCAATCAGCAGACAGCAGTG GTACTTATGTGGTATTCTGGCGGAGGGACCAAGCTGACCGT CCTA
EV- D68- 111	43	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CCTTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCATCAGCAGTGGTGATTACTACTGGAGTTGGATCCG CCAGCCCCCAGGGAAGGGCCTGGAGTGGATTGGGTACAT CTATTACAGTGGGAGCACCTACTACAACCCGTCCCTCAAGA GTCGAGTTACCATATCAGTAGACACGTCCAAGAACCAGTTC TCCCTGAAGCTGAGCTCTGTGACTGCCGCAGACACGGCCG TGTATTACTGTGCCAGCCGCTACGGTGACCCGATAGGGGA CAACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCAC CGTCTCCTCA
	44	light	TCCTATGTGCTGACTCAGCCACCCTCAGTGTGAGTGACCC CAGGAAAGACGGCCAGGATTACCTGTGGGGGAAACAACAT TGGACTTAAAAGTGTCTTCTGGTACCAGGAGAGGCCAGAC CAGGCCCCCTGTGGTGGTCATCTATTATGATAGCGCCCGG CCTCAGGGATCCCTGAGCGAATCTCTGGCTCCAAGTCTGG GAACACGGCCACCCTGACCATCACCAAGGTGCAAGCCGG GGATGAGGCCGACTATTTTGTGAGGTGTGGGATAGTAGT CGTAATCATCCGGTCTTCGGCGGAGGGACCAAACTGACCG TCCTC
EV- D68- 114	45	heavy	GAGGTGCAACTGTTGGAGTCGGGGGGAGGCTTGGTGCAG CCGGGGGGGTCCCTGAGACTCTCCTGCGCAGCCTCCGGA TTCAGGTTTAGCTTCTATGGCATGACCTGGGTCCGCCAGG CTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTAGTGG TACTGGTGCTACCAGAACTGCGCAGACTCCGTGAAGGGC CGGTTACCATCTCCAGAGACAACTCCAAGAACACGCTGT ACCTGCAAATGGACAGCCTGAGAGTCGACGACACGGCCGT TTTTTATTGTGTGAGACGGTTCCTGATGACCACGGTGACAA GCTTTGACTCTTGGGGCCAGGGAACCCTGGTCACCGTCTC CTCA
	46	light	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTC CTGGACAGTCAGTCACCATCTCCTGCACTGGAACCAGCAG TGATGTTGGTGGTTATAACTTTGTCTCCTGGTACCAACAAC ACCCGGGCAAAGCCCCCAAACCTCATGATTTTTGATGTCACT GGGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCA AGTCTGGCAACACGGCCTCCCTGACCATCGCTGGGCTCCA GGCTGAAGATGAGGCTGATTATTATTGTGGCGCATATGCG GGCTTTAACGCTCTTTTCGGCGGAGGGACCAAACTGACCG TCCTA

EV-D68-116	47	heavy	GAGGTGCAGCTGGTGCAGTCGGGGGGAGGCTTGGTCCGG CCGGGGGGGGTCCGTGAGACTCTCCTGTGTAGCCTCTGGAT TCCCCTTCAATATGTTTTGGATGGGCTGGGTCCGCCAGACT CCAGGGAAGGGACTGGAGTGGGTGGCCAATATAAAACAG GATGGCAGTGAGAAATACTATGTGCGACTCCGTGAAGGGCC GATTCGCCATCTCCAGAGACAATGCCAAGAACTCTCTCTTT CTTCAAATGGACAGCCTGAGTGTGCGGGGACACGGCCATCT ATTATTGTGTCAGAGAGGGGGTGC GAAGGGTCGTCTGACG TAGTACCGGTTACTTCGACTTTTGGGGCCAGGGACAGCTG GTCACCGTCTCCTCA
	48	light	TCCTATGAGCTGACACAGCCACCCTCGATGTCAGTGTCCC CAGGACAAACGGCCAGGATCACCTGCTCTGGAGATGCAGT GCCAATAAAATATGTTTATTGGTACCAACAGAGGTCAGGCC AGGCCCCCTGTATTAGTCATCTATGAAGACGACAGACGACC CTCCGGGATCTCTGAGAGATTCTCTGGCTCCAGTTCAGGG ACAACGGCCACCTTGACTATCACTGGGGCCAGGTGGAGG ATGAAGGTGACTACTATTGCTATTCAACAGACAGTAGTGGT TATCAGAGAGCGTTCGGCGGGGGGACCACGCTGACCGTC CTA
EV-D68-150	49	heavy	CGGGTGCAGCTGCAGGAGTCGGCCCCAGGACTGGTGAGG CCTTCAGAGACCCTGTCCCTCACCTGCAGTGTCTCTGGTG GCCCCATCAGCAATGGTCCTTATTACTGGAGCTGGATCCG CCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATTCATC TTTTACAGTGGGAGCACCAACTACAACCCGTCCCTCCGGG GGCGCGTAACCATGGCAGTGGACACGTCTAAGAACCAGTT CTCCCTGAGGCTGAACTCTGTGACTGCCGCGGACACGGCC GTTTATTACTGTGCGAGACATGTGGTGA CTGCGTCGGGGT GGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA
	50	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTGGCACCGACTTAGCCTGGTACCAACAGAAACCT GGCCAGGCTCCCAGGGTCCTCATCTATGATGCATTCAAGA GGGCCACTGGCATCCCAGCCAGGTTCACTGGCAGTGGGT CTGGGACAGAGTTCACCTCTCACCATCAGCAGCCTCGAGCC TGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAGGT GGCCTCCCCCGTACACTTTTGGCCAGGGGACCAAGCTGGA GATCAAA
EV-D68-151	51	heavy	CGGGTGCAGCTGCAGGAGTCGGCCCCAGGACTGGTGAGG CCTTCAGAGACCCTGTCCCTCACCTGCAGTGTCTCTGGTG GCCCCATCAGCAATGGTCCTTATTACTGGAGCTGGATCCG CCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATTCATC TTTTACAGTGGGAGCACCAACTACAACCCGTCCCTCCGGG GGCGCGTAACCATGGCAGTGGACACGTCTAAGAACCAGTT CTCCCTGAGGCTGAACTCTGTGACTGCCGCGGACACGGCC GTTTATTACTGTGCGAGACATGTGGTGA CTGCGTCGGGGT GGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA
	52	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTGGCACCGACTTAGCCTGGTACCAACAGAAACCT GGCCAGGCTCCCAGGGTCCTCATCTATGATGCATTCAAGA GGGCCACTGGCATCCCAGCCAGGTTCACTGGCAGTGGGT CTGGGACAGAGTTCACCTCTCACCATCAGCAGCCTCGAGCC

			TGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAGGT GGCCTCCCCCGTACACTTTTGGCCAGGGGACCAAGCTGGA GATCAAA
EV- D68- 152	53	heavy	CAGGTGCATCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGCAGACTCTGGAG TCACCTTCAGTGACAATGCTTTGTAAGTGGGTCCGCCAGGCT CCAGGCAAGGGGCTGGAGTGGGTCCGAGTTATCTCATATG ATGGAAGCAGTAGATACTACGCAGACTCCGTGAGGGGCGG GTTACCATATCCAGAGACAATTCCAAGGACACGCTGTATC TGCAAATGAACAGACTGAGAGCTGAGGACACGGCTATTTAT TACTGTGCGAGAGTACAGCGGATTACTATGAGAGTAGTG GCAAGGTGTTTTGGGGCCAGGGAGCCCTGGTCGTCTCTC CTCA
	54	light	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATC TGTGGGGGACAGAGTCTCCATCACTTGCCGGGCCAGTCAG AGTGTTAGGAGCTGGTTGGCCTGGTATCAGCACAAACCAG GGAAAGCCCCCTAACTCCTGATCTATAAGGCGTCTAGTTTA GAAAGTGGGGTCCCATCAAGGTTGAGCGGCAGTGGATCTG GGACAGAATTCACCTCTCACCATCAGCAGCCTGCAGGCTGA TGATTTTGCAACTTATTACTGCCAACAGTATCAGACTTTTTCT CTGGACGTTCCGGCCAAGGGACCACGGTGGAAGTCAAA
EV- D68- 154	55	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGCACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCATCAGTGATCACTACTGGAGCTGGATCCGGCAGCC CCCAGGGAAGGGACTGGAGTGGATTGGCTACATCTATACC AGTGGGACCACCAACTACAACCCCTCCCTCAAGAGTCGAG TCACCATATCAGTAGACACATCCAAGAAGCAGTTCTCCCTG AATCTGAGGTCTGTGACCGCCGCAGACACGGCCGTGTATT ACTGTGCGAGAAGTCTAGAAACGGTGATCCGTTTCTACTAC TACCACTACATGGACGTCTGGGGCAAAGGGACCACGGTGA TCGTCTCATCA
	56	light	GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCAC CCCTGGAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAG AGCCTCCTGCAGAGTGATGGGTACAGCTATTTGGATTGGT ACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTA TTTGGGTTCTAATCGGGCCTCCGGGGTCCCTGACAGGTTCT AGTGTCATTGGATCAGGCACATATTTTAACTGAAAATCAG CAGAGTGGAGGCTGAGGATGTTGGCGTTTATTTCTGCATG CAAGCTCTACAACTCCGTGGACGTTCCGGCCAAGGGACCA AGGTGGAAATCAAA
EV- D68- 155	57	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTGCTGGCG GCTCCATCGGTGATTACCACTGGAAGTGGATCCGGCAGCC CGCCGGGAAGGGGCTGGAGTGGATTGGGCGTATACATAG CAGTGGGAACACTGACTACAACCCCTCCCTCAAGAGTCGA GTCACCATGTCAGTAGACACGTCCAAGAACCAGTTCTCCCT GAAACTGAGGTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCGAGGCAAAATGTTTTTGATATCTGGGGCCAAGG GACAATGGTCACCGTCTCTTCA
	58	light	GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTCTGT CTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCAGCCA GAGTGTTTTATTAGCTCCAACAATAAGAACTACTTAGCTTG GTACCAGCAGAAACCAGGACAGCCTCCTAAGTTGCTCATTT ACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATT

			CAGTGGCAGCGGGTCTGAGACAGATTTCACTCTCACCATC AGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTTCTGTCA GCAATTTTATACTACTCCGCTCACTTTTCGGCGGAGGGACCA AGGTGGAGATCAAA
EV- D68- 156	59	heavy	CAGGTACAGATGCAGGAGTCGGGCCCAGGGCTGGTGAAG GCTTCGGAGACCCTGTCCCTCACCTGCAGTGTCTCTGGTA TCTCCATCAATAACTACTATTGGAGTTGGTTCCGCCAGCCC CCCGGGAAGGGCCTGGAGTGGATCGGATATGTCTATTCTA CTGGGAGTTCCAAGTACAATCCCTCCCTCGAGCGTCGAGC CACCATGTCAGTAGACACGTCCAACAACAACCTTCTCCCTGA GGCTGACGTCTGTGACCACTGCGGACACGGCCGTCTACTA CTGTGCGCGGGGGAGTATGCCGCATATCTGGGGCCAGGG CCTCCTGGTCACCGTCTCCTCA
	60	light	CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCCGGGACCC CCGGACAGAGGGTCACCATCTCCTGTTCTGGTAGCACCTC CAACATCGAGACTAATTATGTATACTGGTACCAGCAGGTCC CAGGAACGGCCCCCAAGCCCCTCGTCTATAGGAATGATCA GCGCCCCCTCGGGGGTCCCTGACCGATTCTCTGGCTCCAAG TCTGGCACCTCGGCCTCCCTGGTCATCAGTGGGCTCCGGA CCGAGGATGAGGCTGCTTATTATTGTGCAGCTTGGGATGA CAGTCTGAAAGCTCCGGTCTTCGGAGCTGGGACCAAGGTC GCCGTCTC
EV- D68- 157	61	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGATAAAG CCGGGGGGGTCCCTTAGACTCTCCTGTGCAGCCTCTGGAA TCACTTTTCAGTAACGCCTGGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAATGGGTTGGCCGTATTGAAAGC AAAATTGACGGTGGGACAATAGACTACGCTACACCCGTGA AAGGCAGATTCACCATCTCAAGAGATGATTCAAAAAACACG CTGTACCTGCAAATGAACAGCCTGAAAACCGAGGACACAG CCGTCTATTACTGTACCACAGACCAGGGCTACTATGATAGA AGTGGTTATTGGGTCTCGGGAACCACTTTGACTACTGGG GCCAGGGAATCCTGGTCACCGTCTCCTCA
	62	light	CAGTCTGTGCTGACGCAGCCGCCCTCAGTGTCTGGGGCC CCAGGGCTGAGGGTCACCATCTCCTGTACTGGGAGCAGCA CCAACATCGGGGCAGGTTATGATGTACACTGGTACCAGCA CCTTCCAGGAACAGCCCCAACTCCTCATCTATGGTAACA GCAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTC CAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTC CAGGCTGACGATGCGGCTGATTATTACTGCCAGTCCTATG ACAGAAGCCTGAGTACTTATGTCTTCGGAACCTGGGACCAA GGTCACCGTCCTA
EV- D68- 158	63	heavy	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGCTATGCCATGAACTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGGTATTAGTGGT ACTACAGGTAGCACATACTACGCAGACTCCGTGAAGGGCC GGTTCACCATCTCCAGAGACAATTCCAAGAACACGGTGCAT CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTAT ATTACTGTGCGAAAGATTCTCACTCCATGATAGTAGTTGAT CATGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCG TCTCTTCA
	64	light	TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCC CAGGACAGACGGCCAGGATTACCTGTGGGGGAAACAATAT TGGAACATAAAGTGTGCACTGGTACCAGCAGAGGCCAGGC

			CAGGCCCTGTGCTGGTCTATGATGATAGCGACCGGC CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGG GAACACGGGCCACCCTGACCATCAGCAGGGTCTGAAGCCGG GGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTTAT AATGTTTATTATGTCTTCGGAACAGGGACCAAGGTCACCGT CCTA
EV- D68- 159	65	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGGGGCCTGGTCAAG CCGGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTACTTATATCATGACCTGGGTCCGCCAGGCC CCAGGGAGGGGGCTGGAGTGGGTCTCATCCATTAGTACCA GTAGTGTTTACACATTCTATGCAGATTCACTGAAGGGCCGG TTCACCATCTCCAGAGACAACGCCAAGAATTCACTGTATCT GCAGATGAACAGCCTGAGAGCCGACGACACGGCTGTTTAT TACTGTGCGAGGGAAGAAGGGTTTCGAGCTTATAACCTATA CTGGGGGCCAGGGAACCCTGGTCACTGTCTCCTCA
	66	light	CAGTCTGTGCTGACTCAGCCACCGTCAGCGTCTGGGACCC CCGGGCAGAGGGTCACCATCTCTTGTCTGGAAGCAGCTC CAACATCGAATACAATTATGTTTACTGGTACCAGAAATCCC AGGAACGGCCCCCAAACCTCCTCATCTATAAAAATAATCAGC GGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTC TGGCACCTCAGCCTCCCTGGCCATTAGTGGGCTCCGGTCC GAGGATGAGGGTGATTATTACTGTGCAGCATGGGATGACA TCCTGAGTGGTGTGGTTTTTCGGCGGGGGGACCAAGCTGAC CGTCCTC
EV- D68- 160	67	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCAGAACTCTCCTGTGCAGCCTCTGGAT TCACGTTCAGTAGGTTTGGTATGCACTGGGTCCGCCAGGC TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATTTCTTT GATGGAAGTAATAGATACTACGCAGACTCCGTGAAGGGC GATTCACCATCACCAGAGACAATTCCAAGAACACATTGTAT CTGCAAATGAACAACCTGAGACCTGAGGACACGGCTGTAT ATTACTGCGCAGAGATTGGGATAGGCTGGTTCTGTTCCGGC GGTTGGCTACTGGGGCCAGGGAACCCTGGTCAGCGTCTC CTCA
	68	light	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTC CTGGACAGTCAGTCACCATCTCCTGCACTGGAACCAGTAAT GATGTTGGTGGTTATAACTTTGTCTCCTGGTATCAGCAACA CCCAGGCAAAGCCCCCAAACCTAATGATTTTTGATGTCATTA GGCGGCCCTCAGGGGTCCCTGGTCGCTTCTCTGGCTCCAA GTCTGGCGACACGGCCTCCCTTATCATCTCTGGACTCCAG GCTGAGGATGAGGCTGATTATTACTGCTGCTCATATGCAG GCACCTACACCTGGGTATTTCGGCGCAGGGACCACTGAC CGTCCTA
EV- D68- 161	69	heavy	CAGGTGCACCTGCAGGAGTCGGGCCACGACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCGTCAGCACTGCCACTTACTACTGGAGCTGGATCCG GCAGTCCCCAGGGAGGGGACTGGAGTGGATAGGATATATC TATTCCAGTGGTAACACCAACTACAACCCTCCCTTAAGAG TCGAGTCACCATTTCTTTAGACACGCCCAACAACCAGCTCT CCCTGACGTTGACCTCTGTGACCGCTGCGGACACGGCCAT TTATTATTGTGAGAGGCGCTTACGTATTCTGAGTATTGAGA GGAACCTACTACGCTATGGACGTCTGGGGCCAAGGGACCCC GGTCACCGTCTCCTCA

	70	light	GAAGTTGTGTTGACGCAGACTCCAGGCACCCTGTCTTTGT CTCCGGGGGAAGGAGCCACCCTCTCCTGCAGGGCCAGTC AGAGGGTTGTCAACAACCTACTTAGCCTGGTACCAGCAGAG AGCTGGCCAGGCTCCCAGGCTCCTCATTTTTGGTGCATCC AACAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGCAGTG GGTCTGGGACAGACTTCACTCTCACCATCAGGAAGCTGGA GCCCCAAGATTTTGCAGTGTATTACTGTCAACAATATGGTA GCCCCGTGGACGTTCCGGCCACGGGACCAAGGTGGAAATGA AA
EV- D68- 162	71	heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAGTTATGCTATGCATTGGGTCCGCCAGGCT CCAGGCAGGGGGCTGGAGTGGGTGGCAGTTATATCATATG ATGCAAGCAAGAAATACCACGCAGACTCCGTGAAGGGCCG ATTCACAATCTCCAGAGACAGTTCCAAGAACACGCTGTTTC TGCAAATGAATAGCCTGAAACCTGAGGACACGGCTGTGTA TACTGTGCGAGAGATCATGTCCCCCACAAGGATTGCAGT GATGGTAATTGCCACTCGGACTACGGTATGGACGTCTGGG GCCAAGGGACCACGGTCACCGTCTCCTCA
	72	light	GACATCCAGATGACCCAGTGCCTTCCACTATGTCTGCTTC TGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCAG AGTATTAGTAAGTGGTTGGCCTGGTATCAGCAGAAGCCAG GGAAAGCCCCCTAACTCCTGATCTATAAGGCGTCTACCTTA CAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTG GGACAGAATTCACTCTCACCATCAACAGCCTGCAGCCTGAT GATTTTGAACCTTATTACTGCCAACAGCATAATAGTTATTCG TACACTTTTGGCCAGGGGACCAAGGTGGAGATCAAG
EV- D68- 163	73	heavy	CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGC CTGGGGCCTCAGTGAAGATTTCTTGCAAGGCTTCTGGATA CTCCTTCACTAACTTTGCTGTGCATTGGGTGCGCCAGGCC CCCGGACAAAGACTTGAGTGGATGGGATGGATCAACCCTG GCAATAGAAACACAAAGTATTCACACAACCTTTCAGGGCAGA GTCACCATTAACAGGGACACATCCGCGAACACAGCCTACA TGGAAGTGAAGCAGCCTGAGATCTCAAGACACGGCTGTGTA TACTGTGCGAGACTTCCGATAGCAGCAGCTGGCAGGGGC TGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCC
	74	light	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTATCAGCACCTACTTAGCCTGGTACCAGCAGAGA CCTGGCCAGGCTCCCAGGGTCCTCATCTATGATGTATCCA CCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGCAGTG GGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGA GCCTGAAGATTTTGCAGTCTATTTCTGTCAACAGTATGGTA GTTCAACCGGCGACGTTCCGGCCAAGGGACCAAGGTGGAAAT CAAA
EV- D68- 164	75	heavy	GAAGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAATACTTATGGCATGAACTGGGTCCGCCAGGCT CCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTAGTG CCACCACTACCTTCTACTACGCAGACTCTGTGAGGGGCCG ATTCACCATCTCCAGAGACAATGCCAAGAATTCATATTTCT GCACATGAAGAGCCTGAGAGACGAAGACACGGCAGTTTAT TACTGTGCGAGAGTCTATACTATGCTTCGCGGAGCGAGTAT

			GGACGTCTGGGGCCACGGGACCACGGTCACCGTCTCCTC A
	76	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTGGGACCTACCTGGCCTGGTACCAACAGAAACCT GGCCAGGCTCCCAGGCTCCTCATCTATGATTACGCCAACA GGGCCACTGGCATCCCAGCCCGGTTTCAAGTGGCAGTGGGT CTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCC TGAAGATTTTGCAGTTTATTACTGTCAGCTGCGCATCACCT GGCCTCCTATATTCACCTTCGGCCCTGGGACCAAAGTGGA TGTCAAA
EV- D68- 165	77	heavy	CAGGTGCACCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG TCTGGGGCCTCAGTGAAGGTCTCCTGCAAGACTTTTGGAT ACACCTTCACCGCCTACTATATGCACTGGGTGCGACAGGC CCCTGGACAGGGGCCTGAGTGGATGGGATGGATCAACCC TATCAGTGGTGGCACAACTATGCACAGAAGTTTCAGGGC AGGGTCTCCATGACCAGGGACACGTCCATCAGCACAGCCT ACATGGGCCTGAGCAGGCTGAGACCTGACGACACGGCCG TCTATTACTGTGCGAGAGTGAAGTGTAGTAGTGCCAACTGC TATGGGAACCTTGACTACTGGGGCCAGGGTACCCTGGTCA CCGTCTCCTCA
	78	light	CAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGGTCTC CTGGAGAGTCAAGTACCATCTCCTGCACTGGAACCAGCAG TGACGTTGGTGGTTATACTATGTCTCCTGGTACCAACAAC ACCCAGGCAAAGCCCCCAAACCTCATGATTTATGAGGTCACT AAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCA AGTCTGGCAACACGGCCTCCCTGACCGTCTCTGGGCTCCA GGCTGAGGATGAGGCTGATTATTACTGCAGCTCATATGCA GGCAGCAACAATTTGGTATTTCGGCGGAGGGACCAAGCTGA CCGTCTCCTCA
EV- D68- 166	79	heavy	GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAG CCCGGTGAGTCTCTGAAGATCTCCTGTAAGGGTTCTGGAT ACAGGTTTACCAACTACCGGATCGGCTGGGTGCGCCAGAT GCCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCC CGGTGGCTCTGATACCAGATACAGTCCGTCCCTCCAAGGC CAGGTCACCATGTCAAGTCGACAAGTCCATCAGCACCGCCT ACCTGATGTGGAGCAGCCTGAAGGCCTCGGACACCGCCAT GTATTACTGTGCTCGACAGACCACTCAAAATAGTGGCTACG ATAGATGGTTTGACTCCTGGGGCCAGGGAACCCACGTAC CGTCTCCTCA
	80	light	CAGTCTGTAAGTCACTCAGCCACCCTCAGCGTCTGGGACCC CCGGGCAGAGGGTACCATCTCTTGTCTGGAAGCACCTC CAGCATCGGAAGTAATATTGTAAATTGGTACCAACACCTCC CAGGAACGGCCCCCAAACCTCCTCATCTATATTAATAATCAG CGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGT CTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTC TGAGGATGAGGCTGACTATTACTGTGCAGCATGGGATGAC AGCCTGAATGGTTGGGTGTTTCGGCGGAGGGACCAAGCTG ACCGTCCTG
EV- D68- 181	81	heavy	GAGGTGCAGGTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAA TCACCTTTAGCAGGCATACTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG GAGTGGTGGTAGCACATATCATGCAGACTCCGTGAAGGGC

			CGCTTCACCATCTCCAGAGACAGTTCCAAGAGCACGCTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGT TTATTACTGTGCGATCTCCGTGCCATTATTACGATTTTTGGA GTGGTTTTCAACACCCTTTTGACTTCTGGGGCCAGGGAACC CTGGTCACCGTCTCCTCA
	82	light	GAAATAGTGATGACGCAGTCTCCAGCCTCCCTGTCTGTGT CTCCAGGGGAAAGAGTCACCCTCTCCTGCAGGGCCAGTCA GAGTGTGGGCAGCACCTTAGCCTGGTACCAGCACAAACCT GGCCAGGCTCCCAGGCTCCTCATCTCTGGTGCATCCACCA GGGCCACTGGTGTCCCAGCCAGGTTCAAGTGGCAGTGGGT CTGGGACAGAGTTCACTCTCACCATCAGCAGTCTGCAGTC TGAAGATTTTGCAGTTTACTACTGTCACCAGTATATTAAGT GCCTCCGTGGACGTTCCGCCAAGGGACCAAGGTGGAAT CAAA
EV- D68- 183	83	heavy	GAGGTGCGGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAA CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAATACATATTCCATGAGCTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTGCGCTCCATTAGTAGTA CCGGAAGTTACATATACAATGCAGACTCACTGAAGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTTTC TGCAAATGAACAGCCTGAGAGTCGAAGACACGGCTGTGTA TACTGTGTGAGATTCACCATGACTACAGTGACTAACTTTG ACTCATGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
	84	light	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTC CTGGACAGTCAGTCACCATCTCCTGCACTGGAACCAGCAG TGATGTTGGTGCTTATAGCTATGTCTCCTGGTACCAACAAC ACCCAGGCAAAGCCCCAACTCATGATTTATGATGTCTAC AGGCGGCCCTCAGGGGTCCCTGGTCTGCTTCTCTGGCTCCA AGTCTGGCAACACGGCCTCCCTGACCGTCTCTGGGCTCCA GGCTGAGGATGAGGCTGATTACTACTGCTGCTCACATGCA GGCAGTCACACCTGGGTGTTCCGGCGGAGGGACCAAGGTG ACCGTCCTA
EV- D68- 185	85	heavy	CAGCTGCAGGTGGTGGCGTCTGGGGGAGGCGTGGTCCAG CCTGGGAGGTCCCTGAGACTCTCCTGTAAAGCCTCTGGAT TCACGTTCAACCAATTATGGCATGCACTGGGTTCGCCAGGC GCCAGGCAAGGGGCTGGAGTGGGTGGCTTTTATATCATAC GATGGAGGTAATAAATTTTATGCAGACTCTGTGAAGGGCCG ATTCACCATCTCCAGAGACAATTCCAGGAACACGGTTTATC TGCAAATGAACAGCCTGAGAGTGGCGGACACGGCTATGTA TACTGTCCGAAGGTCATCCCCACCCGTATTATGATAGTA GTGGTGATGATGCTTTTATATCTGGGGCCAAGGGACAAT GGTCGCCATTTCTTCA
	86	light	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCTGGGCCAGTCA GAGTATTAGCCGCAACTTAGCCTGGTATCAGCAAAAACCTG GCCAGGCTCCCCGACTCCTCATCTATGGTGCATCCACCAG GGCCACTGGTATCCCCGCCAAGTTCAGTGGCAGTGGGTCT GGGACAGACTTCACTCTACCGTCAGCAGCCTGCAGTCTG AAGACCTTGCAGTTTATTACTGTCAGCAGTATAGTAAGTTG CCTATCACCTTCGGCCAAGGGACACGACTGGAGATTAAGT CAAA
EV- D68- 200	87	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAG CCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCCCCTTCAGTAGTTATAGCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATACATAAGTGGT

			AGTGGTGGTGACATATACTACGCAGACTCTGTGAAGGGCC GATTCACCATCTCCAGGGACAATGCCAGGAACTCACTGTCT CTGCAAATGAACAGCCTGAGAGCCGACGACACGGCTGTGT ATTACTGTGCGAGAGGGCTGGTAGCAACAACCTGGTACAAG GTACTTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTC TCCTCA
	88	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTAGGAGTTACTTAGCCTGGTACCAACAGAGACCT GGCCAGGCTCCCAGGCTCCTCATCTACGATGCATCCAACA GGGCCACTGGCATCCCAGTCAGGTTCACTGGCAGTGGGT CTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCC TGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCTACT GGCCTCCGTTCACTTTTCGGCGGAGGGACCAAGGTGGAGAT CAAA
EV- D68- 208	89	heavy	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCGGGGTCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAG GCACCTTCAGGAGGTTTGCTATCAGCTGGGTGCGACAGGC CCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCT ATCCTAGGTAGAGGAAAGTACGCACAGAAGTTCCAGGGCA GAGTCAGGATTACCGCGGACGAATCCACGAGCACAGCCTA CATGGAGCTGAGCAGCCTGAGATCTGAAGACACGGCCGTG TATTACTGTGCGAGATTTATTTTCGACAGCCTCCTATGTTCC GGGGACCTTCGAGGACGTCTGGGGCCAAAGGGACACGGT CACCGTCTCCTCA
	90	light	GACATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAACCT GGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACA GGGCCGCTGGCATCCCAGCCAGGTTCACTGGCAGTGGGT CTGAGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCC TGAAGATGTTGCGGTTTATTACTGTCAGCAGCGTAGCGACT GGCCTCCGGGGACTTTTGGCCAGGGGACCAACGTGGAGA TCAAA
EV- D68- 210	91	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGGAACTATAACATCAACTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTAGTA CTGGTAGTTACATACTACGCAGATTTAGTGAAGGGCCGA TTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCT GCAAATGAACAGCCTGAGAGTCGAGGACACGGCTGTATAT TACTGTGCGGAATGGTTAGGAATACGGTGACTGCCTTTG ACTACTGGGGCCAGGGAACCCTGGTCTCCGTCTCCTCA
	92	light	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTC CTGGCCAGTCGATCACCATCTCCTGCACTGGAACCAGCAG TGATGTTGGTGGCTATAACTTTGTCTCCTGGTACCAACAAC AGCCAGGCAGAGCCCCCAAACCTCCTTATTTATGAAGTCATT AAGCGGCCCTCAGGGGTTTCTGATCGCTTCTCTGGCTCCA AGTCTGGCGACACGGCCTCCCTGACAATCTCTGGGCTCCA GGCTGAGGACGAGGCTGATTATTACTGCTGCTCATATGGG GGTAACAACCTTTGGATGTTTCGGCGGAGGGACCATGCTGA CCGTCCTA
	93	heavy	CAGGTCCAGGTGGTACAGTCTGGGGCTGAGATGAAGAAGC CTGGGGCCTCAGTGAAGGTCTCCTGCAAGGTTTCCGGATA

EV-D68-219			CAGGCTCATTGATTTACCCCTTGCACTGGGTGCGACAGGCT CCTGGAAAAGGGCTTGAGTGGATGGGACTTTTTGATCCTG AAAAGGCTGAAGCCATCTACTCACAGAAATCCAGGACAAG GTCACCATAAGCGAGGACACATCTATCGACACAGCCTACAT GGAAGTGAACAGCCTGCGCTCTGAAGACACGGCCGTCTAC TACTGTGCAACTTGGGGAGTTGAGGTGGTGAATGGGAGAA GGGACTACTTTGACTCCTGGGGCCAGGGAACCCTGGTCAC CGTCTCCTCA
	94	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTCAGTGTCCC CAGGACAGACGGCCAGGATCACATGCTATGCAGATGTGTT GTCAAACCAATATACTTACTGGTATCAACAGAAGCCAGGCC AGGCCCCCTGTGTTGGTGATATATAAAGACACTGAGAGGCC CTCAGGGATCCCTGAGCGATTTGCTGGCTCCAGCTCAGGG ACAACAGTCACCTTAGTCATCAATGGAGTCCGGGCAGAGG ACGAGGCTTACTATTACTGTCAATCAGCCGACAACACCAGA ATTACGGTTTTTCGGCGGAGGGACCAAGCTGTCCGTCCTA
EV-D68-220	95	heavy	GGGGTGCAGCTGGTGGAGTCTGGGGGAGGATTGATACAG CCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAGTTTTGAAATGAACTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTTTTACATATTAGTACTA GTGGTAGTACCATATACTACGCAGACTCTGTCAAGGGCCG ATTCACCATCTCCAGAGACAACGCCAGGAACTCACTGTCTC TGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTTTA TACTGTGCGAGAGACGTGAGGGATTGTAGTGCTCTTACG TGCCCCCGAAGGGGAGATGCTTTTGATTTCTGGGGCCGTG GGACAAGGGTCACCGTCTCTTCA
	96	light	GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCAC CCTTGACAGTCGGCCTCCATCTCCTGCAGGTCTAGTCAA AGCCTCGTATACAGTGATGGAAACACCTACTTGAATTGGTT TCAGCAGAGGCCAGGCCAATCTCCAAGGCGCCTAATTTAT AAGGTTTCTAACCGGGACTCTGGGGTCCCAGACAGATTCA GCGGCAGTGGGTGAGGCACTGATTTCACTGAAAATCAG CAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATG CAAGGTACACACTGGCCTCGCACTTTTCGGCCCTGGGACCA AAGTGGATATCAAA
EV-D68-221	97	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCAGTTTCAGTGTCTATCCCATGAACTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGGTCTCATCCATAAGTAGTA GTAGTCGTTACATATCCTACGCAGACTCACTGAGGGGTGCG AATCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATC TGCAAATGAACAGCCTGAGAGTCGAGGACACGGCTGTGTA TACTGTGTGAAAGTCGGGGGTAGCAAACACCAATACTACT TTGACTACTGGGGCCAGGGATCCCTGGTCACCGTCTCCTC A
	98	light	CAGTCTGTGCTGACTCAGCCACCCTCAACGTCTGGGATCC CCGGGCAGACGGTCACCATCTCTTGTCTGGAAGCAGGTC CAACATCGGAAGTTATACTGTAACTGGTACGAGCAACTCC CAGGAACGGCCCCCAAACCTCGTCATCTTTAATAATAATCAG CGTCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGT CTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTC TGAGGGTGAAGCTGATTATTACTGTGCAGCATGGGATGAC AGCCTGAATGGTGTGGTTTTTCGGCGGAGGGACCAAGCTGA CCGTCCTA

EV-D68-224	99	heavy	CAGGTTTCAGTTGGTGCAGTCTGGAGCTGAGGTGAAGAGGC CTGGGGCCCTCAGTGAAGGTCTTCTGCAAGGCTTCTGGTTA CACCTTTACGAATTATGACATCATCTGGGTGCGACAGGCC CTGGACAAGGGCTTGAGTGGGTGGGCTGGATCAGCACTTA CAATGGTAACACAACTATGAACAGAACCTCCAGGGCAGA GTCACCATGACCACAGACACATCCACGAGCACAGCCTACA TGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTTTA CTATTGTGCGAGAGAGCGTTGTAGTACTAGTACCTGCTATA GTCGTTATGCTGACTACTGGGGCCAGGGAACCCCTGGTCAC CGTCTCCTCA
	100	light	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATC TGTGGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAG AGCATTAAATATCTATTTGAATTGGTATCAGCAAAAACCGGG GAAAGCCCCTAAGGTCTGATCTATGCTGCATCCAGTTTGC AAAGTGGGGTCCCATCAAGGTTCCGTGGCAGTGGGTCTGG GACAGATTTTCATCCTCACCATCAGCAGTCTGCAACCTGAAG ACTTTGCAACCTACTACTGTCAACAGAGTTACAGGTCCCCT CGGACGTTCCGGCCAAGGGACCGAGGTGGAAATCAAA
EV-D68-225	101	heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGCACAG CCTGGAGAGTCCATGAGACTCTCCTGTGTAGCCTCTGGAT TCACCTTAAGTCGTTATGAAGTGAAGTGGGTCCGCCAGGC TCCAGGGAAGGGGCTAGAGTGGCTTTTCATACATTAGCAGT GGTGGTCCTTCCATATACTACGCAGACTCTGTGAAGGGCC GATTCACGATCTCCAGAAACAGCGCCGAGAACTCACTGGA ACTACAAATGTCCACCCTGAGGACCGAGGACACGGCTGTT TATTATTGTATGAGAGAGGGTCTTACTTATTATGATAGTACT ATTTGGGGCCAGGGAACCCCTGGTCGCGCTCTCCTCA
	102	light	CAGAATGTGCTGACTCAATCGCCCTCTGCCTCTGCCTCCCT GGGAGCCTCGGTCAAACCTCACCTGCACTCTGAACAGTGGG CACAGCAGATACGCCATCGCATGGCATCAACATCAGCCAC AGAGGGGGCCCTCGGTTCTGATGAAGATTAATAGTGATGG CAGACACATCAGGGGGGACGGCATCTCTGATCGCTTCTCA GGCTCCGCCTCTGGGGCTGAGCGTCATCTCACCATCTCCA GCCTCCAGCCTGAGGATGAGGCTGACTATTATTGTCAGAC CTGGGGCACTGGCTTTCGGGTGTTTCGGCGGAGGGACCAA ACTGACCGTCCTA
EV-D68-227	103	heavy	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTGATGAATATGCCATGCACTGGGTCCGGCAAGTT CCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGA ATGGTGGTAGCAAAGGCTATGCGGACTCTGTGAAGGGCCG ATTCACCATCTCCAGAGATAACGCCAGGTATCCCTGTCTC TGCAAATGAACAGTCTGAGAACTGAGGACACGGCCTTATAT TACTGCGCAAAGATGATTACGAGGGGGCTGGTTTTGATAT CTGGGGCCAAGGGACAGTGGTCACCGTCTCTTCA
	104	light	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTGTTAGCAGCTACTTAGGCTGGTACCAACAGAAATCTG GCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAG GGCCACTGGCATCCCAGCCAGGTTCACTGGCAGTGGGTCT GGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTG AAGATTTTGCAATTTATTACTGTCAGCAGCGTAGCAACTGG CCTATCACCTTCGGCCAAGGGACACGACTGGAGATTA

EV-D68-228	105	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGCT ATTTAATTAGCAATGGTTACTACTGGGGCTGGATCCGGCAG TCCCCCGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATT ATACTAGGGACACCTACTACAACCTGGTCCCTCAAGAGTCGA ATCACCATATCAGTGGACACGTCCTCAAGAAACAGTTCTCCCT GAAGTTGTATTCTGTGACCGCCGCAGACACGGCCGTCTAT TACTGTGTGAGACATGAGGGTCTTGCAATGATGGAAGCT GTTACGGCTCGTTTCGTTGACAACCTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCA
	106	light	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATC TGTAGGAGACAGAGTGACCCTCACTTGTCCGGCGAGTCAG GATATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAACCAG GGAAAGCCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTG CAAAGTGGGGTCCCATCAAGGTTGAGCGGCAGTGGATCTG GGACACATTTCACTCTCACCATCAGCAGCCTGCAGCCTGA AGATTTTCGCAACTTACTTTTGTCAACAGGCTGACAGTTTCAT CACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA
EV-D68-231	107	heavy	GAGGTGCAACTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGATT TCACCTTCAGCAGCTACACCATGGCCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTTGAATGGGTCTCATCTATTAGTGGT GATGGTGTAGCACAAAAACGCAGACTCCGTGAAGGGCC GATTCTCCGTCTCCAGAGACAATTCCAAGAACACACTTTTT CTGCAACTGAACAGTCTGAGAGCCGAGGACACGGCCCTTTT ATTACTGTGCGAGGGGGGGGACCTTCCATAACTGGTACTT CGATCTCTGGGGCCGTGGCGTCTTGGTCACTGTCTCCTCA
	108	light	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGT CTCCAGGGGAAACAGCCACCCTCTCCTGCAGGGCCAGTCA GAGTATTGGCGACAACCTTAGCCTGGTATCAGCAGAAACCT GGCCAGGCTCCCAGGCTCCTCATCTCTGGTGCATCCACAA GGGCCACTGATTTCCAGCCAGGTTCCGTGGCAGTGGGTG TGGGACAGAGTTCACTCTCACCATCAGCAGCCTGCAGTCT GAAGACTTTGCAGTTTATTACTGTCAGCAGTATAAAAACTG GCCTCGGACGTTCCGGCCGAGGGACCAAGGTGGAAGTCAG A
EV-D68-234	109	heavy	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCACCTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCGGAGTGGGTCTCAGGTATTAGTGG TAGTGGTGGTAGCACAACTACGCAGACTCTGCGAAGGGC CGGTTCAACATCTCCAGAGACAATTCCAAGAACACGCTGTA TCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTA TATTATTGTGCGAAAGGGACCATTACTTACTCTACTACTAC ATGGCCGTCTGGGGCAAAGGGACCAACGGTCACCGTCTCCT CA
	110	light	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTC TCCAGGGGAAAGAGGCACCCTCTCCTGCAGGGCCAGTCA GAGTGTAGGAGCAGCTACTTAGCCTGGTACCAGCAGAGA CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCA GCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTG GGTCTGGGACAGACTTCACGCTCACCATCGGCAGACTGGA GCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTA

			CCTCAATAACTTTTCGGCGGAGGGACCAAGGTGGAGATCAA A
EV- D68- 235	111	heavy	CAGGTCCAGGTGGTACAATCTGGGGCTGAGATGAGGAAGC CTGGGGCCTCAGTGAGGGTCTCCTGCAAGGTTTCCGGATA CAGGCTCACTGATTTACCCCTTGCACTGGGTGCGACAGGCT CCTGGAAAAGGGCTTGAATGGATTGGATTTGTTGATCTTGA AAAGCGCGAAATCATCTACGCACAGAAATTTTCAGGGCAAA GTCACCATAACCGAGGACACATCTGCAGACACCGCCTACA TGGAAGTGAACAGCCTGCGATCTGAAGACACGGCCGCTA CTACTGTGCAACTTGGGGAATTGAGGTGGTGAATGGGAGG GACGAATTCCTTGACTCCTGGGGCCAGGGAACCCCTGGTCT CCGTCTCCTCA
	112	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTCAGTGTCCC CAGGACAGACGGCCAGGATCACATGCTATGCAGATGTGTT GTCAAAGCAATATACTTACTGGTATCAGCAGAAGCCAGGCC AGGCCCTGTGTTGGTGATATATAAAGACACTGAGAGGCC CTCAGGGATCCCTGAGCGATTTGCTGGCTCCAGCTCAGGG ACAACAGTCACCTTGATCATCAATGGAGTCCGGACAGAGG ACGAGGCGTACTATTACTGTCAATCAGCCGACACCAGAATT ACGGTTTTTCGGCGGAGGGACCAAGCTGTCCGTCCTA
EV- D68- 236	113	heavy	CAGGTCCAGGTGGTCCAGTCTGGGGCTGAGATGAAGAAG CCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGTTTCCGGAT ATAGTCTCAGTGATTTACCCCTTGCACTGGGTGCGACAGGCT CCTGGAAAAGGGCTTGAAGTGGATGGGACTTTTTGATCCTAT AAACGGTGAATCATCTACGCACAGACATTCCAGGGCAAA GTCACCATAAGCGAGGACACATCGATAGACACAGCCTACA TGGAAGTCAACAGCCTGCGATCTGAAGACACGGCCGCTGTA CTATTGTGCAACTTGGGGAGTTGCGGTGGTGAAGTGGGAGA AGGGACTACTTTGACTCCTGGGGCCAGGGAACCCCTGGTCA CCGTCTCCTCA
	114	light	TCCTATGAGCTGACACAGCCACCCTCGGTGTCAGTGTCCC CAGGACAGACGGCCAGGATCACATGCTATGCAGCTGTATT GTCAAACCAATATACTTACTGGTATCAACAGAAGCCAGGCC AGGCCCTGTGTTGGTGATATATAAAGACACTGAGAGGCC CTCAGGGATCCCTGAGCGATTTGCTGGCTCCAGCTCAGGG ACAACAGTCACCTTGATCATCAATGGAGTCCGGACAGAGG ACGAGGCTTACTATTACTGTCAAACAGCCGACACCAATAT ACGGTTTTTCGGCGGAGGGACCAAGCTGTCCGTCCTA
EV- D68- 241	115	heavy	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAG CCTTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCATCACCAGTGGTGATTACTACTGGAATTGGATCCGC CAGCCCCCAGGGAAGGGCCTGGAGTGGATTGGGTACATC TATCACAGTGGGACCACCTACTACAACCCGTCCCTCAAGA GTCGAGTTACCATATCAGTAGACACGTCCAAGAACAGGTTT TCCCTGAAGTTGTCCTCTGTGACTGCCGCAGACACGGCCG TGTATTTTTGTGCCAGAGCCTACGCTTATGAATTTTGGAGC GGTTACCCTAACTGGTTTCGACCCCTGGGGCCTGGGAACCC TGGTCACCGTCTCATCA
	116	light	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATC TGTTGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAG CGCATTAGTACCTATGTAAATTGGTATCAGGTGAAAGCAGG GACAGCCCCTAAGGTCCTGATCTATGCTGCGTCCAGTTTG CAAAGTGGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTG GGACAGATTTCACTCTCACCATTGTCAGTCTACAACCTGAA

			GATTTTACAACCTACTTCTGTCAACAGAGTTACAGTCCCC GTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA
EV- D68- 247	117	heavy	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCCAG CCTGGTAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGGT TCATCTTCAATAGATATGCCATGCACTGGGTCCGCCAGGCT CCAGGCAAGGGGCTCGAGTGGGTGGCTCTTATATCATATG ATGGAATTAATAAATATTACGCAGACTCCGTGAAGGGCCGA TTCTCCATCTCCAGAGACAATTCCAAGAGTACGCTGTATCT GCAAATGAACAGCCTCAGAGCTGAGGACACGGCTATCTTT TACTGTGCGAGAGGACTAGGATATTGTAGTGGTACCGGTG GTAGCTGTACACCTTTGAATATTGGGGCCAGGGAATCCT GGTCACCGTCTCCTCA
	118	light	GACATCCAGATGACCCAGTCTCCACCCTCCCTGTCTGCAT CTGTTGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCA GAGCATTAAAGAAATATTTAAATTGGTATCAGCAGAAACCAG GGAATGCCCCTAAGCTCCTCATCTATGGCGCATCCAATTTG CAAAGTGGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTG GGACAGATTTTCGCTCTCTCCATCAGCAGTCTGGAAACTGAA GATTTTGCAACTTACTACTGTCAACAGAGTGACAGTGCCCC TCCCACCTTCGGCGGAGGGACCAAGGTGGAGTTCAAA
EV- D68- 254	119	heavy	CAGGTGCAGCTGCAACAGCGGGGCGCAGGGCTGTTGAAG CCCTCGGAGACCCCTGTCCCTTACCTGCGAAATCTATGGTG CATCCCTCAATGATTACGACTGGACCTGGATCCGCCAGCC CCCAGGGAAGAGGCTGGAGTGGATTGGGGTCATCAATCGT CGTGACACTGTTGACTACAACCCGTCCCTCAAGAGTCGGG TCACCCTCTCACTTGACACGTCCAAGAACCAACTTTCCCTG AGTCTGAGTTCTGTGACCGCCGCGGACACGGCTGTTTATT ACTGTGTGAGAGTCCCACGTGCGGGGCTTTGAAGGGTCTTT CGGATTTTGTGATGATACTGCCTGCCGCTACGGGCATACC TGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCT CCTCA
	120	light	GACATTGAGATGACCCAGTCTCCTTCCTCCCTGTCTGCATC TGTTGGCGACAGAGTCACCATCACTTGCCGGGCAAGTCAG AGTATTAGAGATTATTTAAATTGGTATCAACAAAGACCAGG GAAAGCCCCCTAAAGTCCTGATCTTTGCTGGTTCCCGTTTGG AAAGTGGGGTCCCATCGAGGTTTAGAGGCCGTGGATCTGG GACAGAATTCATCTCACCATCAGCGATCTGCAACCTGAG GATTTTGCAACTTACTACTGTCAACAGAGTTACCTTACACCT CCGACATTCGGCCAAGGGACCAACCGTCGATATCAAA
EV- D68- 260	121	heavy	CAGGTCACCTTGAAGGAGTCTGGTCCTGTGCTGGTGAAC CCACAGAGACCCCTCACGCTGACCTGCACCGTCTCTGGATT CTCACTCCGCAATGGTAGAATGGGTGTGAGCTGGATCCGT CAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGACACATTT TTGCGAGTGACGAAAAATCTTACAGTACATCTCAGAGGACC AGGCTCTCCATCTCCAGGGACACCTCCAAAAGCCAAGTGG TCCTTAGCATGACCGACATGGACCCTGTGGACACAGCCAC ATATTACTGTGCGCGGATTTTGAAGTTTGGGACAATGAGGG CCGCATACTACTTTGACTACTGGGGCCAGGGAGCCCTGGT CCCCGTCTCCTCA
	122	light	TCCTATGTGCTGACTCAGCCACCCTCAGTGTGAGTGGCCC CAGGAAAGACGGCCAGGATTACCTGTGGGGGAATCAACAT TGGAATTAGAACTGTACACTGGTACCAGCAGAAGCCAGGC CAGGCCCCCTATGTTGGTCATCTATTATGATAGCGACCGGC CCTTAGGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGG

			GAACACGGGCCACCCTGACCATCAGCAGGGTCTGAAGCCGG GGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGT AGTGATCATGTTGTATTTCGGCGGAGGGACCAAGCTGACCG CCCTA
EV- D68- 266	123	heavy	CAGGTGCACTTGGTGGAGTCTGGGGGAGGCTTGGTCAAG CCTGGAGGGTCCCTGAGACTCTCCTGTGCAGTTTCTGGAT TCACCTTCAGTGACTIONACTACATGAGCTGGATCCGCCAGGC TCCGGGGAAGGGACTGGAGTGGCTTTTCATACATTAGTAGT AGTGGTAGTACCATATACTACGCAGACTCTATGAAGGGCC GATTCACCATCTCCAGGGACAACGCCAGGAACCTCACTCTAT CTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCCTTTT ATTACTGTGCGGGGTCAAAGGTTGGCTATACTACTGGTCTG AAGGAACTGGTACTTCGATCTCTGGGGCCGTGGCACCCCTG GTCACCTGTCTCCTCA
	124	light	CAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGGTCTC CTGGACAGTCACTCACCATCTCCTGCACTGGAACCAGCAG TGACGTTGGTGCTTATAACTATGTCTCCTGGTACCAACAGC ACCCAGGCAAAGCCCCCAAACCTCATGATTTATGAGGTCACT AAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCA AGTCTGGCAACACGGCCTCTCTGACCGTCTCTGGGCTCCA GGCTGAGGATGAGGCTGATTATTACTGCAGCTCATATGCA GGCAACAACAATTTAGTCTTCGGCGGAGGGACCAAGCTGA CCGTCCTA
EV- D68- 269	125	heavy	CAGGTGCACTTGGTGCAGTCTGGGTCTGAGGTGAGGAAAC CTGGGGCCTCAGTGAACATTCACTGTAAGGCATCTGGATT CACTTTACCGACTTCTATTTACACTGGGTGCGACAGGCC CTGGACAAGGGCTTGAGTGGATGGGGATAATCAACCCTGA AACCAGGTGAGACAACCTACTCACAGAAGTTTCAGGGCAGA GTCACCATGACCAGGGACACGTCCACGAGTGTAGTGAATC TGGAAGTGAGGAGCCTGAGATCTGAGGACACGGCCATATA TTACTGTGCGAGAGATCTCGTTGTCGTAGTCCCCGTTGAAA TGTCTCGGCGTGCCCTTTGACATTTGGGGCCAAGGGATTAT GGTCACAGTCTCCTCA
	126	light	TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCC CAGGACAGACGGCCAGGATTCCCTGTGGGGGCAACAACAT TGAACGTAAAAGTGTCCACTGGTACCAGCAGAGGCCAGGC CAGGCCCTGTGTTGGTCTCTATGATGATACTGTCCGGC CCTCAGGTATCCCTGAGCGATTCTCTGGCTCCAACTCCGG GAGCACGGCCACCCTGACCATCAGCAGGGTTCGGAGCCGG GGATGAGGGCGACTATTATTGTCAGGTGTGGGACAGCACC ACTGACCATGGGGTCTTCGGCGGAGGGACCAAGCTGACC GTCCTA
EV- D68- 271	127	heavy	CGGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGGCCTCAGTGAAGGTCTCCTGTGCACTTCTGGAT ACATTTTCAGCGCCTACTACATCTATTGGGTGCGACAGGCC CCTGGACAAGGACTTGAGTGGATGGGACGGATGAACGCTA AGAGTGGAGGCGCAAACACTGCACAGCAGTTTCAGGGCAG ACTCACCATGACCAGGGACATGTCCGTCAGCACAGCCTAC ATGGAAGTGAAGGCTGCGATCGGACGACACGGCCGTC TATTATTGTGCGAGAGACTATAGGGATGACTACATGTGGGG GAGTTATCGGCCTTTAGACTACTGGGGCCAGGGAACCCTG GTCACCGTCTCCTCA
	128	light	GAAGTTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTC TCCAGGGGAAAGAGCCTCCCTCTCCTGCAGGGCCAGTCAG

			AGTGTTAGCGGCTACTTAGCCTGGTACCAACACAAACCTG GCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAG GGCCGCTGGCATCCCAGCCAGGTTCCGTGGCAGTGGGTC TGGGACAGACTTCACTCTCACCATCAGCAGCCTGGAGCCT GAAGATTTTGCAGTTTATTTCTGTCAGCAGCGTAGCAACGG GCTCACTTTCGGCGGAGGGACCAAGGTCGAGATCAAA
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TABLE 2 – PROTEIN SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	SEQ ID NO:	Chain	Variable Sequence
EV-D68-37	129	heavy	QVQLVESGGGVVQPGRSLRLSCAASGFIHSRYALHWVRQ APGKGLDWVAVISYDARNSYYTDSVKGRFTISRDNKNTL FLQMNSLRADDTAVYYCARPTLPYSNNWYAPEYWGQGTL VTVSS
	130	light	SYELTQPPSVSVSPGQTARITCSGDALPKKYASWYQQKSG QAPVLVIYEDTKRPSGIPERFSGSSSGTMTLTISGAQVED ESDYCCSSTDSSGNPVLFGGGTKLTVL
EV-D68-40	131	heavy	EVQLVESGGDLVQPGGSLRLSCAASGFSFSSYAMAWVRQ APGKGLQWVSSISGNGNGRSYADSLKGRFTTSKDLKYTL YLQMNNLRPEDTAIYYCAKVVRIAAVLYYFDYWGPQTQVT VSS
	132	light	EIVLTQSPATLSLSPGERATLSCRASQSVSTYLAWYQQKP GQAPRLLIYEASTRATGIPARFSGSGSGTDFTLISSLEPED FAVYHCQQRSSWPITFGQGTRLEIE
EV-D68-41	133	heavy	DVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMTWVRQ ALGKGLEWVSSISGSGGLTYFAHSVKGRLTISRDNKNTLY LQMSSLRAEDTAVYYCARVKSTTGTTALVFDIWGQGTMTV VSS
	134	light	QTVVTQEPSFSVSPGGTVTLTCGLSSGSVSSSYPSWYQ QTPGQAPRTLIIYSINRRSSGVPDRFSGFILGNKAALTIRGAQ ADDES DYCCGLYMGSGIWIFGGGTKLTVL
EV-D68-43	135	heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFINYGMHWVRQ APGKGLEWVAVISNDGSYNVDADSVKGRFTISRDNKSNKV YLQMNSLRPEDTAVYFCAKDKHGDFDYGVVDVWGQGTTV TVSP
	136	light	DVQMTQSPSSVSASIGDRVTITCRAGQGSSWLAWYQQKP GKAPKLLIYAASNLSQGVPSRFSGSGSGTDFTLTISLQPE DFGTYCQQQADSFPRTFGQGTKVEIK
EV-D68-46	137	heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSYGIHWVRQ APGKGLEWVAVISYDGSNTYAPFVNGRFTISRDNKNTL YLQMNSLRADDTAVYYCARRRPGSFPLCDYWGGQALVT VSS
	138	light	DIQMTQSPSTMSASVGDRTITCRASQSISKWLAWYQQKP GKAPKLLIYKASTLQTGVPSRFSGSGSGTEFTLTINSLQPD DFATYYCQQHNSYSYTFGQGTKVEIK
EV-D68-48	139	heavy	QVQLVQSGAEVKKPGSSVKVSKASGGSFSLTIHWVRQA PGQGLEWMGGHIPFGTTNYALKFQGRVTITADKTTSTAYM ELSSLRSEDTAIYYCARMYSGHGVDVWGQGLTVTVSS
	140	light	EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQHQP GQAPRLLIYDASNRAKGIPARFSGSGSGTDFTLTISLEPED FAVYYCQQRSTWPPGMFGQGTREIK

EV-D68-71	141	heavy	QLQLQESGPGLVKHSETLSLTCTVSGGSISSGFYYWGWIR QPPGKGLEWIGTIYDSGRTYDNPSLKSRTVISADTSKKQFS LTLRSATAADTAVYFCARHLTHLYGDYVTPDALDIWQGGT MVTVSS
	142	light	EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSFLAWYQQK PGQAPRLLIYGASSRATGIPDRFRGSGSGTDFLTISRLEP EDFAVYYCQQYSNSRLTFGQGGTKVEIK
EV-D68-72	143	heavy	EVQLVESGGGLVQPGGSLRLSCAASGFTFTTYSMNWVRQ APGKGLEWISYISSGSSNIYYADSVKGRFTISRDNANKNSLNL QMSSLRDEDTAVYYCARAHGRIVNSGVVISRFPDWGQGIL VTVSS
	144	light	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQL HPGKAPKLMIFEVTYRPSGVSNRFSGSKSGNTASLTISGLQ AEDEADYFCSSYTTSNTLVVFGGGTKLTVL
EV-D68-74	145	heavy	QVQLVQSGAEVKKPGASVKVSCRTSGYTFTAYYMHWVRQ APGQGPEWMGRINPSSGGAQYAQKFQGRVTMTRDTSIST TYMTLSGLTSDDTAVFYCARMGCRSDRCYSTNYNFDQWG QGTLVTVSS
	146	light	SYVLTQPPSVSVAPGQTARIPCGGNNIGTKSVHWYQQKPG QAPVLVVSNDSDRPSGIPERFSGSKSGNTATLTISRVEAGD EADYYCQVWDSGIDVVFGGGTKLTVL
EV-D68-75	147	heavy	EVQLVESGGGLVKPGGSLRLSCAASGFTISPYGMNWVRQ APGKGLEWVSFISSSSRYTYADSVKGRFTISRDNANKNSLS LQMNSLRAEDTAVYYCARERGHSTSSSYFDSWGQGTLT VSS
	148	light	SYVLTQPPSVSLAPGKTARITCGGNNIGTKTVSWYQQKPG QAPVLVMYYDSDRPSGIPERFSGSNSGNTATLTINRVEAG DEADYYCRVWDSDDHRVFGGGTKLTVL
EV-D68-76	149	heavy	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWISWVRQ APGKGLEWVGRIQTKTDGGTTDYAAPVKGRFTISRDDSKN TLYLQMNSLKTEDTALYYCSTGPYYDYDTSGYPQPFDYWG QGTLVTVSS
	150	light	SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKP GQSPVLVIYQDTKRPSGIPERFSGSNSGNTATLTISGTQAM DEADYYCQAWDSSTVVFSGGGTKLTVL
EV-D68-80	151	heavy	EVQMVESGGGLVKPGGSLRLSCSVSGFDFSRYTMNWFR QAPGEGLEWVSSISSTSLYTFYADSVKGRFSISRDNAQGS LSLQMSSLRPEDTAVYYCARVVGPAELDYWGQGVLTVS S
	152	light	VTQLTQSPSSLSASVGDRVITICRASQDIGVDLWGFQQR GKAPKLLIYGASRLQSGVPSRFSGRGSGTFFTLTISLQPE DFTTYFCLQDYNYPWTLGQGGTGVGK

EV-D68-84	153	heavy	QVHLVQSGSAVRKPGASVKVSCKASGYTFTDYYIHWVRQ APGQGLEWMGWINPKTGGSNYTQRFQARVTMTWDTST AYMELSRRLRSDDTAVYYCARAGRNGYDYWGQGLTVTVSS
	154	light	SYELTQPPSVSVSPGQTARITCSADALPKQYAYWYQQKPG QAPVLMYQDTERPSGIPERFSGSSSGTTVTLTISGVQAED EADYYCQSGDSSGTYLVFGGGTKLTVL
EV-D68-85	155	heavy	EVQLLES GGGLVQPGGSLRLSCAASGFKFRNYAMTWVRQ APGKGLEWVSTITSGGST EYADAVKGRFIISRDN SKNTLYL QMNSLRADDTAVYYCTVPWGN YNDYVSDYWGQGLTVPV SS
	156	light	EVVLTQSPATLSLSPGQRATLSCRASQRVGN SLAWYQQK PGQAPSLLIYDASKRATGIPARFSGSGSGTDFTLTIISESE DFAVYYCHQHSTWPRGTFGQGTRLEIK
EV-D68-88	157	heavy	QVHLVESGGGVVQPGRSLRLSCAASGFIFSRYP MHWVRQ APGKGLEWVALISYDGN NKYYADSVKGRFTISRDN SKNTL FLQMNSLRAEDTAVYYCARHFLPYSSSWYQGFNYWGQGI LTVTVSS
	158	light	NFMLTQPHSVSESPGKTVTISCTRSSGSIATNYVQWYQQR PGSSPTPIIFEDSQRP SGVPDRFSGSIDSSSNSASLTISGLR TDDEADYYCQSYDNSNRAVVF GGGTKLTVL
EV-D68-89	159	heavy	QVQLVESGGGVVQPGRSLRLSCEASGFLFSRYGMHWVR QAPGKGLDWVAVISYDGN KKKYYADSVKGRFTISRDN SKNT LYLQVNSLRVEDTAVYYCARGVPYGD TLTGLVYWGQGLV TVSS
	160	light	NFMLTQPHSVSESPGKTVTISCTRSSGTIASNYVQWYQQR PGSAPTTVIYEDNQRP SGVPDRFSGSIDSSSNSASLTISGL KTEDEADYYCQSYDNSDRVF GGGTKLTVL
EV-D68-95	161	heavy	QVTLKESGPVLVKPTETLTCTVSGFSLRNARMGVSWIR QPPGKALEWLAHIFSNDEKSYNTSLKSRLSISKDTSKSQVV LTMTSMDPLDTATYFCARLLVAGTFLPSHYFDYWGQGILVT VSS
	162	light	SYVLTQPPSVSVTPGKTARITCGGNNIGLKS VFWYQERPD QAPVVVIYYDSARPSGIPERISGSKSGNTATLTITRVEAGDE ADYFCQVWDSSRNHPVF GGGTKLTVL
EV-D68-97	163	heavy	ELQLVESGGGLVQPGGSLRLSCAASGFTFSTYSMNWVRQ APGKGLEWVS YISSSSSSTIQYADSVKGRFTISRDN AKNSLY LQMNSLRAEDTAVYYCTRQVGADFSGRGFDYWGQGLLT VSS
	164	light	SYELTQPPSVSVSPGQTATITCFGDKLGDKYACWYQQKPG QSPVLVIYQDSKRPSGIPERFSGSKSGNTATLTISGTQAMD EADYYCQAWDSSTAVF GGGTKLTVL

EV-D68-98	165	heavy	EVQLVESGGGLVQPGGSLRLSCAASGFKFSVYALSWVRQ APGKGGLEWISYISSSGSTIYYSDSVKGRFTISRDNVGNLSLFV QMNSLRAEDTGIYYCATARHITNDGFDIWGQGTMVIVSS
	166	light	DIQLTQSPSFLSASVGDRVITICRASQGISRFLAWYQQKPG KAPKLLIYASATLQRGVPSRFSGSGFGTDFTLTISSLQPEDF ATYYCQQLNSHPRMFTFGPGTTVDIK
EV-D68-105	167	heavy	QVQLQESGPGLVKPSSETLSLTCAVSGYLISNGYYWGWIQR PPGKGGLEWIGSIYHTRSTYYNPSLKSRSVISVDTSKNRFSL RLRSVTAADTAFFYYCARGPGHGYGDDDCYAYYFDQWGQ GTPVTVSP
	168	light	DIQMTQSPSSVSSSVGDRVITICRASQGISNWLAWYQQNP GKAPKLLIYDASSLQSGVPSRFSGSGSGTDFTLTINSLQPE DFATYYCQQANSFPFTFGPGTKVDIK
EV-D68-110	169	heavy	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRFAISWVRQ APGQGLQWMGGILPIFGTANYAQKFQGRVTITADTSTSTA YMELSSLRSEDATVYYCARSLPYCTNDVCSNQNTFDYWG QGTLVTVSS
	170	light	SYELTQPPSVSVSPGQAARITCSGDALPKQYAYWYQQKP GQAPVLVIYEDNKRPSGIPERFSGSSSGTTVTLTISGVQAE DEADYYCQSADSSGTYYVFGGGTKLTVL
EV-D68-111	171	heavy	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIR QPPGKGGLEWIGYIYYSGSTYYNPSLKSRTISVDTSKNQFS LKLSSVTAADTAFFYYCASRYGDPIDGNWFDWPWGQGTLLTV SS
	172	light	SYVLTQPPSVSVTPGKTARITCGGNNIGLKSVMFYQERPD QAPVVVIYYDSARPSGIPERISGSKSGNTATLTITRVEAGDE ADYFCQVWDSSRNHPVFGGGTKLTVL
EV-D68-114	173	heavy	EVQLLES GGGLVQPGGSLRLSCAASGFRFSFYGMTWVRQ APGKGGLEWVSSISGTGATRCADSVKGRFTISRDNKNTL YLQMDSL RVDDTAVFYCVRRFPMTTVTSFDSWGQGTLLVT VSS
	174	light	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNFVSWYQ QHPGKAPKLMIFDVTGRPSGVPDRFSGSKSGNTASLTIAAG LQAEDEADYYCGAYAGFNALFGGGTKLTVL
EV-D68-116	175	heavy	EVQLVQSGGGGLVRPGGSVRLSCVASGFPMFMFWMGWVR QTPGKGGLEWVANIKQDGSEKYYVDSVKGRFAISRDNKNS LFLQMDSL SVGDTAIYYCVREGVRRVVVRSTGYFDFWGQ GQLVTVSS
	176	light	SYELTQPPSMVSPGQTARITCSGDAVPIKYVYWYQQRSG QAPVLVIYEDDRRPSGISERFSGSSSGTTATLTITGAQVED EGDYCYSTDSSGYQRAFGGGTTLTVL

EV-D68-150	177	heavy	RVQLQESAPGLVRPSETLSLTCSVSGGPISNGPYYWSWIR QHHPGKGLEWIGFIFYSGSTNYPNPSLRGRVTMAVDTSKNQF SLRLNSVTAADTAVYYCARHVV TASGWFDPPWGQGLTVTV SS
	178	light	EIVLTQSPATLSLSPGERATLSCRASQSVGTDLAWYQQKP GQAPRVLIYDAFKRATGIPARFSGSGSGTEFTLTISSELEPED FAVYYCQQRSRWPPPYTFGQGTKLEIK
EV-D68-151	179	heavy	RVQLQESAPGLVRPSETLSLTCSVSGGPISNGPYYWSWIR QHHPGKGLEWIGFIFYSGSTNYPNPSLRGRVTMAVDTSKNQF SLRLNSVTAADTAVYYCARHVV TASGWFDPPWGQGLTVTV SS
	180	light	EIVLTQSPATLSLSPGERATLSCRASQSVGTDLAWYQQKP GQAPRVLIYDAFKRATGIPARFSGSGSGTEFTLTISSELEPED FAVYYCQQRSRWPPPYTFGQGTKLEIK
EV-D68-152	181	heavy	QVHLVESGGGVVQPGRSLRLSCADSGVTFSDNALYWVRQ APGKGLEWVAVISYDGSSRYADSVRGRFTISRDNKSDL YLQMNRLRAEDTAIYYCARVTADYYESSGKVFWGQGLV VSS
	182	light	DIQMTQSPSTLSASVGDRVSITCRASQSVRSWLAWYQHHP GKAPKLLIYKASSLESQVPSRFSGSGSGTEFTLTISSLQAD DFATYYCQYQTFSWTFGQGTVEIK
EV-D68-154	183	heavy	QVQLQESGPALVKPSETLSLTCTVSGGSISDHYWSWIRQP PGKGLEWIGYIYTS GTTNYPNPSLKSRTISVDTSKKQFSLNL RSVTAADTAVYYCARSLTVIRFYYHYMDVWGKGTIVV S
	184	light	DIVMTQSPSLPVTGPGEPAISCRSSQSLQSDGYSYLDWY LQKPGQSPQLLIYLGSNRASGVPDRFSVIGSGTYFTLKISR VEAEDVGVYFCMQALQTPWTFGQGTKVEIK
EV-D68-155	185	heavy	QVQLQESGPGLVKPSETLSLTCTVAGGSIGDYHWNWIRQP AGKGLEWIGRIHSSGNTDYNPSLKSRTMSVDTSKNQFSL KLRSVTAADTAVYYCARQNVFDIWWGQGTMTVTVSS
	186	light	DIVMTQSPDSLALSLGERATINCKSSQSVLFSSNNKNYLAW YQQKPGQPPKLLIYWASTRESGVPDRFSGSGSETDFTLTIS SLQAEDVAVYFCQYFTTPTLTFGGGTKVEIK
EV-D68-156	187	heavy	QVQMQESGPGLVKASETSLTCSVSGISINNYWSWFRQP PGKGLEWIGYVYSTGSSKYNPSLERRATMSVDTSNNFSL RLTSVTTADTAVYYCARGSMPHIWGQGLLVTVSS
	188	light	QSVLTQPPSASGTPGQRTISCSGSTSNIETNYVYWYQQV PGTAPKPLVYRNDQRPSPGVPDRFSGSKSGTSASLVISGLR TEDEAAYYCAAWDDSLKAPVFGAGTKVAVL
	189	heavy	EVQLVESGGGLIKPGGSLRLSCAASGITFSNAWMSWVRQA PGKGLEWVGRIESKIDGGTIDYATPVKGRFTISRDDSKNTL

EV-D68-157			YLQMNSLKTEDTAVYYCTTDQGYDRSGYWVVGNHFDYWGQGILVTVSS
	190	light	QSVLTQPPSVSGAPGLRVITISCTGSSTNIGAGYDVHWYQHLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQADDAADYYCQSYDRSLSTYVFGTGKVTVL
EV-D68-158	191	heavy	EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSGISGTTGSTYYADSVKGRFTISRDN SKNTVHLQMNSLRAEDTAVYYCAKDSHSMIVVDHAFDIWGQGTMTVTVSS
	192	light	SYVLTQPPSVSVAPGQTARITCGGNNIGTKSVHWYQQRPGQAPVLVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSYNVHYVFGTGKVTVL
EV-D68-159	193	heavy	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTYIMTWVRQAPRGLEWVSSISTSSVYTFYADSLKGRFTISRDN AKNSVYLQMNSLRADDTAVYYCAREEGFRAYNLYWGQGT LTVTVSS
	194	light	QSVLTQPPSASGTPGQRTVITSCSGSSNIEYNVYVYQKFPGTAPKLLIYKNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEGDYYCAAWDDILSGVVFGGGTKLTVL
EV-D68-160	195	heavy	QVQLVESGGGVVQPGRSQKLSCAASGFTFSRFGMHWVRQAPGKGLEWVAVISFDGSNRYADSVKGRFTITRDNSKNTLYLQMNNLRPEDTAVYYCARDWDLVRSAVG YWGQGT LVS SVSS
	196	light	QSALTQPRSVSGSPGQSVTISCTGTSNDVGGYNFVSWYQQHPGKAPKLMIFDVIRPSGVPGRFSGSKSGDTASLIISGLQAEDEADYYCCSYAGTYTWVFGAGTT LTVL
EV-D68-161	197	heavy	QVHLQESGPRLVKPSETLSLTCTVSGGSVSTATYYWSWIRQSPGRGLEWIGYIYSSGNTNYPNPSLKSRTVITSLDTPNNQLSLTLTSVTAADTAIYYCERRLRILSIERNYYAMDVWGQGTPTVTVSS
	198	light	EVVLTQTPGTLSLSPGEGATLSCRASQRVVNNYLAWYQQRAGQAPRLLIFGASNRATGIPDRFSGSGSGTDFTLTIRKLEPEDFAVYYCQQYGSPWTFGHGTVEMK
EV-D68-162	199	heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGGRGLEWVAVISYDASKKYHADSVKGRFTISRDS SKNTLFLQMNSLKPEDTAVYYCARDHVPPKDCSDGNCHSDYGM DVWGQGT TTVTVSS
	200	light	DIQMTQSPSTMSASVGDRTITCRASQSISKWLAWYQQKPGKAPKLLIYKASTLQTGVPSRFSGSGSGTEFTLTINSLQPD FATYYCQQHNSYSYTFGQGT KVEIK
	201	heavy	QVQLVQSGAEVKKPGASVKISCKASGYSTNFVHWVRQAPGQRLEWMGWINPGNRNTKYSHNFQGRVTITRDT SANT

EV-D68-163			AYMELSSLRSDTAVYYCARLPAAAGRGWFDWPWGQGT LTVSS
	202	light	EIVLTQSPGTLSSLSPGERATLSCRASQSVISTYLAWYQQRP GQAPRVLIYDVSTRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYFCHQYQGSSPATFGQGTKVEIK
EV-D68-164	203	heavy	EVQLVESGGDLVQPGGSLRLSCAASGFTFNTYGMNWVRQ APGKGLEWVSYISSATTTFFYYADSVRGRFTISRDNKNSLF LHMKSLRDEDTAVYYCARVYTMRLRGASMDVWGHGTTVT VSS
	204	light	EIVLTQSPATLSSLSPGERATLSCRASQSVGTYLAWYQQKP GQAPRLLIYDSANRATGIPARFSGSGSGTDFTLTISSEPED FAVYYCQLRITWPPIFTFGPGTKVDVK
EV-D68-165	205	heavy	QVHLVQSGAEVKKSGASVKVSCKTFGYTFTAYYMHWVRQ APGQGPEWMGWINPISGGTNYAQKFQGRVSMTRDTSIST AYMGLSRLRPDDTAVYYCARVKCSSANCYGNFDYWGQG TLVTVSS
	206	light	QSALTQPPSASGSPGESVTISCTGTSSDVGGYNYVSWYQ QHPGKAPKLMIYEVSKRPSGVPDRFSGSKSGNTASLTVSG LQAEDEADYYCSSYAGSNNLVFGGGTKLTVL
EV-D68-166	207	heavy	EVQLVQSGAEVKKPGESLKISCKGSGYRFTNYRIGWVRQM PGKGLEWMGIYPGGS DTRYSPSLQGQVTMSVDKSISTAY LMWSSLKASDTAMYYCARQTTQNSGYDRWFDSWGQGT HVTSS
	208	light	QSVLTQPPSASGTPGQRTVISCSTSSIGSNIVNWYQHLP GTAPKLLIYINNQRPSGVPDRFSGSKSGTSASLAISGLQSE DEADYYCAA WDDSLNGWVFGGGTKLTVL
EV-D68-181	209	heavy	EVQVLES GGGLVQPGGSLRLSCAASGITFSRHTMSWVRQ APGKGLEWVSAISGSGGSTYHADSVKGRFTISRDSKSTL YLQMNSLR AEDTAVYYCAISVPLLRFLEWFQHPFDWGGQ GLTVTVSS
	210	light	EIVMTQSPASLSVSPGERVTLSCRASQSVGSTLAWYQHHP GQAPRLLISGA STRATGVPARFSGSGSGTEFTLTISLQSE DFAVYYCHQYINWPPWTFGQGTKVEIK
EV-D68-183	211	heavy	EVRLVESGGGLVKPGGSLRLSCAASGFTFNTYSMSWVRQ APGKGLEWVASISSTGSIYNADSLKGRFTISRDNKNSLF LQMNSLRVEDTAVYYCVRFTMTTVTNFDSWGQGT LTVSS
	212	light	QSALTQPRSVSGSPGQSVTISCTGTSSDVGAYSIVSWYQ QHPGKAPKLMIYDVYRRPSGVPGRFSGSKSGNTASLTVSG LQAEDEADYYCCSHAGSHTWVFGGGTKVTVL
	213	heavy	QLQVVASGGGVVQPGRLRLSCKASGFTFTNYGMHWVR QAPGKGLEWVAFISYDGGNKFYADSVKGRFTISRDN SRNT

EV-D68-185			VYLMNSLRVADTAMYYCPKVIHPYDSSGDDAFDIWGG GTMVAISS
	214	light	EIVMTQSPATLSVSPGERATLSCWASQSI SRNLAWYQQKP GQAPRLLIYGASTRATGIPAKFSGSGSGTDFTLT VSSLQSE DLAVYYCQQYSKLPITFGQGTRLEIK
EV-D68-200	215	heavy	EVQLVESGGGLVQPGGSLRLSCAASGFPFSSYSMSWVRQ APGKGLEWVSYSISGSGGDIYYADSVKGRFTISRDNARNSL SLQMNSLRADDTAVYYCARGLVATTGTRYFDYWGQGT L V TVSS
	216	light	EIVLTQSPATLSLSPGERATLSCRASQSVRSYLA WYQQRP GQAPRLLIYDASN RATGIPVRFSGSGSGTDFTLT ISSLEPED FAVYYCQQRSYWPPFTFGGGTKVEIK
EV-D68-208	217	heavy	QVQLVQSGAEVKKPGSSVKVSCKASGGTFRRFAISWVRQ APGQGLEWMGGIIPILGRGKYAQKFQGRVRITADESTSTAY MELSSLRSEDTAVYYCARFISTASYVPGTFEDVWGQGT T V TVSS
	218	light	DIVLTQSPATLSLSPGERATLSCRASQSVSSYLA WYQQKP GQAPRLLIYDASNRAAGIPARFSGSGSETDFTLT ISSLEPED VAVYYCQQRSDWPPGTFGQGTNVEIK
EV-D68-210	219	heavy	EVQLVESGGGLVKPGGSLRLSCAASGFTFRNYNINWVRQ APGKGLEWVSSISSTGSYIHYADLVKGRFTISRDN AKNSLY LQMNSLRVEDTAVYYCARMVRNTVTAFDYWGQGT L VSV S
	220	light	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQ QPGRAPKLLIYEVIKRPSGVSDRFSGSKSGDTASLTISGLQ AEDEADYYCCSYGGNNSWMFGGGTMLTVL
EV-D68-219	221	heavy	QVQVVQSGAEMKKPGASVKVSCKVS GYRLIDLPLHWVRQ APGKGLEWMGLFDPEKAEAIYSQKFQDKVTISEDTSIDTAY MELNSLRSEDTAVYYCATWGVEVVNGRRDYFDSWGQGT LVT VSS
	222	light	SYELTQPPSVSVSPGQTARITCYADVLSNQYTYWYQQKPG QAPVLVIYK DTERPSGIPERFAGSSSGTTVTLVINGVRAED EAYYYCQSADNTRITVFGGGTKLSVL
EV-D68-220	223	heavy	GVQLVESGGGLIQPGGSLRLSCAASGFTFSSFEMNWVRQ APGKGLEWVS YISTSGSTIYYADSVKGRFTISRDNARNSL S LQMNSLRAEDTAVYYCARDVRDCSALTCPRRGDAFDFWG RGTRVT VSS
	224	light	DVVMTQSPLSLPVTLGQSASISCRSSQSLVYSDGNTYLNW FQQRPGQSPRRLLIYKVSNRDSGVPDRFSGSGSGTDFTLKI SRVEAEDVG VYYCMQGT HWPRTFGPGTKVDIK
	225	heavy	EVQLVESGGGLVKPGGSLRLSCAASGF SFSVYPMNWVRQ APGKGLEWVSSISSSSRYISYADSLRGRITISRDN AKNSLYL

EV-D68-221			QMNSLRVEDTAVYYCVKVGGSKHQYYFDYWGGQSLVTVS S
	226	light	QSVLTQPPSTSGIPGQTVTISCSGSRNIGSYTVNWYEQLP GTAPKLVIFNNNQRPSPGVDPDRFSGSKSGTSASLAISGLQSE GEADYYCAAWDDSLNGVVFGGGTKLTVL
EV-D68-224	227	heavy	QVQLVQSGAEVKRPGASVKVFCKASGYTFTNYDIIWVRQA PGQGLEWVGWISTYNGNTNYEQNLQGRVTMTTDTSTSTA YMELRSLRSDDTAVYYCARERCSTSTCYSRYADYWGGQT LTVVSS
	228	light	DIQMTQSPSSLSASVGDRVITICRASQSINIYLNWYQQKPG KAPKVLIIYAASSLQSGVPSRFRGSGSGTDFILTISLQPEDF ATYYCQQSYRSPRTFGQGTEVEIK
EV-D68-225	229	heavy	EVQLVESGGGLAQPGESEMRSLSCVASGFTLSRYEVNWVRQ APGKGLEWLSYISSGGPSIYYADSVKGRFTISRNSAENSLE LQMSTLRTEDTAVYYCMREGLTYDSTIWGGQGTAVVSS
	230	light	QNVLTQSPSASASLGASVKLTCTLNSGHSRYAIAWHQHQP QRGPRFLMKINSDGRHIRGDGISDRFSGSASGAERHLTISS LQPEDEADYYCQTWGTGFRVFGGGTKLTVL
EV-D68-227	231	heavy	EVQLVESGGGLVQPGRLRLSCAASGFTFDEYAMHWVRQ VPGKGLEWVSGISWNGGSKGYADSVKGRFTISRDNARYS LSLQMNSLRTEDTALYYCAKDDYEGAGFDIWGGQGTVVTVS S
	232	light	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLGWYQQKS GQAPRLIIYGASSRATGIPARFSGSGSGTDFLTISLQPED FAIYYCQQRSNWPITFGQGTREIK
EV-D68-228	233	heavy	QVQLQESGPGLVKPSETLSLTCTVSGYLISNGYYWGWIQR SPGKGLEWIGSIYYTRDTYYNWSLKSRTISVDTSKKQFSLK LYSVTAADTAVYYCVRHEGSCNDGSCYGSFVDNWGGQGT LTVVSS
	234	light	DIQMTQSPSSVSASVGDRVTLTCRASQDISSWLAWYQQK PGKAPKLLIIYAASSLQSGVPSRFRSGSGSGTHFTLTISLQ EDFATYFCQQADSFITFGGGTKVEIK
EV-D68-231	235	heavy	EVQLLES GGGLVQPGGSLRLSCAASDFTFSSYTMAWVRQ APGKGLEWVSSISGDGVSTKNADSVKGRFSVSRDNSKNTL FLQLNSLRAEDTAFYYCARGGTFHNWYFDLWGRGVLTV SS
	236	light	EIVMTQSPATLSVSPGETATLSCRASQSIGDNLAWYQQKP GQAPRLIIYGASTRATDFPARFRGSGSGTEFTLTISLQSE DFAVYYCQQYKNWPRTFGRGTKVEVR
	237	heavy	EVQLLES GGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQ APGKGP EWVSGISGSGGSTNYADSAKGRFTISRDN SKNTL

EV-D68-234			YLQMNSLRAEDTAVYYCAKGTITYSYYYMAVWGKGTTVTVSS
	238	light	EIVLTQSPGTLISLSPGERGTLSRASQSVSRSSYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTIGRLEPEDFAVYYCQQYGTSITFGGGTKVEIK
EV-D68-235	239	heavy	QVQVVQSGAEMRKPGASVRVSCVKVSGYRLTDLPLHWVRQAPGKGLEWIGFVDLEKREIIYAQKFQGKVTITEDTSADTAYMELNSLRSED TAVYYCATWGIEVVNGRDEFFDSWGQGTLVSVSS
	240	light	SYELTQPPSVSVSPGQTARITCYADVLSKQYTYWYQQKPGQAPVLVIYK DTERPSGIPERFAGSSSGTTVTLIINGV RTEDEAYYYCQSADTRITVFGGGTKLSVL
EV-D68-236	241	heavy	QVQVVQSGAEMKKPGASVKVSCVKVSGYSLSDLPLHWVRQAPGKGLEWMGLFDPINGEIIYAQTFQGKVTISEDTSIDTAYMELNSLRSED TAVYYCATWGVAVVSGRRDYFDSWGQGTLVTVSS
	242	light	SYELTQPPSVSVSPGQTARITCYAAVLSNQYTYWYQQKPGQAPVLVIYK DTERPSGIPERFAGSSSGTTVTLIINGV RTEDEAYYYCQTADTKYTVFGGGTKLSVL
EV-D68-241	243	heavy	QVQLQESGPGLVKPSQTLSTCTVSGGSITSGDYWNWIRQPPGKGLEWIGYIYHSGTTYNPSLKSRTISVDTSKNRFS LKLSSVTAADTAVYFCARAYAYEFWSGYPNWFDPWGLGLTVTVSS
	244	light	DIQMTQSPSSLSASVGDRVTITCRASQRISTYVNWYQVKA GTAPKVLIIYAASSLQTGVPSRFSGSGSGTDFTLTIVSLQPE DFTTYFCQQSYSPPWTFGGGTKVEIK
EV-D68-247	245	heavy	QVQLVESGGGLVQPGRSLRLSCAASGFIFNRYAMHWVRQAPGKGLEWVALISYDGINKYYADSVKGRFSISRDNSTLYLQMNSLRAEDTAIFYCARGLG YCSGTGGSCPTFEYWGGQGLVTVSS
	246	light	DIQMTQSPSSLSASVGDRVTITCRASQSIKKYLNWYQQKPGNAPKLLIYGASNLQTGVPSRFSGSGSGTDFA LSISSLETE DFATYYCQQSDSAPPTFGGGTKVEFK
EV-D68-254	247	heavy	QVQLQQRGAGLLKPSETLSLTCEIYGASLNDYDWTWIRQPGKRLEWIGVINRRD TVDYNPSLKSRTLSLDTSKNQLSLS LSSVTAADTAVYYCVRVPRRGFE GSF GFCDDTACRYGHTWFDPWGQGT LVTVSS
	248	light	DIQMTQSPSSLSASVGDRVTITCRASQSIRDYLNWYQQRP GKAPKVLIFAGSRLES GVP SRFRGRGSGTEFTLTISDLQPE DFATYYCQQSYLTPPTFGGGTTVDIK
	249	heavy	QVTLKESGPVLVKPTETLTCTVSGFSLRN GRMGVSWIRQPPGKALEWLAHIFASDEKSYSTSQRTRLSISRDTSKSQVV

EV-D68-260			LSMTDMDPVDATATYYCARILKFGTMRAAYYFDYWGGAL VPVSS
	250	light	SYVLTQPPSVSVAPGKTARITCGGINIGIRTVHWYQQKPGQ APMLVIYYDSRPLGIPERFSGSKSGNTATLTISRVEAGDE ADYYCQVWDSSSDHVVFGGGTKLTAL
EV-D68-266	251	heavy	QVHLVESGGGLVKPGGSLRLSCAVSGFTFSDYYMSWIRQ APGKGLEWLSYISSSGSTIYYADSMKGRFTISRDNARNSLY LQMNSLRVEDTAFYYCAGSKVGYYTTGRRNWFYDLWGRG TLVTVSS
	252	light	QSALTQPPSASGSPGQSVTISCTGTSSDVGAYNYVSWYQ QHPGKAPKLMIYEVTKRPSGVPDRFSGSKSGNTASLTVSG LQAEDEADYYCSSYAGNNNLVFGGGTKLTVL
EV-D68-269	253	heavy	QVQLLQSGSEVRKPGASVNIHCKASGFTFTDFYLHWVRQA PGQGLEWMGIINPETGETTYSQKFQGRVTMTTRDTSTSVVN LEVRSLRSEDTAIYYCARDLVVVVPVEMSRRAFDIWGGIM VTVSS
	254	light	SYVLTQPPSVSVAPGQTARIPCGGNNIERKSVHWYQQR GQAPVLVYDDTVRPSGIPERFSGSNSGSTATLTISRVGAG DEGDYYCQVWDSTTDHGVFGGGTKLTVL
EV-D68-271	255	heavy	RVQLVQSGAEVKKPGASVKVSCQTSGYIFSAYYIYWVRQA PGQGLEWMGRMNAKSGGANTAQQFQGRLTMTDRMSVS TAYMELSRLRSDDTAVYYCARDYRDDYMWGSYRPLDYW GQGTLVTVSS
	256	light	EVVLTQSPATLSLSPGERASLSCRASQSVSGYLAWYQHQP GQAPRLLIYDASNRAAGIPARFRGSGSGTDFTLTISSELEPE DFAVYFCQQRSNGLTFGGGKVEIK

TABLE 3 – CDR HEAVY CHAIN SEQUENCES

Clone	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
EV- D68-37	GFIFSRYA (257)	ISYDARNS (258)	ARPTLPYSNNWYAPEY (259)
EV- D68-40	GFSFSSYA (260)	ISGNGNGR (261)	AKVVRIAAVLYYFDY (262)
EV- D68-41	GFTFSNYA (263)	ISGSGGLT (264)	ARVKSTTGTTALVFDI (265)
EV- D68-43	GFTFINYG (266)	ISNDGSYN (267)	AKDKHGDYDYGVVDV (268)
EV- D68-46	GFTFSSYG (269)	ISYDGSDN (270)	ARRRPGSFPGLCDY (271)
EV- D68-48	GGFSRSLT (272)	HIPIFGTT (273)	ARMYSGHDGVVDV (274)
EV- D68-71	GGSISSGFYY (275)	IYDSGRT (276)	ARHLTHLYGDYVTPDALDI (277)
EV- D68-72	GFTFTTYS (278)	ISSGSSNI (279)	ARAHGRIVNSGVVISRFDP (280)
EV- D68-74	GYTFTAYY (281)	INPSSGGA (282)	ARMGCRSDRCYSTNYNFDQ (283)
EV- D68-75	GFTISPYG (284)	ISSSSRYT (285)	ARERGHSTSSSYFDS (286)
EV- D68-76	GFTFSNAW (287)	IQTKTDGGTT (288)	STGPYYDTSGYPQPFDY (289)
EV- D68-80	GFDFSRYT (290)	ISSTSLYT (291)	ARVVGPAELDY (292)
EV- D68-84	GYTFTDYY (293)	INPKTGGS (294)	ARAGRNGYDY (295)
EV- D68-85	GFKFRNYA (296)	ITSGGST (297)	TVPWGNYN DYVSDY (298)
EV- D68-88	GFIFSRYP (299)	ISYDGNNK (300)	ARHFLPYSSSWYQGFNY (301)
EV- D68-89	GFLFSRYG (302)	ISYDGNKK (303)	ARGVPYGDTLTGLVY (304)
EV- D68-95	GFSLRNARMG (305)	IFSNDEK (306)	ARLLVAGTFLPSHYFDY (307)
EV- D68-97	GFTFSTYS (308)	ISSSSSTI (309)	TRQVGADFSGRGFDY (310)
EV- D68-98	GFKFSVYA (311)	ISSSGSTI (312)	ATARHITNDGFDI (313)
EV- D68- 105	GYLISNGYY (314)	IYHTRST (315)	ARGPGHCYGD DDCYAYYFDQ (316)
EV- D68- 110	GGTFSRFA (317)	ILPIFGTA (318)	ARSLPYCTNDVCSNQNTFDY (319)
	GGSISSGDYY	IYSGST	ASRYGDP IGDNW FDP

EV-D68-111	(320)	(321)	(322)
EV-D68-114	GFRFSFYG	ISGTGATR	VRRFPMTTVTSFDS
	(323)	(324)	(325)
EV-D68-116	GFPFNMFV	IKQDGSEK	VREGVRRVVVRSTGYFDF
	(326)	(327)	(328)
EV-D68-150	GGPISNGPYY	IFYSGST	ARHVVTASGWFDV
	(329)	(330)	(331)
EV-D68-151	GGPISNGPYY	IFYSGST	ARHVVTASGWFDV
	(332)	(333)	(334)
EV-D68-152	GVTFSDNA	ISYDGSSR	ARVTADYYESSGKVF
	(335)	(336)	(337)
EV-D68-154	GGISDHY	IYTSITT	ARSLETVIRFYHYHYMDV
	(338)	(339)	(340)
EV-D68-155	GGSIGDYH	IHSSGNT	ARQNVFDI
	(341)	(342)	(343)
EV-D68-156	GISINYY	VYSTGSS	ARGSMPI
	(344)	(345)	(346)
EV-D68-157	GITFSNAW	IESKIDGGTI	TTDQGYDRSGYWVGNNHFDY
	(347)	(348)	(349)
EV-D68-158	GFTFSSYA	ISGTTGST	AKDSHSMIVVDHAFDI
	(350)	(351)	(352)
EV-D68-159	GFTFSTYI	ISTSSVYT	AREEGFRAYNLY
	(353)	(354)	(355)
EV-D68-160	GFTFSRFG	ISFDGSNR	ARDWDRLVRSVAGY
	(356)	(357)	(358)
EV-D68-161	GGSVSTATYY	IYSSGNT	ERRLRILSIERNYYAMDV
	(359)	(360)	(361)
EV-D68-162	GFTFSSYA	ISYDASKK	ARDHVPPKDCSDGNCHSDYGMDV
	(362)	(363)	(364)
EV-D68-163	GYSFTNFA	INPGNRNT	ARLPAAAGRGWFDP
	(365)	(366)	(367)
EV-D68-164	GFTFNTYG	ISSATTTT	ARVYTMLRGASMDV
	(368)	(369)	(370)

EV-D68-165	GYTFTAYY	INPISGGT	ARVKCSSANCYGNFDY
	(371)	(372)	(373)
EV-D68-166	GYRFTNYR	IYPGGSDT	ARQTTQNSGYDRWFDS
	(374)	(375)	(376)
EV-D68-181	GITFSRHT	ISGSGGST	AISVPLLRFLEWFQHPFDF
	(377)	(378)	(379)
EV-D68-183	GFTFNTYS	ISSTGSYI	VRFTMTTVTNFDS
	(380)	(381)	(382)
EV-D68-185	GFTFTNYG	ISYDGGNK	PKVIPHPYYDSSGDDAFDI
	(383)	(384)	(385)
EV-D68-200	GFPFSSYS	ISGSGGDI	ARGLVATTGTRYFDY
	(386)	(387)	(388)
EV-D68-208	GGTFRRFA	IIPILGRG	ARFISTASYVPGTFEDV
	(389)	(390)	(391)
EV-D68-210	GFTFRNYN	ISSTGSYI	ARMVRNTVTAFDY
	(392)	(393)	(394)
EV-D68-219	GYRLIDL	FDPEKAEA	ATWGVVNVNGRRDYFDS
	(395)	(396)	(397)
EV-D68-220	GFTFSSFE	ISTSGSTI	ARDVRDCSALTCPRRGDAFDF
	(398)	(399)	(400)
EV-D68-221	GFSFSVYP	ISSSSRYI	VKVGGSKHQYYFDY
	(401)	(402)	(403)
EV-D68-224	GYTFTNYD	ISTYNGNT	ARERCSTSTCYSRYADY
	(404)	(405)	(406)
EV-D68-225	GFTLSRYE	ISSGGPSI	MREGLTYDSTI
	(407)	(408)	(409)
EV-D68-227	GFTFDEYA	ISWNGGSK	AKDDYEGAGFDI
	(410)	(411)	(412)
EV-D68-228	GYLISNGYY	IYYTRDT	VRHEGSCNDGSCYGSFVDN
	(413)	(414)	(415)
EV-D68-231	DFTFSSYT	ISGDGVST	ARGGTFHNWYFDL
	(416)	(417)	(418)
EV-D68-234	GFTFSTYA	ISGSGGST	AKGTITYSYYYMAV
	(419)	(420)	(421)

EV-D68-235	GYRLTDLP	VDLEKREI	ATWGIEVVNGRDEFFDS
	(422)	(423)	(424)
EV-D68-236	GYSLSLDP	FDPINGEI	ATWGVAVVSGRRDYFDS
	(425)	(426)	(427)
EV-D68-241	GGSTITSGDYY	IYHSGTT	ARAYAYEFWSGYPNWFDP
	(428)	(429)	(430)
EV-D68-247	GFIFNRYA	ISYDGINK	ARGLGYCSGTGGSCPTFEY
	(431)	(432)	(433)
EV-D68-254	GASLNDYD	INRRDTV	VRVPRRGFEGSFSGFCDDTACRYGHTWFDP
	(434)	(435)	(436)
EV-D68-260	GFSLRNRMG	IFASDEK	ARILKFGTMRAAYYFDY
	(437)	(438)	(439)
EV-D68-266	GFTFSDYY	ISSSGSTI	AGSKVGYTTGRRNWYFDL
	(440)	(441)	(442)
EV-D68-269	GFTFTDFY	INPETGET	ARDLVVVVPVEMSRRAFDI
	(443)	(444)	(445)
EV-D68-271	GYIFSAYY	MNAKSGGA	ARDYRDDYMWGSYRPLDY
	(446)	(447)	(448)

TABLE 4 – CDR LIGHT CHAIN SEQUENCES

Clone	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)
EV-D68-37	ALPKKY	EDT	SSTDSSGNPVL
	(449)	(450)	(451)
EV-D68-40	QSVSTY	EAS	QQRSSWPIT
	(452)	(453)	(454)
EV-D68-41	SGSVSSSY	SIN	GLYMGSGIWI
	(455)	(456)	(457)
EV-D68-43	QGISSW	AAS	QQADSFPR
	(458)	(459)	(460)
EV-D68-46	QSISKW	KAS	QQHNSYSYT
	(461)	(462)	(463)
EV-D68-48	QSVRSY	DAS	QQRSTWPPGM
	(464)	(465)	(466)
EV-D68-71	QSVSSSF	GAS	QQYSNSRLT
	(467)	(468)	(469)
EV-D68-72	SSDVGGYNY	EVT	SSYTTSNTLVV
	(470)	(471)	(472)
EV-D68-74	NIGTKS	NDS	QVWDSGIDVV
	(473)	(474)	(475)
EV-D68-75	NIGTKT	YDS	RVWDSDTDHRV
	(476)	(477)	(478)
EV-D68-76	KLGDKY	QDT	QAWDSSTVV
	(479)	(480)	(481)
EV-D68-80	QDIGVD	GAS	LQDYNYPWT
	(482)	(483)	(484)
EV-D68-84	ALPKQY	QDT	QSGDSSGTYL
	(485)	(486)	(487)
EV-D68-85	QRVGNS	DAS	HQHSTWPRGT
	(488)	(489)	(490)
EV-D68-88	SGSIATNY	EDS	QSYDNSNRAVV
	(491)	(492)	(493)
EV-D68-89	SGTIASNY	EDN	QSYDNSDRV
	(494)	(495)	(496)
EV-D68-95	NIGLKS	YDS	QVWDSSRNHPV
	(497)	(498)	(499)
EV-D68-97	KLGDKY	QDS	QAWDSSTAV
	(500)	(501)	(502)
EV-D68-98	QGISRF	SAS	QQLNSHPRMFT
	(503)	(504)	(505)
EV-D68-105	QGISNW	DAS	QQANSFPFT
	(506)	(507)	(508)
EV-D68-110	ALPKQY	EDN	QSADSSGTYYV
	(509)	(510)	(511)
EV-D68-111	NIGLKS	YDS	QVWDSSRNHPV
	(512)	(513)	(514)
EV-D68-114	SSDVGGYNF	DVT	GAYAGFNAL
	(515)	(516)	(517)

EV-D68-116	AVPIKY	EDD	YSTDSSGYQRA
	(518)	(519)	(520)
EV-D68-150	QSVGTD	DAF	QQRSRWPPPYT
	(521)	(522)	(523)
EV-D68-151	QSVGTD	DAF	QQRSRWPPPYT
	(524)	(525)	(526)
EV-D68-152	QSVRSW	KAS	QQYQTFSTWT
	(527)	(528)	(529)
EV-D68-154	QSLQSDGYSY	LGS	MQALQTPWT
	(530)	(531)	(532)
EV-D68-155	QSVLFSSNNKNY	WAS	QQFYTTPLT
	(533)	(534)	(535)
EV-D68-156	TSNIETNY	RND	AAWDDSLKAPV
	(536)	(537)	(538)
EV-D68-157	STNIGAGYD	GNS	QSYDRSLSTYV
	(539)	(540)	(541)
EV-D68-158	NIGTKS	DDS	QVWDSYNVHYV
	(542)	(543)	(544)
EV-D68-159	SSNIEYNY	KNN	AAWDDILSGVV
	(545)	(546)	(547)
EV-D68-160	SNDVGGYNF	DVI	CSYAGTYTWV
	(548)	(549)	(550)
EV-D68-161	QRVVNNY	GAS	QQYGGSPWT
	(551)	(552)	(553)
EV-D68-162	QSISKW	KAS	QQHNSYSYT
	(554)	(555)	(556)
EV-D68-163	QSVISTY	DVS	HQYGSSPAT
	(557)	(558)	(559)
EV-D68-164	QSVGTY	DSA	QLRITWPPIFT
	(560)	(561)	(562)
EV-D68-165	SSDVGGYNY	EVS	SSYAGSNNLV
	(563)	(564)	(565)
EV-D68-166	TSSIGSNI	INN	AAWDDSLNGWV
	(566)	(567)	(568)
EV-D68-181	QSVGST	GAS	HQYINWPPWT
	(569)	(570)	(571)
EV-D68-183	SSDVGAYS	DVY	CSHAGSHTWV
	(572)	(573)	(574)
EV-D68-185	QSIERN	GAS	QQYSKLPIT
	(575)	(576)	(577)
EV-D68-200	QSVRSY	DAS	QQRSYWPPFT
	(578)	(579)	(580)
EV-D68-208	QSVSSY	DAS	QQRSDWPPGT
	(581)	(582)	(583)
EV-D68-210	SSDVGGYNF	EVI	CSYGGNNSWM
	(584)	(585)	(586)
EV-D68-219	VLSNQY	KDT	QSADNTRITV
	(587)	(588)	(589)
EV-D68-220	QSLVYSDGNTY	KVS	MQGTHWPRT
	(590)	(591)	(592)
	RSNIGSYT	NNN	AAWDDSLNGVV

EV-D68-221	(593)	(594)	(595)
EV-D68-224	QSINIY	AAS	QQSYRSPRT
	(596)	(597)	(598)
EV-D68-225	SGHSRYA	INSDGRH	QTWGTGFRV
	(599)	(600)	(601)
EV-D68-227	QSVSSY	GAS	QQRSNWPIT
	(602)	(603)	(604)
EV-D68-228	QDISSW	AAS	QQADSFIT
	(605)	(606)	(607)
EV-D68-231	QSIGDN	GAS	QQYKNWPRT
	(608)	(609)	(610)
EV-D68-234	QSVRSSY	GAS	QQYGTSIT
	(611)	(612)	(613)
EV-D68-235	VLSKQY	KDT	QSADTRITV
	(614)	(615)	(616)
EV-D68-236	VLSNQY	KDT	QTADTKYTV
	(617)	(618)	(619)
EV-D68-241	QRISTY	AAS	QQSYSPPWT
	(620)	(621)	(622)
EV-D68-247	QSIKKY	GAS	QQSDSAPPT
	(623)	(624)	(625)
EV-D68-254	QSIRDY	AGS	QQSYLTPPT
	(626)	(627)	(628)
EV-D68-260	NIGIRT	YDS	QVWDSSSDHVV
	(629)	(630)	(631)
EV-D68-266	SSDVGAYNY	EVT	SSYAGNNNLV
	(632)	(633)	(634)
EV-D68-269	NIERKS	DDT	QVWDSTTDHGV
	(635)	(636)	(637)
EV-D68-271	QSVSGY	DAS	QQRSNGLT
	(638)	(639)	(640)

5 All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. More

10 specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

VII. REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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U.S. Patent 3,996,345

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U.S. Patent 4,275,149

U.S. Patent 4,277,437

U.S. Patent 4,366,241

U.S. Patent 4,472,509

U.S. Patent 4,554,101

U.S. Patent 4,680,338

U.S. Patent 4,816,567

U.S. Patent 4,867,973

U.S. Patent 4,938,948

U.S. Patent 5,021,236

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WHAT IS CLAIMED IS:

1. A method of treating a subject infected with enterovirus D68 (EV-D68) or reducing the likelihood of infection of a subject at risk of contracting EV-D68, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
2. The method of claim 1, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.
3. The method of claim 1-2, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identity to as set forth in Table 1.
4. The method of claim 1-2, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
5. The method of claim 1, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
6. The method of claim 1, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
7. The method of claim 1, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
8. The method of claims 1-7, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment, or the antibody is a chimeric antibody or a bispecific antibody.

9. The method of claims 1-8, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation, or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
10. The method of claims 1-9, further comprising treating said subject with a second therapy, such as an anti-viral therapy or an anti-inflammatory therapy.
11. The method of claim 1-10, wherein said antibody or antibody fragment is administered prior to infection or after infection.
12. The method of claim 1-11, wherein said treating treats lung infection and/or neurologic infection, or wherein treating reduces lung infection and/or neurologic infection.
13. The method of claim 1-12, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.
14. A method of detecting enterovirus D68 (EV-D68) infection in a subject comprising:
 - (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (b) detecting EV-D68 in said sample by binding of said antibody or antibody fragment to a EV-D68 antigen in said sample.
15. The method of claim 14, wherein said sample is a body fluid.
16. The method of claims 14, wherein said sample is blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, respiratory droplets or aerosol, tissue scrapings or feces.

17. The method of claims 14-16, wherein detection comprises ELISA, RIA, lateral flow assay or Western blot.
18. The method of claims 14-17, further comprising performing steps (a) and (b) a second time and determining a change in EV-D68 antigen levels as compared to the first assay.
19. The method of claims 14-18, wherein the antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
20. The method of claims 14-18, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.
21. The method of claims 14-18, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.
22. The method of claims 14-18, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
23. The method of claims 14-18, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
24. The method of claims 14-18, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
25. The method of claims 14-24, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

26. A monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
27. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
28. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
29. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.
30. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
31. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
32. The monoclonal antibody of claims 26-31, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
33. The monoclonal antibody of claims 26-31, wherein said antibody is a chimeric antibody, or is bispecific antibody.
34. The monoclonal antibody of claim 26-33, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase

therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.

35. The monoclonal antibody of claim 26-34, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.
36. A hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
37. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
38. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 1.
39. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired variable sequences from Table 1.
40. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
41. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 2.

42. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
43. The hybridoma or engineered cell of claims 36-42, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
44. The hybridoma or engineered cell of claim 36-43, wherein said antibody is a chimeric antibody or a bispecific antibody.
45. The hybridoma or engineered cell of claim 36-43, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
46. The hybridoma or engineered cell of claim 36-45, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.
47. A vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
48. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
49. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

50. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.
51. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
52. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
53. The vaccine formulation of claims 47-52, wherein at least one of said antibody fragments is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
54. The vaccine formulation of claims 47-52, wherein at least one of said antibodies is a chimeric antibody or is bispecific antibody.
55. The vaccine formulation of claims 47-54, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
56. The vaccine formulation of claims 47-55, wherein at least one of said antibodies or antibody fragments further comprises a cell penetrating peptide and/or is an intrabody.
57. A vaccine formulation comprising one or more expression vectors encoding a first antibody or antibody fragment according to claims 26-34.

58. The vaccine formulation of claim 57, wherein said expression vector(s) is/are Sindbis virus or VEE vector(s).
59. The vaccine formulation of claims 57-58, formulated for delivery by needle injection, jet injection, or electroporation.
60. The vaccine formulation of claim 57, further comprising one or more expression vectors encoding for a second antibody or antibody fragment, such as a distinct antibody or antibody fragment of claims 26-34.
61. A method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with enterovirus D68 comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
62. The method of claim 61, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.
63. The method of claim 61-62, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identity to as set forth in Table 1.
64. The method of claim 61-62, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
65. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
66. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.

67. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
68. The method of claims 61-67, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
69. The method of claims 61-68, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE, DHS or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
70. The method of claims 61-67 wherein said antibody is a chimeric antibody or a bispecific antibody.
71. The method of claim 61-70, wherein said antibody or antibody fragment is administered prior to infection or after infection.
72. The method of claim 61-71, wherein said subject is a pregnant female, a sexually active female, or a female undergoing fertility treatments.
73. The method of claim 61-72, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.
74. The method of claim 61, wherein the antibody or antibody fragment increases the size of the placenta as compared to an untreated control.

75. The method of claim 61, wherein the antibody or antibody fragment reduces viral load and/or pathology of the fetus as compared to an untreated control.
76. A method of determining the antigenic integrity, correct conformation and/or correct sequence of an enterovirus D68 antigen comprising:
- (a) contacting a sample comprising said antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (b) determining antigenic integrity, correct conformation and/or correct sequence of said antigen by detectable binding of said first antibody or antibody fragment to said antigen.
77. The method of claim 76, wherein said sample comprises recombinantly produced antigen.
78. The method of claim 76, wherein said sample comprises a vaccine formulation or vaccine production batch.
79. The method of claims 76-78, wherein detection comprises ELISA, RIA, western blot, a biosensor using surface plasmon resonance or biolayer interferometry, or flow cytometric staining.
80. The method of claims 76-79, wherein the first antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
81. The method of claims 76-79, wherein said first antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.
82. The method of claims 76-79 wherein said first antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.

83. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
84. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
85. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
86. The method of claims 76-85, wherein the first antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
87. The method of claims 76-86, further comprising performing steps (a) and (b) a second time to determine the antigenic stability of the antigen over time.
88. The method of claims 76-87, further comprising:
- (c) contacting a sample comprising said antigen with a second antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (d) determining antigenic integrity of said antigen by detectable binding of said second antibody or antibody fragment to said antigen.
89. The method of claim 88, wherein the second antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
90. The method of claim 89, wherein said second antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.

91. The method of claim 89, wherein said second antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.
92. The method of claims 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
93. The method of claim 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
94. The method of claim 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
95. The method of claim 89, wherein the second antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
96. The method of claim 89, further comprising performing steps (c) and (d) a second time to determine the antigenic stability of the antigen over time.
97. A human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody binds to EV-D68 across at least two viral clades.
98. A human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody binds to EV-D68 VP1, VP2 and VP3.
99. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same of claim 98, wherein said antibody binds to EV-D68 VP1 DE loop.

100. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same of claim 98, wherein said antibody binds to EV-D68 VP2 EE loop.
101. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same of claim 98, wherein said antibody binds to EV-D68 VP3 N-terminal loop.
102. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same of claim 98, wherein said antibody binds to (a) EV-D68 VP3 N-terminal loop and EV-D68 VP2 EE loop, or (b) EV-D68 VP2 EE loop and EV-D68 VP1 DE loop, or (c) EV-D68 VP1 DE loop and EV-D68 VP3 N-terminal loop, or (d) EV-D68 VP3 N-terminal loop, EV-D68 VP1 DE loop and EV-D68 VP2 EE loop.

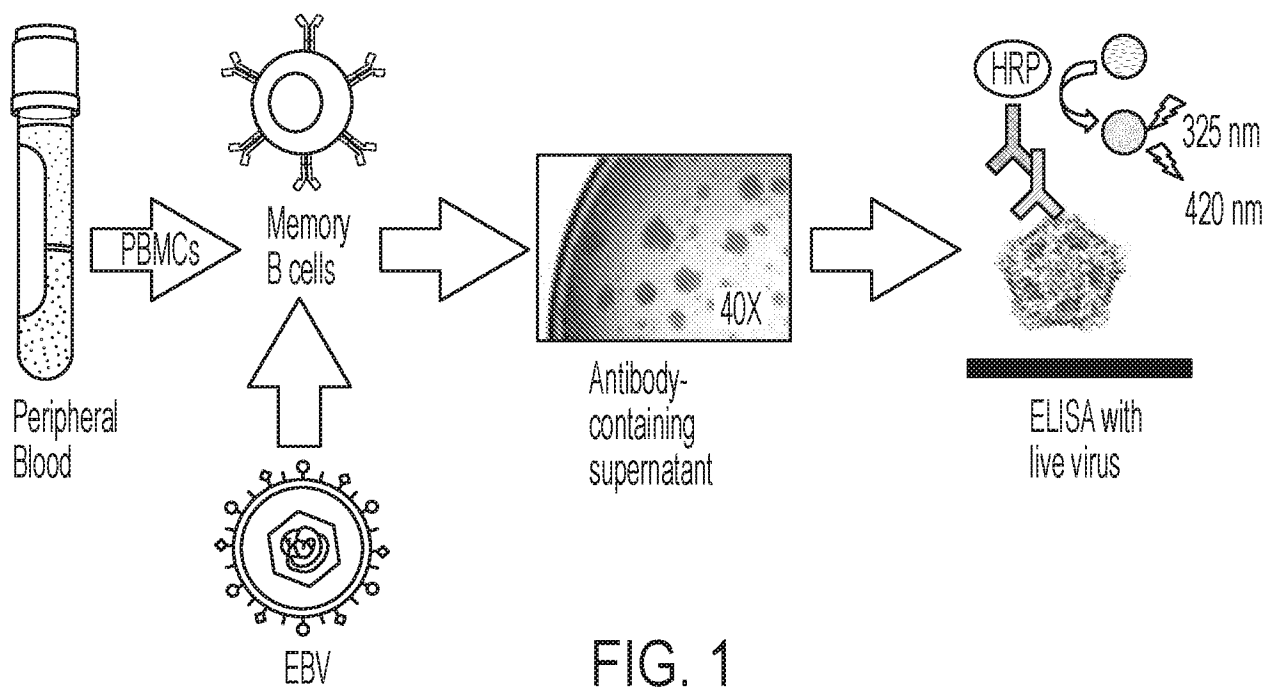


FIG. 1

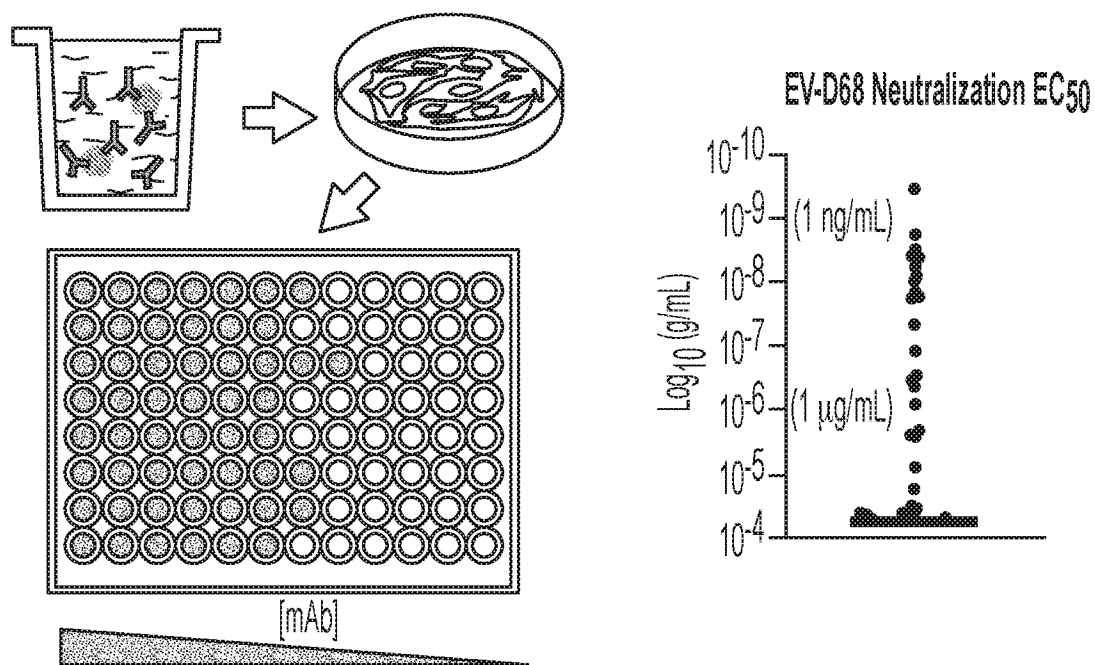


FIG. 2

Cross-reactive binding among clades is variable

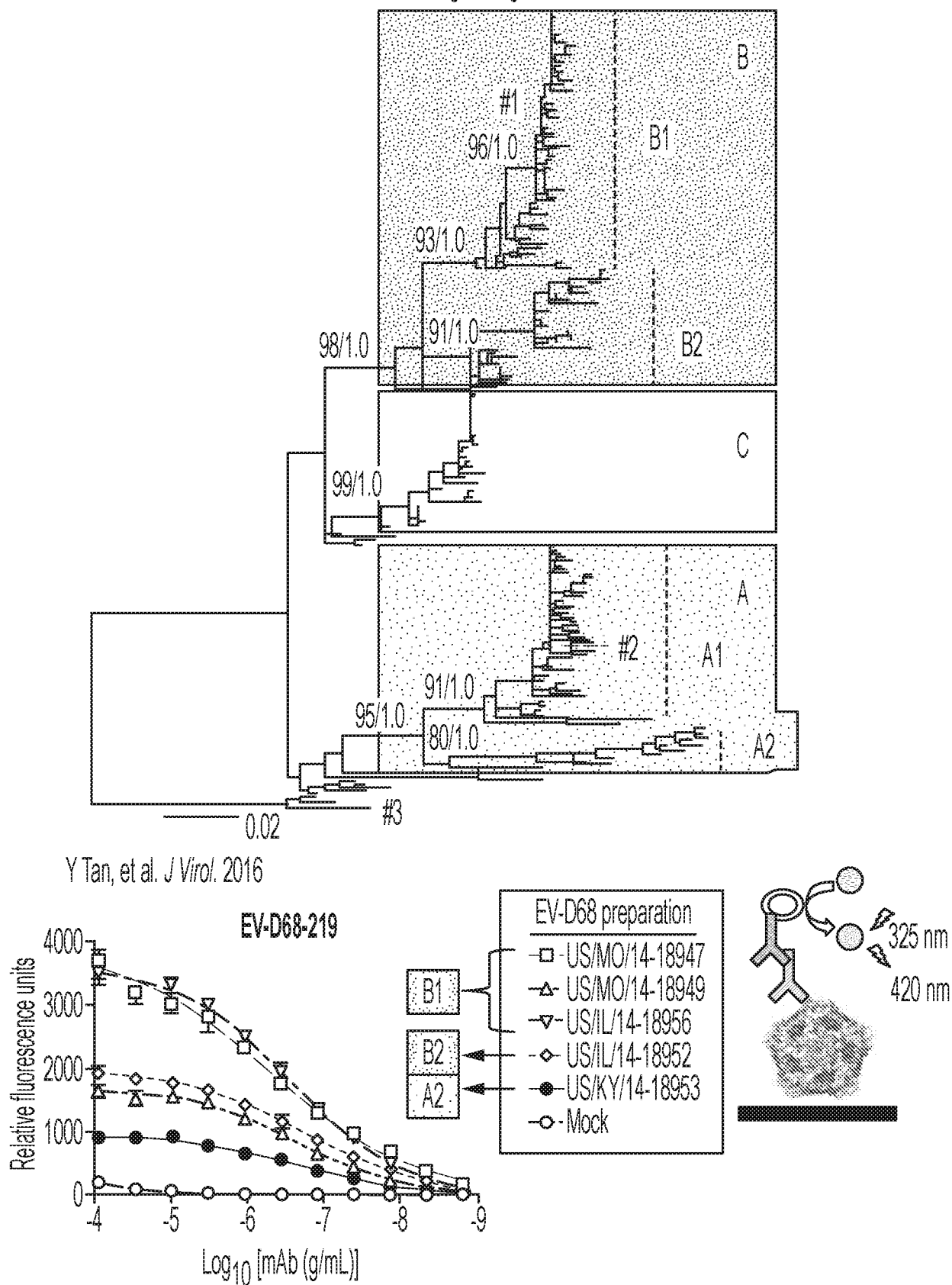


FIG. 3

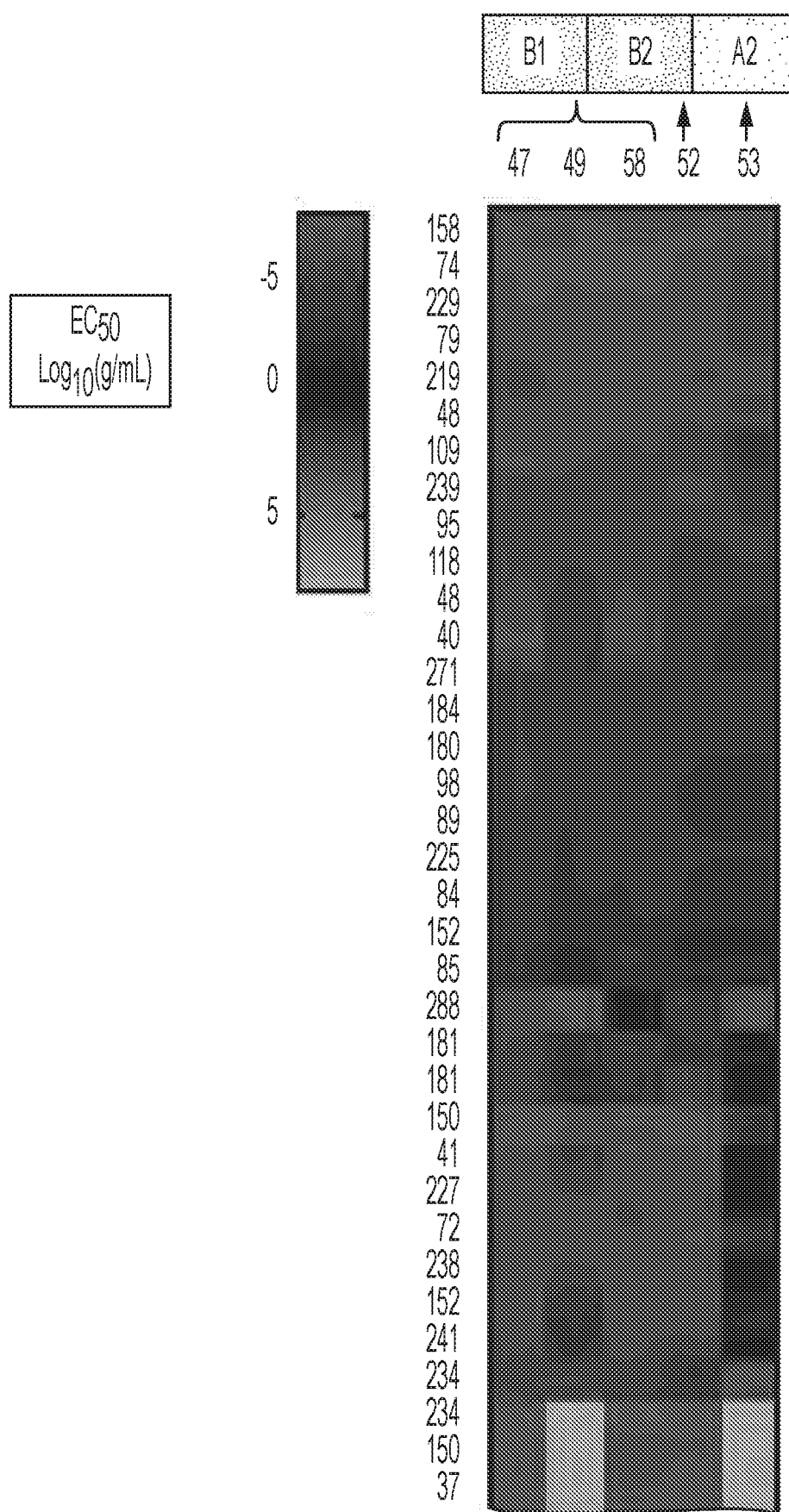


FIG. 3
CONTINUED
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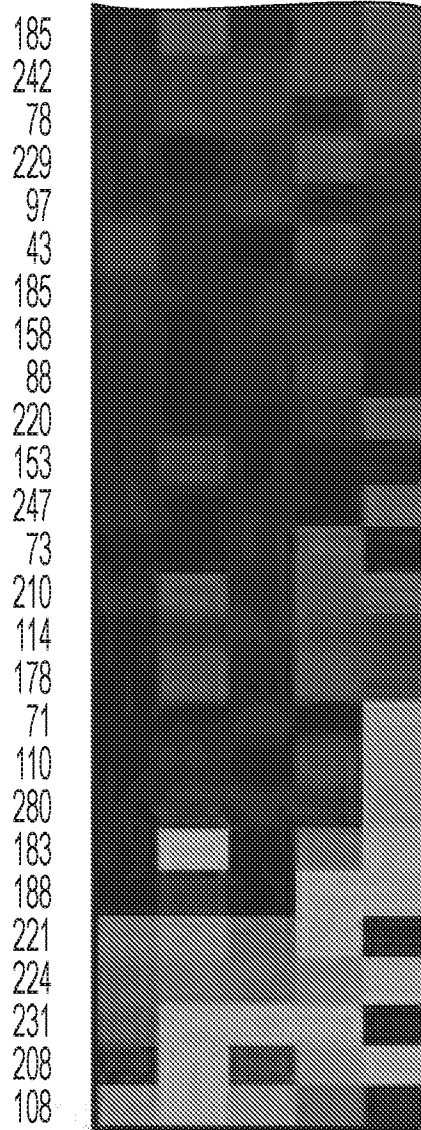


FIG. 3
CONTINUED

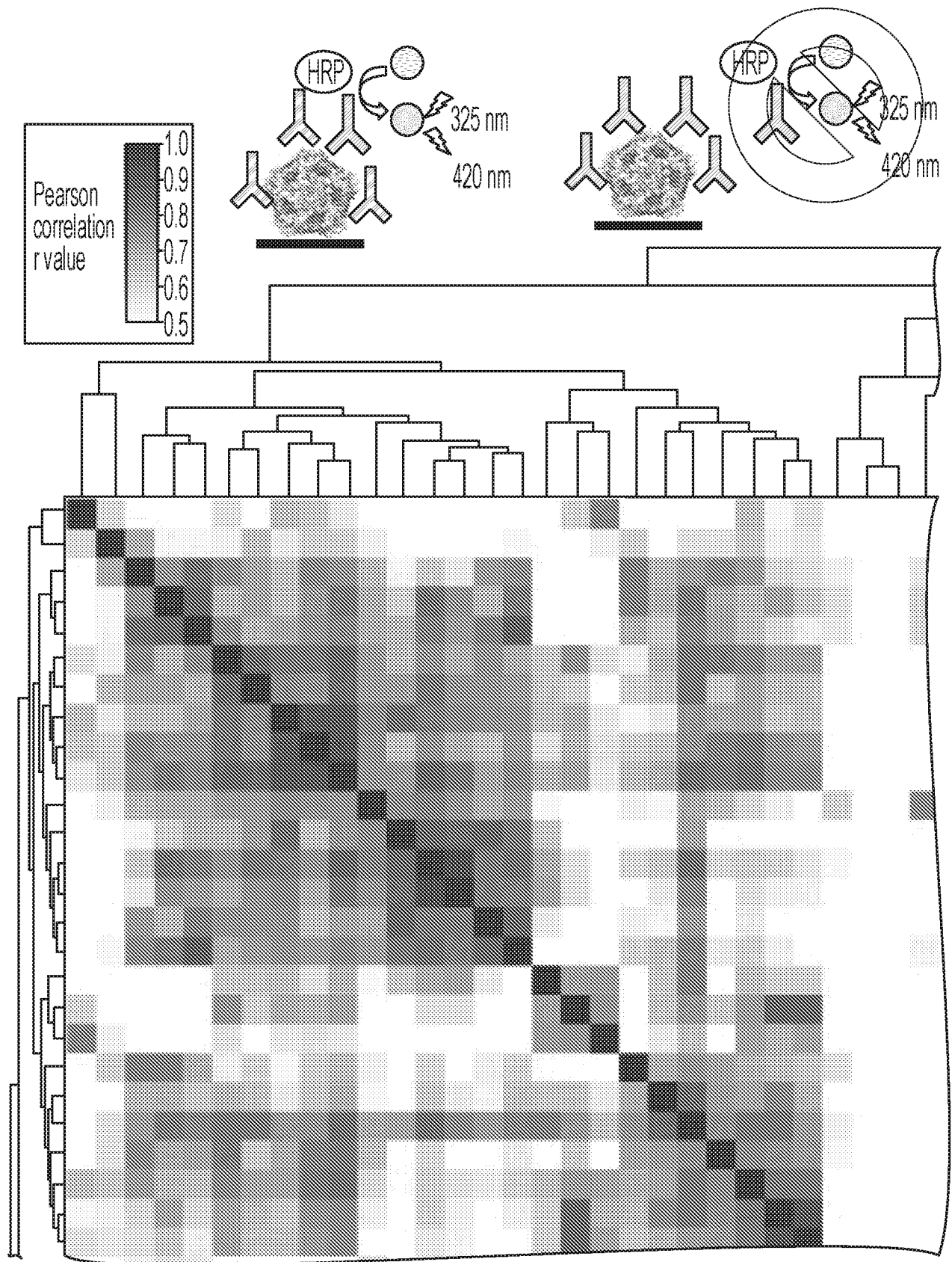


FIG. 4

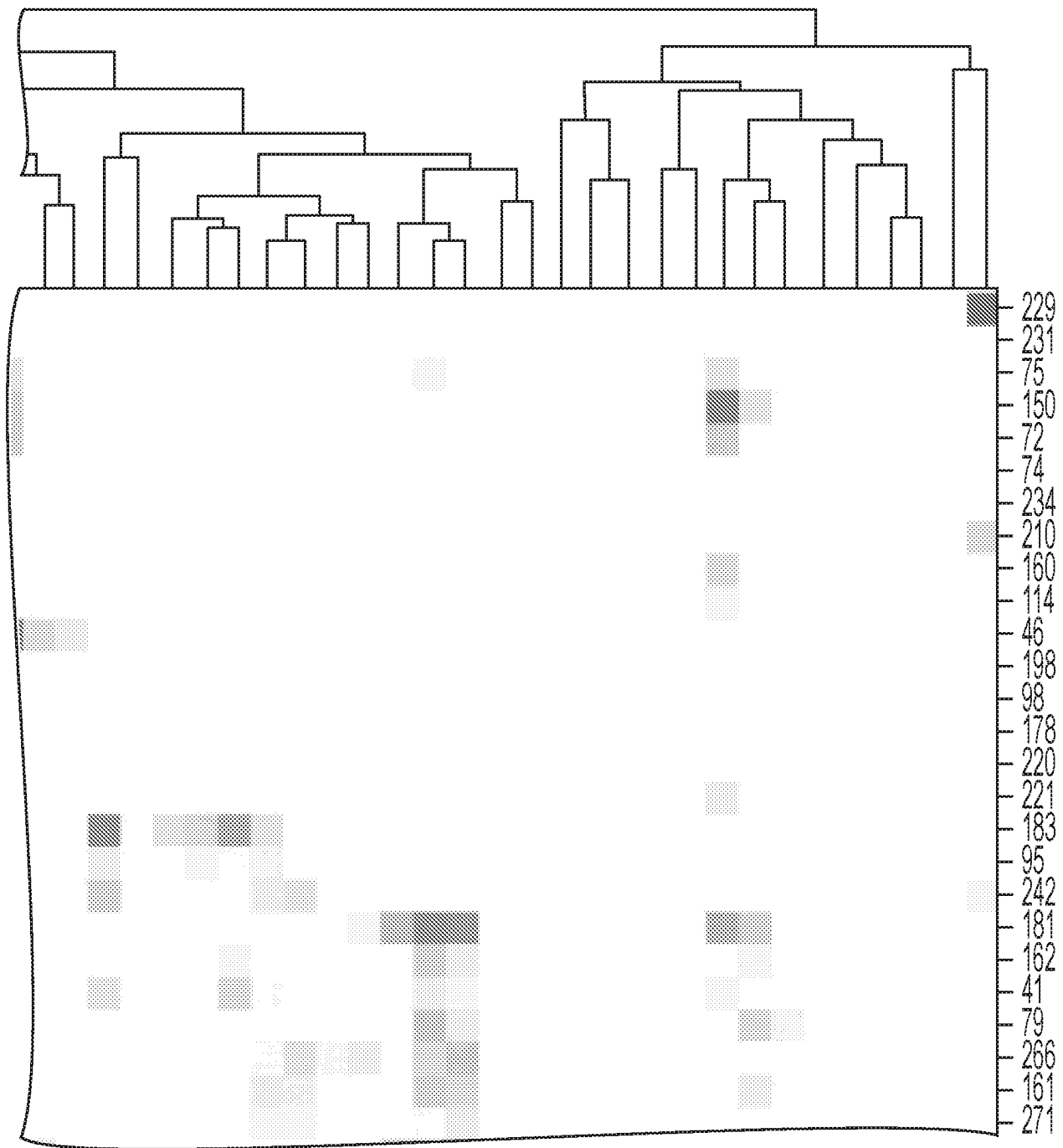


FIG. 4
CONTINUED

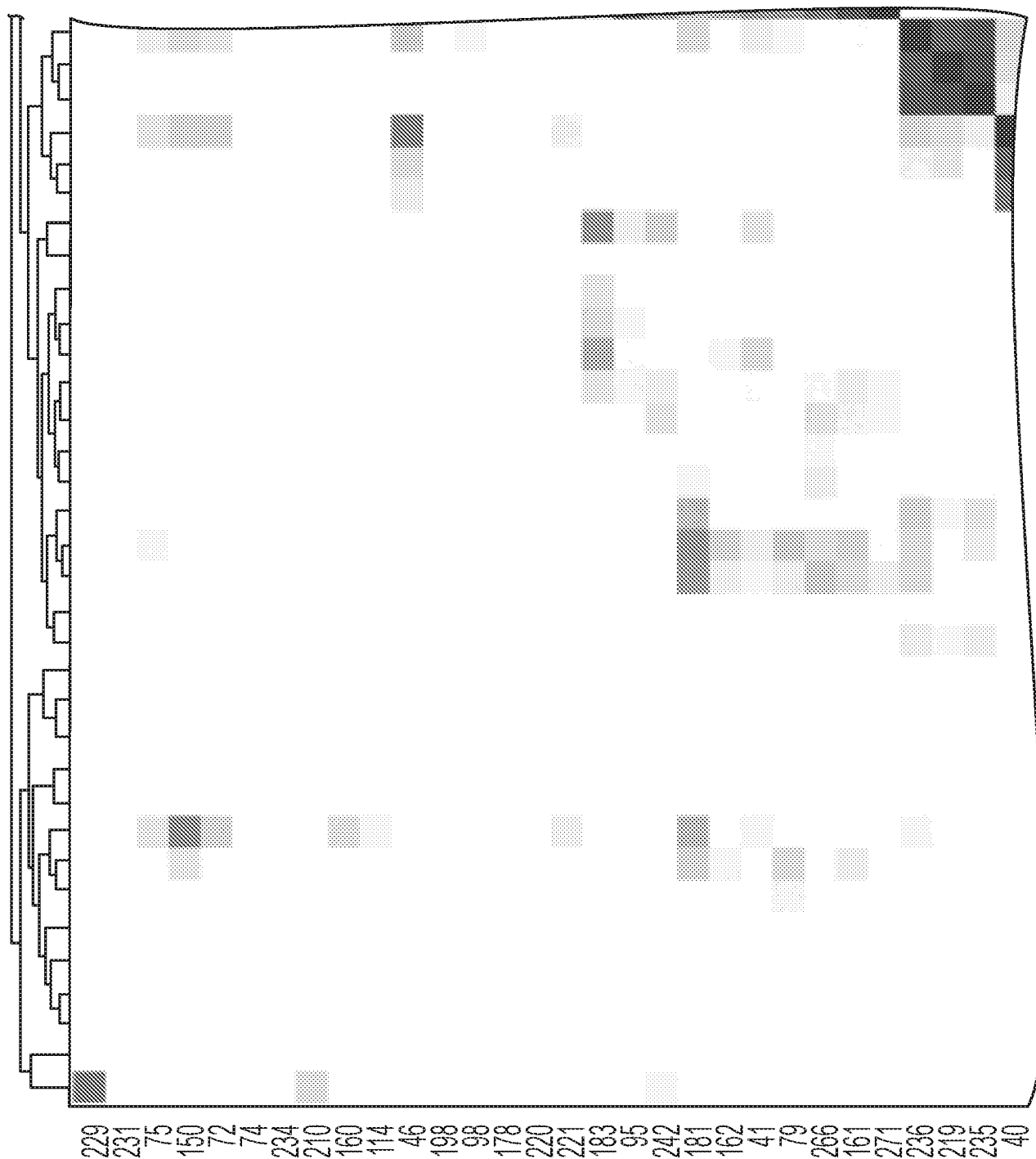


FIG. 4
CONTINUED

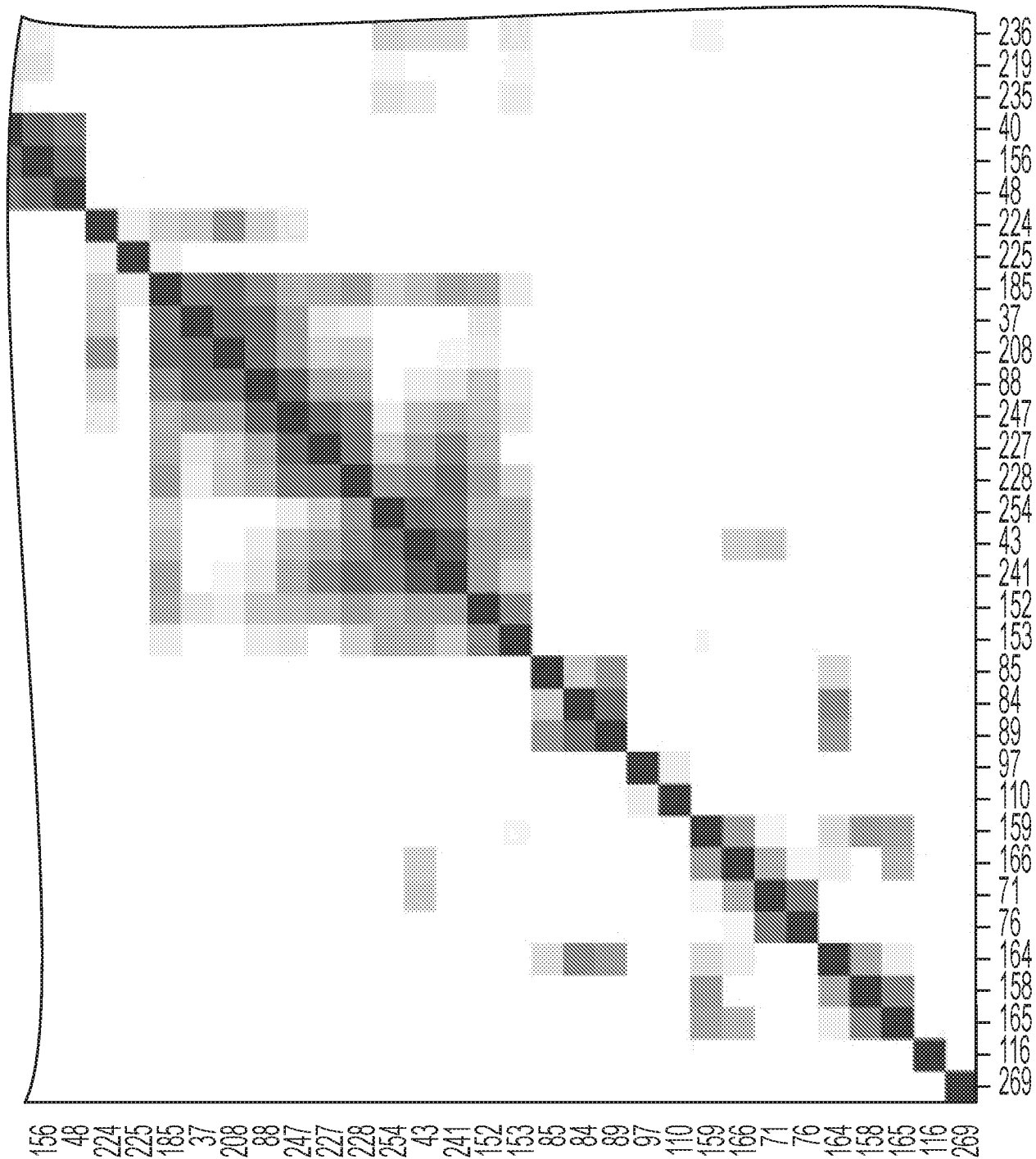


FIG. 4
CONTINUED

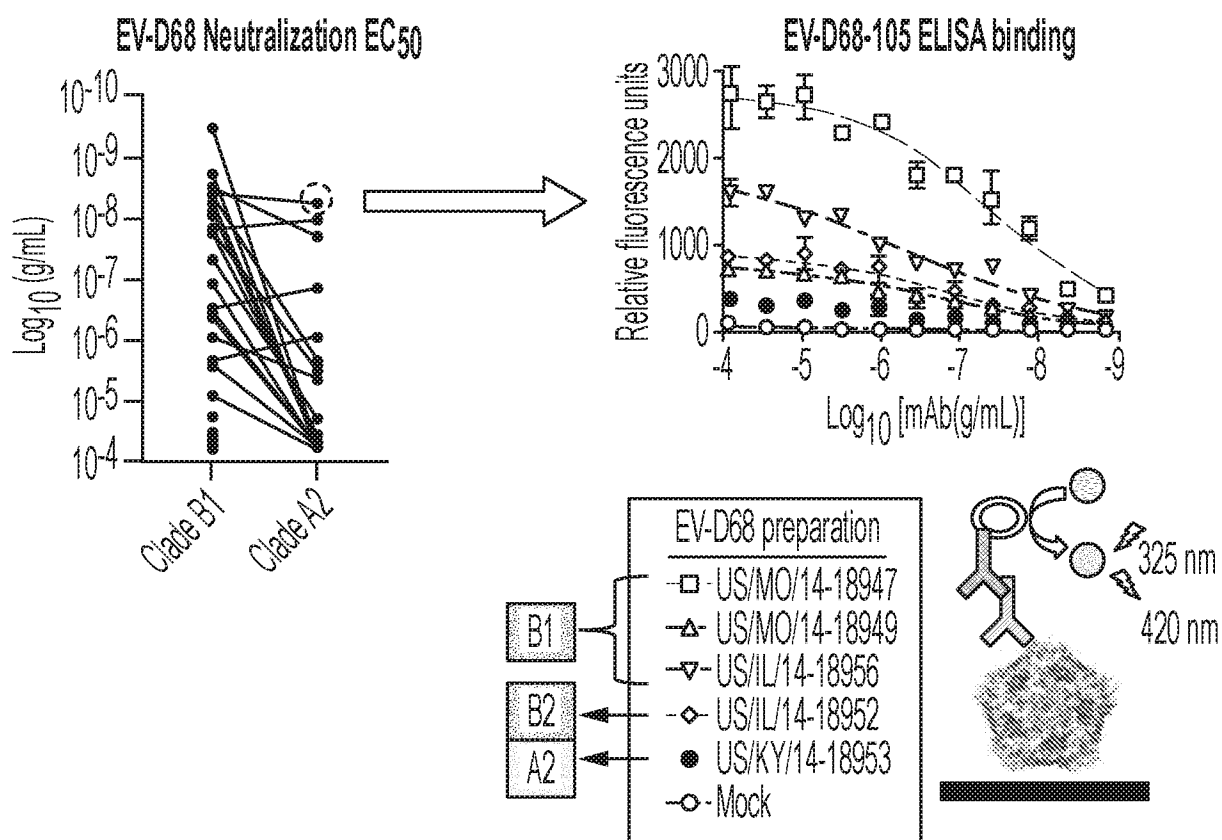


FIG. 5

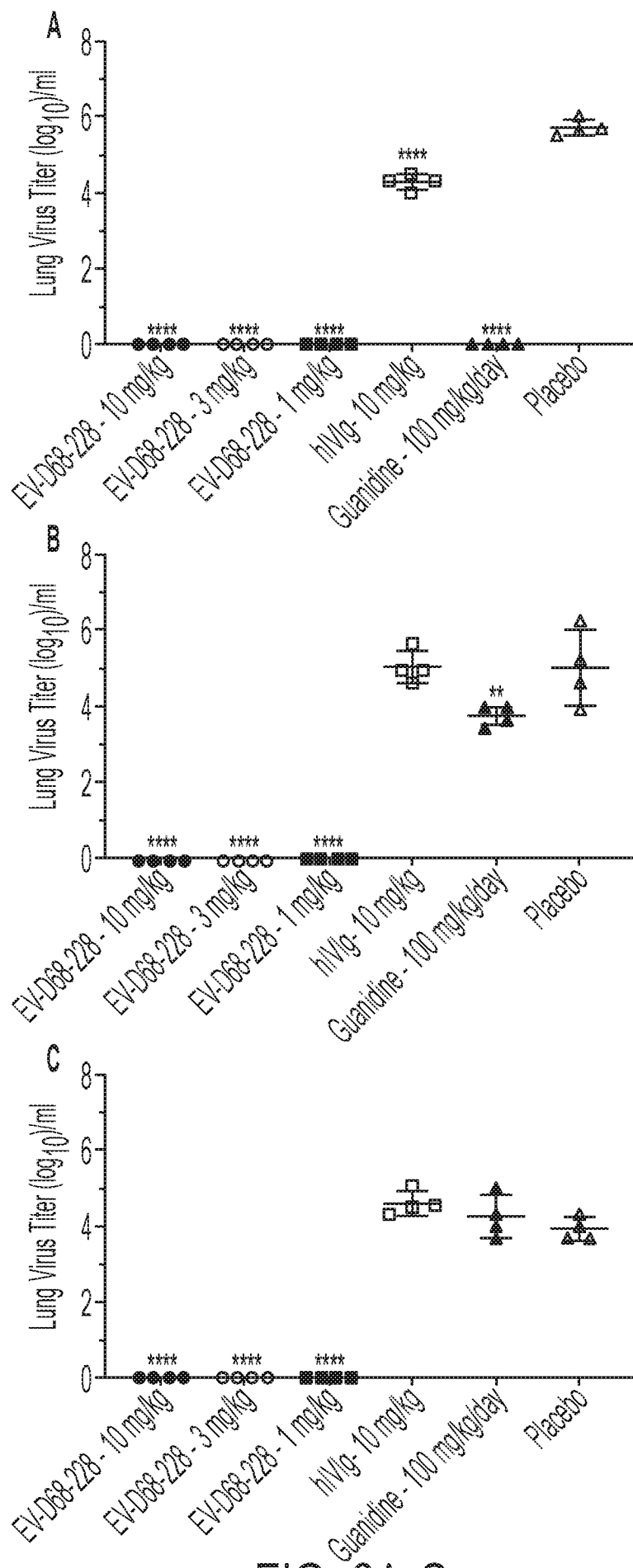


FIG. 6A-C

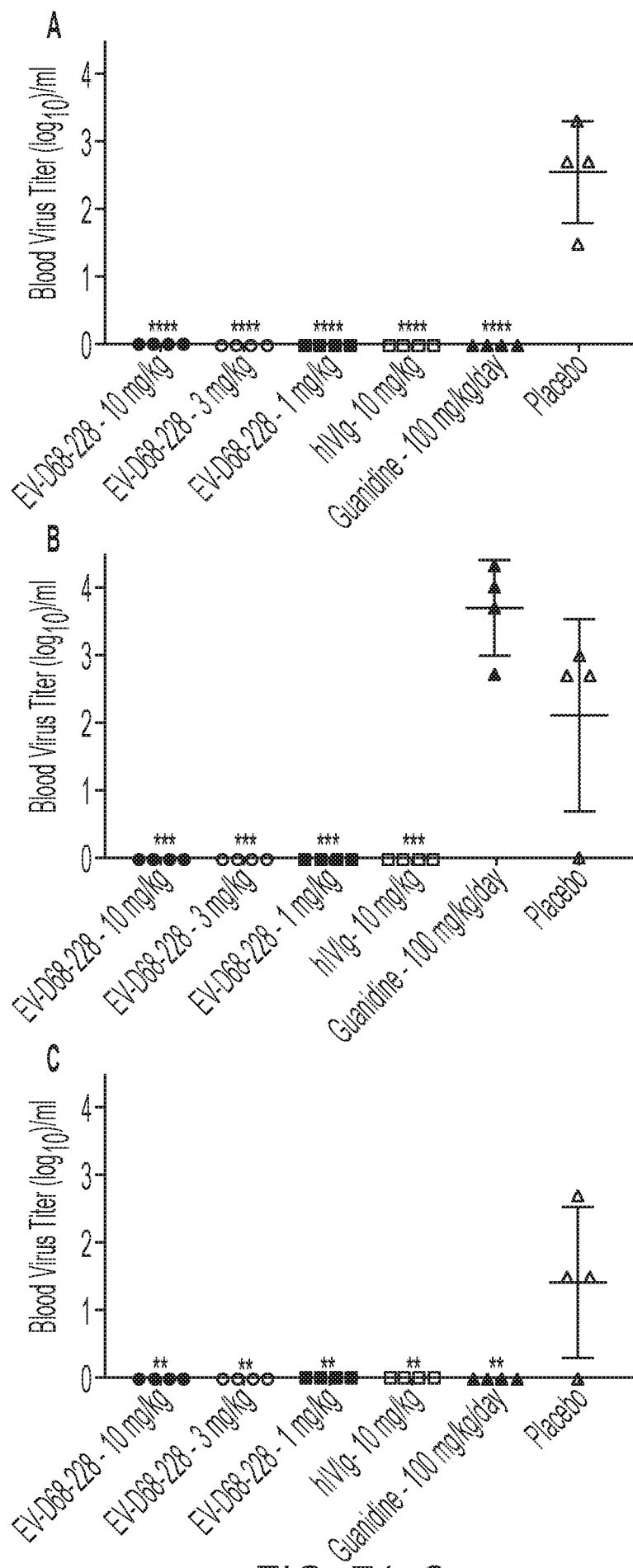


FIG. 7A-C

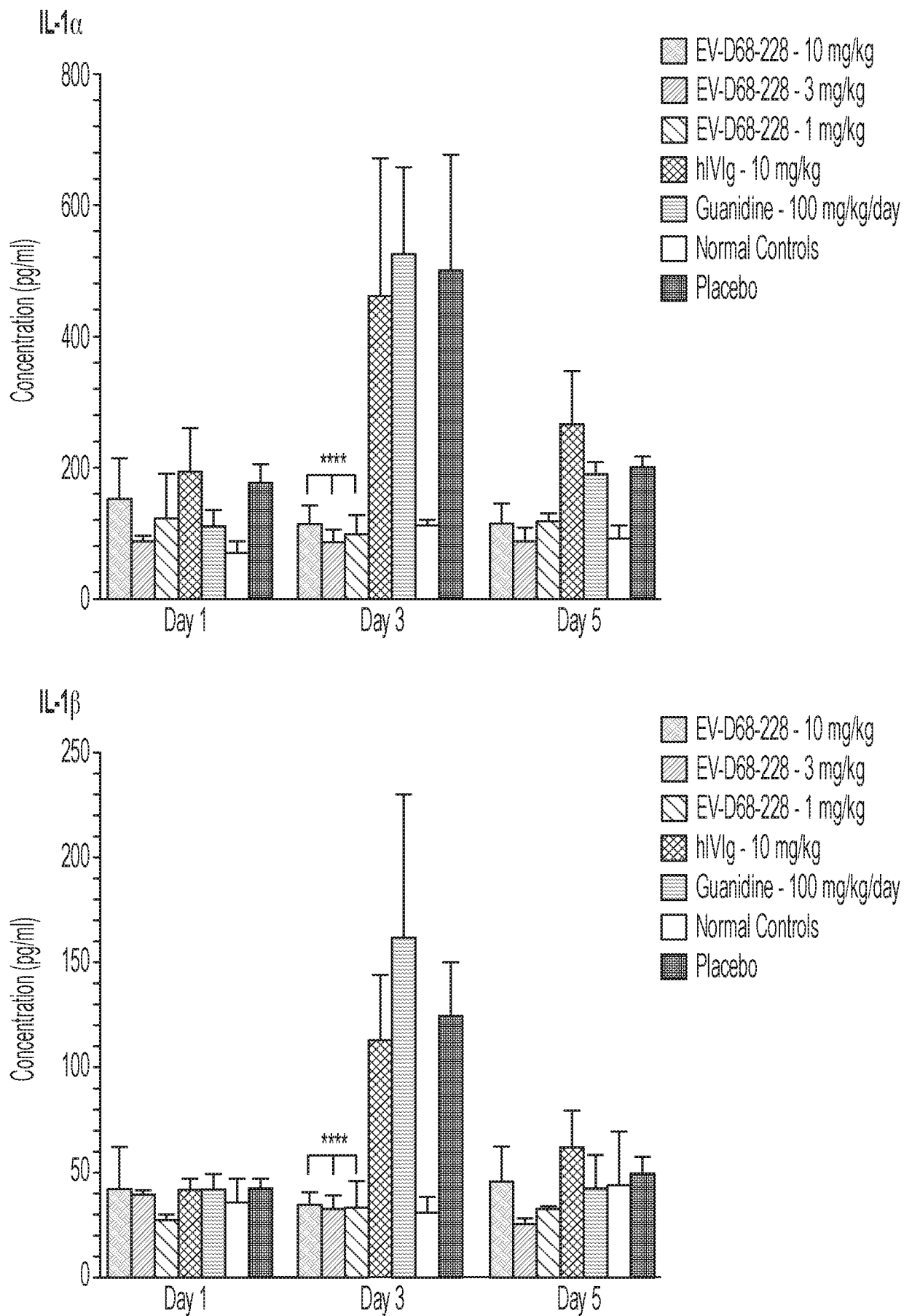


FIG. 8

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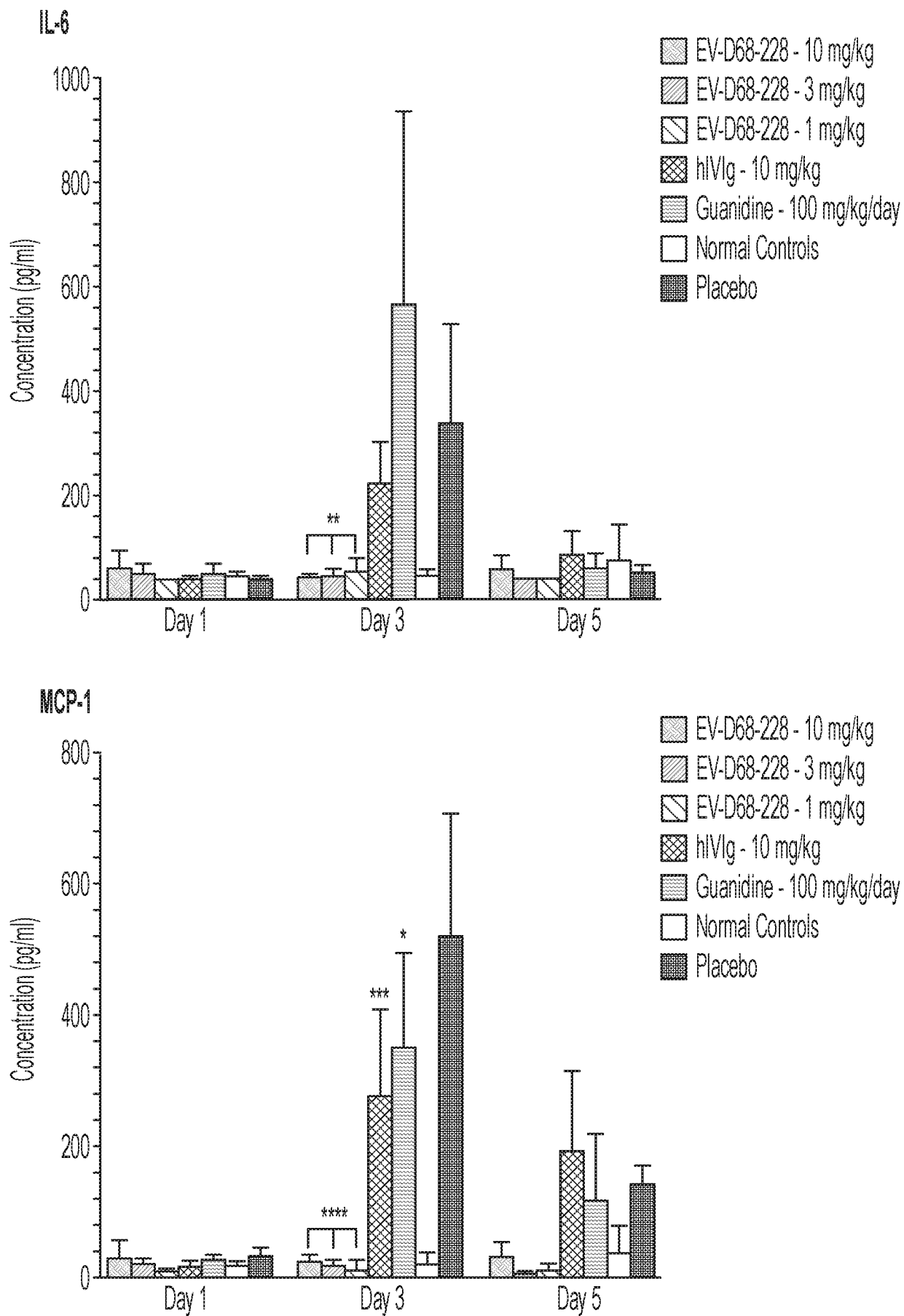


FIG. 9

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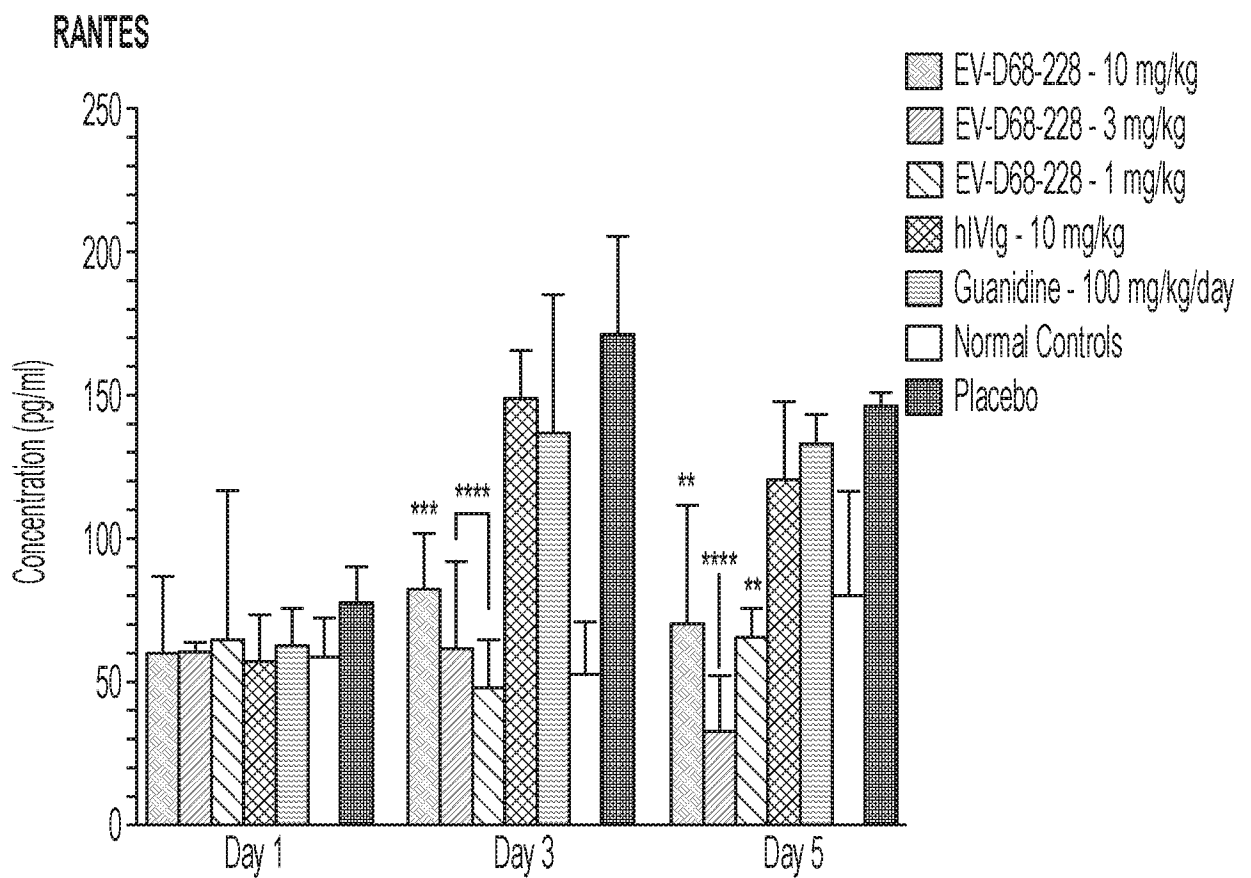


FIG. 10

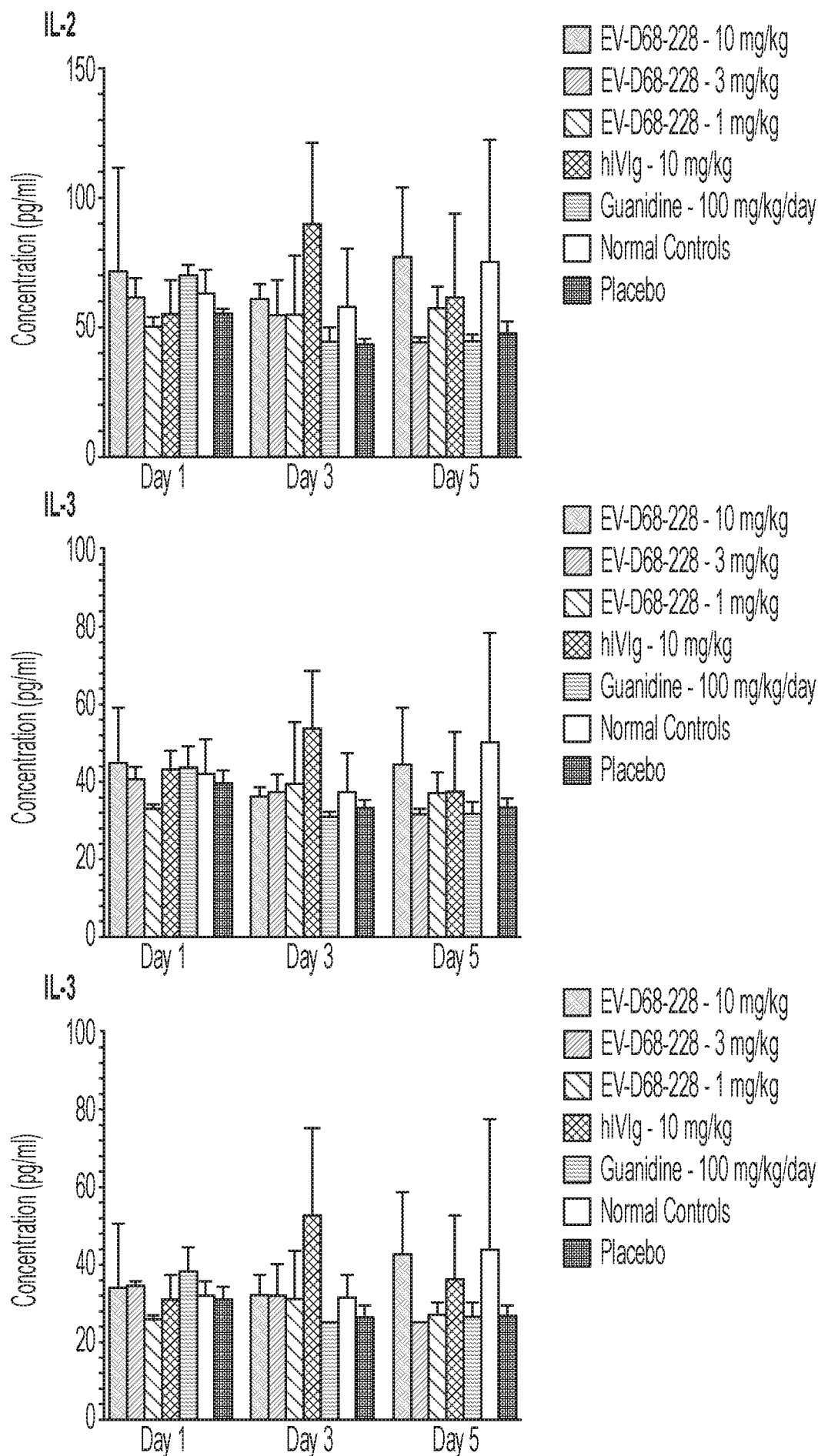


FIG. 11

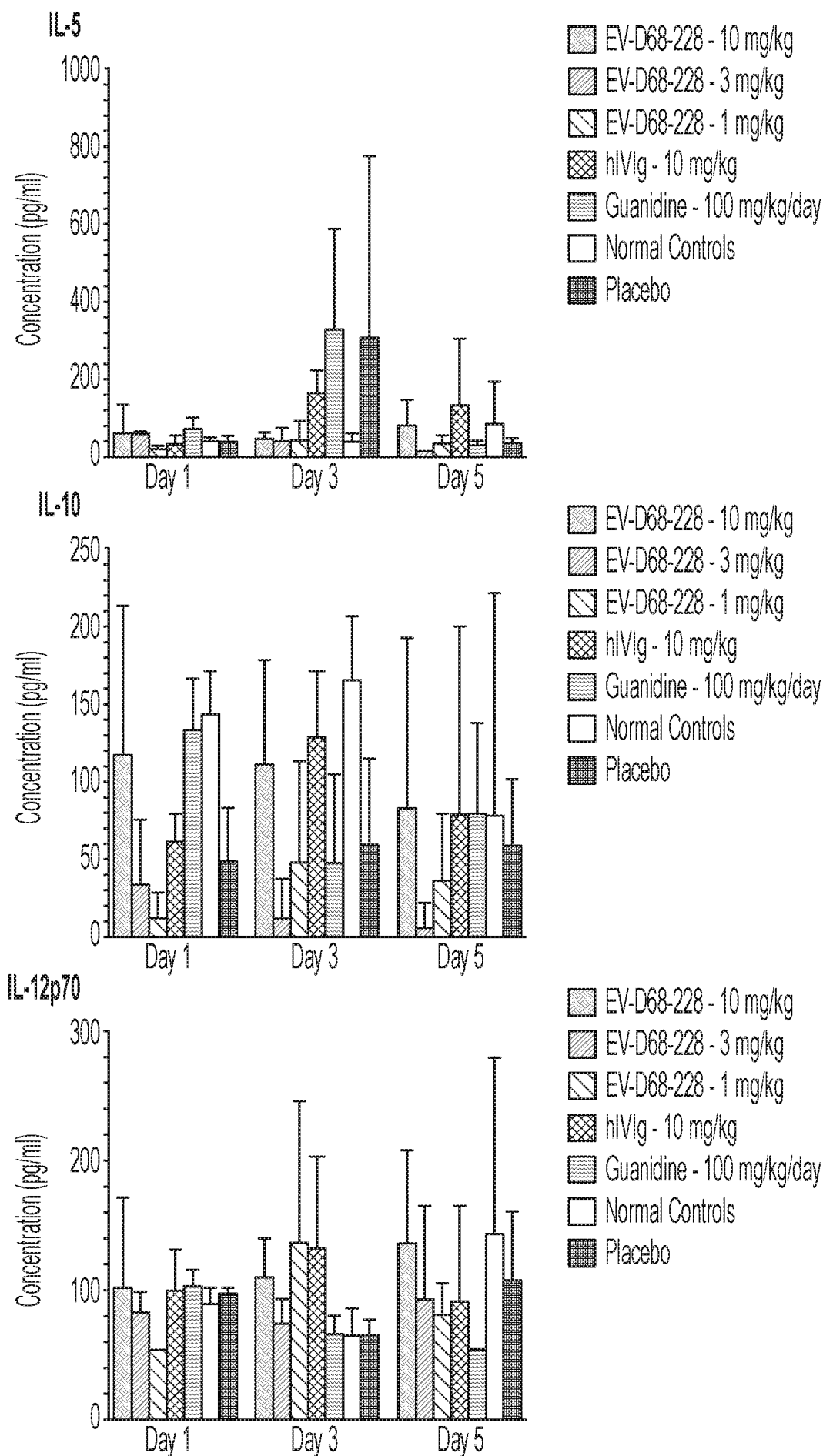


FIG. 12

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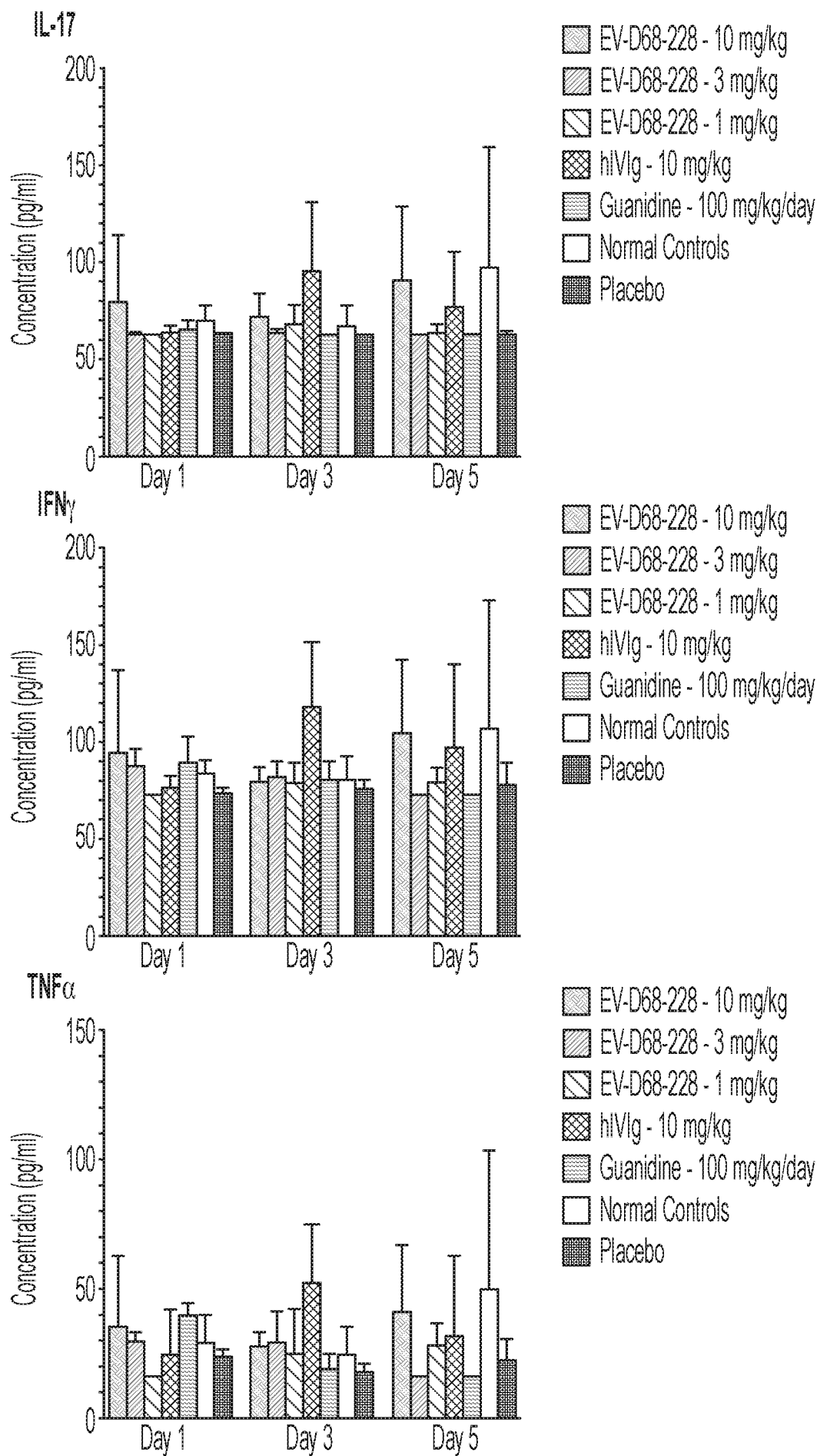
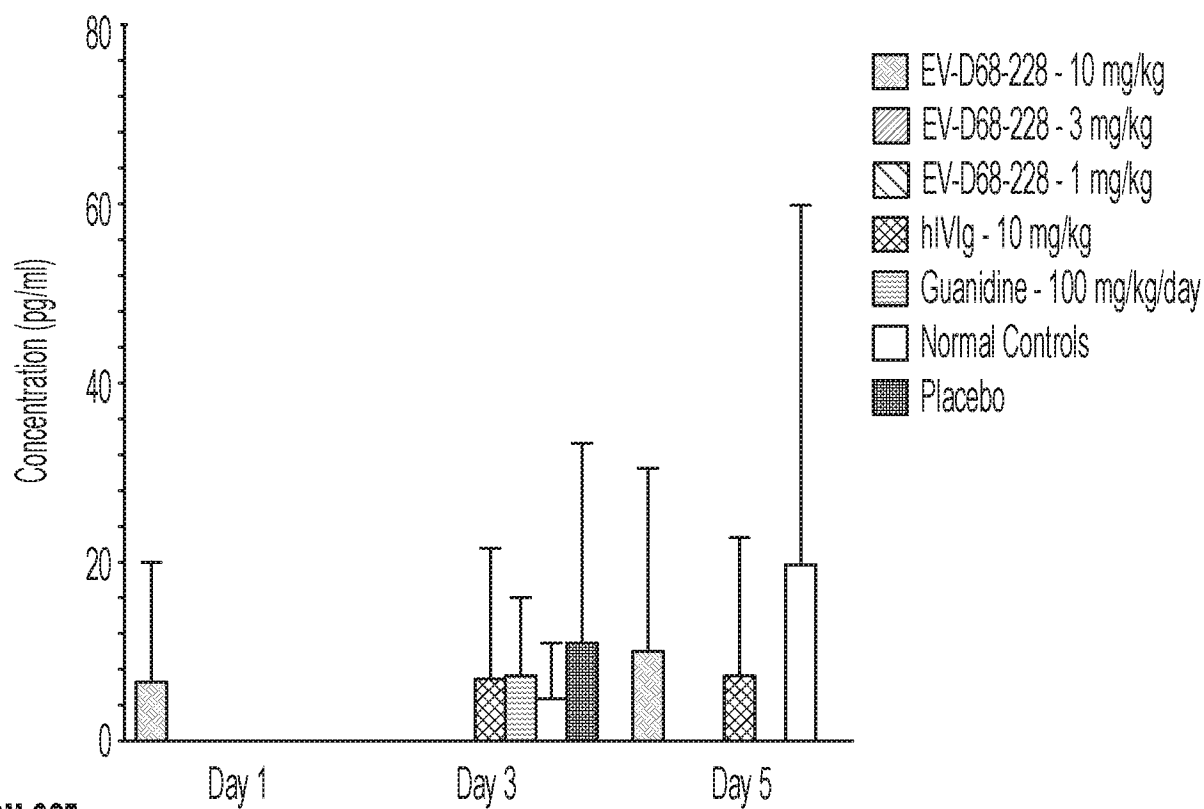


FIG. 13

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MIP-1 α 

GM-CSF

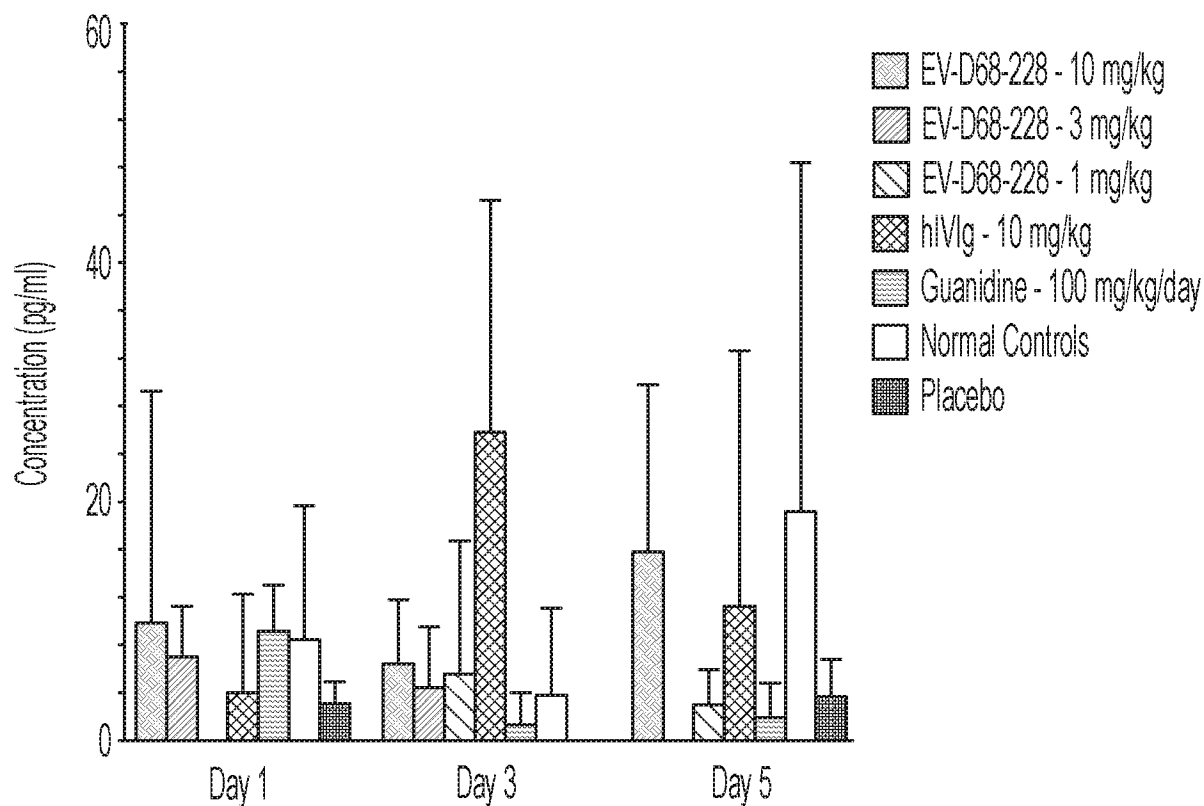


FIG. 14

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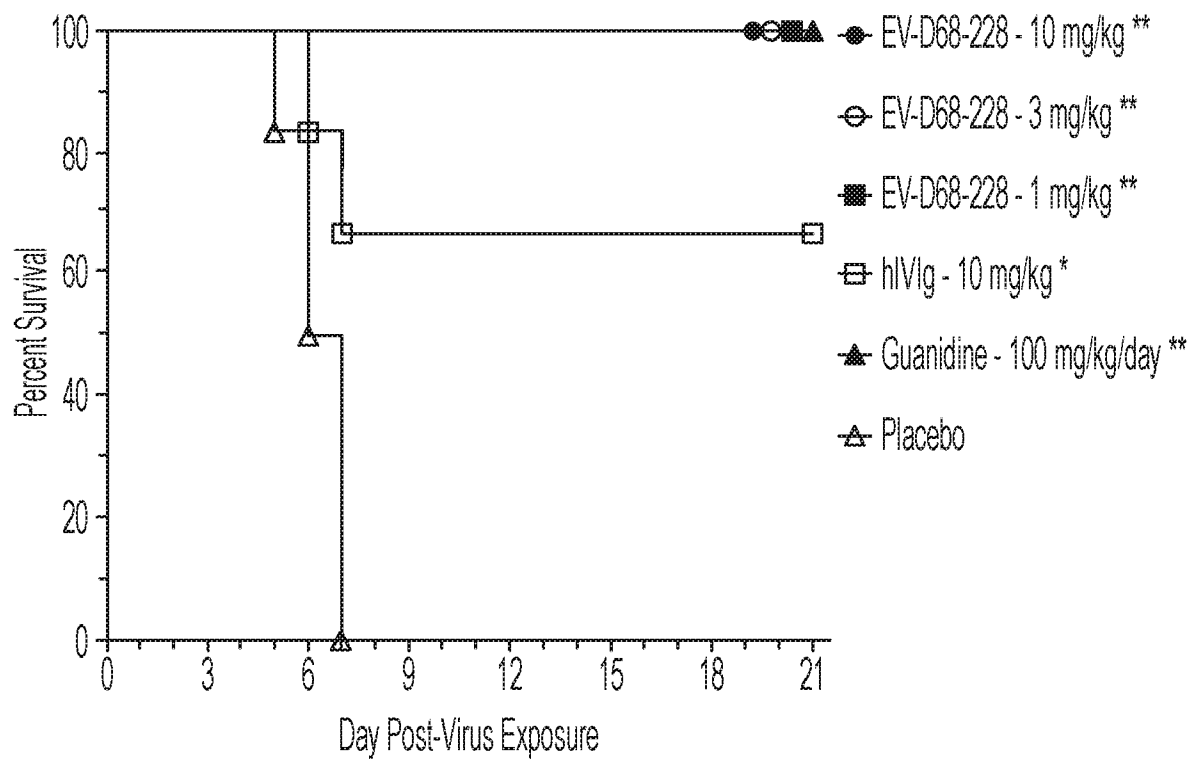


FIG. 15

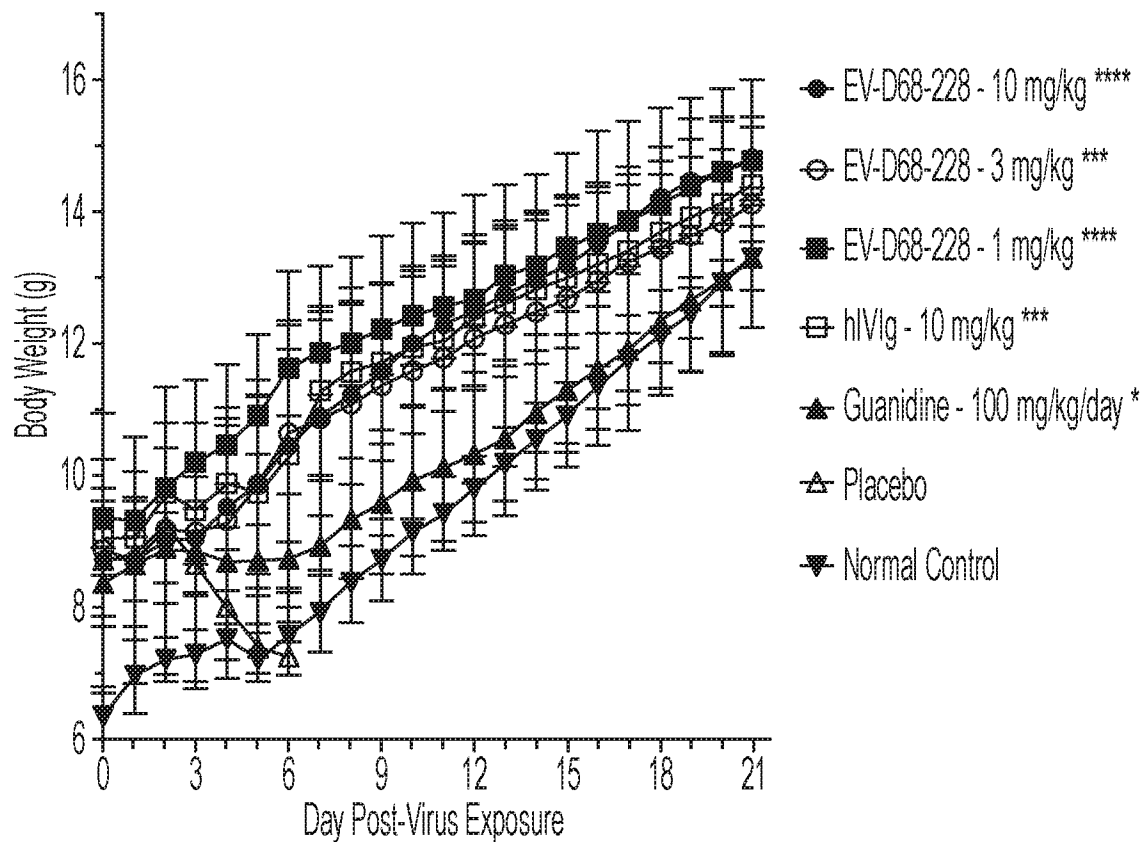


FIG. 16

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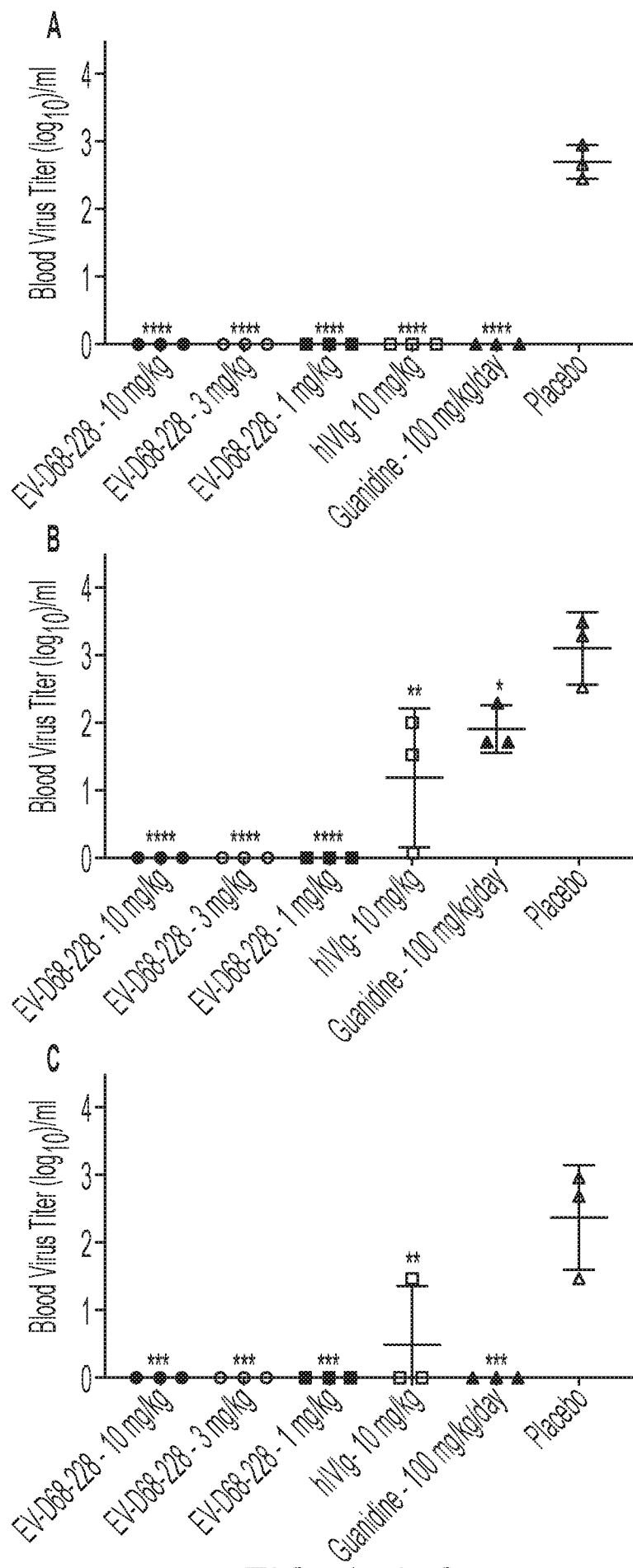


FIG. 17A-C

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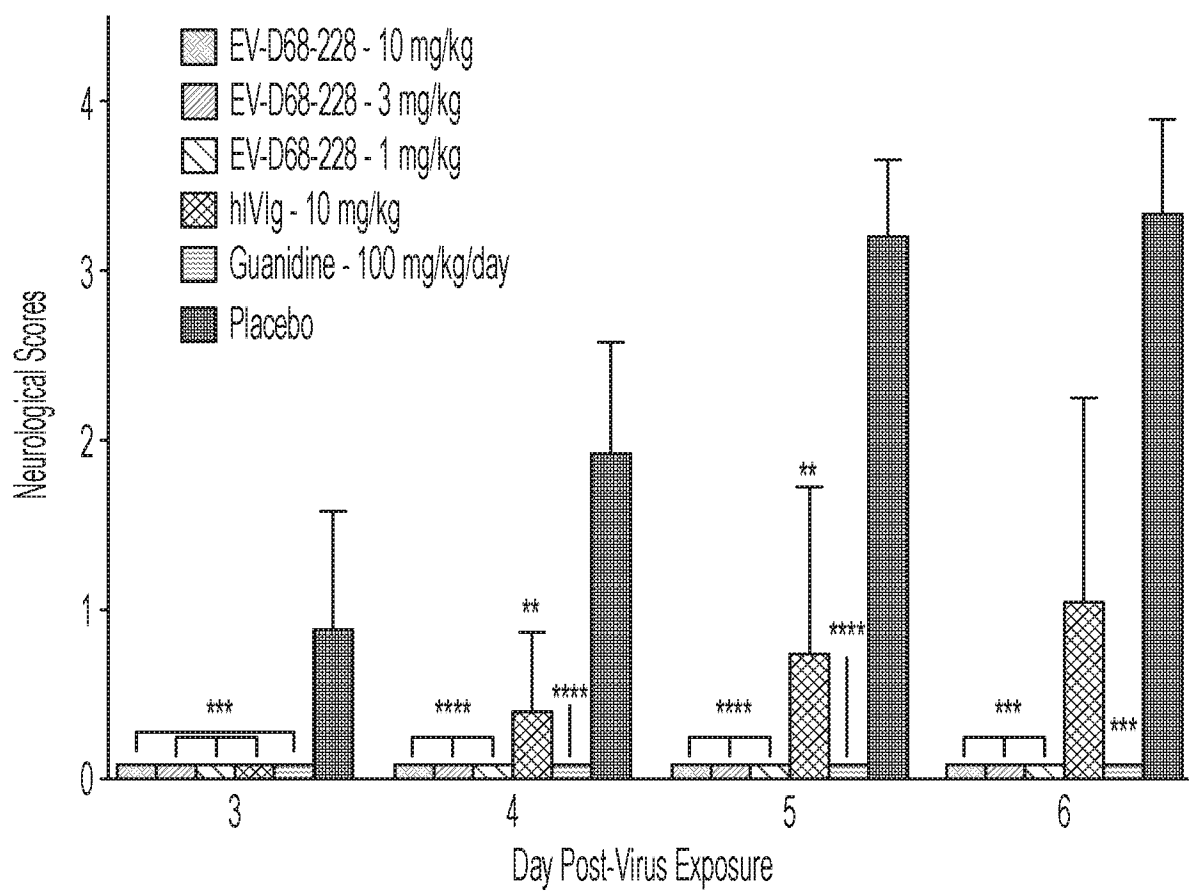


FIG. 18

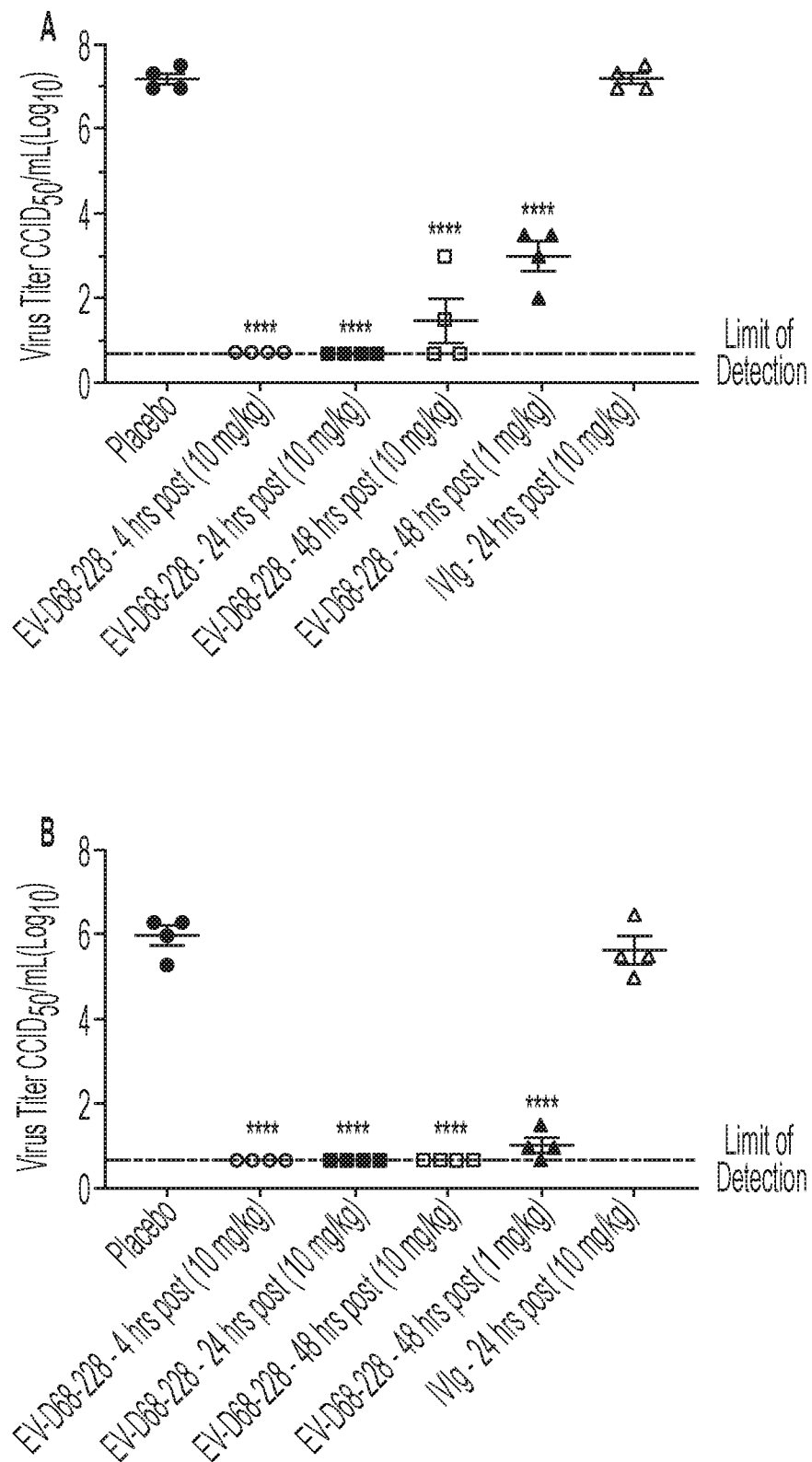


FIG. 19A-B

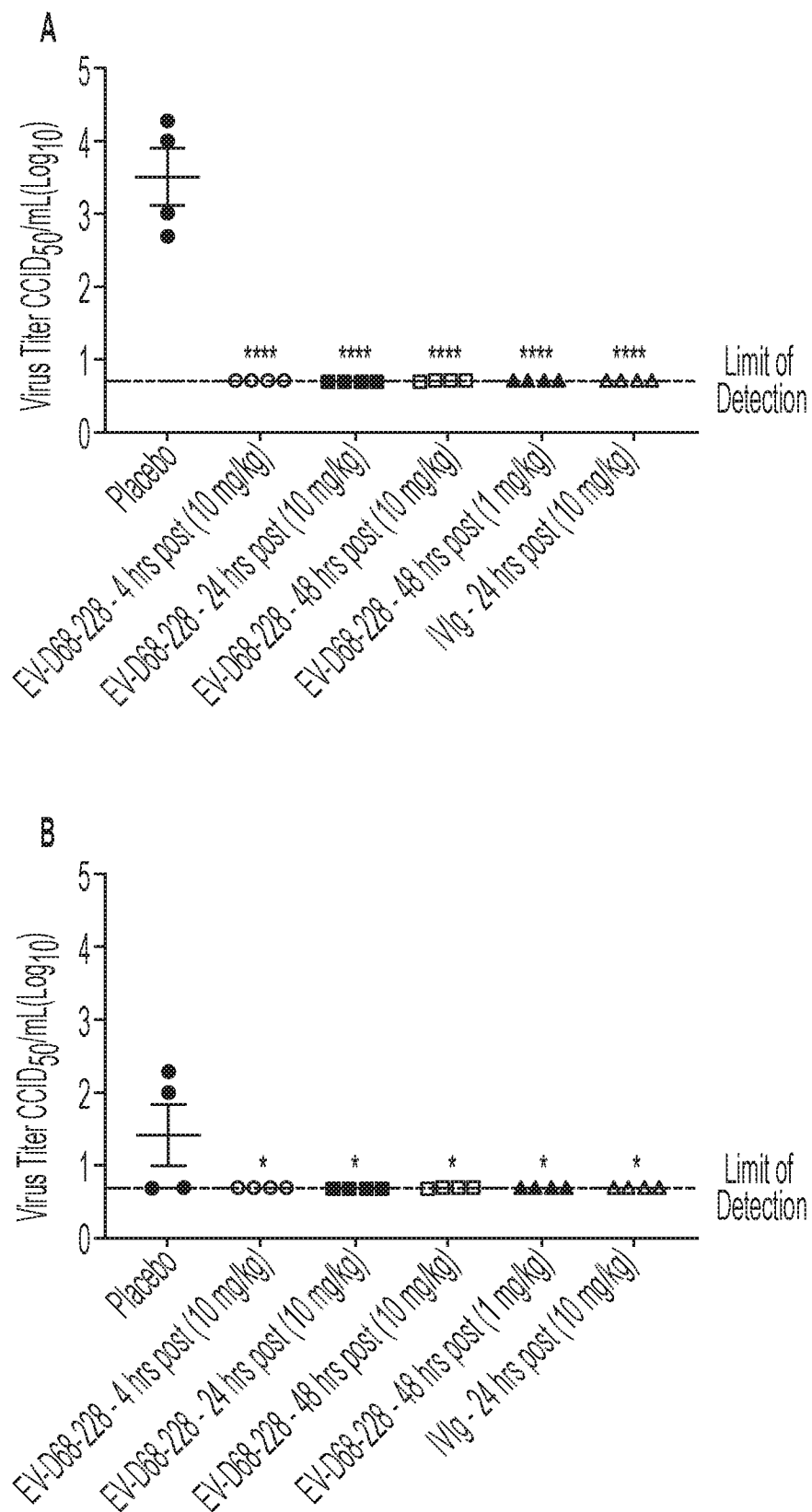


FIG. 20A-B

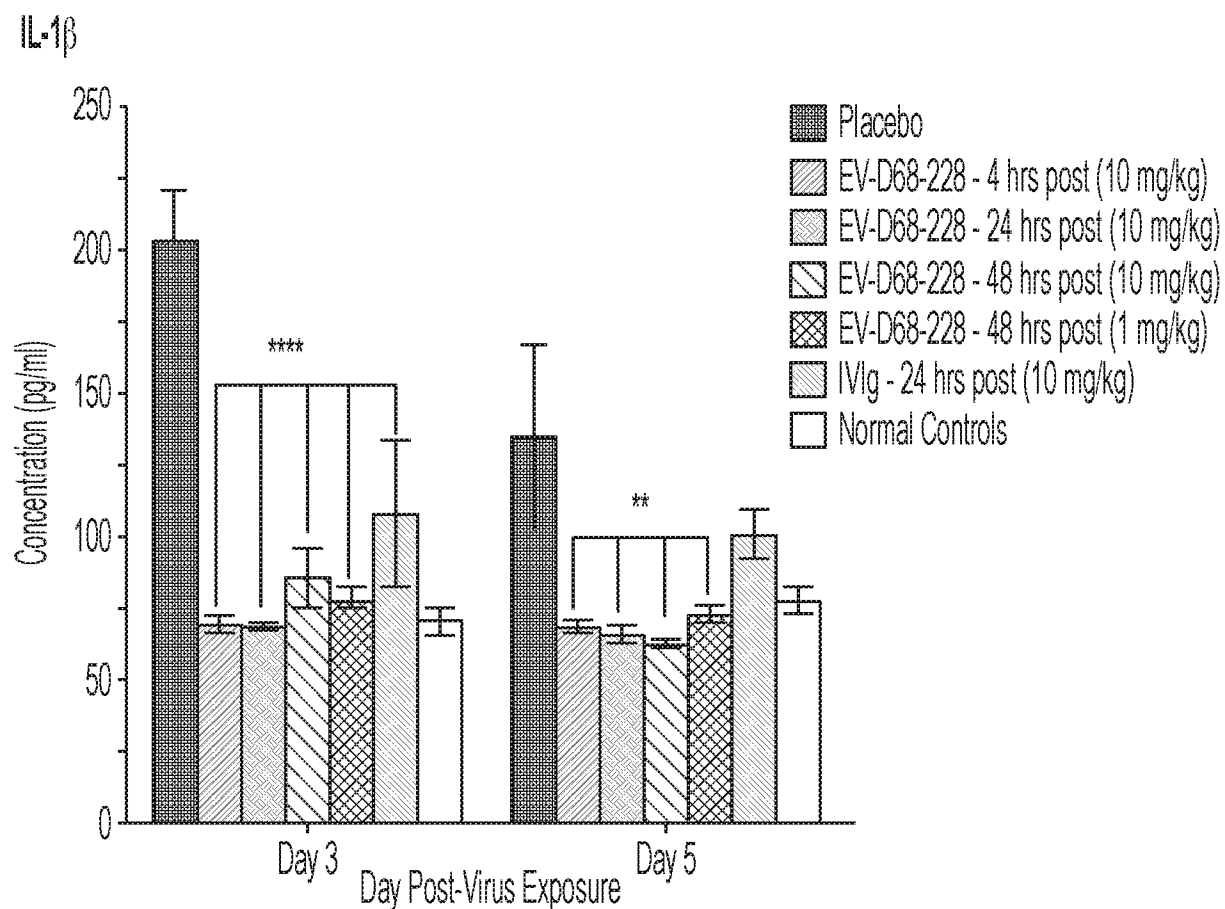
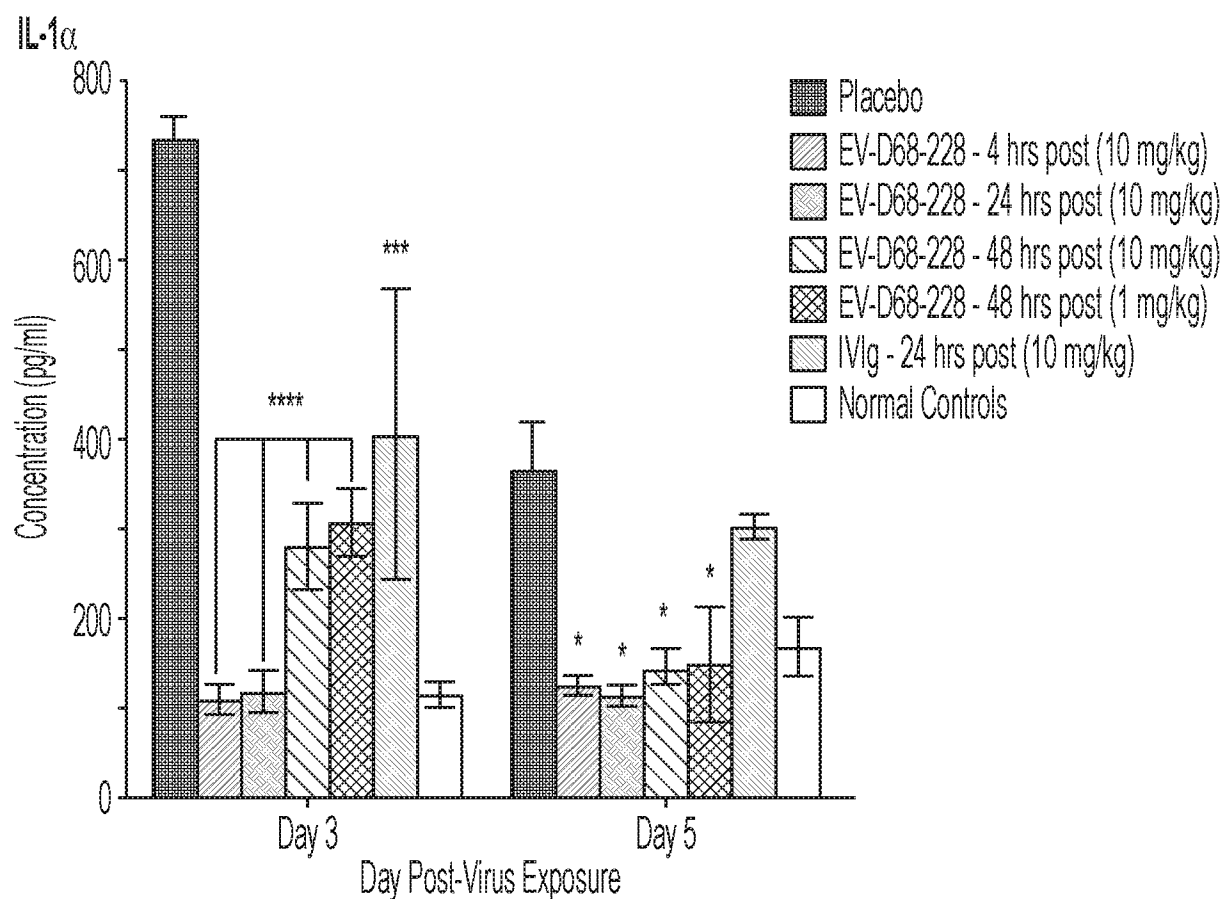


FIG. 21

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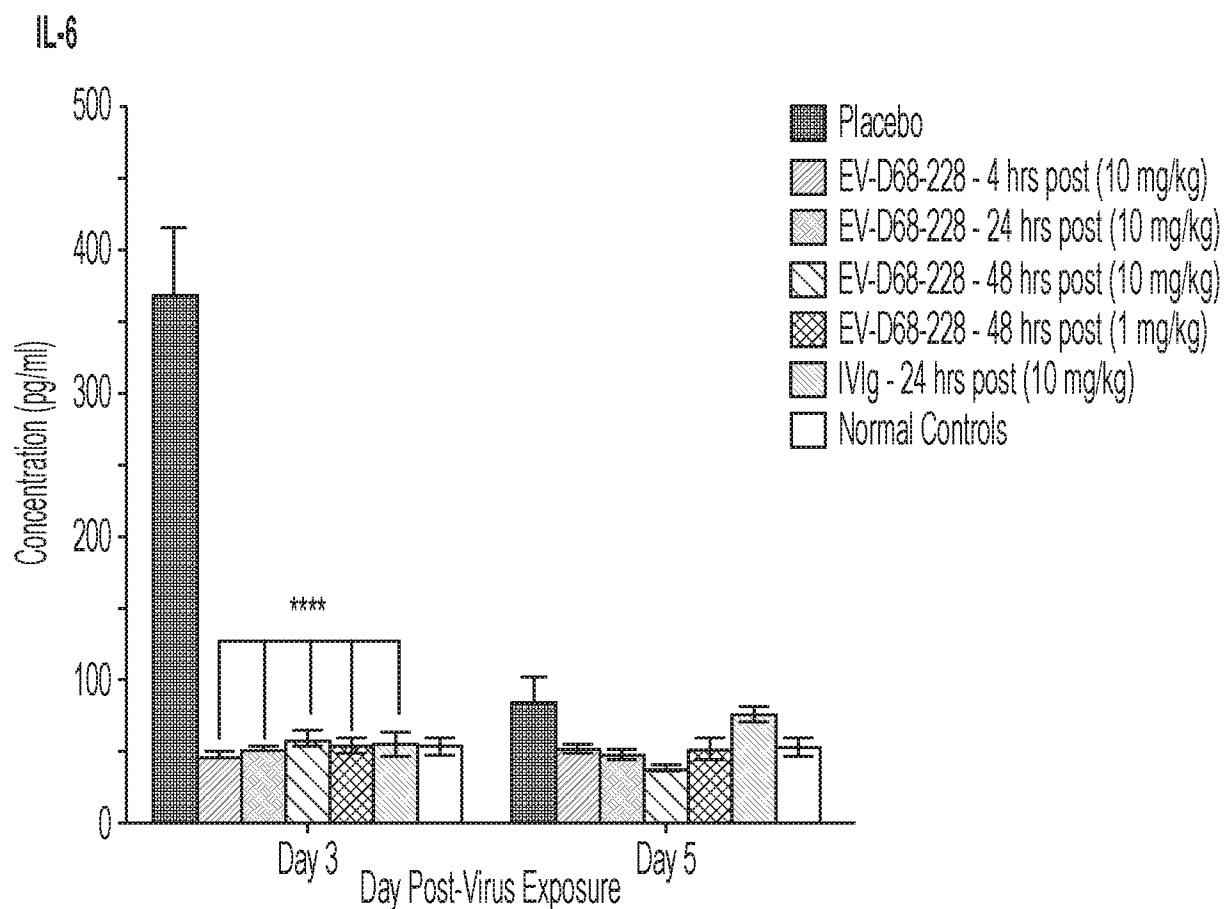
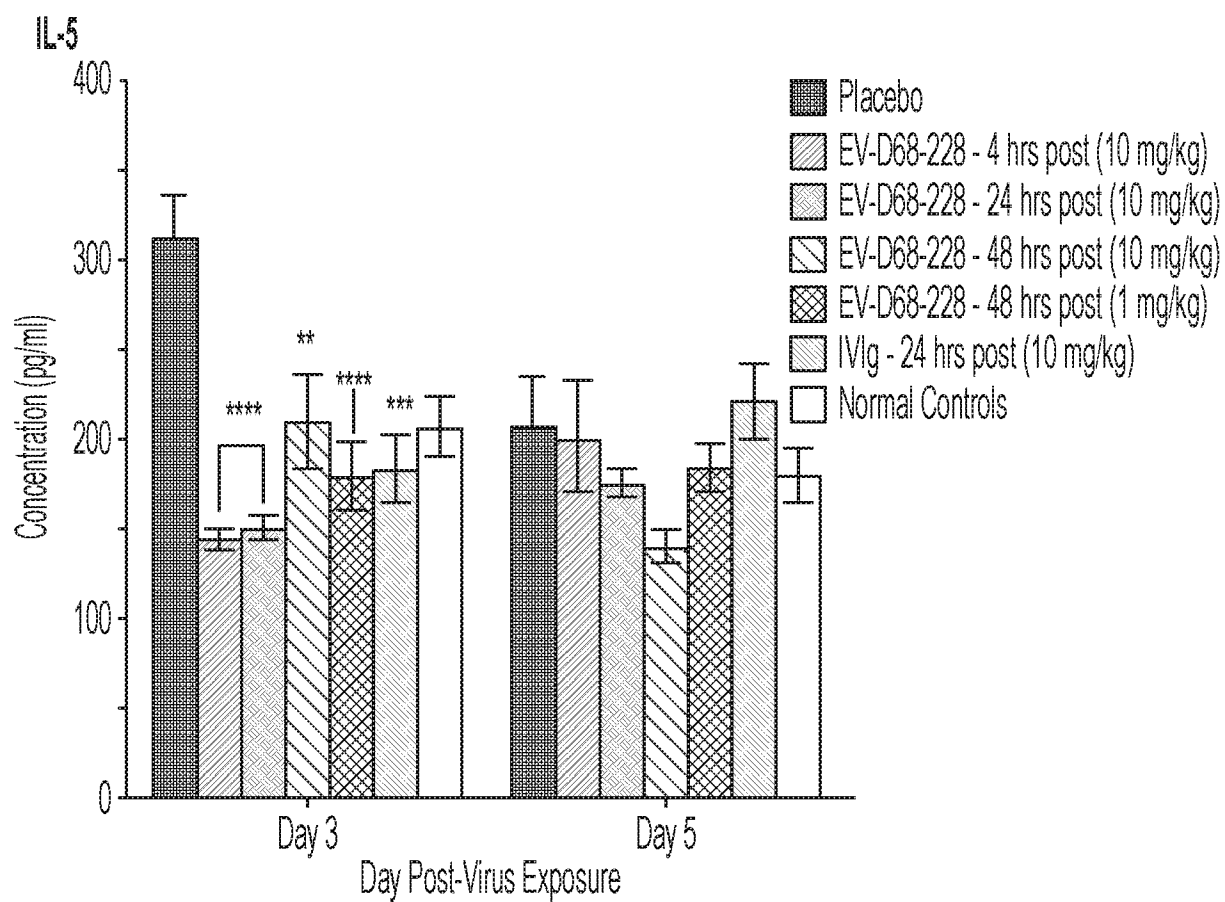


FIG. 22

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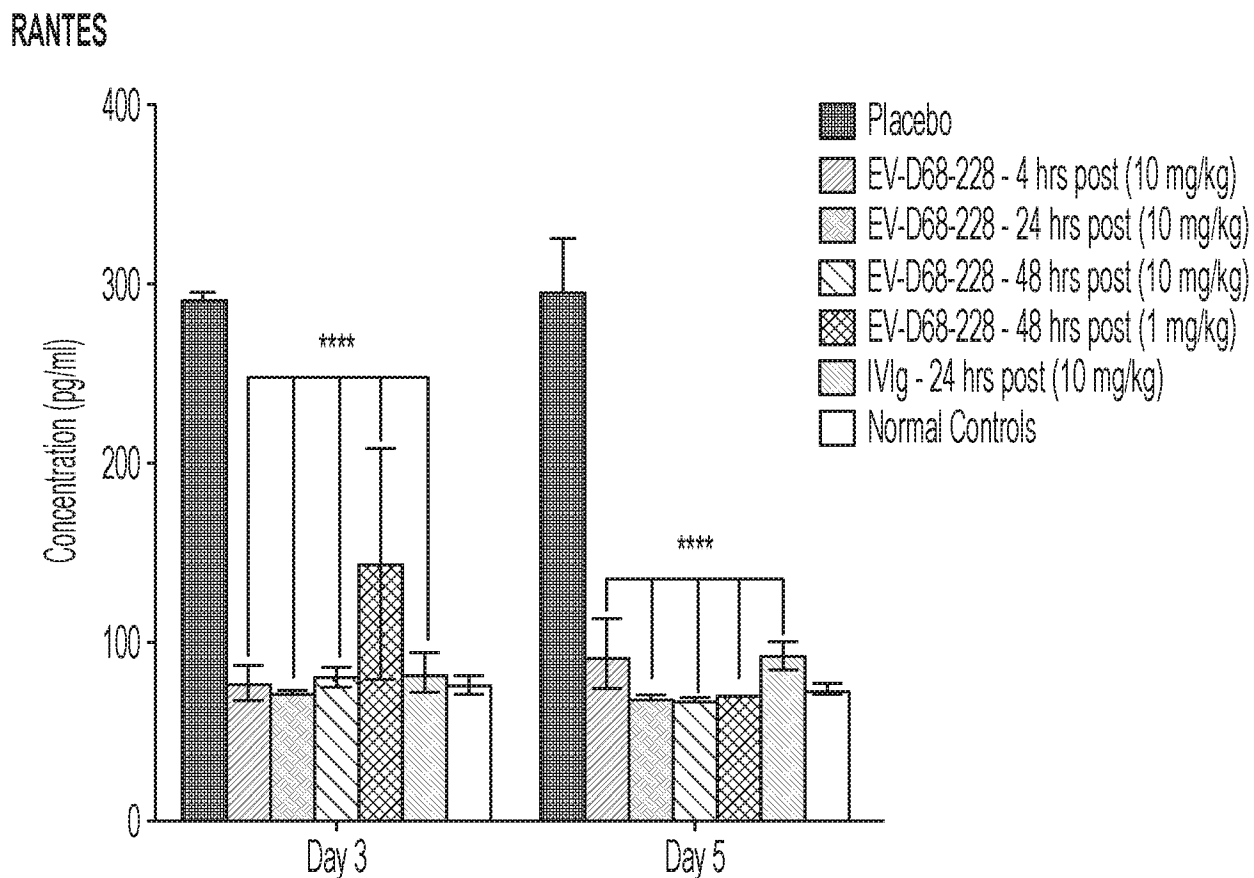
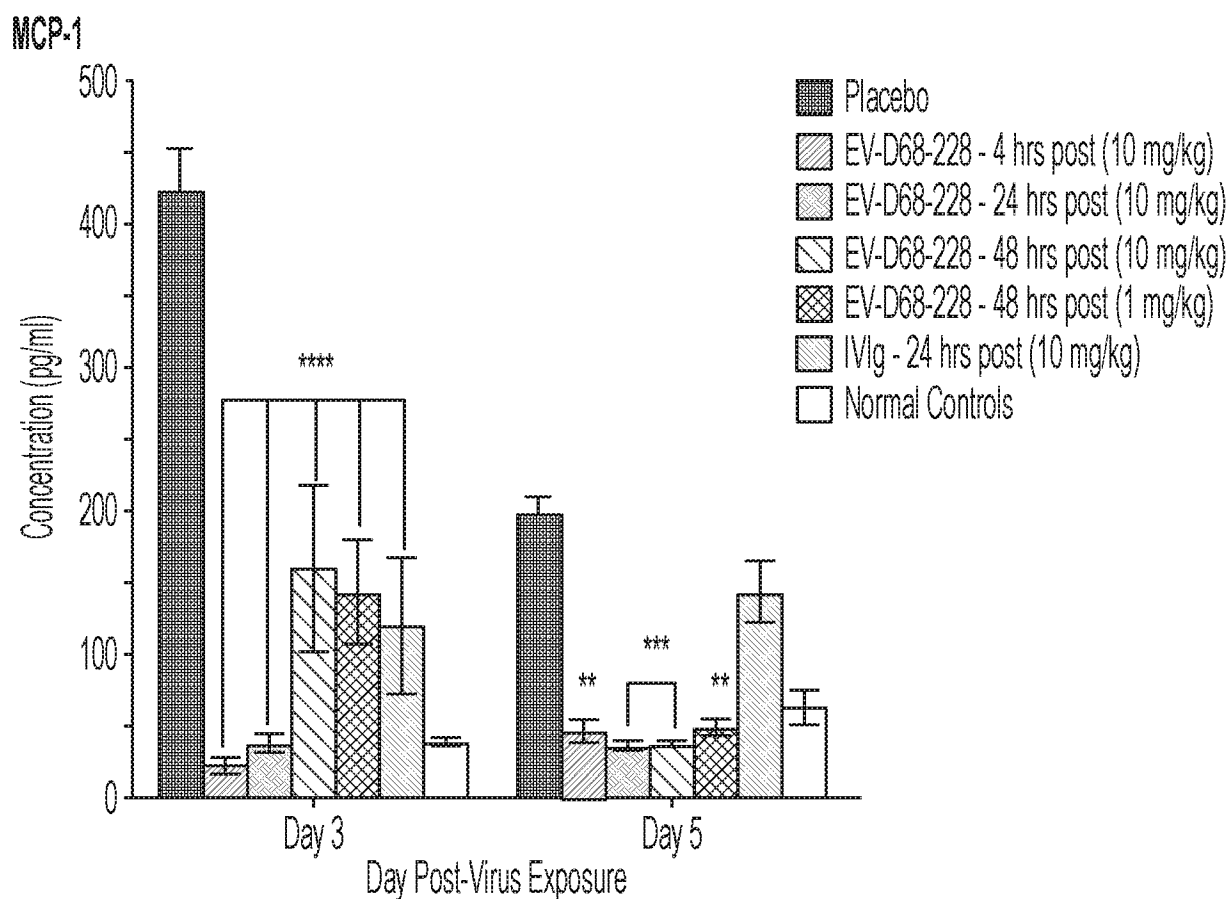


FIG. 23

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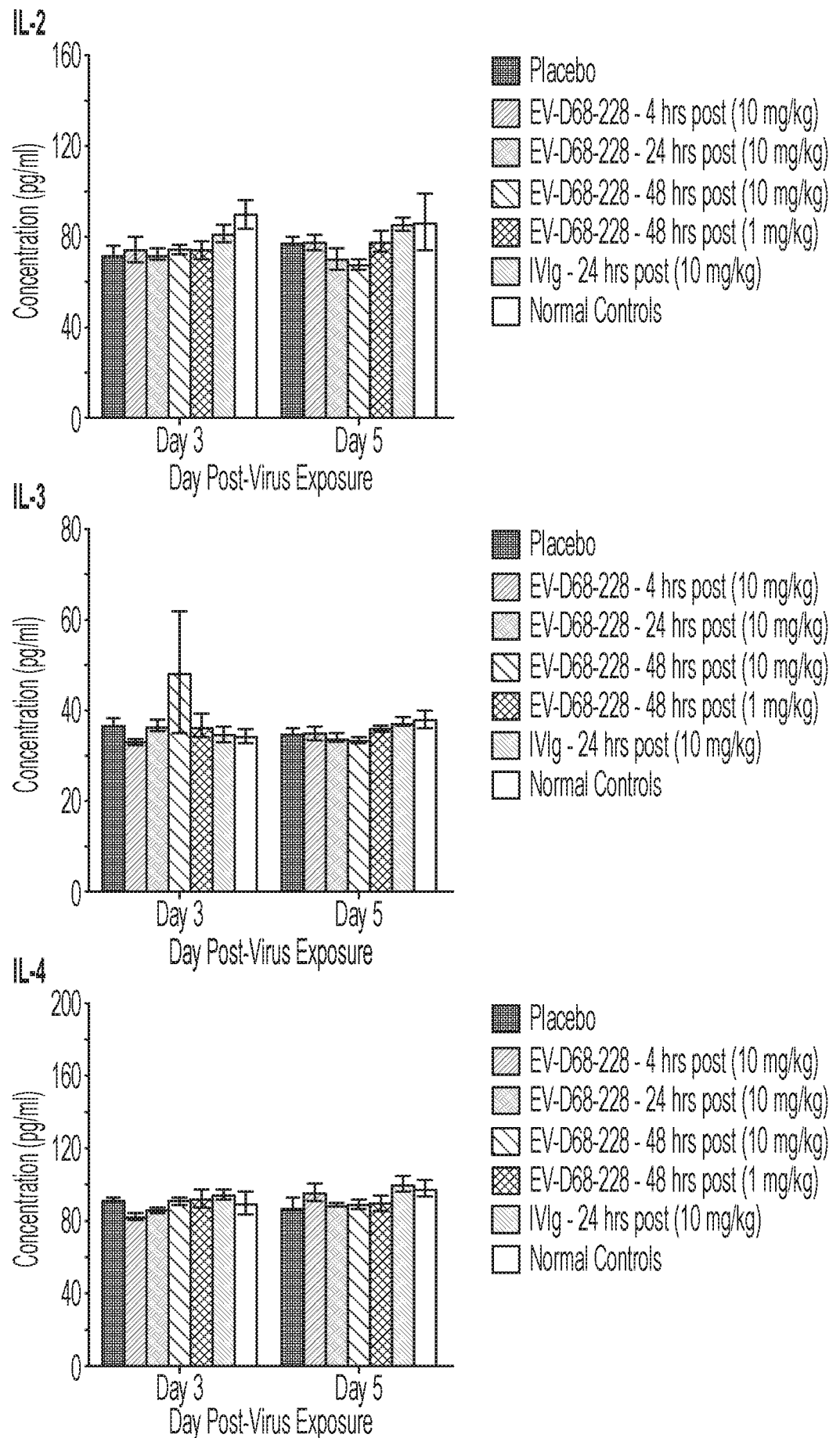


FIG. 24

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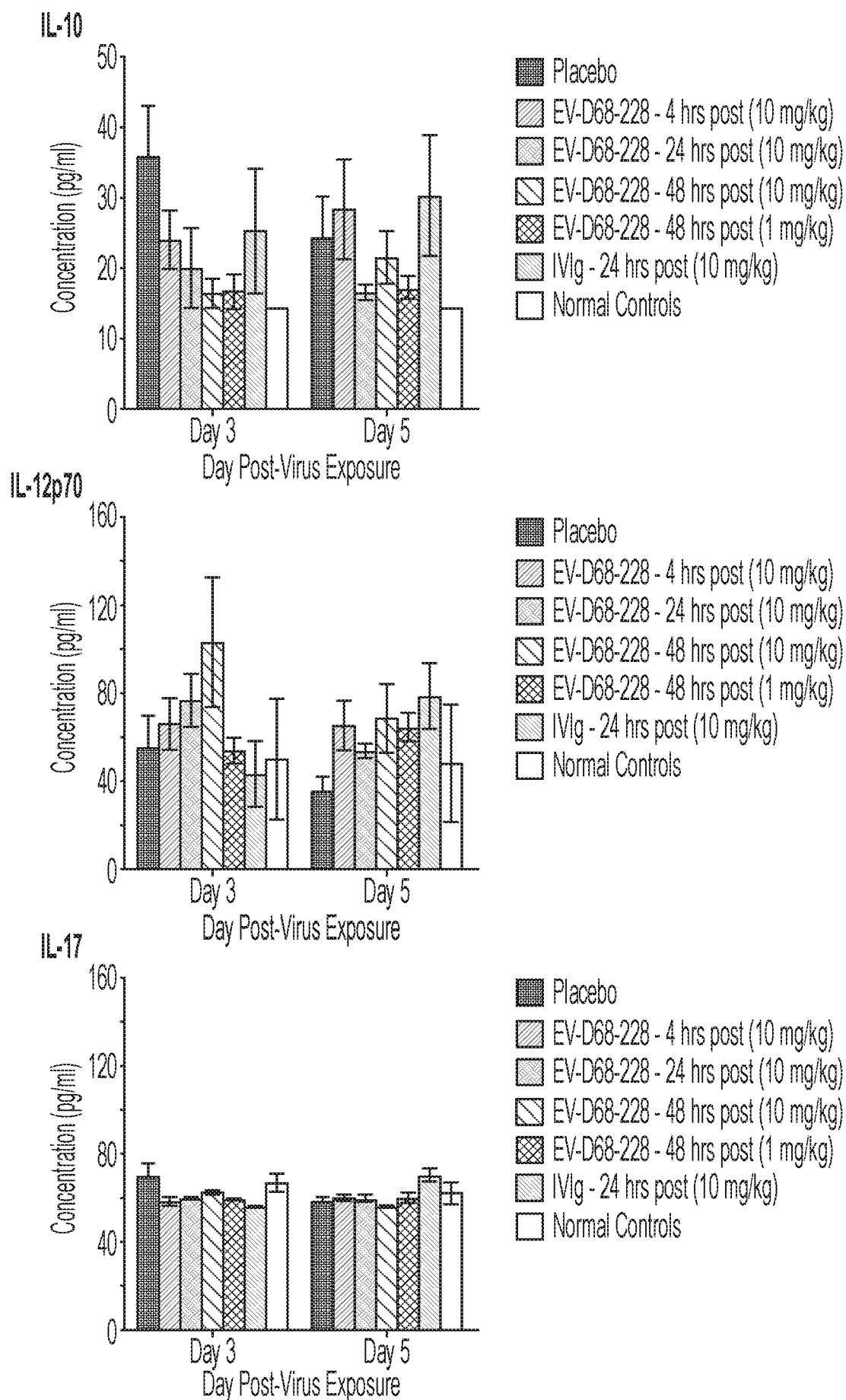


FIG. 25

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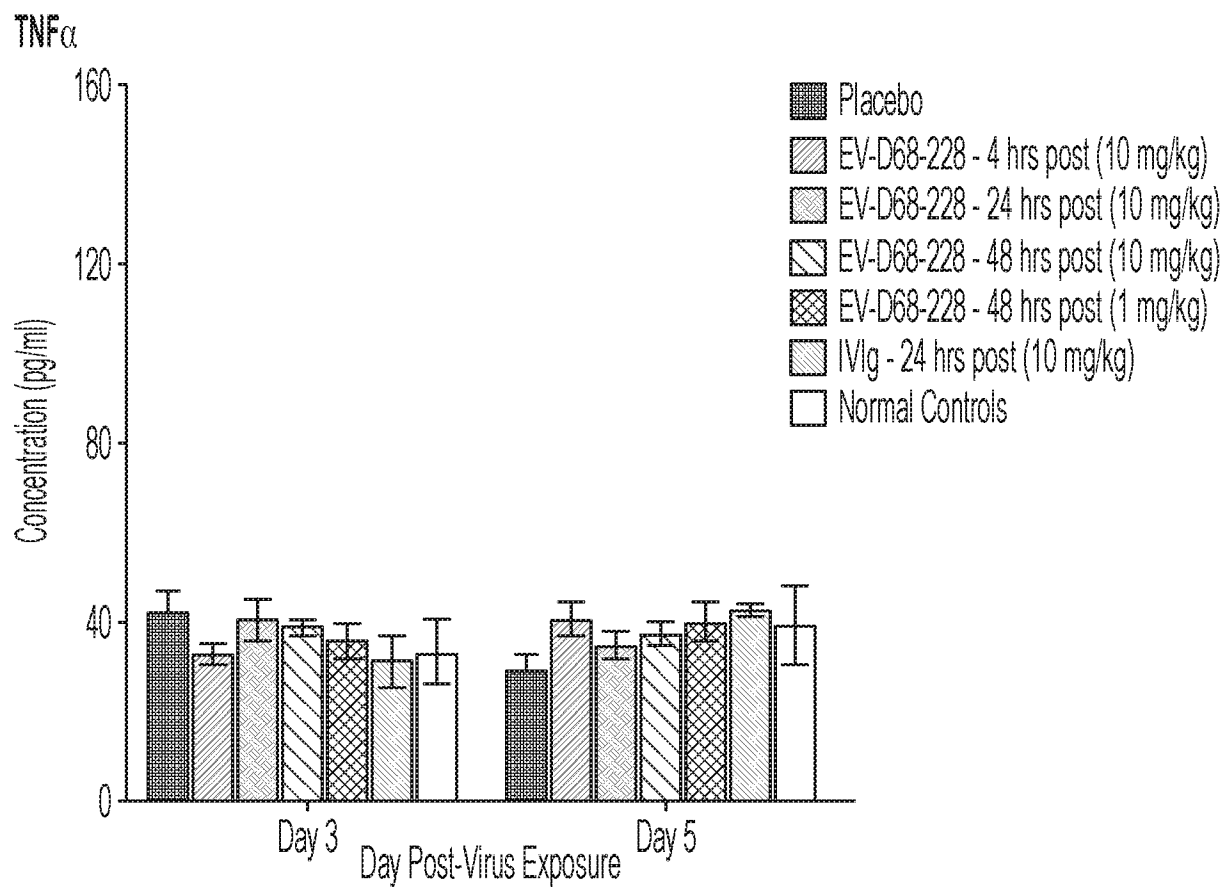
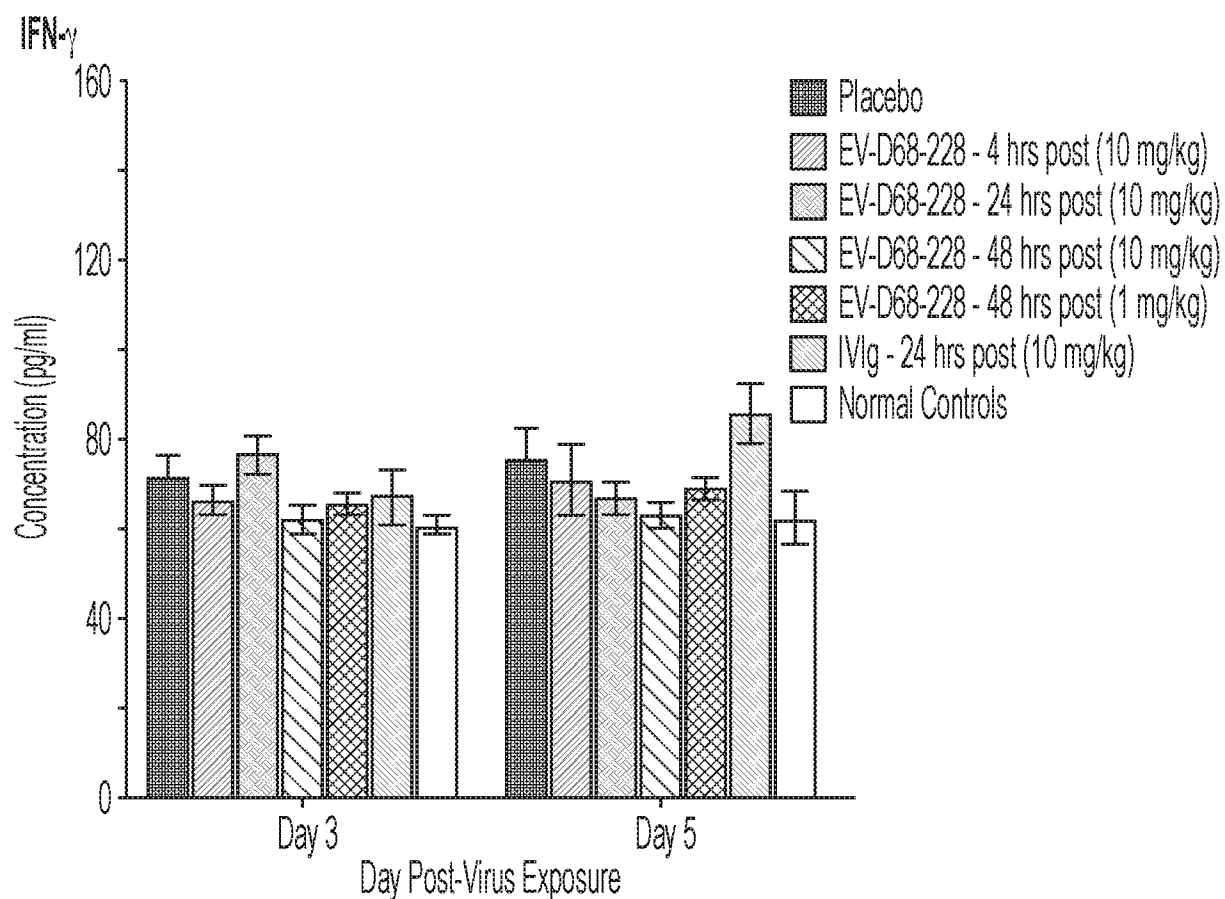


FIG. 26

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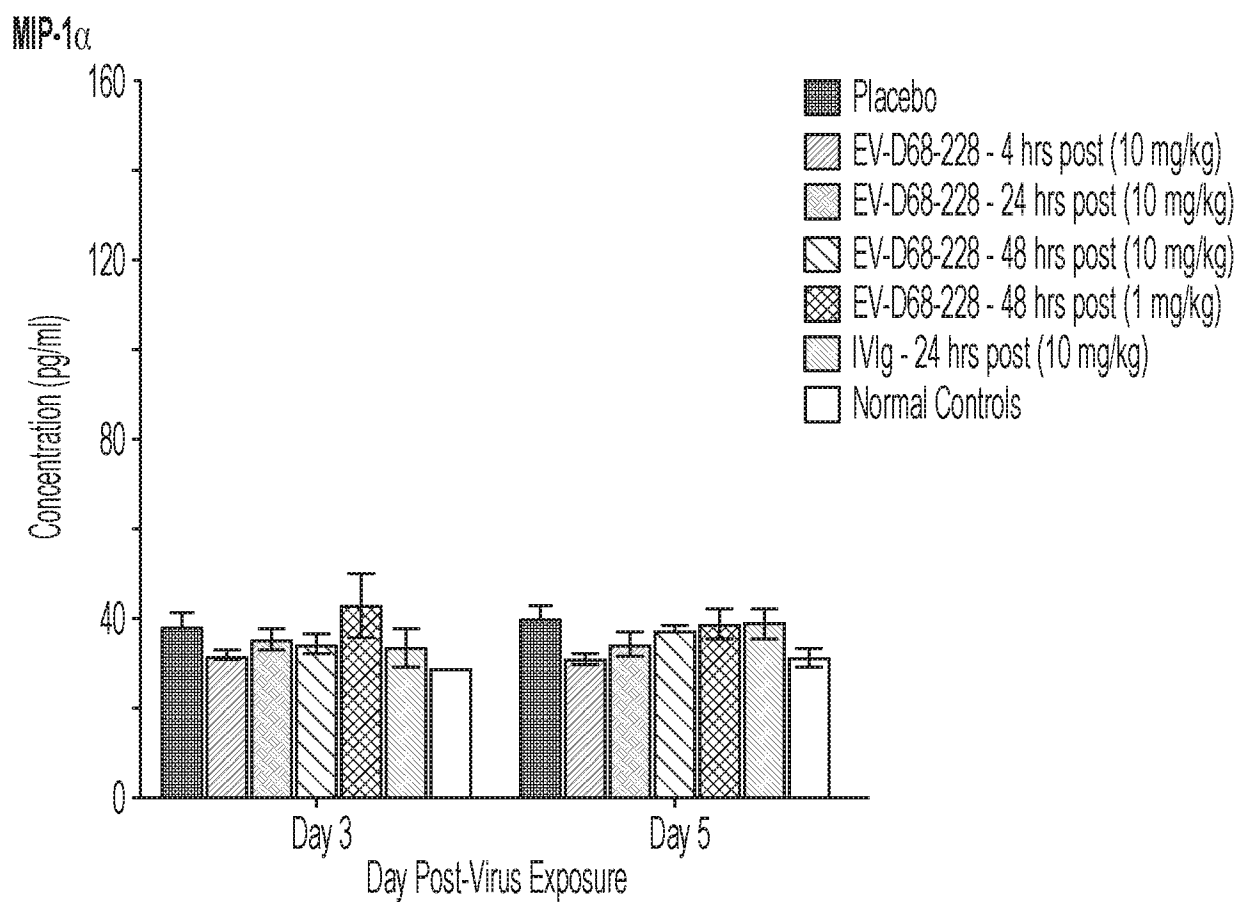


FIG. 27

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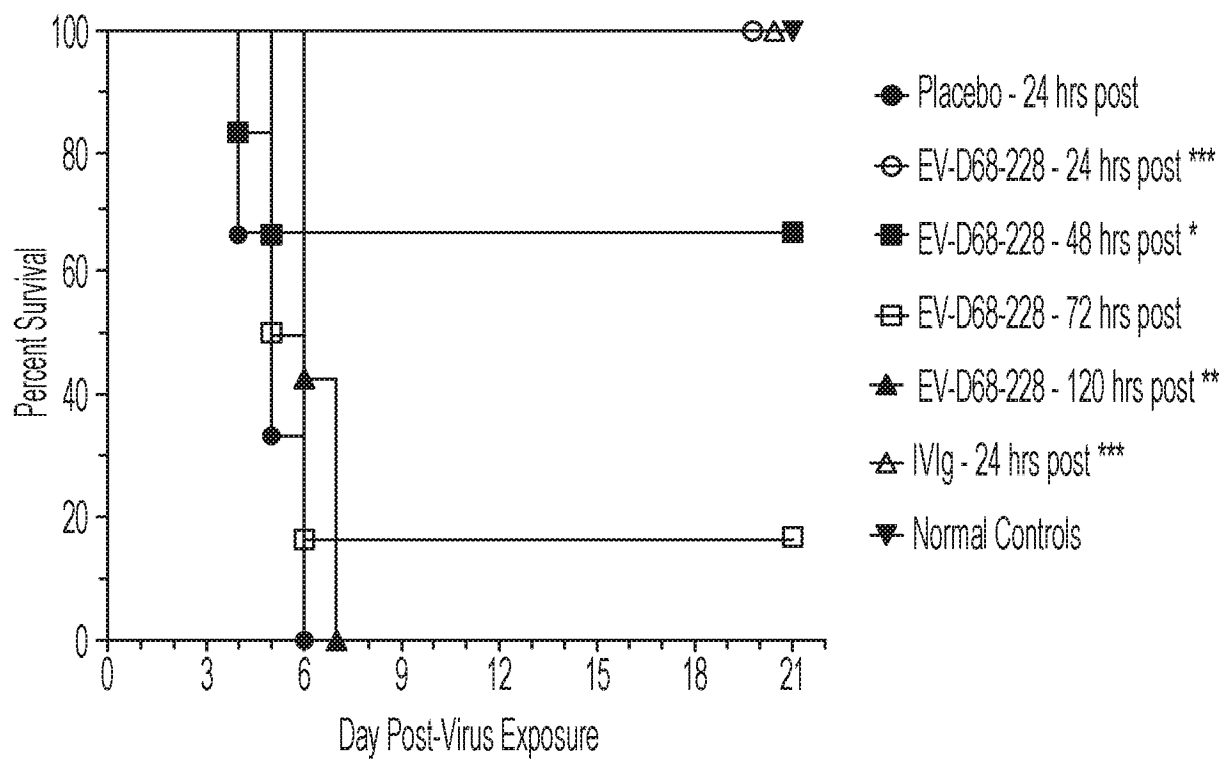


FIG. 28

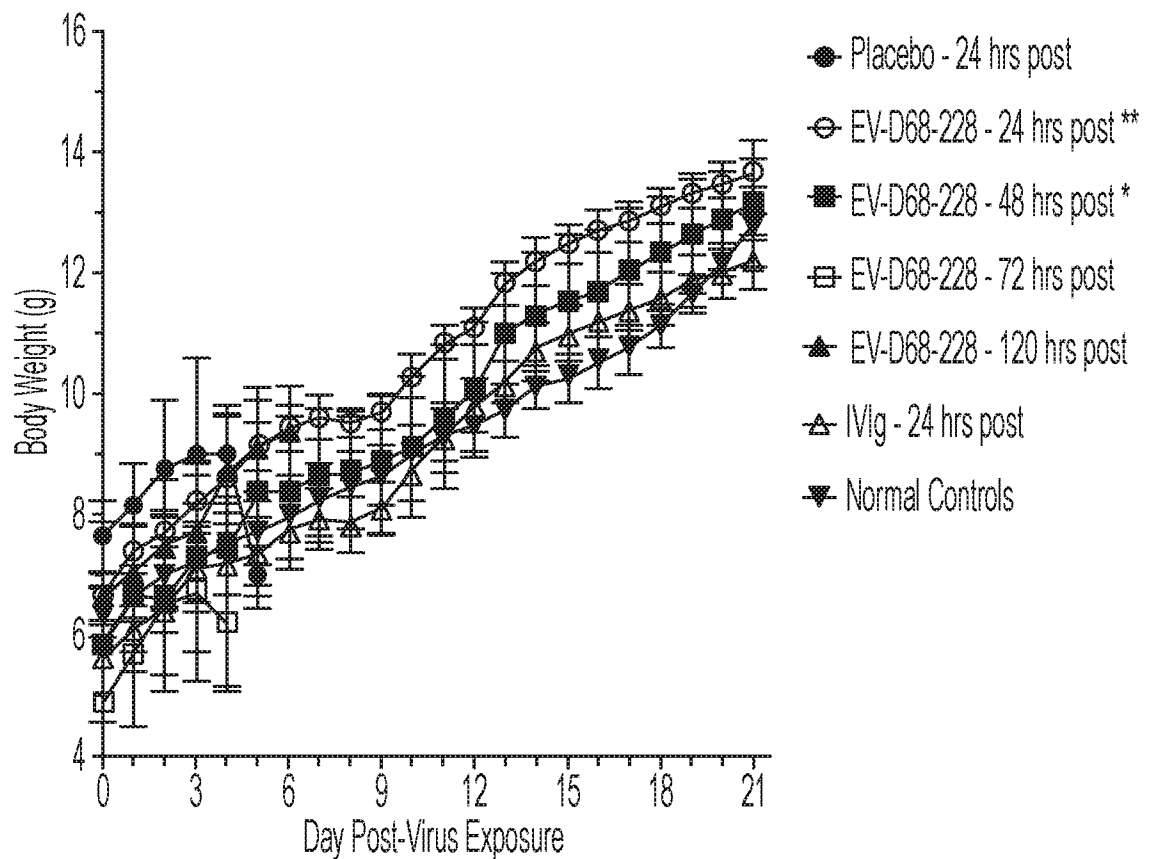


FIG. 29

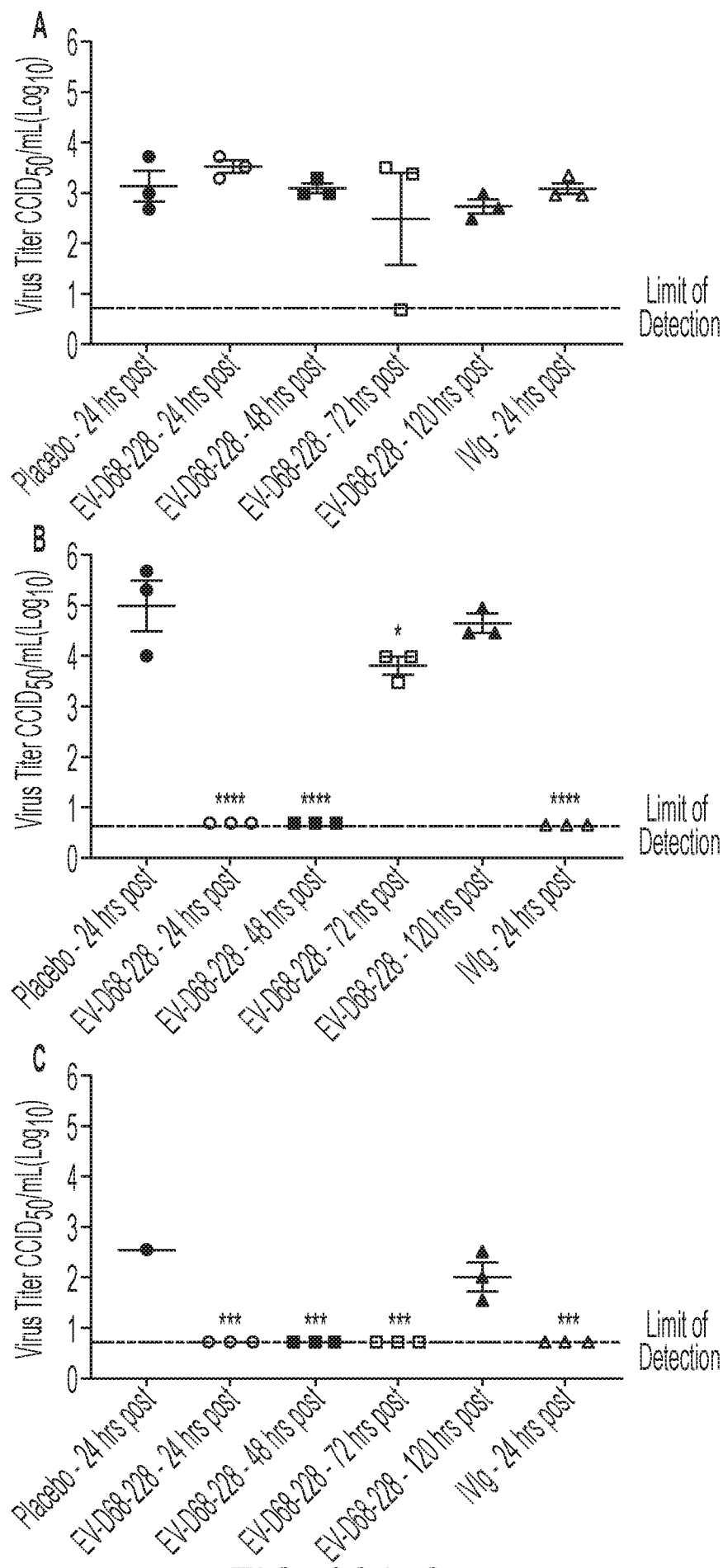


FIG. 30A-C

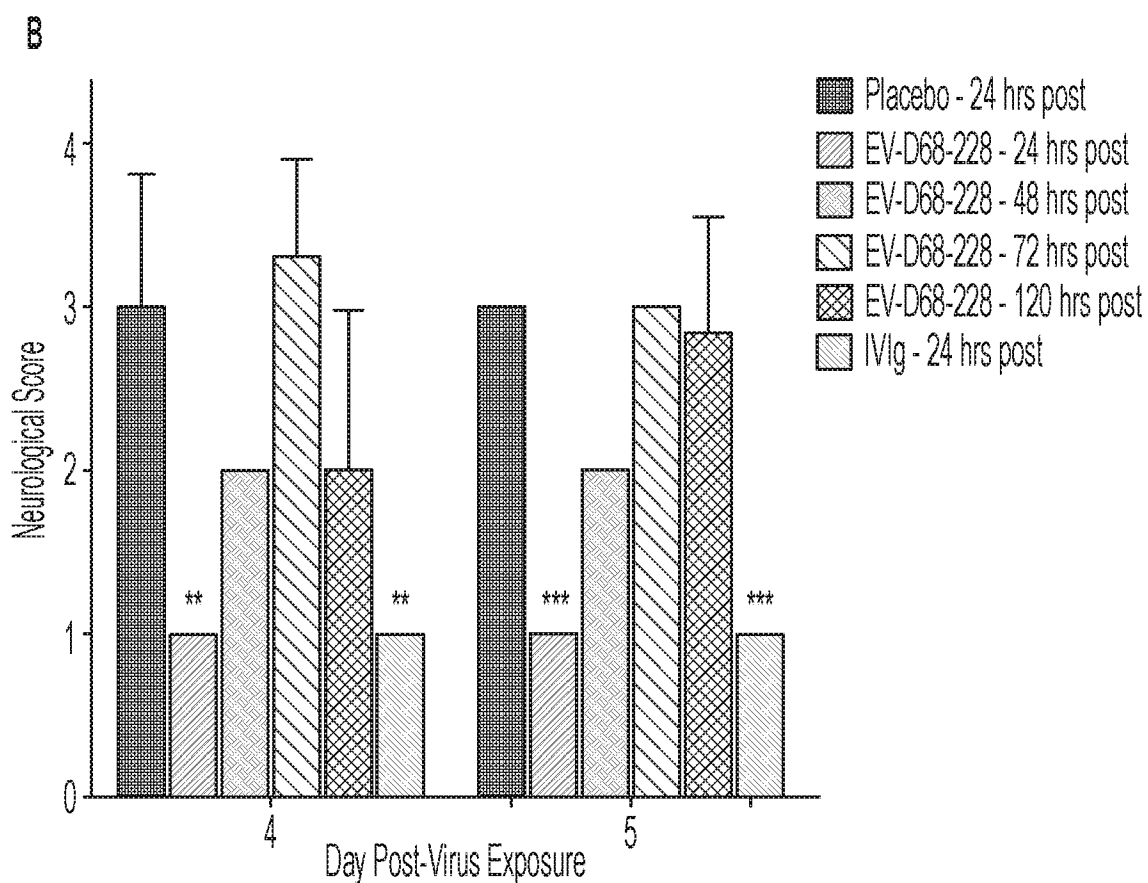
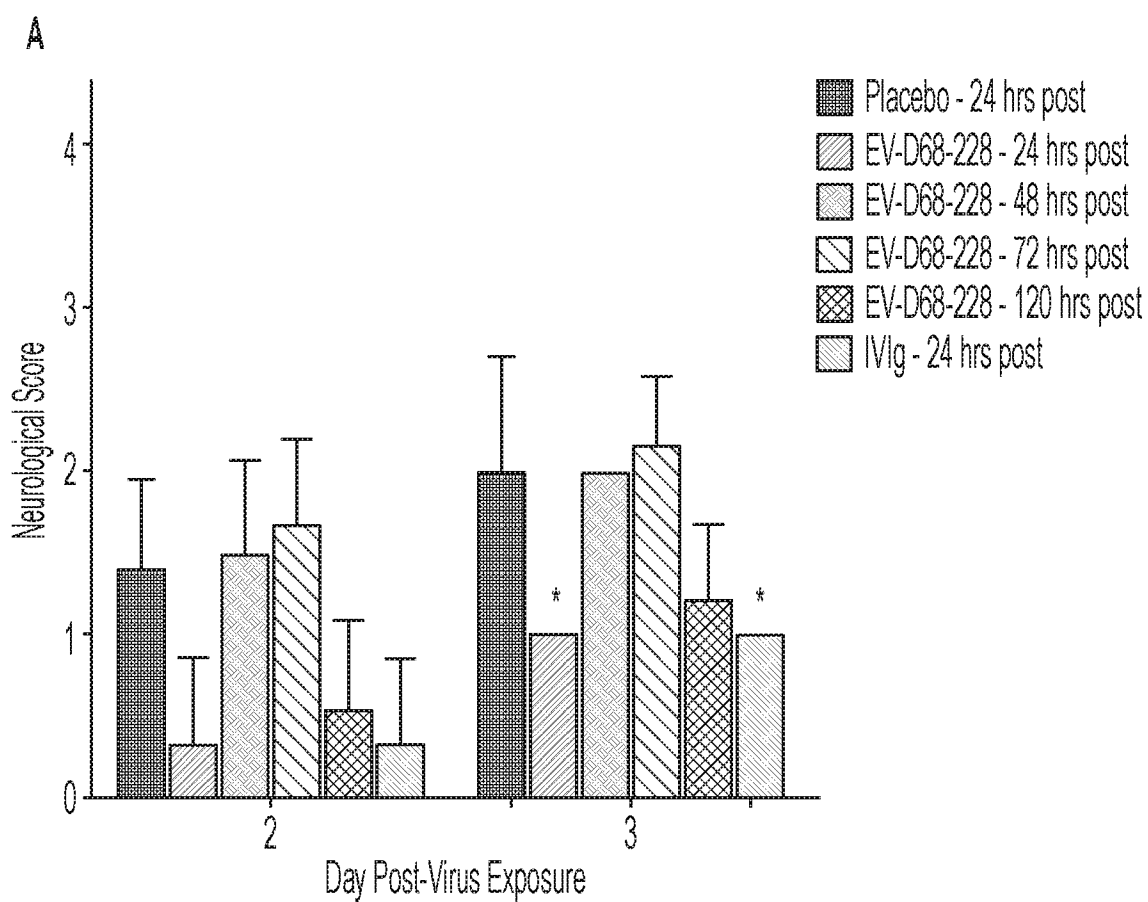


FIG. 31A-B

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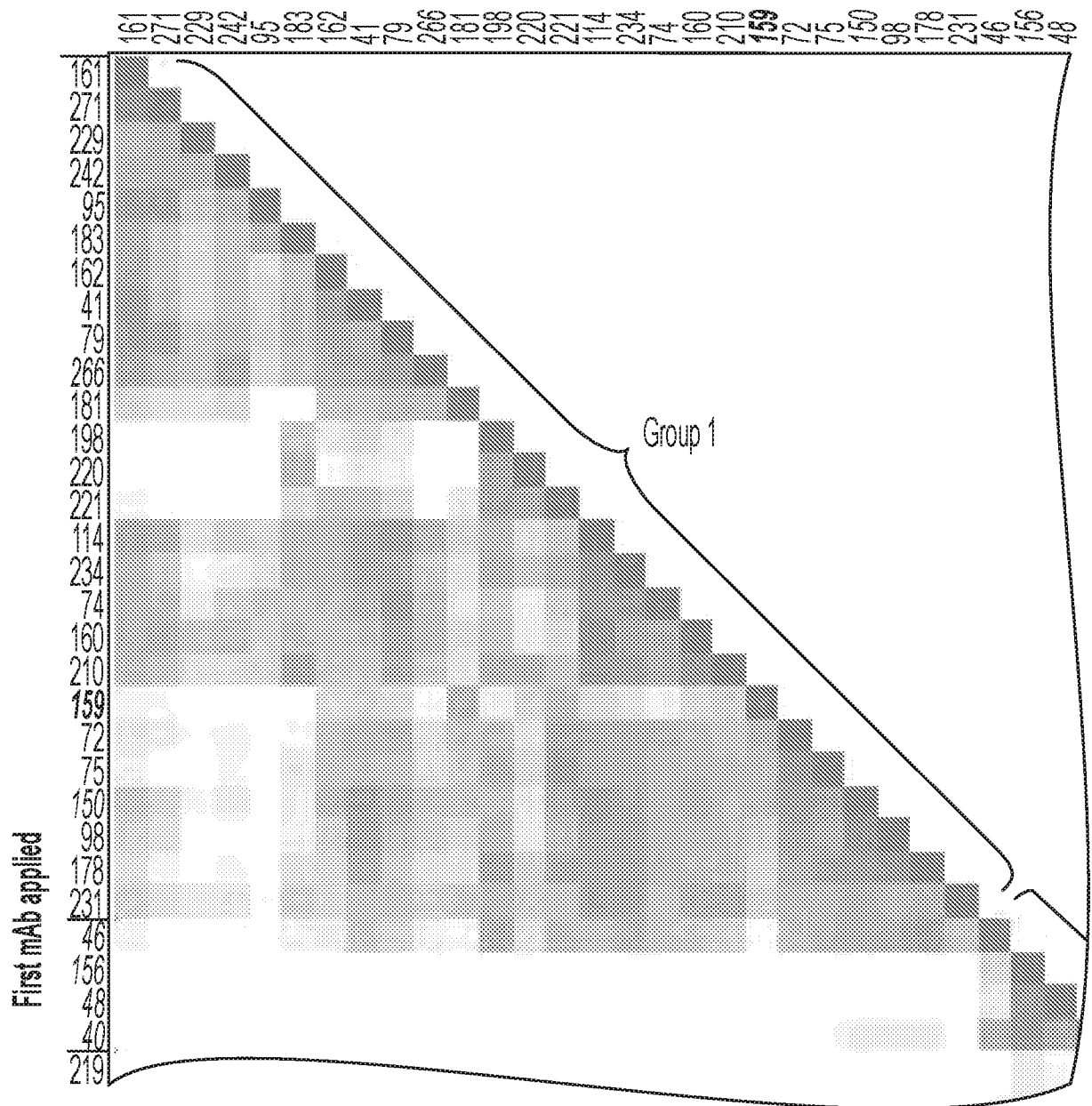


FIG. 32

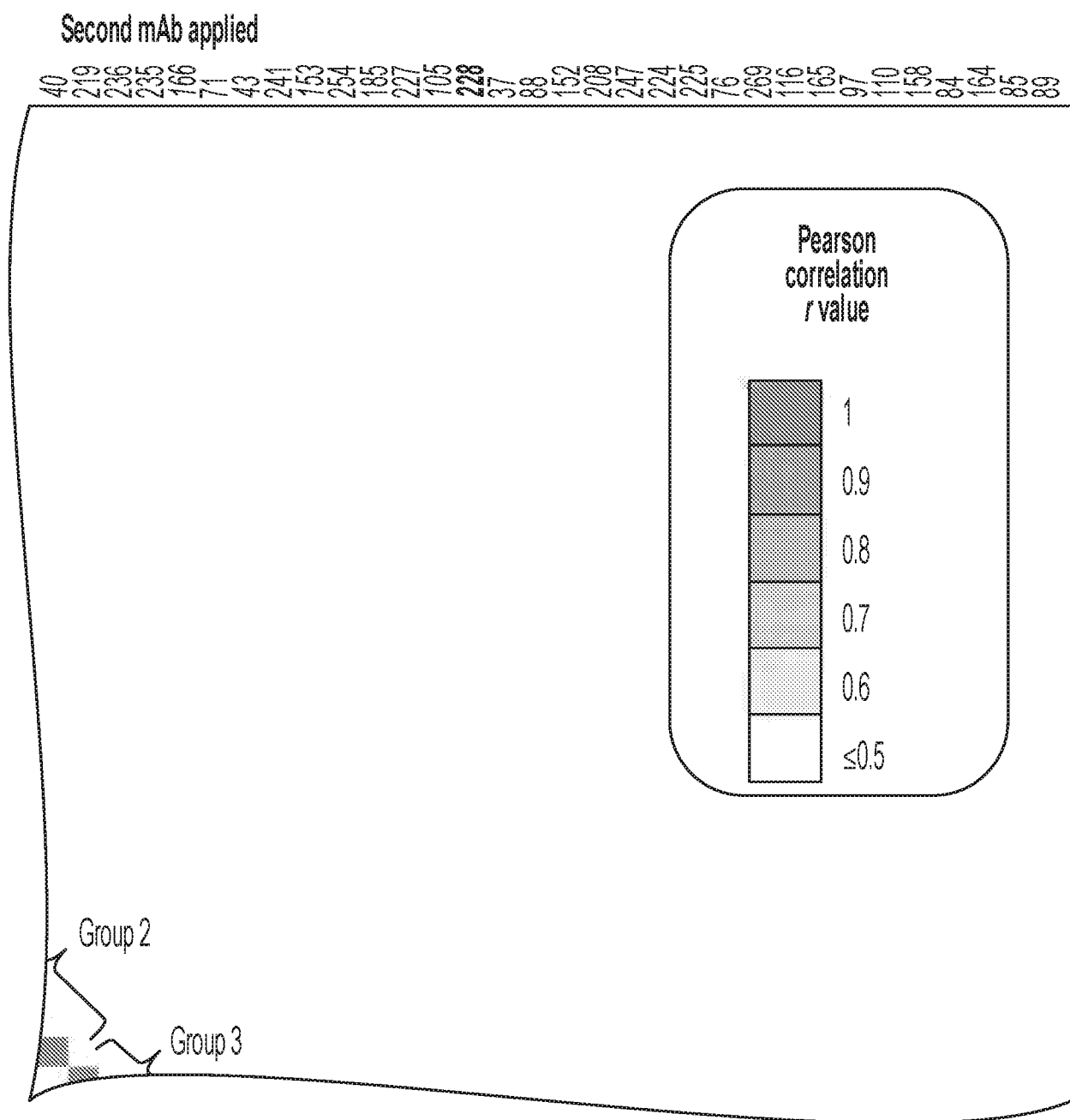


FIG. 32
CONTINUED

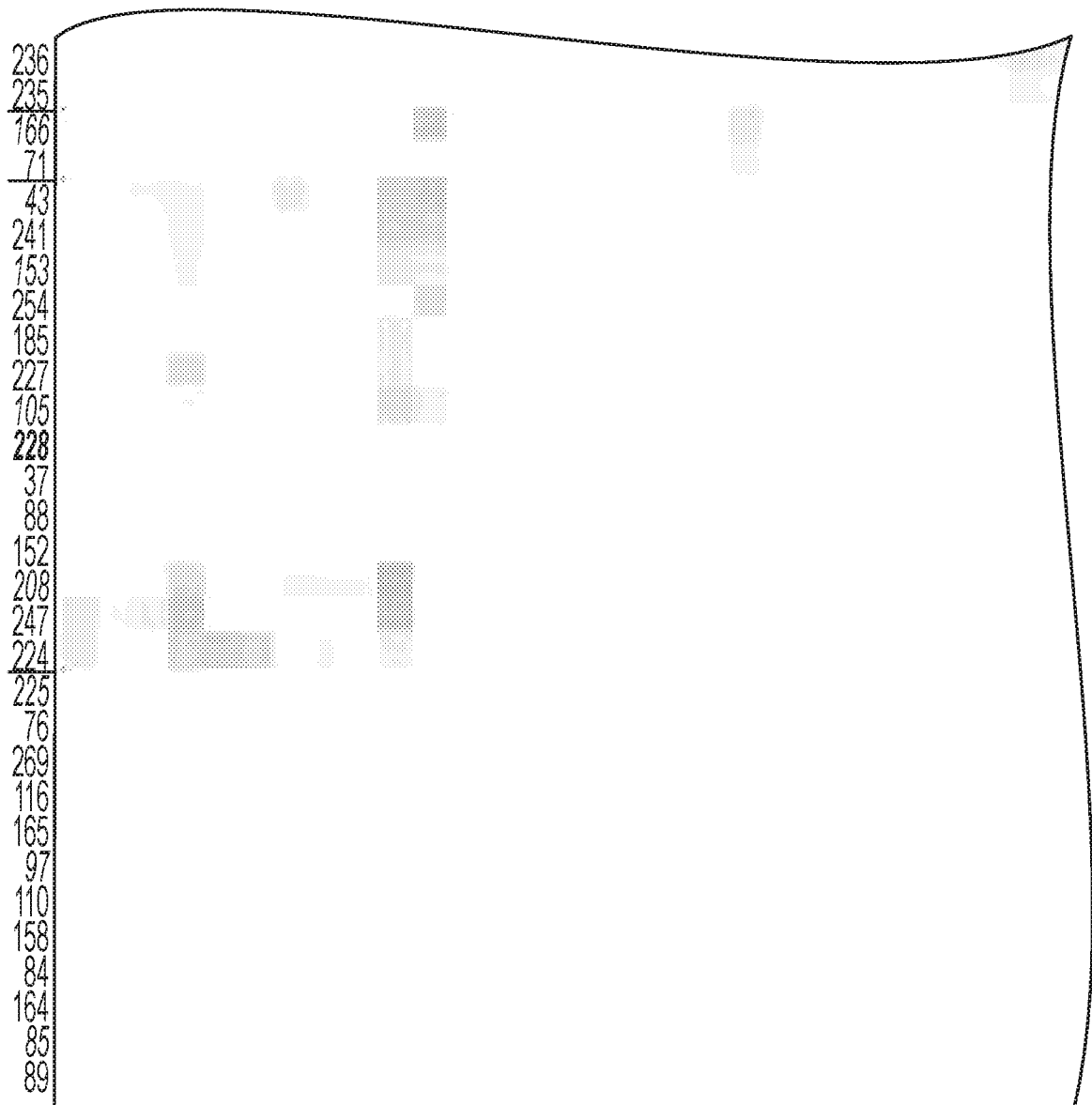


FIG. 32
CONTINUED

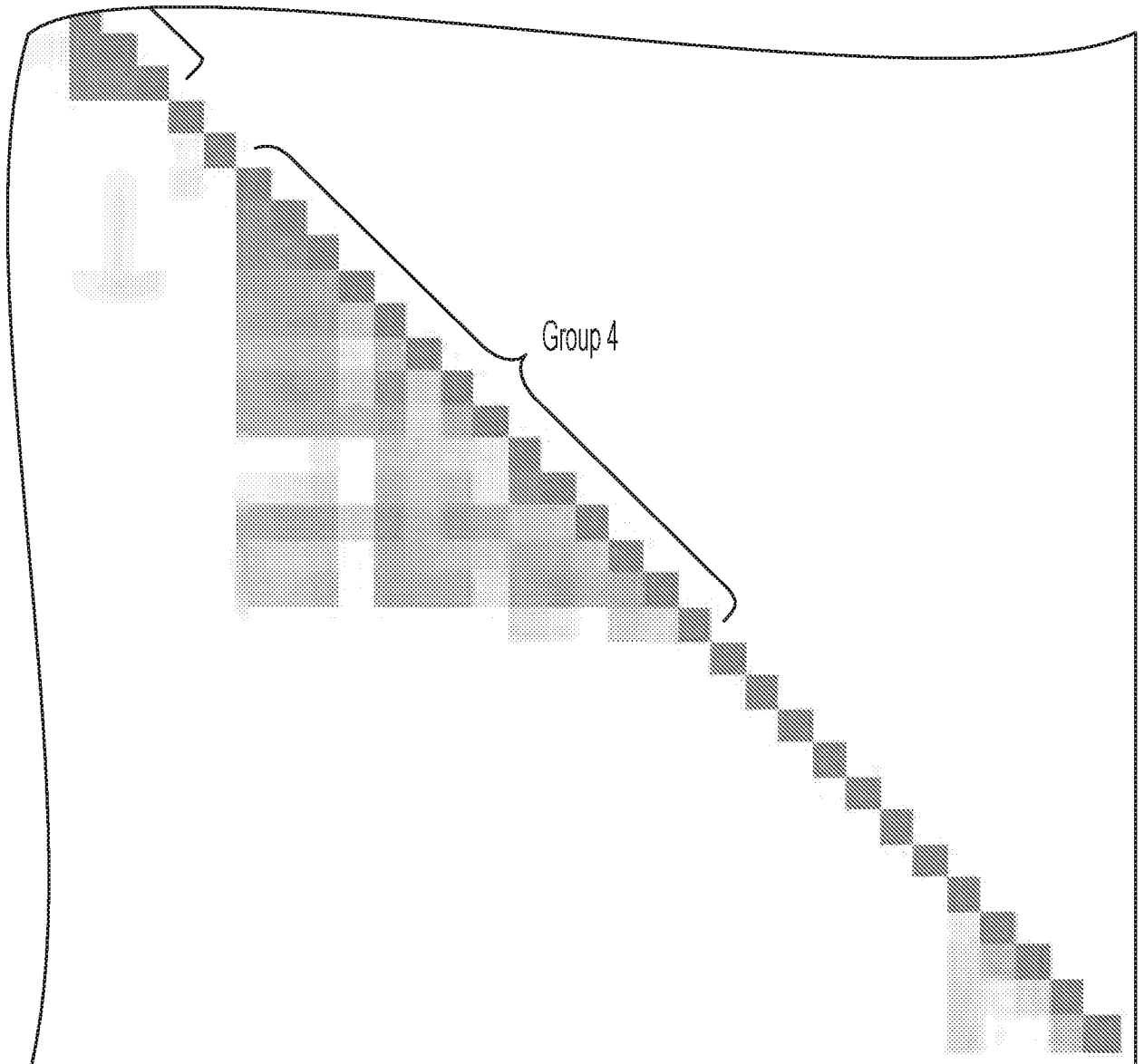
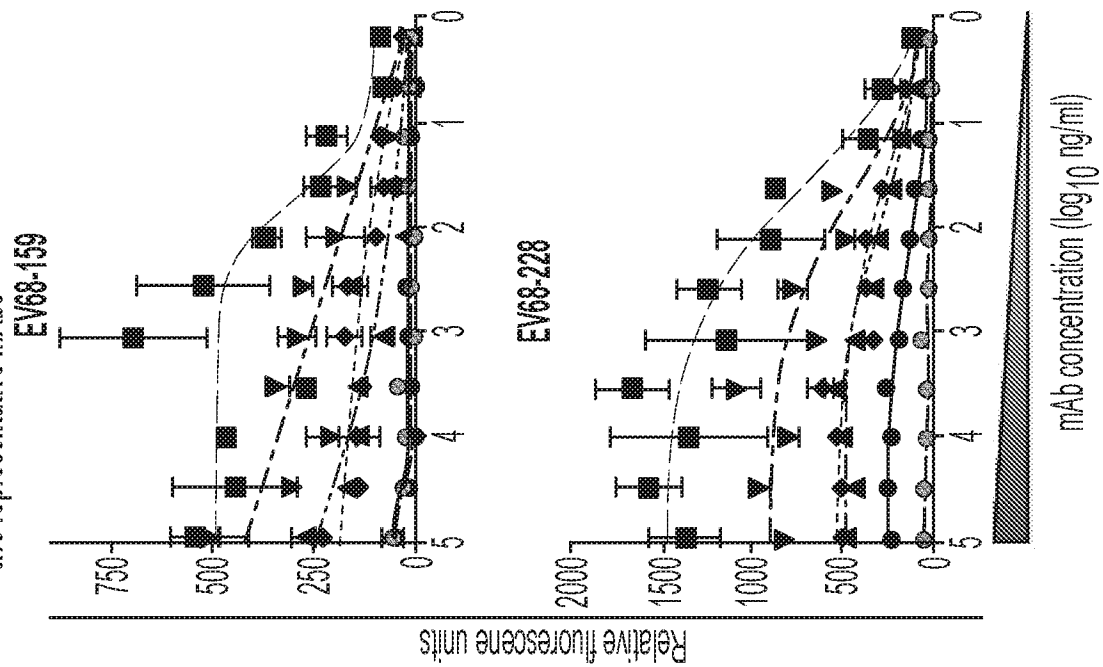


FIG. 32
CONTINUED

FIG. 33A-C



198	210	220	221
229	234	271	
40	219	153	
48	235	105	
46	236	228	
156		43	
		208	
		152	
2	3		

FIG. 33A-C
CONTINUED

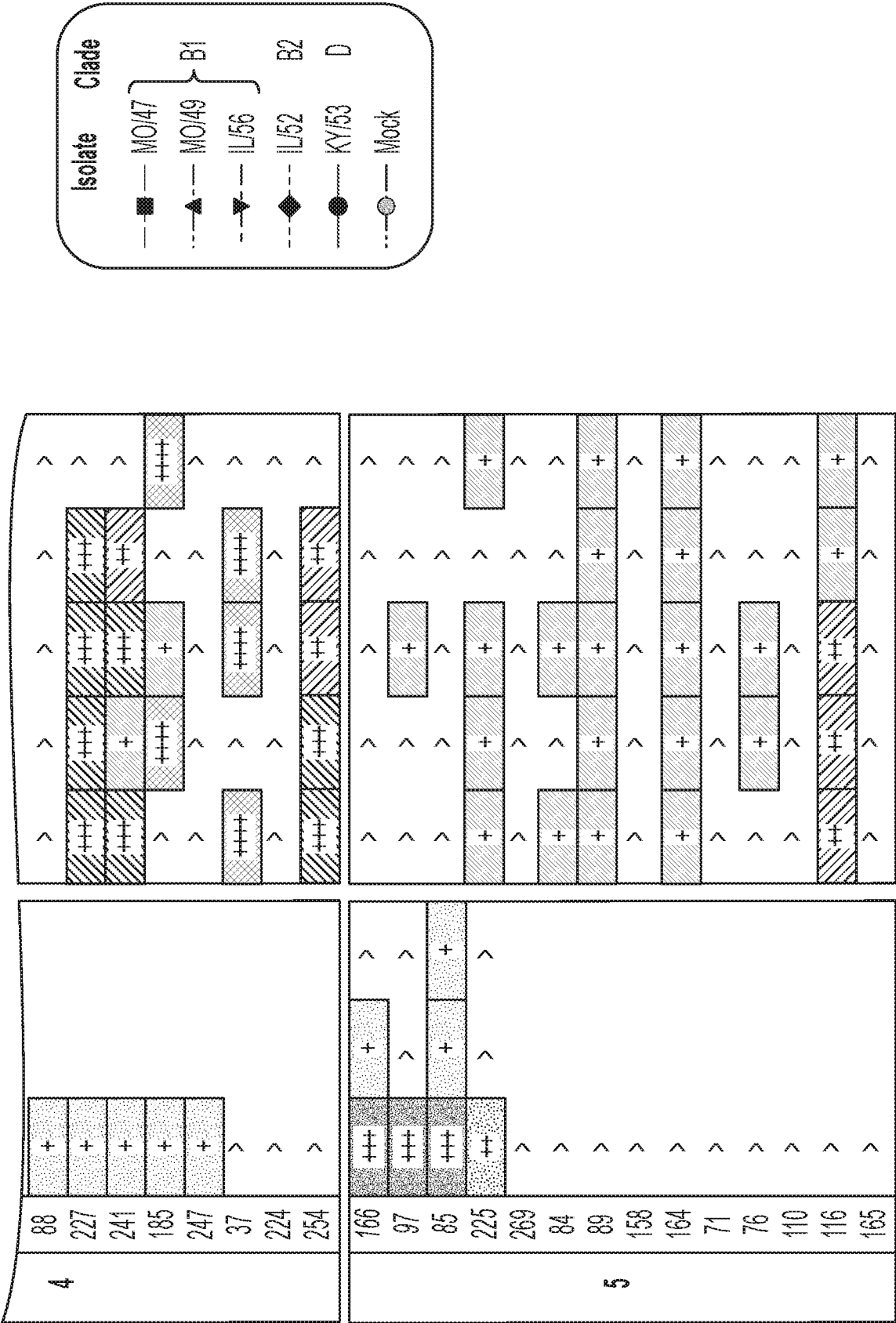
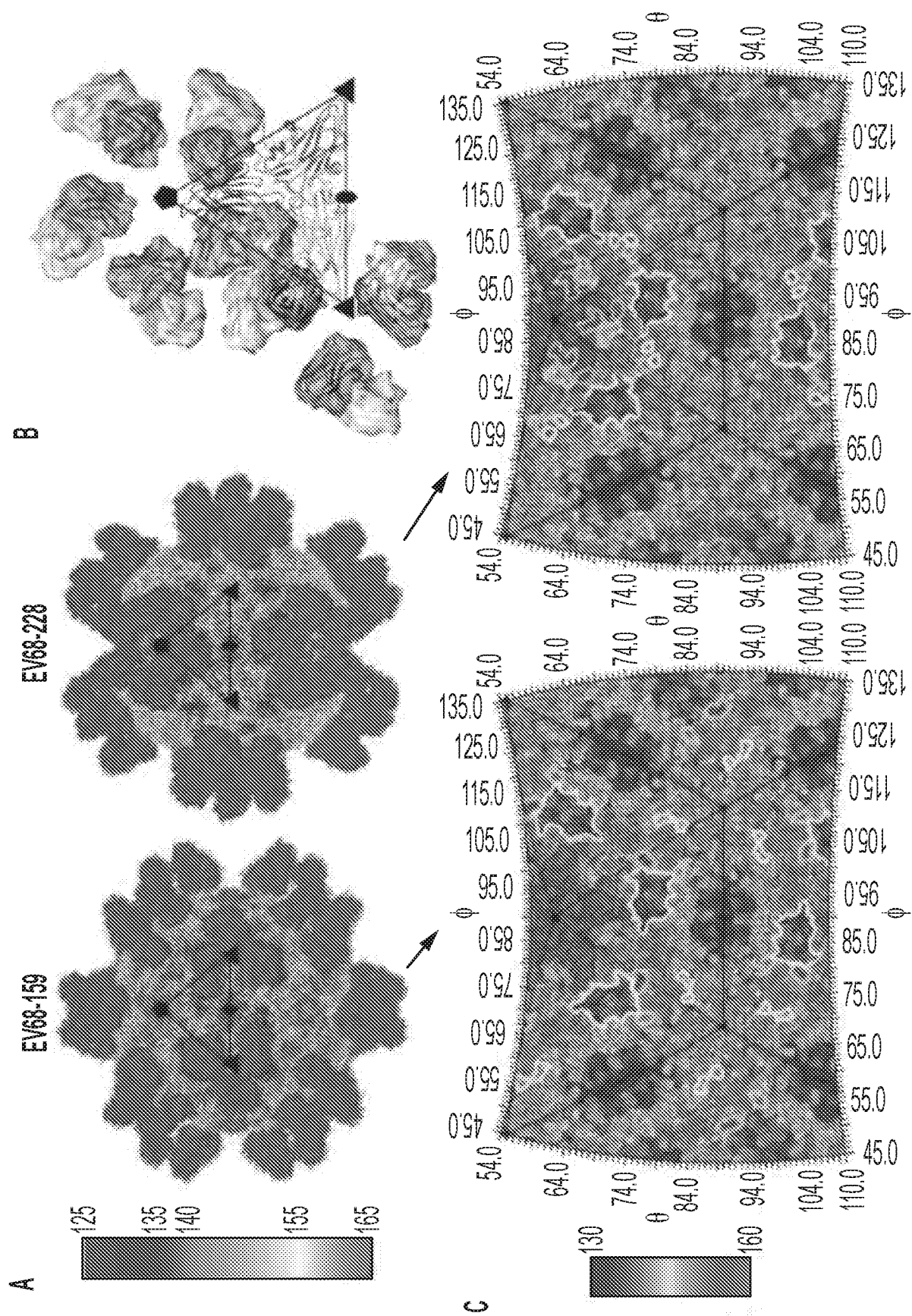


FIG. 33A-C
CONTINUED



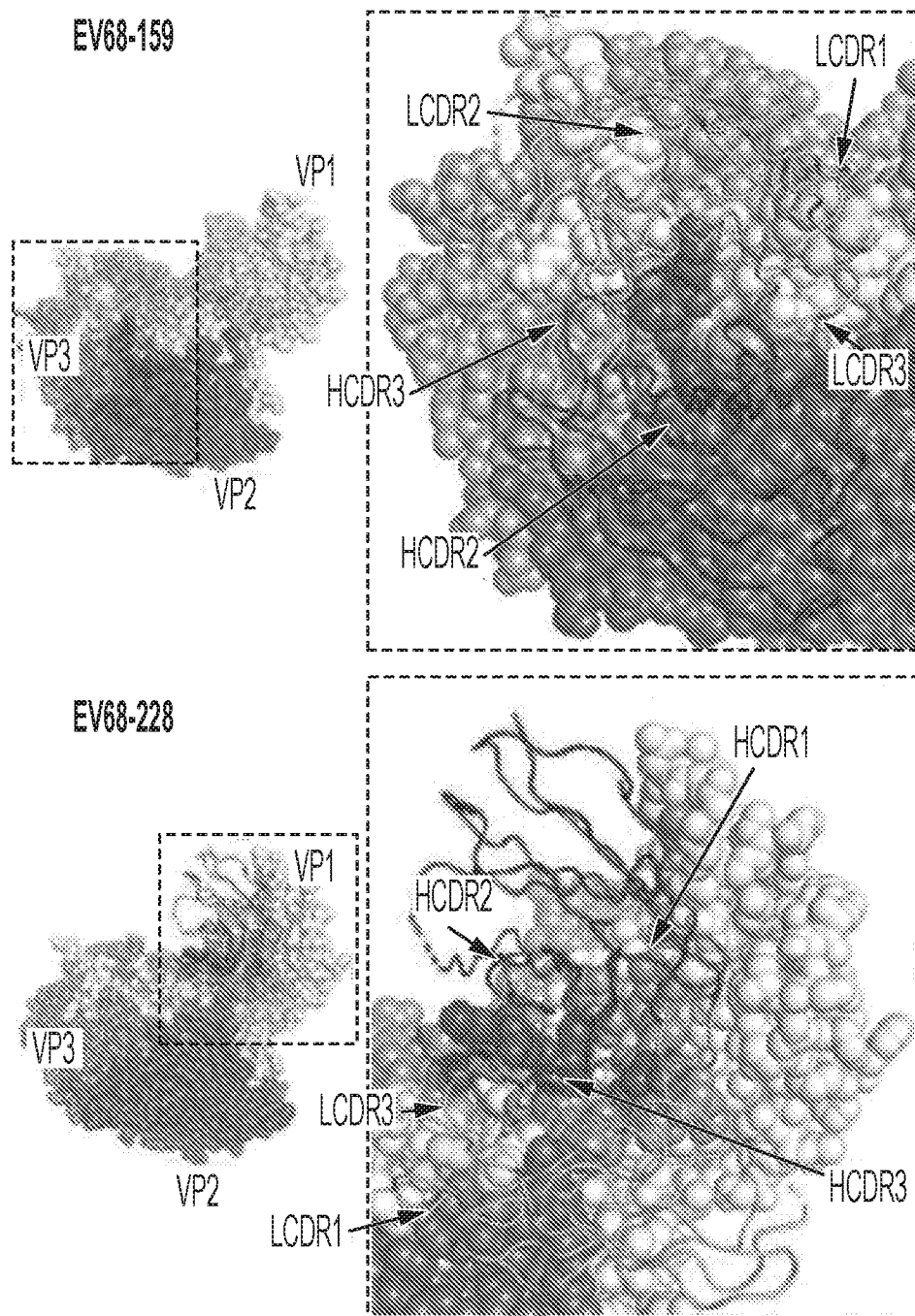


FIG. 35

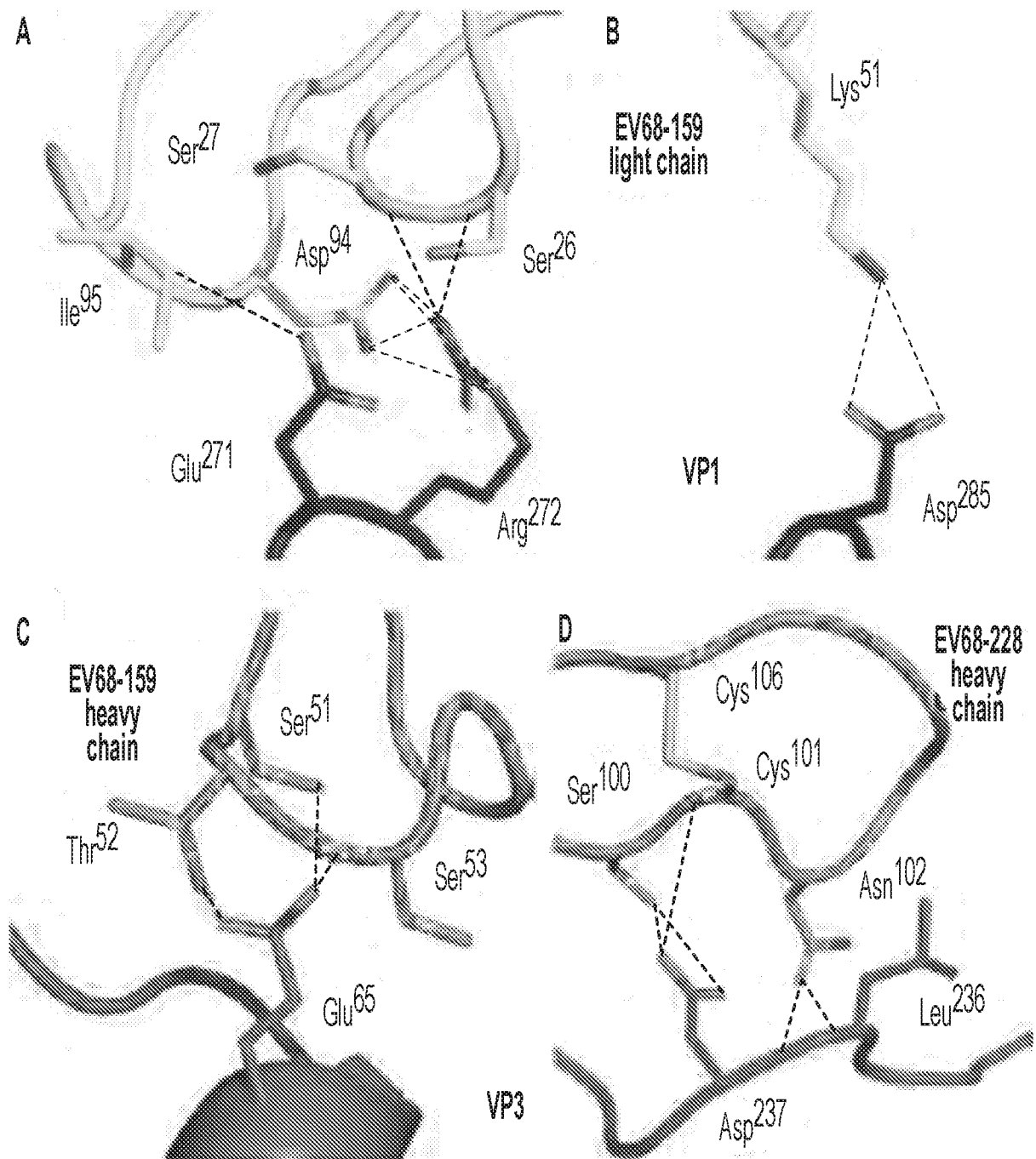


FIG. 36A-D

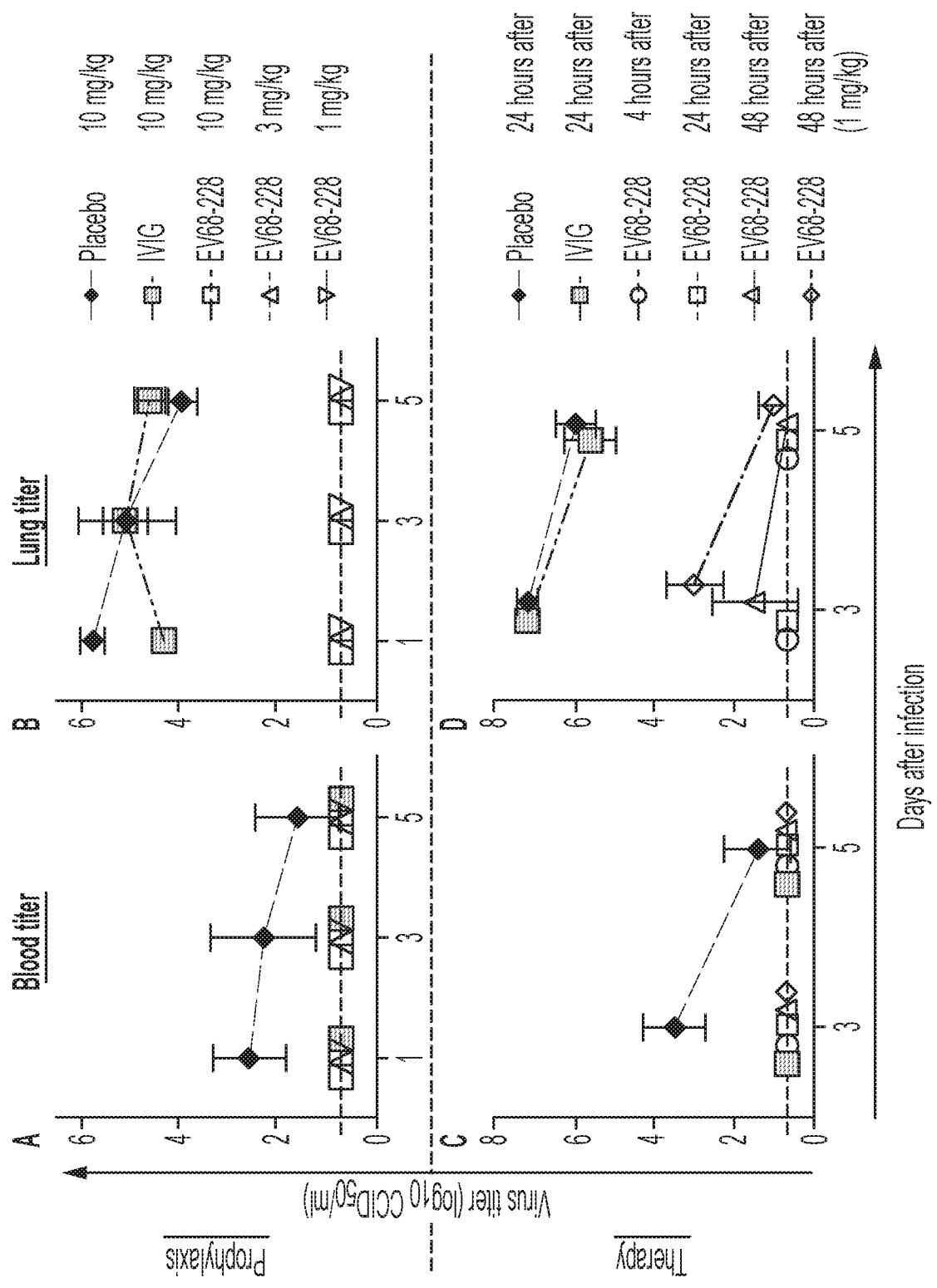


FIG. 37A-D

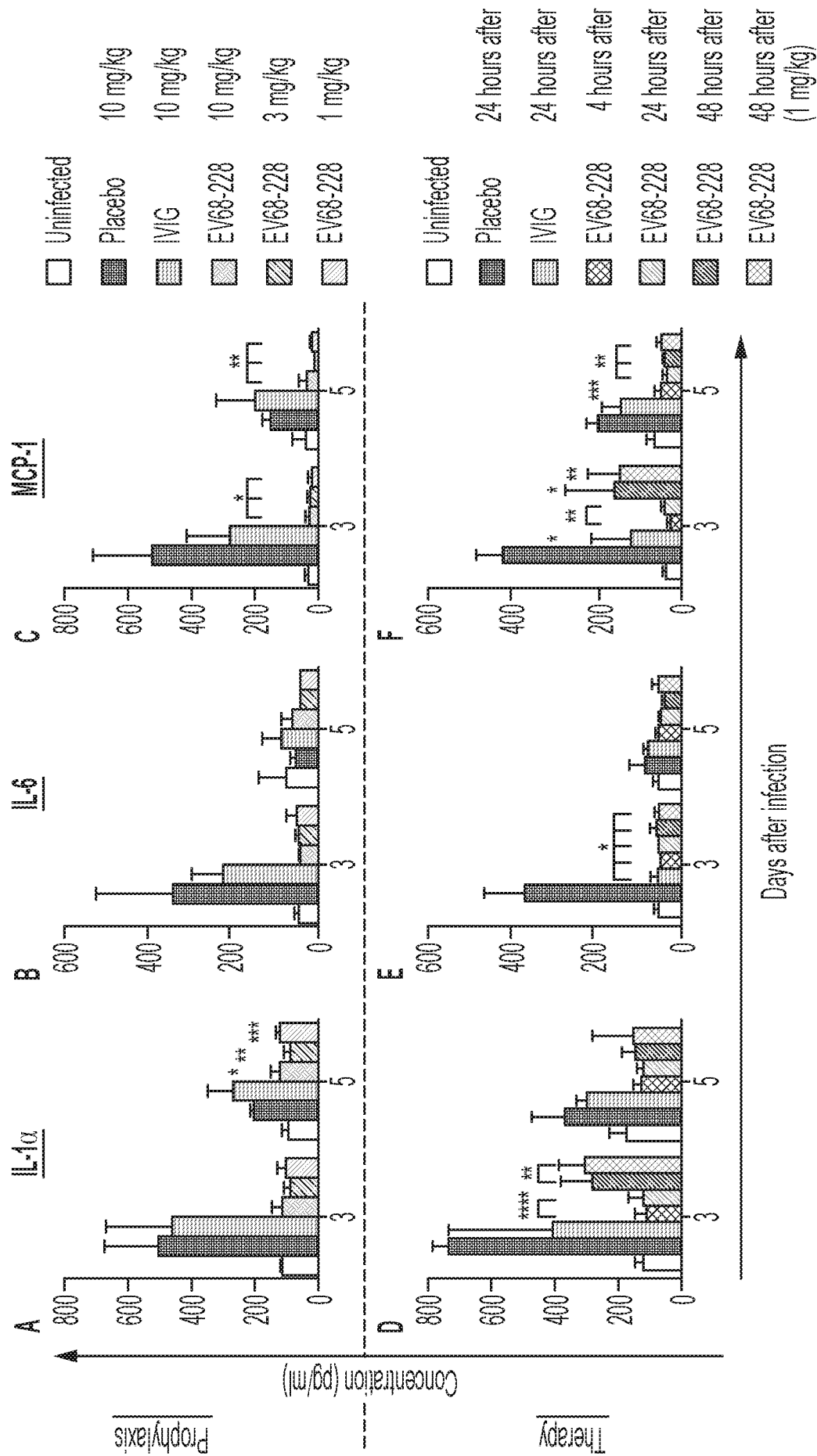


FIG. 38A-F

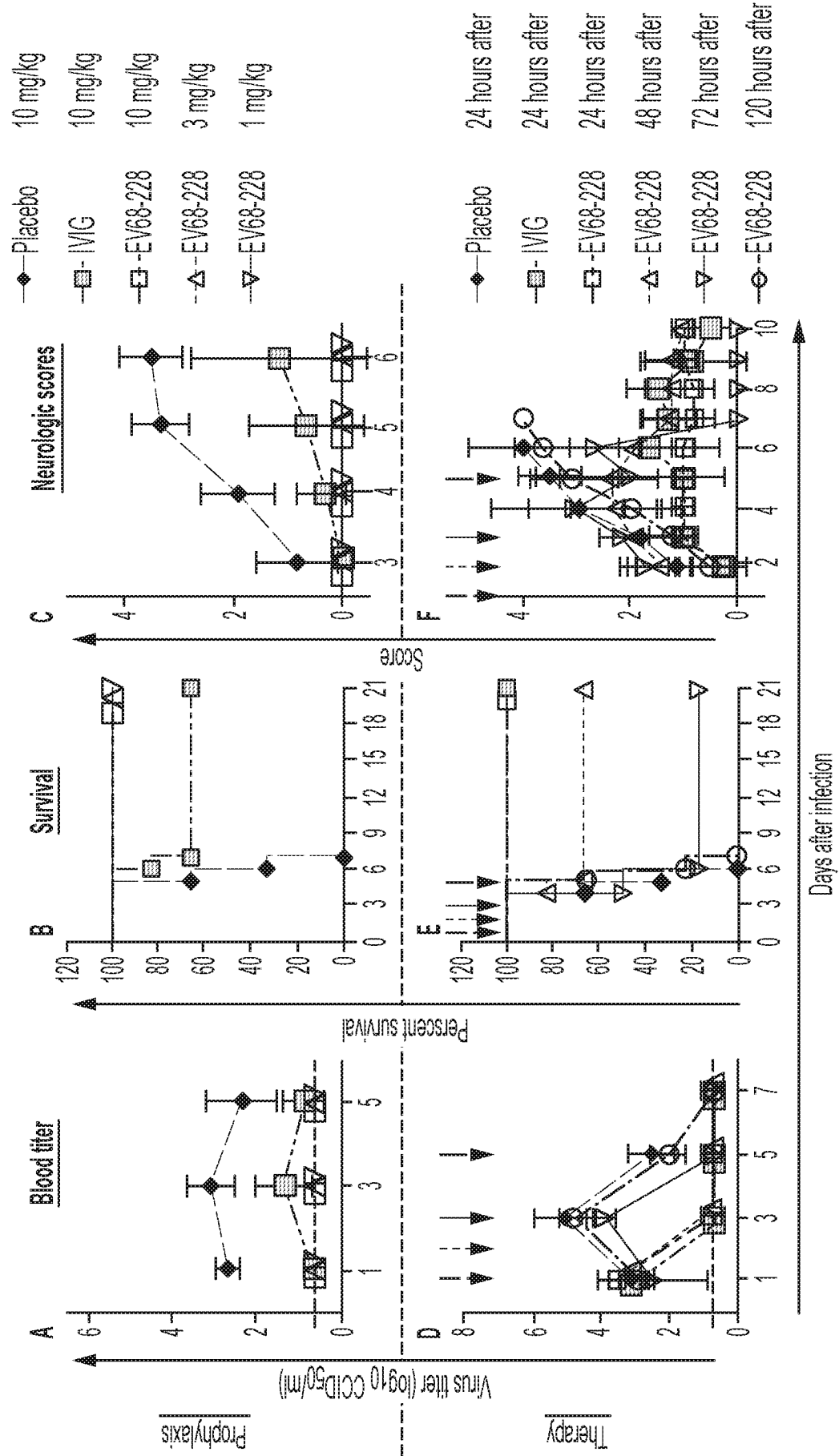


FIG. 39A-F

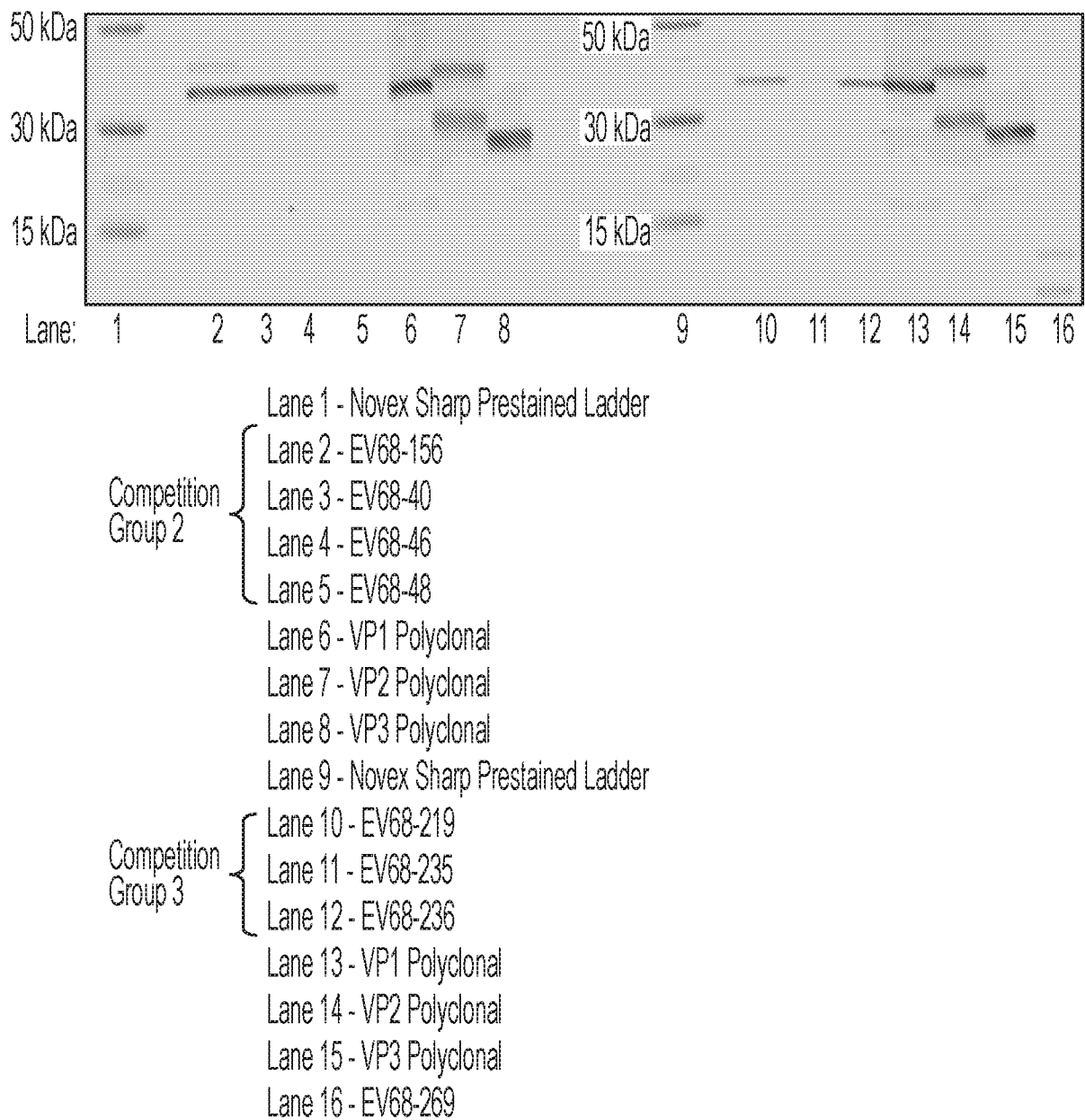


FIG. 40

Neutralization IC ₅₀		Binding EC ₅₀					
Competition group		Clade and isolate tested					
mAb	B1 IL/56	D KY/53	N/A Fermon	B1 MO/47	B1 MO/49	B1 IL/56	D KY/53
159	0.32	>	38,000	21	>	180	>
150	16	28,000	2,800	12	56	36	>
95	100	>	>	4,700	2,700	4,800	3,200
266	250	70	>	57	14	>	30
41	2,400	>	>	73	390	59	>
98	2,400	>	>	13,000	68,000	52,000	>
231	6,700	>	>	>	>	>	>
178	16,000	>	>	>	>	>	>
162	27,000	>	>	>	>	>	>
161	>	>	>	52	2,300	94	>
242	>	>	>	1,700	>	50,000	>
72	>	>	>	>	>	26,000	>
74	>	>	>	160	150	290	>
75	>	>	>	23	16	13	140
79	>	>	>	>	>	>	>
114	>	>	>	100	180	140	380
160	>	>	>	>	>	>	>
181	>	>	>	7,500	45,000	16,000	>
183	>	>	>	1,400	18,000	2,500	>
				>	>	>	>

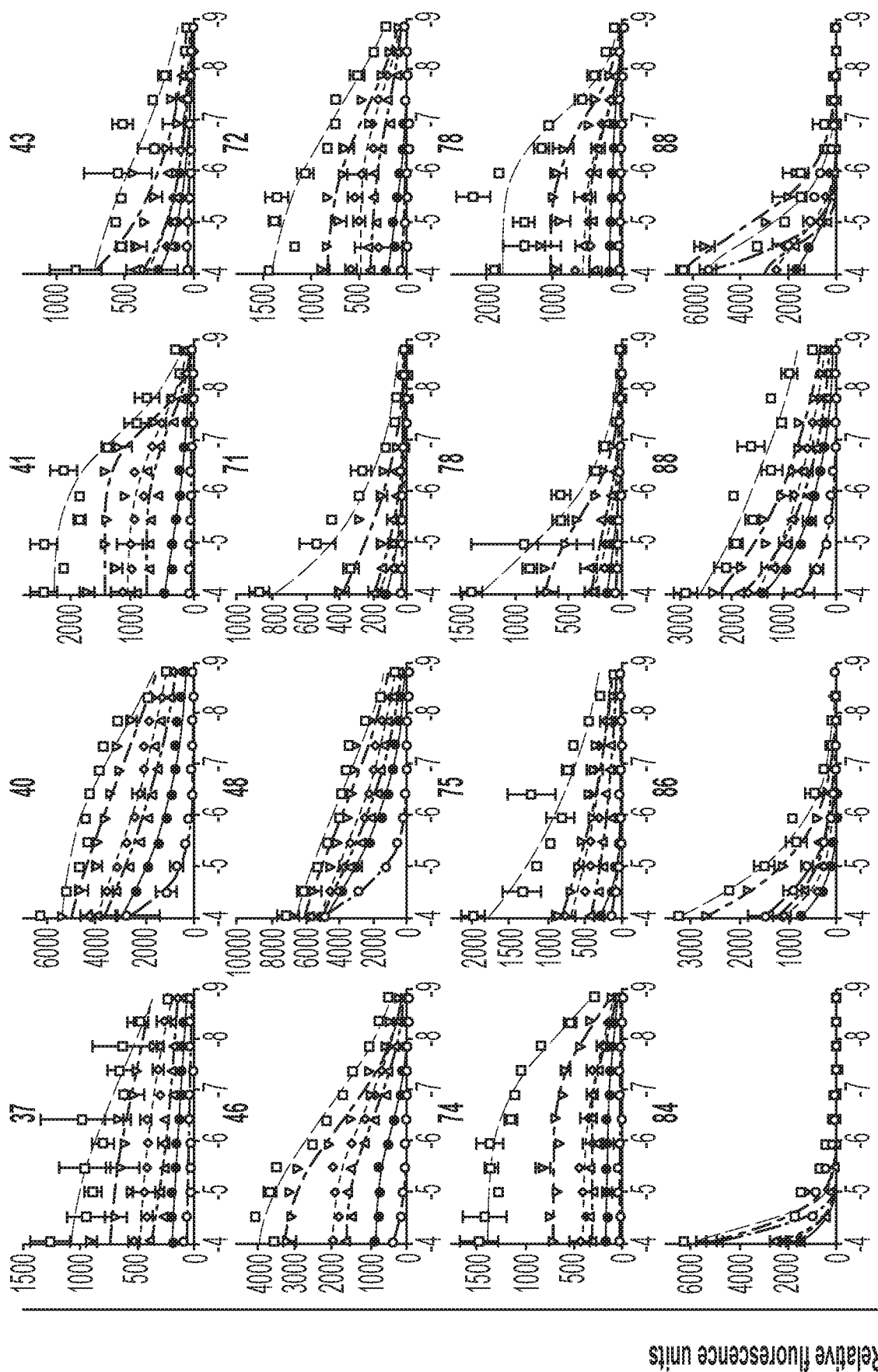
FIG. 41

198	>		>	>	>	>	>	1	1	K
210	>		>	>	>			1	1	L
220	>		>	>	>	23,000		1	1	K
221	>		>	>	>			3	1	L
229	>		>	>	>	65		3	1	K
234	>		>	>	5.5	6.5		3	1	K
271	>		>	31,000	13,000	56,000	2,800	5	3	K
			6.7	8,600						
40	8.2	>	3,300	26	65,000	170	18,000	5	3	K
48	11	5.2	>	210	20,000	940	11,000	5	3	K
46	42	16,000	>	390	500	680	320	5	3	K
156	370	>	>	64	40	51	36	3	1	L
										VP1
219	>			1,100	300	340	370	1	3	L
235	>			1,400	940	1,100	310	3	3	L
236	>			400	650	340	200	3	3	L
										VP1
153	1.6	>	>	>	>	>	>	2	1	L
105	3.1	12	11	45	430	320	620	5	1	K
228	3.7	5.2	52	55	82	78	54	3	1	K
43	3.7	1,700	2,200	1,700	>	>	>	5	1	K
208	790	4,200	36,000	>	>	>	>	1	1	K
152	1,700	740	19,000	70,000	>	>	>	1	3	K

FIG. 41
CONTINUED

4	88	30,000	>	>	>	>	>	3	3	L
	227	32,000	350	740	480	400	>	3	3	K
	241	38,000	840	13,000	820	1,900	>	3	3	K
	185	38,000	>	11	28,000	>	92	1	1	K
	247	40,000	>	>	>	>	>	5	1	K
	37	>	17	>	14	13	>	5	3	L
	224	>	>	>	>	>	>	3	1	K
	254	>	420	980	2,000	5,500	>	5	1	K
5	166	5.5	48,000	>	>	>	>	1	3	L
	97	73	>	56,000	>	>	>	5	1	L
	85	14	3,000	>	>	>	>	3	3	K
	225	310	>	62,000	73,000	65,000	100,000	3	1	L
	269	>	>	>	>	>	>	5	1	L
	84	>	>	55,000	69,000	>	>	3	1	L
	89	>	>	40,000	55,000	27,000	43,000	3	1	L
	158	>	>	>	>	>	>	4	1	L
	164	>	>	30,000	30,000	32,000	26,000	11	1	K
	71	>	>	>	>	>	>	1	1	K
	76	>	>	13,000	16,000	>	>	1	3	L
	110	>	>	>	>	>	>	12	1	L
	116	>	>	1,200	4,200	1,700	17,000	1	3	L
	165	>	>	>	>	>	>	1	1	L
										VP4?

FIG. 41
CONTINUED



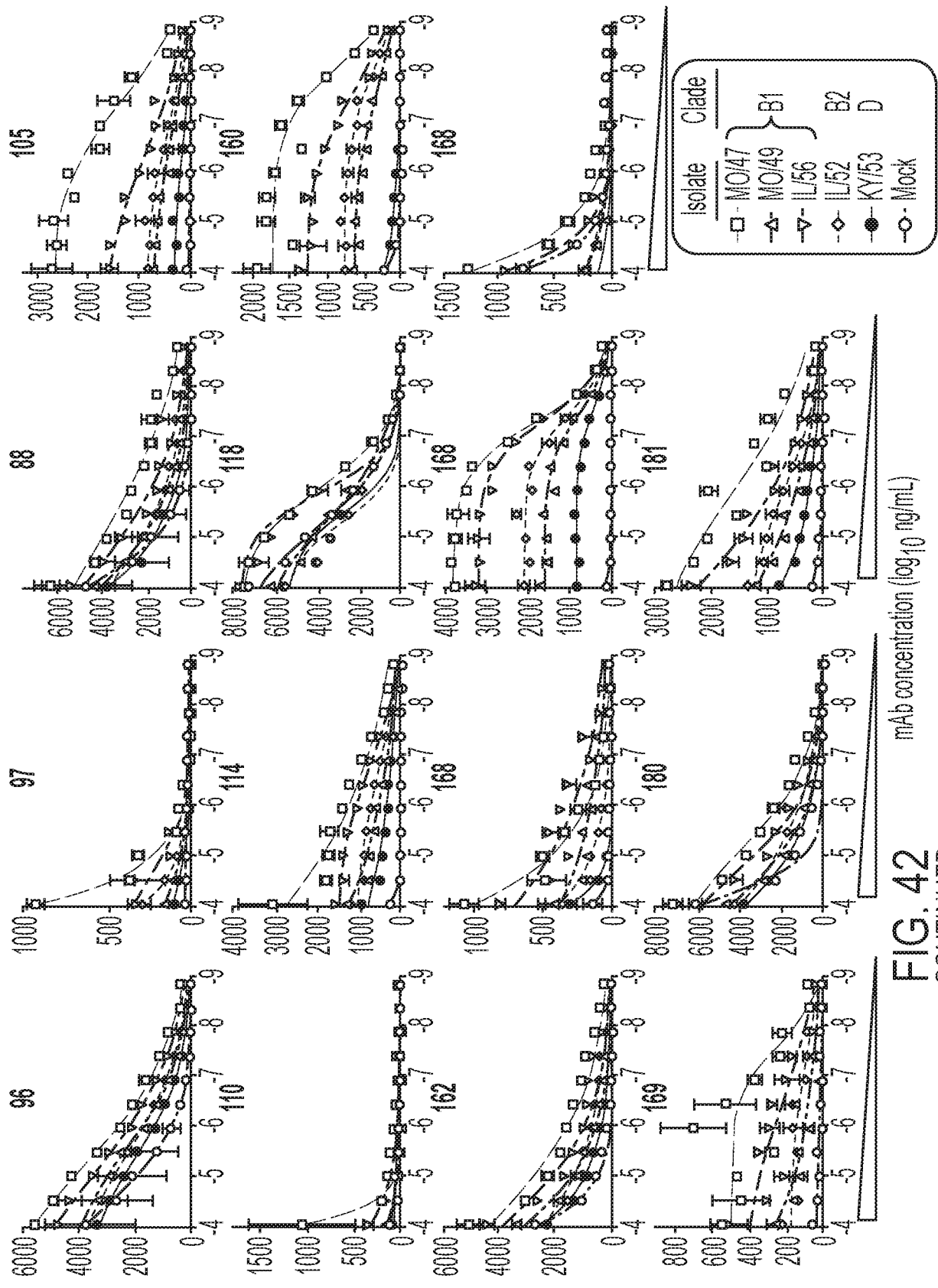


FIG. 42
CONTINUED

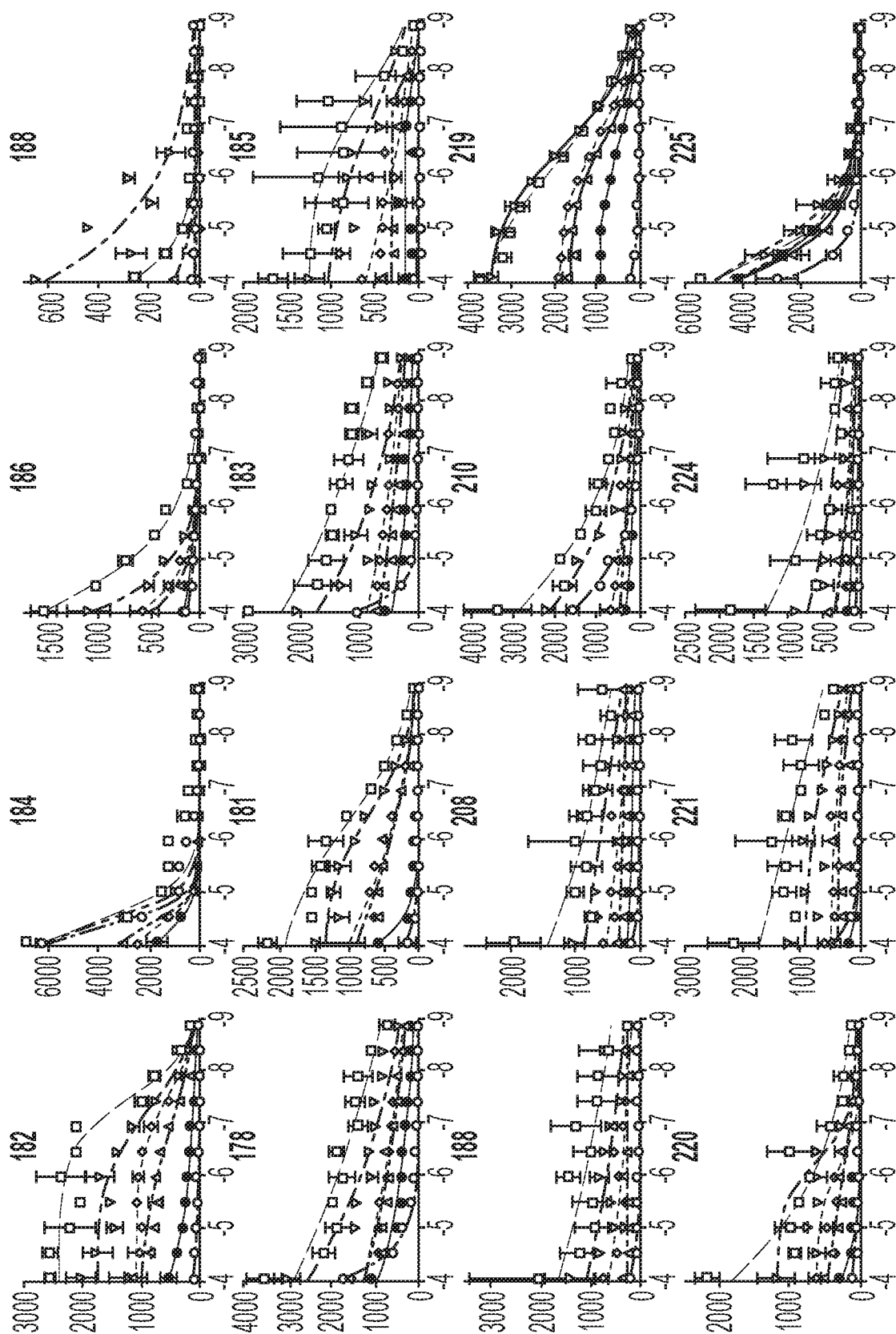


FIG. 42
CONTINUED

Relative fluorescence units

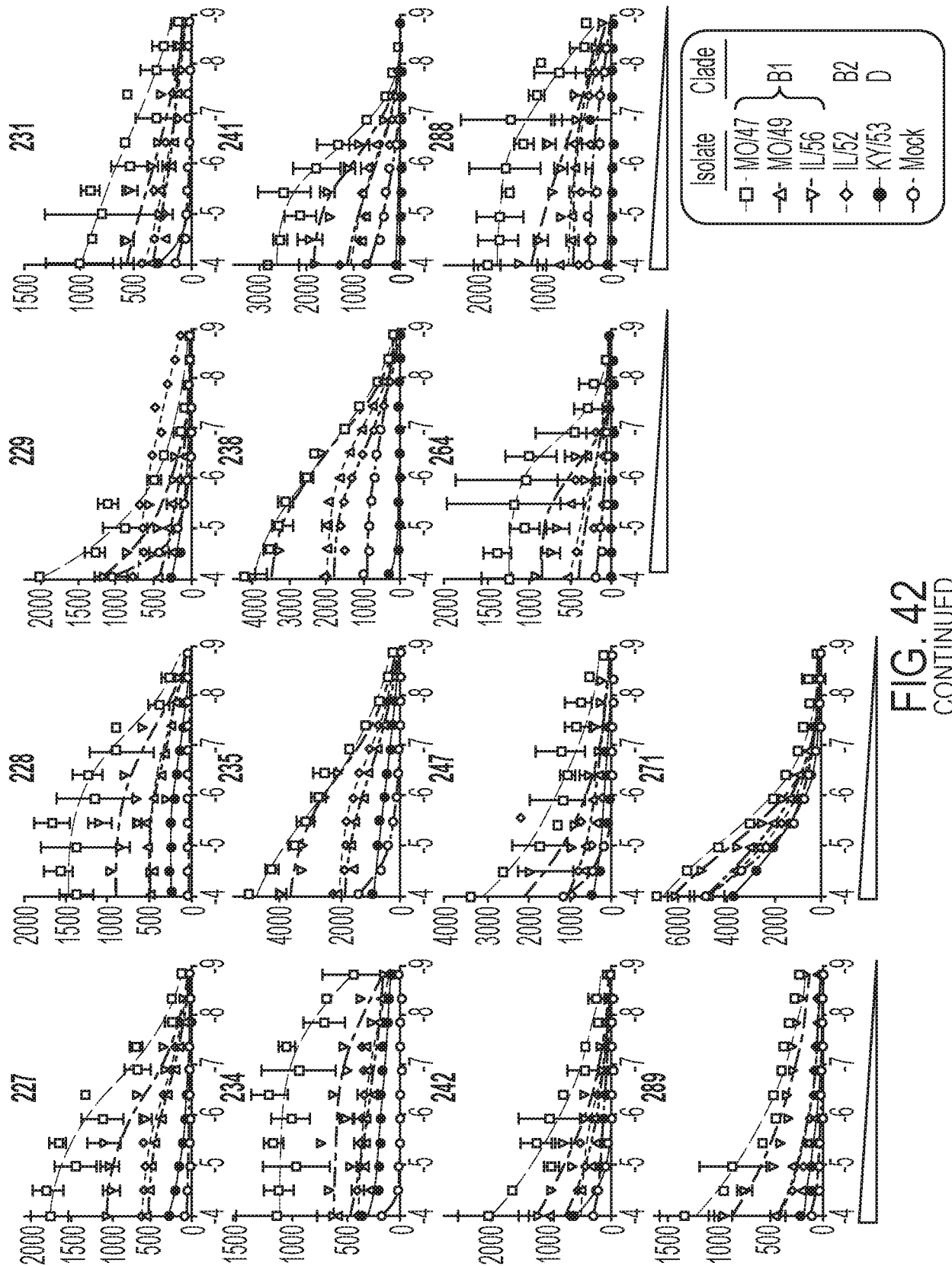


FIG. 42
CONTINUED

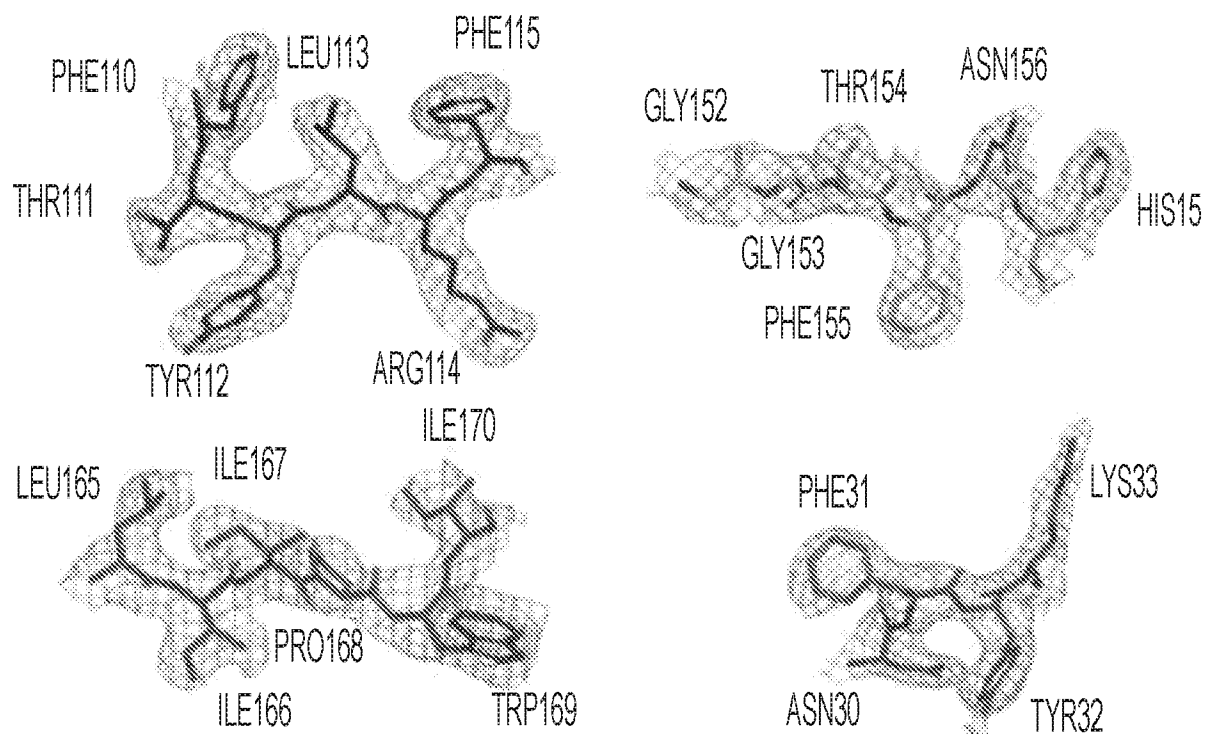


FIG. 43

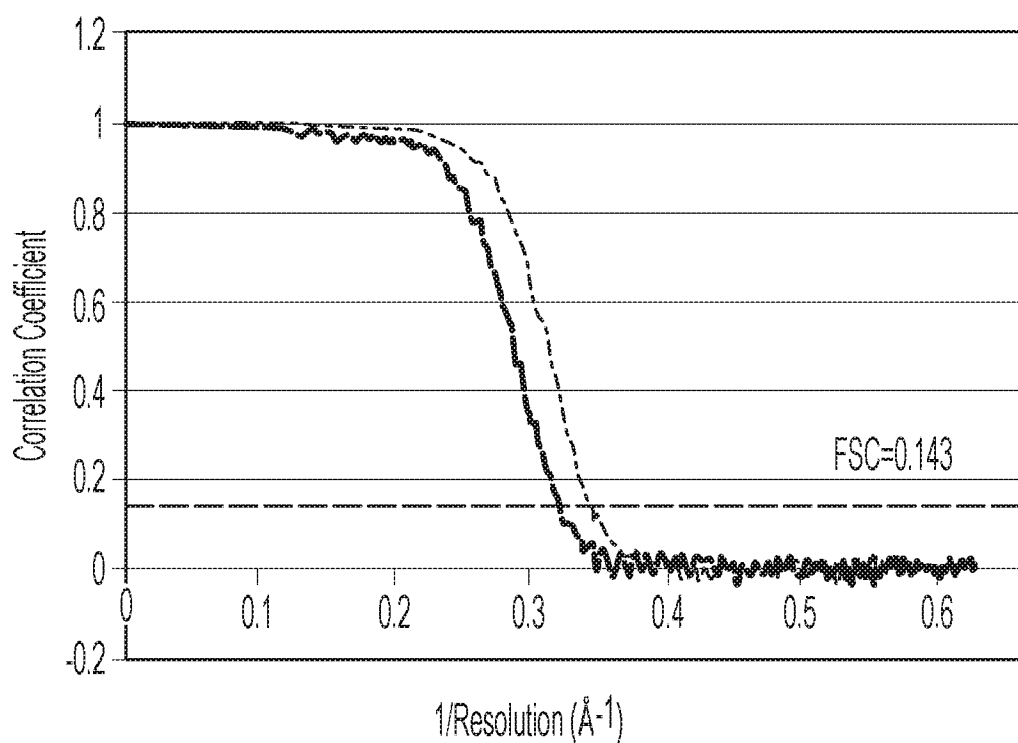


FIG. 44

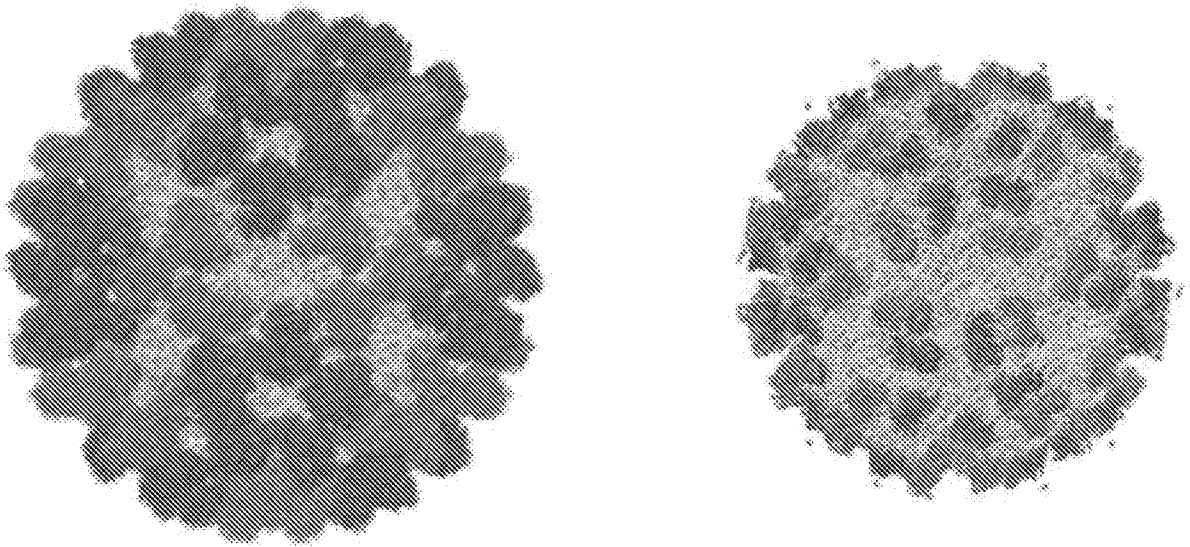


FIG. 45

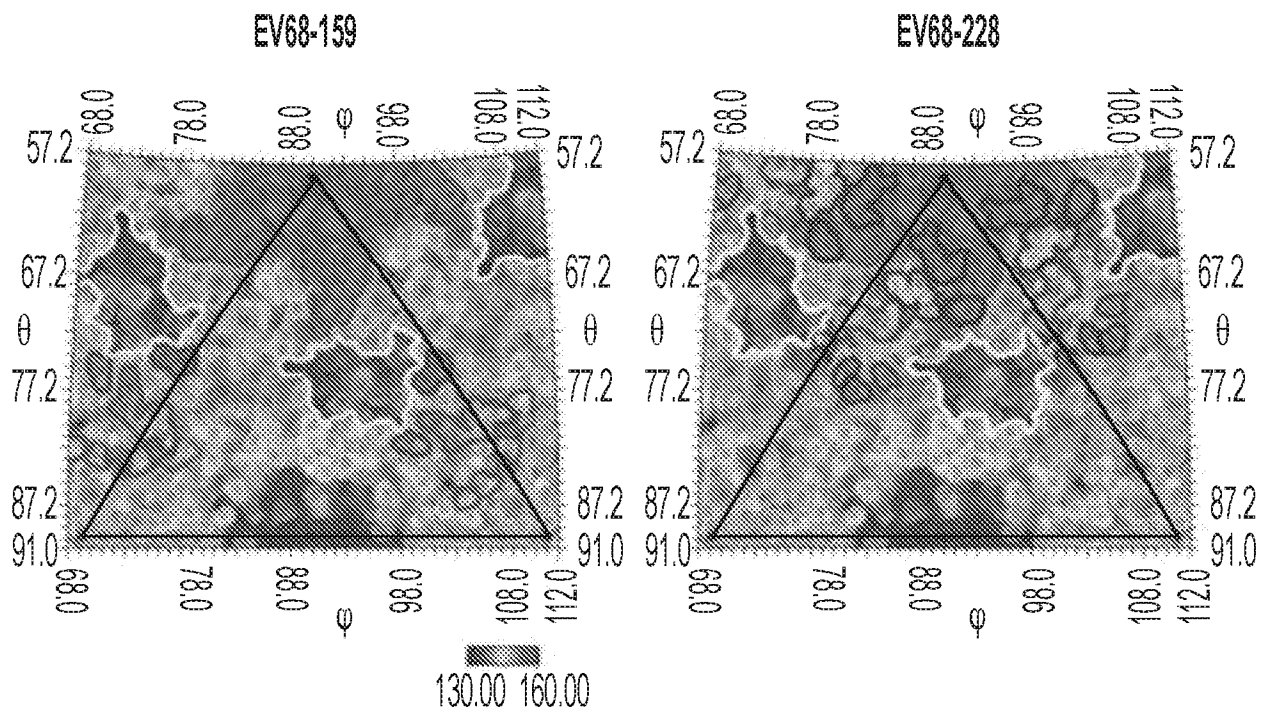


FIG. 46

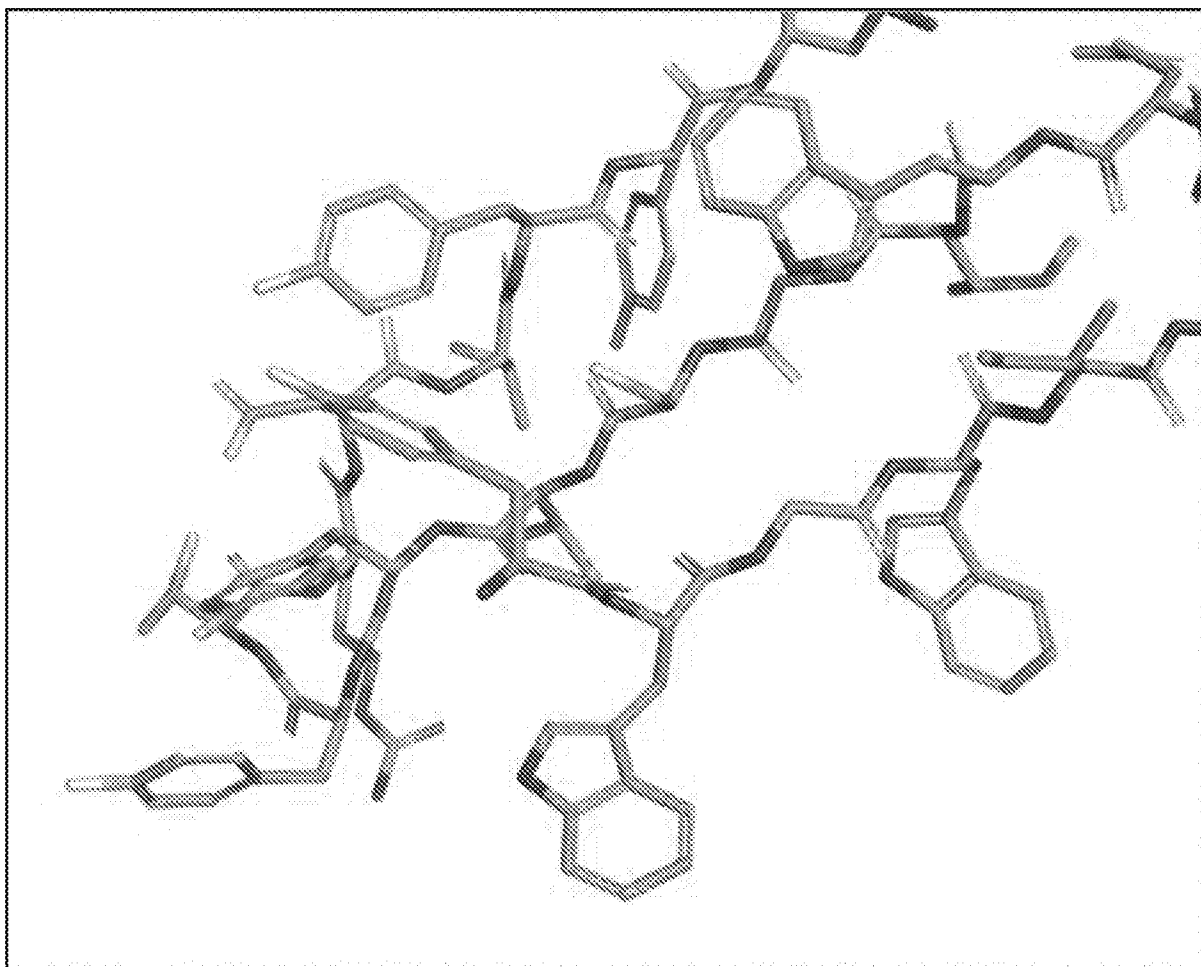


FIG. 47

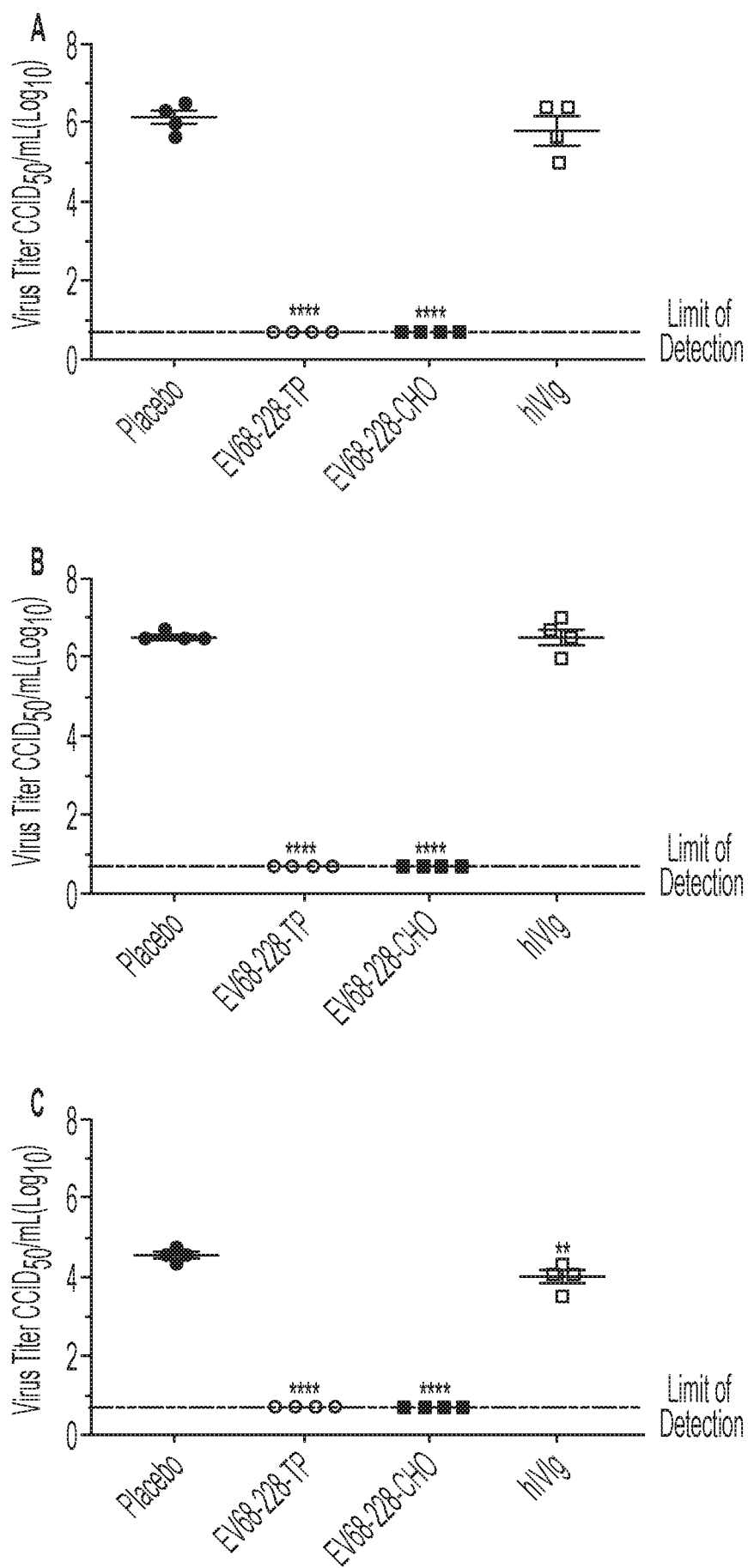


FIG. 48A-C

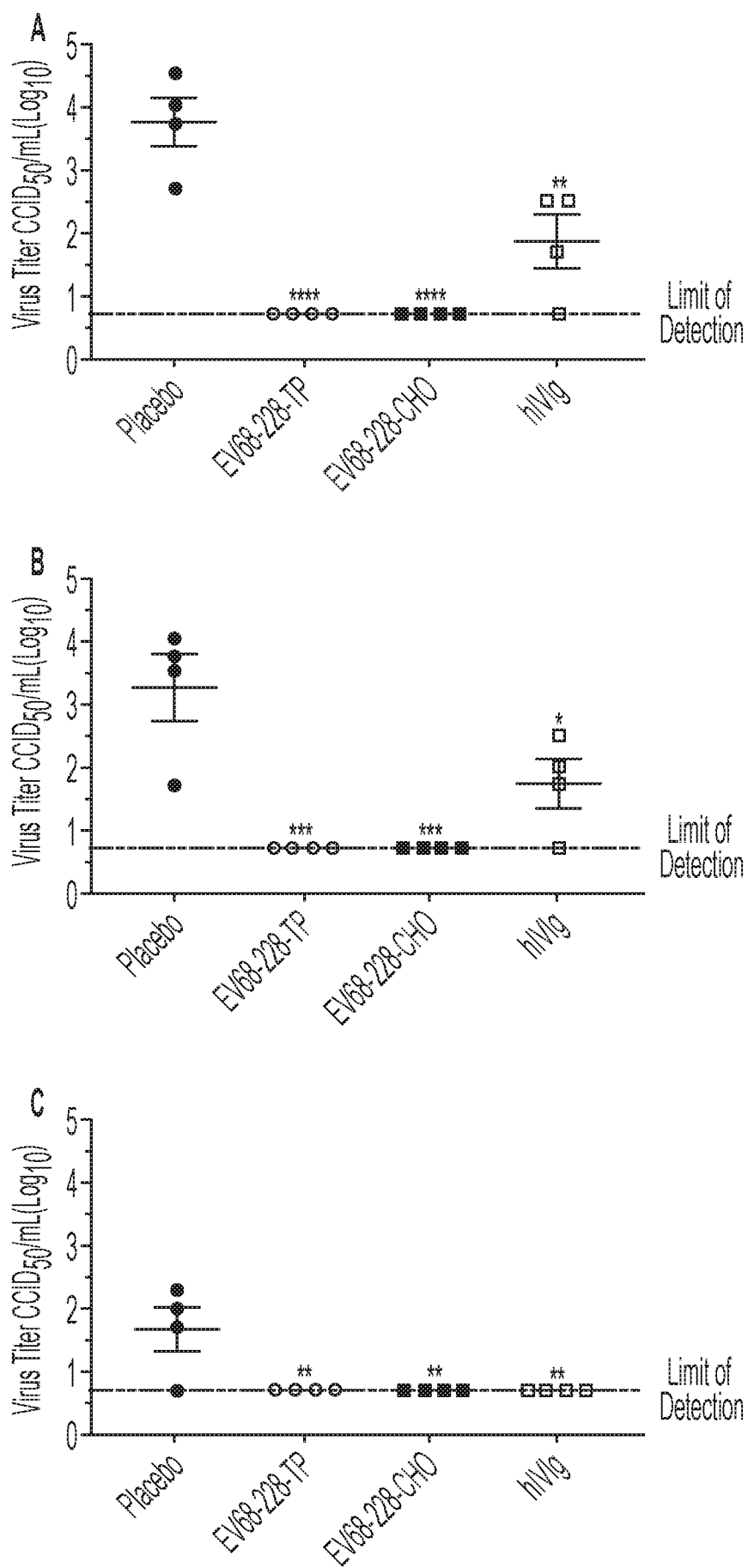


FIG. 49A-C

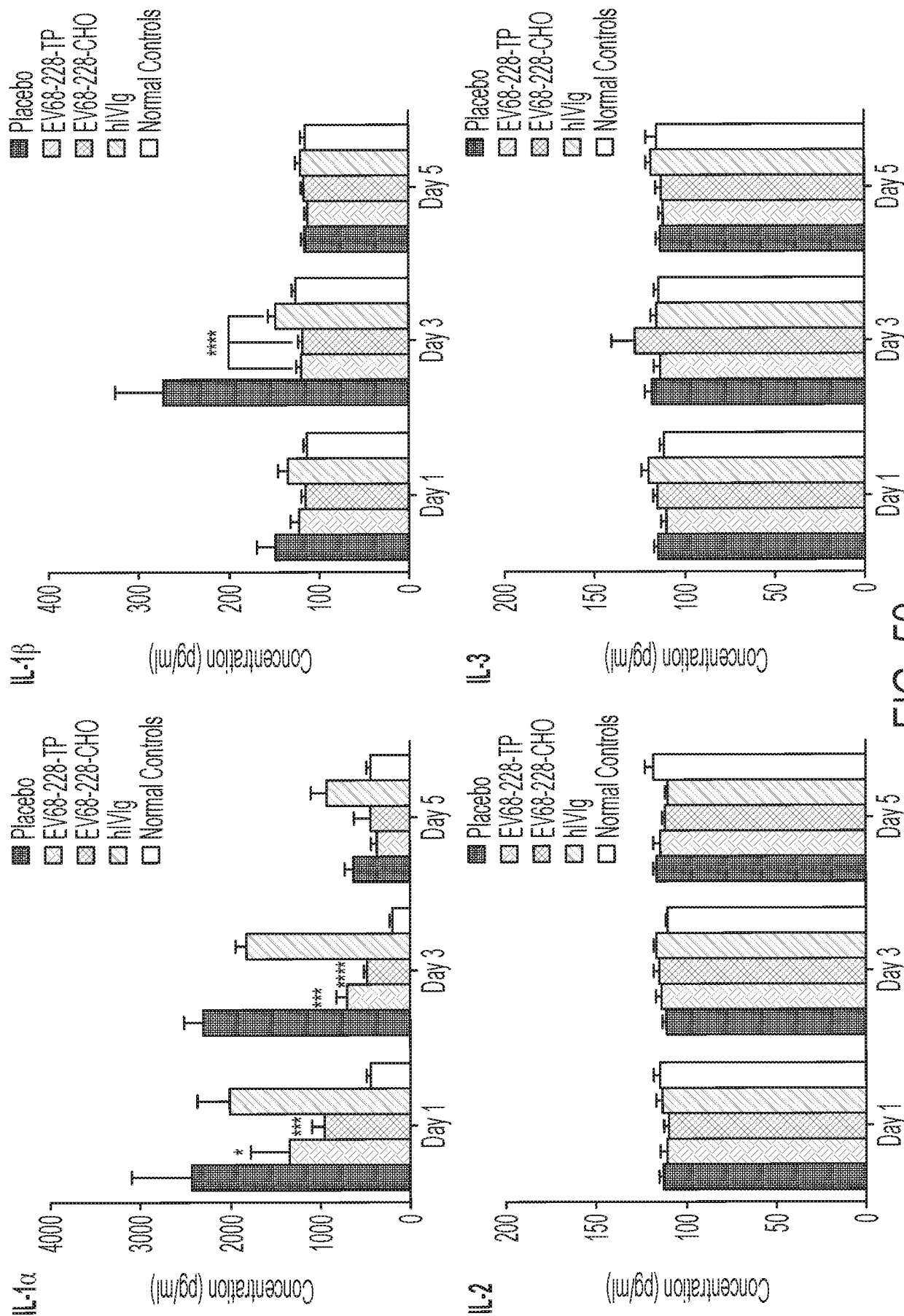


FIG. 50

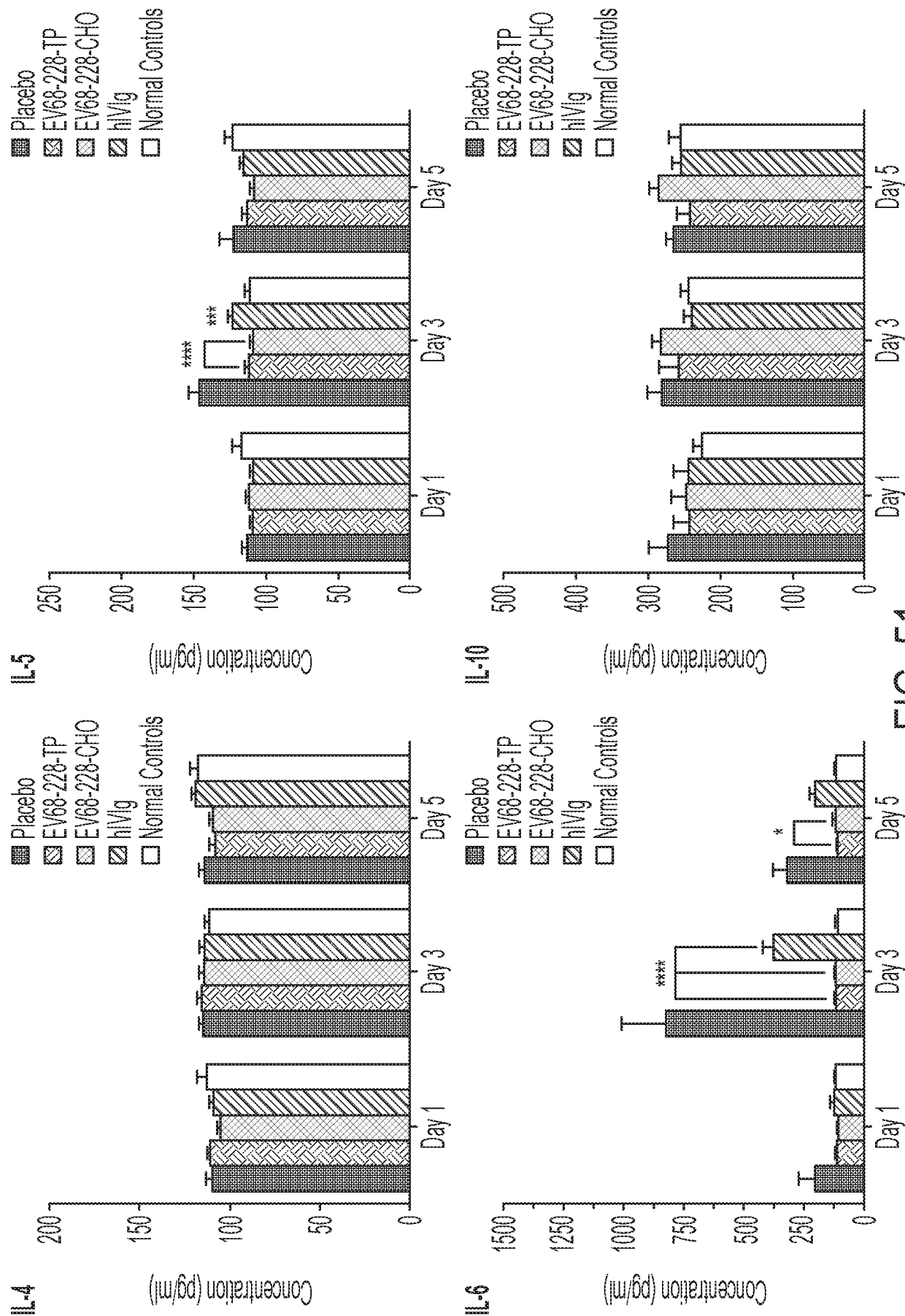


FIG. 51

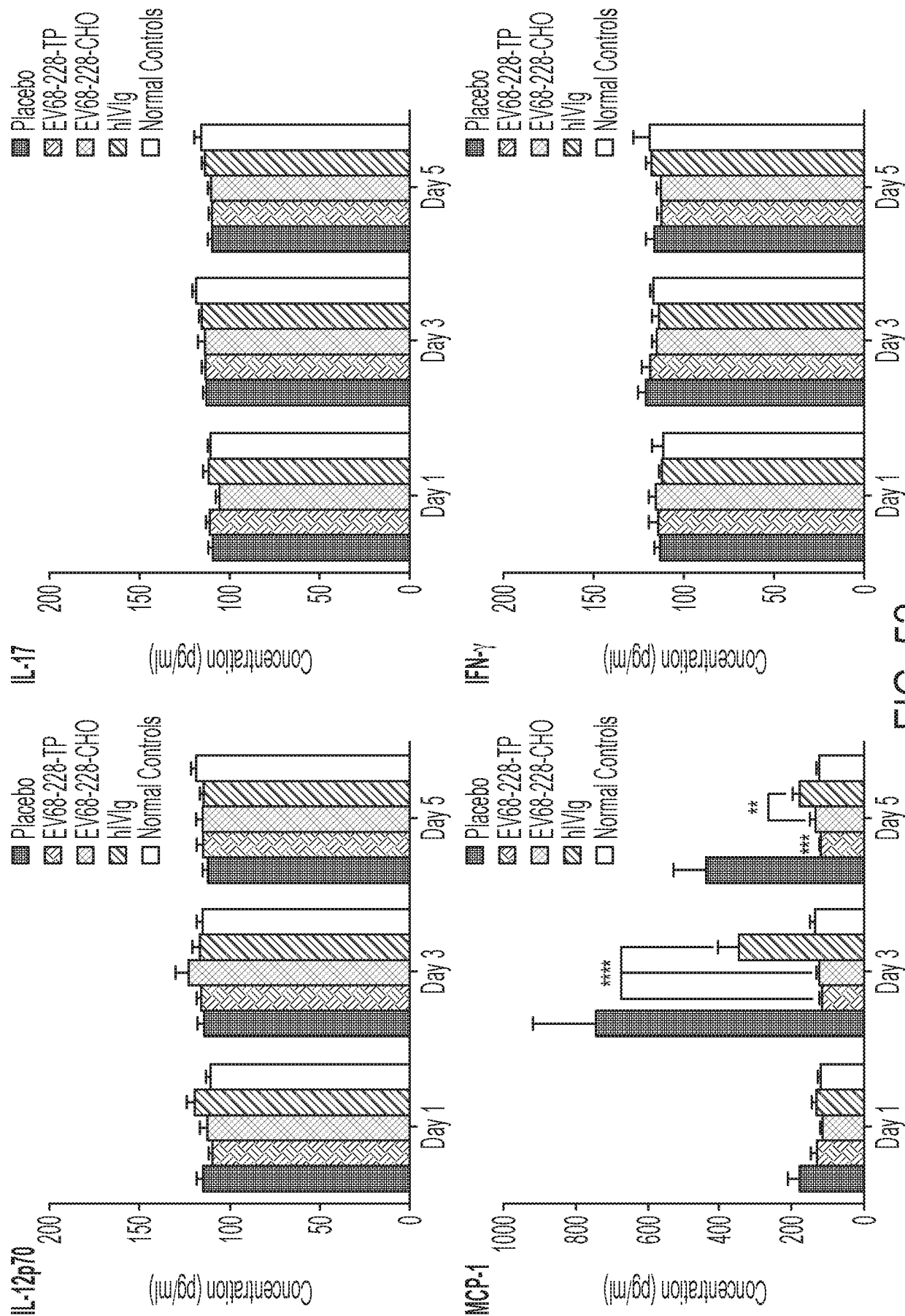


FIG. 52

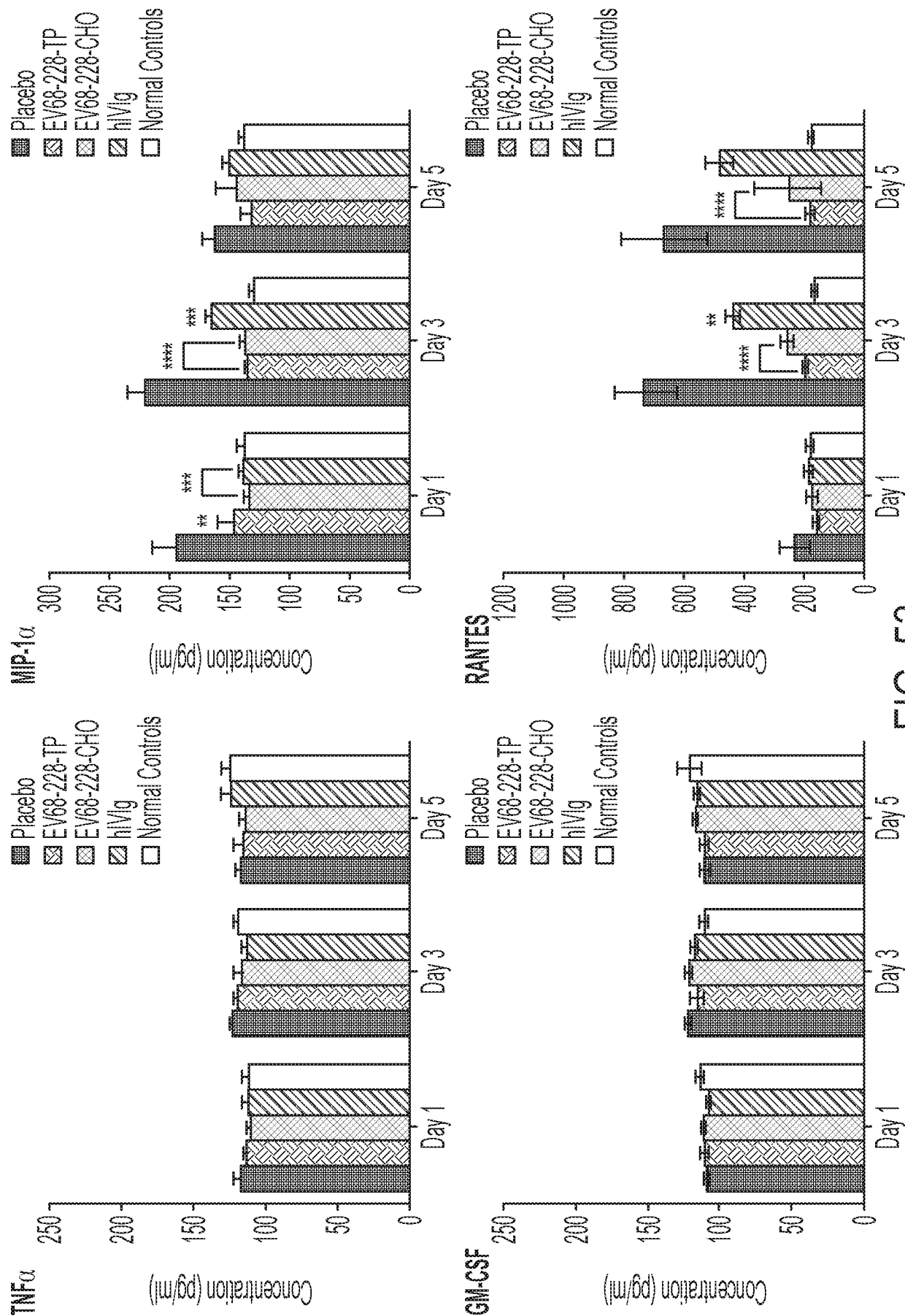


FIG. 53

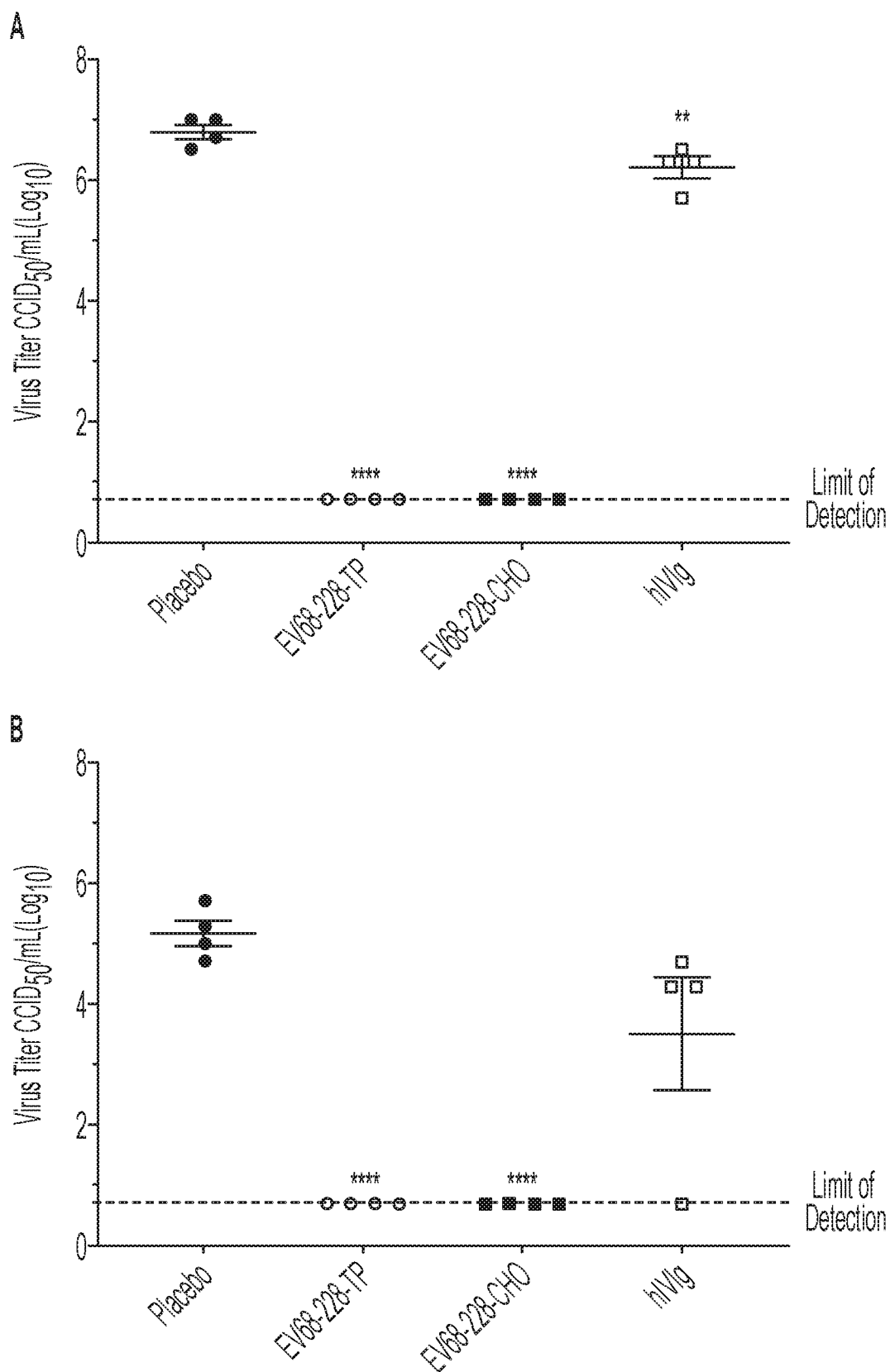


FIG. 54A-B

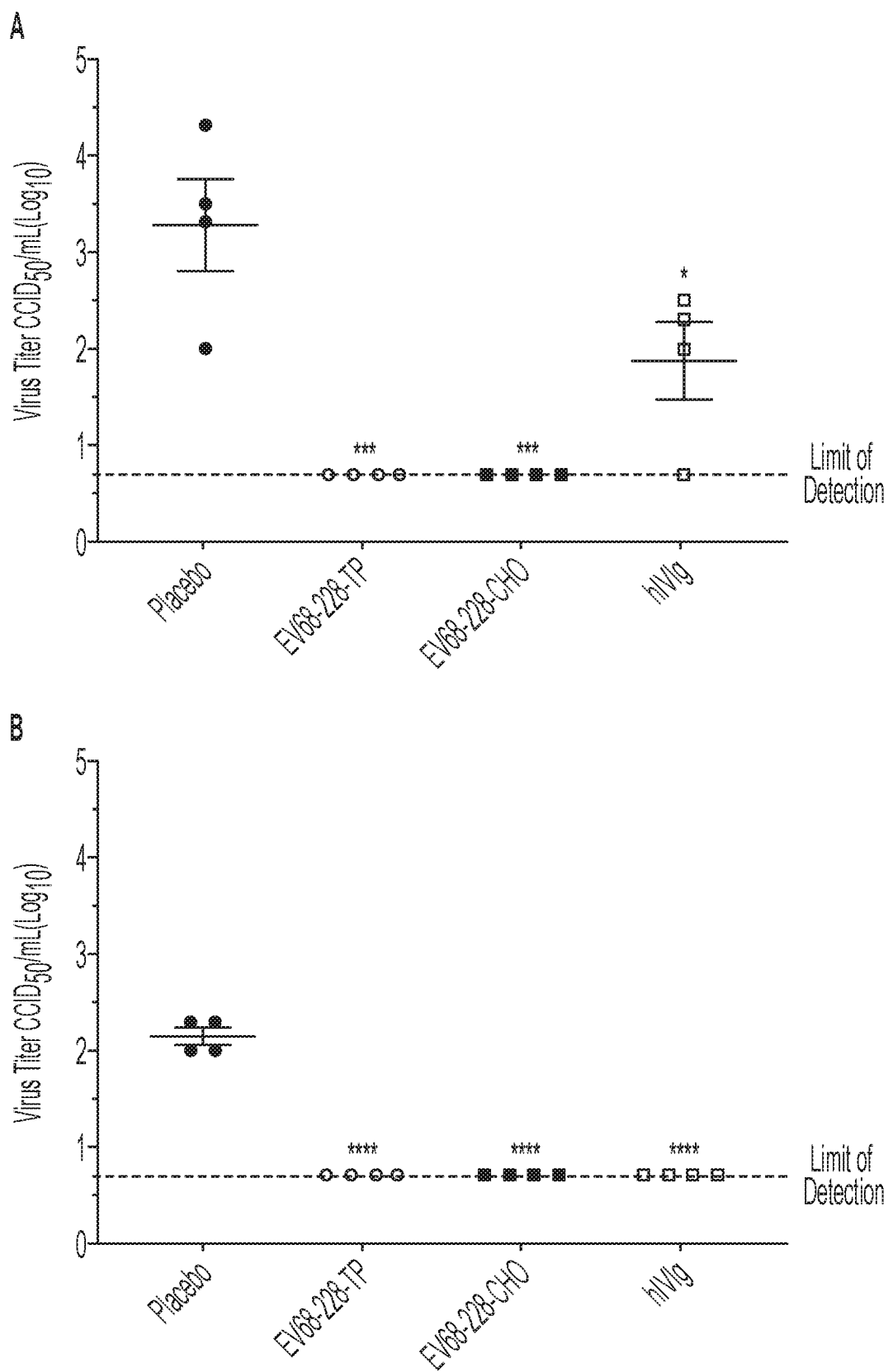


FIG. 55A-B

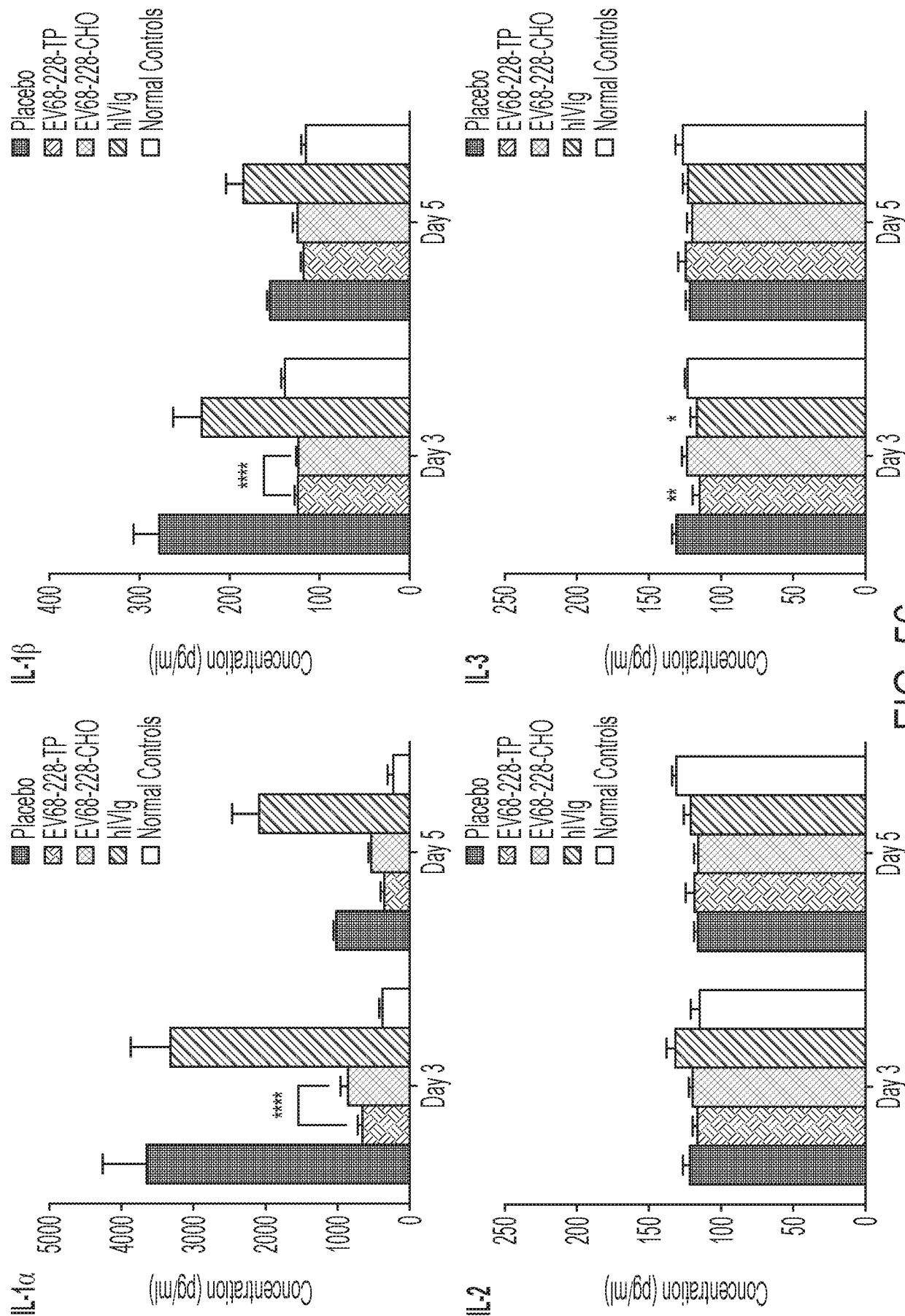


FIG. 56

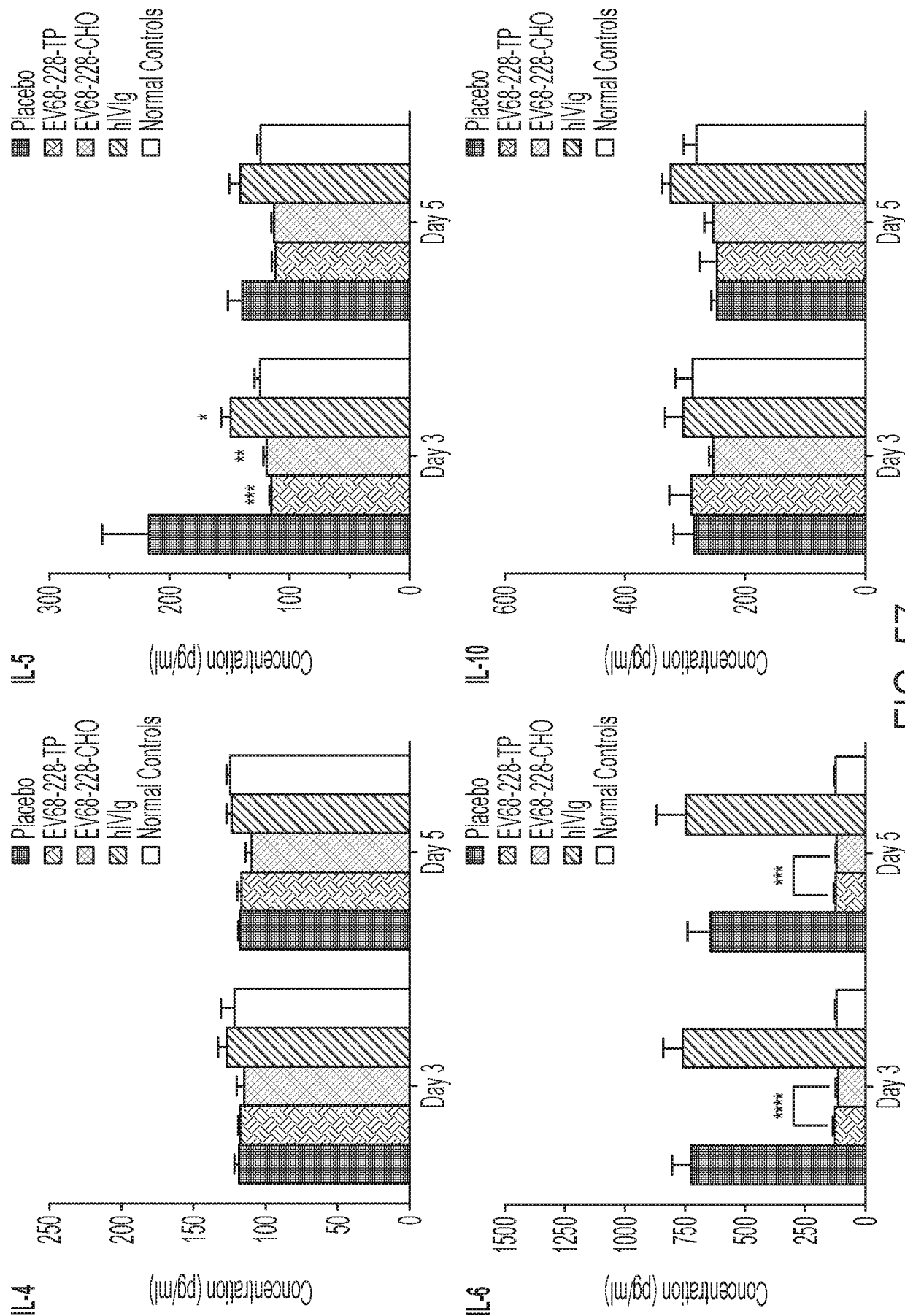


FIG. 57

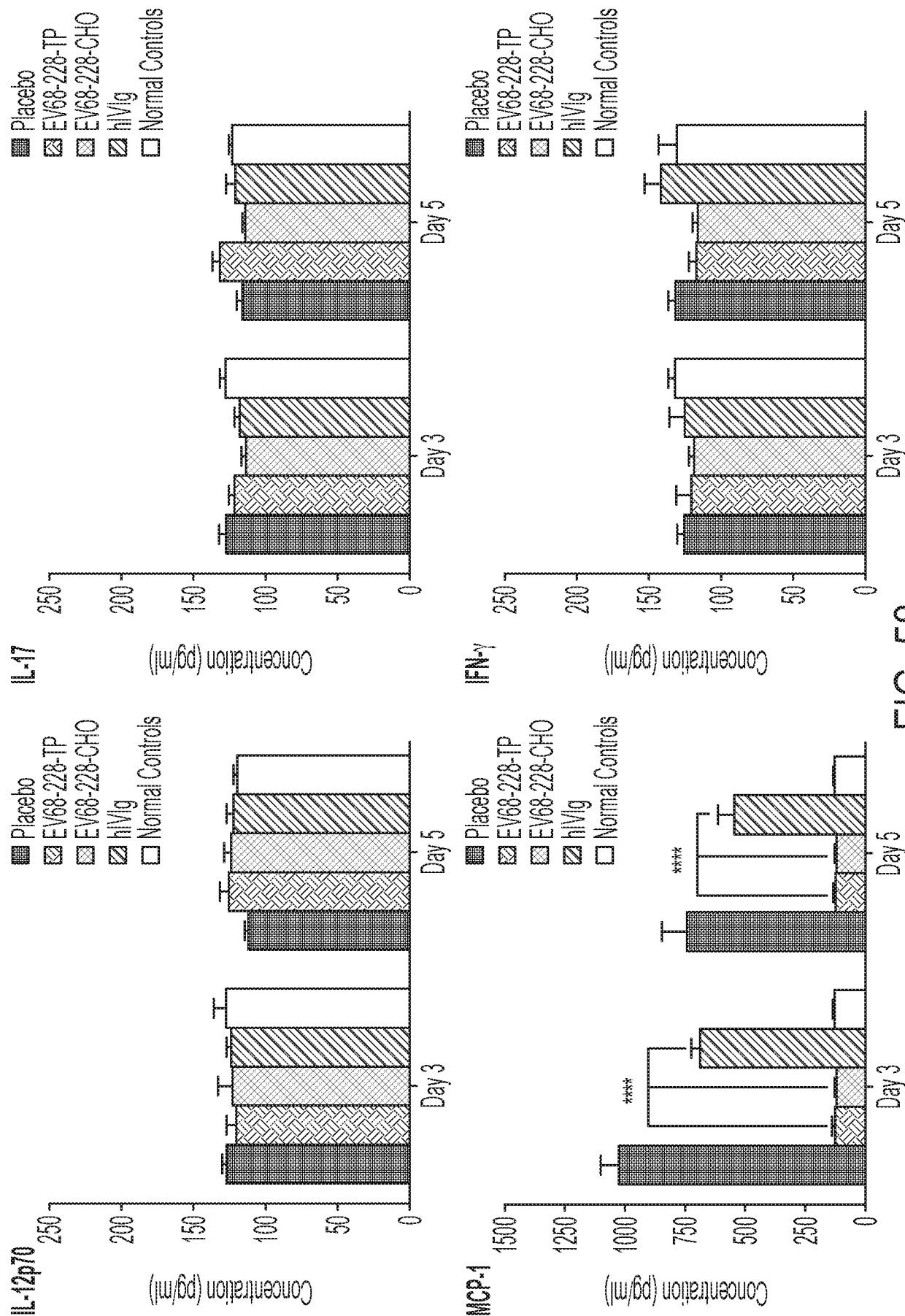


FIG. 58

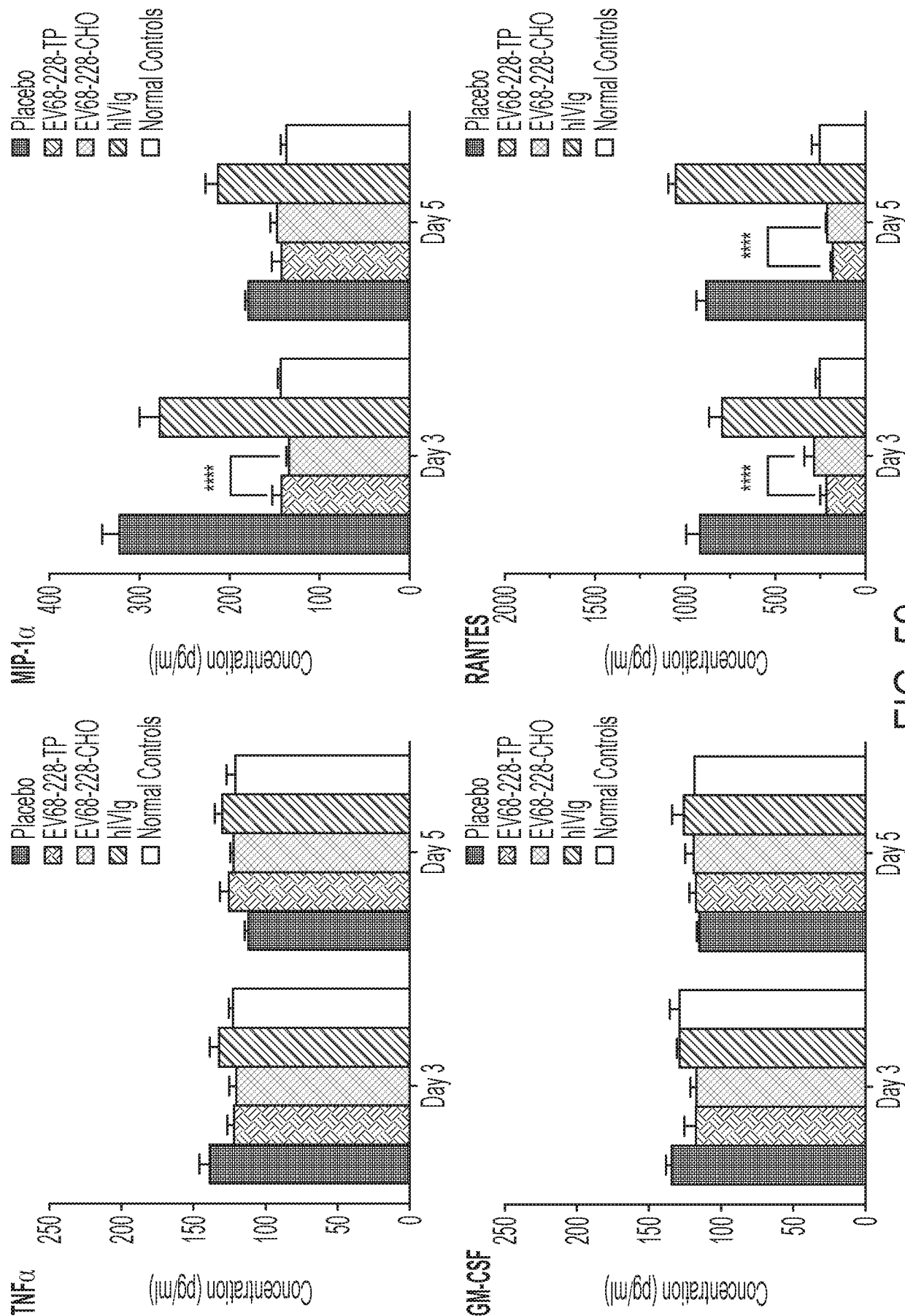


FIG. 59

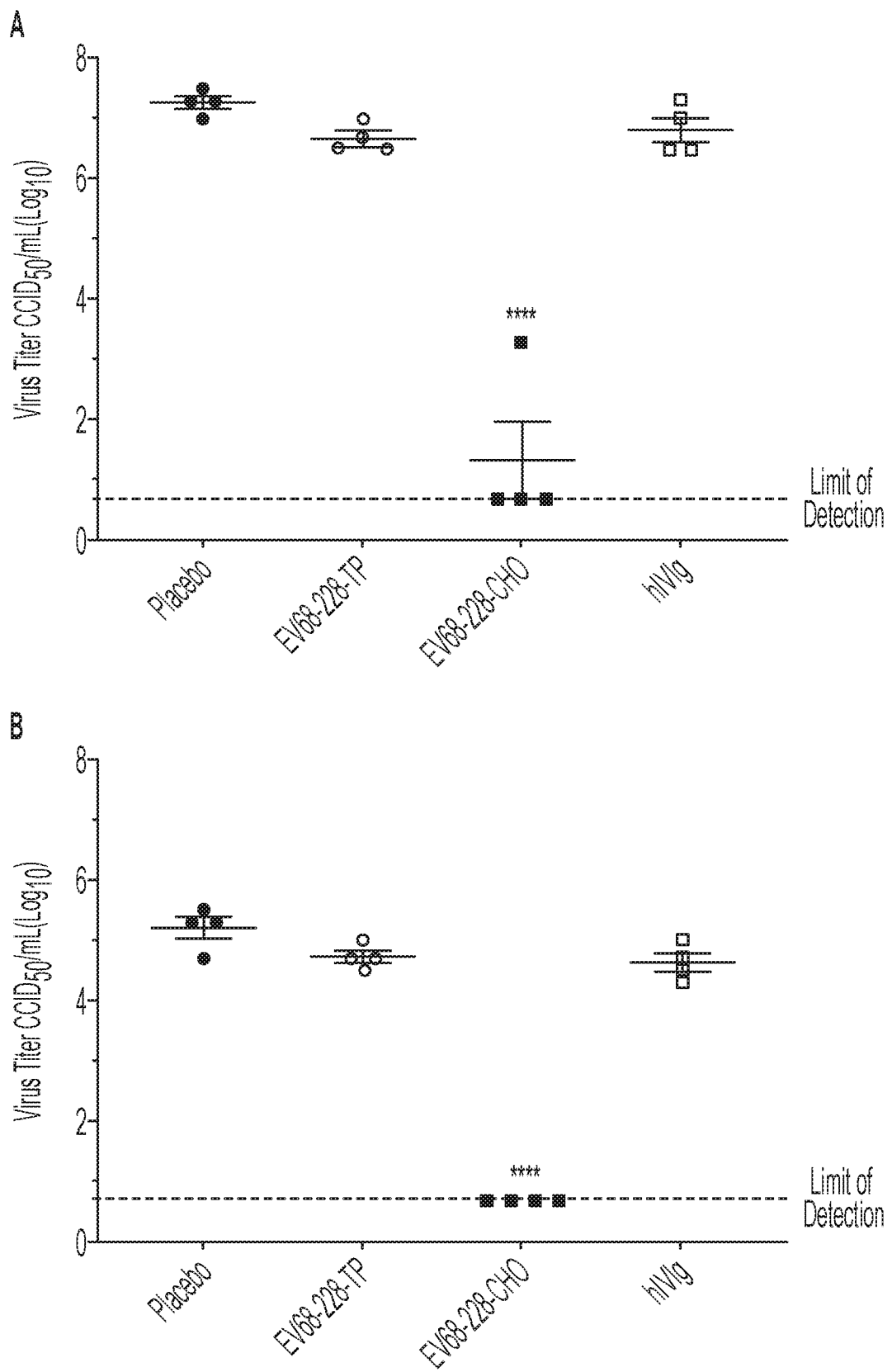


FIG. 60A-B

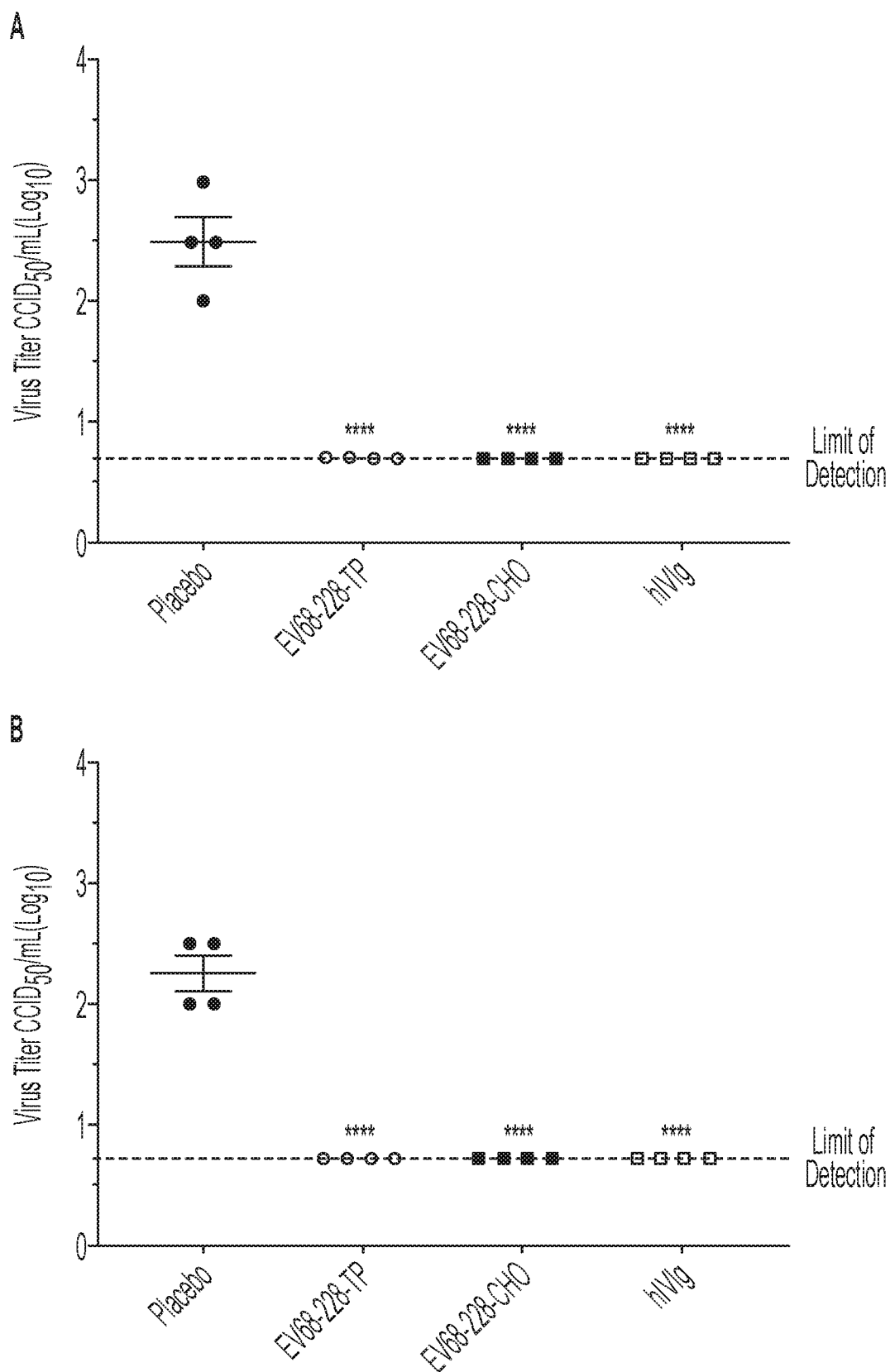


FIG. 61A-B

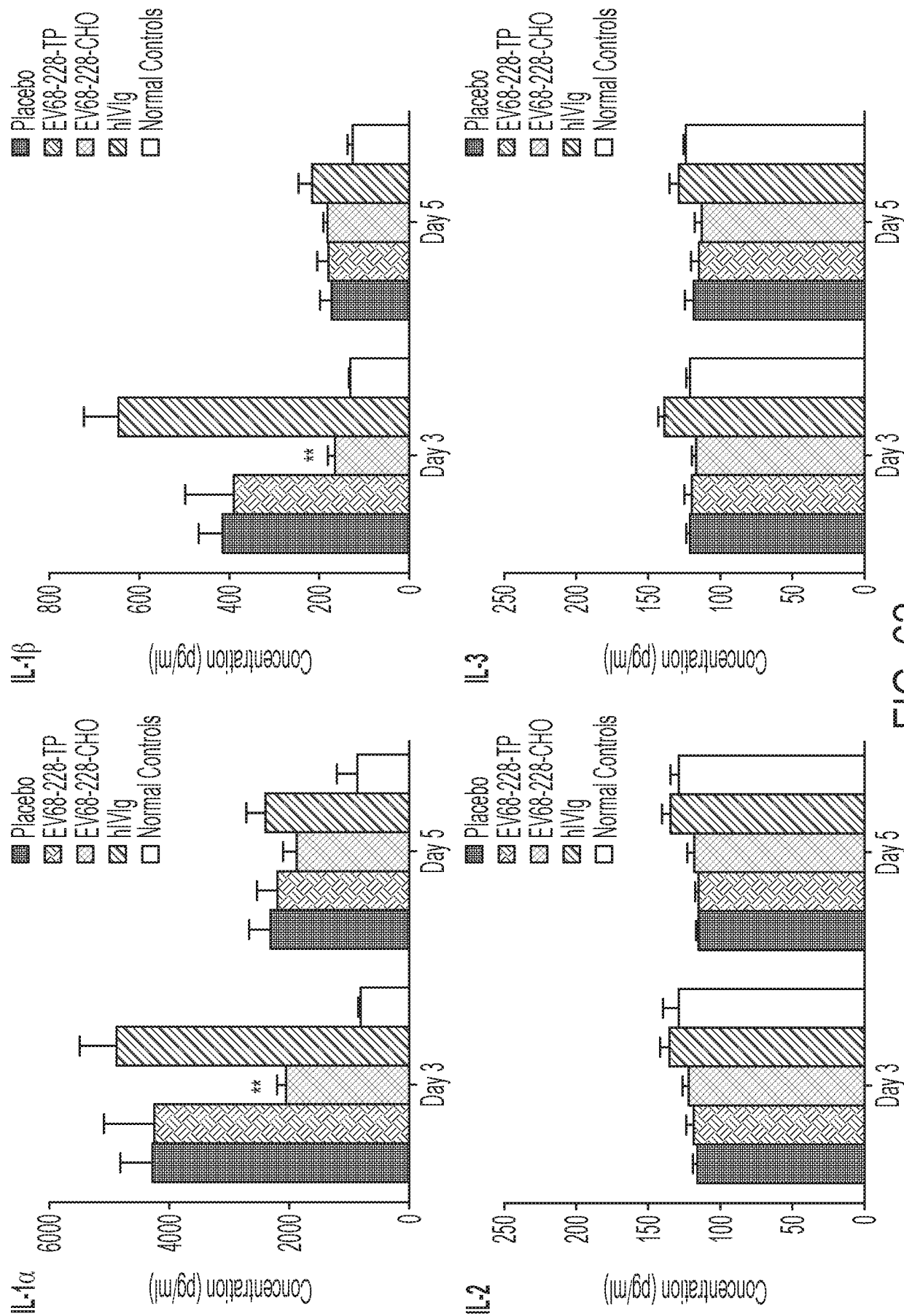


FIG. 62

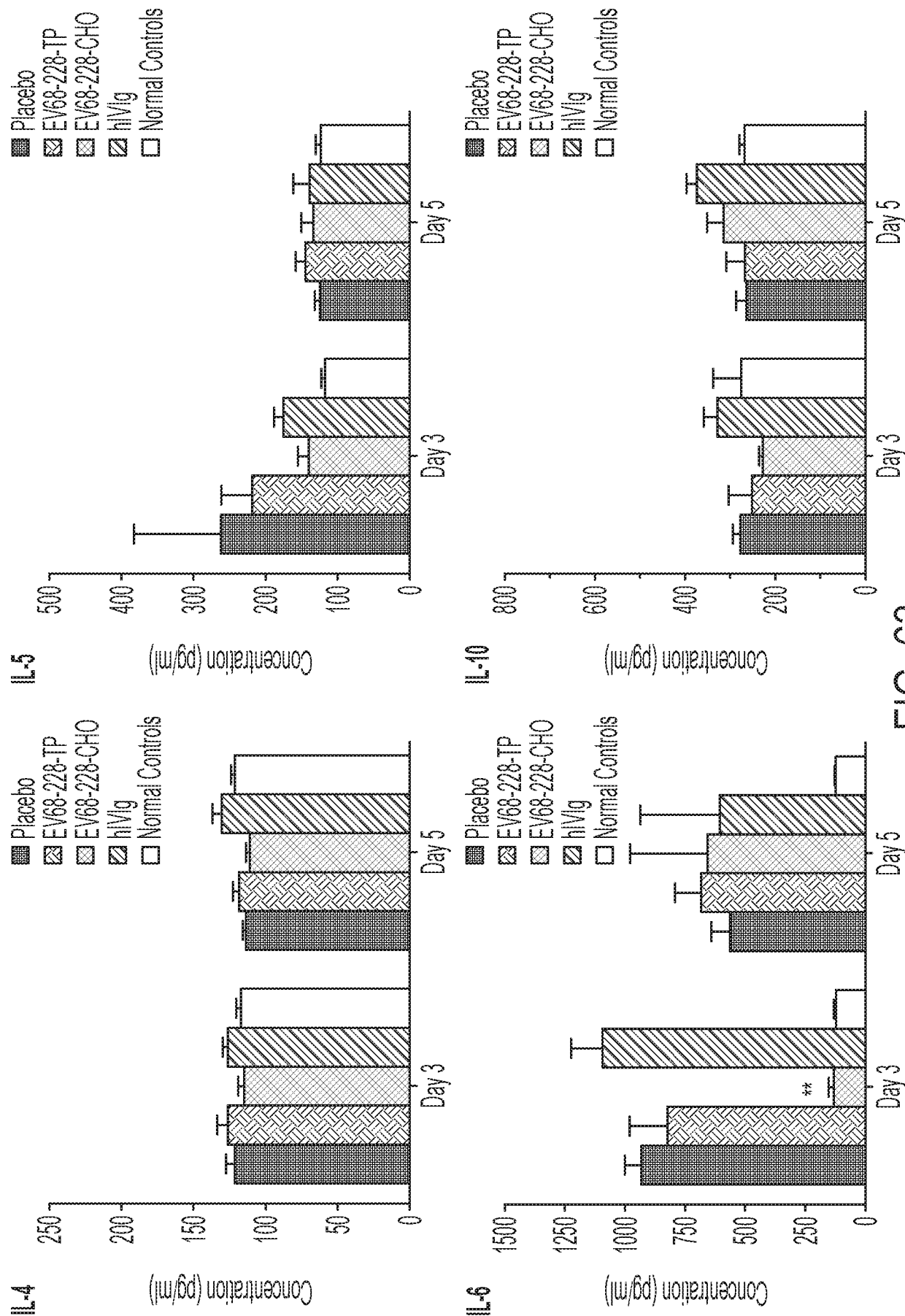


FIG. 63

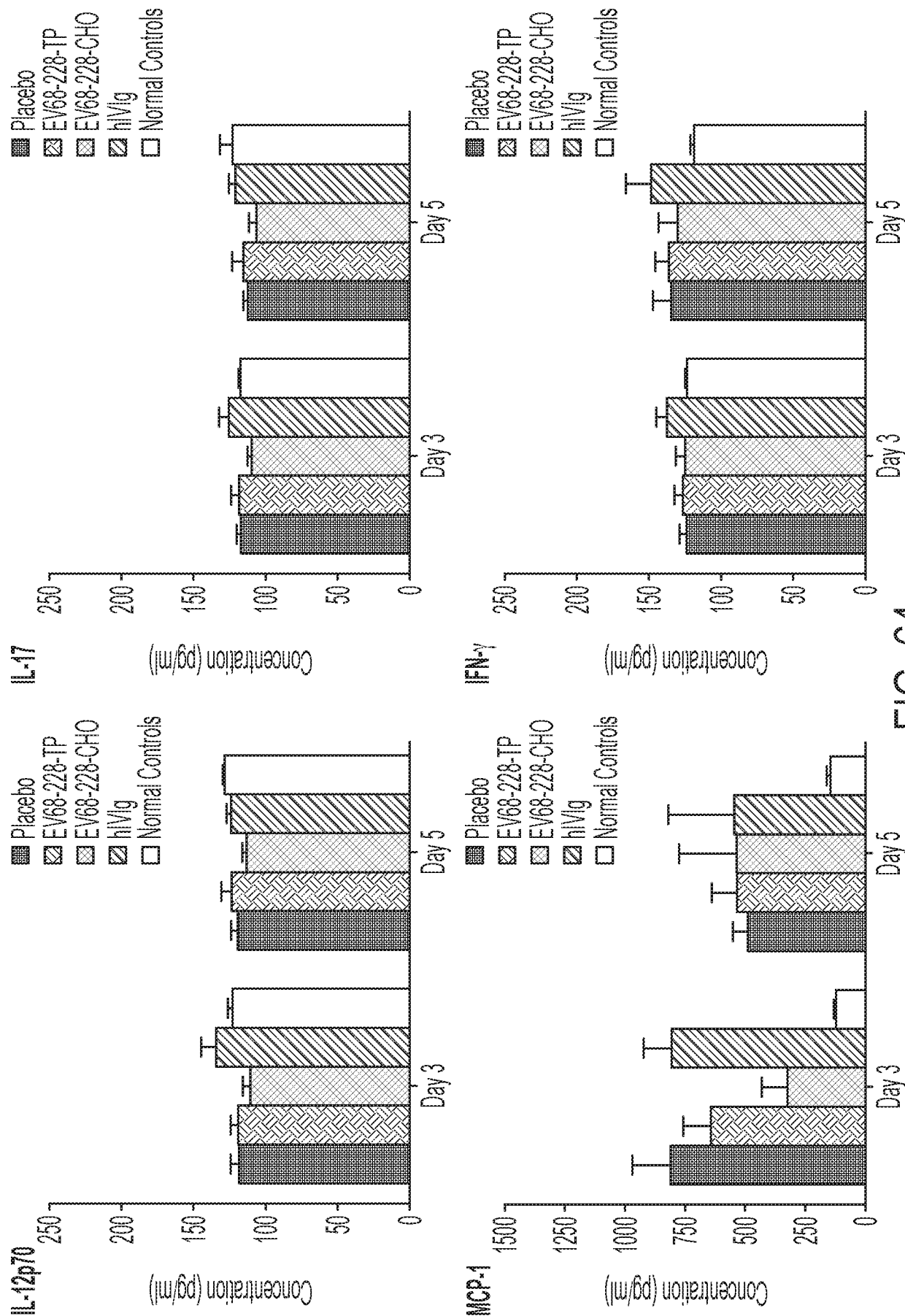


FIG. 64

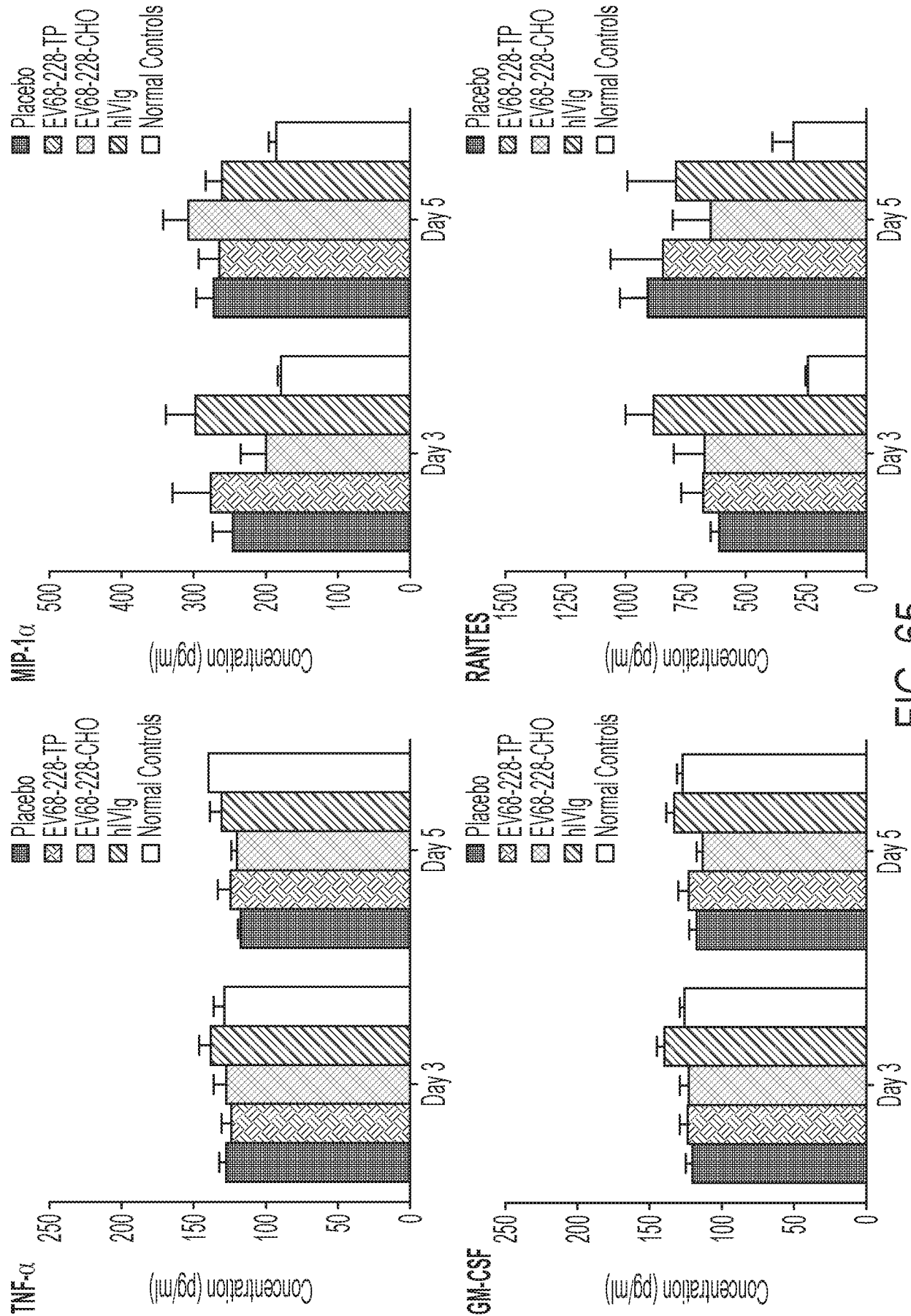


FIG. 65

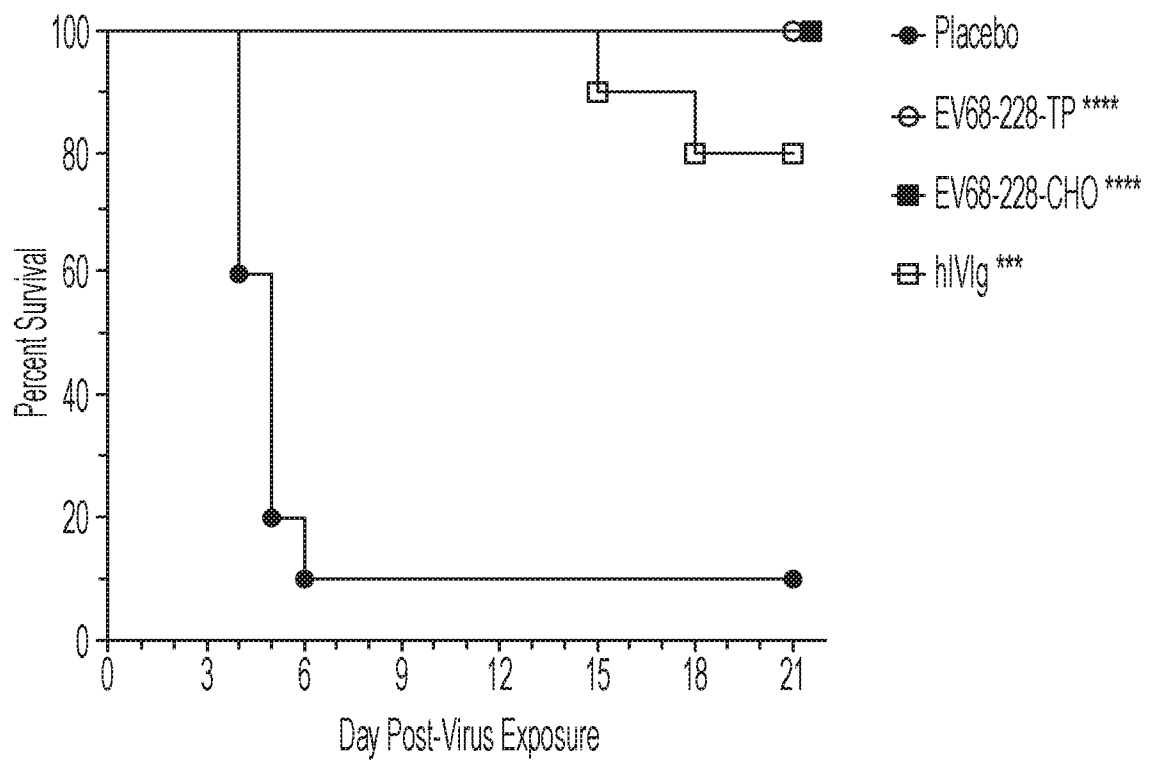


FIG. 66

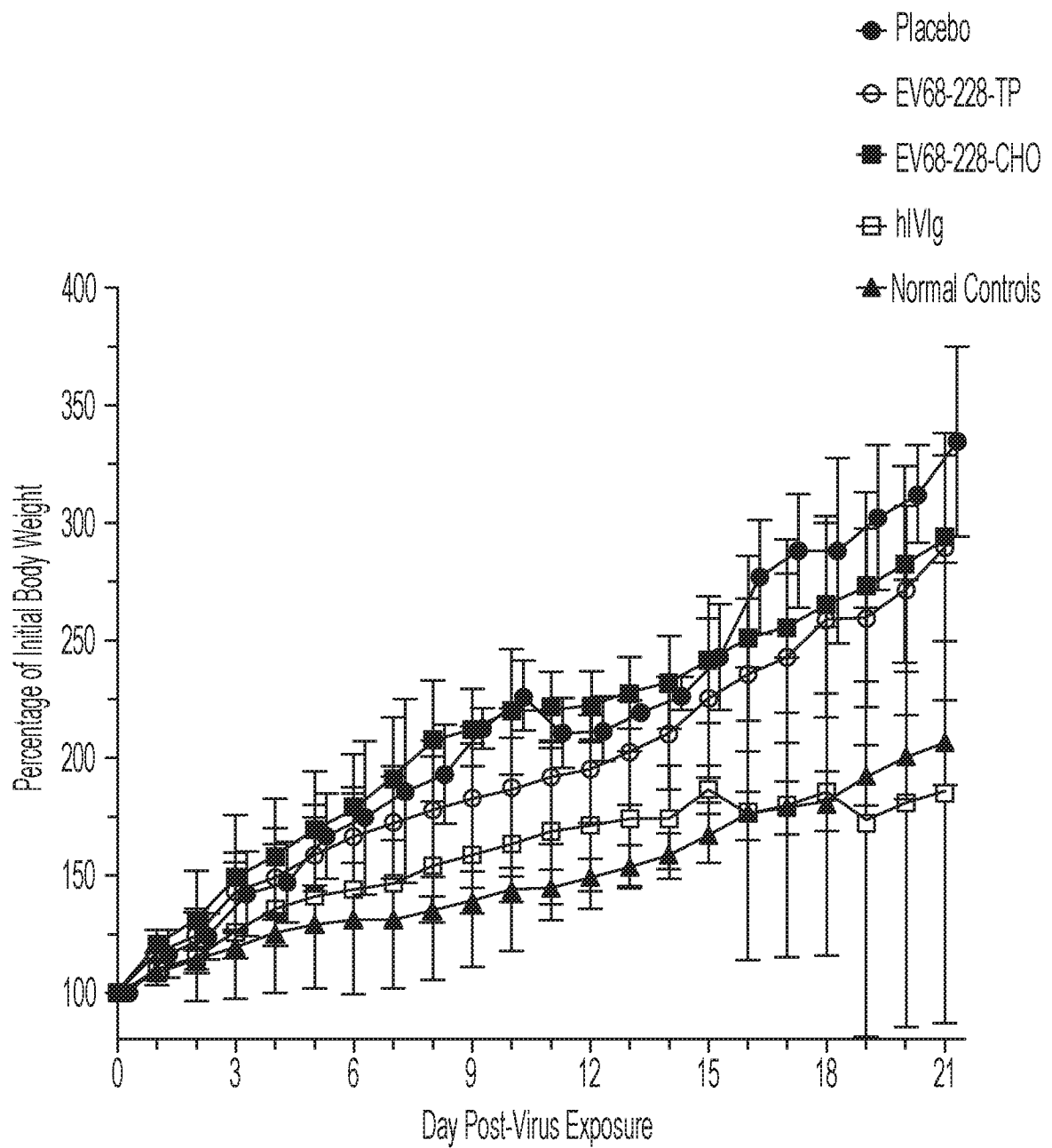


FIG. 67

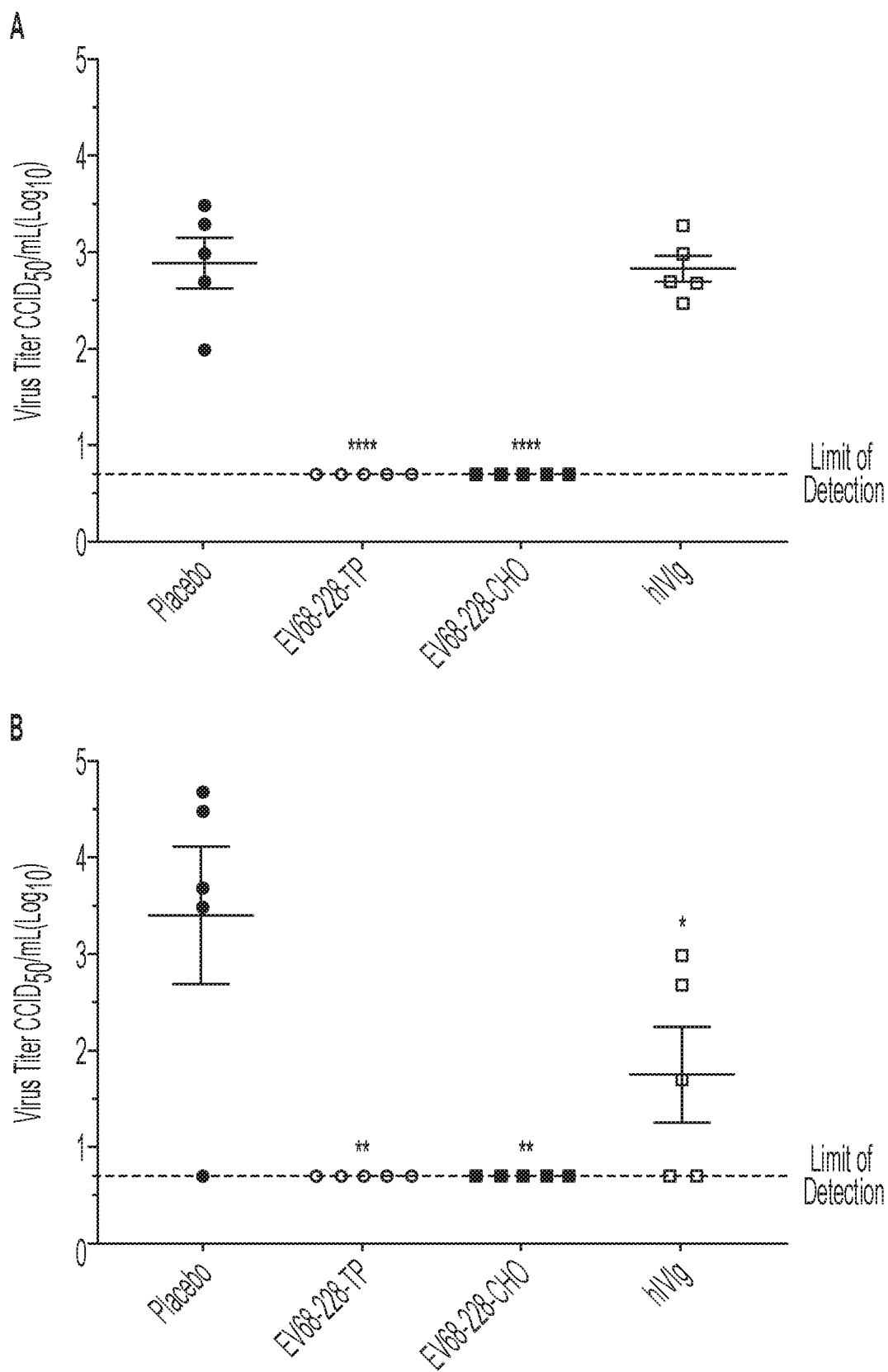


FIG. 68A-B

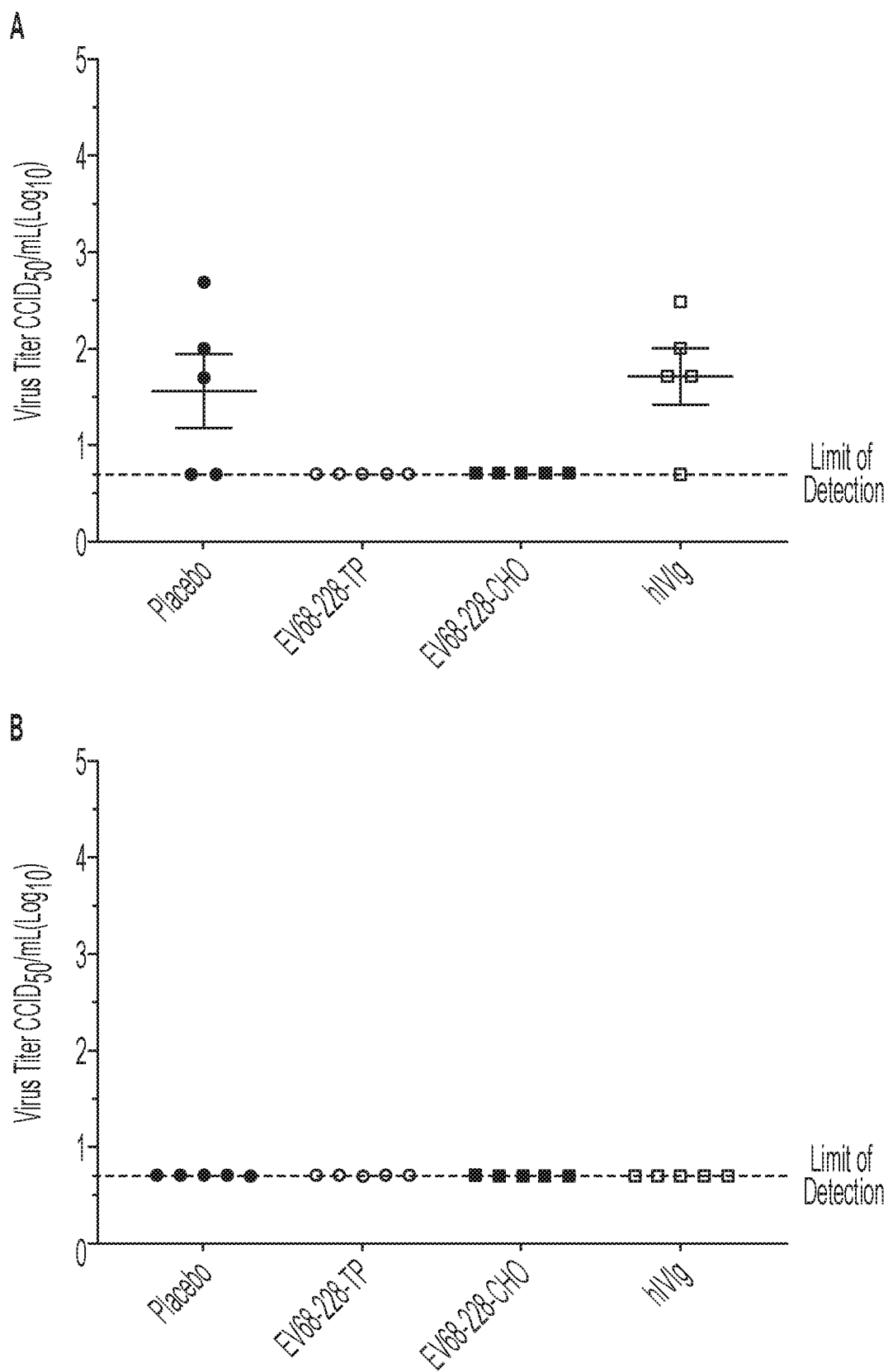


FIG. 69A-B

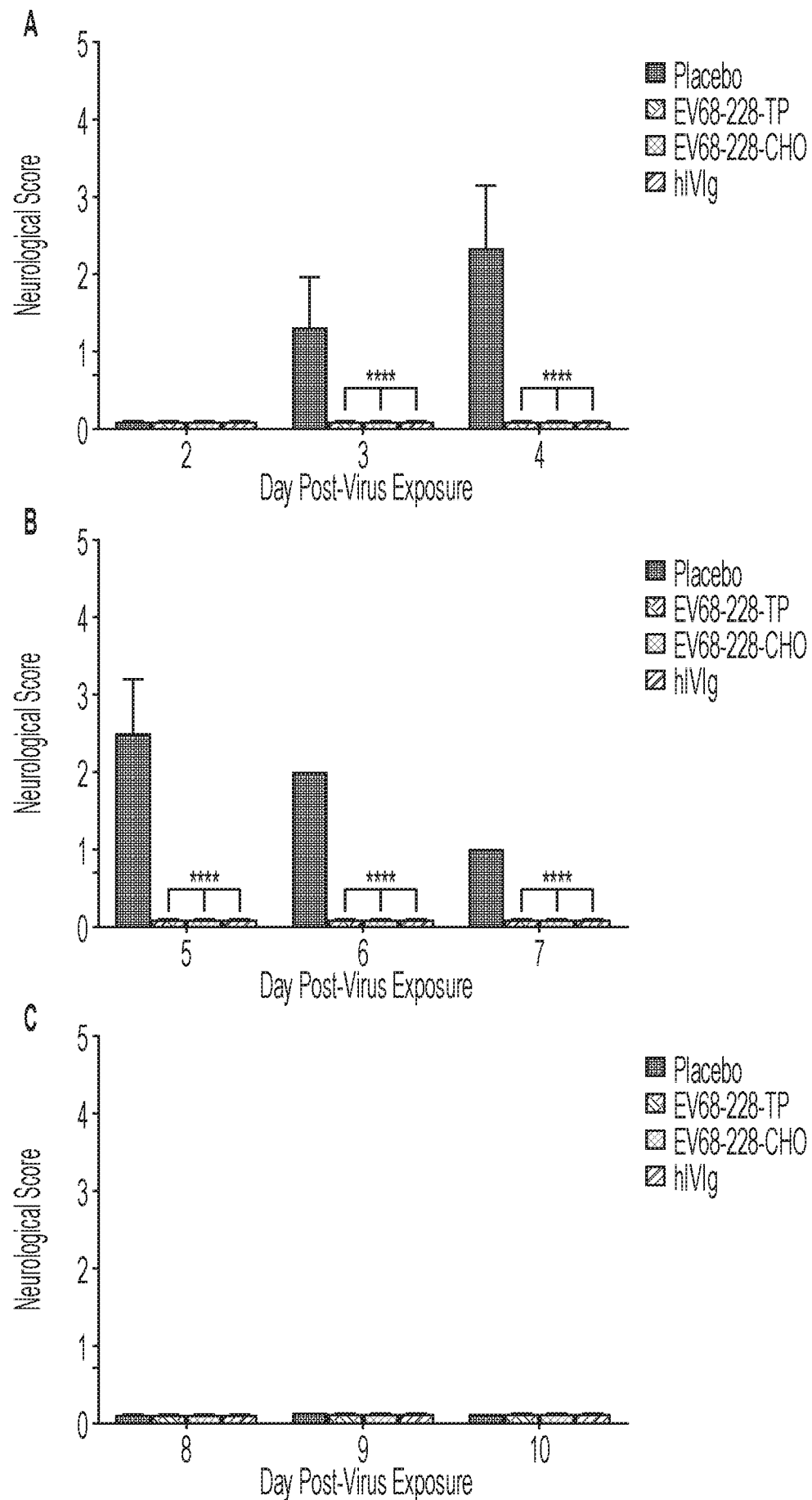


FIG. 70A-C

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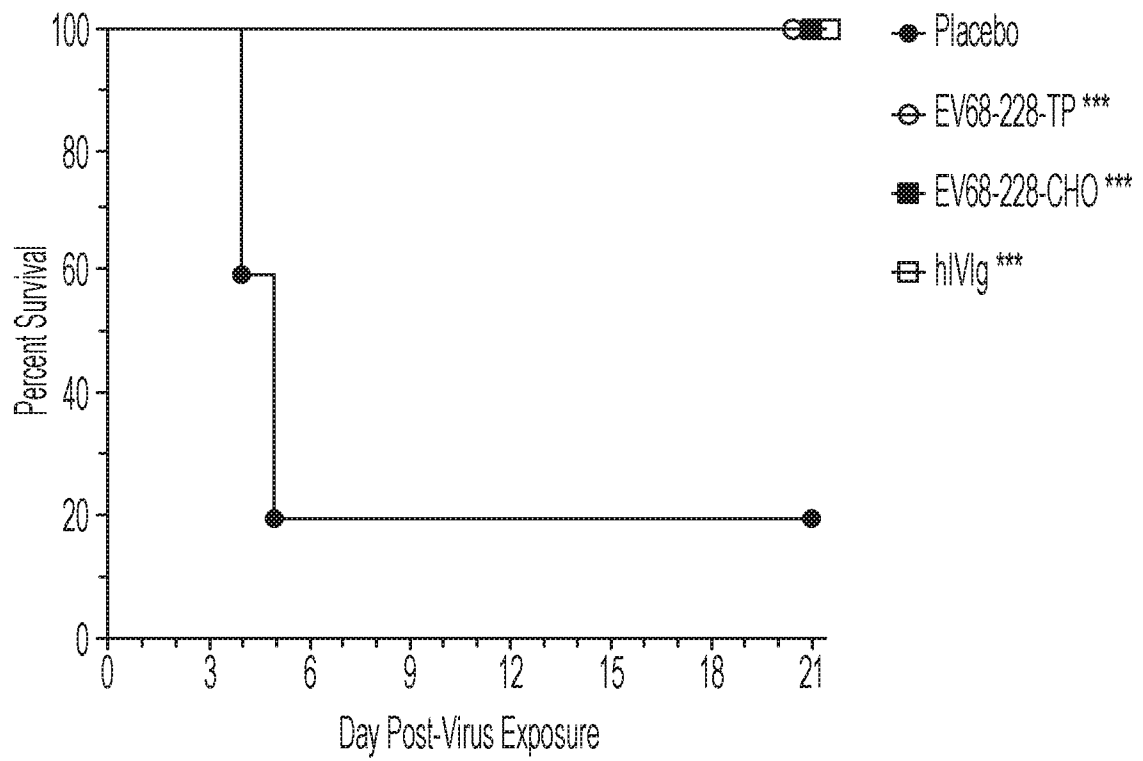


FIG. 71

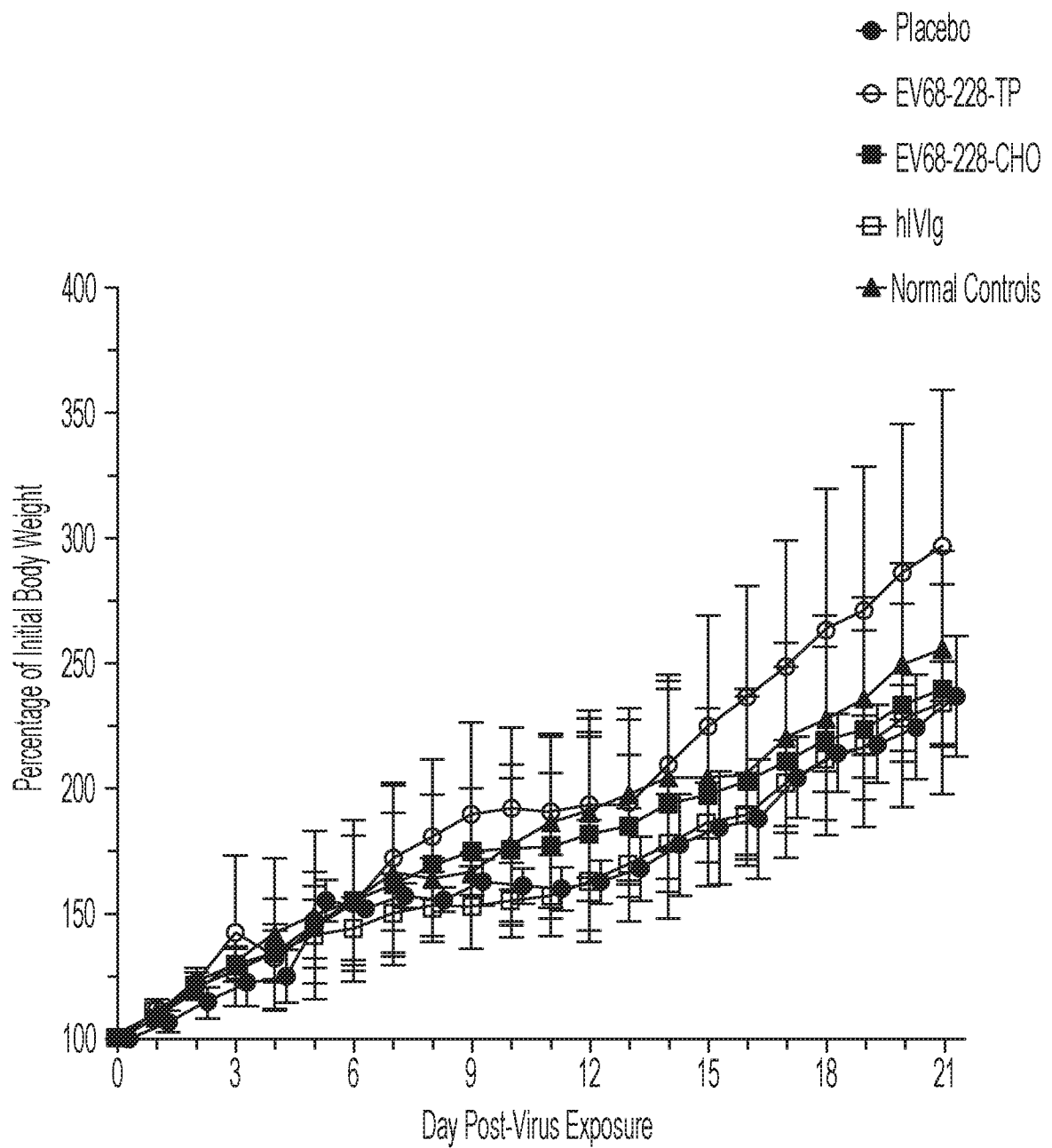
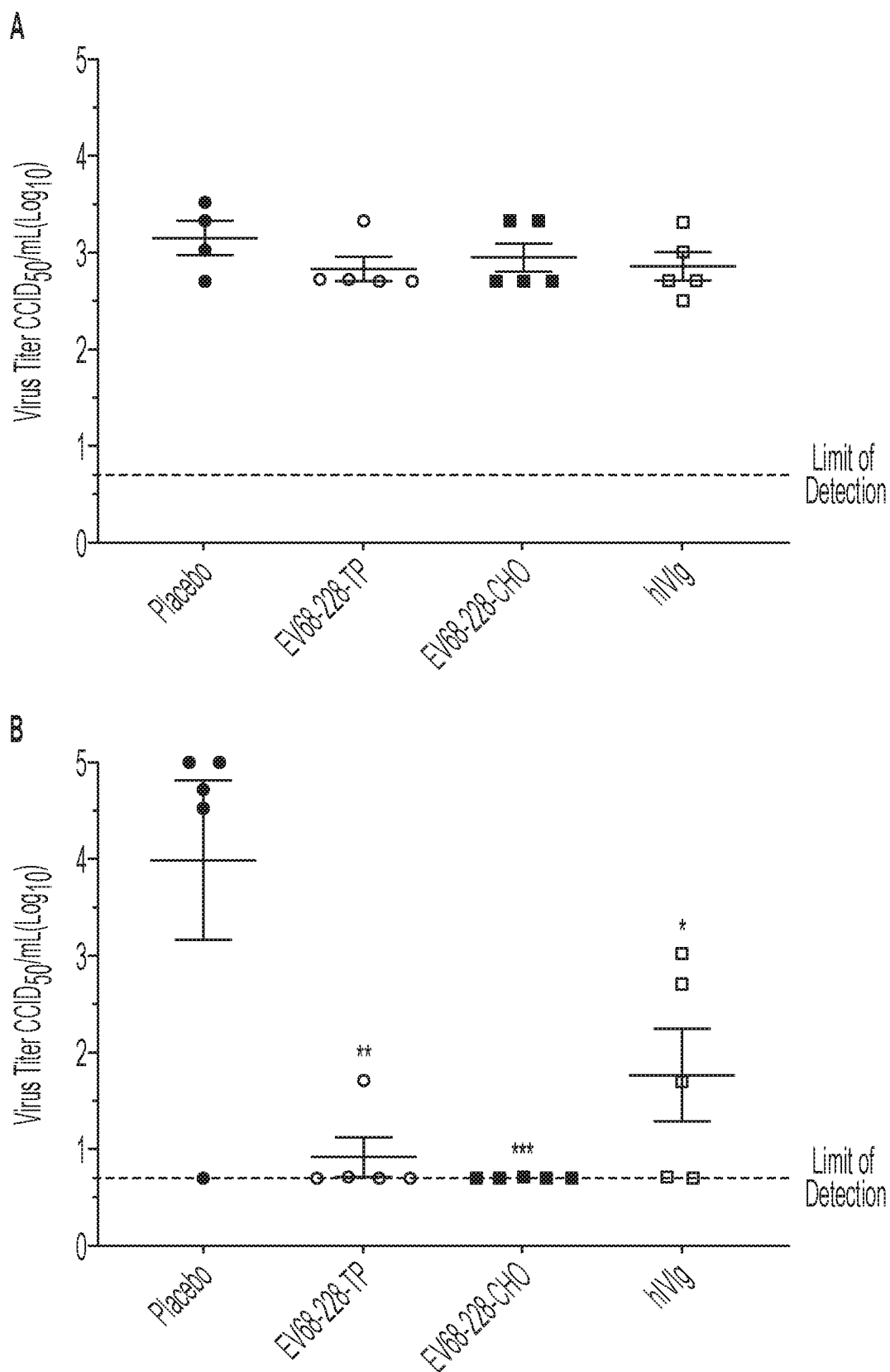


FIG. 72



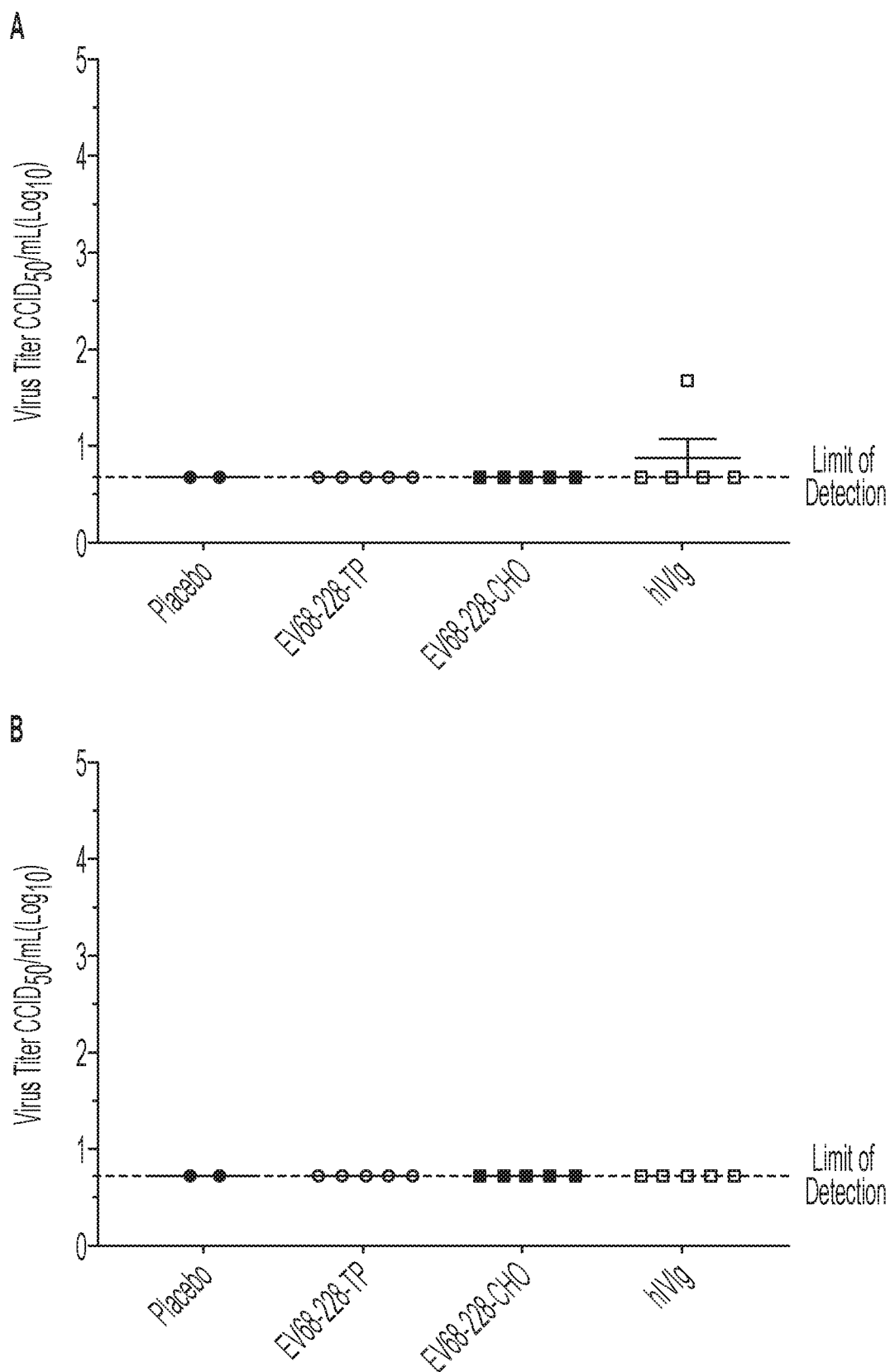


FIG. 74A-B

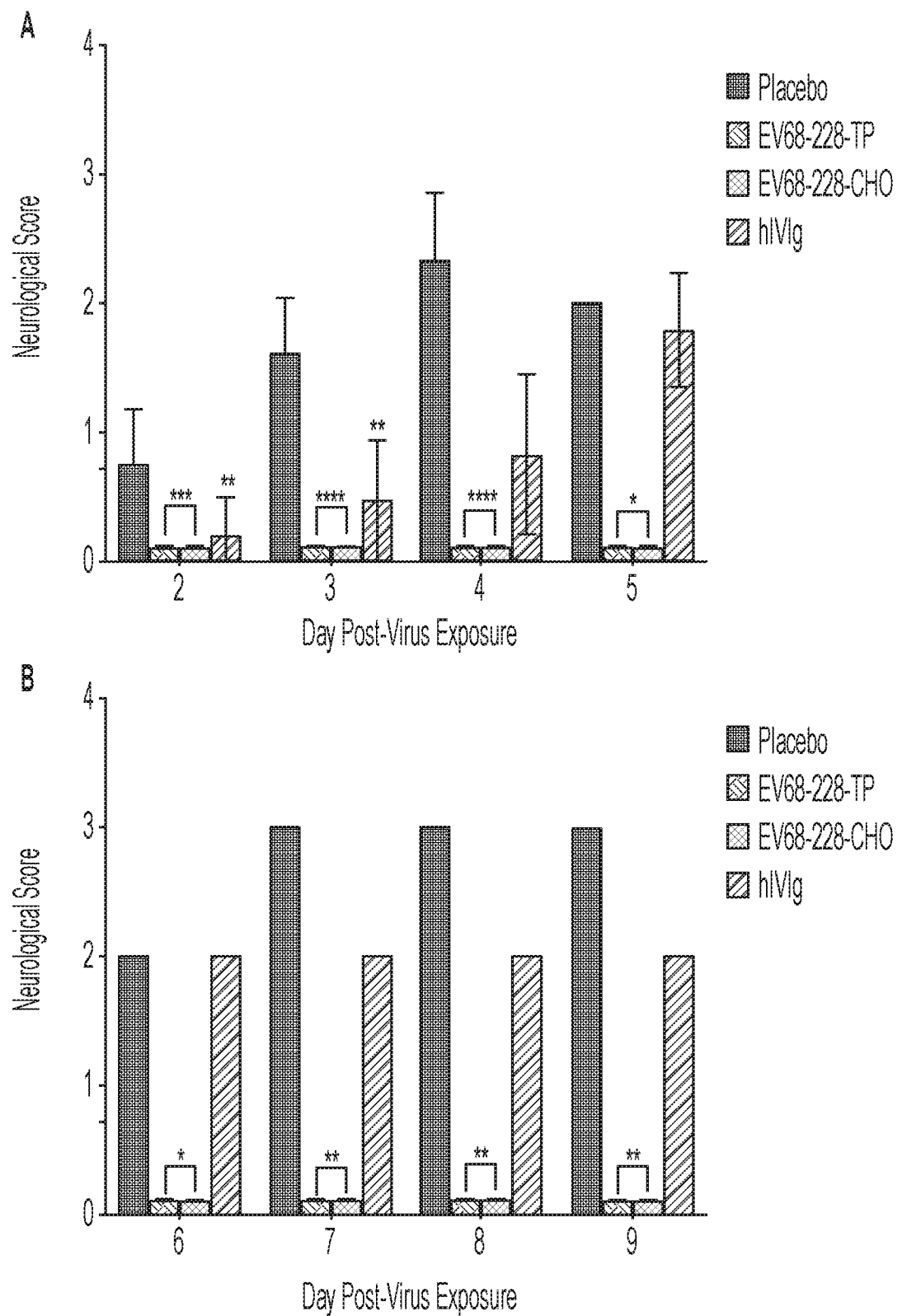


FIG. 75A-B

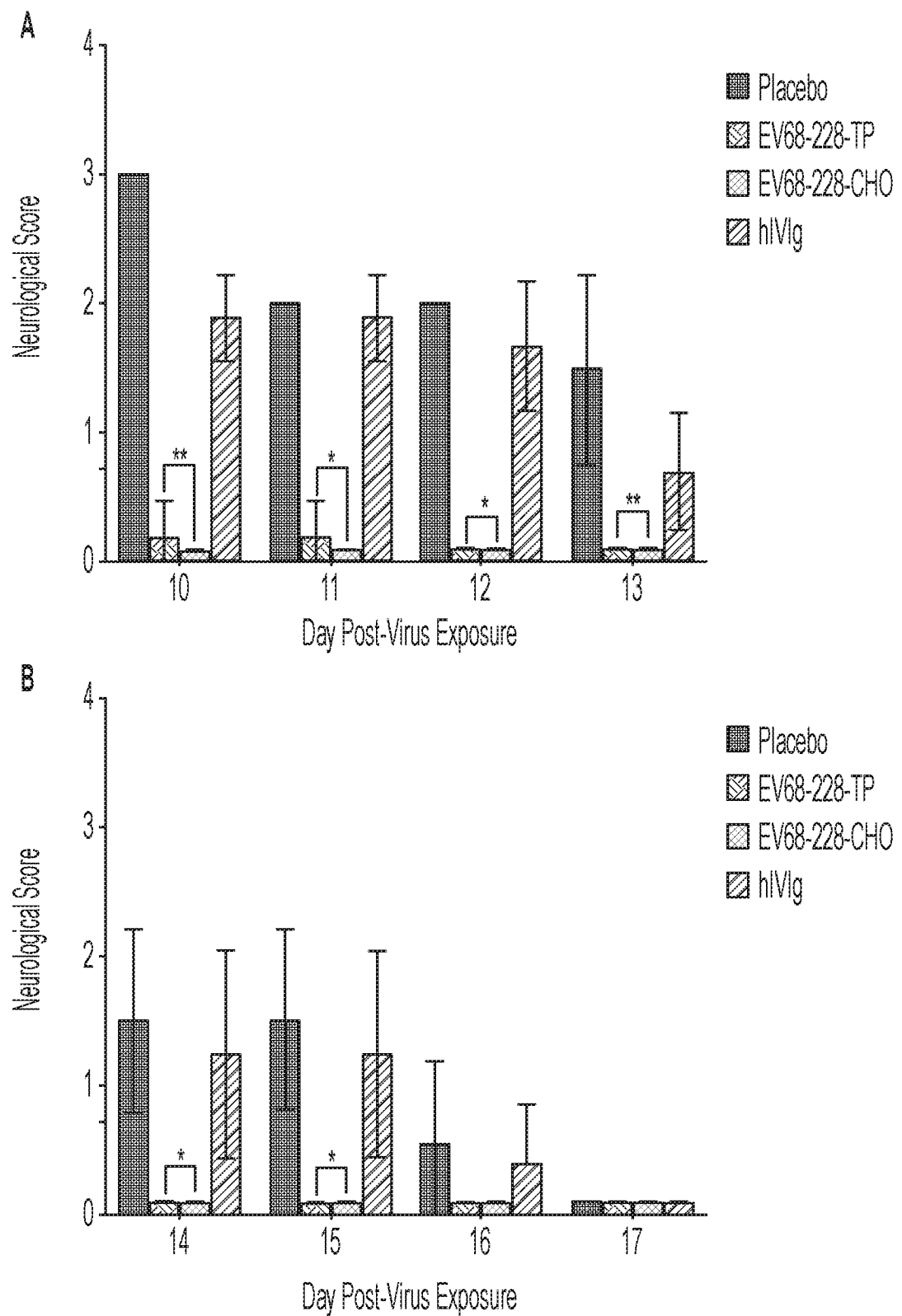


FIG. 76A-B

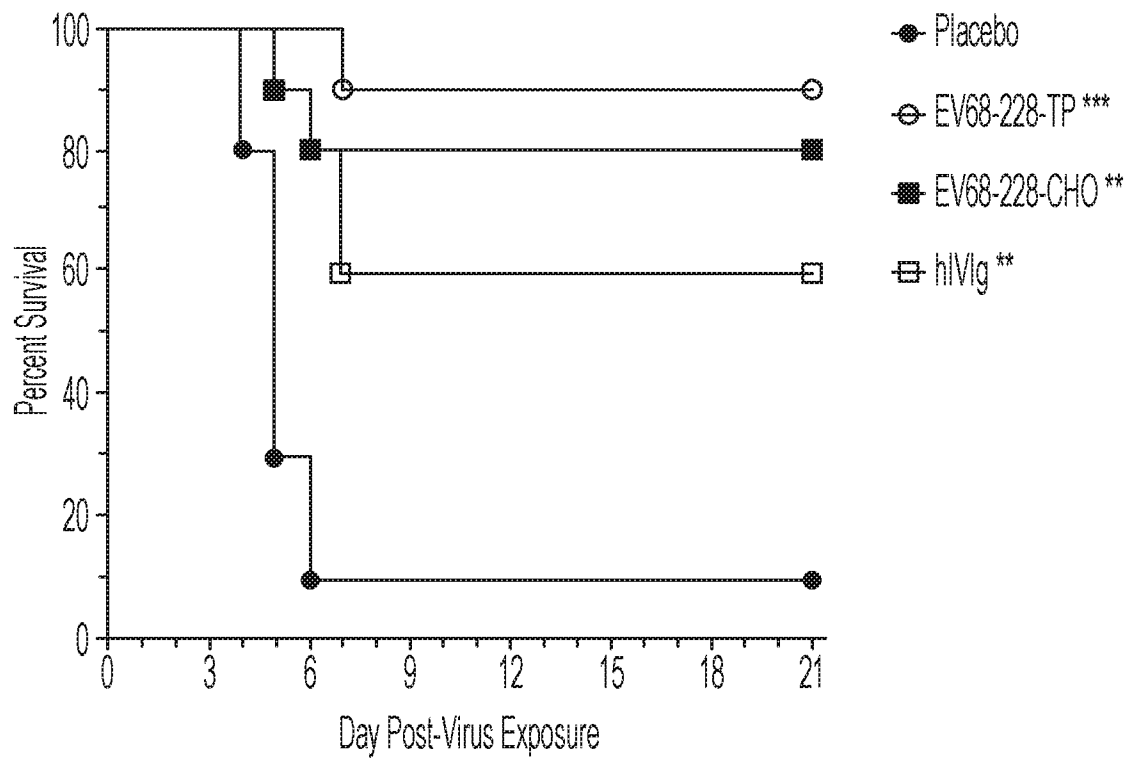


FIG. 77

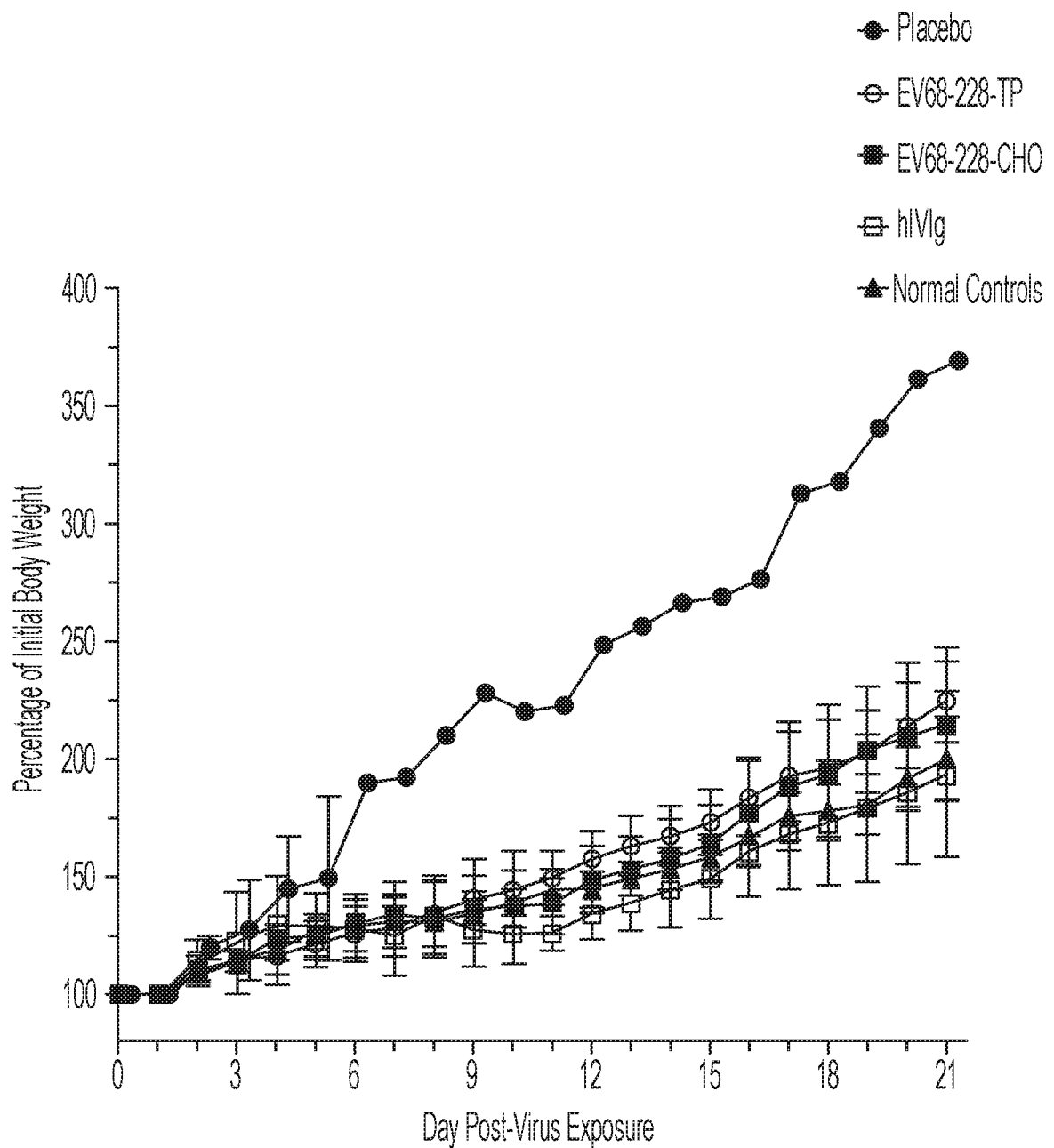


FIG. 78

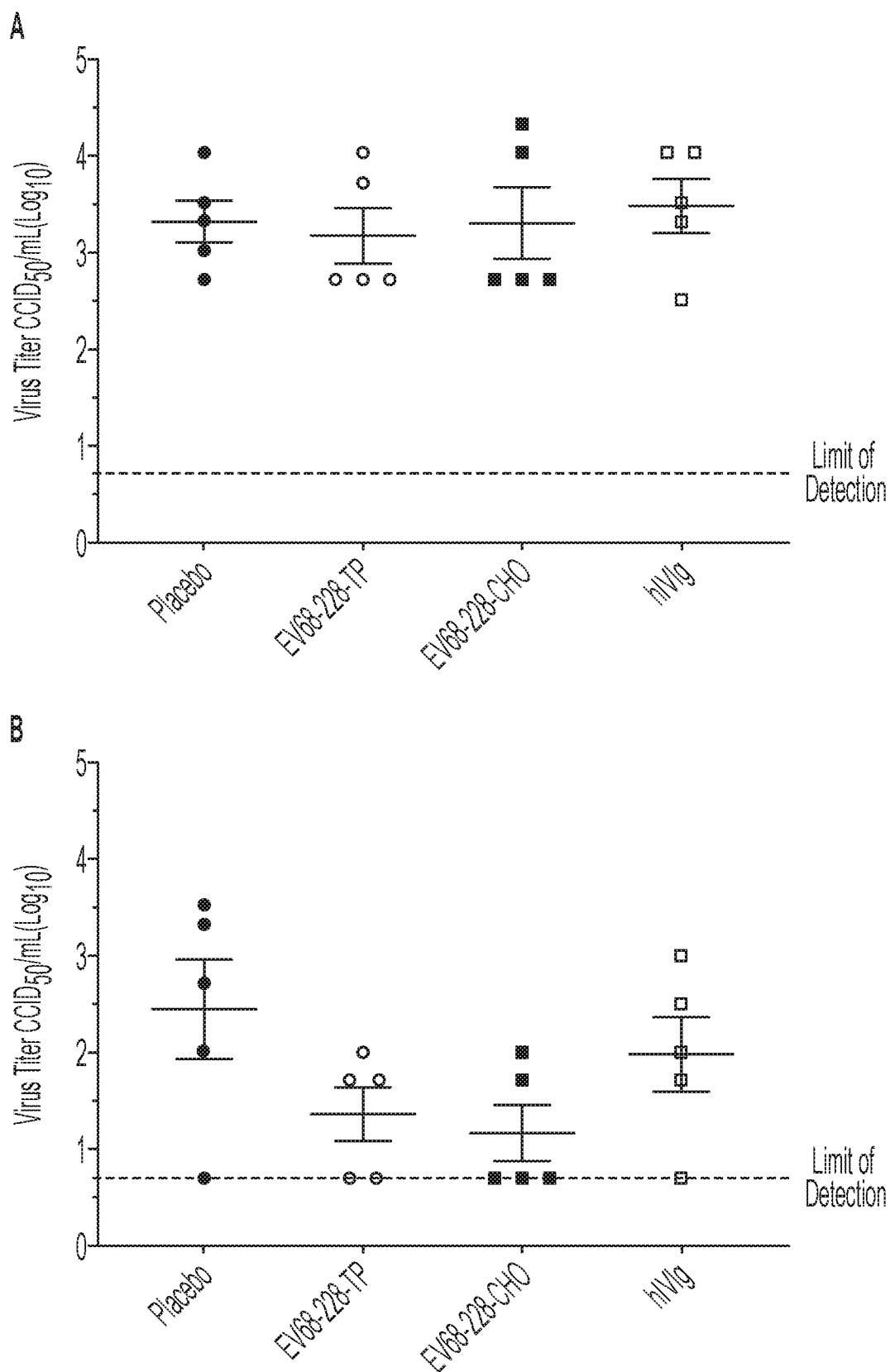


FIG. 79A-B

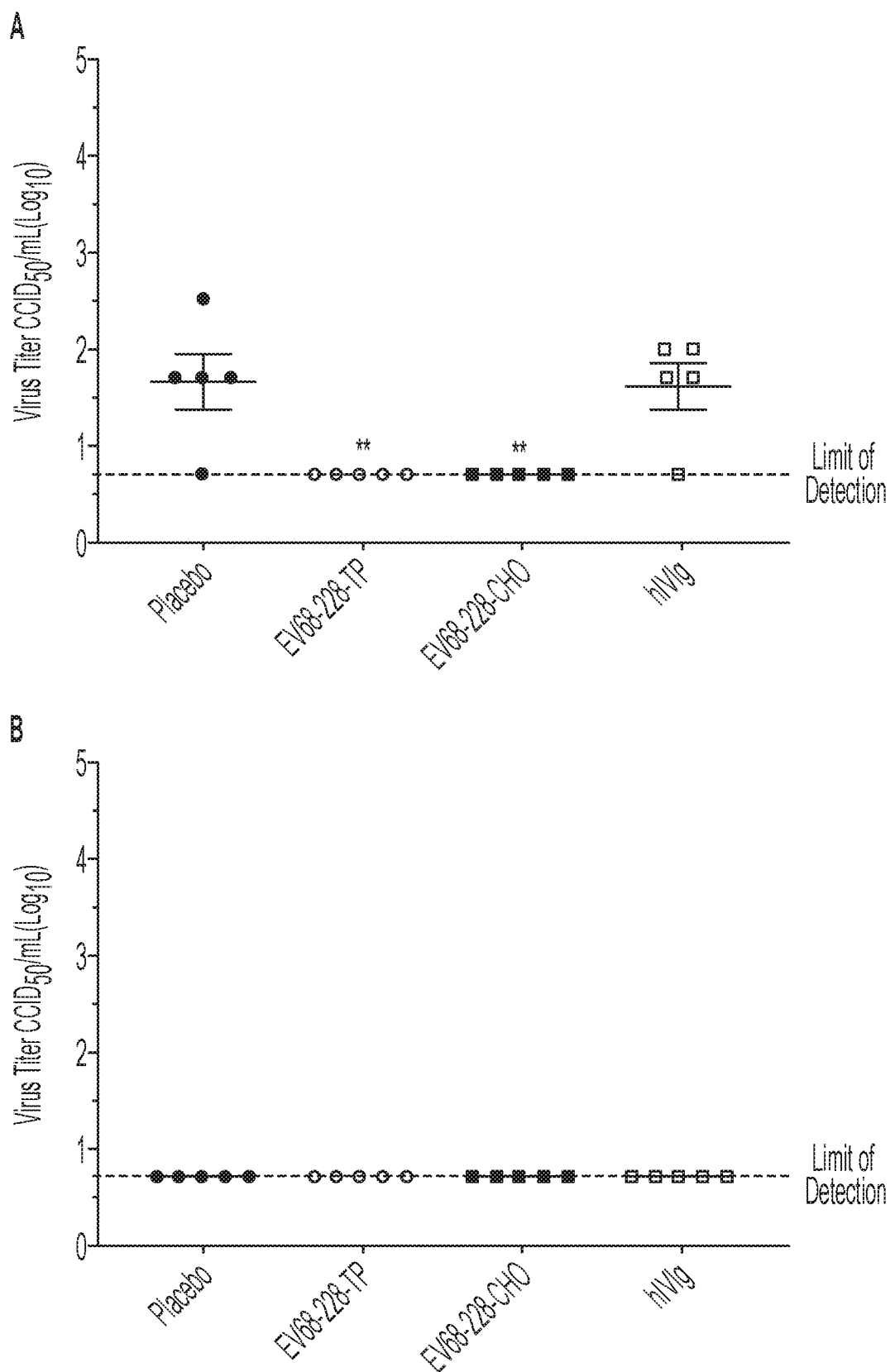


FIG. 80A-B

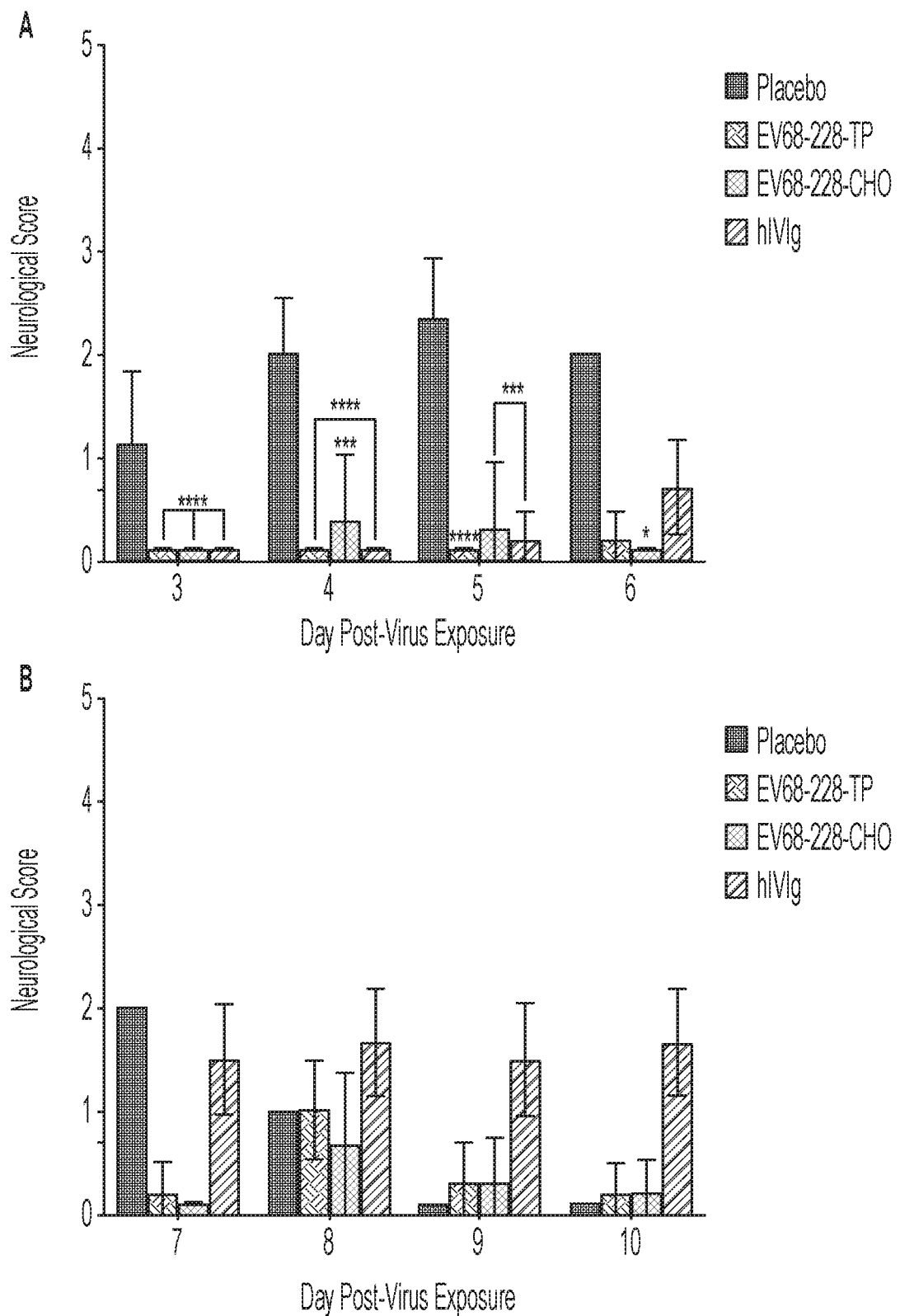


FIG. 81A-B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/043415

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.:
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:

see additional sheet

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 an 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.:

1-102(partially)

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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/043415

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/10 G01N33/53 A61P31/14
ADD. A61K39/00

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)

C07K G01N A61K A61P

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

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

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHENG QINGBING ET AL: "Atomic structures of enterovirus D68 in complex with two monoclonal antibodies define distinct mechanisms of viral neutralization", NATURE MICROBIOLOGY, NATURE PUBLISHING GROUP UK, LONDON, vol. 4, no. 1, 5 November 2018 (2018-11-05), pages 124-133, XP037114932, DOI: 10.1038/S41564-018-0275-7 the whole document ----- -/--	1-102



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

12 October 2020

Date of mailing of the international search report

14/12/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Scheffzyk, Irmgard

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/043415

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUIWEN ZHENG ET AL: "A Novel Neutralizing Antibody Specific to the DE Loop of VP1 Can Inhibit EV-D68 Infection in Mice", THE JOURNAL OF IMMUNOLOGY, vol. 201, no. 9, 3 October 2018 (2018-10-03), pages 2557-2569, XP055737978, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.1800655 the whole document -----	1-102

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-102(partially)

Subject-matter relating to antibody D68-37, i.e. SEQ.ID.NOs
129/130

2-64. claims: 1-102(partially)

Subject-matter relating to antibodies having VH/VL sequences
as shown in SEQ.ID.NOs. 131/132- 255/256
