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

(19) World Intellectual Property Organization
International Bureau

(10) International Publication Number
WO 2023/225637 A1

(43) International Publication Date
23 November 2023 (23.11.2023)

(51) International Patent Classification:
A61K 39/12 (2006.01) *A61P 31/14* (2006.01)

(21) International Application Number:
PCT/US2023/067226

(22) International Filing Date:
19 May 2023 (19.05.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/365,015 19 May 2022 (19.05.2022) US

(71) Applicants: **THE UNITED STATES OF AMERICA, as represented by the Secretary, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health, 6701 Rockledge Drive, Suite 700, MSC 7788, Bethesda, Maryland 20892-7788 (US). **HDT BIO** [US/US]; 1616 Eastlake Ave E., Suite 280, Seattle, Washington 98102 (US).

(72) Inventors: **HAWMAN, David**; 903 S. 4th Street, Rm 28/2A120, Hamilton, Montana 59840 (US). **FELDMANN, Heinz**; 903 S. 4th Street, Hamilton, Montana 59840 (US). **ERASMUS, Jesse Hong-Sae**; 1616 Eastlake Ave., Suite 280, Seattle, Washington 98102 (US).

(74) Agent: **GOFF, Christopher M.** et al.; Armstrong Teasdale LLP, 7700 Forsyth Blvd., Suite 1800, St. Louis, Missouri 63105-1847 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ,

(54) **Title:** REPLICATING RNA VACCINE FOR CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

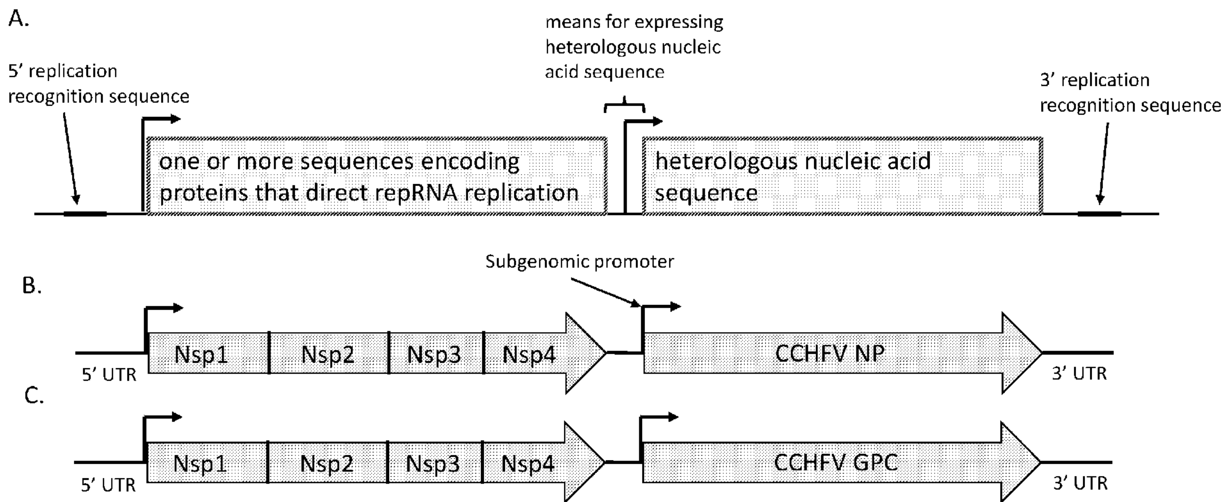


FIG. 1

(57) **Abstract:** Described herein are constructs, compositions, and methods for eliciting an immune response against CCHFV. In particular, the disclosure relates to self-replicating RNAs expressing encoding at least one heterologous polypeptide comprising an epitope that elicits an immune response against CCHFV. The disclosure also relates to compositions and nanoparticles comprising the disclosed self-replicating RNAs, and the use of such nanoparticles and compositions to elicit an immune response against CCHFV in an individual, thereby protecting the individual from infection with CCHFV.

WO 2023/225637 A1

[Continued on next page]

RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

REPLICATING RNA VACCINE FOR CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

[0001] STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH & DEVELOPMENT

[0002] Inventions disclosed herein were funded in part by support under Grant Application ID MCDC2204-011 from the U.S. Government. The U.S. government has certain rights in this invention.

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to vaccines and immunogenic compositions for Crimean-Congo Hemorrhagic Fever Virus (CCHFV). More particularly, the present disclosure relates to a replicating RNA-based vaccine, or an immunogenic composition, that expresses at least one polypeptide comprising one or more epitopes capable of eliciting an immune response against CCHFV, and the use of such vaccines and/or immunogenic compositions for protecting individuals against infection by CCHFV.

BACKGROUND

[0004] Crimean-Congo hemorrhagic fever (CCHF) is a deadly hemorrhagic fever having a high mortality rate. The disease results from infection of an individual by Crimean-Congo hemorrhagic fever virus (CCHFV), which is a tick-borne bunyavirus endemic in Southern and Eastern Europe, Africa, the Middle East, and Asia. Geographically, case distribution is consistent with the range of Hyalomma genus ticks, the main reservoir of CCHFV, and is likely to expand due to climate change. Humans may be infected from tick bites, through contact with infected animals or animal tissue. Nosocomial human-to-human transmission has also been described primarily for healthcare workers. Initial symptoms of CCHF include acute onset of a non-specific febrile illness consisting of sudden fever, myalgia, diarrhea, nausea, and vomiting (1). The hemorrhagic phase is characterized by large areas of severe bruising and uncontrolled bleeding throughout the body; among hospitalized patients, case fatality rates have ranged from 9-50%.

[0005] The general approach in treatment of patients with CCHF depends on the severity of the clinical manifestation, and mainly consists of managing fluid and electrolyte imbalances. Early diagnosis and supportive care in the form of blood, platelet, and plasma replacement may be lifesaving, especially in patients with hemorrhagic manifestation. However, even with such treatment, mortality rates may be high.

[0006] Historically, vaccines have consisted of attenuated or killed pathogens. However, the production of such vaccines can be difficult and time consuming, and depending on the virulence of the pathogen, may require special facilities, thereby increasing cost and potential risk of accidental exposure. Thus, modern research has focused on new types of vaccines that can be produced quickly and safely.

[0007] Recent vaccine development for SARS-CoV-2 has highlighted the advantages of RNA-based vaccines. Such vaccines are inexpensive, easy to produce, and the antigenic components can be switched quickly, thereby altering the vaccine to elicit an immune response against evolving mutant pathogens. However, general RNA-based vaccines may still suffer from insufficient production of antigen and the fact that RNA may be rapidly degraded once in the cell.

[0008] One type of RNA vaccine is a self-replicating RNA vaccine. Such vaccines, which are based on self-replicating RNA viruses, offer advantages over general RNA vaccines. For example, RNA vaccines deposit their RNA directly into the cytoplasm, where they can immediately start producing the antigenic protein. Moreover, because such vaccines are self-replicating, the RNA encoding the antigenic protein is amplified, resulting in a larger stimulus to the immune response. Further, replication of the RNA vaccine results in the production of double-stranded RNA (dsRNA) as well as replication complexes, which may provide an inherent adjuvant function for the vaccine.

[0009] To date, there has not been a widely available and efficacious vaccine or therapeutic for CCHF, resulting in the World Health Organization listing CCHFV as a high priority pathogen for development of antiviral countermeasures. The present disclosure provides an easy to produce and efficacious vaccine for preventing CCHFV infections.

SUMMARY

[0010] The present disclosure provides an alphavirus replicon RNA (repRNA)-based vaccine that may be used to elicit an immune response against Crimean-Congo hemorrhagic fever virus (CCHFV) in an individual. Such vaccine may protect an individual against infection with CCHFV.

[0011] One aspect of the disclosure is an alphavirus replicon RNA (repRNA) comprising at least one heterologous nucleotide sequence encoding a polypeptide

comprising at least one epitope that elicits an immune response against CCHFV, and a means for controlling expression of a nucleotide sequence operably linked to the at least one heterologous nucleotide sequence. In some aspects, the alphavirus, repRNA may comprise two or more heterologous nucleotide sequences, each of the two or more heterologous sequences encoding a polypeptide comprising at least one epitope that elicits an immune response against CCHFV, wherein each of the two or more heterologous nucleotide sequences is operably linked to a means for controlling expression of a nucleotide sequence. The two or more heterologous nucleotide sequences may be in-frame, such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the means of controlling expression of a nucleotide sequence encodes a fusion protein comprising the two or more heterologous proteins encoded by the two or more heterologous nucleotides sequences. A polynucleotide sequence, which may encode a linker peptide, may be present between the two or more heterologous nucleotide sequences, such that the heterologous proteins encoded by the heterologous nucleotide sequences are in frame with each other and with the peptide linker. The linker peptide may comprise an enzymatic cleavage site which may be a self-cleaving peptide, for example, a 2A peptide, a T2A peptide, a P2A peptide, a E2A peptide, or an F2A peptide. The polynucleotide sequence may encode an internal ribosome entry site (IRES), such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the means to express a nucleotide sequence contains an IRES site. The two or more heterologous nucleotide sequences may be joined by a polynucleotide sequence encoding an IRES. The two or more heterologous nucleotide sequence may, independently, be operably linked to different means for controlling expression of a nucleotide sequence.

[0012] The alphavirus may be Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), Eastern equine encephalitis virus (EEEV), Semliki Forest Virus (SFV), Sindbis virus (SV) or Chikungunya virus (CHKV). The repRNA may be a self-replicating repRNA. The repRNA may comprise one or more nucleotide sequences encoding one or more proteins that direct replication of the repRNA. The repRNA may comprise cis RNA sequences that are recognized by the one or more encoded proteins and that enable the encoded proteins to direct replication of the repRNA. The cis RNA sequences may comprise 5' replication sequences and/or 3' replication sequences, which may be present in a 5' alphavirus UTR and a 3' alphavirus UTR, respectively. The elements of the alphavirus repRNA may be arranged as follows: 5'

replication recognition sequence, one or more coding sequences encoding one or more proteins that direct replication of the repRNA, 3' replication recognition sequences.

[0013] The one or more coding sequences may comprise a single open reading frame (ORF) encoding a polyprotein comprising two or more of the one or more encoded proteins. The one or more encoded proteins may comprise an alphavirus nonstructural (ns) protein1 (nsP1), an alphavirus nsP2 protein, an alphavirus nsP3 protein and/or an alphavirus nsP4 protein, and such proteins may be from a species of alphavirus that differs from the species of alphavirus from which the 5' replication sequences and/or the 3' replication sequences' RNA sequences originate. In some aspects, such proteins may be from the same species of alphavirus from which the 5' replication sequences and/or the 3' replication sequences' RNA sequences originate.

[0014] In some aspects, the means for controlling expression of a nucleotide sequence may comprise an internal ribosome entry site or a subgenomic promoter, which may be an alphavirus subgenomic promoter. The alphavirus subgenomic promoter may direct transcription of an operably linked heterologous sequence from a negative strand copy of the repRNA. In some aspects, the subgenomic promoter may be the alphavirus 26s promoter. In some aspects, the alphavirus repRNA may comprise nucleotide elements ordered 5' to 3' as follows: i) the 5' replication recognition sequence; ii) the one or more nucleotide sequences encoding the one or more proteins that direct replication of the repRNA; iii) the means for controlling expression of a nucleotide sequence; iv) the at least one heterologous nucleotide sequence; and, v) the 3' replication recognition sequence. In some aspects, the repRNA may comprise a poly(A) tract.

[0015] In some aspects, the encoded polypeptide may comprise an epitope from a CCHFV protein, and the epitope may comprise at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous amino acid residues from the CCHFV protein. The CCHFV protein may comprise an amino sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. The CCHFV protein may comprise an amino sequence at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to a protein selected from the group consisting of CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the CCHFV polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. The CCHFV polypeptide comprises an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0016] One aspect of the disclosure is a vector, which may be a plasmid or a viral vector, encoding an alphavirus repRNA of the disclosure. In such vector, expression of the alphavirus repRNA may be under control of a promoter that can initiate transcription of the repRNA *in vitro* or *in vivo*. The promoter may be a T7 promoter, an Sp6 promoter, a lac promoter, or a trp promoter.

[0017] One aspect of the disclosure is a method of producing a repRNA of the disclosure, comprising incubating a vector of the disclosure under conditions suitable for transcription of the encoded repRNA. The vector may be linearized prior to transcription of the encoded repRNA. The transcribed repRNA, which may optionally be purified first, may be incubated with a capping enzyme so that the repRNA is capped.

[0018] One aspect of the disclosure is a nanoparticle comprising an alphavirus repRNA of the disclosure. The nanoparticle may comprise viral capsid proteins encapsulating the alphavirus repRNA. The nanoparticle may comprise a cationic nanocarrier (CNC), which may comprise a lipid inorganic nanoparticle. The nanoparticle may comprise a hydrophobic oil base, which may comprise one or more components selected from the group consisting of squalene, sorbitan monostearate (Span) 60, polyoxyethylene sorbitan monooleate 80 (Tween® 80), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) chloride, oleic acid–coated iron oxide nanoparticles, and sodium citrate dihydrate.

[0019] One aspect of the disclosure is an immunogenic composition comprising a repRNA of the disclosure or a nanoparticle of the disclosure.

[0020] One aspect of the disclosure is a method of stimulating or inducing an immune response in an individual, the method comprising administering to the individual a repRNA of the disclosure, a nanoparticle of the disclosure, or an immunogenic composition of the disclosure.

[0021] One aspect of the disclosure is a method of protecting an individual against infection by CCHFV, the method comprising administering to the individual a repRNA of the disclosure, a nanoparticle of the disclosure, or an immunogenic composition of the disclosure.

[0022] One aspect of the disclosure is a kit comprising a repRNA of the disclosure, a nanoparticle of the disclosure, or an immunogenic composition of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIGS. 1A-1C illustrate repRNAs of the disclosure. FIG. 1A provides a schematic illustrating the general structure of a repRNA of the disclosure. FIGS. 1B & 1C illustrate examples of a repRNA encoding a CCHFV NP (FIG. 1B) and a repRNA encoding CCHFV GCP (FIG. 1C).

[0024] FIGS. 2A-2E illustrate that vaccination with a repRNA of the disclosure elicits cellular and humoral immunity to CCHFV. FIG2A illustrates the timeline of the study. FIG. 2B shows the level of total CCHFV-specific IgG, and specific isotypes of CCHFV-specific antibody, respectively, in mice on Day 0. The dashed line indicates background absorbance of wells that did not receive serum. FIG. 2D shows the serum neutralization activity as measured using a neutralization assay against the infectious CCHFV strain Hoti. The dashed lines indicate the limit of detection and statistical significance calculated using one-way ANOVA with Dunnett's multiple comparison test. FIGS. 2E-2G show CCHFV-specific T-cell responses using an IFNγ ELISpot assay. Cumulative spot-forming cells (SFCs) against peptide pools spanning the entire NP or GPC, the mitogen concavalin A (CA), or DMSO vehicle alone (veh) are shown. Statistical comparisons calculated using a two-way ANOVA with Dunnett's multiple comparison test

(FIGS. 2C-2E). FIGS. 2E & 2F show heat maps showing the distribution of measured IFNγ SFCs against NP (FIG. 2E) or GPC (FIG. 2F) peptide pools. ns>0.05, ***P<0.001. FIS 2B-2E data shown as mean plus standard deviation.

[0025] FIGS. 3A-3L show the ability of serum from repRNA-vaccinated mice to recognize CCHFV infected cells. Serum was collected from vaccinated mice and pooled. L929 cells were mock-infected (FIGS. 3A-3D) of infected with CCHFV strain Hoti at an MOI of 1(FIGS. 3E-3L). After 24 hours (hrs.) cells were fixed with paraformaldehyde either left unpermeabilized (FIHS. 3I-3L) or permeabilized with saponin (FIGS. 3A-3H). Sera was applied at 1:500 and bound antibody detected using goat anti-mouse IgG conjugated to AlexaFluor488. Cells were counterstained with Hoechst 33342, and imaged using by laser scanning confocal microscopy.

[0026] FIGS. 4A-4E illustrate that vaccination with a repRNA of the disclosure confers significant protection against CCHFV. Groups of WT mice (n=8 per group) given the indicated prime-boost vaccinations were treated with MAR1-5A3 to blockade type I IFN and then infected with CCHFV strain UG3010. FIG. 4A shows weight loss in mice following infection. FIG. 4B shows survival following infection, while FIG. 4C shows body temperature following infection. Statistical comparisons were calculated using a two-way ANOVA with Dunnett's multiple comparison test (FIG. 4A) or Log-Rank test with Bonferonni's correction for multiple comparisons (FIG. 4B). Statistical significance compared to sham vaccinated animals is shown with symbols: *(repNP), # (repGPC) and + (repNP + repGPC). Viral loads in tissues was quantified using qRT-PCR. Dashed line indicates limit of detection (FIG. 4D). N=6 mice/groups statistical comparisons were calculated using a one-way ANOVA with Turkey's multiple comparison test. ns P>0.05, **P<0.05, ***P<0.001, ****P<0.0001 (FIGS. 4A, 4C, 4D). Data shown as mean plus standard deviation.

[0027] FIG. 5 illustrates that vaccination protects against liver pathology in CCHFV infected mice. Groups of WT mice (n=8 per group) given the indicated prime-boost vaccinations were treated with MAR1-5A3 to blockade type I IFN and then infected with CCHFV strain UG3010. On Day 5 p.i., mice were euthanized, livers collected, and formalin fixed. Sections were H&E stained or probed for the presence of viral antigen using immunohistochemistry (IHC). Representative images for each group are shown as 100X or 400X (inset) magnification and scale bars indicate 100 μm or 20 μm respectively.

[0028] FIGS. 6A& 6B show that a single immunization with a repRNA of the disclosure induces humoral and cellular immunity to CCHFV. Groups of 4 WT mice were vaccinates with the indicated cumulative doses of repNP+repGPC RNA in prime-boost regimen as before or in a prime-only regimen four weeks prior to challenge. FIGS. 6A and 6B show vaccine induced immune responses on Day 0 as measured by ELISA (FIG. 6A; IgG response to whole virion) or IFNγ ELISpot (FIG. 6B). Dashed line indicates background absorbance of wells that did not receive serum. Statistical values were calculated using a two-way ANOVA using Turkey's multiple comparison tests. The summary P value of each vaccine group individually compared against sham-vaccinated animals is also shown. FIG. 6B shows CCHFV-specific T-cell responses measured using IFN ELISpot and cumulative SFCs against the NP or GPC peptide pools. Indicated statistical comparisons were calculated using a two-way ANOVA with Turkey's multiple comparisons test. ns $P>0.05$, $*P<0.05$, $**P<0.01$, $***P<0.001$. Data shown as mean plus standard deviation.

[0029] FIGS. 7A-7E show that prime-only vaccination protects against CCHFV challenge. Groups of WT mice (n=8 per group) given the indicated vaccinations were treated with MAR1-5A3 to blockade type I IFN and then challenged with CCHFV strain UG3010. FIG. 7A shows weight loss in mice following infection. FIG. 7B shows survival following infection, while FIG. 7C shows body temperature following infection. Statistical comparisons were calculated using a two-way ANOVA with Dunnett's multiple comparison test (FIG. 7A) or Log-Rank test with Bonferonni's correction for multiple comparisons (FIG. 7B). Viral loads in indicted tissues were quantified using qRT-PCR (FIG. 7D) or TCID50 assay (FIG. 7E) and statistical comparison between sham-vaccinated mice and rep-vaccinated mice were calculated using one-way ANOVA with Dunnett's multiple comparisons test. Statistical comparisons between sham-vaccinated end every repNP-vaccinated group were significant. N=6 per group. ns $P>0.05$, $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$ (FIGS. 7A, 7C, 7D, 7E). Data shown as mean plus standard deviation.

[0030] FIGS. 8A & 8B show anamnestic antibody responses. At indicated time points post-infection, serum was collected from animals receiving indicated doses of repNP +repGPC. CCHFV-specific antibody responses were quantified by whole-virion ELISA. Indicated statistical comparisons were calculated with two-way ANOVA with

Sidak's multiple comparisons test. ns P>0.05, **P<0.01, ****P<0.0001. Data shown as mean plus standard deviation.

[0031] FIGS. 9A-9G show that prime-only repNP vaccination protects against high-dose CCHFV challenge. On day -28 mice were vaccinated with 1 μg of repNP via the IM route. On Day 0, immune responses from the vaccine were measured by ELISA (FIG9A) and IFN-γ ELISpot (FIG. 9B). Mice were then treated with MAR1-5A3 to suppress type I IFN responses and challenged with 10,000 TCID50 of CCHFV strain UG3010 via the IP route. Mice were monitored daily and weight loss (FIG. 9C), survival (FIG. 9D) and body temperature (FIG. 9E) recorded. On Day 4 Post-infection, a group of mice were euthanized and viral loads in the blood, liver and spleen measured by qRT-PCR (FIG. 9F) or TCID50 assay (FIG. 9G). P values were calculated using Welch's T-test (FIGS. 9A, 9F and 9G), or two-way ANOVA with Sidak's multiple comparison test (FIGS. 9B & 9C) and Log-rank test (FIG. 9D). **P<0.01, ***P<0.001, ****P<0.0001.

[0032] FIGS. 10A-10E show that humoral immunity is required for protection from CCHFV challenge. FIG. 10A outlines the study protocol. Briefly, groups of WT or B-cell deficient μMT mice were vaccinated with 1 ug of repNP+repGPC RNA or sham vaccinated in prime-only vaccination four weeks prior to CCHFV challenge. On days -5 and -2 relative to CCHFV challenge, groups of repNP+repGPC vaccinated mice were treated with an isotype control or antibodies to deplete CD4 T-cells (α-CD4), CD8 T-cells (α-CD8 or both (α-CD4/α-CD8). FIG. 10A shows weight loss in mice following infection. FIG. 10B shows survival following infection, while FIG. 10C shows body temperature following infection. N=16 for sham and 6-8 mice per other groups. Statistical comparisons were calculated using a two-way ANOVA with Dunnett's multiple comparison test (FIG. 10B) or Log-Rank test with Bonferonni's correction for multiple comparisons (FIG. 10C). Viral loads in indicted tissues were quantified using qRT-PCR (FIG. 10E) and indicated statistical comparisons were calculated using one-way ANOVA with Dunnett's multiple comparisons test. N=6 per group (FIGS. 10B, 10D, and 10E). Data shown as mean plus standard deviation. *P<0.05, **P<0.01, ***P<0.001.

[0033] FIGS. 11A-11C show depletion of T-cells and absence of CCHFV-specific antibody in μMT mice. On Day 0, CCHFV-specific antibody was measured by whole virion ELIS (FIG. 11A). N=4 mice per group. Dashed line indicates background absorbance of wells receiving no serum and indicates statistical comparisons calculated using

one-way ANOVA with Dunnett's multiple comparison test. Efficacy and specificity of T-cell depletion in the spleen was measured by flow-cytometry on Day 0 (FIG> 11B). Cells were gated to exclude debris, doublets, and non-viable cells. CD3+ T-cells were defined as CD45+CD3+B220- and CD4+ or CD8+ T-cells were defined as CD45+CD3+B220 and CD4+ or CD8+, respectively. N=4 per group and indicated statistical comparisons calculated using two-way ANOVA with Dunnett's multiple comparison test. Effect of B-cell deficiency or T-cell depletion on CCHFV-specific T-cell responses was measured by IFNγ ELISpot against the immunodominant peptide pools identified for the CCHFV NP and GPC protein (FIG. 11C). N=4 mice per group and indicated statistical comparisons calculated using two-way ANOVA with Dunnett's multiple comparison test. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (FIGS. 11A-C) Data shown as means plus standard deviation.

[0034] FIGS. 12A-12H show the effect of NK-cell depletion on the ability of repRNA to protect against CCHFV. FIG. 12A illustrates the general timeline of the study. WT C57BL6/J mice were (A) vaccinated with 1ug repNP on day -28 relative to CCHFV challenge with strain UG3010 on day 0. Mice were treated with NK1.1 depletion antibody or isotype on day -2 relative to challenge and every third day thereafter. FIG.12B shows the level of CCHFV-specific IgG as measured via whole virion ELISA while FIG. 12C shows T-cell responses as measured via IFNy-ELISpot; total spot forming units (SFCs) against CCHFV Np peptide pool 4, concanavalin A (ConA), and DMSO (Veh) are shown. FIG. 12D shows the number of NK cells present on Day 9, as determined using FLOW cytometry and the log transformation of the number of NK cells (CD45+Cd3-CD11b+CD49b+) normalized to the spleen in isotype treated and NK1.1 treated mice is reported. FIG. 12E shoes the viral loads in the blood, liver, and spleen as determined via $TCID_{50}$ assay. Dashed line represents limit of detection of assay. FIGS. 12F and 12G show the percent weight loss (12F) and survival(12G) over the course of study. FIG. 12H shows the anamnestic response to whole virion CCHFV antigen using an ELISA at days 9 and 14 post infection. Day 0 is duplicated on graph for visual comparison between groups.

[0035] FIG. 13 Schematic diagram showing two possible configurations for a repRNA of the disclosure.

[0036] FIGS. 14A-14G illustrate that repGPC-T2A-NP elicits equivalent cellular but not humoral immune response compared to repNP + repGPC. FIG. 14A illustrates a timeline of the experimental setup. FIGS. 14B-15D show the resulting antibody

response evaluated via (114B) endpoint titers measured using a whole virion IgG ELISA, (14C) recombinant antigen (rAg) ELISA to the CCHFV nucleoprotein (NP) and mature glycoproteins (Gn and Gc), and (14D) neutralization assay using infectious virus. Dashed lines indicate limit of detection. FIGS. 14E-14G show the cellular immune response evaluated via IFNγ ELISpot with cumulative responses against either NP or GPC (14E) and heat maps (FIGS. 14F & 14G) showing the distribution of cellular responses to peptide pools spanning the entire CCHFV glycoprotein precursor (GPC) and NP. Significance was calculated using one-way ANOVA; ns P > 0.05, **** P < 0.0001. Data shown as mean plus standard deviation.

[0037] FIGS15A-D show that vaccination with repGPC-T2A-NP partially protects against lethal CCHFV challenge. Mice vaccinated with Sham, repGPC-T2A-NP, or repGPC + repNP RNA were treated with MAR1-5A3 antibody and infected with a lethal dose of 100 TCID50 CCHFV strain UG3010. FIG. 15A shows daily body weight. FIG. 15B shows survival until day 14 post-infection (p.i.). FIG. 15C shows viral loads in blood, liver tissue and spleen tissue, at 5-days p.i., as measured via qRT-PCR. FIG. 15D shows viral loads in blood, liver tissue and spleen tissue, at 5-days p.i., as measured by infectious virus via tissue culture infectious dose 50 (TCID50). Dashed lines indicate limit of detection. Significance was calculated using one-way ANOVA; ns P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, **** P < 0.0001. Data shown as mean plus standard deviation.

[0038] FIGS. 16A & 16B illustrate the repGPC-T2A-NP anamnestic immune responses. At D14 p.i., surviving groups of mice vaccinated with repGPC-T2A-NP or repGPC + repNP RNA and challenged with a lethal dose of CCHFV strain UG3010 were evaluated for anamnestic immune response. FIG. 16A shows the antibody response assessed via rNP, rGc, and rGn IgG ELISAs. FIG. 16B shows the antibody response assessed via neutralization assay using infectious CCHFV strain Hoti. For rELISAs, comparison of D0 and D14 antibody titers at serum dilution 1:800 is shown. Dashed lines indicate limit of detection. Significance was calculated using one-way ANOVA; ns P > 0.05, ***P < 0.001. Data shown as mean plus standard deviation.

[0039] FIGS. 17A-G show that repNP + repGc-sol or repGc-teth elicits robust humoral and cellular immunity. WT C57BL6/J mice were vaccinated with 1ug of Sham RNA or repNP RNA plus repGc-sol, repGn-sol, repGc-teth, or repGPC RNA prime-boost on days -56 and -28 relative to lethal CCHFV challenge. On D0, groups of mice (N=6)

were evaluated for immune response to CCHFV Hoti. FIG. 17A illustrates the experimental design. FIGS. 17B-17D show the antibody response as determined by (17B) whole virion IgG ELISA, (17C) rNP, rGn, and rGc ELISA and (17D) neutralization assay using infectious virus. Dashed lines indicate limit of detection. FIGS. 17E-17G show the cellular immune response was as determined via IFNγ ELISpot shown as (17E) cumulative SFCs or (17F & 17G) heat maps of cellular responses to peptide pools spanning the entire CCHFV GPC and NP. DMSO vehicle (Veh) is also shown. Sham and repGPC + repNP group data is duplicated from Figures 1&2 for comparison. Significance was calculated using one-way ANOVA; ns $P > 0.05$, $**P < 0.01$, $**** P < 0.0001$. Data shown as mean plus standard deviation.

[0040] FIGS. 18A-18D illustrate that repNP + repGc-teth confers equivalent protection to repNP + repGPC vaccination. Mice vaccinated with sham RNA or repNP RNA plus repGc-sol, repGn-sol, repGc-teth, or repGPC RNA were treated with MAR1-5A3 antibody on D0 and infected with a lethal dose of 100 $TCID_{50}$ CCHFV strain UG3010. FIG. 18A shows daily weight change. FIG. 18B shows survival until D14 p.i.. On D5 p.i., groups of mice (N=6) were evaluated for control of viral loads via qRT-PCR (FIG. 18C) and infectious virus via $TCID_{50}$ (FIG. 18D). Dashed lines indicate limit of detection. Sham and repGPC + repNP group data is duplicated from Figures 1&2 for comparison. Significance was calculated using one-way ANOVA; $***P < 0.001$, $**** P < 0.0001$. Data shown as mean plus standard deviation.

[0041] FIGS. 19A & 19B show the anamnestic immune response elicited by engineered repGPC-variants. At D14 p.i., surviving groups of mice vaccinated with repNP RNA plus repGc-sol, repGn-sol, repGc-teth, or repGPC RNA and challenged with a lethal dose of CCHFV strain UG3010 were evaluated for anamnestic immune response. FIGS. 19A & 19B show the antibody response as determined via (FIG.19A) rNP, rGc, and rGn IgG ELISAs and (FIG.19B) neutralization assay using infectious CCHFV strain Hoti. For rELISAs, comparison of D0 and D14 antibody titers at serum dilution 1:800 is shown, dashed lines indicate limit of detection. repGPC + repNP group data is duplicated from FIG.16 for comparison. Significance was calculated using a one-way ANOVA; ns $P > 0.05$, $*P < 0.05$. Data shown as mean plus standard deviation.

[0042] FIGS. 20A-20I show that removal of V5 tag from repNP does not affect vaccine immunogenicity or efficacy. WT C57BL6/J mice were vaccinated prime-only with 1ug of sham, repNP, or repNP(ΔV5) RNA on day -28 relative to CCHFV challenge.

FIG. 20A illustrates the experimental design. FIGS. 20 B-E show the immune response on D0, as determined by (FIG.20B) rNp IgG ELISA, (FIG.20C) neutralization assay, and (FIGS.20E & 20F) IFNy ELISpot shown as (FIG.20D) cumulative SFCs or (FIG.20E) heat map of cellular responses to peptide pools spanning the entire CCHFV NP. DMSO vehicle (Veh) is also shown. On D0, groups of mice were treated with MAR1-5A3 and infected with a lethal dose of CCHFV strain UG3010. FIG.20F shows daily weight change. FIG. 20G shows survival until D14 p.i.. On D5 p.i., groups of mice (N=6) were evaluated for control of viral loads. FIGS. 20H & 20I show viral loads as determined by qRT-PCR (FIG.20H) and infectious virus via $TCID_{50}$ in indicated tissues (FIG.20I). repNP(ΔV5) data is duplicated from figure 5 and 6 for comparison. Dashed lines indicate limit of detection. Significance was calculated using one-way ANOVA; ns P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, **** P < 0.0001. Data shown as mean plus standard deviation.

[0043] FIGS21A-21G show that bivalent repGcteth-NP RNA and mixed synthesis repGc-teth + repNP RNA elicit robust humoral and cellular immunity. WT C57BL6/J mice were vaccinated with 1ug of sham, repNP(ΔV5), repGc-teth, mixed synthesis (repGc-teth and repNP RNA produced in a single reaction), or bivalent (repGcteth-NP) RNA prime-only on day-28 relative to CCHFV challenge. FIG. 21A illustrates the experimental design. On D0, groups of mice (N=6) were evaluated for immune response to CCHFV Hoti. FIGS. 21B-21D show the antibody response as determined by (FIG.21B) rNp IgG ELISA, (FIG.21C) rGn and rGc ELISA and (FIG.21D) neutralization assay using infectious virus. Dashed lines indicate limit of detection. FIGS> 21E-21G show the cellular immune response as determined by IFNy ELISpot shown as (FIG.21E) cumulative SFCs or (FIGS. 21F & 21G) heat maps of cellular responses to peptide pools spanning the entire CCHFV GPC and NP. DMSO vehicle (Veh) is also shown. Sham and repNP(ΔV5) group data are duplicated from FIG.20 for comparison. Significance was calculated using one-way ANOVA; ns P > 0.05, *P < 0.05, **** P < 0.0001. Data shown as mean plus standard deviation.

[0044] FIGS.22A-22D show that bivalent and mixed synthesis repRNA protect against lethal CCHFV challenge. Mice prime-only vaccinated with sham, repNP(ΔV5), repGc-teth, mixed synthesis, or bivalent RNA on day -28 were treated with a MAR1-5A3 antibody and infected with a lethal dose of CCHFV strain UG3010 on D0. FIG.22A dhows daily weight change. FIG. 22B shows survival until day 14. On D5, groups

of mice (N=6) were evaluated for control of viral loads FIGS.22C & 22d show viral loads as determined by (FIG.22C) qRT-PCR and (FIG.22D) infectious virus via $TCID_{50}$ in indicated tissues. Sham and repNP(ΔV5) group data are duplicated from FIG.20 for comparison. Dashed lines indicate limit of detection. Significance was calculated using one-way ANOVA; ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **** $P < 0.0001$. Data shown as mean plus standard deviation.

[0045] FIGS. 23 A & 23B show the amnestic response to repGc-teth, bivalent, and mixed synthesis repRNA. At D14 p.i., surviving groups of mice vaccinated with sham, repGc-teth, mixed synthesis RNA or bivalent repGcteth-NP and challenged with a lethal dose of CCHFV strain UG3010 were evaluated for anamnestic immune response. FIGS. 21A & 21 B show the antibody response as determined by (FIG.23A) rNP, rGc, and rGn IgG ELISAs and (FIG.23B) neutralization assay using infectious CCHFV strain Hoti. For rELISAs, comparison of D0 and D14 antibody titers at serum dilution 1:1600 or 1:800 is shown, dashed lines indicate limit of detection. Sham and repNP(ΔV5) group data from D0 is duplicated from FIG.20 for comparison. Significance was calculated using a one-way ANOVA; ns $P > 0.05$, *$P < 0.05$. Data shown as mean plus standard deviation.

DETAILED DESCRIPTION

[0046] The present disclosure relates to vaccines for CCHFV. Such vaccines comprise an RNA replicon (repRNA) encoding one or more proteins that elicit a protective immune response against CCHFV. These proteins may be related to, or be, proteins from CCHFV. Accordingly, one aspect of the present disclosure may be practiced by providing a repRNA encoding a polypeptide that elicits an immune response against CCHFV. The repRNA may be associated with a nanocarrier, which may be administered to an individual to vaccinate the individual against CCHFV.

[0047] Before specific, exemplary embodiments of the present disclosure are further described, it is to be understood that this disclosure is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the claims.

[0048] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, a nucleic acid molecule refers to one or more nucleic acid molecules. As such, the terms “a”, “an”, “one or more” and “at least one” can be used interchangeably. Similarly, the terms “comprising”, “including” and “having” can be used interchangeably. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements or use of a “negative” limitation.

[0049] Publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0050] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Terms and phrases, which are common to the various aspects disclosed herein, are defined below.

[0051] As used herein, “RNA replicon”, “replicon RNA”, “repRNA molecule”, “repRNA”, and the like, may be used interchangeably, and refer to a positive (+) strand RNA molecule having a 5’ end and a 3’ end, and comprising elements that allow the RNA molecule to direct its own replication (amplification), the elements comprising, at a minimum, a 5’ untranslated region (UTR) comprising 5’ replication recognition sequences, a 3’ UTR comprising 3’ replication recognition sequences, and one or more coding sequences encoding one or more proteins that, either individually or together (e.g., as part of a complex), recognize the 5’ and 3’ replication recognition sequences and thereby direct replication of the repRNA molecule. Thus, a repRNA may be referred to as “self-

replicating" (e.g., a self-replicating repRNA). In certain aspects, and, optionally, the 5' UTR may comprise a ribosome entry site (RES), an example of which is an internal RES (IRES)). In certain aspects, elements in repRNA molecules of the disclosure may be present in the following order: 5' UTR, one or more coding sequences encoding one or more proteins that direct replication of the repRNA molecule, 3' UTR. In certain aspects, the 5' and 3' UTRs may be alphavirus UTRs. In certain aspects, the 5' and 3' UTRs may, but need not, be from the same alphavirus. In certain aspects, the 5' UTR IRES may be from an alphavirus. In certain aspects, the repRNA may contain additional elements, such as, at least one heterologous nucleotide sequence encoding at least one heterologous protein operably linked to at least one means for controlling expression of the at least one heterologous nucleotide sequence, and/or a polyadenosine (polyA) tract. As used herein, the term "heterologous" means that the nucleotide sequence, or protein, being referenced is from an organism that is different than the organisms from which the other elements of the repRNA were obtained. For example, nucleotide sequences from CCHFV in repRNA made using nucleotide sequences from an alphavirus, would be considered heterologous to the alphavirus sequences.

[0052] In certain aspects, the at least one heterologous nucleotide sequence may be positioned 3' of the 3' end of the one or more coding sequences encoding one or more proteins directing replication of the repRNA. The at least one means of controlling expression of a nucleotide sequence may, but need not, be positioned 5' of the at least one heterologous nucleotide sequence. In certain aspects, the at least one means of controlling expression of a nucleotide sequence may generally be positioned between the 3' end of the one or more coding sequences encoding one or more proteins that direct replication of the repRNA and the 5' end of the at least one heterologous nucleic acid sequence. Thus, in certain aspects, the elements may be ordered as follows: 5' UTR, one or more coding sequences encoding one or more proteins that direct replication of the repRNA molecule, at least one means of controlling expression of a nucleotide sequence, at least one heterologous nucleotide sequence, 3' UTR. In certain aspects, the elements may be ordered as follows: 5' UTR, at least one means of controlling expression of a nucleotide sequence, at least one heterologous nucleotide sequence, one or more coding sequences encoding one or more proteins that direct replication of the repRNA molecule, 3' UTR. Those of skill in the art will recognize that other arrangements of elements are possible, as long as the elements described

herein are present and function and function to achieve the results described herein. An exemplary general repRNA of the disclosure is illustrated in FIG. 1.

[0053] In certain aspects, the repRNA may contain two or more heterologous nucleotide sequences, each encoding a heterologous protein. In certain aspects, the two or more nucleotide sequences may be under control of one or more means for controlling expression of a nucleotide sequence. In certain aspects, the two or more nucleotide sequences may encode the same protein, or they may encode two or more unique proteins. In certain aspects, each individual heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may encode a unique protein. In certain aspects, the two or more heterologous nucleotide sequences may be under control of the one means for controlling expression of a nucleotide sequence. For example, a single means for controlling expression of a nucleotide sequence, such as a promoter, may be positioned 5'of two heterologous nucleotide sequences such that transcription from the promoter produces a nucleic acid molecule comprising the coding sequences of both heterologous nucleotide sequences. In certain aspects, the two or more heterologous nucleotide sequences may be in-frame, such that the nucleic acid molecule (e.g., mRNA) produced by transcription from the single promoter encodes a fusion protein comprising the two or more heterologous proteins encoded by the two or more heterologous nucleotides sequences. In certain aspects, a polynucleotide sequence encoding a linker peptide may be positioned, in-frame, between the two or more heterologous nucleotide sequences, such that the resulting fusion protein comprises a linker peptide between the two heterologous proteins. The terms "linker peptide", "linker" and "spacer" may be used interchangeably and refer to short (e.g., 6-30 amino acids) amino acid sequences that separate two proteins or protein domains. Linker peptides are not typically, but may be, derived from the proteins, or domains, they separate. In certain aspects, linker peptides may be cleavable (i.e., contain an enzyme cleavage site). In certain aspects, a linker peptide may be a self-cleaving peptide, such as the 2A class of peptides. Examples of self-cleaving peptides useful for practicing the disclosure include, but are not limited to, 2A peptides, T2A peptide, P2A peptide, E2A peptide, and F2A peptide. An example of a repRNA encoding a fusion protein comprising a self-cleaving peptide is illustrated in the top FIG.13 (i.e., repGPC-T2A-NP). Thus, in certain aspects, the two or more heterologous nucleotide sequences are joined by a polynucleotide encoding a linker, such that polynucleotide and the two or more heterologous nucleotide sequences are in-frame.

[0054] In certain aspects, a polynucleotide sequence coding an internal ribosome entry site (IRES) may be positioned between the two or more heterologous nucleotide sequences, such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the single means for controlling expression of a nucleotide sequence contains an IRES site. It should be noted that while the term "coding" is typically used to refer to a protein encoded by a nucleic acid molecule, in the context of a genetic element, such as an IRES, the present disclosure may use "coding" to refer to a nucleotide sequence that is the complement of an IREs sequence. Thus, in certain aspects, the two or more heterologous nucleotide sequences are joined by a polynucleotide sequence encoding an IRES.

[0055] In certain aspects, each heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may be operably linked to separate means to control expression of a nucleotide sequence. For example, in a repRNA of the disclosure comprising two heterologous nucleotide sequences, a first heterologous nucleotide sequence may be operably linked to a first promoter that controls expression of the first heterologous nucleotide sequence, and a second heterologous nucleotide sequence may be operably linked to a second promoter that controls expression of the second heterologous nucleotide sequence. In such aspect, the second promoter may control expression of the second heterologous nucleotide sequence independent of the first promoter. In certain aspects, the one or more means for controlling expression of two or more heterologous nucleotide sequences, may be the same, or they may differ from one another. For example, in a repRNA containing two heterologous nucleotide sequences, each independently, operably linked to a separate promoter, the promoters may the same type of promoter (e.g., an alphavirus sub-genomic promoter) or the promoters may differ from one another (e.g., one promoter may be an alphavirus sub-promoter, while the other may be a mammalian promoter). An example of a repRNA comprising two heterologous nucleotide sequences, each under control of a separate means to control expression of a heterologous nucleotide sequence, is illustrated in FIG. 13, bottom (i.e., repGcteh-NP).

[0056] In some aspects of the disclosure, the repRNA may be an alphavirus repRNA. "Alphavirus repRNA", "alphavirus RNA replicon", "alphavirus replicon RNA", and the like, refer to a repRNA in which the 5' UTR containing the 5' replication recognition sequences, the 3' UTR containing the 3' replication recognition sequences and the one or

more coding sequences encoding proteins that recognize the 5' and 3' replication recognition sequences, at least, originate from an alphavirus genome. "Originate" is used herein to mean the origin (e.g., Eastern equine encephalitis virus (EEEV, Venezuelan equine encephalitis virus (VEEV), etc.) of a nucleotide or amino acid sequence. It should be understood that while a sequence may have its origin in a particular strain or species of virus, it does not necessarily mean that the sequence was physically obtained from the viral genome. Once a desired sequence within a viral genome is known, it may, for example, be copied (e.g., using cloning techniques, PCR, etc.), from the genome, it may be obtained from a vector (e.g., a plasmid) containing the sequence, or it may be produced synthetically. It should be noted that not all elements in a repRNA molecule of the disclosure need originate from the same species of alphavirus. For example, a repRNA may comprise 5' and/or 3' replication recognition sequences from one species of alphavirus (e.g., EEE), and coding sequences encoding proteins that direct replication of the virus from another species of virus (e.g., Western equine encephalitis virus (WEEV). Elements from any species of alphavirus may be used to produce an alphavirus repRNA, as long as the elements work together to achieve the desired results described herein.

[0057] In certain aspects, an alphavirus repRNA of the disclosure may comprise a 5' UTR and/or 3' UTR from an alphavirus genome, In certain aspects, an alphavirus repRNA of the disclosure may comprise alphavirus 5' and/or 3' replication recognition sequences. In certain aspects, alphavirus repRNA molecules of the disclosure do not contain nucleic acid sequences encoding functional alphavirus structural proteins (e.g., capsid, E, E2). In certain aspects, alphavirus repRNA molecules of the disclosure do not contain any nucleic acid sequences encoding alphavirus structural proteins.

[0058] "Alphavirus", as used herein, has its standard meaning in the art and refers to a genus of viruses, all of which are members of the Togaviridae family. Examples of alphaviruses for producing repRNAs of the disclosure included, but re not limited to, include EEEV, VEEV, Everglades virus, Mucambo virus, Pixuna virus, WEEV, Sindbis virus (SV), South African arbovirus 86 (S.A.AR86) Semliki Forest virus (SFV), Middleburg virus (MIDV), Chikungunya virus (CHKV), O'nyong-nyong virus, Ross River virus (RRV), Barmah Forest virus, Getah virus, Sagiyama virus, Bebaru virus, Mayaro virus, Una virus, Aura virus, Whataroa virus, Babanki virus, Kyzylagach virus, Highlands J virus, Fort Morgan virus, Ndumu virus, and Buggy Creek virus. The genome of all alphaviruses

comprises a single-stranded, messenger-sense RNA, modified at the 5′-end with a methylated cap and at the 3′-end with a variable-length poly (A) tract.

[0059] Within each named alphavirus, strains and/or subtypes are known. For example, several strains of the WEEV are known. Within the known strains of VEEV, subtypes have been recognized. For example, the Trinidad Donkey strain is in subtype IA/B, and related subtypes include IC and IE. Virulent VEEV strains have been isolated during mosquito-borne epizootic encephalomyelitis in equids in tropical and sub-tropical areas of the New World. The Trinidad Donkey strain is one of the virulent, epizootic strains, and it was passaged serially in tissue culture to create a live, attenuated strain (Berge et al. Amer. J Hyg. 73:209-218 (1961)) known as TC-83. This strain, containing multiple attenuating mutations (Kinney et al. 1989 Virology 170:19-30 (1989); with correction noted in Kinney et al. J Virol 67(3):1269-1277 (1993)), elicits VEEV-specific neutralizing antibodies in most humans and equines and has been used successfully as a vaccine. Any strain of alphavirus may be used as the genetic background for producing an alphavirus repRNA of the disclosure. In some aspects of the disclosure, the TC-83 strain of VEEV may be used as the genetic background for producing an alphavirus repRNA of the disclosure.

[0060] As stated above, repRNA molecules of the disclosure comprise one or more coding sequences encoding one or more proteins that, either individually or together (e.g., as part of a complex), recognize the 5' and 3' replication recognition sequences and thereby direct replication of the RNA molecule. The one or more proteins may comprise alphavirus nonstructural proteins (nsP), such as, alphavirus nsP1, alphavirus nsP2, alphavirus nsP3, alphavirus nsP4, or variants thereof. In some aspects, the one or more coding sequence comprises a single open reading frame (ORF) encoding a polyprotein comprising alphavirus nonstructural proteins (e.g., nsp1, nsp2, nsp3, and nsp4) of the disclosure. Such polyprotein may be proteolytically cleaved within a cell to produce the individual nonstructural proteins. Nonstructural proteins encoded by repRNA molecules of the disclosure may be from any alphavirus protein and, as noted above, may, but need not, originate from the same species of alphavirus from which the 5'and 3' replication recognition sequences originate. Nonstructural proteins encoded by repRNA molecules of the disclosure may be variants (e.g., natural sequence variants, mutant proteins) of alphavirus nonstructural proteins nsP1, nsP2, nsP3, and/or nsP4, provided the variant nonstructural proteins can recognize the 5'and 3' replication recognition sequences and direct replication of the repRNA molecule.

Nonstructural proteins of the disclosure (e.g., nsp1, nsP2, nsP3 and/or, nsP4) may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to wild type (wt) alphavirus nonstructural proteins, such as wt nsP1, wt nsP2, wt nsP3, and/or wt nsP4. As used herein, a "wt protein", "wt polypeptide", and the like, refer to a naturally occurring protein or polypeptide, such as that found in an alphavirus isolated from a natural environment. In some aspects, nsp1 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a wt alphavirus nsp1. In some aspects, nsp2 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a wt alphavirus nsp2. In some aspects, nsp3 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a wt alphavirus nsp3. In some aspects, nsp4 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a wt alphavirus nsp4. In some aspects, nsp1 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. In some aspects, nsp2 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. In some aspects, nsp3 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. In some aspects, nsp4 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12.

[0061] In certain aspects, the at least one heterologous nucleotide sequence, or the two or more heterologous nucleotide sequences, in a repRNA of the disclosure may encode a polypeptide that comprises at least one epitope from a CCHFV protein. It should be understood that in repRNAs of comparing two or more heterologous nucleotide sequences, each of the two or more nucleotide sequences may encode a unique protein

comprising a unique epitope. As used herein, “epitope” refers to a sequence of at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16, contiguous amino acids, that by itself or as part of a larger sequence, binds an antibody generated in response to such sequence or stimulates a cellular immune response. There is no critical upper limit to the length of the epitope, which may comprise nearly the full-length sequence of the protein from which it originates, or even a fusion protein comprising two or more epitopes from one of more CCHFV proteins. An epitope for use herein is not limited to the exact sequence of the portion of the parent protein from which it is originates. There are many known strains of CCHFV, and the virus retains the ability to continue to adapt. Thus, the term “epitope” encompasses sequences identical to the native sequence, as well as modifications of native sequence (either natural or man-made), such as deletions, additions, and substitutions, which may, but need not, be conservative substitutions. The epitope may be from any protein, providing the epitope elicits an immune response against CCHFV. In some aspects, the epitope is from a CCHFV protein. Suitable CCHFV proteins from which to derive epitopes include, but are not limited to, the CCHFV nucleocapsid protein (NP), the CCHFV glycoprotein precursor complex (GPC), the CCHFV N-terminal mucin-like domain (MLD) protein, the CCHFV GP38 protein, the CCHFV Gn protein, the CCHFV NSm protein, the CCHFV Gc protein, the CCHFV NSs protein. In certain aspects of the disclosure, the epitope is from a CCHFV GPC, an alphavirus NP, or an alphavirus Gc protein.

[0062] In certain aspects, a polypeptide encoded by the at least one heterologous nucleotide sequence, or the two or more heterologous nucleotide sequences, may comprise at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous amino acid residues from a protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In certain aspects, the encoded polypeptide may comprise at least 10, 20, 30, 40 or 50 contiguous amino acid residues from a protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In certain aspects, the encoded polypeptide may comprise at least 10, 20, 30, 40 or 50 contiguous amino acid residues from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In

certain aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In certain aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18**.** In certain aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18**.** In certain aspects, the encoded polypeptide may comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0063] In certain aspects, a label is joined to the encoded polypeptide. Such a label allows the expressed polypeptide to be identified as needed. Examples of labels that may be joined to the encoded polypeptide include, but are not limited to, an epitope tag, an enzyme conjugate, and a fluorescent label.

[0064] As used herein, a "means for controlling expression of a nucleotide sequence refers to a genetic element (e.g., nucleotide sequence) that control expression of a cis-connected nucleotide sequence. Examples of such means include, but are not limited to promoters, enhancers, repressors, and the like. The terms "control expression of", "affecting expression of", "drive expression of", and the like, are used in reference to the genetic element and indicate that the genetic element directs transcription of a nucleotide (nucleic acid) sequence operably linked to the genetic element. "Operably linked" refers to an arrangement of a genetic element and a nucleotide sequence wherein the genetic element is

positioned so that it controls expression of a cis-linked nucleotide sequence. For example, one example of a “means for controlling expression” is a promoter sequence (“promoter”). A given promoter operably linked to a nucleotide sequence can affect the expression of the nucleotide sequence when it is in the cis position to the nucleotide sequence and the proper transcriptional molecules (e.g., enzymes, transcription factors, etc.) are present. The “means” (e.g., promoter) need not be directly abutting the nucleotide sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences may be present between the promoter sequence and the nucleotide sequence, and the promoter sequence can still be considered “operably linked” to the nucleotide sequence. Any means (e.g., promoter) suitable for expressing the heterologous nucleic acid sequence from the repRNA may be operably linked to the heterologous nucleotide sequence.

[0065] One example of a promoter useful for practicing aspects of the disclosure is a subgenomic promoter, such as the subgenomic promoter present in the genome of alphaviruses (alphavirus subgenomic promoter). The term “alphavirus subgenomic promoter” refers to a nucleic acid sequence of alphavirus origin that, together with required viral and cellular polymerase(s) and other factors, permits transcription of an RNA molecule of less than genome length. The alphavirus (alphaviral) subgenomic promoter, originates from the alphavirus genome, more specifically in the region between the nonstructural and structural protein ORFs in an alphavirus genome, and normally directs transcription of the alphavirus subgenomic mRNA. Typically, the alphavirus subgenomic promoter consists of a core sequence that provides most promoter-associated activity, as well as flanking regions (e.g., extended or native promoter) that further enhance the promoter-associated activity. For example, in the case of the alphavirus prototype, Sindbis virus, the normal subgenomic junction region promoter typically begins at approximately nucleotide number 7579 and continues through at least nucleotide number 7612 (and possibly beyond). At a minimum, nucleotides 7579 to 7602 are believed to serve as the core sequence necessary for transcription of the subgenomic fragment.

[0066] It will be understood by those skilled in the relevant art that, because of the method by which alphaviruses regulate transcription of the structural gene, the subgenomic promoter sequence present in the (+) stranded alphavirus genome is not functional. However, replication of the (+) stranded alphavirus genome produces a negative (-) stranded nucleic acid molecule, including the sequence complementary to the region

between the nonstructural and structural ORFs and containing the subgenomic promoter sequence. It is the complementary sequence in the (-) strand that contains the functional subgenomic promoter sequence. The subgenomic promoter in the (-) strand copy of the alphavirus genome functions to promote transcription of the cis nucleic acid sequence, resulting in production of a subgenomic mRNA encoding the alphavirus structural proteins. It must be understood that the above discussion of structural gene transcription is in the context of a complete alphavirus genome and is intended to illustrate the regulatory mechanism of the subgenomic promoter.

[0067] As stated earlier, repRNA molecules of the disclosure do not contain coding sequences for alphavirus structural proteins. In fact, in some aspects, a repRNA of the disclosure may be produced by replacing the coding sequence for the alphavirus structural proteins with the at least one heterologous nucleotide sequence or the two or more heterologous nucleotide sequences. In the present disclosure, the (-) strand copy of the repRNA functions to promote transcription of the heterologous nucleotide sequence. Thus, it should be clear from the above that the sequence in a repRNA of the disclosure referred to as “subgenomic promoter” may be the complement of the actual sequence of the functional alphavirus subgenomic promoter, which corresponds to the region in a minus-strand RNA copy that promotes transcription initiation of the positive-strand subgenomic mRNA. However, for ease of discussion regarding repRNA structure, the term “subgenomic promoter” will be used to refer to the sequence in the repRNA that is operably linked to the at least one heterologous nucleotide sequence, or the two or more heterologous nucleotide sequences, the complement of which drives transcription of heterologous-nucleotide-encoding mRNA from a (-) strand copy of the repRNA.

[0068] In some aspects, repRNA molecules of the disclosure may comprise one or more “attenuation mutations”, an attenuating mutation being a nucleotide deletion, addition, or substitution of one or more nucleotide(s), that results in a reduction or loss of virulence in a live virus containing the mutation relative to the appropriate wild-type alphavirus. Several examples of attenuating mutations have been previously described in U.S. Pat. Nos. 5,792,462 and 6,156,558, both of which are incorporated herein by reference in their entirety. In one aspect, the repRNA may comprise a mutation at a nucleotide position corresponding to position 3 in the 5’ UTR of the Trinidad donkey (TRD) strain of VEEV. In some aspects, the repRNA may comprise a mutation at a nucleotide position

corresponding to position 1696 of the TRD genome. Such mutation may be a C to A substitution mutation. Such mutation may alter the amino acid at position 16 of an nsP2 protein, and specifically cause an Ala to Asp substitution, at position 16 of a nsp2 protein.

[0069] One aspect of the disclosure is an alphavirus repRNA comprising at least one heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope from CCHFV, and a means for controlling expression of a nucleotide sequence operably linked to the at least one heterologous nucleotide sequence, wherein the epitope elicits an immune response against CCHFV. In some aspects, the epitope may comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16, contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the encoded polypeptide may comprise an amino acid sequence at

least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0070] In certain aspects, the alphavirus repRNA may comprise two or more heterologous nucleotide sequences, each heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope from CCHFV, and one or more means for controlling expression of a nucleotide sequence operably linked to the two or more heterologous nucleotide sequence, wherein the at least one epitope elicits an immune response against CCHFV. In certain aspects, the epitope encoded by each heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may be the same epitope, or they may differ from one another. In certain aspects, the epitope encoded by each heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may be from the same protein, or they may be from different proteins. In some aspects, each epitope may comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16, contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In certain aspects, the polypeptide encoded by each heterologous nucleotide sequence may be the same polypeptide, or they may differ from one another. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects,

the encoded polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0071] In certain aspects, the two or more heterologous nucleotide sequences in the alphavirus repRNA may be under control of one means for controlling expression of a nucleotide sequence (e.g., one promoter). In certain aspects, the two or more heterologous nucleotide sequences may be in-frame, such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the one means encodes a fusion protein comprising the two or more heterologous proteins encoded by the two or more heterologous nucleotides sequences. In certain aspects, the repRNA comprises a polynucleotide sequence, which may encode a linker peptide between the two or more heterologous nucleotide sequences, such that the heterologous proteins encoded by the heterologous nucleotide sequences are in frame with each other and with the peptide linker. In certain aspects, the linker peptide may comprise an enzymatic cleavage site. cleavable (i.e., contain an enzyme cleavage site. In certain aspects, the linker peptide may be a self-cleaving peptide, such as a 2A peptide, a T2A peptide, a P2A peptide, a E2A peptide, or F2A peptide. In certain aspects, the polynucleotide sequence may encode an internal ribosome entry site (IRES), such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the one means contains an IRES site. In certain aspects, the two or more heterologous nucleotide sequences are

joined by a polynucleotide sequence encoding an IRES. In certain aspects, each heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may be independently, operably linked to a separate means to control expression of a heterologous nucleotide sequence. In certain aspects, the separate means operably linked to teach of the two or more heterologous nucleotide sequences, may be the same, or they may differ from one another.

[0072] In some aspects, the means for controlling expression of a nucleotide sequence may comprise an alphavirus subgenomic promoter. In some aspects, the alphavirus repRNA may comprise one or more coding sequences encoding one or more alphavirus nonstructural proteins of the disclosure. In some aspects, the one or more alphavirus nonstructural proteins may comprise an alphavirus nsp1 of the disclosure, an alphavirus nsp2 of the disclosure, an alphavirus nsp3 of the disclosure, and/or an alphavirus nsp4 of the disclosure. In some aspects, the one or more coding sequences may encode an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. The nsp1 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. The nsp2 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. The nsp3 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. The nsp4 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12. In some aspects, the one or more coding sequence comprise a single ORF encoding a polyprotein comprising or more alphavirus nonstructural proteins of the disclosure. In some aspects, the polyprotein may comprise an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. In some aspects, the polyprotein may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to SEQ ID NO:13 or 14. In some aspects, the heterologous nucleic acid sequence may be positioned 3' of the end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins. In some aspects, the alphavirus subgenomic promoter may be positioned between the 3' end of the one or more nucleotide sequences

encoding the one or more alphavirus nonstructural proteins and 5' of the 5' end of the heterologous nucleotide sequence. In some aspects, the repRNA may comprise alphavirus 5' and/or 3' untranslated regions (UTRs). In some aspects, the alphavirus repRNA may comprise a polyA tract. In some aspects, the alphavirus may be selected from the group consisting of EEEV, VEEV, WEEV, SINV, SFV, MIDV, CHKV, and RRV.

[0073] One aspect of the disclosure is an alphavirus repRNA comprising an alphavirus subgenomic promoter operably linked to at least one heterologous nucleotide sequence encoding a polypeptide comprising an amino acid sequence at least 70% identical to a protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein of the disclosure; and, one or more coding sequences encoding an alphavirus nsp1, nsp2, nsp3 and nsp4 of the disclosure. In some aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the encoded polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the nsp1 may comprise an amino acid sequence least 70%, at least

75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. In some aspects, the nsp2 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. In some aspects, the nsp3 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. In some aspects, the nsp4 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12. In some aspects, the one or more coding sequences comprise a single ORF encoding a polyprotein comprising the nsp1, nsp2, nsp3, and nsp4 proteins. In some aspects, the polyprotein may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to SEQ ID NO:13 or 14. In some aspects, the heterologous nucleic acid sequence may be positioned 3' of the end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins. In some aspects, the alphavirus subgenomic promoter may be positioned between the 3' end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins and 5' of the 5' end of the heterologous nucleotide sequence. In some aspects, the repRNA may comprise alphavirus 5' and/or 3' untranslated regions (UTRs). In some aspects, the alphavirus repRNA may comprise a polyA tract. In some aspects, the alphavirus may be selected from the group consisting of EEEV, VEEV, WEEV, SINV, SFV, MIDV, CHKV, and RRV.

[0074] In certain aspects, the alphavirus repRNA may comprise two or more heterologous nucleotide sequences, each heterologous nucleotide sequence encoding a polypeptide at least 70% identical to a protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein of the disclosure, wherein the alphavirus subgenomic promoter is operably linked to at least one of the two or more heterologous nucleotide sequences, wherein the at least one epitope elicits an immune response against CCHFV. In certain aspects, the polypeptide encoded by each heterologous nucleotide sequence may be the same polypeptide, or they may differ from one another. In some aspects, the polypeptide encoded by each of the two or more heterologous

nucleotide sequences may comprise, independently, at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the encoded polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the polypeptide encoded by each of the two or more heterologous nucleotide sequences may comprise, independently, an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0075] In certain aspects, the two or more heterologous nucleotide sequences are under control of one alphavirus subgenomic promoter. In certain aspects, the two or more heterologous nucleotide sequences may be in-frame, such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the one alphavirus subgenomic promoter encodes a fusion protein comprising the two or more heterologous proteins encoded by the two or more heterologous nucleotides sequences. In certain aspects, aspects,

the repRNA comprises a polynucleotide sequence, which may encode a linker peptide between the two or more heterologous nucleotide sequences, such that the heterologous proteins encoded by the heterologous nucleotide sequences are in frame with each other and with the peptide linker. In certain aspects, the linker peptide may comprise an enzymatic cleavage site. cleavable (i.e., contain an enzyme cleavage site. In certain aspects, the linker peptide may be a self-cleaving peptide, such as a 2A peptide, a T2A peptide, a P2A peptide, a E2A peptide, or F2A peptide. In certain aspects, the polynucleotide sequence may encode an internal ribosome entry site (IRES), such that a nucleic acid molecule (e.g., mRNA) produced by transcription from the one means contains an IRES site. In certain aspects, the two or more heterologous nucleotide sequences are joined by a polynucleotide sequence encoding an IRES. In certain aspects, each heterologous nucleotide sequence of the two or more heterologous nucleotide sequences may be independently, operably linked to an alphavirus subgenomic promoter.

[0076] One aspect of the disclosure is a vector encoding a repRNA of the disclosure. The vector may be any vector capable of encoding and, optionally, expressing a repRNA of the disclosure. Examples of vectors useful for encoding or expressing a repRNA of the disclosure include, but are not limited to, plasmids and virus vectors. The repRNA may be encoded by a polynucleotide sequence, to which may be operably linked a promoter that drives expression of the polynucleotide sequence. Any promoter may be used to drive expression of the polynucleotide sequence, and such promoters may include promoters useful for *in vivo* expression or *in vitro* (e.g., a transcription reaction in a tube). Examples of promoters useful for driving expression of the polynucleotide sequence include, but are not limited to, a T7 promoter, an SP6 promoter, a trp promoter, a cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EF1a) promoter, and a phosphoglycerate kinase (PGC) promoter.

[0077] One aspect of the disclosure is a method of producing a repRNA of the disclosure, the method comprising incubating a vector of the disclosure under conditions suitable for transcription of the encoded repRNA, such that the repRNA is produced. It will be understood by a person skilled in the art that "suitable conditions" will depend on, at least, the type of vector encoding the repRNA as well as the promoter used to drive expression of the encoded repRNA. Using such information, a person skilled in the art will be able to

determine suitable conditions for expression of the repRNA. In some aspects, the vector (e,.g., a plasmid) may be linearized (e.g., by enzymatic digestion) prior to transcription of the encoded repRNA. In some aspects, the suitable conditions may, but need not, comprise co-translational capping reagents. Once the repRNA has been expressed, it may, but need not, be purified using any suitable techniques known in the art. Following expression, and optionally purification, the repRNA may be incubated in a capping reaction, thereby producing a capped repRNA.

[0078] repRNA molecules of the disclosure may be packaged with a nanoparticle useful for administering the repRNA to cells, either *in vitro* or *in vivo*. Thus, one aspect of the disclosure is a nanoparticle comprising a repRNA of the disclosure. The repRNA may comprise an alphavirus subgenomic promoter operably linked to a heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope from CCHFV, wherein the epitope elicits an immune response against CCHFV. In some aspects, the epitope may comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16, contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0079] In some aspects, the means of controlling expression of a nucleotide sequence may comprise an alphavirus subgenomic promoter. In some aspects, the alphavirus repRNA may comprise one or more coding sequences encoding one or more alphavirus nonstructural proteins of the disclosure. In some aspects, the one or more alphavirus nonstructural proteins may comprise an alphavirus nsp1 of the disclosure, an alphavirus nsp2 of the disclosure, an alphavirus nsp3 of the disclosure, and/or an alphavirus nsp4 of the disclosure. In some aspects, the one or more coding sequences may encode an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. The nsp1 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. The nsp2 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. The nsp3 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. The nsp4 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12. In some aspects, the one or more coding sequence comprise a single ORF encoding a polyprotein comprising or more alphavirus nonstructural proteins of the disclosure. In some aspects, the polyprotein may comprise an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. In some aspects, the polyprotein may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to SEQ ID NO:13 or 14. In some aspects, the heterologous nucleic acid sequence

may be positioned 3' of the end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins. In some aspects, the alphavirus subgenomic promoter may be positioned between the 3' end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins and 5' of the 5' end of the heterologous nucleotide sequence. In some aspects, the repRNA may comprise alphavirus 5' and/or 3' untranslated regions (UTRs). In some aspects, the alphavirus repRNA may comprise a polyA tract. In some aspects, the alphavirus may be selected from the group consisting of EEEV, VEEV, WEEV, SINV, SFV, MIDV, CHKV, and RRV.

[0080] One type of nanoparticle with which a repRNA molecule of the disclosure may be packaged is a viral particle, or pseudoviral particle, comprising capsid proteins. Alphavirus genomic RNA is normally packaged into viral particles using a process that requires capsid proteins and a packaging signal sequence (encapsidation sequence) present in the genome. Thus, in some aspects of the disclosure, the repRNA molecule may comprise an alphavirus packaging signal sequence. If the repRNA is not being packaged into capsid-containing particles, no packaging signal is needed. Thus, in some aspects, the repRNA may lack a functional packaging signal sequence.

[0081] Another type of nanoparticle with which a repRNA of the disclosure may be packaged is a nanocarrier. "Nanocarrier" refers to a nanomaterial that can form a nanoparticle that associates with other material, such as a repRNA, thereby acting as a carrier for the other material. In certain aspect, the nanocarrier complexes with the repRNA. In certain aspects, the nanocarrier encapsulates the repRNA. Nanocarriers may comprise materials such as, inorganic particles (e.g., gold, iron, magnetic particles), metallic nanoparticles (e.g., zinc oxide, titanium oxide, platinum, selenium, gadolinium, palladium, cerium dioxide), quantum dots, nanocrystals, dendrimers, polymers, or lipid-based carriers (e.g., liposomes). In certain aspects, the nanocarrier may comprise a cationic nanocarrier (CNC). In certain aspects, the nanoparticle may comprise a CNC complexed with repRNA of the disclosure. The nanoparticle may comprise a hydrophilic oil base. The nanoparticle may comprise squalene, sorbitan monostearate (Span) 60, and polyoxyethylene sorbitan monooleate 80 (Tween® 80). The nanoparticle may comprise squalene, sorbitan monostearate (Span) 60, polyoxyethylene sorbitan monooleate 80 (Tween® 80), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) chloride, oleic acid–coated iron oxide nanoparticles, and sodium citrate dihydrate. The CNC may comprise a lipid inorganic

nanoparticle (LION), which may, but need not, comprise a superparamagnetic iron oxide (Fe3O4) (SPIO) nanoparticle embedded in a hydrophobic oil base. The hydrophobic oil base may comprise squalene, sorbitan monostearate (Span) 60, polyoxyethylene sorbitan monooleate 80 (Tween® 80), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) chloride, oleic acid–coated iron oxide nanoparticles, and sodium citrate dihydrate, thereby forming a LION. In some aspects, the LION may comprise squalene (37.5 mg/ml), Span 60 (37 mg/ml), Tween 80 (37 mg/ml), DOTAP chloride (30 mg/ml), 15-nm oleic acid–coated iron oxide nanoparticles (0.2 mg/ml), and 10 mM sodium citrate dihydrate.

[0082] In certain aspects, the nanoparticle (e.g., LION) may be produced by combining the iron oxide nanoparticles with the oil phase (squalene, Span 60, and DOTAP) and sonicating for 30 min in a 65°C water bath. Separately, the aqueous phase, containing Tween 80 and sodium citrate dihydrate solution in water, may be prepared by continuous stirring until all components were dissolved. The oil and aqueous phases may then be mixed and emulsified using a homogenizer. The resulting crude colloid may be subsequently processed by passaging through a microfluidizer at 137895 kPa until the Z-average hydrodynamic diameter, measured by dynamic light scattering reaches 50 ± 5 nm with a 0.2 polydispersity index. The resulting LION may be filtered with a 200-nm pore-size polyethersulfone filter.

[0083] One aspect of the disclosure is an immunogenic composition (vaccine composition, vaccine) comprising a repRNA molecule of the disclosure, a vector encoding a repRNA of the disclosure, or a nanoparticle of the disclosure. In such compositions, the repRNA may comprise an alphavirus subgenomic promoter operably linked to a heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope from CCHFV, wherein the epitope elicits an immune response against CCHFV. An "immunogenic composition" is a composition that comprises an antigenic molecule (or nucleic acid molecule encoding an antigenic molecule) where administration of the composition to an individual results in development in the subject of a humoral and/or a cellular and/or mucosal immune response to the antigenic molecule. With regard to the present disclosure, the polypeptide encoded by the heterologous nucleic acid sequence may be considered the antigenic molecule. In some aspects, the epitope may comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16, contiguous amino acid residues from a CCHFV protein selected

from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In some aspects, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0084] In some aspects, the means of controlling expression of a nucleotide sequence may comprise an alphavirus subgenomic promoter. In some aspects, the alphavirus repRNA may comprise one or more coding sequences encoding one or more alphavirus nonstructural proteins of the disclosure. In some aspects, the one or more alphavirus nonstructural proteins may comprise an alphavirus nsp1 of the disclosure, an alphavirus nsp2 of the disclosure, an alphavirus nsp3 of the disclosure, and/or an alphavirus nsp4 of the

disclosure. In some aspects, the one or more coding sequences may encode an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. The nsp1 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. The nsp2 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. The nsp3 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. The nsp4 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12. In some aspects, the one or more coding sequence comprise a single ORF encoding a polyprotein comprising or more alphavirus nonstructural proteins of the disclosure. In some aspects, the polyprotein may comprise an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. In some aspects, the polyprotein may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to SEQ ID NO:13 or 14. In some aspects, the heterologous nucleic acid sequence may be positioned 3' of the end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins. In some aspects, the alphavirus subgenomic promoter may be positioned between the 3' end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins and 5' of the 5' end of the heterologous nucleotide sequence. In some aspects, the repRNA may comprise alphavirus 5' and/or 3' untranslated regions (UTRs). In some aspects, the alphavirus repRNA may comprise a polyA tract. In some aspects, the alphavirus may be selected from the group consisting of EEEV, VEEV, WEEV, SINV, SFV, MIDV, CHKV, and RRV.

[0085] An immunogenic composition of the disclosure may be introduced directly into a recipient subject using any suitable route of delivery, such as injection (e.g., intramuscular (IM)) inhalation, oral ingestion, intranasal or any other parenteral or mucosal route of administration. Immunogenic compositions of the disclosure may comprise additional ingredients that help preserve the integrity of the activity of the immunogenic components of the composition, and/or aid in delivery/administration of the composition. Examples of such ingredients include, but are not limited to, stabilizers, buffers, sugars (e.g.,

saccharose, glucose, fructose, dextranes, dextranesulphate, and trehalose), sugar alcohols (e.g., Xylite/Xylitole, Mannite/Mannitol, Sorbite/Sorbitol, and Glycerol), amino acids (e.g., L-glutamine, arginine, cysteine, and lysine), phosphate buffers, Tris buffers, TE (Tris/EDTA), TEN (Tris/NaCl/EDTA), Earle's salt solution, tartaric acid, Pluronic F 68, and Tween 80.

[0086] One aspect of the disclosure is a method of eliciting an immune response in an individual, the method comprising administering to the individual a repRNA of the disclosure, a vector of the disclosure, a nanoparticle of the disclosure, or an immunogenic composition of the disclosure. The terms "individual", "subject", and "patient" are well-recognized in the art and are herein used interchangeably to refer to any animal susceptible infection with CCHFV. Examples include, but are not limited to, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; and birds, including ostriches. The terms individual, subject, and patient by themselves, do not denote a particular age, sex, race, and the like. Thus, individuals of any age, whether male or female, are intended to be covered by the present disclosure and include, but are not limited to the elderly, adults, children, babies, infants, and toddlers. Likewise, the methods of the present disclosure can be applied to any race, including, for example, Caucasian (white), African-American (black), Native American, Native Hawaiian, Hispanic, Latino, Asian, and European. Administration of a repRNA, a immunogenic composition, or nanoparticle of the disclosure may be through any suitable route of administration. Suitable routes of administration include, but are not limited to, injection (e.g., intramuscular (IM), intravenous (IV), subcutaneous (SC)), inhalation, oral ingestion, intranasal or any other parenteral or mucosal route of administration.

[0087] One aspect is a method of protecting an individual against infection by CCHFV, the method comprising administering to the individual a repRNA of the disclosure, a vector of the disclosure, a nanoparticle of the disclosure, or an immunogenic composition of the disclosure. The repRNA, nanoparticle, or immunogenic composition of the disclosure may be administered using any suitable route of administration, which may comprise injection (e.g., intramuscular (IM), intravenous (IV), subcutaneous (SC)), inhalation, oral ingestion, intranasal or any other parenteral or mucosal route of

administration. “Protecting an individual against infection by CCHFV” does not necessarily mean stopping CCHFV from infecting (i.e., entering a cell of) an individual. As used herein, the term refers to eliciting an immune response against CCHFV in an individual such that the individual does not experience any infection-related signs and/or symptoms, or experiences a level of CCHFV-infection-related signs and symptoms that are reduced relative to the level of CCHFV-infection-related signs and symptoms experienced by an individual to whom has not been protected against infection by CCHFV. Preferably, the severity of, incidence of, and/or duration of clinical signs of infection by CCHFV are reduced at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, and even up to 100% in subjects treated with or having received a vaccine or immunogenic composition described herein. Examples of such signs and symptoms include, but are not limited to, neck pain and/or stiffness, headache, fever, high fever, joint pain, stomach pain, vomiting, red eyes, flushing, petechia, tachycardia, lymphadenopathy, and myalgia.

[0088] One aspect is a method of vaccinating an individual against infection by CCHFV, the method comprising administering to the individual a repRNA of the disclosure, a vector of the disclosure, an immunogenic composition of the disclosure, or a nanoparticle of the disclosure.

[0089] One aspect is a method of reducing the amount of CCHFV (viral load) in an individual, the method comprising administering to the individual a repRNA of the disclosure, a vector of the disclosure, an immunogenic composition of the disclosure, or a nanoparticle of the disclosure. In some aspects, the viral load is reduced in the blood. In some aspects, the viral load is reduced in lymphoid tissue. In some aspects, the viral load is reduced in an organ selected from the liver or spleen.

[0090] One aspect is a repRNA, an immunogenic composition, or a nanoparticle of the disclosure for use in eliciting an immune response against CCHFV in an individual.

[0091] One aspect is a repRNA, an immunogenic composition, or a nanoparticle of the disclosure for use in protecting an individual against infection by CCHFV.

[0092] One aspect is a repRNA, an immunogenic composition, or a nanoparticle of the disclosure for use in vaccinating an individual CCHFV in an individual.

[0093] One aspect is a repRNA, an immunogenic composition, or a nanoparticle of the disclosure for use in reducing the load of CCHFV in an individual.

[0094] In these methods and uses, a repRNA, a immunogenic composition, or a nanoparticle of the disclosure may be administered using any suitable route of administration, which may comprise injection (e.g., intramuscular (IM), intravenous (IV), subcutaneous (SC)), inhalation, oral ingestion, intranasal or any other parenteral or mucosal route of administration.

[0095] In these methods and uses of the disclosure, the repRNA may comprise a heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope from a CCHFV protein, operably linked to a means for controlling expression of a nucleotide sequence, wherein the epitope elicits an immune response against CCHFV. In these methods and uses, the epitope may comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16, contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In these methods and uses, the polypeptide may comprise at least 20, at least 30, at least 40 or at least 50 contiguous amino acid residues from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In these methods and uses, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In these methods and uses, the polypeptide may comprise a CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In these methods and uses, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%,

identical to an at least 50, at least 100, at least 150, or at least 200 contiguous amino acid sequence from a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18. In these methods and uses, the polypeptide may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:18.

[0096] In these methods and uses, the means for controlling expression of a nucleotide sequence may comprise an alphavirus subgenomic promoter. In some aspects, the alphavirus repRNA may comprise one or more coding sequences encoding one or more alphavirus nonstructural proteins of the disclosure. In some aspects, the one or more alphavirus nonstructural proteins may comprise an alphavirus nsp1 of the disclosure, an alphavirus nsp2 of the disclosure, an alphavirus nsp3 of the disclosure, and/or an alphavirus nsp4 of the disclosure. In some aspects, the one or more coding sequences may encode an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. The nsp1 may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:9. The nsp2 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:10. The nsp3 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:11. The nsp4 may comprise an amino acid sequence least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:12. In some aspects, the one or more coding sequence comprise a single ORF encoding a polyprotein comprising or more alphavirus nonstructural proteins of the disclosure. In some aspects, the polyprotein may comprise an alphavirus nsp1, nsp2, nsp3, and/or nsp4 protein of the disclosure. In some aspects, the polyprotein may comprise an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identical to SEQ ID NO:13 or 14. In some aspects, the heterologous nucleic acid

sequence may be positioned 3' of the end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins. In some aspects, the alphavirus subgenomic promoter may be positioned between the 3' end of the one or more nucleotide sequences encoding the one or more alphavirus nonstructural proteins and 5' of the 5' end of the heterologous nucleotide sequence. In some aspects, the repRNA may comprise alphavirus 5' and/or 3' untranslated regions (UTRs). In some aspects, the alphavirus repRNA may comprise a polyA tract. In some aspects, the alphavirus may be selected from the group consisting of EEEV, VEEV, WEEV, SINV, SFV, MIDV, CHKV, and RRV.

[0097] These methods and uses may comprise administering between about 0.05 μg and about 1.5 mg of a repRNA of the disclosure, between about 0.05 μg about 5 μg, or between about 0.01 mg and about 1.5 mg, to the individual. These methods and uses may comprise administering a repRNA of the disclosure to the individual at a dosage in the range of about 0.00014 mg/kg to about 0.015 mg/kg. In some methods and uses, a repNA of the disclosure, may be administered once, or a repRNA of the disclosure may be administered using a prime-boost regime disclosed herein. For example, a first dose of a repRNA may be administered at an initial time, and a second dose of a repRNA administered one, two, three or four weeks later.

[0098] These methods and uses may comprise administering more than one type of repRNA to an individual. For example, a method or use of the disclosure may comprise administering to an individual a first repRNA encoding a polypeptide comprising a first epitope from a first CCHFV protein, and a second repRNA encoding a polypeptide comprising a second epitope from a second CCHFV protein, wherein the first and second CCHFV protein may not be the same CCHFV protein. In such methods and uses, the first and second proteins may be selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein. In such methods, the first and second epitopes may be epitopes from the same CCHFV protein. In such methods and uses, the first and second epitopes may be epitopes from different CCHFV proteins. In such methods and uses, the first and second repRNAs may be administered at separate times, or they may be administered at the same time. In such methods and uses, the first and second repRNAs may be administered as naked RNA, as part of an immunogenic composition of the disclosure, or as part of a nanoparticle of the disclosure. In such methods and uses, the

first repNA may encode a polypeptide comprising an epitope from an alphavirus GPC, and the second repRNA may encode a polypeptide comprising an epitope from an alphavirus NP.

[0099] One aspect of the disclosure is a kit comprising a repRNA of the disclosure, a vector of the disclosure, an immunogenic composition of the disclosure, and/or a nanoparticle of the disclosure. A kit of the disclosure may comprise additional components, such as, buffers, diluents, filters, needles, syringes, a lyophilized formulation of the repRNA, immunogenic composition, and/or nanoparticle, along instructions for its reconstitution and/or use. The kit may comprise instructions, or a label, on or associated with the kit that indicates directions for use. For example, the label may indicate that the lyophilized formulation is to be reconstituted to specific concentrations. The kit may comprise a statement associated with the contents of the kit stating that the contents therein are useful or intended for eliciting an immune response against CCHFV.

[0100] This written description uses examples to disclose the disclosure, including the best mode, and to enable any person skilled in the art to practice the disclosure, including making and using any devices or systems and performing any incorporated methods. The patentable scope of the disclosure is defined by the claims, and may include other examples that occur to those skilled in the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural elements with insubstantial differences from the literal languages of the claims.

[0101] EXAMPLES

[0102] Materials and Methods

[0103] *Vaccine.* repRNAs were constructed using standard cloning techniques and sequences for NP and a codon-optimized GPC from CCHFV strain Hoti (accession #s MH483984 and MH483985, respectively) were fused to a 3' V5- or HA-epitope tag, respectively, to facilitate protein expression studies. A generalized schematic of exemplary repRNAs is shown in FIGS. 1A-1C. Vaccine RNA was synthesized *in vitro* and was complexed to CNC as described previously (Erasmus et al., Sci. Transl. med. 12, Aug. 5, 2020).

[0104] *Mice, Vaccinations, and Infection.* Wild-type C57BL6/J (stock #00664) mice or μMT mice on the C57BL6/J background (stock #002288) were purchased

from Jackson Laboratories. Male and female mice were used for all studies and were all approximately 8-weeks of age at time of vaccination. Mice were vaccinated via a single 50μL intramuscular injection to the hind limb. Vaccination appeared well tolerated and no adverse events attributable to the vaccine were observed during the studies. At time of CCHFV challenge, mice received 2.5mg MAR1-5A3 (Leinco) and indicated dose of CCHFV strain UG3010 via intraperitoneal injections. Body temperature was recorded using a Unified Information Devices telemetry system and UID Mouse Matrix reader plates. Mice were implanted with telemetry transponders (UCT-2112, UID) via subcutaneous implantation and mice allowed to recover for at least one-week prior to CCHFV challenge. Data was recorded continuously with a zone interval of 250ms, 2 cycles per series and a 1s series delay. Data reported as mean of readings collected during 12-hour intervals corresponding to vivarium light-dark cycles.

[0105] *Viral Stock.* CCHFV strain UG3010 was originally provided by Eric Bergeron, Centers for Disease Control and Prevention. On site, UG3010 was grown and titrated on SW13 cells in L-15 (ATCC) supplemented with 2% fetal bovine serum, 2 mM L-glutamine, 50 μg/mL penicillin, and 50 μg/mL streptomycin. Virus stock was sequenced via Illumina-based sequencing to confirm identity and exclude contamination. Sequence identity was confirmed compared to Genbank accession numbers DQ211650, DQ211637, DQ211624.

[0106] *In vivo depletions.* Control mice were treated with 200 μg of rat IgG2b isotype (clone 1-2) while T-cell depletion mice were treated with 200μg α-CD4 (clone GK1.5), α-CD8 (clone 2.43), or both diluted in neutral pH sterile phosphate buffered saline (PBS) and administered via 100uL intraperitoneal (IP) injections on day −5 and -2 relative to CCHFV-challenge. On day 0, a group of mice were euthanized for evaluation of depletion efficacy via flow cytometry.

[0107] *ELISA.* An in-house ELISA using whole CCHFV Hoti antigen was used to quantify CCHFV-specific antibodies.

[0108] *Western Blot and Immunofluorescence Assay.* BHK21 cells were transfected with repNP or repGPC RNA using TransIT-mRNA Transfection Kit (Mirus) and incubated overnight. The next day, cell lysate was harvested using Thermofisher RIPA Lysis and Extraction Buffer and Roche complete protease-inhibitor tablets and recommended protocols. Samples were mixed 1:1 with Laemlli buffer (10% SDS, 0.1M DTT), heated for

10min at 99°C, briefly incubated on ice, and loaded onto a 12-well Biorad Criterion TGX precast Gel 10% alongside Biorad Precision Plus Protein Dual Color Standards. After transfer to nitrocellulose membrane, blots stained with primary antibody mouse anti-V5 (Invitrogen) for NP or mouse anti-HA (Thermofisher) for GPC, washed and incubated with anti-mouse HRP (Jackson Immunoresearch). Blot was washed and imaged using supersignal west pico PLUS chemiluminescent substrate (Fisher Scientific) and Proteinsimple FluorChem E Imager. For IFA, cells were washed, fixed in 1% PFA in DPBS for 10minutes, permeabilized and incubated with the respective mouse anti-V5 or mouse anti-HA antibody. Cells were then washed, incubated with secondary Goat anti-Mouse IgG (H+L) conjugated to Alexa Fluor 488 (Thermofisher), washed and imaged on Biorad ZOE fluorescent cell imager.

[0109] *IFNγ ELISpot.* An IFNγ ELISpot to evaluate CCHFV-specific T-cell responses was performed as previously described (*45*) using a mouse single-color IFNγ kit (Cellular Technologies Limited). Briefly, fresh splenocytes were diluted in CTL-Test medium, 300,000 - 500,000 cells per well were plated and stimulated with 1 µg/ml each peptide of 15-mer peptides overlapping by 11 amino acids derived from the Hoti NP or GPC sequence. Peptides were synthesized (Genscript) and dissolved in dimethyl sulfoxide (DMSO; Hybrimax grade [Sigma]) and pooled with 19 to 30 peptides per pool. As positive control, cells were stimulated with concanavalin A (Life Technologies) while negative controls were stimulated with CTL-Test medium containing DMSO vehicle alone. Cells were incubated for 24 h at 37°C and developed according to manufacturer protocols. Spots were counted and analyzed using an S6 Universal Analyzer (CTL) and SFCs normalized to 10^6 splenocytes.

[0110] *Quantitative Reverse-Transcription PCR (qRT-PCR).* RNA was extracted from blood and tissue samples using Qiagen Qiamp viral RNA-mini-isolation kit and Qiagen RNeasy mini-isolation kit, respectively, and provided protocols. Viral RNA was quantified using Qiagen Quantifast one-step qRT-PCR master mix and primers and probe specific for the CCHFV NP:

[0111] (Forward: 5' AAAATGAAGAAGGCACTCCTGAG3' (SEQ ID NO. 15);

[0112] Reverse: 5'GCAGACACCCATTTCACTGATTCT3' (SEQ ID NO. 16) ; and,

[0113] probe 5' CCAATGAAGTGGGGAAAGAA 3' (SEQ ID NO. 17) with a 5' 6-FAM, and an internal and 3' quencher. Primers and probes were purchased from IDT. Reaction was run on a Quantstudio 5 RT-PCR system (ThermoFisher) with cycling conditions as follows: initial hold of 50 °C 10 min, initial denaturation of 95 °C 5 min, and 40 cycles of 95 °C 15s, 50 °C for 20s and 72 °C for 1s. *In vitro* transcribed RNA standards with known copy number were prepared in house, diluted, and run alongside samples for quantification. No template controls were included in each run.

[0114] *Median Tissue Culture Infectious Dose ($TCID_{50}$) Assay.* Infectious virus was quantified on SW13 cells. SW13 cells were plated in 96-well tissue culture plates in L-15 media supplemented with 10% fetal bovine serum, 2mM L-glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin. Blood was initially diluted 1:10 in PBS without Ca2+/Mg2+ before subsequent 1:10 dilutions in L-15 media supplemented with 2% fetal bovine serum, 2mM L-glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin (dilution media). Lung and spleen samples were weighed and then homogenized in 1mL dilution media with sterile bead before subsequent 1:10 dilutions as with blood. 100uL of each dilution was transferred to SW13 cells. SW13 with sample were incubated at 37°C for 5 days before CPE was read. Lung and spleen titers normalized to mg of tissue. Negative samples were set to 0.5 Log(TCID50/mg). TCID50 was calculated using the Reed & Muench method.

[0115] *Neutralization Assay.* SW13 cells were plated in L-15 Media (ATCC) supplemented with 2% fetal bovine serum, 2 mM L-glutamine, 50 µg/mL penicillin, and 50 µg/mL streptomycin for 80-90% confluency at time of assay on 96-well tissue culture plates. Sera was inactivated at 56°C for 30 minutes and serially diluted 1:2 in triplicate starting with a 1:10 dilution in infection media (LT-15 as above with 2% FBS). CCHFV Hoti was diluted to contain 120 $TCID_{50}$ and added 1:1 to sera dilutions and incubated at 37°C for one hour after which 100uL sera-virus mixture was deposited onto SW13 cells and incubated at 37°C. Serum toxicity was checked after 24h and CPE on Day 5.

[0116] *Histology.* Tissues were fixed in 10% Neutral Buffered Formalin x2 changes, for a minimum of 7 days. Tissues were placed in cassettes and processed with a Sakura VIP-6 Tissue Tek, on a 12-hour automated schedule, using a graded series of ethanol, xylene, and ParaPlast Extra. Embedded tissues are sectioned at 5 µm and dried

overnight at 42 °C prior to staining. Sections were stained with hematoxylin and eosin or specific anti-CCHFV immunoreactivity was detected using a rabbit anti-CCHFV N protein antibody (IBT Bioservices cat#04-0011) at a 1:2000 dilution. The secondary antibody was the ImPress VR anti-rabbit IgG polymer (Vector Laboratories cat# MP-6401). The tissues were processed for immunohistochemistry using the Discovery Ultra automated stainer (Ventana Medical Systems) utilizing the ChromoMap DAB kit (Roche Tissue Diagnostics cat#760-159). Scoring was performed by a pathologist blinded to study groups.

[0117] Example 1. This example demonstrates that vaccination of mice with repNP, repGPC, ad repNPr_repGPC induces significant B and T cell responses

[0118] Mice were vaccinated with replicon RNAs expressing either the CCHFV strain Hoti NP (repNP) or GPC (repGPC) or both (repNP + repGPC). An RNA expressing the irrelevant green fluorescent protein (sham) was used as control. *In vitro* protein expression of each antigen was confirmed by both western blot and immunofluorescence. The immunogenicity of the rep constructs was then evaluated in WT C57BL6/J mice vaccinated using a prime-boost regimen (FIG. 2A). Vaccinations and challenge were conducted in 4-week intervals with 2.5μg of each RNA, or 5μg total RNA for repNP + repGPC, complexed to CNC. Mice were vaccinated with an intramuscular (IM) injection after which groups of mice were analyzed for homologous CCHFV-specific responses at day 0 p.i. by ELISA, immunofluorescence assay (IFA), virus neutralization and ELISpot. As measured by ELISA, four-weeks after the second immunization, mice vaccinated with repNP and repNP + repGPC had robust CCHFV-specific IgG titers compared to sham vaccinated animals (FIG. 2B). Consistent with repRNA mimicking an authentic viral infection, the CCHFV-specific IgG response was largely comprised of IgG2c, IgG2b and to a lesser degree IgG1 isotypes (FIG. 2C). In contrast, repGPC vaccinated animals had low levels of CCHFV-specific IgG (FIGS. 2B & 2C). To confirm the ELISA findings, an immunofluorescence assay (IFA) was performed on CCHFV-infected cells (FIGS. 3A-L). Pooled sera from repNP vaccinated mice robustly labeled intracellular antigen in CCHFV infected cells while sera from repGPC-only vaccinated labeled CCHFV-infected cells with reduced intensity compared to sera from repNP animals (FIGS. 3A-3L). Little reactivity was observed when sera from repNP or repGPC vaccinated animals was used to label non-permeabilized cells, consistent with the intracellular localization of these antigens.

Homologous neutralizing titers in any vaccinated group were low, and the increases were not significant compared to sham-vaccinated mice (FIG. 2D).

[0119] The ability of repNP and/or repGPC vaccination to elicit cellular immunity was evaluated by IFNγ ELISpot assay using splenocytes stimulated by overlapping peptide pools spanning the entire CCHFV Hoti NP or GPC (FIGS. 2E-2G). The ELISpot data showed that the rep-expressed NP antigen only weakly elicited a non-significant T-cell response against NP peptides compared to sham-vaccinated animals (FIG. 2E). Similarly, repNP + repGPC vaccinated animals had no significant increase in spot-forming cells (SFCs) against the NP peptide pools (FIG. 2E). In contrast, the rep-expressed GPC antigen elicited robust cellular immunity with repGPC and repNP + repGPC vaccinated animals having significant increases in IFNγ SFCs against the GPC peptide pools (FIG. 2E). Against NP, the T-cell response in repNP and repNP + GPC vaccinated animals was directed against peptide pools 2 (aa 101 – 211) and 4 (aa 301 – 411) (FIG. 2F). In repGPC vaccinated animals IFNγ T cell responses were directed towards GPC peptide pools 9 and 10 (FIG. 2G) which span from aa 961 to 1211 comprising the NSm and N-terminal half of Gc. Cumulatively, that analyses of repNP and repGPC vaccinated mice demonstrated that repNP vaccination primarily elicited a robust but non-neutralizing antibody response while repGPC elicited primarily cellular immunity against epitopes in the CCHFV NSm and Gc proteins.

[0120] Example 2. This example demonstrates that vaccination with repNP and repNP + repGPC protect against heterologous CCHFV challenge

[0121] The efficacy of the repRNA vaccines against lethal heterologous challenge with 100 $TCID_{50}$ of CCHFV strain UG3010 was evaluated. Compared to the vaccine antigens derived from CCHFV strain Hoti, at the amino-acid level, CCHFV strain UG3010 differs by 4.5% in the NP, 25.6% across the whole GPC and 14% specifically in the Gn and Gc glycoproteins. This mismatch between vaccine antigens and CCHFV challenge strain provided a stringent model to test efficacy of repRNA vaccination. Mice were treated with the type I IFN receptor blockade antibody MAR1-5A3 at time of CCHFV challenge to block type I IFN signaling and render mice susceptible to lethal UG3010 infection. This transient type I IFN suppression CCHF model was used rather than vaccination of genetically type I IFN deficient mice (e.g. $IFNAR^{-/-}$) to avoid confounding factors of type I IFN deficiency on development of immune responses to vaccination, particularly self-amplifying RNA vaccines. Most sham-vaccinated animals infected with

UG3010 began losing weight on day +3, and exhibited hyperthermia followed by hypothermia and death by day +7 (FIGS. 4A-4C). One sham-vaccinated animal exhibited delayed disease, not showing clinical disease until day +7, and ultimately survived the infection (FIGS. 4A-4C). Over the 14-day challenge, both repNP and repNP + repGPC vaccinated animals had no evidence of clinical disease (FIGS.4A-4C) and all vaccinated animals survived the infection ($P < 0.001$) (FIG. 4B). In contrast, repGPC vaccinated animals began to lose weight starting at day 4 p.i. (FIG. 4A), had evidence of hypothermia beginning on day 5 (FIG. 4C) and 5 of 8 repGPC vaccinated animals succumbed to the infection by day 7 p.i (FIG. 4B). Surviving sham and repGPC vaccinated animals began recovering weight after days 9 and 6 p.i., respectively (FIG. 4A). These data demonstrate that repNP vaccination could protect against clinical disease following lethal heterologous CCHFV challenge.

[0122] Consistently, viral loads as measured by qRT-PCR and $TCID_{50}$ assay in the blood, liver, and spleen at day 5 p.i. showed that both repNP and repNP + repGPC vaccinated animals had significantly lower viral loads compared to either repGPC vaccinated or sham vaccinated animals (FIGS. 4D & 4E). Consistent with clinical disease and death in repGPC vaccinated animals, although repGPC vaccinated animals had slightly but significantly reduced infectious virus in the blood (FIG. 4E), viral loads in the liver and spleen were similar to sham-vaccinated mice (FIGS. 4D & 4E) suggesting that repGPC vaccination alone could not control the CCHFV infection. By qRT-PCR, mice vaccinated with both repNP + repGPC had significantly lower viral loads in the liver and spleen compared to repNP alone (FIG. 4D), suggesting that repGPC vaccination could contribute to controlling CCHFV replication when in combination with repNP. However, no infectious virus was detected in either group (FIG. 4E) demonstrating that repNP alone conferred robust protection against viral replication.

[0123] Consistent with the viral load data, histological analyses of formalin fixed liver and spleen sections from CCHFV-infected mice at day 5 p.i. showed little-to-no evidence of pathology nor viral antigen in repNP and repNP + repGPC vaccinated animals (FIG. 5). In contrast, sham vaccinated mice developed multifocal to coalescing coagulative necrosis of hepatocytes admixed with small numbers of viable and degenerative neutrophils and fewer macrophages. Remaining hepatocytes often demonstrated lipid-type vacuolar degeneration (FIG. 5). The spleens from these mice had extensive necrosis and loss of lymphocytes from the white pulp, as well as mild reticuloendothelial hyperplasia of the red

pulp. Immunohistochemical analysis revealed viral antigen in the majority of hepatocytes, Kupffer cells, splenic reticuloendothelium and both hepatic and splenic endothelial cells (FIG. 5). Compared to sham-vaccinated animals, repGPC vaccinated animals had similar hepatic and splenic lesions but levels of viral antigen were reduced (FIG. 5). Together these data demonstrate that repNP provides robust protection against lethal CCHFV challenge.

[0124] Example 3. This example demonstrates that dose-down prime-only and prime-boost NP + GPC vaccinations induce significant B and T cell responses

[0125] The results thus far demonstrated that while repNP and repNP + repGPC vaccinations conferred protection against clinical disease following CCHFV infection, repNP + repGPC vaccination conferred the greatest control over viral replication. Thus, further studies were conducted to evaluate repNP + repGPC in dose-down studies and whether prime-boost vaccination was necessary for protection. Mice were vaccinated with 5μg, 1μg, and 0.1μg total repNP + repGPC RNA complexed to CNC in both prime-only and prime-boost schedules. As before, prime-boost mice were vaccinated 4 weeks apart or just 4 weeks prior to challenge (prime-only) and groups of mice evaluated for immunological response to vaccination on day 0 p.i., viral loads on day 5 p.i., and survival to day 14 p.i. On day 0, all groups had significantly increased IgG titers compared to sham vaccinated animals (FIG. 6A) demonstrating that a single immunization with as low as 0.1μg of repRNA (0.05μg each RNA) could elicit significant CCHFV-specific IgG responses. In prime-only animals, the 5μg and 1μg groups had similar titers while the titer for 0.1μg animals was significantly decreased compared to both higher dose groups (FIG. 6A). In prime-boost animals, the 5μg group had significantly higher titers compared to 1μg and 0.1μg groups (FIG. 6A). Interestingly, when comparing mice vaccinated with similar doses in prime-only to prime-boost regimens there was no significant ($P > 0.05$) difference in CCHF-specific IgG (FIG. 6A) indicating that boosting at 4 weeks did not significantly increase antibody responses. Similar to previous data, cellular immunity as measured by IFNγ ELISpot against NP was weak and compared to sham vaccinated animals, no significant increases in SFCs were observed when splenocytes from any vaccination group were stimulated with NP peptides (FIG. 6B). In contrast, compared to sham vaccinated animals, significant ($P < 0.05$) responses to GPC were observed in the 5μg and 1μg prime-only and 5μg prime-boost groups (FIG. 6B). Similar to the ELISA data, there was no significant difference ($P > 0.05$) between prime-only and prime-boost ELISpot responses further suggesting that boosting at 4-weeks

may not increase immunological response to the vaccine. Together, these data demonstrate that a single vaccination with as low as 50ng of each repRNA is sufficient to confer CCHFV-specific immunity.

[0126] Example 4. This example demonstrates that low dose repNP + repGPC RNA vaccinations protect against heterologous CCHFV challenge in both prime-only and prime-boost vaccine schedules

[0127] To further investigate the relationship between RNA dose, dosing schedule and protective efficacy of vaccination against CCHFV challenge, 5μg, 1μg, and 0.1μg vaccinated groups of mice were treated with MAR1-5A3 and challenged with 100 $TCID_{50}$ of CCHFV UG3010 as before. Mice vaccinated with 5μg or 1μg of repRNA in either prime-only and prime-boost regimens had no signs of clinical disease and 100% of mice survived the infection ($p < 0.001$) (FIGS. 7A-7C). Although the 0.1μg prime-only group had a slight decrease in body weight around days 5 and 6 p.i. animals recovered to ~100% initial starting weight by day 7 (FIG. 7A). One mouse in each of the groups vaccinated with 0.1μg in either prime-only or prime-boost regimens succumbed on day 6 p.i. (FIG. 7A) but survival was significantly improved compared to sham-vaccinated animals ($p < 0.001$). At day 5 p.i., as measured by both qRT-PCR and $TCID_{50}$ assay, all repRNA vaccinated animals had significantly decreased viral loads in the blood, liver, and spleen (FIGS. 7D & 7E) demonstrating that a single low-dose immunization could confer robust control of CCHFV replication. Overall, most repRNA-vaccinated animals had no detectable viral RNA or infectious virus in the blood, liver, and spleen (FIG. 7D) and infectious virus was only detected in the blood and spleen of one animal in the prime-boost 0.1μg group (FIG. 7E). Cumulatively, these data show that a single vaccination with as little as 0.1μg repRNA confers near sterile protection against CCHFV challenge.

[0128] To further evaluate control of CCHFV infection, serum obtained on day 14 p.i. of surviving mice was evaluated to determine if CCHFV challenge of vaccinated mice resulted in an anamnestic antibody response. Consistent with mild clinical disease and trending increases in viral RNA loads, the 0.1μg prime-only and prime-boost groups had a significant anamnestic response evidenced by increased CCHFV-specific IgG response compared to day 0 (FIGS. 8A & 8B). In contrast, and consistent with robust control of the CCHFV challenge, neither the 5μg nor the 1μg groups experienced a significant increase in CCHFV-specific IgG at day 14 compared to day 0 (FIGS. 8A & 8B). Cumulatively, these

data show that a single immunization with as little as 0.1µg repRNA confers significant protection against in CCHFV with higher doses conferring near sterile control of CCHFV replication.

[0129] Example 5. This example demonstrates that prime-only repNP vaccination protects against high-dose CCHFV challenge

[0130] To determine if prime-only protection was achievable using just repNP, a cohort of mice were sham vaccinated or received a single vaccination with 1µg repNP. After four weeks, immune responses to the vaccine were evaluated via ELISA and ELIspot (FIGS. 9A & 9B). The results showed that a single immunization with repNP was immunogenic with significant CCHFV-specific IgG and CCHFV-specific T-cell responses. To stringently evaluate vaccine protection, mice were challenged with a high dose of CCHFV UG3010 (10,000 $TCID_{50}$). Mice vaccinated with a single dose of repNP were significantly protected from disease showing little-to-no weight loss nor death (FIGS. 9C & 9D). Two mice in the repNP-vaccinated group showed transient weight loss on days 6 and 7 (peak weight loss 7 – 11%) (FIG. 9C) that was also associated with transient hypothermia (FIG. 9E) suggesting protection was incomplete in these mice. Nevertheless, all mice in the repNP-vaccinated group survived. Consistent with protection from clinical disease, compared to sham-vaccinated animals, as measured by both qRT-PCR and $TCID_{50}$ assay, repNP-vaccinated animals had significantly reduced viral loads in the blood, liver and spleen at day +4 p.i. (FIGS. 9F & 9G). Together these data demonstrate that a single immunization with repNP alone can protect against a high-dose CCHFV challenge.

[0131] Example 6. This example demonstrates that humoral responses are sufficient to confer protection against lethal CCHFV challenge after single-dose repNP + repGPC vaccination

[0132] The data thus far suggested that repNP and repGPC elicit distinct immune responses, primarily humoral and cellular responses, respectively. These data together with the efficacy data showing that repNP but not repGPC alone can confer protection against even high dose CCHFV challenge suggested that humoral immunity is the primary mediator of vaccine-induced protection. This hypothesis was tested through vaccination of mice lacking B-cells or depletion of T-cell populations at time of CCHFV challenge. To evaluate the contribution of humoral immunity, groups of B-cell deficient mice (µMT) were vaccinated four weeks prior to CCHFV challenge with 1µg of repNP + repGPC

in a prime-only regimen (FIG. 10A). To evaluate the contribution of T-cells in vaccinated mice to control of CCHFV challenge, mice were treated with antibodies to deplete $CD4^+$ T-cells (α-CD4), $CD8^+$ T-cells (α-CD8), or both (α-CD4/α-CD8) just prior to CCHFV challenge on days -5 and -2 relative to CCHFV challenge. B- and T-cell deficient mice were compared against both sham and repNP + repGPC vaccinated WT mice. Depletion of T cells as well as verification of absent CCHFV-specific antibody in μMT mice was confirmed at day 0 (FIGS. 11A-11C). Notably, the ELISpot data showed that depletion of $CD8^+$ but not $CD4^+$ T-cells abolished IFNγ responses against the GPC peptide pool (FIG. 11C) suggesting that repRNA vaccination elicits primarily $CD8^+$ T cells specific for the CCHFV GPC. Together these data confirmed that the approach successfully and specifically abolished vaccine-induced humoral or cellular immunity to CCHFV.

[0133] The mice were then challenged with CCHFV strain UG3010 as before. Vaccinated WT mice or vaccinated mice depleted of either $CD4^+$ or $CD8^+$ T-cells had no clinical disease and 100% of mice survived the CCHFV challenge (FIGS. 10B-10D). In the α-CD4/α-CD8 group, two animals began losing weight between day 4 and 7 p.i. and exhibited a maximal weight loss of 14 & 19% on day 9 p.i. before beginning to recover after day 10 p.i. (FIG. 10B). These data suggest that protection against CCHFV may be incomplete in mice lacking both $CD4^+$ and $CD8^+$ T-cells. In contrast, compared to WT vaccinated mice, all μMT mice exhibited clinical disease evidenced by significant ($P < 0.05$) weight loss on days 4 – 9 p.i, significantly elevated body temperature on days 4 – 7 p.i. and 5 of 8 mice succumbed to disease (FIGS. 10B-10D). However, compared to sham-vaccinated controls, clinical disease was delayed, and survival significantly improved in vaccinated μMT mice (FIGS. 10B-10D) indicating that vaccine-induced cellular immunity can contribute to control of the CCHFV infection. Consistent with the overt clinical disease and death in μMT mice, quantification of viral RNA at day 5 p.i. demonstrated that μMT mice failed to control the infection with significantly increased viral RNA loads compared to vaccinated WT mice and viral loads similar to sham-vaccinated mice ($P > 0.05$) (FIG. 10E). In the blood, mice depleted of T-cells had no significant increase in viral RNA loads suggesting that T-cells are dispensable for control of viremia (FIG. 10E). However, in the liver and spleen a trend towards increasing viral RNA loads compared to vaccinated WT mice was observed (FIG. 10E), suggesting that T-cells may contribute to control of viral replication in these tissues.

Cumulatively, these studies demonstrate that repRNA-induced humoral immunity is essential for protection from clinical disease and control of viral replication while vaccine-induced cellular immunity alone is only partially protective against lethal disease in CCHFV-infected mice.

[0134] Example 7. Role of NK cell and ADCC in repRNA-mediated protection

[0135] To evaluate the role of NK cells and antibody-dependent cellular cytotoxicity (ADCC) in the repNP-mediated protection, the vaccine was evaluated to determine if it could protect mice that had been depleted of NK cells. Mice were vaccinated with 1ug of repNP on day -28 and then depleted of NK cells via anti-NK1.1. antibody (FIG.12A). As expected, mice vaccinated with repNP had robust antibody response and a trending increase in CCHFV specific T cells at day 0 (FIGS. 12B & 12C). Depletion of NK cells by NK1.1 antibody was confirmed on day 9 post infection (FIG. 12D). On day 5, a group of mice were necropsied and viral loads quantified by TCID50 assay. repNP-vaccinated mice with intact NK cells or depleted of NK cells had similar robust control of the viral challenge with no infectious virus measured in any tissue evaluated (FIG. 12E). Consistent with robust control of virus repNP vaccination conferred 100% survival and maintenance of weight throughout the course of study in both vaccinated groups, regardless of presence of NK cells. Lastly, infection did not induce an anamnestic antibody response by end of study suggestive of robust control of the virus even in mice depleted of NK cells. Cumulatively, these data indicate that NK cells are dispensable for repNP-mediated protection from lethal CCHFV challenge.

[0136] Example 8. repGPC-T2A-NP vaccination elicits robust T-cell but, not B-cell response.

[0137] To determine if a bivalent repRNA expressing both NP and GPC could be protective a repRNA expressing the full-length GPC and NP separated by a T2A self-cleaving peptide sequence under a single alphavirus sub-genomic promoter (repGPC-T2A-NP) was created. To evaluate immunogenicity of this single RNA *in vivo*, wild-type C57BL6/J mice were vaccinated with 1μg of sham (expressing an irrelevant green fluorescent protein (GFP) antigen), repGPC-T2A-NP, or repNP + repGPC RNA complexed to LION. Vaccinations were administered intramuscularly (IM) prime-boost, with 4-week intervals between vaccinations (FIG. 14A). Previous work had shown that this vaccination

scheme and dose was sufficient to elicit complete protection and robust humoral and cellular immunity. On day 0, the immune response to vaccination was evaluated by whole-virion ELISA, recombinant antigen ELISA, neutralization assay, and ELISpot using CCHFV Hoti antigens which are homologous to vaccine antigens. Surprisingly, compared to repNP + repGPC, repGPC-T2A-NP had a significantly diminished antibody response to whole virus (FIG. 14B), largely due to diminished antibody responses to the CCHFV NP (FIG. 14C). Further, neither vaccination induced anti-Gn antibodies and there was only a slight, and non-significant, increase in anti-Gc antibodies (FIG. 14C). Nevertheless, previously it had been shown that as little as 50ng of repNP is sufficient to elicit robust protective antibody responses after a single immunization and thus diminished antibody responses even after two vaccinations with 1μg of this bivalent RNA was unexpected. Similar to previous studies, vaccine elicited antibody responses had little-to-no neutralizing activity, consistent with the low titers of anti-Gc antibodies (FIG.14D). Interestingly, the bivalent RNA conferred slightly higher neutralizing activity compared to individual RNAs (FIG. 14D), but this was not statistically significant. T-cell responses were also evaluated via IFNγ ELISpot using splenocytes stimulated with overlapping peptides covering the entire CCHFV NP and GPC. Both vaccinations induced significantly increased T-cell responses against the CCHFV GPC compared to sham vaccination (FIG.14E). These responses were directed against peptide pools 9 and 10 which span the end of the Nsm and N-terminal domain of the Gc (FIG. 14F). Neither vaccine induced significantly increased T-cell responses to the CCHFV NP (FIG. 14G). Overall, although both vaccinations elicited similar T-cell responses towards the CCHFV GPC, repGPC-T2A-NP had a diminished anti-NP antibody response compared to repNP + repGPC. Consistent with diminished immunogenicity, immunofluorescence assay (IFA) showed that cells transfected with repGPC-T2A-NP had diminished NP and GPC expression compared to cells transfected with individual repRNAs expressing each antigen. This may be due to inherent issues in translation around the T2A self-cleavage site necessary to release mature NP or due to the large size of the construct expressing both NP and full-length GPC.

[0138] Example 9. repGPC-T2A-NP confers partial protection against lethal CCHFV challenge.

[0139] This example compares the efficacy of repGPC-T2A-NP vs. repGPC + repNP vaccinations against a lethal CCHFV challenge. 4-weeks post boost

vaccination, groups of mice were treated with 2.5mg of MAR1-5A3 antibody to block the type I IFN response and render mice susceptible to CCHFV infection and disease. In order to avoid confounding factors associated with immunocompromised IFNAR-/- mice, the MAR1-5A3 infection model was utilized so that vaccine-mediating immune responses could develop in fully immunocompetent, wild-type mice. Mice were infected intraperitoneally (IP) with 100 $TCID_{50}$ of CCHFV strain UG3010 which differs from vaccine antigens in amino acid sequence by 4.5% in the NP, 25.6% across the whole GPC and 14% specifically in the Gn and Gc glycoproteins. This heterologous challenge provides a stringent evaluation of vaccine efficacy. Mice were monitored daily for weight loss and survival until end of study on D14 p.i.. In the sham-vaccinated group, weight loss began on D3 p.i. and continued until mice had succumbed to disease by D6 p.i. (FIGS. 15A & 15B). This was associated with high viral loads and infectious virus in the blood, liver and spleen at day 5, shortly before the mice succumbed (FIGS. 15C & 15D). Similar to previous studies, none of the repNP + repGPC mice experienced weight loss and vaccination conferred 100% protection against lethal disease (FIGS. 15A & 15B). No infectious virus and little-to-no viral RNA was measured in the blood, liver and spleen of repNP + repGPC vaccinated mice (FIGS. 15A-15D). In contrast, repGPC-T2A-NP vaccination only partially protected from CCHFV disease with 3 of 8 mice exhibiting weight loss and 2 of 8 mice succumbing (FIGS. 15A & 15B). Consistently, these mice had significantly higher viral loads in the blood, liver and spleen compared to repNP + repGPC vaccination, although viral loads were still significantly decreased compared to sham vaccination (FIG. 15C). Consistent with breakthrough clinical disease, three repGPC-T2A-NP mice had detectable infectious virus in these tissues as well (FIG. 15D).

[0140] To further evaluate viral control and provide insight into whether immunity that develops after viral challenge may contribute to protection, the anamnestic antibody and T-cell response in mice that survived the infection was evaluated. Consistent with the breakthrough clinical disease and diminished control of viral replication in repGPC-T2A-NP vaccinated animals, this group had a significant anamnestic antibody response with significant increases in both anti-NP and anti-Gc antibody titers at D14 compared to D0 (FIG. 16A). In contrast, repNP + repGPC vaccinated mice, which were protected, did not experience a significant increase in anamnestic antibody titers against either antigen, although some mice in the group had increased anti-Gc antibodies (FIG. 16A). Little-to-no

response was measured against the CCHFV Gn indicating that the anti-Gn immune responses may not contribute to protection from CCHFV in this model (FIG. 16A). Interestingly, although there was a trending increase in neutralizing antibodies in both groups at D14, this was not significant, indicating that de novo development of a neutralizing antibody response after CCHFV challenge is also dispensable for protection from CCHFV in this model (FIG. 16B). Together, these data suggest that antibodies against the viral glycoproteins, either present at time of challenge or primed by vaccination and boosted by viral infection are dispensable for protection against lethal CCHFV challenge. Cumulatively, the immunology and viral challenge data indicate that repGPC-T2A-NP confers only partial protection against lethal CCHFV infection, likely due to the diminished anti-NP antibody response (FIG. 14B & 14C).

[0141] Example. 10 Results: repNP + engineered GPC variants, repGc-sol and repGc-teth, elicit robust B and T cell responses.

[0142] It was hypothesized that the diminished antibody response and efficacy of repGPC-T2A-NP may be due to size and complexity of the CCHFV GPC. Thus, the inventors sought to refine the protective epitope within GPC that confers the robust protection observed in repNP + repGPC vaccination. Three engineered GPC variants were tested including soluble versions of both the Gn and Gc (Gc-sol, Gn-sol) and a membrane tethered version of Gc (Gc-teth) that retained the Gc transmembrane domain. Mice were vaccinated with 1ug of repNP + repGc-sol, repNP + repGn-sol, and repNP + repGc-teth and compared with the responses in sham and repNP + repGPC vaccinated mice (FIGS. 14 and 15). Vaccinations were administered IM prime-boost 4 weeks apart and on D0 (4 weeks post boost) groups of mice were analyzed for homologous CCHFV-specific immune responses via ELISA, neutralization assay, and ELISpot (FIG. 17A). Compared to sham vaccinated animals, all groups induced significant and robust IgG antibody titers (FIG. 17B). Specifically, these antibodies were primarily directed against NP with similar titers in all groups (FIG. 17C). Interestingly, the repGc-teth vaccination induced significantly higher anti-Gc antibody titers compared to sham while repGc-sol and repGPC vaccinations elicited only slight but non-significant increases of anti-Gc antibody (FIG. 17C). Neither the repGn-sol nor repGPC groups induced anti-Gn antibody (FIG. 17C). Consistent with previous studies, antibodies in all groups had little to no neutralizing activity (FIG. 17D). In addition, CCHFV-specific T-cell responses were measured in repGc-sol, repGc-teth, and repGPC

vaccinated groups while, the repGn-sol vaccination did not elicit a measurable T-cell response (FIGS. 17E & 17F). As before, none of the vaccines stimulated a strong NP T-cell response; peptide pools 2 and 4 showed the strongest response but this was not significant compared to sham (FIGS. 17E & 17G). Overall, both the repNP + repGc-sol and repNP + repGc-teth vaccinations elicited immune responses equivalent to the original repNP + repGPC vaccination and, although repNP + repGn-sol had equivalent B-cell stimulation, this vaccine did not stimulate a T-cell response.

[0143] Example 11. repGc-teth is the minimal protective epitope of repGPC when administered with repNP (repNP + repGc-teth).

[0144] This example demonstrates how immune responses to the repNP + repGPC engineered variants protect against lethal CCHFV challenge. Vaccinated mice were challenged on D0 with 100 $TCID_{50}$ of CCHFV UG3010 immediately after treatment with 2.5mg MAR1-5A3 and monitored daily to D14 p.i.. Sham mice began to experience significant weight loss on D3 p.i. and all mice had succumbed to disease by D6 p.i. (FIGS. 18A & 18B). In contrast, all other groups of mice were protected from weight loss and had 100% survival (FIGS. 18A & 18B). This is consistent with previous work showing that repNP could confer protection from disease and death on its own. Instead, it was found that inclusion of the GPC antigen led to enhanced control of viral RNA and infectious virus in the blood, liver, and spleen. Thus, a group of mice at D5 p.i. was evaluated for control of viral replication in these tissues. Interestingly, all groups had little to no viremia with only one mouse in the repGc-sol and repGn-sol groups having detectable viral RNA by qRT-PCR (FIGS. 18C & 18D). Viral RNA loads in the tissues illustrated larger differences in efficacy with nearly all mice in the repNP + repGc-sol group having detectable viral RNA loads in the liver and spleen (FIG. 18C). The repGn-sol group also had diminished control of viral replication with 3 of 6 mice having detectable viral RNA in the liver compared to only 1 of 6 mice vaccinated with repGPC (FIG. 18C). On the other hand, the data demonstrate that vaccination with repNP + repGc-teth performed equivalent to the repNP + repGPC vaccine with only 1 mouse in each group positive by qRT-PCR in the liver and spleen (FIG. 18C).

[0145] Liver and spleen tissue was evaluated for infectious virus, and it was found that all sham-vaccinated mice had infectious virus in these tissues. In contrast, all repNP + repGPC, repGc-teth, and repGn-sol groups had no infectious virus detected in the blood, liver, or spleen, with the repGc-sol group having 1 mouse positive for low amounts

of infectious virus in the liver (FIG. 18D). This is consistent with previous data showing that repNP vaccination alone can control infectious virus burden in these key tissues and that immunity against GPC further enhances control of viral replication as measured by reduction of viral RNA loads by qRT-PCR [9]. Interestingly, only the repNP + repGc-teth group experienced an increase in anamnestic antibody responses against the NP and, unexpectedly, the Gn although these increases in titers were low (FIG. 19A). None of the groups had increased anamnestic anti-Gc antibody responses, although titers did trend higher compared to D0 (FIG. 19A). Further, the repGn-sol group was the only group to have significantly increased neutralizing antibody titers on D14 compared to D0 (FIG. 19B). Nonetheless, based on the robust efficacy of repNP and little-to-no development of anti-Gn or Gc antibodies after infection, the data suggest neutralizing antibodies are dispensable for protection against CCHFV in this model. Overall, while all vaccinations prevented weight loss, conferred 100% survival, and significantly reduced viral loads, only the repNP + repGc-teth vaccination conferred protection equivalent to repNP + repGPC.

[0146] Example 12. repNP lacking the V5-epitope tag confers equivalent protection to epitope tagged repNP.

[0147] Previous work showed that prime-only vaccination with repNP RNA protects against weight loss and confers 100% survival with significantly decreased viral loads and no detectable infectious virus after CCHFV challenge. However, this repNP vaccine encoded a CCHFV NP with a C-terminal V5 epitope tag. In planning for further clinical development, the V5 epitope tag was removed from repNP, resulting in the repNP(ΔV5) construct. This construct was evaluated for whether it would provide similar protection as the previous repNP vaccine. repNP(ΔV5) vaccination resulted in similar non-neutralizing, anti-NP antibody titers and T-cell responses to repNP after a single dose (FIGS. 20A-20E) and, during lethal CCHFV challenge, protected against weight loss while conferring 100% survival and significant control of viral loads with no infectious virus in the blood, liver, and spleen (Suppl. FIGS. 20F-20I). Remarkably, repNP(ΔV5) vaccination conferred trending lower viral RNA loads compared to repNP in the blood and liver with significantly lower viral loads in the spleen, suggesting that removal of the V5 epitope tag may have increased efficacy of repNP vaccination (FIG. 20H).

[0148] Example 13. Results: Bivalent repGcteth-NP and mixed synthesis repNP + repGc-teth RNA elicit robust B and T cell responses.

[0149] With the protective region of repGPC narrowed to repGc-teth and confirmation that removal of the epitope tag on repNP did not negatively impact efficacy, a bivalent RNA approach to vaccination was tested. In addition to using the Gc-teth epitope to reduce the size of the expressed antigen, the new bivalent repGcteth-NP RNA (bivalent) contained two alphavirus subgenomic promoters driving synthesis of independent mRNAs for Gc-teth and NP. This is in contrast to repGPC-T2A-NP which produced a single mRNA that is cleaved during translation. Also evaluated were vaccines produced by a mixed synthesis reaction (mixed synth.) that produces individual repGc-teth and repNP RNAs in one synthesis reaction thus simplifying manufacturing.

[0150] These new constructs and syntheses methods were evaluated by vaccinating wild-type mice with a single immunization of 1μg of sham RNA, mixed synthesis, or bivalent RNA. A prime only approach was evaluated, as previous work demonstrated that prime-only vaccination with repNP alone can confer protection. Since the repGc-teth vaccine successfully induced significant antibodies against the Gc protein (FIG. 17C), mice were also vaccinated with repGc-teth alone, to determine if this response was sufficient for protection. 4 weeks post-prime, groups of mice were evaluated for homologous CCHFV-specific immune responses (FIG. 21A) as above by ELISA, neutralization assay, and ELISpot. Both the mixed synthesis and bivalent RNAs induced significantly increased anti-NP antibody titers slightly lower but similar to repNP(ΔV5) vaccination (FIG. 21B). In addition, both of these vaccinations also induced higher anti-Gc antibodies although only the bivalent was significant compared to sham (FIG. 21C). However, consistent with previous data, repGc-teth vaccination did induce a low yet significant increase in anti-Gc antibodies (FIG. 21C). Further, repGc-teth, mixed synthesis, and bivalent RNA vaccinations induced significant T-cell response against the GPC peptide pool 10 which spans the N-terminus of CCHFV-Gc (FIGS. 21E & 21F). As before, none of the vaccinations induced a significant T-cell response against the NP (FIGS. 21E & 21G). Overall, both the mixed synthesis and bivalent RNA approaches induced significant anti-NP antibodies, similar to repNP(ΔV5) and CCHFV-specific T-cell responses similar to repGc-teth vaccination alone and previous repNP + repGPC vaccination. Furthermore, the data also demonstrate that the single, bivalent RNA approach can elicit immune responses against two distinct antigens.

[0151] Example 13. Results: Bivalent repGcteth-NP and mixed synthesis RNA vaccinations protect against lethal CCHFV challenge.

[0152] To test the efficacy of the new bivalent construct and mixed synthesis RNA, mice vaccinated with sham, repNP(ΔV5), repGc-teth, mixed synthesis, and bivalent RNA with a lethal dose of CCHFV strain UG3010 and MAR1-5A3 antibody treatment were challenged, challenged 4 weeks post prime vaccination, and monitored daily for weight loss and survival out to D14 p.i.. All mice in the repNP(ΔV5), mixed synthesis, and bivalent RNA groups were protected from weight loss and had 100% survival (FIGS. 22A & 22B). In contrast, despite measurable anti-Gc antibodies, mice vaccinated with repGc-teth, were only partially protected with 6/7 mice experiencing weight loss and only ~20% survival (2/7 mice surviving disease) (FIGS. 22A & 22B). At D5 p.i., all groups had significantly lower viremia but, only the repNP(ΔV5), mixed synthesis, and bivalent groups had significantly reduced viral loads in the liver and spleen (FIG. 22C). Remarkably, both the repNP(ΔV5) and mixed synthesis groups had no detectable viral RNA loads in the blood and spleen (FIG. 22C). Although two mice vaccinated with repGc-teth did not have detectable viral RNA loads in the tissues, viral RNA loads overall were not significantly decreased compared to sham vaccinated animals (FIG. 22C). In addition to reduced viral RNA loads, no infectious virus was detected in the blood, liver, or spleen for repNP(ΔV5), mixed synthesis, or bivalent vaccinations (FIG. 22D). While the repGc-teth group had no infectious virus in the blood, two mice were positive in the liver and spleen and although decreased compared to sham, this was only significant in the liver (FIG. 22D). Post-infection, no significant increases were observed in neutralizing or anti-Gc antibodies but both the repNP(ΔV5) and bivalent groups experienced a significant increase in anti-Gn antibodies, although these titers were very low (FIGS.23A & 23B). As before, this indicates that neutralizing and anti-glycoprotein antibodies are dispensable for protection in this model. Interestingly, although there were no significant increases in anti-NP antibodies post-infection, the repNP(ΔV5) group experienced a significant decrease in antibody titers however, anti-NP antibodies did persist in the mixed synthesis and bivalent groups (FIG. 23A). Cumulatively, the data suggest that repGc-teth vaccination alone confers partial protection while further demonstrating that vaccination with repNP alone can confer nearly complete protection against lethal CCHFV challenge. The data also suggest that protection

can be conferred by vaccination with repNP and repGc-teth either in a bivalent construct or, by a mixed synthesis reaction yielding two individual repRNAs.

WHAT IS CLAIMED IS:

1. A self-replicating, alphavirus replicon RNA (repRNA) comprising a heterologous nucleotide sequence encoding a polypeptide comprising at least one epitope that elicits an immune response against Crimean-Congo hemorrhagic fever virus (CCHFV), and a means for controlling expressing of
5 the heterologous nucleotide sequence operably linked to the heterologous nucleotide sequence.

2. The alphavirus repRNA of claim 1, wherein the epitope is from a CCHFV protein.

3. The alphavirus repRNA of claim 2, wherein the CCHFV protein comprise an amino sequence at least 70% identical to an at least 50, at least 100, at least 150,
5 or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein, a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein

4. The alphavirus repRNA of claim 1, wherein the alphavirus, repRNA comprises two or more heterologous nucleotide sequences, each of the two or more heterologous sequences encoding a polypeptide comprising at least one epitope that elicits an immune response against CCHFV, wherein each of the two or
5 more heterologous nucleotide sequences are operably linked to a means of controlling expression of a nucleotide sequence.

5. The alphavirus repRNA of claim 4, wherein the epitope is from a CCHFV protein.

6. The alphavirus repRNA of claim 5, wherein the CCHFV protein comprise an amino sequence at least 70% identical to an at least 50, at least 100, at least 150,
5 or at least 200 contiguous amino acid sequence from a protein selected from the group consisting of CCHFV protein selected from the group consisting of a CCHFV NP, a CCHFV GPC, a CCHFV MLD protein, a CCHFV GP38 protein,

a CCHFV Gn protein, a CCHFV NSm protein, a CCHFV Gc protein, and a CCHFV NSs protein.

7. The alphavirus repRNA of any one of claims 4-6, wherein the two or more heterologous nucleotide sequences are in frame, and wherein control of expression of the two or more heterologous nucleotide sequences are operably linked to the same means for controlling expression of the two or more
5 heterologous nucleotide sequences, such that the nucleic acid molecule produced by transcription from the promoter encodes a fusion protein comprising the polypeptides encoded by the two or more heterologous nucleotide sequences.

8. The alphavirus repRNA of claim 7, wherein the fusion protein comprises a linker peptide between the two or more heterologous proteins, the fusion protein comprising an enzymatic cleavage site.

9. The alphavirus repRNA of claim 4, wherein each of the two or more heterologous nucleotide sequences are operably linked to separate means to control expression of a heterologous nucleotide sequence.

10. The alphavirus repRNA, wherein the two or more heterologous nucleotide sequence encode an alphavirus NP protein and an alphavirus Gc protein.

11. The alphavirus repRNA of any one of claims 1-10, wherein the means to control expression of a heterologous nucleotide sequence is an alphavirus sub-genomic promoter, optionally alphavirus 26S sub-genomic promoter.

12. A vector encoding the alphavirus repRNA of any one of claims 1-11.

13. A nanoparticle comprising the alphavirus repRNA of any one of claims 1-12.

14. A method of eliciting an immune response in an individual, comprising administering to the individual the repRNA of any one of claims 1-11, the vector of claim 12, or the nanoparticle of claim 13.

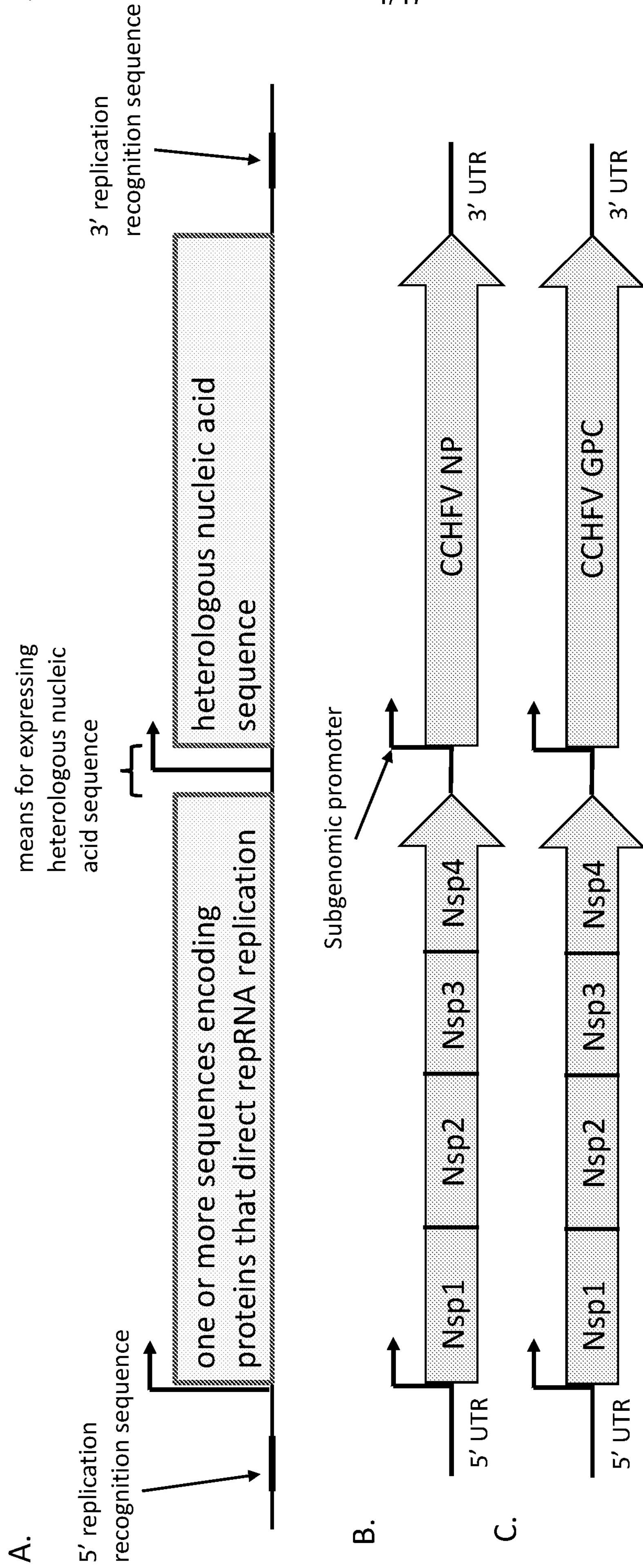


FIG. 1

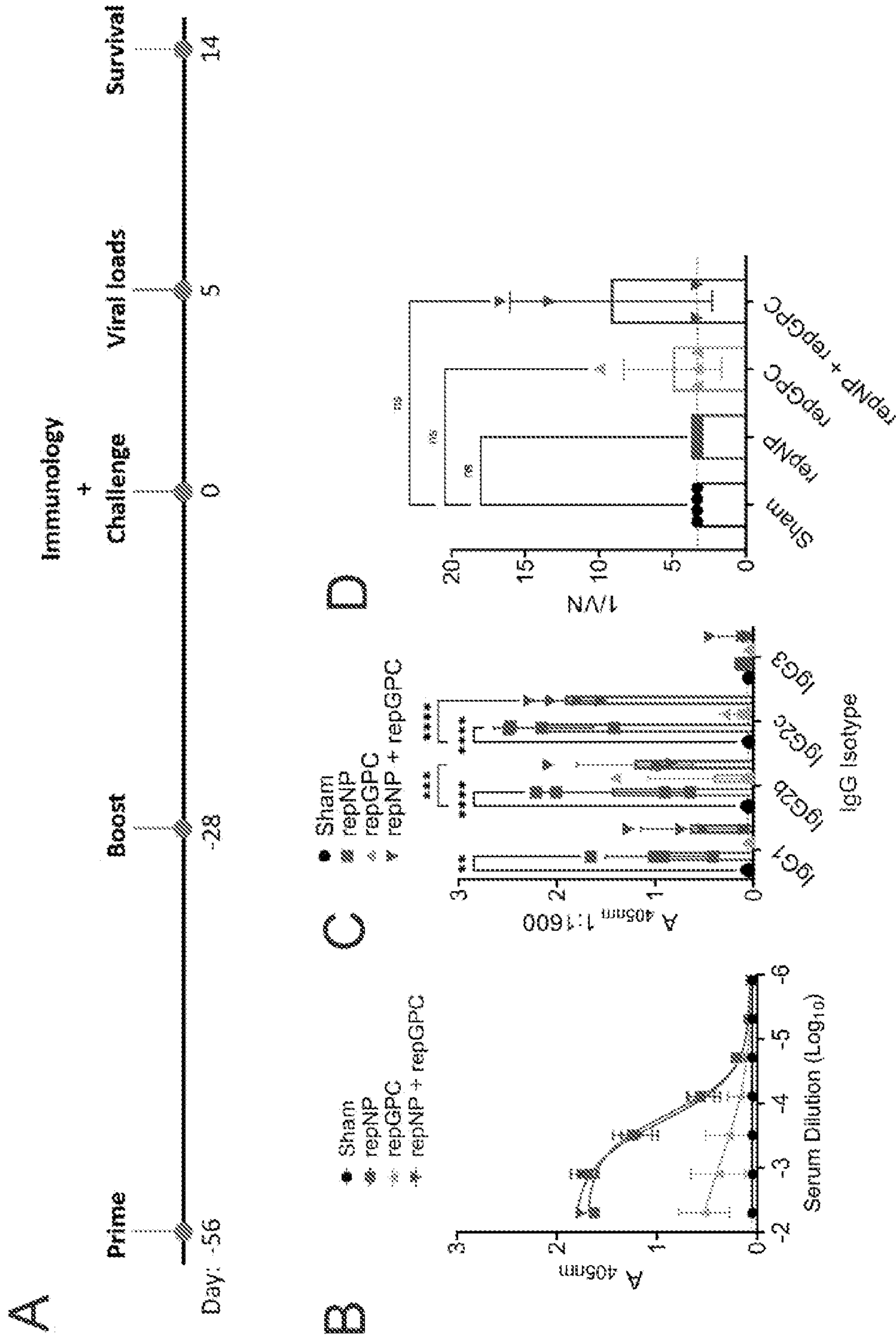


FIG. FIGS. 2A-2D

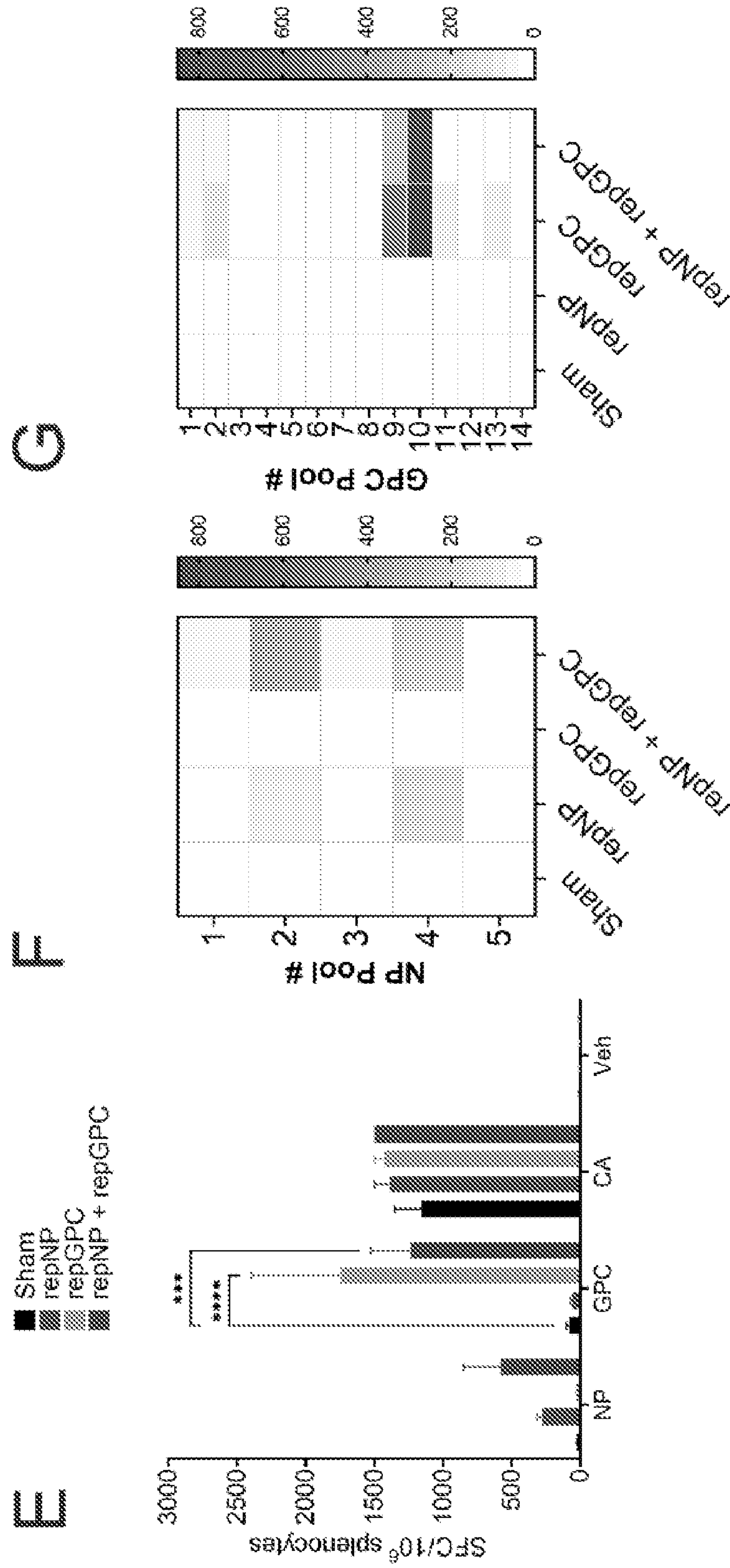
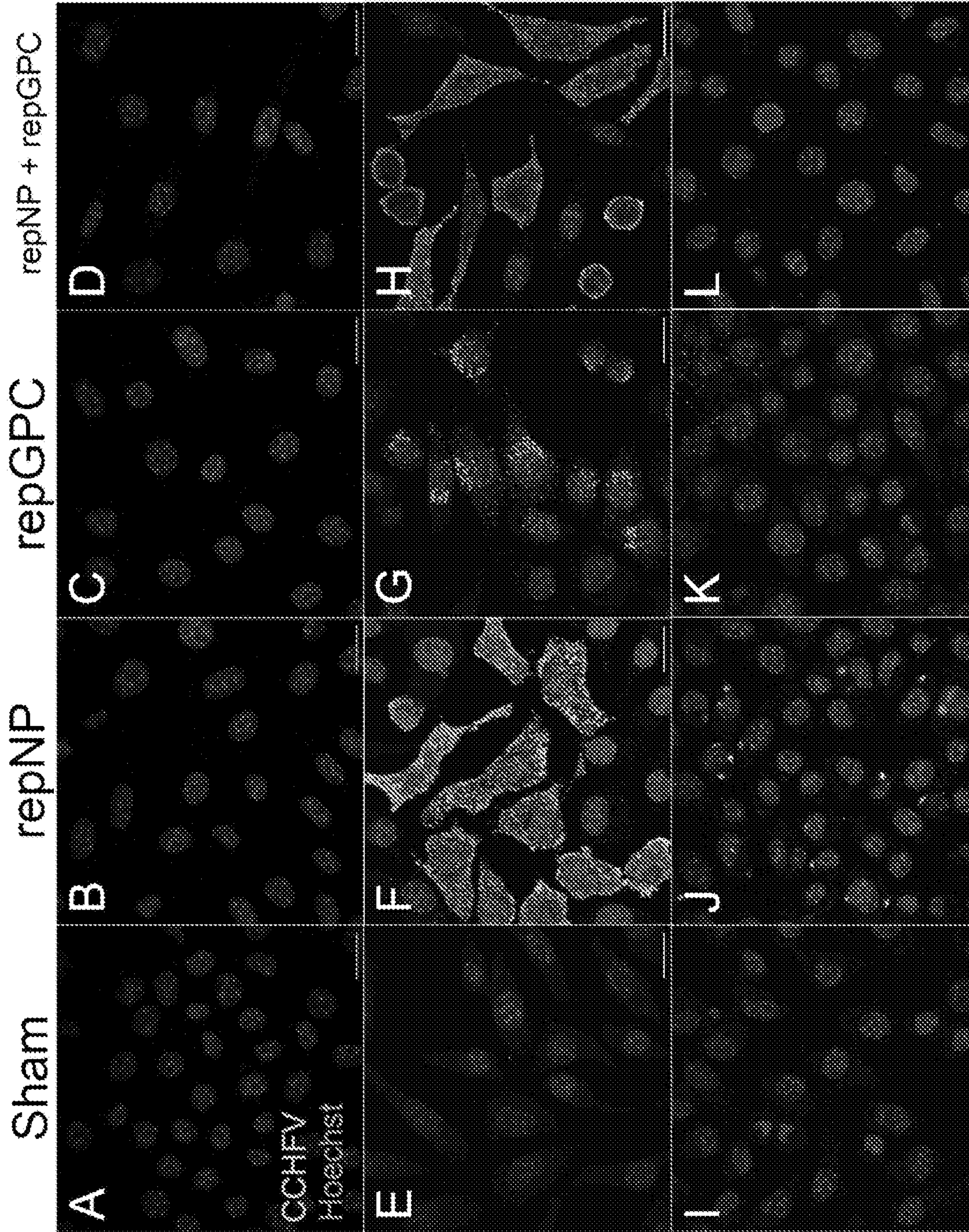
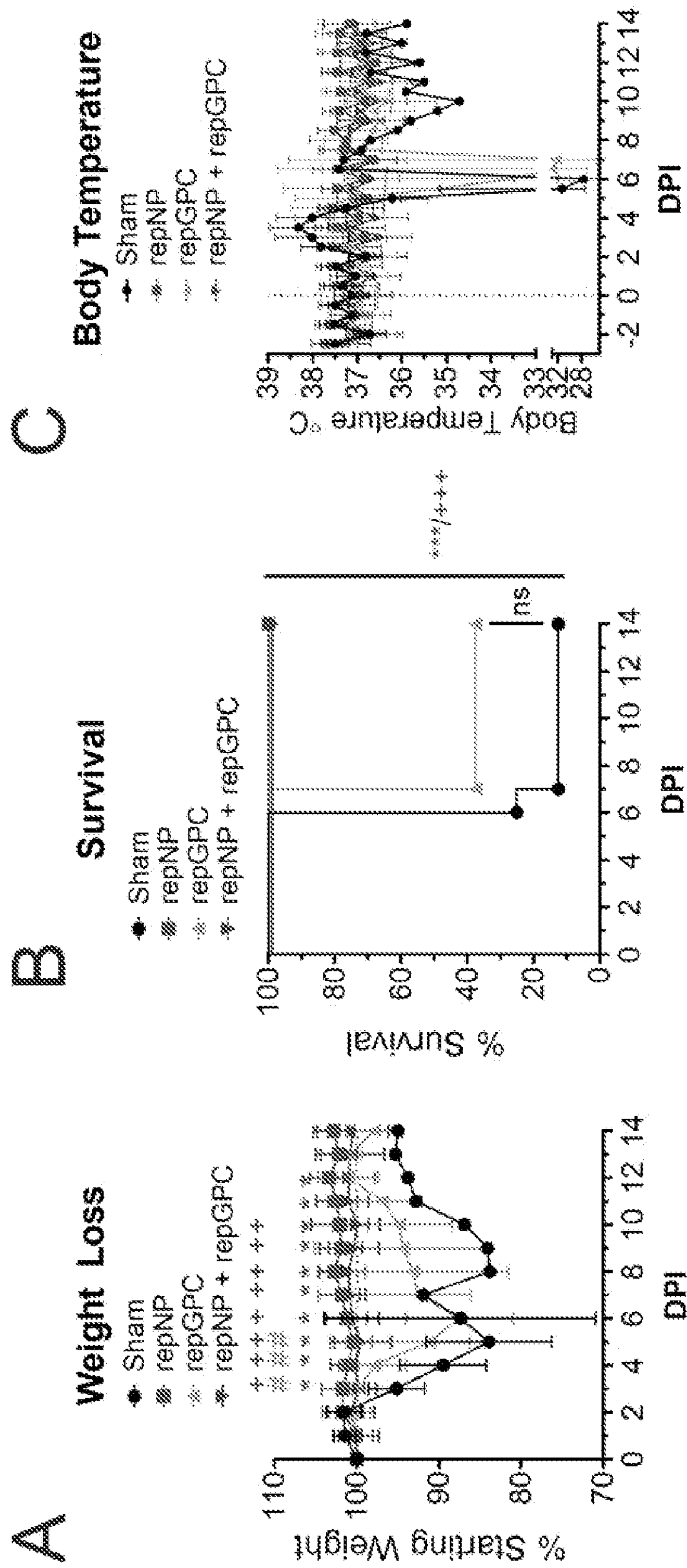


FIG. FIGS. 2E-2G



FIGS. 3A-3L



FIGS. 4A-4C

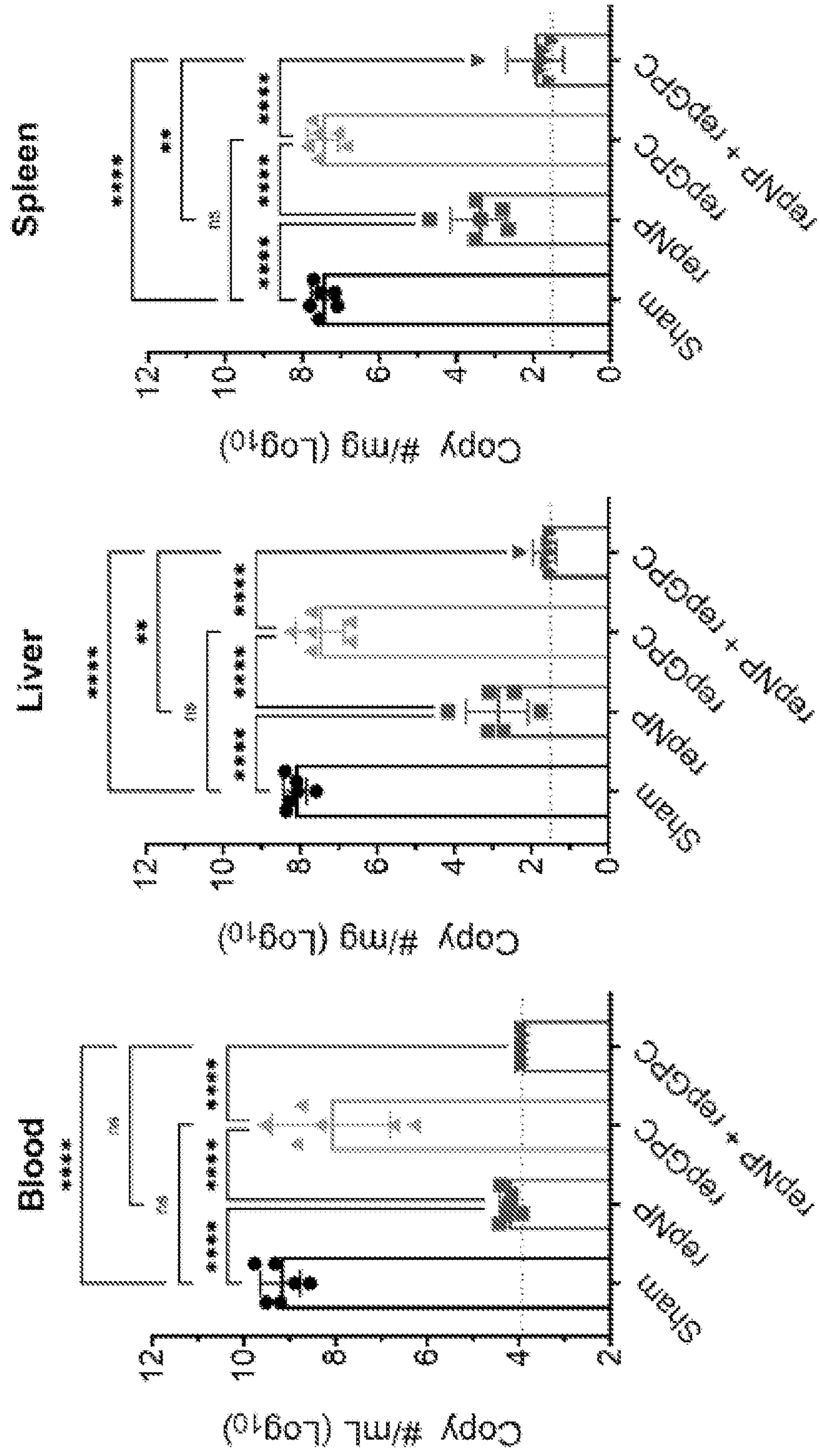


FIG. 4D

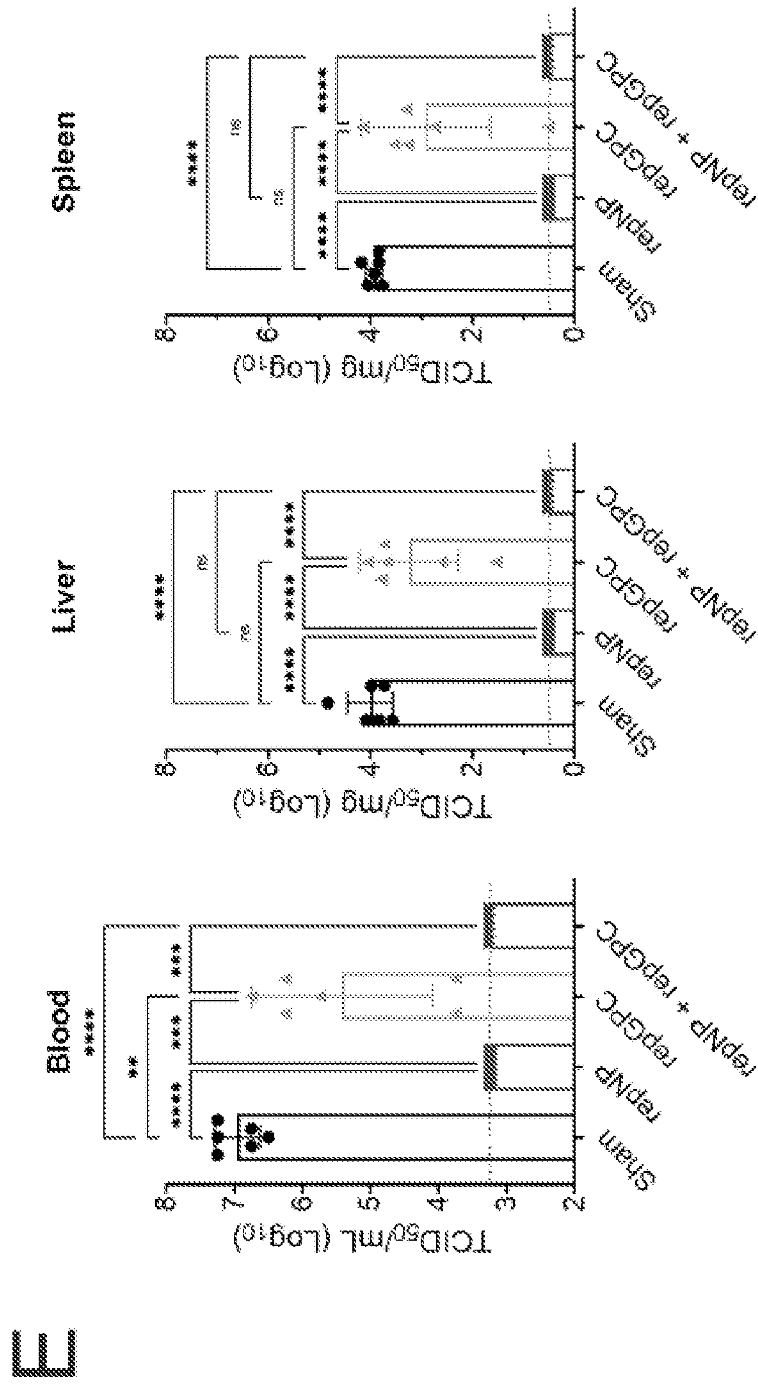


FIG. 4E

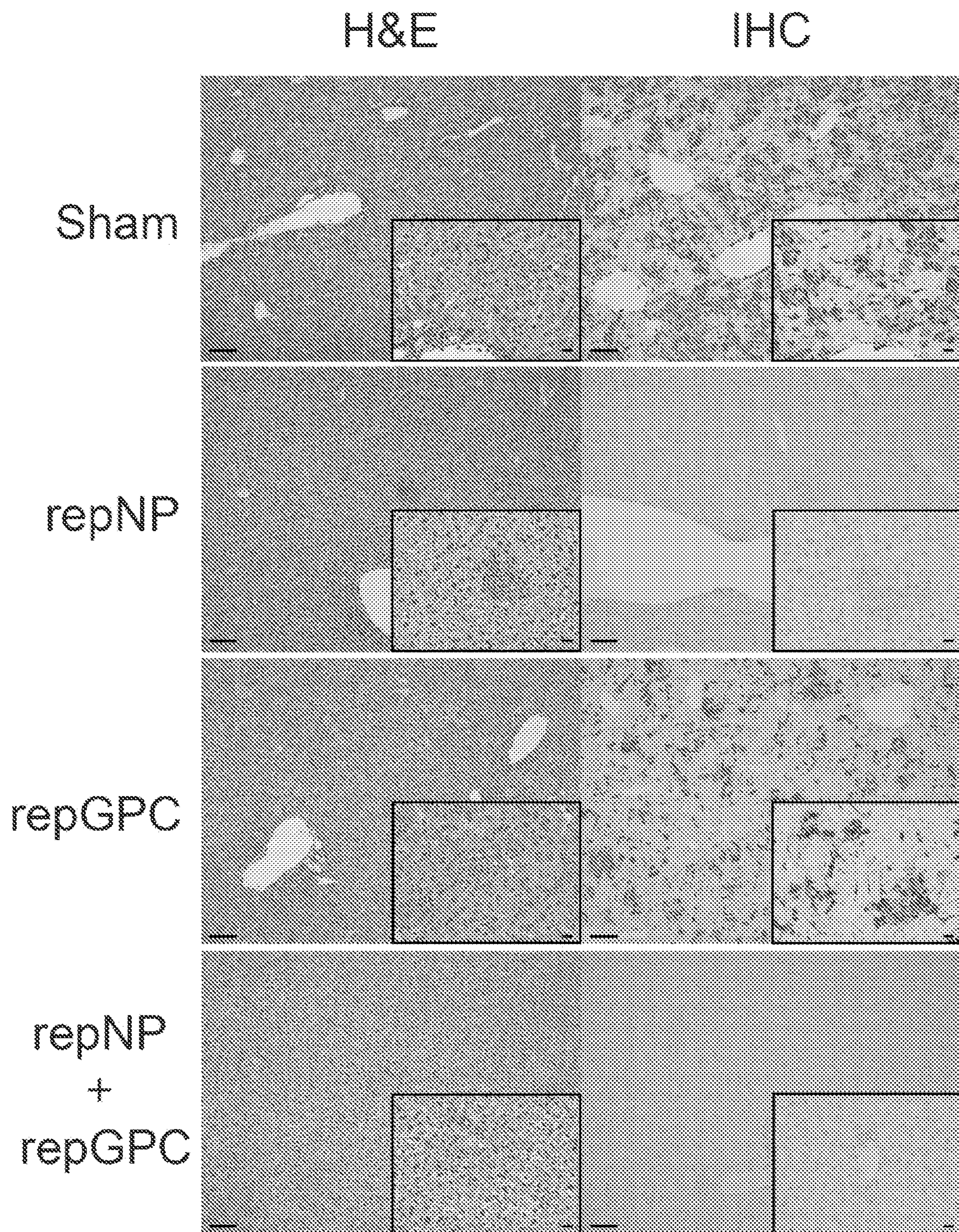
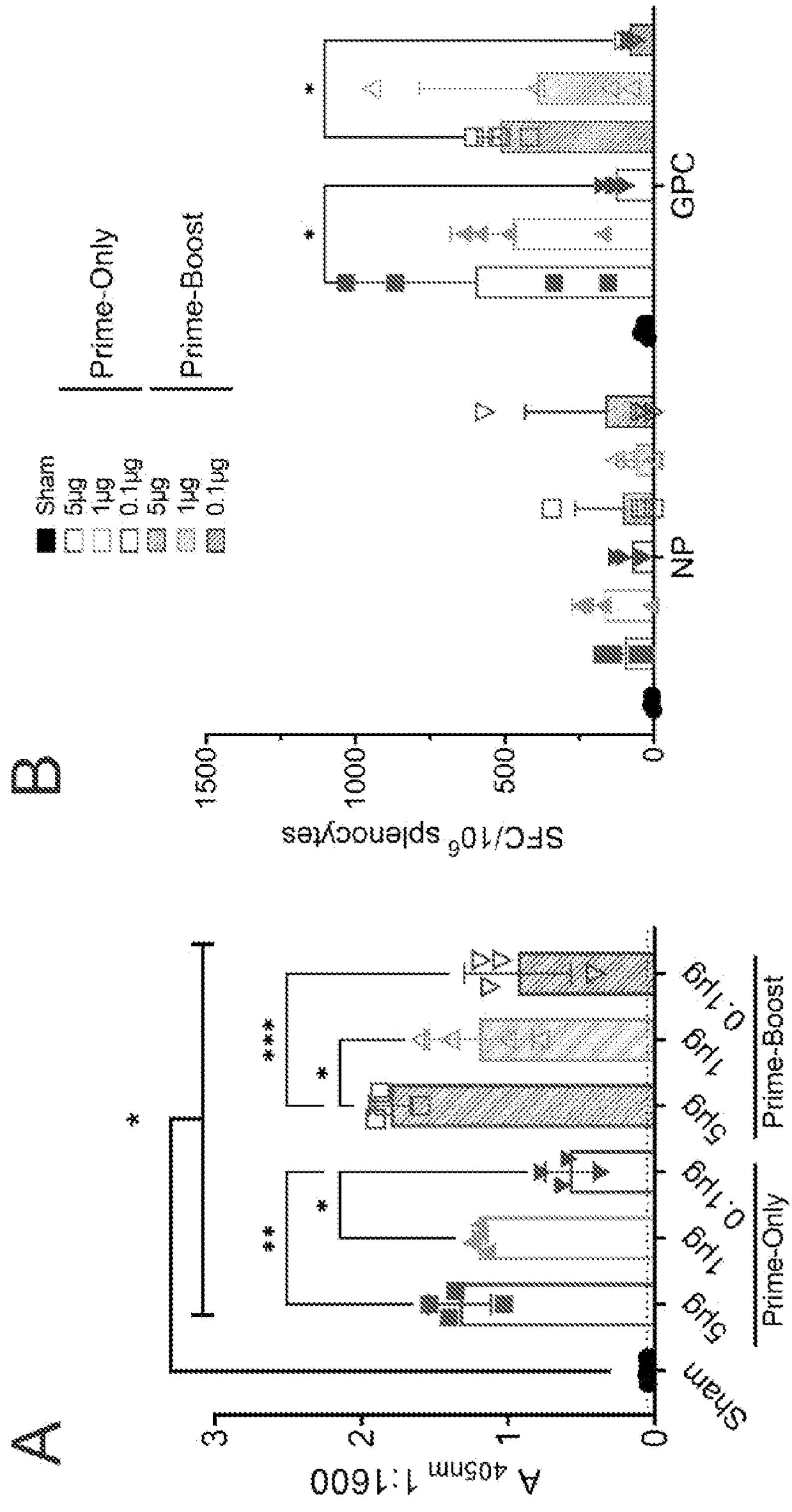
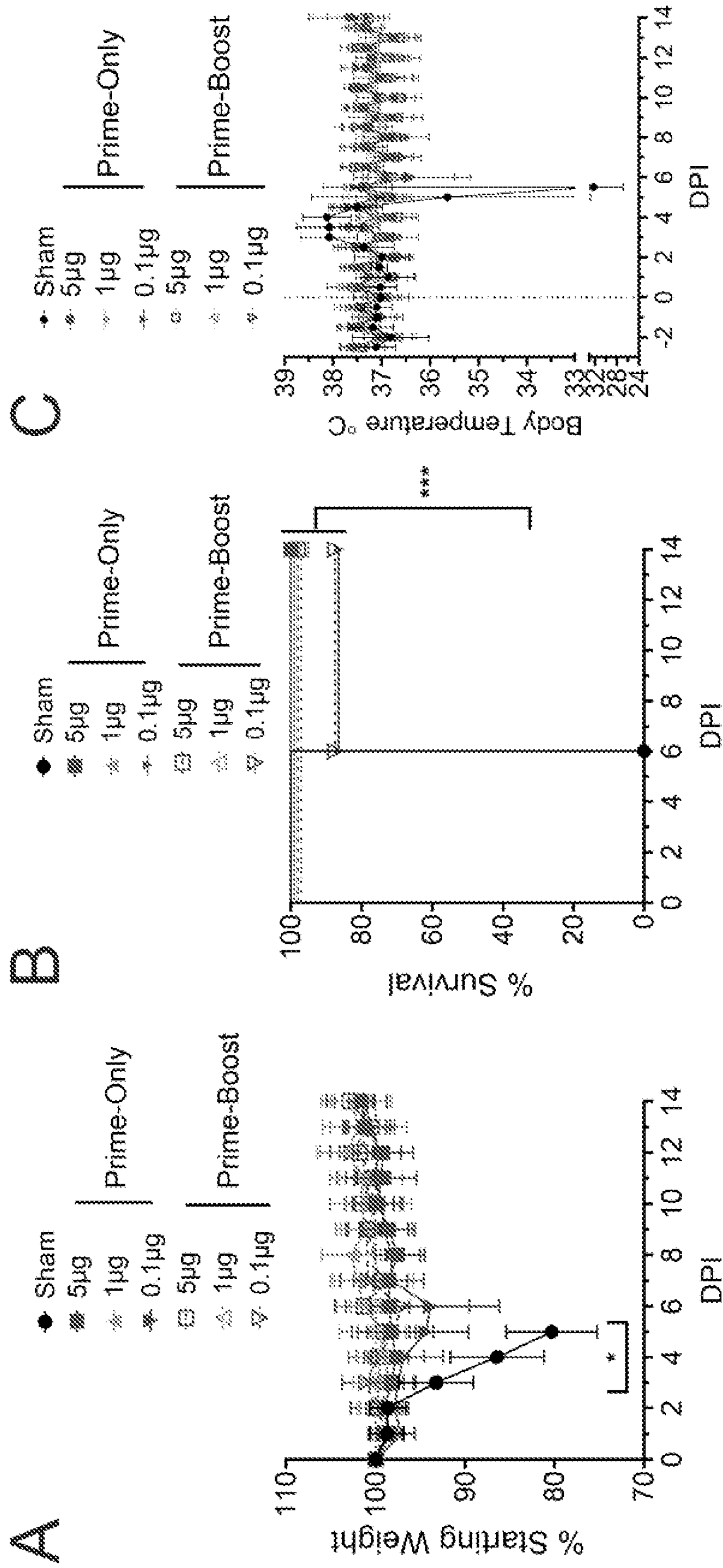


FIG.5



FIGS. 6A & 6B



FIGS. 7A-7C

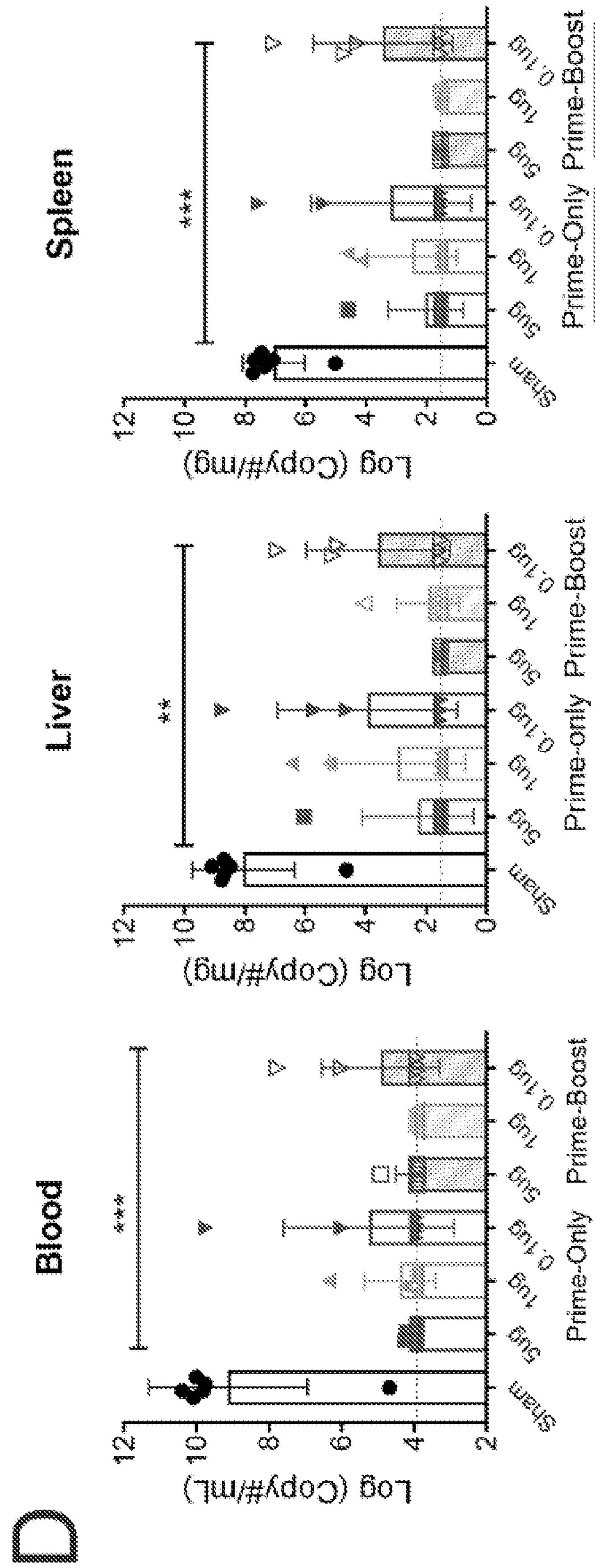


FIG. 7D

E

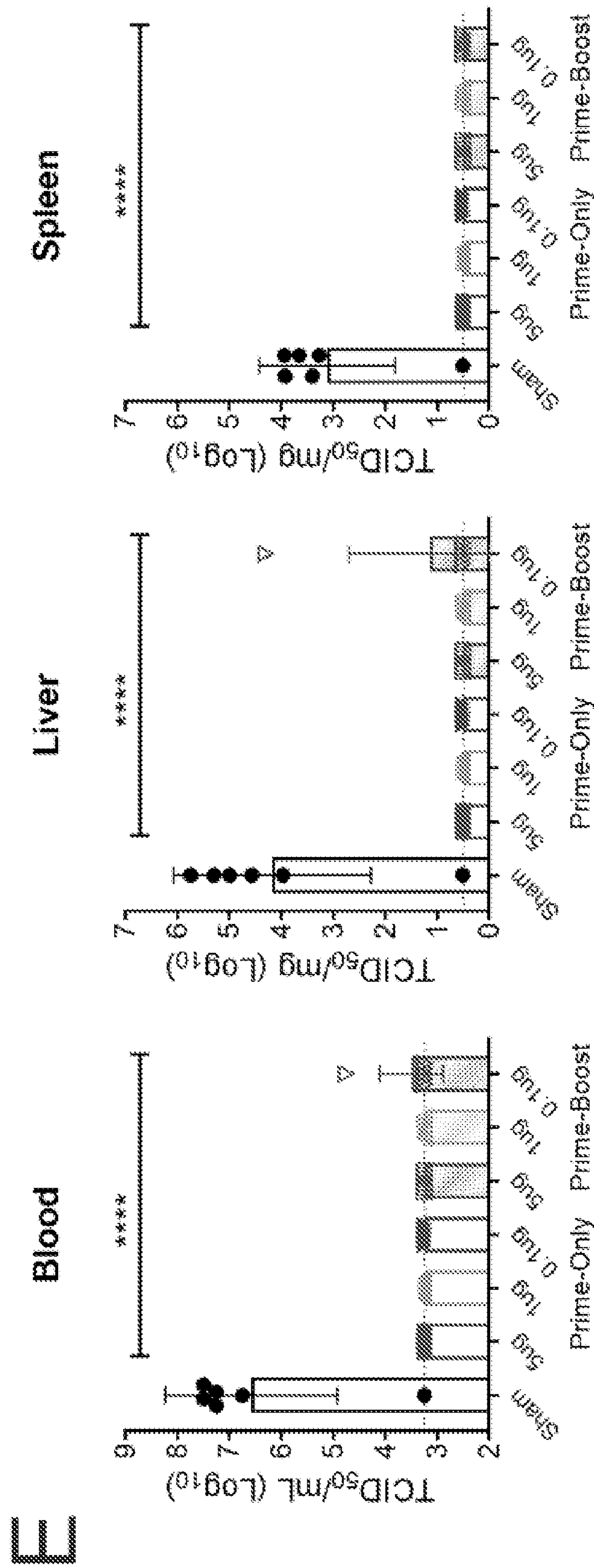
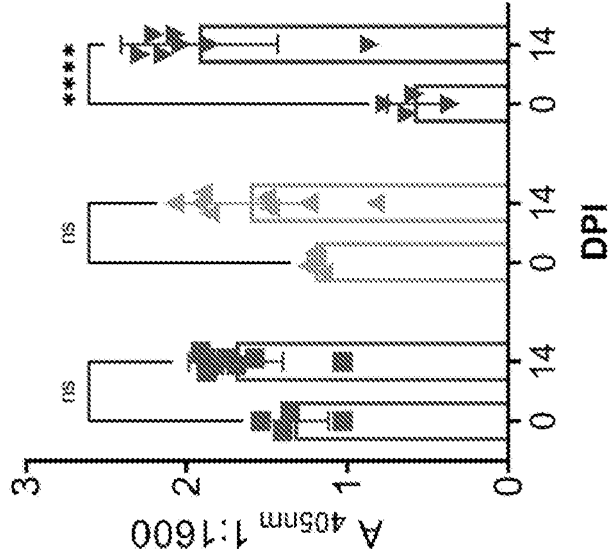
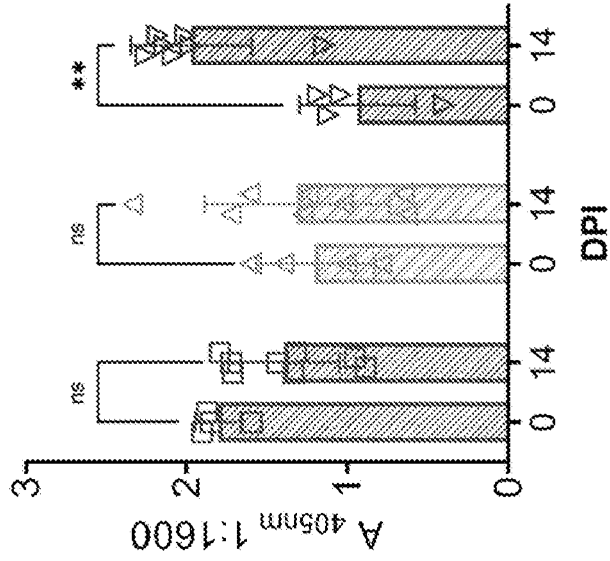
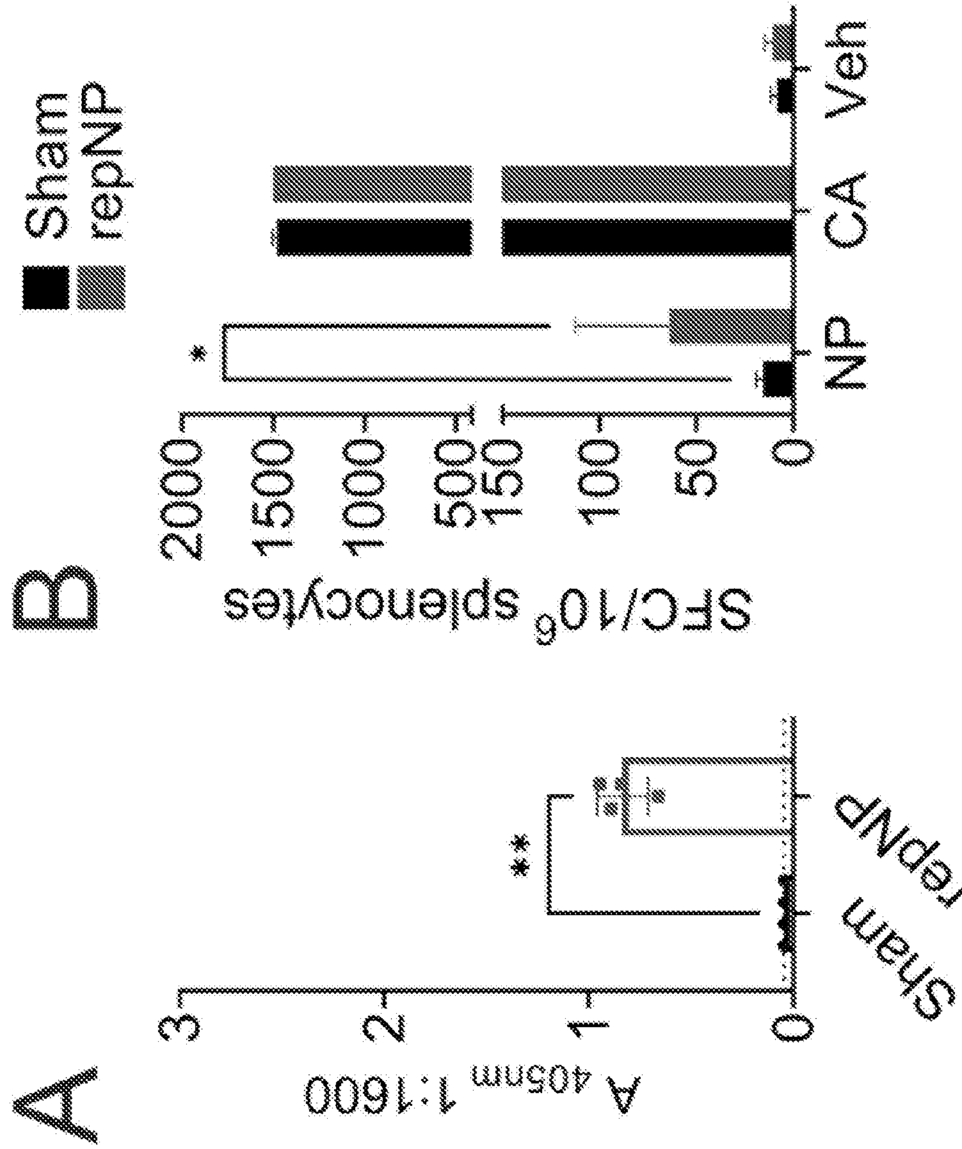


FIG. 7E

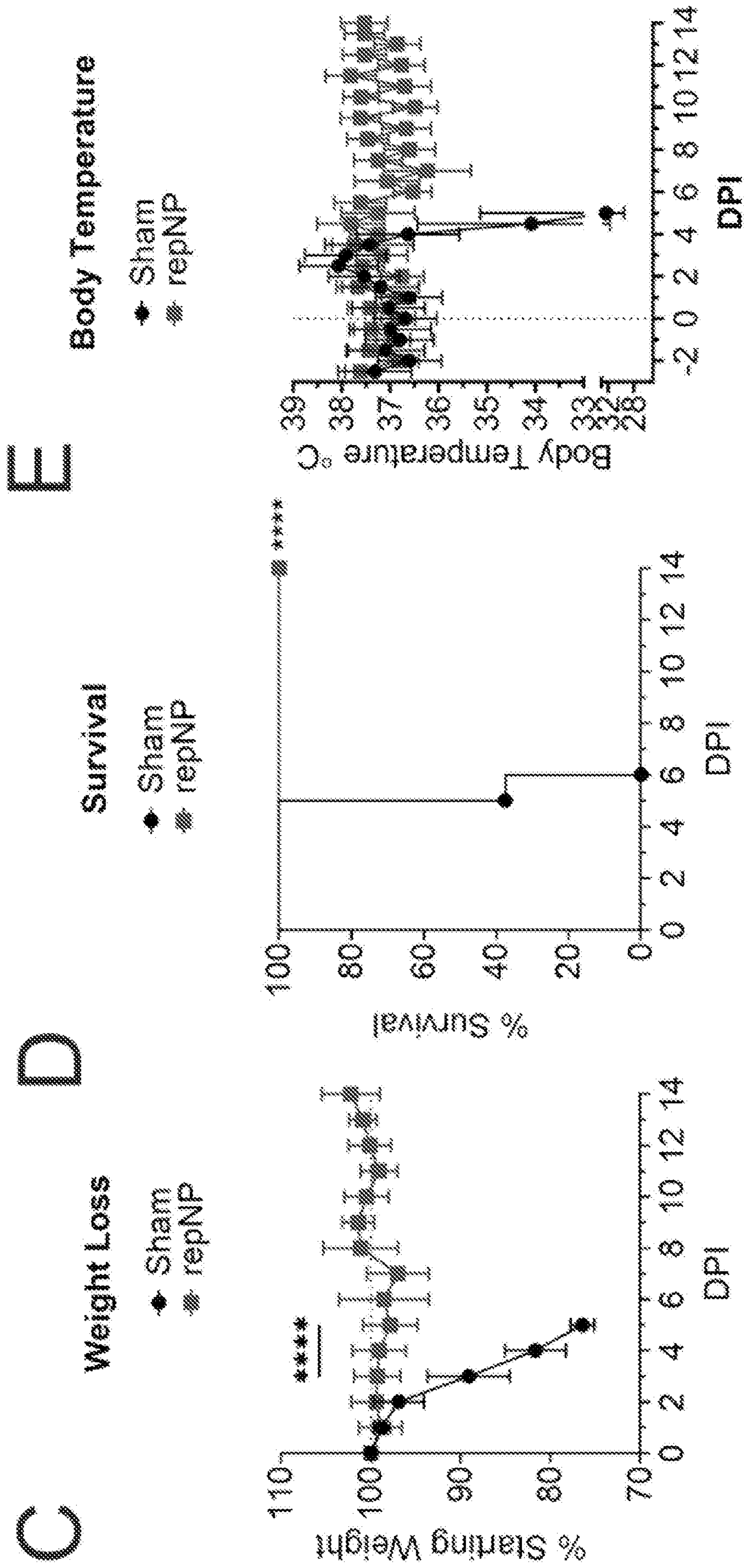




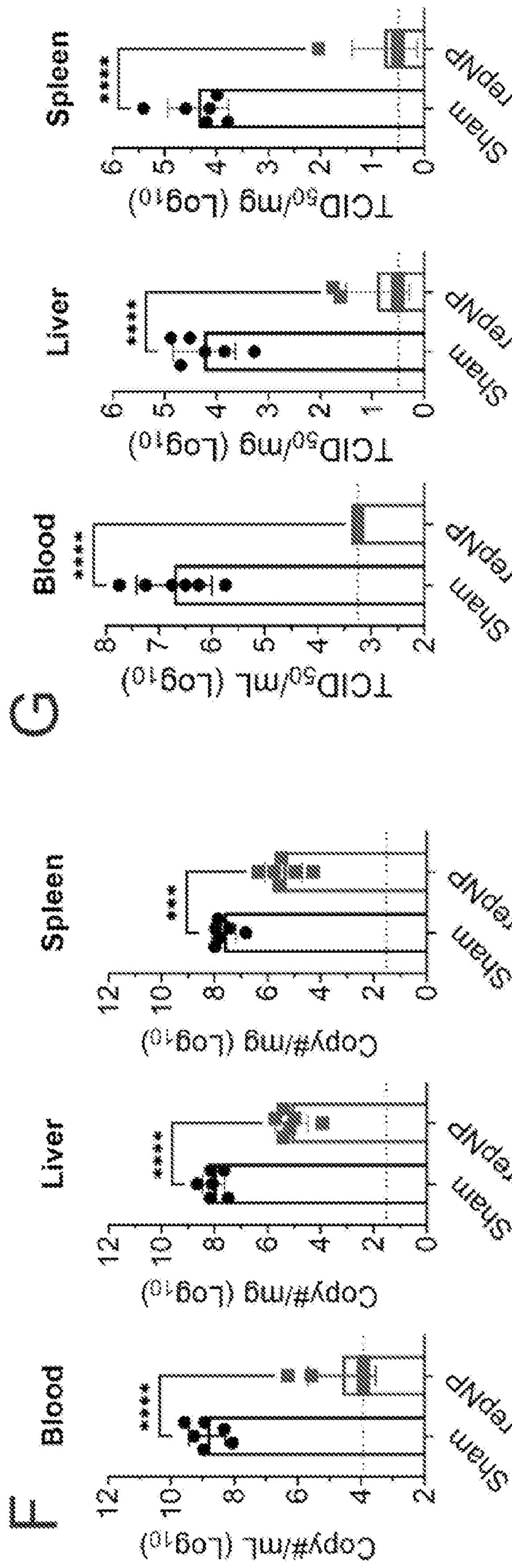
FIGS.8A & 8B



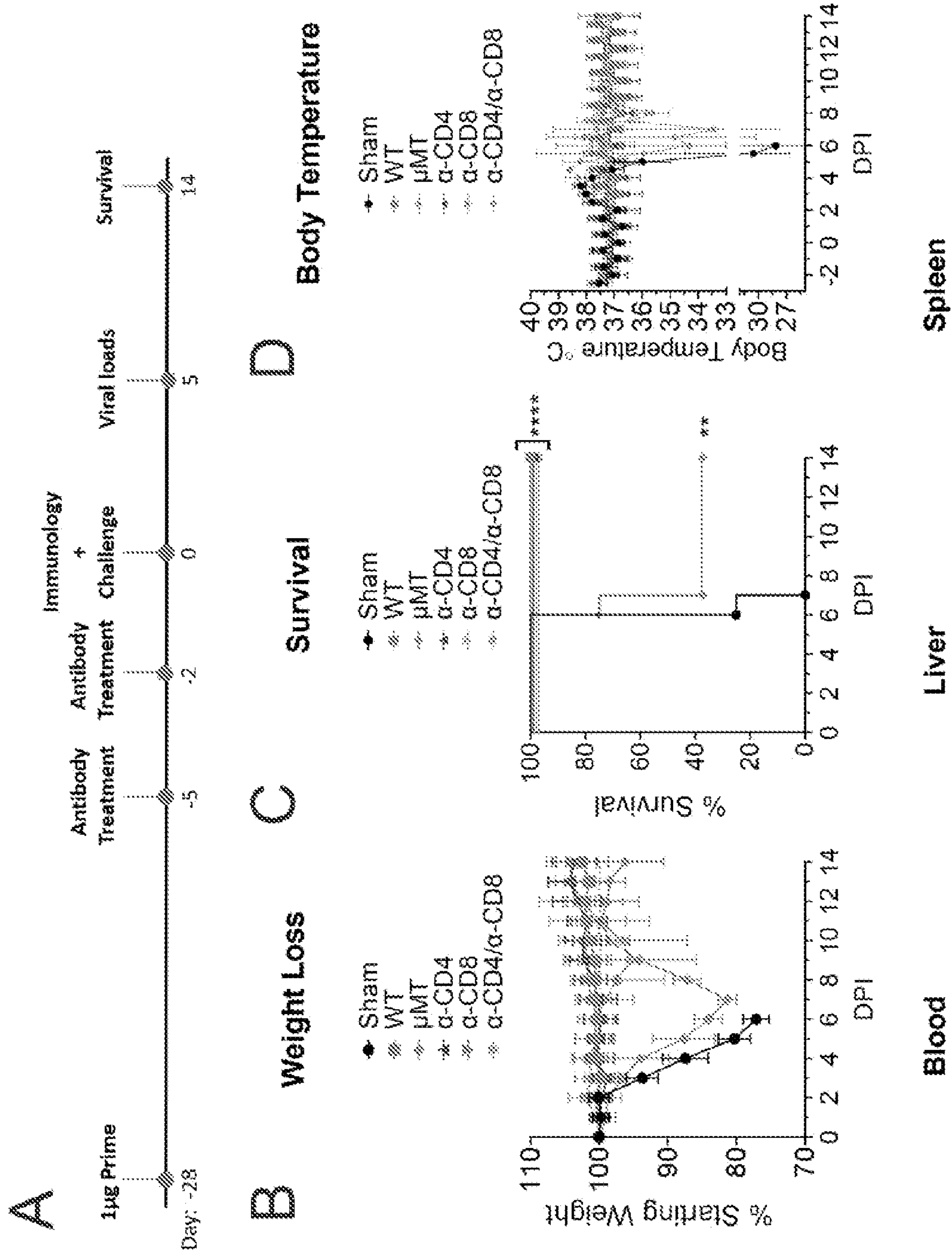
FIGS. 9A & 9B



FIGS. 9C-9E



FIGS. 9F & 9G



FIGS. 10A-10D

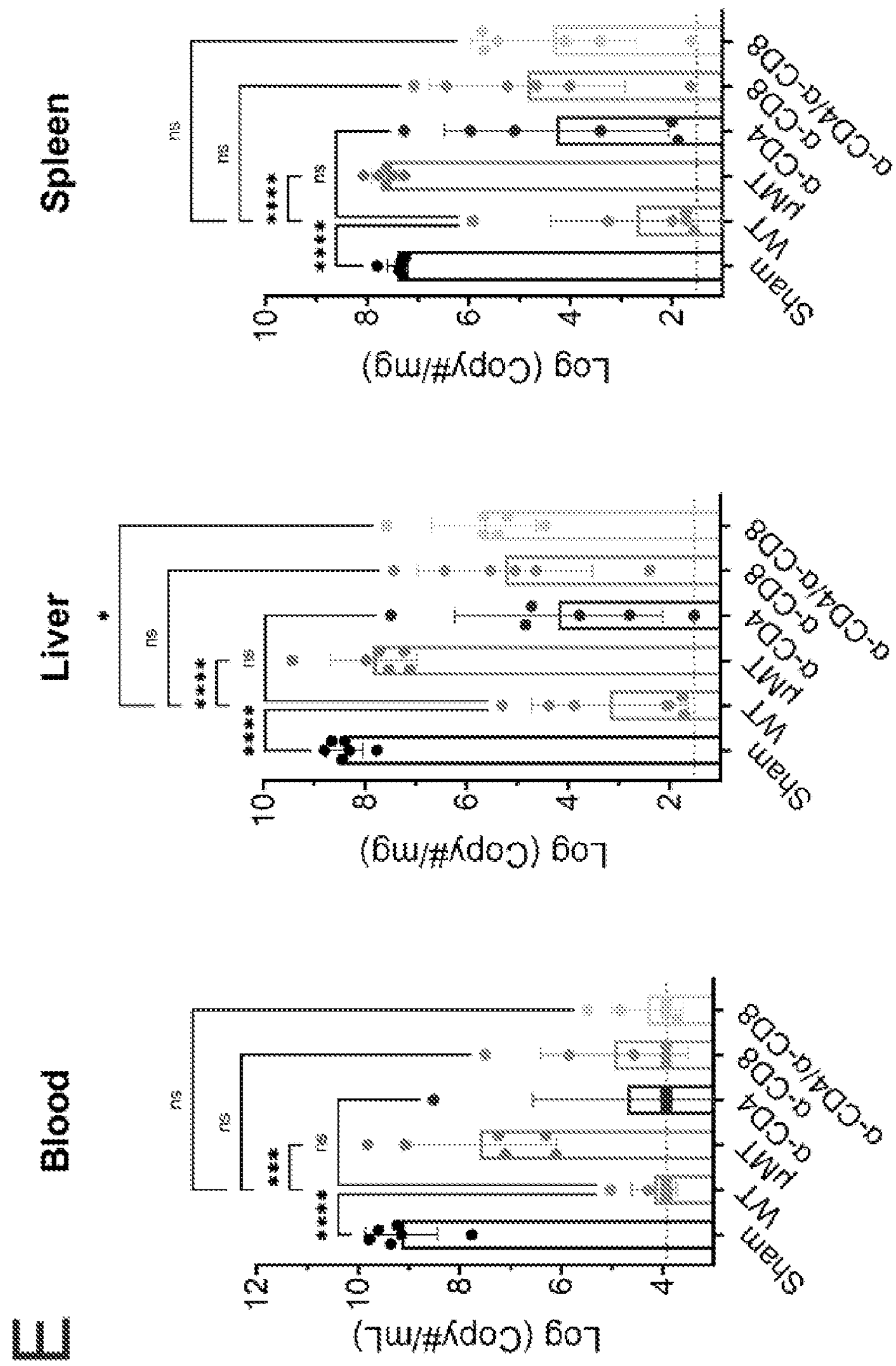
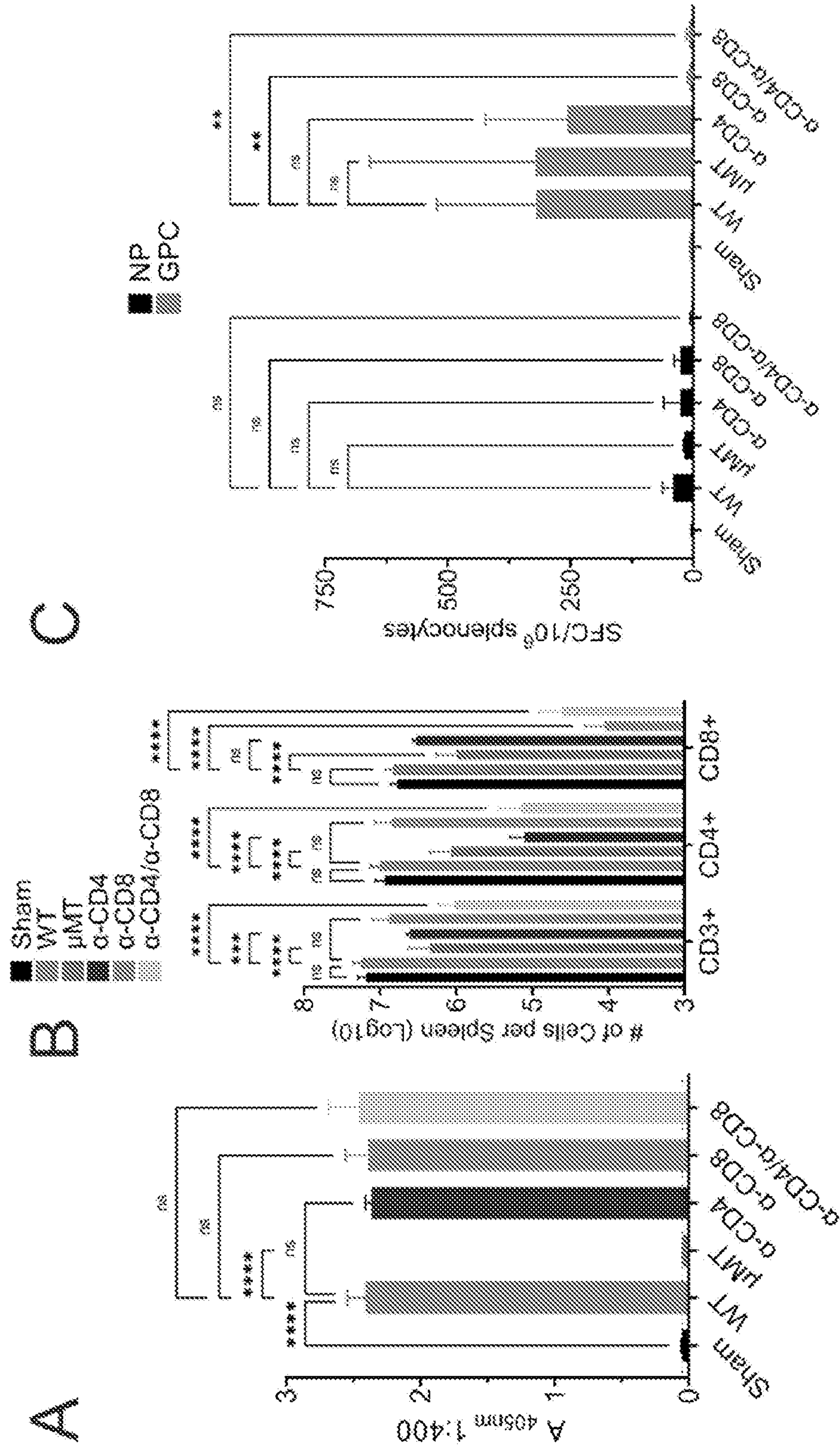
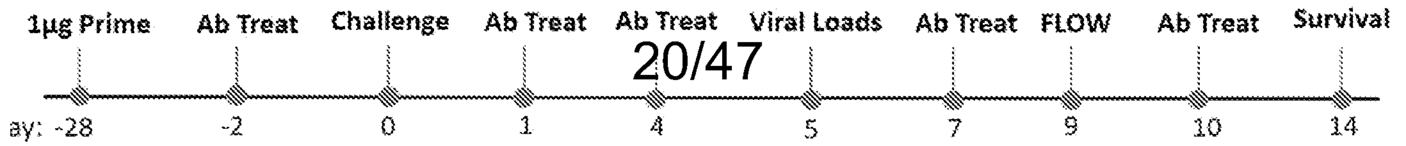
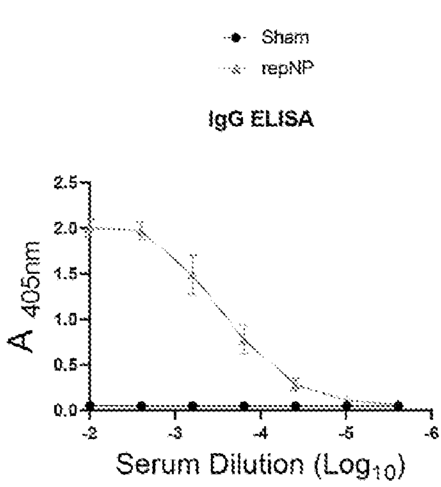


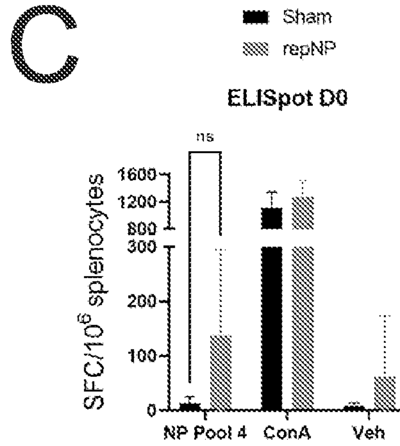
FIG. 10E

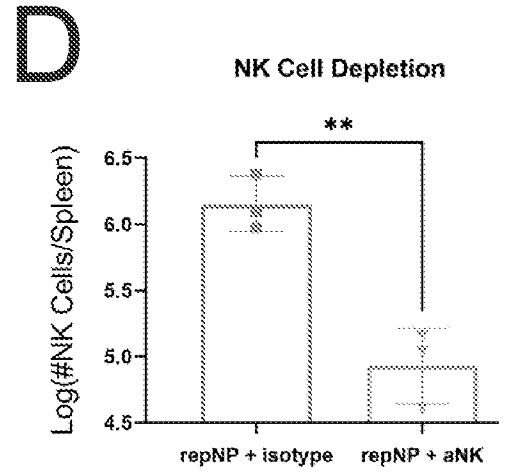


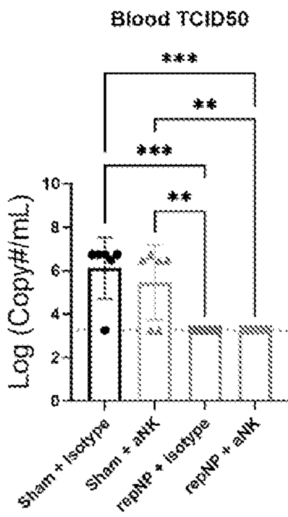
FIGS. 11A-11C

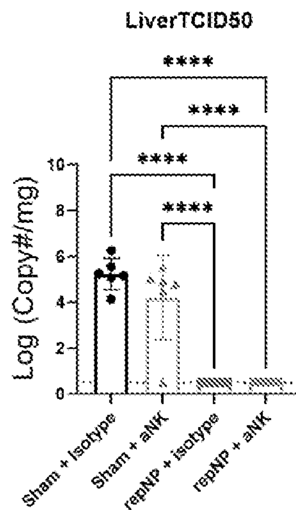


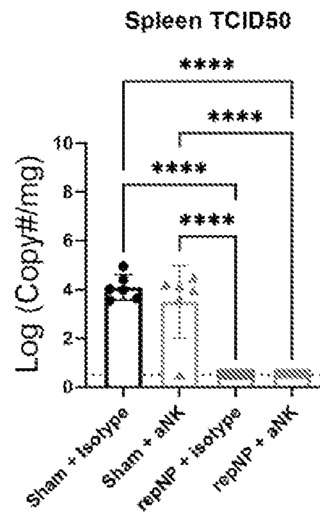


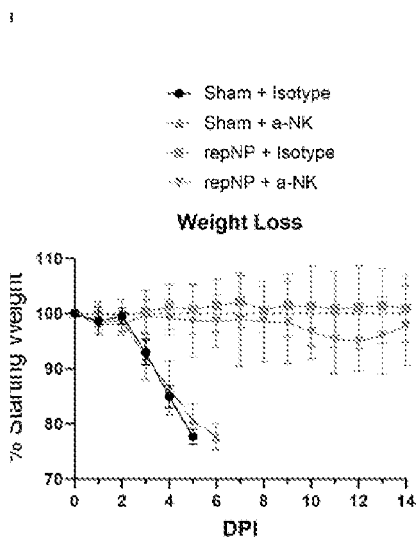


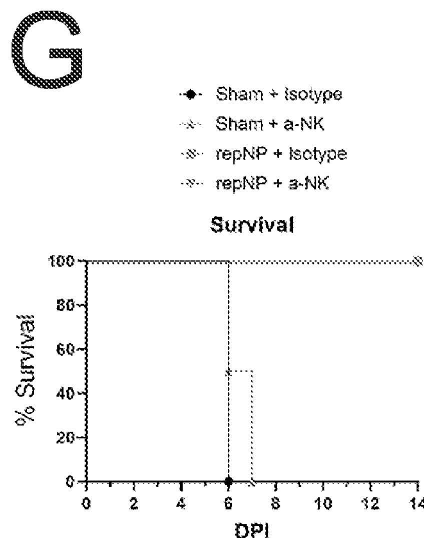


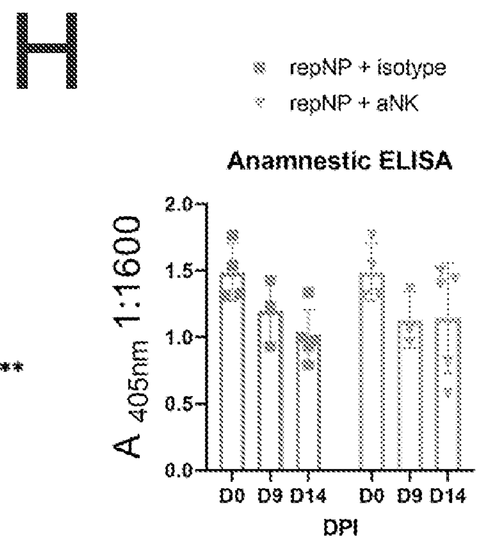












FIGS. 12A-12H

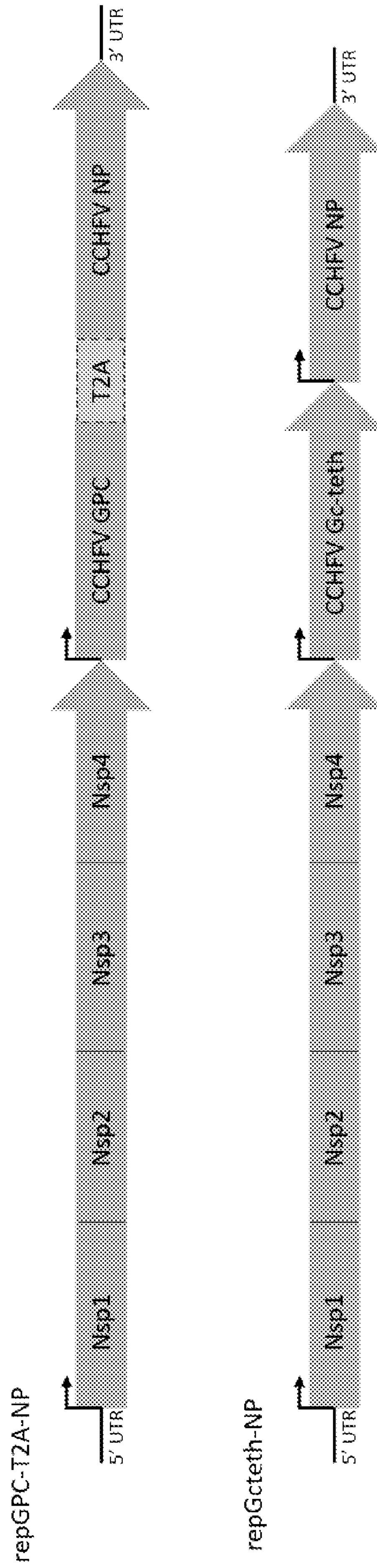
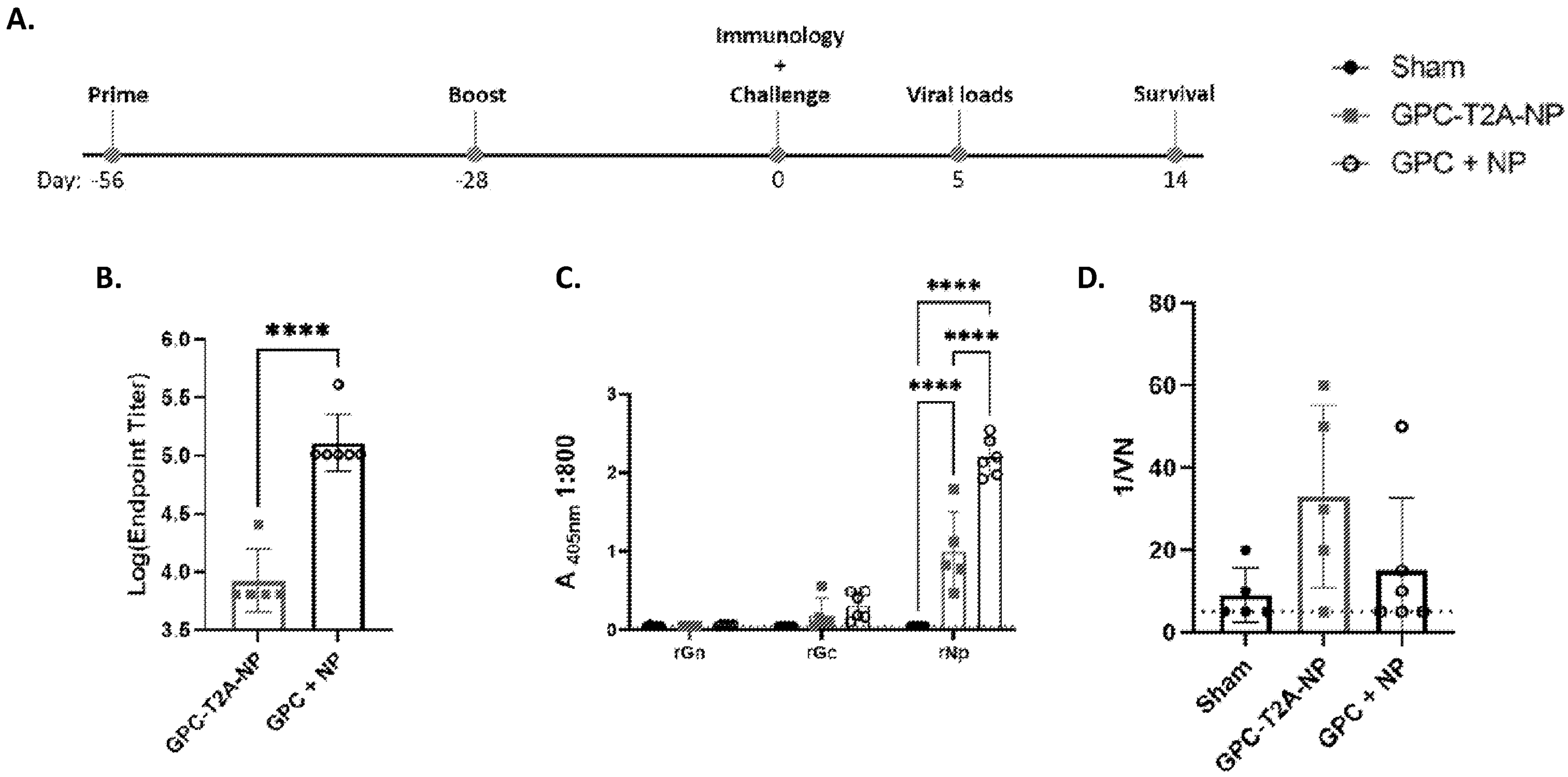
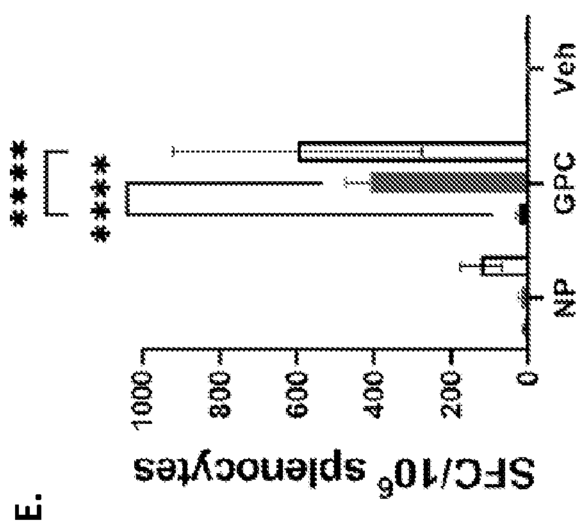


FIG13

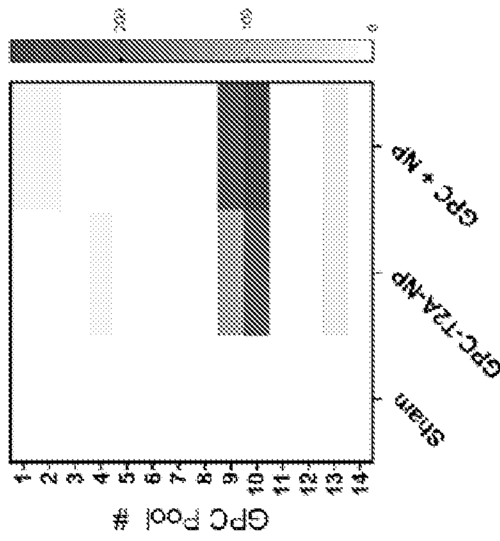


FIGS. 14A-D

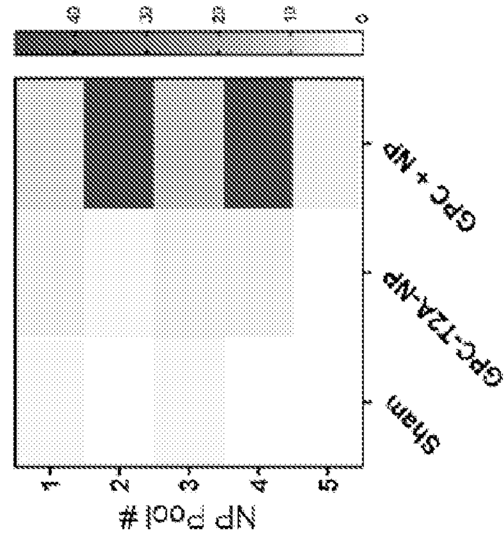
E.



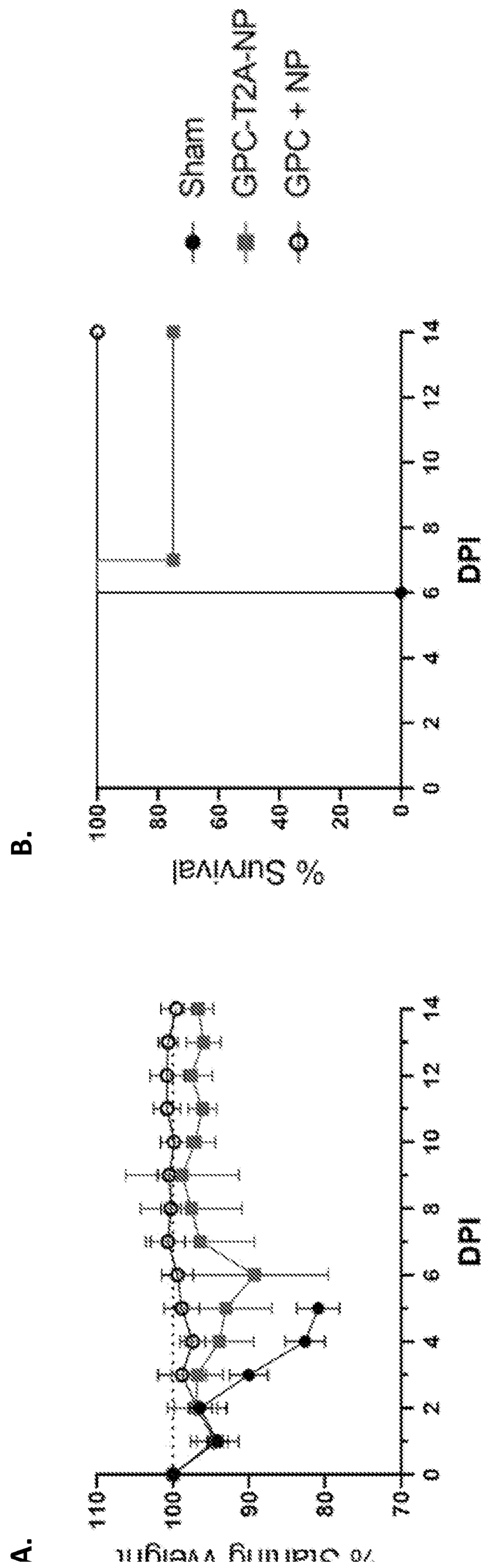
F.



G.



FIGS. 14E-G



FIGS. 15A & 15B

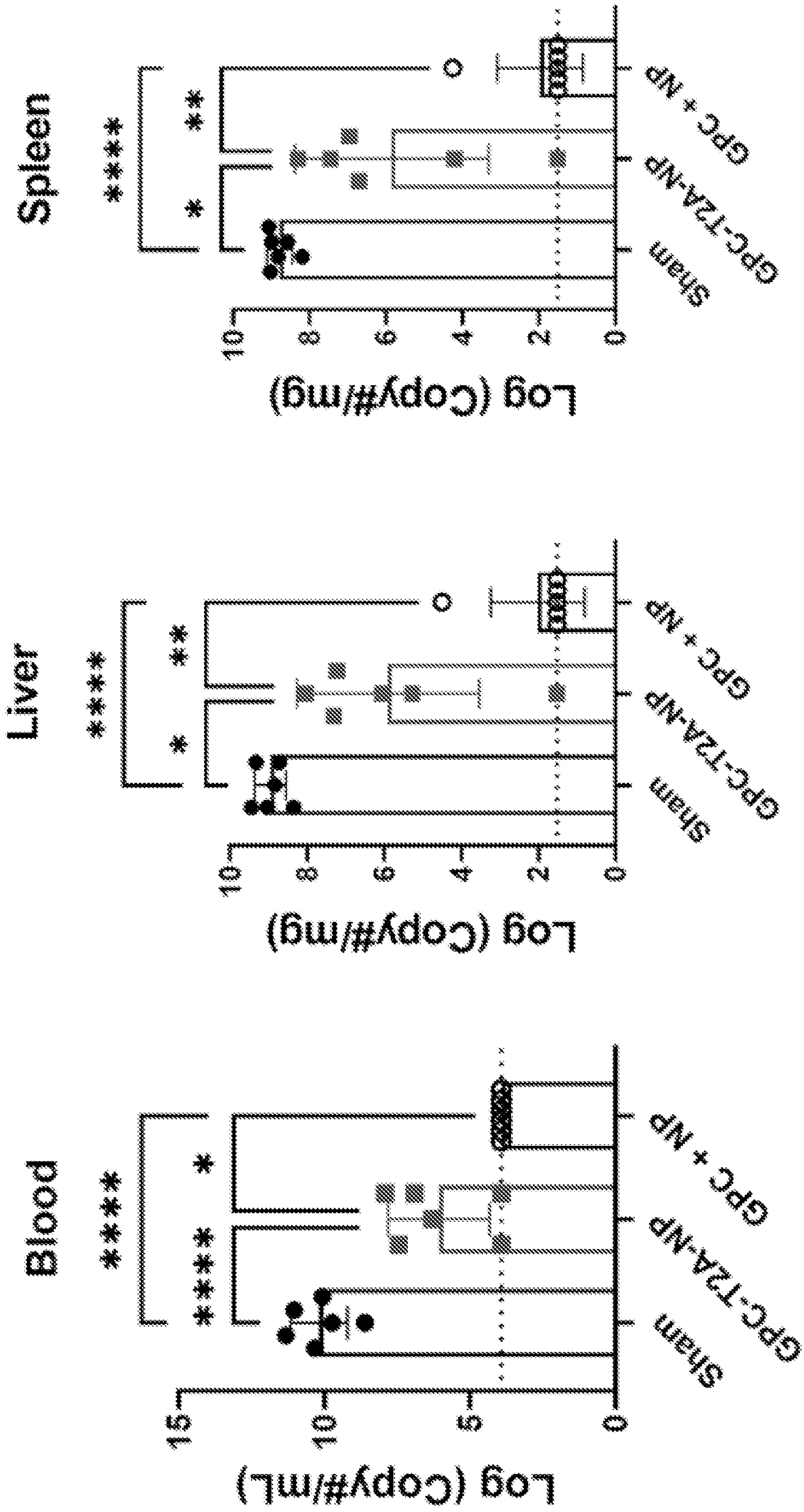


FIG.15C

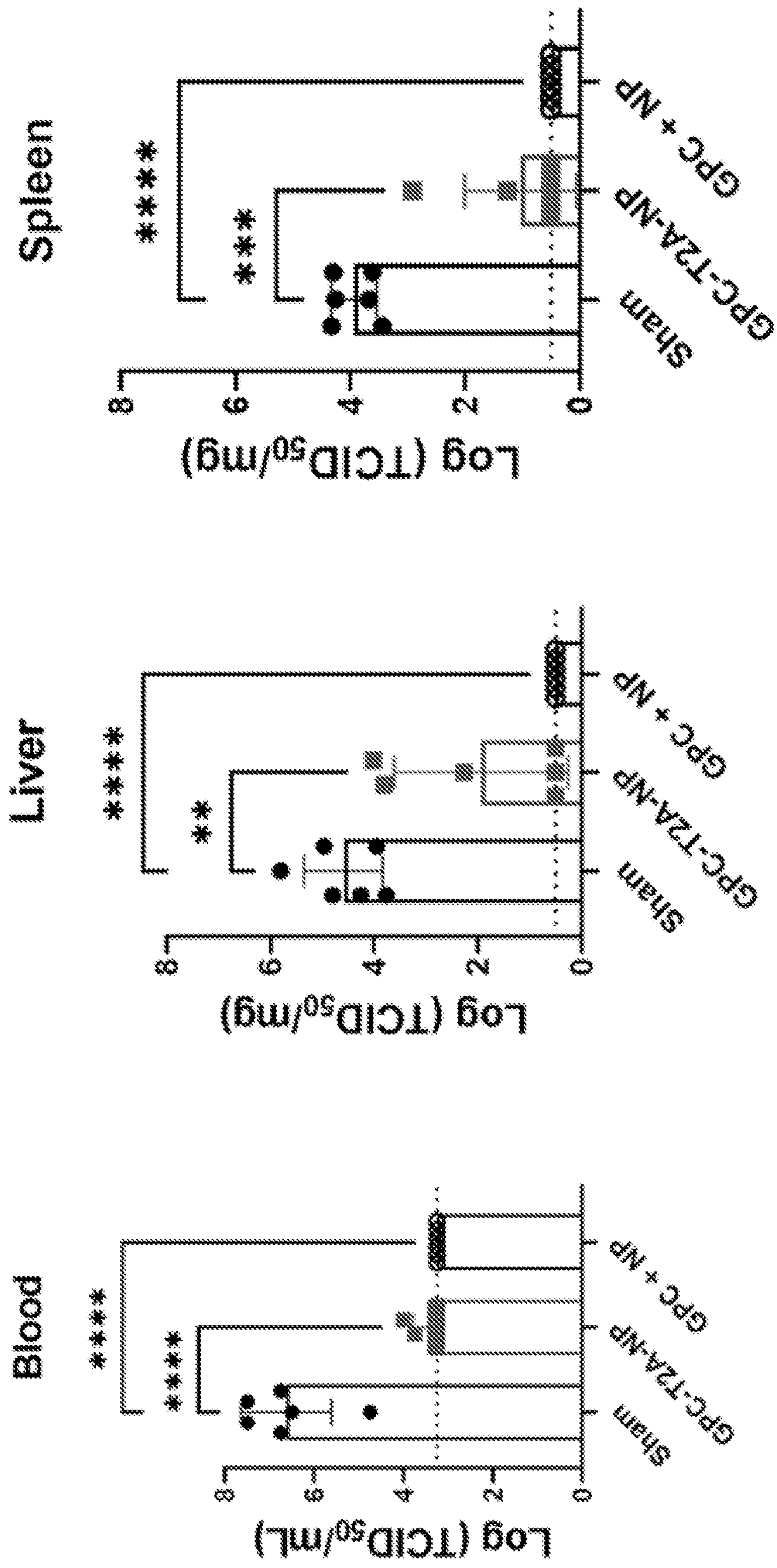


FIG.15D

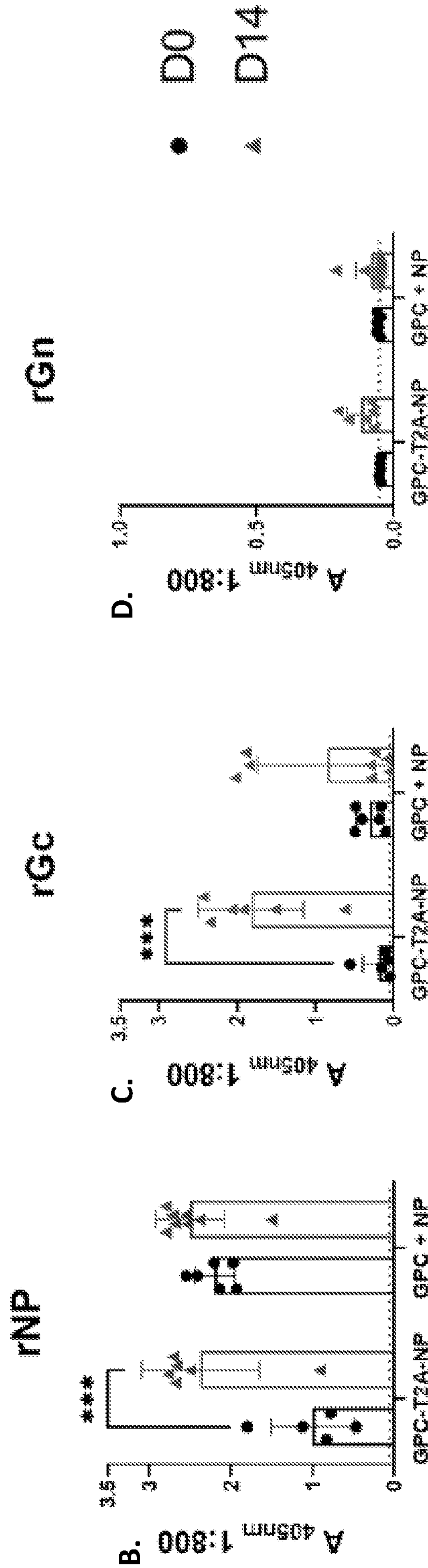


FIG. 16A

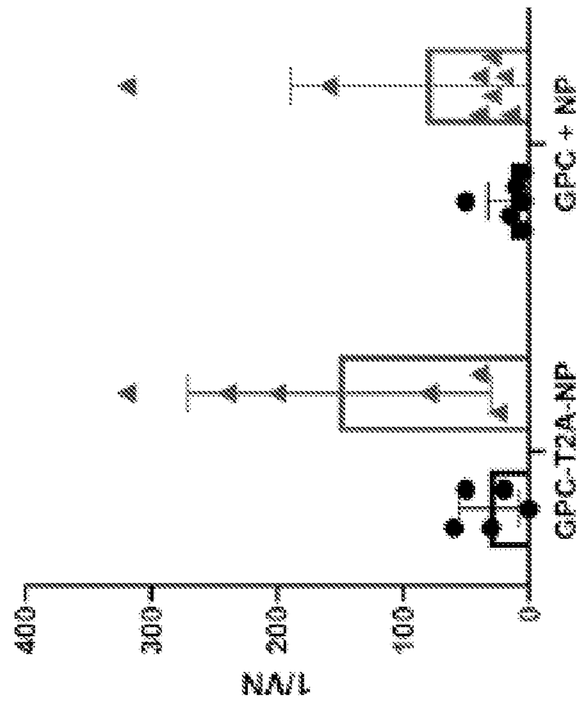
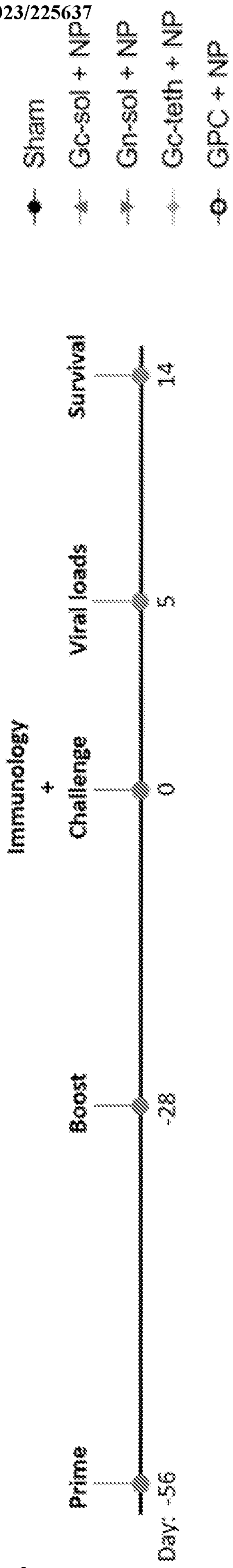
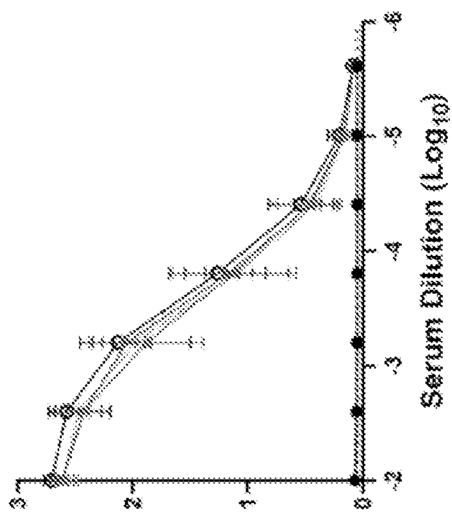
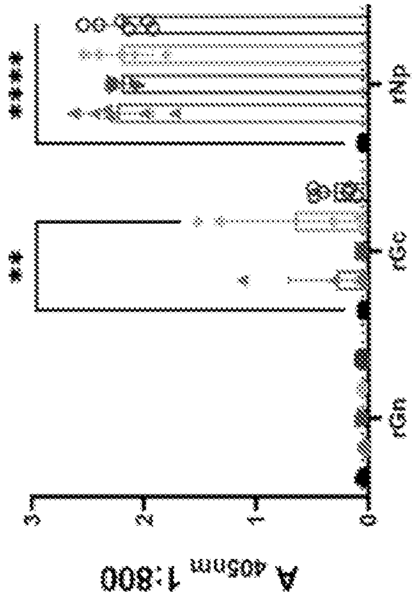
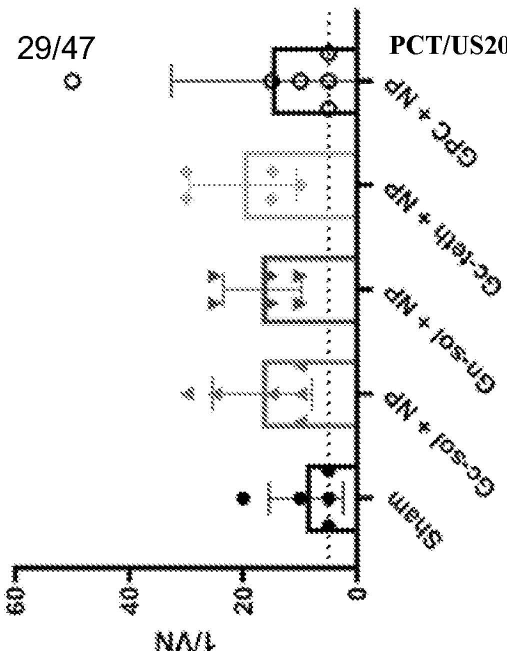


FIG. 16B

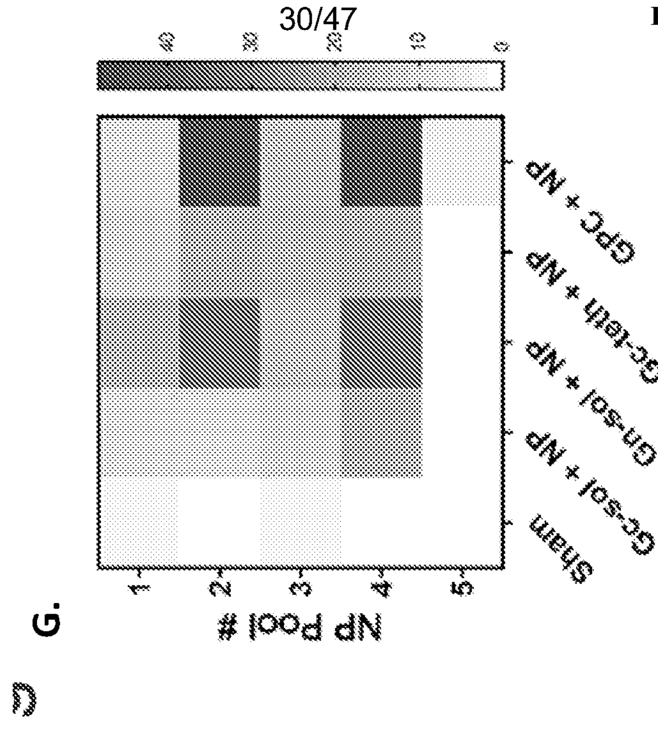




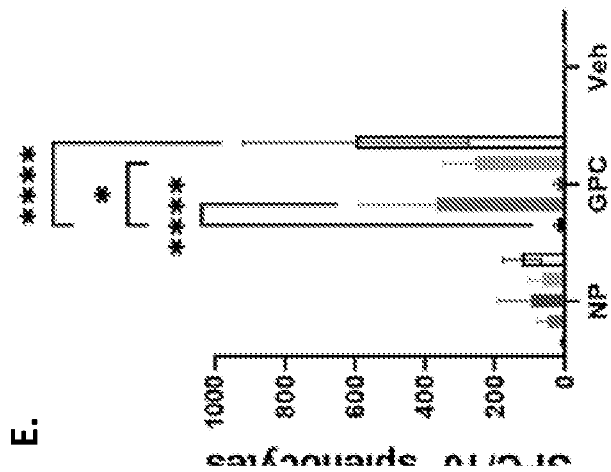




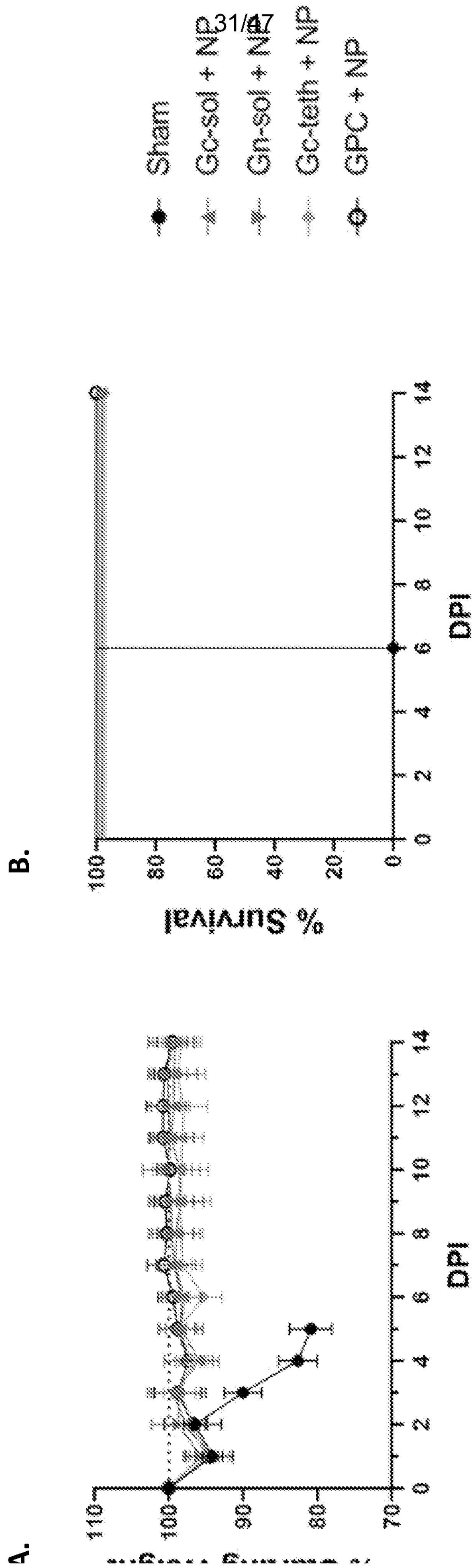
FIGS.17A-17D



F.
GPC Pool #
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Sham
Gc-sol + NP
Gn-sol + NP
Gc-teth + NP
GPC + NP
300
200
100
0



FIGS.17E-17G



FIGS. 18A & 18B

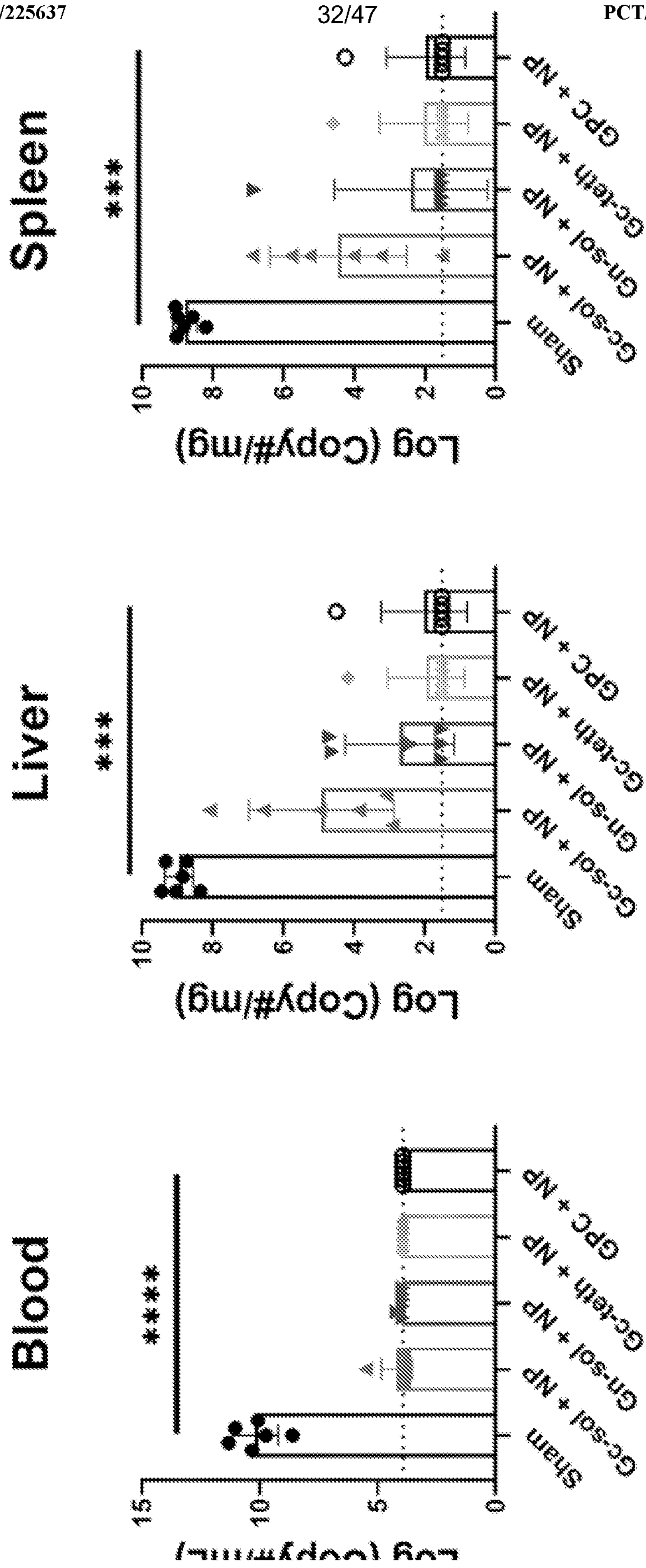


FIG. 18C

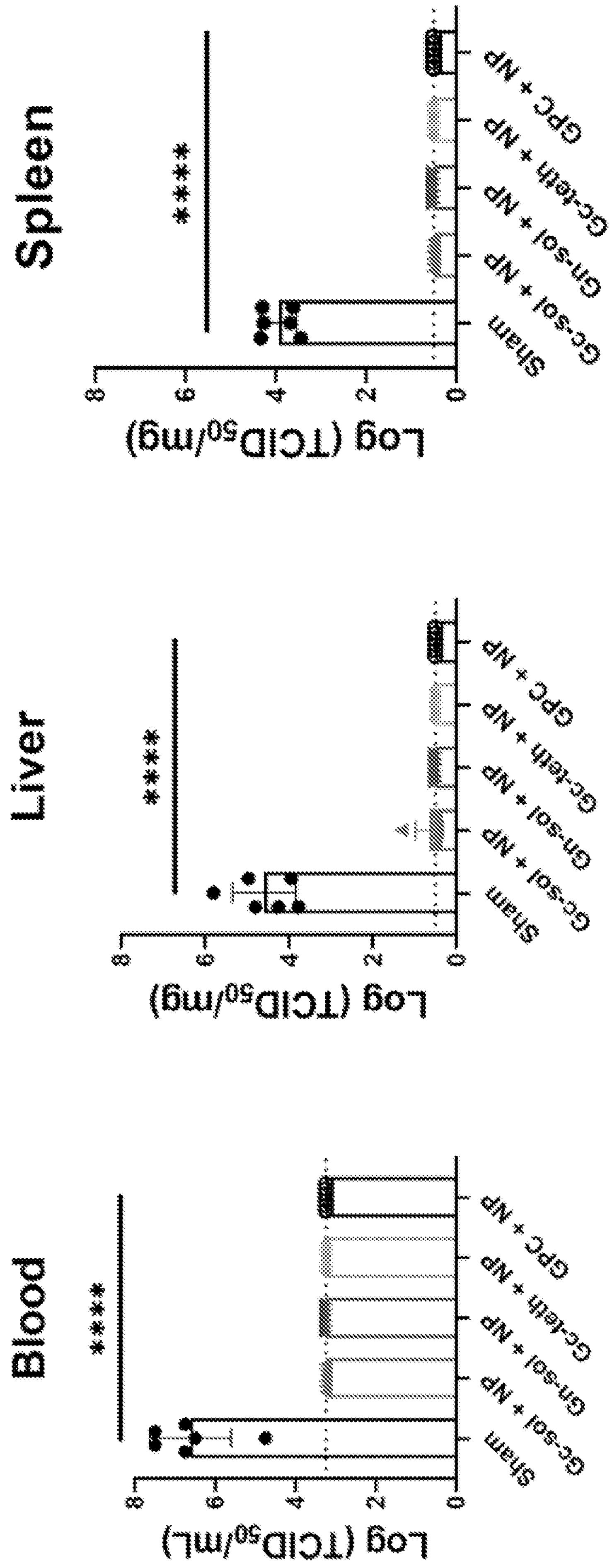


FIG. 18D

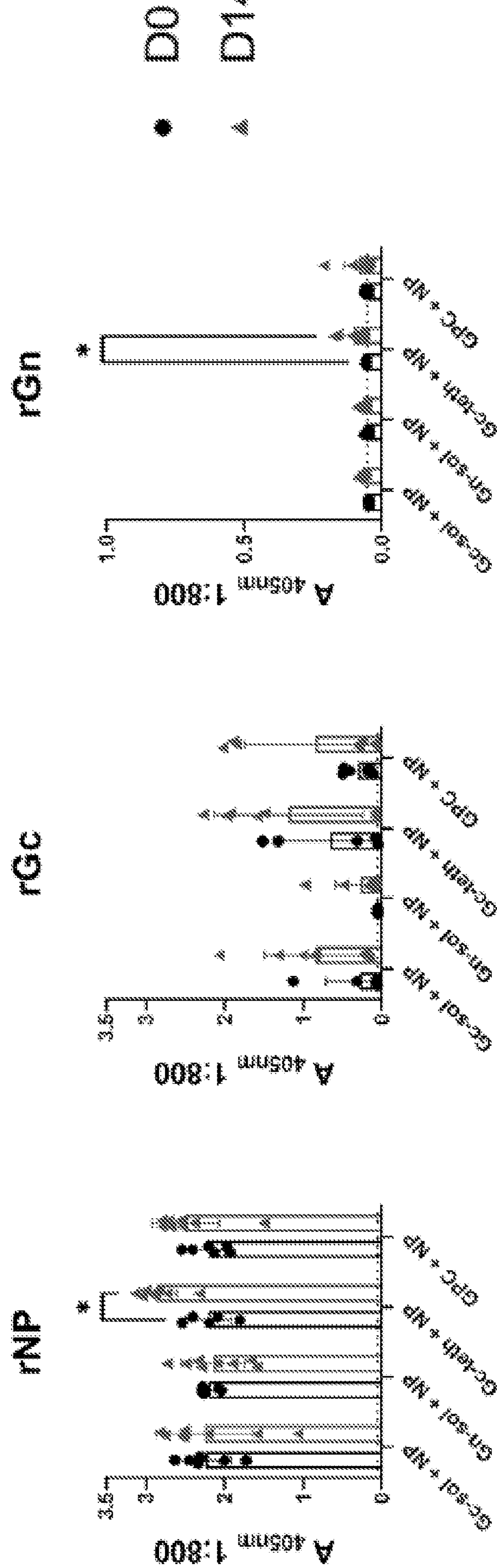


FIG.19A

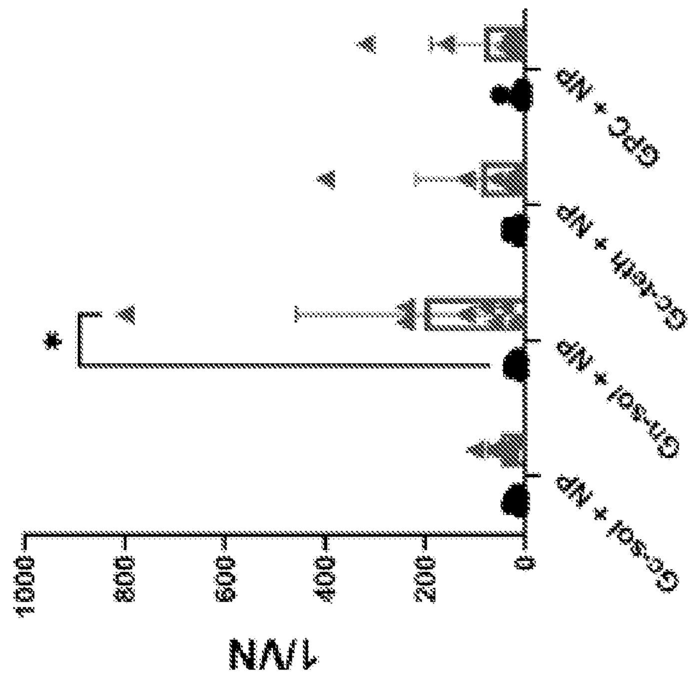
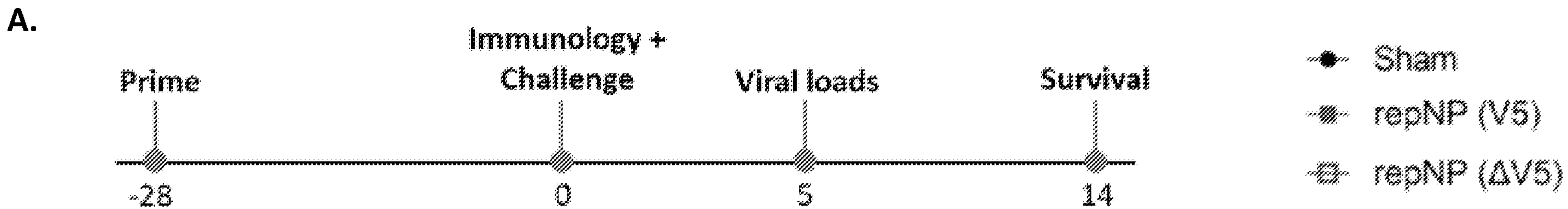
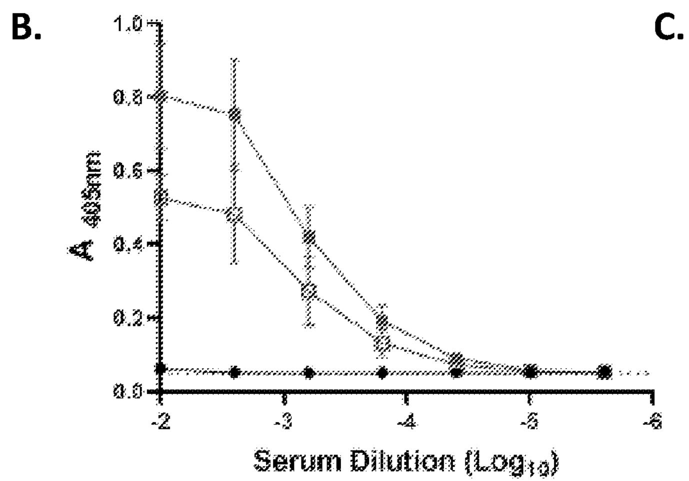


FIG.19B

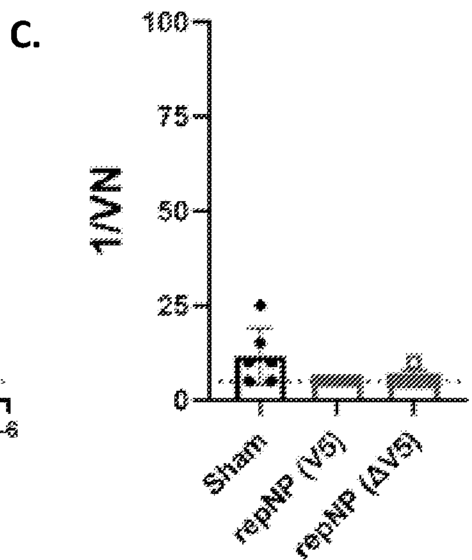
A.



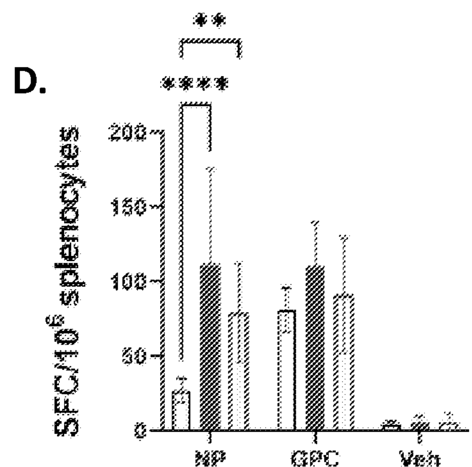
B.



C.



D.



FIGS.20A-20D

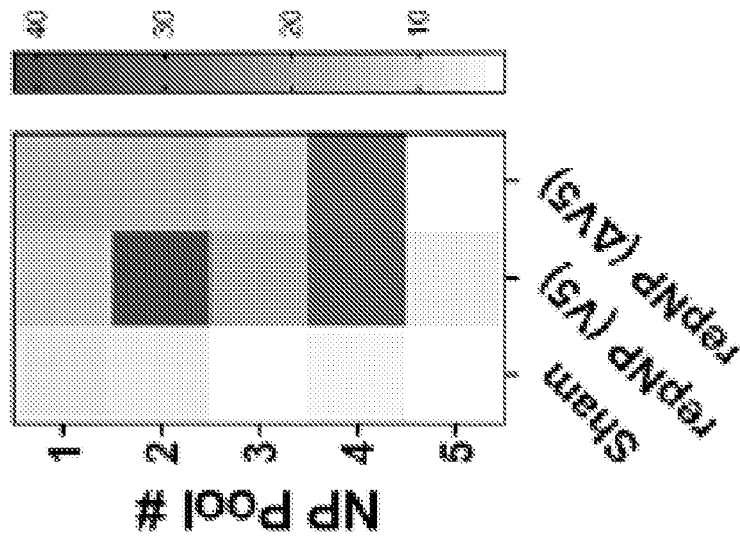
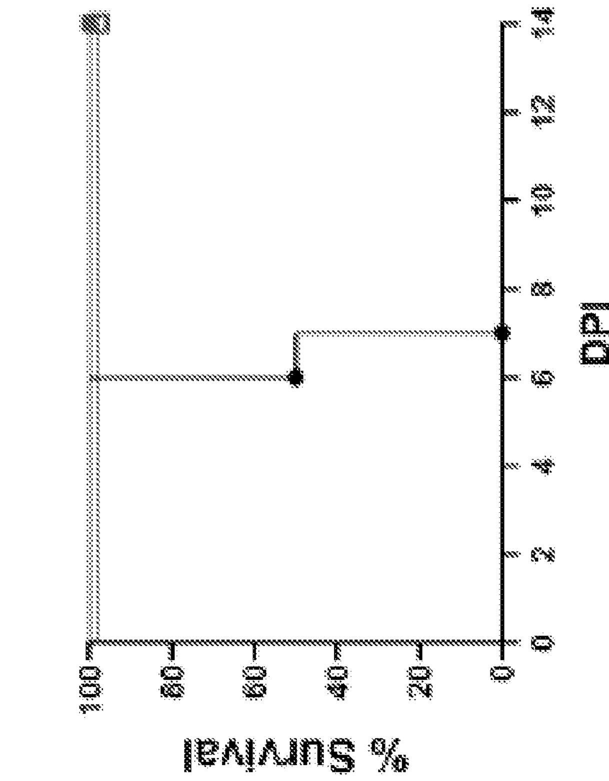
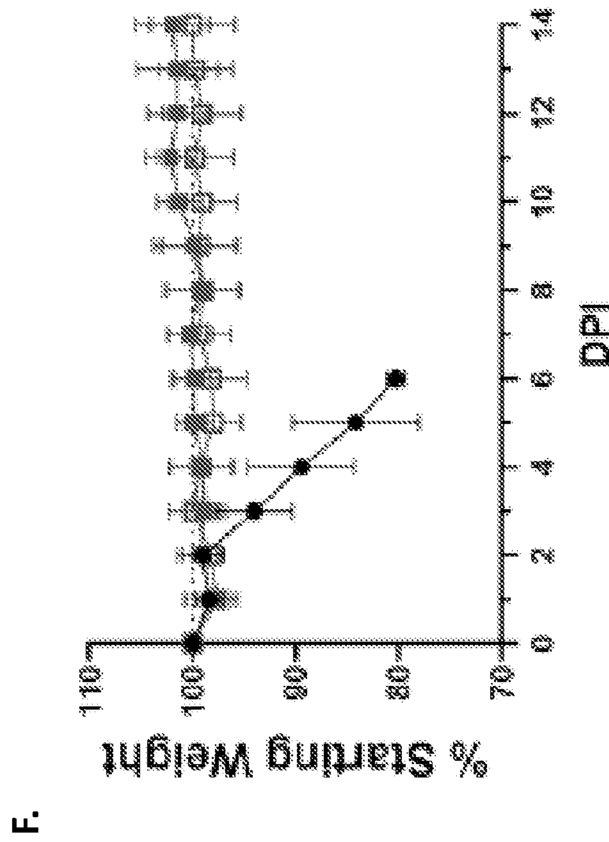


FIG.20E

G.



F.



FIGS.20F & 20G

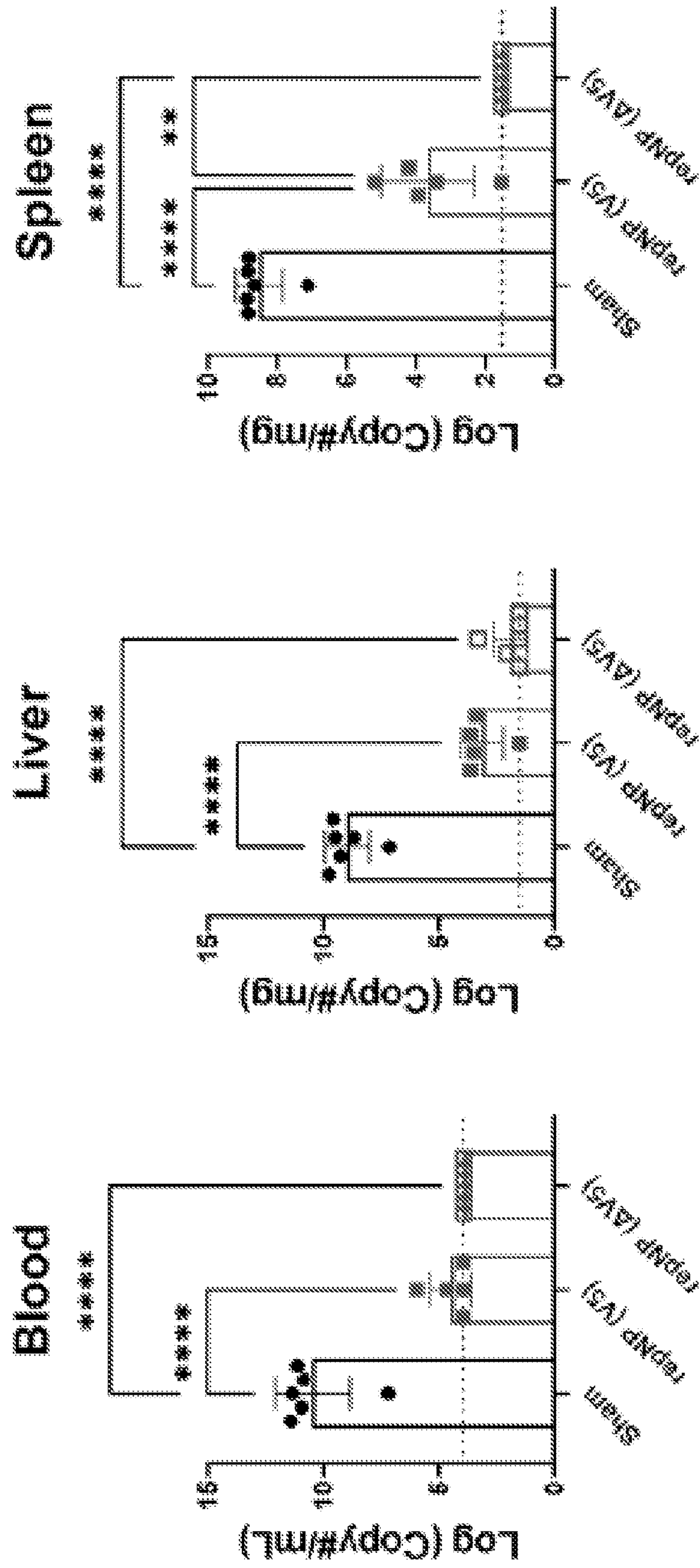


FIG.20H

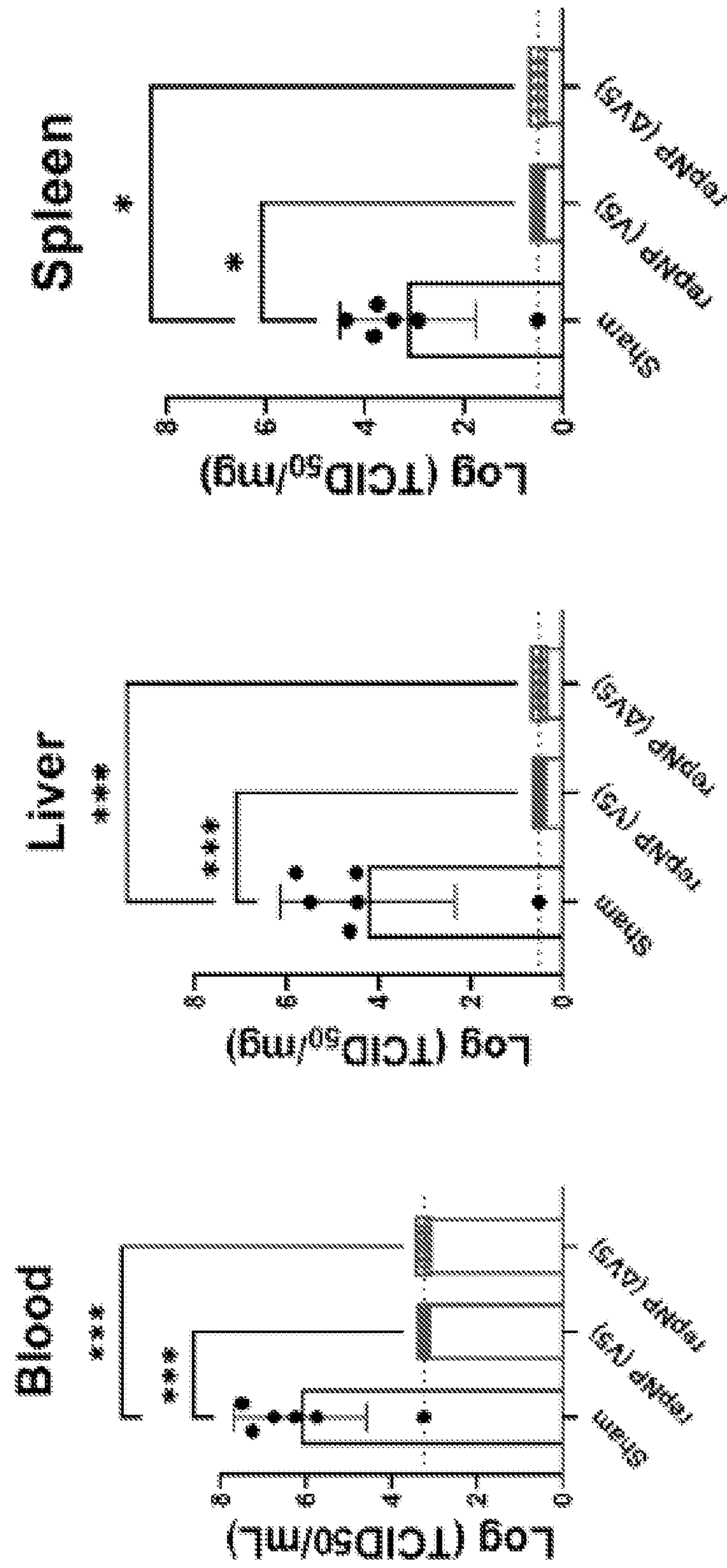
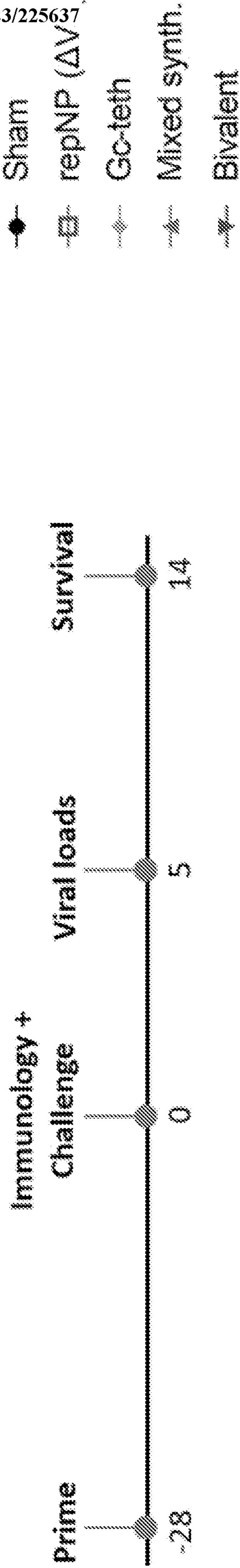
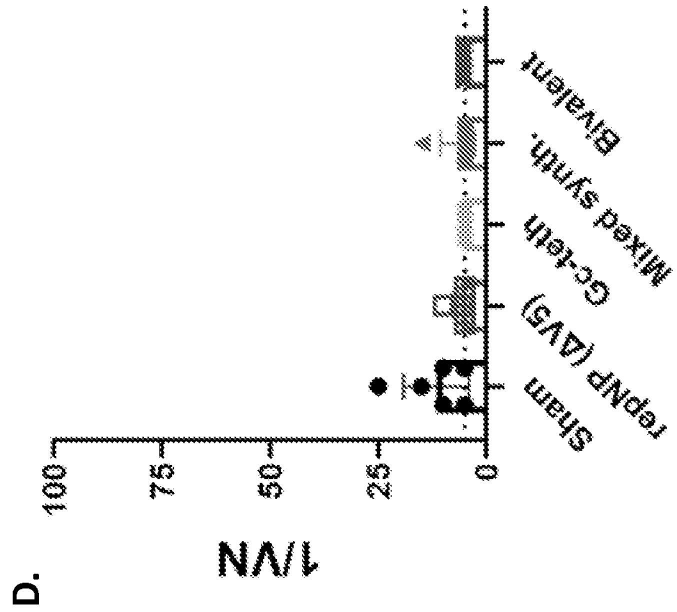
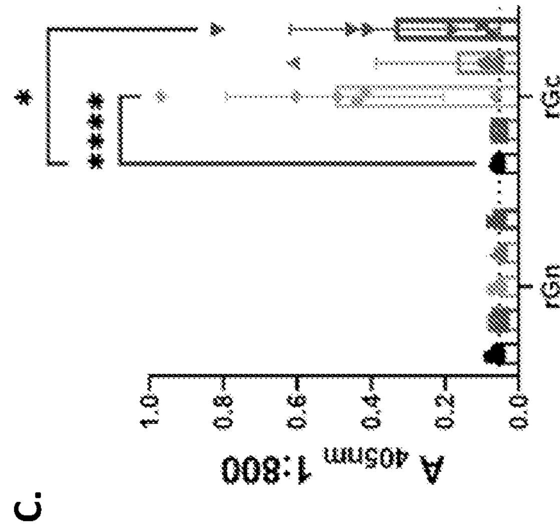


FIG.20I

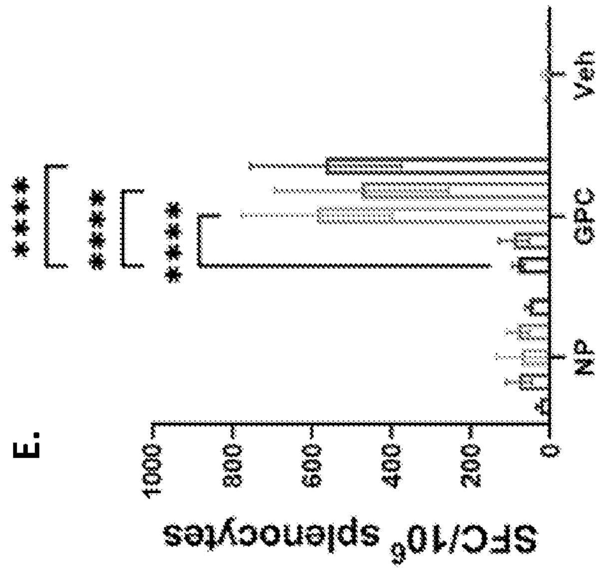




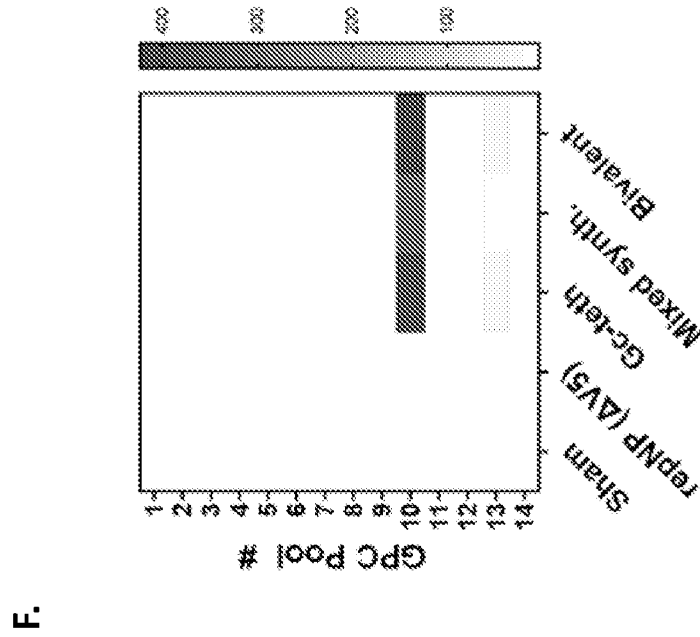


FIGS.21A-21D

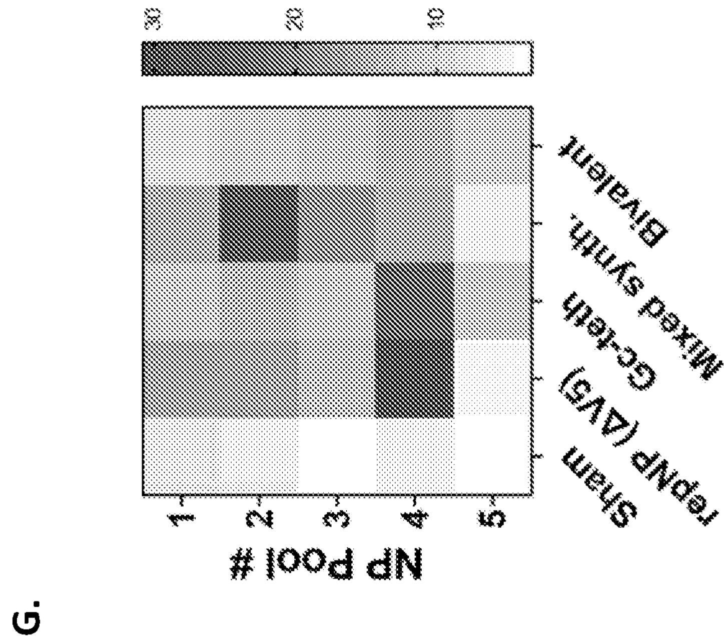
E.



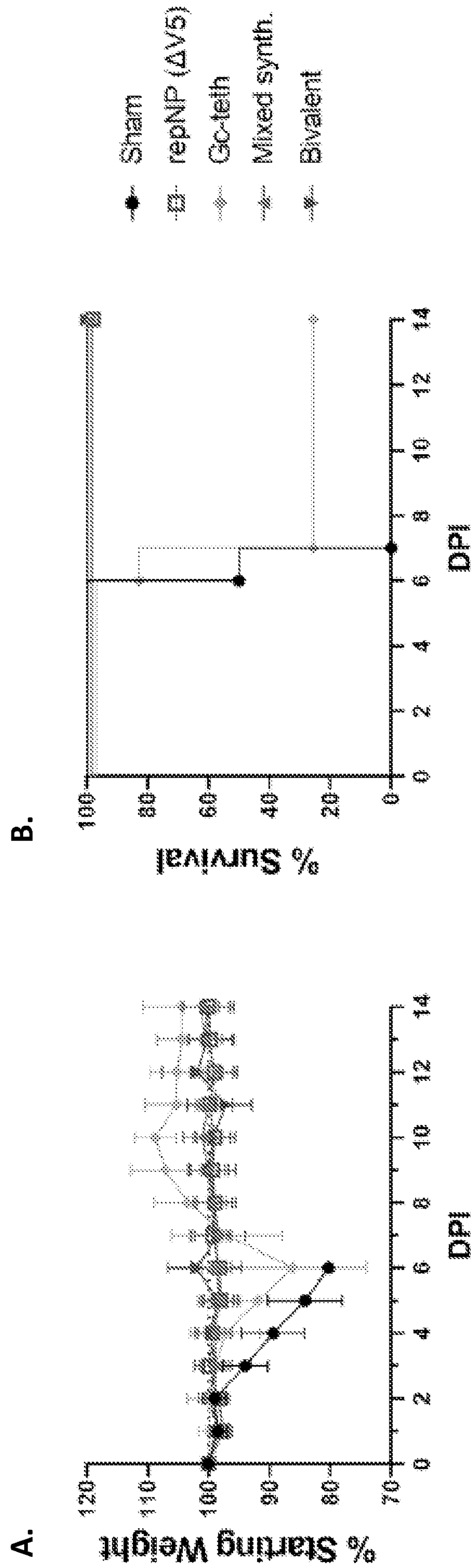
F.



G.



FIGS.21E-21G



FIGS. 22A & 22B

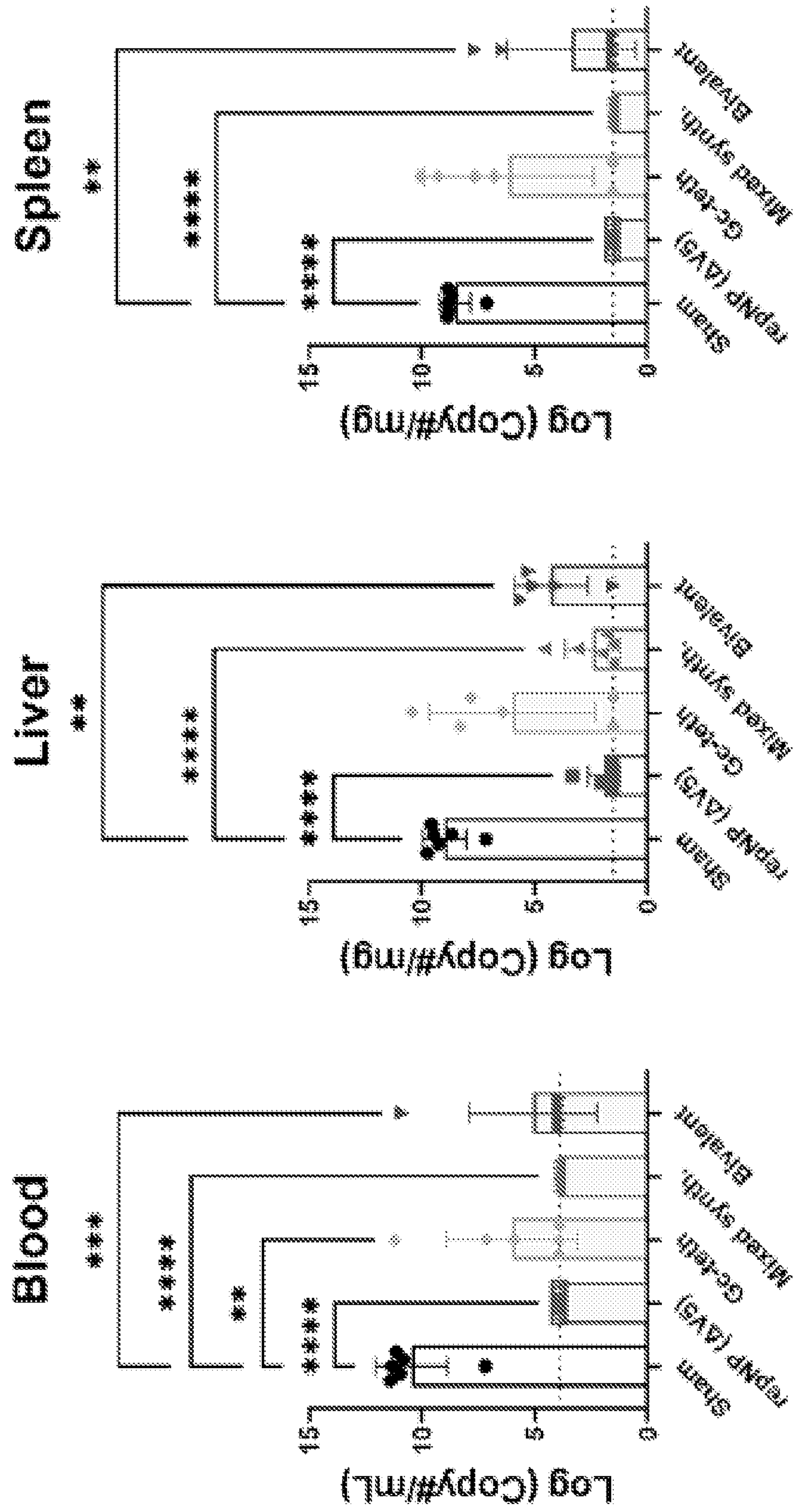


FIG.22C

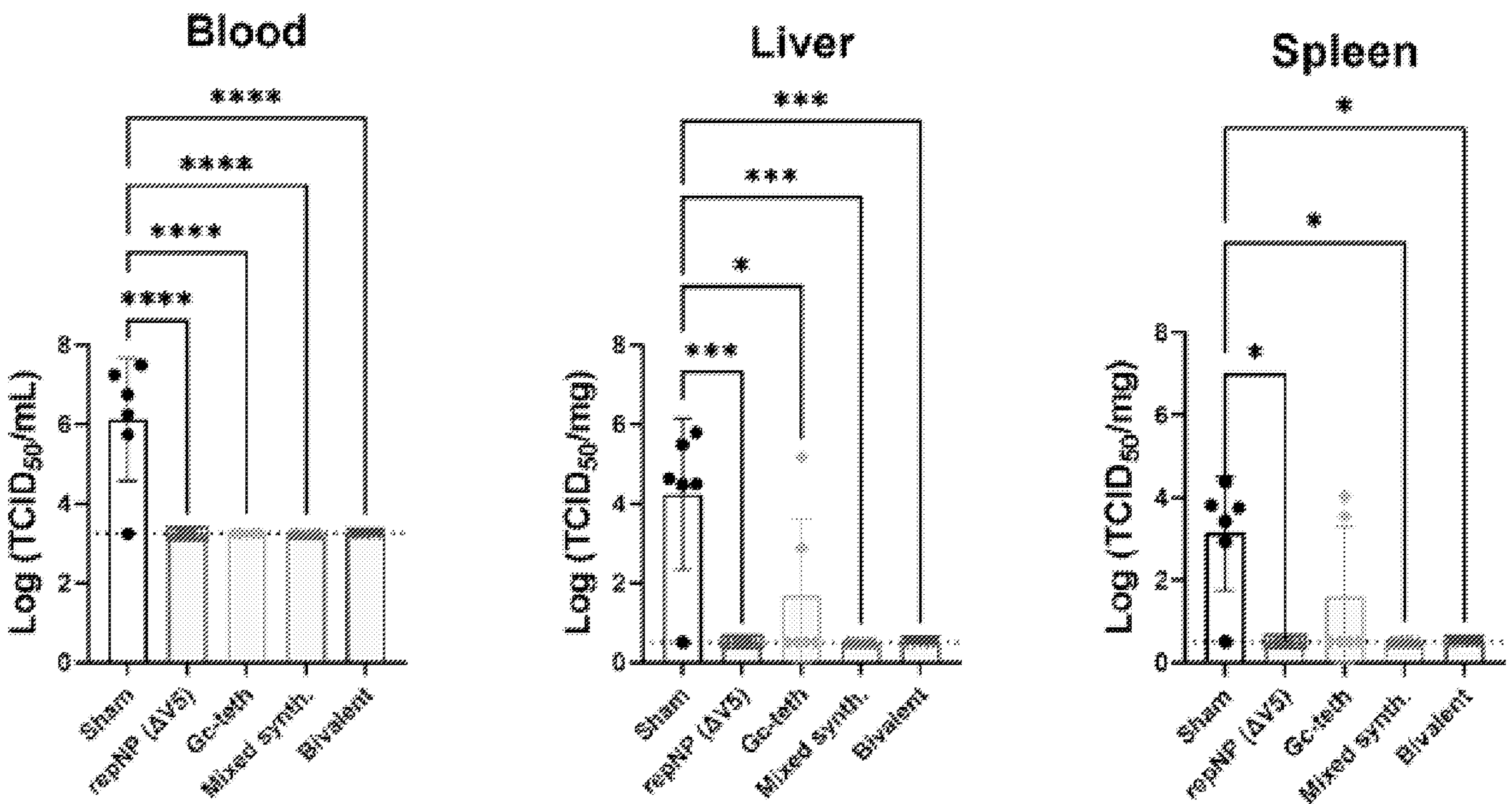


FIG.22D

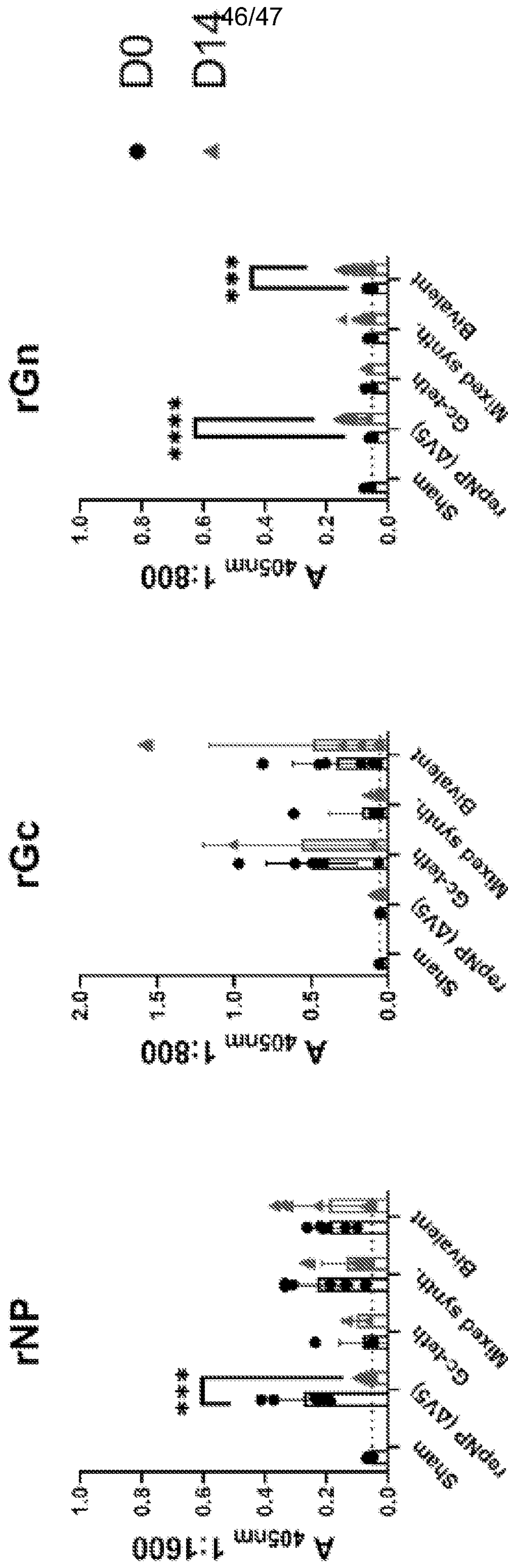


FIG.23A

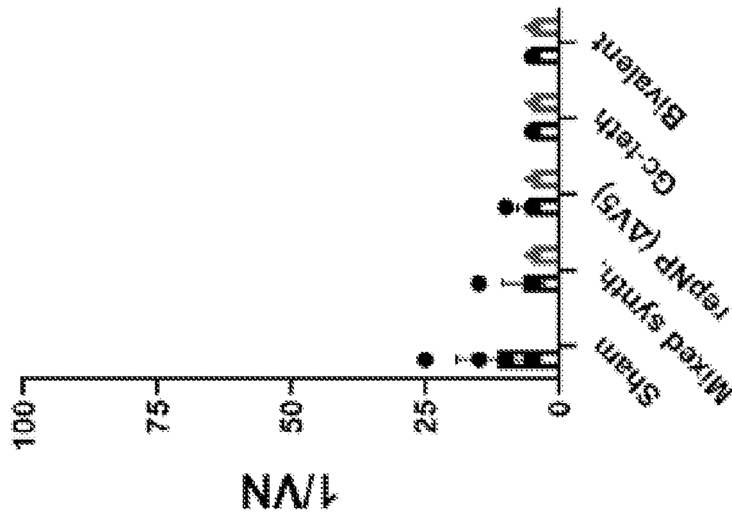


FIG.23B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/067226

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61P31/14
ADD.

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)
A61K A61P C12N

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/085660 A2 (ALPHAVAX INC [US]; SMITH JONATHAN F [US] ET AL.) 7 October 2004 (2004-10-07) page 12; claims 1,2,13,23,52 -----	1-14
A	HAWMAN DAVID W ET AL: "A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model", NATURE MICROBIOLOGY, vol. 6, no. 2, 30 November 2020 (2020-11-30), pages 187-195, XP037349615, DOI: 10.1038/S41564-020-00815-6 page 189 - page 191 -----	1-14

-/--

[x] Further documents are listed in the continuation of Box C.

[x] 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	Date of mailing of the international search report
14 September 2023	22/09/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mattugini, Nicola

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JESSE H. ERASMUS ET AL: "An Alphavirus -derived replicon RNA vaccine induces SARS-CoV-2 neutralizing antibody and T cell responses in mice and nonhuman primates", SCIENCE TRANSLATIONAL MEDICINE, vol. 12, no. 555, 20 July 2020 (2020-07-20), page eabc9396, XP055743966, ISSN: 1946-6234, DOI: 10.1126/scitranslmed.abc9396 page 2; figure 1a -----	1-14
A	SHRIVASTAVA NEHA ET AL: "Identification of functional epitopes of structural proteins and in-silico designing of dual acting multiepitope anti-tick vaccine against emerging Crimean-Congo hemorrhagic fever virus", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, ELSEVIER AMSTERDAM, NL, vol. 151, 30 May 2020 (2020-05-30), XP086205633, ISSN: 0928-0987, DOI: 10.1016/J.EJPS.2020.105396 [retrieved on 2020-05-30] the whole document -----	1-14
A	MARKO ZIVCEC ET AL: "Molecular Insights into Crimean-Congo Hemorrhagic Fever Virus", VIRUSES, vol. 8, no. 4, 21 April 2016 (2016-04-21), page 106, XP055676857, CH ISSN: 1999-4915, DOI: 10.3390/v8040106 the whole document -----	1-14
A	DOWALL STUART D ET AL: "Development of vaccines against Crimean-Congo haemorrhagic fever virus", VACCINE, vol. 35, no. 44, 4 July 2017 (2017-07-04), pages 6015-6023, XP085234197, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2017.05.031 the whole document -----	1-14
	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LEVENTHAL SHANNA S. ET AL: "Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge", EBIOMEDICINE, vol. 82, 27 July 2022 (2022-07-27), page 104188, XP93081863, NL ISSN: 2352-3964, DOI: 10.1016/j.ebiom.2022.104188 the whole document -----	1-14

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/067226

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

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

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/067226

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004085660 A2	07-10-2004	AU 2004223477 A1	07-10-2004
		BR PI0408424 A	21-03-2006
		CA 2518546 A1	07-10-2004
		CN 1791678 A	21-06-2006
		DK 1608762 T3	07-04-2014
		EP 1608762 A2	28-12-2005
		ES 2453344 T3	07-04-2014
		HK 1084692 A1	04-08-2006
		IL 170607 A	27-09-2011
		JP 5016305 B2	05-09-2012
		JP 5572144 B2	13-08-2014
		JP 2006520602 A	14-09-2006
		JP 2012050450 A	15-03-2012
		KR 20060006780 A	19-01-2006
		KR 20130008652 A	22-01-2013
		MX PA05010007 A	10-03-2006
		NZ 542353 A	31-07-2008
		PL 1608762 T3	30-06-2014
		US 2007166820 A1	19-07-2007
		WO 2004085660 A2	07-10-2004
		ZA 200507153 B	31-05-2006