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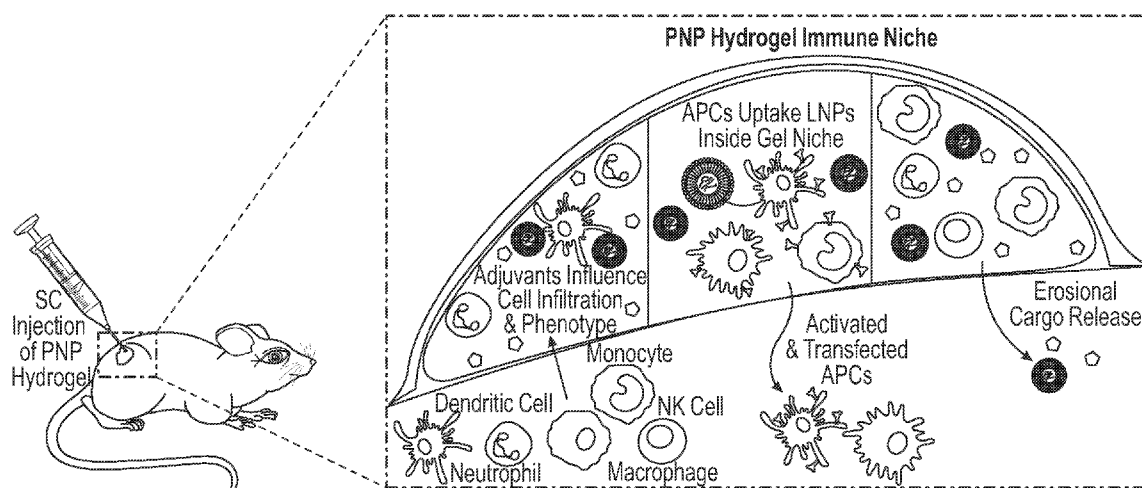


FIG. 5

(57) Abstract: Compositions and methods for delivery of nucleic acid therapeutics are disclosed herein. In some embodiments, a composition for treating a subject includes a dynamic hydrogel, and a nucleic acid therapeutic encapsulated by the dynamic hydrogel. The dynamic hydrogel can include a polymer and a plurality of nanoparticles. The polymer can be non-covalently crosslinked with the plurality of nanoparticles.

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COMPOSITIONS AND METHODS FOR DELIVERY OF NUCLEIC ACID THERAPEUTICS

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 63/518,375, filed August 9, 2023, and U.S. Provisional Application No. 63/569,662, filed March 25, 2024, the disclosures of each which are incorporated by reference herein in their entirety.

TECHNICAL FIELD

[0002] The present technology generally relates to drug delivery, and in particular, to compositions and methods for delivery of nucleic acid therapeutics.

BACKGROUND

[0003] Vaccines are among the most effective medical interventions in history. The eradication of smallpox, near eradication of poliomyelitis, and vast decreases in diphtheria, measles, and rubella are testaments to the ability of vaccines to transform disease burden worldwide. It is estimated that vaccines have prevented 103 million cases of disease in the United States since 1924, and save 2.5 million lives worldwide per year.

[0004] Innovations in vaccine design have the potential to improve current vaccines and pave the way for creating new vaccines. Traditional vaccine design is based on using attenuated or inactivated live viruses, which provide cues to the immune system to create an immune memory without causing illness in patients. These whole pathogen vaccines do not allow for targeted immune responses because they contain multiple antigens and innate immune-activating molecules. In contrast, subunit vaccines are composed of a purified antigen (often a protein) from the microorganism and an adjuvant to stimulate the immune system. These vaccines drive highly specific antigen targeting, and remove many of the safety challenges associated with using whole microorganisms. Subunit vaccines have become more widely used for infectious diseases, though they have limited ability to produce robust and persistent immune responses for many target diseases. The failure of subunit vaccines to elicit

a sufficiently strong immune response likely arises, in part, from inappropriate temporal control over antigen presentation and adjuvant mediated activation.

[0005] Nucleic acid vaccination involves introducing genetic material (e.g., mRNA or plasmid DNA) encoding an antigen into a subject that is expressed by the subject's own cells to stimulate an immune response in the subject against the antigen. The advantages of nucleic acid vaccination include improved safety, cost-effectiveness, ease of large-scale manufacturing, and versatility. However, conventional approaches for delivering nucleic acid vaccines typically rely on the route of administration and properties of the vaccine carrier to passively target the nucleic acid vaccine to immune cells and immune system organs. Consequently, a significant proportion of the delivered vaccine may be taken up by non-immune cells, which may reduce vaccination efficacy and produce undesirable side effects such as injection site pain.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Many aspects of the present disclosure can be better understood with reference to the following drawings. The components in the drawings are not necessarily to scale. Instead, emphasis is placed on illustrating clearly the principles of the present disclosure.

[0007] FIG. 1 illustrates polymer nanoparticle (PNP) hydrogels formed through the interactions of PEG-PLA nanoparticles (NPs) and dodecyl-modified hydroxypropyl methylcellulose polymers (HPMC-C12).

[0008] FIGS. 2A and 2B illustrate preparation of PNP hydrogels. FIG. 2A schematically illustrates PNP hydrogels composed of HPMC-C12 and PEG-PLA NPs. Cargo, including mRNA/LNP vaccines and molecular adjuvants, can be easily mixed with the polymer components to form a dynamic hydrogel. FIG. 2B are photographs of a process for preparing PNP hydrogels. Hydrogel components are loaded into two syringes, attached with an elbow, and mixed to form a homogenous material, pre-loaded in a syringe and ready for administration.

[0009] FIGS. 3A–3E illustrate mechanical properties of PNP hydrogels formulated with 0.5 wt% HPMC-C12 and 5 wt% PEG-PLA NPs (“PNP-0.5-5”). FIG. 3A is a graph illustrating frequency response of PNP-0.5-5 hydrogels. Frequency shear rheology showed that PNP-0.5-5 material was solid-like across time scales and mRNA/LNPs (50 $\mu\text{g mL}^{-1}$) did not alter this. FIG. 3B is a graph illustrating yield behavior of PNP-0.5-5 hydrogels. Stress-ramp data showed that the static yield stress, defined as the intersection of tangent lines for the

plateau and yielding regimes, for the PNP-0.5-5 hydrogel was not impacted by LNPs. FIG. 3C is a graph illustrating shear thinning behavior of PNP-0.5-5 hydrogels. High-to-low shear rheology demonstrated that shear thinning properties were not impacted by LNP incorporation. FIG. 3D is a graph illustrating flow and depot recovery of PNP-0.5-5 hydrogels. Step-shear rheology showed that PNP hydrogel viscosity dropped under high shear (10 s⁻¹) and recovered rapidly at low shear (1 s⁻¹) over repeated cycles and was not impacted by LNPs. FIG. 3E is a photograph showing that the PNP-0.5-5 hydrogel was injectable through a 26G needle and formed a solid-like depot following injection.

[0010] FIG. 4 is a graph illustrating the luminescent signal from RAW-Blue macrophages dosed with luciferase mRNA/LNPs in either PBS bolus or PNP hydrogel after storage in that condition for up to 30 days at 4 °C. Hydrogels did not impact transfection or stability of mRNA/LNPs. Data shown as mean ± SD, n = 3–4, and statistics are multiple unpaired two-tailed t-tests run in GraphPad Prism with false discovery rate (FDR) correction using a two-stage step-up method.

[0011] FIG. 5 is a schematic of immune niche in PNP hydrogels including mRNA/LNPs. PNP hydrogels injected subcutaneously allow immune cell infiltration and interaction with loaded cargo. Incorporation of various adjuvants influences the immune niche. Cells within the niche take up mRNA/LNPs, express the delivered protein, and migrate to initiate immune responses in the draining lymph nodes.

[0012] FIGS. 6A–6I illustrate characterization of in vivo immune niche in PNP hydrogels. FIG. 6A is a schematic showing the study design. PNP hydrogels loaded with mCherry mRNA/LNPs injected subcutaneously were excised at days 3 and 7 for dissociation and analysis of cells with flow cytometry. FIG. 6B provides photographs of PNP hydrogels on days 3 and 7. The PNP hydrogels formed an excisable depot with no evidence of fibrosis or foreign body response. FIG. 6C is a graph showing counts of CD45⁺ leukocytes per gel. Robust infiltration was observed on both days, with increased infiltration with adjuvant cargo. Data shown as mean ± SEM, n = 6. Statistical values shown are *p* values obtained from general linear model (GLM) fitting and Tukey HSD multiple comparison test in JMP. FIG. 6D provides graphs illustrating changes in cell type populations visualized with UMAPs. FIG. 6E is a graph illustrating quantification of cell types in each hydrogel niche as percentages of all gated CD45⁺ cells. Data shown as mean ± SEM, n = 6. FIG. 6F provides graphs illustrating counts of each cell type per PNP hydrogel depot. Data shown as mean ± SEM, n = 6. FIG. 6G shows percentages of CD45⁺ gated cells for each cell type. Data shown as mean, n = 6. FIG. 6H is a

UMAP visualization showing relation of Ly6C^{hi} macrophage subpopulation to macrophages and monocytes. FIG. 6I is a UMAP visualization of dendritic cell (DC) subpopulations highlighting differences between classical DCs (cDC1s and 2s) and monocyte-derived DCs (mDCs).

[0013] FIGS. 7A–7I illustrate characterization of mRNA expression in PNP hydrogel immune niche. FIG. 7A is a schematic showing that cells infiltrate injected PNP hydrogels loaded with mCherry mRNA/LNPs in vivo, take up LNPs, and express reporter mCherry protein. These cells can be quantified on days 3 and 7 following hydrogel excision with flow cytometry. FIG. 7B provides representative flow plots showing mCherry signal in CD45⁺ cells. FIGS. 7C and 7D are graphs showing the count of mCherry expressing CD45⁺ leukocytes per gel (FIG. 7C) and those cells as a percentage of all CD45⁺ cells (FIG. 7D). Adjuvants increased the absolute number and percentage of CD45⁺ cells expressing the delivered mRNA. Data shown as mean $\pm$ SEM, $n = 6$. Statistical values shown are p values obtained from GLM fitting and Tukey HSD multiple comparison test in JMP. FIG. 7E is a graph showing quantification of cell types in the mCherry⁺ subniche per hydrogel as percentages of all gated CD45⁺ mCherry⁺ cells. Data shown as mean $\pm$ SEM, $n = 6$. FIG. 7F provides graphs showing counts of mCherry⁺ cells of each cell type per PNP hydrogel depot. Data shown as mean $\pm$ SEM, $n = 6$. FIG. 7G shows percentages of mCherry⁺ CD45⁺ gated cells for each cell type. Data shown as mean, $n = 6$. FIG. 7H is a graph showing the ratio of the proportion each cell type makes up of the mCherry⁺ subniche to its percentage of the full CD45⁺ niche. Values of one indicate a cell is represented equally in the mCherry⁺ subniche as in the full gel. Values under one indicate fewer cells are mCherry⁺ than expected based on that cell type's percentage of the CD45⁺ niche, and vice versa for values over one (overrepresented in mCherry⁺ subniche). Data shown as mean $\pm$ SEM, $n = 6$. FIG. 7I provides graphs showing percentages of mCherry⁺ cells for each cell type. Data shown as mean $\pm$ SEM, $n = 6$.

[0014] FIGS. 8A–8E illustrate characterization of alternative PNP formulations. FIGS. 8A–8C are graphs illustrating frequency response, yield behavior, and shear thinning behavior of PNP-0.5-5 and PNP-1-5 hydrogels, respectively. Frequency shear rheology showed that the PNP-15 material was stiffer than PNP-0.5-5 and solid-like across time scales. FIG. 8D is a graph illustrating luminescent signal from RAW-Blue macrophages dosed with luciferase mRNA LNPs in either PBS bolus, PNP-0.5-5, or PNP-1-5 hydrogels. Data shown as mean $\pm$ SD, $n = 8$, and statistics are multiple unpaired two-tailed student t-tests run in GraphPad Prism with false discovery rate (FDR) correction using a two-stage step-up method. FIG. 8E is a

graph illustrating antibody titers over time in mice immunized with 0.25 µg Moderna Spikevax monovalent (WH1) in PBS bolus, PNP-0.5-5, or PNP-1-5 hydrogel at week 0 and boosted with 0.25 µg Moderna Spikevax bivalent in PBS bolus (bolus and PNP-0.5-5 groups) or PNP-1-5 at week eight. Serum was collected and analyzed for anti-spike (WH1) antibodies via ELISA. Data shown as mean ± SEM, n = 5–6.

[0015] FIGS. 9A–9H illustrate humoral response to hydrogel-based mRNA/LNP vaccines. FIG. 9A is a schematic of the study design. Mice were immunized with 0.25 µg commercially available bivalent SARS-CoV-2 mRNA vaccine (0.125 µg each variant) in PBS bolus or PNP-0.5-5 hydrogel with or without 1 µg 3M-52 or 20 µg MPLAs at week 0 and boosted with a homologous boost at week eight. Serum collected at designated time points was analyzed for anti-spike (Wuhan-Hu-1) antibodies via ELISA, as well as for other viral variant anti-spike antibodies, IgG subtypes, and neutralizing capacity. A separate cohort of animals primed and boosted in the same fashion were euthanized two weeks post-boost and spleens harvested for ELISpot evaluation of spike specific (WH1) T cells. FIGS. 9B–9D are graphs illustrating anti-WH1 spike IgG endpoint titers for all animals at week eight (FIG. 9B), week 26 (FIG. 9C), and over time (FIG. 9D). FIG. 9E is a graph illustrating area under the curve of antibody titer from week 0 to 26 per animal. FIG. 9F is a graph illustrating decay half-life of antibody titer post-boost derived from parametric bootstrapping of titers following each treatment group's post-boost peak. Data shown as mean ± SEM, n = 1000 simulations. FIG. 9G is a graph illustrating percent infectivity of BA.4/5 pseudotyped lentivirus at week 13 post-prime and 1:50 serum dilution. FIG. 9H is a graph illustrating spike specific (WH1) IFN-γ producing splenocytes, as a proxy for T cells, at week two post-boost. Data shown as mean ± SEM, n = 4–6. Statistical values shown are *p* values obtained from GLM fitting and Tukey HSD multiple comparison test in JMP. In FIG. 9D, comparisons shown are to bolus control.

[0016] FIGS. 10A–10G illustrate breadth and functionality of response to hydrogel-based mRNA/LNP vaccines. FIG. 10A is a graph illustrating IgG isotype endpoint titers at week 16 post-prime. FIG. 10B provides graphs illustrating anti-spike endpoint ELISA titers against different variants at week 16 post-prime. FIG. 10C is a graph illustrating ratio of IgG2c to IgG1 antibody isotypes indicating Th1 (higher values) or Th2 (lower values) skewing. FIG. 10D is a radar plot of absolute IgG endpoint titer for each variant. Broader and larger petals indicate improved breadth and consistency of response. FIG. 10E is a graph illustrating quantification of FIG. 10D, where each data point is the average of n = 5–6 animals against a single spike variant. Data shown as mean ± SD. FIG. 10F is a graph illustrating ratio of endpoint

IgG titer for each variant compared with WH1 as a metric of consistency and breadth of humoral response. FIG. 10G is a phylogenetic tree showing lineage of variants assessed. Unless otherwise written, data shown as mean $\pm$ SEM, $n = 5-6$. Statistical values shown are p values obtained from GLM fitting and Tukey HSD multiple comparison test in JMP.

[0017] FIGS. 11A–11J illustrate germinal center response to hydrogel-based mRNA/LNP vaccines. FIG. 11A is a schematic of the study design. Mice were immunized with bivalent SARS-CoV-2 mRNA vaccine in PBS bolus or PNP-0.5-5 hydrogel with or without adjuvants and lymph nodes harvested at weeks 1, 2, and 4. FIGS. 11B and 11C are week one representative flow plots and quantification of activated B cells ($B220^+ MHCII^+ CD86^+$) (FIG. 11B) and light and dark zone B_{GC} cells ($B220^+ CD95^- GL7^+ CD38^- CD86^+ CXCR4^-$ and $CD86^- CXCR4^+$) (FIG. 11C). FIGS. 11D–11F provide representative flow plots of B_{GC} cells ($B220^+ CD95^- GL7^+$) (FIG. 11D), spike-specific B_{GC} cells ($CD38^- Spike^{++}$) (FIG. 11E), and T follicular helper cells (T_{FH} , $CD19^- CD3^+ CD4^+ CXCR5^+ PD-1^+$) (FIG. 11F). Percentages of parent population except $Spike^{++}$ B_{GC} cells, which are of grandparent. FIG. 11G provides graphs illustrating quantification of B_{GC} cell percentage of all B cells. FIG. 11H provides graphs illustrating counts of $Spike^{++}$ B_{GC} cells. FIG. 11I provides graphs illustrating quantification of T_{FH} cell percentage of all $CD4^+$ T cells. FIG. 11J provides graphs illustrating ratio of B_{GC} cells to T_{FH} cells as a metric of T_{FH} cell help quality. All data shown as mean $\pm$ SEM, $n = 5-10$. Statistical values shown are p values obtained from GLM fitting and Tukey HSD multiple comparison test in JMP.

[0018] FIGS. 12A and 12B illustrate that mRNA LNPs encapsulated in PNP-0.5-5 hydrogels can be combined with immune cell attractant chemokines. Mice were vaccinated subcutaneously with 0.25 μ g Spikevax monovalent mRNA LNPs in 100 μ L PNP-0.5-5 hydrogel either alone (blue), or with 3 μ g GM-CSF (orange) or 3 μ g FLT3L (red). Mice were boosted with 0.25 μ g Spikevax bivalent mRNA LNPs in the same gel formulation as the prime at week eight (black arrows). FIG. 6A is a graph illustrating anti-Spike IgG titers for wildtype SARS-CoV-2 Spike trimer. Data is presented as individual mice with mean $\pm$ SEM ($n=5-6$). FIG. 6B is a graph illustrating anti-Spike IgG titers for omicron variants B.1.1.529 (squares) and BA.4/BA.5/BA.5.2 (triangles) measured four weeks post-prime (W4) and post-boost (W12) via ELISA. Data is presented as mean $\pm$ SEM.

[0019] FIGS. 13A–13D illustrate that PNP hydrogels supported in vivo immune niche formation and cellular composition could be skewed with chemokines. Mice were vaccinated subcutaneously with 100 μ L PNP-0.5-5 hydrogel alone (grey) or with 0.25 μ g Spikevax

bivalent mRNA LNPs alone (blue) or combined with 3 μ g GM-CSF (orange) or 3 μ g FLT3L (red). Hydrogel depots were explanted at days 3 and 7 post-prime and single cells were isolated and analyzed via flow cytometry. FIG. 13A illustrates the percent composition of CD45⁺ leukocytes. Data is presented as the mean of n=3. FIG. 13B is a graph of the total CD45⁺ leukocyte count per 100 μ L gel depot. FIG. 13C is a graph of total antigen presenting cell (APC) count per 100 μ L gel depot. FIG. 13D is a graph of APC percentage of CD45⁺ leukocytes. Data for FIGS. 13B–13D are presented as mean $\pm$ SEM (n=3).

[0020] FIGS. 14A and 14B illustrate that chemokines in combination with adjuvants altered the humoral response to mRNA LNPs encapsulated in PNP-0.5-5 hydrogels. FIG. 14A is a graph illustrating anti-spike IgG titers for wildtype SARS-CoV-2 spike trimer measured via ELISA. Addition of GM-CSF to adjuvanted PNP hydrogels delivering mRNA LNP vaccine showed slight decreases in titers post boost (week eight, black arrows). FIG. 14B is a graph illustrating area under the curve (AUC) of endpoint titer weeks 1–16 calculated for each mouse using GraphPad Prism and plotted. Data in FIGS. 14A and 14B is presented as individual mice with mean $\pm$ SEM (n=6).

DETAILED DESCRIPTION

[0021] The present technology relates to compositions and methods for delivery of nucleic acid therapeutics, such as mRNA lipid nanoparticles (LNPs). In some embodiments, for example, the present disclosure provides a composition for treating a subject, where the composition includes a dynamic hydrogel composed of a polymer (e.g., a hydrophobically modified cellulose derivative) and a plurality of nanoparticles (e.g., amphiphilic polymeric nanoparticles). The polymer can be non-covalently crosslinked with the plurality of nanoparticles, thus conferring shear-thinning, self-healing, and/or viscoelastic properties to the dynamic hydrogel. The composition can further include a nucleic acid therapeutic, such as a nucleic acid vaccine (e.g., a mRNA vaccine), encapsulated by the dynamic hydrogel. In some embodiments, the nucleic acid therapeutic is composed of a nucleic acid molecule combined with a delivery vector (e.g., a lipid or polymer vector), and the dynamic hydrogel is formulated to avoid disrupting the interactions between the nucleic acid molecule and the delivery vector, such as by reducing the stiffness, storage modulus, and/or yield stress of the dynamic hydrogel, and/or altering the chemistry of the components of the dynamic hydrogel.

[0022] Upon administration of the composition to the subject, the dynamic hydrogel can form a cohesive depot in vivo that retains the nucleic acid therapeutic at the administration

site for a desired treatment period (e.g., 7 to 10 days). Moreover, the dynamic hydrogel can limit access to the nucleic acid therapeutic to target cells that are capable of infiltrating into the hydrogel network, thereby reducing or preventing uptake of the nucleic acid therapeutic by nontarget cells. In embodiments where the nucleic acid therapeutic is a nucleic acid vaccine, the target cells can be immune cells, such as antigen-presenting cells (e.g., dendritic cells, macrophages, and/or B cells). For example, a SARS-CoV-2 mRNA LNP vaccine can be co-encapsulated in the dynamic hydrogel with immune activating cargo to direct key immune cells to the depot niche and promote more cell-specific uptake of the vaccine compared to standard intramuscular injection.

[0023] The embodiments of the present technology can provide numerous advantages compared to conventional therapeutic products and treatment approaches. For instance, conventional approaches for delivery of nucleic acid therapeutics, such as mRNA LNPs, generally rely on the route of administration (e.g., intramuscular, intravenous) and properties of the LNPs (e.g. charge, PEGylation) to passively target certain organs or lymph nodes. Little focus has been placed on technologies to deliver the LNPs or other nucleic acid carriers themselves to cells of interest with higher precision and less manipulation of the carrier formulation. In contrast, the present technology provides a modular platform that can be used to incorporate different nucleic acid therapeutic modalities and allows better control over their delivery to cell populations of interest. The compositions described herein can retain nucleic acid therapeutics at the administration site for improved local delivery in cases where a specific organ or tumor is targeted. Moreover, the compositions herein can attract target cells to the depot niche for more cell-specific delivery of the nucleic acid therapeutic, without requiring chemical or other modifications to the nucleic acid therapeutic to incorporate targeting capability.

[0024] Additionally, conventional hydrogel-based depot technologies typically exhibit several critical shortcomings, including complicated manufacturing, poor formulation stability, challenging administration, and burst release that can contribute to poor tolerability of the therapy. In contrast to conventional covalently crosslinked hydrogels, the dynamic hydrogels of the present technology are formed through strong yet dynamic physical interactions. As a result, these materials can address the shortcomings of other hydrogel-based depot technologies by exhibiting: (i) mild formulation requirements favorable for facile formulation with therapeutic cargo, and for maintaining stability of the therapeutic cargo during manufacturing and storage; (ii) shear-thinning properties allowing for straightforward injectability through

standard syringes and needles; (iii) rapid self-healing of hydrogel structure and depot formation to avoid burst release of the therapeutic cargo; (iv) sufficiently high yield stress to form a robust depot that persists under the normal stresses of the subcutaneous space following administration; (v) controlled delivery of therapeutic cargo over clinically desirable timeframes; and/or (vi) biodegradability.

[0025] Embodiments of the present disclosure will be described more fully hereinafter with reference to the accompanying drawings in which example embodiments are shown. Embodiments of the claims may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. The examples set forth herein are non-limiting examples and are merely examples among other possible examples.

[0026] The headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed present technology. Embodiments under any one heading may be used in conjunction with embodiments under any other heading.

I. Dynamic Hydrogels

[0027] The present technology utilizes dynamic hydrogels that can serve as a versatile platform for controlled release of therapeutic cargo, such as the nucleic acid therapeutics described in Section II below. In some embodiments, the dynamic hydrogels exhibit dynamic behavior, such as shear-thinning behavior, self-healing behavior, and/or highly tunable viscoelastic mechanical properties. The shear-thinning, self-healing, and/or viscoelastic properties of the dynamic hydrogels can result from non-covalent, supramolecular interactions between the components of the hydrogel (e.g., polymers and nanoparticles, as described further below). The non-covalent interactions can include physical crosslinking, which may encompass various types of crosslinking arising from weak physical interactions such as hydrogen bonding, hydrophobic interactions, ionic interactions, van der Waals interactions, host-guest interactions, crystal formation, physical entanglement, or combinations thereof. The non-covalent interactions can allow for the formation of dynamic, reversible crosslinks between components of the hydrogel that are capable of dissociating and reforming, e.g., spontaneously and/or in response to applied stress.

[0028] The dynamic hydrogels described herein can provide many advantages for therapeutic applications. For instance, the dynamic hydrogels described herein can exhibit high drug loading capacity, gentle conditions for encapsulation of biologic cargo, sustained delivery of cargo, and/or mechanical tunability. However, unlike traditional covalently crosslinked

hydrogels, the dynamic hydrogels herein can be easily administered via techniques such as direct injection, catheter delivery, spreading, or spraying, due to their shear-thinning and/or self-healing properties. Additionally, the dynamic hydrogels herein can exhibit unique dynamic network rearrangements that provide highly tunable release characteristics for the therapeutic cargo. The dynamic hydrogels provided herein can also be synthesized in a straight-forward, cost-effective manner that is easily scalable.

A. Polymer Nanoparticle Hydrogels

[0029] In some embodiments, the dynamic hydrogels described herein are polymer nanoparticle (PNP) hydrogels. PNP hydrogels are a type of supramolecular hydrogel formed from non-covalent interactions between polymers and nanoparticles. A PNP hydrogel can self-assemble rapidly upon mixing of a polymer solution with a nanoparticle solution. Self-assembly of the PNP hydrogel network can occur when polymers are linked together by adsorption of segments of the polymer chains onto the surfaces of the nanoparticles through multivalent, transient interactions. PNP hydrogel formation can be an entropy-driven process in which solvent molecules (e.g., water) solvating the polymer chains and nanoparticle surfaces are released into the bulk solution upon binding of the polymer chains to the nanoparticle surfaces, thus producing large gains in translational entropy. The interactions between the polymers and nanoparticle surfaces can be transient and reversible, thus allowing the PNP hydrogel to flow under applied shear stress, followed by rapid self-healing when the stress is relaxed.

[0030] The PNP hydrogels described herein can be composed of any suitable combination of polymers and nanoparticles that are capable of interacting non-covalently with each other to form crosslinks with the desired dynamic behavior. In some embodiments, the nanoparticle and polymer are selected to have a sufficiently strong affinity to produce efficient crosslinking. That is, the free energy gain (ϵ) resulting from the adsorption of a polymer chain to the surface of a nanoparticle can be greater than or comparable to the thermal energy ($k_B T$). In addition, the average number of interactions per polymer chain and particle can be greater than 2 to achieve percolation of the hydrogel network. Moreover, to favor polymer bridging of multiple nanoparticles (as opposed to polymer wrapping around individual particles), the nanoparticle diameter can be comparable to or less than the persistence length of the polymer chains. When some or all of these criteria are met, the nanoparticles can serve as crosslinkers between the polymer chains, while the polymer chains can bridge many different particles, thus

enabling hydrogel formation. In some embodiments, the modulus (G) of the PNP hydrogel is related to the number of dynamic hydrogel interactions per unit volume (n) and the energy associated with each interaction ($\alpha k_B T$) according to the following relation: $G \approx n \alpha k_B T$. In some embodiments, the nanoparticle surfaces are hydrophobic, such that the adsorption of the polymer chains to the nanoparticle surfaces are at least partially influenced by the general level of hydrophobicity along the polymer chain (e.g., the size and/or number of hydrophobic groups attached to the polymer chain).

[0031] For example, as shown in FIG. 1, the PNP hydrogels described herein can be formed through dynamic interactions between hydrophobically-modified cellulose derivatives and nanoparticles, such as dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12) and biodegradable polymeric nanoparticles composed of poly(ethylene glycol)-block-poly(lactic acid) (PEG-PLA). The polymers can bridge between nanoparticles and dynamically interact with the nanoparticle surfaces. Additional examples of nanoparticles and polymers suitable for use in the PNP hydrogels herein are provided in Sections I.A.1 and I.A.2 below, respectively.

[0032] The PNP hydrogels described herein can be differentiated from conventional drug delivery systems that include nanoparticles embedded in a covalently crosslinked hydrogel. Such conventional systems typically include gel-forming polymers that are covalently crosslinked with each other to form the gel network, while the nanoparticles serve as an optional additive that plays no role in gel formation, and thus can be freely substituted with other additives or omitted altogether. In contrast, the PNP hydrogels herein may be specifically formed through the interactions between the nanoparticles and polymers. In some embodiments, the polymers and nanoparticles used in the PNP hydrogels herein each independently do not form a gel alone, or are not used at concentrations where the polymer alone or the nanoparticle alone form a gel, such that gel formation occurs only when the polymer and nanoparticle are combined.

[0033] In some embodiments, the PNP hydrogels herein include one or more polymers combined with one or more nanoparticles, such that the loss modulus of a solution of the one or more polymers and the loss modulus of a solution of the one or more particles are each greater than their respective storage moduli at an angular frequency within a range from 0.1 rad/s to 100 rad/s (e.g., 10 rad/s) as measured by oscillatory shear rheometry in the linear viscoelastic region. The storage modulus of the PNP hydrogel produced by combining the one or more polymers with the one or more particles may be greater than the loss modulus of the PNP hydrogel at an angular frequency within a range from 0.1 rad/s to 100 rad/s (e.g., 10 rad/s).

as measured by oscillatory shear rheometry in the linear viscoelastic region. In some embodiments, the dynamic shear viscosity of the PNP hydrogel at a shear rate within a range from 0.1 s^{-1} to 100 s^{-1} (e.g., 10 s^{-1}) is greater than the sum of the dynamic shear viscosity of the solution of the one or more polymers and dynamic shear viscosity of the solution of the one or more nanoparticles at the shear rate within the range from 0.1 s^{-1} to 100 s^{-1} . For example, the dynamic shear viscosity of the PNP hydrogel can be greater than the sum of the dynamic shear viscosities of the polymer solution and the nanoparticle solution by a multiplicative factor within a range from 2 to 100,000, 2 to 1000, 2 to 100, or 2 to 10.

[0034] The PNP hydrogels described herein can include any concentration of polymers and nanoparticles suitable for providing desired hydrogel properties. For instance, higher polymer concentrations can produce PNP hydrogels with a higher stiffness and/or slower degradation rate. Higher nanoparticle concentrations can produce PNP hydrogels with a higher viscosity, stiffness, and yield stress, and/or slower degradation rate. The hydrogel properties may depend not only on the overall amount of solid content in the hydrogel, but also on the stoichiometry of polymer content to nanoparticle content. For example, increasing the nanoparticle concentration at a constant polymer concentration can produce a hydrogel having a more solid-like rheological response (e.g., lower tan delta), increased strain-to-yield, and increased yield stress. Increasing the polymer concentration at a constant nanoparticle concentration can produce a hydrogel having a more liquid-like rheological response (e.g., higher tan delta and greater frequency dependency of the storage modulus) and reduced strain-to-yield.

[0035] In some embodiments, the PNP hydrogels described herein include at least 0.25 wt%, 0.5 wt%, 0.75 wt%, 1 wt%, 1.5 wt%, 2 wt%, 2.5 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt % polymer; and/or at least 0.5 wt%, 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 12 wt%, or 15 wt% nanoparticles. Alternatively or in combination, the concentration of polymer within the PNP hydrogel can be within a range from 0.5 wt% to 5 wt%, 0.5 wt% to 4 wt%, 0.5 wt% to 3 wt%, 0.5 wt% to 2 wt%, 0.5 wt% to 1 wt%, 1 wt% to 5 wt%, 1 wt% to 4 wt%, 1 wt% to 3 wt%, 1 wt% to 2 wt%, 2 wt% to 5 wt%, 2 wt% to 4 wt%, 2 wt% to 3 wt%, 3 wt% to 5 wt%, 3 wt% to 4 wt%, or 4 wt% to 5 wt%; and/or the concentration of nanoparticles within the PNP hydrogel can be within a range from 1 wt% to 12 wt%, 1 wt% to 10 wt%, 1 wt% to 8 wt%, 1 wt% to 5 wt%, 1 wt% to 3 wt%, 3 wt% to 12 wt%, 3 wt% to 10 wt%, 3 wt% to 8 wt%, 3 wt% to 5 wt%, 5 wt % to 12 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, 8 wt% to 12 wt%, 8 wt% to 10 wt%, or 10 wt% to 12 wt%. The

nomenclature “X-Y hydrogel” or “X:Y hydrogel” is used herein to refer to a hydrogel having X wt% polymer and Y wt% nanoparticles.

[0036] In some embodiments, the PNP hydrogels herein are prepared by simple mixing of the polymers, nanoparticles, therapeutic cargo, and any optional additives. For example, the PNP hydrogel can be prepared by forming a polymer solution (e.g., by dissolving the polymer in an aqueous solvent such as water or a buffered solution such as phosphate-buffered saline (PBS)), forming a nanoparticle solution (e.g., by suspending the nanoparticles in an aqueous solvent), and forming a solution containing the therapeutic cargo (e.g., by dissolving or suspending the therapeutic cargo in an aqueous solvent). The solutions can then be combined, optionally with external agitation, to form the PNP hydrogel including the therapeutic cargo.

1. Nanoparticles

[0037] The PNP hydrogels described herein can include a plurality of nanoparticles. The nanoparticles can be any suitable shape, such as spheres, cubes, rods, tubes, plates, fibers, etc. The nanoparticles can have a mean particle size (e.g., diameter) within a range from 1 nm to 1000 nm, 1 nm to 500 nm, 1 nm to 250 nm, 1 nm to 150 nm, 1 nm to 100 nm, 1 nm to 50 nm, 1 nm to 25 nm, 1 nm to 10 nm, 10 nm to 1000 nm, 10 nm to 500 nm, 10 nm to 250 nm, 10 nm to 150 nm, 10 nm to 100 nm, 10 nm to 50 nm, 10 nm to 25 nm, 25 nm to 1000 nm, 25 nm to 500 nm, 25 nm to 250 nm, 25 nm to 150 nm, 25 nm to 100 nm, 25 nm to 50 nm, 50 nm to 1000 nm, 50 nm to 500 nm, 50 nm to 250 nm, 50 nm to 150 nm, 50 nm to 100 nm, 100 nm to 1000 nm, 100 nm to 500 nm, 100 nm to 250 nm, 100 nm to 150 nm, 150 nm to 1000 nm, 150 nm to 500 nm, 150 nm to 250 nm, 250 nm to 1000 nm, 250 nm to 500 nm, or 500 nm to 1000 nm. In some embodiments, the nanoparticles have a mean particle size less than or equal to 500 nm, 250 nm, 150 nm, 140 nm, 130 nm, 120 nm, 110 nm, 100 nm, 90 nm, 80 nm, 70 nm, 60 nm, 50 nm, 40 nm, 30 nm, 20 nm, or 10 nm. As described herein, to facilitate hydrogel formation, the mean particle size of the nanoparticles can be similar to or less than the persistence length of the polymer in the PNP hydrogel, such as less than or equal to 125%, 110%, 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the persistence length of the polymer. As used herein, “mean particle size” may refer to the statistical mean particle size (e.g., diameter) of the particles in the PNP hydrogel composition. The diameter of an essentially spherical particle may refer to the physical or hydrodynamic diameter. The diameter of a non-spherical particle may refer to the hydrodynamic diameter or to the largest linear

distance between two points on the surface of the particle. Mean particle size can be measured using methods known in the art, such as dynamic light scattering.

[0038] The nanoparticles can be made out of a single material or can be made out of a combination of multiple different materials (e.g., two, three, four, five, or more different materials). The material(s) can be biodegradable and/or biocompatible. For example, in some embodiments, the nanoparticles are made partially or entirely out of one or more biodegradable and/or biocompatible polymers. Generally, biodegradable polymers can degrade by enzymatic hydrolysis, exposure to water in vivo, surface erosion, and/or bulk erosion. Biodegradable polymers can include synthetic polymers, naturally occurring polymers, or combinations thereof. Examples of synthetic biodegradable polymers include polyhydroxy acids (e.g., poly(lactic acid), poly(glycolic acid)), polyanhydrides, poly(ortho)esters, polyesters, polyurethanes, poly(butyric acid), poly(valeric acid), poly(caprolactone), poly(hydroxybutyrate), poly(lactide-co-glycolide), poly(lactide-co-caprolactone), poly(ethylene-co-maleic anhydride), poly(ethylene maleic anhydride-co-L-dopamine), poly(ethylene maleic anhydride-co-phenylalanine), poly(ethylene maleic anhydride-co-tyrosine), poly(butadiene-co-maleic anhydride), poly(butadiene maleic anhydride-co-L-dopamine) (pBMAD), poly(butadiene maleic anhydride-co-phenylalanine), poly(butadiene maleic anhydride-co-tyrosine), and combinations (e.g., mixtures, copolymers) thereof. Examples of naturally occurring biodegradable polymers include polysaccharides (e.g., cellulose, alginate, collagen, chitosan, hyaluronic acid, starch, agarose, agar, xanthan gum), proteins (e.g., collagen, fibrin, albumin, zein, gelatin), and derivatives thereof (e.g., derivatives of cellulose such as cellulose nanocrystals, cellulose nanofibers), and combinations thereof.

[0039] Alternatively or in combination, the nanoparticles can be made partially or entirely out of one or more non-biodegradable polymers. Examples of non-biodegradable polymers include polystyrenes, polyalkylene glycols, poly(meth)acrylates, poly(meth)acrylamides, polyalkylenes (e.g., polyethylene, polyvinyls, poly(vinyl acetate), poly(ethylene terephthalate)), and combinations thereof.

[0040] The polymer(s) used to form the nanoparticles herein can have any suitable molecular weight, such as a molecular weight (e.g., number-average molecular weight (M_n)) within a range from 500 Da to 10,000 kDa, 1 kDa to 1000 kDa, or 10 kDa to 100 kDa. As used herein, "molecular weight" may refer to the relative average chain length of the bulk polymer, and can be estimated or characterized in various ways including gel permeation chromatography (GPC) and capillary viscometry. GPC molecular weights are reported as the

number-average molecular weight (M_n) as opposed to the weight-average molecular weight (M_w). Capillary viscometry provides estimates of molecular weight (M_v) as the inherent viscosity determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

[0041] In some embodiments, the nanoparticles are made partially or entirely out of one or more inorganic materials, such as clays (e.g., silicates) or other types of minerals (e.g., sulfides, oxides, halides, carbonates, sulfates, phosphates, apatites), or combinations thereof. Alternatively or in combination, the nanoparticles can be made partially or entirely out of one or more metals, such as gold, silver, copper, platinum, palladium, ruthenium, or combinations thereof. Optionally, the nanoparticles can be made partially or entirely out of carbon nanotubes (e.g., single-walled or multi-walled nanotubes), graphene, graphene oxide, or other ultrathin single crystals, including black phosphorous and boron based nanosheets.

[0042] In some embodiments, the nanoparticles are core-shell particles (also known as “core-corona particles”). A core-shell particle can have a core containing or formed from a first material, and a shell or corona containing or formed from a second, different material. For example, a core-shell particle can include at least two polymers, such that the core is made from a first polymer, and the shell or corona is made from a second, different polymer. As another example, the core-shell particle can include a single block copolymer, such that the core is made from a first block of the block copolymer, and the shell or corona can be made from a second block of the block copolymer. In some embodiments, one or both of the components of the core-shell particle is a non-polymeric material.

[0043] A core-shell particle can be composed of two compositionally disparate phases, of which one (either the core or shell/corona) is hydrophobic and the other (core or shell/corona) is hydrophilic. Suitable hydrophobic components include polyamides (e.g., poly(amino acids)), polyesters (e.g., poly(lactic acid), poly(caprolactone)), polypropylene oxides, polystyrenes, and combinations thereof. Suitable hydrophilic components include polysaccharides, proteins, polyamides (e.g., poly(amino acids)), naturally occurring polymers, synthetic polymers, and combinations thereof. Suitable block copolymers include combinations of polyethylene glycol and polyesters (e.g., PEG-PLA, poly(ethylene glycol)-block-poly(caprolactone) (PEG-PCL)) and combinations of polyethylene glycol and polypropylene glycol (e.g., poloxamers). In some embodiments, the core-shell particle is composed of an amphiphilic polymer including (1) one or more hydrophobic polymers selected from polyanhydrides, poly(ortho)esters, polyesters, polyurethanes, and/or copolymers thereof,

and (2) one or more hydrophilic polymers selected from polysaccharides, proteins, poly(amino acids), and/or polyalkylene oxides.

[0044] Alternatively, the nanoparticles can be homogenous nanoparticles. A homogenous nanoparticle can be uniformly formed from a single material, or can be formed from multiple materials that are not separated into disparate phases within the particle as in core-shell particles.

[0045] The nanoparticles can be prepared using techniques known in the art. The technique to be used can depend on a variety of factors, including the materials used to form the nanoparticles, the desired size range of the resulting nanoparticles, and suitability for the material to be encapsulated. Examples of suitable techniques include, but are not limited to, solvent evaporation, solvent removal, hot melt microencapsulation, spray drying, phase inversion, polyelectrolyte condensation, single and double emulsion (e.g., probe sonication), nanoparticle molding, and electrostatic self-assembly.

[0046] The concentration of the nanoparticles in the PNP hydrogel can be varied to produce the desired hydrogel properties. In some embodiments, for example, the concentration of the nanoparticles in the PNP hydrogel is within a range from 1 wt% to 15 wt%, 2 wt% to 12 wt%, 3 wt% to 10 wt%, 5 wt% to 8 wt%, 5 wt% to 15 wt%, 5 wt% to 10 wt%, 10 wt% to 15 wt%, or 10 wt% to 12 wt%. The concentration of the nanoparticles in the PNP hydrogel can be about 1 wt%, about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, about 8 wt%, about 9 wt%, about 10 wt%, about 11 wt%, about 12 wt%, about 13 wt%, about 14 wt%, or about 15 wt%. In some embodiments, the concentration of the nanoparticles in the PNP hydrogel can be greater than or equal to 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 11 wt%, 12 wt%, 13 wt%, or 14 wt%. Alternatively or in combination, the concentration of the nanoparticles in the PNP hydrogel can be less than or equal to 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 11 wt%, 12 wt%, 13 wt%, 14 wt%, or 15 wt%.

2. Polymers

[0047] The PNP hydrogel can be formed when the nanoparticles are mixed with and interact with one or more polymers. The shear-thinning and/or self-healing properties of the PNP hydrogel can be derived from reversible, non-covalent interactions between the nanoparticles and the polymer chains, as described herein. The PNP hydrogel can include a single type of polymer or can include a combination of multiple different polymers (e.g., two,

three, four, five, or more different polymers). The polymer(s) can be biodegradable and/or biocompatible. The polymer(s) can include naturally occurring polymers, synthetic polymers, or derivatives or combinations thereof. Examples of naturally occurring polymers include polysaccharides (e.g., cellulose, alginate, collagen, chitosan, hyaluronic acid, starch, agarose, agar, xanthan gum), proteins (e.g., collagen, fibrin, albumin, zein, gelatin), and combinations thereof. Examples of synthetic polymers include polyacrylamide, poly(lactic acid), polyethylene glycol, polyethylene glycol-co-propylene glycol (PEO-PPO), poly(acrylates) (e.g., poly(2-hydroxyethyl methacrylate)), and combinations thereof. In some embodiments, the PNP hydrogel includes a derivative of a naturally occurring polymer, such as a cellulose derivative. Examples of cellulose derivatives include hydroxypropylmethylcellulose (HPMC), hydroxyethyl cellulose (HEC), hydroxypropylcellulose (HPC), ethylcellulose (EC), methylcellulose (MC), hydroxyethylmethylcellulose (HEMC), carboxymethylcellulose (CMC), carboxymethyl ethyl cellulose (CMEC), and combinations thereof.

[0048] In some embodiments, the PNP hydrogels herein include at least one polymer that is modified with a hydrophobic moiety. Hydrophobic modification of polymers may increase the energy associated with each polymer nanoparticle interaction ($\alpha k_B T$), thereby increasing the modulus of the dynamic hydrogel given the same number of interactions per unit volume. Such modification may facilitate favorable interactions between the hydrophobic moiety on the polymer chain and the hydrophobic core of the nanoparticle, thereby enhancing the adsorption energy of the polymer to the nanoparticles. The hydrophobic moiety can include a plurality of carbon atoms (e.g., from 2 to 50 carbon atoms, 2 to 30 carbon atoms, or 2 to 18 carbon atoms), and can be a saturated molecule or an unsaturated molecule. Examples of hydrophobic moieties that may be used include, but are not limited to, alkyl moieties (e.g., C4 to C18 alkyls such as butyl (–C4), hexyl (–C6), octyl (–C8), decyl (–C10), dodecyl (–C12), tetradecyl (–C14), pentadecyl (–C15), hexadecyl (–C16), heptadecyl (–C17), octadecyl (–C18)), alkenyl moieties (e.g., oleyl, linoleyl), aryl moieties (e.g., phenyl, benzyl, pyryl, naphthyl, anthracene), and cycloalkyl moieties (e.g., adamantyl, cyclohexyl, cholesterol). In some embodiments, the degree of modification of the polymer (e.g., percentage of reactive groups on the polymer have been functionalized with the hydrophobic moiety) is within a range from 1% to 50%, 5% to 30%, 5% to 25%, or 10% to 15%. For example, the degree of modification can be about 5%, 10%, 15%, 20%, or 25%.

[0049] The concentration of the polymer(s) in the PNP hydrogel can be varied to produce the desired hydrogel properties (e.g., stiffness, storage modulus, degradation rate). In

some embodiments, for example, the concentration of the polymer(s) in the PNP hydrogel is within a range from 0.25 wt% to 10 wt%, 0.5 wt% to 5 wt%, 0.5 wt% to 2 wt%, 1 wt% to 5 wt%, or 1 wt% to 2 wt%. The concentration of the polymer(s) in the PNP hydrogel can be about 0.1 wt%, 0.25 wt%, 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt%. In some embodiments, the concentration of the polymer(s) in the PNP hydrogel can be greater than or equal to 0.25 wt%, 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, or 4.5 wt%. Alternatively or in combination, the concentration of the polymer(s) in the PNP hydrogel can be less than or equal to 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt%.

3. Additional Components

[0050] The PNP hydrogels herein can optionally include one or more additional components to facilitate gel formation and/or modify the properties of the hydrogel. For example, the PNP hydrogels herein can include at least one enhancer compound that enhances the interactions between the polymers and nanoparticles, e.g., by providing bridging-type non-covalent interactions between the polymers and nanoparticles. In some embodiments, a portion of an enhancer compound interacts non-covalently with the polymer and a second portion of the enhancer compound interacts non-covalently with the nanoparticle. Non-limiting examples of such interactions include ionic interactions such as cationic/anionic interactions, electrostatic interactions, and hydrogen bonding interactions.

[0051] For example, in embodiments where the polymer is negatively charged at physiological pH (e.g., hyaluronic acid, carboxymethyl cellulose), a cationic surfactant can be used to enhance adsorption of the anionic polymer to the nanoparticles via electrostatic interactions. Examples of positively charged surfactants include cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium iodide, cetyltrimethylammonium fluoride, and cetyltrimethylammonium chloride. Conversely, in embodiments where the polymer is positively charged at physiological pH (e.g., chitosan, aminopolysaccharides, poly(lysine), cationic acrylate polymers, cationic vinyl polymers), an anionic surfactant can be used to enhance adsorption of the cationic polymer to the nanoparticles via electrostatic interactions. Examples of negatively charged surfactants include sodium dodecyl sulfate, sodium stearate, and charged fatty acid surfactants.

[0052] In some embodiments, molecular recognition between at least two compounds can provide the enhancement. For example, the adsorption of polymers, such as polysaccharides, to nanoparticles can be enhanced by an enhancer compound which includes a carbohydrate in one portion of the enhancer and a polymer tail that interacts with the nanoparticle.

[0053] The concentration of the enhancer compound can be varied to produce the desired effect on hydrogel formation. In some embodiments, for example, the concentration of the enhancer compound in the PNP hydrogel is within a range from 0.25 wt% to 10 wt%, 0.5 wt% to 5 wt%, 0.5 wt% to 2 wt%, 1 wt% to 5 wt%, or 1 wt% to 2 wt%. The concentration of the enhancer compound in the PNP hydrogel can be about 0.1 wt%, 0.25 wt%, 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt%. In some embodiments, the concentration of the enhancer compound in the PNP hydrogel can be greater than or equal to 0.25 wt%, 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, or 4.5 wt%. Alternatively or in combination, the concentration of the enhancer compound in the PNP hydrogel can be less than or equal to 0.5 wt%, 0.75 wt%, 1 wt%, 1.25 wt%, 1.5 wt%, 1.75 wt%, 2 wt%, 2.25 wt%, 2.5 wt%, 2.75 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt%. Optionally, the PNP hydrogel may not include any enhancer compounds.

B. Hydrogel Properties

[0054] The dynamic hydrogels described herein (e.g., the PNP hydrogels of Section I.A) can exhibit favorable physical and biological properties that contribute to their efficacy as drug delivery platforms. The properties of the dynamic hydrogels herein can be tuned in various ways, such as by modifying the types of components used to form the hydrogel (e.g., polymers, nanoparticles, and/or additional components as previously described in Section I.A; and/or the therapeutic cargo carried by the hydrogel as described below in Section II), the concentrations of the components, and/or the chemical functionalities of the components. Accordingly, the properties of the dynamic hydrogels herein can be adapted to the particular therapeutic application, such as forming a stable and/or persistent depot when delivered in vivo, providing a desired release profile for the therapeutic cargo (e.g., short-term release versus long-term release), providing a desired release mechanism for the therapeutic cargo (e.g., diffusion-based release versus erosion-based release), compatibility with a desired route of administration (e.g., injecting, infusing, spraying, spreading), biodegradability, biocompatibility, and/or allowing

for cellular infiltration. Any reference herein to a property of a dynamic hydrogel may refer to the property of the dynamic hydrogel without any therapeutic cargo (e.g., a PNP hydrogel composed only of polymers and nanoparticles), the property of the dynamic hydrogel including the therapeutic cargo (e.g., a PNP hydrogel including polymers, nanoparticles, and the encapsulated therapeutic cargo), or both, unless otherwise stated or otherwise evident from the context.

[0055] The storage modulus (G') of the dynamic hydrogel can correlate to the overall stiffness of the hydrogel, which in turn can dictate the time scale of degradation of the hydrogel (e.g., hydrogels having a higher storage modulus may be stiffer and degrade more slowly than gels having a lower storage modulus). Accordingly, in embodiments where the therapeutic cargo of the dynamic hydrogel is released primarily or entirely via an erosion-based mechanism, the release rate of the therapeutic cargo can be tuned by adjusting the storage modulus of the hydrogel (e.g., a higher storage modulus can produce a slower degradation rate and thus a slower release rate of the therapeutic cargo, while a lower storage modulus can produce a higher degradation rate and thus a faster release rate of the therapeutic cargo). For example, in embodiments where the dynamic hydrogel is a PNP hydrogel, the storage modulus of the PNP hydrogel can be increased or decreased by increasing or decreasing the polymer concentration, and/or by increasing or decreasing the nanoparticle concentration. In some embodiments, the dynamic hydrogels herein have a storage modulus within a range from 1 Pa to 10,000 Pa, 1 Pa to 5000 Pa, 1 Pa to 2500 Pa, 1 Pa to 1000 Pa, 1 Pa to 500 Pa, 1 Pa to 200 Pa, 1 Pa to 10 Pa, 10 Pa to 10,000 Pa, 10 Pa to 5000 Pa, 10 Pa to 2500 Pa, 10 Pa to 1000 Pa, 10 Pa to 500 Pa, 10 Pa to 200 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 50 Pa to 10,000 Pa, 50 Pa to 5000 Pa, 50 Pa to 2500 Pa, 50 Pa to 1000 Pa, 50 Pa to 500 Pa, 50 Pa to 200 Pa, 50 Pa to 100 Pa, 100 Pa to 10,000 Pa, 100 Pa to 5000 Pa, 100 Pa to 2500 Pa, 100 Pa to 1000 Pa, 100 Pa to 500 Pa, 100 Pa to 200 Pa, 200 Pa to 10,000 Pa, 200 Pa to 5000 Pa, 200 Pa to 2500 Pa, 200 Pa to 1000 Pa, 200 Pa to 500 Pa, 500 Pa to 10,000 Pa, 500 Pa to 5000 Pa, 500 Pa to 2500 Pa, 500 Pa to 1000 Pa, 1000 Pa to 10,000 Pa, 1000 Pa to 5000 Pa, 1000 Pa to 2500 Pa, 2500 Pa to 10,000 Pa, 2500 Pa to 5000 Pa, or 5000 Pa to 10,000 Pa. The storage modulus can be measured, for example, using an oscillatory shear test in a parallel plate rheometer at an angular frequency of 10 rad/s, a strain within the linear viscoelastic region of the hydrogel (e.g., 1% strain), and a temperature of 25 °C.

[0056] The yield stress (τ_y) of the dynamic hydrogel can correlate to the ability of the hydrogel to form and maintain a cohesive depot in vivo (e.g., materials lacking a yield stress

may flow rather than forming a cohesive depot). The dynamic hydrogels herein can exhibit little or no flow when subjected to stresses below the yield stress. When subjected to stresses above the yield stress, the dynamic hydrogels can flow, corresponding to a significant drop in observed viscosity (e.g., a decrease of at least one or two orders of magnitude). In embodiments where the dynamic hydrogel is a PNP hydrogel, the yield stress can be increased or decreased by increasing or decreasing the nanoparticle concentration, respectively. In some embodiments, the dynamic hydrogels herein have a yield stress within a range from 0.1 Pa to 1000 Pa, 0.1 Pa to 500 Pa, 0.1 Pa to 200 Pa, 0.1 Pa to 100 Pa, 0.1 Pa to 50 Pa, 0.1 Pa to 20 Pa, 0.1 Pa to 10 Pa, 0.1 Pa to 1 Pa, 1 Pa to 1000 Pa, 1 Pa to 500 Pa, 1 Pa to 200 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 10 Pa, 10 Pa to 1000 Pa, 10 Pa to 500 Pa, 10 Pa to 200 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 20 Pa, 20 Pa to 1000 Pa, 20 Pa to 500 Pa, 20 Pa to 200 Pa, 20 Pa to 100 Pa, 20 Pa to 50 Pa, 50 Pa to 1000 Pa, 50 Pa to 500 Pa, 50 Pa to 200 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 200 Pa, 200 Pa to 1000 Pa, 200 Pa to 500 Pa, or 500 Pa to 1000 Pa. The yield stress can be measured, for example, using a stress ramp or stress sweep (e.g., from 1 Pa to 100 Pa, or from 1 Pa to 1000 Pa) in a parallel plate rheometer at a temperature of 25 °C to identify the stress at which the hydrogel exhibits a drop in viscosity.

[0057] The tan delta of the dynamic hydrogel (the ratio of the loss modulus (G'') over the storage modulus (G') ($\tan(\delta) = G''/G'$)) can describe the overall viscoelasticity of the hydrogel (e.g., lower tan delta values correspond to more solid-like behavior, higher tan delta values correspond to more liquid-like behavior), and can correlate to the degradation rate of the hydrogel. In some embodiments, the dynamic hydrogels herein have a tan delta less than or equal to 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1. The tan delta can be within a range from 0.1 to 1, 0.1 to 0.5, 0.1 to 0.3, 0.2 to 1, 0.2 to 0.5, or 0.5 to 1. The tan delta can be measured, for example, using an oscillatory shear test in a parallel plate rheometer at an angular frequency of 10 rad/s, a strain within the linear viscoelastic region of the hydrogel (e.g., 1% strain), and a temperature of 25 °C.

[0058] In some embodiments, the dynamic hydrogels herein exhibit shear-thinning behavior, in that the viscosity of the dynamic hydrogel decreases with increasing shear rate and/or shear stress. Shear-thinning behavior can be advantageous, for example, to allow the dynamic hydrogel to be administered via injection. In some embodiments, the viscosity of the gel decreases with increasing shear rate at a shear rate within a range from 0.1 s⁻¹ to 1000 s⁻¹, for example, as observed on an oscillatory rheometer (e.g., a parallel plate rheometer) at 25 °C. In some embodiments, the dynamic hydrogels herein have a viscosity within a range from 10

mPa-s to 2000 mPa-s, 10 mPa-s to 1000 mPa-s, 10 mPa-s to 500 mPa-s, 10 mPa-s to 200 mPa-s, 10 mPa-s to 100 mPa-s, 10 mPa-s to 50 mPa-s, 50 mPa-s to 2000 mPa-s, 50 mPa-s to 1000 mPa-s, 50 mPa-s to 500 mPa-s, 50 mPa-s to 200 mPa-s, 50 mPa-s to 100 mPa-s, 100 mPa-s to 2000 mPa-s, 100 mPa-s to 1000 mPa-s, 100 mPa-s to 500 mPa-s, 100 mPa-s to 200 mPa-s, 200 mPa-s to 2000 mPa-s, 200 mPa-s to 1000 mPa-s, 200 mPa-s to 500 mPa-s, 500 mPa-s to 2000 mPa-s, 500 mPa-s to 1000 mPa-s, or 1000 mPa-s to 2000 mPa-s at a shear rate of 1000 s^{-1} . The viscosity can be less than 10,000 mPa-s, 1000 mPa-s, or 100 mPa-s at a shear rate of 1000 s^{-1} . The viscosity can be measured, for example, using steady shear measurements in a parallel plate rheometer at a temperature of $25\text{ }^{\circ}\text{C}$.

[0059] In some embodiments, the dynamic hydrogels herein exhibit self-healing behavior. Self-healing may refer to a process in which a gel that exhibits reduced resistance to flow when subjected to an external stress regains some or all of its rigidity and/or strength after the external stress is removed. Self-healing behavior can be advantageous, for example, to allow the dynamic hydrogel to form a cohesive depot after administration via injection and/or to limit burst release. In some embodiments, the dynamic hydrogels herein stop flowing and recover their mechanical properties in no more than 5 seconds, 10 seconds, 30 seconds, 1 minute, 2 minutes, 3 minutes, 5 minutes, or 10 minutes after the external stress is removed. Optionally, the modulus and/or viscosity of the dynamic hydrogel can recover to at least 90% of the initial value before application of the external stress within 5 minutes in a step-strain measurement (conducted with strains of 0.5% and 500%) or step-shear measurement (conducted with shear rates of 0.1 s^{-1} and 100 s^{-1}), respectively, on an oscillatory rheometer.

[0060] In some embodiments, the dynamic hydrogels herein exhibit viscoelastic behavior, in that the storage modulus (G') of the hydrogel is dominant over the loss modulus (G'') at some point, for example, as observed in an oscillatory frequency sweep measurement in a range from 0.1 rad/s to 100 rad/s on an oscillatory rheometer performed in the linear viscoelastic region, yet the hydrogel exhibits complete stress relaxation following application of a constant strain of 500% within 15 minutes.

[0061] In some embodiments, the dynamic hydrogels described herein are biocompatible. A biocompatible material can be a material that is, along with any metabolites or degradation products thereof, generally non-toxic to the subject, and do not cause any significant adverse effects to the subject, at concentrations resulting from the degradation of the administered materials. A biocompatible material can be a material that does not elicit a significant inflammatory or immune response when administered to a subject.

[0062] In some embodiments, the dynamic hydrogels described herein are biodegradable. A biodegradable material can be a material that will degrade or erode under physiological conditions to smaller units or chemical species that are capable of being metabolized, eliminated, or excreted by the subject. For example, upon in vivo administration to a subject, the dynamic hydrogel can dissolve as the non-covalent bonds dissociate. The degradation rate of the dynamic hydrogel can be varied as desired, e.g., depending on the desired release profile for the therapeutic cargo. In some embodiments, following in vivo administration, the dynamic hydrogels are designed to persist at the administration site (e.g., remain as a cohesive depot) for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 21 days, 28 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, or 12 months. Alternatively or in combination, the dynamic hydrogels herein can persist at the administration site for no more than 12 months, 9 months, 6 months, 5 months, 4 months, 3 months, 2 months, 1 month, 28 days, 21 days, 14 days, 13 days, 12 days, 11 days, 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day.

II. Compositions for Delivery of Nucleic Acid Therapeutics and Associated Methods

[0063] In some embodiments, the present technology provides compositions for delivery of nucleic acid therapeutics for preventing and/or treating a disease or condition in a subject. The composition can include a dynamic hydrogel and a nucleic acid therapeutic encapsulated by the dynamic hydrogel. The dynamic hydrogel can exhibit shear-thinning behavior that allows for facile administration via injection, as well as self-healing behavior that allows for formation of a cohesive depot that delivers the nucleic acid therapeutic over a desired treatment period. In some embodiments, upon formation of a depot in vivo, access to the nucleic acid therapeutic is primarily or entirely limited to cells that are capable of infiltrating into the depot, such as immune cells. This approach can be used to provide selective delivery of the nucleic acid therapeutic to a target cell population, which may be beneficial for enhancing therapeutic efficacy and/or reducing off-target effects.

A. Nucleic Acid Therapeutics

[0064] The dynamic hydrogels herein can be used to encapsulate and deliver a wide variety of nucleic acid therapeutics. A nucleic acid therapeutic can include one or more nucleic acid molecules that exert a therapeutic effect in vivo. For instance, the nucleic acid molecule can encode a gene product (e.g., a protein or peptide) that, when expressed by a cell in the

subject, provides the therapeutic effect. Alternatively or in combination, the nucleic acid molecule itself may provide the therapeutic effect. In some embodiments, the nucleic acid therapeutic is an RNA-based therapeutic including one or more RNA molecules, such as messenger RNA (mRNA), small interfering RNA (siRNA), short hairpin RNA, microRNA, circular RNA, self-amplifying RNA, CRISPR-CAS guide RNA, ribozymes, riboswitches, RNA aptamers, or combinations thereof. RNA-based therapeutics such as mRNA can provide certain advantages in some instances, such as not requiring nuclear entry for activity and improved safety due to inability to integrate the host genome. Alternatively or in combination, the nucleic acid therapeutic can be a DNA-based therapeutic including one or more DNA molecules, such as plasmid DNA, DNA oligonucleotides, deoxyribozymes, DNA aptamers, or combinations thereof. DNA-based therapeutics may provide certain advantages in some instances, such as improved stability and longer-term activity.

1. Delivery Vectors

[0065] In some embodiments, the nucleic acid therapeutic includes a delivery vector (also known as a “delivery vehicle” or “carrier”) that encapsulates, binds to, or otherwise is combined with the nucleic acid molecule. The delivery vector can improve the delivery efficiency, and thus, the therapeutic efficacy, of the nucleic acid molecule. For example, naked nucleic acids typically cannot readily traverse the hydrophobic lipid cell membrane due to their large molecular size, hydrophilicity, and anionic charge. Moreover, following endocytosis, naked nucleic acid molecules are generally trafficked to acidic lysosomes and degraded, with only a small proportion being released into the cytoplasmic space. Additionally, certain types of nucleic acids such as mRNA are intrinsically unstable and susceptible to degradation by extracellular nucleases. Delivery vectors can overcome some or all of these delivery barriers, such as by packaging the nucleic acid molecule into a compact format, protecting the nucleic acid molecule from degradation, facilitating cellular uptake, and/or enabling endosomal escape and cytoplasmic delivery.

[0066] In some embodiments, the delivery vector is a nonviral delivery vector, which can alleviate immunogenicity and safety concerns (e.g., insertional mutagenesis) associated with viral vectors. Nonviral delivery vectors can use one or more materials that form complexes with a nucleic acid molecule, such as synthetic materials, natural materials, or suitable combinations thereof. Examples of materials that can be used in formulating nonviral delivery

vectors include, but are not limited to, lipids, polymers, peptides (e.g., protamine), proteins, small molecules, metals, and combinations thereof (e.g., hybrid polymer-lipid vectors).

[0067] For example, the delivery vector can be a lipid vector, such as a micelle, a liposome, or lipid nanoparticle (LNP). A micelle can be composed of a lipid monolayer, while a liposome can be composed of a lipid bilayer. A LNP can be composed of multiple lipid layers, and can include microdomains of lipid and nucleic acid. The lipid-based vector (e.g., LNP, liposome, or micelle) can include one or more lipids, such as synthetic lipids, naturally occurring lipids, or suitable combinations thereof.

[0068] In some embodiments, the lipid-based vector includes at least one cationic and/or ionizable lipid. The cationic and/or ionizable lipid can bind to the nucleic acid molecule via electrostatic interactions. The cationic and/or ionizable lipid can also enhance intracellular delivery and/or endosomal escape. Examples of cationic and/or ionizable lipids that may be used include: 1,2-dioleoyl-3-trimethylammonium propane (DOTAP); N,N-dimethyl-2,3-dioleoyloxypropylamine (DODMA); 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA); 3-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol); dimethyldioctadecylammonium bromide (DDAB); 1,2-dioleoyl-3-dimethylammonium-propane (DODAP); 1,2-diacyloxy-3-dimethylammonium propane; 1,2-dialkyloxy-3-dimethylammonium propane; dioctadecyldimethyl ammonium chloride (DODAC); 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA); 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC); 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE); 2,3-dioleoyloxy-N-[2(spermine carboxamide)ethyl]-N,N-dimethyl-1-propanamium trifluoroacetate (DOSPA); 1,2-dilinoleoyloxy-N,N-dimethylaminopropane (DLinDMA); 1,2-dilinenyloxy-N,N-dimethylaminopropane (DLinDMA); dioctadecylamidoglycyl spermine (DOGS); 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane (CLinDMA); 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxyl]-3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy)propane (CpLinDMA); N,N-dimethyl-3,4-dioleoyloxybenzylamine (DMOBA); 1,2-N,N'-dioleoylcarbamyl-3-dimethylaminopropane (DOcarbDAP); 2,3-dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP); 1,2-N,N'-dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP); 1,2-dilinoleoylcarbamyl-3-dimethylaminopropane (DLinCDAP); 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA); 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-K-XTC2-DMA); 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA);

heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA); N-(2-Hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (DMRIE); (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(cis-9-tetradecenyl-oxy)-1-propanaminium bromide (GAP-DMORIE); (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (GAP-DLRIE); (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (GAP-DMRIE); N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (PAE-DMRIE); N-(4-carboxybenzyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminium (DOBAQ); 2-({8-[(3p)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA); 1,2-dimyristoyl-3-dimethylammonium-propane (DMDAP); 1,2-dipalmitoyl-3-dimethylammonium-propane (DPDAP); N1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-amino-propyl)amino]butylcarboxamido)ethyl]-3,4-di[oleoyloxy]-benzamide (MVL5); 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOEPC); 2,3-bis(dodecyloxy)-N-(2-hydroxyethyl)-N,N-dimethylpropan-1-ammonium bromide (DLRIE); N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-aminium bromide (DMORIE); di((Z)-non-2-en-1-yl) 8,8'-(((2(dimethylamino)ethyl)thio)carbonyl)azanediyl)dioctanoate (ATX); N,N-dimethyl-2,3-bis(dodecyloxy)propan-1-amine (DLDMA); N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-amine (DMDMA); di((Z)-non-2-en-1-yl)-9-((4-(dimethylaminobutanoyl)oxy)heptadecanedioate (L319); N-dodecyl-3-((2-dodecylcarbamoyl-ethyl)-{2-[(2-dodecylcarbamoyl-ethyl)-2-{(2-dodecylcarbamoyl-ethyl)-[2-(2-dodecylcarbamoyl-ethylamino)-ethyl]-amino}-ethylamino]propionamide (lipidoid 98N12-5); 1-[2-[bis(2-hydroxydodecyl)amino]ethyl]-[2-[4-[2-[bis(2-hydroxydodecyl)amino]ethyl]piperazin-1-yl]ethyl]amino]dodecan-2-ol (lipidoid C12-200); (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine (L608); N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine; and 9-heptadecanyl 8-{{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate (SM-102).

[0069] Optionally, the lipid-based vector can include one or more additional lipids that can improve therapeutic efficacy, such as by facilitating encapsulation and/or complexation, enhancing cellular uptake, promoting endosomal escape, improving biocompatibility, and/or improving bioavailability. In some embodiments, the lipid-based vector includes a phospholipid, which can facilitate formation of a lipid bilayer structure. The phospholipid can be a phosphatidylcholine, a phosphatidylethanolamine, a phosphatidylglycerol, a phosphatidic

acid, a phosphatidylserine, or a sphingomyelin. Examples of phospholipids that may be used include distearoylphosphatidylcholine (DSPC); dioleoylphosphatidylcholine (DOPC); dimyristoylphosphatidylcholine (DMPC); dipentadecanoylphosphatidylcholine; dilauroylphosphatidylcholine; dipalmitoylphosphatidylcholine (DPPC); diarachidoylphosphatidylcholine (DAPC); dibehenoylphosphatidylcholine (DBPC); ditricosanoylphosphatidylcholine (DTPC); dilignoceroylphosphatidylcholine (DLPC); palmitoyl-oleoyl-phosphatidylcholine (POPC); 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC); 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC); 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC); dioleoylphosphatidylethanolamine (DOPE); distearoyl-phosphatidylethanolamine (DSPE); dipalmitoyl-phosphatidylethanolamine (DPPE); dimyristoyl-phosphatidylethanolamine (DMPE); dilauroyl-phosphatidylethanolamine (DLPE); and diphytanoyl-phosphatidylethanolamine (DPyPE).

[0070] In some embodiments, the lipid-based vector includes a sterol, such as cholesterol or a cholesterol derivative, which can increase lipid bilayer stability. Examples of cholesterol derivatives include a cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, and tocopherol.

[0071] In some embodiments, the lipid-based vector includes a PEG-functionalized lipid, which can improve biocompatibility, improve bioavailability, and/or limit non-specific uptake. Examples of PEG-functionalized lipids that may be used include 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159), 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000-DMG), and 1,2-distearoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000-DSG).

[0072] For example, a LNP can include four types of lipids: a cationic and/or ionizable lipid, a phospholipid, a sterol, and a PEG-functionalized lipid. When combined, the lipids can self-assemble to form the LNP. The LNP can have any suitable particle size, such as a diameter within a range from 10 nm to 1000 nm, 10 nm to 800 nm, 10 nm to 500 nm, 10 nm to 400 nm, 10 nm to 300 nm, 10 nm to 200 nm, 10 nm to 100 nm, 10 nm to 50 nm, 50 nm to 1000 nm, 50 nm to 800 nm, 50 nm to 500 nm, 50 nm to 400 nm, 50 nm to 300 nm, 50 nm to 200 nm, 50 nm to 100 nm, 100 nm to 1000 nm, 100 nm to 800 nm, 100 nm to 500 nm, 100 nm to 400 nm, 100 nm to 300 nm, 100 nm to 200 nm, 200 nm to 1000 nm, 200 nm to 800 nm, 200 nm to 500 nm, 200 nm to 400 nm, 200 nm to 300 nm, 300 nm to 1000 nm, 300 nm to 800 nm, 300 nm to 500 nm, 300 nm to 400 nm, 400 nm to 1000 nm, 400 nm to 800 nm, 400 nm to 500 nm, 500 nm to

1000 nm, 500 nm to 800 nm, or 800 nm to 1000 nm. The size of the LNP can be sufficiently large to allow for physical entrapment within the dynamic hydrogel, as described further herein.

[0073] As another example, the delivery vector can be a polymer vector. The polymer vector can include one or more polymers, such as synthetic polymers, naturally occurring polymers, or a combination thereof. In some embodiments, the polymer vector includes at least one cationic and/or ionizable polymer that complexes the nucleic acid molecule via electrostatic interactions. Examples of cationic and/or ionizable polymers include polyethyleneimine (PEI), poly(L-lysine) (PLL), poly(β -amino esters) (PBAEs), poly(2-dimethylaminoethyl methacrylate) (DMAEMA), chitosan, polyaspartamides, poly(glycidyl amines), poly(amidoamines), or combinations (e.g., copolymers or mixtures) thereof. Optionally, the polymer vector can include one or more additional polymers that provide additional functionality, such as PEG to enhance biocompatibility. The additional polymer(s) can be copolymerized with, conjugated to, or mixed with the cationic and/or ionizable polymer.

[0074] When combined, the nucleic acid molecule and delivery vector can aggregate with each other to form a complex. The nucleic-acid delivery vector complex can have a size (e.g., mean diameter) that is greater than the mesh size of the dynamic hydrogel. For example, the mesh size of the dynamic hydrogel can be less than or equal to 2 nm, 2.5 nm, 3 nm, 3.5 nm, 4 nm, 4.5 nm, or 5 nm, and/or within a range from 2 nm to 5 nm. The size of the nucleic acid-delivery vector complex can be within a range from 10 nm to 1000 nm, 10 nm to 800 nm, 10 nm to 500 nm, 10 nm to 400 nm, 10 nm to 300 nm, 10 nm to 200 nm, 10 nm to 100 nm, 10 nm to 50 nm, 50 nm to 1000 nm, 50 nm to 800 nm, 50 nm to 500 nm, 50 nm to 400 nm, 50 nm to 300 nm, 50 nm to 200 nm, 50 nm to 100 nm, 100 nm to 1000 nm, 100 nm to 800 nm, 100 nm to 500 nm, 100 nm to 400 nm, 100 nm to 300 nm, 100 nm to 200 nm, 200 nm to 1000 nm, 200 nm to 800 nm, 200 nm to 500 nm, 200 nm to 400 nm, 200 nm to 300 nm, 300 nm to 1000 nm, 300 nm to 800 nm, 300 nm to 500 nm, 300 nm to 400 nm, 400 nm to 1000 nm, 400 nm to 800 nm, 400 nm to 500 nm, 500 nm to 1000 nm, 500 nm to 800 nm, or 800 nm to 1000 nm. In such embodiments, the nucleic acid-delivery vector complex can be encapsulated in the dynamic hydrogel via physical entrapment by the hydrogel network. Accordingly, in vivo delivery of the nucleic acid-delivery vector complex may rely primarily upon erosion of the dynamic hydrogel and/or infiltration of cells into the dynamic hydrogel, as described further below.

[0075] The formulation of the dynamic hydrogel can be selected to avoid interfering with the complexation of the nucleic acid molecule with the delivery vector. In some instances, stiffer dynamic hydrogels may require more vigorous mixing during hydrogel formation, which

may result in disruption of the nucleic acid-delivery vector complex. Weaker dynamic hydrogels can therefore be advantageous for preserving the integrity of the complexes and thus may exhibit improved therapeutic activity compared to stiffer dynamic hydrogels. Weaker dynamic hydrogels may also promote cellular infiltration into the hydrogel, which can be beneficial for targeting the nucleic acid therapeutic to a target cell population as described further below. Additionally, although weaker dynamic hydrogels may also degrade faster than stiffer dynamic hydrogels, this may be acceptable or even advantageous in some instances, such as to promote cell infiltration into the hydrogel and/or when delivering relatively unstable nucleic acids such as mRNA, as discussed further below.

[0076] For example, in some embodiments, the storage modulus of the dynamic hydrogel encapsulating the nucleic acid-delivery vector complex is less than or equal to 500 Pa, 250 Pa, 200 Pa, 150 Pa, 100 Pa, 75 Pa, 50 Pa, 25 Pa, or 10 Pa. The storage modulus can be within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa. The storage modulus can be measured, for example, using an oscillatory shear test in a parallel plate rheometer at an angular frequency within a range from 0.1 rad/s to 100 rad/s (e.g., 10 rad/s), a strain within the linear viscoelastic region of the hydrogel (e.g., 1% strain), and a temperature of 25 °C.

[0077] Dynamic hydrogels having a lower stiffness and storage modulus can also exhibit a lower yield stress. The yield stress of the dynamic hydrogel encapsulating the nucleic acid-delivery vector complex can be less than or equal to 100 Pa, 75 Pa, 50 Pa, 25 Pa, 10 Pa, 5 Pa, 1 Pa, 0.5 Pa, or 0.1 Pa. The yield stress can be within a range from 0.1 Pa to 100 Pa, 0.1 Pa to 50 Pa, 0.1 Pa to 25 Pa, 0.1 Pa to 10 Pa, 0.1 Pa to 5 Pa, 0.1 Pa to 1 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5 Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa. The yield stress can be measured, for example, using a stress ramp or stress sweep (e.g., from 0.1 Pa to 100 Pa, or from 0.1 Pa to 1000 Pa) in a parallel plate rheometer at a temperature of 25 °C to identify the stress at which the hydrogel exhibits a drop in viscosity.

[0078] The stiffness, storage modulus, and/or yield stress of a dynamic hydrogel can be reduced by decreasing the solid content of the hydrogel. For instance, in embodiments where the dynamic hydrogel encapsulating the nucleic acid-delivery vector complex is a PNP

hydrogel, the PNP hydrogel can include no more than 2 wt%, 1 wt%, 0.8 wt%, 0.5 wt%, or 0.25 wt% polymer; and/or no more than 10 wt%, 8 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, or 2 wt% nanoparticles. The polymer concentration can be within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%. The nanoparticle concentration can be within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%. In some embodiments, the PNP hydrogel is a 0.25-2 hydrogel, 0.25-5 hydrogel, a 0.25-8 hydrogel, a 0.25-10 hydrogel, a 0.5-2 hydrogel, a 0.5-5 hydrogel, a 0.5-8 hydrogel, a 0.5-10 hydrogel, a 0.8-2 hydrogel, a 0.8-5 hydrogel, a 0.8-8 hydrogel, a 0.8-10 hydrogel, a 1-2 hydrogel, a 1-5 hydrogel, a 1-8 hydrogel, or a 1-10 hydrogel.

[0079] Alternatively or in combination, the chemistry of the components of the dynamic hydrogel can be selected to avoid disrupting the nucleic acid-delivery vector complex. For instance, in embodiments where the dynamic hydrogel includes a polymer (e.g., HPMC) modified with a hydrophobic moiety, the hydrophobic moiety can be selected to reduce interactions with lipid vectors such as LNPs. In some embodiments, hydrophobic moieties that have shorter lipid chains (e.g., C6 instead of C12) can exhibit less lipid-like characteristics and thus may be less likely to disrupt the complexation between the nucleic acid molecule and the lipid vector.

[0080] In some embodiments, the nucleic acid therapeutic is delivered by a viral delivery vector. The viral delivery vector can include a polynucleotide expression cassette including a nucleic acid molecule as described herein. The viral delivery vector can be derived from viruses from any mammalian species, such as humans and non-human primates (e.g., gorillas, rhesus macaques). The viral delivery vector may be derived from a retrovirus, an adenovirus, an adeno-associated virus, a herpes virus, a vaccinia virus, a poxvirus, an alphavirus, a gamma retrovirus, a polyoma virus, a lentivirus, a paramyxovirus (e.g., Newcastle disease virus, respiratory syncytial virus, parainfluenza type 5 virus), or a vesicular stomatitis virus, for example. The viral delivery vector may be encapsulated in a dynamic hydrogel as described herein, e.g., via physical entrapment within the hydrogel network.

2. Nucleic Acid Vaccines

[0081] In some embodiments, the nucleic acid therapeutic is a nucleic acid vaccine (e.g., an RNA vaccine or a DNA vaccine). The nucleic acid vaccine can include at least one nucleic acid molecule (e.g., mRNA or plasmid DNA) that encodes one or more antigens. The

nucleic acid molecule can encode the entire antigen (e.g., a whole protein) or a portion of the antigen (e.g., a protein fragment or peptide sequence). Optionally, the nucleic acid molecule can encode a fusion protein that includes multiple antigens as part of a single polypeptide (e.g., multiple copies of the same antigen, multiple different antigens). In some embodiments, the nucleic acid vaccine includes a single type of nucleic acid molecule that encodes for a single antigen. In other embodiments, however, the nucleic acid vaccine can include two or more different nucleic acid molecules that encode for two or more different antigens. The different antigens can be different antigens for the same disease, antigens for different diseases, or a combination thereof.

[0082] The nucleic acid molecule(s) of the nucleic acid vaccine can be complexed with a delivery vector (e.g., a lipid or polymer vector), as described herein. For instance, the nucleic acid molecule can be a mRNA and the delivery vector can be a LNP, such that the nucleic acid vaccine is a mRNA LNP vaccine. In other embodiments, however, different types of nucleic acid molecules and/or delivery vectors can be used.

[0083] In some embodiments, the nucleic acid vaccine is used to prevent and/or treat an infectious disease. Accordingly, the nucleic acid molecule of the nucleic acid vaccine can encode one or more antigens of a pathogen associated with the infectious disease, such as a viral protein, a bacterial protein, a parasite protein, or a fungal protein, or a fragment thereof. Examples of pathogens that may be targeted by the nucleic acid vaccines herein include viruses, such as alphaviruses (e.g., chikungunya virus, Eastern equine encephalitis virus, Ross River virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), coronaviruses (e.g., SARS-CoV-1, SARS-CoV-2, MERS), filoviruses (e.g., Ebola virus, Marburg virus, Sudan virus), flaviviruses (e.g., dengue virus, yellow fever virus), hepatitis viruses (e.g., hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus), herpes simplex virus (HSV) (e.g., HSV-1, HSV-2), human immunodeficiency virus (HIV), human papillomavirus (HPV), influenza virus (e.g., serotype A (group 1 and 2), serotype B), measles virus, mumps virus, paramyxoviruses (e.g., Hendra virus, Nipah virus), poliovirus, rabies virus, respiratory syncytial virus (RSV), rubella virus, varicella-zoster virus, variola virus, West Nile virus; and/or other microbes, such as *Bacillus anthracis*, *Bordetella pertussis*, *Burkholderia cepacia*, *Clostridium botulinum*, *Clostridium tetani*, *Corynebacterium diphtheria*, Group B *Streptococcus*, *Haemophilus influenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella boydii*, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella*

sonnei, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Vibrio cholera*, *Yersinia enterocolitica*, *Giardia duodenalis*, *Plasmodium falciparum*, *Plasmodium vivax*, *Toxoplasma gondii*, and *Trypanosoma brucei*. Examples of infectious diseases that may be prevented and/or treated using the nucleic acid vaccines described herein include anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes (e.g., oral herpes, genital herpes), Hendra virus disease, HIV/AIDs, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, Middle East respiratory syndrome (MERS), mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, severe acute respiratory syndrome (SARS), smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, and yersiniosis.

[0084] In some embodiments, the nucleic acid vaccine is used to prevent and/or treat cancer. Accordingly, the nucleic acid molecule of the nucleic acid vaccine can encode one or more antigens that are expressed by tumor cells of the cancer. Examples of cancers that may be prevented and/or treated by the nucleic acid vaccines herein include biliary tract cancer, bladder cancer, brain cancer (e.g., glioblastomas, medulloblastomas), breast cancer, cervical cancer, choriocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia (e.g., acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia), liver cancer, lymphoma (e.g., Hodgkin's disease, non-Hodgkin lymphoma), lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer (e.g., renal cell adenocarcinoma, nephroblastoma), sarcoma (e.g., fibrosarcoma, leiomyosarcoma, liposarcoma, rhabdomyosarcoma, osteosarcoma), skin cancer (e.g., basal cell carcinoma, squamous cell carcinoma, melanoma), testicular cancer, and thyroid cancer.

[0085] In some embodiments, the nucleic acid vaccine is a tolerogenic vaccine that is used to treat and/or prevent an autoimmune disease or condition. Accordingly, the nucleic acid molecule of the nucleic acid vaccine can encode one or more autoantigens, such that administration of the nucleic acid vaccine to the subject induces immunological tolerance to the autoantigen(s). Examples of autoimmune diseases or conditions that can be prevented and/or treated by the nucleic acid vaccines herein include arthritis (e.g., psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis), diabetes, inflammatory bowel disease (e.g.,

Crohn's disease, ulcerative colitis), multiple sclerosis (MS) (e.g., relapsing-remitting MS, secondary-progressive MS, primary-progressive MS), myasthenia gravis, pemphigus (e.g., pemphigus vulgaris, pemphigus foliaceus), psoriasis, system lupus erythematosus, and transplant rejection.

[0086] Optionally, a nucleic acid vaccine can be codelivered with at least one additional therapeutic agent that enhances the efficacy of the nucleic acid vaccine, such as a small molecule drug, peptide, protein, polysaccharide, nucleic acid, cell, or combinations thereof. For example, the therapeutic agent(s) can include one or more adjuvants, such a lipid-based adjuvant (e.g., a bacterial lipopolysaccharide-based adjuvant such as monophosphoryl lipid A (MPLA), an emulsion-based adjuvant such as MF59), a saponin-based adjuvant (e.g., QS-21), a polynucleotide adjuvant (e.g., cytosine phosphoguanine (CpG), immunomodulatory GpG, polyinosinic-polycytidylic acid (poly(I:C))), a metal-based adjuvant (e.g., an aluminum-based adjuvant such as amorphous aluminum hydroxyphosphate sulfate, aluminum hydroxide, aluminum phosphate, or potassium aluminum sulfate (alum)), or a combination thereof. In some embodiments, the adjuvant is a Toll-like receptor (TLR) agonist, such a TLR7/8 agonist (e.g., an imidazoquinoline such as R848, 3M052), a TLR1/2 agonist (e.g., Pam3CSK4), or a TLR2/6 agonist (e.g., Pam2CSK4). In some embodiments, the adjuvant is a NOD1 agonist, a NOD2 agonist (e.g., L18-MDP), or a NOD1/2 agonist (e.g., murabutide). The adjuvant can be a small molecule or peptide that has been functionalized with a lipid (e.g., 3M052, Pam3CSK4, Pam2CSK4, L18-MDP)

[0087] As another example, the therapeutic agent(s) can include one or more immunomodulatory molecules that influence the activity of immune cells, such as antigen-presenting cells (APCs), T cells, and/or B cells. For instance, the therapeutic agent(s) can include one or more immunostimulatory molecules that recruit and/or activate immune cells, and/or one or more checkpoint inhibitors that prevent inhibition of immune cells activity. Examples of such molecules include cytokines (e.g., GM-CSF, FLT3L, IL-2, IL-10, IL-7, IL-12, IL-15, IL-21), chemokines (e.g., CCL1, CCL2, CCL3, CCL5, CCL 7, CCL8, CCL13, CCL17, CCL22, CXCL8, CXCL9, CXCL10, CXCL11), and antibodies (e.g., anti-CD40, anti-PD1). Alternatively, in embodiments where the nucleic vaccine is a tolerogenic vaccine, the therapeutic agent(s) can include one more immunosuppressive and/or anti-inflammatory molecules that facilitate the induction of immunological tolerance. Examples of such molecules include cytokines (e.g., IL-1Ra, IL-2, IL-4, IL-10, IL-11, IL-13, TGF β), small molecule drugs (e.g., glucocorticoids such as prednisone, dexamethasone, and hydrocortisone), and antibodies

(e.g., anti-CD20, anti-TNF α). Optionally, the therapeutic agent(s) can include a combination of at least one adjuvant and at least one immunomodulatory molecule.

[0088] The therapeutic agent(s) can be encapsulated in the dynamic hydrogel via physical entrapment, interactions with hydrogel components (e.g., hydrophobic interactions), or suitable combinations thereof. For instance, therapeutic agents including a lipophilic moiety (e.g., lipid-based and/or lipid-functionalized adjuvants) can adhere to hydrophobic components of the hydrogel (e.g., hydrophobic side chains of the polymer and/or hydrophobic surfaces of the nanoparticles). Optionally, the therapeutic agent(s) can be administered to the subject separately from the nucleic acid therapeutic via any suitable administration route (e.g., parenteral or non-parenteral administration).

[0089] In embodiments where the therapeutic agent is a peptide or a protein (e.g., a cytokine, chemokine, or antibody), a second nucleic acid therapeutic including a second nucleic acid molecule (e.g., mRNA or plasmid DNA) encoding the therapeutic agent can be administered to the subject. The second nucleic acid therapeutic can include a delivery vector for the second nucleic acid molecule as described herein, such as a lipid vector (e.g., a LNP). The second nucleic acid therapeutic can be encapsulated in the dynamic hydrogel together with the nucleic acid vaccine, can be encapsulated in a separate dynamic hydrogel, or can be administered to the subject via another administration route. Accordingly, following administration of the second nucleic acid therapeutic to the subject, the therapeutic agent encoded by the second nucleic acid molecule can be expressed and can exert its therapeutic effect in combination with the nucleic acid vaccine.

[0090] In some embodiments, the nucleic acid vaccine can be codelivered with another type of vaccine, such as a subunit (e.g., protein) vaccine, a live attenuated vaccine, an inactivated vaccine, or a toxoid vaccine. In such embodiments, the nucleic acid vaccine and the other vaccine can be encapsulated together in the dynamic hydrogel, the nucleic acid vaccine can be encapsulated in a first dynamic hydrogel and the other vaccine can be encapsulated in a second dynamic hydrogel, or the nucleic acid vaccine can be encapsulated in a first dynamic hydrogel while the other vaccine is administered to the subject via another administration route. The nucleic acid vaccine and the other vaccine can be administered concurrently, the nucleic acid vaccine can be administered before the other vaccine, or the other vaccine can be administered before the nucleic acid vaccine.

[0091] Although certain embodiments herein are described in connection with nucleic acid vaccines, the nucleic acid therapeutics described herein can be used for other types of therapeutic applications. For example, the nucleic acid therapeutics herein can be used for gene therapy, such as by providing a nucleic acid molecule (e.g., mRNA or plasmid DNA) having a normal copy of one or more genes that are missing or defective in the subject. As another example, the nucleic acid therapeutics herein can be used for gene silencing, such as by providing a nucleic acid molecule (e.g., siRNA) that inhibits expression of one or more genes in the subject. In a further example, the nucleic acid therapeutics herein can be used for gene editing, such as by providing a nucleic acid molecule that encodes a gene editing system (e.g., CRISPR-Cas9 system) and/or a nucleic acid molecule that encodes a guide sequence for the gene editing system (e.g., guide RNA) in order to modify one or more genes in the subject.

B. Compositions and Methods

[0092] In some embodiments, the present technology provides compositions including a dynamic hydrogel and one or more nucleic acid therapeutics (e.g., a nucleic acid vaccine) encapsulated by the dynamic hydrogel. The dynamic hydrogel carrying the nucleic acid therapeutic can be any of the dynamic hydrogels described in Section I above. For example, the dynamic hydrogel can be a PNP hydrogel composed of a polymer and a plurality of nanoparticles that interact non-covalently with each other, as previously discussed in Section I.A. The dynamic hydrogel can exhibit shear-thinning, self-healing, and/or viscoelastic properties resulting from non-covalent, supramolecular interactions between the hydrogel components, as described above in Section I.B. In embodiments where the nucleic acid therapeutic is a nucleic acid-delivery vector complex (e.g., a mRNA LNP complex), the dynamic hydrogel can be formulated to avoid disrupting the complex, as discussed herein.

[0093] The composition can include any suitable amount of the nucleic acid therapeutic (e.g., nucleic acid molecule and delivery vector) for providing the desired therapeutic effect. For example, the composition can include at least 0.01 μ g, 0.05 μ g, 0.1 μ g, 0.25 μ g, 0.5 μ g, 1 μ g, 2 μ g, 5 μ g, 10 μ g, 15 μ g, 20 μ g, 25 μ g, 30 μ g, 35 μ g, 40 μ g, 45 μ g, 50 μ g, 60 μ g, 70 μ g, 80 μ g, 90 μ g, 100 μ g, 125 μ g, 150 μ g, 175 μ g, or 200 μ g of the nucleic acid therapeutic. Alternatively or in combination, the composition can include no more than 200 μ g, 175 μ g, 150 μ g, 125 μ g, 100 μ g, 90 μ g, 80 μ g, 70 μ g, 60 μ g, 50 μ g, 45 μ g, 40 μ g, 35 μ g, 30 μ g, 25 μ g, 20 μ g, 15 μ g, 10 μ g, 5 μ g, 2 μ g, 1 μ g, 0.5 μ g, 0.25 μ g, 0.1 μ g, or 0.05 μ g of the nucleic acid therapeutic. The amount of the nucleic acid therapeutic in the composition can be within a range from 0.01 μ g to 200 μ g, 0.01 μ g to 150 μ g, 0.01 μ g to 100 μ g, 0.01 μ g to 50 μ g, 0.01 μ g

to 40 μg , 0.01 μg to 30 μg , 0.01 μg to 20 μg , 0.01 μg to 10 μg , 0.01 μg to 5 μg , 0.01 μg to 1 μg , 0.01 μg to 0.5 μg , 0.01 μg to 0.25 μg , 0.01 μg to 0.1 μg , 0.25 μg to 200 μg , 0.25 μg to 150 μg , 0.25 μg to 100 μg , 0.25 μg to 50 μg , 0.25 μg to 40 μg , 0.25 μg to 30 μg , 0.25 μg to 20 μg , 0.25 μg to 10 μg , 0.25 μg to 5 μg , 0.25 μg to 1 μg , 0.25 μg to 0.5 μg , 0.5 μg to 200 μg , 0.5 μg to 150 μg , 0.5 μg to 100 μg , 0.5 μg to 50 μg , 0.5 μg to 40 μg , 0.5 μg to 30 μg , 0.5 μg to 20 μg , 0.5 μg to 10 μg , 0.5 μg to 5 μg , 0.5 μg to 1 μg , 1 μg to 200 μg , 1 μg to 150 μg , 1 μg to 100 μg , 1 μg to 50 μg , 1 μg to 40 μg , 1 μg to 30 μg , 1 μg to 20 μg , 1 μg to 10 μg , 1 μg to 5 μg , 5 μg to 200 μg , 5 μg to 150 μg , 5 μg to 100 μg , 5 μg to 50 μg , 5 μg to 40 μg , 5 μg to 30 μg , 5 μg to 20 μg , 5 μg to 10 μg , 10 μg to 200 μg , 10 μg to 150 μg , 10 μg to 100 μg , 10 μg to 50 μg , 10 μg to 40 μg , 10 μg to 30 μg , 10 μg to 20 μg , 20 μg to 200 μg , 20 μg to 150 μg , 20 μg to 100 μg , 20 μg to 50 μg , 20 μg to 40 μg , 20 μg to 30 μg , 30 μg to 200 μg , 30 μg to 150 μg , 30 μg to 100 μg , 30 μg to 50 μg , 30 μg to 40 μg , 40 μg to 200 μg , 40 μg to 150 μg , 40 μg to 100 μg , 40 μg to 50 μg , 50 μg to 200 μg , 50 μg to 150 μg , 50 μg to 100 μg , 100 μg to 200 μg , 100 μg to 150 μg , or 150 μg to 200 μg . The amount of the nucleic acid therapeutic in the composition can be approximately 0.01 μg , 0.05 μg , 0.1 μg , 0.25 μg , 0.5 μg , 1 μg , 2 μg , 5 μg , 10 μg , 15 μg , 20 μg , 25 μg , 30 μg , 35 μg , 40 μg , 45 μg , 50 μg , 60 μg , 70 μg , 80 μg , 90 μg , 100 μg , 125 μg , 150 μg , 175 μg , or 200 μg .

[0094] The compositions herein can be administered to the subject via any suitable route, such as a parenteral route. For example, in some embodiments, the composition is administered to the subject via injection (e.g., subcutaneous injection or intramuscular injection). The shear-thinning properties of the dynamic hydrogel can allow for facile delivery of the hydrogel and the encapsulated therapeutic cargo via injection. Injection of the composition can be performed using any suitable tubular device having a lumen configured for delivery of a hydrogel, such as needles (e.g., hypodermic needles, surgical needles, infusion needles), injector pens, catheters, trocars, cannulas, tubing, etc. The composition can be injected into any suitable site in the subject's body, such as an arm, thigh, abdomen, or buttock. The composition can be formulated to have a volume that is sufficiently small for injection, such as a volume less than or equal to 2 mL, 1.75 mL, 1.5 mL, 1.25 mL, 1 mL, 0.75 mL, 0.5 mL, or 0.25 mL. In some embodiments, the composition is administered as a single injection at a single injection site, while in other embodiments, the composition can be administered as multiple injections at the same or different injection sites.

[0095] In some embodiments, the self-healing properties of the dynamic hydrogel cause the dynamic hydrogel to form a cohesive depot at the injection site. The formation of a

depot can control the release of the nucleic acid therapeutic. For instance, in embodiments where the nucleic acid therapeutic is physically entrapped in the dynamic hydrogel, the nucleic acid therapeutic may be released from the depot primarily or entirely via erosion of the hydrogel, rather than via diffusion out of the hydrogel. Accordingly, the release rate of the nucleic acid therapeutic can be controlled by tuning the degradation rate of the hydrogel, such as by modifying the stiffness of the hydrogel as described elsewhere herein.

[0096] Alternatively or in combination, the formation of a depot can be used to target the nucleic acid therapeutic to a target cell population. The target cell population can include one or more cell types that, upon taking up the nucleic acid therapeutic, produce a desired therapeutic effect (e.g., preventing and/or treating a disease). In some embodiments, the dynamic hydrogel forms a mesh network that retains the nucleic acid therapeutic within the interior of the hydrogel. The mesh network can allow certain target cells to infiltrate into the hydrogel to take up the nucleic acid therapeutic, while excluding nontarget cells from accessing the nucleic acid therapeutic. The time frame of release of the nucleic acid therapeutic from the dynamic hydrogel can be slower than the time frame of cell infiltration into the hydrogel, such that the nucleic acid therapeutic is taken up primarily or entirely by cells that infiltrate into the hydrogel, rather than by cells external to the hydrogel. This approach can enhance the therapeutic efficacy of the nucleic acid therapeutic and/or reduce side effects associated with uptake of the nucleic acid therapeutic by nontarget cells.

[0097] For instance, in embodiments where the nucleic acid therapeutic is a nucleic acid vaccine (e.g., a mRNA LNP vaccine), immune cells can infiltrate into the dynamic hydrogel, while nonimmune cells at the injection site (e.g., muscle cells, skin cells, adipocytes, fibroblasts) can be mostly or entirely excluded from the hydrogel. The immune cells can include APCs, such as dendritic cells (e.g., cDC1s, cDC2s, iDCs), monocytes, macrophages, and B cells. Selective targeting of nucleic acid vaccines to APCs may improve vaccine efficacy, while also reducing side effects, such as injection site pain, that may be attributable to nonspecific uptake of the vaccine by nonimmune cells. In addition to promoting selective uptake of the nucleic acid vaccine by APCs, the dynamic hydrogel can optionally serve as a local immunological niche by recruiting other types of immune cells, such as T cells (e.g., cytotoxic T cells, helper T cells (such as Th1 cells, Th2 cells, and Th17 cells), regulatory T cells, memory T cells, $\gamma\delta$ T cells), natural killer (NK) cells, neutrophils, basophils, eosinophils, and other myeloid and non-myeloid cells). As described herein, the dynamic hydrogel can

optionally be loaded with one or more therapeutic agents, such as adjuvants, cytokines, and/or chemokines, that recruit and/or activate immune cells.

[0098] The composition can be administered to the subject according to any suitable timing. For instance, the composition can be administered on a periodic basis, such as once per week, once per 2 weeks, once per 4 weeks, once per month, once per 2 months, once per 3 months, once per 4 months, once per 5 months, once per 6 months, once 9 months, once per year, once per 2 years, once per 5 years, or once per 10 years. In embodiments where the composition is a nucleic acid vaccine, the nucleic acid vaccine can be administered according to a vaccination protocol. The vaccination protocol may include a priming dose followed by one or more boost doses (e.g., one, two, three, or more boost doses). The interval between the priming dose and first boost dose can be within a range from 1 week to 12 weeks, 1 week to 10 weeks, 1 week to 8 weeks, 2 weeks to 10 weeks, 2 weeks to 8 weeks, 2 weeks to 4 weeks, 3 weeks to 8 weeks, 4 weeks to 8 weeks, or 1 year to 2 years. The interval between subsequent boost doses can be within a range from 1 month to 12 months, 1 month to 6 months, 1 month to 3 months, 2 months to 6 months, 2 months to 3 months, 2 months to 4 months, 3 months to 6 months, 3 months to 4 months, 4 months to 6 months, 1 year to 2 years, 2 years to 5 years, or 5 years to 10 years. The dosage of the nucleic acid vaccine can be the same for the priming dose and the boost dose(s), or can be different (e.g., the priming dose can be a higher dosage than the boost dose(s)). In embodiments where multiple boost doses are used, each boost dose can have the same dosage, or some or all of the boost doses can have different dosages. Optionally, the vaccination schedule can include the priming dose only, without any boost doses.

[0099] The compositions herein can be configured to deliver a therapeutically effective amount of the nucleic acid therapeutic over a desired treatment period, which can be an amount that is effective to prevent the subject from contracting the disease or condition, and/or to ameliorate or prevent a symptom of a disease or condition in the subject. For example, the treatment period can be at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 28 days, 35 days, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, 100 days, 110 days, 120 days, 150 days, 180 days, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or 1 year. The treatment period can be approximately 2 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, or 12 months.

[0100] In embodiments where the nucleic acid therapeutic includes a relatively unstable nucleic acid molecule, the composition can deliver the nucleic acid therapeutic for a shorter treatment period. For instance, RNA molecules such as mRNA may spontaneously degrade even when encapsulated within the dynamic hydrogel (e.g., due to hydrolysis from the presence of water molecules in the hydrogel). Accordingly, to ensure that the nucleic acid molecule is delivered before it degrades, the dynamic hydrogel can be configured to deliver the nucleic acid therapeutic for a shorter treatment period, such as a treatment period less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day. The treatment period can be within a range from 1 day to 14 days, 1 day to 12 days, 1 day to 10 days, 1 day to 7 days, 4 days to 12 days, 5 days to 10 days, 7 days to 14 days, 7 days to 10 days, 8 days to 12 days, or 8 days to 10 days.

[0101] The treatment period can be the same or similar as the time period in which the dynamic hydrogel remains as a cohesive depot at the injection site, and can therefore be shortened by increasing the degradation rate of the dynamic hydrogel. In some embodiments, the dynamic hydrogel has a relatively low stiffness, storage modulus, and/or yield stress to produce a faster degradation rate and, thus, a shorter treatment period. Weaker hydrogels can also be beneficial for enhancing cellular infiltration and/or avoiding disruption of nucleic acid-delivery vector complexes, as described herein.

[0102] For example, in some embodiments, the storage modulus of the dynamic hydrogel is less than or equal to 500 Pa, 250 Pa, 200 Pa, 150 Pa, 100 Pa, 75 Pa, 50 Pa, 25 Pa, or 10 Pa. The storage modulus can be within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa. The storage modulus can be measured, for example, using an oscillatory shear test in a parallel plate rheometer at an angular frequency within a range from 0.1 rad/s to 100 rads/s (e.g., 10 rad/s), a strain within the linear viscoelastic region of the hydrogel (e.g., 1% strain), and a temperature of 25 °C.

[0103] Alternatively or in combination, the yield stress of the dynamic hydrogel can be less than or equal to 100 Pa, 75 Pa, 50 Pa, 25 Pa, 10 Pa, 5 Pa, 1 Pa, 0.5 Pa, or 0.1 Pa. The yield stress can be within a range from 0.1 Pa to 100 Pa, 0.1 Pa to 50 Pa, 0.1 Pa to 25 Pa, 0.1 Pa to 10 Pa, 0.1 Pa to 5 Pa, 0.1 Pa to 1 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5 Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa,

10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa. The yield stress can be measured, for example, using a stress ramp or stress sweep (e.g., from 0.1 Pa to 100 Pa, or from 0.1 Pa to 1000 Pa) in a parallel plate rheometer at a temperature of 25 °C to identify the stress at which the hydrogel exhibits a drop in viscosity.

[0104] The stiffness, storage modulus, and/or yield stress of a dynamic hydrogel can be reduced by decreasing the solid content of the hydrogel. For instance, in embodiments where the dynamic hydrogel is a PNP hydrogel, the PNP hydrogel can include no more than 2 wt%, 1 wt%, 0.8 wt%, 0.5 wt%, or 0.25 wt% polymer; and/or no more than 10 wt%, 8 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, or 2 wt% nanoparticles. The polymer concentration can be within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%. The nanoparticle concentration can be within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%. In some embodiments, the PNP hydrogel is a 0.25-2 hydrogel, 0.25-5 hydrogel, a 0.25-8 hydrogel, a 0.25-10 hydrogel, a 0.5-2 hydrogel, a 0.5-5 hydrogel, a 0.5-8 hydrogel, a 0.5-10 hydrogel, a 0.8-2 hydrogel, a 0.8-5 hydrogel, a 0.8-8 hydrogel, a 0.8-10 hydrogel, a 1-2 hydrogel, a 1-5 hydrogel, a 1-8 hydrogel, or a 1-10 hydrogel.

[0105] In some embodiments, the present technology provides methods for treating a subject by administering a composition as described herein. The composition can prevent and/or treat a disease or condition by producing a desired therapeutic effect in the subject, such as alleviation of symptoms, a reduction in the severity of the disease or condition, inhibiting an underlying cause of the disease or condition, steadying the disease or condition in a non-advanced state, delaying the progress of a disease or condition, improving or alleviating the disease or condition, and/or preventing the subject from contracting a disease or condition.

[0106] For example, the disease or condition can be an infectious disease, such as anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes (e.g., oral herpes, genital herpes), Hendra virus disease, HIV/AIDS, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, MERS, mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, SARS, smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, and yersiniosis.

[0107] In some embodiments, a method of preventing and/or treating an infectious disease includes administering a composition of the present technology to a subject in need thereof. The composition can be administered to the subject via injection (e.g., a subcutaneous or intramuscular injection). The composition can include a dynamic hydrogel (e.g., a PNP hydrogel) encapsulating a therapeutically effective amount of a nucleic acid therapeutic for preventing and/or treating the infectious disease. For instance, in embodiments where the composition includes a nucleic acid vaccine (e.g., a mRNA LNP vaccine), the therapeutically effective amount can be an amount sufficient to elicit an immune response in the subject that prevents and/or treats the infectious disease. Optionally, the composition can include at least one additional therapeutic agent, such as an adjuvant, cytokine, and/or chemokine. The components of the dynamic hydrogel can be selected to provide injectability, formation of a cohesive depot in vivo, controlled release of the nucleic acid therapeutic, and/or selective infiltration of the depot by a target cell population (e.g., immune cells such as APCs). For example, the treatment period can be less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day. In some embodiments, the dynamic hydrogel is a PNP hydrogel (e.g., composed of a hydrophobically modified cellulosic derivative (e.g., HPMC-C12) and a plurality of amphiphilic nanoparticles (e.g., PEG-PLA nanoparticles). The PNP hydrogel can be a 0.25-2 hydrogel, 0.25-5 hydrogel, a 0.25-8 hydrogel, a 0.25-10 hydrogel, a 0.5-2 hydrogel, a 0.5-5 hydrogel, a 0.5-8 hydrogel, a 0.5-10 hydrogel, a 0.8-2 hydrogel, a 0.8-5 hydrogel, a 0.8-8 hydrogel, a 0.8-10 hydrogel, a 1-2 hydrogel, a 1-5 hydrogel, a 1-8 hydrogel, or a 1-10 hydrogel.

[0108] As another example, the disease or condition can be a cancer, such as biliary tract cancer, bladder cancer, brain cancer (e.g., glioblastomas, medulloblastomas), breast cancer, cervical cancer, choriocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia (e.g., acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia), liver cancer, lymphoma (e.g., Hodgkin's disease, non-Hodgkin lymphoma), lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer (e.g., renal cell adenocarcinoma, nephroblastoma), sarcoma (e.g., fibrosarcoma, leiomyosarcoma, liposarcoma, rhabdomyosarcoma, osteosarcoma), skin cancer (e.g., basal cell carcinoma, squamous cell carcinoma, melanoma), testicular cancer, or thyroid cancer.

[0109] In some embodiments, a method of preventing and/or treating cancer includes administering a composition of the present technology to a subject in need thereof. The

composition can be administered to the subject via injection (e.g., a subcutaneous or intramuscular injection). The composition can include a dynamic hydrogel (e.g., a PNP hydrogel) encapsulating a therapeutically effective amount of a nucleic acid therapeutic for preventing and/or treating the cancer. For instance, in embodiments where the composition includes a nucleic acid vaccine (e.g., a mRNA LNP vaccine), the therapeutically effective amount can be an amount sufficient to elicit an immune response in the subject that prevents and/or treats the cancer. Optionally, the composition can include at least one additional therapeutic agent and/or a second nucleic acid therapeutic encoding the at least one additional therapeutic agent, such as an adjuvant, a cytokine, a chemokine, and/or a checkpoint inhibitor. The components of the dynamic hydrogel can be selected to provide injectability, formation of a cohesive depot in vivo, controlled release of the nucleic acid therapeutic, and/or selective infiltration of the depot by a target cell population (e.g., immune cells such as APCs). For example, the treatment period can be less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day. In some embodiments, the dynamic hydrogel is a PNP hydrogel (e.g., composed of a hydrophobically modified cellulosic derivative (e.g., HPMC-C12) and a plurality of amphiphilic nanoparticles (e.g., PEG-PLA nanoparticles). The PNP hydrogel can be a 0.25-2 hydrogel, 0.25-5 hydrogel, a 0.25-8 hydrogel, a 0.25-10 hydrogel, a 0.5-2 hydrogel, a 0.5-5 hydrogel, a 0.5-8 hydrogel, a 0.5-10 hydrogel, a 0.8-2 hydrogel, a 0.8-5 hydrogel, a 0.8-8 hydrogel, a 0.8-10 hydrogel, a 1-2 hydrogel, a 1-5 hydrogel, a 1-8 hydrogel, or a 1-10 hydrogel.

[0110] In a further example, the disease or condition can be an autoimmune disease or condition, such as arthritis (e.g., psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis), inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis), multiple sclerosis (MS) (e.g., relapsing-remitting MS, secondary-progressive MS, primary-progressive MS), myasthenia gravis, pemphigus (e.g., pemphigus vulgaris, pemphigus foliaceus), psoriasis, system lupus erythematosus, or transplant rejection.

[0111] In some embodiments, a method of preventing and/or treating an autoimmune disease or condition includes administering a composition of the present technology to a subject in need thereof. The composition can be administered to the subject via injection (e.g., a subcutaneous or intramuscular injection). The composition can include a dynamic hydrogel (e.g., a PNP hydrogel) encapsulating a therapeutically effective amount of a nucleic acid therapeutic for preventing and/or treating the autoimmune disease. For instance, in embodiments where the composition includes a tolerogenic nucleic acid vaccine (e.g., a mRNA

LNP vaccine), the therapeutically effective amount can be an amount sufficient to elicit immunological tolerance in the subject. Optionally, the composition can include at least one additional therapeutic agent and/or a second nucleic acid therapeutic encoding the at least one additional therapeutic agent, such as an immunosuppressive molecule. The components of the dynamic hydrogel can be selected to provide injectability, formation of a cohesive depot in vivo, controlled release of the nucleic acid therapeutic, and/or selective infiltration of the depot by a target cell population (e.g., immune cells such as APCs). For example, the treatment period can be less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day. In some embodiments, the dynamic hydrogel is a PNP hydrogel (e.g., composed of a hydrophobically modified cellulosic derivative (e.g., HPMC-C12) and a plurality of amphiphilic nanoparticles (e.g., PEG-PLA nanoparticles). The PNP hydrogel can be a 0.25-2 hydrogel, 0.25-5 hydrogel, a 0.25-8 hydrogel, a 0.25-10 hydrogel, a 0.5-2 hydrogel, a 0.5-5 hydrogel, a 0.5-8 hydrogel, a 0.5-10 hydrogel, a 0.8-2 hydrogel, a 0.8-5 hydrogel, a 0.8-8 hydrogel, a 0.8-10 hydrogel, a 1-2 hydrogel, a 1-5 hydrogel, a 1-8 hydrogel, or a 1-10 hydrogel.

[0112] In some embodiments, the present technology provides methods for preparing a composition for treating a subject as described herein. The method can include combining the components of a dynamic hydrogel (e.g., polymer and nanoparticles) with a nucleic acid therapeutic, thus forming a dynamic hydrogel encapsulating the nucleic acid therapeutic. The combining of the hydrogel components and nucleic acid therapeutic can be performed using simple mixing under gentle conditions, such as physiological pH (e.g., pH 7.0 to 7.4) at room temperature (e.g., 25 °C) or physiological temperature (e.g., 37 °C). Optionally, in embodiments where the nucleic acid therapeutic includes a nucleic acid molecule complexed with a delivery vector (e.g., a lipid vector or a polymer vector), the mixing can be sufficiently gentle to avoid disrupting the nucleic acid-delivery vector complex.

[0113] In some embodiments, the composition is prepared no more than 1 hour, 30 minutes, 15 minutes, 10 minutes, 5 minutes, 2 minutes, or 1 minute before administering the composition to the subject. Alternatively or in combination, the composition can be prepared at least 1 minute, 2 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, or 1 hour before administering the composition to the subject. The dynamic hydrogel can be mostly or fully formed before the composition is administered to the subject. For example, the dynamic hydrogel can be sufficiently crosslinked (e.g., non-covalently crosslinked) to exhibit the shear-

thinning, self-healing, and/or viscoelastic properties described herein before the composition is administered to the subject.

[0114] In some embodiments, the present technology provides kits for preparing a composition as described herein. The kit can include a solution containing a nucleic acid therapeutic (e.g., a solution containing nucleic acid molecules combined with a delivery vector) and one or more solutions containing the components of a dynamic hydrogel (e.g., a solution containing a polymer and a solution containing nanoparticles, or a single solution containing a polymer and nanoparticles). The solutions can be provided in tubes, bottles, ampoules, syringes, or any other suitable storage container. In some embodiments, the solutions each independently include a suitable pharmaceutically acceptable diluent. The pharmaceutically acceptable diluent can be any diluent that does not substantially produce adverse reactions, e.g., toxic, allergic, or immunological reactions, when administered to a subject. Examples of pharmaceutically acceptable diluents include, but are not limited to, saline, Ringer's solution, dextrose solution, phosphate buffered saline, water, or a combination thereof. The pharmaceutically acceptable diluent can include an isotonicity imparting agent, such as sodium chloride, potassium chloride, or monosodium phosphate. The pharmaceutically acceptable diluent can include a buffer, such as bicarbonate, TRIS, HEPES, MOPS, CHES, CHAPS, or phosphate buffered saline. The pharmaceutically acceptable diluent can include stabilizers and/or preservatives, as appropriate. Additional examples and details of pharmaceutically acceptable diluents can be found in Martin, Remington's Pharmaceutical Sciences, 21st Ed., Mack Publ. Co., Easton, Pa. (2005), which is incorporated herein by reference in its entirety.

Examples

[0115] The present technology is further illustrated by the following non-limiting examples.

Example 1: Preparation of mRNA LNP Loaded PNP Hydrogels

[0116] This example describes a process for preparing PNP hydrogels loaded with mRNA LNPs.

[0117] *Materials:* Hydroxypropylmethylcellulose (HPMC, meets USP testing specifications), N-methyl-2-pyrrolidone (NMP), 1-dodecylisocyanate, N,N-diisopropylethylamine (DIPEA), acetone, monomethoxy-PEG (5 kDa), diazobicycloundecene (DBU), acetic acid, diethyl ether, hexanes, dimethyl sulfoxide (DMSO), acetonitrile were

purchased from Sigma-Aldrich and used as received. Dichloromethane (DCM) was purchased from Sigma-Aldrich and further dried via cryo-distillation. Lactide was purchased from Sigma-Aldrich and recrystallized from ethyl acetate (dried over sodium sulfate) three times.

[0118] *HPMC-C12 synthesis:* HPMC-C12 was prepared as follows. HPMC (1.0 g) (SEC MALS: M_w ($\bar{M}$) = 372.4 kDa (1.43)) was dissolved in NMP (40 mL) at room temperature with stirring. Once the polymer had completely dissolved, the reaction was brought to 50 °C and a solution of 1-dodecylisocyanate (0.5 mmol) in NMP (5 mL) was added dropwise, followed by DIPEA (catalyst, 125 μ L). The reaction was maintained at 50 °C for 30 minutes, then heat was shut off and mixture was left stirring overnight at room temp. The solution was then precipitated from acetone and HPMC-C12 was purified by dialysis against MilliQ water for 3-4 days (MWCO 3.5 kDa) and lyophilized, yielding HPMC-C12 as a white amorphous powder. The polymer was dissolved at 20 mg mL⁻¹ in sterile PBS, pH 7.4, prior to use in hydrogels.

[0119] *PEG-PLA synthesis:* PEG-PLA was prepared and analyzed as follows. Recrystallized lactide (10 g) was fully dissolved in cryo-distilled DCM (45 mL) under N₂ (g) with mild heating. Methoxy poly(ethylene glycol) (5 kDa; 2.5 g) was heated to 100 °C under vacuum for 1–2 h, allowed to cool under N₂, and then dissolved in cryodistilled DCM (5 mL). Once dissolved, the full PEG solution was added to the lactide solution under N₂ and mixed with hand swirling. A solution of DBU (150 μ L cryodistilled DBU per 1 mL cryodistilled DCM) was prepared and 500 μ L added to the lactide-PEG solution under N₂. The reaction was swirled by hand and allowed to react for 8 min before quenching with acetic acid (~2 drops in 500 μ L acetone). The PEG-PLA copolymer was precipitated from excess 50:50 mixture ethyl ether and hexanes, collected, and dried under vacuum to yield a white amorphous powder. DMF GPC: M_w ($\bar{M}$) = 24.5 kDa (1.13).

[0120] *PEG-PLA nanoparticle (NP) preparation:* NPs were prepared and analyzed as follows. Briefly, a solution (1 mL) of PEG-PLA in 25:75 DMSO:acetonitrile (50 mg mL⁻¹) was added dropwise to water (10 mL) at a stir rate of 600 rpm. NPs were purified by ultracentrifugation over a filter (MWCO 10 kDa; Millipore Amicon Ultra-15) followed by resuspension in PBS to a final concentration of 200 mg mL⁻¹. NP size and dispersity were characterized by DLS (Wyatt DynaPro PlateReader-II; average diameter = 34.1 nm, PDI = 0.05).

[0121] *PNP hydrogel formulation:* HPMC-C12 was dissolved at 2 wt% in PBS and loaded into a 1 mL luer-lock syringe. A 20 wt% solution of PEG-PLA NPs in PBS was added

to a solution of PBS with or without mRNA/LNPs and/or adjuvant depending on formulation, and loaded into a second 1 mL syringe. The two syringes were connected with a female-female luer lock elbow, with care to avoid air at the interface of the HPMC-C₁₂ and nanoparticle solution, and gently mixed until a homogenous PNP hydrogel was formed. Hydrogels were formulated with final concentrations of 0.5 or 1 wt% HPMC-C₁₂ and 5 wt% NPs, denoted PNP-0.5-5 or PNP-1-5.

[0122] FIGS. 2A and 2B illustrate preparation of PNP hydrogels. PNP hydrogels were formed through simple mixing of HPMC-C₁₂ and PEG-PLA NPs. These components interact in a multivalent, non-covalent fashion to form an injectable shear-thinning and self-healing dynamic hydrogel. Multiple cargos can be admixed into the hydrogel, including commercially available mRNA vaccines, other mRNA/LNPs, and adjuvants.

Example 2: Rheological Characterization of mRNA LNP Loaded PNP Hydrogels

[0123] This example describes rheological characterization of PNP hydrogels loaded with mRNA LNPs.

[0124] *PNP hydrogel rheological characterization:* Rheological characterization was performed on PNP hydrogels with or without Moderna Spikevax monovalent (50 µg mL⁻¹) using a TA Instruments DHR-2 stress-controlled rheometer. All experiments were performed using a 20 mm diameter serrated plate geometry at 25 °C with a 500 µm gap. Frequency sweep measurements were performed at a constant 1% strain in the linear viscoelastic regime. Stress sweeps were performed from low to high with steady state sensing and yield stress values defined as the stress at the intersection of lines tangent to the plateau and the yielding regimes. Flow sweeps were performed from high to low shear rates. Step shear experiments were performed by alternating between a low shear rate (0.1 s⁻¹; 60 s) and a high shear rate (10 s⁻¹; 30 s) for three cycles.

[0125] Rheology studies were performed to characterize how commercially available SARS-CoV-2 mRNA vaccine impacted mechanical properties of the PNP hydrogels. Frequency sweep oscillatory shear rheology showed the selected formulation, PNP-0.5-5 (0.5 wt% HPMC-C₁₂; 5 wt% NPs), was solid-like over tested frequencies and was not impacted by mRNA/LNP incorporation (FIG. 3A). Further, stress ramp experiments showed clear pre-yielded and yielded regimes with a yield stress of approximately 3 Pa (3.33 Pa for PNP hydrogel only, 2.89 Pa for PNP hydrogel with mRNA/LNPs) (FIG. 3B). These results indicated these materials would form disk-like depots in the subcutaneous space. High-to-low shear-

ramp and step-shear experiments showed that PNP-0.5-5 hydrogels were shear-thinning and capable of repeatedly returning to a high viscosity state following high-shear events, indicating the hydrogel could be injected through standard needles and recover as a solid-like depot following injection (FIGS. 3C–3E).

Example 3: In Vitro Efficacy of mRNA LNP Loaded PNP Hydrogels

[0126] This example describes in vitro characterization of the efficacy of PNP hydrogels loaded with mRNA LNPs.

[0127] *In vitro LNP transfection:* RAW-Blue cells (InvivoGen, raw-sp) were cultured at 37 °C with 5% CO₂ in DMEM supplemented with high glucose, L-glutamine, sodium pyruvate (Cytiva, SH30243.FS), 10% heat inactivated fetal bovine serum (Cytiva, SH30396.03HI), and penicillin (100 U mL⁻¹)/streptomycin (100 µg mL⁻¹) (Cytiva, SV30010). 100 k cells were plated in each well of a tissue culture treated 96-well plate in 200 µL media and allowed to adhere for 24 h. After 24 h, media was aspirated, replaced with 180 µL fresh media, and 20 µL of PNP hydrogel or PBS with 5 µg mL⁻¹ luciferase mRNA/LNPs was injected through a 26-gauge needle into each well (n=3–4 wells) for a final concentration of 0.1 µg per well. The plate was incubated for 24 hours and transfection efficiency measured using Bright-Glo™ Luciferase Assay System (Promega). Briefly, 100 µL of BrightGlo reagent was added to each well and, after a 3 minute incubation, 180 µL from each well was transferred to a white 96 well plate and luminescence was read on a Synergy H1 Microplate Reader (BioTek Instruments).

[0128] Having shown mRNA/LNPs did not disrupt hydrogel material properties (Example 2), in vitro transfection studies were performed to confirm that mRNA/LNPs were unimpacted by loading within PNP hydrogels. mRNA/LNP transfection efficiency in vitro was assessed with luciferase-encoding mRNA/LNPs mimicking the lipid formulation used in Moderna's Spikevax vaccine. In these assays, the luciferase mRNA/LNPs were diluted into PBS or incorporated into PNP-0.5-5 hydrogels and stored at 4 °C for 0, 1, 7, and 30 days before delivering to RawBlue macrophages and measuring transfection efficiency via bioluminescence after 24 hours (FIG. 4). Transfection efficiency remained unchanged for mRNA/LNPs formulated into PNP hydrogels compared with delivery in bolus at all tested storage times. These stability data agreed with the product stability for Moderna Spikevax (4 °C for 30 days) and demonstrated PNP hydrogel-based formulations did not impede transfection capacity of the mRNA/LNPs.

Example 4: In Vivo Efficacy of mRNA LNP Loaded PNP Hydrogels

[0129] This example describes in vivo characterization of the efficacy of PNP hydrogels loaded with mRNA LNPs.

[0130] mRNA delivered in LNPs rose to the forefront of vaccine candidates during the COVID-19 pandemic due in part to scalability, adaptability, and potency. Yet there remain critical areas for improvements of these vaccines in durability and breadth of humoral responses. This example explores a modular strategy to target mRNA/LNPs to antigen presenting cells with an injectable PNP hydrogel depot technology which recruits key immune cells and forms an immunological niche in vivo. This niche was characterized on a single cell level and was found to be highly tunable through incorporation of adjuvants like MPLAs and 3M-52. Delivering commercially available SARS-CoV-2 mRNA vaccines in PNP hydrogels improves the durability and quality of germinal center reactions, and the magnitude, breadth, and durability of humoral responses. The tunable immune niche formed within PNP hydrogels effectively skews immune responses based on encapsulated adjuvants, creating opportunities to precisely modulate mRNA/LNP vaccines for various indications from infectious diseases to cancers.

[0131] Over the last three decades, mRNA technology has grown as a therapeutic modality for treatment and prevention of various diseases. It has been developed for protein replacement as well as vaccination against infectious diseases and cancers. During the COVID-19 pandemic, mRNA delivered in LNPs rose to the forefront of vaccine candidates due to its safety profile, rapid and affordable scalability, and adaptability to emergent variants of concern. Moderna and Pfizer-BioNTech mRNA vaccines proved highly potent and were instrumental in curbing the spread of SARS-CoV-2. Yet there remain critical areas for improvement of mRNA vaccines, including stability for simpler storage, transport, and deployment in under-resourced parts of the world, as well as enhanced durability and breadth of humoral immune responses. This example describes an approach to enhancing mRNA/LNP vaccines by targeting of the mRNA/LNPs to antigen presenting cells (APCs), adjuvanting with molecular adjuvants, and prolonging antigen availability.

[0132] APCs are key immune cells responsible for processing and presenting antigen to train the adaptive arm of the immune system—T and B cells—and a desirable target for mRNA/LNP transfection. Macrophages and dendritic cells (DCs) infiltrating the site of administration, as well as off-target adipocytes, fibroblasts, and epithelial cells in these tissues,

can take up mRNA and express protein following intramuscular (IM) injection in non-human primates (NHPs), with peak expression 24 hours post-injection. While antigen produced by non-APCs could contribute to a meaningful immune response, improving antigen production and delivery to professional APCs is expected to be beneficial for priming both B and T cell responses. APC targeting may be improved by modifying LNPs with targeting moieties like antibodies or mannose, as well as altering lipid chemistries to bias their biodistribution. While these strategies may demonstrate improved cell-type targeting, they often involve cumbersome large-scale screening campaigns and complex lipid chemistries that can alter LNP bioactivity since the lipids are known to contribute to transfection efficiency and adjuvanting effects of the LNPs. Moreover, each newly designed lipid will be regulated as a novel chemical entity, thereby limiting translatability. mRNA/LNP vaccines could benefit from a more modular method to target APCs and limit off-target cell transfection without alterations to the existing LNP chemistries.

[0133] In addition to better APC targeting, adjuvanting mRNA/LNP vaccines could improve the overall magnitude and quality of the vaccine response. Adjuvants are immunostimulants which can drive potent immune responses, as well as tailor a response toward T helper-1 (Th1), Th2, or other phenotypes important in mounting successful responses against different diseases. Yet the impact of adjuvants on mRNA vaccines remains poorly characterized. Excessive stimulation of innate cells can reduce mRNA expression and negatively impact vaccine efficacy. In some cases, however, incorporation of pathogen-associated molecular pattern (PAMP) adjuvants like toll-like receptor agonists (TLRas) can improve mRNA vaccine efficacy, particularly TLR2/6a, 7/8a, and 9a. Conventional techniques for incorporating PAMP adjuvants generally require lipid modification, reformulation, or even use of a different delivery modality entirely, and are not readily applicable within existing mRNA/LNP vaccines. Furthermore, the need to modify LNPs to incorporate adjuvants severely limits which adjuvants can be used and compromises the ability to directly compare impacts of adjuvants across platforms. An off-the-shelf technique allowing modular selection of single or combination adjuvants may be especially beneficial to cater to different disease needs as mRNA vaccines expand in use to other infectious diseases, anti-cancer vaccines, and even tolerogenic vaccines.

[0134] Finally, extended delivery of antigen can improve germinal center reactions and overall vaccine responses to subunit vaccines. However, sustained delivery is difficult to achieve with mRNA vaccines as mRNA expression is transient and mRNA is prone to

hydrolysis and degradation following injection, even with advances in base modifications and LNP delivery vehicles. Conventional efforts have pursued prolonged antigen availability through increased expression with self-amplifying RNA (saRNA), but LNPs for saRNA can be difficult to formulate due to the larger mRNA required to encode the target antigen as well as the replication machinery. A strategy to extend the expression and availability of antigen with standard mRNA vaccines could further improve their efficacy.

[0135] This example describes a modular strategy to adjuvant mRNA/LNPs and target their uptake by APCs via encapsulation in an injectable, dynamic hydrogel depot technology known to attract and activate key immune cells in vivo in a local immunologic niche. The strategy leverages PNP hydrogels formed through dynamic, non-covalent interactions between hydrophobically-modified hydroxypropylmethylcellulose (HPMC-C12) and poly(ethylene glycol)-block-poly(lactic acid) nanoparticles (PEG-PLA NPs). These materials are biocompatible and inert, yet they attract immune cells and form an immune niche in vivo when loaded with inflammatory cargo like vaccines and adjuvants. While these hydrogels have a small effective mesh size capable of entrapping diverse molecular cargo, their dynamic crosslinks allow cells to exert forces and actively migrate into the material and interact with loaded cargos. It was hypothesized these materials would promote targeting of mRNA/LNPs to infiltrating APCs, as well as allow for admixing of different adjuvants for a modular, off-the-shelf platform to study and compare adjuvanting of mRNA vaccines. The ability to admix different adjuvants allows for tuning of the inflammatory microenvironment where APCs encounter the mRNA/LNPs across a spectrum of inflammatory states, from highly inflammatory for anti-cancer applications to tolerogenic for applications in diabetic “inverse” vaccines. It was also hypothesized that sequestering mRNA/LNPs within the hydrogel would slow the diffusion and penetration of RNases and other enzymes, allowing for extended delivery and expression of mRNA, improving the immune response.

[0136] First, the plasticity of the PNP hydrogel immunologic niche was demonstrated in vivo in response to mRNA/LNPs alone or with two separate TLRs, synthetic monophosphoryl lipid A (synthetic MPLA, denoted MPLAs; TLR4a) or 3M-52 (TLR7/8a). Flow cytometry analyses revealed clear skewing of the cellular milieu based on encapsulated cargo, with transient infiltration of different immune cells such as neutrophils, monocytes, or natural killer cells influenced by different cargos. Using a model mRNA cargo, the mRNA expression in this in vivo niche was examined and it was found that key APCs actively take up LNPs and produce the encoded protein. The impact of distinct PNP hydrogel-based immune

niches on the humoral and cellular responses to a commercially available SARS-CoV-2 mRNA vaccine compared with a clinical control bolus administration was investigated. Both PNP hydrogels alone and with co-encapsulated adjuvants improved the magnitude, durability, and breadth of humoral immune responses, the functionality of the antibodies produced, and the systemic T cell response. The lymph node germinal center reactions underlying these improvements in vaccine response was further investigated and it was found that PNP hydrogels, particularly when adjuvanted, increased the magnitude and quality of the germinal center reaction to mRNA/LNP vaccines.

[0137] *Mice and vaccination:* All animal studies were performed in accordance with the National Institutes of Health (NIH) guidelines, with the approval of the Stanford Administrative Panel on Laboratory Animal Care. Seven to eight week old female C57BL/6 were purchased from Charles River and housed in the animal facility at Stanford University. Mice were shaved and injected through a 26-gauge needle subcutaneously on the right flank with 100 μ L of either bolus or gel vaccine under brief isoflurane anesthesia. Unless noted otherwise, mice were injected within five weeks of arriving at Stanford. Blood was collected weekly from tail veins. For flow cytometry, mice were euthanized under CO₂ and organs such as inguinal lymph node (LN) and spleen were explanted or hydrogels were explanted from the subcutaneous space.

[0138] *Vaccine and other formulations:* Vaccine primes and boosts (eight weeks after prime) contained 0.25 μ g Moderna Spikevax bivalent (WH1 and BA.4/5, 0.125 μ g each variant) per dose in either bolus (PBS) or in PNP hydrogels with or without 20 μ g per dose MPLAs (Invivogen, vac-mpls) or 1 μ g per dose 3M-52 (AAHI). Spikevax was obtained from the Stanford Hospital Pharmacy and stored at 4 °C until use within seven days of first septa puncture, or frozen at -20 °C and only thawed once before use. For gel infiltration studies, 1 μ g per dose of mCherry encoding mRNA/LNPs was incorporated into PNP hydrogels and dosed. mRNA/LNPs encoding mCherry or firefly luciferase were prepared via nanoprecipitation and commercially available lipids mimicking the Moderna Spikevax formulation. Briefly, SM-102 (BroadPharm), DSPC (Avanti Polar Lipids), cholesterol (Avanti Polar Lipids), and PEG-DMG 2000 (Avanti Polar Lipids) were dissolved in ethanol in the molar ratio 50:10:38.5:1.5 to make a lipid solution. mRNA with 5-methoxyuridine (5moU) modification encoding mCherry or firefly luciferase (TriLink) was dissolved in citrate buffer (pH 3). LNPs were formulated by mixing lipid and mRNA solutions in the ratio 1:3 (v/v) at 12 mL min⁻¹ using an Ignite NanoAssemblr (Precision Nanosystems). LNPs were dialyzed overnight against 20 mM Tris

acetate and 8% (w/v) RNase-free sucrose (VWR) using 3,500 MWCO dialysis cassettes (Thermo Fisher), aliquoted, and stored at -80 °C until use.

[0139] *Flow cytometry of PNP hydrogel immune infiltrate:* Mice were shaved and injected subcutaneously on the right flank with 100 μ L PNP-0.5-5 hydrogels with 1 μ g dose⁻¹ mCherry mRNA/LNPs, with or without MPLAs or 3M-52. At three and seven days after injection, mice were euthanized by CO₂ and hydrogel depots extracted and placed in microcentrifuge tubes with 750 μ L FACS buffer (PBS, 3% heat inactivated FBS, 1 mM EDTA). Hydrogels were mechanically disrupted to single cell suspensions using Kimble BioMasherIIIs (DWK Life Sciences). Suspensions were passed through a 70 μ m cell filter (Celltreat, 229484) into 15 mL Falcon tubes, spun at 500 rcf for 5 minutes, resuspended in PBS, and counted using acridine orange/propidium iodide cell viability stain (Vitascientific, LGBD10012) and a Luna-FL dual Fluorescence cell counter (Logos biosystems). 1 million live cells per sample were transferred to a 96-well conical bottom plate (Thermo Scientific, 249570) and stained.

[0140] *Mouse serum ELISAs:* Antigen-specific IgG endpoint titers were measured using an endpoint ELISA. MaxiSorp plates (Thermo Scientific, 449824) were coated with SARS-CoV-2 Spike trimer (Sino Biological, 40589-V08H4) or variant trimer (Sino Biological, 40589-V08H32, 40589-V08H26, 40589-V08H45, 40589-V08H55, 40589-V08H58, or 40634-V08B) at 2 μ g mL⁻¹ in PBS at 4 °C overnight and subsequently stored at -80 °C. Plates were thawed for 1-2 h at room temperature, washed 5 times with 300 μ L per well wash solution (PBS with 0.05% Tween 20), and blocked with diluent buffer (PBS with 1% bovine serum albumin) for 2 h. All incubation steps were at room temperature on a rotator and plates were washed 5 times with wash solution between each step. Serum dilutions were prepared during the blocking step in a conical bottom plate (Thermo Scientific, 249570) in diluent buffer starting at 1:100 (1 μ L serum into 99 μ L diluent buffer) and serially diluted 4-fold. Following blocking and washing, serum dilutions were transferred to antigen-coated plate, 50 μ L per well, and incubated for 2 hr. Goat anti-mouse IgG Fc HRP (Invitrogen, A16084) was diluted from glycerol stock (1 mg mL⁻¹) into diluent buffer (1:10,000) and 50 μ L added to each well and incubated for 1 h. For isotype ELISAs, goat anti-mouse IgG1 or IgG2c heavy chain HRP (Abcam, ab97240 or ab97255) were diluted 1:20,000 from vendor stock solution. Plates were developed for 6 minutes with high sensitivity TMB substrate (Abcam, ab171523) and reaction was stopped with 1 N HCl. Absorbance was read at 450 nm with a Synergy H1 microplate reader (BioTek Instruments). Data were analyzed in GraphPad Prism and fit with a five point

asymmetric sigmoidal curve with constraints $S > 0$, $top < 4$, and $bottom = 0.48$ (background absorbance value). The endpoint titer was defined as the serum dilution value at which the absorbance reached 2x the background value, or 0.1, interpolated from the curve fits in GraphPad Prism. For isotype plates, background absorbance was set to 0.094 and 0.66 for IgG1 and 2c respectively and endpoint was defined at 0.2. Samples below endpoint absorbance at 1:100 dilution were designated below the limit of detection and the endpoint titer defined as a dilution value of 25.

[0141] *Pseudotyped lentivirus production:* SARS-CoV-2 spike-pseudotyped lentiviruses encoding a luciferase-ZsGreen reporter were produced in HEK293F cells by co-transfection of five plasmids. The five plasmids include a packaging vector (pHAGE-Luc2-IRES-ZsGreen), a plasmid encoding the SARS-CoV-2 spike (HDM-SARS2-spike-delta21, Addgene, 155130) and three helper plasmids (pHDM-Hgpm2, pHDM-Tat1b and pRC-CMV_Rev1b). BA.4/5 mutations include T19I, $\Delta 24-26$, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. 50 mL of cells were diluted to a density of approximately $3-4 \times 10^6$ cells per mL. Transfection mixture was prepared by adding five plasmids (50 μ g of packaging vector, 17 μ g of SARS-CoV-2-encoding plasmid and 11 μ g of each helper plasmid) to 5 mL of Expi-free medium, followed by the dropwise addition of BioT transfection reagent (150 μ L, Bioland Scientific) with vigorous mixing. After 10 min incubation at room temperature, the transfection mixture was transferred to HEK293F cells. D-glucose (4 g L⁻¹, Sigma-Aldrich) and valproic acid (3 mM, Acros Organics) were then added to the cells immediately post-transfection to increase recombinant protein production. The cells were harvested 3–5 days after transfection by spinning the cultures at 300g for 5 min. The supernatant was filtered through a 0.45 μ m filter and 0.5 mL of 1mM HEPES was added to neutralize the pH. Viral stocks were aliquoted and flash-frozen in liquid nitrogen. They were stored at -80°C and titrated before further use.

[0142] *Serum neutralization assays against pseudoviruses:* Antisera were heat inactivated (56°C , 30–60 min) before neutralization assays. Neutralization against SARS-CoV-2 BA.4/5 was analyzed in HeLA-ACE2/TMPRSS2 cells. One day before infection (day 0), cells were seeded at 8,000 cells per well in white-walled, white-bottom, 96-well plates (Thermo Fisher or Greiner Bio-One). On day 1, antisera were serially diluted in D10 media and mixed 1:1 with pseudoviruses for 1-2 hrs at 37°C before being transferred to cells. The

pseudovirus mixture contained SARS-CoV-2 BA.4/5, D10 media and polybrene (1:500). Assays were read out with luciferase substrates 2 d after infection by removing the media from the wells and adding 80 μ L of a 1:1 dilution of BriteLite in DPBS (BriteLite Plus, Perkin Elmer). Luminescence values were measured using a microplate reader (BioTek SynergyTM HT or Tecan M200). Percent infection was normalized on each plate. Neutralization assays were performed in technical duplicates.

[0143] *ELISpot*: Spike-specific IFN- γ producing splenocytes were evaluated using a Mouse IFN- γ Single color ELISpot Kit (CTL) and CTL counter (Immunospot S6 Ultra M2). Spleens were harvested from mice two weeks post-boost, disrupted to single cell suspensions with frosted glass slides in CTL test media (CTLT-005) supplemented with 1% L-glutamine (termed CTL-g), passed through 70 μ m cell filters into 15 mL Falcon tubes, and spun at 400 rcf for 4 minutes. Red blood cells were lysed with 1 mL ACK lysis buffer (Gibco, Thermo Scientific, A1049201) for 2 minutes and reaction was quenched with 9 mL CTL-g. Samples were spun at 400 rcf for 4 minutes, resuspended in CTL-g, counted, and plated in a low-bind 96-well conical bottom plate. Samples were transferred to pre-coated ELISpot plates and stimulated for 24 h at 37 °C with either 5 μ g peptide⁻¹ mL⁻¹ SARS-CoV-2 pan-spike peptides (JPT Peptide Technologies, PM-WCPV-S-1), 1 μ g mL⁻¹ concanavalin A (ConA, Sigma-Aldrich, C0412-5MG) as a positive control, or CTL-g as a negative control. Cells were at a final concentration of 200 k in negative wells, 400 k in ConA wells, and both 200 k and 400 k in peptide wells. After 24 h, spots were developed following manufacturer's instructions.

[0144] *Flow cytometry of lymph nodes*: Draining inguinal lymph nodes were harvested from mice at weeks one, two, and four post-prime. LNs were disrupted, filtered, and counted as previously described in hydrogel preparation and 1 million live cells per sample were transferred to a 96-well conical bottom plate and stained.

[0145] *Staining protocol and panels*: LN and hydrogel samples were first stained with 100 μ L Live/Dead Fixable Near-IR (Thermo Scientific, L34975) for 30 minutes at room temperature, quenched with 100 μ L FACS buffer, and spun at 935 rcf for 2 minutes. Samples were then incubated with 50 μ L anti-mouse CD16/CD32 (1:50 dilution; BD, 553142) for 5 minutes on ice before incubating with 50 μ L full antibody stain for 40 minutes on ice for hydrogel samples and 60 minutes for lymph node samples. Samples were spun and resuspended in 50 - 70 μ L FACS buffer and run on the BD FACSymphony A5 SORP in the Stanford Shared FACS Facility. Data was analyzed in FlowJo.

[0146] LN full antibody stain included anti-CD184 (1:400 dilution; BV421; Fisher Scientific, 50-605-189), anti-CD138 (1:400; BV605; Fisher Scientific, 50-207-1399), anti-CD86 (1:400; BV785; Fisher Scientific, NC1188484), anti-CD279 (1:400; PE-Cy7; BioLegend, 135215), anti-CD19 (1:200; PerCP/Cy5.5; Fisher Scientific, 50-113-0313), anti-CD95 (1:200; BV650; Fisher Scientific, BDB740507), anti-CD38 (1:200; BUV737; BD, 741748), anti-CD4 (1:200; BUV805; BD, 612900), anti-I-A/I-E (1:200; BV510; BioLegend, 107636), anti-GL7 (1:100; AF488; Fisher Scientific, 50-711-897), anti-CD3 (1:100; AF700; Fisher Scientific, 50-162-375), anti-CD45R (1:100; BUV395; BD, 563793), anti-CD185 (1:50; BV711; Fisher Scientific, 50-207-1644), anti-spike tetramer (20nM; AF647), and anti-spike tetramer (20nM; PE). Tetramers were prepared on ice by adding AF647- or PE-Streptavidin (Thermo Scientific, S32357 or BD, 554061) to 6 μ M biotinylated wildtype SARS-CoV-2 spike trimer (Sino Biological, 40589-V27B-B) in five steps, once every 20-60 min, for a final molar ratio of 4:1:1 spike protein to dye and concentration of 0.5 μ M.

[0147] Hydrogel full antibody stain included anti-I-A/I-E (1:800 dilution; FITC; BioLegend, 107605), anti-CD45 (1:800; AF700; BioLegend, 103127), anti-Ly6C (1:400; BV570; BioLegend, 128029), anti-XCR1 (1:200; AF647; BioLegend, 148213), anti-F4/80 (1:200; BV421; BioLegend, 123137), anti-Ly6G (1:200; BV711; BioLegend, 127643), anti-CD3e (1:200; PerCP-eFluor710; Thermo Scientific, 46-0033-82), anti-CD19 (1:200; PE-Cy7; BioLegend, 115519), anti-CD11c (1:200; PE; BioLegend, 117307), anti-CD11b (1:100; BV510; Fisher Scientific, 50-112-9846), and anti-NK1.1 (1:100; BV605; BioLegend, 108753).

[0148] *Statistical Analysis:* For in vivo experiments, animals were cage blocked in all experiments except the humoral study and data presented as mean $\pm$ SEM. Comparisons between multiple groups were conducted with the general linear model (GLM) and Tukey HSD test in JMP, accounting for cage blocking. In order to normalize the variance and meet requirements for statistical tests used, flow cytometry data presented as percentages (e.g. Bgc cells percent of B cells) were transformed using the equation $y = \ln(x/(100-x))$, where x is the original data point plotted and y the data statistics are run on. Comparisons between two groups were conducted with multiple unpaired two-tailed student t-tests run in GraphPad Prism with false discovery rate (FDR) correction using two-stage step-up method of Benjamini, Krieger. Select p values are shown in the text and figures and all p values are in the supporting information.

[0149] The in vivo studies described herein leveraged the unique mechanical properties and modularity of PNP hydrogels to form an immune niche and selectively deliver

mRNA/LNPs to recruited APCs. Following subcutaneous injection in mice, migratory immune cells are recruited to the injected hydrogel depot, where they can exert forces and infiltrate the dynamically crosslinked material (FIG. 5). Once cells enter the PNP hydrogel depot, they encounter the mRNA/LNPs and can be transfected before migrating to the draining lymph node to initiate germinal center reactions. It was hypothesized that cell populations in the hydrogel immune niche could be skewed by incorporating different inflammatory cargo such as potent TLR4a (MPLAs) or TLR7/8a (3M-52) adjuvants. These potent adjuvants have shown promise in other vaccine formulations and clinical vaccines (e.g., the human papillomavirus vaccine by GSK is adjuvanted with ASO4 comprising MPL/Alum). Additionally, both adjuvants have lipid moieties which promote retention in the PNP hydrogel.

[0150] One unique feature of PNP hydrogels is that their nano-scale mesh size allows retention of small cargos, yet their dynamic crosslinks allow immune cells to migrate into the material in response to inflammatory cargo. Flow cytometry was employed to phenotype the immune niche within the PNP hydrogel depot over time in response to mRNA/LNPs with and without MPLAs or 3M-52 adjuvants. C57BL/6 mice were injected subcutaneously on the flank with 100 μ L of PNP hydrogel containing 1 μ g mCherry mRNA/LNPs, either alone or with adjuvants, and hydrogel depots were excised at days three and seven for single cell flow cytometry (FIG. 6A). The hydrogels clearly formed persistent depots without signs of fibrosis or foreign body response on both excision days (FIG. 6B). The hydrogels contained over a million CD45⁺ leukocytes on day three (1.35 M), and more in adjuvant-loaded hydrogels (2.23 M with MPLAs and 2.27 M with 3M-52; $p = 0.71$ for MPLAs and 0.57 for 3M-52 compared to LNP-only hydrogels). PNP hydrogels with mRNA/LNPs hosted almost double the cells found in empty PNP hydrogels (0.78 M), likely due to the inherent immunogenicity of mRNA/LNPs, and PNP hydrogels loaded with Moderna Spikevax showed comparable infiltration to ones loaded with mCherry mRNA/LNP. Over time, cells migrate out of PNP hydrogels and the depot dissolves away. On day seven there were fewer, but still a measurable number of, cells in LNP-only hydrogels (0.20 M), while adjuvanted hydrogels maintained more cells (0.34 M with MPLAs and 0.65 M with 3M-52) (FIG. 6C). The hydrogel depots measured approximately 100 mm³ on day three and 70 – 75 mm³ on day seven, indicating a cell density of 3–23 M mL⁻¹. For reference, the average cell density in lymph nodes across animals studied for germinal center responses later in this work was ~110 M mL⁻¹. Impressively, the PNP hydrogel depots sustained cell densities up to a quarter of that of lymph nodes undergoing active vaccine responses.

[0151] The plasticity of the PNP cell niche across time and with the different adjuvant cargos was examined using uniform manifold approximation and projection (UMAP) (FIG. 6D). Overlaying the generated UMAP with gated cell populations, it was found cell types fell within clear UMAP clusters and observed distinct and dramatic differences among groups and time points. These differences were quantified with the percentage for each cell type of the total CD45⁺ cells, as well as cell counts per gel (FIGS. 6E–6G). On day three, there were clear differences across groups in neutrophil, DC, and monocyte populations. Neutrophils composed 59% and 42% of CD45⁺ cells in the MPLAs and 3M-52 groups, but only 24% in LNP-only hydrogels. There were more monocytes in adjuvanted groups compared to LNP-only hydrogels, significantly so for 3M-52 hydrogels ($p=0.01$). Surprisingly, fewer DCs were observed in 3M-52 hydrogels compared to LNP-only and MPLAs hydrogels ($p=0.21$ and 0.66 respectively). Different subpopulations and phenotypes of macrophages and DCs were also visualized on the UMAPs, with monocyte-derived macrophages (Ly6C^{hi}) and DCs (mDCs) clustering nearer to monocytes (FIGS. 6H and 6I). By day seven, all cell types in LNP-only hydrogels had decreased, with neutrophils composing 2% and macrophages 55% of all CD45⁺ cells. While macrophage counts in all PNP hydrogel groups were similar, the percentage these cells comprised of CD45⁺ cells, and their phenotypes, were distinct. Macrophages made up 27% of MPLAs hydrogels and spanned the UMAP spectrum, while they made up 19% of 3M-52 hydrogels and fell almost entirely within the monocyte-derived region of the UMAP. In contrast, LNP-only macrophages were 55% of the CD45⁺ cells and primarily Ly6C^{lo}. Adjuvanted hydrogels retained substantially more neutrophils and monocytes at day seven ($p=0.085$ and $p=0.24$ for each cell type respectively in 3M-52 hydrogels). MPLAs hydrogels exhibited a slight increase in DCs with a larger population of inflammatory mDCs compared to LNP-only hydrogels. Finally, an influx of natural killer (NK cells) was observed into adjuvanted groups, especially 3M-52 hydrogels, where they were found to constitute 28% of CD45⁺ cells. Overall, these data clearly indicate that immune cells migrate to injected PNP hydrogel depots and form highly plastic and tunable inflammatory niches in response to different adjuvant cargos. The ability of PNP hydrogel materials to retain vaccine cargo while enabling cellular infiltration is one which can be leveraged to target mRNA/LNPs to key APCs under specific conditions, such as being surrounded by unique cellular milieu whose inflammatory state can be skewed with different cargos.

[0152] Following characterization of the highly plastic cell niche within the PNP hydrogel depot, studies were performed to investigate if cells within the niche took up LNPs

and expressed the delivered mCherry mRNA (FIG. 7A). Cells isolated from hydrogels on days three and seven were mCherry⁺ by flow cytometry (FIG. 7B). The count and proportion of CD45⁺ mCherry⁺ cells were quantified, and it was observed that 3.8, 2.2, and 6.9 ($\times 10^3$) cells expressed mCherry on day three in LNP-only, MPLAs, and 3M-52 hydrogels respectively, making up 0.3, 0.1, and 0.3% of all CD45⁺ cells. The PNP hydrogels clearly prolonged mRNA expression through day seven, with significantly more mCherry⁺ cells in adjuvanted groups compared to the LNP-only group, up to 12.3 ($\times 10^3$) in 3M-52 hydrogels (2.1% of all CD45⁺ cells, $p = 0.0008$ and 0.0002 compared to LNP-only by count and percentage respectively) (FIGS. 7C and 7D). Further examining the cell subtypes expressing delivered mRNA, it was observed that mCherry⁺ cells were overwhelmingly APCs – DCs, monocytes, macrophages – across all gel groups (FIGS. 7E–7G). Interestingly, monocytes composed 48% and 76% of mCherry⁺ cells in MPLAs and 3M-52 hydrogels, whereas DCs composed 22% and only 1% for each group respectively.

[0153] Considering the obvious differences observed in overall CD45⁺ cell niche compositions, yet relatively similar mCherry⁺ subniche compositions across groups, we sought to examine if certain cell types were specifically enriched in the mCherry⁺ subniche. To do this, we took the ratio of each cell type percentage of mCherry⁺ cells divided by the same cell type's percentage of all CD45⁺ cells (FIG. 7H). In this way, cell types which compose the same percentage of mCherry⁺ cells as they do all CD45⁺ cells would have a ratio of one. Similarly, ratios less than one would indicate fewer of that cell type are mCherry⁺ than expected given their frequency in the overall CD45⁺ niche, and vice versa for values over one (cell types enriched for mRNA expression). From this we observed APCs were overrepresented in the mCherry⁺ subniche while neutrophils and NK cells, despite composing a large fraction of the overall CD45⁺ cell niche, were underrepresented in the mCherry⁺ subniche. Looking at the fraction of each cell type that was expressing mCherry, less than 0.07% of NK cells and 0.76% of neutrophils expressed mCherry across all groups while up to 2.1, 4.5, and 5.3% of macrophages, monocytes, and DCs respectively expressed mCherry on day seven (FIG. 7I). These observations indicated that mRNA/LNPs incorporated into PNP hydrogels successfully transfected APCs, a key cell type for training adaptive immune responses. Further, the transfected cell types did not change substantially with different adjuvant cargo, whereas the surrounding cell niche did, providing a means to independently tune the context in which APCs express and present antigen. This approach presents a key opportunity when considering

mounting different types of immune responses, such as anti-cancer or tolerogenic, where the context of antigen processing and presentation is critical.

[0154] Studies were performed to evaluate the impact of PNP hydrogels with and without adjuvants (and their corresponding cell niches) on the response to an mRNA/LNP vaccine. The modularity of PNP hydrogels allows simple incorporation of commercially available vaccines, like Moderna's Spikevax vaccine encoding the full spike protein of SARS-CoV-2, in a simple off-the-shelf manner with and without adjuvants. First, two material formulations were evaluated, PNP-0.5-5 and PNP-1-5, to assess any effects of material mechanical properties on the humoral response (FIGS. 8A–8E). PNP-1-5 hydrogels were an order of magnitude stiffer than PNP-0.5-5 hydrogels, resulting in a more persistent depot with slower release kinetics and potentially limited cellular infiltration at early time points. As mRNA degrades rapidly by hydrolysis, the softer materials performed better *in vitro* and *in vivo*, so subsequent studies were performed with the previously characterized PNP-0.5-5 formulation.

[0155] 0.25 µg mRNA/LNPs (Moderna Spikevax bivalent vaccine encoding WH1 and BA.4/.5) were delivered in a 100 µL subcutaneous injection in either PBS, the current clinical standard, or PNP-0.5-5 hydrogel with or without MPLAs or 3M-52. Mice were dosed at weeks zero (prime) and eight (homologous boost) and serum was collected for antibody titer evaluation over six months (FIG. 9A). A separate cohort of animals was immunized and T cell response was evaluated via IFN-γ ELISpot at week ten (two-weeks post-boost). Serum titer ELISAs against WH1 spike protein at week eight post-prime showed that 5 in 6 animals exhibited measurable titers in the PNP hydrogel group compared to only 1 in 6 in the bolus control group (FIG. 9B). Additionally, PNP hydrogel including 3M-52 produced significantly higher titers compared to both bolus and PNP hydrogel only ($p=0.43$ and 0.093 respectively). As early as week two, 3M-52 adjuvanted PNP hydrogel exhibited 4.5-fold improved antibody titers ($p=0.29$) while 3M-52 adjuvanted bolus had no observable benefit compared to unadjuvanted bolus ($p=0.59$). At four months post-boost, animals in adjuvanted groups exhibited higher titers compared to bolus controls, and animals that received PNP hydrogel with 3M-52 exhibited titers nearly an order of magnitude higher than those that received bolus control or LNP-only hydrogel vaccines ($p = 0.17$ and 0.099 respectively) (FIG. 9C). Antibody titers throughout the study showed that mRNA/LNP vaccine delivery in PNP hydrogels improved titers, particularly post-prime, and that PNP hydrogels adjuvanted with 3M-52 enhanced titers at all time points and produced substantively higher overall antibody exposure

as measured by area under the curve (AUC) compared to the bolus control ($p=0.71$) (FIGS. 9D and 9E).

[0156] Considering durability is a concern with current commercial mRNA/LNP vaccines, an average antibody half-life was extracted using parametric bootstrapping on titer data following the post-boost peak (week 10 for bolus and week 12 for hydrogel groups, $n=1000$ runs) (FIG. 9F). The PNP hydrogels increased antibody decay half-life by 11% over the bolus control, and adjuvanted PNP hydrogel groups increased antibody decay half-lives by 143% and 68% (MPLAs and 3M-52 respectfully, $p<0.0001$) over the bolus control. These enhancements represent a shift in half-life from one month for bolus delivery to upwards of 2.5 months for adjuvanted PNP hydrogels.

[0157] Next, the functionality of the antibody response as well as the T cell response was examined. SARS-CoV-2 BA.4/5 pseudotyped lentivirus neutralization assays were performed at week thirteen post-prime (week five post-boost). No significant difference in infectivity between bolus or LNP-only hydrogels was found, and a trend toward enhanced neutralization (i.e., lower infectivity) in adjuvanted PNP hydrogel groups was observed (FIG. 9G). PNP hydrogels with 3M-52 exhibited a mean infectivity of 29.3% compared to 41.4 – 51.4% for other groups. Furthermore, only PNP hydrogel groups yielded infectivities below 10% at this dilution (2, 1, and 2 animals out of 6 treated animals for PNP only, with MPLAs, and with 3M-52, respectively). Next, functional T cell responses to WH1 spike peptides were assessed via ELISpot for IFN- γ producing splenocytes, a proxy for IFN- γ producing T cells, at week ten post-prime (week two post-boost). Vaccine delivery in PNP hydrogels alone was again comparable to bolus while adjuvanting with 3M-52 produced a more robust T cell response (FIG. 9H). Altogether, the humoral and cellular responses to SARS-CoV-2 mRNA/LNP vaccines were improved with delivery in PNP hydrogels, and especially so with adjuvanted PNP hydrogels, whereas no improvement was achieved with standard bolus adjuvanting.

[0158] Another important aspect of a vaccine response, particularly against highly mutable viruses like SARS-CoV-2, is the breadth of humoral responses. Antibody isotypes and endpoint titers were evaluated at week sixteen post-prime against seven SARS-CoV-2 variants of concern, as well as SARS-CoV-1 (FIGS. 10A and 10B). Interestingly, all groups exhibited similar IgG1 titers, with PNP hydrogel groups showing modestly higher IgG1 titers compared to bolus, but only PNP hydrogels with 3M-52 produced measurable IgG2c titers in a majority of animals (4 in 6 treated animals). The IgG2c titer for PNP hydrogels with 3M-52 was

significantly higher than that of PNP hydrogel only, as well as the bolus control ($p=0.37$ and 0.12 , respectively). This observation was further reflected in the IgG2c/IgG1 ratio, a proxy for Th1 and Th2 skewing (FIG. 10C). While PNP hydrogels exhibited comparable Th2 skewing to the bolus control, incorporation of adjuvants like 3M-52 elicited a clear skewing toward Th1. Since protection against different infectious diseases can be better mediated by different Th1/2 skewing depending on the disease, the ability to tailor the response in a simple and modular way by just changing the admixed adjuvant co-encapsulated within the PNP hydrogel is a significant benefit.

[0159] Additionally, PNP hydrogel delivery of mRNA/LNP vaccines improved the breadth of antibody titers, particularly in adjuvanted PNP hydrogel groups. A petal/radar plot of anti-spike titers across variants is one way to quickly visualize increased breadth (FIG. 10D). The PNP hydrogel group exhibited a slightly larger and broader petal than the bolus control, indicating an overall increase in antibodies against the tested variants not included in the vaccine, as well as a more consistent response across those variants. PNP hydrogel comprising 3M-52 elicited an even larger, broader petal than PNP hydrogel alone or with MPLAs. Of note, PNP hydrogels also reduced the number of non-responders to some variants like B.1.1.529 and SARS-CoV-1 over standard bolus vaccination, and PNP hydrogels with 3M-52 produced measurable anti-SARS-CoV-1 titers in all six animals. The breadth of the humoral immune response was further quantified with an absolute breadth index, composed of the average titer for animals in one treatment group against a single variant those animals were not vaccinated against (FIG. 10E). This breadth index was slightly increased for PNP hydrogels alone and comprising MPLAs compared to bolus, and significantly higher for PNP hydrogels with 3M-52 ($p<0.0001$). The quality of breadth was also evaluated using a relative ratio comparing antibody titer for each variant to the titer against wildtype WH1 (FIG. 10F). This breadth ratio analysis revealed that PNP hydrogel groups elicited more consistent responses, with ratios closer to one for all variants, indicating not just higher absolute titers but anti-variant titers closer to those against wildtype WH1 despite not being represented in the vaccine. The variants tested span CDC variants of concern and are genetically distinct within the Omicron clade (FIG. 10G).

[0160] To better understand the origin of the observed improvements in durability, breadth, and T cell responses imparted by PNP hydrogel delivery of mRNA/LNP vaccines with and without adjuvants, flow cytometry was used to probe the germinal center responses in draining lymph nodes at weeks one, two, and four following prime vaccination with $0.25\ \mu\text{g}$

Moderna Spikevax bivalent vaccine (WH1 and BA.4/5) (FIG. 11A). Within the first week it was observed that PNP hydrogel delivery elicited a higher proportion of activated B cells (MHCII⁺ CD86⁺), which was further increased with adjuvants and significantly so with 3M-52 ($p=0.0003$) (FIG. 11B). A significantly higher light zone to dark zone (LZ:DZ) ratio was also found after one week for PNP hydrogel alone ($p=0.09$) and adjuvanted with 3M-52 ($p=0.0003$) compared to bolus vaccination (FIG. 11C). These improvements observed only a week after prime injection in PNP hydrogel groups were interesting considering the slow release of cargo would lead to expectations of slower germinal center kinetics compared to bolus. Without wishing to be bound by theory, it is hypothesized these early increases in lymph node and germinal center reactions result from the unique advantage of the in vivo immune niche formed within the PNP hydrogel depots and an early boost in the response from APCs transfected in the PNP niche.

[0161] The germinal center reaction was more specifically examined by quantifying germinal center B (B_{GC}) cells, including WH1 spike-specific B_{GC} cells, and T follicular helper (T_{FH}) cells (FIGS. 11D–11F). PNP hydrogels showed comparable or increased percentages of B_{GC} cells to bolus controls at all time points, with 0.29 – 0.69% in bolus and 0.56 – 0.94% in PNP hydrogel animals (FIG. 11G). Adjuvanting with MPLAs provided a substantive increase over bolus or delivery in PNP hydrogels alone, with B_{GC} cells comprising up to 1.19% of B cells ($p=0.14$ and 0.67 at week two, $p=0.50$ and 0.15 at week four). PNP hydrogel adjuvanted with 3M-52 exhibited further significant improvements, up to 1.79% of B cells at week two, compared to bolus ($p=0.0066$ and 0.27 at weeks two and four), as well as a substantive benefit over unadjuvanted PNP hydrogel ($p=0.083$ and 0.087). The B_{GC} cell percentages were integrated over time and PNP hydrogels were observed to increase the total B_{GC} cell exposure (AUC) by 36%, and PNP hydrogel groups adjuvanted with MPLAs and 3M-52 further increased this to 44% and 149% ($p=0.19$ for 3M-52) compared to bolus. B_{GC} cells were quantified for those that were specific for the mRNA encoded antigen, WH1 SARS-CoV-2 spike protein. Spike-specific B_{GC} cells were indeed found to be increased in PNP hydrogel groups, significantly so in PNP hydrogels adjuvanted with 3M-52 ($p=0.33$ to bolus), and as early as week one, with persistence observed across the four weeks (FIG. 11H). Interestingly, PNP hydrogels comprising MPLAs led to significantly more spike-specific B_{GC} cells at the later time point (week 4) compared to bolus ($p=0.28$), while the improvement in PNP hydrogel with 3M-52 was observed primarily in the earlier weeks.

[0162] T_{FH} cells exhibited an earlier response than B_{GC} cells, with higher percentages of T_{FH} cells at week one for PNP hydrogel (FIG. 11I). T_{FH} cells included about 1% of $CD4^{+}$ helper T cells for the PNP hydrogel group, but only 0.7 – 0.87% for the bolus group and upward of 1 – 1.22% and 0.94 – 1.37% for the PNP hydrogel groups adjuvanted with MPLAs and 3M-52. PNP hydrogels comprising 3M-52 maintained significantly higher percentages of T_{FH} cells than bolus at weeks one and two ($p=0.0073$ and 0.0032). PNP hydrogels comprising MPLAs also produced a substantively higher percentage of T_{FH} cells at week two than bolus ($p=0.63$), and this enhancement continued to increase through week four up to 1.37%, following a similar kinetic trend seen in the spike-specific B_{GC} cell counts across vaccine groups. Integration over time corroborated that PNP hydrogels increased T_{FH} cell percentage and overall exposure, and this was further increased for PNP hydrogels with adjuvants.

[0163] The ratio of B_{GC} cells to T_{FH} cells can be used as a metric for the quality of T_{FH} cell help, which was evaluated at weeks two and four (week one was excluded due to the weaker B_{GC} response) (FIG. 11J). More animals exhibited consistently higher quality T_{FH} cell help in the PNP hydrogel group at week two, with 4 in 10 animals at or above the third quartile compared with only 2 in 10 animals in the bolus group, which also had a lower third quartile value. Adjuvanting of the PNP hydrogels with MPLAs or 3M-52 further improved the quality of T_{FH} cell help, both in magnitude (average ratio) as well as number of animals experiencing better help (i.e., the spread of animals above the average). Week four ratios showed a slight waning, but adjuvanted PNP hydrogel delivery prolonged significantly better quality T_{FH} cell help compared to bolus ($p=0.10$ and 0.19 for MPLAs and 3M-52) and PNP hydrogel alone ($p=0.21$ and 0.46 for MPLAs and 3M-52). It was clear PNP hydrogels, particularly PNP hydrogels adjuvanted with 3M-52, improved the magnitude, duration, and quality of the germinal center reaction to mRNA/LNP vaccines, which reflected improvements in antibody titers, breadth, and durability found earlier.

[0164] These results demonstrate that delivering a commercially available SARS-CoV-2 mRNA/LNP vaccine within an injectable PNP hydrogel depot led to improved humoral and cellular immune responses driven by increased and prolonged germinal center reactions. PNP hydrogels enable facile formulation of off-the-shelf mRNA/LNPs with various adjuvant cargos, create tunable immunological niches in vivo with different adjuvant cargos, and promote transfection of APCs within the niche without any modifications to the LNPs. The incorporation of mRNA/LNPs into PNP hydrogel materials was characterized and it was shown that formulation in this way does not impact material properties nor LNP transfection

efficiency. The cellular milieu within the PNP hydrogel depot in vivo was characterized and determined to be highly plastic and tunable based on incorporation of adjuvant cargo. It was shown that key APCs within this niche preferentially expressed delivered mRNA. Following this characterization, it was shown that commercial mRNA/LNP vaccine delivery in a PNP hydrogel niche with and without adjuvants improved the efficacy of Moderna Spikevax over clinically relevant bolus controls. Finally, the germinal center reactions driving these observed improvements were investigated and PNP hydrogel delivery was found to have increased the magnitude, duration, and quality of these reactions.

[0165] In contrast to chemical strategies to target LNPs to APCs, this approach does not require altering LNPs and can be readily applied to commercially available mRNA/LNP vaccines. Furthermore, these PNP hydrogels may be more widely applicable to other mRNA and nucleic acid delivery platforms like poly(β -amino esters) (PBAEs) or viral vectors. Interestingly, the best humoral response and most active germinal center reactions were found in animals immunized with PNP hydrogels adjuvanted with 3M-52, a potent TLR7/8 agonist, yet this hydrogel niche was found to host the fewest DCs and instead comprised substantially more transfected monocytes than other tested groups (FIGS. 7A–7I and 9A–9H). It was initially hypothesized better vaccine responses would positively correlate with the presence of DCs in the PNP hydrogel niche; however, these data indicate this is not necessarily the case. Indeed, there is little data characterizing cellular recruitment to the injection site or understanding correlations between injection site cellular milieu and strong or specific mounted immune responses. This shortfall is due in part to fundamental difficulties in measuring cells within the injection site, particularly in the subcutaneous space. While some research has attempted to characterize the immune infiltrate following intramuscular or intradermal injections, these studies have relied heavily on imaging modalities and immunohistochemistry approaches requiring biopsies of an injection site which can be difficult to find reproducibly. One of the advantages of the PNP platform is that it allows for the study of injection site reactions with single cell resolution in a way previously not possible. Additional processing, like single cell sequencing and cytokine profiling, may be used to further elucidate differences at the injection site which may correlate to overall shifts in the downstream immune responses.

[0166] While adjuvants are known to improve the immune response to protein antigens, adjuvanting mRNA vaccines is complicated and under-characterized, involving a balanced approach as excessive immune activation and cytokine production can hamper mRNA translation. Adjuvants, particularly TLR agonists, exhibit a wide range of physiochemical

properties, from small molecules to large single or double stranded nucleic acid chains, which make their interactions with mRNA vaccines difficult to study in a systematic or modular fashion. It is advantageous to more thoroughly understand the impact of adjuvants on mRNA vaccines, as well as to find adjuvants which improve and tailor vaccine responses for different antigens or diseases. Conventional approaches to adjuvanting mRNA vaccines have focused on incorporating adjuvants into LNPs, often requiring modifications to both the adjuvant and the particle formulation and are generally nontransferable to other adjuvant molecules. The PNP hydrogel platform provides a unique means to independently incorporate adjuvants alongside mRNA/LNPs, allowing for unprecedented control over adjuvanting mRNA vaccines and investigating the impact on immune responses. In this work, it was found that TLR4 activation by MPLAs has little benefit over PNP hydrogel delivery alone, but TLR7/8 activation by 3M-52 significantly improves the magnitude and quality of vaccine responses. Interestingly, PNP hydrogels alone are rather inert and do not modulate T-helper skewing (FIG. 10A–10G) like other depot technologies such as Alum, which produces a strong Th2-skewed response even when combined with Th1-skewing adjuvants like 3M-52.

[0167] This approach may be extended to other adjuvants targeting different TLRs, such as TLR1/2a (Pam3CSK4), TLR9a (CpG), or STING (cGAMP), as well as combinations of adjuvants shown in other contexts to afford synergistic immune activation. Additionally, the PNP hydrogel platform enables encapsulation of other immune activating cargo in a plug-and-play manner, like protein chemokines or cytokines, that would otherwise be difficult or impossible to co-deliver with mRNA/LNPs without modification. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a small protein which should drive infiltration of more DCs into the PNP material and could be combined with other adjuvants. Cytokines like IL-10 or IL-2 could also be incorporated to induce a more tolerogenic niche in the PNP hydrogel. The modularity of this platform can effectively skew the immunological niche and subsequent response based on encapsulated molecular cargos, creating opportunities beyond improving infectious disease vaccines to directing improved cytotoxic responses in cancer vaccines or suppressive responses in tolerogenic applications.

[0168] Follow up work can be performed to evaluate the kinetics of the immune niche and mRNA expression in greater depth and at additional time points, as well as investigate the kinetics of antigen and cellular accumulation in the lymph nodes. Furthermore, this work could be conducted in Ai14/Cre mice to allow analysis of all cells which translate delivered mRNA, not just the ones currently expressing enough protein to be detected. This would further allow

decoupling of cellular expression of encoded protein from the possibility that cells within the PNP niche are phagocytosing protein made by other transfected cells in the niche.

[0169] Taken together, the presented work shows that PNP hydrogel delivery of mRNA/LNPs is a promising strategy to employ a tunable immunomodulatory platform for a variety of applications, ranging from probing fundamental questions about injection site cellular reactions to mRNA vaccines, to improving vaccine efficacy against infectious diseases without altering LNP chemistries, and even potentially tailoring pro-inflammatory or tolerogenic responses.

Example 5: In Vivo Efficacy of mRNA LNP Vaccine and Chemokine Loaded PNP Hydrogels

[0170] This example describes in vivo characterization of the efficacy of PNP hydrogels loaded with mRNA LNPs and immune cell attractant chemokines.

[0171] *Vaccine formulations:* Vaccine primes and boosts (eight weeks after prime) contained 0.25 µg Spikevax bivalent mRNA LNPs per dose with varying concentrations of adjuvants or chemokines in either bolus form (in PBS) or in PNP hydrogels (n=5–6). Spikevax was stored at 4 °C until use within seven days of first septa puncture. Adjuvant and chemokine were dosed at 20 µg per dose MPLAs (Invivogen, vac-mpls), 5 µg per dose 3M052 (AAHI), or 3 µg per dose GM-CSF (PeproTech, 315-03). To prepare PNP hydrogels, HPMC-C12 was dissolved at 2 wt% in PBS and loaded into a 1-mL Luer-lock syringe. A 20 wt% solution of PEG-PLA nanoparticles in PBS was added to a solution of PBS with adjuvant, chemokine, and/or mRNA LNPs depending on formulation, and loaded into a second 1-mL syringe. The two syringes were connected with a female-female Luer lock elbow, with care to avoid air at the interface of the HPMC-C12 and nanoparticle solution, and gently mixed until a homogenous PNP hydrogel was formed. Hydrogels were formulated with final concentrations of 0.5 wt% HPMC-C12 and 5 wt% NPs (PNP-0.5-5 hydrogel).

[0172] *Mice and vaccination:* Seven- to eight-week-old female C57BL/6 mice were purchased from Charles River. Mice were shaved and injected through a 26-gauge needle subcutaneously on the right flank with 100 µL of either bolus or PNP hydrogel vaccine under brief isoflurane anesthesia. Unless noted otherwise, mice were injected within five weeks of arrival. Blood was collected weekly from tail veins. For flow cytometry, mice were euthanized under CO₂ and organs such as inguinal lymph node (LN) and spleen were explanted or hydrogels were explanted from the subcutaneous space.

[0173] *Mouse serum ELISAs:* Antigen-specific IgG endpoint titers were measured using an endpoint ELISA. MaxiSorp plates (Thermo Scientific, 449824) were coated with SARS-CoV-2 Spike trimer (Sino Biological, 40589-V08H4) at 2 µg/mL in PBS at 4 °C overnight and subsequently stored at -80 °C. Plates were thawed for 1–2 hr at room temperature, washed 5 times with 300 µL per well of wash solution (PBS with 0.05% Tween 20), and subsequently blocked with diluent buffer (PBS with 1% bovine serum albumin) for 2 hr. All incubation steps were at room temperature on a rotator and plates were washed 5 times with wash solution in between each step. Serum dilutions were prepared during the blocking step in a conical bottom plate (Thermo Scientific, 249570) in diluent buffer. Dilutions started at 1:100 (1 µL serum into 99 µL diluent buffer) and were serially diluted 4-fold or 5-fold (25 or 20 µL into 75 or 80 µL). Following blocking and washing, serum dilutions were transferred to antigen-coated plate, 50 µL per well, and incubated for 2 hr. Goat anti-mouse IgG Fc HRP (Invitrogen, A16084) was diluted from glycerol stock (1 mg/mL) into diluent buffer (1:10,000) and 50 µL added to each well and incubated for 1 hr. Plates were developed for 6 minutes with high sensitivity TMB substrate (Abcam, ab171523) and reaction was stopped with 1 N HCl. Absorbance was read at 450 nm with a Synergy H1 microplate reader (BioTek Instruments). Data were analyzed in GraphPad Prism and fit with a five-point asymmetric sigmoidal curve with constraints $S > 0$, $top < 4$, and $bottom = 0.048$ (background absorbance value). The endpoint titer was defined as the serum dilution value at which the absorbance reached 0.1 and was interpolated from the curve fits in GraphPad Prism. Samples below 0.1 absorbance at 1:100 dilution were designated below the limit of detection and the endpoint titer defined as a dilution value of 25.

[0174] *Flow cytometry of lymph nodes:* Draining inguinal lymph nodes (LNs) were harvested from mice at weeks one, two, and four after prime injection. LNs were disrupted to single cell suspensions in 700 µL FACS buffer (PBS, 3% heat inactivated FBS, 1 mM EDTA) using Kimble BioMasherIIs (DWK Life Sciences). Suspensions were passed through a 70-µm cell filter (Celltreat, 229484) into 15-mL Falcon tubes, spun at 500 rcf for 5 minutes, resuspended in PBS, and counted using acridine orange/propidium iodide cell viability stain (Vitascientific, LGBD10012) and a Luna-FL dual Fluorescence cell counter (Logos biosystems). 1 million cells per sample were transferred to a 96-well conical bottom plate (Thermo Scientific, 249570) and stained.

[0175] *Flow cytometry of PNP hydrogel immune infiltrate:* Female C57BL/6 mice eight weeks of age were shaved and injected subcutaneously on the right flank with 100 µL

PNP-0.5-5 hydrogels with 1 µg/dose mCherry mRNA LNPs, with or without MPLAs or 3M052. At three and seven days after injection, mice were euthanized by CO₂ and hydrogel depots dissected and placed in microcentrifuge tubes with 750 µL FACS buffer. Hydrogels were mechanically disrupted, filtered, and counted as previously described for lymph node preparation. 1 million cells per sample were transferred to a 96-well conical bottom plate and stained.

[0176] *Staining protocol and panels:* LN and hydrogel samples were first stained with 100 µL Live/Dead Fixable Near-IR (Thermo Scientific, L34975) for 30 minutes at room temperature, quenched with 100 µL FACS buffer, and spun down (935 ref for 2 minutes). Samples were then incubated with 50 µL anti-mouse CD16/CD32 (1:50 dilution; BD, 553142) for 5 minutes on ice before incubating with 50 µL full antibody stain for 40 minutes on ice for hydrogel samples and 60 minutes for lymph node samples. Samples were spun and resuspended in 50–70 µL FACS buffer. Samples were run on the BD FACSymphony A5 SORP and data analyzed in FlowJo.

[0177] LN full antibody stain included anti-CD184 (1:400 dilution; BV421; Fisher Scientific, 50-605-189), anti-CD138 (1:400; BV605; Fisher Scientific, 50-207-1399), anti-CD86 (1:400; BV785; Fisher Scientific, NC1188484), anti-CD279 (1:400; PE-Cy7; BioLegend, 135215), anti-CD19 (1:200; PerCP/Cy5.5; Fisher Scientific, 50-113-0313), anti-CD95 (1:200; BV650; Fisher Scientific, BDB740507), anti-CD38 (1:200; BUV737; BD, 741748), anti-CD4 (1:200; BUV805; BD, 612900), anti-I-A/I-E (1:200; BV510; BioLegend, 107636), anti-GL7 (1:100; AF488; Fisher Scientific, 50-711-897), anti-CD3 (1:100; AF700; Fisher Scientific, 50-162-375), anti-CD45R (1:100; BUV395; BD, 563793), anti-CD185 (1:50; BV711; Fisher Scientific, 50-207-1644), anti-spike tetramer (20nM; AF647), and anti-spike tetramer (20nM; PE). Tetramers were prepared on ice by adding AF647- or PE-Streptavidin (Thermo Scientific, S32357 or BD, 554061) to 6 µM biotinylated wildtype SARS-CoV-2 spike trimer (Sino Biological, 40589-V27B-B) in five steps, once every 20–60 minutes, for a final molar ratio of 4.1 : 1 spike protein to dye.

[0178] Hydrogel full antibody stain included anti-I-A/I-E (1:800 dilution; FITC; BioLegend, 107605), anti-CD45 (1:800; AF700; BioLegend, 103127), anti-Ly6C (1:400; BV570; BioLegend, 128029), anti-XCR1 (1:200; AF647; BioLegend, 148213), anti-F4/80 (1:200; BV421; BioLegend, 123137), anti-Ly6G (1:200; BV711; BioLegend, 127643), anti-CD3e (1:200; PerCP-eFluor710; Thermo Scientific, 46-0033-82), anti-CD19 (1:200; PE-Cy7;

BioLegend, 115519), anti-CD11c (1:200; PE; BioLegend, 117307), anti-CD11b (1:100; BV510; Fisher Scientific, 50-112-9846), and anti-NK1.1 (1:100; BV605; BioLegend, 108753).

[0179] FIGS. 12A and 12B illustrate that mRNA LNPs encapsulated in PNP-0.5-5 hydrogels can be combined with immune cell attractant chemokines. Mice were vaccinated subcutaneously with 0.25 μ g Spikevax monovalent mRNA LNPs in 100 μ L PNP-0.5-5 hydrogel either alone (blue), or with 3 μ g GM-CSF (orange) or 3 μ g FLT3L (red). Mice were boosted with 0.25 μ g Spikevax bivalent mRNA LNPs in the same gel formulation as the prime at week eight (black arrows). FIG. 12A is a graph of anti-Spike IgG titers for wildtype SARS-CoV-2 Spike trimer measured via ELISA, showing that chemokines improved titers post-boost. Data is presented as individual mice with mean $\pm$ SEM (n=5–6). FIG. 12B is a graph of anti-Spike IgG titers for omicron variants B.1.1.529 (squares) and BA.4/BA.5/BA.5.2 (triangles) measured four weeks post-prime (W4) and post-boost (W12) via ELISA, showing equivalent vaccine breadth for all groups. Data is presented as mean $\pm$ SEM.

[0180] FIGS. 13A–13D illustrate that PNP hydrogels supported in vivo immune niche formation and cellular composition could be skewed with chemokines. Mice were vaccinated subcutaneously with 100 μ L PNP-0.5-5 hydrogel alone (grey) or with 0.25 μ g Spikevax bivalent mRNA LNPs alone (blue) or combined with 3 μ g GM-CSF (orange) or 3 μ g FLT3L (red). Hydrogel depots were explanted at days 3 and 7 post-prime and single cells were isolated and analyzed via flow cytometry. FIG. 13A illustrates the percent composition of CD45⁺ leukocytes, showing the plasticity of the cellular niche based on chemokine cargo and time point. Data is presented as mean of n=3. FIG. 13B is a graph of the total CD45⁺ leukocyte count per 100 μ L gel depot, showing that GM-CSF attracted the highest number of leukocytes, followed by FLT3L, at both time points. FIG. 13C is a graph of total APC count per 100 μ L gel depot, showing that GM-CSF attracted far more APCs on day 3 and maintained higher counts on day 7. FIG. 13D is a graph of APC percentage of CD45⁺ leukocytes, showing that GM-CSF promoted almost 40% APC composition on day 3 and all groups had 50% or higher APC composition on day 7. APCs were defined as cDC1s (CD45⁺ Lin[−] CD11c⁺ MHCII⁺ Ly6C[−] F4/80[−] CD11b[−] CD103⁺), cDC2s (CD45⁺ Lin[−] CD11c⁺ MHCII⁺ Ly6C[−] F4/80[−] CD11b⁺ CD103[−]), iDCs (CD45⁺ Lin[−] CD11c⁺ MHCII⁺ Ly6C⁺ CD11b⁺), macrophages (CD45⁺ Lin[−] CD11c⁺ MHCII⁺ Ly6C⁺ F4/80⁺), and B cells (CD45⁺ CD19⁺), with Lin[−] defined as CD19[−] CD3[−] CD335[−] Ly6G[−]. Data for FIGS. 7B–7D are presented as mean $\pm$ SEM (n=3).

[0181] FIGS. 14A and 14B illustrate that chemokines in combination with adjuvants altered the humoral response to mRNA LNPs encapsulated in PNP-0.5-5 hydrogels. FIG. 14A

is a graph illustrating anti-spike IgG titers for wildtype SARS-CoV-2 spike trimer measured via ELISA. Addition of GM-CSF to adjuvanted PNP hydrogels delivering mRNA LNP vaccine showed slight decreases in titers post boost (week eight, black arrows). FIG. 14B is a graph illustrating area under the curve (AUC) of endpoint titer weeks 1–16 calculated for each mouse using GraphPad Prism and plotted. Data in FIGS. 14A and 14B is presented as individual mice with mean $\pm$ SEM (n=6).

[0182] Overall, these studies demonstrate that chemokines can be used in PNP hydrogels to modulate the subset of immune cells that are recruited and activated.

Additional Examples

[0183] Additional examples of aspects of the present technology are described below as numbered clauses (1, 2, 3, etc.) for convenience. These are provided as examples and do not limit the subject technology.

[0184] Clause 1. A composition for treating a subject, the composition comprising:

a dynamic hydrogel comprising a polymer and a plurality of nanoparticles, wherein the polymer is non-covalently crosslinked with the plurality of nanoparticles; and

a nucleic acid therapeutic encapsulated by the dynamic hydrogel, wherein the nucleic acid therapeutic comprises a nucleic acid molecule complexed with or incorporated in a delivery vector.

[0185] Clause 2. The composition of Clause 1, wherein the nucleic acid molecule comprises an RNA molecule.

[0186] Clause 3. The composition of Clause 2, wherein the RNA molecule is a mRNA molecule.

[0187] Clause 4. The composition of Clause 1, wherein the nucleic acid molecule comprises a DNA molecule.

[0188] Clause 5. The composition of Clause 4, wherein the DNA molecule is a plasmid DNA molecule.

[0189] Clause 6. The composition of any one of Clauses 1 to 5, wherein the delivery vector comprises a lipid vector.

[0190] Clause 7. The composition of Clause 6, wherein the lipid vector comprises a lipid nanoparticle (LNP).

[0191] Clause 8. The composition of Clause 6 or 7, wherein the lipid vector comprises one or more of a cationic lipid, an ionizable lipid, a phospholipid, a sterol, or a polyethylene glycol (PEG)-functionalized lipid.

[0192] Clause 9. The composition of any one of Clauses 1 to 5, wherein the delivery vector comprises a polymer vector.

[0193] Clause 10. The composition of any one of Clauses 1 to 5, wherein the delivery vector comprises a viral vector.

[0194] Clause 11. The composition of any one of Clauses 1 to 10, wherein the nucleic acid therapeutic comprises a nucleic acid vaccine.

[0195] Clause 12. The composition of Clause 11, wherein the nucleic acid vaccine comprises a mRNA vaccine.

[0196] Clause 13. The composition of Clause 12, wherein the mRNA vaccine is a mRNA LNP vaccine.

[0197] Clause 14. The composition of any one of Clauses 11 to 13, wherein the nucleic acid vaccine comprises at least one nucleic acid molecule encoding an antigen for an infectious disease.

[0198] Clause 15. The composition of Clause 14, wherein the infectious disease is anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes, Hendra virus disease, HIV/AIDs, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, MERS, mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, SARS, smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, or yersiniosis.

[0199] Clause 16. The composition of any one of Clauses 11 to 13, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an antigen for a cancer.

[0200] Clause 17. The composition of Clause 16, wherein the cancer is biliary tract cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, choriocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia, liver

cancer, lymphoma, lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, testicular cancer, or thyroid cancer.

[0201] Clause 18. The composition of any one of Clauses 11 to 17, further comprising at least one additional therapeutic agent encapsulated by the dynamic hydrogel, wherein the at least one additional therapeutic agent comprises one or more of an adjuvant, a cytokine, a chemokine, or a checkpoint inhibitor.

[0202] Clause 19. The composition of Clause 18, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a lipid-based adjuvant, a saponin-based adjuvant, a polynucleotide adjuvant, or a metal-based adjuvant.

[0203] Clause 20. The composition of Clause 18 or 19, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a Toll-like receptor agonist.

[0204] Clause 21. The composition of any one of Clauses 11 to 13, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an autoantigen for an autoimmune disease or condition.

[0205] Clause 22. The composition of Clause 21, wherein the autoimmune disease or condition is arthritis, inflammatory bowel disease, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, system lupus erythematosus, or transplant rejection.

[0206] Clause 23. The composition of any one of Clauses 1 to 22, wherein the composition comprises from 0.1 µg to 200 µg, 0.25 µg to 1 µg, 1 µg to 10 µg, 10 µg to 50 µg, 50 µg to 150 µg, or 100 µg to 200 µg of the nucleic acid therapeutic.

[0207] Clause 24. The composition of any one of Clauses 1 to 23, wherein the polymer comprises a hydrophobically-modified polysaccharide.

[0208] Clause 25. The composition of Clause 24, wherein the hydrophobically-modified polysaccharide comprises a hydrophobically-modified cellulose derivative.

[0209] Clause 26. The composition of Clause 25, wherein the hydrophobically-modified cellulose derivative is dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12).

[0210] Clause 27. The composition of any one of Clauses 1 to 26, wherein the plurality of nanoparticles comprises a plurality of polymeric nanoparticles.

[0211] Clause 28. The composition of Clause 27, wherein the plurality of polymeric nanoparticles comprises a plurality of amphiphilic polymeric nanoparticles.

[0212] Clause 29. The composition of Clause 27 or 28, wherein the plurality of polymeric nanoparticles comprises a plurality of poly(ethylene glycol)-block-poly(lactic acid) (PEG-PLA) nanoparticles.

[0213] Clause 30. The composition of any one of Clauses 1 to 29, wherein a concentration of the polymer in the dynamic hydrogel is within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%.

[0214] Clause 31. The composition of any one of Clauses 1 to 30, wherein a concentration of the plurality of nanoparticles in the dynamic hydrogel is within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%.

[0215] Clause 32. The composition of any one of Clauses 1 to 31, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a storage modulus within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa when measured at 25 °C over an angular frequency of 0.1 rad/s to 100 rad/s within a linear viscoelastic region of the dynamic hydrogel.

[0216] Clause 33. The composition of any one of Clauses 1 to 32, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a yield stress within a range from 0.1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5 Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa when measured at 25 °C.

[0217] Clause 34. The composition of any one of Clauses 1 to 33, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a viscosity less than 10,000 mPa-s when measured at 25 °C at a shear rate of 1000 s⁻¹.

[0218] Clause 35. The composition of any one of Clauses 1 to 34, wherein the composition is configured for administration via injection.

[0219] Clause 36. The composition of any one of Clauses 1 to 35, wherein, upon administration to the subject, the composition delivers the nucleic acid therapeutic to the subject over a treatment period less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day.

[0220] Clause 37. The composition of any one of Clauses 1 to 36, wherein, upon administration to the subject, the composition forms a depot that permits infiltration by a target cell and inhibits infiltration by a nontarget cell.

[0221] Clause 38. The composition of Clause 37, wherein the target cell comprises an immune cell.

[0222] Clause 39. The composition of Clause 38, wherein the immune cell comprises an antigen-presenting cell (APC).

[0223] Clause 40. The composition of any one of Clauses 37 to 39, wherein the nontarget cell comprises a nonimmune cell.

[0224] Clause 41. The composition of Clause 40, wherein the nonimmune cell comprises one or more of a muscle cell, a skin cell, an adipocyte, or a fibroblast.

[0225] Clause 42. A method of treating a subject, the method comprising:
administering a composition to the subject, wherein the composition comprises:
a dynamic hydrogel comprising a polymer and a plurality of nanoparticles,
wherein the polymer is non-covalently crosslinked with the plurality of
nanoparticles, and
a nucleic acid therapeutic encapsulated by the dynamic hydrogel, wherein the
nucleic acid therapeutic comprises a nucleic acid molecule complexed
with or incorporated in a delivery vector.

[0226] Clause 43. The method of Clause 42, wherein the nucleic acid molecule comprises an RNA molecule.

[0227] Clause 44. The method of Clause 43, wherein the RNA molecule is a mRNA molecule.

[0228] Clause 45. The method of Clause 42, wherein the nucleic acid molecule comprises a DNA molecule.

[0229] Clause 46. The method of Clause 45, wherein the DNA molecule is a plasmid DNA molecule.

[0230] Clause 47. The method of any one of Clauses 42 to 46, wherein the delivery vector comprises a lipid vector.

[0231] Clause 48. The method of Clause 47, wherein the lipid vector comprises a lipid nanoparticle (LNP).

[0232] Clause 49. The method of Clause 47 or 48, wherein the lipid vector comprises one or more of a cationic lipid, an ionizable lipid, a phospholipid, a sterol, or a polyethylene glycol (PEG)-functionalized lipid.

[0233] Clause 50. The method of any one of Clauses 42 to 46, wherein the delivery vector comprises a polymer vector.

[0234] Clause 51. The method of any one of Clauses 42 to 46, wherein the delivery vector comprises a viral vector.

[0235] Clause 52. The method of any one of Clauses 42 to 51, wherein the nucleic acid therapeutic comprises a nucleic acid vaccine.

[0236] Clause 53. The method of Clause 52, wherein the nucleic acid vaccine comprises a mRNA vaccine.

[0237] Clause 54. The method of Clause 53, wherein the mRNA vaccine is a mRNA LNP vaccine.

[0238] Clause 55. The method of any one of Clauses 52 to 54, wherein the nucleic acid vaccine comprises at least one nucleic acid molecule encoding an antigen for an infectious disease, and treating the subject comprises preventing or treating the infectious disease.

[0239] Clause 56. The method of Clause 55, wherein the infectious disease is anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes, Hendra virus disease, HIV/AIDS, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, MERS, mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, SARS, smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, or yersiniosis.

[0240] Clause 57. The method of any one of Clauses 52 to 54, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an antigen for a cancer, and treating the subject comprises preventing or treating the cancer.

[0241] Clause 58. The method of Clause 57, wherein the cancer is biliary tract cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, choriocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia, liver cancer, lymphoma, lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, testicular cancer, or thyroid cancer.

[0242] Clause 59. The method of any one of Clauses 52 to 58, wherein the composition further comprises at least one additional therapeutic agent encapsulated by the dynamic hydrogel, and wherein the at least one additional therapeutic agent comprises one or more of an adjuvant, a cytokine, or a chemokine.

[0243] Clause 60. The method of Clause 59, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a lipid-based adjuvant, a saponin-based adjuvant, a polynucleotide adjuvant, or a metal-based adjuvant.

[0244] Clause 61. The method of Clause 59 or 60, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a Toll-like receptor agonist

[0245] Clause 62. The method of any one of Clauses 52 to 54, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an autoantigen for an autoimmune disease or condition.

[0246] Clause 63. The method of Clause 62, wherein the autoimmune disease or condition is arthritis, inflammatory bowel disease, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, system lupus erythematosus, or transplant rejection.

[0247] Clause 64. The method of any one of Clauses 42 to 63, wherein the composition comprises from 0.1 μg to 200 μg , 0.25 μg to 1 μg , 1 μg to 10 μg , 10 μg to 50 μg , 50 μg to 150 μg , or 100 μg to 200 μg of the nucleic acid therapeutic.

[0248] Clause 65. The method of any one of Clauses 42 to 64, wherein the polymer comprises a hydrophobically-modified polysaccharide.

[0249] Clause 66. The method of Clause 65, wherein the hydrophobically-modified polysaccharide comprises a hydrophobically-modified cellulose derivative.

[0250] Clause 67. The method of Clause 66, wherein the hydrophobically-modified cellulose derivative is dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12).

[0251] Clause 68. The method of any one of Clauses 42 to 67, wherein the plurality of nanoparticles comprises a plurality of polymeric nanoparticles.

[0252] Clause 69. The method of Clause 68, wherein the plurality of polymeric nanoparticles comprises a plurality of amphiphilic polymeric nanoparticles.

[0253] Clause 70. The method of Clause 68 or 69, wherein the plurality of polymeric nanoparticles comprises a plurality of poly(ethylene glycol)-block-poly(lactic acid) (PEG-PLA) nanoparticles.

[0254] Clause 71. The method of any one of Clauses 42 to 70, wherein a concentration of the polymer in the dynamic hydrogel is within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%.

[0255] Clause 72. The method of any one of Clauses 42 to 71, wherein a concentration of the plurality of nanoparticles in the dynamic hydrogel is within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%.

[0256] Clause 73. The method of any one of Clauses 42 to 72, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a storage modulus within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa when measured at 25 °C over an angular frequency of 0.1 rad/s to 100 rad/s within a linear viscoelastic region of the dynamic hydrogel.

[0257] Clause 74. The method of any one of Clauses 42 to 73, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a yield stress within a range from 0.1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5 Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa when measured at 25 °C.

[0258] Clause 75. The method of any one of Clauses 42 to 74, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a viscosity less than 10,000 mPa-s when measured at 25 °C at a shear rate of 1000 s⁻¹.

[0259] Clause 76. The method of any one of Clauses 42 to 75, wherein the composition is administered via injection.

[0260] Clause 77. The method of any one of Clauses 42 to 76, wherein, upon administration to the subject, the composition delivers the nucleic acid therapeutic to the subject over a treatment period less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day.

[0261] Clause 78. The method of any one of Clauses 42 to 77, wherein, upon administration to the subject, the composition forms a depot that permits infiltration by a target cell and inhibits infiltration by a nontarget cell.

[0262] Clause 79. The method of Clause 78, wherein the target cell comprises an immune cell.

[0263] Clause 80. The method of Clause 79, wherein the immune cell comprises an antigen-presenting cell (APC).

[0264] Clause 81. The method of any one of Clauses 78 to 80, wherein the nontarget cell comprises a nonimmune cell.

[0265] Clause 82. The method of Clause 81, wherein the nonimmune cell comprises one or more of a muscle cell, a skin cell, an adipocyte, or a fibroblast.

Conclusion

[0266] Although many of the embodiments are described above with respect to compositions and methods related to nucleic acid vaccines, the technology is applicable to other applications and/or other approaches, such as other types of gene therapy applications. Moreover, other embodiments in addition to those described herein are within the scope of the technology. Additionally, several other embodiments of the technology can have different configurations, components, or procedures than those described herein. A person of ordinary skill in the art, therefore, will accordingly understand that the technology can have other embodiments with additional elements, or the technology can have other embodiments without several of the features shown and described above with reference to FIGS. 1–14B.

[0267] The descriptions of embodiments of the technology are not intended to be exhaustive or to limit the technology to the precise form disclosed above. Where the context permits, singular or plural terms may also include the plural or singular term, respectively. Although specific embodiments of, and examples for, the technology are described above for

illustrative purposes, various equivalent modifications are possible within the scope of the technology, as those skilled in the relevant art will recognize. For example, while steps are presented in a given order, alternative embodiments may perform steps in a different order. The various embodiments described herein may also be combined to provide further embodiments.

[0268] As used herein, the terms “generally,” “substantially,” “about,” and similar terms are used as terms of approximation and not as terms of degree, and are intended to account for the inherent variations in measured or calculated values that would be recognized by those of ordinary skill in the art. The terms “about” and “approximately,” in reference to a number, are used herein to include numbers that fall within a range of 10%, 5%, or 1% in either direction (greater than or less than) the number unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0269] Moreover, unless the word “or” is expressly limited to mean only a single item exclusive from the other items in reference to a list of two or more items, then the use of “or” in such a list is to be interpreted as including (a) any single item in the list, (b) all of the items in the list, or (c) any combination of the items in the list. As used herein, the phrase “and/or” as in “A and/or B” refers to A alone, B alone, and A and B. Additionally, the term “comprising” is used throughout to mean including at least the recited feature(s) such that any greater number of the same feature and/or additional types of other features are not precluded.

[0270] As used herein, the term “subject” may refer to any animal, including but not limited to, humans and non-human animals (e.g., dogs, cats, cows, horses, sheep, pigs, poultry, fish, crustaceans, etc.).

[0271] To the extent any materials incorporated herein by reference conflict with the present disclosure, the present disclosure controls.

[0272] It will also be appreciated that specific embodiments have been described herein for purposes of illustration, but that various modifications may be made without deviating from the technology. Further, while advantages associated with certain embodiments of the technology have been described in the context of those embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the technology. Accordingly, the disclosure and associated technology can encompass other embodiments not expressly shown or described herein.

CLAIMS

What is claimed is:

1. A composition for treating a subject, the composition comprising:
a dynamic hydrogel comprising a polymer and a plurality of nanoparticles, wherein
the polymer is non-covalently crosslinked with the plurality of nanoparticles;
and
a nucleic acid therapeutic encapsulated by the dynamic hydrogel, wherein the nucleic
acid therapeutic comprises a nucleic acid molecule complexed with or
incorporated in a delivery vector.
2. The composition of claim 1, wherein the nucleic acid molecule comprises an
RNA molecule.
3. The composition of claim 2, wherein the RNA molecule is a mRNA molecule.
4. The composition of claim 1, wherein the nucleic acid molecule comprises a
DNA molecule.
5. The composition of claim 4, wherein the DNA molecule is a plasmid DNA
molecule.
6. The composition of any one of claims 1 to 5, wherein the delivery vector
comprises a lipid vector.
7. The composition of claim 6, wherein the lipid vector comprises a lipid
nanoparticle (LNP).
8. The composition of claim 6 or 7, wherein the lipid vector comprises one or
more of a cationic lipid, an ionizable lipid, a phospholipid, a sterol, or a polyethylene glycol
(PEG)-functionalized lipid.

9. The composition of any one of claims 1 to 5, wherein the delivery vector comprises a polymer vector.

10. The composition of any one of claims 1 to 5, wherein the delivery vector comprises a viral vector.

11. The composition of any one of claims 1 to 10, wherein the nucleic acid therapeutic comprises a nucleic acid vaccine.

12. The composition of claim 11, wherein the nucleic acid vaccine comprises a mRNA vaccine.

13. The composition of claim 12, wherein the mRNA vaccine is a mRNA LNP vaccine.

14. The composition of any one of claims 11 to 13, wherein the nucleic acid vaccine comprises at least one nucleic acid molecule encoding an antigen for an infectious disease.

15. The composition of claim 14, wherein the infectious disease is anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes, Hendra virus disease, HIV/AIDs, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, MERS, mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, SARS, smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, or yersiniosis.

16. The composition of any one of claims 11 to 13, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an antigen for a cancer.

17. The composition of claim 16, wherein the cancer is biliary tract cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, choriocarcinoma, colorectal

cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia, liver cancer, lymphoma, lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, skin cancer, testicular cancer, or thyroid cancer.

18. The composition of any one of claims 11 to 17, further comprising at least one additional therapeutic agent encapsulated by the dynamic hydrogel, wherein the at least one additional therapeutic agent comprises one or more of an adjuvant, a cytokine, a chemokine, or a checkpoint inhibitor.

19. The composition of claim 18, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a lipid-based adjuvant, a saponin-based adjuvant, a polynucleotide adjuvant, or a metal-based adjuvant.

20. The composition of claim 18 or 19, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a Toll-like receptor agonist.

21. The composition of any one of claims 11 to 13, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an autoantigen for an autoimmune disease or condition.

22. The composition of claim 21, wherein the autoimmune disease or condition is arthritis, inflammatory bowel disease, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, system lupus erythematosus, or transplant rejection.

23. The composition of any one of claims 1 to 22, wherein the composition comprises from 0.1 μg to 200 μg , 0.25 μg to 1 μg , 1 μg to 10 μg , 10 μg to 50 μg , 50 μg to 150 μg , or 100 μg to 200 μg of the nucleic acid therapeutic.

24. The composition of any one of claims 1 to 23, wherein the polymer comprises a hydrophobically-modified polysaccharide.

25. The composition of claim 24, wherein the hydrophobically-modified

polysaccharide comprises a hydrophobically-modified cellulose derivative.

26. The composition of claim 25, wherein the hydrophobically-modified cellulose derivative is dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12).

27. The composition of any one of claims 1 to 26, wherein the plurality of nanoparticles comprises a plurality of polymeric nanoparticles.

28. The composition of claim 27, wherein the plurality of polymeric nanoparticles comprises a plurality of amphiphilic polymeric nanoparticles.

29. The composition of claim 27 or 28, wherein the plurality of polymeric nanoparticles comprises a plurality of poly(ethylene glycol)-block-poly(lactic acid) (PEG-PLA) nanoparticles.

30. The composition of any one of claims 1 to 29, wherein a concentration of the polymer in the dynamic hydrogel is within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%.

31. The composition of any one of claims 1 to 30, wherein a concentration of the plurality of nanoparticles in the dynamic hydrogel is within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%.

32. The composition of any one of claims 1 to 31, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a storage modulus within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa when measured at 25 °C over an angular frequency of 0.1 rad/s to 100 rad/s within a linear viscoelastic region of the dynamic hydrogel.

33. The composition of any one of claims 1 to 32, wherein the dynamic hydrogel

encapsulating the nucleic acid therapeutic has a yield stress within a range from 0.1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5 Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa when measured at 25 °C.

34. The composition of any one of claims 1 to 33, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a viscosity less than 10,000 mPa-s when measured at 25 °C at a shear rate of 1000 s⁻¹.

35. The composition of any one of claims 1 to 34, wherein the composition is configured for administration via injection.

36. The composition of any one of claims 1 to 35, wherein, upon administration to the subject, the composition delivers the nucleic acid therapeutic to the subject over a treatment period less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day.

37. The composition of any one of claims 1 to 36, wherein, upon administration to the subject, the composition forms a depot that permits infiltration by a target cell and inhibits infiltration by a nontarget cell.

38. The composition of claim 37, wherein the target cell comprises an immune cell.

39. The composition of claim 38, wherein the immune cell comprises an antigen-presenting cell (APC).

40. The composition of any one of claims 37 to 39, wherein the nontarget cell comprises a nonimmune cell.

41. The composition of claim 40, wherein the nonimmune cell comprises one or more of a muscle cell, a skin cell, an adipocyte, or a fibroblast.

42. A method of treating a subject, the method comprising:
administering a composition to the subject, wherein the composition comprises:
a dynamic hydrogel comprising a polymer and a plurality of nanoparticles,
wherein the polymer is non-covalently crosslinked with the plurality of
nanoparticles, and
a nucleic acid therapeutic encapsulated by the dynamic hydrogel, wherein the
nucleic acid therapeutic comprises a nucleic acid molecule complexed
with or incorporated in a delivery vector.
43. The method of claim 42, wherein the nucleic acid molecule comprises an
RNA molecule.
44. The method of claim 43, wherein the RNA molecule is a mRNA molecule.
45. The method of claim 42, wherein the nucleic acid molecule comprises a DNA
molecule.
46. The method of claim 45, wherein the DNA molecule is a plasmid DNA
molecule.
47. The method of any one of claims 42 to 46, wherein the delivery vector
comprises a lipid vector.
48. The method of claim 47, wherein the lipid vector comprises a lipid
nanoparticle (LNP).
49. The method of claim 47 or 48, wherein the lipid vector comprises one or more
of a cationic lipid, an ionizable lipid, a phospholipid, a sterol, or a polyethylene glycol
(PEG)-functionalized lipid.
50. The method of any one of claims 42 to 46, wherein the delivery vector
comprises a polymer vector.

51. The method of any one of claims 42 to 46, wherein the delivery vector comprises a viral vector.

52. The method of any one of claims 42 to 51, wherein the nucleic acid therapeutic comprises a nucleic acid vaccine.

53. The method of claim 52, wherein the nucleic acid vaccine comprises a mRNA vaccine.

54. The method of claim 53, wherein the mRNA vaccine is a mRNA LNP vaccine.

55. The method of any one of claims 52 to 54, wherein the nucleic acid vaccine comprises at least one nucleic acid molecule encoding an antigen for an infectious disease, and treating the subject comprises preventing or treating the infectious disease.

56. The method of claim 55, wherein the infectious disease is anthrax, botulism, chickenpox, chikungunya fever, cholera, COVID-19, dengue fever, diarrhea, diphtheria, Ebola, encephalitis, gastritis, gastric ulcer, giardiasis, hepatitis, herpes, Hendra virus disease, HIV/AIDs, HPV infection, influenza, listeriosis, Marburg virus disease, malaria, measles, meningitis, meningococcal disease, MERS, mumps, Nipah virus disease, pneumococcal disease, pneumonia, polio, rabies, Ross River fever, RSV infection, rubella, SARS, smallpox, shigellosis, sleeping sickness, streptococcal disease, tetanus, toxoplasmosis, tuberculosis, typhoid fever, West Nile fever, whooping cough, yellow fever, or yersiniosis.

57. The method of any one of claims 52 to 54, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an antigen for a cancer, and treating the subject comprises preventing or treating the cancer.

58. The method of claim 57, wherein the cancer is biliary tract cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, choriocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, leukemia, liver cancer, lymphoma, lung cancer, neuroblastoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer,

renal cancer, sarcoma, skin cancer, testicular cancer, or thyroid cancer.

59. The method of any one of claims 52 to 58, wherein the composition further comprises at least one additional therapeutic agent encapsulated by the dynamic hydrogel, and wherein the at least one additional therapeutic agent comprises one or more of an adjuvant, a cytokine, or a chemokine.

60. The method of claim 59, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a lipid-based adjuvant, a saponin-based adjuvant, a polynucleotide adjuvant, or a metal-based adjuvant.

61. The method of claim 59 or 60, wherein the at least one additional therapeutic agent comprises the adjuvant, and wherein the adjuvant comprises a Toll-like receptor agonist

62. The method of any one of claims 52 to 54, wherein the nucleic acid vaccine comprises a nucleic acid molecule encoding an autoantigen for an autoimmune disease or condition.

63. The method of claim 62, wherein the autoimmune disease or condition is arthritis, inflammatory bowel disease, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, system lupus erythematosus, or transplant rejection.

64. The method of any one of claims 42 to 63, wherein the composition comprises from 0.1 μg to 200 μg , 0.25 μg to 1 μg , 1 μg to 10 μg , 10 μg to 50 μg , 50 μg to 150 μg , or 100 μg to 200 μg of the nucleic acid therapeutic.

65. The method of any one of claims 42 to 64, wherein the polymer comprises a hydrophobically-modified polysaccharide.

66. The method of claim 65, wherein the hydrophobically-modified polysaccharide comprises a hydrophobically-modified cellulose derivative.

67. The method of claim 66, wherein the hydrophobically-modified cellulose derivative is dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12).

68. The method of any one of claims 42 to 67, wherein the plurality of nanoparticles comprises a plurality of polymeric nanoparticles.

69. The method of claim 68, wherein the plurality of polymeric nanoparticles comprises a plurality of amphiphilic polymeric nanoparticles.

70. The method of claim 68 or 69, wherein the plurality of polymeric nanoparticles comprises a plurality of poly(ethylene glycol)-block-poly(lactic acid) (PEG-PLA) nanoparticles.

71. The method of any one of claims 42 to 70, wherein a concentration of the polymer in the dynamic hydrogel is within a range from 0.25 wt% to 1 wt%, 0.25 wt% to 0.8 wt%, 0.25 wt% to 0.5 wt%, 0.5 wt% to 1 wt%, 0.5 wt% to 0.8 wt%, or 0.8 wt% to 1 wt%.

72. The method of any one of claims 42 to 71, wherein a concentration of the plurality of nanoparticles in the dynamic hydrogel is within a range from 2 wt% to 10 wt%, 2 wt% to 8 wt%, 2 wt% to 5 wt%, 5 wt% to 10 wt%, 5 wt% to 8 wt%, or 8 wt% to 10 wt%.

73. The method of any one of claims 42 to 72, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a storage modulus within a range from 1 Pa to 500 Pa, 1 Pa to 250 Pa, 1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 10 Pa to 500 Pa, 10 Pa to 250 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 500 Pa, 25 Pa to 250 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, 50 Pa to 500 Pa, 50 Pa to 250 Pa, 50 Pa to 100 Pa, 100 Pa to 500 Pa, 100 Pa to 250 Pa, or 250 Pa to 500 Pa when measured at 25 °C over an angular frequency of 0.1 rad/s to 100 rad/s within a linear viscoelastic region of the dynamic hydrogel.

74. The method of any one of claims 42 to 73, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a yield stress within a range from 0.1 Pa to 100 Pa, 1 Pa to 50 Pa, 1 Pa to 25 Pa, 1 Pa to 10 Pa, 1 Pa to 5 Pa, 5 Pa to 100 Pa, 5 Pa to 50 Pa, 5

Pa to 25 Pa, 5 Pa to 10 Pa, 10 Pa to 100 Pa, 10 Pa to 50 Pa, 10 Pa to 25 Pa, 25 Pa to 100 Pa, 25 Pa to 50 Pa, or 50 Pa to 100 Pa when measured at 25 °C.

75. The method of any one of claims 42 to 74, wherein the dynamic hydrogel encapsulating the nucleic acid therapeutic has a viscosity less than 10,000 mPa-s when measured at 25 °C at a shear rate of 1000 s⁻¹.

76. The method of any one of claims 42 to 75, wherein the composition is administered via injection.

77. The method of any one of claims 42 to 76, wherein, upon administration to the subject, the composition delivers the nucleic acid therapeutic to the subject over a treatment period less than or equal to 14 days, 12 days, 10 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day.

78. The method of any one of claims 42 to 77, wherein, upon administration to the subject, the composition forms a depot that permits infiltration by a target cell and inhibits infiltration by a nontarget cell.

79. The method of claim 78, wherein the target cell comprises an immune cell.

80. The method of claim 79, wherein the immune cell comprises an antigen-presenting cell (APC).

81. The method of any one of claims 78 to 80, wherein the nontarget cell comprises a nonimmune cell.

82. The method of claim 81, wherein the nonimmune cell comprises one or more of a muscle cell, a skin cell, an adipocyte, or a fibroblast.

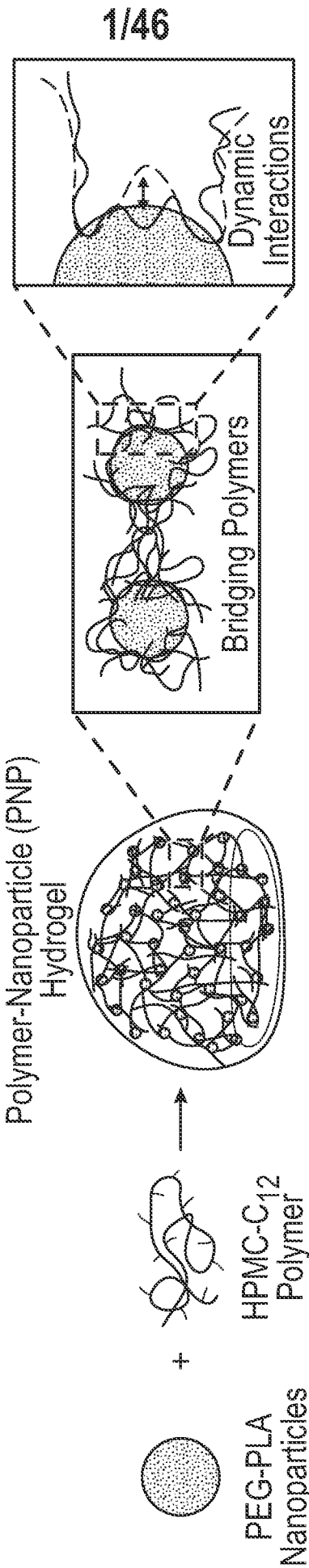
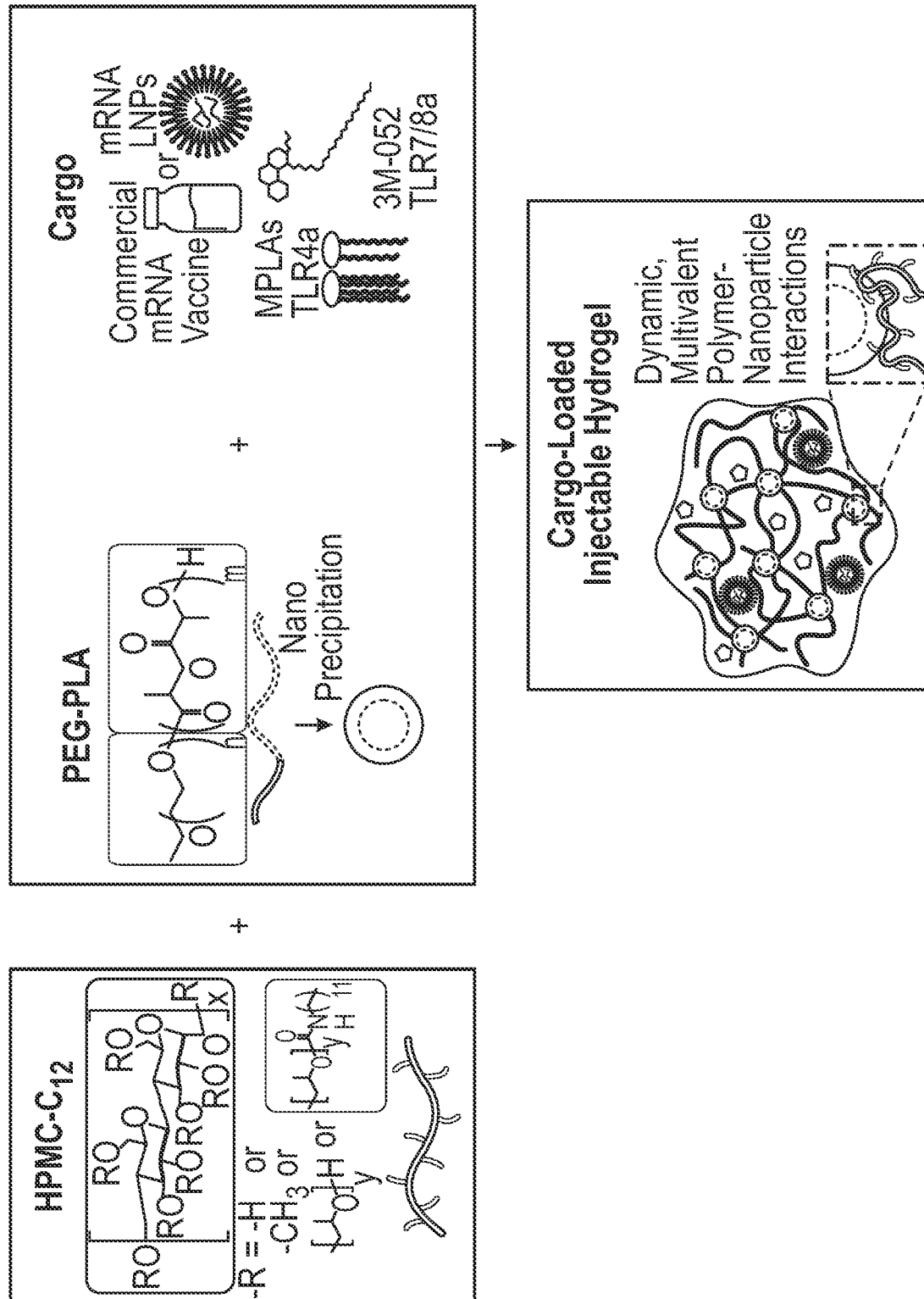


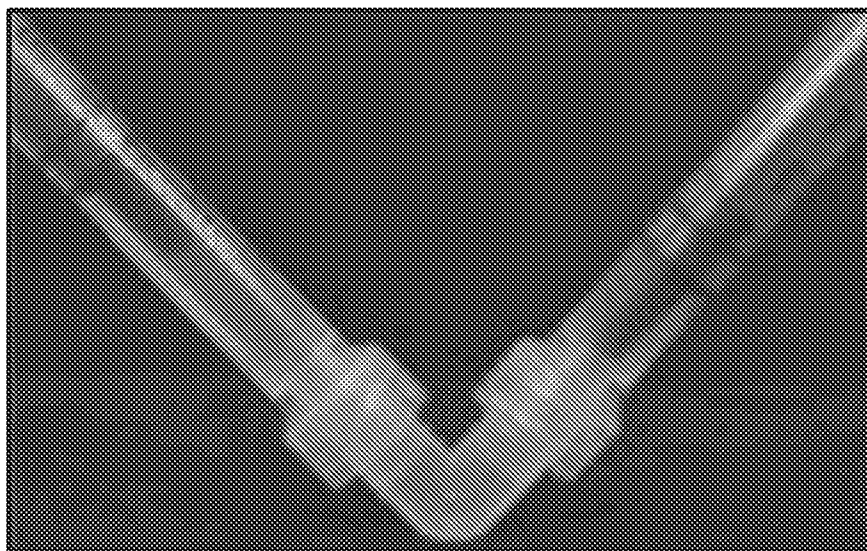
FIG. 1



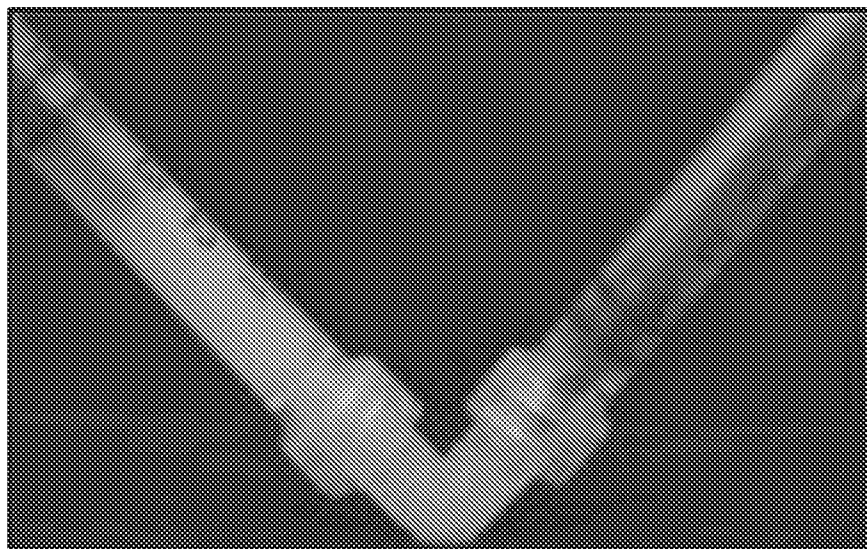
2A
G²
XXXXXX
LL

3/46

Loading

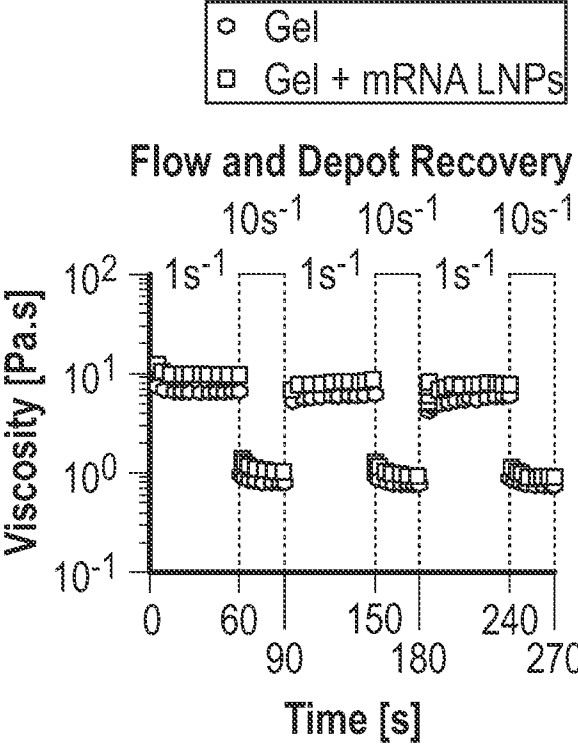
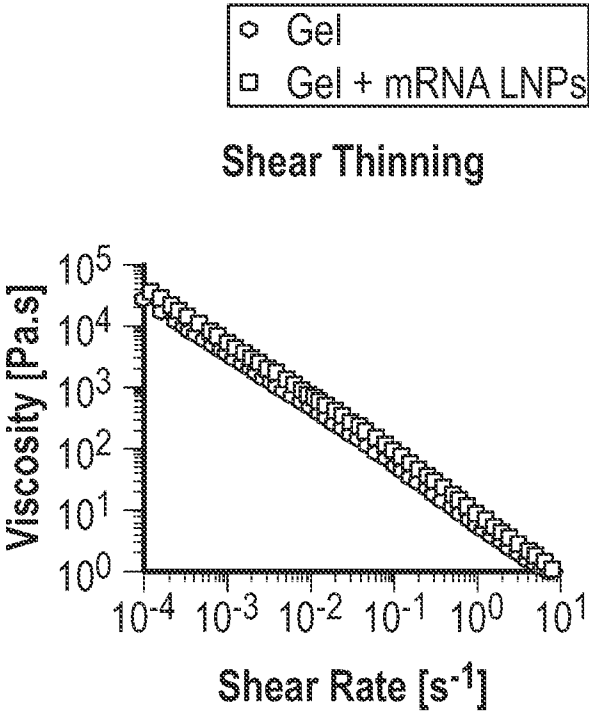
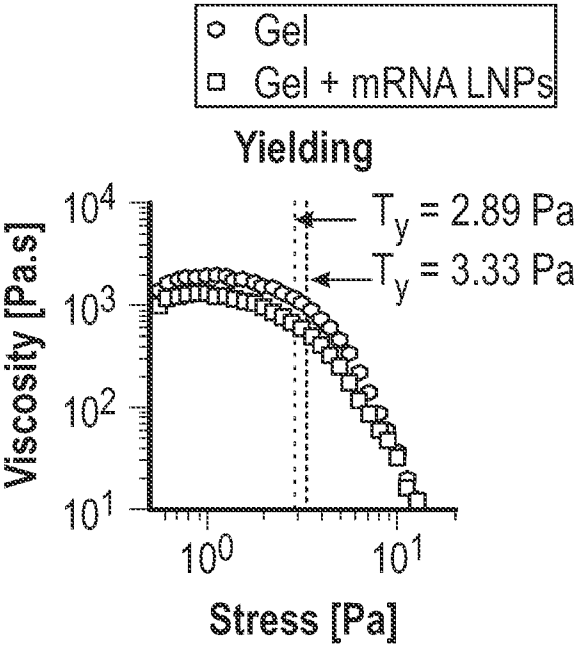
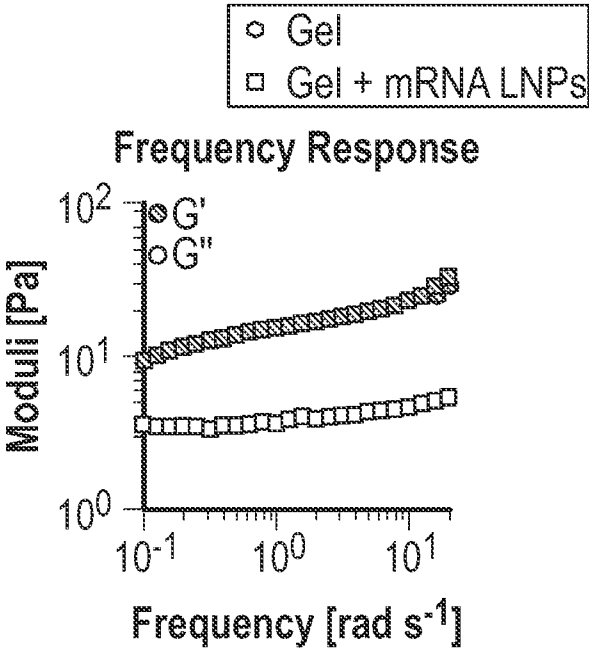


Mixing



Homogeneous Gel

FIG. 2B



Injectable & Depot Forming

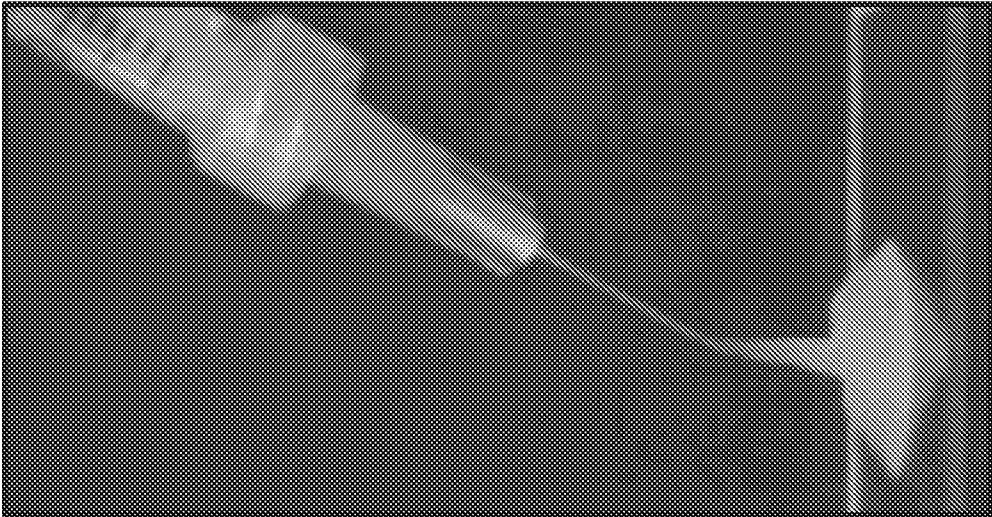


FIG. 3E

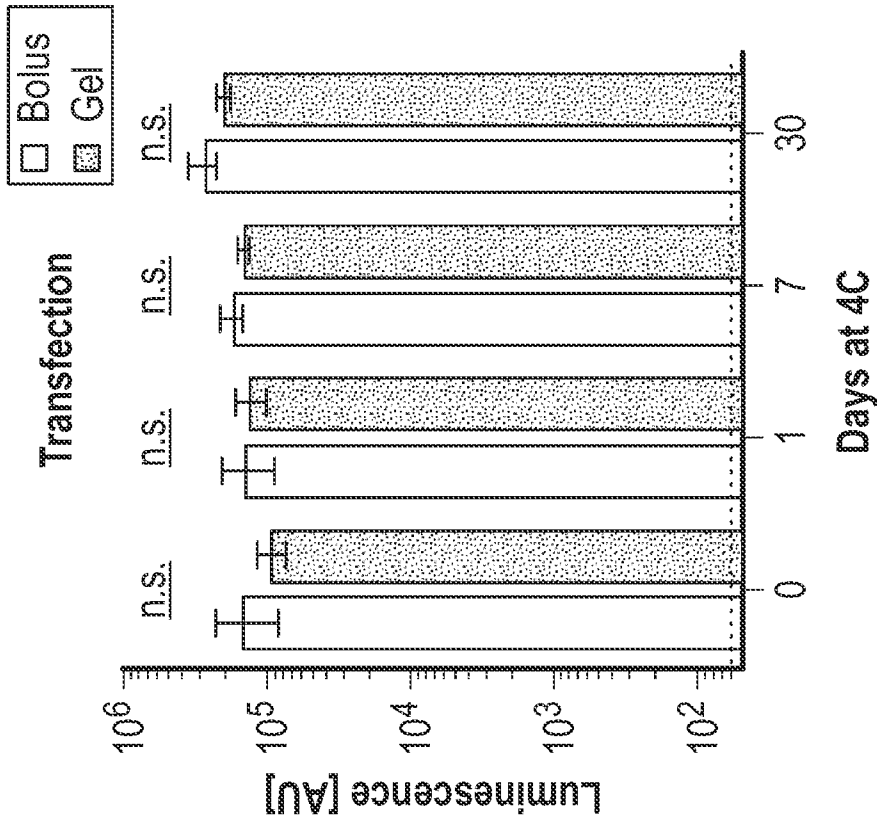


FIG. 4

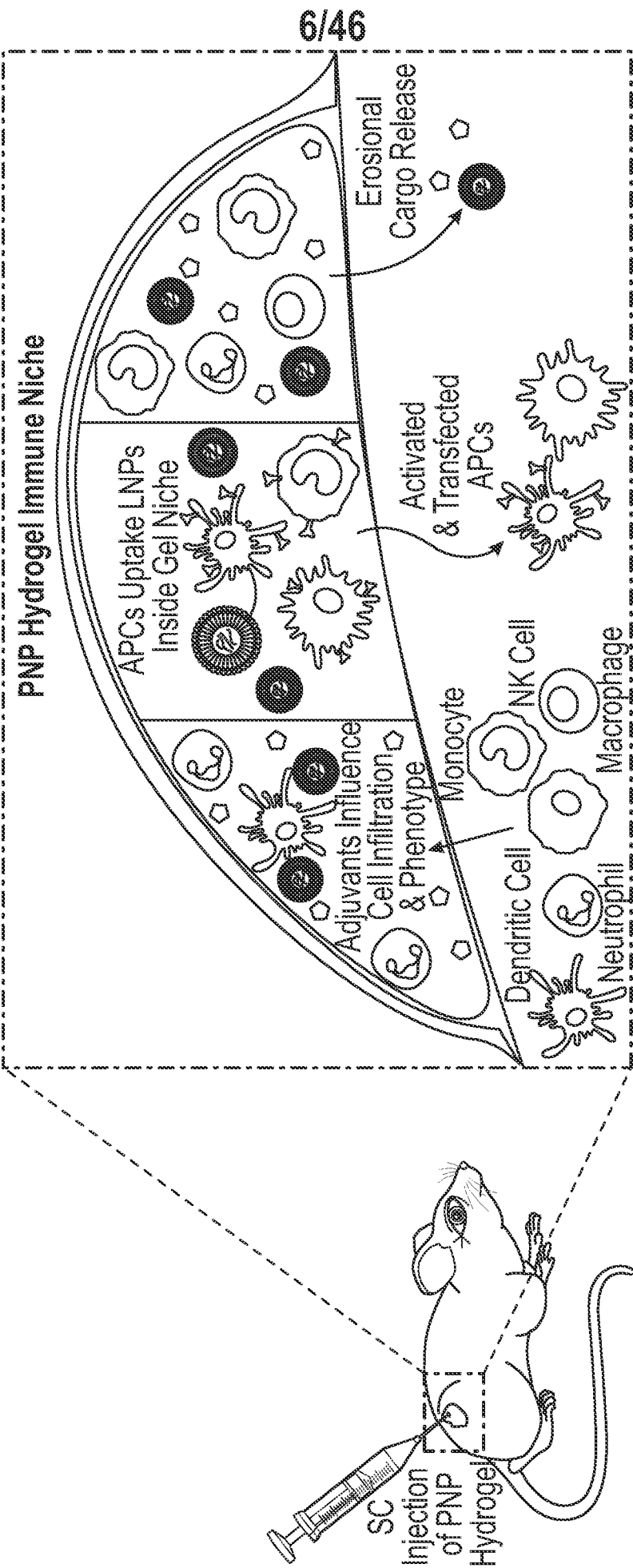
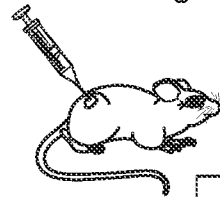


FIG. 5

7/46

1 μ g mCherry mRNA
LNPs in PNP gel



Day 0

3

7

Hydrogel depot harvest
for flow analysis of niche

FIG. 6A

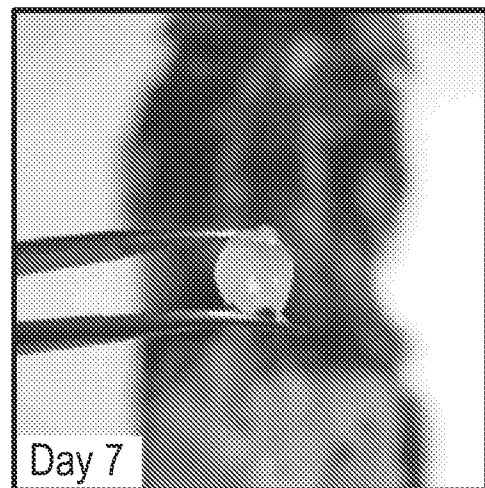
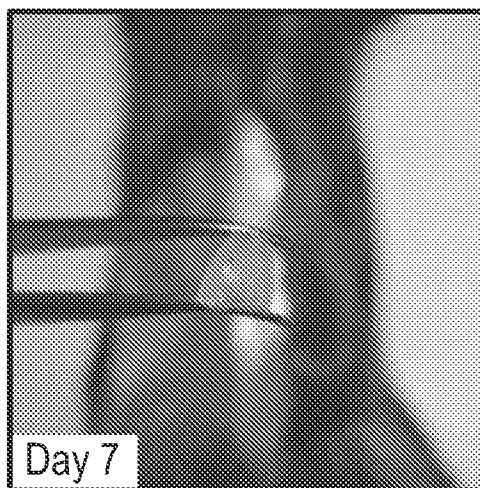
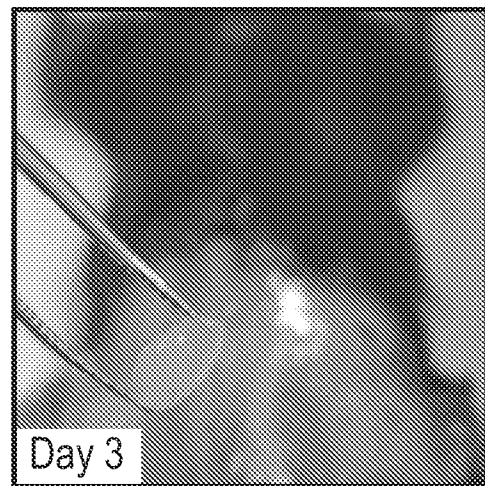
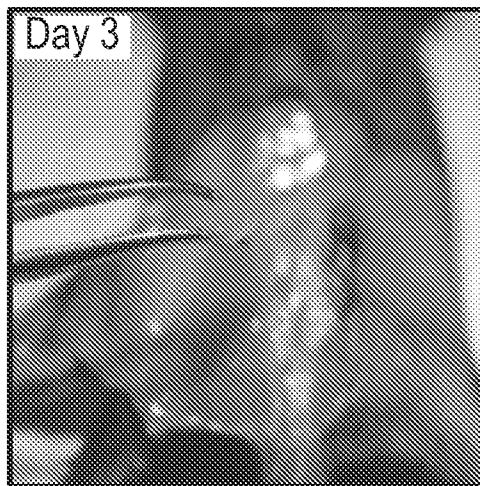


FIG. 6B

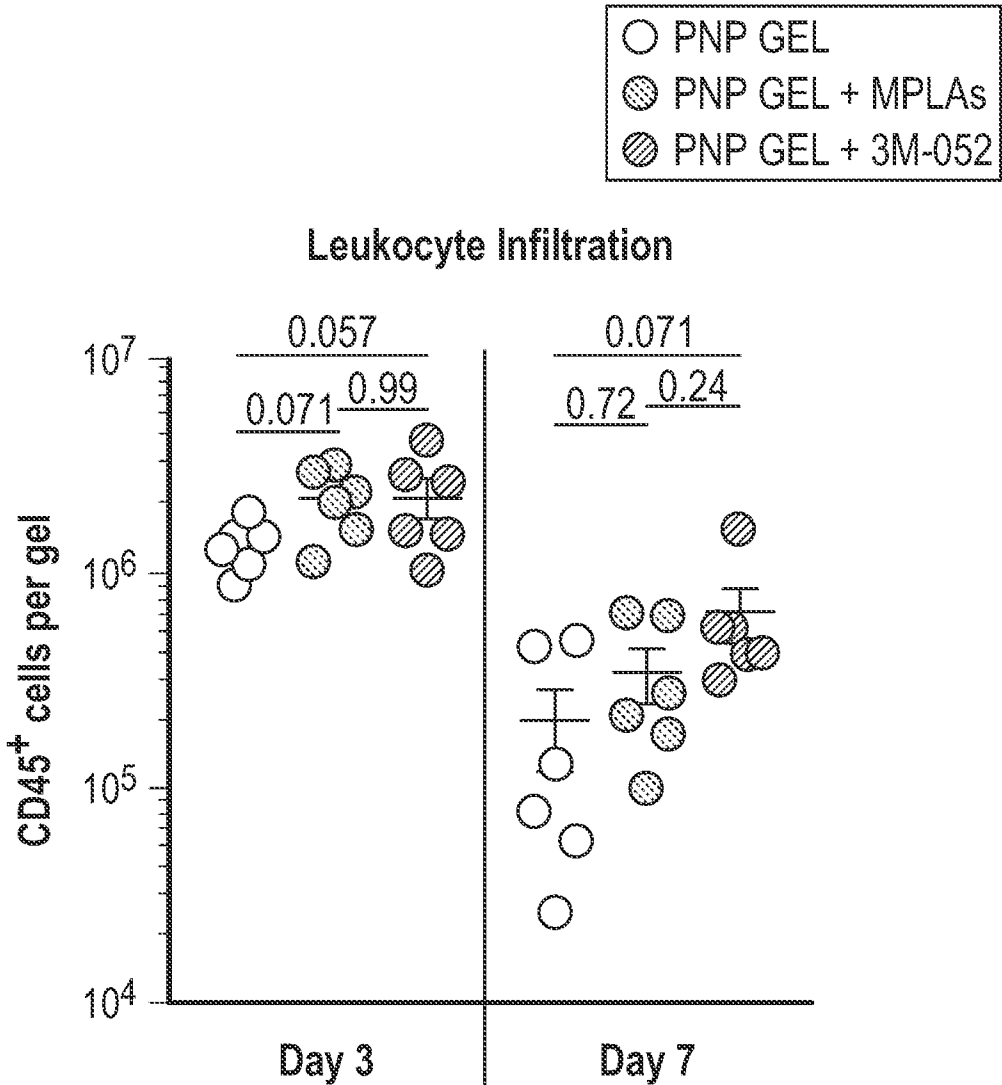


FIG. 6C

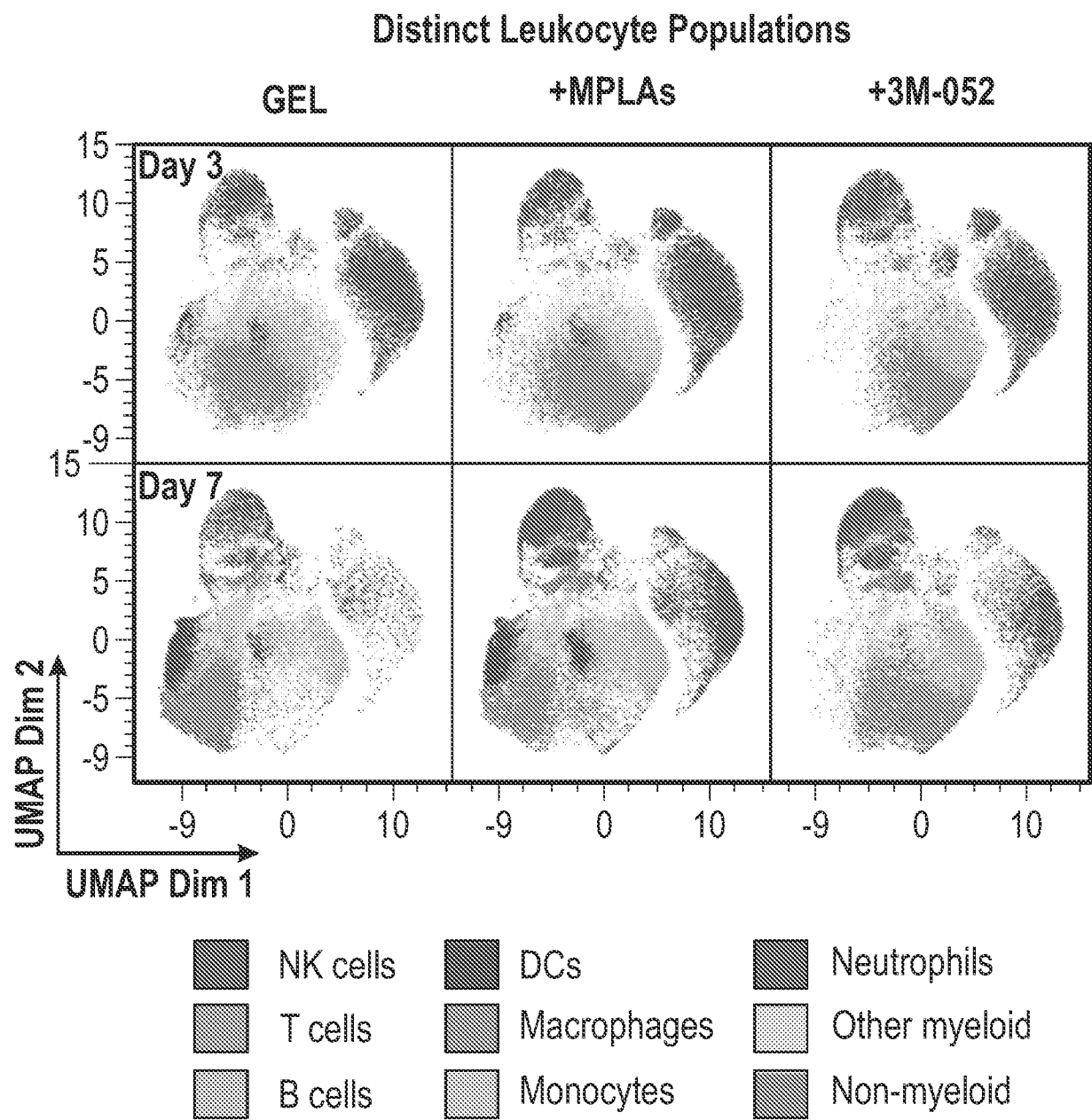


FIG. 6D

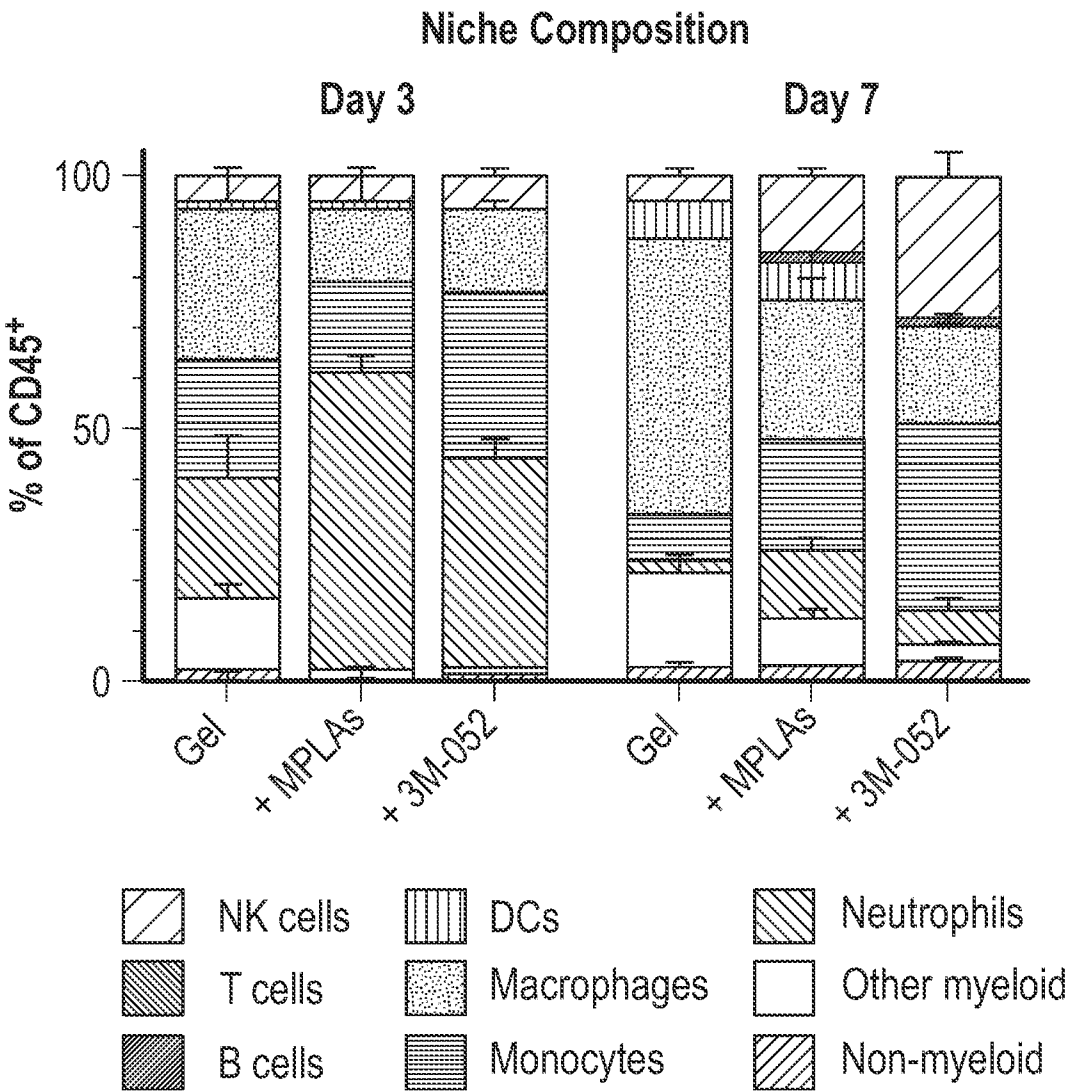


FIG. 6E

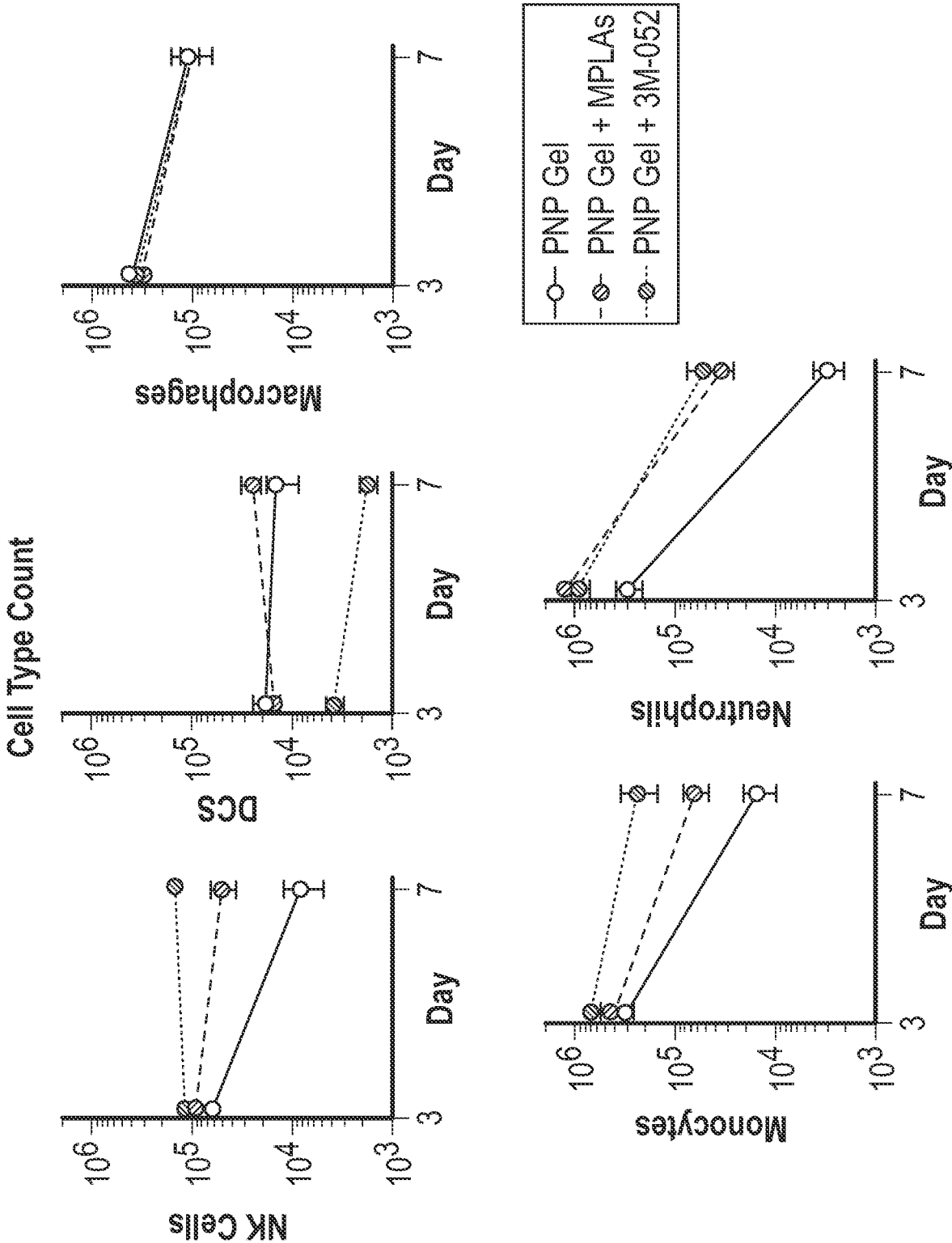


FIG. 6F

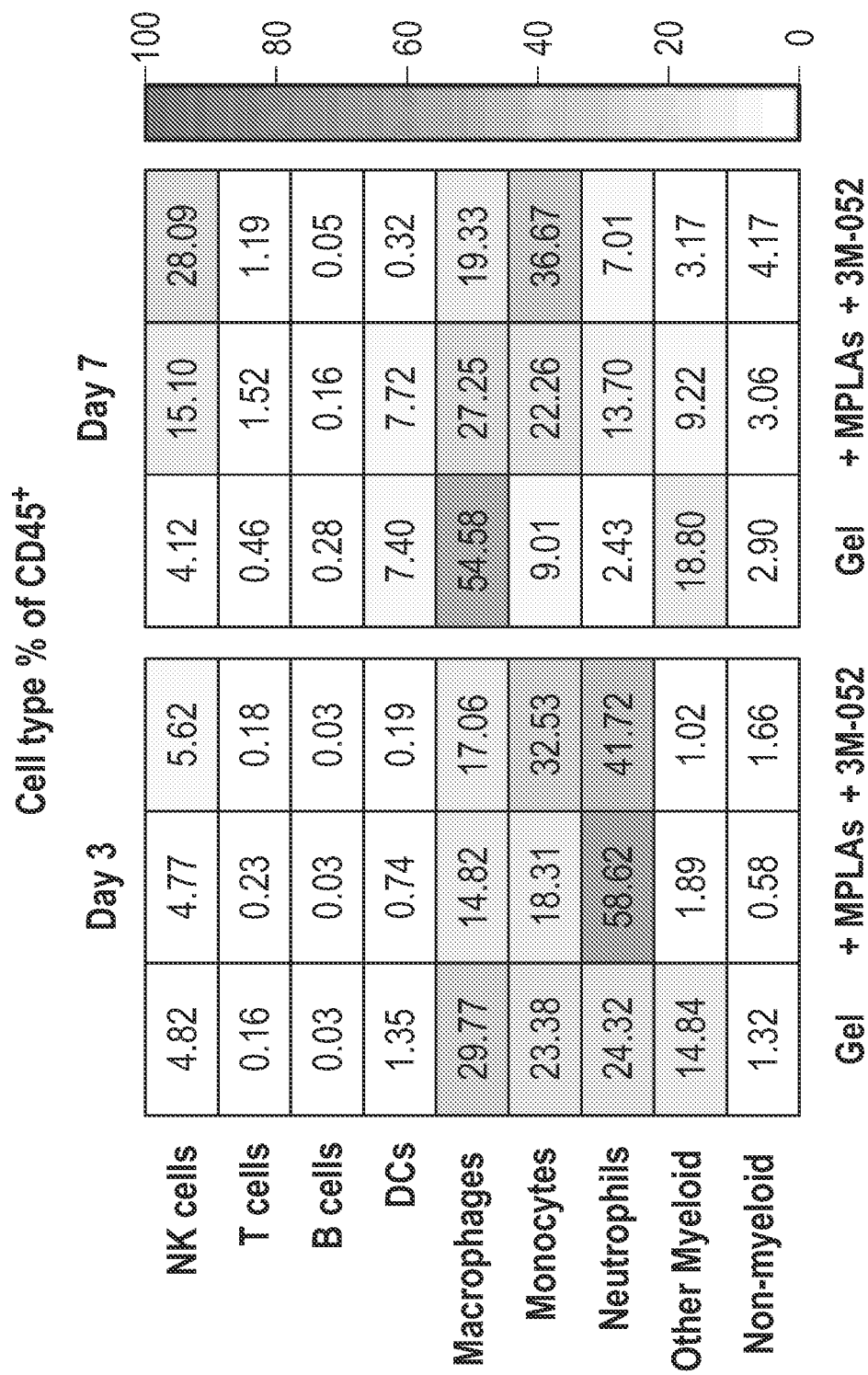


FIG. 6G

13/46

Monocyte/Macrophage Populations

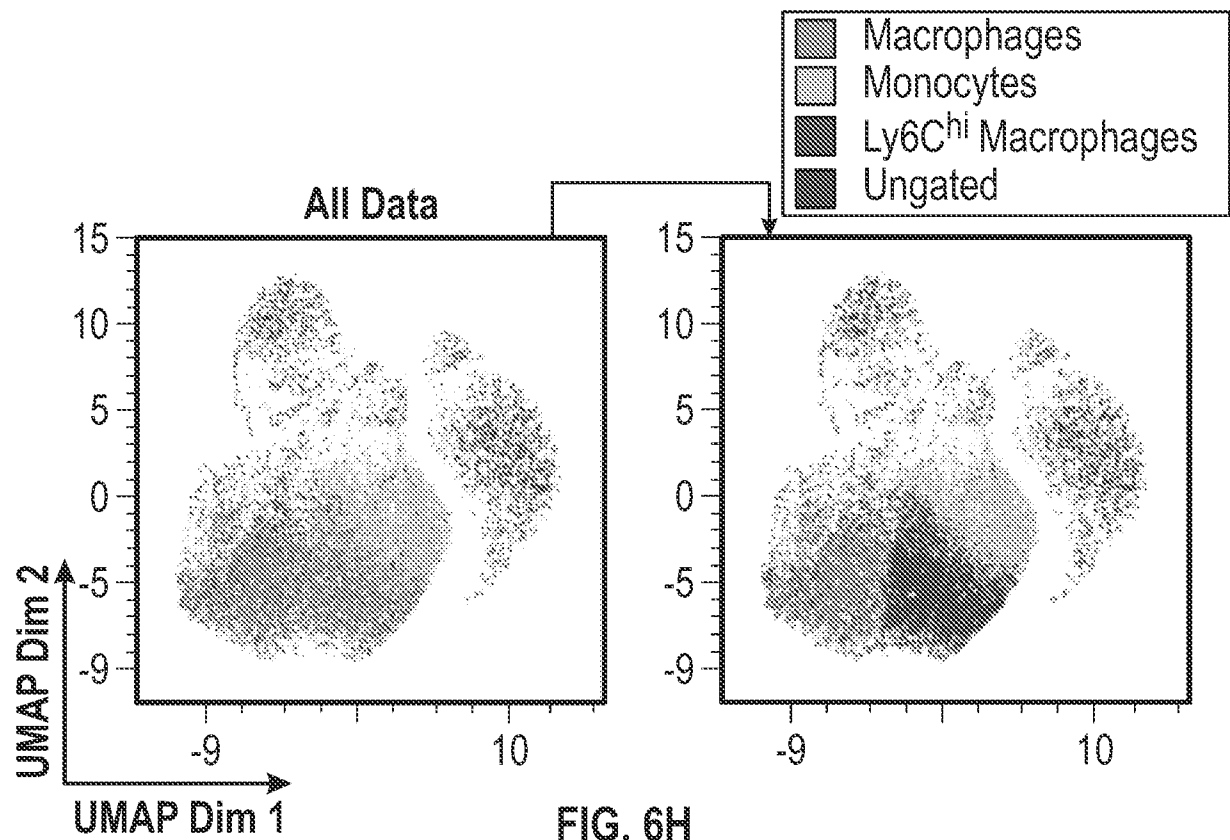


FIG. 6H

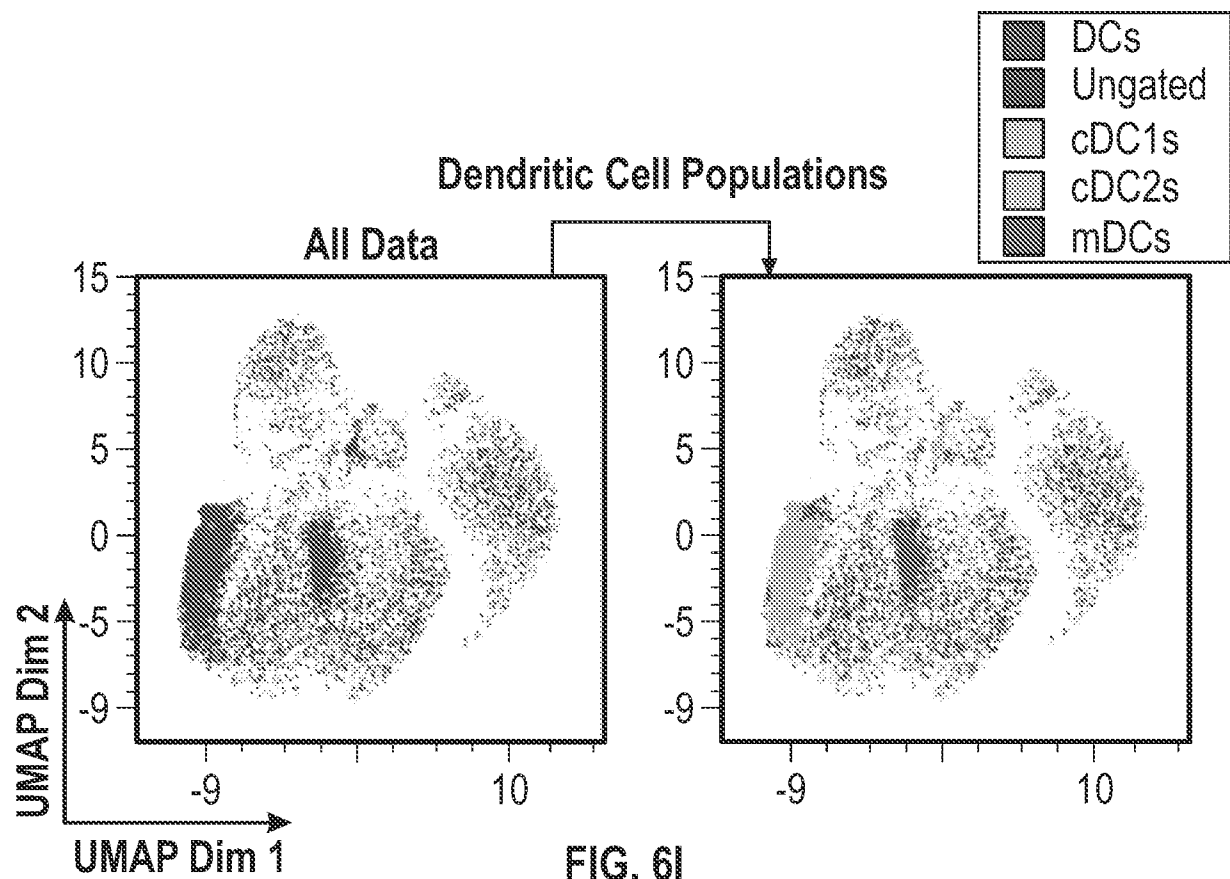


FIG. 6I

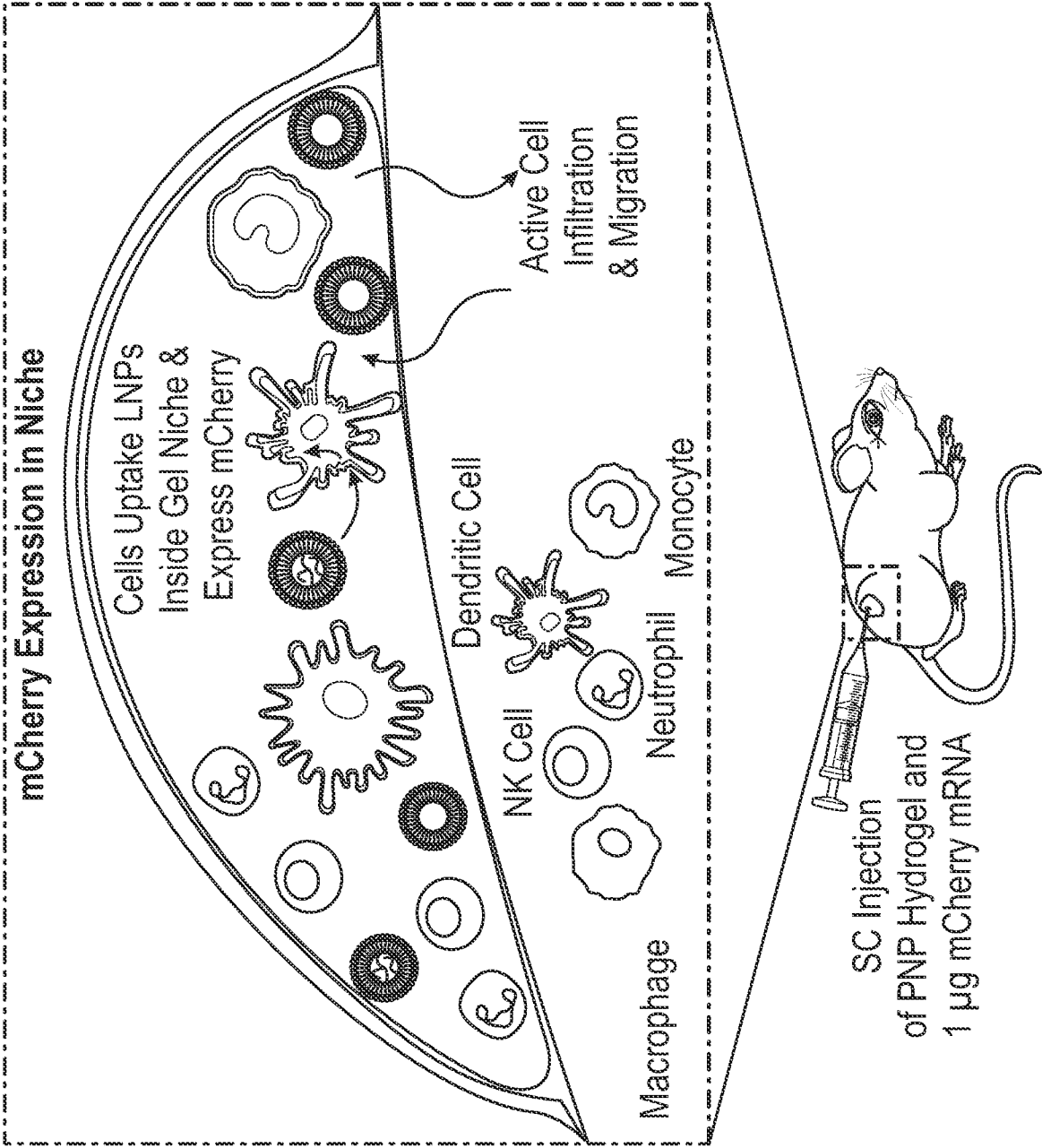


FIG. 7A

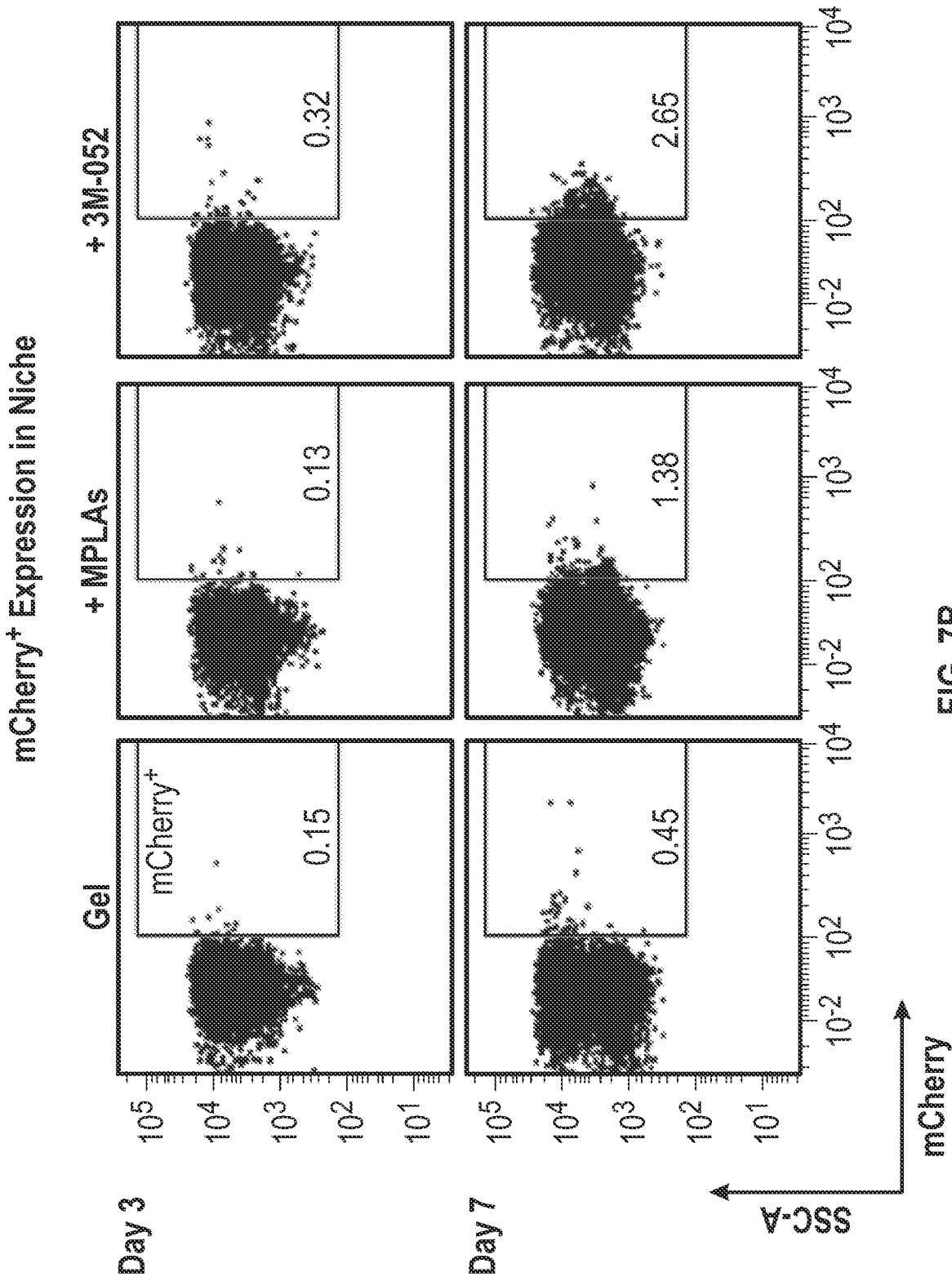


FIG. 7B

16/46

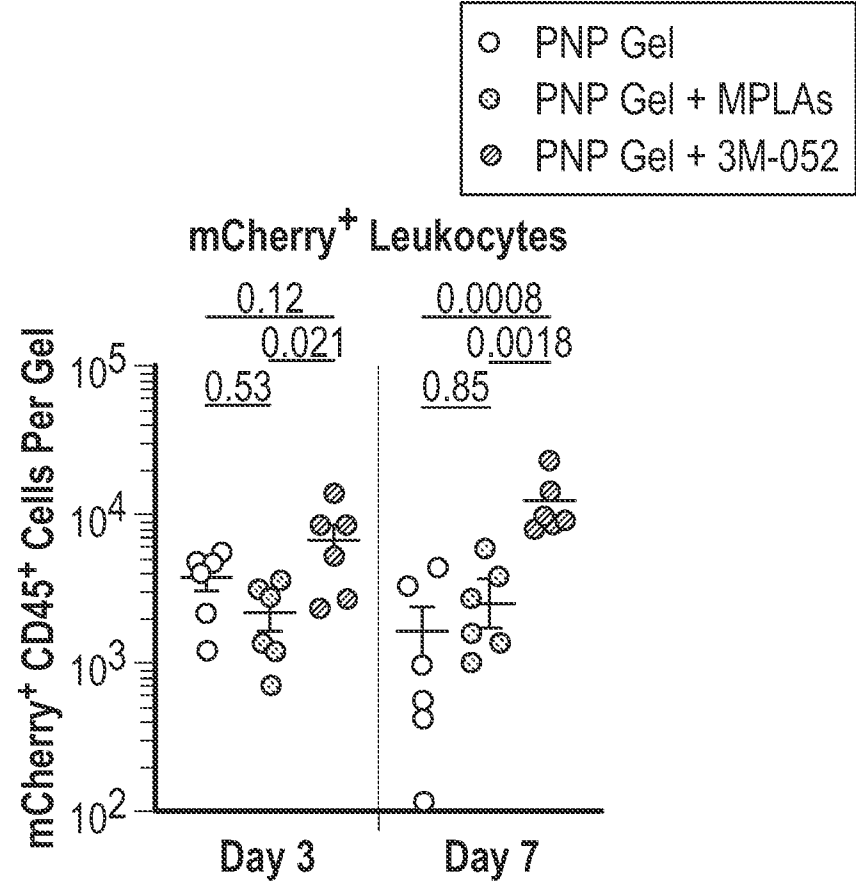


FIG. 7C

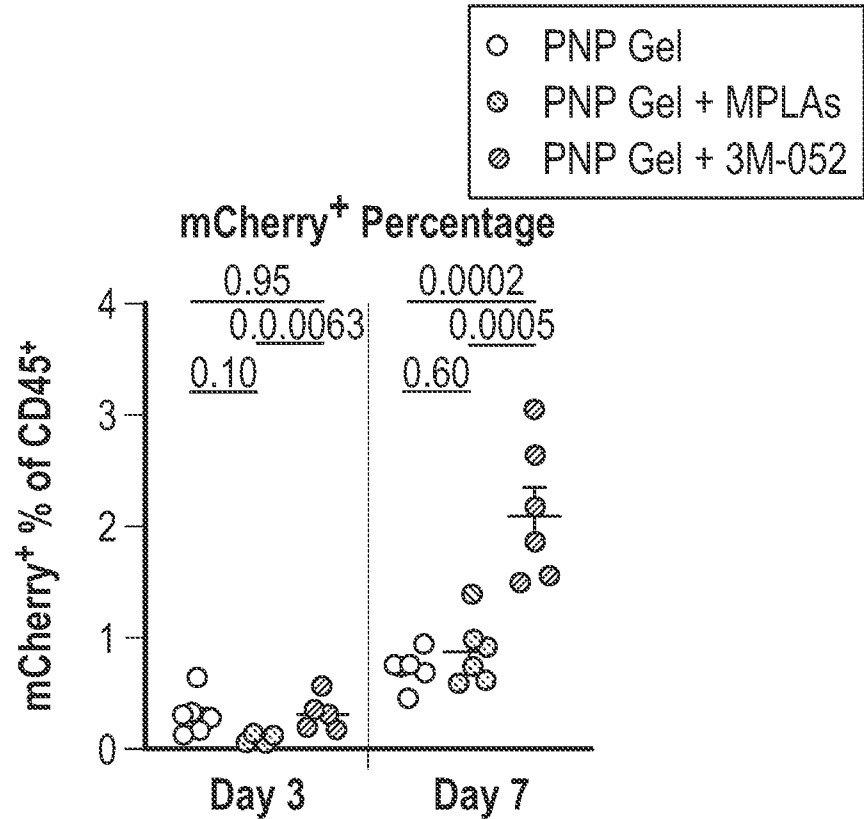


FIG. 7D

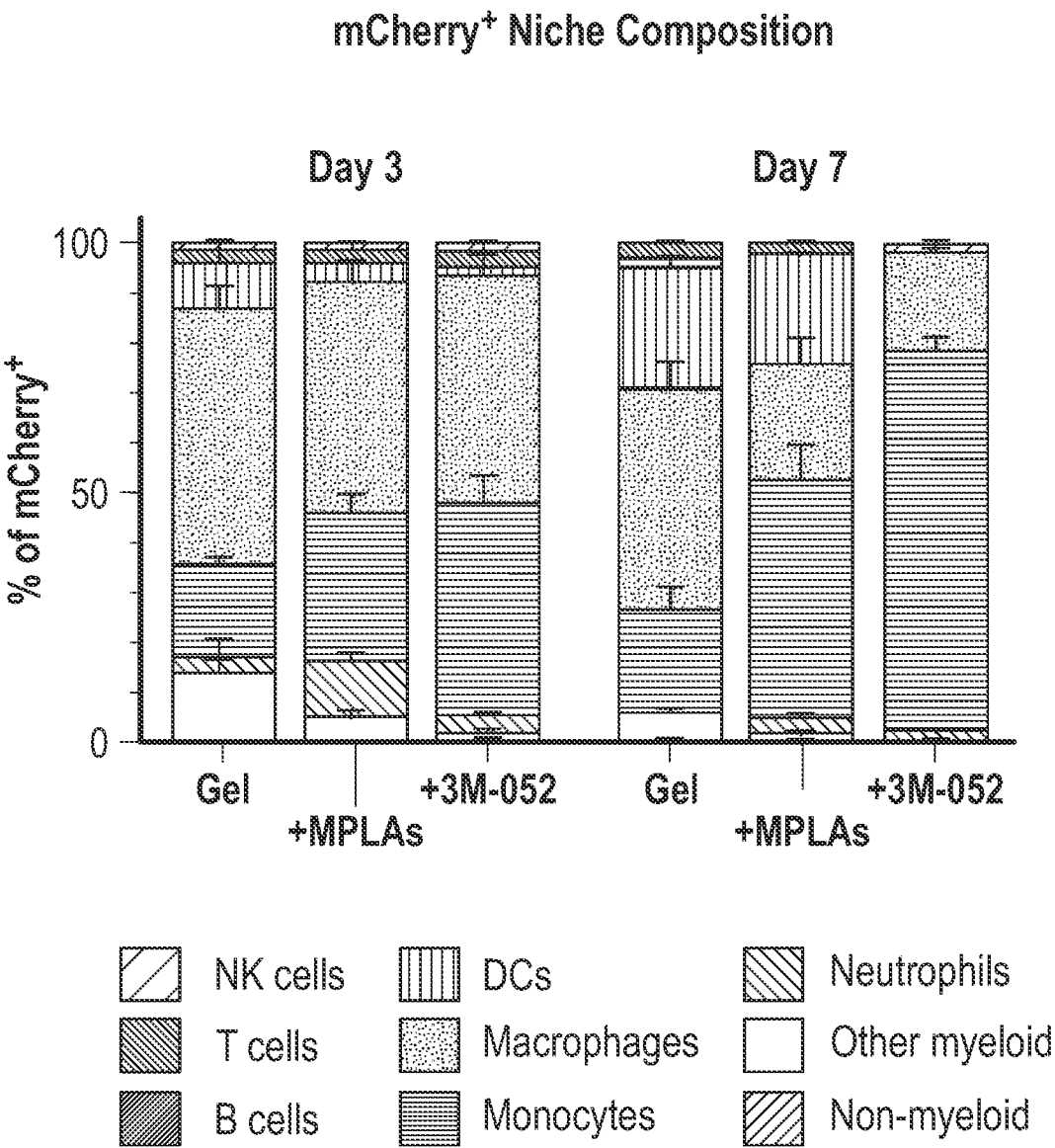


FIG. 7E

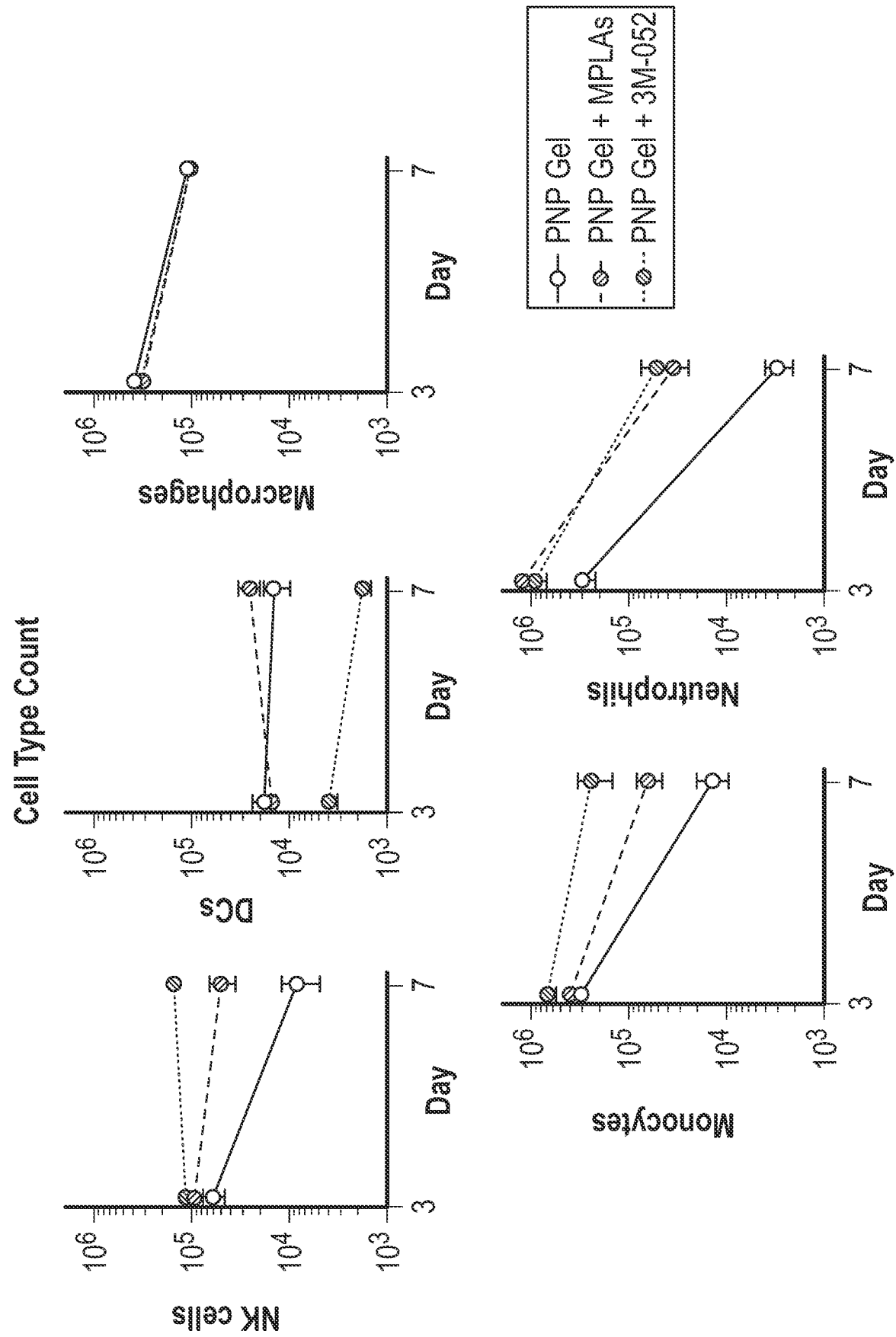


FIG. 7F

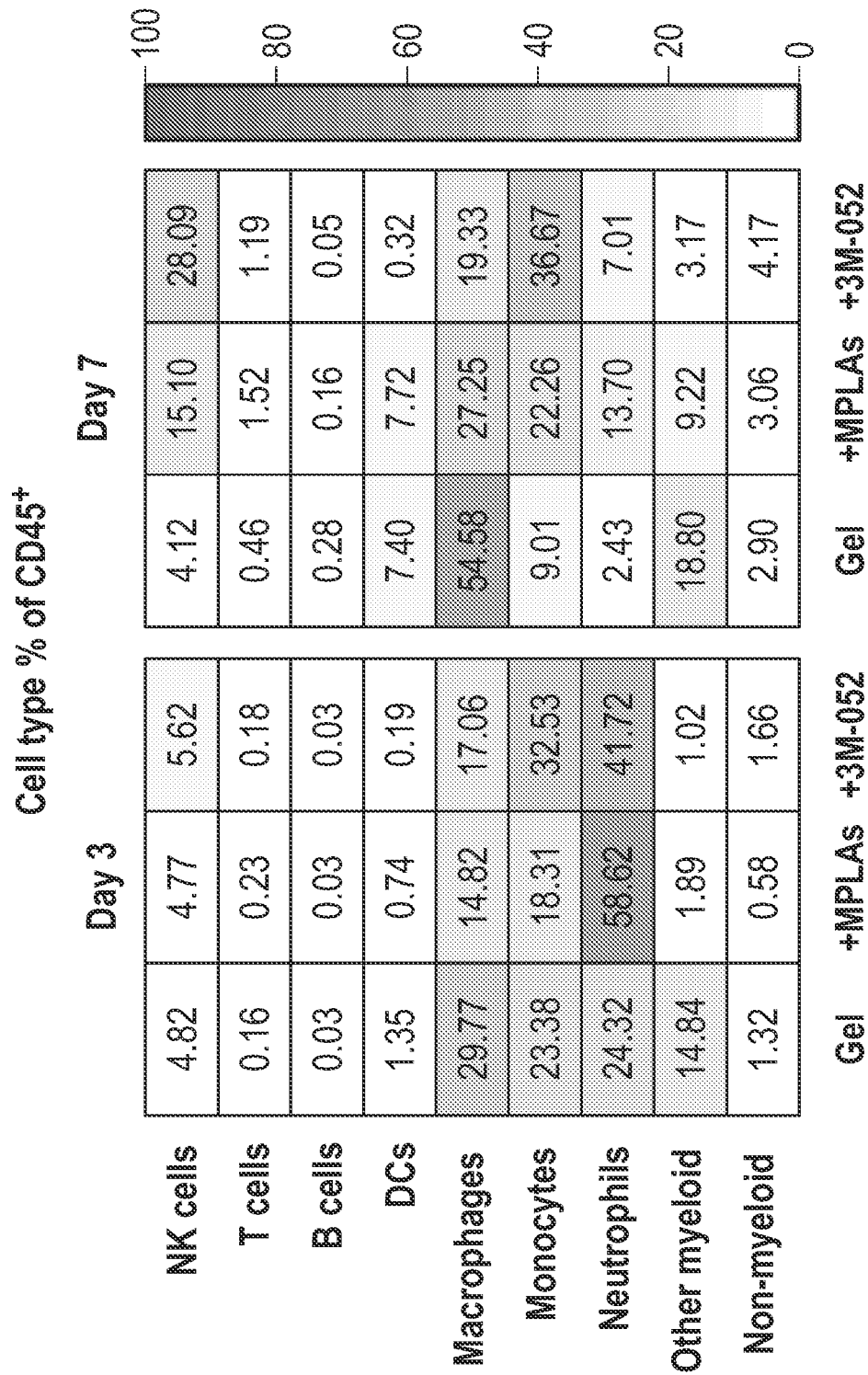


FIG. 7G

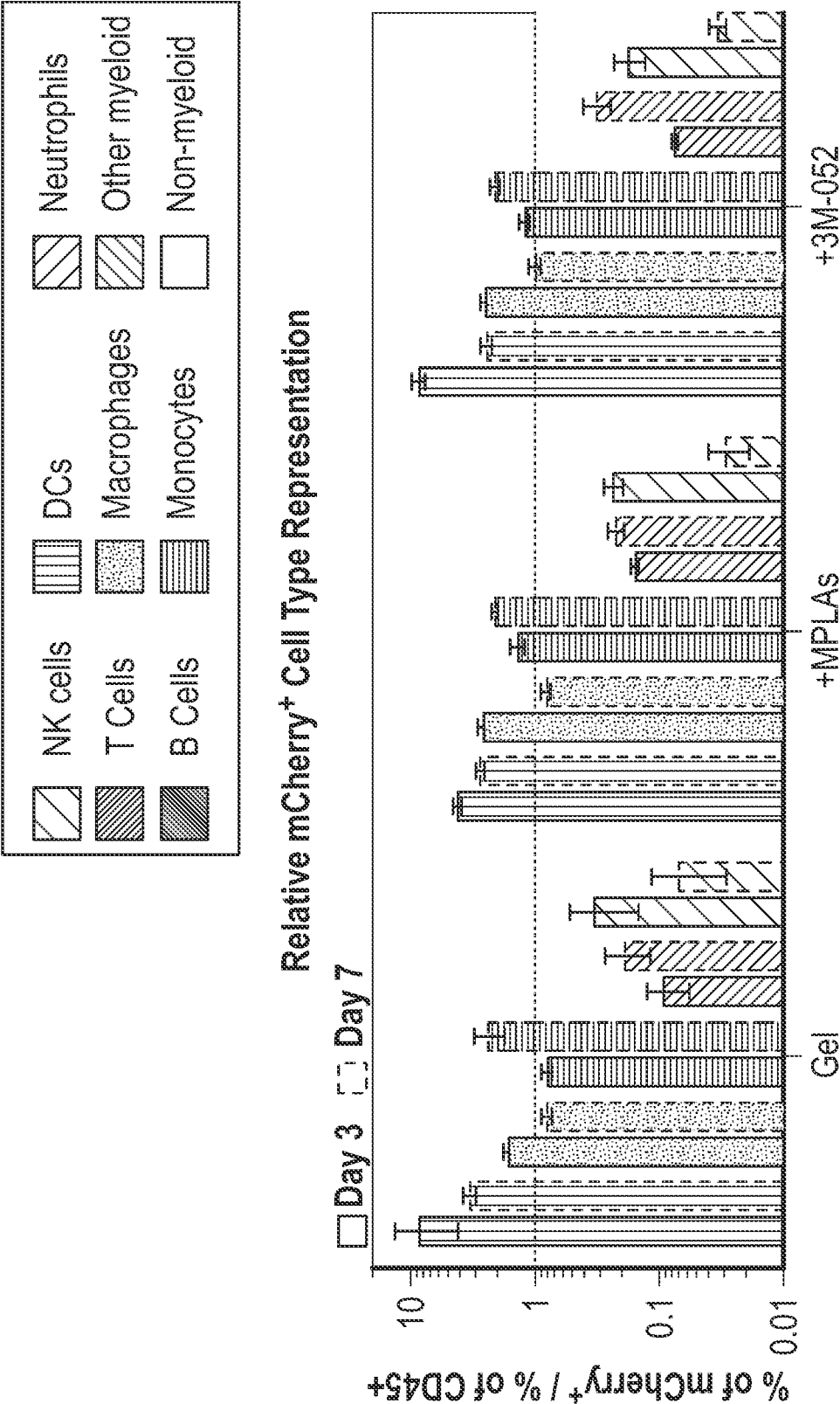
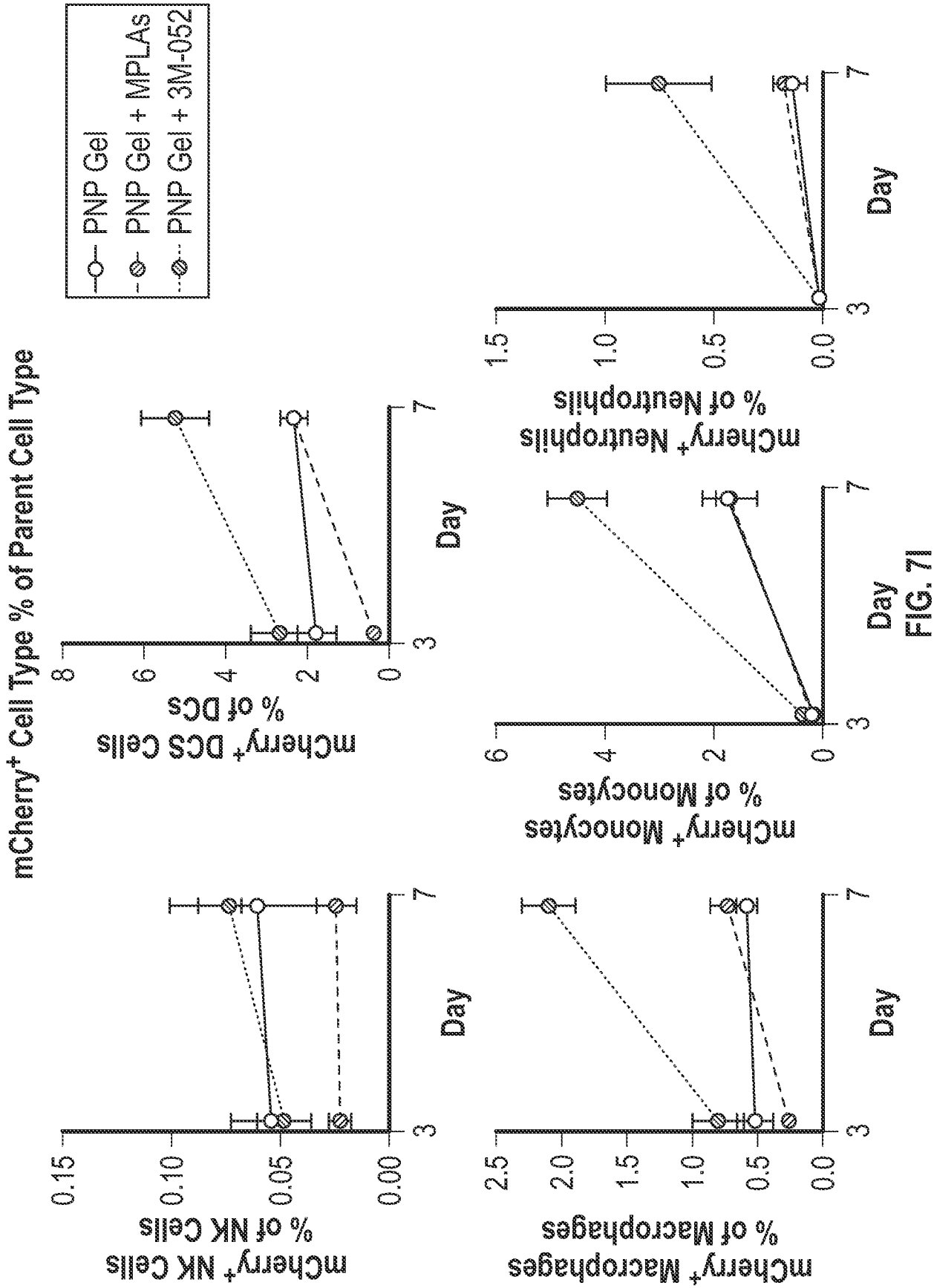
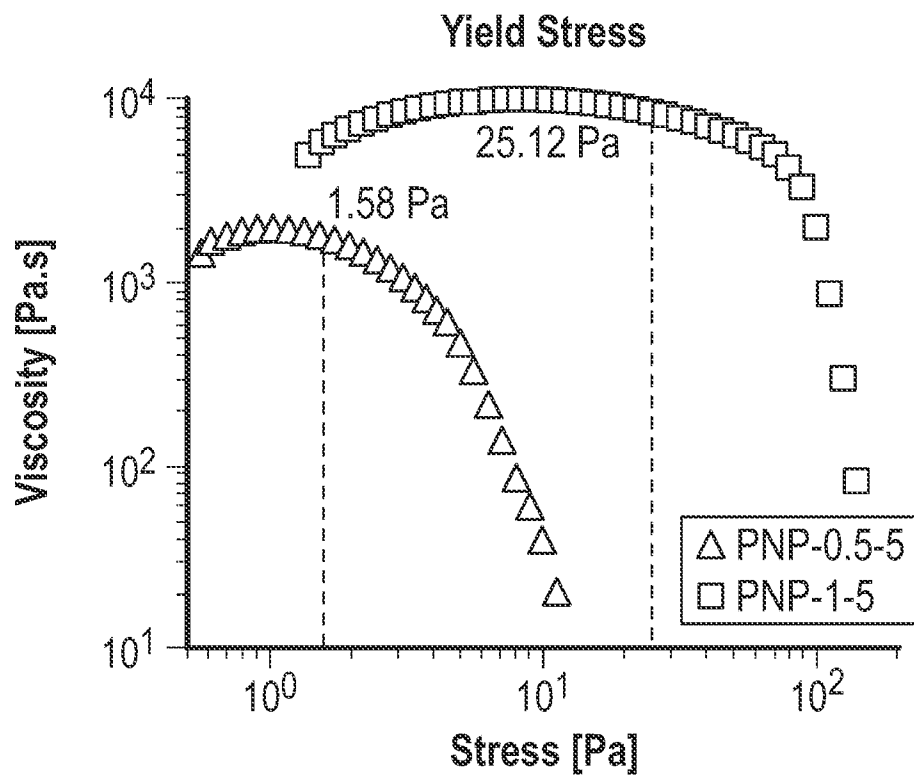
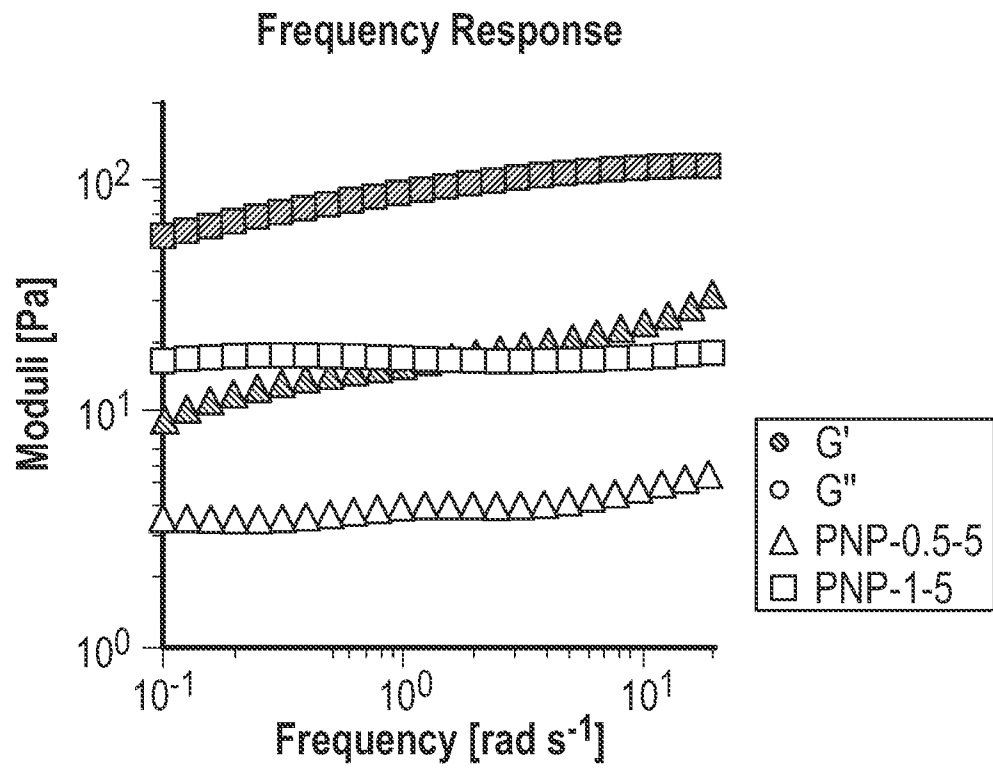


FIG. 7H



22/46



23/46

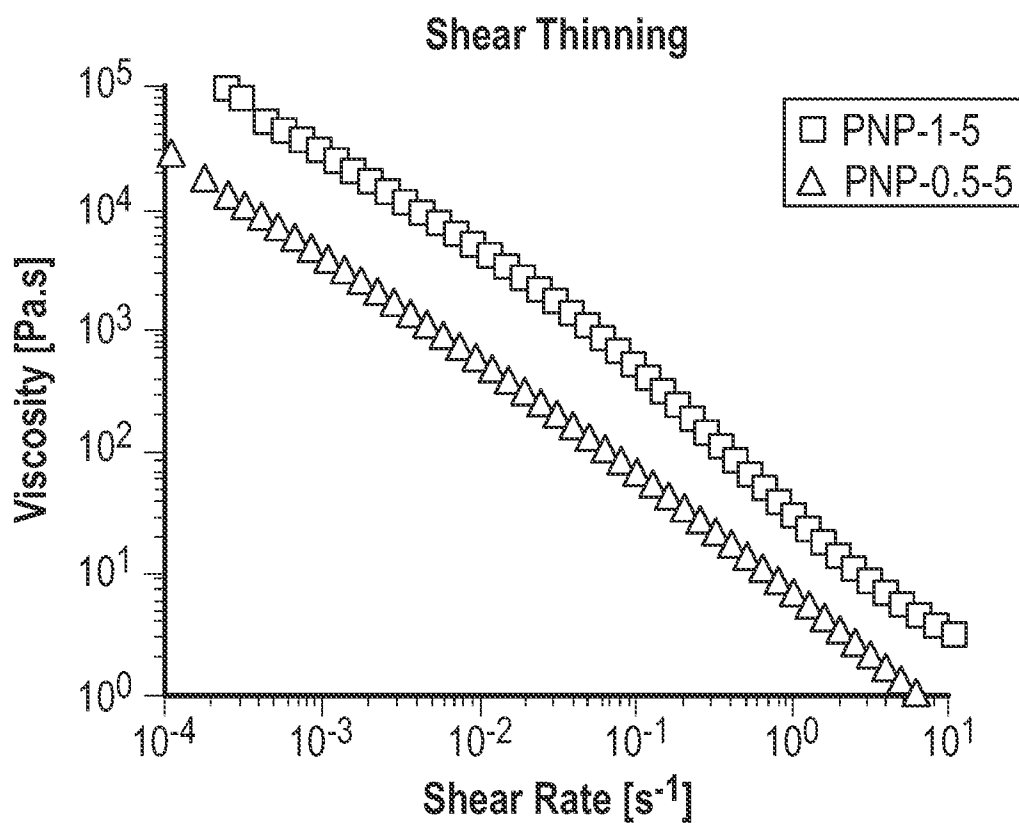


FIG. 8C

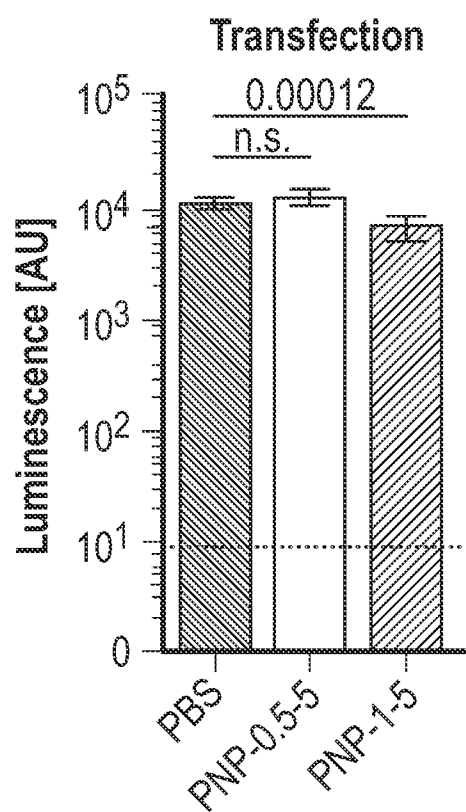


FIG. 8D

24/46

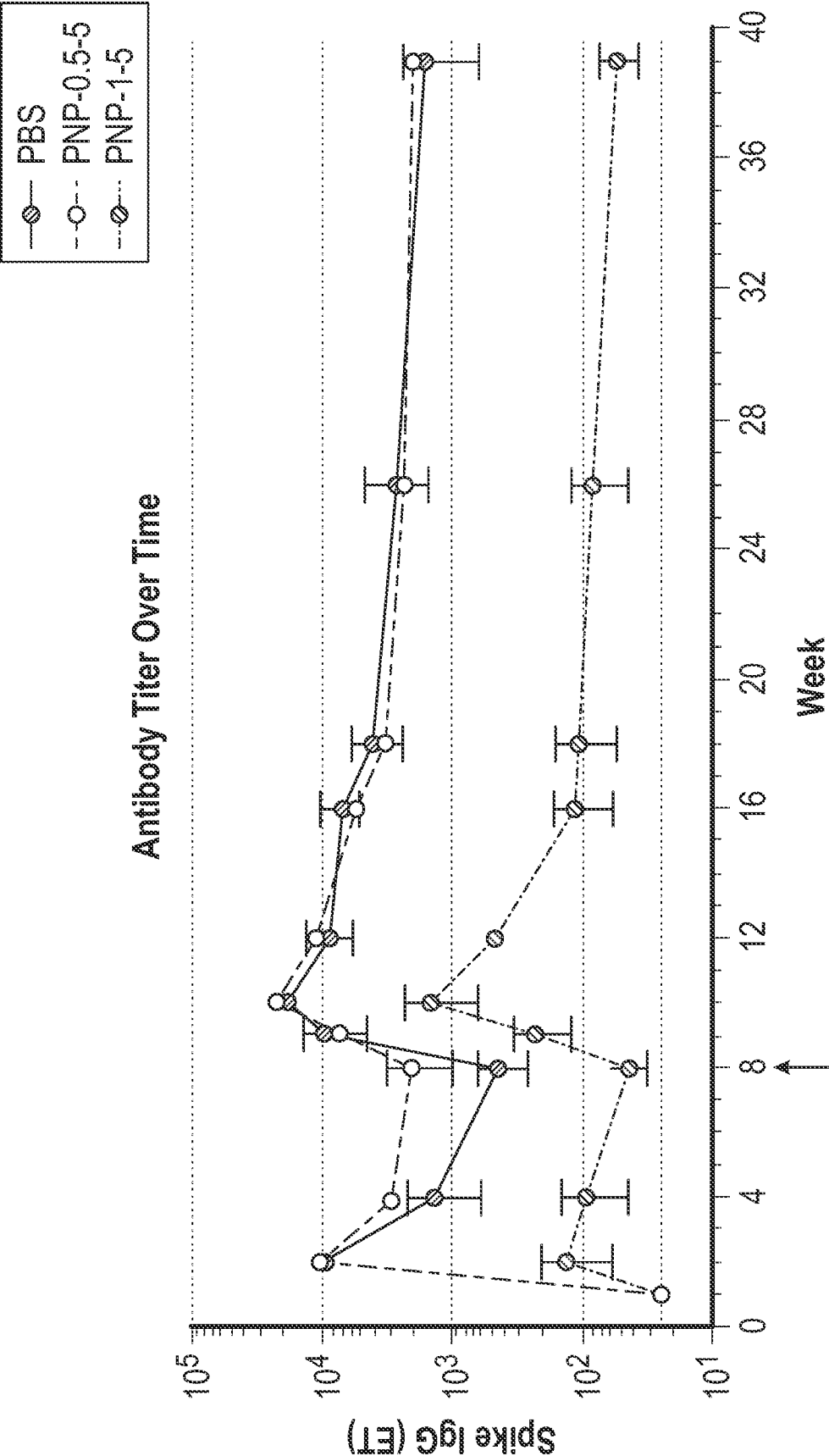


FIG. 8E

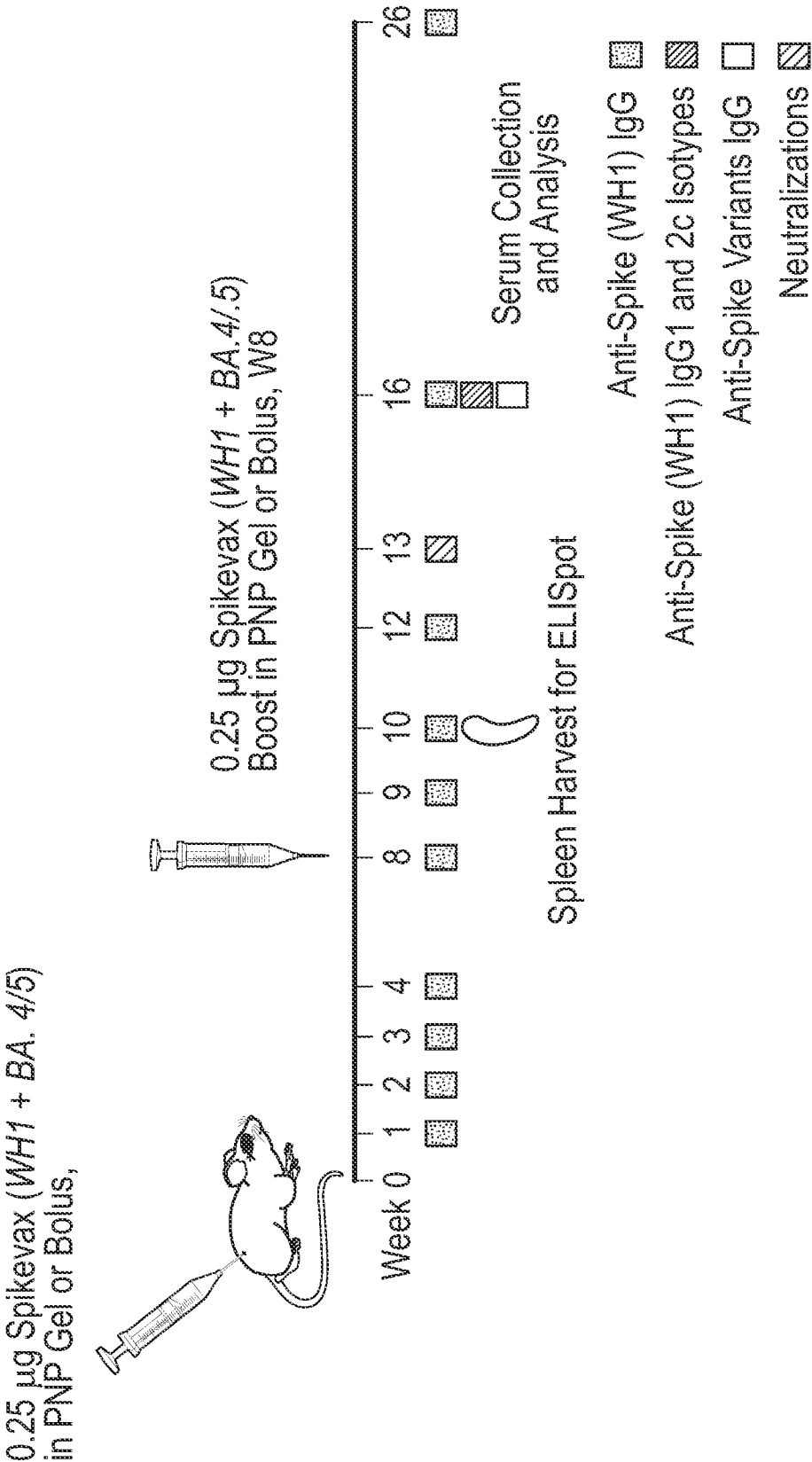


FIG. 9A

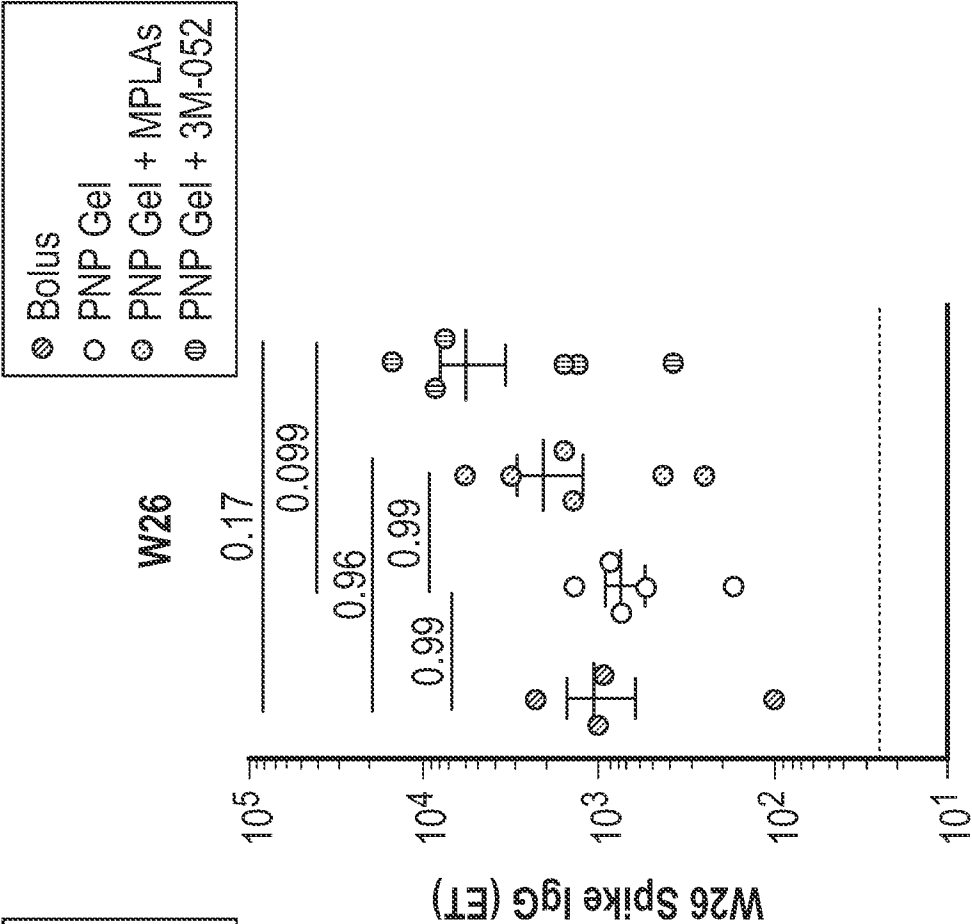


FIG. 9B

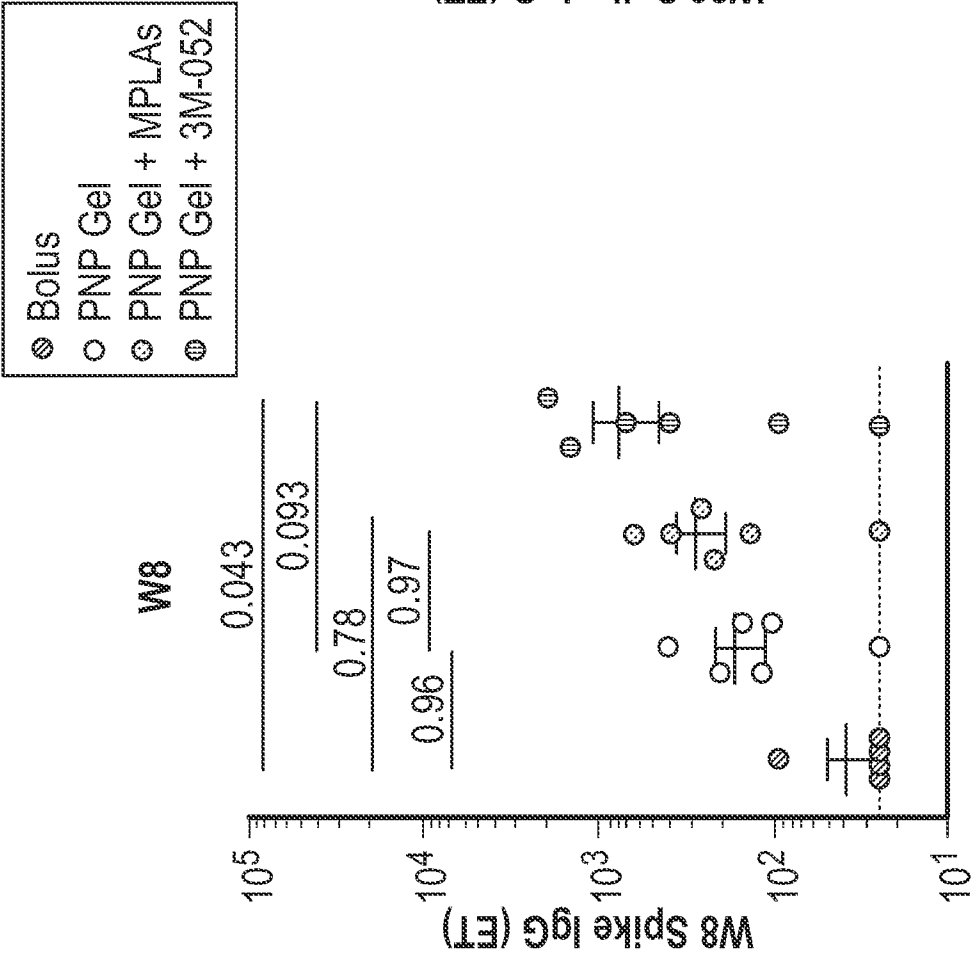
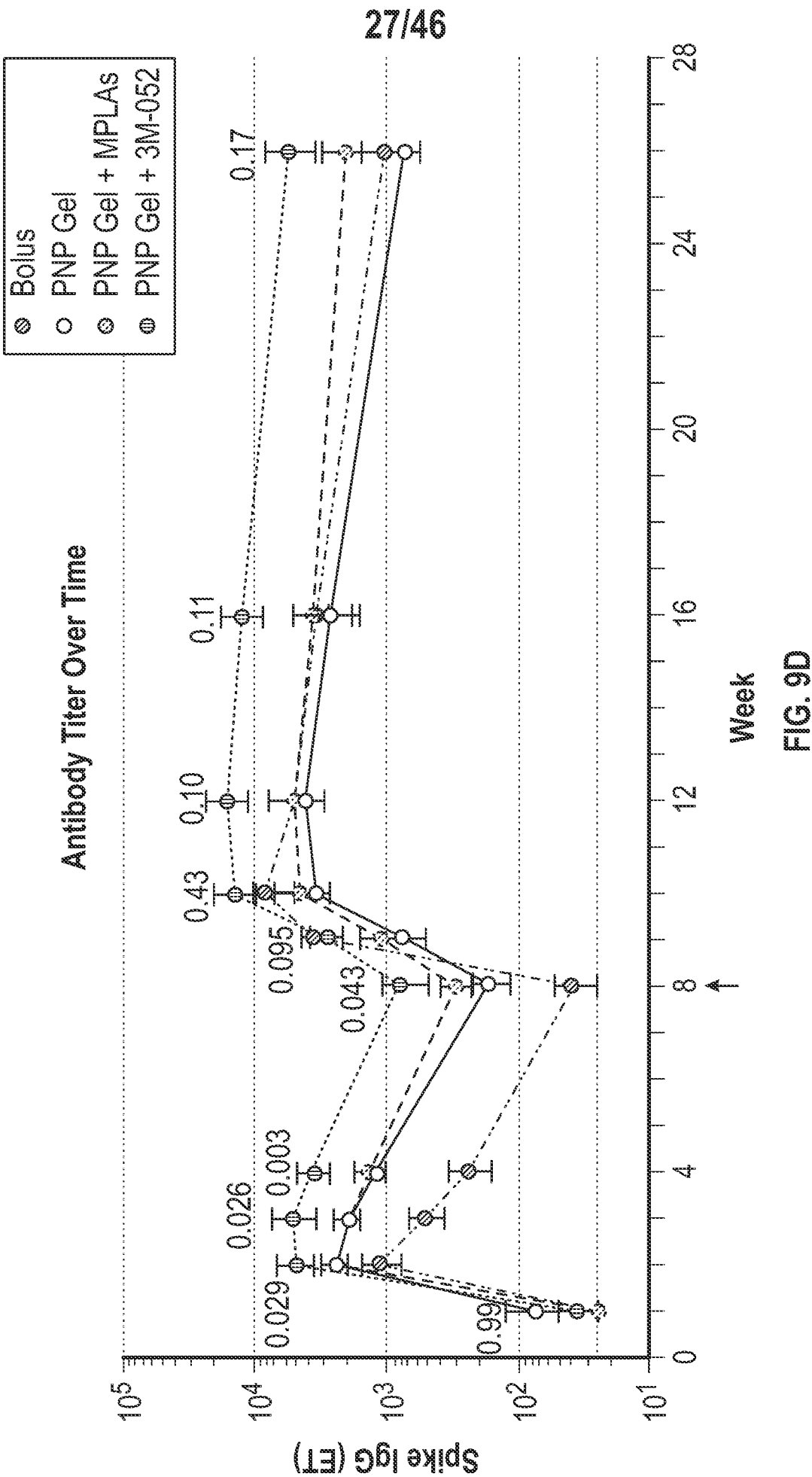


FIG. 9C



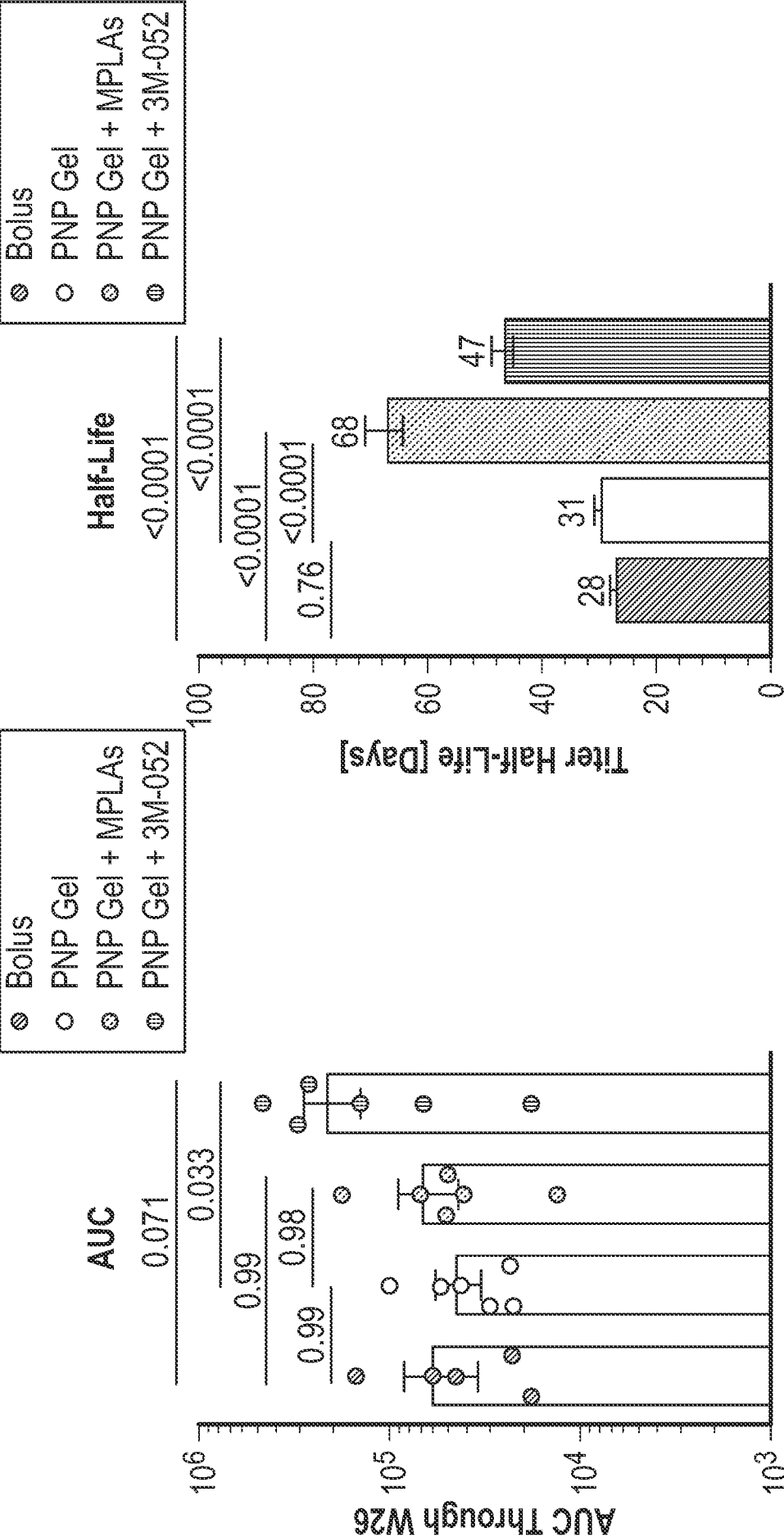


FIG. 9F

FIG. 9E

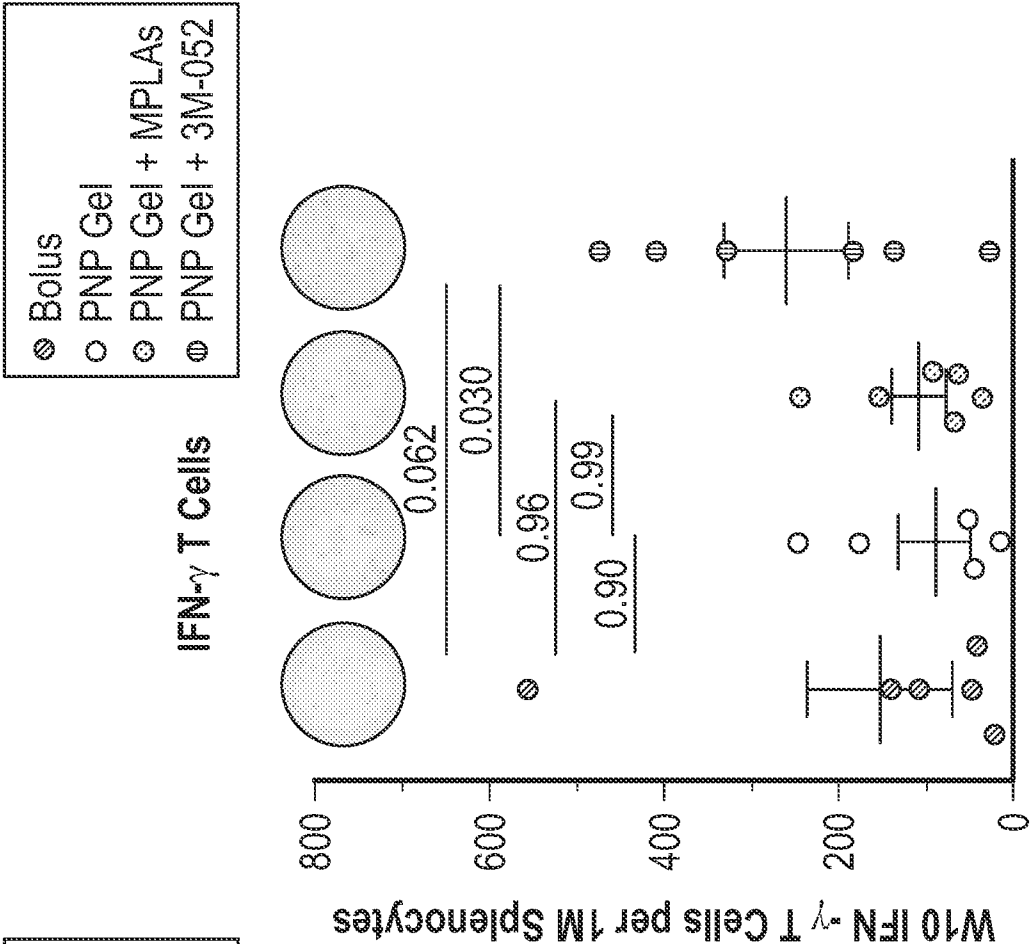


FIG. 9H

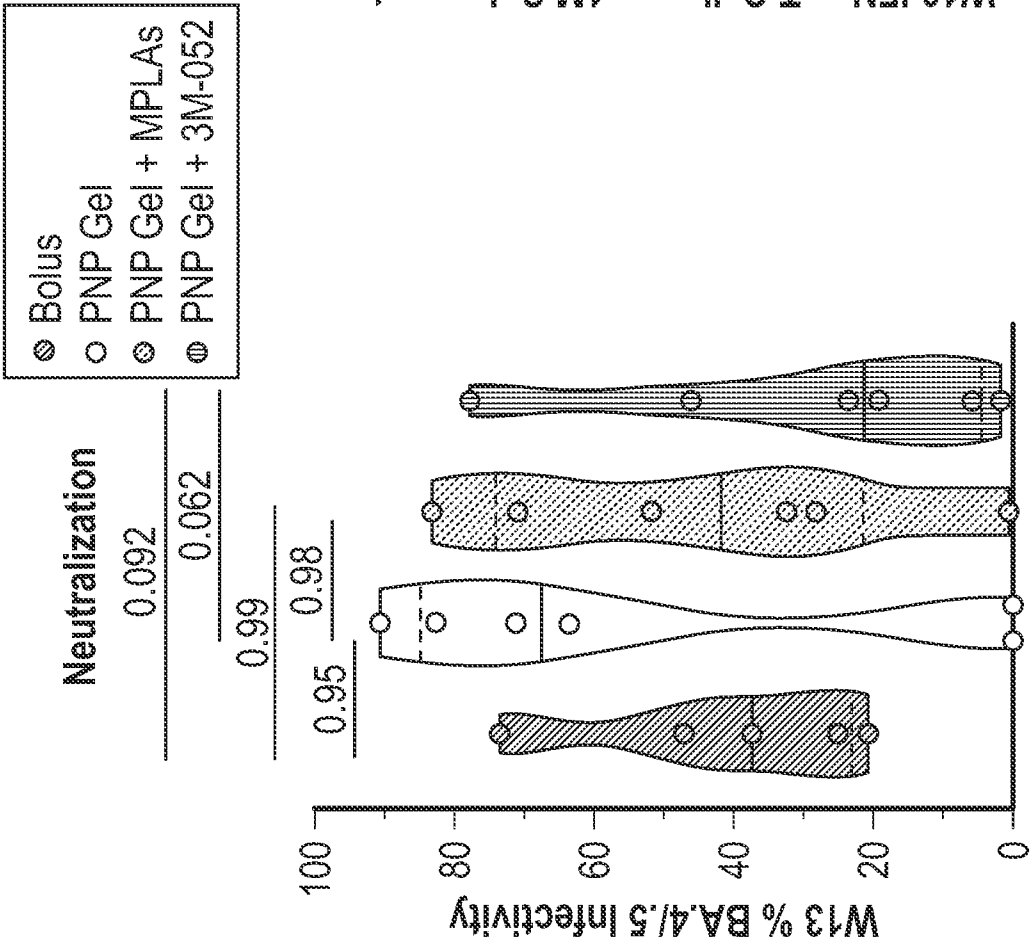


FIG. 9G

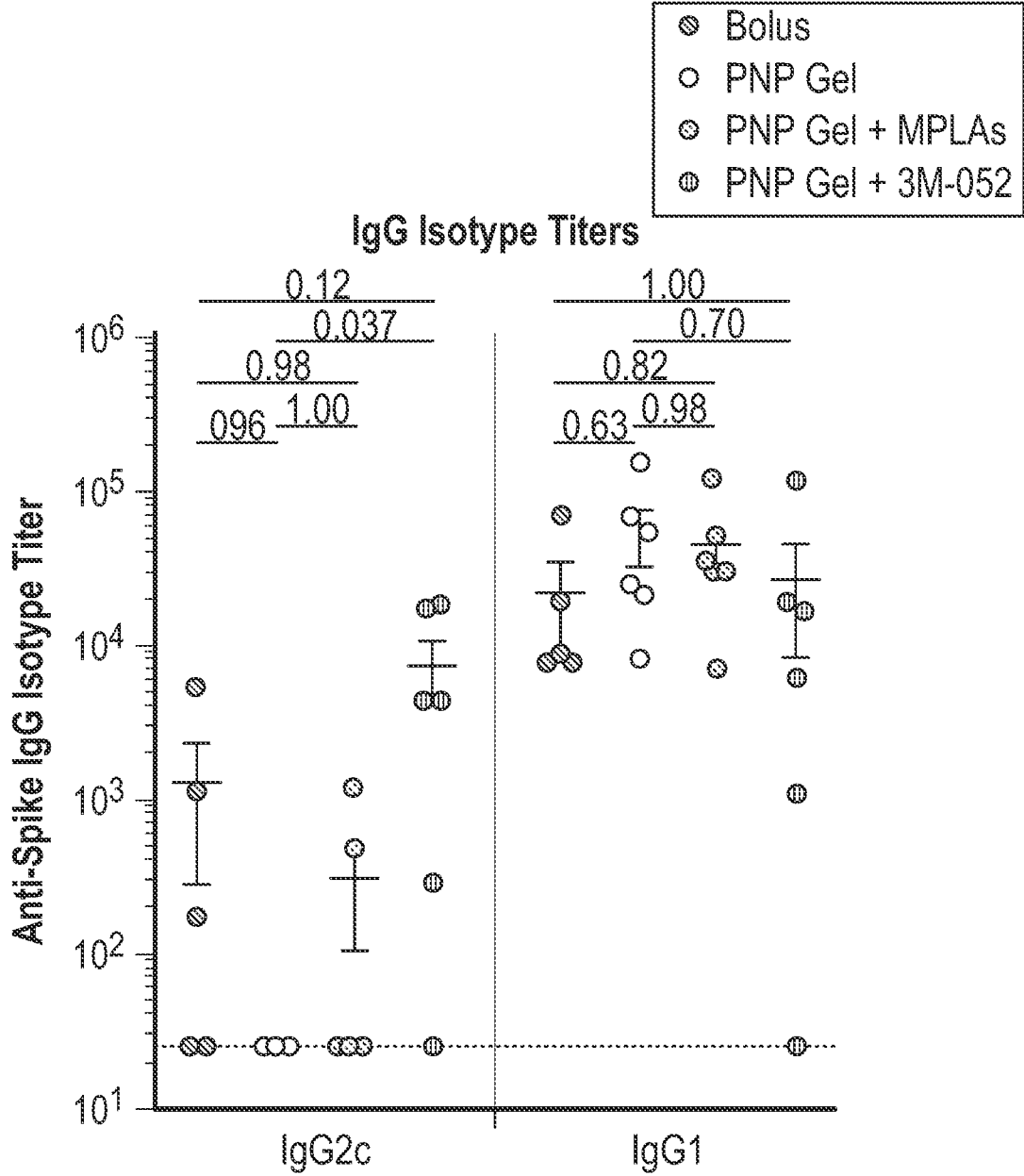


FIG. 10A

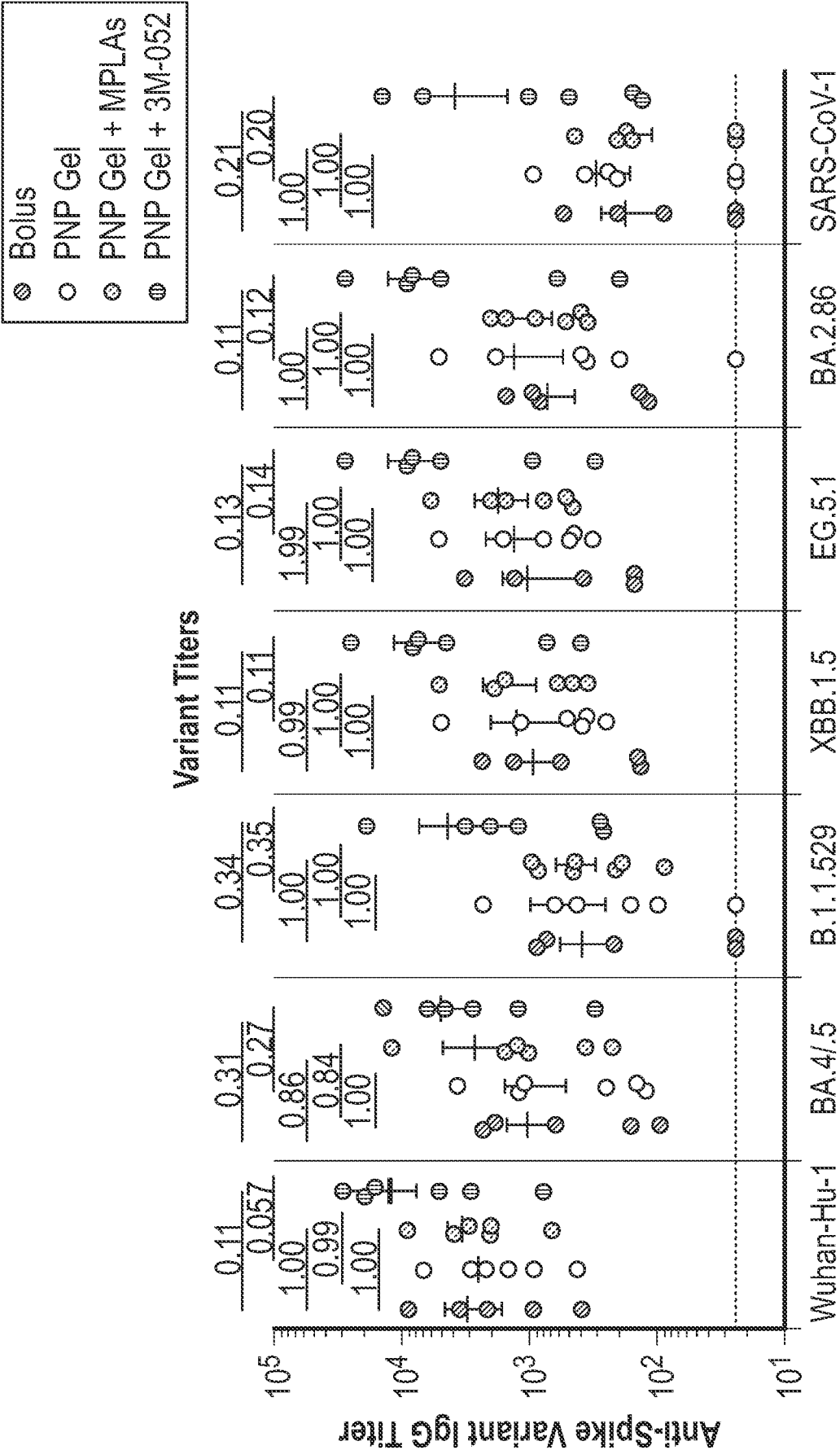


FIG. 10B

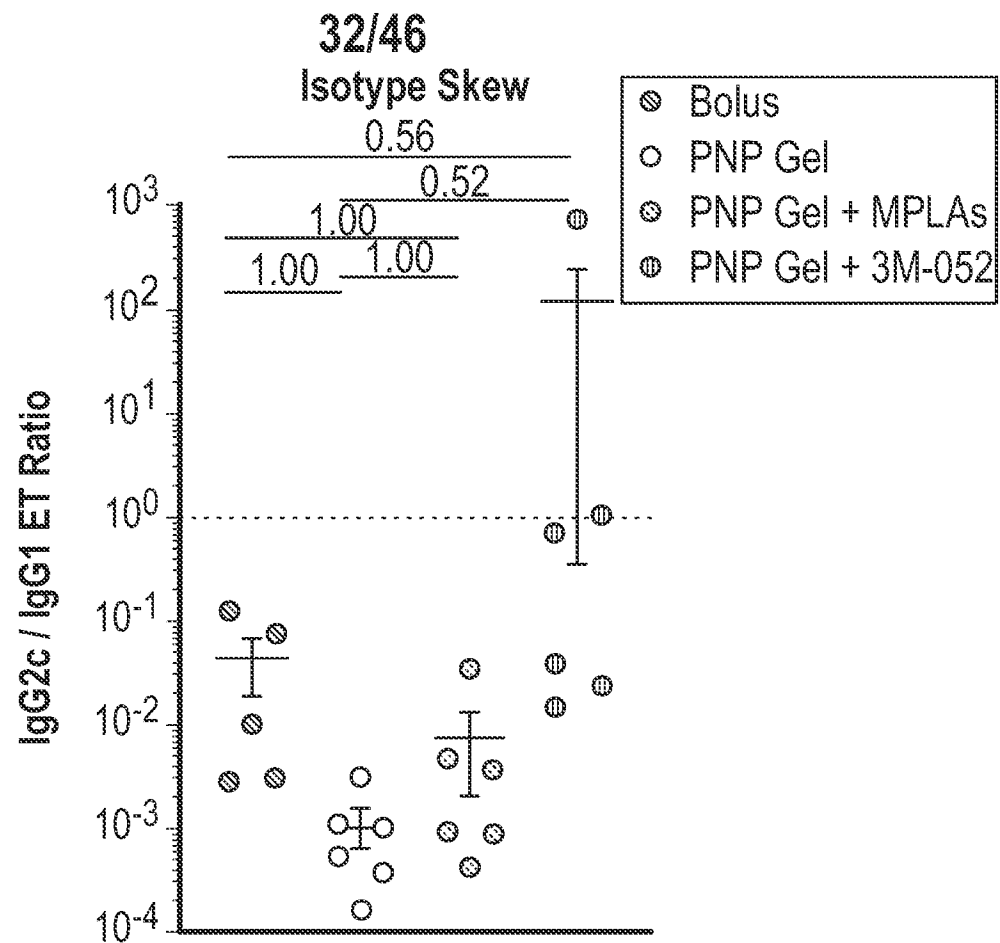


FIG. 10C

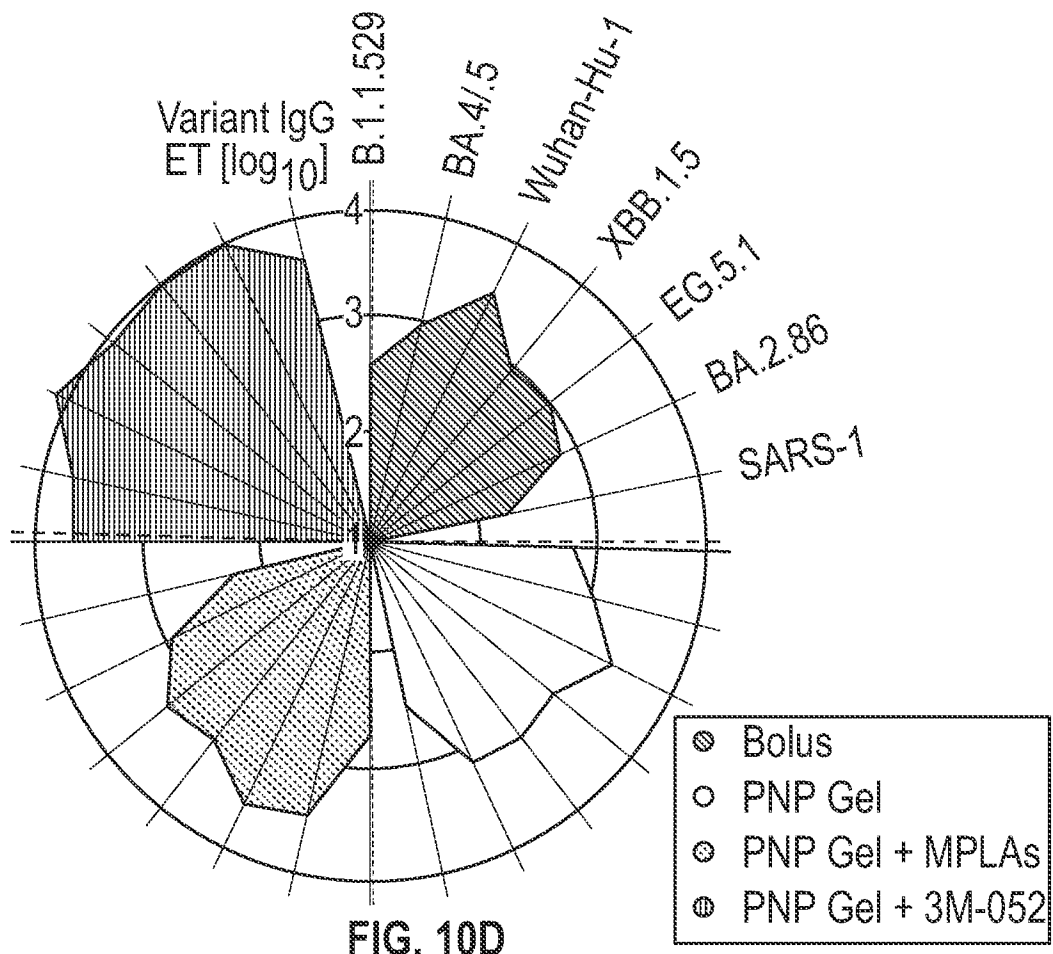


FIG. 10D

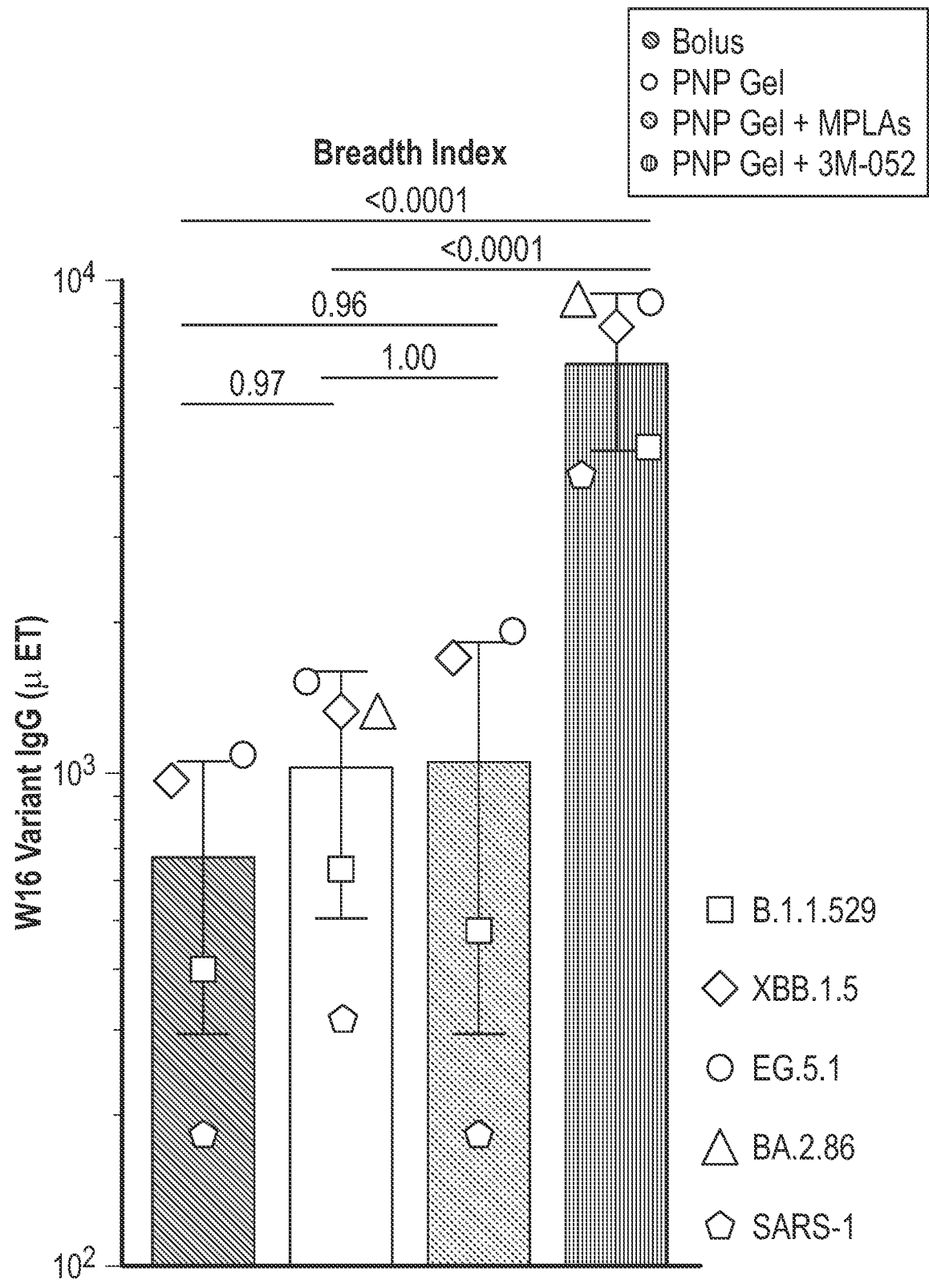


FIG. 10E

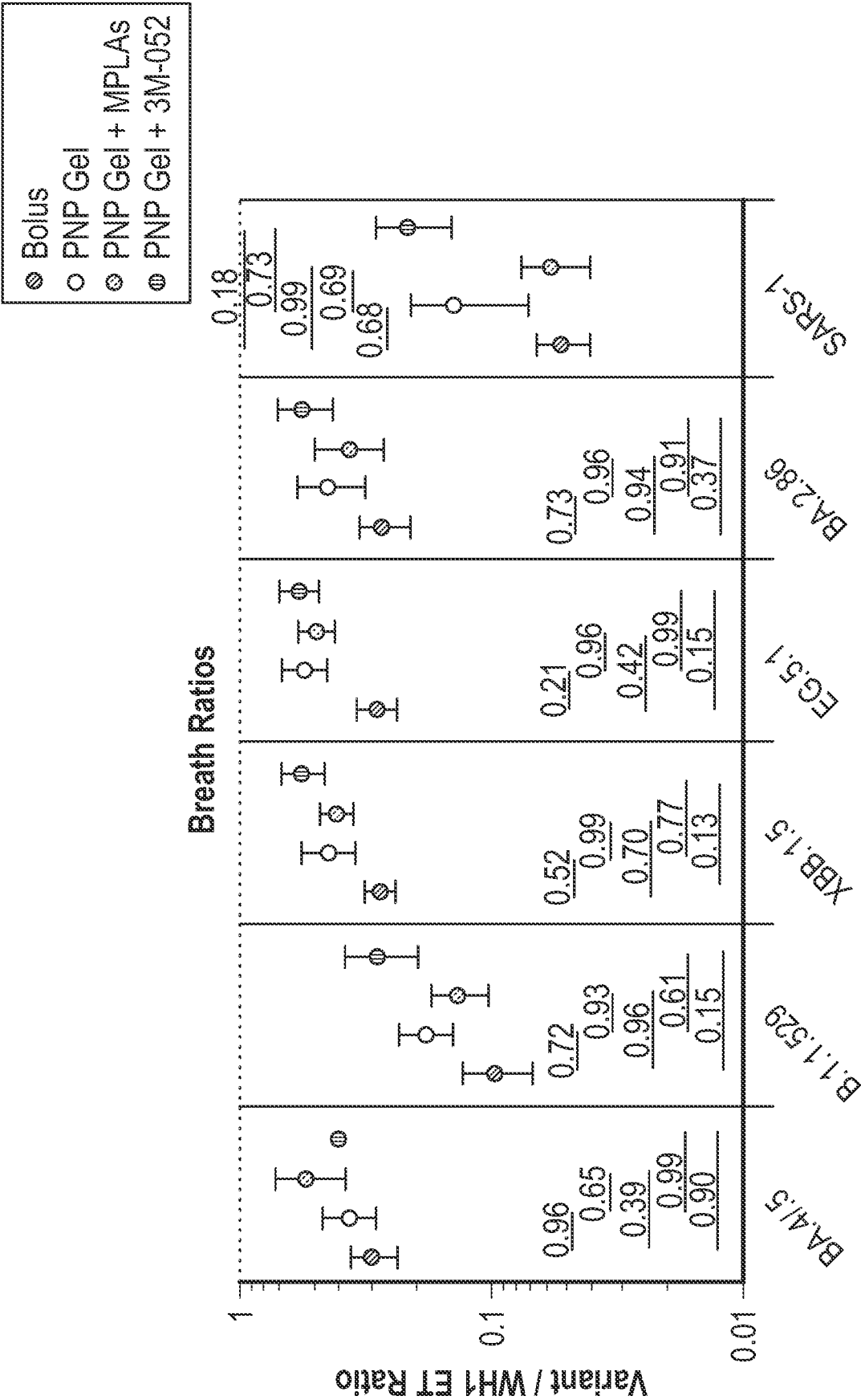


FIG. 10F

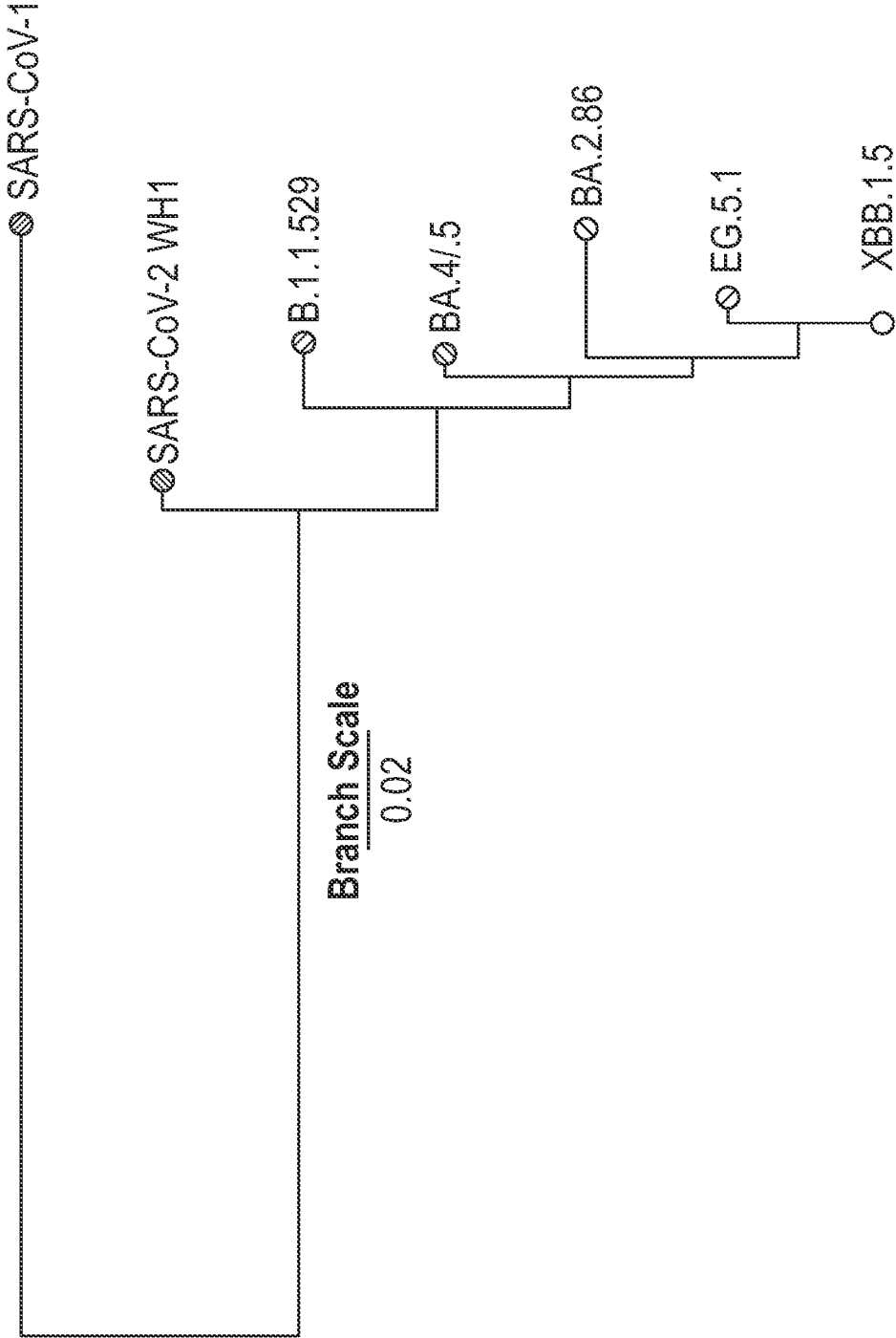


FIG. 10G

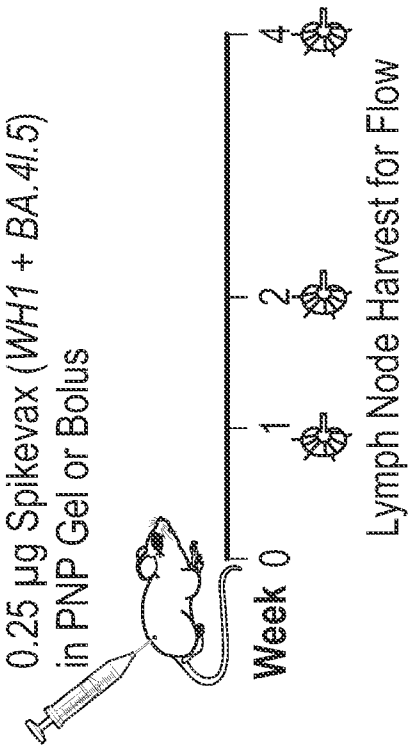


FIG. 11A

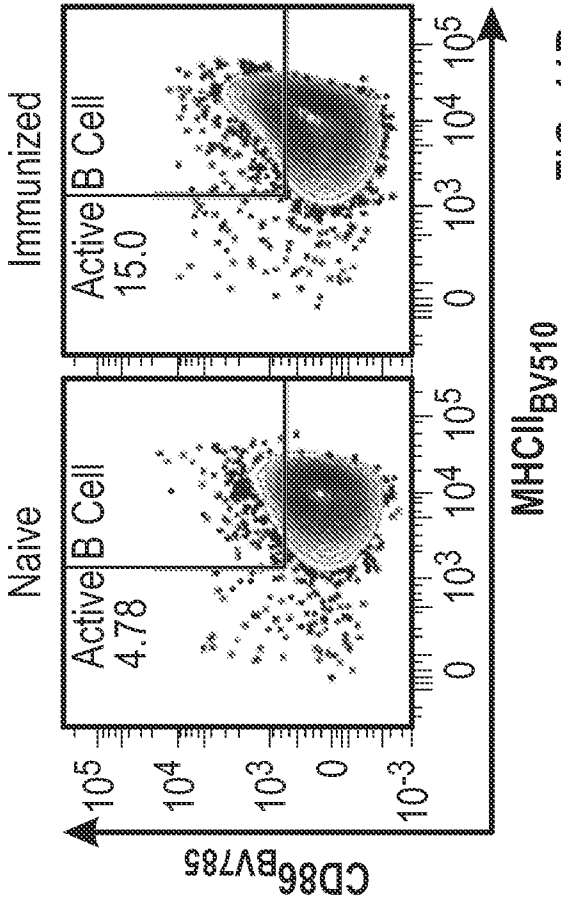
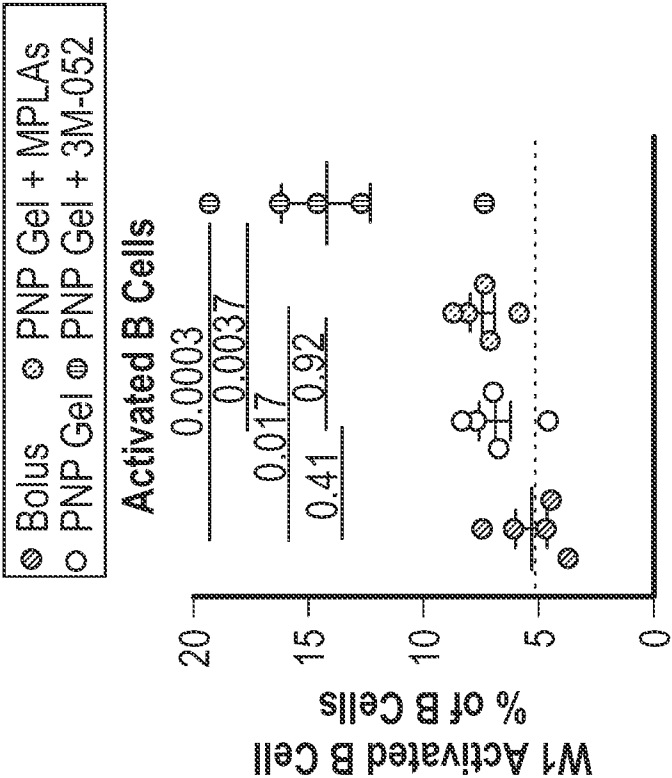


FIG. 11B



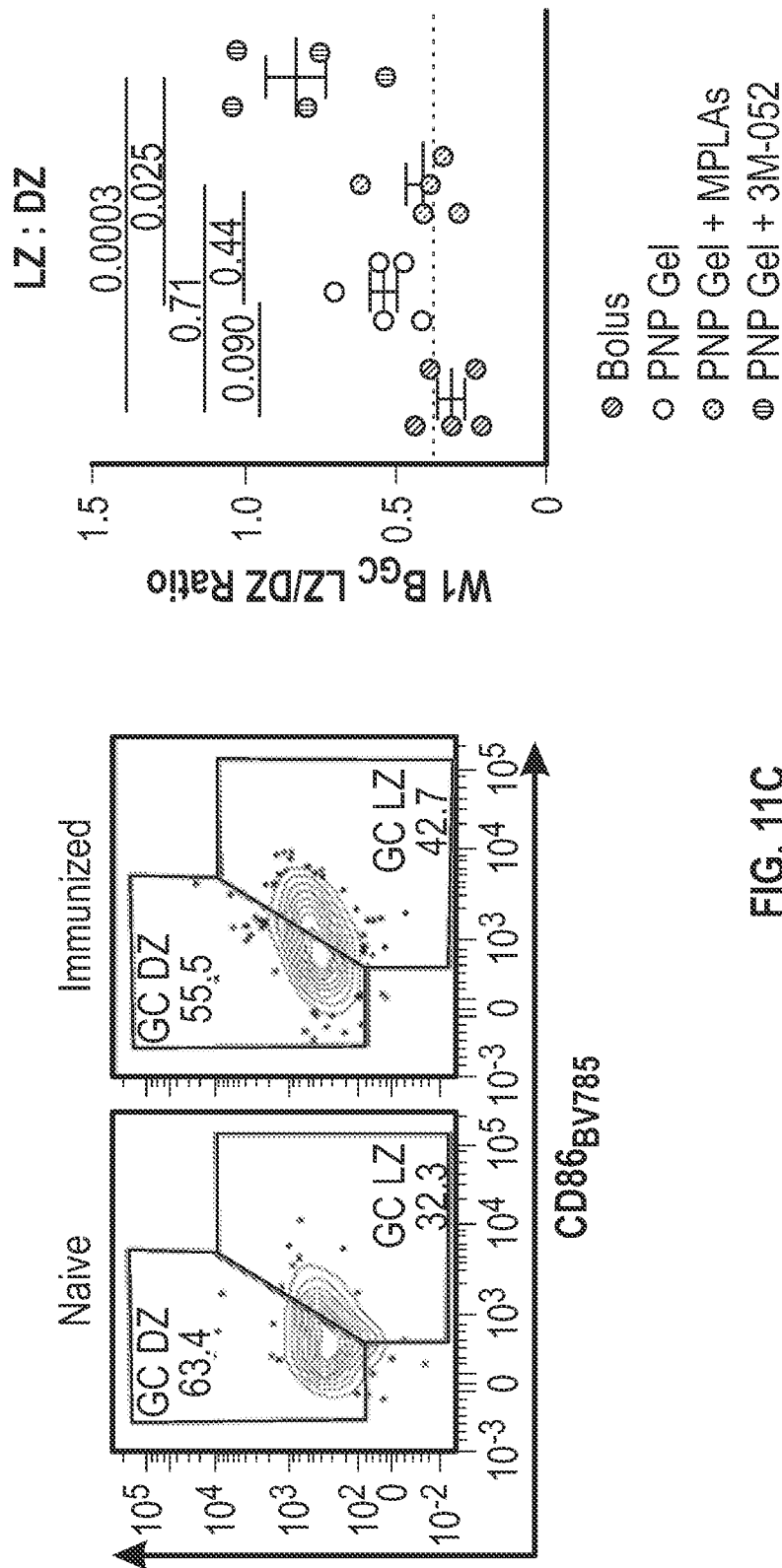


FIG. 11C

38/46

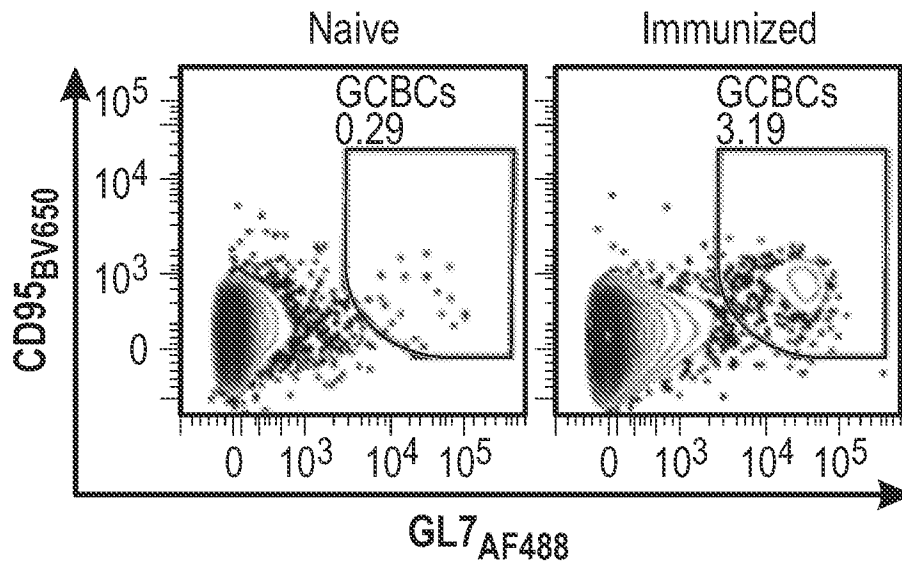


FIG. 11D

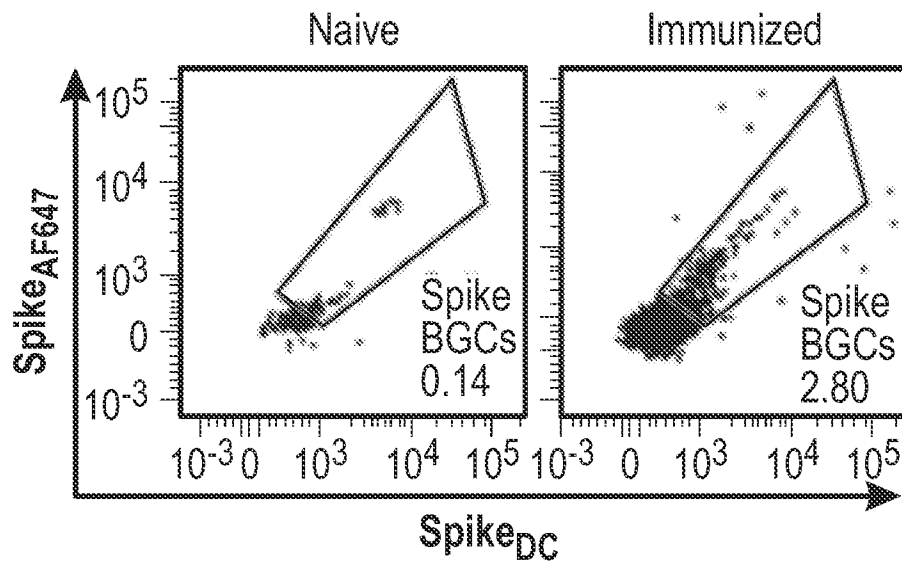


FIG. 11E

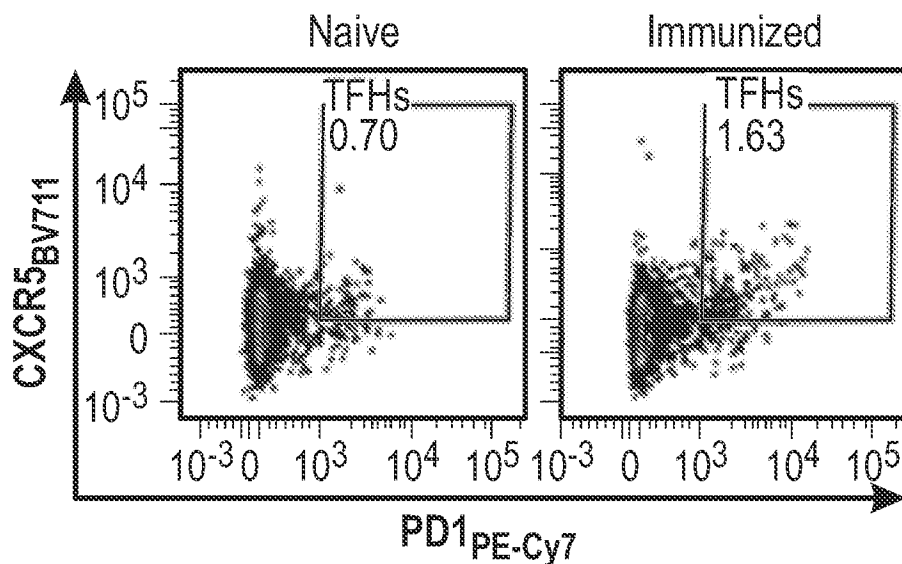


FIG. 11F

39/46

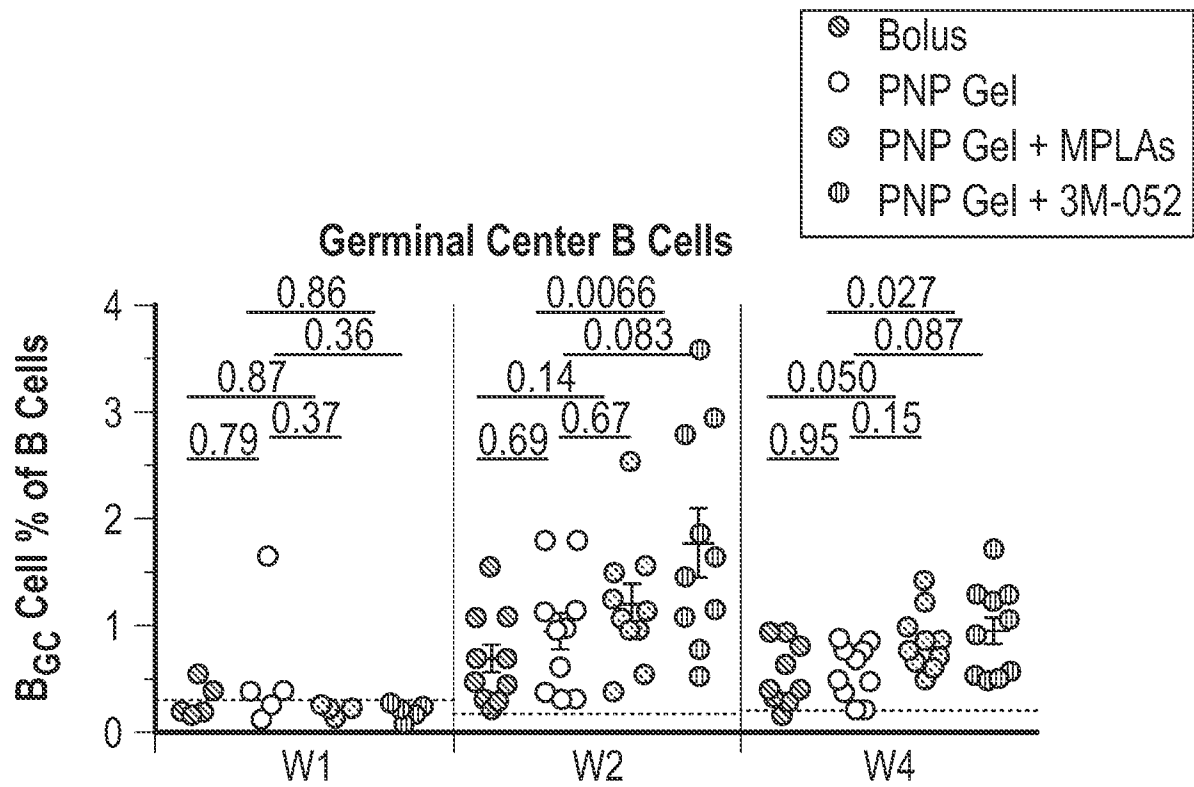


FIG. 11G

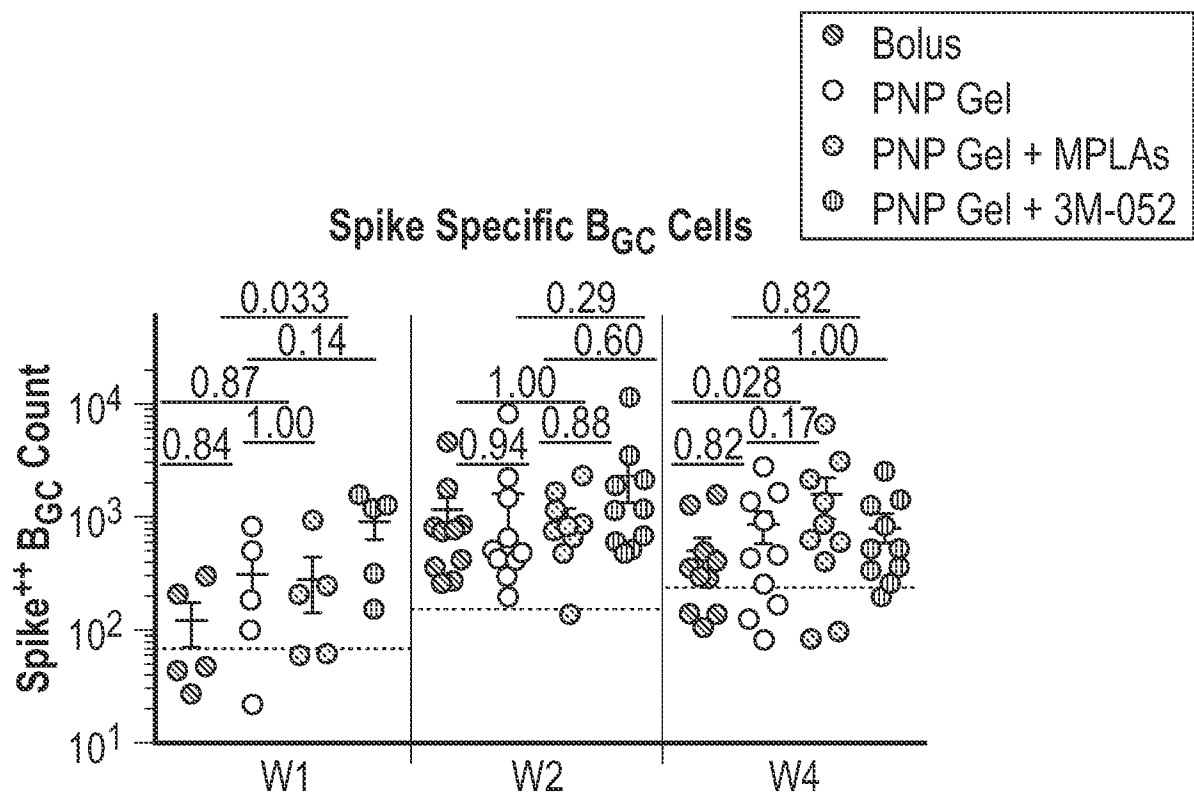


FIG. 11H

40/46

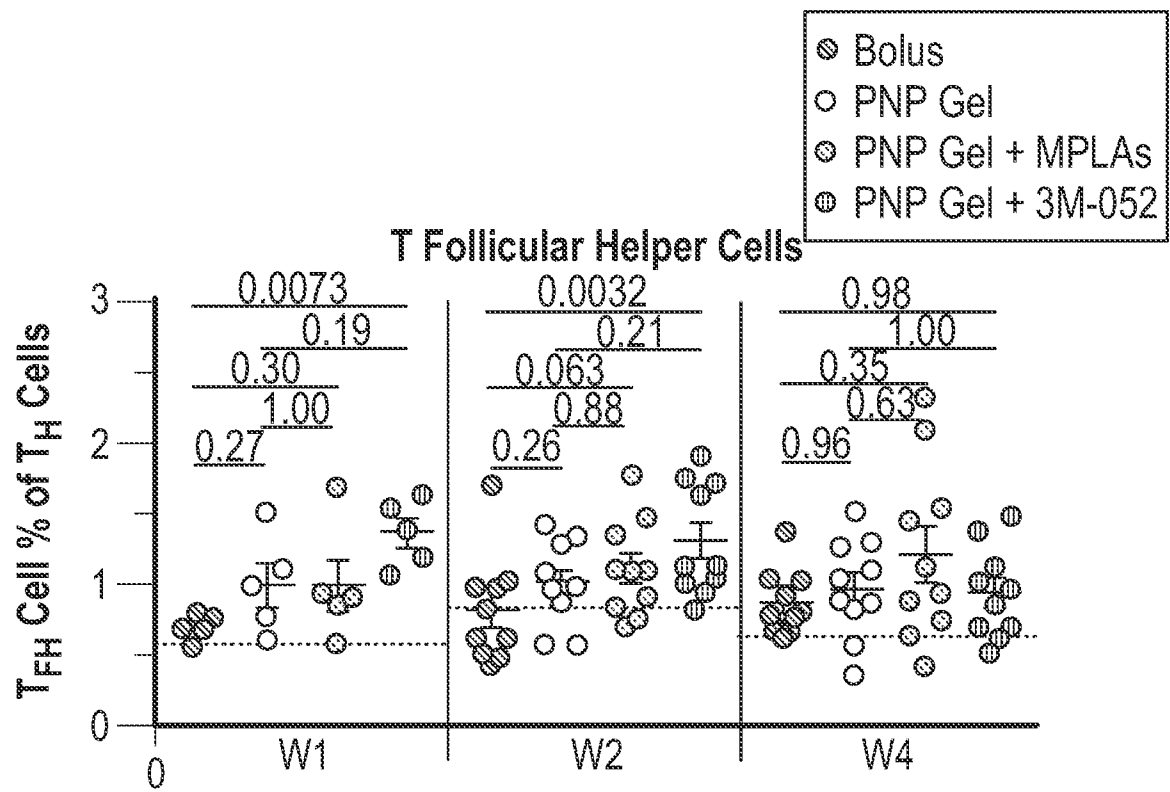


FIG. 11I

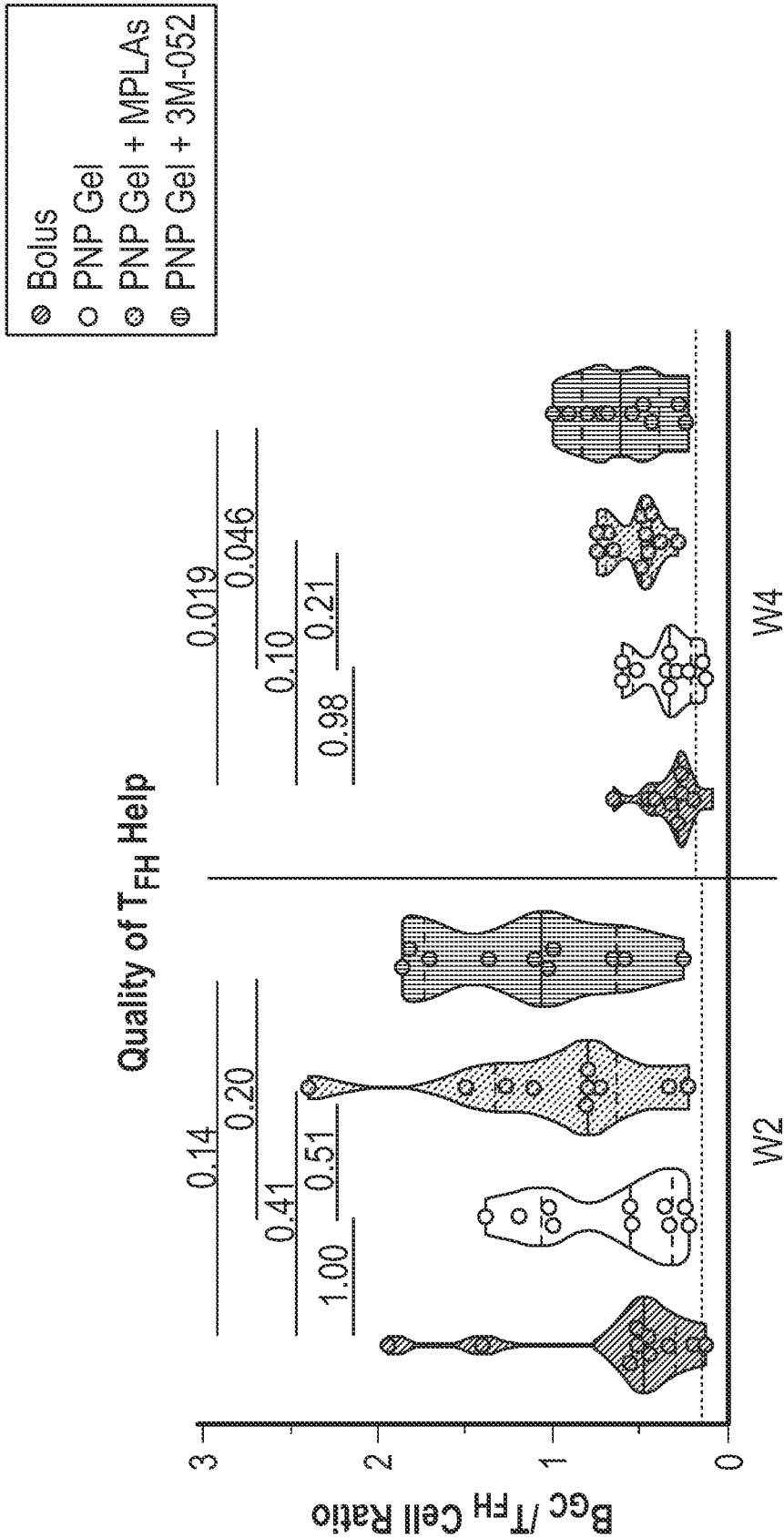


FIG. 11J

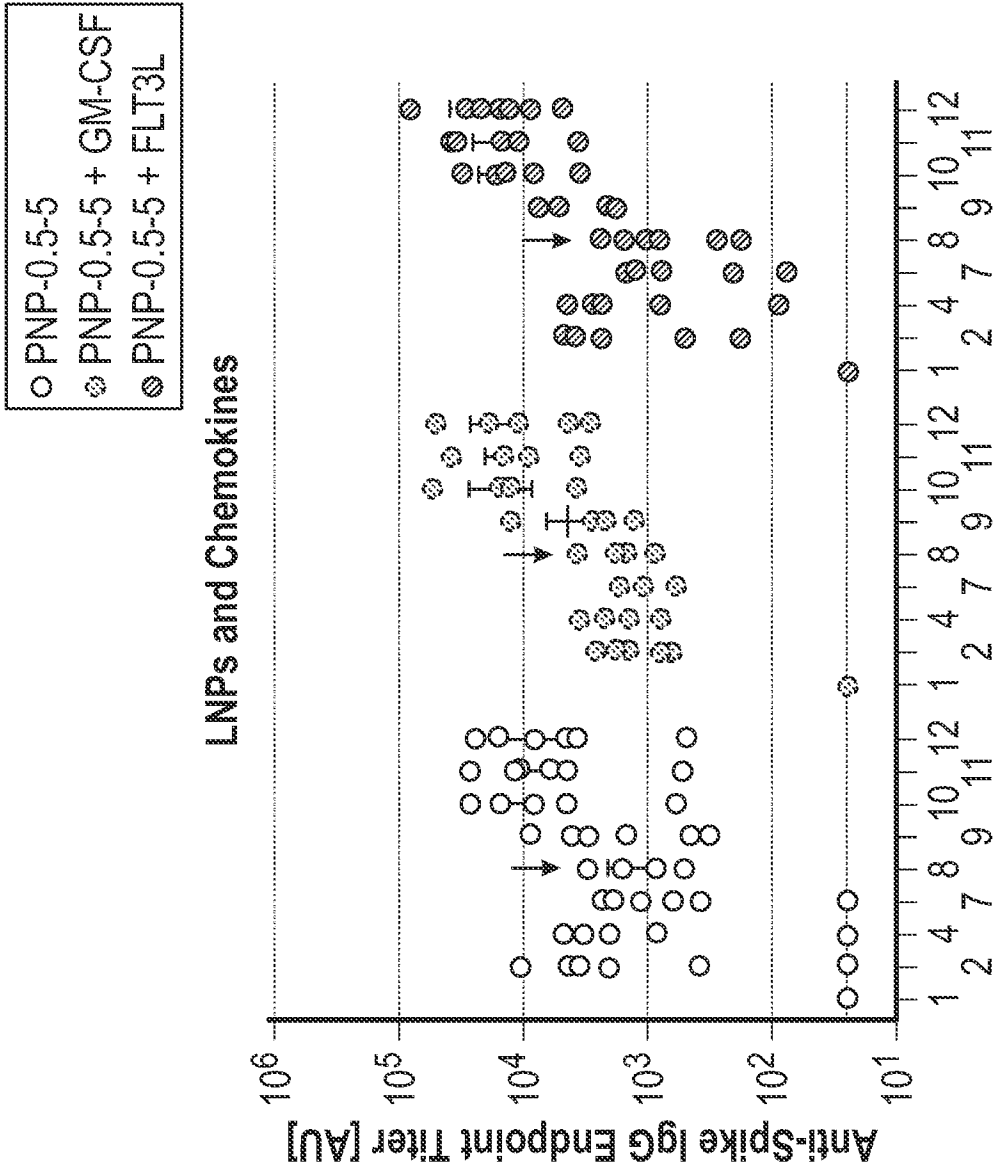


FIG. 12A

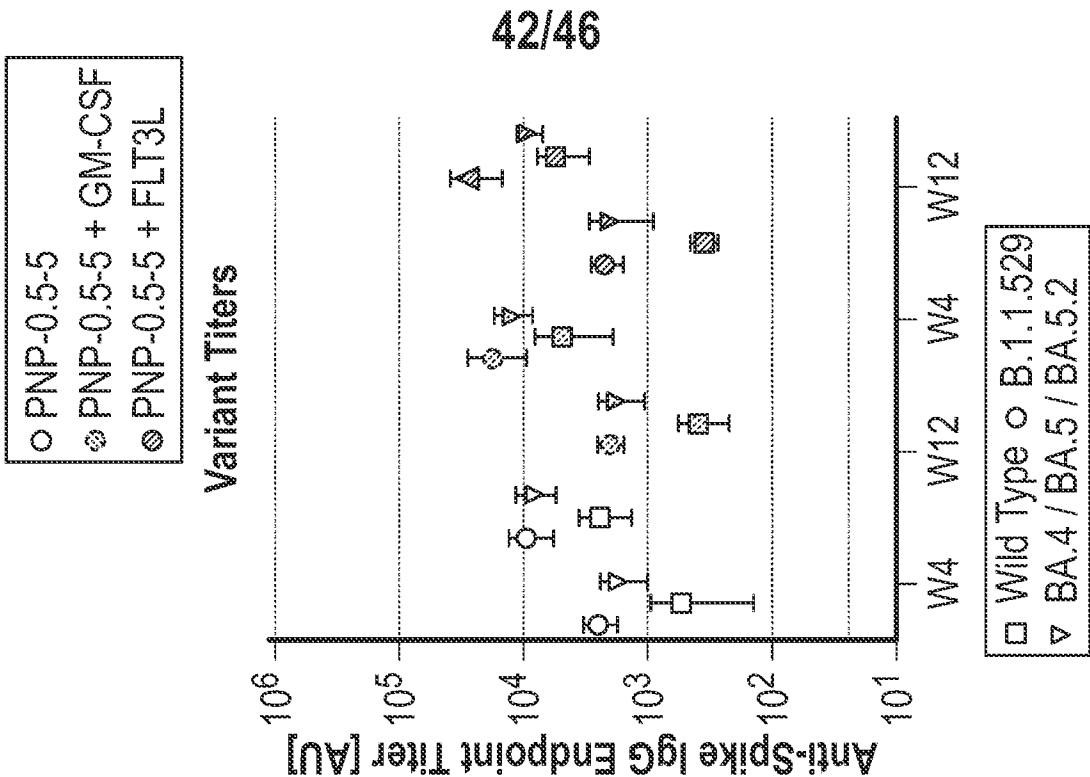


FIG. 12B

CD45+ Leukocyte Composition - Chemokines

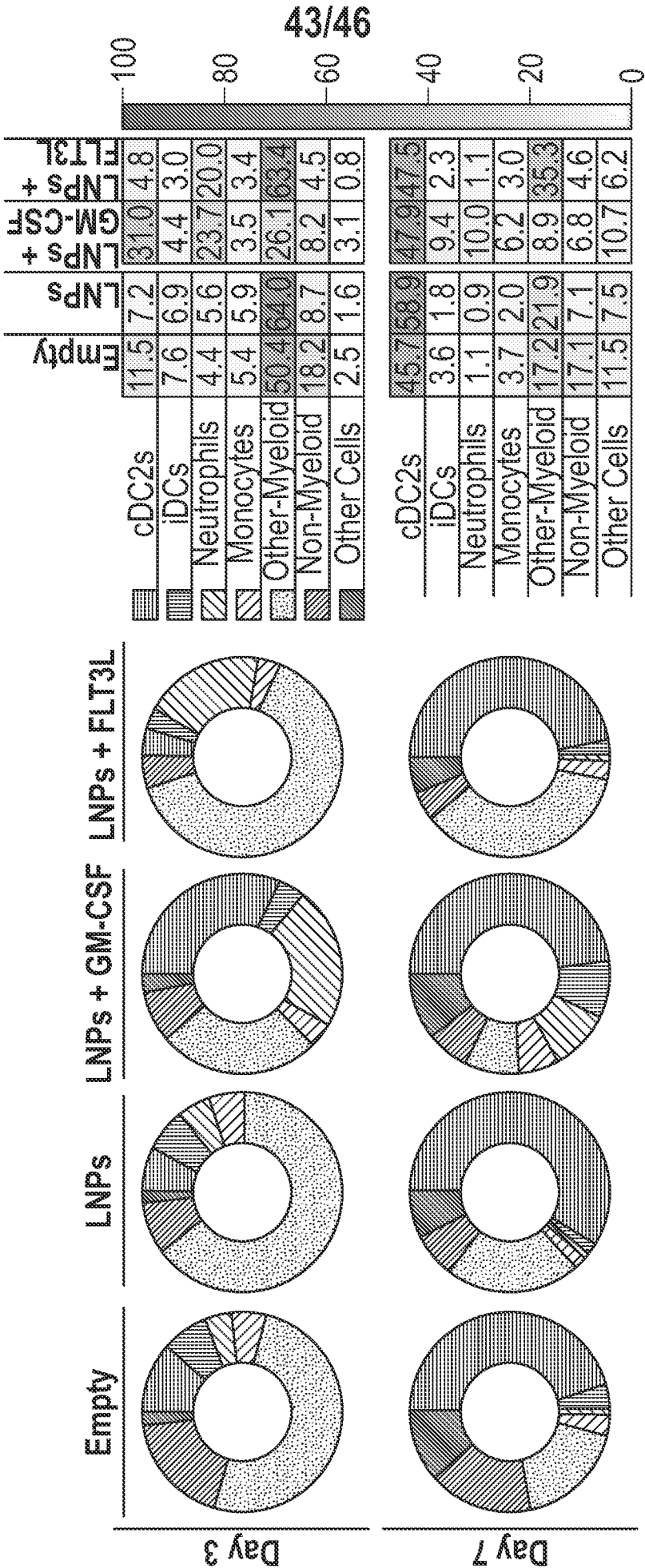


FIG. 13A

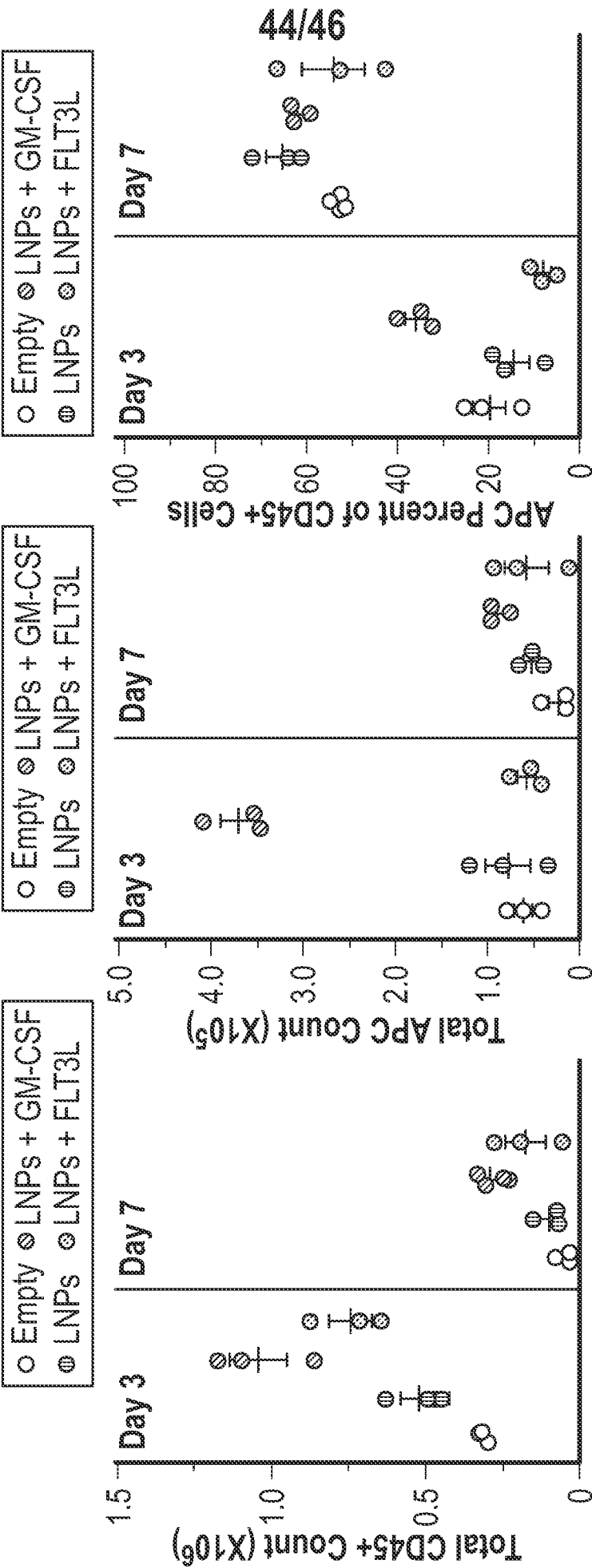
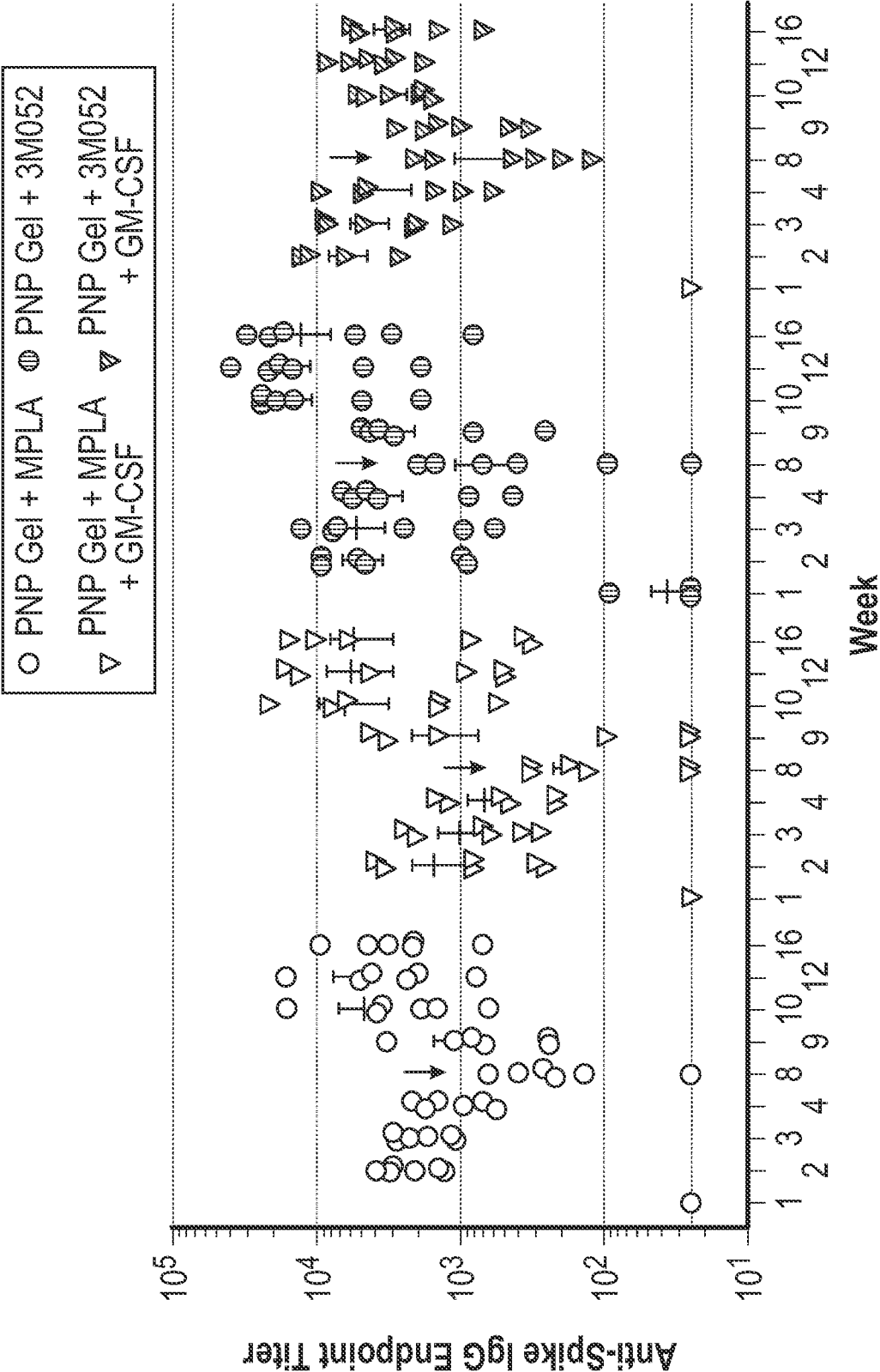


FIG. 13D

FIG. 13C

FIG. 13B



46/46

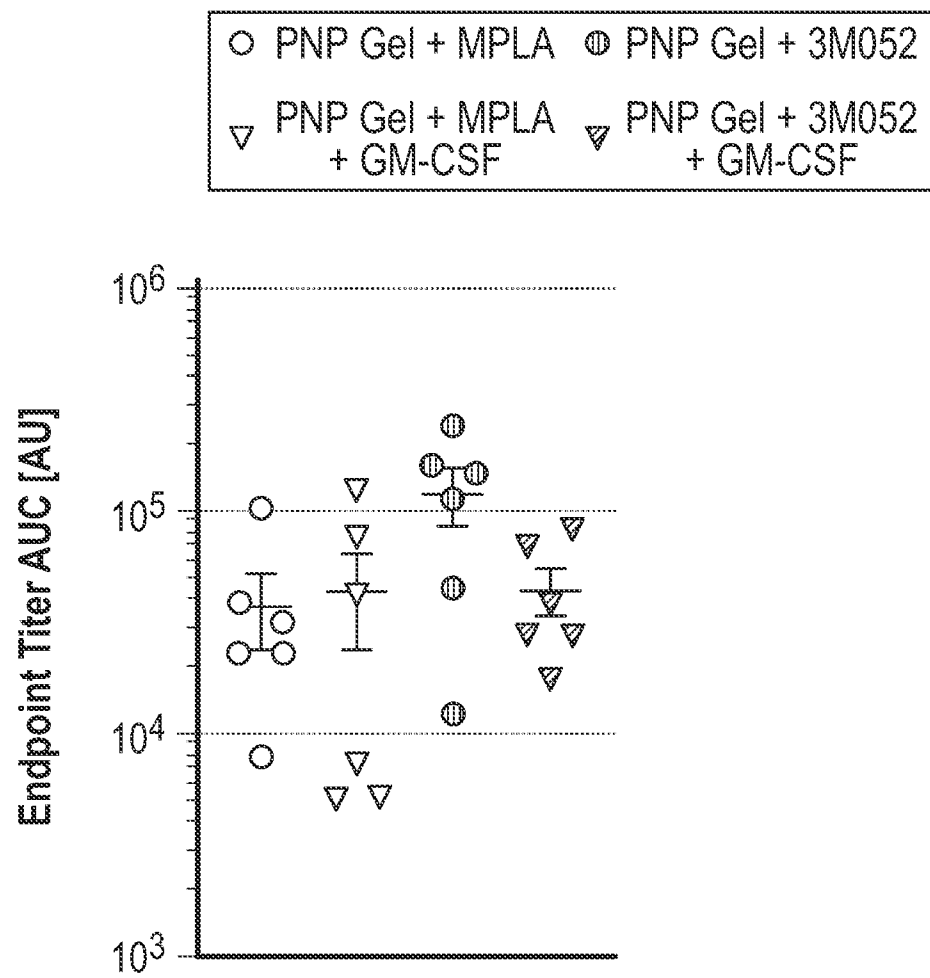


FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/041544

A. CLASSIFICATION OF SUBJECT MATTER					
INV.	A61K9/06	A61K9/127	A61K9/51	A61K39/215	A61K47/10
	A61K47/12	C12N15/88	A61K47/38		
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 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	BEN S OU ET AL: "Broad and Durable Humoral Responses Following Single Hydrogel Immunization of SARS-CoV-2 Subunit Vaccine", ADVANCED HEALTHCARE MATERIALS, WILEY - V C H VERLAG GMBH & CO. KGAA, DE, vol. 12, no. 28, 20 June 2023 (2023-06-20), page n/a, XP072531498, ISSN: 2192-2640, DOI: 10.1002/ADHM.202301495 section 5, pages 10-11 figure 1, page 3 figure 2, page 4 ----- - / - -				1 - 82
☒ Further documents are listed in the continuation of Box C.					
☒ See patent family annex.					
* Special categories of cited documents :					
"A" document defining the general state of the art which is not considered to be of particular relevance			"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		
"E" earlier application or patent but published on or after the international filing date			"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		
"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)			"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		
"O" document referring to an oral disclosure, use, exhibition or other means			"&" document member of the same patent family		
"P" document published prior to the international filing date but later than the priority date claimed					
Date of the actual completion of the international search			Date of mailing of the international search report		
13 November 2024			26/11/2024		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016			Authorized officer Leitner, Nikolaus		

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/041544

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GALE EMILY C. ET AL: "Hydrogel-Based Slow Release of a Receptor-Binding Domain Subunit Vaccine Elicits Neutralizing Antibody Responses Against SARS-CoV-2", ADVANCED MATERIALS, vol. 33, no. 51, 14 October 2021 (2021-10-14), page 2104362, XP093062525, DE ISSN: 0935-9648, DOI: 10.1002/adma.202104362 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/adma.202104362> section 4, pages 10-11 figure 1, page 3 figure 2, page 5 -----	1-82
X	WO 2022/192438 A1 (UNIV LELAND STANFORD JUNIOR [US]) 15 September 2022 (2022-09-15) paragraph 0094, page 18 paragraph 0155-0161, page 32-34 figures 4 and 6-9 -----	1-82
X	US 2023/173059 A1 (APPEL ERIC A [US] ET AL) 8 June 2023 (2023-06-08) paragraph 22, page 2 paragraph 0291-292 page 31 figures 8, 13B-C and 14-15 -----	1-82
A	ZHONG RUIBO ET AL: "Hydrogels for RNA delivery", NATURE MATERIALS, vol. 22, no. 7, 20 March 2023 (2023-03-20), pages 818-831, XP093221942, London ISSN: 1476-1122, DOI: 10.1038/s41563-023-01472-w Retrieved from the Internet: URL:https://www.nature.com/articles/s41563-023-01472-w.pdf> the whole document -----	1-82

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/041544

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			EP	4304672	A1	17-01-2024			
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			WO	2021231942	A1	18-11-2021			
