



(51) International Patent Classification:

A61K 39/00 (2006.01) A61P 31/00 (2006.01)

A61K 39/39 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/US2021/040676

(22) International Filing Date:

07 July 2021 (07.07.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/048,789 07 July 2020 (07.07.2020) US

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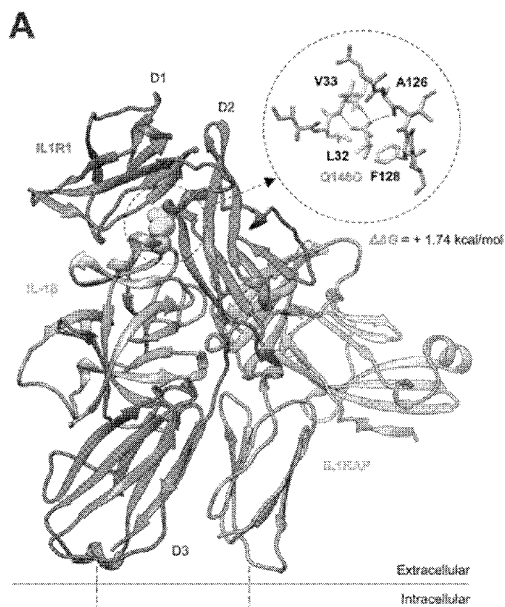
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS,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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: IMMUNOSTIMULATORY ADJUVANTS

(57) Abstract: The present invention relates, in part, to vaccine compositions, adjuvants, chimeric proteins, or chimeric protein complexes and their use as vaccines or therapeutic agents. The present invention further relates to methods of vaccination or treatment of various diseases.

FIG. 20



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

IMMUNOSTIMULATORY ADJUVANTS

FIELD

The present invention relates, in part, to new vaccine compositions and methods for vaccination and their use in the treatment of infectious diseases including, e.g., influenza and severe acute respiratory syndrome.

5

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/048,789, filed on July 7, 2020, the entire contents of which are incorporated herein.

SEQUENCE LISTING

10 The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety. A computer readable format copy of the Sequence Listing (filename: ORN-074PC_ST25.txt, date created: July 2, 2021; file size: 220,680 bytes).

BACKGROUND

15 Vaccination is one of the most effective preventative health tools available against infectious diseases. A vaccine helps the body's immune system to recognize and fight pathogens like viruses or bacteria, which then provides safety from the diseases they cause. Vaccination aims to generate a strong immune response to an administered antigen and provide long-term protection against the disease. Often, however, an antigen alone is insufficient to stimulate protective immunity in a vaccine and needs an adjuvant.

20 Vaccine adjuvants are compounds that enhance the specific immune responses against a desired antigen. Currently, several hundred natural and synthetic compounds are known to have adjuvant activity but only alum salts and AS04 are licensed for use in humans in the United States. Vaccine adjuvants based on alum are low in cost and potent in raising neutralizing antibodies. Unfortunately, alum-supplemented vaccines score poorly in inducing antigen-specific CD8⁺ T cell responses. In contrast to alum, agonists of pattern-recognition receptors (PRRs), such as monophosphoryl lipid A (MPLA), can potently promote cellular immunity, which is desirable. One of the PRRs that plays an essential role in connecting innate and adaptive immunity is the NLRP3 inflammasome. NLRP3 inflammasome assembly can
25 be triggered by a variety of pathogen- and damage-associated molecular patterns, leading to the activation of the cysteine protease caspase-1 and the subsequent cleavage of immature pro-IL-1 β to active IL-1 β , which can ultimately be released by different innate immune cells. The NLRP3 inflammasome and its product interleukin-1 β (IL-1 β) are pivotal mediators of cellular immune responses, yet, overactivation of these systems leads to side effects, which hamper clinical applications.

30 Accordingly, there remains a need for safe and effective vaccine compositions and adjuvants that can effectively stimulate a subject's immune response and exhibit minimal toxicity.

SUMMARY

One aspect of the present application is related to a vaccine composition comprising: (a) an adjuvant, and (b) an antigen that is suitable for inducing an immune response. The adjuvant comprises a chimeric protein or chimeric protein complex comprising: (i) a wild type or mutant IL-1 β (which is an example of a signaling agent as described herein), (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii).

The connector comprises: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii); and/or (2) a flexible linker that connects (i) and (ii), wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor.

In some embodiments, the adjuvants, the chimeric proteins, or the chimeric protein complexes described herein comprise IL-1 β mutants (which is an example of a cytokine/signaling agent that may also be used in the present invention) with reduced biological activity that is coupled to one or more targeting moieties. In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes of the present invention include AcTakines (Activity-on-Target cytokines) that have one or more mutated cytokines that remain inactive *en route* through the body and only reveal their full agonistic activity upon target cell binding. In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes target a mutant IL-1 β to CD8 $^{+}$ T cells (sometimes referred to as CD8 α AcTaleukin-1/ALN-1). *In vivo*, this CD8 α ALN-1 can act as an adjuvant that potently promotes the CD8 $^{+}$ T cell response to antigens, with a significantly reduced toxicity profile compared to wild-type (WT) IL-1 β .

In some embodiments, the present vaccine adjuvants as described herein have the capacity to safely promote activation, expansion and memory differentiation of CD8 $^{+}$ T cells.

The present disclosure also concerns, in part, to the finding that adjuvants, chimeric proteins, or chimeric protein complexes comprising wild type IL-1 β , or variants thereof, exhibit substantially reduced IL-1 β activity compared to wild type IL-1 β . This reduced IL-1 β -activation signaling activity, however, can be induced and/or restored at a target cell when directed to such a cell through a targeting moiety, which binds to an antigen or receptor of interest. Surprisingly, the induced IL-1 β activity at a target cell, achieved through targeting of adjuvants, chimeric proteins, or chimeric protein complexes comprising IL-1 β , or variants thereof, may be similar or greater at the target cell than that of wild type IL-1 β .

Importantly, the adjuvants, chimeric proteins, and chimeric protein complexes comprising mutant IL-1 β described herein, exhibit substantial and surprising selectivity for target cells versus non-target cells, and substantially more than, for example, achieved with targeted wild type IL-1 β chimeric protein(s). In summary, a unique combination of highly

potent and highly cell target-selective signaling activation can be achieved with the adjuvants, chimeric proteins, or chimeric protein complexes described herein.

In some embodiments, the loss in affinity and/or activity of wild type IL-1 β for its receptor, e.g., IL-1 receptor, can be induced and restored upon directing or targeting of the adjuvant, the chimeric protein, or the chimeric protein complex comprising IL-1 β to a target cell through a targeting moiety. In some embodiments, the induction and restoration of IL-1 β -mediated activation at a target cell may reach a level that is similar to or higher than activation achieved with wild type (non-chimeric) IL-1 β .

In some embodiments, the IL-1 β is modified, *i.e.*, it is a variant and comprises one or more mutations in IL-1 β . In some embodiments, the one or more mutations reduce the biological activity of the IL-1 β (sometimes referred to as "attenuated by mutation"). For example, the one or more mutations may reduce the affinity and/or activity of the IL-1 β for IL-1 receptor. In an embodiment, the IL-1 receptors comprise the IL-1R1 and IL-1RACP.

In an embodiment, the modified IL-1 β comprises one or more mutations that reduce its affinity and/or activity for IL-1R1. In another embodiment, the modified IL-1 β comprises one or more mutations that reduce its affinity and/or activity for IL-1R2. In an embodiment, the modified IL-1 β comprises one or more mutations that reduce its affinity and/or activity for IL-1R1 and comprises one or more mutations that reduce its affinity and/or activity for IL-1R2. In some embodiments, the loss in affinity and/or activity of the modified IL-1 β ("attenuated by mutation") for a receptor, e.g., IL-1R1, IL-1R2, can be induced and restored upon directing or targeting of the adjuvant, the chimeric protein, or the chimeric protein complex comprising the modified IL-1 β to a target cell through a targeting moiety.

In some embodiments, the adjuvant, the chimeric protein, and the chimeric protein complex, including Fc-based chimeric protein complex, comprises one or more additional signaling agents or cytokines, e.g., without limitation, an interferon, an interleukin, and a tumor necrosis factor, that may be modified. In various embodiments, the adjuvant, the chimeric protein, or chimeric protein complex, including Fc-based chimeric protein complex, of the invention provides improved safety and/or therapeutic activity and/or pharmacokinetic profiles (e.g., increased serum half-life) compared to an untargeted and/or unmodified IL-1 β or an unmodified, wild type IL-1 β .

In various embodiments, the adjuvants, the chimeric proteins, or the chimeric protein complexes, including Fc-based chimeric protein complexes, comprise one or more targeting moieties which have recognition domains (e.g. antigen recognition domains, including without limitation various antibody formats, inclusive of single-domain antibodies) which specifically bind to a target (e.g. antigen, receptor) of interest. In various embodiments, the targeting moieties have recognition domains that specifically bind to a target (e.g. antigen, receptor) of interest, including those found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, T regulatory cells (Tregs), natural killer (NK) cells, natural killer T (NKT) cells, macrophages (e.g. M1 and M2 macrophages), B cells, B regulatory (Breg) cells, neutrophils, monocytes, myeloid derived cells, and dendritic cells. In various embodiments,

the targeting moieties have recognition domains that specifically bind to a target (e.g. antigen, receptor) of interest, including those found on one or more endothelial cells, epithelial cells, mesenchymal cells, stromal cells or other cell types that are characteristic of and/or unique for specific organs and/or tissues, including those specifically associated with disease. In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) of interest and effectively recruit one or more immune cells.

In some embodiments, the adjuvants, the chimeric proteins, or the chimeric protein complexes, including Fc-based chimeric protein complexes, may recruit an immune cell, e.g., an immune cell that can cause an anti-infective effect, or modulate other immune cells, to a site of action. In some embodiments, the adjuvants, the chimeric proteins, or the chimeric protein complexes, including Fc-based chimeric protein complexes, may modulate an immune cell at a site of action, or recruit an immune cell to a site of action.

In one aspect, the present invention is related to a method for vaccinating a subject against an infectious disease, comprising administering: (a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a wild type or mutant IL-1 β (which is an example of a signaling agent as described herein), (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) an antigen which is suitable for inducing an immune response.

Another aspect of the invention is related to a method for vaccinating a subject against an influenza infection, comprising administering: (a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a wild type or mutant IL-1 β (which is an example of a signaling agent as described herein), (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) an influenza antigen which is suitable for inducing an immune response.

Yet another aspect of the invention is related to a method for vaccinating a subject against a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection comprising administering: (a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a wild type or mutant IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more

mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) a SARS-CoV-2 antigen which is suitable for inducing an immune response.

5 Another aspect of the invention is related to a method for treating a subject afflicted with an infectious disease, comprising administering a chimeric protein or chimeric protein complex, comprising: (i) a wild type or mutant IL-1 β IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the
10 Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor.

In various embodiments, the adjuvants, the chimeric proteins, and the chimeric protein complexes find use in the vaccination against or treatment of various diseases or disorders, such as infections. The present invention
15 encompasses various methods of treatment or various methods of vaccination against such diseases or disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-F, 2A-H, 3A-H, 4A-D, 5A-F, 6A-J, 7A-D, 8A-F, 9A-J, 10A-F, 11A-L, 12A-L, 13A-F, 14A-L, 15A-L, 16A-J, 17A-J, 18A-F, and 19A-F show various non-limiting illustrative schematics of the Fc-based chimeric protein complexes of the present invention. In embodiments, each schematic is a composition of the present invention. Where applicable
20 in the figures, "TM" refers to a "targeting moiety" as described herein, "SA" refers to a "signaling agent" as described herein, "—|—" is an optional "linker" as described herein, the two long parallel rectangles are human Fc domains, e.g. from IgG1, from IgG2, or from IgG4, as described herein and optionally with effector knock-out and/or stabilization mutations as also described herein, and the two long parallel rectangles with one having a protrusion and the other having an indentation are human Fc domains, e.g. from IgG1, from IgG2, or from IgG4 as described herein, with knob-
25 in-hole and/or ionic pair (a/k/a charged pairs, ionic bond, or charged residue pair) mutations as described herein and optionally with effector knock-out and/or stabilization mutations as also described herein.

FIGs. 1A-F show illustrative homodimeric 2-chain complexes. These figures show illustrative configurations for the homodimeric 2-chain complexes.

FIGs. 2A-H show illustrative homodimeric 2-chain complexes with two targeting moieties (TM) (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In embodiments, the constructs shown in the box (i.e., Figs. 2G and 2H) have signaling agent (SA) between TM1 and TM2 or between TM1 and Fc.
30

FIGs. 3A-H show illustrative homodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable. In embodiments, the constructs shown in the box (*i.e.*, Figs. 3G and 3H) have TM between SA1 and SA2 or TM at N- or C-terminus.

5 **FIGs. 4A-D** show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely the TM on the knob chain of the Fc and the SA on hole chain of the Fc.

FIGs. 5A-F show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with both TMs on the knob chain of the Fc and with SA on hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are
10 interchangeable. In some embodiments, TM1 and TM2 can be identical.

FIGs. 6A-J show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with TM on the knob chain of the Fc and with a SA on the hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In these orientations and/or configurations, one SA is on the knob chain and one SA is on the hole chain. In embodiments, the position of SA1 and SA2 are interchangeable.

15 **FIGs. 7A-D** show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely the SA on the knob chain of the Fc and the TM on hole chain of the Fc.

FIGs. 8A-F show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with SA on the knob chain of the Fc and both TMs on hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are
20 interchangeable. In some embodiments, TM1 and TM2 can be identical.

FIGs. 9A-J show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with SA on the knob chain of the Fc and TM on hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In these orientations and/or configurations, one SA is on the knob chain and one SA is on the hole chain. In embodiments, the position of SA1 and SA2 are interchangeable.

25 **FIGs. 10A-F** show illustrative heterodimeric 2-chain complexes with TM and SA on the same chain, namely the SA and TM both on the knob chain of the Fc.

FIGs. 11A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the knob chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable.
30 In some embodiments, TM1 and TM2 can be identical.

FIGs. 12A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the knob chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 13A-F show illustrative heterodimeric 2-chain complexes with TM and SA on the same chain, namely the SA and TM both on the hole chain of the Fc.

FIGs. 14A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties are present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In embodiments, TM1 and TM2 can be identical.

FIGs. 15A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 16A-J show illustrative heterodimeric 2-chain complexes with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments) and with SA on knob Fc and TM on each chain. In embodiments, TM1 and TM2 can be identical.

FIGs. 17A-J show illustrative heterodimeric 2-chain complexes with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments) and with SA on hole Fc and TM on each chain. In embodiments, TM1 and TM2 can be identical.

FIGs. 18A-F show illustrative heterodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments) and with split SA and TM chains: SA on knob and TM on hole Fc.

FIGs. 19A-F show illustrative heterodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments) and with split SA and TM chains: TM on knob and SA on hole Fc.

FIGs. 20A-G shows that Q148G is an IL-1 β mutant with strongly reduced biological activity that can be reactivated upon targeting using CD8 α sdAbs (single domain antibodies). Panel A shows a model of the IL-1R complex, consisting of the IL-1R1 (red, domains D1 – D3) and IL-1RAP (orange) receptor subunits together with the IL-1 β ligand, showing a detail of the Q148G mutation. Panel B shows NF- κ B-driven luciferase reporter gene expression in HEK-Blue-IL1R cells. NF- κ B activity is normalized to background and expressed as fold induction. WT IL-1 β activity is shown as reference. Each data point represents the mean of four independent experiments $\pm$ s.e.m. Panel C. Blueprint of the CD8 α ALN-1 design: IL-1 β Q148G is coupled by a 20xGGS linker to a sdAb binding mouse CD8 α . The Bcl10 sdAb fusion is used as an untargeted control. The C-terminal 9xHis-tag allows for protein purification. Panel D shows a representative SDS-PAGE protein gel demonstrating purity after recombinant production in HEK-293F cells. Panel E shows a proof-of-concept for CD8 α ALN-1 targeting using NF- κ B-driven luciferase reporter gene expression in HEK-Blue-IL1R cells, transiently transfected with or without CD8 α . The activities of WT IL-1 β and CD8 α WT IL-1 β are

shown as reference. Each data point represents the mean of at least five independent experiments $\pm$ s.e.m. Panel F shows nuclear translocation of NF- κ B's p65 subunit in HEK-Blue-IL1R cells, assessed by confocal microscopy. HEK-Blue-IL1R cells were either transiently transfected with CD8 α or empty plasmid DNA, mixed in a 1:1-ratio and plated 24h prior to stimulation. Nuclei were stained with DAPI, NF- κ B p65 Ser-536 and CD8 α were stained with antibodies conjugated with AlexaFluor 647 or AlexaFluor 488 fluorochromes, respectively. The activities of vehicle, WT IL-1 β and untargeted ALN-1 are included as controls. The scale bar (white) indicates 50 μ m. Panel G shows induction of NF- κ B-, p38 MAPK- and AP-1-driven target genes in human 132 1N1 astrocytes transiently transfected with CD8 α or an irrelevant target protein, evaluated by RT-qPCR. Data are normalized to WT IL-1 β -induced gene expression and bars represent the mean $\pm$ s.e.m. of two independent experiments. In each set of histograms, the left bar represents CD8 α ALN-1 (CD8 α ⁺ cells); the middle bar represents Untargeted ALN-1 (CD8 α ⁺ cells); and the right bar represents CD8 α ALN-1 (irrelevant target cells). See also FIG. 27.

FIGs. 21A-H shows that CD8 α ALN-1 promotes antigen-dependent proliferation and activation. Panel A shows flow cytometric detection of CD8 α ALN-1 binding *in vitro* within different immune cell populations (spleen) from WT or IL-1R1^{-/-} C57BL/6 mice. In the set of histograms, the bars represent, from left to right: Vehicle; WT IL-1 β ; CD8 α ALN-1; and Bcl10 ALN-1. Panel B shows flow cytometric detection of CD8 α ALN-1 binding *in vitro* within the XCR1⁺ cDC population (spleen) from WT or IL-1R1^{-/-} C57BL/6 mice. In the set of histograms, the bars represent, from left to right: WT IL-1 β ; CD8 α ALN-1 and Bcl10 ALN-1. In A and B, controls include vehicle, WT IL-1 β and untargeted Bcl10 ALN-1 binding. Bars represent the mean His-tag⁺ fraction (%) in the annotated cell population $\pm$ s.e.m. of three independent experiments. ****, $p < 0.0001$; ***, $p < 0.001$; *, $p < 0.05$; ns, $p \geq 0.05$ compared with the binding of untargeted Bcl10 ALN-1 by unpaired Student's *t*-test (two-tailed). Panel C shows representative histograms demonstrating binding of CD8 α ALN-1 on CTLs, cDCs and type I cDCs. Binding of vehicle, WT IL-1 β and untargeted Bcl10 ALN-1 are shown as controls. Panel D shows representative histograms showing titration of CD8 α ALN-1 binding on CTLs and cDCs, assessed by flow cytometry. Molecules were titrated in the same concentration range shown in panels (Panel E) and (Panel F). Panel E and Panel F show quantification of CD8 α ALN-1's binding affinity on CTLs and cDCs. Each data point represents the mean fluorescence intensity (MFI) of the His-tag signal in CTL population $\pm$ s.e.m. or the mean His-tag⁺ fraction (%) in the cDC population $\pm$ s.e.m. of three independent experiments. Panel G shows flow cytometry analysis of OT-I proliferation (CFSE dilution, *left*) and activation (CD25 upregulation, *right*) in *in vitro* OT-I co-cultures. OT-I cells were defined as CD3⁺CD4⁺CFSE^{labeled} cells. Each bar represents the mean fold induction (CFSE dilution or CD25 upregulation under treatment conditions relative to the vehicle signal) $\pm$ s.e.m. of at least three independent experiments. ****, $p < 0.0001$; **, $p < 0.01$; *, $p < 0.05$; ns, $p \geq 0.05$ by one-way ANOVA with Tukey's multiple comparisons test. Panel H shows representative histograms illustrating OT-I proliferation and upregulation of CD25 in the divided OT-I subset. See also FIG. 28 and 29.

FIGs. 22A-J shows that CD8 α ALN-1 induces CD8 $^{+}$ T cell proliferation and effector functions in response to antigen *in vivo*. Panel A shows a schematic representation of the adoptive transfer experiment. C57BL/6 mice received an intravenous (i.v.) transfer of OT-I cells. OVA (100 μ g) was delivered intraperitoneally (i.p.) one day later. Together with OVA, mice received three treatments with either LPS (25 μ g), WT IL-1 β (5 μ g), CD8 α ALN-1 or untargeted Bcl10 ALN-1 (10 μ g) (i.p. every 24h). Flow cytometry was performed one day after the last treatment. Panel B shows representative flow cytometry histograms illustrating enhanced OT-I proliferation in C57BL/6 recipient mice upon treatment with OVA and CD8 α ALN-1. Controls include the OT-I response without OVA (PBS) and with OVA alone or combined with LPS, WT IL-1 β or untargeted Bcl10 ALN-1. Panel C and Panel D show quantification of OT-I proliferation in C57BL/6 (Panel C) or C57BL/6 IL-1R1 $^{-/-}$ (Panel D) recipient mice, visualized as stacked histograms. Individual stacks show the mean percentages of total proliferating OT-I cells in a certain stage of cell division $\pm$ s.e.m. Shown is a pool of two independent experiments with $n = 6$ (Panel C) or 10 (Panel D) mice/group combined. **, $p < 0.01$; *, $p < 0.05$; ns, $p \geq 0.05$ for comparison of cell frequencies in the ultimate (sixth) fraction of division by Kruskal-Wallis test with Dunn's multiple comparisons test (Panel C) or unpaired Mann-Whitney U test (two-tailed) (Panel D). Panel E shows CD44/CD62L expression as a measure of CTL activation in spleens of C57BL/6 recipient mice. Each bar represents mean percentages of CTLs with the CD44 $^{+}$ CD62L $^{-}$ phenotype $\pm$ s.e.m. of a pool of two independent experiments with $n = 6$ mice/group combined. *, $p < 0.05$ by one-way ANOVA with Tukey's multiple comparisons test. Panel F shows schematic representation of the *in vivo* killing experiment. C57BL/6 mice received one i.p. OVA (100 μ g) treatment together with three treatments with either LPS (25 μ g), WT IL-1 β (5 μ g), CD8 α ALN-1 or untargeted Bcl10 ALN-1 (10 μ g) (i.p. every 24h). One week after OVA delivery, a 1:1-mixture of splenocytes (either CTV high labeled and SIINFEKL-loaded or CTV low labeled and non-loaded) was i.v. transferred. Flow cytometry to assess SIINFEKL-directed cytolytic activity was performed one day *post-transfer*. Panel G shows representative histograms illustrating SIINFEKL-directed cytolytic activity in C57BL/6 mice induced upon treatment with OVA and CD8 α ALN-1. Controls include the response without OVA (PBS) and with OVA alone or combined with LPS, WT IL-1 β or untargeted Bcl10 ALN-1. Panel H shows quantification of SIINFEKL-specific target cell lysis. Panel I shows one week after initial OVA immunization, induction of SIINFEKL-specific CD8 $^{+}$ T cell responses in spleens of treated mice were measured by IFN- γ ELISPOT and quantified. Panel J shows representative ELISPOT pictures are shown, depicting the number of spots after treatment with: i. OVA alone, ii. OVA and WT IL-1 β , iii. OVA and CD8 α ALN-1 and iv. OVA and untargeted Bcl10 ALN-1. In (Panel H) and (Panel I), data points represent individual mice $\pm$ s.e.m. of a pool of two independent experiments with $n = 10$ mice/group combined. ****, $p < 0.0001$; ***, $p < 0.001$; *, $p < 0.05$; ns, $p \geq 0.05$ by Kruskal-Wallis test with Dunn's multiple comparisons test. See also FIG. 30 and 31.

FIGs. 23A-I shows that systemic treatment of mice with antigen in combination with CD8 α ALN-1 is completely free of toxicity. Panel A shows a schematic representation of the toxicity experiment. C57BL/6 mice received one i.p. OVA (100 μ g) treatment together with three treatments with either WT IL-1 β (5 μ g) or CD8 α ALN-1 (10 μ g) (i.p. every 24h).

Controls include mice treated with PBS or OVA alone. Tail vein blood was sampled 6h after the first treatment and body weight was tracked over time. Panel B and Panel C show change in body weight over time (Panel B) or after three days of treatment (Panel C). Data points represent the mean (Panel B) or individual mice (Panel C) $\pm$ s.e.m. of a representative of two independent experiments with $n = 5$ mice/group. Panels D-I show hematological analysis of fresh EDTA-coated blood (Panels E-I) or plasma derived from this blood (Panel D), showing systemic IL-6 levels (Panel D), platelet counts (Panel E) and mean platelet volumes (Panel F), total white blood cell counts (Panel G), lymphocyte counts (Panel H) and neutrophil counts (Panel I). Data points represent individual mice $\pm$ s.e.m. of a pool of two independent experiments with $n = 10$ mice/group combined. ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, $p \geq 0.05$ by one-way ANOVA with Tukey's multiple comparisons test (Panel C) and (Panel G) or by Kruskal-Wallis test with Dunn's multiple comparisons test (Panel D), (Panel E), (Panel F) and (Panel H). See also FIG. 32.

FIGs. 24A-I shows that an influenza vaccine adjuvanted with CD8 α ALN-1 protects mice against viral infection. Panel A shows a schematic representation of the prime-boost prophylactic influenza vaccination experiment. BALB/c mice were immunized intramuscularly (i.m.) with X47 WIV (15 μ g), either alone or combined with SAS adjuvant (15 μ g i.m. together with WIV), WT IL-1 β (5 μ g), CD8 α ALN-1, untargeted Bcl10 ALN-1 or CD8 α hIFN α 2 (10 μ g i.v. 24h post-WIV). An identical boost treatment was delivered two weeks later. A heterosubtypic pH1N1 virus that shares strongly conserved T cell epitopes with X47 WIV was used to infect the mice two weeks later (intranasally (i.n), 2xLD₅₀). Panel B shows a change in body weight over time (left) or after nine days of infection (right) of mice challenged i.n. with a high inoculum of pH1N1 influenza virus. Data points represent the mean (left) or individual mice (right) $\pm$ s.e.m. of a representative of two independent experiments with $n = 6$ mice/group. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$ by one-way ANOVA with Tukey's multiple comparisons test. Panel C shows a Kaplan-Meier curve, representing survival of mice during pH1N1 influenza virus infection. Each data point represents the mean survival (%) $\pm$ s.e.m. of a representative of two independent experiments with $n = 6$ mice/group. *, $p < 0.05$ by log-rank testing. Panels D-I shows change in body weight (%) over time of virus-infected mice for each individual treatment, including WIV alone (Panel D) or combined with SAS (Panel E), WT IL-1 β (Panel F), CD8 α ALN-1 (Panel G), untargeted Bcl10 ALN-1 (Panel H) and CD8 α hIFN α 2 (Panel I).

FIGs. 25A-C shows that the protective antiviral effect of CD8 α ALN-1 correlates with the induction of strong and long-lasting influenza-specific T cell responses in lung and lymphoid tissues. Panel A shows induction of NP-specific CD8 $^{+}$ and CD4 $^{+}$ T cell responses in spleens of vaccinated mice two weeks after boost administration, measured by IFN- γ ELISPOT. Below the graph, representative ELISPOT pictures are shown, depicting the number of spots after treatment with: i. WIV alone, ii. WIV and SAS, iii. WIV and WT IL-1 β , iv. WIV and CD8 α ALN-1, v. WIV and untargeted Bcl10 ALN-1 and vi. WIV and CD8 α hIFN α 2. In the graph above, bars represent the mean $\pm$ s.e.m. of a representative of two independent experiments with $n = 5$ mice/group. ****, $p < 0.0001$; **, $p < 0.01$; ns, $p \geq 0.05$ by Kruskal-Wallis test with Dunn's multiple comparisons test (CD8 ELISPOT) or one-way ANOVA with Tukey's multiple comparisons test

(CD4 ELISPOT). Undetectable responses are indicated by ####. Panel B shows the detection of NP-specific CD8⁺ T cells in the lung-draining LNs (upper histograms) and lung parenchyma (lower histograms) of mice vaccinated with WIV and WT IL-1 β or CD8 α ALN-1, seven days *post*-pH1N1 influenza A virus infection. In the NP-pentamer⁺ CTL population, T_{CM}, T_{EM}, and T_{RM} cells are identified in LNs and/or lung parenchyma. Bars on the right represent the mean $\pm$ s.e.m. of a representative of two independent experiments with $n = 5$ mice/group. *, $p < 0.05$; ns, $p \geq 0.05$ by unpaired Student's *t*-test (two-tailed). In each set of histograms, the left bar represents WIV + CD8 ALN-1 and the right bar represents WIV + WT IL-1 β . Panel C shows flow cytometric detection of NP-specific CD8⁺ T cells in the lungs of surviving mice that were vaccinated with WIV and WT IL-1 β or CD8 α ALN-1, 50 days *post*-pH1N1 influenza A virus infection. *Left*: Representative histograms showing the fraction of NP-pentamer⁺ cells in the CD8⁺ T cell population. *Right*: Bars represent the mean $\pm$ s.e.m. of one experiment with $n = 5$ (WT IL-1 β) or 6 (CD8 α ALN-1) mice/group. ns, $p \geq 0.05$ by unpaired Student's *t*-test (two-tailed). See also FIG. 33.

FIGs. 26A-D shows that the transcriptional landscape of CD8⁺ T cells isolated from vaccinated mice during influenza virus infection supports the cellular adjuvant effect of CD8 α ALN-1. Panel A shows volcano plots showing all genes found differentially up- (blue) or downregulated (red) in CD8⁺ T cells sorted from lung parenchyma (left) or lung-draining mediastinal LNs (right) of mice vaccinated with WIV and WT IL-1 β (up) or CD8 α ALN-1 (down), compared with mice treated with WIV alone. Significance is indicated by a False Discovery Rate (*FDR*) < 0.05 and an absolute log₂ *fold*-change > 1 . Panel B shows heat maps of all statistically significant DEGs (differentially expressed genes) identified in CD8⁺ T cells isolated from lungs (left map) or draining LNs (right map) of mice vaccinated with WIV and WT IL-1 β (left columns) or CD8 α ALN-1 (right column). Cells summarize the log₂ *fold*-change in the gene expression level of $n = 3$ mice/group, compared to treatment with WIV alone. Clusters indicate DEGs shared between (i.) or unique for treatment with WT IL-1 β (iii.) and/or CD8 α ALN-1 (ii.). Within these clusters, genes are organized by increasing *FDR*. Panel C shows top five of up- and downregulated GO biological processes, clustered using the DAVID bioinformatics tool based on the lists of DEGs identified in lungs and draining LNs. Panel D shows a graphical representation of the subcellular localization of gene products with a known association with regulation of CD8⁺ T cell activation and memory development (based on literature). See also FIG. 34.

FIGs. 27A-C. Panel A shows that the CD8 α sdAb does not interfere with CD8⁺ T cell activation *in vitro*. The CD8 α sdAb was tested (20 μ M top concentration and 1:5 serial dilution) *in vitro* using the OT-I co-culture system. OT-I activation was assessed by flow cytometry after 72h of culture by evaluating proliferation (CTV dilution). Staining with CD3 and CD4 antibodies. OT-I cells were detected as single cells (based on FSC/SSC) that stained CD3⁺CD4⁺CTV^{labeled}. *Left*: Quantification of OT-I proliferation. Shown is mean proliferation under treatment conditions relative to vehicle $\pm$ s.e.m. of one experiment. Treatment with inhibitory antibody is shown as control. *Right*: Representative histograms illustrating unaltered OT-I proliferation in the presence of the CD8 α sdAb. Panel B shows heat maps showing gene expression (relative to vehicle) of *IL8*, *A20*, *JUN*, *DUSP*, *NFKBIA* and *ICAM1* in CD8 α ⁺ 132 1N1 human

astrocytes upon stimulation with WT IL-1 β , CD8 α ALN-1 or untargeted ALN-1. Representative heat maps of two independent experiments. Panel C shows the biological activity of CD8 α ALN-1 upon targeting is dependent on the level of target antigen expression. NF- κ B-driven luciferase reporter gene expression induced by CD8 α ALN-1 (1 nM) in HEK-Blue-IL1R cells, transiently transfected with CD8 α (50 ng top concentration and 1:5 serial dilution). *Top*: NF- κ B activity is normalized to β -galactosidase activity and expressed as fold induction compared to the activity of WT IL-1 β (1 nM) for every tested DNA concentration. Each data point represents the mean of two independent experiments $\pm$ s.e.m. Untargeted Bcl10 ALN-1 (1 nM) is included as control. *Bottom*: Representative Western Blot image of total HEK-Blue-IL1R cell lysates, demonstrating decreasing CD8 α protein (37 kDa) levels with decreasing levels of plasmid DNA. Tubulin (50 kDa) is included as loading control. Staining with primary antibodies against Flag-tag (Sigma F7425) and tubulin (Sigma T6199). Secondary detection with HRP-conjugated antibodies against rabbit and mouse IgG (GE Healthcare). Detection by chemiluminescence using the Amersham Imager 680 (GE Healthcare).

FIGs. 28A-E. Panels A-D show the gating strategy for the detection of CD8 α ALN-1 binding in C57BL/6 (IL-1R1 $^{-/-}$) splenocyte pools. Staining with LIVE/DEAD, CD19, CD3, CD4, CD11b, CD11c, XCR1 and anti-His-tag antibodies. Panel E *Left*: CD8 α ALN-1 does not bind NK cells in a mixed pool of WT C57BL/6 splenocytes, whereas strong CTL targeting is present. Bars represent the mean His-tag signal (MFI) in the annotated cell populations $\pm$ s.e.m. of two independent experiments. Controls include vehicle, WT IL-1 β and untargeted Bcl10 ALN-1 binding. Representative histograms are included below the graph. Panel E *Right*: Gating strategy for this analysis. Staining with LIVE/DEAD, CD19, CD3, CD4, NK1.1 (clone PK136, 108707, BioLegend) and anti-His-tag antibodies. NK cells were identified as CD19-CD3-NK1.1 $^{+}$. In the set of histograms, the bars represent, from left to right: Vehicle; WT IL-1 β (1nM); CD8 α ALN-1 (1 nM); and Bcl10 ALN-1 (1nM).

FIGs. 29A-D. Panel A shows the gating strategy for OT-I coculture experiments. Staining with CD3, CD4 and CD25 antibodies and detection of CD3 $^{+}$ CD4 $^{+}$ CFSE $^{\text{labeled}}$ OT-I cells. Proliferation is calculated as a measure of CFSE dilution. CD25 upregulation was evaluated in the proliferated OT-I subset. Panel B shows OT-I proliferation induced by treatment with CD8 α ALN-1 in the absence (left) and presence (right) of SIINFEKL on BM-DCs. Bars represent mean $\pm$ s.e.m of three independent experiments. **, $p < 0.01$ by unpaired Student's t -test (two-tailed). Panel C shows flow cytometry analysis of OT-I proliferation (CFSE dilution, left) and activation (CD25 upregulation, right) in *in vitro* OT-I co-cultures with IL-1R1 $^{-/-}$ BM-DCs. OT-I cells were defined as CD3 $^{+}$ CD4 $^{+}$ CFSE $^{\text{labeled}}$ cells. Each bar represents the mean fold induction of treatment vs. vehicle $\pm$ s.e.m. of two independent experiments. Panel D shows ELISA detection of IFN- γ and TNF in the conditioned supernatant of OT-I cocultures with WT BM-DCs. Each bar represents the mean fold induction of treatment vs. vehicle $\pm$ s.e.m. of at least two independent experiments. Treatments with vehicle, inhibitory antibody and WT IL-1 β are included as controls. In each set of histograms, the bars represent, from left to right: Vehicle; Inhibitory antibody; WT IL-1 β ; and CD8 α ALN-1.

FIGs. 30A-F. Panel A shows the gating strategy for the detection of OT-I activation and proliferation after transfer in C57BL/6 recipient mice. Staining of splenocytes with CD19, CD3, CD4, CD62L, CD44 and CD45.1 antibodies and OVA-pentamer. Panels B-E show the treatment with OVA and CD8 α ALN-1 promotes OT-I proliferation and increases the amount of OT-I cells in lymphoid and peripheral organs compared with OVA treatment alone. Experiment performed according to Panel A with two treatment groups: OVA (100 μ g) alone or combined with CD8 α ALN-1 (10 μ g). Staining with LIVE/DEAD, CD19, CD3, CD4, CD8 and CD45.1. OT-I cells were detected in the single cell population (based on FSC/SSC) as LIVE/DEAD-CD19-CD3⁺CD4-CD8⁺CD45.1⁺ cells. Displayed are the relative (% in the CD8⁺ T cell population) and absolute (counts in the CD8⁺ T cell population) amounts of OT-I cells in LNs (Panel B), spleen (Panel C), lungs (Panel D) and liver (Panel E). Data points represent individual mice $\pm$ s.e.m. of one experiment with $n = 5$ mice/group. ns, $p \geq 0.05$; *, $p < 0.05$ by unpaired Mann-Whitney U test (two-tailed). Panel F. Representative dot plots illustrating simultaneous upregulation of CD44 and downregulation of CD62L on CTLs induced by CD8 α ALN-1 treatment. In each set of histograms, the left bar represents OVA alone and the right bar represents OVA + CD8 α ALN-1.

FIGs. 31A-B. Panel A shows splenocyte labeling for the *in vivo* killing assay experiment. Cells were labeled with a high or low intensity of CTV and loaded with SIINFEKL (CTV^{high}) or left unloaded (CTV^{low}). Peptide presentation is detected with a SIINFEKL in H-2kB antibody. Panel B shows gating strategy for the detection of adoptively transferred target cells in the spleens of acceptor mice and measurement of SIINFEL-directed cytotoxicity.

FIGs. 32A-C. Panel A shows white blood cell counts in C57BL/6 mice treated i.p. with OVA (100 μ g), either alone or combined with CD8 α ALN-1 (daily i.p. treatment with 10 μ g for three consecutive days). Tail vein blood was sampled 6h after every administration and analyzed on the Hemavet 950FS system. Panel B shows the gating strategy for the detection of different lymphocyte populations in mouse peripheral blood. Staining with LIVE/DEAD and CD45, CD19, CD3, CD4, CD8 and NK1.1 antibodies. Panel C shows flow cytometry analysis for measurement of absolute cells counts in mouse peripheral blood, sampled at the indicated timepoints as described in (Panel A). Day 4 means sampling 24h after the last treatment. In the single (based on FSC/SSC) living (LIVE/DEAD⁻) cell population, we identified leukocytes (CD45⁺), B cells (CD45⁺CD19⁺CD3⁻), T cells (CD45⁺CD19⁻CD3⁺), CD4⁺ T cells (CD45⁺CD19⁻CD3⁺CD4⁺CD8⁻), CD8⁺ T cells (CD45⁺CD19⁻CD3⁺CD4⁻CD8⁺) and NK cells (CD45⁺CD19⁻CD3⁻NK1.1⁺). Data points represent individual mice $\pm$ s.e.m. of one experiment with $n = 5$ mice/group in (Panel A) or $n = 6$ mice/group in (Panel C). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, $p \geq 0.05$ by two-way repeated measures ANOVA with Sidak's multiple comparisons test. In each set of histograms, the left bar represents OVA alone and the right bar represents OVA + CD8 α ALN-1.

FIGs. 33A-C. Panels A and B show the gating strategy for the detection of NP-specific CTLs in lung-draining mediastinal LNs (Panel A) or lung parenchyma (Panel B) one week *post*-influenza infection. Staining with LIVE/DEAD, CD45, CD3, CD4, CD8, CD44, CD62L, CD127 and CD69 antibodies and NP-pentamer. Panel C. Gating strategy for

the detection of long-lasting NP-specific CTLs in lung parenchyma 50 days *post*-influenza infection. Staining with LIVE/DEAD, CD45, CD3, CD4 and CD8 antibodies and NP-pentamer. Panels on the right indicate the cleanliness of the NP pentamer staining, which is shown to be selective for CD8⁺ T cells.

FIGs. 34A-C. Panels A-C show the gating strategy for sorting CD8⁺ T lymphocytes from the lung parenchyma (Panel A) and lung-draining LNs (Panel B) of influenza-infected mice one week *post*-influenza A virus challenge. Staining with LIVE/DEAD, CD45, CD3, CD4 and CD8 antibodies. Panel C shows venn diagrams representing the numbers of DEGs shared between (i.) or unique for treatment with WT IL-1 β (iii.) and/or CD8 α ALN-1 (ii.). Upregulated genes are printed in blue, whereas downregulated genes are printed in red. In CD8⁺ T cells isolated from lung, 63 differentially expressed genes (DEGs) (24 up- and 39 downregulated) were shared between WT IL-1 β and CD8 α ALN-1-vaccinated mice. Both WT IL-1 β and CD8 α ALN-1 had non-redundant effects on gene modulation in lung, as evidenced by 19 DEGs (12 up- and 7 downregulated) unique to WT IL-1 β and 44 DEGs (14 up- and 30 downregulated) unique to CD8 α ALN-1 treatment. In CD8⁺ T cells sorted from draining LNs, 34 DEGs (12 up- and 22 downregulated) were shared between mice that received WT IL-1 β or CD8 α ALN-1 as adjuvant. Here, we identified 15 DEGs (8 up- and 7 downregulated) modulated by WT IL-1 β only and 16 DEGs (7 up- and 9 downregulated) uniquely regulated upon CD8 α ALN-1-treatment.

DETAILED DESCRIPTION

One aspect of the present application is related to a vaccine composition comprising: (a) an adjuvant, and (b) an antigen that is suitable for inducing an immune response. The adjuvant comprises a chimeric protein or chimeric protein complex comprising: (i) a wild type or mutant IL-1 β (which is an example of a signaling agent as described herein), (ii) one or more targeting moieties, said targeting moieties comprising recognition domains, which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii).

In embodiments, the connector comprises: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii); and/or (2) a flexible linker that connects (i) and (ii), wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor.

In embodiments, the adjuvant is a nucleic acid, which encodes the chimeric protein or chimeric protein complex. In embodiments, the nucleic acid is an mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine. In embodiments, the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.

In some embodiments, the vaccine composition of the present invention further comprises an aluminum gel or salt. In embodiments, the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum

sulfate. In some embodiments, the adjuvant is a nucleic acid encoding the chimeric protein or chimeric protein complex as described herein.

In some embodiments, the additional adjuvant is selected from, oil-in-water emulsion formulations, saponin adjuvants, ovalbumin, toll like receptors ligands, Freund's Adjuvant, cytokines, and chitosans. Illustrative additional adjuvants include, but are not limited to: (1) ovalbumin (e.g. ENDOFIT), which is often used for biochemical studies; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as, for example, Model HOy microfluidizer (Microfluidics, Newton, Mass.), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, (c) RIBI adjuvant system (RAS), (RIBI IMMUNOCHEM, Hamilton, MO.) containing 2% Squalene, 0.2% Tween 80, and, optionally, one or more bacterial cell wall components from the group of monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), including MPL+CWS (DETOX™); and (d) ADDAVAX (Invitrogen); (3) saponin adjuvants, such as STIMULON (Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (by way of non-limiting example, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; (6) chitosans and other derivatives of chitin or poly-N-acetyl-D-glucosamine in which the greater proportion of the N-acetyl groups have been removed through hydrolysis (see, e.g., European Patent Application 460 020, which is hereby incorporated by reference in its entirety, disclosing pharmaceutical formulations including chitosans as mucosal absorption enhancers; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition, e.g., monophosphoryl lipid A. In other embodiments, the additional adjuvant is one or more of an aluminum salt or gel, a pattern recognition receptors (PRR) agonist, CpG ODNs and imidazoquinolines. In some embodiments, the additional adjuvant is one or more of cyclic [G(3',5')pA(3',5')p] (e.g. 3'3'-cGAMP VACCIGRADE); cyclic [G(2',5')pA(3',5')p]2'3' (e.g. 2'3' cGAMP VACCIGRADE); cyclic [G(2',5')pA(2',5')p] (e.g. 2'2'-cGAMP VACCIGRADE); cyclic diadenylate monophosphate (e.g. c-di-AMP VACCIGRADE); cyclic diguanylate monophosphate (e.g. c-di-GMP VACCIGRADE); TLR7 agonist-imidazoquinolines compound (e.g. TLR7 agonists, such as, for example, Gardiquimod VACCIGRADE, Imiquimod VACCIGRADE, R848 VACCIGRADE); lipopolysaccharides (e.g. TLR4 agonists), such as that from E. coli 0111:B4 strain (e.g. LPS-EB VACCIGRADE); monophosphoryl lipid A (e.g. MPLA-SM VACCIGRADE and MPLA Synthetic VACCIGRADE); N-glycolylated muramyl dipeptide (e.g. N-Glycolyl-MDP VACCIGRADE); CpG ODN, class A and/or CpG ODN, class B and/or CpG ODN, class C (e.g. ODN 1585 VACCIGRADE, ODN 1826 VACCIGRADE, ODN 2006 VACCIGRADE, ODN 2395 VACCIGRADE); a triacylated lipoprotein (e.g. Pam3CSK4 VACCIGRADE); Polyinosine-polycytidylic acid (e.g.

Poly(I:C) (HMW) VACCIGRADE); and cord factor (*i.e.* mycobacterial cell wall component trehalose 6,6' dimycolate (TDM,)) or an analog thereof (*e.g.* TDB VACCIGRADE, TDB-HS15 VACCIGRADE). In some embodiments, the additional adjuvant is a TLR agonist (*e.g.* TLR1, and/or TLR2, and/or TLR3, and/or TLR4, and/or TLR5, and/or TLR6, and/or TLR7, and/or TLR8, and/or TLR9, and/or TLR10, and/or TLR11, and/or TLR12, and/or TLR13), a nucleotide-binding oligomerization domain (NOD) agonist, a stimulator of interferon genes (STING) ligand, or related agent. In some embodiments, the adjuvant is a ligand for toll like receptors (TLR) including endotoxin derived compounds, CpG, and flagellin.

In some embodiments, the additional adjuvant is one or more of a mineral adjuvant, gel-based adjuvant, tensoactive agent, bacterial product, oil emulsion, particulated adjuvant, fusion protein, and lipopeptide. Other mineral salt adjuvants, besides the aluminum adjuvants described elsewhere, include salts of calcium (*e.g.* calcium phosphate), iron and zirconium. Other gel-based adjuvants, besides the aluminum gel-based adjuvants described elsewhere, include Acemannan. Tensoactive agents include Quil A, saponin derived from an aqueous extract from the bark of *Quillaja saponaria*; saponins, tensoactive glycosides containing a hydrophobic nucleus of triterpenoid structure with carbohydrate chains linked to the nucleus, and QS-21. Bacterial products include cell wall peptidoglycan or lipopolysaccharide of Gram-negative bacteria (*e.g.* from *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulosum*, *Bordetella pertussis* and *Neisseria meningitidis*), N-acetyl muramyl-L-alanyl-D-isoglutamine (MDP), different compounds derived from MDP (*e.g.* threonyl-MDP), lipopolysaccharides (LPS) (*e.g.* from the cell wall of Gram-negative bacteria), trehalose dimycolate (TDM), and DNA containing CpG motifs. Oil emulsions include FIA, Montanide, Adjuvant 65, Lipovant, the montanide family of oil-based adjuvants, and various liposomes. Among particulated and polymeric systems, poly (DL-lactide-coglycolide) microspheres have been extensively studied and find use herein.

Further, in some embodiments, cytokines are an adjuvant of the present invention (*e.g.* IFN- γ and granulocyte-macrophage colony stimulating factor (GM-CSF)). Also carbohydrate adjuvants (*e.g.* inulin-derived adjuvants, such as, gamma inulin, alammulin (a combination of γ -inulin and aluminum hydroxide), and polysaccharides based on glucose and mannose, such as glucans, dextrans, lentinans, glucomannans and galactomannans) find use in the present invention. In some embodiments, adjuvant formulations are useful in the present invention and include alum salts in combination with other adjuvants such as Lipid A, alammulin, immunostimulatory complexes (ISCOMS), which are virus like particles of 30–40 nm and dodecahedral structure, composed of Quil A, lipids, and cholesterol.

In some embodiments, the additional adjuvants are described in Jennings *et al.* Adjuvants and Delivery Systems for Viral Vaccines-Mechanisms and Potential. In: Brown F, Haaheim LR, (eds). *Modulation of the Immune Response to Vaccine Antigens. Dev. Biol. Stand.*, Vol. 92. Basel: Karger 1998; 19–28 and/or Sayers *et al.* J Biomed Biotechnol. 2012; 2012: 831486, and/or Petrovsky and Aguilar, *Immunology and Cell Biology* (2004) 82, 488–496 the contents of which are hereby incorporated by reference in their entireties.

In various embodiments, the present adjuvants may be part of live and attenuated, or killed or inactivated, or toxoid, or subunit or conjugate vaccines.

In some embodiments, the vaccine or vaccine composition of the present invention causes an improvement in adjuvant properties relative to a vaccine comprising the antigen and the aluminum gel or salt alone. In various embodiments, the vaccine and/or adjuvant described herein causes a broader, more diverse, more robust and longer lasting immunostimulatory effect than the vaccine comprising the antigen and the aluminum gel or salt alone and/or the adjuvant comprising the aluminum gel or salt alone.

In some embodiments, the described vaccine, vaccine composition, and/or described adjuvant causes immunostimulation of one or more of T_{H1} and T_{H2} -mediated immune response. In some embodiments, the described vaccine, vaccine composition, and/or described adjuvant causes immunostimulation of both of T_{H1} and T_{H2} -mediated immune response. In some embodiments, the described vaccine, vaccine composition, and/or described adjuvant causes immunostimulation of T_{H1} -mediated immune response at levels greater than a vaccine comprising the antigen and the aluminum gel or salt alone or an adjuvant comprising the aluminum gel or salt alone.

In various embodiments, the present vaccine composition is part of the following vaccines (e.g. the antigens of these vaccines may be used as the antigen of the present vaccines): DTP (diphtheria-tetanus-pertussis vaccine), DTaP (diphtheria-tetanus-acellular pertussis vaccine), Hib (Haemophilus influenzae type b) conjugate vaccines, Pneumococcal conjugate vaccine, Hepatitis A vaccines, Poliomyelitis vaccines, Yellow fever vaccines, Hepatitis B vaccines, combination DTaP, Tdap, Hib, Human Papillomavirus (HPV) vaccine, Anthrax vaccine, and Rabies vaccine.

In some embodiments, the adjuvant or vaccine composition as described herein has (a) low toxicity; (b) an ability to stimulate a long-lasting immune response against the antigen; (c) substantial stability; (d) an ability to elicit a humoral immune response and/or a cell-mediated immunity to the antigen; (e) a capability of selectively interacting with populations of antigen presenting cells; (f) an ability to specifically elicit T_{H1} and/or T_{H2} cell-specific immune responses to the antigen; and/or (g) an ability to selectively increase appropriate antibody isotype levels against antigens, the isotype optionally being IgA, when administered to a patient.

T_{H1} -mediated immune response (or "Type 1 response") largely involves interaction with macrophages and CD8+ T cells and may be linked to interferon- γ , TNF- β , interleukin-2, and interleukin-10 production. The T_{H1} -mediated immune response promotes cellular immune system and maximizes the killing efficacy of the macrophages and the proliferation of cytotoxic CD8+ T cells. The T_{H1} -mediated immune response also promotes the production of opsonizing antibodies (e.g. IgG, IgM and IgA). The Type 1 cytokine IFN- γ increases the production of interleukin-12 by dendritic cells and macrophages, and via positive feedback, IL-12 stimulates the production of IFN- γ in helper T cells, thereby promoting the T_{H1} profile. Interferon- γ also inhibits the production of cytokines such as interleukin-4, a cytokine associated with the Type 2 response, and thus it also acts to preserve its own response. T_{H2} -mediated immune response (or "Type 2

response") largely involves interaction with B-cells, eosinophils, and mast cells and may be linked to interleukin-4, interleukin-5, interleukin-6, interleukin-9, interleukin-10, and interleukin-13. T_{H2}-mediated immune response promotes humoral immune system and may stimulate B-cells into proliferation, induce B-cell antibody class switching, and increase neutralizing antibody production (e.g. IgG, IgM and IgA as well as IgE antibodies). Other functions of the Type 2 response include promoting its own profile using two different cytokines. Interleukin-4 acts on helper T cells to promote the production of T_{H2} cytokines, while interleukin-10 (IL-10) inhibits a variety of cytokines including interleukin-2 and IFN- γ in helper T cells and IL-12 in dendritic cells and macrophages.

In some embodiments, the adjuvant or vaccine composition stimulates a CD8⁺ T cell response to the antigen, when administered to a patient. In embodiments, the adjuvant or vaccine composition does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient. In some embodiments, the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient. In some embodiments, the methods described herein are where the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

In some embodiments, the present invention is related to a vaccine composition comprising a wild type IL-1 β , e.g. with an amino acid sequence of SEQ ID NO: 1, or a variant having at least about 95%, or at least about 97%, or at least about 99% identity thereto, and a targeting moiety that comprises a recognition domain that recognizes and/or binds CD8.

In some embodiments, the present invention is related to a vaccine composition comprising a mutant IL-1 β that comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1, or a variant having at least about 95%, or at least about 97%, or at least about 99% identity thereto, and a targeting moiety that comprises a recognition domain that recognizes and/or binds CD8.

In some embodiments, the antigen of the present invention is a protein or an antigenic fragment of a protein. In some embodiments, the antigen is a nucleic acid encoding a protein or an antigenic fragment of a protein. In embodiments, the nucleic acid which is an antigen or which encodes a protein or an antigenic fragment of a protein can be an mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine. In embodiments, the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.

Interleukin-1 β or a Mutant Thereof

In one aspect, the present invention provides a vaccine composition comprising a chimeric protein or a chimeric protein complex that includes a wild type or engineered/mutant interleukin-1 β . IL-1 β is a proinflammatory cytokine and an important immune system regulator. It is a potent activator of CD4 T cell responses, increases proportion of Th17 cells and expansion of IFN γ and IL-4 producing cells. IL-1 β is also a potent regulator of CD8⁺ T cells, enhancing antigen-

specific CD8⁺ T cell expansion, differentiation, migration to periphery and memory. IL-1 receptors comprise IL-1R1 and IL-1RACP. Binding to and signaling through the IL-1R1 constitutes the mechanism whereby IL-1 β mediates many of its biological (and pathological) activities. IL-1R2 can function as a decoy receptor, thereby reducing IL-1 β availability for interaction and signaling through the IL-1R1.

5 In various embodiments, the present invention provides a vaccine composition that comprises a chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes, that include the mutant IL-1 β fused to one or more targeting moieties. In some embodiments, the mutant IL-1 β is human IL-1 β . In some embodiments, the mutant IL-1 β has low affinity and/or activity for IL-1 receptor. In some embodiments, the mutant IL-1 β has substantially reduced or ablated affinity and/or activity for IL-1 receptor. In some embodiments, the low affinity or activity of mutant IL-1 β at
10 the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

In an embodiment, the wild type IL-1 β has the amino acid sequence of:

IL-1 beta (mature form, wild type) (SEQ ID NO: 1)

APVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVWFMSFVQGEES
15 NDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWIYSTSQAENMPVFLGGTKGGQDITDFTMQFVSS

In some embodiments, the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1. In some embodiments, the mutant IL-1 β has a deletion of amino acids at positions 52-54 which produces a modified human IL-1 β with reduced binding affinity for type I IL-1R and reduced biological activity. See, for
20 example, WO 1994/000491, the entire contents of which are hereby incorporated by reference. In some embodiments, the mutant IL-1 β has one or more substitution mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, N245Q (where X can be any change in amino
25 acid, e.g., a non-conservative change), which exhibit reduced binding to IL-1R, as described, for example, in WO2015/007542 and WO/2015/007536, the entire contents of which is hereby incorporated by reference (numbering base on the human IL-1 β sequence, Genbank accession number NP_000567, version NP-000567.1, GI: 10835145). In some embodiments, the modified human IL-1 β may have one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q,
30 E221S and E221K. In an embodiment, the modified human IL-1 β comprises the mutations Q131G and Q148G. In an embodiment, the modified human IL-1 β comprises the mutations Q148G and K208E. In an embodiment, the modified human IL-1 β comprises the mutations R120G and Q131G. In an embodiment, the modified human IL-1 β comprises the mutations R120G and H146A. In an embodiment, the modified human IL-1 β comprises the mutations R120G and

H146N. In an embodiment, the modified human IL-1 β comprises the mutations R120G and H146R. In an embodiment, the modified human IL-1 β comprises the mutations R120G and H146E. In an embodiment, the modified human IL-1 β comprises the mutations R120G and H146G. In an embodiment, the modified human IL-1 β comprises the mutations R120G and K208E. In an embodiment, the modified human IL-1 β comprises the mutations R120G, F162A, and Q164E.

5 In one embodiment, the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.

In various embodiments, the mutations allow for IL-1 β to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmodified or unmutated, *i.e.*, the wild type form of IL-1 β (*e.g.* comparing IL-1 β in a wild type form versus a modified (*e.g.* mutant) form). In some embodiments, the mutations that attenuate or reduce binding or affinity include those mutations that

10 substantially reduce or ablate binding or activity. In some embodiments, the mutations that attenuate or reduce binding or affinity are different than those mutations which substantially reduce or ablate binding or activity. Consequentially, in various embodiments, the mutations allow for IL-1 β to have improved safety, *e.g.* have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated, *i.e.* wild type, IL-1 β (*e.g.* comparing IL-1 β in a wild type form versus a modified (*e.g.* mutant) form).

15 In various embodiments, IL-1 β is modified to have one or more mutations that reduce its binding affinity or activity for one or more of its receptors. In some embodiments, IL-1 β is modified to have one or more mutations that substantially reduce or ablate binding affinity or activity for the receptors. In some embodiments, the activity provided by the wild type IL-1 β is agonism at the receptor (*e.g.* activation of a cellular effect at a site of therapy). For example, the wild type IL-1 β may activate its receptor. In such embodiments, the mutations result in the modified IL-1 β to have reduced or

20 ablated activating activity at the receptor. For example, the mutations may result in the modified IL-1 β to deliver a reduced activating signal to a target cell or the activating signal could be ablated.

In some embodiments, the reduced affinity or activity of IL-1 β at the receptor is restorable by attachment with one or more of the targeting moieties. In other embodiments, the reduced affinity or activity of IL-1 β at the receptor is not substantially restorable by the activity of one or more of the targeting moieties.

25 In various embodiments, the chimeric proteins of the present invention reduce off-target effects because the IL-1 β has mutations that weaken or ablate binding affinity or activity at a receptor. In various embodiments, this reduction in side effects is observed relative with, for example, the wild type IL-1 β . In various embodiments, the IL-1 β is active on target cells because the targeting moiety(ies) compensates for the missing/insufficient binding (*e.g.*, without limitation and/or avidity) required for substantial activation. In various embodiments, the modified IL-1 β is substantially inactive *en route*

30 to the site of therapeutic activity and has its effect substantially on specifically targeted cell types that greatly reduces undesired side effects.

In various embodiments, substantially reducing or ablating binding or activity at the receptor causes the therapeutic effect of IL-1 β to improve as there is a reduced or eliminated sequestration of the therapeutic chimeric proteins away from the site of therapeutic action. For instance, in some embodiments, this obviates the need of high doses of the present vaccine compositions that compensate for loss at the other receptor. Such ability to reduce dose further provides a lower likelihood of side effects.

In various embodiments, the modified IL-1 β comprises one or more mutations that cause IL-1 β to have reduced, substantially reduced, or ablated affinity, e.g. binding (e.g. K_D) and/or activation. In various embodiments, the reduced affinity at IL-1 β 's receptor allows for attenuation of activity (inclusive of agonism or antagonism). In such embodiments, the modified IL-1 β has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the receptor relative to the wild type IL-1 β . In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower relative to the wild type IL-1 β .

In various embodiments, the modified IL-1 β comprises one or more mutations that reduce the endogenous activity of IL-1 β to about 75%, or about 70%, or about 60%, or about 50%, or about 40%, or about 30%, or about 25%, or about 20%, or about 10%, or about 5%, or about 3%, or about 1%, e.g., relative to the wild type IL-1 β . In some embodiments, the modified IL-1 β comprises one or more mutations that cause IL-1 β to have reduced affinity for its receptor that is lower than the binding affinity of the targeting moiety(ies) for its(their) receptor(s). In some embodiments, this binding affinity differential is between IL-1 β /receptor and targeting moiety/receptor on the same cell. In some embodiments, this binding affinity differential allows for mutant IL-1 β to have localized, on-target effects and to minimize off-target effects that underlie side effects that are observed with wild type IL-1 β . In some embodiments, this binding affinity is at least about 2-fold, or at least about 5-fold, or at least about 10-fold, or at least about 15-fold lower, or at least about 25-fold, or at least about 50-fold lower, or at least about 100-fold, or at least about 150-fold.

Receptor binding activity may be measured using methods known in the art. For example, affinity and/or binding activity may be assessed by Scatchard plot analysis and computer-fitting of binding data (e.g. Scatchard, 1949) or by reflectometric interference spectroscopy under flow through conditions, as described by Brecht *et al.* (1993), the entire contents of all of which are hereby incorporated by reference.

In various embodiments the modified IL-1 β comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the known wild type IL-1 β (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments the modified IL-1 β comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any of amino acid sequences of the wildtype IL-1 β disclosed herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments, the modified IL-1 β comprises an amino acid sequence having one or more amino acid mutations. In some embodiments, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations. In some embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions.

“Conservative substitutions” may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

As used herein, “conservative substitutions” are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices.

As used herein, “non-conservative substitutions” are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

In various embodiments, the substitutions may also include non-classical amino acids (e.g. selenocysteine, pyrrolysine, *N*-formylmethionine β -alanine, GABA and δ -Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general).

In some embodiments, the reduced affinity or activity of the modified IL-1 β at the therapeutic receptor is inducible or restorable by attachment to a targeting moiety or upon inclusion of a targeting moiety in a chimeric protein or a chimeric protein complex, e.g., a Fc-based chimeric protein complex as disclosed herein. In some embodiments, the activity of IL-1 β is reduced or attenuated by virtue of its fusion with another protein, including, in some instances, by fusion with targeting moieties as described herein. In other embodiments, the activity of IL-1 β is reduced or attenuated by modifying the IL-1 β , e.g., by introducing mutations as described herein. In some embodiments, attenuation of the activity can be restored by attaching the IL-1 β to a targeting moiety or by the action of the attached targeting moiety. In embodiments, the targeting moiety—by virtue of its attachment or by its activity—induces IL-1 β ’s activity. In some embodiments, the reduced affinity or activity at the receptor is inducible or restorable by attachment with one or more of the targeting moieties as described herein or upon inclusion in the chimeric protein complexes, such as Fc-based chimeric protein complex disclosed herein.

Targeting Moiety

In various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes, such as Fc-based chimeric protein complexes of the present invention comprise one or more targeting moieties having recognition domains which specifically bind to a target (e.g. antigen or receptor of interest). In some embodiments, the adjuvants, chimeric proteins,

or chimeric protein complexes, may comprise two, three, four, five, six, seven, eight, nine, ten or more targeting moieties. In illustrative embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes comprise two or more targeting moieties. In such embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes can target two different cells (e.g. to make a synapse) or the same cell (e.g. to get a more concentrated signaling agent effect).

In various embodiments, the target (e.g. antigen, receptor) of interest can be found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophages (e.g. M1 or M2 macrophages), B cells, Breg cells, dendritic cells, or subsets thereof. In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) of interest and effectively, directly or indirectly, recruit one of more immune cells. In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes may directly or indirectly recruit an immune cell, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a therapeutic effect). In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes may directly or indirectly recruit an immune cell, e.g. an immune cell that can kill an infectious agent and/or suppress an infection, to a site of action.

In various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes such as Fc-based chimeric protein complexes have targeting moieties having recognition domains which specifically bind to a target (e.g. antigen, receptor) which is part of a non-cellular structure. In some embodiments, the antigen or receptor is not an integral component of an intact cell or cellular structure. In some embodiments, the antigen or receptor is an extracellular antigen or receptor. In some embodiments, the target is a non-proteinaceous, non-cellular marker, including, without limitation, nucleic acids, inclusive of DNA or RNA, such as, for example, DNA released from necrotic cells or extracellular deposits such as cholesterol.

In some embodiments, the target (e.g. antigen, receptor) of interest is part of the non-cellular component of the stroma or the extracellular matrix (ECM) or the markers associated therewith. As used herein, stroma refers to the connective and supportive framework of a tissue or organ. Stroma may include a compilation of cells such as fibroblasts/myofibroblasts, glial, epithelia, fat, immune, vascular, smooth muscle, and immune cells along with the extracellular matrix (ECM) and extracellular molecules. In various embodiments, the target (e.g. antigen, receptor) of interest is part of the non-cellular component of the stroma such as the extracellular matrix and extracellular molecules. As used herein, the ECM refers to the non-cellular components present within all tissues and organs. The ECM is composed of a large collection of biochemically distinct components including, without limitation, proteins, glycoproteins, proteoglycans, and polysaccharides. These components of the ECM are usually produced by adjacent cells and secreted into the ECM via exocytosis. Once secreted, the ECM components often aggregate to form a complex network of macromolecules. In various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes of the invention comprises a targeting moiety that recognizes a target (e.g., an antigen or receptor or non-proteinaceous molecule) located on any component of the ECM. Illustrative components of the ECM include, without

limitation, the proteoglycans, the non-proteoglycan polysaccharides, fibers, and other ECM proteins or ECM non-proteins, *e.g.* polysaccharides and/or lipids, or ECM associated molecules (*e.g.* proteins or non-proteins, *e.g.* polysaccharides, nucleic acids and/or lipids).

In some embodiments, the targeting moiety recognizes one or more ECM proteins including, but not limited to, a
5 tenascin, a fibronectin, a fibrin, a laminin, or a nidogen/entactin.

In various embodiments, the targeting moiety may bind to the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or any other naturally occurring or synthetic analogs, variants, or mutants of any of the targets described herein. In various embodiments, the targeting moiety may bind to any forms of the proteins described herein, including monomeric, dimeric, trimeric, tetrameric, heterodimeric, multimeric and associated forms.

10 In various embodiments, the targeting moiety may bind to any post-translationally modified forms of the proteins described herein, such as glycosylated and/or phosphorylated forms.

In various embodiments, the targeting moiety comprises an antigen recognition domain that recognizes extracellular molecules such as DNA. In some embodiments, the targeting moiety comprises an antigen recognition domain that recognizes DNA. In an embodiment, the DNA is shed into the extracellular space from necrotic or apoptotic cells or
15 other diseased cells.

In some embodiments, the adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes of the invention may have two or more targeting moieties that bind to non-cellular structures. In some embodiments, there are two targeting moieties and one targets a cell while the other targets a non-cellular structure. In various embodiments, the targeting moieties can directly or indirectly recruit cells, such as disease cells
20 and/or effector cells. In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes are capable of, or find use in methods involving, shifting the balance of immune cells in favor of immune attack of an infection. For instance, the adjuvants, chimeric proteins, or chimeric protein complexes can shift the ratio of immune cells at a site of clinical importance in favor of cells that can kill and infectious agent and/or suppress an infection (*e.g.* T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophages (*e.g.* M1 macrophages), B cells, dendritic cells, or subsets thereof) and in opposition to cells that reduce immunity (*e.g.* myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs); tumor associated neutrophils (TANs), M2 macrophages, tumor associated macrophages (TAMs), or subsets thereof). In some embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes are capable of increasing a ratio of effector T cells to regulatory T cells.

30 For example, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with T cells. In some embodiments, the recognition domains directly or indirectly recruit T cells. In an embodiment, the recognition domains specifically bind to effector T cells. In some embodiments, the recognition domain directly or indirectly recruits effector T cells, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or

more disease cell or cell to be modulated for a therapeutic effect). Illustrative effector T cells include cytotoxic T cells (e.g. $\alpha\beta$ TCR, CD3⁺, CD8⁺, CD45RO⁺); CD4⁺ effector T cells (e.g. $\alpha\beta$ TCR, CD3⁺, CD4⁺, CCR7⁺, CD62Lhi, IL-7R/CD127⁺); CD8⁺ effector T cells (e.g. $\alpha\beta$ TCR, CD3⁺, CD8⁺, CCR7⁺, CD62Lhi, IL-7R/CD127⁺); effector memory T cells (e.g. CD62Llow, CD44⁺, TCR, CD3⁺, IL-7R/CD127⁺, IL-15R⁺, CCR7low); central memory T cells (e.g. CCR7⁺, CD62L⁺, CD27⁺; or CCR7hi, CD44⁺, CD62Lhi, TCR, CD3⁺, IL-7R/CD127⁺, IL-15R⁺); CD62L⁺ effector T cells; CD8⁺ effector memory T cells (TEM) including early effector memory T cells (CD27⁺ CD62L⁻) and late effector memory T cells (CD27⁻ CD62L⁻) (TemE and TemL, respectively); CD127⁽⁺⁾CD25^(low/-) effector T cells; CD127⁽⁻⁾CD25⁽⁻⁾ effector T cells; CD8⁺ stem cell memory effector cells (TSCM) (e.g. CD44^(low)CD62L^(high)CD122^(high)sca⁽⁺⁾); TH1 effector T-cells (e.g. CXCR3⁺, CXCR6⁺ and CCR5⁺; or $\alpha\beta$ TCR, CD3⁺, CD4⁺, IL-12R⁺, IFN γ R⁺, CXCR3⁺), TH2 effector T cells (e.g. CCR3⁺, CCR4⁺ and CCR8⁺; or $\alpha\beta$ TCR, CD3⁺, CD4⁺, IL-4R⁺, IL-33R⁺, CCR4⁺, IL-17RB⁺, CRT2⁺); TH9 effector T cells (e.g. $\alpha\beta$ TCR, CD3⁺, CD4⁺); TH17 effector T cells (e.g. $\alpha\beta$ TCR, CD3⁺, CD4⁺, IL-23R⁺, CCR6⁺, IL-1R⁺); CD4⁺CD45RO⁺CCR7⁺ effector T cells, ICOS⁺ effector T cells; CD4⁺CD45RO⁺CCR7⁽⁻⁾ effector T cells; and effector T cells secreting IL-2, IL-4 and/or IFN- γ .

Illustrative T cell antigens of interest include, for example (and inclusive of the extracellular domains, where applicable): CD8, CD3, SLAMF4, IL-2R α , 4-1BB/TNFRSF9, IL-2 R β , ALCAM, B7-1, IL-4 R, B7-H3, BLAME/SLAMFS, CEACAM1, IL-6 R, CCR3, IL-7 R α , CCR4, CXCR1/IL-S RA, CCR5, CCR6, IL-10R α , CCR 7, IL-10 R β , CCR8, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13 R α 1, IL-13, CD3, CD4, ILT2/CDS5j, ILT3/CDS5k, ILT4/CDS5d, ILT5/CDS5a, Luteal α 4/CD49d, CDS, Integrin α E/CD103, CD6, Integrin α M/CD 11 b, CDS, Integrin α X/CD11c, Integrin β 2/CDIS, KIR/CD15S, CD27/TNFRSF7, KIR2DL1, CD2S, KIR2DL3, CD30/TNFRSF8, KIR2DL4/CD15Sd, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CDS3, Leukotriene B4-R1, CDS4/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ , Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fc γ RIII/CD16, TIM-6, TNFR1/TNFRSF1A, Granulysin, TNF RIII/TNFRSF1B, TRAIL R1/TNFRSF10A, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAILR3/TNFRSF10C, IFN- γ R1, TRAILR4/TNFRSF10D, IFN- γ R2, TSLP, IL-1 R1 and TSLP R. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative T cell antigens.

By way of non-limiting example, in various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes have a targeting moiety directed against a checkpoint marker expressed on a T cell, e.g. one or more of PD-1, CD28, CTLA4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, TIM3, and A2aR.

For example, in some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with B cells. In some embodiments, the recognition domains directly or indirectly recruit B cells, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a

therapeutic effect). Illustrative B cell antigens of interest include, for example, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138, CDw150, CS1, and B-cell maturation antigen (BCMA). In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative B cell antigens.

By way of further example, in some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with Natural Killer cells. In some embodiments, the recognition domains directly or indirectly recruit Natural Killer cells, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative Natural Killer cell antigens of interest include, for example TIGIT, 2B4/SLAMF4, KIR2DS4, CD155/PVR, KIR3DL1, CD94, LMIR1/CD300A, CD69, LMIR2/CD300c, CRACC/SLAMF7, LMIR3/CD300LF, DNAM-1, LMIR5/CD300LB, Fc-epsilon RII, LMIR6/CD300LE, Fc-gamma RI/CD64, MICA, Fc-gamma RIIB/CD32b, MICB, Fc-gamma RIIC/CD32c, MULT-1, Fc-gamma RIIA/CD32a, Nectin-2/CD112, Fc-gamma RIII/CD16, NKG2A, FcRH1/IRTA5, NKG2C, FcRH2/IRTA4, NKG2D, FcRH4/IRTA1, NKp30, FcRH5/IRTA2, NKp44, Fc-Receptor-like 3/CD16-2, NKp46/NCR1, NKp80/KLRF1, NTB-A/SLAMF6, Rae-1, Rae-1 alpha, Rae-1 beta, Rae-1 delta, H60, Rae-1 epsilon, ILT2/CD85j, Rae-1 gamma, ILT3/CD85k, TREM-1, ILT4/CD85d, TREM-2, ILT5/CD85a, TREM-3, KIR/CD158, TREML1/TLT-1, KIR2DL1, ULBP-1, KIR2DL3, ULBP-2, KIR2DL4/CD158d and ULBP-3. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative NK cell antigens.

Also, in some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with macrophages/monocytes. In some embodiments, the recognition domains directly or indirectly recruit macrophages/monocytes, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative macrophages/monocyte antigens of interest include, for example SIRP1a, B7-1/CD80, ILT4/CD85d, B7-H1, ILT5/CD85a, Common beta Chain, Integrin alpha 4/CD49d, BLAME/SLAMF8, Integrin alpha X/CD11c, CCL6/C10, Integrin beta 2/CD18, CD155/PVR, Integrin beta 3/CD61, CD31/PECAM-1, Latexin, CD36/SR-B3, Leukotriene B4 R1, CD40/TNFRSF5, LIMP3/SR-B2, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68, LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163, LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2, EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc-gamma RI/CD64, Osteopontin, Fc-gamma RIIB/CD32b, PD-L2, Fc-gamma RIIC/CD32c, Siglec-3/CD33, Fc-gamma RIIA/CD32a, SIGNR1/CD209, Fc-gamma RIII/CD16, SLAM, GM-CSF R alpha, TCCR/WSX-1, ICAM-2/CD102, TLR3, IFN-gamma RI, TLR4, IFN-gamma R2, TREM-1, IL-1 RII, TREM-2, ILT2/CD85j, TREM-3, ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF 4, IL-10 R alpha, ALCAM, IL-10 R beta, AminopeptidaseN/ANPEP, ILT2/CD85j, Common beta Chain, ILT3/CD85k, Clq R1/CD93, ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, Integrin alpha 4/CD49d, CCR5, Integrin alpha M/CD11b, CCR8, Integrin alpha X/CD11c, CD155/PVR, Integrin beta 2/CD18, CD14, Integrin beta 3/CD61, CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4-

R1, CD68, LIMP11SR-B2, CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, LMIR3/CD300LF, Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1, LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1, MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc-γ RI/CD64, PSGL-1, Fc-γ RIICD16, RP105, G-CSF R, L-Selectin, GM-CSF R α, Siglec-3/CD33, HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-1, IL-6 R, TREM-2, CXCR1/IL-8 RA, TREM-3 and TREML1/TLT-1. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative macrophage/monocyte antigens.

Also, in some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with dendritic cells. In some embodiments, the recognition domains directly or indirectly recruit dendritic cells, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative dendritic cell antigens of interest include, for example, CLEC9A, XCR1, RANK, CD36/SRB3, LOX-1/SR-E1, CD68, MARCO, CD163, SR-A1/MSR, CD5L, SREC-1, CL-PI/COLEC12, SREC-II, LIMP11SRB2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-IBB Ligand/TNFSF9, IL-12/IL-23 p40, 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6Ckine, ILT3/CD85k, 8-oxo-dG, ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, lutealrin α 4/CD49d, Aαg, Integrin β 2/CD18, AMICA, Langerin, B7-2/CD86, Leukotriene B4 RI, B7-H3, LMIR1/CD300A, BLAME/SLAMF8, LMIR2/CD300c, C1q R1/CD93, LMIR3/CD300LF, CCR6, LMIR5/CD300LB CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4-a, CD43, MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR, CD97, NCAMLI, CD2F-10/SLAMF9, Osteoactivin GPNMB, Chern 23, PD-L2, CLEC-1, RP105, CLEC-2, CLEC-8, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC/CLEC4C, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc-γ R1/CD64, TLR3, Fc-γ RIIB/CD32b, TREM-1, Fc-γ RIIC/CD32c, TREM-2, Fc-γ RIIA/CD32a, TREM-3, Fc-γ RIIC/CD16, TREML1/TLT-1, ICAM-2/CD102 and Vanilloid R1. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative DC antigens.

In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) on immune cells selected from, but not limited to, megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, myeloid cells, monocytes, eosinophils, or subsets thereof. In some embodiments, the recognition domains directly or indirectly recruit megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, myeloid cells, monocytes, eosinophils, or subsets thereof, e.g., in some embodiments, to a therapeutic site (e.g. a locus with one or more disease cell or cell to be modulated for a therapeutic effect). In some embodiments, the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with megakaryocytes and/or thrombocytes. Illustrative megakaryocyte and/or thrombocyte antigens of interest include, for

example, GP IIb/IIIa, GPIb, vWF, PF4, and TSP. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative megakaryocyte and/or thrombocyte antigens.

5 In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with erythrocytes. Illustrative erythrocyte antigens of interest include, for example, CD34, CD36, CD38, CD41a (platelet glycoprotein IIb/IIIa), CD41b (GPIIb), CD71 (transferrin receptor), CD105, glycophorin A, glycophorin C, c-kit, HLA-DR, H2 (MHC-II), and Rhesus antigens. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these illustrative erythrocyte antigens.

10 In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with mast cells. Illustrative mast cells antigens of interest include, for example, SCFR/CD117, Fc ϵ RI, CD2, CD25, CD35, CD88, CD203c, C5R1, CMAI, FCERIA, FCER2, TPSAB1. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these mast cell antigens.

15 In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with basophils. Illustrative basophils antigens of interest include, for example, Fc ϵ RI, CD203c, CD123, CD13, CD107a, CD107b, and CD164. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these basophil antigens.

20 In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with neutrophils. Illustrative neutrophils antigens of interest include, for example, 7D5, CD10/CALLA, CD13, CD16 (FcRIII), CD18 proteins (LFA-1, CR3, and p150, 95), CD45, CD67, and CD177. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these neutrophil antigens.

In some embodiments, the recognition domains specifically bind to a target (e.g. antigen, receptor) associated with eosinophils. Illustrative eosinophils antigens of interest include, for example, CD35, CD44 and CD69. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of these eosinophil antigens.

25 In various embodiments, the recognition domain may bind to any appropriate target, antigen, receptor, or cell surface markers known by the skilled artisan. In some embodiments, the antigen or cell surface marker is a tissue-specific marker. Illustrative tissue-specific markers include, but are not limited to, endothelial cell surface markers such as ACE, CD14, CD34, CDH5, ENG, ICAM2, MCAM, NOS3, PECAMI, PROCR, SELE, SELP, TEK, THBD, VCAMI, VWF; smooth muscle cell surface markers such as ACTA2, MYH10, MYH11, MYH9, MYOCD; fibroblast (stromal) cell surface markers such as ALCAM, CD34, COL1A1, COL1A2, COL3A1, FAP, PH-4; epithelial cell surface markers such as CDID, K6IRS2, KRT10, KRT13, KRT17, KRT18, KRT19, KRT4, KRT5, KRT8, MUC1, TACSTD1; neovasculature markers such as CD13, TFNA, Alpha-v beta-3 ($\alpha_v\beta_3$), E-selectin; and adipocyte surface markers such as ADIPOQ, FABP4, and RETN. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes

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binds one or more of these antigens. In various embodiments, a targeting moiety of the adjuvants, chimeric proteins, or chimeric protein complexes binds one or more of cells having these antigens.

By way of non-limiting example, in various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to CD8, SLAMF4, IL-2 R α , 4-1BB/TNFRSF9, IL-2 R β , ALCAM, B7-1, IL-4 R, B7-H3, BLAME/SLAMFS, CEACAM1, IL-6 R, CCR3, IL-7 R α , CCR4, CXCR1/IL-8 RA, CCR5, CCR6, IL-10R α , CCR 7, IL-10 R β , CCR8, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13 R α 1, IL-13, CD3, CD4, ILT2/CDS5j, ILT3/CDS5k, ILT4/CDS5d, ILT5/CDS5a, Lutein α 4/CD49d, CDS, Integrin α E/CD103, CD6, Integrin α M/CD 11 b, CDS, Integrin α X/CD11c, Integrin β 2/CD18, KIR/CD15S, CD27/TNFRSF7, KIR2DL1, CD2S, KIR2DL3, CD30/TNFRSF8, KIR2DL4/CD15Sd, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFRSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CDS3, Leukotriene B4-R1, CDS4/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ , Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFRSF6, TIM-4, Fc γ RIII/CD16, TIM-6, TNFR1/TNFRSF1A, Granulysin, TNF RIII/TNFRSF1B, TRAIL RI/TNFRSF10A, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAILR3/TNFRSF10C, IFN- γ R1, TRAILR4/TNFRSF10D, IFN- γ R2, TSLP, IL-1 R1, or TSLP R.

By way of non-limiting example, in various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to CLEC-9A, XCR1, RANK, CD36/SR-B3, LOX-1/SR-E1, CD68, MARCO, CD163, SR-A1/MSR, CD5L, SREC-1, CL-PI/COLEC12, SREC-II, LIMP1/SR-B2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-1BB Ligand/TNFRSF9, IL-12/IL-23 p40, 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6CKine, ILT3/CD85k, 8-oxo-dG, ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, Lutein α 4/CD49d, Aag, Integrin β 2/CD18, AMICA, Langerin, B7-2/CD86, Leukotriene B4 RI, B7-H3, LMIR1/CD300A, BLAME/SLAMF8, LMIR2/CD300c, Clq R1/CD93, LMIR3/CD300LF, CCR6, LMIR5/CD300LB, CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4-a, CD43, MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR, CD97, NCAM1, CD2F-10/SLAMF9, Osteoactivin GPNMB, Chern 23, PD-L2, CLEC-1, RP105, CLEC-2, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc γ R1/CD64, TLR3, Fc γ RIIB/CD32b, TREM-1, Fc γ RIIC/CD32c, TREM-2, Fc γ RIIA/CD32a, TREM-3, Fc γ RIII/CD16, TREML1/TLT-1, ICAM-2/CD102, or Vanilloid R1.

By way of non-limiting example, in various embodiments, the adjuvants, chimeric proteins, or chimeric protein complexes have (i) a targeting moiety directed against a monocyte/macrophage, for example, mediated by targeting to SIRP1a, B7-1/CD80, ILT4/CD85d, B7-H1, ILT5/CD85a, Common β Chain, Integrin α 4/CD49d, BLAME/SLAMF8, Integrin α X/CD11c, CCL6/C10, Integrin β 2/CD18, CD155/PVR, Integrin β 3/CD61, CD31/PECAM-1, Latexin, CD36/SR-

B3, Leukotriene B4 R1, CD40/TNFRSF5, LIMP1/CD300A, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68, LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163, LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2, EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc-γ RI/CD64, Osteopontin, Fc-γ RIIB/CD32b, PD-L2, Fc-γ RIIC/CD32c, Siglec-3/CD33, Fc-γ RIIA/CD32a, 5 SIGNR1/CD209, Fc-γ RIIC/CD16, SLAM, GM-CSF R α, TCCR/WSX-1, ICAM-2/CD102, TLR3, IFN-γ RI, TLR4, IFN-γ R2, TREM-I, IL-1 RII, TREM-2, ILT2/CD85j, TREM-3, ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF 4, IL-10 R α, ALCAM, IL-10 R β, AminopeptidaseN/ANPEP, ILT2/CD85j, Common β Chain, ILT3/CD85k, C1q R1/CD93, ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, CD206, Integrin α 4/CD49d, CCR5, Integrin α M/CD11b, CCR8, Integrin α X/CD11c, CD155/PVR, Integrin β 2/CD18, CD14, Integrin β 3/CD61, CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4-R1, CD68, 10 LIMP1/CD300A, CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, CD163, LMIR3/CD300LF, Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1, LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1, MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc-γ RI/CD64, PSGL-1, Fc-γ RIIC/CD16, RP105, G-CSF R, L-Selectin, GM-CSF R α, Siglec-3/CD33, HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-I, IL-6 R, TREM-2, CXCR1/IL-8 RA, TREM-3, or TREML1/TLT-1.

15 In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a T cell and one or more targeting moieties directed against the same or another T cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a T cell and one or more targeting moieties directed against a B cell. In one embodiment, the present adjuvants, 20 chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a T cell and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a T cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as 25 Fc-based chimeric protein complex comprises one or more targeting moieties against a T cell and one or more targeting moieties directed against a NK cell.

In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against the same or another B cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric 30 protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against a T cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein

complex comprises one or more targeting moieties against a B cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a B cell and one or more targeting moieties directed against a NK cell.

5 In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against the same or another dendritic cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against a T cell. In one
10 embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against a B cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a dendritic cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present adjuvants, chimeric
15 proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a dendritic cell and one or more targeting moieties directed against a NK cell.

In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against the same or another macrophage. In one embodiment, the present adjuvants, chimeric
20 proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against a T cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against a B cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes
25 such as Fc-based chimeric protein complex comprises one or more targeting moieties against a macrophage and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a macrophage and one or more targeting moieties directed against a NK cell.

In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric
30 protein complex comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against the same or another NK cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against a T cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex

comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against a B cell. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against an NK cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against an NK cell and one or more targeting moieties directed against a dendritic cell.

In some embodiments, the adjuvants, chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention comprises one or more targeting moieties having recognition domains that bind to a target (e.g. antigen, receptor) of interest including those found on one or more cells selected from adipocytes (e.g., white fat cell, brown fat cell), liver lipocytes, hepatic cells, kidney cells (e.g., kidney parietal cell, kidney salivary gland, mammary gland, *etc.*), duct cells (of seminal vesicle, prostate gland, *etc.*), intestinal brush border cells (with microvilli), exocrine gland striated duct cells, gall bladder epithelial cells, ductulus efferens nonciliated cells, epididymal principal cells, epididymal basal cells, endothelial cells, ameloblast epithelial cells (tooth enamel secretion), planum semilunatum epithelial cells of vestibular system of ear (proteoglycan secretion), organ of Corti interdental epithelial cells (secreting tectorial membrane covering hair cells), loose connective tissue fibroblasts, corneal fibroblasts (corneal keratocytes), tendon fibroblasts, bone marrow reticular tissue fibroblasts, nonepithelial fibroblasts, pericytes, nucleus pulposus cells of intervertebral disc, cementoblasts/cementocytes (tooth root bonelike ewan cell secretion), odontoblasts/odontocytes (tooth dentin secretion), hyaline cartilage chondrocytes, fibrocartilage chondrocytes, elastic cartilage chondrocytes, osteoblasts/osteocytes, osteoprogenitor cells (stem cell of osteoblasts), hyalocytes of vitreous body of eye, stellate cells of perilymphatic space of ear, hepatic stellate cells (Ito cell), pancreatic stelle cells, skeletal muscle cells, satellite cells, heart muscle cells, smooth muscle cells, myoepithelial cells of iris, myoepithelial cells of exocrine glands, exocrine secretory epithelial cells (e.g., salivary gland cells, mammary gland cells, lacrimal gland cells, sweat gland cells, sebaceous gland cells, prostate gland cells, gastric glad cells, pancreatic acinar cells, pneumocytes), a hormone secreting cells (e.g., pituitary cells, neurosecretory cells, gut and respiratory tract cells, thyroid gland cells, parathyroid glad cells, adrenal gland cells, Leydig cells of testes, pancreatic islet cells), keratinizing epithelial cells, wet stratified barrier epithelial cells, neuronal cells (e.g., sensory transducer cells, autonomic neuron cells, sense organ and peripheral neuron supporting cells, and central nervous system neurons and glial cells such as interneurons, principal cells, astrocytes, oligodendrocytes, and ependymal cells).

Targeting Moiety Formats

In various embodiments, the targeting moiety of the vaccine composition, adjuvants, chimeric protein, or chimeric protein complex described herein is a protein-based agent capable of specific binding, such as an antibody or derivatives thereof. In an embodiment, the targeting moiety comprises an antibody. In some embodiments, the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell. In some

embodiments, the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof. In one embodiment, the immune cell is a T cell.

In various embodiments, the antibody is a full-length multimeric protein that includes two heavy chains and two light chains. Each heavy chain includes one variable region (*e.g.*, V_H) and at least three constant regions (*e.g.*, CH₁, CH₂ and CH₃), and each light chain includes one variable region (V_L) and one constant region (C_L). The variable regions determine the specificity of the antibody. Each variable region comprises three hypervariable regions also known as complementarity determining regions (CDRs) flanked by four relatively conserved framework regions (FRs). The three CDRs, referred to as CDR1, CDR2, and CDR3, contribute to the antibody binding specificity. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody.

In some embodiments, the targeting moiety comprises antibody derivatives or formats. In some embodiments, the targeting moiety of the vaccine composition described herein is a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (cysteine knot protein, knottin), a DARPin; a Tetranectin; an Affibody; a Transbody; an Anticalin; an AdNectin; an Affilin; a Microbody; a peptide aptamer; an alterases; a plastic antibodies; a phylomer; a stradobodies; a maxibodies; an evibody; a fynomer, an armadillo repeat protein, a Kunitz domain, an avimer, an atrimer, a probody, an immunobody, a triomab, a troybody; a pepbody; a vaccibody, a UniBody; affimers, a DuoBody, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, or a synthetic molecule, as described in US Patent Nos. or Patent Publication Nos. US 7,417,130, US 2004/132094, US 5,831,012, US 2004/023334, US 7,250,297, US 6,818,418, US 2004/209243, US 7,838,629, US 7,186,524, US 6,004,746, US 5,475,096, US 2004/146938, US 2004/157209, US 6,994,982, US 6,794,144, US 2010/239633, US 7,803,907, US 2010/119446, and/or US 7,166,697, the contents of which are hereby incorporated by reference in their entireties. See also, Storz MAbs. 2011 May-Jun; 3(3): 310–317.

In one embodiment, the targeting moiety comprises a single-domain antibody, such as VHH from, for example, an organism that produces VHH antibody such as a camelid, a shark, or a designed VHH. VHHs are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. VHH technology is based on fully functional antibodies from camelids that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH₂ and CH₃). VHHs are commercially available under the trademark of NANOBODY or NANOBODIES.

In an embodiment, the targeting moiety comprises a VHH. In some embodiments, the VHH is a humanized VHH or camelized VHH.

In some embodiments, the VHH comprises a fully human VH domain, *e.g.* a HUMABODY (Crescendo Biologics, Cambridge, UK). In some embodiments, fully human VH domain, *e.g.* a HUMABODY is monovalent, bivalent, or trivalent. In some embodiments, the fully human VH domain, *e.g.* a HUMABODY is mono- or multi-specific such as

monospecific, bispecific, or trispecific. Illustrative fully human VH domains, e.g. a HUMABODIES are described in, for example, WO 2016/113555 and WO2016/113557, the entire disclosure of which is incorporated by reference.

In various embodiments, the targeting moiety is a protein-based agent capable of specific binding to a cell receptor, such as a natural ligand for the cell receptor. In various embodiments, the cell receptor is found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophages (e.g. M1 macrophages), B cells, dendritic cells, or subsets thereof. In some
5 embodiments, the cell receptor is found on megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, eosinophils, or subsets thereof.

In some embodiments, the targeting moiety is a natural ligand such as a chemokine. Illustrative chemokines that may
10 be included in the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention include, but are not limited to, CCL1, CCL2, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, CX3CL1, HCC-4, and LDGF-PBP.

In some embodiments, the targeting moiety is a natural ligand, such as, FMS-like tyrosine kinase 3 ligand (Flt3L) or a truncated region thereof (e.g., which is able to bind Flt3). In some embodiments, the targeting moiety is an extracellular domain of Flt3L. In some embodiments, the targeting moiety comprising a Flt3L domain, wherein the Flt3L domain is a single chain dimer, optionally where one Flt3L domain is connected to the other Flt3L domain via one or more linkers, wherein the linker is a flexible linker. In some embodiments, the targeting moiety of the present invention comprises
15 Flt3L domain, wherein the Flt3L domain is a single chain dimer and an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain. In some embodiments, the targeting moiety recognizes CD20. In some embodiments, the targeting moiety recognizes PD-L1. In some embodiments, the targeting moiety recognizes Clec9A. In some embodiments, the targeting moiety comprises a recognition domain that recognizes
20 and/or binds CD8 or CD4.
25

In an exemplary embodiment, the present vaccines, adjuvants, chimeric proteins, or chimeric protein complexes comprise a targeting moiety directed against CD8. In various embodiments, the targeting moiety directed against CD8 is a protein-based agent capable of specific binding to CD8 without functional modulation (e.g. partial or complete
30 neutralization) of CD8. In various embodiments, the chimeric protein of the invention comprises a targeting moiety having an antigen recognition domain that recognizes an epitope present on the CD8 α and/or β chains. In an embodiment, the antigen-recognition domain recognizes one or more linear epitopes on the CD8 α and/or β chains. As used herein, a linear epitope refers to any continuous sequence of amino acids present on the CD8 α and/or β

chains. In another embodiment, the antigen-recognition domain recognizes one or more conformational epitopes present on the CD8 α and/or β chains. As used herein, a conformation epitope refers to one or more sections of amino acids (which may be discontinuous) which form a three-dimensional surface with features and/or shapes and/or tertiary structures capable of being recognized by an antigen recognition domain.

5 In various embodiments, the present vaccines, adjuvants, chimeric proteins, or chimeric protein complexes comprise a targeting moiety that may bind to the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or any other naturally occurring or synthetic analogs, variants, or mutants of human CD8 α and/or β chains. In various embodiments, the targeting moiety directed against CD8 may bind to any forms of the human CD8 α and/or β chains, including monomeric, dimeric, heterodimeric, multimeric and associated forms. In an embodiment, 10 the targeting moiety directed against CD8 may bind to the monomeric form of CD8 α chain or CD8 β chain. In another embodiment, the targeting moiety directed against CD8 may bind to a homodimeric form comprised of two CD8 α chains or two CD8 β chains. In a further embodiment, the targeting moiety directed against CD8 may bind to a heterodimeric form comprised of one CD8 α chain and one CD8 β chain.

In an embodiment, the CD8 binding agent comprises a targeting moiety which is an antibody. In various embodiments, 15 the antibody is a full-length multimeric protein that includes two heavy chains and two light chains as described elsewhere herein. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody.

In some embodiments, the present vaccines, adjuvants, chimeric proteins, or chimeric protein complexes comprise a targeting moiety directed against CD8 which is a single-domain antibody, such as a VHH. The VHH may be derived 20 from, for example, an organism that produces VHH antibody such as a camelid, a shark, or the VHH may be a designed VHH. VHHs are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. VHH technology is based on fully functional antibodies from camelids that lack light chains. These heavy-chain antibodies contain a single variable domain (V_HH) and two constant domains (CH2 and CH3). VHHs are commercially available under the trademark of NANOBODY or NANOBODIES. In an embodiment, 25 the present chimeric protein comprises a VHH.

In some embodiments, the present vaccines, adjuvants, chimeric proteins, or chimeric protein complexes comprise a targeting moiety directed against CD8 which is a VHH comprising a single amino acid chain having four "framework regions" or FRs and three "complementary determining regions" or CDRs. As used herein, "framework region" or "FR" refers to a region in the variable domain, which is located between the CDRs. As used herein, "complementary 30 determining region" or "CDR" refers to variable regions in VHHs that contains the amino acid sequences capable of specifically binding to antigenic targets.

In various embodiments, the targeting moiety directed against CD8 comprises a VHH having a variable domain comprising at least one CDR1, CDR2, and/or CDR3 sequences.

In some embodiments, the targeting moiety comprises anti-CD8 antibody as described in WO 2019033043, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD8 antibody comprises
5 at least one heavy chain variable region comprising the amino acid sequence of CDR H1: GFNIKDTYIH (SEQ ID NO: 37); CDR H2: RIDPANDNTLYASKFQG (SEQ ID NO: 38); CDR H2: RIDPANDNTLYARKFQG (SEQ ID NO: 39); CDR H3: GRGYGYVFDH (SEQ ID NO: 40); or CDR H3: TRGYGYVFDT (SEQ ID NO: 41).

In some embodiments, the anti-CD8 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDR L1: SISQY (SEQ ID NO: 42); CDR L1: SISKY (SEQ ID NO: 43); CDR L2: SGSTLQ (SEQ ID
10 NO: 44); CDR L3: HNENPL (SEQ ID NO: 45); CDR L3: HNEFPV (SEQ ID NO: 46); CDR L3: HNEFPP (SEQ ID NO: 47); CDR L3: VNEFPP (SEQ ID NO: 48); CDR L3: VNEFPV (SEQ ID NO: 49).

In some embodiments, the targeting moiety comprises anti-CD8 antibody as described in WO2019023148, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD8 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDR H1: GFIFSNYG (SEQ ID NO:
15 50); CDR H2: IWYDGSNK (SEQ ID NO: 51); CDR H3: ARSYDMLTGSGDYGL (SEQ ID NO: 52). In some embodiments, the anti-CD8 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDR L1: QDITNY (SEQ ID NO: 53); CDR L2: GAS (SEQ ID NO: 553); CDR L3: QQYNNYPLT (SEQ ID NO: 54).

In some embodiments, the targeting moiety comprises anti-CD8 antibody as described in WO2015184203, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD8 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDR H1: SGYTGTDYNMH (SEQ ID
20 NO: 55); CDR H2: YIYPYTGGTGYNQKFKN (SEQ ID NO: 56); CDR H1: DFGMN (SEQ ID NO: 57); CDR H2: LIYYDGSNKFY (SEQ ID NO: 58); CDR H3: PHYDGYHHFFDS (SEQ ID NO: 59). In some embodiments, the anti-CD8 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDR L1:
25 RASEVDSYDNLMLH (SEQ ID NO: 60); CDR L2: LASNLES (SEQ ID NO: 61); CDR L3: QQNNEDPYT (SEQ ID NO: 62); CDR L1: KGSQDINNYLA (SEQ ID NO: 63); CDR L2: NTDILHT (SEQ ID NO: 64); CDR L3: YQYNNGYT (SEQ ID NO: 65).

In some embodiments, the targeting moiety comprises anti-CD8 antibody as described in WO2018170096, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD8 antibody comprises
30 at least one heavy chain variable region comprising the amino acid sequence of CDR H1: GYTFTSY (SEQ ID NO: 66); CDR H2: DPSDNY (SEQ ID NO: 67); CDR H3: PKSAYAFDVGGYAMDY (SEQ ID NO: 68). In some embodiments, the anti-CD8 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDR L1: RTSENIDSYLT (SEQ ID NO: 69); CDR L2: AATLLAD (SEQ ID NO: 70); CDR L3: QHYYSTPWT (SEQ ID NO: 71).

In some embodiments, the targeting moiety comprises anti-CD8 antibody as described in WO2014164553, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD8 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDR H1: GFNIKD (SEQ ID NO: 72); CDR H2: RIDPANDNT (SEQ ID NO: 73); CDR H3: GYGYVFDH (SEQ ID NO: 74). In some embodiments, the anti-CD8 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDR L1: RTSRISQYLA (SEQ ID NO: 75); CDR L2: SGSTLQS (SEQ ID NO: 76); CDR L3: QQHNENPLT (SEQ ID NO: 77).

In some embodiments, the CDR1 sequence is selected from:

GFTFDDYAMS (SEQ ID NO:148) or

GFTFDDYAIG (SEQ ID NO:149).

10 In some embodiments, the CDR2 sequence is selected from:

TINWNGGSAEYAEPVKG (SEQ ID NO:150) or

CIRVSDGSTYYADPVKG (SEQ ID NO:151).

In some embodiments, the CDR3 sequence is selected from:

KDADLVWYNLS (SEQ ID NO:152) or

15 KDADLVWYNLR (SEQ ID NO:153) or

AGSLYTCVQSIVVVPARPYDMDY (SEQ ID NO:154).

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:150, and SEQ ID NO:152.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:150, and SEQ ID NO:153.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:150, and SEQ ID NO:154.

20 In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:151, and SEQ ID NO:152.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:151, and SEQ ID NO:153.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:148, SEQ ID NO:151, and SEQ ID NO:154.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:150, and SEQ ID NO:152.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:150, and SEQ ID NO:153.

25 In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:150, and SEQ ID NO:154.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:151, and SEQ ID NO:152.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:151, and SEQ ID NO:153.

In various embodiments, the CD8 targeting moiety comprises SEQ ID NO:149, SEQ ID NO:151, and SEQ ID NO:154.

In various embodiments, the CD8 targeting moiety comprises an amino acid sequence selected from the following sequences:

R3HCD27 (SEQ ID NO:155)

5 QVQLQESGGGSVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSTI
NWNGGSAEYAEPVKGRFTISRDNKNTVYLQMNSLKLEDTAVYYCAKDADLV
WYNLSTGQGTQVTVSSAAAYPYDVPDYGS

or

R3HCD129 (SEQ ID NO:156)

10 QVQLQESGGGLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSTI
NWNGGSAEYAEPVKGRFTISRDNKNTVYLQMNSLKLEDTAVYYCAKDADLV
WYNLRTGQGTQVTVSSAAAYPYDVPDYGS

or

R2HCD26 (SEQ ID NO:157)

15 QVQLQESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCIR
VSDGSTYYADPVKGRFTISSDNKNTVYLQMNSLKPEDAAVYYCAAGSLYTCV
QSIVVVPARPYDMDYWGKGTQVTVSSAAAYPYDVPDYGS.

In various embodiments, the targeting moiety comprises an amino acid sequence described in US Patent Publication No. 2014/0271462, the entire contents of which are incorporated by reference. In various embodiments, the the CD8 targeting moiety comprises an amino acid sequence described in Table 0.1, Table 0.2, Table 0.3, and/or Figures 1A-12I of US Patent Publication No. 2014/0271462, the entire contents of which are incorporated by reference. In various embodiments, the the CD8 targeting moiety comprises a HCDR1 of a HCDR1 of SEQ ID NO: 158 or 159 and/or a HCDR2 of HCDR1 of SEQ ID NO: 158 or 159 and/or a HCDR3 of HCDR1 of SEQ ID NO: 158 or 159 and/or a LCDR1 of LCDR1 of SEQ ID NO: 160 and/or a LCDR2 of LCDR1 of SEQ ID NO: 160 and/or a LCDR3 of LCDR1 of SEQ ID NO: 160, as provided below.

SEQ ID NO: 158:

30 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu
Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Ile His Trp Val Arg Gln Ala
Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Asp Pro Ala Asn Asp Asn Thr Leu Tyr
Ala Ser Lys Phe Gln Gly Arg Ala Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Gly Arg Gly Tyr
Gly Tyr Tyr Val Phe Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser.

SEQ ID NO: 159:

5 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile
Ser Cys Lys Val Ser Gly Phe Asn Ile Lys Asp Thr Tyr Ile His Trp Val Gln Gln Ala
Pro Gly Lys Gly Leu Glu Trp Met Gly Arg Ile Asp Pro Ala Asn Asp Asn Thr Leu Tyr
Ala Ser Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr
Gly Tyr Tyr Val Phe Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser.

SEQ ID NO: 160:

10 Asp Val Gln Ile Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
Ile Thr Cys Arg Thr Ser Arg Ser Ile Ser Gln Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
Gly Lys Val Pro Lys Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln His Asn Glu Asn Pro Leu Thr Phe Gly
Gly Gly Thr Lys Val Glu Ile Lys.

15 In various embodiments, the CD8 binding agent comprises a VHH having a variable domain comprising at least one CDR1, CDR2, and/or CDR3 sequences.

In some embodiments, the CDR1 sequence is selected from:

GRSFSSYTLA (SEQ ID NO:162); GRTFSSYTMG (SEQ ID NO:163); GRTFSSYIMG (SEQ ID NO:164);
GRTFSSYTMG (SEQ ID NO:165); GRTSGRTFSSYTMG (SEQ ID NO:166); GRTFSSYAMG (SEQ ID NO:167);
20 GLTFSNYIMG (SEQ ID NO:168); GRTFSSYTMG (SEQ ID NO:169); GRTFSSDTMG (SEQ ID NO:170);
GLTFSNYIMG (SEQ ID NO:171); GFTLDYYGIG (SEQ ID NO:172); GHTFSSYTMG (SEQ ID NO:173); GRTFSSYVIG
(SEQ ID NO:174); GFAFDGYAIG (SEQ ID NO:175); GFAFGFFDMT (SEQ ID NO:176); GRTFSNYVIG (SEQ ID
NO:177); GSIFSINVMG (SEQ ID NO:178); GRTFSNINVIG (SEQ ID NO:179); GHTFSSYTMG (SEQ ID NO:180);
GRTFSTYPVG (SEQ ID NO:181); GRTFSNYAMG (SEQ ID NO:182); GRTFSDYRMG (SEQ ID NO:183);
25 GLTFSNYIMA (SEQ ID NO:184); GRTFSNSVMG (SEQ ID NO:185); GRTFSSYIIG (SEQ ID NO:186); GRTFSSYVMG
(SEQ ID NO:187); GGTFSSNYVMG (SEQ ID NO:188); GRTFSNYGIG (SEQ ID NO:189); GFTFDDYAIA (SEQ ID
NO:190); GRTFSSYTVA (SEQ ID NO:191); GFPFDDYAIA (SEQ ID NO:192); GRTFSSYVMG (SEQ ID NO:193);
GRTLSSNPMA (SEQ ID NO:194); GFTFDNYAIG (SEQ ID NO:195); GRAFSSYFMG (SEQ ID NO:196);
TPTFSSYNMG (SEQ ID NO:197); GFTFDDYAIA (SEQ ID NO:198); GGTFSGYIMG (SEQ ID NO:199); GRSFSSYTIA
30 (SEQ ID NO:200); GFSSDDYTIG (SEQ ID NO:201); GFTFDDYTIG (SEQ ID NO:202); GFSSDDYTIG (SEQ ID
NO:203); GFTFDQYTIA (SEQ ID NO:204); GRTFSSYAMA (SEQ ID NO:205); GFAFDGYAIG (SEQ ID NO:206);
GFSSDDYTIA (SEQ ID NO:207); GFSSDDYTIG (SEQ ID NO:208); GFTFDDYTIG (SEQ ID NO:209); GFSSDDYTIG

(SEQ ID NO:210); GFSSDDYTIG (SEQ ID NO:211); GFSFDDYAIA (SEQ ID NO:212); GFSSDDYTIG (SEQ ID NO:213); GFTGNDLAIG (SEQ ID NO:214); GFSSDDYTIA (SEQ ID NO:215); EGTLSYGIG (SEQ ID NO:216); GFSSDDYTIA (SEQ ID NO:217); GFTFDDYAIA (SEQ ID NO:218); GLSSDDYTIG (SEQ ID NO:219); GLSSDDYTIG (SEQ ID NO:220); GFSSDDYTIG (SEQ ID NO:221); GFSFDDYTIG (SEQ ID NO:222); GFTFDDYAIA (SEQ ID NO:223); GFTFDDYAIG (SEQ ID NO:224); GFTFGDYTIG (SEQ ID NO:225); EGTFSSYGIG (SEQ ID NO:226); GFSSDDYTIG (SEQ ID NO:227); GVSIGDYNIG (SEQ ID NO:228); GFTFDDYTIA (SEQ ID NO:229); GFTFDDYTIA (SEQ ID NO:300).

In some embodiments, the CDR2 sequence is selected from:

ASITWGGGNTY (SEQ ID NO:331); AATVWTGAGTV (SEQ ID NO:332); AAIGWSADITV (SEQ ID NO:333);
 10 AFIDWSGGGTY (SEQ ID NO:334); ATITWGGGSTY (SEQ ID NO:335); AAISWSGGPTV (SEQ ID NO:336);
 AAITWGGGSTV (SEQ ID NO:337); AAITWSGVSTV (SEQ ID NO:338); GAIMWSGAFTH (SEQ ID NO:339);
 AAITWGGGSTV (SEQ ID NO:340); SCISSSDRNTY (SEQ ID NO:341); AFIDWSGGGTY (SEQ ID NO:342);
 AVITWSGDSTY (SEQ ID NO:343); ACISSKDGSTY (SEQ ID NO:344); SGINSIGGSTT (SEQ ID NO:345);
 AVTWSGDSTY (SEQ ID NO:346); AKITNFGITS (SEQ ID NO:347); SFISWISDITY (SEQ ID NO:348);
 15 AFIDWSGGGTY (SEQ ID NO:349); AVILWSGVSTY (SEQ ID NO:350); AAIVWSGGSTY (SEQ ID NO:351);
 AAISSSGYHTY (SEQ ID NO:352); SCISSPDGSTY (SEQ ID NO:353); AAVLWSGVSTA (SEQ ID NO:354);
 VAITWDGSATT (SEQ ID NO:355); AAIGWNGGITY (SEQ ID NO:356); GFITWSGASTY (SEQ ID NO:357);
 AGINWSGESAD (SEQ ID NO:358); SCIERSDGSTY (SEQ ID NO:359); SCISNTDSSTY (SEQ ID NO:360);
 SCISNTDSSTY (SEQ ID NO:371); AQISWSAGSIY (SEQ ID NO:372); AGMSWNPMPAV (SEQ ID NO:373);
 20 SCISRSDGSTY (SEQ ID NO:374); ANIGWTGDMTY (SEQ ID NO:375); AAIWWSGSMTY (SEQ ID NO:376);
 SCISNTDSSTY (SEQ ID NO:377); AANTWSGGPTY (SEQ ID NO:378); SCISSDGSTG (SEQ ID NO:379);
 SCYSSSDGSTG (SEQ ID NO:380); SCISSDGSTG (SEQ ID NO:381); GCIKSSDGTTG (SEQ ID NO:382);
 SCISNTDSSTY (SEQ ID NO:383); AAIWSAGSTY (SEQ ID NO:384); SCISSKEGSTY (SEQ ID NO:385);
 SCISSSDGSTG (SEQ ID NO:386); SCYSSRDGTTG (SEQ ID NO:387); SCISSDGSTG (SEQ ID NO:388);
 25 SCYSSSDGSTG (SEQ ID NO:389); SCFSSSDGSTG (SEQ ID NO:390); SCISNTDSSTF (SEQ ID NO: 391);
 SCYSSSDGSTG (SEQ ID NO:392); SCISNTDSSTY (SEQ ID NO:393); SCISSDGSTG (SEQ ID NO:394);
 GGINWSGDSTD (SEQ ID NO:395); SCFSSSDGSAG (SEQ ID NO:396); SCISNTDSSTY (SEQ ID NO:397);
 SCFSTRDGNAG (SEQ ID NO:398); SCFSSRDGSTG (SEQ ID NO:399); SCFSSRDGSTG (SEQ ID NO:400);
 SCISSDGSTG (SEQ ID NO:401); SCISNTDSSTY (SEQ ID NO:402); SCISSPDGSTY (SEQ ID NO:403);
 30 SCYSSSDGNTG (SEQ ID NO:404); GGINWSGDSTD (SEQ ID NO:405); SCFSSSDGSTG (SEQ ID NO:406);
 SCISSGDGTTY (SEQ ID NO:407); SCISSDGSTG (SEQ ID NO:408); SCISSDGSTG (SEQ ID NO:409); and
 SSISRSDGSTY (SEQ ID NO: 410).

In some embodiments, the CDR3 sequence is selected from:

AKGLRNSDWDLRRGYEYDY (SEQ ID NO:411); ADQASVPPPYGSERYDIASPSEYDY (SEQ ID NO:412);
ANSRAYYSSSYDLGRLASYDY (SEQ ID NO:413); AAQRLGSVTDYTKYDY (SEQ ID NO:414);
ASVKVWAGSGIDISGRNYDY (SEQ ID NO:415); AKRLDYSATDKGVDLSDEYDY (SEQ ID NO:416);
AAGSGRLRDLKVGQNYDY (SEQ ID NO:417); ADSPRTYSSGSVNLEDGSEYDY (SEQ ID NO:418);
5 VIPGRGSALPIDVGKSDEYDY (SEQ ID NO:419); AAGASGRLRDLKVGQNYDY (SEQ ID NO:420);
ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:421); AAQRLGSVTDYTKYDY (SEQ ID NO:422);
AIPPRAYSGGSYSLKDQSKYDY (SEQ ID NO:423); ADGNVWSPPICSSAGPPPGGMDY (SEQ ID NO:424);
KSRSSYSNN (SEQ ID NO:425); AMPPRAYTGRSVSLKDQSKYDY (SEQ ID NO:426); LDTTGWGPPPYQY (SEQ ID
NO:427); AHPPDPSRGGEWRLQTPSEYDY (SEQ ID NO:428); AAQRLGSVTDYTKYDY (SEQ ID NO:429);
10 VPRSHFTTAQDMGQDMGAPSWYDY (SEQ ID NO:430); AVLIRYSSGGYQGLSDANEYDY (SEQ ID NO:431);
VVKYLSGSYSYAGQYNF (SEQ ID NO:432); ADFNVWSPPICGSVGPPPGGMDY (SEQ ID NO:433);
AHESTYSSGTYYLTDPRIYVY (SEQ ID NO:434); AVPARGLTMDLENSDIYDH (SEQ ID NO:435);
AATLQVTGSYYDLSTVDIYDN (SEQ ID NO:436); ATLFRSNGPKDLSSGYEYDY (SEQ ID NO:437);
AGESGVWVGGLDY (SEQ ID NO:438); VGSANSGEFRFGWVLKPDLYNY (SEQ ID NO:439);
15 ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:440); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:441);
ERGYAYCSDGCRQTQDYDY (SEQ ID NO:442); GAARAWWSGSYDYTRMNNYDY (SEQ ID NO:443);
AETSADSGEFRFGWVLKPSLYDY (SEQ ID NO:444); AAGSAYSGSYWNITMAANYDY (SEQ ID NO:445);
AQRIFGAQPMDLSDYDY (SEQ ID NO:446); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:447);
ARDYRGIKDLKGDYDY (SEQ ID NO:448); ADFNVWSPPICGSIWYGPPPRGMDY (SEQ ID NO:449);
20 ADSNVWSPPICGSRWYGPPPGGMAY (SEQ ID NO:450); ADFNVWSPPICGSNWYGPPPGGMDY (SEQ ID NO:451);
ADFNWSPPICGSIWYGPPPGGMDY (SEQ ID NO:452); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:453);
ARIITVATMRDSDYDY (SEQ ID NO:454); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:455);
ADSNVWSPPICGRTWYGPPPGGMDY (SEQ ID NO:456); ADFNVWSPPICGSIWYGPPPGGMAY (SEQ ID NO:457);
ADFNWSPPICGSNWYGPPPGGMDY (SEQ ID NO:458); ADFNVWSPPICGSSWYGPPPGGMDY (SEQ ID NO:459);
25 ADFNVWSPPICGSRWYGPPPGGMEY (SEQ ID NO:460); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:461);
ADFNWSPPICGSRWYGPPPGGMAY (SEQ ID NO:462); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:463);
ADSNVWSPPICGKTWYGPPPGGMDY (SEQ ID NO:464); AGESGVWVGGLDY (SEQ ID NO:465);
ADSNVWSPPICGSTWYGPPPGGMAY (SEQ ID NO:466); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:467);
ADFNWSPPICGSRWYGPPPGGMDY (SEQ ID NO:468); ADFNVWSPPICGSRWYGPPPGGMDY (SEQ ID NO:469);
30 ADFNVWSPPICGSRWYGPPPGGMDY (SEQ ID NO:470); ADFNVWSPPICGSIWYGPPPGGMDY (SEQ ID NO:471);
ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:472); ADFNVWSPPICGSVGPPPGGMDY (SEQ ID NO:473);
ADFNWSPPICGSSWYGPPPGGMAY (SEQ ID NO:474); AGESGVWVGGLDY (SEQ ID NO:475);
ADFNWSPPICGSSWYGPPPGGMEY (SEQ ID NO:476); ADGNVWSPPICGSAGPPPGGMDY (SEQ ID NO:477);
ADFNWSPPICSSNWYGPPPRGMDY (SEQ ID NO:478); ADFNVWSPPICGSIWYGPPPRGMDY (SEQ ID NO:479).

In various embodiments, the CD8 binding agent comprises an amino acid sequence selected from the following sequences:

1CDA 7 (SEQ ID NO:480)

QVQLQESGGGLVQAGGSLRLSCAASGRSFSSYTLAWFRQAPGKEREFVASITWGGGNTYYPD SVKGRFTISRDDAK
5 NTVYLQMNSLKPEDTAVYYCAAKGLRNSDWDLRRGYEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 12 (SEQ ID NO:481)

QVQLQESGGGLVQDGGSLRLSCAFSGRTFSSYTMGWFRQGPGKEREFVAATVWTGAGTVYADSVKGRFTISRDN
KNTVYLQMNSLRPEDTAVYYCAADQASVPPPYGSERYDIASPSYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

10 1CDA 14 (SEQ ID NO:482)

QVQLQESGGGLVQAGASLRLSCAASGRTFSSYIMGWFRQAPGKEREFVAAIGWSADITVYADSVKGRFTISRDN
NMVYLQMNSLNPEDTAVYYCAANSRAYSSSYDLGRLASYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 15 (SEQ ID NO:483)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYTMGWFRQAPGKEREFVAFIDWSGGGTYYDDSVKGRFTISRDN
15 ENTVYLQMNLEPEDTAVYYCAAQRLGSVTDYTKYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 17 (SEQ ID NO:484)

QVQLQESGGGLVQAGGSLRLSCAASGRTSGRTFSSYTMGWFRQAPGKEREFVATITWGGGSTYYADSVKGRFTIS
RDNANNTVYLQMNSLKPEDTAVYYCAASVKVWAGSGIDISGRNYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

20 1CDA 18 (SEQ ID NO:485)

QVQLQESGGGLVQPGGSLRLSCLASGRTFSSYAMGWFRQAPGKEREFVAAISWSGGPTVYADHVKGRFTISRDN
KNTVYLQVNSLKPEDTADYYCAAKRLDYSATDKGVDLSDEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 19 (SEQ ID NO:486)

QVQLQESGGGLVQAGDSLRLSCAASGLTFSNYIMGWFRQAPGKEREFVAAITWGGGSTVYADSVVEGRFTISR
25 NTVSLQMNSLLPEDTAVYYCAAAGGSGRLRDLKVGQNYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 24 (SEQ ID NO:487)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYTMGWFRQAPGREREFVAAITWSGVSTVYTD SVKGRFTVSRDNA
KNTVYLQMNSLKPEDTAVYYCAADSPPRTYSSGSVNLEDGSEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH
;

1CDA 26 (SEQ ID NO:488)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSDTMGWFRQAPGKEREFVGAIMWSGAFTHYADSVKGRFTISRDN
KNTVYLQMNALKPEDTAVYYCAVIPGRGSALPIDVGKSDEYEWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 28 (SEQ ID NO:489)

5 QVQLQESGGGLVQAGDSLRLSCAASGLTFSNYIMGWFRQAPGKEREFVAAITWGGGSTVYADSVGRFTISRDTG
NTVSLQMNSLPEDTAVYYCAAAGASGRLRDLKVGQNYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 37 (SEQ ID NO:490)

QVQLQESGGGLVQAGGSLRLSCAGSGFTLDYYGIGWFRQAPGKEREGVSCISSDRNTYYADSVKGRFTISGDN
NTVYLQMNLLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

10 1CDA 43 (SEQ ID NO:491)

QVQLQESGGGLVQAGGSLRLSCVASGHTFSSYTMGWFRQAPGKEREFVAFIDWSGGGTYANSVKGRFTISRDN
ENTVYLQMNLLKPEDTAVYYCAAQRLGSVTDYTKYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 45 (SEQ ID NO:492)

15 QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYVIGWFRQAPGKEREFVAVITWSGDSTYSSDSLKGRFTISRDN
NTVYLQMNLLNPEDTAVYYCAAIPPRAYSGGSYSLKDQSKYEWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 47 (SEQ ID NO:493)

QVQLQESGGGLVQAEGSLKLSGISGFADGYAIGWFRQAPGKEREGVACISSKDGSTYYADSVKGRFTMSVDKTK
TVYLQMSSLKPEDTAVYYCAADGNVWSPPICSSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 48 (SEQ ID NO:494)

20 QVQLQESGGGLVQPGGSLTLSCAASGFAGFFDMTWVRQAPGKLEWVSGINSIGGSTTYADSVKGRFTISRDN
NELYLQMNLLKPDPTAVYYCAKSRSSYSNNWRPPGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 58 (SEQ ID NO:495)

QVQLQESGGGLVQARGSLTLSCAASGRTFSSNYVIGWFRQAPGEEREFVAVTWSGDSTYSSDSLKGRFTISRDN
NTVYLQMNLLNPEDTAVYYCAAMPPrAYTGRSVSLKDQSKYEWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

25 1CDA 65 (SEQ ID NO:496)

QVQLQESGGGLVQPGGSLRLSCAASGSIFSINVMGWYRQTPGKERELVAKITNFGITSYADSAQGRFTISRGN
VYLQMNLLKPEDTAVYYCNLDTTGWGPPPYQYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 68 (SEQ ID NO:497)

QVQLQESGGGLVQAGASLRLSCAASGRTFSNYNVGWFRQAPGKEREFVSFISWISDITYYSDSVKGRFIISRDNAKN
MVYQLQMNSLKPEDTAVYYCAAHPDPSRGGEWRLQTPSEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 73 (SEQ ID NO:498)

QVQLQESGGGLVQAGGSLRLSCAASGHTFSSYTMGWFRQAPGKEREFVAFIDWSGGGTYADSVKGRFTISRDN
5 ENTVYLQMNNLKPEDTAVYYCAAQRLGSVTDYTKYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 75 (SEQ ID NO:499)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSTYPVGWFRQAPGKEREFVAVILWSGVSTYYADSVKGRFTISRDN
NTVYLQMDSLKPEDTAVYYCAVPRSHFTTAQDMGQDMGAPSWYEWGQGTQVTVSSAAAYPYDVPDYGSHHHHH
H;

10 1CDA 86 (SEQ ID NO:500)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVAAIVWSGGSTYYADSVKGRFTISRDN
KNTVYLQMNSLKPEDTAVYYCAAVLIRYYSGGYQGLSDANEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 87 (SEQ ID NO:501)

QVQLQESGGGLVQAGASLRLSCSASGRTFSDYRMGWFRQAPGKEREWVAAISSSGYHTYYADSVKGRFTISRDN
15 KNTGYLQMSSLKPEDTAVYYCAVVKYLSGSYSYAGQYNFWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 88 (SEQ ID NO:502)

QVQLQESGGGLVQAGDSLKLSCAASGLTFSNYIMAWFRQAPGKEREGVSCISSPDGSTYYADSVKGRFTISSDN
NTVYLQMNSLKPEDTAVYYCAADFNVWSPPICGSGVPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 89 (SEQ ID NO:503)

20 QVQLQESGGGLVQAGGSLRLSCAASGRTFSNSVMGWFRQPPGKEREFVAAVLWSGVSTAYADSVKGRFTISRDN
KNTVYLQMNNLKPDDTAVYYCAAHESTYYSGTYLTDPRRYVYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

1CDA 92 (SEQ ID NO:504)

QVQLQESGGGLVQAGGSLRLSCVGDGRTFSSYIIGWFRQAPGNEREFVVAITWDGSATTYADSVKGRFTVSRDS
NTAYLQMNSLKPEDTAVYYCAAVPARGLTMDLENSDIYDHWGRGTQVTVSSAAAYPYDVPDYGSHHHHHH;

25 1CDA 93 (SEQ ID NO:505)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQALGKEREFVAAIGWNGGITYYADSVKGRFAISRDN
NTVYLQMNSLKPEDTAVYYCAAATLQVTGSYYLDLSTVDIYDNWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 1 (SEQ ID NO:506)

QVQLQESGGGLVQAGGSLRLSCAASGGTFSNYVMGFRQAPGKEREFVGFITWSGASTYYADSVKGRFTISRDN
ENTVYLQMNSLKPEDTAVYYCAATLFRSNGPKDLSSGYEYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 5 (SEQ ID NO:507)

QVQLQESGGGLVQAGDSLRLTCTASGRTFSNYGIGWFRQAPGKEREFVAGINWSGESADYSVKGRFTISRDN
5 NTVYLQMNSLKPEDTAVYYCAAGESGVWVGGLDYWXQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 22 (SEQ ID NO:508)

QVQLQESGGGLVQAGGSLRLSCAASGFTFDDYAIWFRQAPGKEREGVSCIERSDGSTYYADSVKGRFTISSDN
NTVYLQMNSLKPEDTAVYYCAVGSANSGEFRFGWVLKPDLYNYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 28 (SEQ ID NO:509)

10 QVQLQESGGGLVQAGGSLRLSCTASGRTFSSYTVWFRQSPGKEREGISCISNTDSSTYYADSVKGRFTISSDN
TVHLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 62 (SEQ ID NO:510)

QVQLQESGGGLVQPGGSLRLSCATFGFPDDYAIWFRQAPGKEREGVSCISNTDSSTYYADSVKGRFTISSDN
TVHLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

15 2CDA 68 (SEQ ID NO:511)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYVMGFRQAPGKEREFVAQISWSAGSIYYADSVKGRFTISDN
RTVYLQMNSLKPEDTAVYYCAERGYAYCDDGCQRTQDYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 73 (SEQ ID NO:512)

QVQLQESGGGLVQAGGSLRLSCAASGRTLSSNPMWFRQAAGKEREFVAGMSWNPMPAVYADSVKGRFTISR
20 AENTVYLQMNSLKPEDTAVYYCAGAAAWWSGSYDYTRMNNYDYWGPQTQVTVSSAAAYPYDVPDYGSHHHHHH
;

2CDA 74 (SEQ ID NO:513)

QVQLQESGGGLVQAGGSLRLSCAVSGFTFDNYAIGWFRQAPGKEREGVSCISRSDGSTYYADSVRGRFTISSDN
NTVYLQMNSLKPEDTAVYYCAAETSADSGEFRFGWVLKPSLYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

25 2CDA 74 (C50S) (SEQ ID NO:514)

QVQLQESGGGLVQAGGSLRLSCAVSGFTFDNYAIGWFRQAPGKEREGVSSISRSDGSTYYADSVRGRFTISSDN
NTVYLQMNSLKPEDTAVYYCAAETSADSGEFRFGWVLKPSLYDYWGQGTQVTVSS;

2CDA 75 (SEQ ID NO:515)

QVQLQESGGGLVQAGGSLRLSCAASGRAFSSYFMGWFRQTPGKEREFVANIGWTGDMTYADSVKGRFTISRDN
KNTVYLQMNSLKPEDTAVYYCAAAGSAYSGSYWNITMAANYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 77 (SEQ ID NO:516)

QVQLQESGGGLVQAGGSLRLSCAASTPTFSSYNMGWFRQAPGKEREFVAIIWGSMTYYADSMKGRFTVSIDNAK
5 NTVYLQMNSLKPEDTAVYYCAAQRIFGAQPMDLSDYEWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 81 (SEQ ID NO:517)

QVQLQESGGGLVQAGGSLRLSCATFGFTDDYAIWFRQAPGKEREGISCISNTDSSTYYADSVKGRFTISSDS
TVHLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 87 (SEQ ID NO:518)

10 QVQLQESGGGLVQAGGSLRLSCKASGGTFSGYIMGWFRQAPGKEREFVAANTWSGGPTYSDSVKGRFTISRDN
KNTVYLQMNTLKPEDTAVYQCAARDYRGIKDLKGDYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 88 (SEQ ID NO:519)

QVQLQESGGGLVQAGDSLKLSCATSGRSFSSYTIWFRQAPGKEREGISCISSDGSTGYADSVRGRFTISSDNAKNT
VYLQMNSLKPEDTAVYYCAADFNWSPPICGSIWYGPPPRGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

15 2CDA 89 (SEQ ID NO:520)

QVQLQESGGGLVQAGGYLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCYSSSDGSTGFADSVKGRFTISSDNAK
NTVYLQMNNLRPEDTAVYYCAADSNVWSPPICGSRWYGPPPGGMAYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

2CDA 91 (SEQ ID NO:521)

20 QVQLQESGGGLAQVGGSLRLSCTASGFTDDYTIGWFRQAPGKEREGISCISSDGSTGYADSVKGRFTISSDNAKNT
VYLQMNSLKPEDTAVYYCAADFNWSPPICGSNWYGPPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 92 (SEQ ID NO:522)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGIGCIKSSDGTGYADSVKGRFTISSDNAK
NTVYLQMNSLKPEDTAVYYCAADFNWSPPICGSIWYGPPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHH

25 H;

2CDA 93 (SEQ ID NO:523)

QVQLQESGGGLAQAGGSLRLSCAASGFTFDQYTIWFRQAPGKEREGVSCISNTDSSTYYADSVKGRFTISSDNAK
NTVYLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 94 (SEQ ID NO:524)

QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYAMAWFRQAPGKEREFVAAIAWSAGSTYYADSVKGRFAISRDN
ENTVYLQMNSLKPEDTAVYYCAARIITVATMRDSDYDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

2CDA 95 (SEQ ID NO:525)

5 QVQLQESGGGLVQAGGSLRLSCAASGFAFDGYAIGWFRQAPGKEREGVSCISSKEGSTYYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 3 (SEQ ID NO:526)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIWFRAPGKEREGISCISSDGSTGYADSVKGRFTITSDSAKN
TVYLQMNSLKPEDTAVYYCAADSNVWSPPICGRTWYGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHH
H;

10 3CDA 8 (SEQ ID NO:527)

QVQLQESGGGLVQPGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCISSRDGTTGYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAADFNWSPPICGSIWYGPPPGGMAYWGQGTQVTVSSAAAYPYDVPDYGSHHHHH
H;

3CDA 11 (SEQ ID NO:528)

15 QVQLQESGGGLVQAGGSLRLSCAASGFTFDDYTIGWFRQAPGKEREGISCISSDGSTGYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAADFNWSPPICGSNWYGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 18 (SEQ ID NO:529)

20 QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCISSDGSTGYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAADFNWSPPICGSSWYGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 19 (SEQ ID NO:530)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCFSSSDGSTGFADSVKGRFTISSDNA
KNTVYLEMNSLKPEDTAVYYCAADFNWSPPICGSRWYGPPPGGMEYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

25 3CDA 21 (SEQ ID NO:531)

QVQLQESGGGLVQAGGSLRLSCATFGFSFDDYIAWFRQAPGKEREGISCISNTDSSTFYADSVKGRFTISSDNA
KNTVHLQMNSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 24 (SEQ ID NO:532)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCYSSSDGSTGFADSVKGRFTISSDNAK
NTVYLQMNSLRPEDTAVYYCAADFNVWSPPICGSRWYGGPPGGMAYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 28 (SEQ ID NO:533)

5 QVQLQESGGGLVQVGGSLRLSCTISGFTGNDLAIGWFRQAPGKDQREGISCISNTDSSTYYADSVKGRFTISSDNAK
NTVHLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 29 (SEQ ID NO:534)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIWFRRAPGKEREGISCISSSDGSTGYADSVKGRFTISSDNAK
TVYLQMTSLKPEDTAVYYCAADSNVWSPPICGKTWYGGPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHH
10 H;

3CDA 31 (SEQ ID NO:535)

QVQLQESGGGLVQAGDSLRLSCAGSEGLSSYGIGWFRQAPGKEREFVGGINWSGDSTDYADSVKGRFTISRDSA
KNTVYLQMNSLRPEDTAVYYCAAGESGVWVGGLDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 32 (SEQ ID NO:536)

15 QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIWFRRAPGKEREGISCFSSSDGSAGYADSVKGRFTVSSDNAK
NTVYLQMNSLRPEDTAVYYCAADSNVWSPPICGSTWYGGPPGGMAYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 33 (SEQ ID NO:537)

QVQLQESGGGLVQAGGSLRLSCATSGFTFDDYAIWFRQAPGKEREGVSCISNTDSSTYYADSVKGRFTISSDNAK
20 TVYLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 37 (SEQ ID NO:538)

QVQLQESGGGLVQAGGSLRLSCEVSGLSDDYTIGWFRQAPGKEREGFSCFSTRDGNAGYADSVKGRFTISSDNA
KNTVYLQMNNLRPEDTAVYYCAADFNVWSPPICGSRWYGGPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHH
HHH;

25 3CDA 40 (SEQ ID NO:539)

QVQLQESGGGLVQAGGSLRLSCEVSGLSDDYTIGWFRQAPGKKREGFSCFSSRDGSTGYADSVKGRFTISSDNAK
NTVYLQMNSLRPEDTAVYYCAADFNVWSPPICGSRWYGGPPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 41 (SEQ ID NO:540)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGFSCFSSRDGSTGYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAADFNVWSPPICGSRWYGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHH
HHH;

3CDA 48 (SEQ ID NO:541)

5 QVQLQESGGGLVQAGGSLRLSCAASGFSDDDYTIGWFRQVPGKEREGISCISSDGSTGYADSVKGRFTISSDNAKNT
VYLQINSLKPEDTAVYYCAADFNVWSPPICGSIWYGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 57 (SEQ ID NO:542)

QVQLQESGGGLVQAGGSLRLSCATFGFTDDYAIWFRQAPGKEREGISCISNTDSSTYYADSVKGRFTISSDNAKN
TVHLQMSSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

10 3CDA 65 (SEQ ID NO:543)

QVQLQESGGGLVQAGGSLXLSCAASGFTDDYAIWFRQAPGKEREGVSCISSPDGSTYYADSVKGRFTISSDNAK
NTVYLQMNSLKPEDTAVYYCAADFNVWSPPICGSVGPMPGGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 70 (SEQ ID NO:544)

15 QVQLQESGGGLVQAGASLRLSCKASGFTFGDYTIGWFRQAPGKEREGISCYSSSDGNTGYADSVKGRFTISSDNAK
NTVYLQMNSLRPEDTAVYYCAADFNVWSPPICGSSWYGPPPGMAYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 73 (SEQ ID NO:545)

QVQLQESGGGLVQAGDSLRLSCAGSEGTFSSYGIGWFRQAPGKEREFVGGINWSGDSTDYADSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAVYYCAAGESGVWVGGLDYWGQGTQVTVSSAAAYPYDVPDYGSHHHHHH;

20 3CDA 83 (SEQ ID NO:546)

QVQLQESGGGLVQAGGSLRLSCAASGFSSDDYTIGWFRQAPGKEREGISCFSSSDGSTGFADSVKGRFTISSDNAT
NTVYLQMNSLKPEDTAVYYCAADFNVWSPPICGSSWYGPPPGGMEYWGKGTQVTVSSAAAYPYDVPDYGSHHHH
HH;

3CDA 86 (SEQ ID NO:547)

25 QVQLQESGGGLVQAGDSLRLSCTASGVSIGDYNIGWFRQAPGKEREGVSCISSGDGTTYTDSVKGRFTISTDNAKN
TVYLQMNSLKPEDTAVYYCAADGNVWSPPICGSAGPPPGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH;

3CDA 88 (SEQ ID NO:548)

QVQLQESGGGLVQAGGSLRLSCAASGFTFDDYTIWFRQAPGGKEREKISCISSDGSTGYADSVKGRFTISSDNAKN
MVYLQMNSLKPEDTALYYCAADFNVWSPPICSSNWyGPPPRGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH
H; or

3CDA 90 (SEQ ID NO:549)

5 QVQLQESGGGLVQAGGSLRLSCAASGFTFDDYTIWFRQAPGGKEREKISCISSDGSTGYADSVRGRFTISSDNAKNT
VYLQMNSLKPEDTAVYYCAADFNVWSPPICGSIWYGPPPRGMDYWGKGTQVTVSSAAAYPYDVPDYGSHHHHHH.

In various exemplary embodiments, the CD8 binding agent comprises an amino acid sequence selected from any one of the above sequences without the terminal histidine tag sequence (*i.e.*, HHHHHH, SEQ ID NO: 550).

10 In some embodiments, the CD8 binding agent comprises an amino acid sequence selected from any one of the above sequences without the HA tag (*i.e.*, YPYDVPDYGS; SEQ ID NO: 551).

In some embodiments, the CD8 binding agent comprises an amino acid sequence selected from any one of the above sequences without the AAA linker.

15 In some embodiments, the CD8 binding agent comprises an amino acid sequence selected from any one of the above sequences without the AAA linker, HA tag, and terminal histidine tag sequence (*i.e.*, AAAYPYDVPDYGSHHHHHH; SEQ ID NO: 552).

20 In some embodiments, the targeting moiety is a CD4 binding agent that is a VHH comprising a single amino acid chain having four "framework regions" or FRs and three "complementary determining regions" or CDRs. As used herein, "framework region" or "FR" refers to a region in the variable domain that is located between the CDRs. As used herein, "complementary determining region" or "CDR" refers to variable regions in VHHs that contains the amino acid sequences capable of specifically binding to antigenic targets. In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2020082045, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: GYTFTAHI (SEQ ID NO: 78); CDRH2: IKPQYGAV (SEQ ID NO: 79); or CDRH3: AR (SEQ ID NO: 554). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region
25 comprising the amino acid sequence of CDRL1: QGVGSD (SEQ ID NO: 80); CDRL2: HTS; or CDRL3: QVLQF (SEQ ID NO: 81).

30 In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2018170096, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: GYTFTSN (SEQ ID NO: 82); CDRH2: YPRSGN (SEQ ID NO: 83); or CDRH3: RVPYFDH (SEQ ID NO: 84). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: KASQSVGNVA (SEQ ID NO: 85); CDRL2: YASNRYT (SEQ ID NO: 86); or CDRL3: QQHYSSPFT (SEQ ID NO: 87).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2016156570, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one CDR1 comprising the amino acid sequence of: GYWMY (SEQ ID NO: 88); CDR1: SYSMG (SEQ ID NO: 89); CDR1: FNAMEG (SEQ ID NO: 90); or CDR1: VMG (SEQ ID NO: 555). In some embodiments, the anti-CD4 antibody comprises at least one CDR2 comprising the amino acid sequence of CDR2: AISPGGGSTYYPDSVK (SEQ ID NO: 91); CDR2: AISWSGDETSYADSVK (SEQ ID NO: 92); CDR2: TIARAGATKYADSVKG (SEQ ID NO: 93); or CDR2: AVRWSSTGIYYTQYAD (SEQ ID NO: 94). In some embodiments, the anti-CD4 antibody comprises at least one CDR3 comprising the amino acid sequence of CDR3: SLTATHTYEYDY (SEQ ID NO: 95); CDR3: DRWWRPAGLQWDY (SEQ ID NO: 96); CDR3: RVFDLPNDY (SEQ ID NO: 97); or CDR3: DTYNNSNPARDGYDF (SEQ ID NO: 98).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2012145238, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: AYVIS (SEQ ID NO: 99); CDRH2: EIYPGSGSSYYNEKFKG (SEQ ID NO: 100); or CDRH3: SGDGSKFVY (SEQ ID NO: 101). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: KASQSDYCGDSYMN (SEQ ID NO: 102); CDRL2: VASNLES (SEQ ID NO: 103); or CDRL3: QQSLQDPPT (SEQ ID NO: 104).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2008134046, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: GYTFTSYVIH (SEQ ID NO: 105); CDRH2: YINPYNDGTDYDEKFK (SEQ ID NO: 106); or CDRH3: EKDNATGAWFAY (SEQ ID NO: 107). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: KSSQSLLYSTNQKNY (SEQ ID NO: 108); CDRL2: WASTRES (SEQ ID NO: 109); or CDRL3: QQYYSYRT (SEQ ID NO: 110).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2009012944, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: SYVIH (SEQ ID NO: 111); CDRH1: GFTFSNYAMS (SEQ ID NO: 112); or CDRH2: AISDHSTNTYYP (SEQ ID NO: 113); CDRH3: EKDNATGAWFAY (SEQ ID NO: 114); or CDRH3: ARKYGGDYDPF (SEQ ID NO: 115). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: KSSQSLLYSTNQKNYL (SEQ ID NO: 116); CDRL1: KSSQSLLYSTNQKNYL (SEQ ID NO: 117); CDRL1: KASQDINNY (SEQ ID NO: 118); CDRL2: WASTRES (SEQ ID NO: 119); CDRL2: YTSTLQPGVPS (SEQ ID NO: 120); CDRL3: QQYYSYRT (SEQ ID NO: 121); or CDRL3: YDNLLF (SEQ ID NO: 122).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2004005350, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: TFGVH (SEQ ID NO: 123); CDRH1: TAGVH (SEQ ID NO: 124); or CDRH1: TFGVA (SEQ ID NO: 125); CDRH2: VIWRSGITDYNVPFMS (SEQ ID NO: 126); CDRH2: VIARSGITDYNVPFMS (SEQ ID NO: 127); CDRH2: VIWASGITDYNVPFMS (SEQ ID NO: 128); CDRH3: NDPGTGFAY (SEQ ID NO: 129); CDRH3: NDPGTGAAY (SEQ ID NO: 130); or CDRH3: NDPGTGFAA (SEQ ID NO: 131). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: RASENIYSYLA (SEQ ID NO: 132); CDRL1: RASENIYSALA (SEQ ID NO: 133); CDRL2: DAKTLAE (SEQ ID NO: 134); CDRL3: QHHYGNPPT (SEQ ID NO: 135); CDRL3: QHAYGNPPT (SEQ ID NO: 136); or CDRL3: QHHAGNPPT (SEQ ID NO: 137).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2004083247, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain variable region comprising the amino acid sequence of CDRH1: DYVIN (SEQ ID NO: 138); CDRH2: EIYPGSGSDYYNENLKD (SEQ ID NO: 139); or CDRH3: KGENGNSLAFAY (SEQ ID NO: 140). In some embodiments, the anti-CD4 antibody comprises at least one light chain variable region comprising the amino acid sequence of CDRL1: QSVDDYDGSYMN (SEQ ID NO: 141); CDRL2: AASNLES (SEQ ID NO: 142); or CDRL3: QQSIQDPCT (SEQ ID NO: 143).

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2014100139, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain comprising the following amino acid sequence:

Anti-CD4 antibody MV1, Heavy Chain

MEWSGVFMFLSVTAGVHSQVQLQQSGPEVVKPGASVKMSCKASGYTFTSYVIHWVRQKPGQG
LDWIGYINPYNDGTDYDEKFKGKATLTSDTSTSTAYMELSSLRSEDTAVYYCAREKDNYATGAWF
AYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEF
EGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNHYTQKSLSLSPGK (SEQ ID NO: 144)

In some embodiments, the anti-CD4 antibody comprises at least one light chain comprising the following amino acid sequence:

Anti-CD4 antibody MV1, Light Chain

MEWSGVFIFLLSVTAGVHSDIVMTQSPDSLAVSLGERVTMNCKSSQSLLYSTNQKNYLAWYQQKP
GQSPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYYRTFGGGTKLEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 145)

In some embodiments, the targeting moiety comprises anti-CD4 antibody as described in WO2004083247, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the anti-CD4 antibody comprises at least one heavy chain comprising the following amino acid sequence:

5 EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQAPGKGLEWIGVISVKSENYGANYAE
SVRGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCSASYRYDVGAFAYGQGTLTVSS (SEQ ID
NO: 146)

In some embodiments, the anti-CD4 antibody comprises at least one light chain comprising the following amino acid sequence:

10 DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSIYWYQQKPGQPPKLLIYLASILESGVPDRFS
GSGSGTDFTLTISSLQAEDVAVYYCQHSRELPTFGGQTKVEIK (SEQ ID NO: 147)

In various embodiments, the vaccine composition comprises targeting moieties in various combinations. In an illustrative embodiment, the vaccine composition may comprise two targeting moieties, wherein both targeting moieties are antibodies or derivatives thereof. In another illustrative embodiment, the vaccine composition may comprise two targeting moieties, wherein both targeting moieties are natural ligands for cell receptors. In a further illustrative embodiment, the vaccine composition may comprise two targeting moieties, wherein one of the targeting moieties is an antibody or derivative thereof, and the other targeting moiety is a natural ligand for a cell receptor.

In various embodiments, the recognition domain of the targeting moiety functionally modulates (by way of non-limitation, partially or completely neutralizes) the target (e.g. antigen, receptor) of interest, e.g. substantially inhibiting, reducing, or neutralizing a biological effect that the antigen has.

In various embodiments, the recognition domain of the targeting moiety binds but does not functionally modulate the target (e.g. antigen, receptor) of interest, e.g. the recognition domain is, or is akin to, a binding antibody. For instance, in various embodiments, the recognition domain simply targets the antigen or receptor but does not substantially inhibit, reduce or functionally modulate a biological effect that the antigen or receptor has. For example, some of the smaller antibody formats described above (e.g. as compared to, for example, full antibodies) have the ability to target hard to access epitopes and provide a larger spectrum of specific binding locales. In various embodiments, the recognition domain binds an epitope that is physically separate from an antigen or receptor site that is important for its biological activity (e.g. the antigen's active site).

Such non-neutralizing binding finds use in various embodiments of the present invention, including methods in which the vaccine composition is used to, directly or indirectly, recruit active immune cells to a site of need via an effector antigen, such as any of those described herein. For example, in various embodiments, the present vaccine compositions may be used to directly or indirectly recruit cytotoxic T cells via CD8 to a site of infection in a method of treating an infection. In such embodiments, it is desirable to directly or indirectly recruit CD8-expressing cytotoxic T cells but not to functionally modulate the CD8 activity.

In various embodiments, the recognition domain of the targeting moiety binds to an immune modulatory antigen (e.g. immune stimulatory or immune inhibitory). In various embodiments, the immune modulatory antigen is one or more of 4-1BB, OX-40, HVEM, GITR, CD27, CD28, CD30, CD40, ICOS ligand; OX-40 ligand, LIGHT (CD258), GITR ligand, CD70, B7-1, B7-2, CD30 ligand, CD40 ligand, ICOS, ICOS ligand, CD137 ligand and TL1A.

- 5 In various embodiments, the recognition domain of the targeting moiety may be in the context of chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex that comprises two recognition domains that have neutralizing activity, or comprises two recognition domains that have non-neutralizing (e.g. binding) activity, or comprises one recognition domain that has neutralizing activity and one recognition domain that has non-neutralizing (e.g. binding) activity.

10 Fc Domains

The fragment crystallizable domain (Fc domain) is the tail region of an antibody that interacts with Fc receptors located on the cell surface of cells that are involved in the immune system, e.g., B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils, and mast cells. In IgG, IgA and IgD antibody isotypes, the Fc domain is composed of two identical protein fragments, derived from the second and third constant domains of the antibody's two heavy chains. In IgM and IgE antibody isotypes, the Fc domain contains three heavy chain constant domains (C_H domains 2–4) in each polypeptide chain.

In some embodiments, the Fc-based chimeric protein of complex the present technology includes a Fc domain. In some embodiments, the Fc domains are from selected from IgG, IgA, IgD, IgM or IgE. In some embodiments, the Fc domains are from selected from IgG1, IgG2, IgG3, or IgG4.

- 20 In some embodiments, the Fc domains are from selected from human IgG, IgA, IgD, IgM or IgE. In some embodiments, the Fc domains are from selected from human IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the Fc domains of the Fc-based chimeric protein complex comprise the CH2 and CH3 regions of IgG. In some embodiments, the IgG is human IgG. In some embodiments, the human IgG is selected from IgG1, IgG2, IgG3, or IgG4.

- 25 In some embodiments, the Fc domains comprise one or more mutations. In some embodiments, the mutation(s) to the Fc domains reduces or eliminates the effector function the Fc domains. In some embodiments, the mutated Fc domain has reduced affinity or binding to a target receptor. By way of example, in some embodiments, the mutation to the Fc domains reduces or eliminates the binding of the Fc domains to FcγR. In some embodiments, the FcγR is selected from FcγRI; FcγRIIa, 131 R/R; FcγRIIa, 131 H/H, FcγRIIb; and FcγRIII. In some embodiments, the mutation to the Fc domains reduces or eliminated binding to complement proteins, such as, e.g., C1q. In some embodiments, the mutation to the Fc domains reduces or eliminated binding to both FcγR and complement proteins, such as, e.g., C1q.

In some embodiments, the Fc domains comprise the LALA mutation to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the LALA mutation comprises L234A and L235A substitutions

in human IgG (e.g., IgG1) (wherein the numbering is based on the commonly used numbering of the CH2 residues for human IgG1 according to EU convention (PNAS, Edelman et al., 1969; 63 (1) 78-85)).

In some embodiments, the Fc domains of human IgG comprise a mutation at 46. to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the mutations are selected from L234A, L234F, L235A, L235E, L235Q, K322A, K322Q, D265A, P329G, P329A, P331G, and P331S.

In some embodiments, the Fc domains comprise the FALA mutation to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the FALA mutation comprises F234A and L235A substitutions in human IgG4.

In some embodiments, the Fc domains of human IgG4 comprise a mutation at one or more of F234, L235, K322, D265, and P329 to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the mutations are selected from F234A, L235A, L235E, L235Q, K322A, K322Q, D265A, P329G, and P329A.

In some embodiments, the mutation(s) to the Fc domain stabilize a hinge region in the Fc domain. By way of example, in some embodiments, the Fc domain comprises a mutation at S228 of human IgG to stabilize a hinge region. In some embodiments, the mutation is S228P.

In some embodiments, the mutation(s) to the Fc domain promote chain pairing in the Fc domain. In some embodiments, chain pairing is promoted by ionic pairing (a/k/a charged pairs, ionic bond, or charged residue pair).

In some embodiments, the Fc domain comprises a mutation at one more of the following amino acid residues of IgG to promote of ionic pairing: D356, E357, L368, K370, K392, D399, and K409.

By way of example, in some embodiments, the human IgG Fc domain comprise one of the mutation combinations in

Table 1 to promote of ionic pairing.

Table 1	
Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
D356K D399K	K392D K409D
E357R L368R	K370D K409D
E357R L368K	K370D K409D
E357R D399K	K370D K409D
E357R	K370D
L368R D399K	K392D K409D
L368K D399K	K392D K409D
L368R D399K	K409D
L368K D399K	K409D
L368R	K409D
L368K	K409D
K370D K409D	E357R D399K
K370D K409D	E357R L368R
K370D K409D	E357R L368K
K370D K409D	E357R D399K
K370D K409D	E357R L368R

Table 1	
Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
K370D K409D	E357R L368K
K370D	E357R
K370D	E357R
K392D K409D	D356K D399K
K392D K409D	L368R D399K
K392D K409D	L368K D399K
K392D K409D	D399K
D399K	K392D K409D
D399K	K409D
K409D	L368R
K409D	L368K
K409D	L368R D399K
K409D	L368K D399K
K409D	L368R
K409D	L368K
K409D	L368R D399K
K409D	L368K D399K
K409D	D399K

In some embodiments, chain pairing is promoted by a knob-in-hole mutations. In some embodiments, the Fc domain comprises one or more mutations to allow for a knob-in-hole interaction in the Fc domain. In some embodiments, a first Fc chain is engineered to express the "knob" and a second Fc chain is engineered to express the complementary "hole." By way of example, in some embodiments, human IgG Fc domain comprises the mutations of **Table 2** to allow for a knob-in-hole interaction.

Table 2	
Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
T366Y	Y407T
T366Y/F405A	T394W/Y407T
T366W	Y407A
T366W	Y407V
T366Y	Y407A
T366Y	Y407V
T366Y	Y407T

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology comprise any combination of the above disclosed mutations. By way of example, in some embodiments, the Fc domain comprises mutations that promote ionic pairing and/or a knob-in-hole interaction. By way of example, in some embodiments, the Fc domain comprises mutations that have one or more of the following properties: promote ionic pairing, induce a knob-in-hole interaction, reduce or eliminate the effector function of the Fc domain, and cause Fc stabilization (e.g. at hinge).

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 3**, which promote ionic pairing and/or promote a knob-in-hole interaction in the Fc domain.

Table 3	
Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
T366W K370D	E357R Y407A
T366W K370D	E357R Y407V
T366W K409D	L368R Y407A
T366W K409D	L368R Y407V
T366W K409D	L368K Y407A
T366W K409D	L368K Y407V
T366W K409D	L368R D399K Y407A
T366W K409D	L368R D399K Y407V
T366W K409D	L368K D399K Y407A
T366W K409D	L368K D399K Y407V
T366W K409D	D399K Y407A
T366W K409D	D399K Y407V
T366W K392D K409D	D399K Y407A
T366W K392D K409D	D399K Y407V
T366W K392D K409D	D356K D399K Y407A
T366W K392D K409D	D356K D399K Y407V
T366W K370D K409D	E357R D399K Y407A
T366W K370D K409D	E357R D399K Y407V
T366W K370D K409D	E357R L368R Y407A
T366W K370D K409D	E357R L368R Y407V
T366W K370D K409D	E357R L368K Y407A
T366W K370D K409D	E357R L368K Y407V
T366W K392D K409D	L368R D399K Y407A
T366W K392D K409D	L368R D399K Y407V
T366W K392D K409D	L368K D399K Y407A
T366W K392D K409D	L368K D399K Y407V
E357R T366W	K370D Y407A
E357R T366W	K370D Y407V
T366W L368R	Y407A K409D
T366W L368R	Y407V K409D
T366W L368K	Y407A K409D
T366W L368K	Y407V K409D
T366W L368R D399K	Y407A K409D
T366W L368R D399K	Y407V K409D
T366W L368K D399K	Y407A K409D
T366W L368K D399K	Y407V K409D
T366W D399K	Y407A K409D
T366W D399K	Y407V K409D
T366W D399K	K392D Y407A K409D
T366W D399K	K392D Y407V K409D
T366W D356K D399K	K392D Y407A K409D
T366W D356K D399K	K392D Y407V K409D
E357R T366W D399K	K370D Y407A K409D
E357R T366W D399K	K370D Y407V K409D

Table 3	
Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
E357R T366W L368R	K370D Y407A K409D
E357R T366W L368R	K370D Y407V K409D
E357R T366W L368K	K370D Y407A K409D
E357R T366W L368K	K370D Y407V K409D
T366W L368R D399K	K392D Y407A K409D
T366W L368R D399K	K392D Y407V K409D
T366W L368K D399K	K392D Y407A K409D

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 4**, which promote ionic pairing, promote a knob-in-hole interaction, or a combination thereof in the Fc domain. In embodiments, the "Chain 1" and "Chain 2" of **Table 4** can be interchanged (e.g. Chain 1 can have Y407T and Chain 2 can have T366Y).

Table 4			
Chain 1 mutation	Chain 2 mutation	Reference	IgG
T366Y	Y407T	Ridgway <i>et al.</i> , 1996 Protein Engineering, Design and Selection, Volume 9, Issue 7, 1 July 1996, Pages 617–62	IgG1
T366Y/F405A	T394W/Y407T	Ridgway <i>et al.</i> , 1996 Protein Engineering, Design and Selection, Volume 9, Issue 7, 1 July 1996, Pages 617–62	IgG1
T366W	Y407A	Atwell <i>et al.</i> , 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368V/Y407A	Atwell <i>et al.</i> , 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	L368A/Y407A	Atwell <i>et al.</i> , 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368A/Y407A	Atwell <i>et al.</i> , 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368G/Y407V	Atwell <i>et al.</i> , 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W/D399C	T366S/L368A/K392C/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
T366W/K392C	T366S/L368A/D399C/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1

S354C/T366W	Y349C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
Y349C/T366W	S354C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
E356C/T366W	Y349C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
Y349C/T366W	E356C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
E357C/T366W	Y349C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
Y349C/T366W	E357C/T366S/L368A/Y407V	Merchant <i>et al.</i> , 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
D339R	K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339R	K409D	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K409D	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K360D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K392D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K	K392D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E357K	K392D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K	K409E/K439D	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E357K	K370D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K/E357K	K370D/K392D/K409E	Gunasekaran <i>et al.</i> , 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
S364H/F405A	Y349T/T394F	Moore <i>et al.</i> , 2011 mAbs, 3:6, 546-557	IgG1
S364H/T394F	Y349T/F405A	Moore <i>et al.</i> , 2011 mAbs, 3:6, 546-557	IgG1
D221R/P228R/K409R	D221E/P228E/L368E	Strop <i>et al.</i> , 2012 JMB Volume 420, Issue 3, 13 July 2012, Pages 204-219	IgG1
C223R/E225R/P228R/K409R	C223E/P228E/L368E	Strop <i>et al.</i> , 2012 JMB Volume 420, Issue 3, 13 July 2012, Pages 204-219	IgG2
F405L	K409R	Labrijn <i>et al.</i> , 2013 PNAS March 26, 2013. 110 (13) 5145-5150	IgG1
F405A/Y407V	T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1

F405A/Y407V	T366I/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
F405A/Y407V	T366L/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
F405A/Y407V	T366L/K392M/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
L351Y/F405A/Y407V	T366L/K392M/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
T350V/L351Y/F405A/Y407V	T350V/T366L/K392M/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
T350V/L351Y/F405A/Y407V	T350V/T366L/K392L/T394W	Von Kreudenstein <i>et al.</i> , 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
K409W	D339V/F405T	Choi <i>et al.</i> , 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K360E	Q347R	Choi <i>et al.</i> , 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K360E/K409W	D339V/Q347R/F405T	Choi <i>et al.</i> , 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
Y349C/K360E/K409W	D339V/Q347R/S354C/F405T	Choi <i>et al.</i> , 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K392A/K409D	E356K/D399K	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
T366W	T366S/L358A/Y407A	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
D339M/Y407A	T336V/K409V	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
D339M/K360D/Y407A	T336V/E345R/Q347R/K409V	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366V/K370Y/K409V	E357D/S364Q/Y407A	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366M/K370Y/K409V	E356G/E357D/S364Q/Y407A	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366M/K370Y/K409V	E357D/S364R/Y407A	Leaver-Fey <i>et al.</i> , 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
And any combination as described in Tables 1-3 of US20150284475A1			

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 5**, which reduce or eliminate FcγR and/or complement binding in the Fc domain. In embodiments, the **Table 5** mutations are in both chains.

Table 5

Chain 1 mutation	Reference	IgG
L234A/L235A	Alegre <i>et al.</i> , 1994 Transplantation 57:1537–1543	IgG1
F234A/L235A	Alegre <i>et al.</i> , 1994 Transplantation 57:1537–1543	IgG4
L235E	Morgan <i>et al.</i> , 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
L235E	Morgan <i>et al.</i> , 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG4
L235A	Morgan <i>et al.</i> , 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
G237A	Morgan <i>et al.</i> , 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
N297H	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG1
N297Q	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG1
N297K	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG3
N297Q	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG3
D265A	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
D270A, V, K	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
K322A, L, M, D, E	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
P329A, X	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
P331A, S, G, X	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
D265A	Idusogie <i>et al.</i> , 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1

L234A	Hezareh <i>et al.</i> , 2001 J. Virol. December 2001 vol. 75 no. 24 12161-12168	IgG1
L234A/L235A	Hezareh <i>et al.</i> , 2001 J. Virol. December 2001 vol. 75 no. 24 12161-12168	IgG1
L234F/L235E/P331S	Oganesyan <i>et al.</i> , 2008 Acta Cryst. (2008). D64, 700-704	IgG1
H268Q/V309L/A330S/P331S	An <i>et al.</i> , 2009 mAbs Volume 1, 2009 - Issue 6, pp. 572-579	IgG1
G236R/L328R	Moore <i>et al.</i> , 2011 mAbs Volume 3, 2011 - Issue 6, pp. 546-557	IgG1
N297G	Couch <i>et al.</i> , 2013 Sci. Transl. Med., 5 (2013) 183ra57, 1-12	IgG1
N297G/D265A	Couch <i>et al.</i> , 2013 Sci. Transl. Med., 5 (2013) 183ra57, 1-12	IgG1
V234A/G237A/P328S/H268A/V309L/A330S/P331S	Vafa <i>et al.</i> , 2014 Methods Volume 65, Issue 1, 1 January 2014, Pages 114-126	IgG2
L234A/L235A/P329G	Lo <i>et al.</i> , 2016 The Journal of Biological Chemistry 292, 3900-3908	IgG1
N297D	Schlothauer <i>et al.</i> , 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457-466	IgG1
<u>S228P</u> /L235E	Schlothauer <i>et al.</i> , 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457-466	IgG4
<u>S228P</u> /L235E/P329G	Schlothauer <i>et al.</i> , 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457-466	IgG4
L234F/L235A/K322Q	Borrok <i>et al.</i> , 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008-1017	IgG1
L234F/L235Q/P331G	Borrok <i>et al.</i> , 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008-1017	IgG1

L234F/L235Q/K322Q	Borrok <i>et al.</i> , 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008–1017	IgG1
L234A/L235A/G237A/P328S/H268A/A330S/P331S	Tam <i>et al.</i> , 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG1
<u>S228P</u> /F234A/L235A	Tam <i>et al.</i> , 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4
<u>S228P</u> /F234A/L235A/G237A/P238S	Tam <i>et al.</i> , 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4
<u>S228P</u> /F234A/L235A/G236V/G237A/P238S	Tam <i>et al.</i> , 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology are homodimeric, *i.e.*, the Fc region in the chimeric protein complex comprises two identical protein fragments.

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology are heterodimeric, *i.e.*, the Fc domain comprises two non-identical protein fragments.

In some embodiments, heterodimeric Fc domains are engineered using ionic pairing and/or knob-in-hole mutations described herein. In some embodiments, the heterodimeric Fc-based chimeric protein complexes have a trans orientation/configuration. In a trans orientation/configuration, the targeting moiety and signaling agent, *e.g.* IL-1 β are, in embodiments, not found on the same polypeptide chain in the present Fc-based chimeric protein complexes.

In some embodiments, the Fc domains includes or starts with the core hinge region of wild-type human IgG1, which contains the sequence Cys-Pro-Pro-Cys. In some embodiments, the Fc domains also include the upper hinge, or parts thereof (*e.g.*, DKTHTCPPC; see WO 2009053368), EPKSCDKTHTCPPC, or EPKSSDKTHTCPPC; see Lo *et al.*, Protein Engineering vol.11 no.6 pp.495–500, 1998)).

Fc-based Chimeric Protein Complexes

The Fc-based chimeric protein complexes of the present technology comprise at least one Fc domain disclosed herein, at least one signaling agent, *e.g.* IL-1 β (SA) disclosed herein, *e.g.* IL-1 β , and at least one targeting moiety (TM) disclosed herein.

It is understood that, the present Fc-based chimeric protein complexes may encompass a complex of two fusion proteins, each comprising an Fc domain.

In some embodiments, the Fc-based chimeric protein complex is heterodimeric. In some embodiments, the heterodimeric Fc-based chimeric protein complex has a trans orientation/configuration. In some embodiments, the heterodimeric Fc-based chimeric protein complex has a cis orientation/configuration.

In some embodiments, heterodimeric Fc domains are engineered using ionic pairing and/or knob-in-hole mutations described herein. In some embodiments, the heterodimeric Fc-based chimeric protein complexes have a trans orientation.

In a trans orientation, the targeting moiety and signaling agent are, in embodiments, not found on the same polypeptide chain in the present Fc-based chimeric protein complexes. In a trans orientation, the targeting moiety and signaling agent are, in embodiments, found on separate polypeptide chains in the Fc-based chimeric protein complexes. In a cis orientation, the targeting moiety and signaling agent are, in embodiments, found on the same polypeptide chain in the Fc-based chimeric protein complexes.

In some embodiments, where more than one targeting moiety is present in the heterodimeric protein complexes described herein, one targeting moiety may be in trans orientation (relative to the signaling agent), whereas another targeting moiety may be in cis orientation (relative to the signaling agent). In some embodiments, the signaling agent and target moiety are on the same ends/sides (N-terminal or C-terminal ends) of an Fc domain. In some embodiments, the signaling agent and targeting moiety are on different sides/ends of a Fc domain (N-terminal and C-terminal ends).

In some embodiments, where more than one targeting moiety is present in the heterodimeric protein complexes described herein, the targeting moieties may be found on the same Fc chain or on two different Fc chains in the heterodimeric protein complex (in the latter case the targeting moieties would be in trans relative to each other, as they are on different Fc chains). In some embodiments, where more than one targeting moiety is present on the same Fc chain, the targeting moieties may be on the same or different sides/ends of a Fc chain (N-terminal or/and C-terminal ends).

In some embodiments, where more than one signaling agent is present in the heterodimeric protein complexes described herein, the signaling agents may be found on the same Fc chain or on two different Fc chains in the heterodimeric protein complex (in the latter case the signaling agents would be in trans relative to each other, as they are on different Fc chains). In some embodiments, where more than one signaling agent is present on the same Fc chain, the signaling agents may be on the same or different sides/ends of a Fc chain (N-terminal or/and C-terminal ends).

In some embodiments, where more than one signaling agent is present in the heterodimeric protein complexes described herein, one signaling agent may be in trans orientation (as relates to the targeting moiety), whereas another signaling agent may be in cis orientation (as relates to the targeting moiety).

In some embodiments, the heterodimeric Fc-based chimeric protein complex does not comprise the signaling agent, e.g. IL-1 β and targeting moiety on a single polypeptide.

In some embodiments, the Fc-based chimeric protein has an improved in vivo half-life relative to a chimeric protein lacking an Fc or a chimeric protein which is not a heterodimeric complex. In some embodiments, the Fc-based chimeric protein has an improved solubility, stability and other pharmacological properties relative to a chimeric protein lacking an Fc or a chimeric protein which is not a heterodimeric complex.

5 Heterodimeric Fc-based chimeric protein complexes are composed of two different polypeptides. In embodiments described herein, the targeting domain is on a different polypeptide than the signaling agent, *e.g.* IL-1 β , and accordingly, proteins that contain only one targeting domain copy, and also only one signaling agent, *e.g.* IL-1 β copy can be made (this provides a configuration in which potential interference with desired properties can be controlled). Further, in embodiments, one targeting domain (*e.g.* VHH) only can avoid cross-linking of the antigen on the cell surface
10 (which could elicit undesired effects in some cases). Further, in embodiments, one signaling agent, *e.g.* IL-1 β may alleviate molecular "crowding" and potential interference with avidity mediated induction or restoration of effector function in dependence of the targeting domain. Further, in embodiments, heterodimeric Fc-based chimeric protein complexes can have two targeting moieties and these can be placed on the two different polypeptides. For instance, in embodiments, the C-terminus of both targeting moieties (*e.g.* VHHs) can be masked to avoid potential autoantibodies
15 or pre-existing antibodies (*e.g.* VHH autoantibodies or pre-existing antibodies). Further, in embodiments, heterodimeric Fc-based chimeric protein complexes, *e.g.* with the targeting domain on a different polypeptide than the signaling agent, *e.g.* IL-1 β (*e.g.* wild type signaling agent, *e.g.* wild type IL-1 β), may favor "cross-linking" of two cell types. Further, in embodiments, heterodimeric Fc-based chimeric protein complexes can have two signaling agent, each on different polypeptides to allow more complex effector responses.

20 Further, in embodiments, heterodimeric Fc-based chimeric protein complexes, *e.g.* with the targeting domain on a different polypeptide than the signaling agent, *e.g.* IL-1 β , combinatorial diversity of targeting moiety and signaling agent, *e.g.* IL-1 β is provided in a practical manner. For instance, in embodiments, polypeptides with any of the targeting moieties described herein can be combined "off the shelf" with polypeptides with any of the signaling agents described herein to allow rapid generation of various combinations of targeting moieties and signaling agents in single Fc-based
25 chimeric protein complexes.

In some embodiments, the Fc-based chimeric protein complex comprises one or more linkers. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects the Fc domain, signaling agent, *e.g.* IL-1 β (s) and targeting moiety(ies). In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each signaling agent, *e.g.* IL-1 β and targeting moiety (or, if more than one targeting moiety, a signaling agent, *e.g.* IL-
30 1 β to one of the targeting moieties). In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each signaling agent, *e.g.* IL-1 β to the Fc domain. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each targeting moiety to the Fc domain. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects a targeting moiety to another targeting moiety. In some

embodiments, the Fc-based chimeric protein complex includes a linker that connects a signaling agent, e.g. IL-1 β to another signaling agent.

In some embodiments, a Fc-based chimeric protein complex comprises two or more targeting moieties. In such embodiments, the targeting moieties can be the same targeting moiety or they can be different targeting moieties.

5 In some embodiments, a Fc-based chimeric protein complex comprises two or more signaling agents. In such embodiments, the signaling agents can be the same targeting moiety or they can be different targeting moieties.

By way of example, in some embodiments, the Fc-based chimeric protein complex comprise a Fc domain, at least two signaling agents (SA), and at least two targeting moieties (TM), wherein the Fc domain, signaling agents, and targeting moieties are selected from any of the Fc domains, signaling agents, and targeting moieties disclosed herein. In some

10 embodiments, the Fc domain is homodimeric.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 1A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 2A-H.

15 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 3A-H.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 4A-D.

20 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 5A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 6A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 7A-D.

25 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 8A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 9A-J.

30 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 10A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 11A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 12A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 13A-F.

5 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 14A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 15A-L.

10 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 16A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 17A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 18A-F.

15 In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 19A-F.

In some embodiments, the signaling agents are linked to the targeting moieties and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

20 In some embodiments, the signaling agents and targeting moieties are linked to the Fc domain, wherein the targeting moieties and signaling agents are linked on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

In some embodiments, the targeting moieties are linked to signaling agents and the signaling agents are linked to the Fc domain on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

25 In some embodiments, the homodimeric Fc-based chimeric protein complex has two or more targeting moieties. In some embodiments, there are four targeting moieties and two signaling agents, the targeting moieties are linked to the Fc domain and the signaling agents are linked to targeting moieties on the same terminus (see FIGS. 2A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to the Fc domain and two targeting moieties are linked to the signaling agents, which are linked to the Fc domain on the same terminus (see FIGS. 2A-H). In some embodiments,
30 the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to each other and one of the targeting moieties of from each pair is linked to the Fc domain on the same terminus and the signaling agents are linked to the Fc domain on the same terminus (see FIGS.

2A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to each other, wherein one of the targeting moieties of from each pair is linked to a signaling agent, *e.g.* IL-1 β and the other targeting moiety of the pair is linked the Fc domain, wherein the targeting moieties linked to the Fc domain are linked on the same terminus (see FIGS. 2A-H). In
5 some embodiments, the Fc domain is homodimeric.

In some embodiments, the homodimeric Fc-based chimeric protein complex has two or more signaling agents. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to each other and one of the signaling agents of from pair is linked to the Fc domain on the same terminus and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 3A-H). In some embodiments, the Fc
10 domain is homodimeric. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to the Fc domain one the same terminus and two of the signaling agents are each linked to a targeting moiety, wherein the targeting moieties are linked to the Fc domain at the same terminus (see FIGs. 3A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to each other and one of the signaling agents of from pair
15 is linked to a targeting moiety and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 3A-H). In some embodiments, the Fc domain is homodimeric.

By way of example, in some embodiments, the Fc-based chimeric protein complex comprise a Fc domain, wherein the Fc domain comprises ionic pairing mutation(s) and/or knob-in-hole mutation(s), at least one signaling agent, *e.g.* IL-1 β , and at least one targeting moiety, wherein the ionic pairing motif and/or a knob-in-hole motif, signaling agent, *e.g.*
20 IL-1 β , and targeting moiety are selected from any of the ionic pairing motif and/or a knob-in-hole motif, signaling agents, and targeting moieties disclosed herein. In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, the signaling agent, *e.g.* IL-1 β is linked to the targeting moiety, which is linked to the Fc domain (see FIGs. 10A-F and 13A-F). In some embodiments, the targeting moiety is linked to the signaling agent, *e.g.* IL-1 β ,
25 which is linked to the Fc domain (see FIGs. 10A-F and 13A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, the signaling agent, *e.g.* IL-1 β and targeting moiety are linked to the Fc domain (see FIGs. 4A-D, 7A-D, 10A-F, and 13A-F). In some embodiments, the targeting moiety and the signaling agent, *e.g.* IL-1 β are linked to different Fc chains on the same terminus (see FIGs. 4A-D and 7A-D). In some embodiments, the targeting moiety
30 and the signaling agent, *e.g.* IL-1 β are linked to different Fc chains on different termini (see FIGs. 4A-D and 7A-D). In some embodiments, the targeting moiety and the signaling agent, *e.g.* IL-1 β are linked to the same Fc chain (see FIGs. 10A-F and 13A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, e.g. IL-1 β and two targeting moieties, the signaling agent, e.g. IL-1 β is linked to the Fc domain and two targeting moieties can be: 1) linked to each other with one of the targeting moieties linked to the Fc domain; or 2) each linked to the Fc domain (see FIGs. 5A-F, 8A-F, 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the targeting moieties are linked on one Fc chain and the signaling agent, e.g. IL-1 β is on the other Fc chain (see FIGs. 5A-F and 8A-F). In some embodiments, the paired targeting moieties and the signaling agent, e.g. IL-1 β are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, a targeting moiety is linked to the Fc domain and the other targeting moiety is linked to the signaling agent, e.g. IL-1 β , and the paired targeting moiety is linked to the Fc domain (see FIGs. 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked to different Fc chains (see FIGs. 16A-J and 17A-J). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked on the same terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, e.g. IL-1 β and two targeting moieties, a targeting moiety is linked to the signaling agent, e.g. IL-1 β , which is linked to the Fc domain, and the unpaired targeting moiety is linked to the Fc domain (see FIGs. 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the paired signaling agent, e.g. IL-1 β and unpaired targeting moiety are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, the paired signaling agent, e.g. IL-1 β and unpaired targeting moiety are linked to different Fc chains (see FIGs. 16A-J and 17A-J). In some embodiments, the paired signaling agent, e.g. IL-1 β and unpaired targeting moiety are linked on the same terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, e.g. IL-1 β and two targeting moieties, the targeting moieties are linked together and the signaling agent, e.g. IL-1 β is linked to one of the paired targeting moieties, wherein the targeting moiety not linked to the signaling agent, e.g. IL-1 β is linked to the Fc domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, e.g. IL-1 β and two targeting moieties, the targeting moieties are linked together and the signaling agent, e.g. IL-1 β is linked to one of the paired targeting moieties, wherein the signaling agent, e.g. IL-1 β is linked to the Fc domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, e.g. IL-1 β and two targeting moieties, the targeting moieties are both linked to the signaling agent, e.g. IL-1 β , wherein one of the targeting moieties is linked to the Fc

domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there is one signaling agent, e.g. IL-1 β and two targeting moieties, the targeting moieties and the signaling agent, e.g. IL-1 β are linked to the Fc domain (see FIGs. 16A-J and 17A-J). In some embodiments, the targeting moieties are linked on the terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked to the Fc domain on the same terminus and the targeting moiety is linked to the Fc domain (see FIGs. 6A-J and 9A-J).

In some embodiments, the signaling agents are linked to the Fc domain on the same Fc chain and the targeting moiety is linked on the other Fc chain (see FIGs. 18A-F and 19A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, a signaling agent, e.g. IL-1 β is linked to the targeting moiety, which is linked to the Fc domain and the other signaling agent, e.g. IL-1 β is linked to the Fc domain (see FIGs. 6A-J, 9A-J, 12A-L, and 15A-L). In some embodiments, the targeting moiety and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains on the same terminus (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains on different termini (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, e.g. IL-1 β are linked to the same Fc chains (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the targeting moiety is linked to a signaling agent, e.g. IL-1 β , which is linked to the Fc domain and the other signaling agent, e.g. IL-1 β is linked to the Fc domain (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling agent, e.g. IL-1 β and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling agent, e.g. IL-1 β and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains on the same terminus (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling agent, e.g. IL-1 β and the unpaired signaling agent, e.g. IL-1 β are linked to different Fc chains on different termini (see FIGs. 6A-J and 9A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and the targeting moiety is linked to one of the paired signaling agents, wherein the targeting moiety is linked

to the Fc domain (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and one of the signaling agents is linked to the Fc domain and the targeting moiety is linked to the Fc domain (see FIGs. 12A-L, 15A-L, 18A-F, and 19A-F). In some embodiments, the paired signaling agents and targeting moiety are linked to the same Fc chain (see FIGs. 12A-L and 15A-L). In some embodiments, the paired signaling agents and targeting moiety are linked to different Fc chains (see FIGs. 18A-F and 19A-F). In some embodiments, the paired signaling agents and targeting moiety are linked to different Fc chains on the same terminus (see FIGs. 18A-F and 19A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are both linked to the targeting moiety, wherein one of the signaling agents is linked to the Fc domain (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and one of the signaling agents is linked to the targeting moiety and the other signaling agent, e.g. IL-1 β is linked to the Fc domain (see FIGs. 12A-L and 15A-L).

In some embodiments, where there are two signaling agents and one targeting moiety, each signaling agent, e.g. IL-1 β is linked to the Fc domain and the targeting moiety is linked to one of the signaling agents (see FIGs. 12A-L and 15A-L). In some embodiments, the signaling agents are linked to the same Fc chain (see FIGs. 12A-L and 15A-L).

In some embodiments, a targeting moiety or signaling agent, e.g. IL-1 β is linked to the Fc domain, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding the targeting moiety, signaling agent, e.g. IL-1 β , or combination thereof, linked as a single nucleotide sequence to an Fc domain can be used to prepare such polypeptides.

Additional Signaling Agents

In one aspect, the present invention provides a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising one or more signaling agents (for instance, an immune-modulating agent) in addition to the IL-1 β or a variant thereof described herein. In illustrative embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise two, three, four, five, six, seven, eight, nine, ten or more signaling agents in addition to the IL-1 β or a variant thereof described herein. In various embodiments, the additional signaling agent is modified to have reduced affinity or activity for one or more of its receptors, which allows for attenuation of activity (inclusive of agonism or antagonism) and/or prevents non-specific signaling or

undesirable sequestration of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex.

In various embodiments, the additional signaling agent is antagonistic in its wild type form and bears one or more mutations that attenuate its antagonistic activity. In various embodiments, the additional signaling agent is antagonistic due to one or more mutations, e.g. an agonistic signaling agent is converted to an antagonistic signaling agent and, such a converted signaling agent, optionally, also bears one or more mutations that attenuate its antagonistic activity (e.g. as described in WO 2015/007520, the entire contents of which are hereby incorporated by reference).

In various embodiments, the additional signaling agent is selected from modified versions of cytokines, growth factors, and hormones. Illustrative examples of such cytokines, growth factors, and hormones include, but are not limited to, lymphokines, monokines, traditional polypeptide hormones, such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and tumor necrosis factor- β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- α ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; osteo inductive factors; interferons such as, for example, interferon- α , interferon- β and interferon- γ (and interferon type I, II, and III), colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as, for example, IL-1 β , IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, and IL-18; a tumor necrosis factor such as, for example, TNF- α or TNF- β ; and other polypeptide factors including, for example, LIF and kit ligand (KL). As used herein, cytokines, growth factors, and hormones include proteins obtained from natural sources or produced from recombinant bacterial, eukaryotic or mammalian cell culture systems and biologically active equivalents of the native sequence cytokines.

In some embodiments, the additional signaling agent is a modified version of a growth factor selected from, but not limited to, transforming growth factors (TGFs) such as TGF- α and TGF- β , epidermal growth factor (EGF), insulin-like growth factor such as insulin-like growth factor-I and -II, fibroblast growth factor (FGF), heregulin, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF).

In an embodiment, the growth factor is a modified version of a fibroblast growth factor (FGF). Illustrative FGFs include, but are not limited to, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, murine FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF23.

In an embodiment, the growth factor is a modified version of a vascular endothelial growth factor (VEGF). Illustrative VEGFs include, but are not limited to, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PGF and isoforms thereof including the various isoforms of VEGF-A such as VEGF₁₂₁, VEGF_{121b}, VEGF₁₄₅, VEGF₁₆₅, VEGF_{165b}, VEGF₁₈₉, and VEGF₂₀₆.

In an embodiment, the growth factor is a modified version of a transforming growth factor (TGF). Illustrative TGFs include, but are not limited to, TGF- α and TGF- β and subtypes thereof including the various subtypes of TGF- β including TGF β 1, TGF β 2, and TGF β 3.

5 In some embodiments, the additional signaling agent is a modified version of a hormone selected from, but not limited to, human chorionic gonadotropin, gonadotropin releasing hormone, an androgen, an estrogen, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone, adrenocorticotrophic hormone, antidiuretic hormone, oxytocin, thyrotropin-releasing hormone, growth hormone releasing hormone, corticotropin-releasing hormone, somatostatin, dopamine, melatonin, thyroxine, calcitonin, parathyroid hormone, glucocorticoids, mineralocorticoids, adrenaline, noradrenaline, progesterone, insulin, glucagon, amylin, calcitriol, calciferol, atrial-
10 natriuretic peptide, gastrin, secretin, cholecystokinin, neuropeptide Y, ghrelin, PYY3-36, insulin-like growth factor (IGF), leptin, thrombopoietin, erythropoietin (EPO), and angiotensinogen.

In some embodiments, the additional signaling agent is an immune-modulating agent, e.g. one or more of an interleukin, interferon, and tumor necrosis factor.

In some embodiments, the additional signaling agent is an interleukin, including for example IL-1 β ; IL-2; IL-3; IL-4; IL-
15 5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-11; IL-12; IL-13; IL-14; IL-15; IL-16; IL-17; IL-18; IL-19; IL-20; IL-21; IL-22; IL-23; IL-24; IL-25; IL-26; IL-27; IL-28; IL-29; IL-30; IL-31; IL-32; IL-33; IL-35; IL-36 or a fragment, variant, analogue, or family-member thereof. Interleukins are a group of multi- functional cytokines synthesized by lymphocytes, monocytes, and macrophages. Known functions include stimulating proliferation of immune cells (e.g., T helper cells, B cells, eosinophils, and lymphocytes), chemotaxis of neutrophils and T lymphocytes, and/or inhibition of interferons.
20 Interleukin activity can be determined using assays known in the art: Matthews *et al.*, in *Lymphokines and Interferons: A Practical Approach*, Clemens *et al.*, eds, IRL Press, Washington, D.C. 1987, pp. 221-225; and Orencole & Dinarello (1989) Cytokine 1, 14-20.

In some embodiments, the signaling agent is a modified version of an interferon such as interferon types I, II, and III. Illustrative interferons, including for example, interferon- α -1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, and 21, interferon- β and
25 interferon- γ , interferon κ , interferon ϵ , interferon τ , and interferon ω .

In embodiments, the additional signaling agent is a type I interferon. In embodiments, the type I interferon is selected from IFN- α 2, IFN α 1, IFN- β , IFN- γ , Consensus IFN, IFN- ϵ , IFN- κ , IFN- τ , IFN- δ , and IFN- ν .

In some embodiments, the additional signaling agent is a modified version of a tumor necrosis factor (TNF) or a protein in the TNF family, including but not limited to, TNF- α , TNF- β , LT- β , CD40L, CD27L, CD30L, FASL, 4-1BBL, OX40L,
30 and TRAIL.

In various embodiments, the additional signaling agent is a modified (e.g. mutant) form of the signaling agent having one or more mutations. In various embodiments, the mutations allow for the modified signaling agent to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced

specific bioactivity relative to unmodified or unmutated, *i.e.* the wild type form of the signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). In various embodiments, the mutations allow for the modified signaling agent to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmodified or unmutated, *i.e.* the unmutated IL-1 β . In some embodiments, the mutations which attenuate or reduce binding or affinity include those mutations which substantially reduce or ablate binding or activity. In some embodiments, the mutations which attenuate or reduce binding or affinity are different than those mutations which substantially reduce or ablate binding or activity. Consequentially, in various embodiments, the mutations allow for the signaling agent to be more safe, *e.g.* have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated, *i.e.* wild type, signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). In various embodiments, the mutations allow for the signaling agent to be safer, *e.g.* have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated interferon, *e.g.* the unmutated sequence of IL-1 β .

In various embodiments, the additional signaling agent is modified to have one or more mutations that reduce its binding affinity or activity for one or more of its receptors. In some embodiments, the signaling agent is modified to have one or more mutations that substantially reduce or ablate binding affinity or activity for the receptors. In some embodiments, the activity provided by the wild type signaling agent is agonism at the receptor (*e.g.* activation of a cellular effect at a site of therapy). For example, the wild type signaling agent may activate its receptor. In such embodiments, the mutations result in the modified signaling agent to have reduced or ablated activating activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced activating signal to a target cell or the activating signal could be ablated. In some embodiments, the activity provided by the wild type signaling agent is antagonism at the receptor (*e.g.* blocking or dampening of a cellular effect at a site of therapy). For example, the wild type signaling agent may antagonize or inhibit the receptor. In these embodiments, the mutations result in the modified signaling agent to have a reduced or ablated antagonizing activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced inhibitory signal to a target cell or the inhibitory signal could be ablated. In various embodiments, the signaling agent is antagonistic due to one or more mutations, *e.g.* an agonistic signaling agent is converted to an antagonistic signaling agent (*e.g.* as described in WO 2015/007520, the entire contents of which are hereby incorporated by reference) and, such a converted signaling agent, optionally, also bears one or more mutations that reduce its binding affinity or activity for one or more of its receptors or that substantially reduce or ablate binding affinity or activity for one or more of its receptors.

In some embodiments, the reduced affinity or activity at the receptor is inducible or restorable by attachment with one or more of the targeting moieties or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In other embodiments, the reduced affinity or activity at the receptor is not substantially inducible or restorable by the activity of one or more of the targeting moieties or upon inclusion in the Fc-based chimeric protein complex disclosed herein.

In various embodiments, the additional signaling agent is active on target cells because the targeting moiety(ies) compensates for the missing/insufficient binding (e.g., without limitation and/or avidity) required for substantial activation. In various embodiments, the modified signaling agent is substantially inactive *en route* to the site of therapeutic activity and has its effect substantially on specifically targeted cell types which greatly reduces undesired side effects.

In some embodiments, the additional signaling agent may include one or more mutations that attenuate or reduce binding or affinity for one receptor (*i.e.*, a therapeutic receptor) and one or more mutations that substantially reduce or ablate binding or activity at a second receptor. In such embodiments, these mutations may be at the same or at different positions (*i.e.*, the same mutation or multiple mutations). In some embodiments, the mutation(s) that reduce binding and/or activity at one receptor is different than the mutation(s) that substantially reduce or ablate at another receptor. In some embodiments, the mutation(s) that reduce binding and/or activity at one receptor is the same as the mutation(s) that substantially reduce or ablate at another receptor. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a modified signaling agent that has both mutations that attenuate binding and/or activity at a therapeutic receptor and therefore allow for a more controlled, on-target therapeutic effect (e.g. relative wild type signaling agent) and mutations that substantially reduce or ablate binding and/or activity at another receptor and therefore reduce side effects (e.g. relative to wild type signaling agent).

In some embodiments, the substantial reduction or ablation of binding or activity is not substantially inducible or restorable with a targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In some embodiments, the substantial reduction or ablation of binding or activity is inducible or restorable with a targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In various embodiments, substantially reducing or ablating binding or activity at a second receptor also may prevent deleterious effects that are mediated by the other receptor. Alternatively, or in addition, substantially reducing or ablating binding or activity at the other receptor causes the therapeutic effect to improve as there is a reduced or eliminated sequestration of the therapeutic chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes away from the site of therapeutic action. For instance, in some embodiments, this obviates the need of high doses of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes that compensate for loss at the other receptor. Such ability to reduce dose further provides a lower likelihood of side effects.

In various embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced, substantially reduced, or ablated affinity, e.g. binding (e.g. K_D) and/or activation (for instance, when the modified signaling agent is an agonist of its receptor, measurable as, for example, K_A and/or EC_{50}) and/or inhibition (for instance, when the modified signaling agent is an antagonist of its receptor, measurable as, for example, K_I and/or IC_{50}), for one or more of its receptors. In various embodiments, the reduced affinity at the signaling agent's receptor allows for attenuation of activity (inclusive of agonism or antagonism). In such embodiments, the modified signaling agent has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about

30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the receptor relative to the wild type signaling agent. In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower relative to the wild type signaling agent (including, by way of non-limitation, relative to the unmutated IL-1 β).

In embodiments wherein the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has mutations that reduce binding at one receptor and substantially reduce or ablate binding at a second receptor, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor. In some embodiments, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor by about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In various embodiments, substantial reduction or ablation refers to a greater reduction in binding affinity and/or activity than attenuation or reduction.

In various embodiments, the additional modified signaling agent comprises one or more mutations that reduce the endogenous activity of the signaling agent to about 75%, or about 70%, or about 60%, or about 50%, or about 40%, or about 30%, or about 25%, or about 20%, or about 10%, or about 5%, or about 3%, or about 1%, *e.g.*, relative to the wild type signaling agent (including, by way of non-limitation, relative to the unmutated IL-1 β).

In various embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity and/or activity for a receptor of any one of the cytokines, growth factors, and hormones as described herein.

In some embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity for its receptor that is lower than the binding affinity of the targeting moiety(ies) for its(their) receptor(s). In some embodiments, this binding affinity differential is between signaling agent/receptor and targeting moiety/receptor on the same cell. In some embodiments, this binding affinity differential allows for the signaling agent, *e.g.* mutated signaling agent, to have localized, on-target effects and to minimize off-target effects that underlie side effects that are observed with wild type signaling agent. In some embodiments, this binding affinity is at

least about 2-fold, or at least about 5-fold, or at least about 10-fold, or at least about 15-fold lower, or at least about 25-fold, or at least about 50-fold lower, or at least about 100-fold, or at least about 150-fold.

Receptor binding activity may be measured using methods known in the art. For example, affinity and/or binding activity may be assessed by Scatchard plot analysis and computer-fitting of binding data (e.g. Scatchard, 1949) or by
5 reflectometric interference spectroscopy under flow through conditions, as described by Brecht *et al.* (1993), the entire contents of all of which are hereby incorporated by reference.

The amino acid sequences of the wild type signaling agents described herein are well known in the art. Accordingly, in various embodiments the additional modified signaling agent comprises an amino acid sequence that has at least
10 about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%,
15 or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the known wild type amino acid sequences of the signaling agents described herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments the additional modified signaling agent comprises an amino acid sequence that has at least
25 about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%,
30 or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any of the sequences disclosed herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or

about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments, the additional modified signaling agent comprises an amino acid sequence having one or more amino acid mutations. In some embodiments, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations.

In some embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions as described herein.

As described herein, the additional modified signaling agents bear mutations that affect affinity and/or activity at one or more receptors. In various embodiments, there is reduced affinity and/or activity at a therapeutic receptor, *e.g.* a receptor through which a desired therapeutic effect is mediated (*e.g.* agonism or antagonism). In various embodiments, the modified signaling agents bear mutations that substantially reduce or ablate affinity and/or activity at a receptor, *e.g.* a receptor through which a desired therapeutic effect is not mediated (*e.g.* as the result of promiscuity of binding). The receptors of any modified signaling agents, *e.g.*, one of the cytokines, growth factors, and hormones as described herein, are known in the art.

Linkers and Functional Groups

In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex optionally comprises one or more linkers. In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex such as Fc-based chimeric protein complex comprises a linker connecting the targeting moiety and the signaling agent (*e.g.*, IL-1 β or a variant thereof). In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex comprises a linker within the signaling agent (*e.g.*, IL-1 β or a variant thereof). In some embodiments, the linker may be utilized to link various functional groups, residues, or moieties as described herein to the vaccine composition, adjuvant, chimeric protein or chimeric protein complex. In some embodiments, the linker is a single amino acid or a plurality of amino acids that does not affect or reduce the stability, orientation, binding, neutralization, and/or clearance characteristics of the binding regions and the binding protein. In various embodiments, the linker is selected from a peptide, a protein, a sugar, or a nucleic acid.

In some embodiments vectors encoding the vaccine composition, adjuvant, chimeric protein, or chimeric protein complex linked as a single nucleotide sequence to any of the linkers described herein are provided and may be used to prepare such vaccine composition, adjuvant, chimeric protein or chimeric protein complex. In embodiments, the substituents of the Fc-based chimeric protein complex are expressed as nucleotide sequences in a vector.

In some embodiments, the linker length allows for efficient binding of a targeting moiety and the signaling agent (*e.g.*, IL-1 β or a variant thereof) to their receptors. For instance, in some embodiments, the linker length allows for efficient binding of one of the targeting moieties and the signaling agent to receptors on the same cell.

In some embodiments the linker length is at least equal to the minimum distance between the binding sites of one of the targeting moieties and the signaling agent to receptors on the same cell. In some embodiments the linker length is at least twice, or three times, or four times, or five times, or ten times, or twenty times, or 25 times, or 50 times, or one hundred times, or more the minimum distance between the binding sites of one of the targeting moieties and the signaling agent to receptors on the same cell.

As described herein, the linker length allows for efficient binding of one of the targeting moieties and the signaling agent to receptors on the same cell, the binding being sequential, e.g. targeting moiety/receptor binding preceding signaling agent/receptor binding.

In some embodiments, there are two linkers in a single chimera, each connecting the signaling agent to a targeting moiety. In various embodiments, the linkers have lengths that allow for the formation of a site that has a disease cell and an effector cell without steric hindrance that would prevent modulation of the either cell.

The invention contemplates the use of a variety of linker sequences. In various embodiments, the linker may be derived from naturally-occurring multi-domain proteins or are empirical linkers as described, for example, in Chichilli *et al.*, (2013), Protein Sci. 22(2):153-167, Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369 and Crasto *et al.*, (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference. In various embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex.

In some embodiments, the linker is a polypeptide. In some embodiments, the linker is less than about 100 amino acids long. For example, the linker may be less than about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long. In some embodiments, the linker is a polypeptide. In some embodiments, the linker is greater than about 100 amino acids long. For example, the linker may be greater than about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long. In some embodiments, the linker is flexible. In another embodiment, the linker is rigid.

In some embodiments directed to chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes having two or more targeting moieties, a linker connects the two targeting moieties to each other and this linker has a short length and a linker connects a targeting moiety and a signaling agent this linker is longer than the

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chimeric protein complexes such as Fc-based chimeric protein complex are known in the art, for example, see *Remington's Pharmaceutical Sciences*, 16th ed., Mack Publishing Co., Easton, Pa. (1980).

In various embodiments, each of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be conjugated and/or fused with another agent to extend half-life or otherwise improve pharmacodynamic and pharmacokinetic properties. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be fused or conjugated with one or more of PEG, XTEN (e.g., as rPEG), polysialic acid (POLYXEN), albumin (e.g., human serum albumin or HAS), elastin-like protein (ELP), PAS, HAP, GLK, CTP, transferrin, and the like. In various embodiments, each of the individual chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is fused to one or more of the agents described in BioDrugs (2015) 29:215–239, the entire contents of which are hereby incorporated by reference.

In some embodiments, the functional groups, residues, or moieties comprise a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). In some embodiments, attachment of the PEG moiety increases the half-life and/or reduces the immunogenicity of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to single domain antibodies such as VHs); see, for example, Chapman, *Nat. Biotechnol.*, 54, 531-545 (2002); by Veronese and Harris, *Adv. Drug Deliv. Rev.* 54, 453-456 (2003), by Harris and Chess, *Nat. Rev. Drug Discov.*, 2, (2003) and in WO04060965, the entire contents of which are hereby incorporated by reference. Various reagents for pegylation of proteins are also commercially available, for example, from Nektar Therapeutics, USA. In some embodiments, site-directed pegylation is used, in particular via a cysteine-residue (see, for example, Yang *et al.*, *Protein Engineering*, 16, 10, 761-770 (2003), the entire contents of which is hereby incorporated by reference). In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex is modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the amino-and/or carboxy-terminus of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex, using techniques known in the art.

In some embodiments, the functional groups, residues, or moieties comprise N-linked or O-linked glycosylation. In some embodiments, the N-linked or O-linked glycosylation is introduced as part of a co-translational and/or post-translational modification.

In some embodiments, the functional groups, residues, or moieties comprise one or more detectable labels or other signal-generating groups or moieties. Suitable labels and techniques for attaching, using and detecting them are known in the art and, include, but are not limited to, fluorescent labels (such as fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine and fluorescent metals such as Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels

(such as luminal, isoluminol, therronic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes, metals, metals chelates or metallic cations or other metals or metallic cations that are particularly suited for use in *in vivo*, *in vitro* or *in situ* diagnosis and imaging, as well as chromophores and enzymes (such as malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase). Other suitable labels include moieties that can be detected using NMR or ESR spectroscopy. Such labeled VHHs and polypeptides of the invention may, for example, be used for *in vitro*, *in vivo* or *in situ* assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays," etc.) as well as *in vivo* diagnostic and imaging purposes, depending on the choice of the specific label.

In some embodiments, the functional groups, residues, or moieties comprise a tag that is attached or genetically fused to the vaccine composition, adjuvant, chimeric protein or chimeric protein complex. In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex may include a single tag or multiple tags. The tag for example is a peptide, sugar, or DNA molecule that does not inhibit or prevent binding of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to its target or any other antigen of interest. In various embodiments, the tag is at least about: three to five amino acids long, five to eight amino acids long, eight to twelve amino acids long, twelve to fifteen amino acids long, or fifteen to twenty amino acids long. Illustrative tags are described for example, in U.S. Patent Publication No. US2013/0058962. In some embodiment, the tag is an affinity tag such as glutathione-S-transferase (GST) and histidine (His) tag. In an embodiment, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex comprises a His tag.

In some embodiments, the functional groups, residues, or moieties comprise a chelating group, for example, to chelate one of the metals or metallic cations. Suitable chelating groups, for example, include, without limitation, diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

In some embodiments, the functional groups, residues, or moieties comprise a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, *i.e.*, through formation of the binding pair. For example, the adjuvant, chimeric protein or chimeric protein complex may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used as a reporter, for example, in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may, for example, also be used to bind the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to a carrier, including carriers suitable for pharmaceutical

purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, *Journal of Drug Targeting*, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention.

Production of Chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein Complex

5 Methods for producing the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention are described herein. For example, DNA sequences encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention (e.g., DNA sequences encoding the signaling agent (e.g., IL-1 β or a variant thereof) and the targeting moiety and the linker) can be chemically synthesized using methods known in the art. Synthetic DNA sequences can be ligated to other appropriate nucleotide sequences, including, e.g., expression control sequences, to produce gene expression constructs encoding the desired chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. Accordingly, in various embodiments, the present invention provides for isolated nucleic acids comprising a nucleotide sequence encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention.

15 Nucleic acids encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention can be incorporated (ligated) into expression vectors, which can be introduced into host cells through transfection, transformation, or transduction techniques. For example, nucleic acids encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention can be introduced into host cells by retroviral transduction. Illustrative host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention. Accordingly, in various embodiments, the present invention provides expression vectors comprising nucleic acids that encode the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention. In various embodiments, the present invention additional provides host cells comprising such expression vectors.

25 Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, e.g., Trp or Tac, and a prokaryotic signal sequence. In another example, if the engineered gene is to be expressed in eukaryotic host cells, e.g., CHO cells, it is first inserted into an expression vector containing for example, a suitable eukaryotic promoter, a secretion signal, enhancers, and various introns. The gene construct can be introduced into the host cells using transfection, transformation, or transduction techniques.

The vaccine composition, adjuvant, chimeric protein or chimeric protein complex such as Fc-based chimeric protein complex of the invention can be produced by growing a host cell transfected with an expression vector encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex under conditions that permit expression of the protein. Following expression, the protein can be harvested and purified using techniques well known in the art, e.g., affinity tags such as glutathione-S-transferase (GST) and histidine tags or by chromatography.

Accordingly, in various embodiments, the present invention provides for a nucleic acid encoding a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention. In various embodiments, the present invention provides for a host cell comprising a nucleic acid encoding a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention.

In various embodiments, IL-1 β , its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IL-1 β or its variant may be expressed *in vivo*, for instance, in a patient. For example, in various embodiments, the IL-1 β , its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IL-1 β or its variant may administered in the form of nucleic acid which encodes for the IL-1 β or its variant or chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising IL-1 β or its variant. In various embodiments, the nucleic acid is DNA or RNA. In some embodiments, the IL-1 β , its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IL-1 β or its variant is encoded by a modified mRNA, *i.e.* an mRNA comprising one or more modified nucleotides. In some embodiments, the modified mRNA comprises one or modifications found in U.S. Patent No. 8,278,036, the entire contents of which are hereby incorporated by reference. In some embodiments, the modified mRNA comprises one or more of m5C, m5U, m6A, s2U, Ψ , and 2'-O-methyl-U. In some embodiments, the present invention relates to administering a modified mRNA encoding one or more of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In some embodiments, the present invention relates to gene therapy vectors comprising the same. In some embodiments, the present invention relates to gene therapy methods comprising the same. In various embodiments, the nucleic acid is in the form of an oncolytic virus, e.g. an adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus or vaccinia.

In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a targeting moiety that is a VHH. In various embodiments, the VHH is not limited to a specific biological source or to a specific method of preparation. For example, the VHH can generally be obtained: (1) by isolating the V_HH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring V_HH domain; (3) by "humanization" of a naturally occurring V_HH domain or by expression of a nucleic acid encoding a such humanized V_HH domain; (4) by "camelization" of a naturally occurring VH domain from any animal species, such as from a mammalian species, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by "camelization" of a "domain antibody" or "Dab" as described in the art, or by expression of a nucleic acid encoding such a camelized VH domain; (6) by using synthetic or semi-

synthetic techniques for preparing proteins, polypeptides or other amino acid sequences known in the art; (7) by preparing a nucleic acid encoding a VHH using techniques for nucleic acid synthesis known in the art, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of one or more of the foregoing.

In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that corresponds to the V_HH domains of naturally occurring heavy chain antibodies directed against a target of interest. In some embodiments, such V_HH sequences can generally be generated or obtained by suitably immunizing a species of Camelid with a molecule of based on the target of interest (*e.g.*, CD8, *etc.*) (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against the target of interest), by obtaining a suitable biological sample from the Camelid (such as a blood sample, or any sample of B-cells), and by generating V_HH sequences directed against the target of interest, starting from the sample, using any suitable known techniques. In some embodiments, naturally occurring V_HH domains against the target of interest can be obtained from naive libraries of Camelid V_HH sequences, for example, by screening such a library using the target of interest or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known in the art. Such libraries and techniques are, for example, described in WO 9937681, WO 0190190, WO 03025020 and WO 03035694, the entire contents of which are hereby incorporated by reference. In some embodiments, improved synthetic or semi-synthetic libraries derived from naive V_HH libraries may be used, such as V_HH libraries obtained from naive V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example, described in WO 0043507, the entire contents of which are hereby incorporated by reference. In some embodiments, another technique for obtaining V_HH sequences directed against a target of interest involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against the target of interest), obtaining a suitable biological sample from the transgenic mammal (such as a blood sample, or any sample of B-cells), using any suitable known techniques. For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02085945 and in WO 04049794 (the entire contents of which are hereby incorporated by reference) can be used.

In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that has been "humanized" *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of the naturally occurring V_HH sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being. This can be performed using humanization techniques known in the art. In some embodiments, possible humanizing substitutions or combinations of humanizing substitutions may be determined by methods known in the art, for example, by a comparison between the sequence of a VHH and the sequence of a naturally occurring human VH domain. In some embodiments, the humanizing substitutions are chosen such that the resulting humanized VHHs still retain advantageous functional properties. Generally, as a result of humanization, the VHHs of the invention may become more "human-like," while still retaining favorable properties such as a reduced

immunogenicity, compared to the corresponding naturally occurring V_HH domains. In various embodiments, the humanized VHHs of the invention can be obtained in any suitable manner known in the art and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_HH domain as a starting material.

5 In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that has been "camelized," *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_HH domain of a heavy chain antibody of a camelid. In some
10 embodiments, such "camelizing" substitutions are inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called Camelidae hallmark residues (see, for example, WO9404678, the entire contents of which are hereby incorporated by reference). In some embodiments, the VH sequence that is used as a starting material or starting point for generating or designing the camelized VHH is a VH sequence from a mammal, for example, the VH sequence of a human being, such as a VH3 sequence. In various embodiments, the camelized VHHs can be obtained in any suitable manner known in the art and thus are not strictly limited to polypeptides that have been
15 obtained using a polypeptide that comprises a naturally occurring VH domain as a starting material.

In various embodiments, both "humanization" and "camelization" can be performed by providing a nucleotide sequence that encodes a naturally occurring V_HH domain or VH domain, respectively, and then changing, in a manner known in the art, one or more codons in the nucleotide sequence in such a way that the new nucleotide sequence encodes a
20 "humanized" or "camelized" VHH, respectively. This nucleic acid can then be expressed in a manner known in the art, so as to provide the desired VHH of the invention. Alternatively, based on the amino acid sequence of a naturally occurring V_HH domain or VH domain, respectively, the amino acid sequence of the desired humanized or camelized VHH of the invention, respectively, can be designed and then synthesized *de novo* using techniques for peptide synthesis known in the art. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring V_HH domain or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized VHH,
25 respectively, can be designed and then synthesized *de novo* using techniques for nucleic acid synthesis known in the art, after which the nucleic acid thus obtained can be expressed in a manner known in the art, so as to provide the desired VHH of the invention. Other suitable methods and techniques for obtaining the VHHs of the invention and/or nucleic acids encoding the same, starting from naturally occurring VH sequences or V_HH sequences, are known in the art, and may, for example, comprise combining one or more parts of one or more naturally occurring VH sequences
30 (such as one or more FR sequences and/or CDR sequences), one or more parts of one or more naturally occurring V_HH sequences (such as one or more FR sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide a VHH of the invention or a nucleotide sequence or nucleic acid encoding the same.

Pharmaceutically Acceptable Salts and Excipients

The vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt. A pharmaceutically acceptable acid addition salt is formed from a pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

Pharmaceutically acceptable salts include, by way of non-limiting example, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphorsulfonate, pamoate, phenylacetate, trifluoroacetate, acrylate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, isobutyrate, phenylbutyrate, α -hydroxybutyrate, butyne-1,4-dicarboxylate, hexyne-1,4-dicarboxylate, caprate, caprylate, cinnamate, glycollate, heptanoate, hippurate, malate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, phthalate, teraphthalate, propiolate, propionate, phenylpropionate, sebacate, suberate, p-bromobenzenesulfonate, chlorobenzenesulfonate, ethylsulfonate, 2-hydroxyethylsulfonate, methylsulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, naphthalene-1,5-sulfonate, xylenesulfonate, and tartarate salts.

The term "pharmaceutically acceptable salt" also refers to a salt of the compositions of the present invention having an acidic functional group, such as a carboxylic acid functional group, and a base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or tri-alkylamines, dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-OH-lower alkylamines), such as mono-, bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxyl-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

In some embodiments, the compositions described herein are in the form of a pharmaceutically acceptable salt.

Pharmaceutical Compositions and Formulations

In various embodiments, the present invention pertains to vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein and a pharmaceutically acceptable carrier or excipient. Any vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein can be administered to a subject as a

component of a composition that comprises a pharmaceutically acceptable carrier or vehicle. Such compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration.

In various embodiments, pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In one embodiment, the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a useful excipient when any agent described herein is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, specifically for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Any agent described herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents. Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

The present invention includes the described vaccine composition, adjuvant, chimeric protein or chimeric protein complex in various formulations. Any inventive vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, gelatin capsules, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, lyophilized powder, frozen suspension, dessicated powder, or any other form suitable for use. In yet another embodiment, the vaccine composition is formulated as a liquid.

Where necessary, the inventive the vaccine composition, adjuvant, chimeric protein or chimeric protein complex can also include a solubilizing agent. Also, the agents can be delivered with a suitable vehicle or delivery device as known in the art. Combination therapies outlined herein can be co-delivered in a single delivery vehicle or delivery device.

The formulations comprising the inventive the vaccine composition, adjuvant, chimeric protein or chimeric protein complex of the present invention may conveniently be presented in unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods generally include the step of bringing the therapeutic agents into association with a carrier, which constitutes one or more accessory ingredients. Typically, the formulations are prepared by uniformly and intimately bringing the therapeutic agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into dosage forms of the desired formulation (e.g., wet or dry granulation, powder blends, etc., followed by tableting using conventional methods known in the art).

In various embodiments, any compositions (and/or additional agents) described herein is formulated in accordance with routine procedures as a composition adapted for a mode of administration described herein.

Routes of administration include, for example: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically. In some embodiments, the vaccine composition, the adjuvant, and/or the antigen are formulated for administration intravenously. In embodiments, the vaccine composition, the adjuvant and/or the antigen are formulated for administration to the lung. In embodiments, the vaccine composition, the adjuvant and/or the antigen are formulated for administration by inhalation. In embodiments, the vaccine composition, the adjuvant and/or the antigen are formulated for administration via aerosol or nebulizer. In some embodiments, the vaccine composition, the adjuvant and/or the antigen are formulated for administration liquid nebulization, dry powder dispersion and meter-dose administration.

Administration can be local or systemic. In some embodiments, the administering is effected orally. In another embodiment, the administration is by parenteral injection. The mode of administration can be left to the discretion of the practitioner, and depends in-part upon the site of the medical condition. In most instances, administration results in the release of any agent described herein into the bloodstream.

In one embodiment, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein is formulated in accordance with routine procedures as a composition adapted for oral administration. Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can comprise one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation.

Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period. Selectively permeable membranes surrounding an osmotically active driving vaccine compositions described herein are also suitable for orally administered compositions. In these latter platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be useful. Oral compositions can include standard excipients such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, and magnesium carbonate. In one embodiment, the excipients are of pharmaceutical grade. Suspensions, in addition to the active compounds, may contain suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, *etc.*, and mixtures thereof.

Dosage forms suitable for parenteral administration (*e.g.* intravenous, intramuscular, intraperitoneal, subcutaneous and intra-articular injection and infusion) include, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (*e.g.* lyophilized composition), which can

be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art. Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

The compositions provided herein, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Any inventive vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein can be administered by controlled-release or sustained-release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,556, each of which is incorporated herein by reference in its entirety. Such dosage forms can be useful for providing controlled-or sustained-release of one or more active ingredients using, for example, hydropropyl cellulose, hydropropylmethyl cellulose, polyvinylpyrrolidone, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled- or sustained-release formulations known to those skilled in the art, including those described herein, can be readily selected for use with the active ingredients of the agents described herein. The invention thus provides single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled- or sustained-release.

Controlled- or sustained-release of an active ingredient can be stimulated by various conditions, including but not limited to, changes in pH, changes in temperature, stimulation by an appropriate wavelength of light, concentration or availability of enzymes, concentration or availability of water, or other physiological conditions or compounds.

In another embodiment, a controlled-release system can be placed in proximity of the target area to be treated, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, *Science* 249:1527-1533) may be used.

Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

Administration and Dosage

5 It will be appreciated that the actual dose of the vaccine composition, adjuvant, chimeric protein or chimeric protein complex to be administered according to the present invention will vary according to the particular dosage form, and the mode of administration. Many factors that may modify the action of the vaccine composition, adjuvant, chimeric protein or chimeric protein complex (e.g., body weight, gender, diet, time of administration, route of administration, rate of excretion, condition of the subject, drug combinations, genetic disposition and reaction sensitivities) can be taken
10 into account by those skilled in the art. Administration can be carried out continuously or in one or more discrete doses within the maximum tolerated dose. Optimal administration rates for a given set of conditions can be ascertained by those skilled in the art using conventional dosage administration tests.

In some embodiments, a suitable dosage of the vaccine composition, adjuvant, chimeric protein or chimeric protein complex is in a range of about 0.01 µg/kg to about 100 mg/kg of body weight of the subject, about 0.01 µg/kg to about
15 10 mg/kg of body weight of the subject, or about 0.01 µg/kg to about 1 mg/kg of body weight of the subject for example, about 0.01 µg/kg, about 0.02 µg/kg, about 0.03 µg/kg, about 0.04 µg/kg, about 0.05 µg/kg, about 0.06 µg/kg, about 0.07 µg/kg, about 0.08 µg/kg, about 0.09 µg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8
20 mg/kg, 1.9 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg body weight, or about 100 mg/kg body weight, inclusive of all values and ranges therebetween.

Individual doses of the vaccine composition, adjuvant, chimeric protein or chimeric protein complex can be administered in unit dosage forms (e.g., tablets, capsules, or liquid formulations) containing, for example, from about
25 1 µg to about 100 mg, from about 1 µg to about 90 mg, from about 1 µg to about 80 mg, from about 1 µg to about 70 mg, from about 1 µg to about 60 mg, from about 1 µg to about 50 mg, from about 1 µg to about 40 mg, from about 1 µg to about 30 mg, from about 1 µg to about 20 mg, from about 1 µg to about 10 mg, from about 1 µg to about 5 mg, from about 1 µg to about 3 mg, from about 1 µg to about 1 mg per unit dosage form, or from about 1 µg to about 50 µg per unit dosage form. For example, a unit dosage form can be about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29, about 30 µg, about 35 µg, about 40 µg, about 45 µg, about 50 µg, about 60 µg, about 70 µg, about 80 µg, about 90 µg, about 0.1 mg, about
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0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg, inclusive of all values and ranges therebetween.

In one embodiment, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex is administered at an amount of from about 1 µg to about 100 mg daily, from about 1 µg to about 90 mg daily, from about 1 µg to about 80 mg daily, from about 1 µg to about 70 mg daily, from about 1 µg to about 60 mg daily, from about 1 µg to about 50 mg daily, from about 1 µg to about 40 mg daily, from about 1 µg to about 30 mg daily, from about 1 µg to about 20 mg daily, from about 01 µg to about 10 mg daily, from about 1 µg to about 5 mg daily, from about 1 µg to about 3 mg daily, or from about 1 µg to about 1 mg daily. In various embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex is administered at a daily dose of about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29, about 30 µg, about 35 µg, about 40 µg, about 45 µg, about 50 µg, about 60 µg, about 70 µg, about 80 µg, about 90 µg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg, inclusive of all values and ranges therebetween.

In accordance with certain embodiments of the invention, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex may be administered, for example, more than once daily (e.g., about two times, about three times, about four times, about five times, about six times, about seven times, about eight times, about nine times, or about ten times daily), about once per day, about every other day, about every third day, about once a week, about once every two weeks, about once every month, about once every two months, about once every three months, about once every six months, or about once every year. In an embodiment, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex is administered about three times a week.

In various embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex may be administered for a prolonged period. For example, the vaccine composition comprising chimeric proteins or chimeric protein complexes may be administered as described herein for at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 9 weeks, at least about 10 weeks, at least about 11 weeks, or at least about 12 weeks. For example, the vaccine composition may be administered for 12 weeks, 24 weeks, 36 weeks or 48 weeks. In some

embodiments, the vaccine composition is administered for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, or at least about 12 months. In some embodiments, the vaccine composition may be administered for at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, or at least about 5 years.

Combination Therapy and Additional Therapeutic Agents

In various embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex of the present invention is co-administered in conjunction with additional therapeutic agent(s). Co-administration can be simultaneous or sequential.

In one embodiment, the additional therapeutic agent and the vaccine composition, adjuvant, chimeric protein or chimeric protein complex of the present invention are administered to a subject simultaneously. The term "simultaneously" as used herein, means that the additional therapeutic agent and the vaccine composition are administered with a time separation of no more than about 60 minutes, such as no more than about 30 minutes, no more than about 20 minutes, no more than about 10 minutes, no more than about 5 minutes, or no more than about 1 minute. Administration of the additional therapeutic agent and the vaccine composition, adjuvant, chimeric protein or chimeric protein complex can be by simultaneous administration of a single formulation (e.g., a formulation comprising the additional therapeutic agent and the vaccine composition) or of separate formulations (e.g., a first formulation including the additional therapeutic agent and a second formulation including the vaccine composition).

Co-administration does not require the therapeutic agents to be administered simultaneously, if the timing of their administration is such that the pharmacological activities of the additional therapeutic agent and the vaccine composition, adjuvant, chimeric protein or chimeric protein complex overlap in time, thereby exerting a combined therapeutic effect. For example, the additional therapeutic agent and the vaccine composition, adjuvant, chimeric protein or chimeric protein complex can be administered sequentially. The term "sequentially" as used herein means that the additional therapeutic agent and the vaccine composition are administered with a time separation of more than about 60 minutes. For example, the time between the sequential administration of the additional therapeutic agent and the vaccine composition can be more than about 60 minutes, more than about 2 hours, more than about 5 hours, more than about 10 hours, more than about 1 day, more than about 2 days, more than about 3 days, more than about 1 week apart, more than about 2 weeks apart, or more than about one month apart. The optimal administration times will depend on the rates of metabolism, excretion, and/or the pharmacodynamic activity of the additional therapeutic agent and the vaccine composition, adjuvant, chimeric protein or chimeric protein complex being administered.

Co-administration also does not require the therapeutic agents to be administered to the subject by the same route of administration. Rather, each therapeutic agent can be administered by any appropriate route, for example, parenterally or non-parenterally.

In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex herein acts synergistically when co-administered with another therapeutic agent. In such embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex and the additional therapeutic agent may be administered at doses that are lower than the doses employed when the agents are used in the context of monotherapy.

5 In some embodiments, inclusive of, without limitation, infectious disease applications, the present invention pertains to anti-infectives as additional therapeutic agents. In some embodiments, the anti-infective is an anti-viral agent including, but not limited to, Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuvirtide, Etravirine, Famciclovir, and Foscarnet. In some
10 embodiments, the anti-infective is an anti-bacterial agent including, but not limited to, cephalosporin antibiotics (cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftibiprole); fluoroquinolone antibiotics (cipro, Levaquin, floxin, tequin, avelox, and norflox); tetracycline antibiotics (tetracycline, minocycline, oxytetracycline, and doxycycline); penicillin antibiotics (amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); monobactam antibiotics (aztreonam); and carbapenem
15 antibiotics (ertapenem, doripenem, imipenem/cilastatin, and meropenem). In some embodiments, the anti-infectives include anti-malarial agents (e.g., chloroquine, quinine, mefloquine, primaquine, doxycycline, artemether/lumefantrine, atovaquone/proguanil and sulfadoxine/pyrimethamine), metronidazole, tinidazole, ivermectin, pyrantel pamoate, and albendazole.

Additional therapeutic agents, e.g. for coronavirus-related methods, include, for example, one or more of acyclovir, ganciclovir, remdesivir; favipiravir; galidesivir; prezcobix; lopinavir and/or ritonavir and/or arbidol; mRNA-1273;
20 recombinant proteins such as agonists, antagonists, blockers, or decoy mimetics of the viral spike protein, or agonists, antagonists, blockers, or decoy mimetics of the ACE2 protein; stem cell-derived exosomes; lopinavir/ritonavir and/or ribavirin and/or IFN-alpha, IFN-beta, IFN-gamma; xiyanning; anti-VEGF-A agents (e.g. Bevacizumab, ranibizumab, aflibercept, and others); fingolimod; carrimycin; hydroxychloroquine chloroquine; darunavir and cobicistat; prednisone, prednisolone, methylprednisolone; fluocinalone, brilacidin; leronlimab (PRO 140); and thalidomide. In various
25 embodiments, the present adjuvants are administered to a patient undergoing treatment with one or more additional therapeutic agents. In various embodiments, additional therapeutic agents include anti-virals, anti-inflammatories, agents that reduce vascular leakage and tissue edema, anti-fibrotic agents. Additional therapeutic agents, include, for example, one or more of acyclovir, ganciclovir, remdesivir; favipiravir; galidesivir; prezcobix; lopinavir and/or ritonavir and/or arbidol; mRNA-1273; recombinant proteins such as agonists, antagonists, blockers, or decoy mimetics of the
30 viral spike protein, or agonists, antagonists, blockers, or decoy mimetics of the ACE2 protein; stem cell-derived exosomes; lopinavir/ritonavir and/or ribavirin and/or IFN-alpha, IFN-beta, IFN-gamma; xiyanning; anti-VEGF-A agents (e.g. Bevacizumab, ranibizumab, aflibercept, and others); fingolimod; carrimycin; hydroxychloroquine chloroquine; darunavir and cobicistat; prednisone, prednisolone, methylprednisolone; fluocinalone, brilacidin; leronlimab (PRO 140); and thalidomide.

In some embodiments, the additional therapeutic agents include convalescent plasma, i.e., plasma from a donor subject (e.g. a human subject) who has recovered from the viral infection, e.g., SARS-CoV-2. In some embodiments, the additional therapeutic agents include plasma from a donor subject (e.g. a human subject) comprising IgG and IgM antibodies directed against a virus disclosed herein that causes an infection or a disease, e.g., SARS-CoV-2.

5 In some embodiments, the present invention relates to combination therapy with one or more chimeric agents described in WO 2013/10779, WO 2015/007536, WO 2015/007520, WO 2015/007542, and WO 2015/007903, the entire contents of which are hereby incorporated by reference in their entireties.

In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex includes derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the composition such that
10 covalent attachment does not prevent the activity of the composition. For example, but not by way of limitation, derivatives include composition that have been modified by, *inter alia*, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin,
15 etc.

In still other embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex further comprise a cytotoxic agent, comprising, in illustrative embodiments, a toxin, a chemotherapeutic agent, a radioisotope, and an agent that causes apoptosis or cell death. Such agents may be conjugated to a composition described herein. The vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein may be modified
20 post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

Illustrative cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine,
25 cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclophosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin
30 (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimetabolic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, teniposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol,

puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine, bleomycin, VEGF antagonists, EGFR antagonists, platins, taxols, irinotecan, 5-fluorouracil, gemcytabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids (e.g., vinblastine, vincristine, vindesine and vinorelbine), mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins (e.g. IL-12 or IL-2), IL-12R antagonists, Toxin conjugated monoclonal antibodies, Erbitux, Avastin, Pertuzumab, anti-CD20 antibodies, Rituxan, ocrelizumab, ofatumumab, DXL625, HERCEPTIN®, or any combination thereof. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the therapeutic agents (e.g. antibodies) to generate cell-type-specific-killing reagents (Youle, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, *et al.*, Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta particles is stably coupled to the vaccine composition comprising chimeric proteins or chimeric protein complexes, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32, Scandium-47, Copper-67, Gallium-67, Yttrium-88, Yttrium-90, Iodine-125, Iodine-131, Samarium-153, Lutetium-177, Rhenium-186 or Rhenium-188, and alpha-emitters such as Astatine-211, Lead-212, Bismuth-212, Bismuth-213 or Actinium-225.

Illustrative detectable moieties further include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, beta-galactosidase and luciferase. Further illustrative fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further illustrative chemiluminescent moieties include, but are not limited to, luminol. Further illustrative bioluminescent materials include, but are not limited to, luciferin and aequorin. Further illustrative radioactive materials include, but are not limited to, Iodine-125, Carbon-14, Sulfur-35, Tritium and Phosphorus-32.

Methods of Treatment or Vaccination

Methods and compositions described herein have application to treating or vaccinating against various diseases and disorders, including, e.g., infectious diseases. Further, any of the disclosed vaccine compositions, adjuvants, chimeric proteins, or chimeric protein complexes may be for use in the treating/vaccinating against, or the manufacture of a medicament for treating, various diseases and disorders, including, infections.

In some embodiments, the vaccine composition, adjuvant, chimeric protein or chimeric protein complex described herein are suitable for vaccinating against, preventing, or mitigating a disease or disorder is an infectious disease. In some embodiments, the disease or disorder is selected from diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles.

One aspect of the present invention is related to a method for vaccinating a subject against an infectious disease, comprising administering: (a) administering an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a mutant IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) an antigen which is suitable for inducing an immune response.

In some embodiments, the infectious disease is an infection with a pathogen, optionally selected from a bacterium, virus, fungus, or parasite. In some embodiments, the virus is: (a) an influenza virus, optionally selected from Type A, Type B, Type C, and Type D influenza viruses, (b) a member of the *Coronaviridae* family, optionally selected from a betacoronavirus, optionally selected from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV, Middle East Respiratory Syndrome-Corona Virus (MERS-CoV), HCoV-HKU1, and HCoV-OC43 or an alphacoronavirus, optionally selected from HCoV-NL63 and HCoV-229E, or (c) a member of Picornaviridae family, optionally selected from Rhinovirus A or Rhinovirus B.

Coronavirus infection 2019 (COVID-19), caused by SARS-CoV-2 (e.g., 2019-nCoV), is a disease thought to be originated from the bat. COVID-19 causes severe respiratory distress and this RNA virus strain has been the cause of a worldwide outbreak that was declared a major threat to public health and worldwide emergency. Phylogenetic analysis of the complete genome of 2019-nCoV revealed that the virus was most closely related (89.1% nucleotide similarity) to a group of SARS-like coronaviruses (genus Betacoronavirus, subgenus Sarbecovirus). Wu et al., A new coronavirus associated with human respiratory disease in China. *Nature*, Feb 3, 2020. 2019-nCoV is thought to spread from person-to-person and the spread may be possible from contact with infected surfaces or objects.

In some embodiments, the virus is SARS-CoV-2. In some embodiments, the antigen is a 2019-nCoV protein, or an antigenic fragment thereof, optionally selected from spike surface glycoprotein, membrane glycoprotein M, envelope protein E, and nucleocapsid phosphoprotein N. In some embodiments, the antigen is the S1 or S2 subunit of the spike surface glycoprotein, or an antigenic fragment thereof.

In an embodiment, the spike surface glycoprotein comprises the amino acid sequence of SEQ ID NO: 31:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSG
 TNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVMIKVCEFQFCNDPFLGVYY
 HKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLP
 5 QGFSALEPLVDLPIGINITRFQTLALHRSYLTGDSGGWTAGAAAYYVGYLQPRTFLLKYNENGTTITDAVD
 CALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRKISNCVA
 DYSLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLPDDFTGCVIA
 WNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGY
 QPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVR
 10 DPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGC
 LIGAEHVNNSECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTT
 EILPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDF
 GGFNFSQILPDPSPKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMI
 QYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSSTASA
 15 LGKLQDVVNQNAQALNTLVKQLSSNFGAIVSVLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI
 RASANLAATKMSECVLGQSKRVDFCGKGYHLSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHGDKAH
 FPREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVWIGVNNVTYDPLQPELDSFKEELDKYFKNHT
 SPDVDLGDISGINASVWNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLC
 CMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLLKGVKLHYT

20 In an embodiment, the membrane glycoprotein precursor M comprises the amino acid sequence of SEQ ID NO: 32:

MADSNGTITVEELKKLLEQWNLVIGFLFTWICLLQFAYANRNRFLYIIKLIFLWLLWPVTLACFVLAAYRINW
 ITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILLNVPLHGTILTRPILLESELVIGAVILRGHLRI
 AGHHLGRCDIKDLPKEITVATSRTLSTYYKLGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ

In an embodiment, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 33:

25 MYSFVSEETGLIVNSVLLFLAFVFLVLTALRLCAYCCNIVNVSLVKPSFYVYSRVKNLNSSRPDLLV

In an embodiment, the nucleocapsid phosphoprotein N comprises the amino acid sequence of SEQ ID NO: 34:

MSDNGPQNQRNAPRITFGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLKFP
 GQGVPIINTSSPDDQIGYYRRATRIRGGDGKMKDLSRWYFYLLGTGPEAGLPYGANKDGIWVATEGAL
 NTPKDHIGTRNPANNAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSRSRNSRNPSTPGSSRGTSPAR
 30 MAGNGGDAALALLLDRLNQLESKMSGKGQQQQGQTVTKSAAEASKKPRQKRTATKAYNVTQAFGRRG
 PEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDKDPNFKD
 QVILLNKHIDAYKTFPTEPKKDKKKKADETQALPQRQKKQQTVTLPAADLDDFSKQLQQSMSSADSTQA

In various embodiments, the subject is afflicted with coronavirus disease 2019 (COVID-19). In additional embodiments, the subject is elderly and/or afflicted with one or more comorbidities, including, but not limited to, hypertension and/or diabetes. A subject afflicted with a coronavirus infection can acquire symptoms including, but not limited to, fever, tiredness, dry cough, aches and pains, shortness of breath and other breathing difficulties, diarrhea, upper respiratory symptoms (e.g. sneezing, runny nose, nasal congestion, cough, sore throat), pneumonia, pneumonia respiratory failure, hepatic and renal insufficiency, acute respiratory distress syndrome (ARDS), and a cytokine imbalance.

In some embodiments, the virus is an influenza virus. In some embodiments, the antigen is an influenza viral antigen, optionally selected from hemagglutinin (HA) protein, matrix 2 (M2) protein, and neuraminidase, or an antigenic fragment thereof.

In some embodiments, the antigens described herein have at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with its wild type sequence. In some embodiments, the spike surface glycoprotein, membrane glycoprotein M, envelope protein E, and nucleocapsid phosphoprotein N have at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with their sequences as shown above.

In various embodiments, the SARS-CoV-2 surface glycoprotein comprises the amino acid sequence of SEQ ID NO:31. In various embodiments, the SARS-CoV-2 fragment comprises amino acid residues F486, N487, Q493, Q498, T500, N501 of the SARS-CoV-2 surface glycoprotein, having the amino acid sequence of SEQ ID NO: 31, that interact with the $\alpha 1$ helix of the ACE2 receptor.

In some embodiments, the SARS-CoV-2 peptide is a fragment of the SARS-CoV-2 RBD or the spike protein, including the wild type or a variant (also referred to as lineages). In some embodiments, the SARS-CoV-2 peptide is a fragment of SEQ ID NO: 31 or a variant thereof. In embodiments, the wild type SARS-CoV-2 coronavirus is the "Wuhan strain."

In embodiments, the present vaccine is pan-antigenic, thus providing immune response to the wild type (e.g., "Wuhan strain") and numerous variants of the coronavirus. In embodiments, the present vaccine comprises one or more peptides of the wild type and/or a variants of the spike proteins, or RBD thereof. Accordingly, in various embodiments, the vaccine includes two or more peptides of a respective variant, lineage, or strain of a coronavirus protein. For example, the variants can include a coronavirus protein having a mutation (e.g., without limitation, a substitution, deletion, or insertion) in any part of the spike, or the RBD thereof, protein, such as in the S1 subunit (e.g., in the RBD of the Spike protein), or in the S2 subunit. In some embodiments, a mutation is in a glycosylation site of the Spike protein.

In some embodiments, the variant (also referred to as lineages) is one or more of B.1.1.7, B.1.351, B.1.617.2, B.1.427, B.1.429, B.1.525, B.1.526, B.1.617.1, B.1.617.3, B.1, B.1.1.28, B.1.2, CAL.20C, B.6, P.1, and P.2 variants and/or any other variants, or antigenic fragments thereof. In some embodiments, the lineages include A.1, A.2, A.3, A.4, A.5, A.6,

A.7, A.8, A.9, B, B.1, B.1.1, B.1.1.1, B.2, B.3, B.4, B.5, B.6, B.7, B.9, B.10, B.11, B.12, B.13, B.14, B.15, B.16, B.17, B.18, B.19, B.20, B.21, B.22, B.23, B.24, B.25, B.26, B.27, C.1, C.2, C.3, D.1, and D2.

In some embodiments, the SARS-CoV-2 variant is B.1.1.7, also known as the Alpha variant. In embodiments, the B.1.1.7 ("Alpha") variant comprises one or more mutations selected from 69del, 70del, 144del, (E484K*), (S494P*),
 5 N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H (K1191N*), relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.351, also known as the Beta variant. In embodiments, the B.1.351 ("Beta") variant comprises one or more mutations selected from D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, and A701V, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment
 10 thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.617.2, also known as the Delta variant. In embodiments, the B.1.617.2 ("Delta") variant comprises one or more mutations selected from T19R, (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

15 In some embodiments, the SARS-CoV-2 variant is P.1, also known as the Gamma variant. In embodiments, the P.1 ("Gamma") variant comprises one or more mutations selected from L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, and T1027I, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.427, also known as the Epsilon variant. In embodiments, the
 20 B.1.427 ("Epsilon") variant comprises one or more mutations selected from L452R and D614G, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.429, also known as the Epsilon variant. In embodiments, the B.1.429 ("Epsilon") variant comprises one or more mutations selected from S13I, W152C, L452R, and D614G, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

25 In some embodiments, the SARS-CoV-2 variant is B.1.525, also known as the Eta variant. In embodiments, the B.1.525 ("Eta") variant comprises one or more mutations selected from A67V, 69del, 70del, 144del, E484K, D614G, Q677H, and F888L, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.526, also known as the Iota variant. In embodiments, the B.1.526 ("Iota") variant comprises one or more mutations selected from L5F, (D80G*), T95I, (Y144-*), (F157S*),
 30 D253G, (L452R*), (S477N*), E484K, D614G, A701V, (T859N*), (D950H*), and (Q957R*), relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is B.1.617.1, also known as the Kappa variant. In embodiments, the B.1.617.1 ("Kappa") variant comprises one or more mutations selected from (T95I), G142D, E154K, L452R, E484Q, D614G, P681R, and Q1071H, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

5 In some embodiments, the SARS-CoV-2 variant is B.1.617.3. In embodiments, the B.1.617.3 variant comprises one or more mutations selected from T19R, G142D, L452R, E484Q, D614G, P681R, D950N, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, the SARS-CoV-2 variant is P.2, also known as the Zeta variant. In embodiments, the P.2 ("Zeta") variant comprises one or more mutations selected from E484K, (F565L*), D614G, and V1176F, relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

10 In some embodiments, a variant is a SARS-CoV-2 protein having a variation in a glycosylation site of a Spike protein.

In some embodiments, a variant is a Spike protein having one or more of D614G, E484K, N501Y, K417N, S477G, and S477N mutations relative to the amino acid sequence of SEQ ID NO: 31 or an antigenic fragment thereof.

In some embodiments, a variant is a Spike protein having a mutation in the RBD of the Spike protein. In some embodiments, the mutation in the RBD of the Spike protein is a mutation in a glycosylation site in the RBD.

15 In some embodiments, a variant is a Spike protein having a mutation outside the RBD of the Spike protein.

Another aspect of the present invention is related to a method for vaccinating a subject against an influenza infection, comprising administering: (a) administering an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a mutant IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) an influenza antigen which is suitable for inducing an immune response.

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25 Yet another aspect of the present invention is related to a method for vaccinating a subject against a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection comprising administering: (a) administering an adjuvant comprising a chimeric protein or chimeric protein complex, comprising: (i) a mutant IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) or (2) a

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flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and (b) a SARS-CoV-2 antigen which is suitable for inducing an immune response.

Another aspect of the invention is related to a method for treating a subject afflicted with an infectious disease, comprising administering a chimeric protein or chimeric protein complex, comprising: (i) a mutant IL-1 β , (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and (iii) a connector between (i) and (ii), the connector being: (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or (2) a flexible linker that connects (i) and (ii); wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor. For example, in embodiments, the invention is related to a method for treating a subject afflicted with an a coronavirus (e.g. SARS-CoV-2) or influenza infection. In such embodiments, the adjuvant is administered to a patient who has a low level or moderate infection and the adjuvant causes a boost to the natural immune response to the infection occurring in the patient.

In some embodiments, the present invention relates to the treatment or vaccination of patients who are naive to antiviral therapy. In other embodiments, the present invention relates to the treatment or vaccination of patients who did not respond to previous antiviral therapy. In some embodiments, the present vaccine compositions may be used to vaccinate relapsed patients.

In various embodiments, the vaccine compositions of the invention provide improved safety compared to, e.g., untargeted IL-1 β or an unmodified, wild type IL-1 β or a modified IL-1 β (e.g., pegylated IL-1 β). In illustrative embodiments, administration of the vaccine composition is associated with minimal side effects such as those side effects associated with the use of the untargeted IL-1 β or an unmodified, wild type IL-1 β or a modified IL-1 β (e.g., influenza-like symptoms, myalgia, leucopenia, thrombocytopenia, neutropenia, depression, and weight loss).

In some embodiments, the vaccine composition of the invention shows improved therapeutic activity compared to untargeted IL-1 β or an unmodified, wild type IL-1 β , or a modified IL-1 β (e.g., pegylated IL-1 β). In some embodiments, the vaccine composition of the invention shows improved pharmacokinetic profile (e.g., longer serum half-life and stability) compared to untargeted IL-1 β or an unmodified, wild type IL-1 β or a modified IL-1 β (e.g., pegylated IL-1 β).

Kits

The invention also provides kits for the administration of any agent described herein. The kit is an assembly of materials or components, including at least one of the compositions described herein. Thus, in some embodiments, the kit contains at least one of the compositions described herein. The exact nature of the components configured in the kit depends on its intended purpose. In one embodiment, the kit is configured for the purpose of treating or vaccinating human subjects.

Instructions for use may be included in the kit. Instructions for use typically include a tangible expression describing the technique to be employed in using the components of the kit to effect a desired outcome, such as to treat an infectious disease or vaccinate against such diseases. Optionally, the kit also contains other useful components, such as, diluents, buffers, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, bandaging materials or other useful paraphernalia as will be readily recognized by those of skill in the art.

The materials and components assembled in the kit can be provided to the practitioner stored in any convenience and suitable ways that preserve their operability and utility. For example, the components can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging materials. In various embodiments, the packaging material is constructed by well-known methods, preferably to provide a sterile, contaminant-free environment. The packaging material may have an external label that indicates the contents and/or purpose of the kit and/or its components.

Definitions

As used herein, "a," "an," or "the" can mean one or more than one.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive and covers both "or" and "and".

Further, the term "about" when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication, e.g., within (plus or minus) 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. For example, the language "about 50" covers the range of 45 to 55.

An "effective amount," when used in connection with medical uses is an amount that is effective for providing a measurable treatment, prevention, or reduction in the rate of pathogenesis of a disease of interest.

As used herein, something is "decreased" if a read-out of activity and/or effect is reduced by a significant amount, such as by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100%, in the presence of an agent or stimulus relative to the absence of such modulation. As will be understood by one of ordinary skill in the art, in some embodiments, activity is decreased and some downstream read-outs will decrease but others can increase.

Conversely, activity is "increased" if a read-out of activity and/or effect is increased by a significant amount, for example by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100% or more, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold,

at least about 10-fold, at least about 50-fold, at least about 100-fold, in the presence of an agent or stimulus, relative to the absence of such agent or stimulus.

As referred to herein, all compositional percentages are by weight of the total composition, unless otherwise specified. As used herein, the word "include," and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the compositions and methods of this technology. Similarly, the terms "can" and "may" and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features.

Although the open-ended term "comprising," as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as "consisting of" or "consisting essentially of."

As used herein, the words "preferred" and "preferably" refer to embodiments of the technology that afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the technology.

The amount of compositions described herein needed for achieving a therapeutic effect may be determined empirically in accordance with conventional procedures for the particular purpose. Generally, for administering therapeutic agents for therapeutic purposes, the therapeutic agents are given at a pharmacologically effective dose. A "pharmacologically effective amount," "pharmacologically effective dose," "therapeutically effective amount," or "effective amount" refers to an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for treating the disorder or disease. An effective amount as used herein would include an amount sufficient to, for example, delay the development of a symptom of the disorder or disease, alter the course of a symptom of the disorder or disease (e.g., slow the progression of a symptom of the disease), reduce or eliminate one or more symptoms or manifestations of the disorder or disease, and reverse a symptom of a disorder or disease. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to about 50% of the population) and the ED₅₀ (the dose therapeutically effective in about 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD₅₀/ED₅₀. In some embodiments, compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from in vitro assays, including, for example, cell culture assays. Also, a dose can be formulated in animal models to

achieve a circulating plasma concentration range that includes the IC₅₀ as determined in cell culture, or in an appropriate animal model. Levels of the described compositions in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

5 In certain embodiments, the effect will result in a quantifiable change of at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 70%, or at least about 90%. In some embodiments, the effect will result in a quantifiable change of about 10%, about 20%, about 30%, about 50%, about 70%, or even about 90% or more. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

10 As used herein, "methods of treatment" are equally applicable to use of a composition for treating the diseases or disorders described herein and/or compositions for use and/or uses in the manufacture of a medicaments for treating the diseases or disorders described herein. This invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1: Q148G is an IL-1 β mutant with strongly reduced biological activity that can be completely reactivated upon targeting using CD8 α sdabs.

15 We generated several human IL-1 β mutants, predicted to have reduced biological activity (Fig. 20A). Of these, the IL-1 β mutant Q148G (FIG. 20A) was selected for further study. Q148 is an important residue for IL-1R1 binding, located in one of two known cytokine-receptor contact areas. Within this interface, 118 Å² of the amino acid's accessible surface becomes buried while it makes multiple connections with the IL-1R1: two direct contacts with F128 and L32 and three hydrogen bonds with the backbone amides of V33 and A126 of the receptor subunit (FIG. 20A). Mutating the glutamine at position 148 to glycine destabilizes the interaction with 1.74 kcal/mol, as predicted by Fold-X. This leads to an approximately 168-fold reduction in activity compared with WT IL-1 β (FIG. 20B), as measured by NF- κ B-driven expression of a luciferase reporter in HEK-293 cells stably transfected with the mouse IL-1R complex (HEK-Blue-IL1R).

20 To locally reactivate IL-1 β Q148G by targeting to cell type-specific surface molecules, we genetically fused the mutant cytokine to a sdAb that is specific for mouse CD8 α , thereby creating the CD8 α ALN-1 (Fig. 20C). This sdAb was found not to impede the activation of CD8⁺ T cells during antigen presentation *in vitro* (FIG. 27A). This is important, as different anti-CD8 antibodies with the potential to interfere with CTL activation have been described before. As a negative control for targeting, we made a genetic fusion with a Bcl10 sdAb, which is directed against bacterial β -lactamase. These fusion proteins were produced in mammalian HEK-F cells and subsequently purified using the built-in 9xHis-tag (FIG. 20D). We measured an approximately 60-fold reduction in the biological activity of WT IL-1 β after N-terminal coupling to the CD8 α sdAb (FIG. 20E), meaning that the specific activity of CD8 α ALN-1 is approximately 9000-fold lower than that of WT. Next, we evaluated CD8 α ALN-1's reactivation-by-targeting on cells transiently transfected with CD8 α . We found that the agonistic activity of CD8 α ALN-1 could be fully restored up to WT level on CD8 α ⁺ cells, while CD8 α

ALN-1 remained completely inactive on cells lacking the sdAb target. This was demonstrated by measuring NF- κ B activation via reporter gene (FIG. 20E) and confocal microscopy (FIG. 20F) experiments in HEK-Blue-IL1R cells. Nuclear translocation of the NF- κ B p65 subunit upon CD8 α ALN-1 treatment occurred only in CD8 α^+ cells, whereas WT IL-1 β induced NF- κ B activation independently of CD8 α expression and untargeted ALN-1 did not show any activity. Additionally, we evaluated IL-1R signaling further downstream by measuring the expression of selected NF- κ B, p38 MAPK and AP-1 target genes via RT-qPCR analysis in human 132 1N1 astrocytes, which endogenously express the IL-1R complex and were transiently transfected with CD8 α or an irrelevant target protein. We found that the induction of most genes (*IL8*, *A20*, *NFKBIA* and *ICAM1*) was only restored upon targeting of CD8 α ALN-1 to CD8 α^+ cells, while the expression of other genes (*JUN* and *DUSP*) was only partially restored (FIG. 20G). Noteworthy, the *JUN*, *DUSP* and *ICAM1* transcripts were less strongly induced upon WT IL-1 β stimulation (Fig. 27B). Moreover, we found that the biological activity of CD8 α ALN-1 upon targeting is dependent on the level of target antigen expression (FIG. 27C).

Altogether, these findings illustrate that Q148G, an IL-1 β mutant with strongly reduced biological activity; can regain WT activity upon its targeting using a CD8 α -specific sdAb.

Example 2: CD8 α ALN-1 promotes antigen-dependent proliferation and activation.

Due to the cross-reactivity of human IL-1 β in mouse we could further evaluate the specificity and affinity of CD8 α ALN-1 on murine splenocytes *in vitro* using flow cytometry (gating strategy in FIGs. 28A-D). In this mixed population of cells, two different cellular subsets specifically bound CD8 α ALN-1: CD4 $^+$ T cells, corresponding to cytotoxic T lymphocytes (CTLs), and conventional DCs (cDCs) (Fig. 21A and Fig. 21C). Furthermore, the cDCs targeted by CD8 α ALN-1 expressed XCR1, identifying them as type I cDCs, which are known to be CD8 α^+ in mice (Figs. 21B-C). We did not observe binding of CD8 α ALN-1 to any other immune cell type tested (Figs. 21A-C), including NK cells (Fig. 28E). No binding could be detected for WT IL-1 β and untargeted Bcl10 ALN-1 (Figs. 21A-C). The importance of this sdAb is confirmed by the observation that CD8 α ALN-1 binding remained intact on IL-1R1 $^{-/-}$ splenocytes (Figs. 21A-B). Titration of the CD8 α sdAb on the CTL and cDC subsets showed that this targeting moiety binds with nanomolar affinity (K_D of 5.6 nM for CTL binding and 1.3 nM for cDC binding) (Figs. 21D-F).

To address the potential adjuvant capacity of CD8 α ALN-1 *in vitro*, we used the OT-I co-culture system. We found that, despite high background proliferation of antigen-exposed OT-I cells, CD8 α ALN-1 (like WT IL-1 β) further promoted SIINFEKL peptide-dependent proliferation of OT-I cells (Figs. 21G-H, *left*; gating strategy in Fig. 29A). This effect completely depended on presentation of antigen by bone marrow-derived DCs (BM-DCs) to OT-I cells (Fig. 29B). Similar results were obtained using IL-1R1 $^{-/-}$ BM-DCs in the co-cultures, suggesting that CD8 α ALN-1 acts directly on the antigen-specific CTLs (Fig. 29C). Moreover, treatment with CD8 α ALN-1 led to an enhanced upregulation of CD25

(IL-2R α) in the dividing OT-I cell subset (Figs. 21G-H, *right*) and augmented release of the effector cytokines tumor necrosis factor (TNF) and IFN- γ , indicative for enhanced CTL activation (Fig. 29D).

In conclusion, we demonstrated that CD8 α ALN-1 can efficiently deliver IL-1 β activity to CD8 $^{+}$ T cells, leading to an enhanced antigen-specific T cell response *in vitro*.

Example 3: CD8 α ALN-1 induces CD8 $^{+}$ T cell proliferation and effector functions in response to antigen *in vivo*.

To investigate whether CD8 α ALN-1 displays cellular adjuvant activity *in vivo*, as was earlier reported for WT IL-1 β , we first performed OT-I adoptive transfer experiments (Fig. 22A; gating strategy in Fig. 30A). In this model, intraperitoneal (i.p.) immunization of mice with OVA alone already resulted in the proliferation of OT-I cells compared with mice treated without antigen (PBS) (Figs. 22B-C). Co-administration of OVA and LPS (used as a positive control adjuvant) further increased OT-I division. Characteristic for this effect is the significant increase in the fraction of OT-I cells in the latest stage of cell proliferation (*i.e.* the sixth CellTrace Violet (CTV) dilution peak) compared with immunization with OVA alone. Similar to the effect of LPS, CD8 α ALN-1 significantly enhanced OVA-induced OT-I proliferation. No significant effect of untargeted Bcl10 ALN-1 was observed when compared with immunization with OVA alone. When using C57BL/6 IL-1R1 $^{-/-}$ recipient mice, the observed CD8 α ALN-1 effect on proliferation remained intact (Fig. 22D), indicating that CD8 α ALN-1 acts directly on the OT-I cells. We found that treatment with OVA and CD8 α ALN-1 increased the fraction of OT-I cells within the total CD8 $^{+}$ T cell population and the absolute numbers of OT-I cells both in lymphoid (lymph nodes (LNs) and spleen) and peripheral (liver and lungs) organs compared with delivery of OVA alone (Figs. 30B-E). Moreover, CTL activation was enhanced in the spleens of mice treated with OVA and CD8 α ALN-1 compared with mice immunized with OVA alone, as indicated by the simultaneous upregulation of CD44 and downregulation of CD62L (Fig. 22E; representative dot plots in Fig. 30F).

We next explored whether CD8 α ALN-1 could also boost endogenous OVA-specific CD8 $^{+}$ T cell activity using an *in vivo* killing assay (Fig. 22F; gating strategy and splenocyte labeling in Figs. 31A-B). No antigen-specific target cell killing was observed in mice immunized with OVA alone, while cytolytic activity was strongly promoted upon co-administration of LPS, WT IL-1 β and CD8 α ALN-1 (Figs. 22G-H). In this assay, CD8 α ALN-1 was found to be equally efficacious as high-dose LPS and WT IL-1 β . Conversely, treatment with OVA and untargeted Bcl10 ALN-1 had no significant effect on target cell killing compared with OVA immunization alone.

ELISPOT analysis revealed an increase in the numbers of SIINFEKL-specific CD8 $^{+}$ T cells that produced IFN- γ in mice that had been treated with OVA and WT IL-1 β or CD8 α ALN-1 compared with mice immunized with OVA alone (Figs. 22I-J). In agreement with the results from the *in vivo* killing experiment, the effect of CD8 α ALN-1 was comparable to that of WT IL-1 β . Some residual activity of the combination of OVA with untargeted Bcl10 ALN-1 was apparent in the ELISPOT. These data demonstrate that CD8 α ALN-1 stimulates antigen-specific CTL responses *in vivo*.

Example 4: Systemic treatment of mice with antigen in combination with CD8 α ALN-1 is completely free of toxicity.

Because WT IL-1 β is well known for its capacity to induce systemic inflammation, we addressed the safety of the immunization strategy described above. As a measure of morbidity, we measured mouse body weight over time and evaluated the systemic release of IL-6 in blood, sampled 6h after the first treatment. In these samples, we also looked for abnormalities in different hematological parameters (Fig. 23A). We found that repeated delivery of WT IL-1 β leads to detrimental side effects, such as severe body weight loss (Figs. 23B-C), systemic release of IL-6 (Fig. 23D) and platelet destruction (Figs. 23E-F), whereas treatment with a corresponding dose of CD8 α ALN-1 had no significant effects on these parameters. Intriguingly, both WT IL-1 β and CD8 α ALN-1 elicit leukopenia (Fig. 23G), which we found is primarily due to a reduction in circulating lymphocytes (Fig. 23H) as the level of neutrophils remains unaltered (Fig. 23I).

We looked further into this leukopenia and observed that, based on Hemavet 950FS measurements, this decrease in circulating lymphocytes is apparent 6h after treatment and is thereafter further maintained when CD8 α ALN-1 is repeatedly administered (Fig. 32A). Using flow cytometry, we further investigated which lymphocyte subpopulations were exactly affected (see Fig. 32B for the gating strategy) and observed drops in absolute counts for all evaluated cell types (Fig. 32C).

Together, these data show that overall wellbeing of mice is improved when IL-1 β activity is restricted to CD8 $^+$ T cells.

Example 5: An influenza vaccine adjuvanted with CD8 α ALN-1 protects mice against viral infection.

Adjuvants promoting T cell responses against conserved influenza epitopes could be critical in the search for a universal influenza vaccine. Therefore, we evaluated the efficacy of CD8 α ALN-1 as an adjuvant in a prime-boost vaccination strategy, using whole-inactivated X47 (H3N2) virus (WIV) as source of viral antigen, to protect against challenge infection with a heterosubtypic H1N1 2009 pandemic (pH1N1) influenza virus, a mouse-adapted derivative from a clinical isolate responsible for the 2009 flu pandemic (Fig. 24A). Protection in this model is expected to depend predominantly on T cell responses as mainly T cell epitopes are conserved between heterosubtypic X47 and pH1N1. We found that all mice vaccinated with CD8 α ALN-1 survived the challenge infection with pH1N1 (Figs. 24B-I). Treatment with WIV and CD8 α ALN-1 was as efficacious as treatment with WIV and WT IL-1 β . Four out of six mice immunized with WIV alone succumbed to the infection. This suggests that CD8 α ALN-1 treatment induces potent cross-protective antiviral immunity. Interestingly, vaccination with CD8 α ALN-1 also outperforms the commercial Sigma Adjuvant System (SAS), an oil-in-water emulsion containing MPLA, for which no significant difference with WIV alone could be observed. Moreover, no protection compared to the WIV only vaccine was observed when using untargeted Bcl10 ALN-1 or a CD8 α sdAb coupled to human IFN α 2. As human IFN α 2 is inactive in mice and has a molecular weight comparable to WT IL-1 β , this molecule is used as control for possible effects via the CD8 α sdAb.

Altogether, these data show that CD8 α ALN-1 can be used as an adjuvant to protect against influenza A virus infection by prime-boost vaccination.

Example 6: The protective antiviral effect of CD8 α ALN-1 correlates with the induction of strong and long-lasting influenza-specific T cell responses in lung and lymphoid tissues

Influenza virus nucleoprotein (NP) is a conserved internal antigen, known for its ability to mount strong and long-lasting T cell responses. Functionally, NP is an RNA-binding protein that encapsulates the viral genome and is required for RNA transcription, replication and viral genome packaging.

We sampled mice on different time points before, during and after pH1N1 virus encounter to evaluate whether vaccination with WIV combined with CD8 α ALN-1 induces NP-specific T cell responses. Two weeks after the boost, we observed a significantly stronger increase in the number of splenic NP-specific IFN- γ producing CD8 $^{+}$ and CD4 $^{+}$ T cells in mice that had been vaccinated with WIV and CD8 α ALN-1 compared with mice immunized with WIV alone (Fig. 25A). CD8 α ALN-1 proved to be as efficacious as WT IL-1 β to induce these responses. The combinations of WIV with either SAS, untargeted Bcl10 ALN-1 or CD8 α hIFN α 2 had no significant effect, which is consistent with their inability to enhance survival.

We further characterized the NP-specific CD8 $^{+}$ T cells, induced upon vaccination with both WT IL-1 β and CD8 α ALN-1, in lung-draining mediastinal LNs and lung parenchyma of mice, one week post-influenza A virus challenge using flow cytometry (Fig. 25B; gating strategy in Figs. 33A-B). In the draining LNs, the bulk of these NP-specific CD8 $^{+}$ T cells showed a CD62L $^{+}$ CD44 $^{+}$ expression pattern, representing a central memory T cell (T_{CM}) pool. A much smaller fraction consisted of CD62L $^{-}$ CD44 $^{+}$ cells, which identify as effector memory T cells (T_{EM}). Consistent with this profile, higher expression of the pro-survival marker CD127 (IL-7R α) was found in the T_{CM} subset compared to the T_{EM} cells. In the functional lung parenchyma, most of the retrieved NP-specific CD8 $^{+}$ T cells were positive for the residency marker CD69, which is indicative for a tissue-resident (T_{RM}) phenotype. A smaller proportion of antiviral CTLs in the lung were CD69 $^{-}$ T_{EM} cells. In both LNs and lung, we found no significant differences for the evaluated parameters between mice treated with WT IL-1 β and CD8 α ALN-1. Moreover, we found that NP-specific CD8 $^{+}$ T cells were still present in the lungs of mice vaccinated with WT IL-1 β and CD8 α ALN-1 by day 50 after pH1N1 infection (Fig. 25C; gating strategy in Fig. 33C).

Example 7: The transcriptional landscape of CD8 $^{+}$ T cells isolated from vaccinated mice during influenza virus infection supports the cellular adjuvant effect of CD8 α ALN-1.

The previous data clearly demonstrate that vaccination of mice with WIV and CD8 α ALN-1 raises potent and long-lasting CD8 $^{+}$ and CD4 $^{+}$ T cell responses, directed against the viral NP antigen. In order to better understand how selective IL-1 β activity on CD8 $^{+}$ T cells establishes these effects, we performed RNA sequencing and looked into transcriptomic changes in CTLs. For this, we sorted CD8 $^{+}$ T cells from the lung parenchyma and lung-draining mediastinal LNs of mice vaccinated with either WIV alone or combined with WT IL-1 β or CD8 α ALN-1, one week after viral challenge (see Figs. 34A-B for the gating strategy).

The transcriptome of CD8⁺ T cells isolated from mice vaccinated with WIV and WT IL-1 β or CD8 α ALN-1 was significantly altered in lung (50 up- and 76 downregulated genes) and to a lesser extent in draining LNs (27 up- and 38 downregulated genes) compared with mice vaccinated with WIV alone (Figs. 26A-B, Fig. 34C). For mice vaccinated with WIV and WT IL-1 β or CD8 α ALN-1, all genes that were found to be significantly up- or downregulated compared to WIV alone vaccinated mice are summarized in Supplementary Table 2.

We summarized the gene ontology (GO) biological processes (BPs) associated with these transcriptome changes by performing DAVID bioinformatics analysis using the differentially expressed genes (DEGs) in lung or draining LNs as input. Among the top-five most upregulated BPs were the inflammatory response and different chemotactic processes, indicative for immune cell recruitment to the site of viral infection (Fig. 26C). Furthermore, this GO analysis indicated that genes associated with the antiviral response to influenza (e.g. *Ifi2712a*, *Isg20*, *Oas1a*, *Isg15*, *Ifit1*, *Oas1* and *Ifit3*) were strongly enriched in mice vaccinated with WIV alone as compared to mice vaccinated with WT IL-1 β or CD8 α ALN-1.

We performed a structured literature search to correlate the identified transcriptional changes in CD8⁺ T cells to the enhanced T cell responses and survival rates after viral challenge observed in mice vaccinated with WT IL-1 β or CD8 α ALN-1. We found that several transcripts with previously reported roles in long-term survival of T cells (e.g. *Cacnb1*, *Bcl2* and *Aqp9* and *Arg1*), installation of peripheral residency (e.g. *Zfp683* or *Hobit*), activity of T cells (e.g. *Pros1* and *Tnfrsf4*) and T cell effector functions (e.g. *Cx3cr1*) were significantly upregulated in CD8⁺ T cells isolated from mice vaccinated with WT IL-1 β and CD8 α ALN-1. On the other hand, downregulation of several *Schlafen*-family genes (e.g. *Slfn 1*, 3, 5 and 8) was found in these cells, which has been described before as a consequence of CTL activation, allowing T cells to exit a quiescent state. We further summarized DEGs with known importance for different CD8⁺ T cell-related processes in Fig. 7D.

Remarkably, in draining LNs from mice vaccinated with WIV and CD8 α ALN-1, we retrieved several genes associated with the immunoglobulin response (e.g. *Igkc*, *Ighg2b* and *Jchain*), mediated by B lymphocytes. These results are probably due to sample contamination with small amounts of B cells (Fig. 26B).

Altogether, these transcriptomics data indicate that WT IL-1 β and CD8 α ALN-1 provoke changes in the transcriptome of CD8⁺ T cells that are in line with the generation of potent and active CTLs both in lung parenchyma and draining LNs.

Example 8: Methods of Examples 1-7.

In silico analyses of the IL-1 β /receptor interactions.

Effects of mutations on the IL-1 β /receptor interactions were predicted using the crystal structure of the human IL-1 β with the extracellular fragments of its receptors (PDB code 1DEP). The average of five calculations with the FoldX 4.0 command PSSM was used to predict the $\Delta\Delta G$ of the IL-1 β Q148G mutant on its interaction with the IL-1R1. Buried

surface area of the mutated residue and contacts of the mutated residue were derived using PDB-ePISA and by visualization of the crystal structure using UCSF Chimera.

Molecular cloning and recombinant protein production and purification.

The Q148G mutation was introduced in the coding sequence of human IL-1 β by site-directed mutagenesis (Q5[®] Hot Start High-Fidelity DNA Polymerase, M0493L, New England BioLabs Inc.) (5'-GAAGGCACTGCATCTGGGTGGCCAGGACATGGAACAGC-3' (forward) (SEQ ID NO: 35); 5'-GCTGTTCCATGTCCTGGCCACCCAGATGCAGTGCCTTC-3' (reverse complement) (SEQ ID NO: 36), primers were synthesized and purified by Eurogentec). Anti-mouse CD8 α and Bcl10 sdAbs were generated by the VIB Protein Service Facility. WT IL-1 β , CD8 α WT IL-1 β , CD8 α ALN-1, Bcl10 ALN-1 and CD8 α hIFN α 2 were constructed in an in-house developed vector (pmTW). Plasmid DNA was purified from the supernatant of DH10B *E. coli* bacteria (18290-015, Thermo Fisher Scientific) after overnight growth (37°C, 175 rpm) by anion exchange chromatography, using the NucleoBond[®] PC 2000 system (740525, Machery Nagel). Proteins were produced in FreeStyle[™] 293-F cells (R79007, Thermo Fisher Scientific), grown in suspension and transfected at a density of 1.2x10⁶ cells/mL in 300 mL of FreeStyle[™] 293 expression medium (12338026, Thermo Fisher Scientific) using the PEI-25k transfection reagent (600 μ g) (23966-2, PolySciences Inc.), complexed with DNA (300 μ g). Additional medium (100 mL) was added 72h *post*-transfection and 48h later the supernatant was collected and filtered. Proteins were purified overnight at 4°C by immobilized metal affinity chromatography (IMAC), using nickel ion-loaded sepharose resins (17526801, GE Healthcare). Resins were washed with two column volumes of 20 mM imidazole (8.14223.0250, Merck Millipore) in PBS (14190-169, Thermo Fisher Scientific) and proteins were eluted with 400 mM imidazole in PBS. Imidazole was exchanged with PBS by gel filtration using PD-10 columns (17-0851-01, GE Healthcare). Protein concentrations were determined by measuring absorbance at 280 nm. Purity was assessed by SDS-PAGE and Instant Blue (EP ISB1L, Expedeon) staining of the protein gel.

Cell lines and culture conditions.

HEK-Blue-IL1R cells (hkb-il1r, Invivogen) and 132 1N1 human astrocytes were cultured in DMEM (41966-052, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (10270-106, Thermo Fisher Scientific), following conditions specified by the provider. All cells were grown at 37°C in a humidified atmosphere containing 5% CO₂ and tested negative for contamination with mycoplasma (Venor[™]GeM Mycoplasma Detection Kit, PCR-based, MP0025, Sigma-Aldrich). No full authentication of the cell lines was performed.

In vitro bio-activity and activation-by-targeting proof of concept experiments.

For reporter gene experiments, HEK-Blue-IL1R cells were plated 24h prior to transfection in 75 cm² flasks (1x10⁶ cells/flask). Cells were transfected with 1 μ g of NF- κ B-3kB-Luc reporter gene DNA, using calcium phosphate precipitation. In the activation-by-targeting experiments, this transfection mix was additionally complemented with 10 μ g of DNA encoding mouse CD8 α or an irrelevant target (pMET7 vector). Transfected cells were plated in 96-black bottom well plates 24h *post*-transfection (1x10⁵ cells/well). Cells were stimulated 24h later with WT IL-1 β , CD8 α WT

IL-1 β or CD8 α ALN-1 in a concentration range. Cells were lysed 6h *post*-stimulation and incubated with luciferase substrate, after which luminescence was measured on an Envision luminometer. NF- κ B activity was calculated as fold induction by normalization to the activity in unstimulated cells (bio-activity experiments) or cells stimulated with WT IL-1 β (activation-by-targeting experiments).

5 **Confocal microscopy.**

For confocal imaging, HEK-Blue-IL1R cells were seeded in 6-well plates (2.5×10^5 cells/well). The next day, cells were transfected with either DNA encoding Flag-tagged mouse CD8 α or empty vector DNA (both 0.5 μ g) using lipofectamine-2000 (11668019, Thermo Fisher Scientific). 24h later, transfected cells were detached using enzyme-free cell dissociation buffer (13151014, Thermo Fisher Scientific), washed and resuspended in DMEM with 10% fetal bovine serum in a 1:1-ratio (50,000 cells/mL). Next, cells (200 μ L/well) were transferred to 8-well chambered coverslips (80826, Ibidi), coated with poly-L-lysine (P4707, Sigma-Aldrich). After 24h, cells were treated for 30 min with vehicle or WT IL-1 β , CD8 α ALN-1 or untargeted ALN-1 (0.5 nM). At the end of the stimulation, cells were rinsed with PBS, containing Ca $^{2+}$ and Mg $^{2+}$ (14040133, Thermo Fisher Scientific) and fixed for 15 min at room temperature in paraformaldehyde in PBS (4%). After three PBS washes, cells were permeabilized with Triton X-100 in PBS (0.1%) for 10 min and blocked in BSA in PBS (1%) for another 10 min at room temperature. Samples were then incubated overnight (4°C) with mouse anti-Flag (2000x diluted) (F3165, Sigma) and rabbit anti-p65 (400x diluted) (sc-372, Santa Cruz) in BSA in PBS (1%). After three washes in PBS, cells were incubated for 30 min at room temperature (dark) with anti-rabbit Alexa 568 (500x diluted) (A10042, Thermo Fisher Scientific) and anti-mouse Alexa 488 (A11029, Thermo Fisher Scientific) (500x diluted) in BSA in PBS (1%). DAPI (2 μ g/ml) was added to the secondary antibody mix to stain the nuclei. Next, cells were washed four times in PBS and covered with propyl gallate mounting medium. Images were acquired using a 60x 1.35 NA objective on an Olympus IX-81 laser scanning confocal microscope and analyzed using Fluoview 1000 software.

RNA isolation and RT-qPCR.

132 1N1 cells were seeded in 6-well plates (2.5×10^5 cells/well). The next day, cells were transfected with 0.5 μ g of DNA encoding mouse CD8 α or an irrelevant target protein using lipofectamine-2000. 24h later transfected cells were detached using trypsin-EDTA (25300096, Thermo Fisher Scientific), washed with PBS and resuspended in DMEM, supplemented with 10% fetal bovine serum (2×10^5 cells/mL). Cells were transferred to 24-well plates (1×10^5 cells/well) and stimulated the next day with vehicle or WT IL-1 β , CD8 α ALN-1 or untargeted ALN-1 (0.5 nM). After 6h of treatment, cells were washed with PBS and total RNA was extracted using the RNeasy Mini Kit (74104, Qiagen). Reverse transcription was performed on 0.5 μ g of total RNA using the PrimeScript RT Reagent kit (RR037A, Takara). For real-time cDNA amplification we used the LightCycler $^{\circ}$ 480 SYBR Green I Mastermix (04707516001, Roche) and the primers listed in Supplementary Table 1. Samples were amplified and analyzed using the LightCycler $^{\circ}$ 480 System

(Roche). Relative quantification of mRNA expression was performed using $\Delta\Delta C_t$ analysis with HPRT as reference gene.

Mice

All mice were housed under pathogen-free conditions in individually ventilated cages, placed in a temperature-controlled environment with a 12h day/12h night cycle. Mice received food and water *ad libitum*. Female C57BL/6 and BALB/c mice were purchased from Charles River Laboratories. OT-I transgenic CD45.1 C57BL/6 Rag2^{-/-} mice and C57BL/6 IL-1R1^{-/-} mice were bred at our own facility. All mice were between 7-12 weeks of age at the start of experiments. All animal experiments followed the guidelines of the Federation of European Laboratory Animal Science Association (FELASA) and were approved by Ethical Committees of Ghent University (ECD 17/80k, ECD 18/127k, ECD 16/07 and ECD 2018/86). For all experiments, mice were allocated to groups randomly. The investigators were not blinded during data collection nor analysis.

Binding studies on murine splenocytes in vitro.

Spleens were isolated from C57BL/6 or C57BL/6 IL-1R1^{-/-} mice, collected in PBS and passed through 70 μ m nylon strainers by mashing. Red blood cells were lysed with a home-made lysis buffer (155 mM NaCl, 12 mM NaHCO₃ and 127 μ M EDTA in PBS pH 7.4). Cells were resuspended in home-made FACS buffer (1% FBS, 0.09% sodium azide and 0.05 mM EDTA in PBS), plated in a 96-well plate (1x10⁶ cells/well) and incubated for 2h at 4°C with WT IL-1 β , CD8 α ALN-1 or Bcl10 ALN-1 (10 nM for type I cDC binding, 1 nM for binding on all other cell subsets, concentration range for titration experiments). Cells were stained in FACS buffer with anti-CD16/32 (100x dilution) (Thermo Fisher Scientific) to block Fc receptors, followed by staining with LIVE/DEAD Fixable Aqua (1000x dilution) (Thermo Fisher Scientific), CD19 AF700 (500x dilution) (clone eBio1D3, 56-0193-82, Thermo Fisher Scientific), CD3 AF700 (clone 17A2, 56-0032-82, Thermo Fisher Scientific) or PE-Cy7 (250x dilution) (clone 145-2C11, 552774, BD Biosciences), CD4 PE (250x dilution) (clone RM4-5, 553048, BD Biosciences), CD11b PE-Cy7 (250x dilution) (clone M1/70, 101215, BioLegend), CD11c APC (100x dilution) (clone N418, 117310, BioLegend), XCR1 PE (100x dilution) (clone ZET, 148204, BioLegend) and His-tag FITC (2000x dilution) (clone 6G2A9, A01620, GenScript). Cells were recorded on a four-laser Attune Nxt flow cytometer (Thermo Fisher Scientific) and data were analyzed using FlowJo software (Treestar). First, we selected single cells based on FSC/SSC and living cells based on negativity for LIVE/DEAD. Within this subset, we described CD19⁺ cells as B cells, CD19/CD3⁺/CD4⁺ cells as CD4⁺ T cells, CD19/CD3⁺/CD4⁻ cells as CD4⁻ T cells (corresponding to CTLs), CD19/CD3/CD11c⁺ as cDCs, CD19/CD3/CD11c⁺/XCR1⁺ cells as type I cDCs and CD19/CD3/CD11b⁺ cells as myeloid cells.

In vitro OT-I co-culture assay.

Bone marrow was isolated from tibias and femurs of C57BL/6 mice by flushing the bones with PBS. Red blood cells were lysed as described above and cells were seeded in 6-well plates (1x10⁵ cells/mL) in RPMI-1640 (61870-044, Thermo Fisher Scientific), supplemented with 5% Fetal Clone I (FCI), recombinant mouse GM-CSF (20 ng/mL), gentamicin (100 μ g/mL) (15710-049, Thermo Fisher Scientific) and 50 μ M β -mercaptoethanol (21985-023, Thermo

Fisher Scientific). After 10 days of culture, mature BM-DCs were plated in 6-wells (2.5×10^6 cells/mL) and pulsed with 10 pM, 100 pM or 1 nM SIINFEKL (OVA₂₅₇₋₂₆₄) (AS-60193-1, AnaSpec) for 1h at 37°C, 5% CO₂. Spleens were isolated from OT-I transgenic CD45.1 C57BL/6 Rag2^{-/-} mice and processed to single cells as described before. CD8⁺ T cells were purified by negative selection using magnetic-activated cell sorting (MACS) (130-104-075, Miltenyi Biotec), following the manufacturer's instructions. These purified OT-I cells express a transgenic T cell receptor (TCR), recognizing SIINFEKL. Cells were subsequently labeled with 5 μ M carboxyfluorescein succinimidyl ester (CFSE) (65-0850-84, Thermo Fisher Scientific) and plated (1×10^5 cells/well) together with the loaded BM-DCs (1×10^4 cells/well) in 96-well plates for 72h, supplemented with inhibitory antibody (2000x dilution) (clone CT-CD8a, MA5-17594, Thermo Fisher Scientific), WT IL-1 β or CD8 α ALN-1 (1 nM). Following this co-culture, cells were stained in FACS buffer with anti-CD16/32, CD3 PE-Cy7, CD4 PE and CD25 APC (100x dilution) (clone PC61.5, 17-0251-81, Thermo Fisher Scientific). Samples were recorded on the Attune Nxt flow cytometer and data were analyzed using FlowJo software. First, we selected single cells based on FSC/SSC. Within this subset, we described OT-I cells as CD3⁺/CD4⁻/CFSE^{labeled}. TNF (DY410, R&D Systems) and IFN- γ (CMC4033, Thermo Fisher Scientific) were detected in the conditioned supernatant by ELISA, following the manufacturer's instructions.

In vivo OT-I proliferation experiments.

CD8⁺ OT-I cells were isolated and MACS-purified as described above. Purified cells were labeled with 5 μ M CellTrace Violet (CTV) (C34557, Thermo Fisher Scientific), according to the manufacturer's instructions, and i.v. adoptively transferred in C57BL/6 recipient mice (1.5×10^6 cells/mouse in 200 μ L PBS). One day *post-transfer*, recipient mice were immunized i.p. with endotoxin-free full-length OVA protein (100 μ g/mouse in 50 μ L PBS) (vac-pova-100, Invivogen). Starting together with the antigen delivery, mice received additional i.p. treatments with LPS (25 μ g/mouse) (tlrl-eklps, Invivogen), WT IL-1 β (5 μ g/mouse), CD8 α ALN-1 (10 μ g/mouse) or Bcl10 ALN-1 (10 μ g/mouse, all in 100 μ L PBS) every 24h for three consecutive days. One day after the last adjuvant treatment, spleens were isolated and processed to single cells as described above and stained in FACS buffer with anti-CD16/32, CD19 AF700, CD3 PE-Cy7 (250x dilution), CD4 PE, CD45.1 BV605 (100x dilution) (clone A20, 110737, BioLegend), SIINFEKL in H-2kB pentamer APC (10 μ L/sample, following the manufacturer's instructions) (F093-4A, ProImmune), CD44 PerCP-Cy5.5 (100x dilution) (clone IM7, 103032, BioLegend) and CD62L PE-Cy7 (100x dilution) (clone MEL-14, 104428, BioLegend). Cells were recorded on the Attune Nxt flow cytometer and data were analyzed using FlowJo software. Single cells were selected based on FSC/SSC. OT-I cells were described as CD19⁻/CD3⁺/CD4⁻/CD45.1⁺/SIINFEKL in H-2kB pentamer⁺/CTV^{labeled}.

In vivo killing experiments.

Splenocytes were isolated from C57BL/6 mice, processed to single cells as described earlier and suspended in RPMI-1640, supplemented with 10% fetal bovine serum. Half of these splenocytes were pulsed with SIINFEKL (10 μ g/mL) for 2h at 37°C, 5% CO₂, while the remaining cells were left unloaded. Peptide-loaded cells were labeled with 5 μ M CTV, according to the manufacturer's instructions, while unloaded cells were labeled with a 10-fold lower concentration

of CTV (500 nM). Both splenocyte pools were subsequently mixed in a 1:1-ratio and i.v. transferred (1×10^7 cells/mouse in 200 μ L PBS) in recipient mice, five days after the last adjuvant treatment (the immunization strategy applied is presented earlier for the *in vivo* OT-I proliferation experiments). One day *post-transfer*, splenocytes were isolated from recipient mice, processed to single-cell suspensions and recorded on the Attune Nxt flow cytometer. Transferred cells were identified as CTV^{labeled} cells after single cell selection using FSC/SSC. Data were analyzed using FlowJo software, allowing to calculate the percentage of antigen-specific killing as: $100 - [100 \times (\%CTV^{high} \text{ treated mice} / \%CTV^{low} \text{ treated mice}) / (\%CTV^{high} \text{ PBS-treated mice} / \%CTV^{low} \text{ PBS-treated mice})]$. Prior to transfer, correct CTV labeling and peptide presentation was accounted using flow cytometry, by staining the splenocyte pools in FACS buffer with a SIINFEKL in H-2kB PE antibody (100x dilution) (clone 25-D1.16, 141603, BioLegend).

Hematological analyses.

Six hours after the initial delivery of OVA and adjuvant, blood was sampled from the tail vein of treated mice, collected in EDTA-coated microcuvette tubes (Sarstedt) and analyzed in a Hemavet 950FS whole blood counter (Drew Scientific). For the analysis of IL-6 in plasma, blood was centrifuged at 14,000xg for 10 min at 4°C, after which the cytokine concentration was determined by ELISA (431302, BioLegend), according to the manufacturer's instruction.

Influenza viruses and viral infection procedures.

X47 (H3N2) and pH1N1 (A/Belgium/145-MA/2009, a mouse-adapted virus derived from a clinical isolate of the H1N1 2009 pandemic virus) were grown on Madin-Darby canine kidney (MDCK) cells in serum-free RPMI-1640, complemented with L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (T1426, Sigma-Aldrich). WIV was prepared as described earlier. BALB/c mice were primed by an i.m. injection of whole-inactivated X47 virus (WIV) (15 μ g/mouse in 50 μ L PBS), followed by an i.v. injection with WT IL-1 β (5 μ g/mouse), CD8 α ALN-1 (10 μ g/mouse), Bcl10 ALN-1 (10 μ g/mouse) or CD8 α hIFN α 2 (10 μ g/mouse, all in 200 μ L PBS) 24h *post-WIV* delivery. SAS adjuvant (15 μ g/mouse) (S6322-1VL, Sigma-Aldrich) was i.m. co-administered with WIV. An identical boost treatment was administered two weeks after priming. Two weeks *post-boost*, mice were challenged i.n. under mild isoflurane anesthesia (Abbott Animal Health) with 2xLD₅₀ of the pH1N1 in 50 μ L PBS. The body weight of the mice was determined daily, during 14 days, after infection and mice that had lost 25% or more of their initial body weight were euthanized. All influenza virus infections were conducted in a biosafety level 2+ accredited animal facility.

IFN- γ enzyme-linked immunospot (ELISPOT) assays.

For the OVA-specific IFN- γ ELISPOT assays, spleens were isolated from C57BL/6 mice seven days after the initial OVA immunization and first adjuvant treatment. For the NP-specific IFN- γ ELISPOT assays, spleens were isolated from BALB/c mice two weeks after the boost treatment. Splenocytes were processed to single-cell suspensions as described above and seeded (2.5×10^5 cells/well) in a 96 well-plate, *pre-coated* with an anti-IFN- γ antibody (CT317-T2, U-CyTech biosciences), in the presence of peptide (5 μ g/mL) for 24h. The ELISPOT was further developed according to the manufacturer's instructions. The peptides used for restimulation of the cells were the MHC-I epitopes OVA₂₅₇₋₂₆₄

(SIINFEKL) and NP₁₄₇₋₁₅₅ (TYQRTRALV) and the MHC-II epitopes NP₂₀₆₋₂₂₉ (FWRGENGRKTRSAYERMCNILKKGK), NP₅₅₋₇₇ (RLIQNSLTIERMVLSAFDERNK) and NP₁₈₂₋₂₀₅ (AVKGVGTMVMELIRMIKRGINDRN) (NP peptides were synthesized and purified by GenScript).

Analyses of T cell status in lungs and draining lymph nodes upon influenza infection.

5 One week *post*-pH1N1 inoculum, mice were euthanized by overdose with ketamine (80 mg/kg) (Eurovet) and xylazine (5 mg/kg) (Bayer) in 500 μ L PBS (i.p.) and perfused with PBS, supplemented with heparin (50 IU/mL) (H5515-100KU, Sigma-Aldrich). Lungs and lung-draining mediastinal LNs were isolated and collected in ice-cold PBS, supplemented with DnaseI (5 IU/mL) (4536282001, Sigma-Aldrich) and liberase (50 μ g/mL) (5401119001, Sigma-Aldrich). Lungs were chopped finely using scissors and further minced mechanically using GentleMACS (Miltenyi Biotec). The obtained
10 cell suspension was incubated for 30 min at 4°C (while rotating) and subjected to another round of GentleMACS mincing. Red blood cells were lysed, as described before and finally cells were passed through 70 μ m nylon strainers. LNs were mashed, passed through 70 μ m nylon strainers and incubated with DNaseI (5 IU/mL) and liberase (50 μ g/mL) in PBS for 30 min at 4°C (while rotating). Single cells isolated from lung and LNs were stained in FACS buffer with anti-CD16/32, LIVE/DEAD Fixable Aqua, CD45 APC-Cy7 (500x dilution) (clone 30-F11, 103116, BioLegend), CD3 PE-Cy7,
15 CD4 BV605 (250x dilution) (clone RM4-5, 100547, BioLegend), CD8 PerCP-Cy5.5 (250x dilution) (clone 53-6.7, 100733, BioLegend), TYQRTRALV in H-2kB pentamer APC (10 μ L/sample, following the manufacturer's instructions) (F098-4A, ProImmune), CD44 BV711 (100x dilution) (clone IM7, 103057, BioLegend), CD62L PE (100x dilution) (clone MEL-14, 104407, BioLegend) and CD127 BV421 (100x dilution) (clone A7R34, 135023, BioLegend) (cells from lung only) or CD69 BV421 (100x dilution) (clone H1.2F3, 104527, BioLegend) (cells from LNs only). Cells were recorded on
20 a five-laser FACSymphony (BD Biosciences) and data were analyzed using FlowJo software. First, we selected single cells based on FSC/SSC and living cells based on negativity for LIVE/DEAD. Within this subset, we identified antiviral CTLs as CD45⁺/CD3⁺/CD4⁺/CD8⁺/TYQRTRALV in H-2kB pentamer⁺ cells. In LNs, T_{CM} cells were further gated as CD44⁺/CD62L⁺ and T_{EM} cells as CD44⁺/CD62L⁻. In lung parenchyma, T_{RM} cells were further gated as CD44⁺/CD62L⁻/CD69⁺ and T_{EM} cells as CD44⁺/CD62L⁻/CD69⁻.

25 ***CD8⁺ T cell sorting and RNA extraction.***

Lungs and lung-draining mediastinal LNs were isolated from mice one week *post*-pH1N1 infection and processed to single-cell suspensions as described above. Single cells were stained in FACS buffer with anti-CD16/32, LIVE/DEAD Fixable Aqua, CD45 eFluor450 (500x dilution) (clone 30-F11, 48-0451-82, Thermo Fisher Scientific), CD3 PE-Cy7, CD4 FITC (250x dilution) (clone RM4-5, 100510, BioLegend) and CD8 PE (500x dilution) (clone 53-6.7, 12-0081-82,
30 Thermo Fisher Scientific). Samples were run on a three-laser FACSMelody (BD Biosciences) and CD8⁺ T cells were sorted out (4°C) of the mixed cell populations and captured in 350 μ L of RLT⁺ lysis buffer, supplemented with β -mercaptoethanol (4°C) (RNeasy Plus Micro Kit, 74034, Qiagen). First, we selected single cells based on FSC/SSC and living cells based on negativity for LIVE/DEAD. We then identified CTLs as CD45⁺/CD3⁺/CD4⁺/CD8⁺ cells. RNA was isolated following the manufacturer's instructions (RNeasy Plus Micro Kit, 74034, Qiagen). The concentration,

quality and integrity of RNA was addressed using an Agilent 2100 Bioanalyzer (Agilent) and only samples with an RNA integrity number (RIN) ≥ 8 , 280/260- and 260/230-values > 1.8 were sent for sequencing.

RNA sequencing, data processing, analysis and statistical identification of DEGs.

The VIB Nucleomics Core performed the library preparation and library pooling, two runs of sequencing using an Illumina NextSeq 500 device, processing and analysis of the sequencing data and the statistical identification of DEGs. For every sample, gene expression levels were first computed. Briefly, the number of reads in the alignments overlapping with gene features were counted for each individual run using the featureCounts R package. This raw count data of both runs was summed, resulting in total merged counts, which were coupled with reference gene annotations. Absent genes, for which all samples had less than 1 counts-per-million, were filtered out. Every sample was corrected for its intrinsic GC-content using full quantile normalization on bins of GC-content. The variation in library size and RNA composition between samples was corrected by full quantile normalization using the EDASeq R package. For each sample the normalized gene counts were divided by the total number of counts (in millions) and for every gene, these scaled counts were divided by the gene length (in kbp), resulting in the number of fragments per kilobase of gene sequence and per million fragments of library size (FPKM values). Statistical identification of DEGs was done by statistical modelling of the data, specifying the design of the experiment as follows: $\log(\text{count}) = \text{WIV alone (LN)} \times \beta_1 + \text{WIV alone (lung)} \times \beta_2 + \text{WT IL-1}\beta \text{ (LN)} \times \beta_3 + \text{WT IL-1}\beta \text{ (lung)} \times \beta_4 + \text{CD8}\alpha \text{ ALN-1 (LN)} \times \beta_5 + \text{CD8}\alpha \text{ ALN-1 (lung)} \times \beta_6$. The edgeR R package, version 3.20.9, was used to estimate all coefficients β for every gene by fitting a negative binomial generalized linear model (GLM), using offsets instead of normalized counts. These model estimates allowed for computing the contrasts of interest, here being (i) the WT IL-1 β effect in lungs and LNs (WIV alone vs. WIV + WT IL-1 β) and (ii) the CD8 α ALN-1 effect in lungs and LNs (WIV alone vs. CD8 α ALN-1). Using edgeR 3.20.9, differential expression (a significant deviation of these contrasts from 0) was tested with a GLM likelihood ratio test. Resulting p -values were corrected for multiple testing with Benjamini-Hochberg to control the FDR . Genes with a $FDR < 0.05$ were selected and considered differentially expressed when the absolute $\log_2$ -ratio > 1 . For every identified DEG, the biological relevance was accounted by checking if the raw counts > 50 . We conducted an extensive literature research to select for DEGs with known roles during T cell homeostasis, activation, metabolism, exhaustion and memory formation (the search consisted of "gene name" AND "CD8 T cell" OR "influenza").

Statistical analyses and data presentation.

Statistical analyses were performed using the GraphPad Prism 8 software (GraphPad Software). Unless stated otherwise, data are presented as mean $\pm$ s.e.m. in all experiments. The number of independent biological replicates or the number of individual mice is shown as " n ". Normality of the data was accounted by Shapiro-Wilk testing at the $\alpha = 0.05$ significance level for every group in the statistical comparison. Normally distributed data sets were compared by unpaired Student's t -testing (two-tailed) for the statistical analysis of differences between two groups or ANOVA (one- or two-way or repeated measurements) with Tukey's or Sidak's multiple comparisons test for the statistical analysis of differences between more than two groups. Non-normally distributed data were compared by unpaired

Mann-Whitney U testing (two-tailed) for the statistical analysis of differences between two groups or Kruskal-Wallis testing with Dunn's multiple comparisons test for the statistical analysis of differences between more than two groups. Log-rank testing was used for the statistical analysis of differences between Kaplan–Meier survival curves. Statistical significance was throughout defined as $p < 0.05$.

5

EQUIVALENTS

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

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Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

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All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

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As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

CLAIMS

What is claimed is:

1. A vaccine composition, comprising
 - (a) an adjuvant, comprising a chimeric protein or chimeric protein complex, comprising:
 - (i) a wildtype or mutant IL-1 β ,
 - (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and
 - (iii) a connector between (i) and (ii), the connector being:
 - (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or
 - (2) a flexible linker that connects (i) and (ii),
 - wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and
 - (b) an antigen which is suitable for inducing an immune response.
2. The vaccine composition of claim 1, wherein the wildtype or mutant IL-1 β is human IL-1 β .
3. The vaccine composition of claim 1 or 2, wherein the low affinity or activity at the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.
4. The vaccine composition of any one of claims 1-3, wherein the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1.
5. The vaccine composition of any one of claims 1-4, wherein the mutant IL-1 β comprises one or more mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, and N245Q, wherein X is any change in amino acid, with respect to the amino acid sequence of SEQ ID NO: 1.
6. The vaccine composition of claim 5, wherein the mutant IL-1 β comprises one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K, with respect to the amino acid sequence of SEQ ID NO: 1.
7. The vaccine composition of claim 5, wherein the mutant IL-1 β comprises any of the following with respect to the amino acid sequence of SEQ ID NO: 1:

Q131G and Q148G;

Q148G and K208E;
R120G and Q131G;
R120G and H146A;
R120G and H146N;
R120G and H146R;
R120G and H146E;
R120G and H146G;
R120G and K208E; and
R120G, F162A, and Q164E.

8. The vaccine composition of claim 5, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.
9. The vaccine composition of any one of claims 1-8, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell.
10. The vaccine composition of claim 9, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.
11. The vaccine composition of claim 10, wherein the immune cell is a T cell.
12. The vaccine composition of any one of claims 1-11, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.
13. The vaccine composition of any one of claims 1-12, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a microprotein (e.g. cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.
14. The vaccine composition of any one of claims 1-13, wherein the chimeric protein or chimeric protein complex further comprises additional cytokines, optionally modified, optionally mutated.
15. The vaccine composition of any one of claims 1-14, wherein the chimeric protein or chimeric protein complex further comprises one or more additional targeting moieties.
16. The vaccine composition of any one of the above claims, wherein the chimeric protein or chimeric protein complex further comprises two signaling agents and/or two targeting moieties or two of both.

17. The vaccine composition of any one of the above claims, wherein the chimeric protein or chimeric protein complex further comprises three signaling agents and/or three targeting moieties or three of both.
18. The vaccine composition of any one of the above claims, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1 and the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.
19. The vaccine composition of any one of the above claims, further comprising an aluminum gel or salt.
20. The vaccine composition of claim 19, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum sulfate.
21. The vaccine composition of any one of the above claims, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, Freund's Adjuvants, cytokines, toll like receptors ligands, and chitosans.
22. The vaccine composition of any one of the above claims, wherein the vaccine composition is suitable for preventing or mitigating a disease or disorder is an infectious disease.
23. The vaccine composition of claim 22, wherein the disease or disorder is an infectious disease.
24. The vaccine composition of claim 23, wherein the infectious disease is an infection with a pathogen, optionally selected from a bacterium, virus, fungus, or parasite.
25. The vaccine composition of claim 24, wherein the pathogen is a virus.
26. The vaccine composition of claim 25, wherein the virus is:
- (a) an influenza virus, optionally selected from Type A, Type B, Type C, and Type D influenza viruses, or
 - (b) a member of the *Coronaviridae* family, optionally selected from
 - (i) a betacoronavirus, optionally selected from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV, Middle East Respiratory Syndrome—Corona Virus (MERS-CoV), HCoV-HKU1, and HCoV-OC43,
 - (ii) an alphacoronavirus, optionally selected from HCoV-NL63 and HCoV-229E, or
 - (iii) a member of Picornaviridae family, optionally selected from Rhinovirus A or Rhinovirus B.
27. The vaccine composition of claim 26, wherein the virus is SARS-CoV-2.
28. The vaccine composition of any one of the above claims, wherein the adjuvant is a nucleic acid encoding the chimeric protein or chimeric protein complex.
29. The vaccine composition of any one of claims 1-28, wherein the antigen is a protein or an antigenic fragment of a protein.

30. The vaccine composition of any one of claims 1-28, wherein the antigen is a nucleic acid encoding a protein or an antigenic fragment of a protein.
31. The vaccine composition of claim 28 or 30, wherein the nucleic acid is mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine.
32. The vaccine composition of claim 30, wherein the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.
33. The vaccine composition of claim 29, wherein the antigen is a 2019-nCoV protein, or an antigenic fragment thereof, optionally selected from spike surface glycoprotein, membrane glycoprotein M, envelope protein E, and nucleocapsid phosphoprotein N.
34. The vaccine composition of claim 33, wherein the spike surface glycoprotein is the S1 or S2 subunit, or an antigenic fragment thereof.
35. The vaccine composition of claim 34, wherein the spike surface glycoprotein comprises the amino acid sequence of SEQ ID NO: 31, membrane glycoprotein precursor M comprises the amino acid sequence of SEQ ID NO: 32, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 33, and the nucleocapsid phosphoprotein N comprises the amino acid sequence of SEQ ID NO: 34, or an amino acid sequence at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with any of the foregoing, or an antigenic fragment of any of the foregoing.
36. The vaccine composition of claim 26, wherein the virus is an influenza virus.
37. The vaccine composition of claim 36, wherein the antigen is an influenza viral antigen, optionally selected from hemagglutinin (HA) protein, matrix 2 (M2) protein, and neuraminidase, or an antigenic fragment thereof.
38. The vaccine composition of claim 23, wherein the disease or disorder is selected from diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles.
39. The vaccine composition of claim 38, wherein the antigen is that of one or more of the following vaccines: DTP (diphtheria-tetanus-pertussis vaccine), DTaP (diphtheria-tetanus-acellular pertussis vaccine), Hib (Haemophilus influenzae type b) conjugate vaccines, Pneumococcal conjugate vaccine, Hepatitis A vaccines, Poliomyelitis vaccines, Yellow fever vaccines, Hepatitis B vaccines, combination DTaP, Tdap, Hib, Human Papillomavirus (HPV) vaccine, Anthrax vaccine, and Rabies vaccine.
40. The vaccine composition of any one of the above claims, wherein the connector between (i) and (ii) is a flexible linker.

41. The vaccine composition of claim 40, wherein the flexible linker is substantially comprised of glycine and serine residues, optionally wherein i) the flexible linker comprises (Gly₄Ser)_n, where n is from about 1 to about 8, or ii) the flexible linker comprises one or more of SEQ ID NOs: 556-571.
42. The vaccine composition of claim 41, wherein the flexible linker is substantially comprised of GGS₂GGSGGGSGGGGS (SEQ ID NO: 10).
43. The vaccine composition of claim 40, wherein the flexible linker is substantially comprised of LE, GGGGS (SEQ ID NO:2), (GGGGS)_n (n=1-4) (SEQ ID NO:2 -SEQ ID NO:5), (Gly)₈ (SEQ ID NO:11), (Gly)₆ (SEQ ID NO:12), (EAAAK)_n (n=1-3) (SEQ ID NO:13 -SEQ ID NO:15), A(EAAAK)_nA (n = 2-5) (SEQ ID NO:16 – SEQ ID NO:19), AEAAAKEAAKA (SEQ ID NO:16), A(EAAAK)₄LEA(EAAAK)₄A (SEQ ID NO:21), PAPAP (SEQ ID NO:22), KESGSVSSEQLAQFRSLD (SEQ ID NO:23), EGKSSGSGSESKST (SEQ ID NO:24), GSAGSAAGSGEF (SEQ ID NO:25), and (XP)_n, with X designating any amino acid, e.g., Ala, Lys, or Glu.
44. The vaccine composition of any one of the above claims, wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.
45. The vaccine composition of claim 44, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.
46. The vaccine composition of claim 44, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.
47. The vaccine composition of claim 46, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.
48. The vaccine composition of any one of the above claims, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.
49. The vaccine composition of claim 48, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.
50. The vaccine composition of claim 48, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.
51. The vaccine composition of any one of the above claims, wherein the one or more mutations to the Fc domain results in the reduction or elimination of the effector function of the Fc domain.
52. The vaccine composition of any one of the above claims, wherein the chimeric protein complex is a heterodimer and has a trans orientation.
53. The vaccine composition of any one of the above claims, wherein the chimeric protein-complex is a heterodimer and has a cis orientation.
54. The vaccine composition of any one of the above claims, wherein the Fc domain comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).

55. The vaccine composition of claim 54, wherein the Fc domain is human IgG1, and optionally contains one or more of L234, L235, K322, D265, P329, and P331 (according to EU numbering).
56. The vaccine composition of any one of the above claims, wherein the chimeric protein complex has an orientation and/or configuration of any one of FIGs. 1A-19F.
57. The vaccine composition of any one of the above claims, wherein the vaccine composition is formulated for administration intravenously.
58. The vaccine composition of any one of the above claims, wherein the vaccine composition is formulated for administration to the lung.
59. The vaccine composition of any one of the above claims, wherein the vaccine composition is formulated for administration by inhalation.
60. The vaccine composition of any one of the above claims, wherein the vaccine composition is formulated for administration via aerosol or nebulizer.
61. The vaccine composition of any one of the above claims, wherein the vaccine composition is formulated for administration liquid nebulization, dry powder dispersion and meter-dose administration.
62. The vaccine composition of any one of the above claims, wherein the adjuvant or vaccine composition has
- (a) low toxicity;
 - (b) an ability to stimulate a long-lasting immune response against the antigen;
 - (c) substantial stability;
 - (d) an ability to elicit a humoral immune response and/or a cell-mediated immunity to the antigen;
 - (e) a capability of selectively interacting with populations of antigen presenting cells;
 - (f) an ability to specifically elicit T_{H1} and/or T_{H2} cell-specific immune responses to the antigen; and/or
 - (g) an ability to selectively increase appropriate antibody isotype levels against antigens, the isotype optionally being IgA,
- when administered to a patient.
63. The vaccine composition of any one of the above claims, wherein the adjuvant or vaccine composition stimulates a CD8⁺ T cell response to the antigen, when administered to a patient.
64. The vaccine composition of any one of the above claims, wherein the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

65. The vaccine composition of any one of the above claims, wherein the adjuvant or vaccine composition does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient.

66. A method for vaccinating a subject against an infectious disease, comprising administering:

(a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising:

(i) a wildtype or mutant IL-1 β ,

(ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and

(iii) a connector between (i) and (ii), the connector being:

(1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or

(2) a flexible linker that connects (i) and (ii);

wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and

(b) an antigen which is suitable for inducing an immune response.

67. The method of claim 66, wherein the adjuvant and antigen are administered concurrently.

68. The method of claim 66, wherein the adjuvant complex and antigen are co-formulated.

69. The method of claim 66, wherein the adjuvant and antigen are administered sequentially.

70. The method of claim 66, wherein the adjuvant and antigen are administered in multiple doses.

71. The method of claim 66, wherein the adjuvant is administered in multiple booster doses and the antigen is administered once.

72. The method of any one of claims 66-71, wherein the wildtype or mutant IL-1 β is human IL-1 β .

73. The method of any one of claims 66-72, wherein the low affinity or activity at the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

74. The method of any one of claims 66-73, wherein the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1.

75. The method of any one of claims 66-74, wherein the mutant IL-1 β comprises one or more mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A,

N224S/K225S, E244K, and N245Q, wherein X is any change in amino acid, with respect to the amino acid sequence of SEQ ID NO: 1.

76. The method of claim 75, wherein the mutant IL-1 β comprises one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K, with respect to the amino acid sequence of SEQ ID NO: 1.

77. The method of claim 75, wherein the mutant IL-1 β comprises any of the following with respect to the amino acid sequence of SEQ ID NO: 1:

Q131G and Q148G;

Q148G and K208E;

R120G and Q131G;

R120G and H146A;

R120G and H146N;

R120G and H146R;

R120G and H146E;

R120G and H146G;

R120G and K208E; and

R120G, F162A, and Q164E.

78. The method of claim 75, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.

79. The method of any one of claims 66-78, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell.

80. The method of claim 79, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

81. The method of claim 80, wherein the immune cell is a T cell.

82. The method of any one of claims 66-81, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

83. The method of any one of claims 66-81, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a

microprotein (e.g. cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

84. The method of any one of claims 66-83, wherein the chimeric protein or chimeric protein complex further comprises additional cytokines, optionally modified, optionally mutated.

85. The method of any one of claims 66-84, wherein the chimeric protein or chimeric protein complex further comprises one or more additional targeting moieties.

86. The method of any one of claims 66-85, wherein the chimeric protein or chimeric protein complex further comprises two signaling agents and/or two targeting moieties or two of both.

87. The method of any one of claims 66-86, wherein the chimeric protein or chimeric protein complex further comprises three signaling agents and/or three targeting moieties or three of both.

88. The method of any one of claims 66-87, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1 and the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

89. The method of any one of claims 66-88, further comprising an aluminum gel or salt.

90. The method of claim 89, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum sulfate.

91. The method of any one of claims 66-90, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, Freund's Adjuvants, cytokines, toll like receptors ligands, and chitosans.

92. The method of claim 91, wherein the infectious disease is an infection with a pathogen, optionally selected from a bacterium, virus, fungus, or parasite.

93. The method of claim 91, wherein the pathogen is a virus.

94. The method of claim 93, wherein the virus is:

(a) an influenza virus, optionally selected from Type A, Type B, Type C, and Type D influenza viruses, or

(b) a member of the *Coronaviridae* family, optionally selected from

(i) a betacoronavirus, optionally selected from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV, Middle East Respiratory Syndrome-Corona Virus (MERS-CoV), HCoV-HKU1, and HCoV-OC43,

(ii) alphacoronavirus, optionally selected from HCoV-NL63 and HCoV-229E, or

(iii) a member of Picornaviridae family, optionally selected from Rhinovirus A or Rhinovirus B.

95. The method of claim 94, wherein the virus is SARS-CoV-2.

96. The method of any one of claims 66-95, wherein the adjuvant is a nucleic acid encoding the chimeric protein or chimeric protein complex.
97. The method of any one of claims 66-96, wherein the antigen is a protein or an antigenic fragment of a protein.
98. The method of any one of claims 66-97, wherein the antigen is a nucleic acid encoding a protein or an antigenic fragment of a protein.
99. The method of claim 98, wherein the nucleic acid is mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine.
100. The method of claim 98, wherein the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.
101. The method of claim 97 or 98, wherein the antigen is a 2019-nCoV protein, or an antigenic fragment thereof, optionally selected from spike surface glycoprotein, membrane glycoprotein M, envelope protein E, and nucleocapsid phosphoprotein N.
102. The method of claim 101, wherein the spike surface glycoprotein is the S1 or S2 subunit, or an antigenic fragment thereof.
103. The method of claim 102, wherein the spike surface glycoprotein comprises the amino acid sequence of SEQ ID NO: 31, membrane glycoprotein precursor M comprises the amino acid sequence of SEQ ID NO: 32, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 33, and the nucleocapsid phosphoprotein N comprises the amino acid sequence of SEQ ID NO: 34, or an amino acid sequence at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with any of the foregoing, or an antigenic fragment of any of the foregoing.
104. The method of claim 94, wherein the virus is an influenza virus.
105. The method of claim 104, wherein the antigen is an influenza viral antigen, optionally selected from hemagglutinin (HA) protein, matrix 2 (M2) protein, and neuraminidase, or an antigenic fragment thereof.
106. The method of any one of claims 66-105, wherein the connector between (i) and (ii) is a flexible linker.
107. The method of claim 106, wherein the flexible linker is substantially comprised of glycine and serine residues, optionally wherein i) the flexible linker comprises $(\text{Gly}_4\text{Ser})_n$, where n is from about 1 to about 8, or ii) the flexible linker comprises one or more of SEQ ID NOs: 556-571.
108. The method of claim 107, wherein the flexible linker is substantially comprised of GGSGGSGGGGSGGGGS (SEQ ID NO: 10).
109. The method of claim 106, wherein the flexible linker is substantially comprised of LE, GGGGS (SEQ ID NO:2), $(\text{GGGGS})_n$ (n=1-4) (SEQ ID NO:2-SEQ ID NO:5), $(\text{Gly})_8$ (SEQ ID NO:11), $(\text{Gly})_6$ (SEQ ID NO:12), $(\text{EAAAK})_n$ (n=1-3) (SEQ ID NO:13-SEQ ID NO:15), $\text{A}(\text{EAAAK})_n\text{A}$ (n = 2-5) (SEQ ID NO:16 – SEQ ID NO:19),

AEAAAKEAAKA (SEQ ID NO:16), A(EAAAK)₄ALEA(EAAAK)₄A (SEQ ID NO:21), PAPAP (SEQ ID NO:22), KESGSVSSEQLAQFRSLD (SEQ ID NO:23), EGKSSGSGSESKST (SEQ ID NO:24), GSAGSAAGSGEF (SEQ ID NO:25), and (XP)_n, with X designating any amino acid, e.g., Ala, Lys, or Glu.

110. The method of any one of claims 66-109, wherein the wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.

111. The method of claim 110, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.

112. The method of claim 110, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.

113. The method of claim 112, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.

114. The method of any one of claims 66-113, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.

115. The method of claim 114, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.

116. The method of claim 114, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.

117. The method of any one of claims 66-116, wherein the one or more mutations to the Fc domain results in the reduction or elimination of the effector function of the Fc domain.

118. The method of any one of claims 66-117, wherein the Fc-based chimeric protein complex is a heterodimer and has a trans orientation.

119. The method of any one of claims 66-118, wherein the Fc-based chimeric protein-complex is a heterodimer and has a cis orientation.

120. The method of any one of claims 66-119, wherein the Fc comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).

121. The method of claim 120, wherein the Fc is human IgG1, and optionally contains one or more of L234, L235, K322, D265, P329, and P331 (according to EU numbering).

122. The method of any one of claims 66-121, wherein the Fc-based chimeric protein complex has an orientation and/or configuration of any one of FIGs. 1A-19F.

123. The method of any one of claims 66-122, wherein the adjuvant and/or the antigen are formulated for administration intravenously.

124. The method of any one of claims 66-122, wherein the adjuvant and/or the antigen are formulated for administration to the lung.

125. The method of any one of claims 66-122, wherein the adjuvant and/or the antigen are formulated for administration by inhalation.

126. The method of any one of claims 66-122, wherein the adjuvant and/or the antigen are formulated for administration via aerosol or nebulizer.

127. The method of any one of claims 66-122, wherein the adjuvant and/or the antigen are formulated for administration liquid nebulization, dry powder dispersion and meter-dose administration.

128. The method of any one of claims 66-127, wherein the adjuvant has

- (a) low toxicity;
 - (b) an ability to stimulate a long-lasting immune response against the antigen;
 - (c) substantial stability;
 - (d) an ability to elicit a humoral immune response and/or a cell-mediated immunity to the antigen;
 - (e) a capability of selectively interacting with populations of antigen presenting cells;
 - (f) an ability to specifically elicit T_{H1} and/or T_{H2} cell-specific immune responses to the antigen; and/or
 - (g) an ability to selectively increase appropriate antibody isotype levels against antigens, the isotype optionally being IgA,
- when administered to a patient.

129. The method of any one of claims 66-128, wherein the adjuvant stimulates a CD8⁺ T cell response to the antigen, when administered to a patient.

130. The method of any one of claims 66-129, wherein the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

131. The method of any one of claims 66-130, wherein the adjuvant does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient.

132. A method for vaccinating a subject against an influenza infection, comprising administering:

- (a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising:
 - (i) a wildtype or mutant IL-1 β ,
 - (ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and
 - (iii) a connector between (i) and (ii), the connector being:
 - (1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or

(2) a flexible linker that connects (i) and (ii);

wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and

(b) an influenza antigen which is suitable for inducing an immune response.

133. The vaccine composition of claim 132, wherein the mutant IL-1 β is human IL-1 β .

134. The method of claim 132 or 133, wherein the low affinity or activity at the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

135. The method of any one of claims 132-134, wherein the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1.

136. The method of any one of claims 132-135, wherein the mutant IL-1 β comprises one or more mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, and N245Q, wherein X is any change in amino acid, with respect to the amino acid sequence of SEQ ID NO: 1.

137. The method of claim 136, wherein the mutant IL-1 β comprises one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K, with respect to the amino acid sequence of SEQ ID NO: 1.

138. The method of claim 136, wherein the mutant IL-1 β comprises any of the following with respect to the amino acid sequence of SEQ ID NO: 1:

Q131G and Q148G;

Q148G and K208E;

R120G and Q131G;

R120G and H146A;

R120G and H146N;

R120G and H146R;

R120G and H146E;

R120G and H146G;

R120G and K208E; and

R120G, F162A, and Q164E.

139. The method of claim 136, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.

140. The method of any one of claims 132-139, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell.

141. The method of claim 140, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

142. The method of claim 141, wherein the immune cell is a T cell.

143. The method of any one of claims 132-142, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

144. The method of any one of claims 132-143, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a microprotein (e.g. cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

145. The method of any one of claims 132-144, wherein the chimeric protein or chimeric protein complex further comprises additional cytokines, optionally modified, optionally mutated.

146. The method of any one of claims 132-145, wherein the chimeric protein or chimeric protein complex further comprises one or more additional targeting moieties.

147. The method of any one of claims 132-146, wherein the chimeric protein or chimeric protein complex further comprises two signaling agents and/or two targeting moieties or two of both.

148. The method of any one of claims 132-147, wherein the chimeric protein or chimeric protein complex further comprises three signaling agents and/or three targeting moieties or three of both.

149. The method of any one of claims 132-148, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1 and the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

150. The method of any one of claims 132-149, further comprising an aluminum gel or salt.

151. The method of claim 150, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum sulfate.

152. The method of any one of claims 132-151, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, Freund's Adjuvants, cytokines, toll like receptors ligands, and chitosans.

153. The method of any one of claims 132-152, wherein the adjuvant is a nucleic acid encoding the chimeric protein or chimeric protein complex.

154. The method of any one of claims 132-153, wherein the antigen is a protein or an antigenic fragment of a protein.
155. The method of any one of claims 132-153, wherein the antigen is a nucleic acid encoding a protein or an antigenic fragment of a protein.
156. The method of claim 155, wherein the nucleic acid is mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine.
157. The method of claim 155, wherein the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.
158. The method of claim 154, wherein the influenza viral antigen is selected from hemagglutinin (HA) protein, matrix 2 (M2) protein, and neuraminidase, or an antigenic fragment thereof.
159. The method of any one of claims 132-158, wherein the connector between (i) and (ii) is a flexible linker.
160. The method of claim 159, wherein the flexible linker is substantially comprised of glycine and serine residues, optionally wherein i) the flexible linker comprises $(\text{Gly}_4\text{Ser})_n$, where n is from about 1 to about 8, or ii) the flexible linker comprises one or more of SEQ ID NOs: 556-571.
161. The method of claim 160, wherein the flexible linker is substantially comprised of GGSGGSGGGGSGGGGS (SEQ ID NO: 10).
162. The method of claim 159, wherein the flexible linker is substantially comprised of LE, GGGGS (SEQ ID NO:2), $(\text{GGGGS})_n$ ($n=1-4$) (SEQ ID NO:2-SEQ ID NO:5), $(\text{Gly})_8$ (SEQ ID NO:11), $(\text{Gly})_6$ (SEQ ID NO:12), $(\text{EAAAK})_n$ ($n=1-3$) (SEQ ID NO:13-SEQ ID NO:15), $\text{A}(\text{EAAAK})_n\text{A}$ ($n = 2-5$) (SEQ ID NO:16 – SEQ ID NO:19), AEAAAKEAAKA (SEQ ID NO:16), $\text{A}(\text{EAAAK})_4\text{ALEA}(\text{EAAAK})_4\text{A}$ (SEQ ID NO:21), PAPAP (SEQ ID NO:22), $\text{KESGSVSSEQLAQFRSLD}$ (SEQ ID NO:23), EGKSSGSGSESKST (SEQ ID NO:24), GSAGSAAGSGEF (SEQ ID NO:25), and $(\text{XP})_n$, with X designating any amino acid, e.g., Ala, Lys, or Glu.
163. The method of any one of claims 132-162, wherein the wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.
164. The method of claim 163, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.
165. The method of claim 163, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.
166. The method of claim 165, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.
167. The method of any one of claims 132-166, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.
168. The method of claim 167, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.

169. The method of claim 167, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.

170. The method of claim 167, wherein the one or more mutations to the Fc domain results in the reduction or elimination of the effector function of the Fc domain.

171. The method of any one of claims 132-170, wherein the Fc-based chimeric protein complex is a heterodimer and has a trans orientation.

172. The method of any one of claims 132-170, wherein the Fc-based chimeric protein-complex is a heterodimer and has a cis orientation.

173. The method of any one of claims 132-172, wherein the Fc comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).

174. The method of claim 173, wherein the Fc is human IgG1, and optionally contains one or more of L234, L235, K322, D265, P329, and P331 (according to EU numbering).

175. The method of any one of claims 132-174, wherein the Fc-based chimeric protein complex has an orientation and/or configuration of any one of FIGs. 1A-19F.

176. The method of any one of claims 132-175, wherein the adjuvant and/or the antigen are formulated for administration intravenously.

177. The method of any one of claims 132-175, wherein the adjuvant and/or the antigen are formulated for administration to the lung.

178. The method of any one of claims 132-175, wherein the adjuvant and/or the antigen are formulated for administration by inhalation.

179. The method of any one of claims 132-175, wherein the adjuvant and/or the antigen are formulated for administration via aerosol or nebulizer.

180. The method of any one of claims 132-175, wherein the adjuvant and/or the antigen are formulated for administration liquid nebulization, dry powder dispersion and meter-dose administration.

181. The method of any one of claims 132-180, wherein the adjuvant has

- (a) low toxicity;
- (b) an ability to stimulate a long-lasting immune response against the antigen;
- (c) substantial stability;
- (d) an ability to elicit a humoral immune response and/or a cell-mediated immunity to the antigen;
- (e) a capability of selectively interacting with populations of antigen presenting cells;
- (f) an ability to specifically elicit T_{H1} and/or T_{H2} cell-specific immune responses to the antigen; and/or

(g) an ability to selectively increase appropriate antibody isotype levels against antigens, the isotype optionally being IgA,

when administered to a patient.

182. The method of any one of claims 132-181, wherein the adjuvant stimulates a CD8⁺ T cell response to the antigen, when administered to a patient.

183. The method of any one of claims 132-182, wherein the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

184. The method of any one of claims 132-183, wherein the adjuvant does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient.

185. A method for vaccinating a subject against a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection comprising administering:

(a) an adjuvant comprising a chimeric protein or chimeric protein complex, comprising:

(i) a wildtype or mutant IL-1 β ,

(ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and

(iii) a connector between (i) and (ii), the connector being:

(1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or

(2) a flexible linker that connects (i) and (ii);

wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor; and

(b) a SARS-CoV-2 antigen which is suitable for inducing an immune response.

186. The method of claim 185, wherein the wildtype or mutant IL-1 β is human IL-1 β .

187. The method of any one of claims 185-186, wherein the low affinity or activity at the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

188. The method of any one of claims 185-187, wherein the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1.

189. The method of any one of claims 185-188, wherein the mutant IL-1 β comprises one or more mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A,

Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, and N245Q, wherein X is any change in amino acid, with respect to the amino acid sequence of SEQ ID NO: 1.

190. The method of claim 189, wherein the mutant IL-1 β comprises one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K, with respect to the amino acid sequence of SEQ ID NO: 1.

191. The method of claim 189, wherein the mutant IL-1 β comprises any of the following with respect to the amino acid sequence of SEQ ID NO: 1:

Q131G and Q148G;

Q148G and K208E;

R120G and Q131G;

R120G and H146A;

R120G and H146N;

R120G and H146R;

R120G and H146E;

R120G and H146G;

R120G and K208E; and

R120G, F162A, and Q164E.

192. The method of claim 189, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.

193. The method of any one of claims 185-192, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell.

194. The method of claim 193, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

195. The method of claim 194, wherein the immune cell is a T cell.

196. The method of any one of claims 185-195, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

197. The method of any one of claims 185-196, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only

antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a microprotein (e.g. cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

198. The method of any one of claims 185-197, wherein the chimeric protein or chimeric protein complex further comprises additional cytokines, optionally modified, optionally mutated.

199. The method of any one of claims 185-198, wherein the chimeric protein or chimeric protein complex further comprises one or more additional targeting moieties.

200. The method of any one of claims 185-199, wherein the chimeric protein or chimeric protein complex further comprises two signaling agents and/or two targeting moieties or two of both.

201. The method of any one of claims 185-200, wherein the chimeric protein or chimeric protein complex further comprises three signaling agents and/or three targeting moieties or three of both.

202. The method of any one of claims 185-201, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1 and the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

203. The method of any one of claims 185-202, further comprising an aluminum gel or salt.

204. The method of claim 203, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum sulfate.

205. The method of any one of claims 185-204, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, Freund's Adjuvants, cytokines, toll like receptors ligands, and chitosans.

206. The method of any one of claims 185-205, wherein the adjuvant is a nucleic acid encoding the chimeric protein or chimeric protein complex.

207. The method of any one of claims 185-206, wherein the antigen is a protein or an antigenic fragment of a protein.

208. The method of any one of claims 185-206, wherein the antigen is a nucleic acid encoding a protein or an antigenic fragment of a protein.

209. The method of claim 208, wherein the nucleic acid is mRNA, optionally comprising one or more non-canonical nucleotides, optionally selected from pseudouridine and 5-methoxyuridine.

210. The method of claim 208, wherein the nucleic acid is DNA, optionally selected from linear DNA, DNA fragments, or DNA plasmids.

211. The method of claim 207, wherein the SARS-CoV-2 antigen is a 2019-nCoV protein, or an antigenic fragment thereof, selected from spike surface glycoprotein, membrane glycoprotein M, envelope protein E, and nucleocapsid phosphoprotein N.

212. The method of claim 211, wherein the spike surface glycoprotein is the S1 or S2 subunit, or an antigenic fragment thereof.

213. The method of claim 212, wherein the spike surface glycoprotein comprises the amino acid sequence of SEQ ID NO: 31, membrane glycoprotein precursor M comprises the amino acid sequence of SEQ ID NO: 32, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 33, and the nucleocapsid phosphoprotein N comprises the amino acid sequence of SEQ ID NO: 34, or an amino acid sequence at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with any of the foregoing, or an antigenic fragment of any of the foregoing.

214. The method of any one of claims 185-213, wherein the connector between (i) and (ii) is a flexible linker.

215. The method of claim 214, wherein the flexible linker is substantially comprised of glycine and serine residues, optionally wherein i) the flexible linker comprises $(\text{Gly}_4\text{Ser})_n$, where n is from about 1 to about 8, or ii) the flexible linker comprises one or more of SEQ ID NOs: 556-571.

216. The method of claim 215, wherein the flexible linker is substantially comprised of GGSGSGGGSGGGGS (SEQ ID NO: 10).

217. The method of claim 214, wherein the flexible linker is substantially comprised of LE, GGGGS (SEQ ID NO:2), $(\text{GGGGS})_n$ ($n=1-4$) (SEQ ID NO:2-SEQ ID NO:5), $(\text{Gly})_8$ (SEQ ID NO:11), $(\text{Gly})_6$ (SEQ ID NO:12), $(\text{EAAAK})_n$ ($n=1-3$) (SEQ ID NO:13-SEQ ID NO:15), $\text{A}(\text{EAAAK})_n\text{A}$ ($n = 2-5$) (SEQ ID NO:16 – SEQ ID NO:19), AEAAAKEAAKA (SEQ ID NO:16), $\text{A}(\text{EAAAK})_4\text{ALEA}(\text{EAAAK})_4\text{A}$ (SEQ ID NO:21), PAPAP (SEQ ID NO:22), $\text{KESGSVSSEQLAQFRSLD}$ (SEQ ID NO:23), EGKSSGSGSESKST (SEQ ID NO:24), GSAGSAAGSGEF (SEQ ID NO:25), and $(\text{XP})_n$, with X designating any amino acid, e.g., Ala, Lys, or Glu.

218. The method of any one of claims 185-217, wherein the wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.

219. The method of claim 218, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.

220. The method of claim 218, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.

221. The method of claim 220, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.

222. The method of any one of claims 185-221, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.

223. The method of claim 222, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.

224. The method of claim 222, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.
225. The method of any one of claims 185-224, wherein the one or more mutations to the Fc domain results in the reduction or elimination of the effector function of the Fc domain
226. The method of any one of claims 185-225, wherein the Fc-based chimeric protein complex is a heterodimer and has a trans orientation.
227. The method of any one of claims 185-225, wherein the Fc-based chimeric protein-complex is a heterodimer and has a cis orientation.
228. The method of any one of claims 185-227, wherein the Fc comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).
229. The method of claim 228, wherein the Fc is human IgG1, and optionally contains one or more of L234, L235, K322, D265, P329, and P331 (according to EU numbering).
230. The method of any one of claims 185-229, wherein the Fc-based chimeric protein complex has an orientation and/or configuration of any one of FIGs. 1A-19F.
231. The method of any one of claims 185-230, wherein the adjuvant and/or the antigen are formulated for administration intravenously.
232. The method of any one of claims 185-230, wherein the adjuvant and/or the antigen are formulated for administration to the lung.
233. The method of any one of claims 185-230, wherein the adjuvant and/or the antigen are formulated for administration by inhalation.
234. The method of any one of claims 185-230, wherein the adjuvant and/or the antigen are formulated for administration via aerosol or nebulizer.
235. The method of any one of claims 185-230, wherein the adjuvant and/or the antigen are formulated for administration liquid nebulization, dry powder dispersion and meter-dose administration.
236. The method of any one of claims 185-235, wherein the adjuvant has
- (a) low toxicity;
 - (b) an ability to stimulate a long-lasting immune response against the antigen;
 - (c) substantial stability;
 - (d) an ability to elicit a humoral immune response and/or a cell-mediated immunity to the antigen;
 - (e) a capability of selectively interacting with populations of antigen presenting cells;
 - (f) an ability to specifically elicit T_{H1} and/or T_{H2} cell-specific immune responses to the antigen; and/or

(g) an ability to selectively increase appropriate antibody isotype levels against antigens, the isotype optionally being IgA,

when administered to a patient.

237. The method of any one of claims 185-236, wherein the adjuvant stimulates a CD8⁺ T cell response to the antigen, when administered to a patient.

238. The method of any one of claims 185-237, wherein the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

239. The method of any one of claims 185-238, wherein the adjuvant does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient.

240. A method for treating a subject afflicted with an infectious disease, comprising administering a chimeric protein or chimeric protein complex, comprising:

(i) a wild type or mutant IL-1 β ,

(ii) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and

(iii) a connector between (i) and (ii), the connector being:

(1) an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain that connects (i) and (ii) and/or

(2) a flexible linker that connects (i) and (ii);

wherein the mutant IL-1 β is characterized by low affinity or activity at the IL-1 receptor.

241. The method of claim 240, wherein the wildtype or mutant IL-1 β is human IL-1 β .

242. The method of any one of claims 240-241, wherein the low affinity or activity at the IL-1 receptor is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

243. The method of any one of claims 240-242, wherein the mutant human IL-1 β has an amino acid sequence of at least 95%, or 97% or 98% identity to SEQ ID NO: 1.

244. The method of any one of claims 240-243, wherein the mutant IL-1 β comprises one or more mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, and N245Q, wherein X is any change in amino acid, with respect to the amino acid sequence of SEQ ID NO: 1.

245. The method of claim 244, wherein the mutant IL-1 β comprises one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K, with respect to the amino acid sequence of SEQ ID NO: 1.

246. The method of claim 244, wherein the mutant IL-1 β comprises any of the following with respect to the amino acid sequence of SEQ ID NO: 1:

Q131G and Q148G;

Q148G and K208E;

R120G and Q131G;

R120G and H146A;

R120G and H146N;

R120G and H146R;

R120G and H146E;

R120G and H146G;

R120G and K208E; and

R120G, F162A, and Q164E.

247. The method of claim 244, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1.

248. The method of any one of claims 240-247, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds an antigen or receptor on a an endothelial cell, epithelial cell, mesenchymal cell, stromal cell, ECM and/or immune cell, organ cell, and/or tissue cell.

249. The method of claim 248, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

250. The method of claim 249, wherein the immune cell is a T cell.

251. The method of any one of claims 240-250, wherein the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.

252. The method of any one of claims 240-250, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a microprotein (e.g. cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a F(ab')₂, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

253. The method of any one of claims 240-252, wherein the chimeric protein or chimeric protein complex further comprises additional cytokines, optionally modified, optionally mutated.
254. The method of any one of claims 240-253, wherein the chimeric protein or chimeric protein complex further comprises one or more additional targeting moieties.
255. The method of any one of claims 240-254, wherein the chimeric protein or chimeric protein complex further comprises two signaling agents and/or two targeting moieties or two of both.
256. The method of any one of claims 240-255, wherein the chimeric protein or chimeric protein complex further comprises three signaling agents and/or three targeting moieties or three of both.
257. The method of any one of claims 240-256, wherein the mutant IL-1 β comprises Q148G, with respect to the amino acid sequence of SEQ ID NO: 1 and the targeting moiety comprises a recognition domain that recognizes and/or binds CD8 or CD4.
258. The method of any one of claims 240-257, further comprising an aluminum gel or salt.
259. The method of claim 258, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and aluminum sulfate.
260. The method of any one of claims 240-259, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, Freund's Adjuvants, cytokines, toll like receptors ligands, and chitosans.
261. The method of claim 260, wherein the infectious disease is an infection with a pathogen, optionally selected from a bacterium, virus, fungus, or parasite.
262. The method of claim 260, wherein the pathogen is a virus.
263. The method of claim 262, wherein the virus is:
- (a) an influenza virus, optionally selected from Type A, Type B, Type C, and Type D influenza viruses, or
 - (b) a member of the *Coronaviridae* family, optionally selected from
 - (i) a betacoronavirus, optionally selected from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV, Middle East Respiratory Syndrome—Corona Virus (MERS-CoV), HCoV-HKU1, and HCoV-OC43 or
 - (ii) an alphacoronavirus, optionally selected from HCoV-NL63 and HCoV-229E, or
 - (iii) a member of Picornaviridae family, optionally selected from Rhinovirus A or Rhinovirus B.
264. The method of claim 263, wherein the virus is SARS-CoV-2.
265. The method of claim 263, wherein the virus is an influenza virus.
266. The method of any one of claims 240-265, wherein the connector between (i) and (ii) is a flexible linker.

267. The method of claim 266, wherein the flexible linker is substantially comprised of glycine and serine residues, optionally wherein i) the flexible linker comprises $(\text{Gly}_4\text{Ser})_n$, where n is from about 1 to about 8, or ii) the flexible linker comprises one or more of SEQ ID NOs: 556-571.

268. The method of claim 267, wherein the flexible linker is substantially comprised of GSGSGSGSGSGSGSGS (SEQ ID NO: 10).

269. The method of claim 266, wherein the flexible linker is substantially comprised of LE, GGGGS (SEQ ID NO:2), $(\text{GGGGS})_n$ (n=1-4) (SEQ ID NO:2-SEQ ID NO:5), $(\text{Gly})_8$ (SEQ ID NO:11), $(\text{Gly})_6$ (SEQ ID NO:12), $(\text{EAAAK})_n$ (n=1-3) (SEQ ID NO:13-SEQ ID NO:15), $\text{A}(\text{EAAAK})_n\text{A}$ (n = 2-5) (SEQ ID NO:16 – SEQ ID NO:19), AEAAAKEAAKA (SEQ ID NO:16), $\text{A}(\text{EAAAK})_4\text{ALEA}(\text{EAAAK})_4\text{A}$ (SEQ ID NO:21), PAPAP (SEQ ID NO:22), $\text{KESGSVSSEQLAQFRSLD}$ (SEQ ID NO:23), EGKSSSGSGSESKST (SEQ ID NO:24), GSAGSAAGSGEF (SEQ ID NO:25), and $(\text{XP})_n$, with X designating any amino acid, e.g., Ala, Lys, or Glu.

270. The method of any one of claims 240-269, wherein the wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.

271. The method of claim 270, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.

272. The method of claim 270, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.

273. The method of claim 272, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.

274. The method of any one of claims 240-273, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.

275. The method of claim 274, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.

276. The method of claim 274, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.

277. The method of any one of claims 240-276, wherein the one or more mutations to the Fc domain results in the reduction or elimination of the effector function of the Fc domain

278. The method of any one of claims 240-277, wherein the Fc-based chimeric protein complex is a heterodimer and has a trans orientation.

279. The method of any one of claims 240-278, wherein the Fc-based chimeric protein-complex is a heterodimer and has a cis orientation.

280. The method of any one of claims 240-279, wherein the Fc comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).

281. The method of claim 280, wherein the Fc is human IgG1, and optionally contains one or more of L234, L235, K322, D265, P329, and P331 (according to EU numbering).

282. The method of any one of claims 240-281, wherein the Fc-based chimeric protein complex has an orientation and/or configuration of any one of FIGs. 1A-19F.

283. The method of any one of claims 240-282, wherein the chimeric protein or chimeric protein complex stimulates a CD8⁺ T cell response to the antigen, when administered to a patient.

284. The method of any one of claims 240-283, wherein the adjuvant or vaccine composition stimulates activation of the IL-1R, when administered to a patient.

285. The method of any one of claims 240-284, wherein the chimeric protein or chimeric protein complex does not substantially cause one or more of fever, neutrophilia and the release of acute phase proteins when administered to a patient.

FIG. 1A

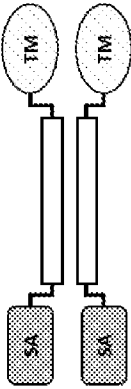


FIG. 1B

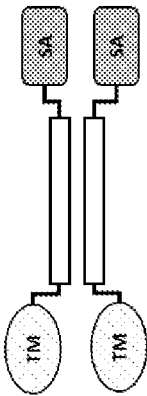


FIG. 1C

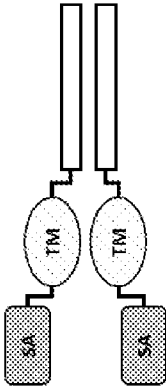


FIG. 1D

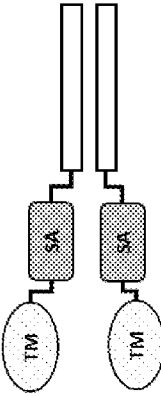


FIG. 1E

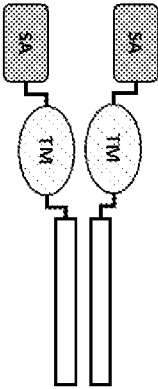


FIG. 1F

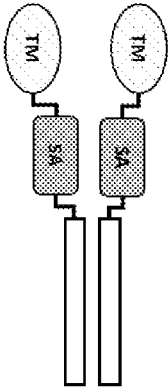


FIG. 2A

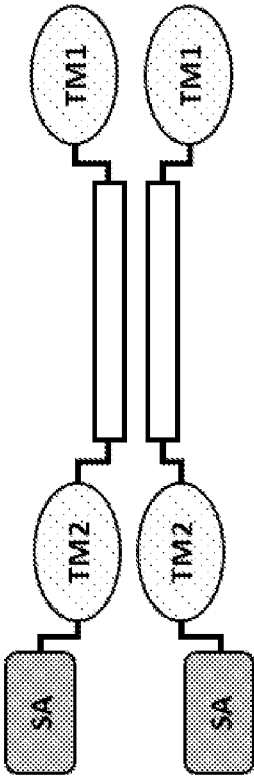


FIG. 2B

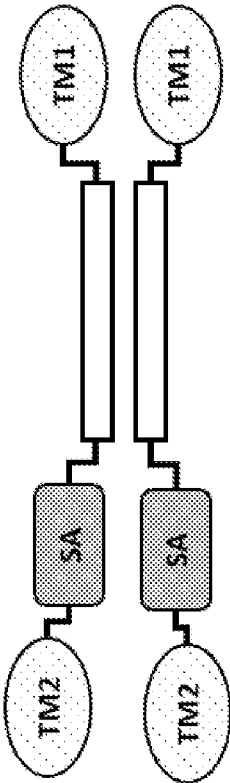


FIG. 2C

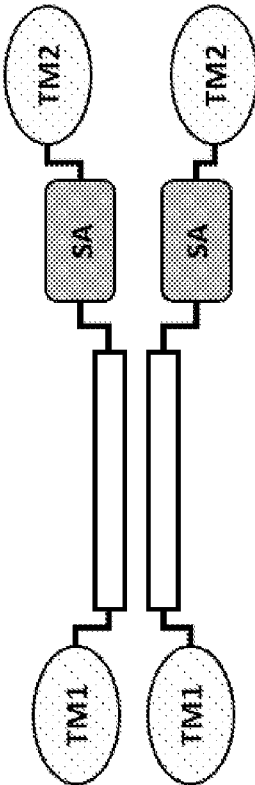


FIG. 2D

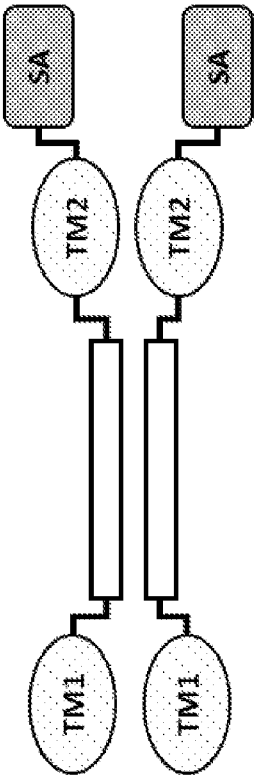


FIG. 2E

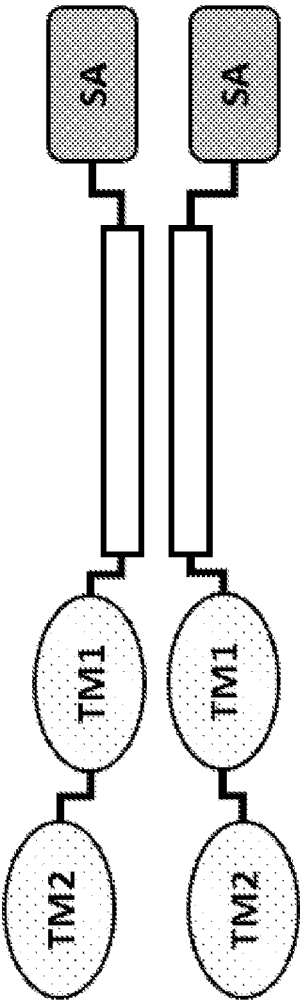


FIG. 2F

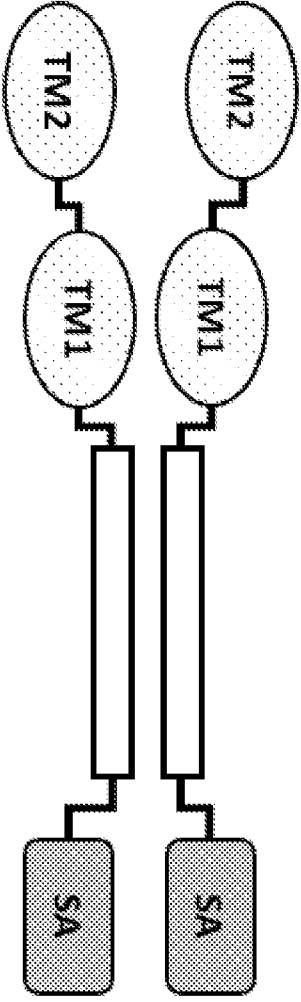


FIG. 2G

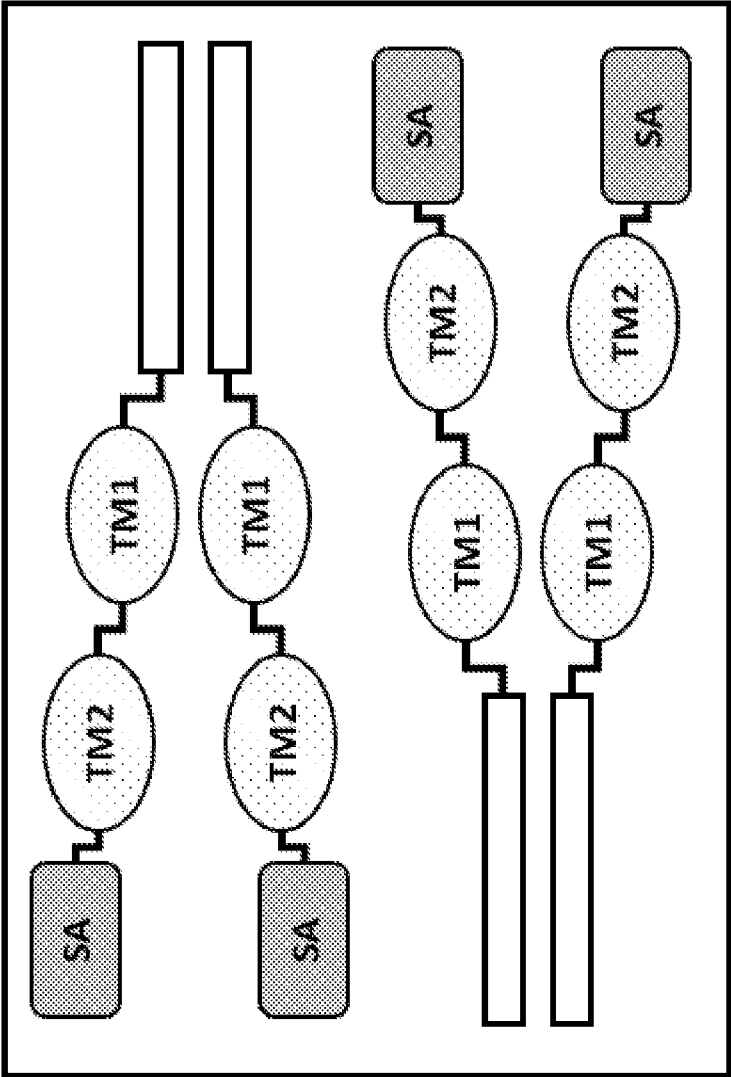


FIG. 2H

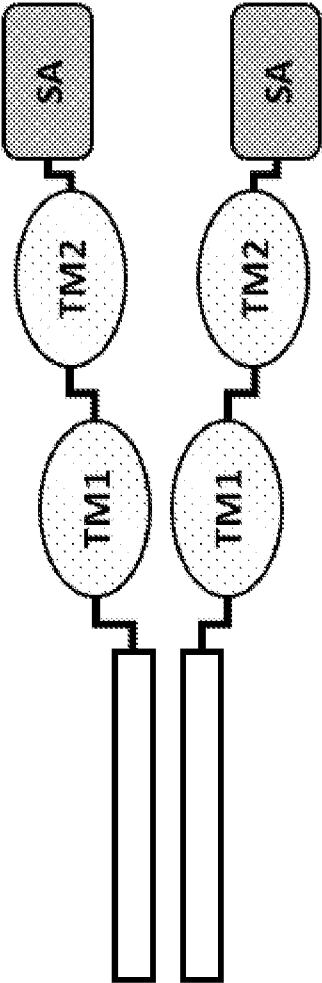


FIG. 3A

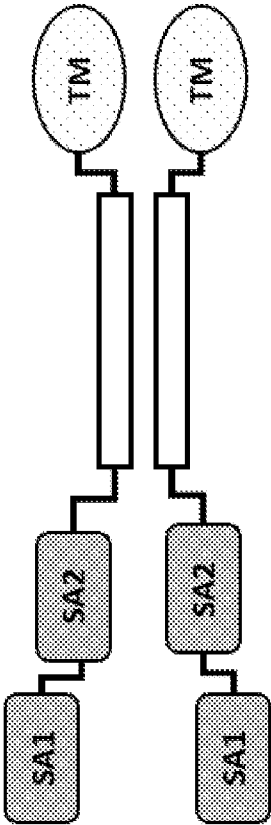


FIG. 3B

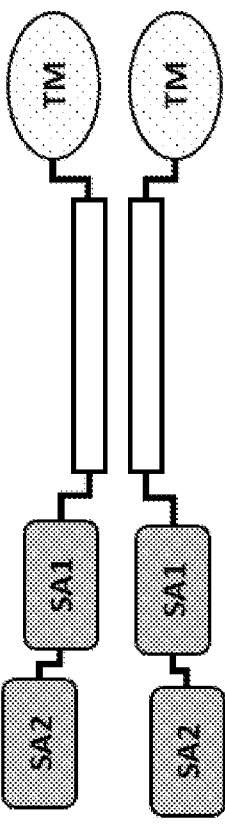


FIG. 3C

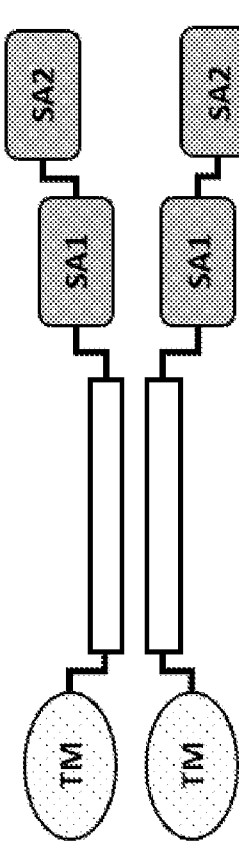


FIG. 3D

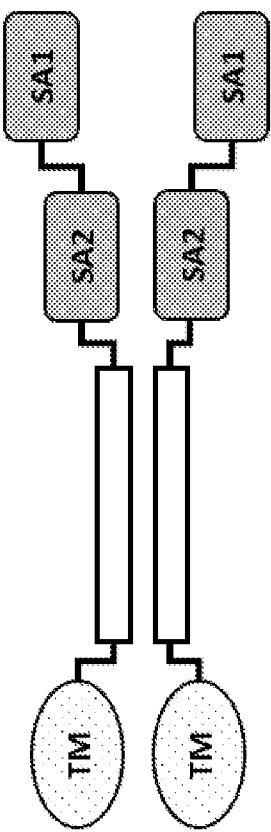


FIG. 3E

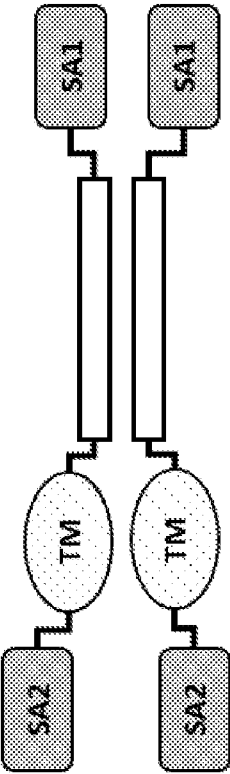


FIG. 3F

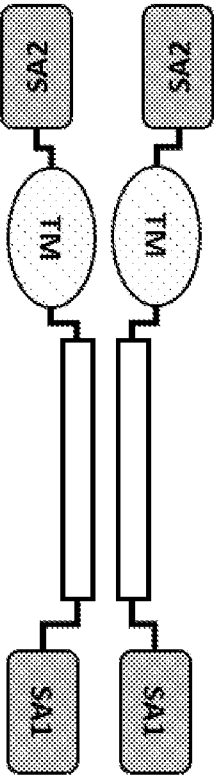


FIG. 3G

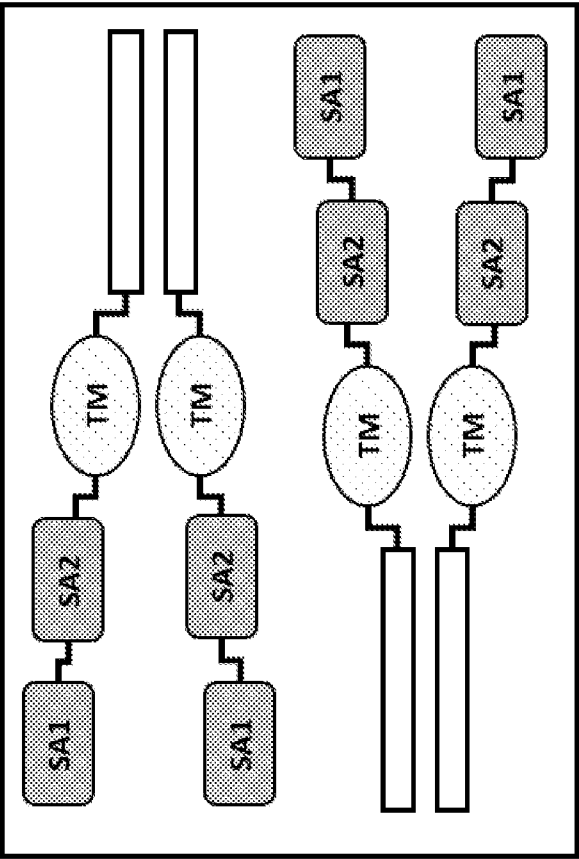


FIG. 3H

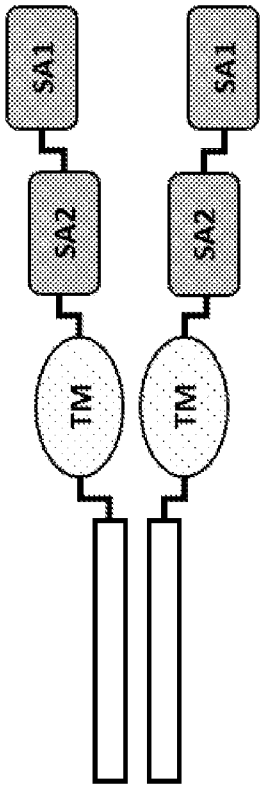


FIG. 4A

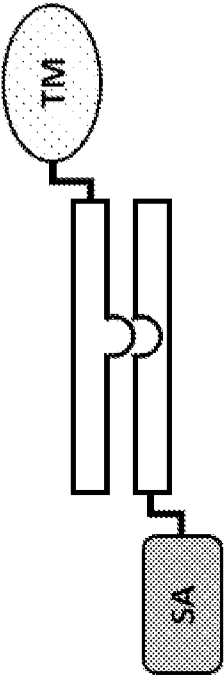


FIG. 4B

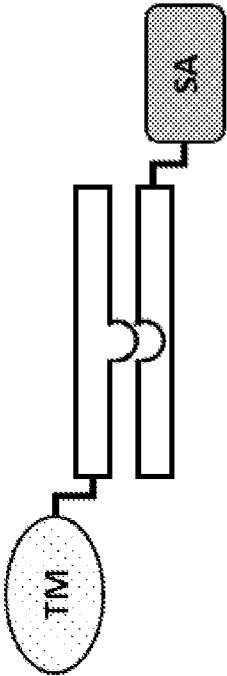


FIG. 4C

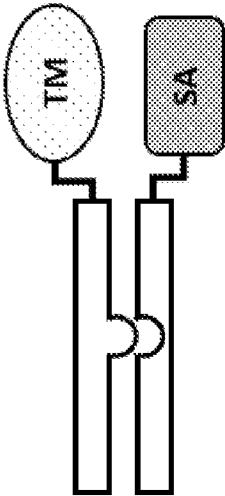


FIG. 4D

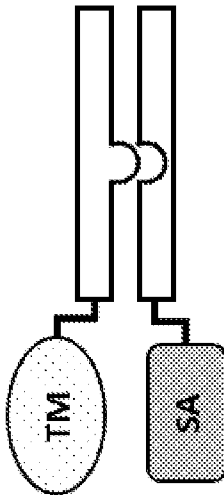


FIG. 5A

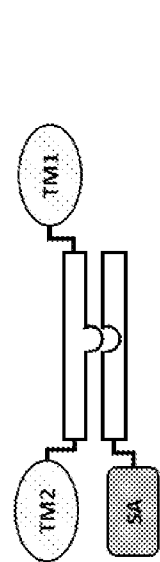


FIG. 5B

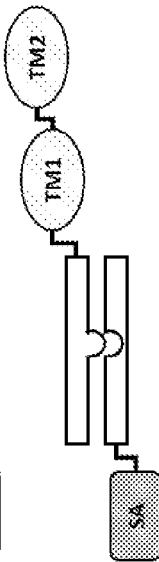


FIG. 5C

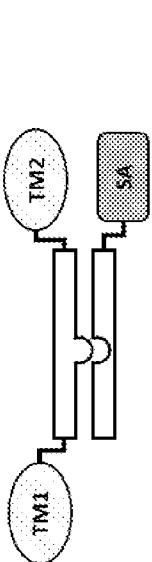


FIG. 5D

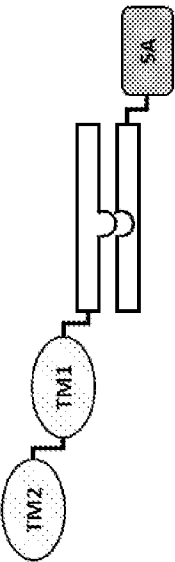


FIG. 5E

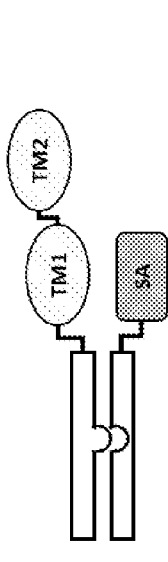


FIG. 5F

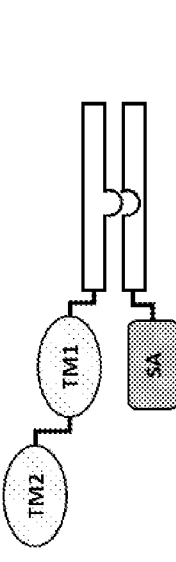


FIG. 6A

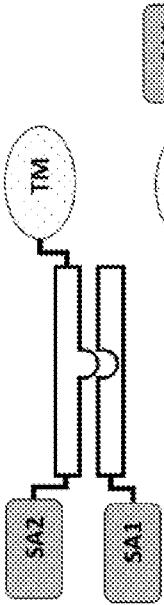


FIG. 6B

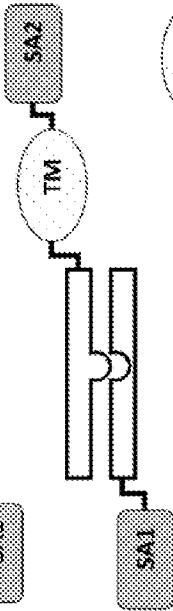


FIG. 6C

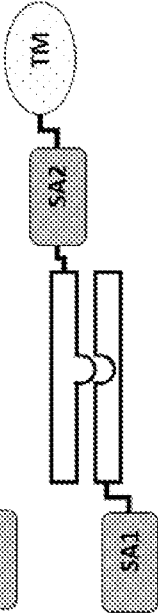


FIG. 6D

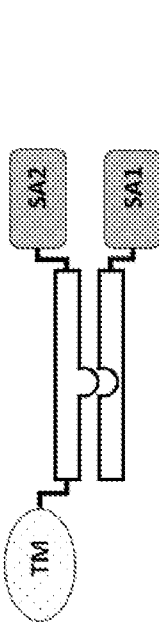


FIG. 6E

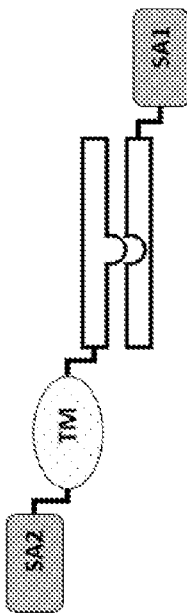


FIG. 6F

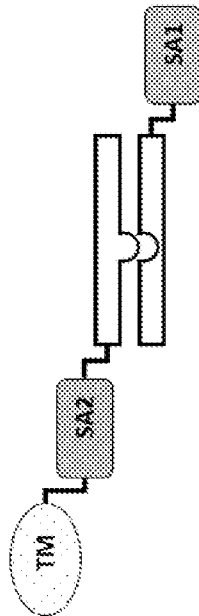


FIG. 6G

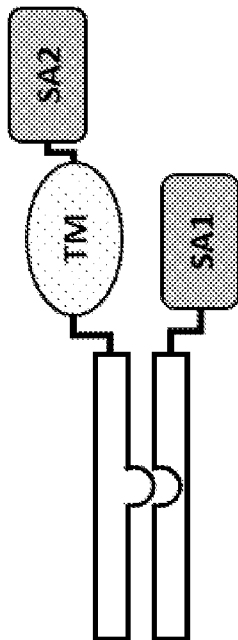


FIG. 6H

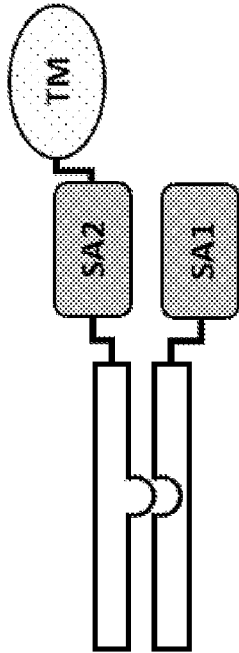


FIG. 6I

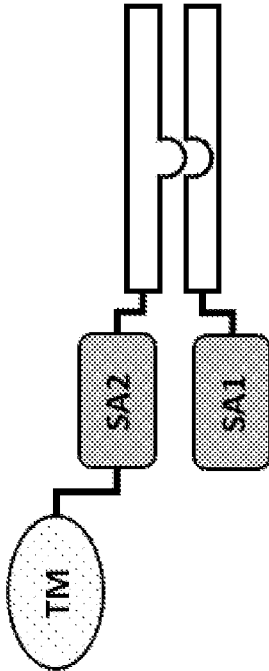


FIG. 6J

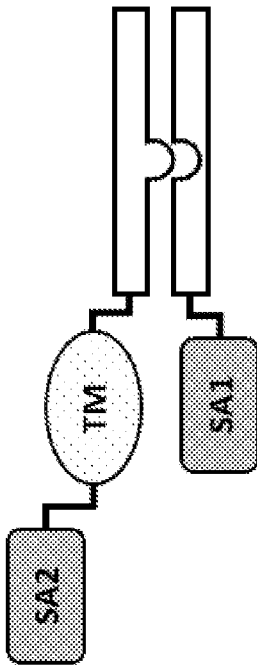


FIG. 7A

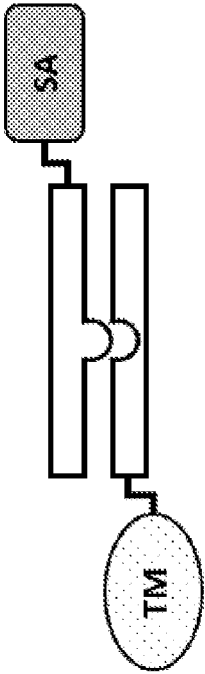


FIG. 7B

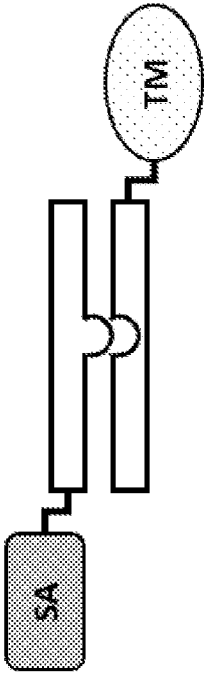


FIG. 7C

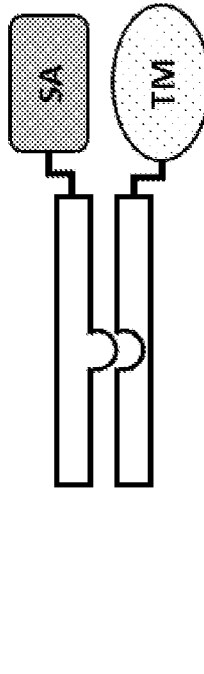


FIG. 7D

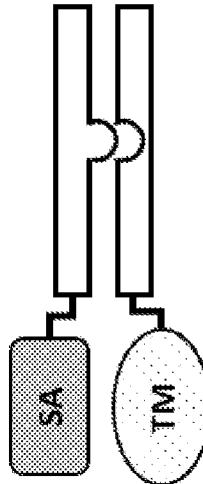


FIG. 8A

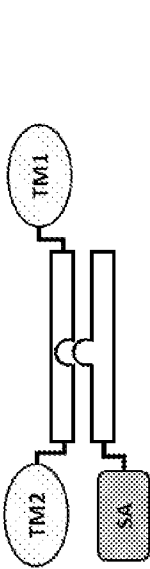


FIG. 8B

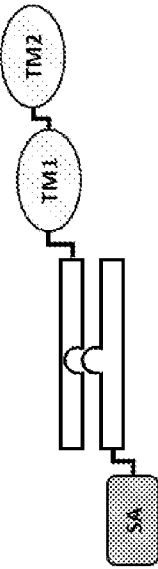


FIG. 8C

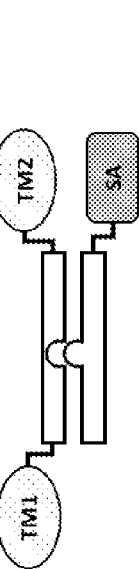


FIG. 8D



FIG. 8E

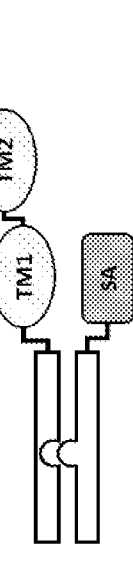


FIG. 8F

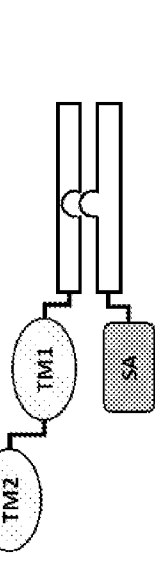


FIG. 9A

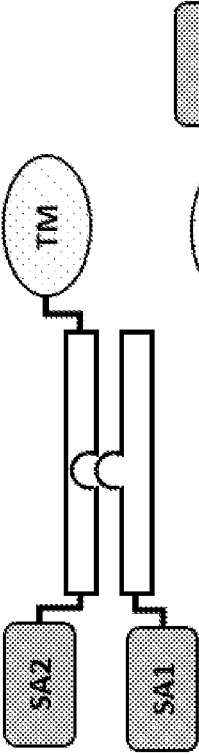


FIG. 9B

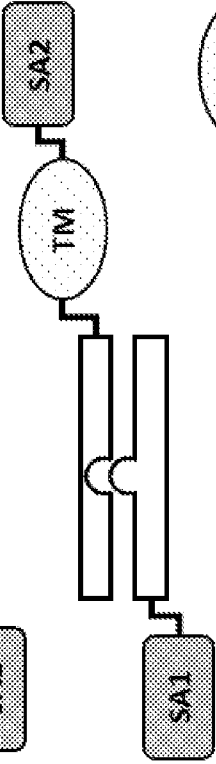


FIG. 9C

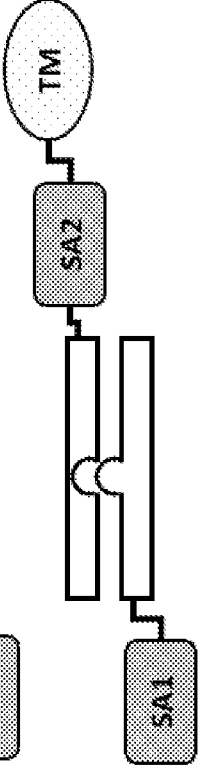


FIG. 9D

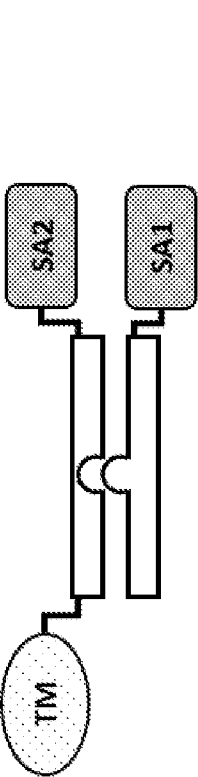


FIG. 9E

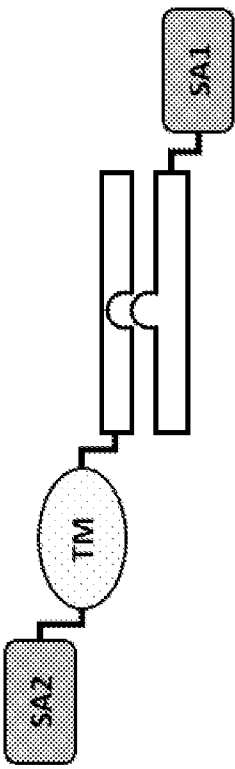


FIG. 9F

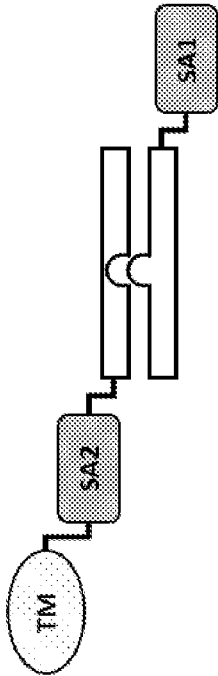


FIG. 9G

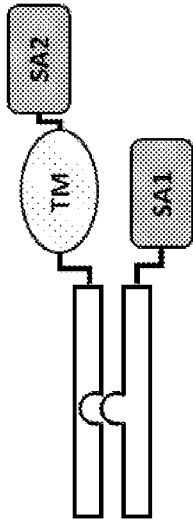


FIG. 9H

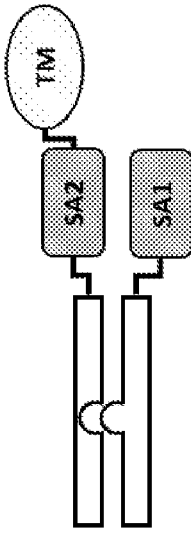


FIG. 9I

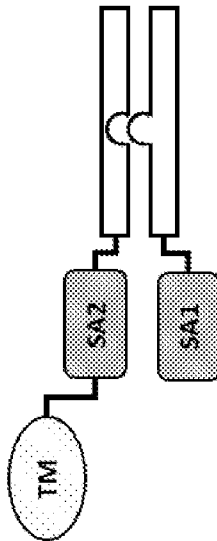


FIG. 9J

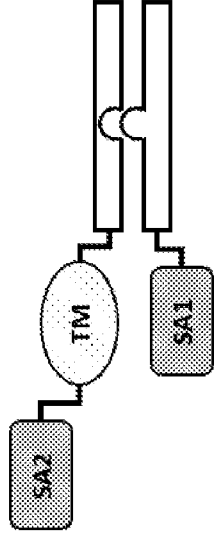


FIG. 10A

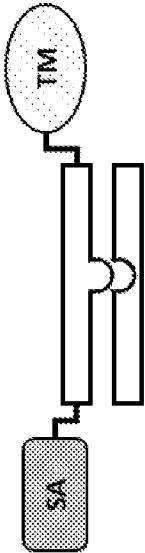


FIG. 10B

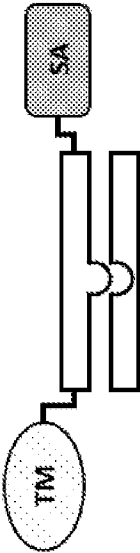


FIG. 10C

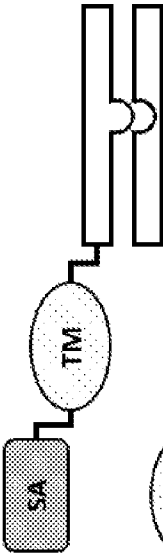


FIG. 10D

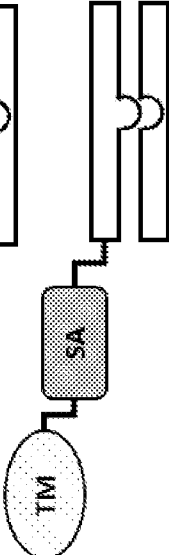


FIG. 10E

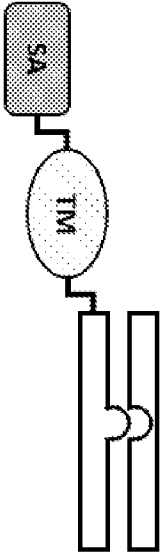


FIG. 10F

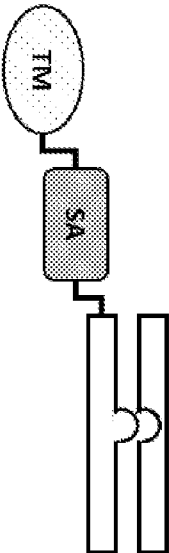


FIG. 11A

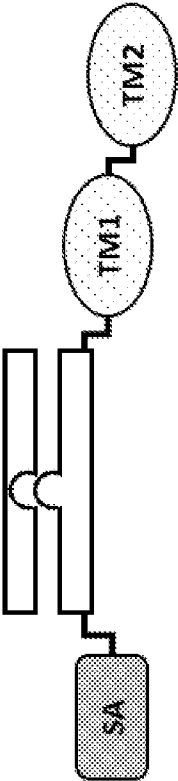


FIG. 11B

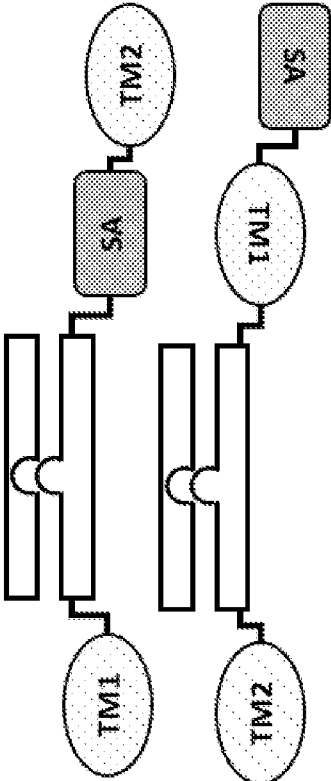


FIG. 11C

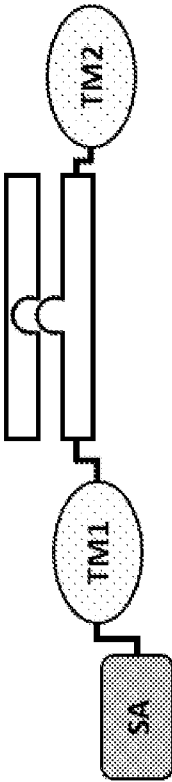


FIG. 11D

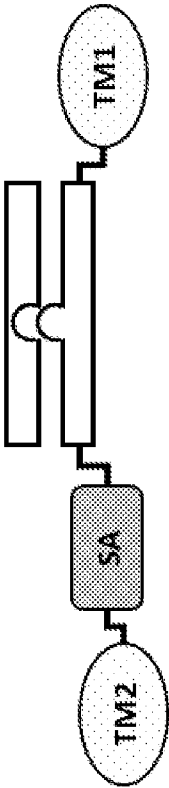


FIG. 11F

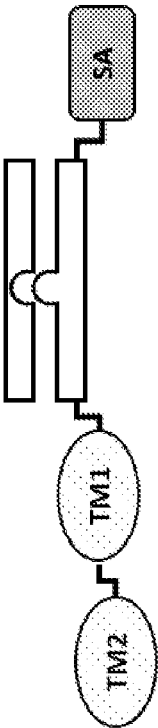


FIG. 11G

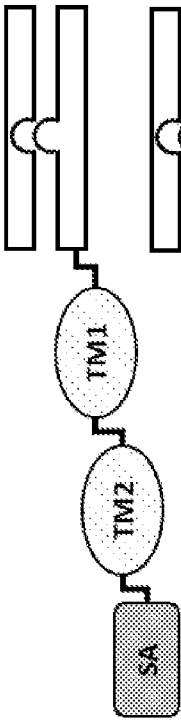


FIG. 11H

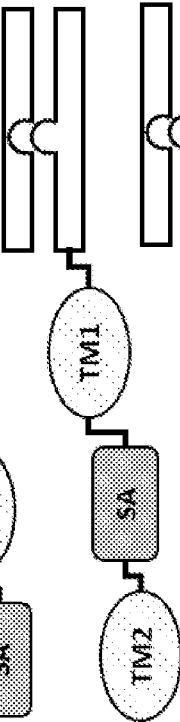


FIG. 11I

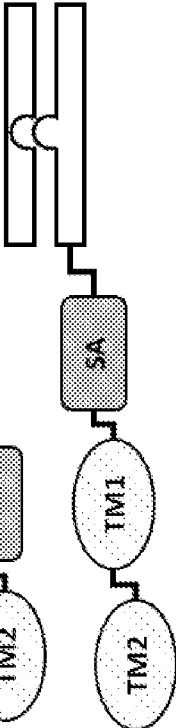


FIG. 11J

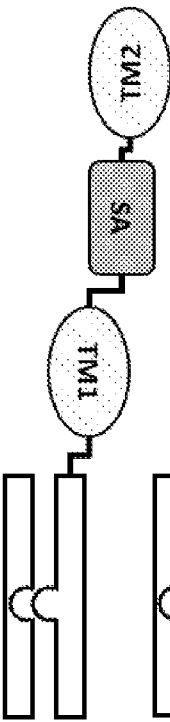


FIG. 11K

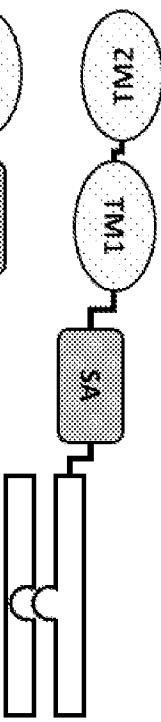


FIG. 11L

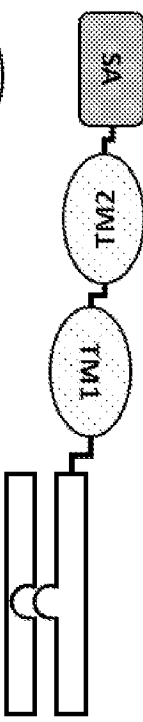


FIG. 12A

FIG. 12B

FIG. 12C

FIG. 12D

FIG. 12E

FIG. 12F

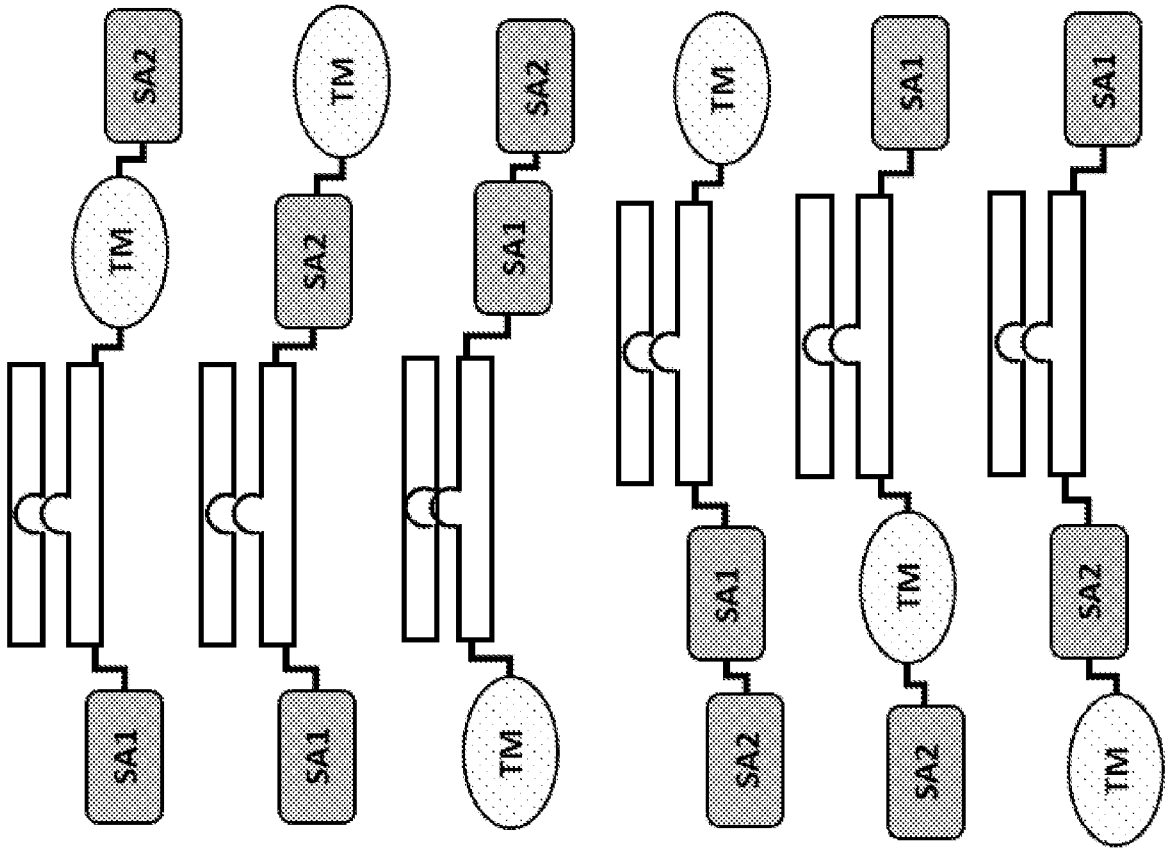


FIG. 12G

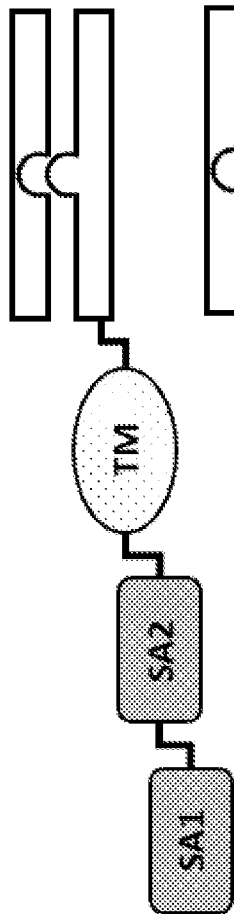


FIG. 12H

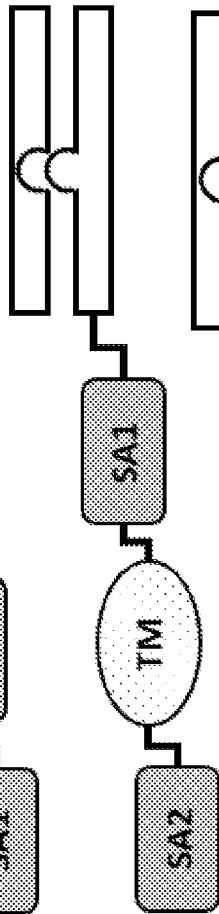


FIG. 12I

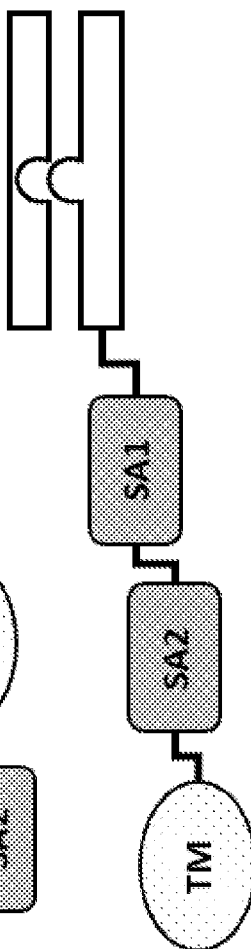


FIG. 12J

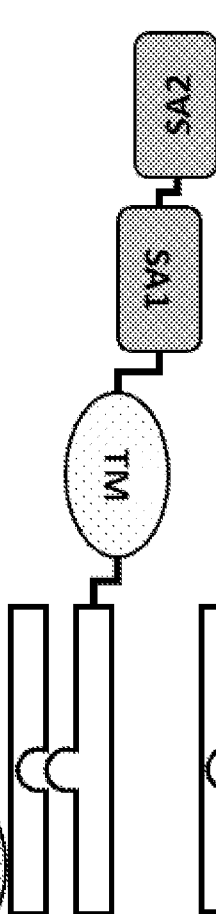


FIG. 12K

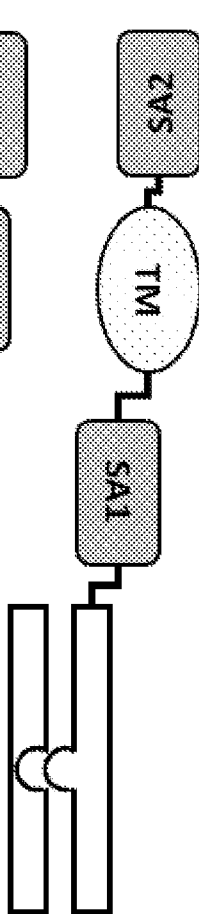


FIG. 12L

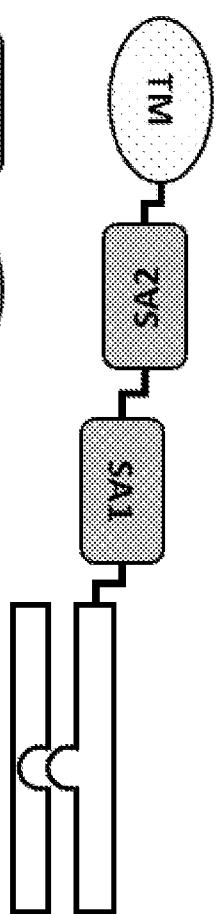


FIG. 13A

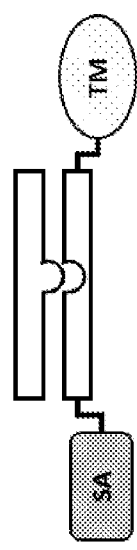


FIG. 13B

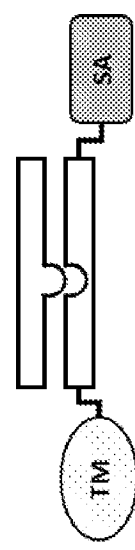


FIG. 13C

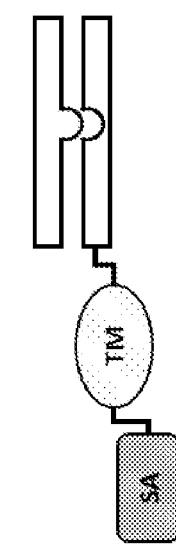


FIG. 13D

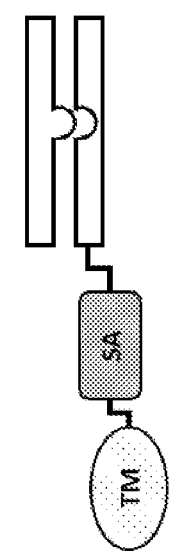


FIG. 13E

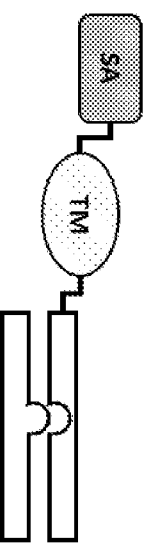


FIG. 13F

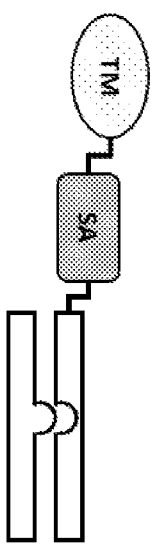


FIG. 14A

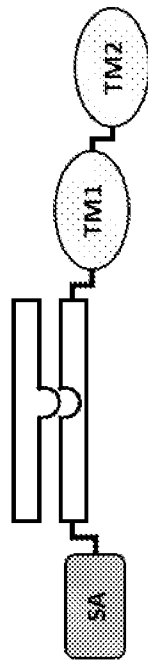


FIG. 14B

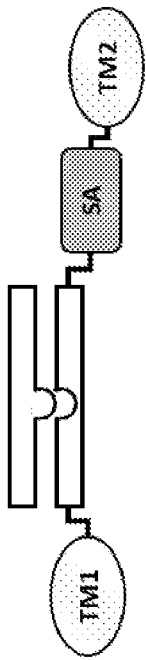


FIG. 14C

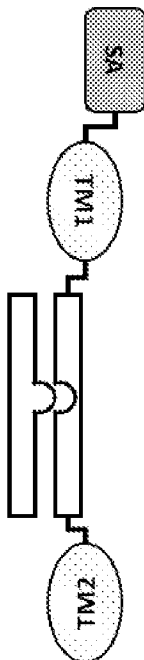


FIG. 14D

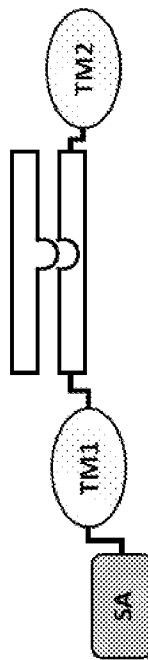


FIG. 14E

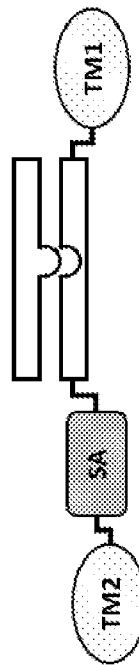


FIG. 14F

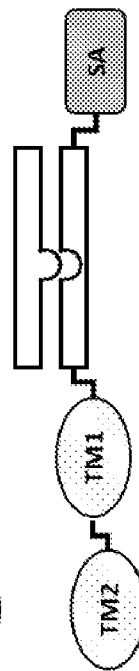


FIG. 14G

FIG. 14H

FIG. 14I

FIG. 14J

FIG. 14K

FIG. 14L

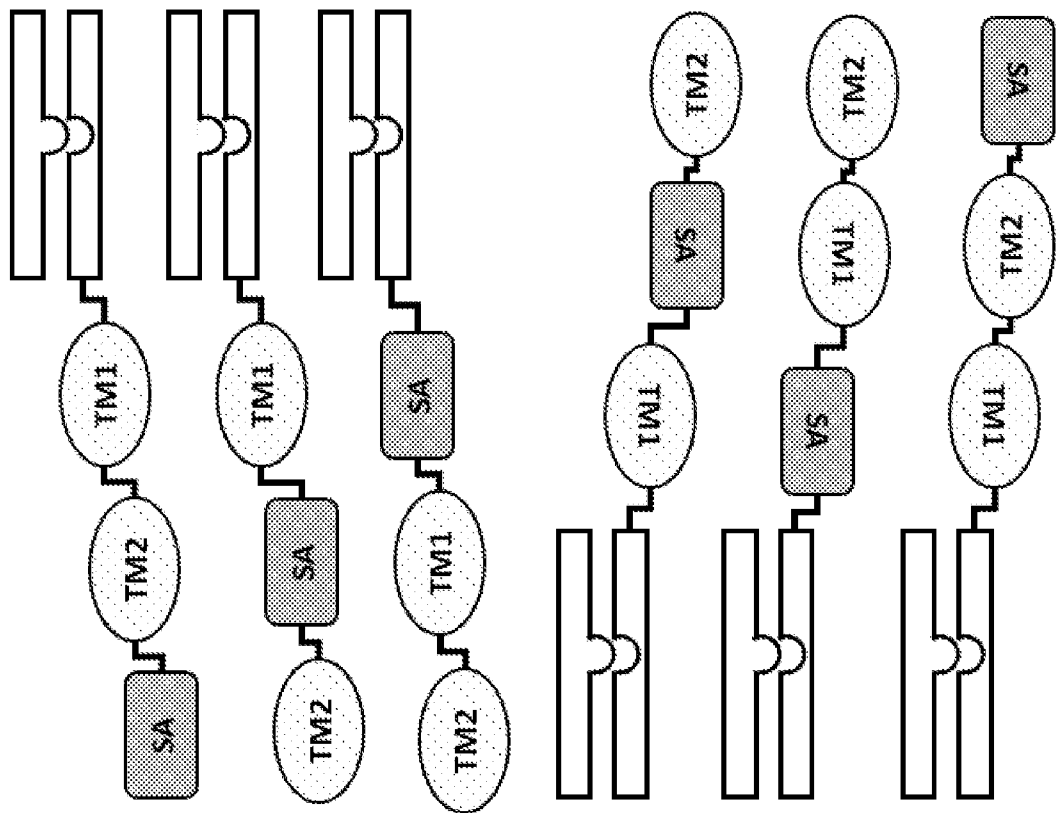


FIG. 15A



FIG. 15B

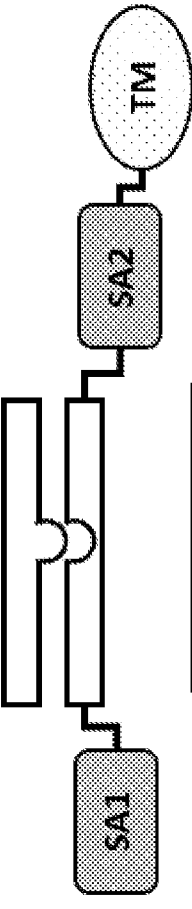


FIG. 15C

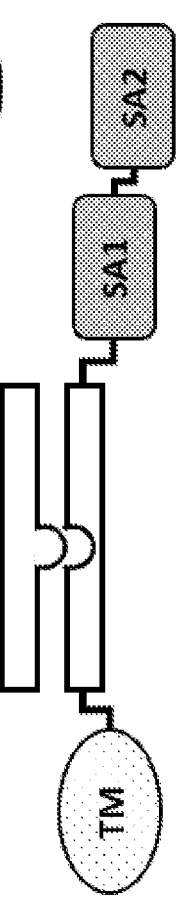


FIG. 15D

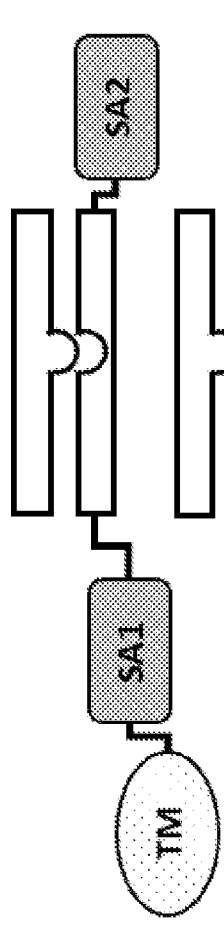


FIG. 15E

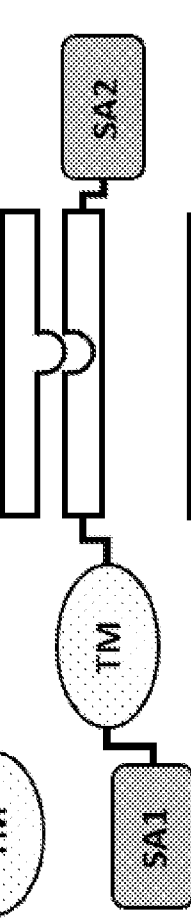


FIG. 15F

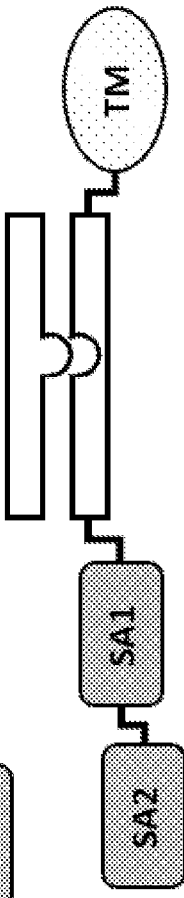


FIG. 15G

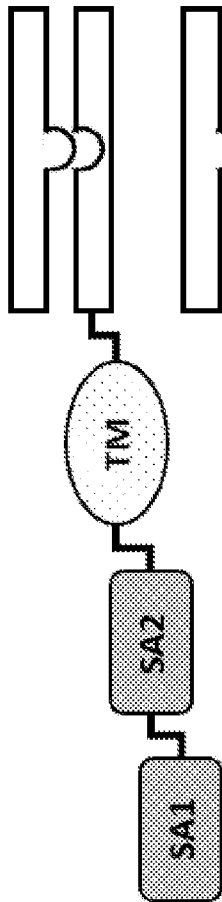


FIG. 15H

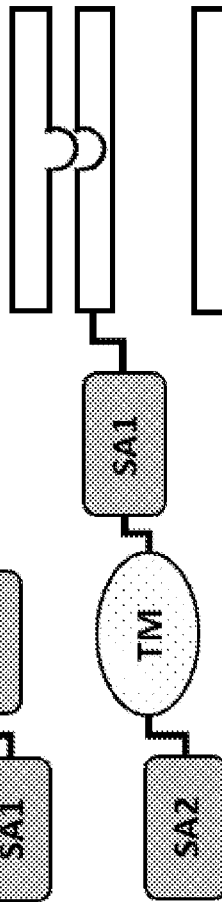


FIG. 15I

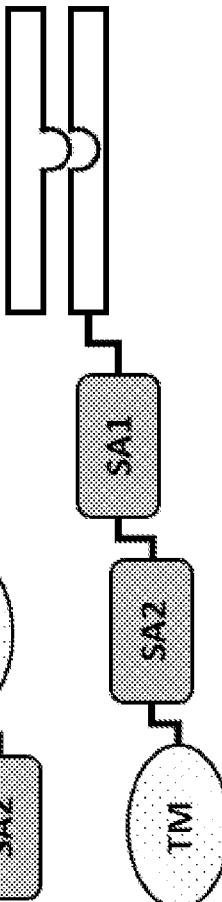


FIG. 15J

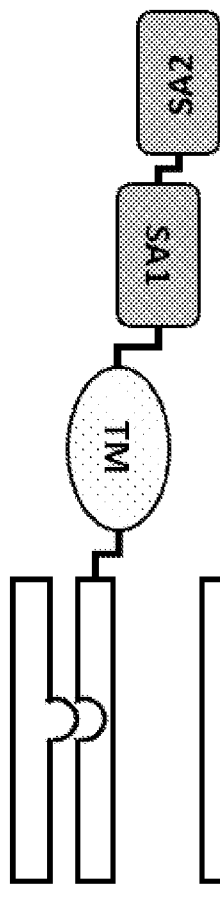


FIG. 15K

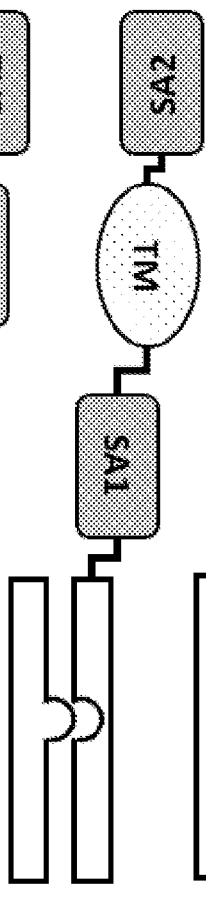


FIG. 15L

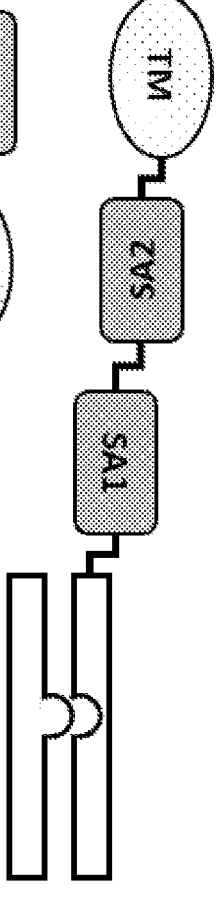


FIG. 16A

FIG. 16B

FIG. 16C

FIG. 16D

FIG. 16E

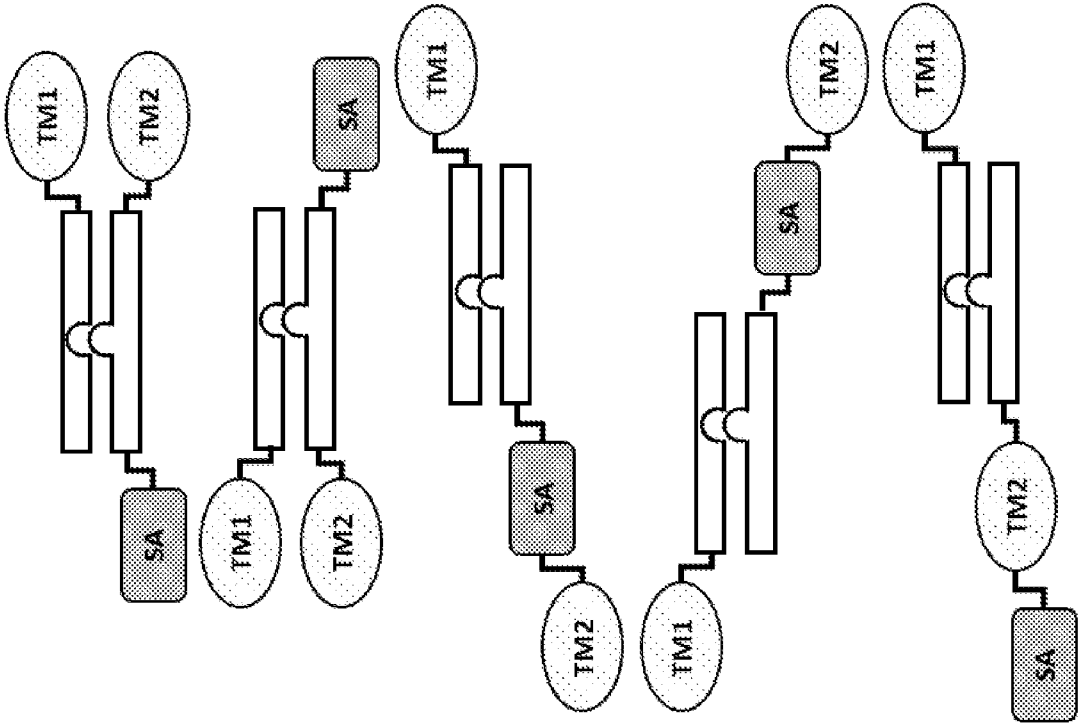


FIG. 16F

FIG. 16G

FIG. 16H

FIG. 16I

FIG. 16J

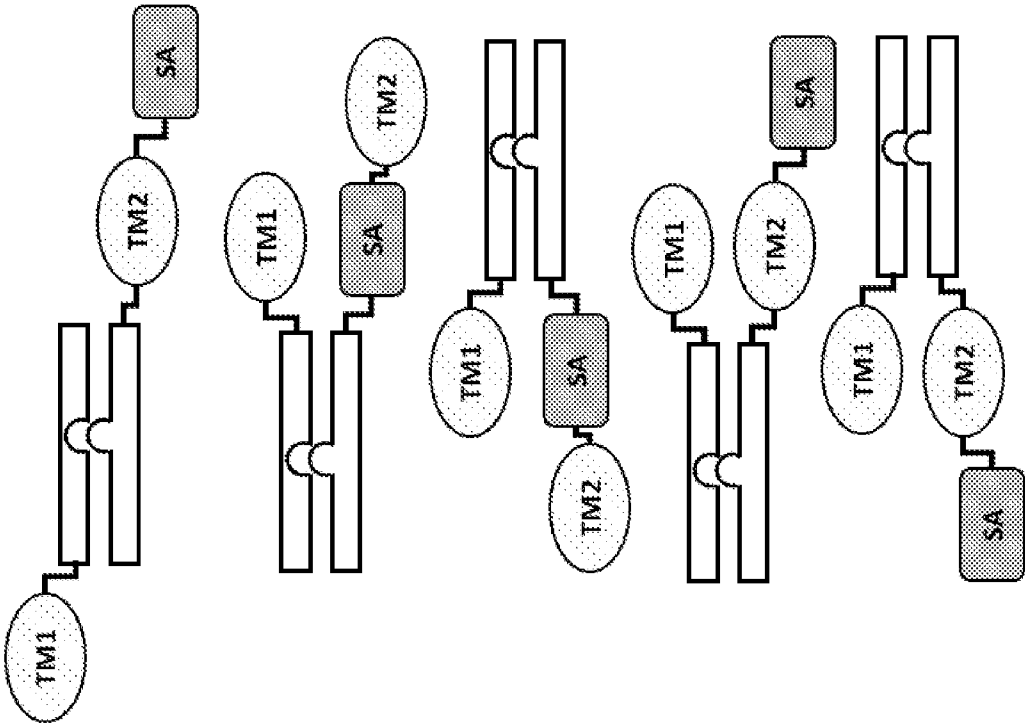


FIG. 17A

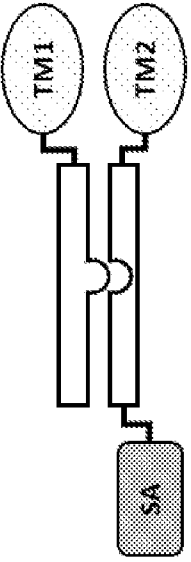


FIG. 17B

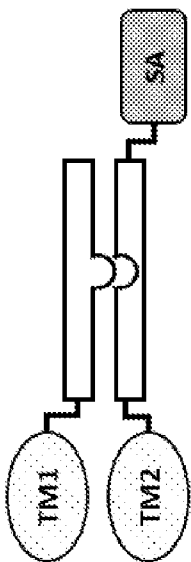


FIG. 17C

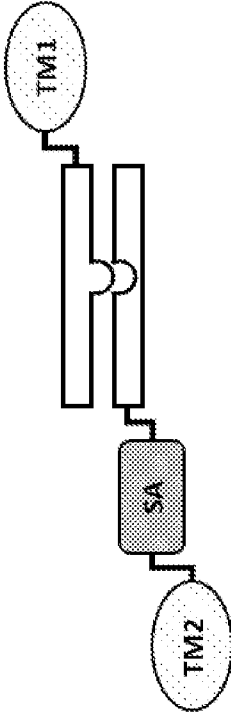


FIG. 17D

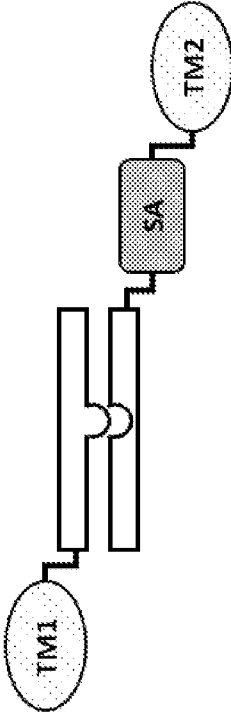


FIG. 17E

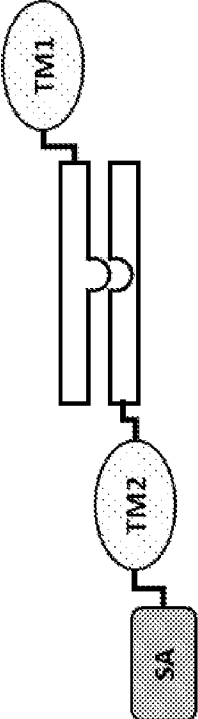


FIG. 17F

FIG. 17G

FIG. 17H

FIG. 17I

FIG. 17J

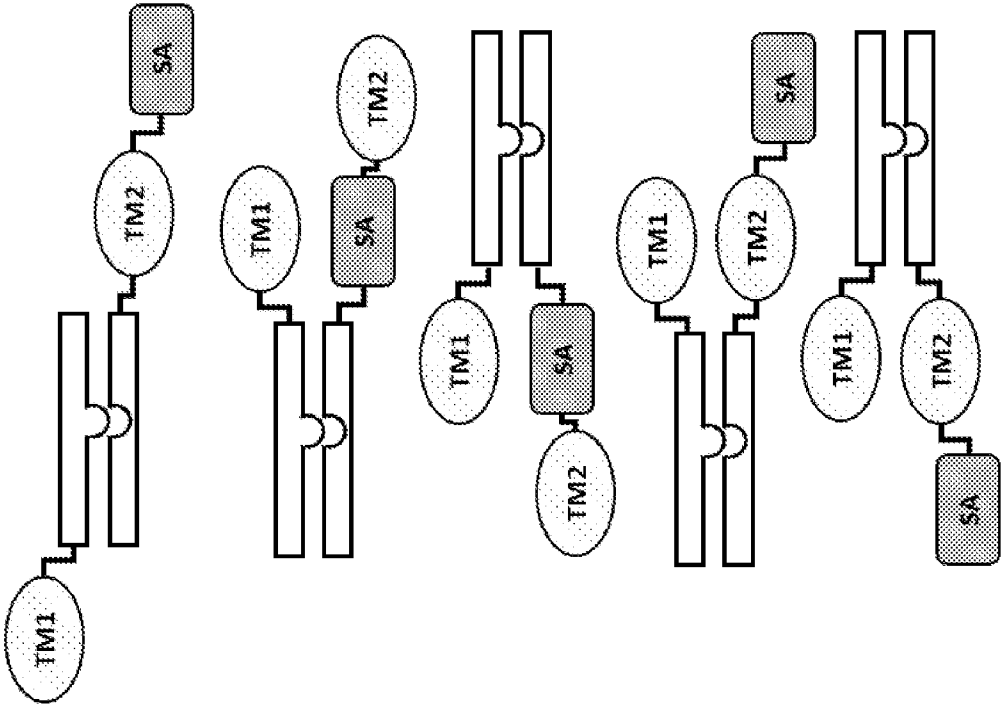


FIG. 18A

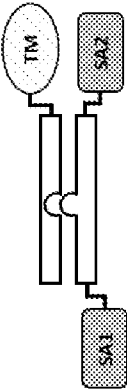


FIG. 18B

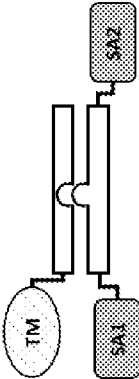


FIG. 18C

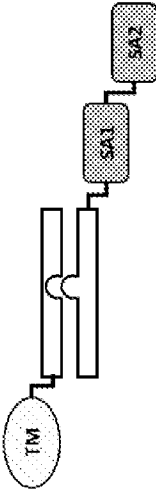


FIG. 18D

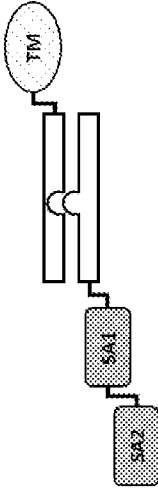


FIG. 18E

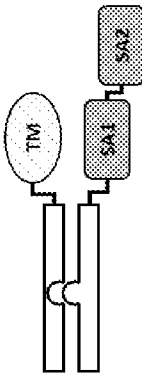


FIG. 18F

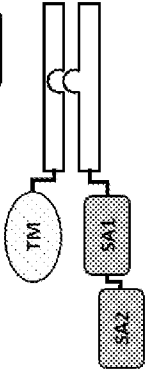


FIG. 19A

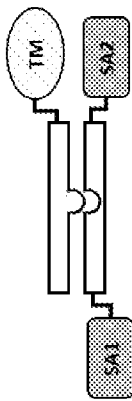


FIG. 19B

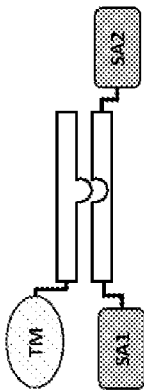


FIG. 19C

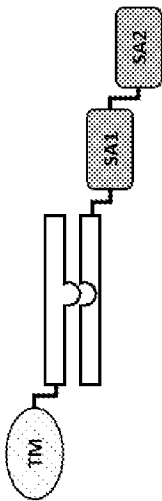


FIG. 19D

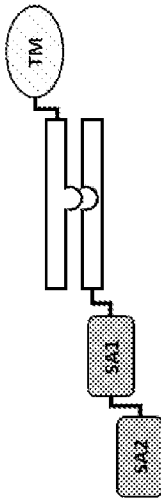


FIG. 19E

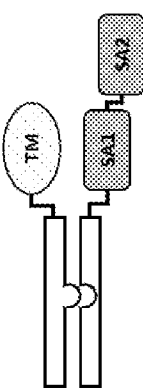


FIG. 19F

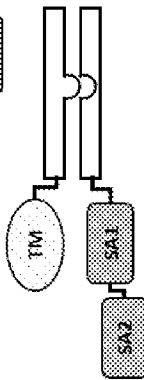


FIG. 20

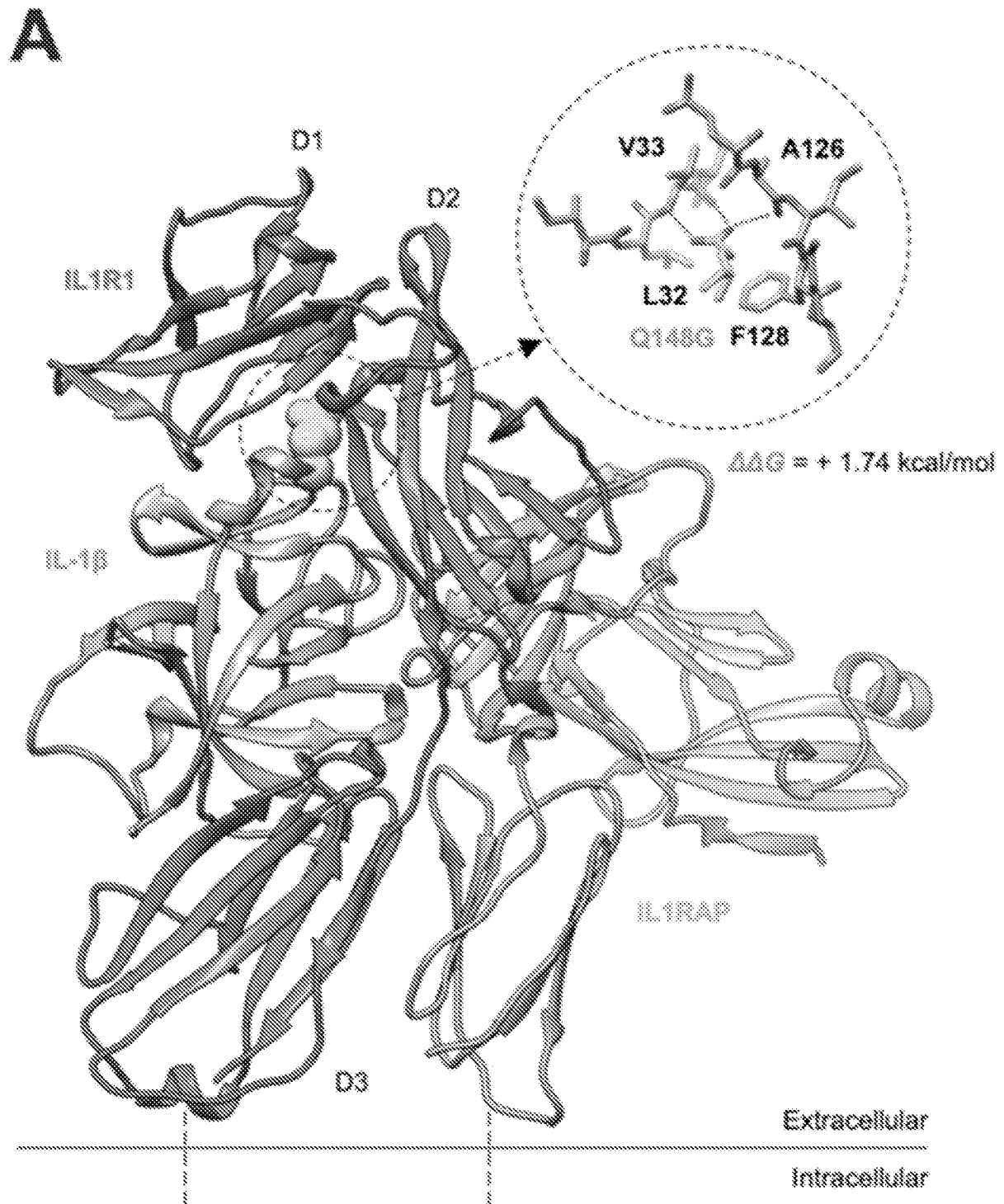


FIG. 20

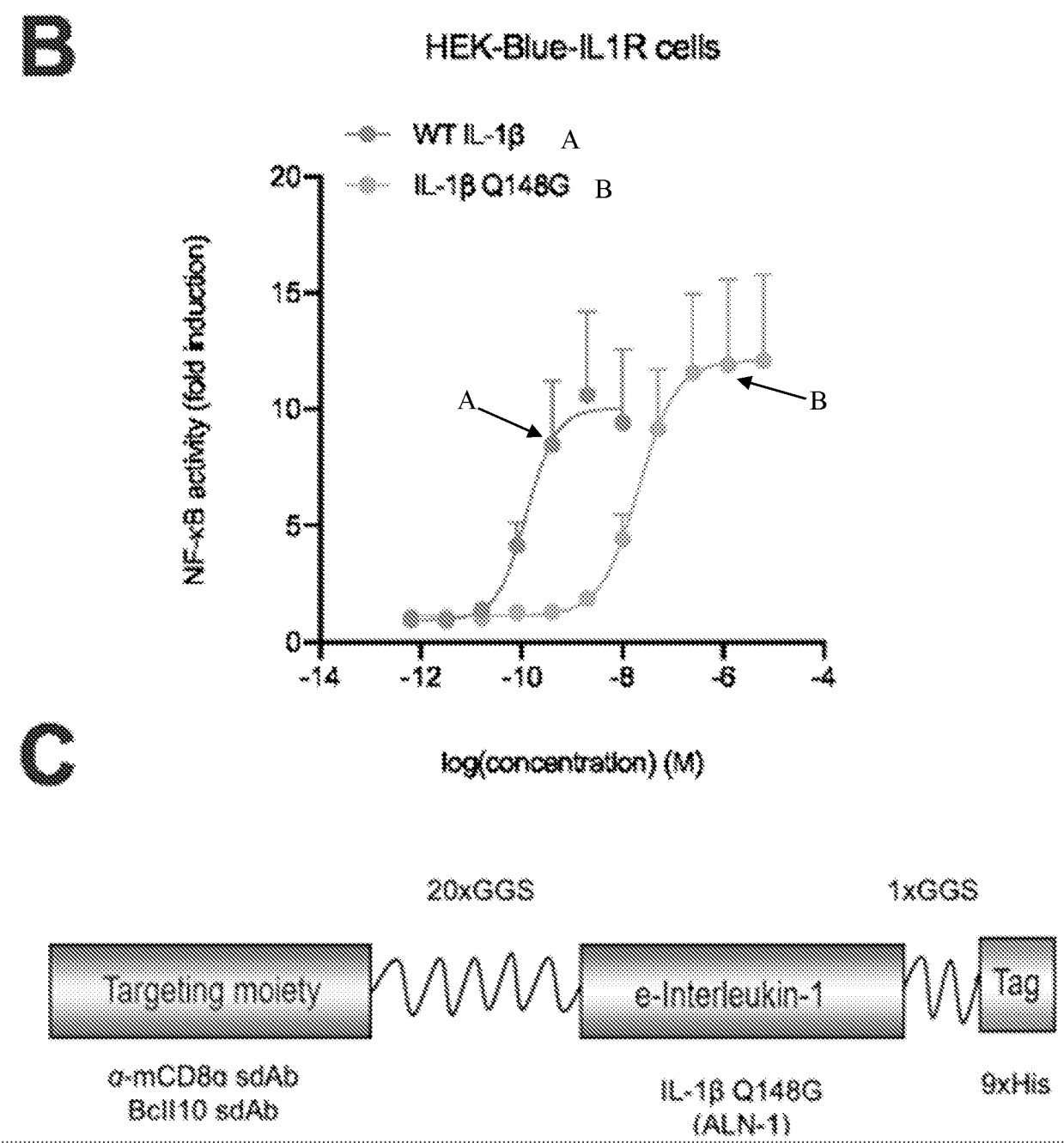


FIG. 20

D

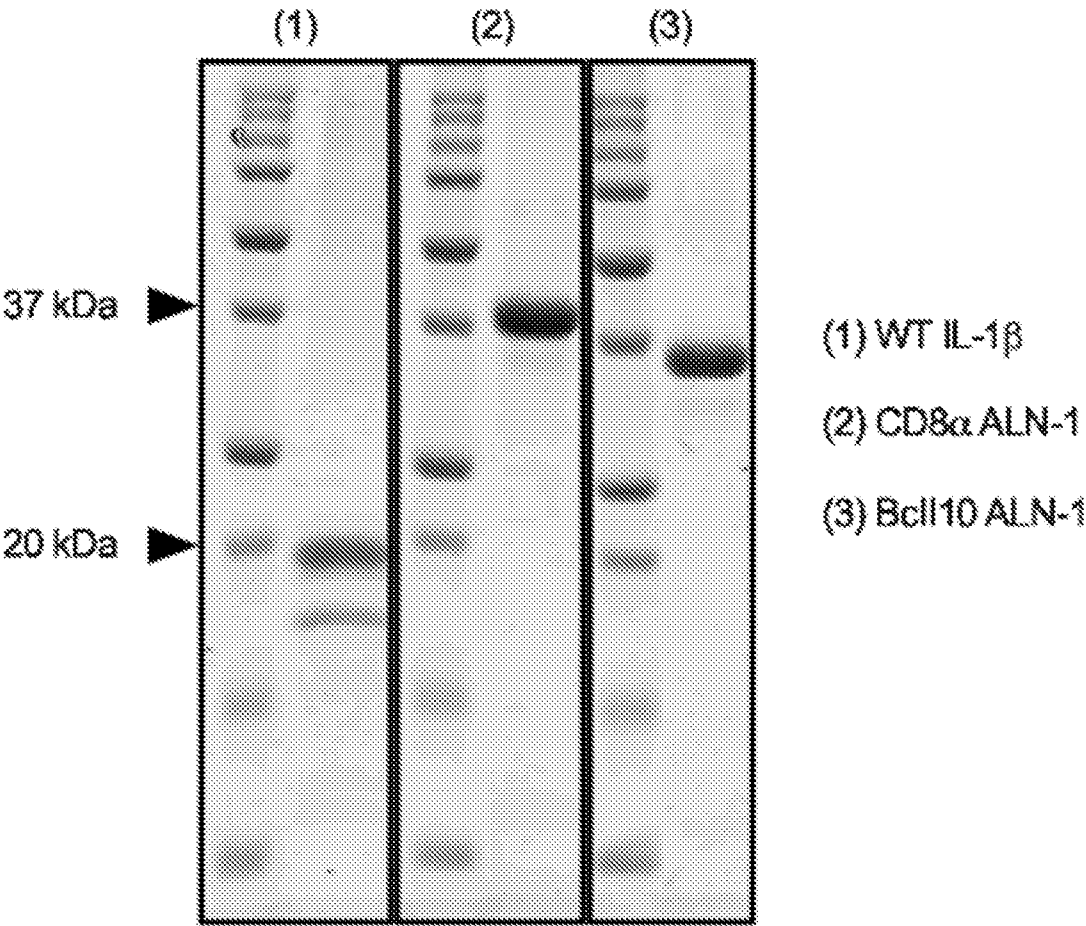


FIG. 20

E

HEK-Blue-IL1R cells

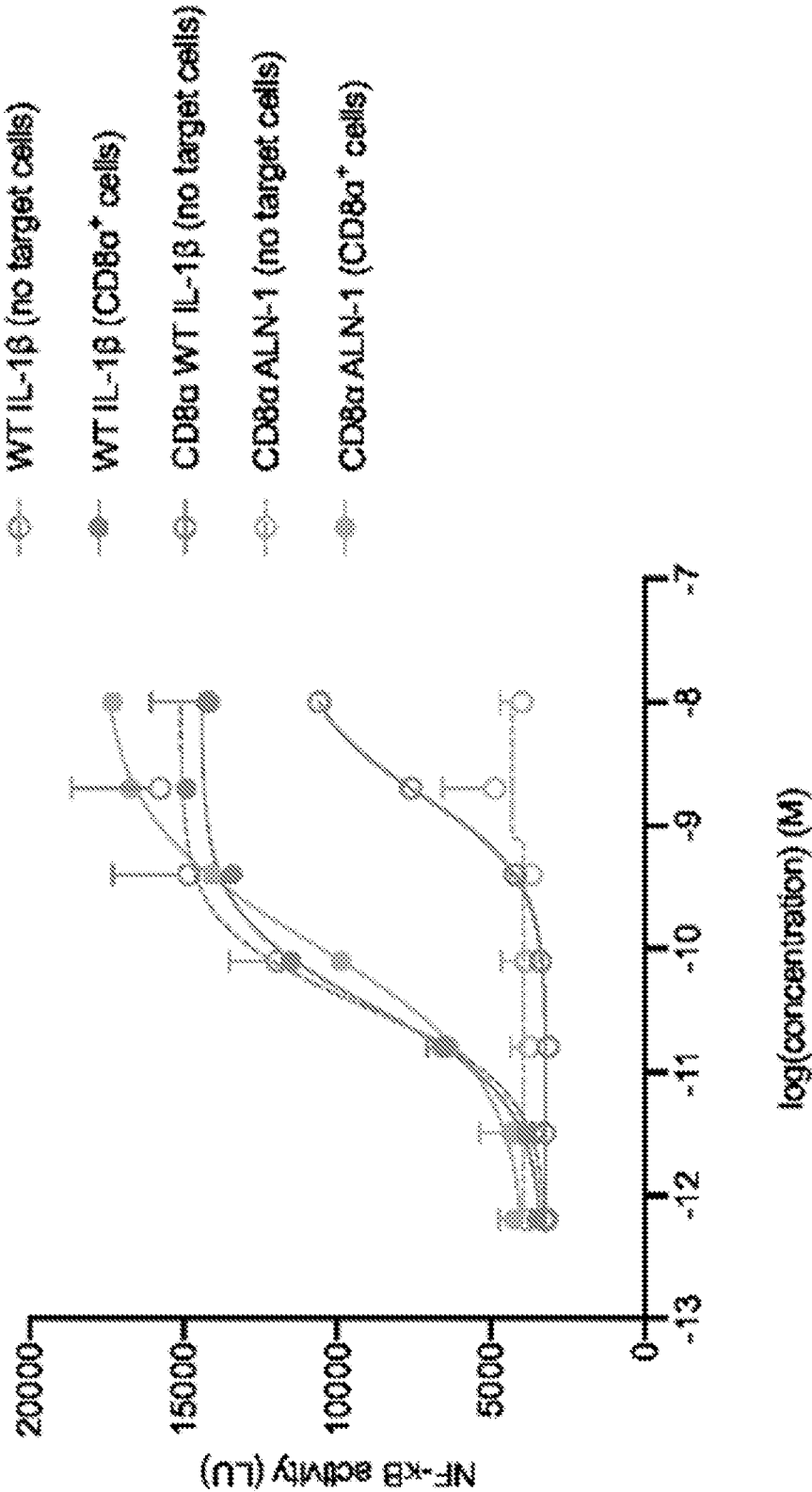


FIG. 20

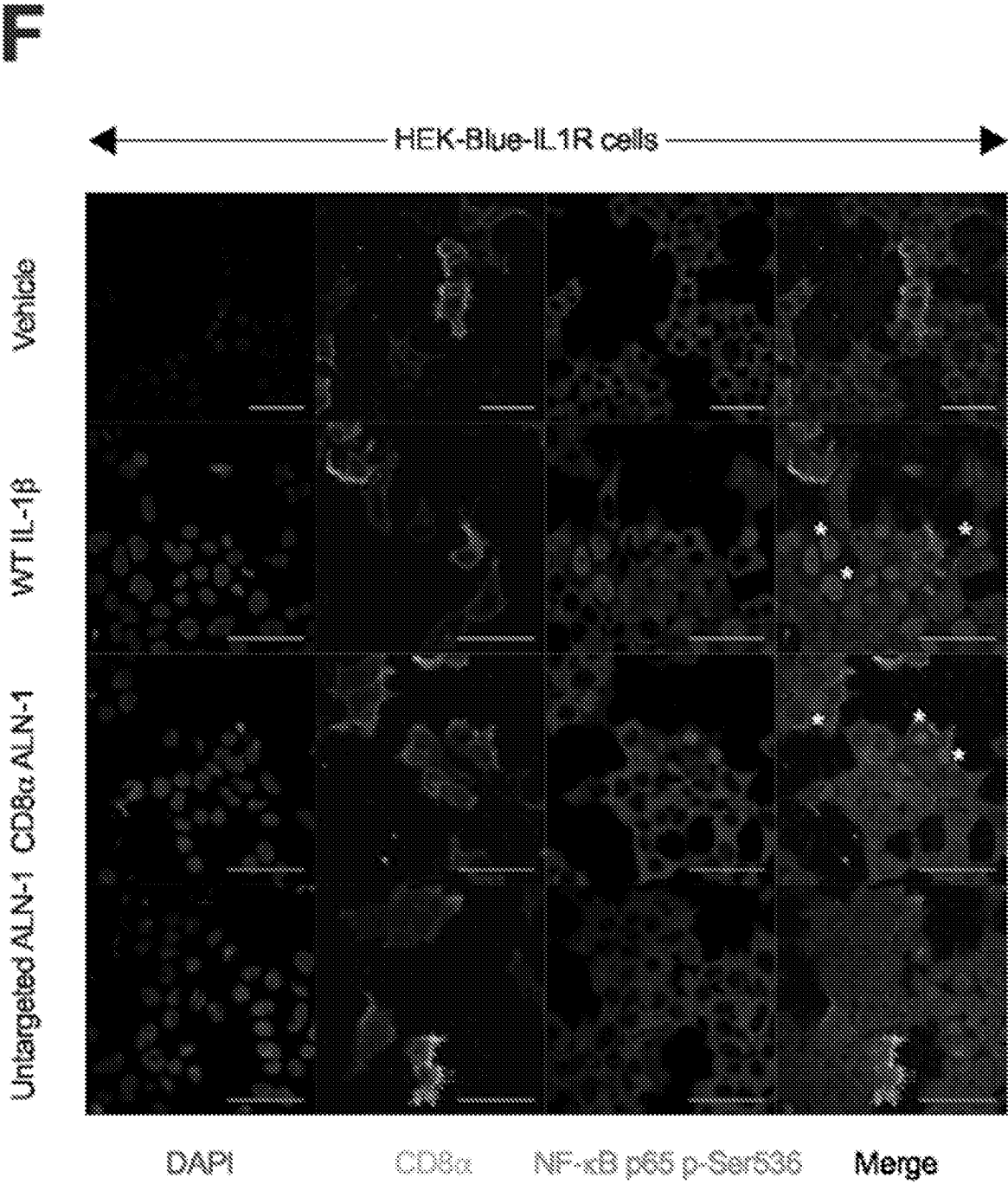


FIG. 20

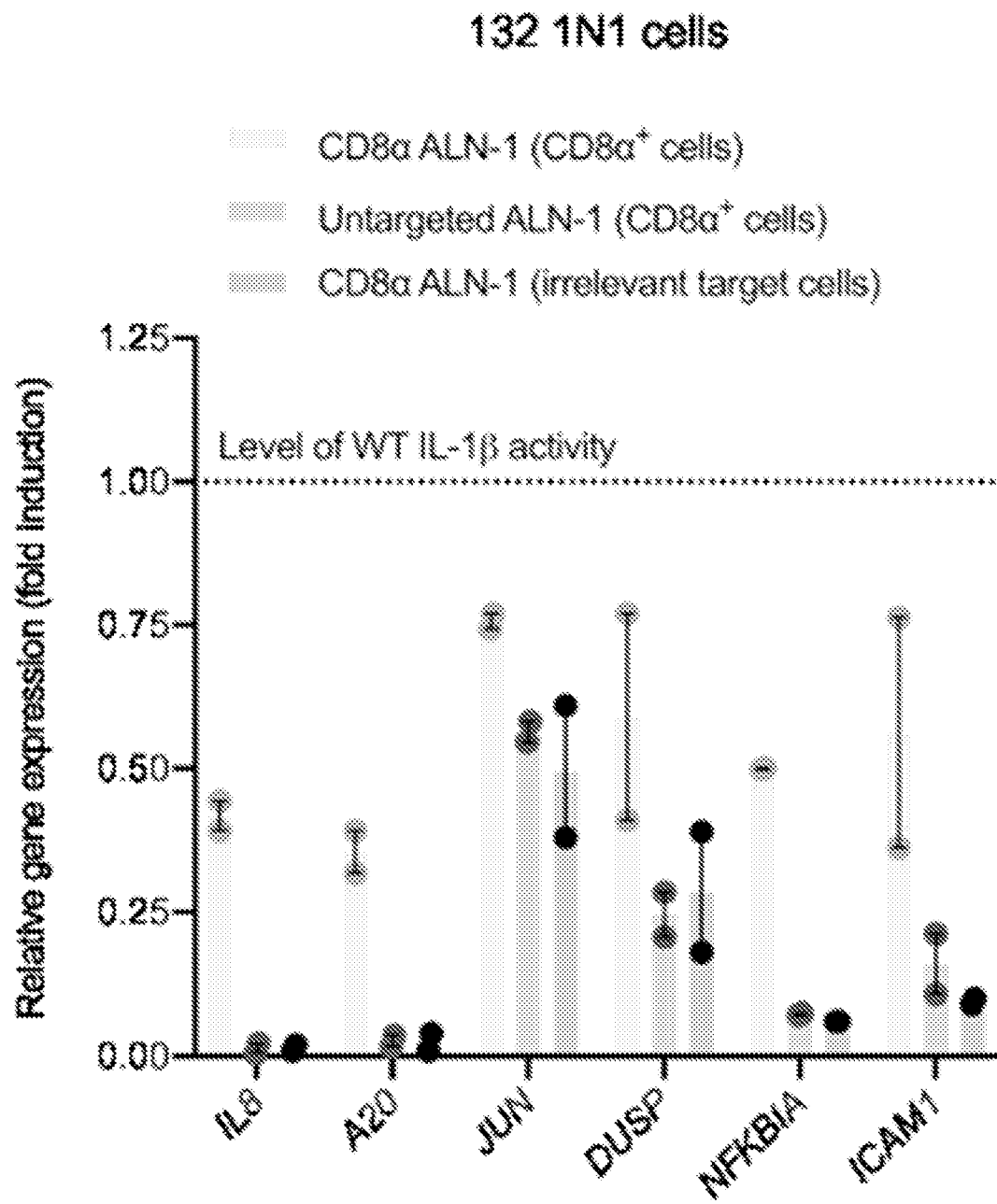
G

FIG. 21

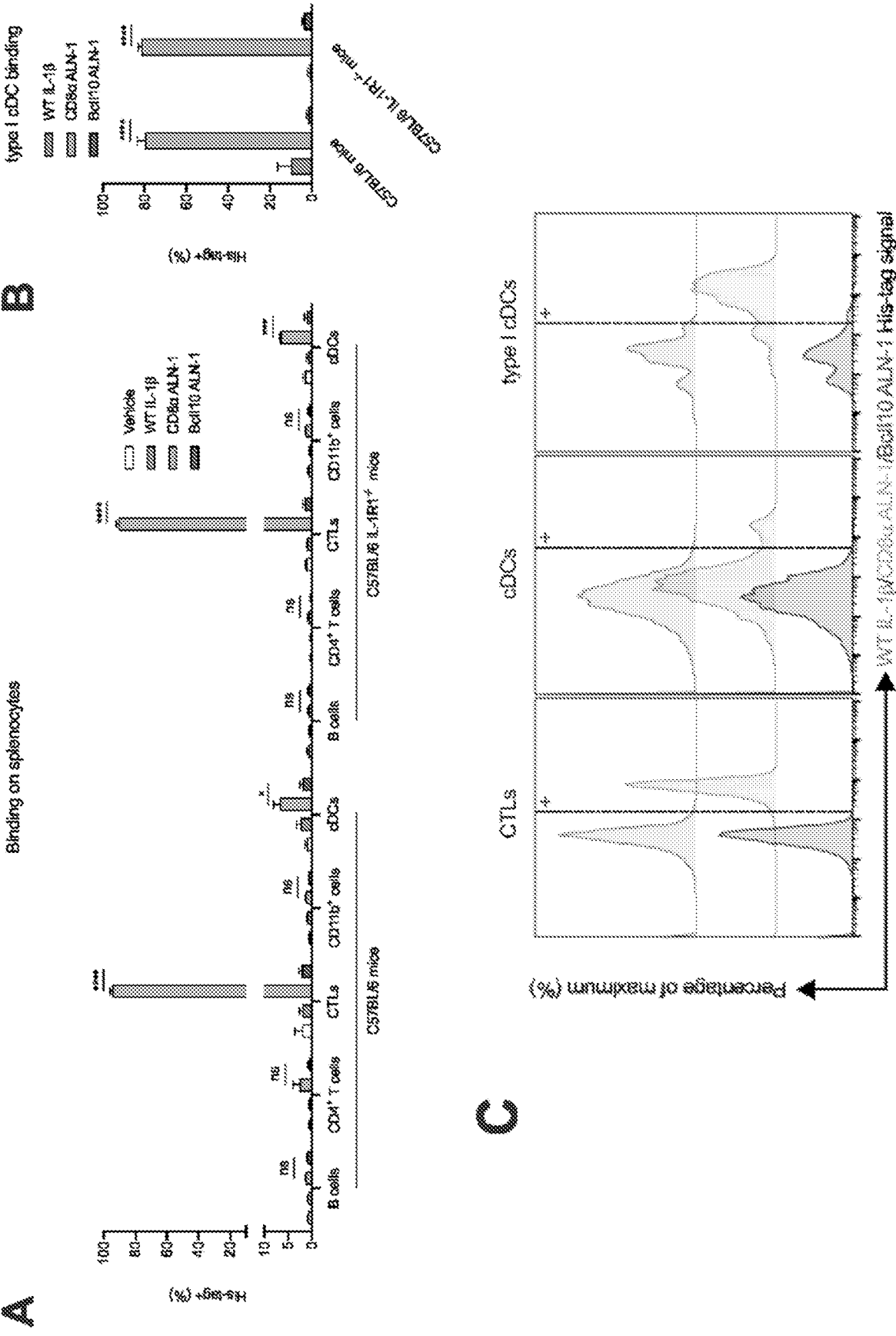


FIG. 21

D

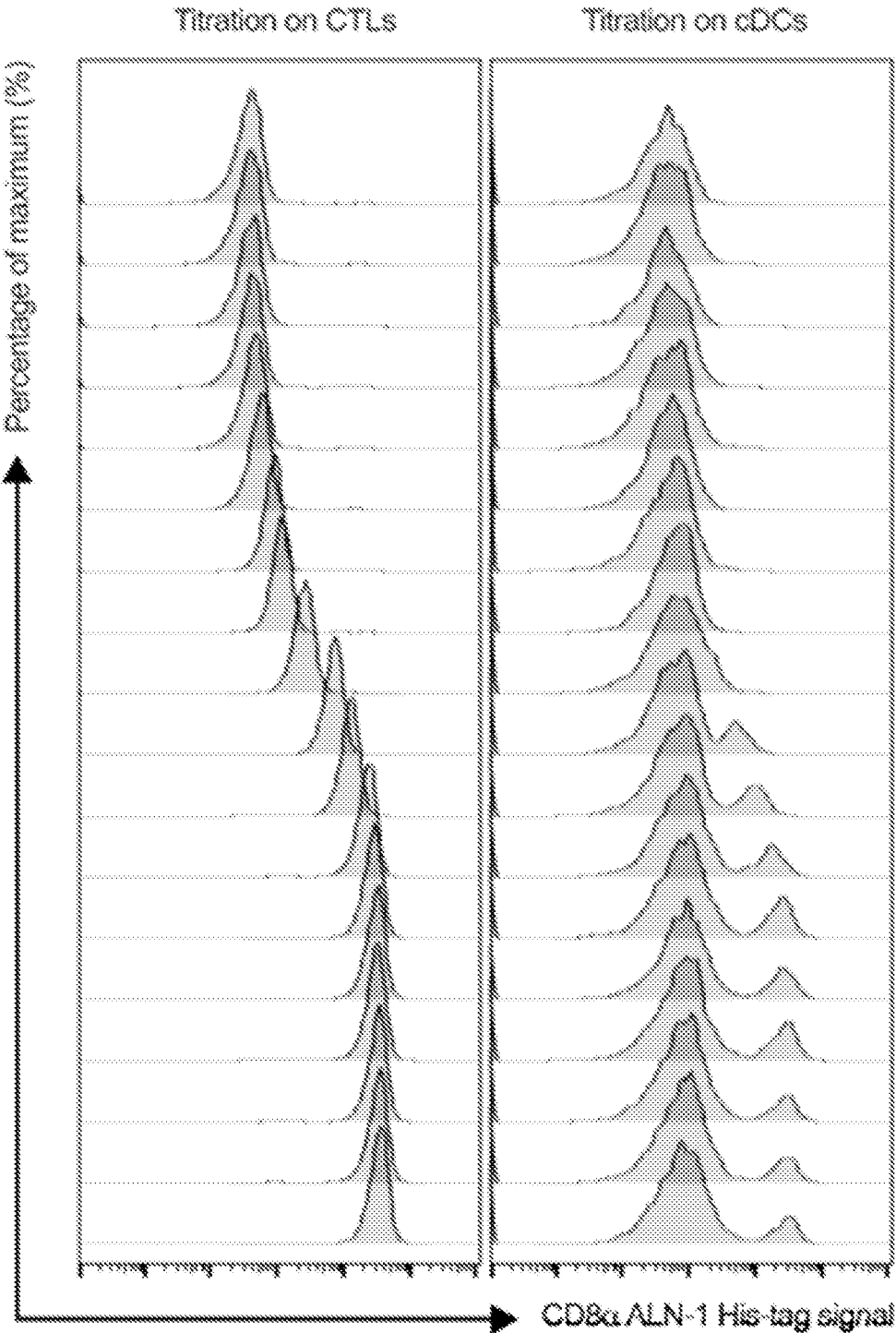


FIG. 21

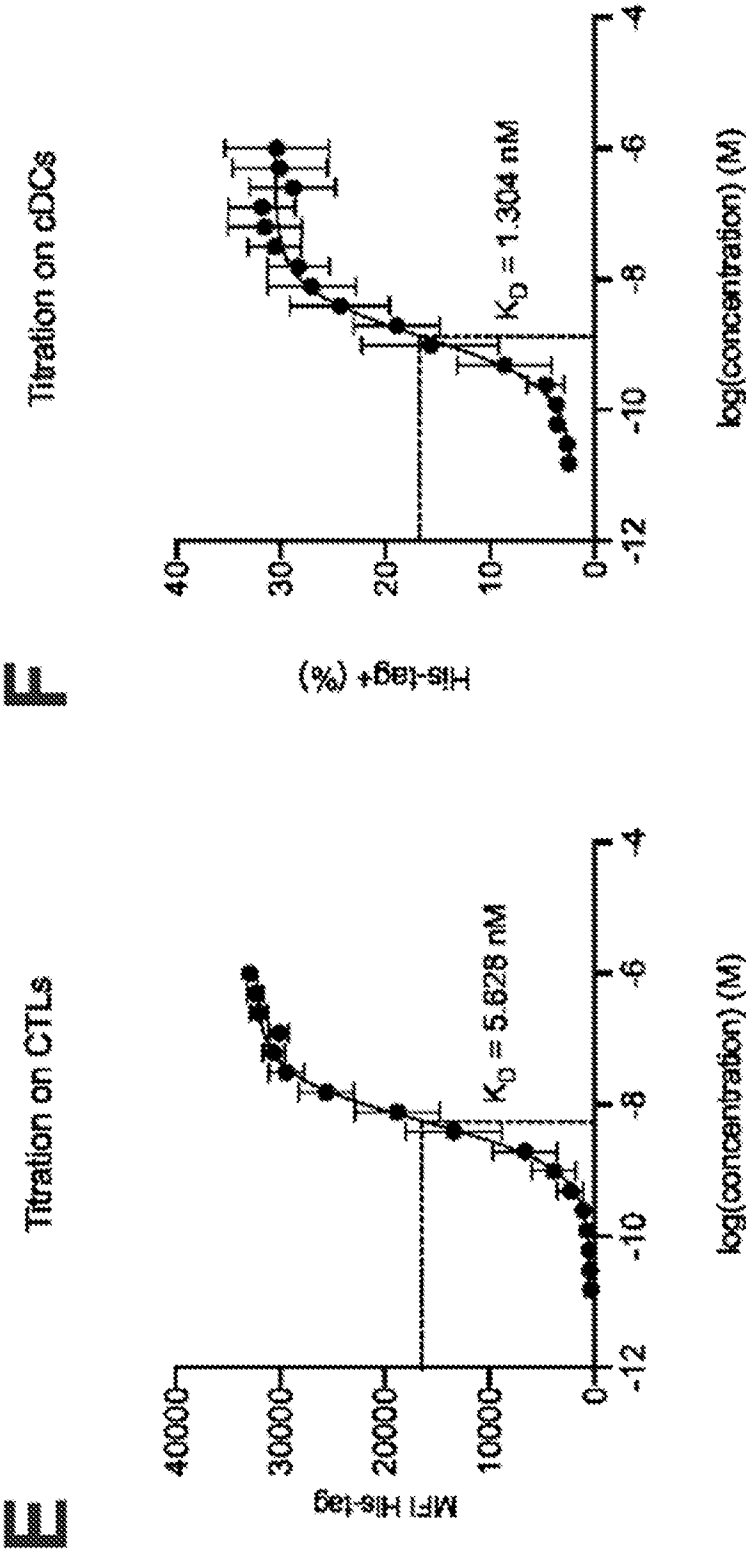


FIG. 21

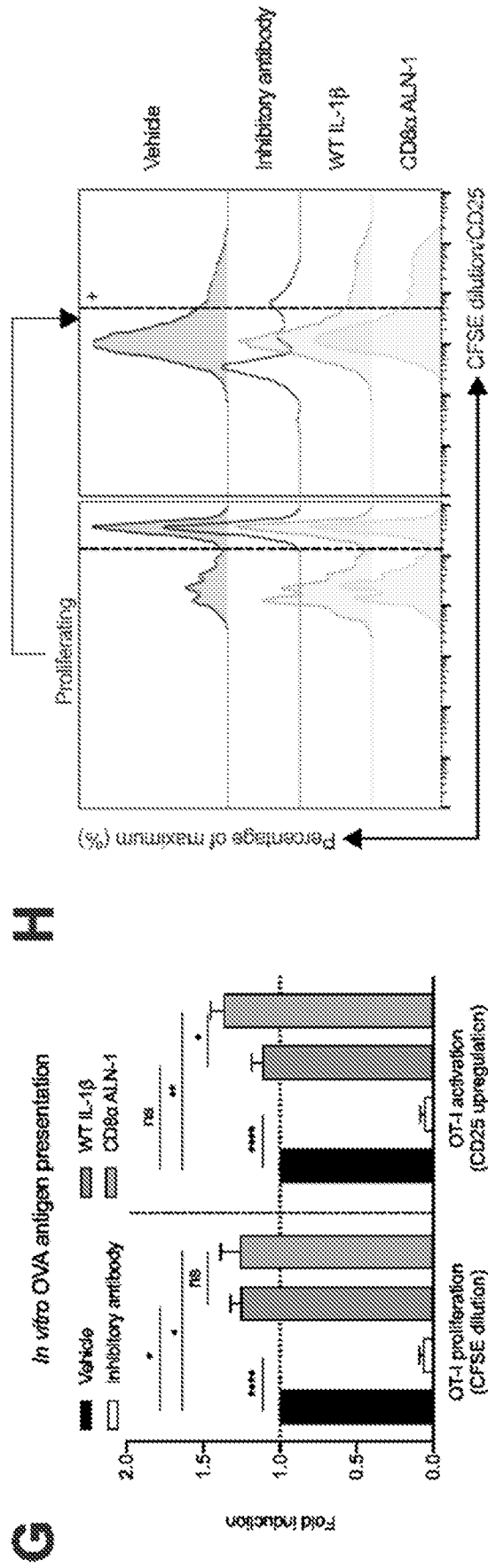


FIG. 22

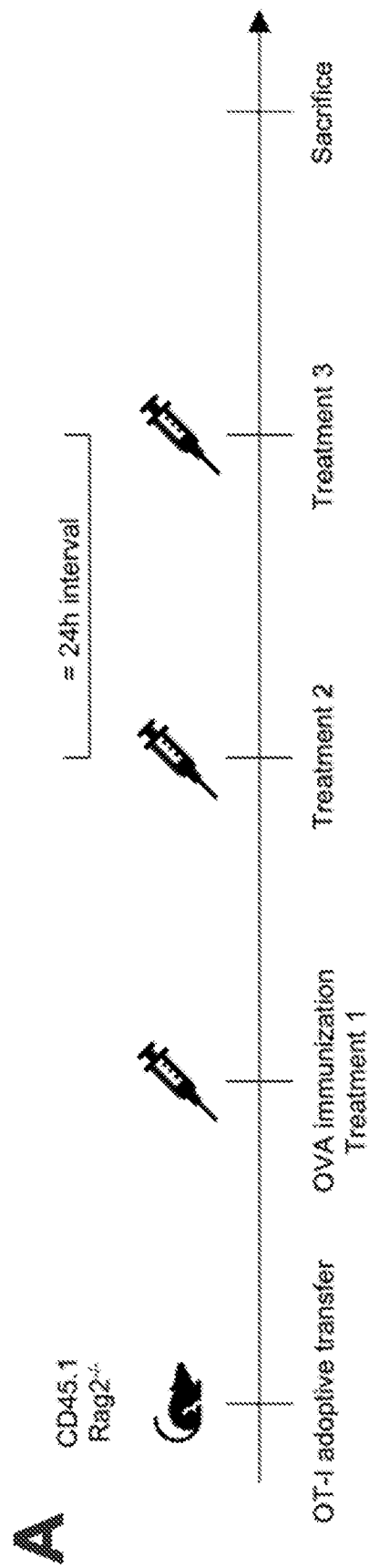
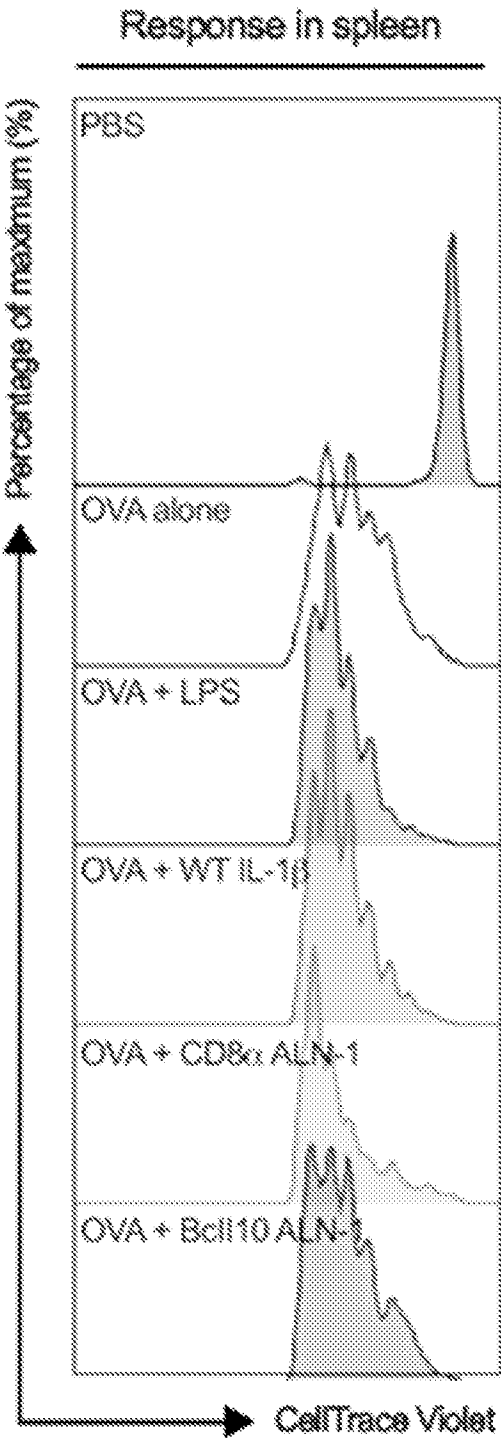


FIG. 22

B



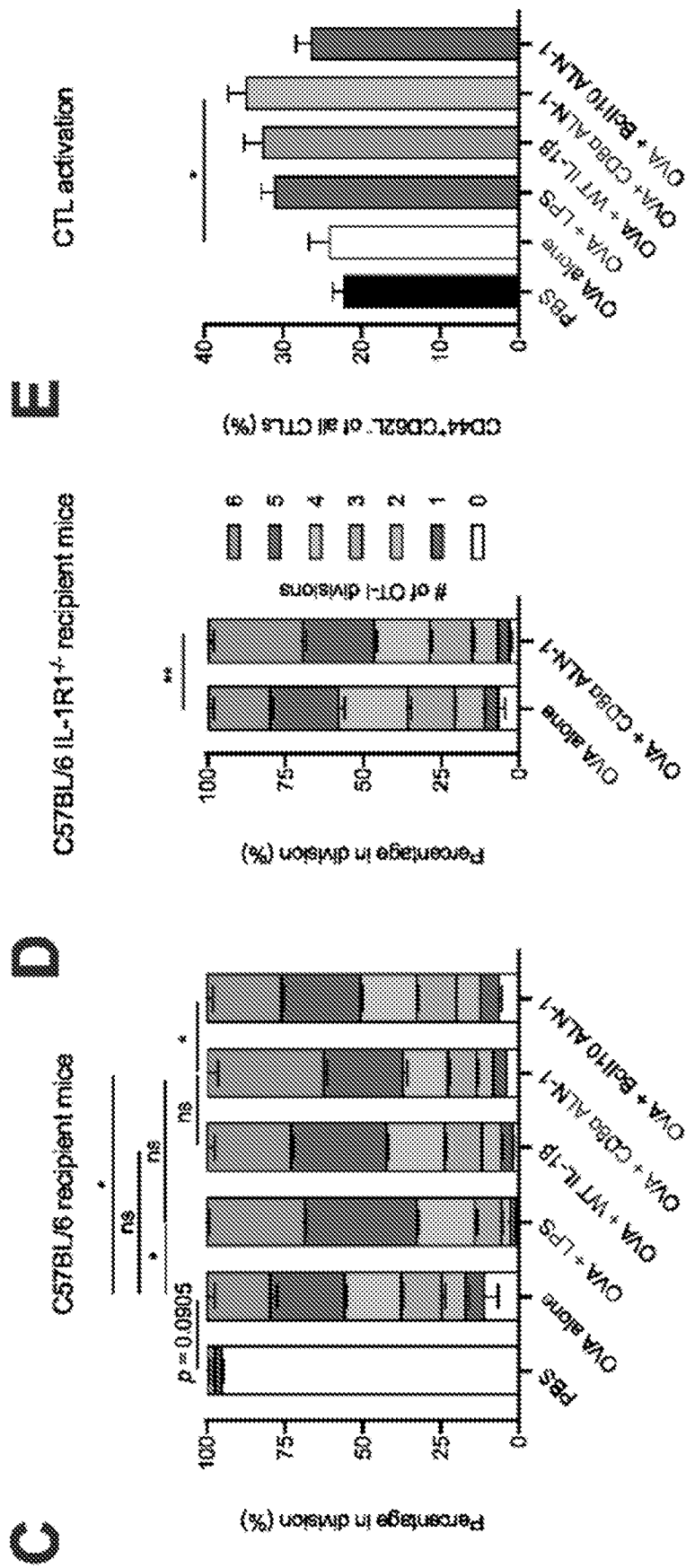
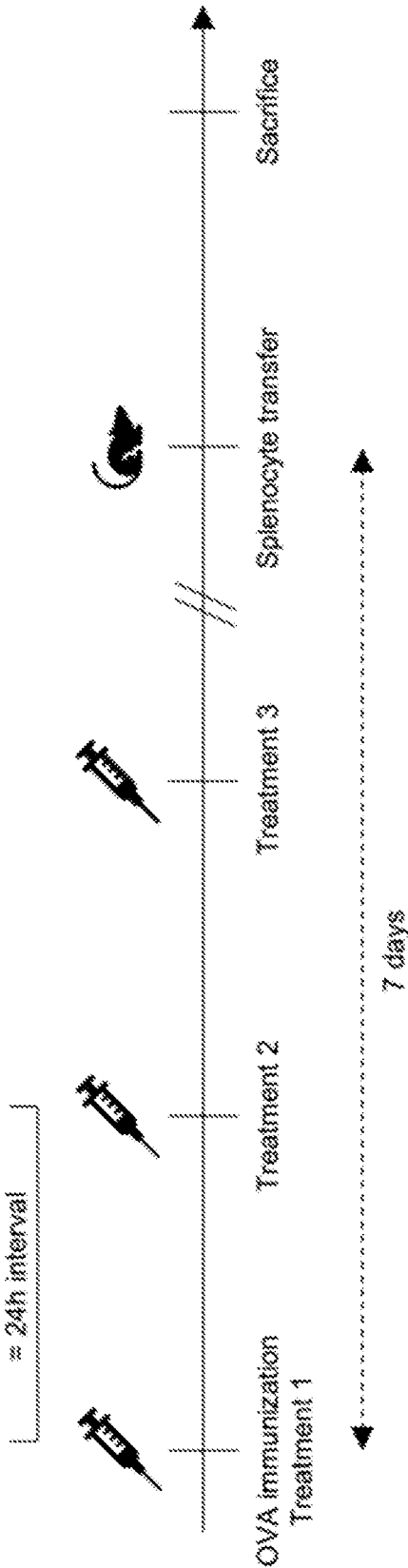
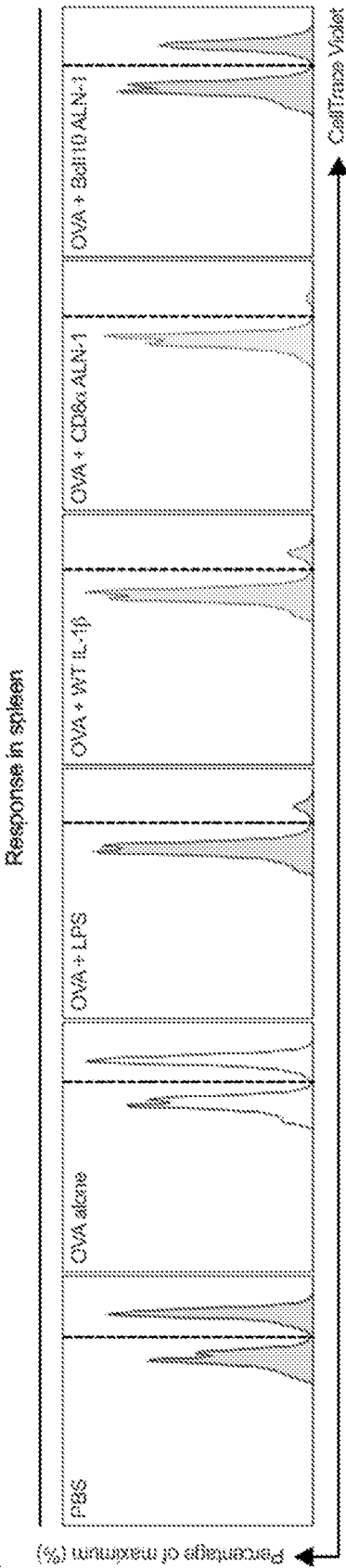


FIG. 22

F



G



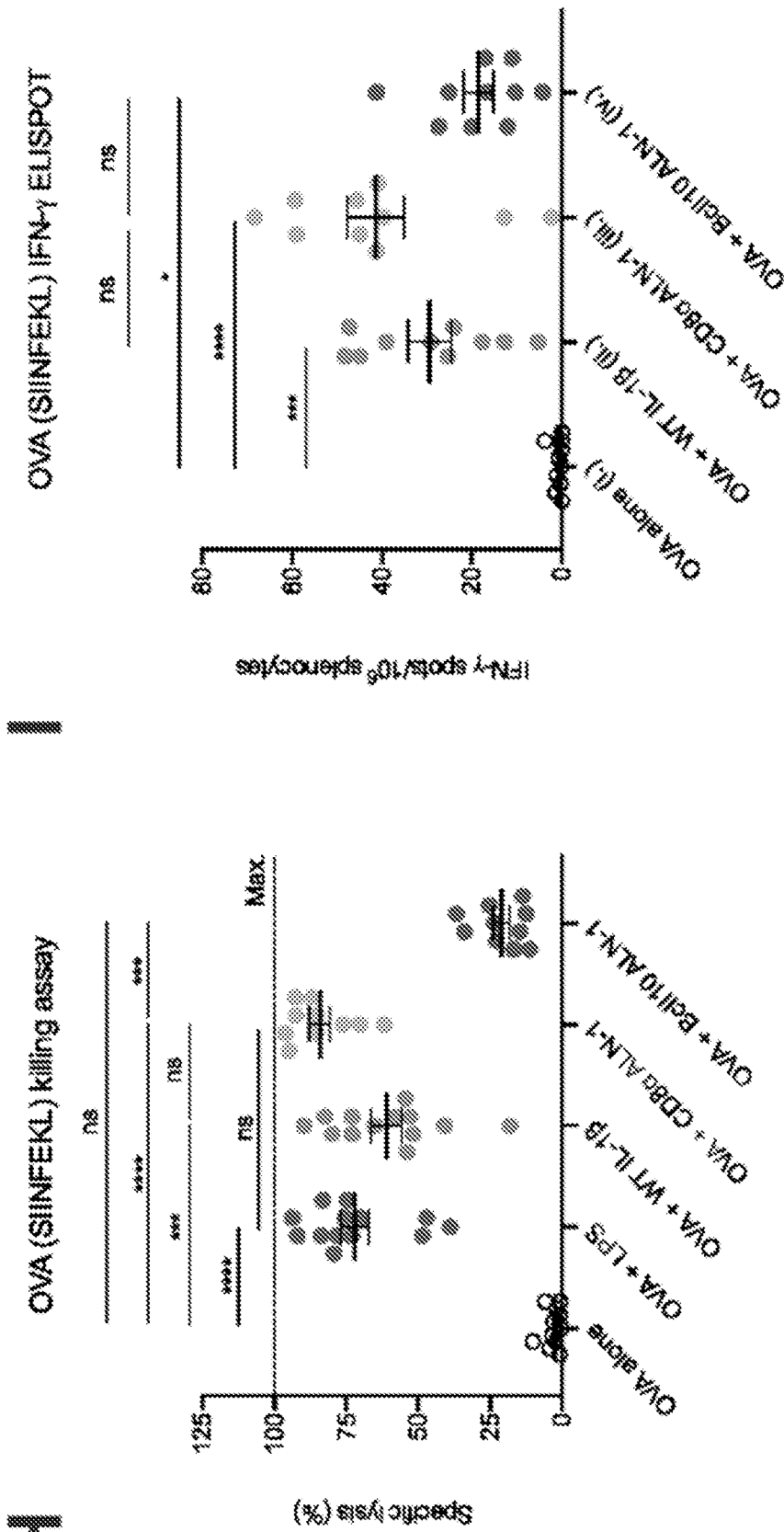


FIG. 22
J

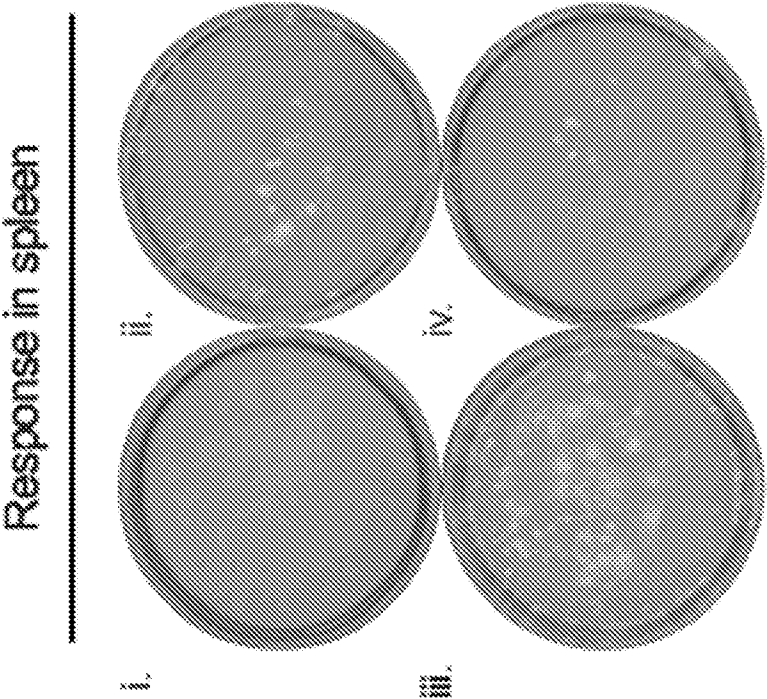


FIG. 23

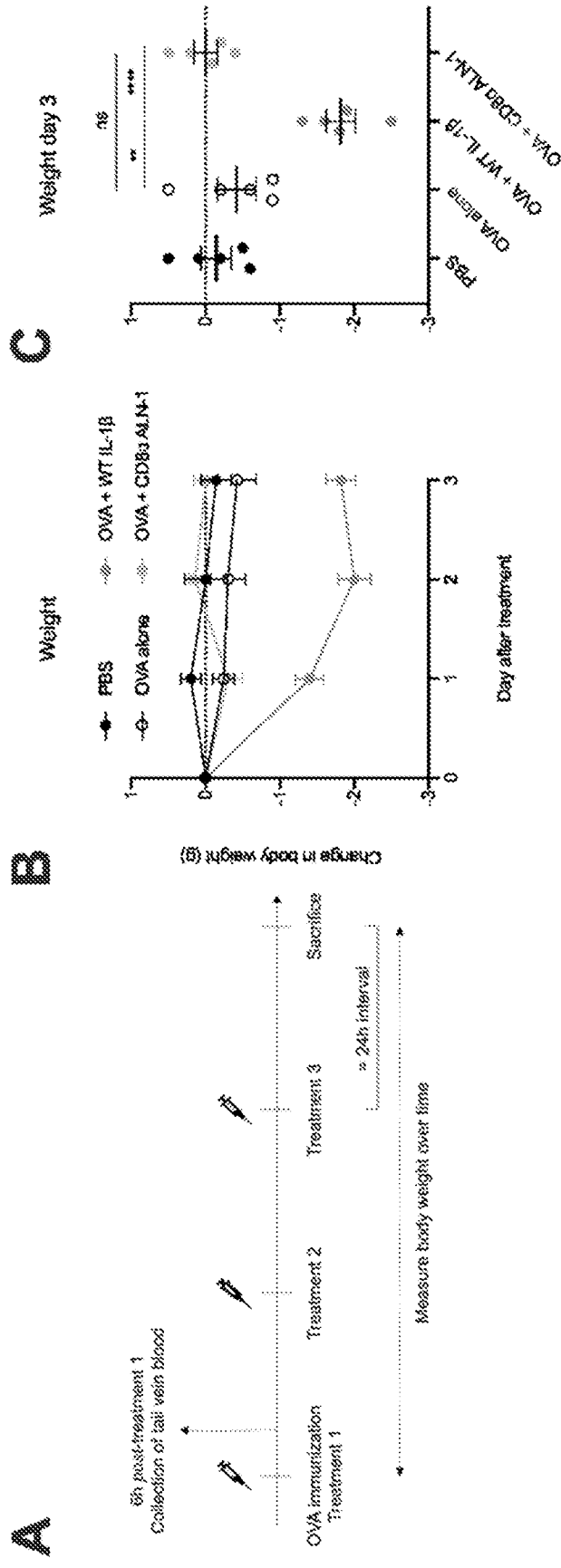


FIG. 23

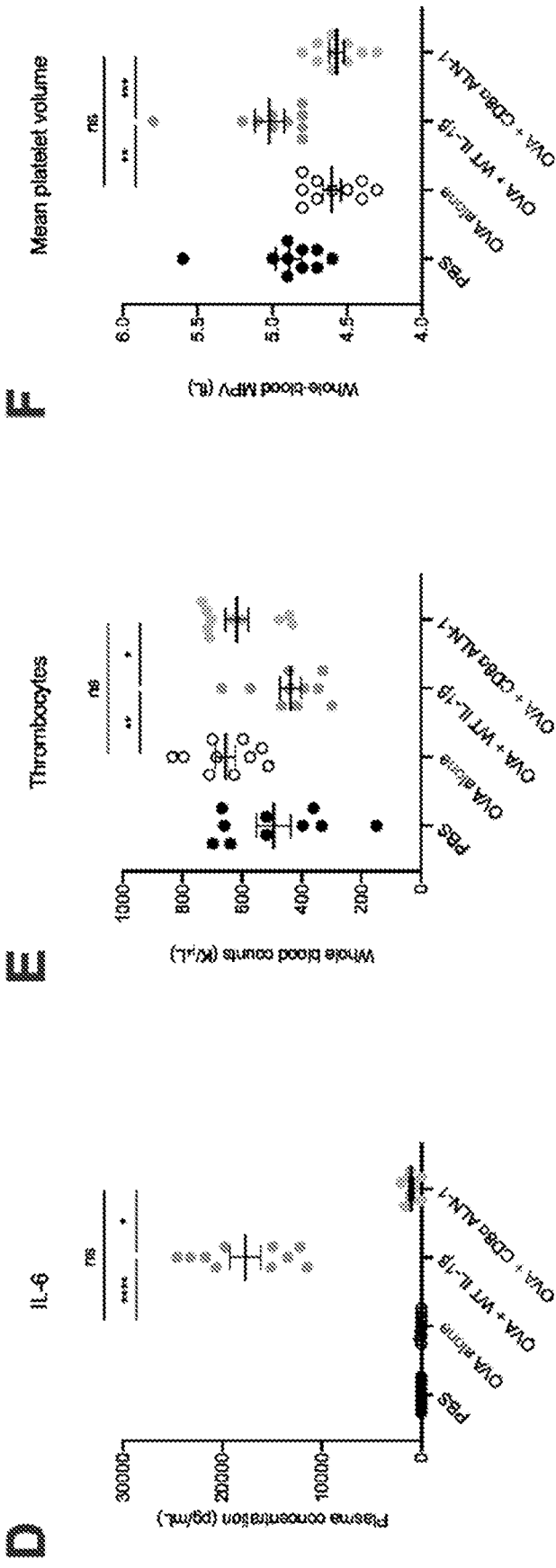


FIG. 23

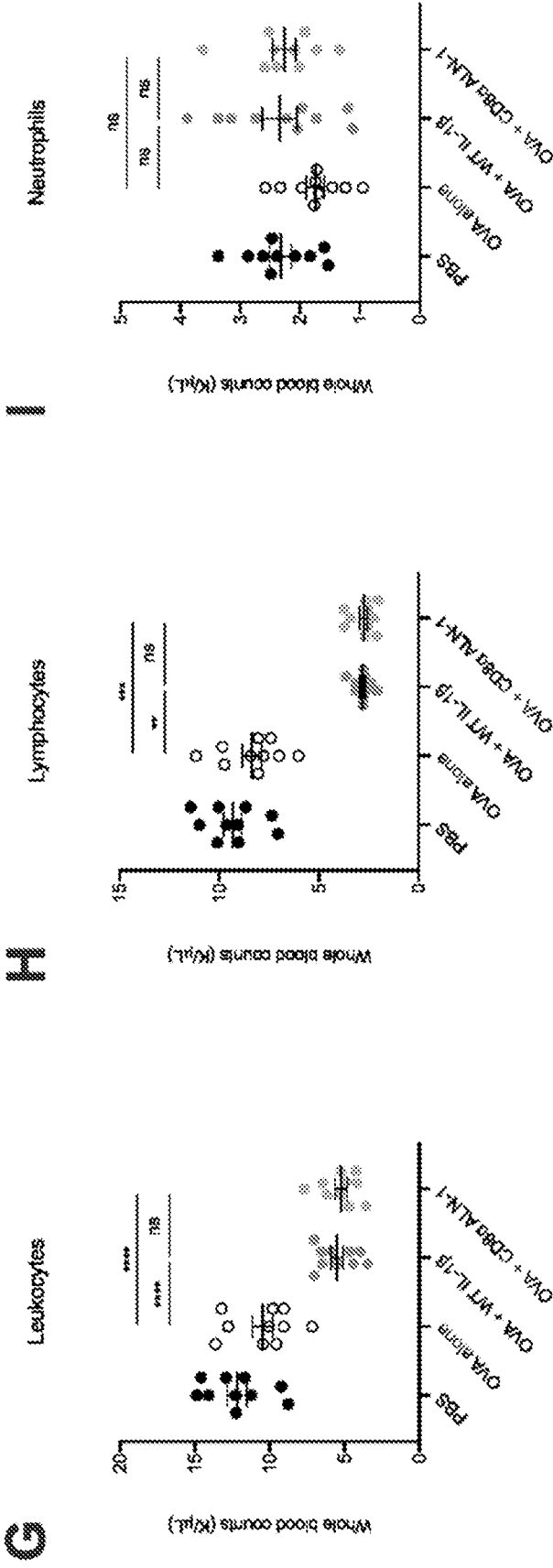


FIG. 24

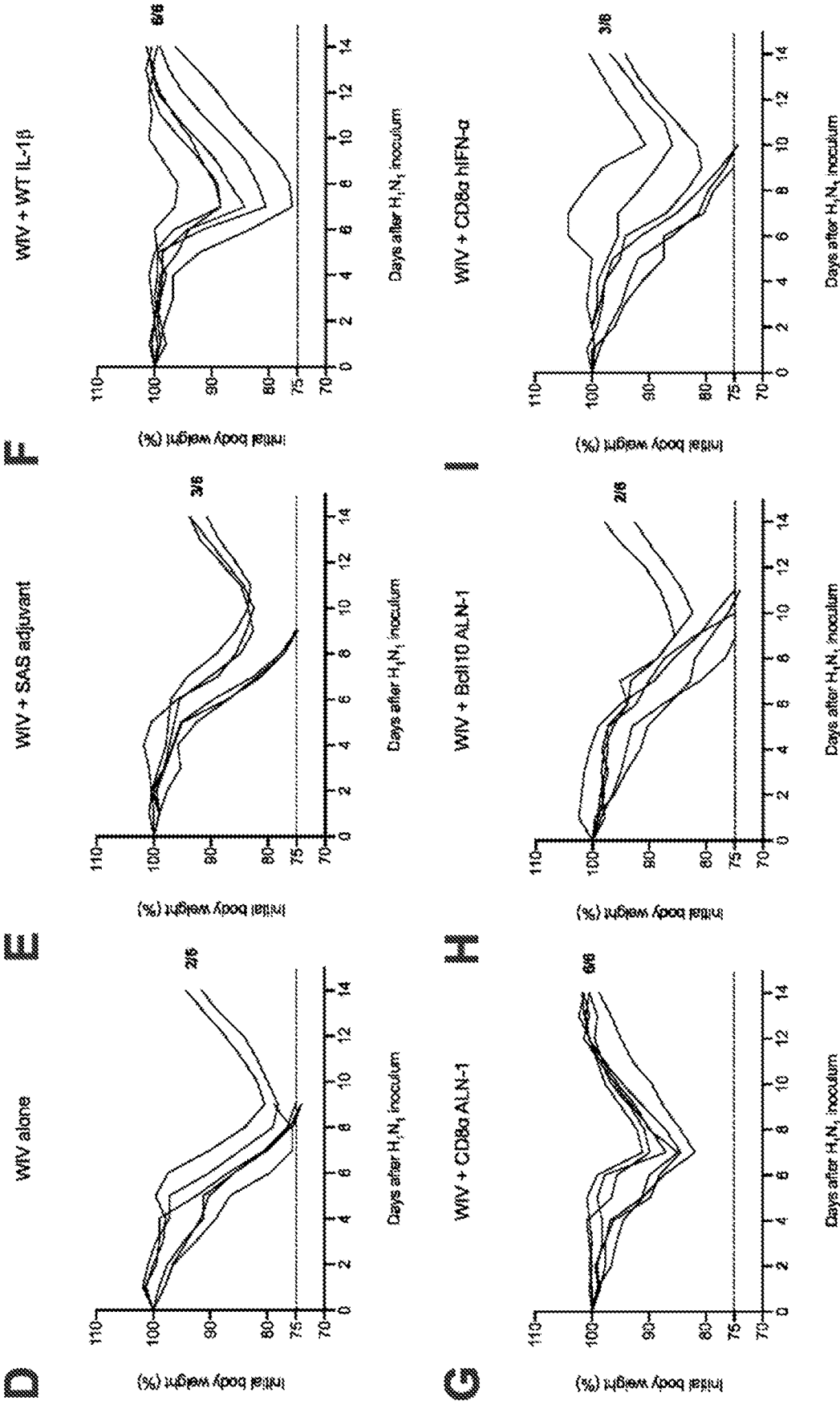


FIG. 25

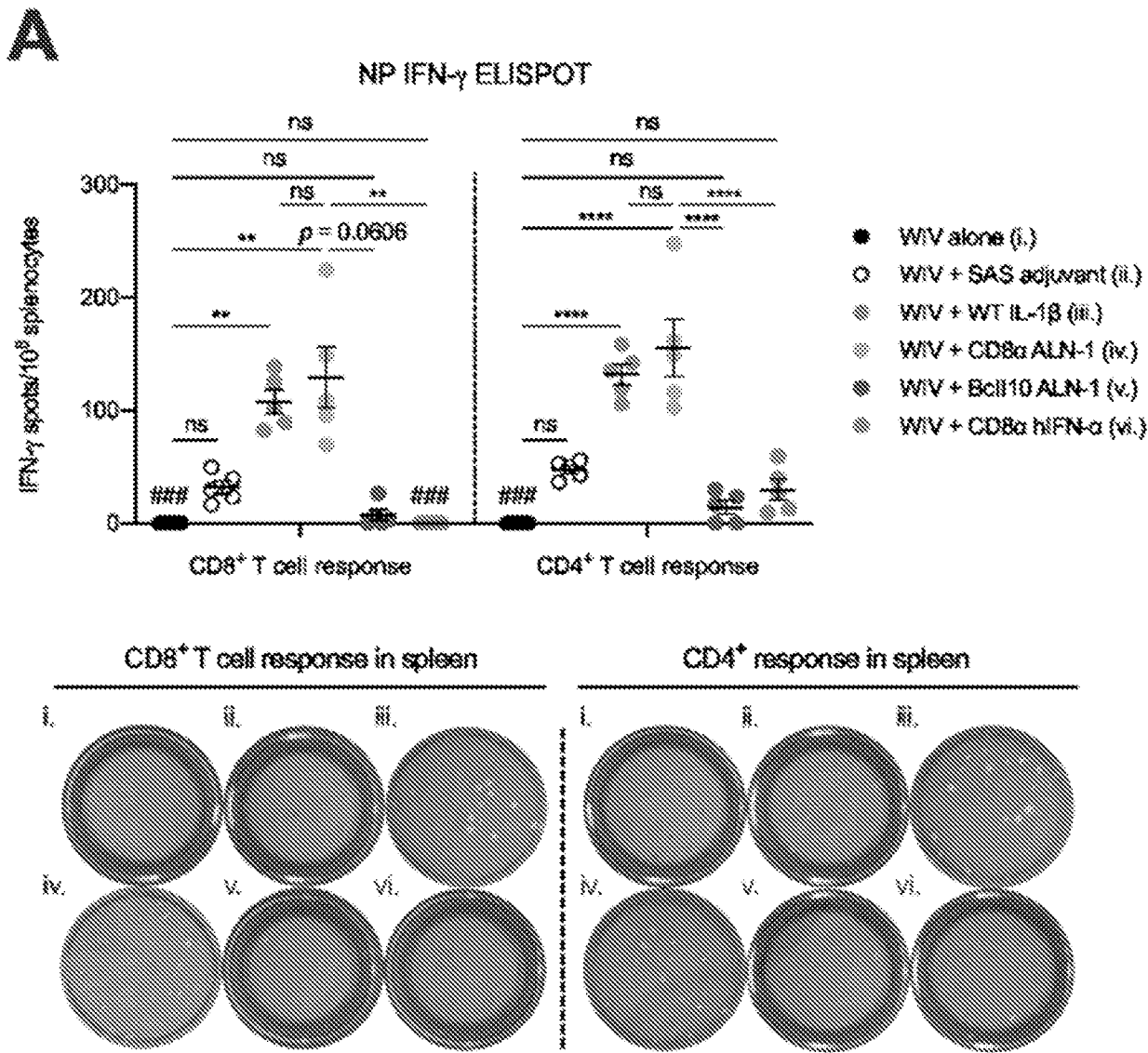


FIG. 25

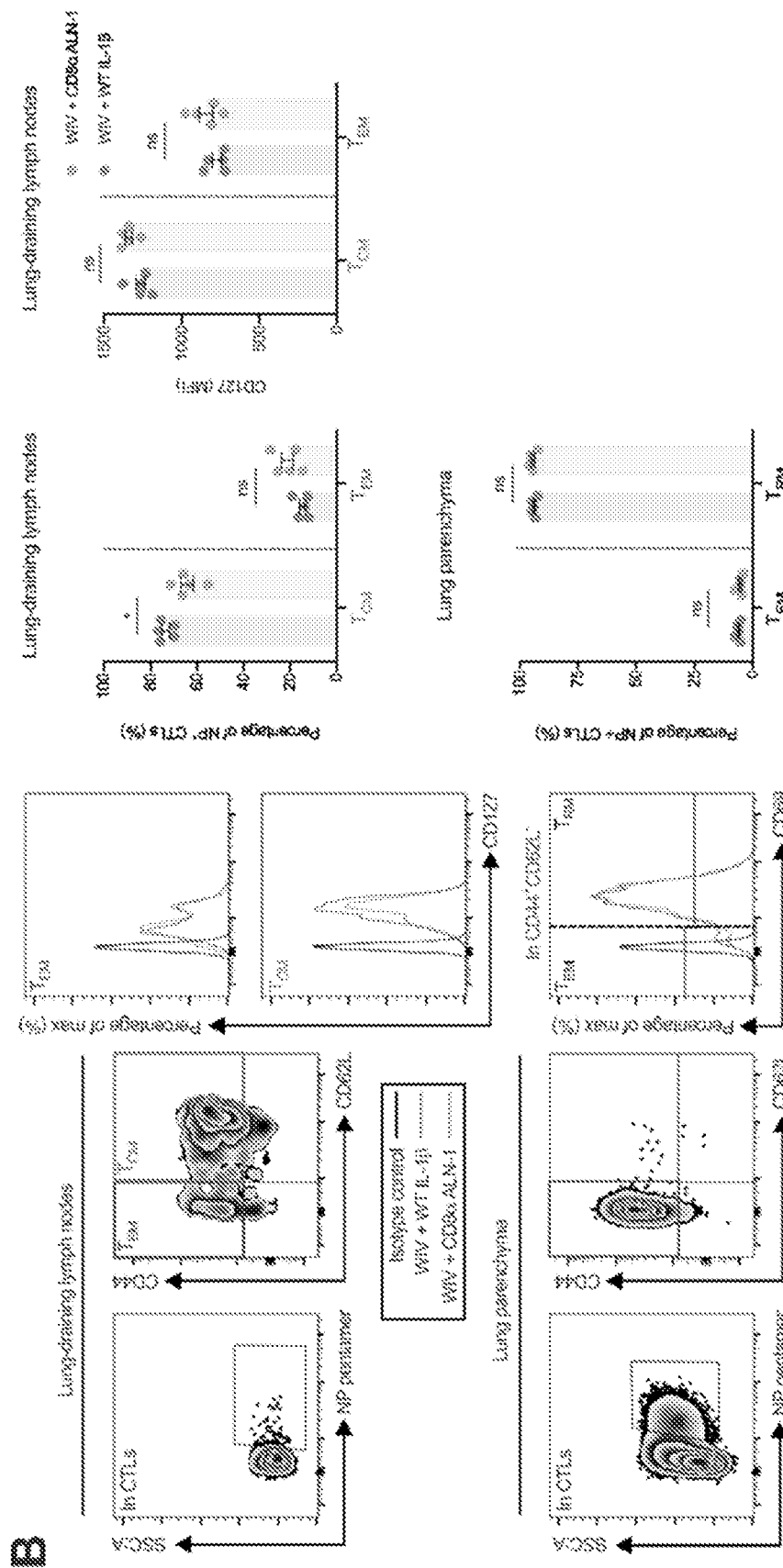


FIG. 25

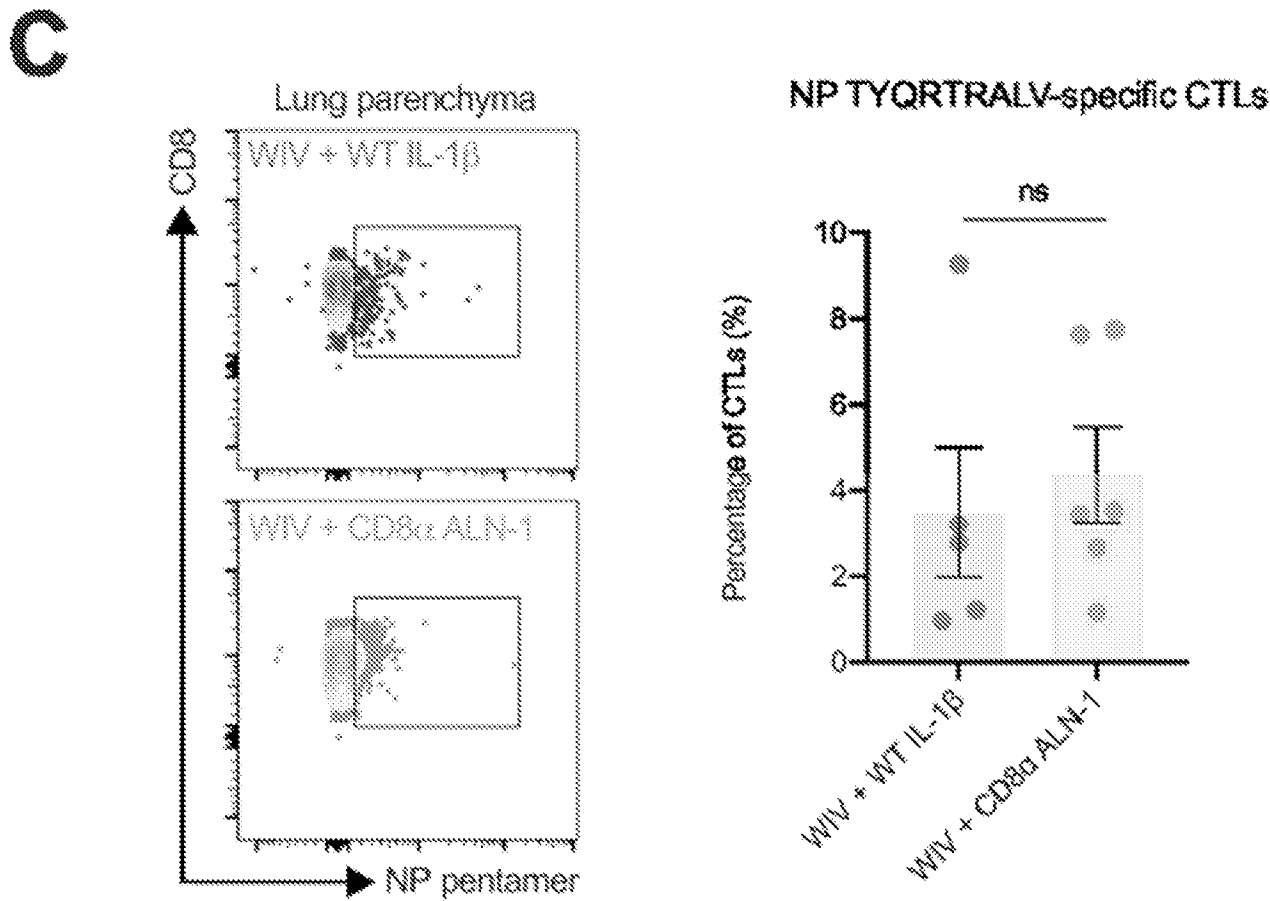


FIG. 26

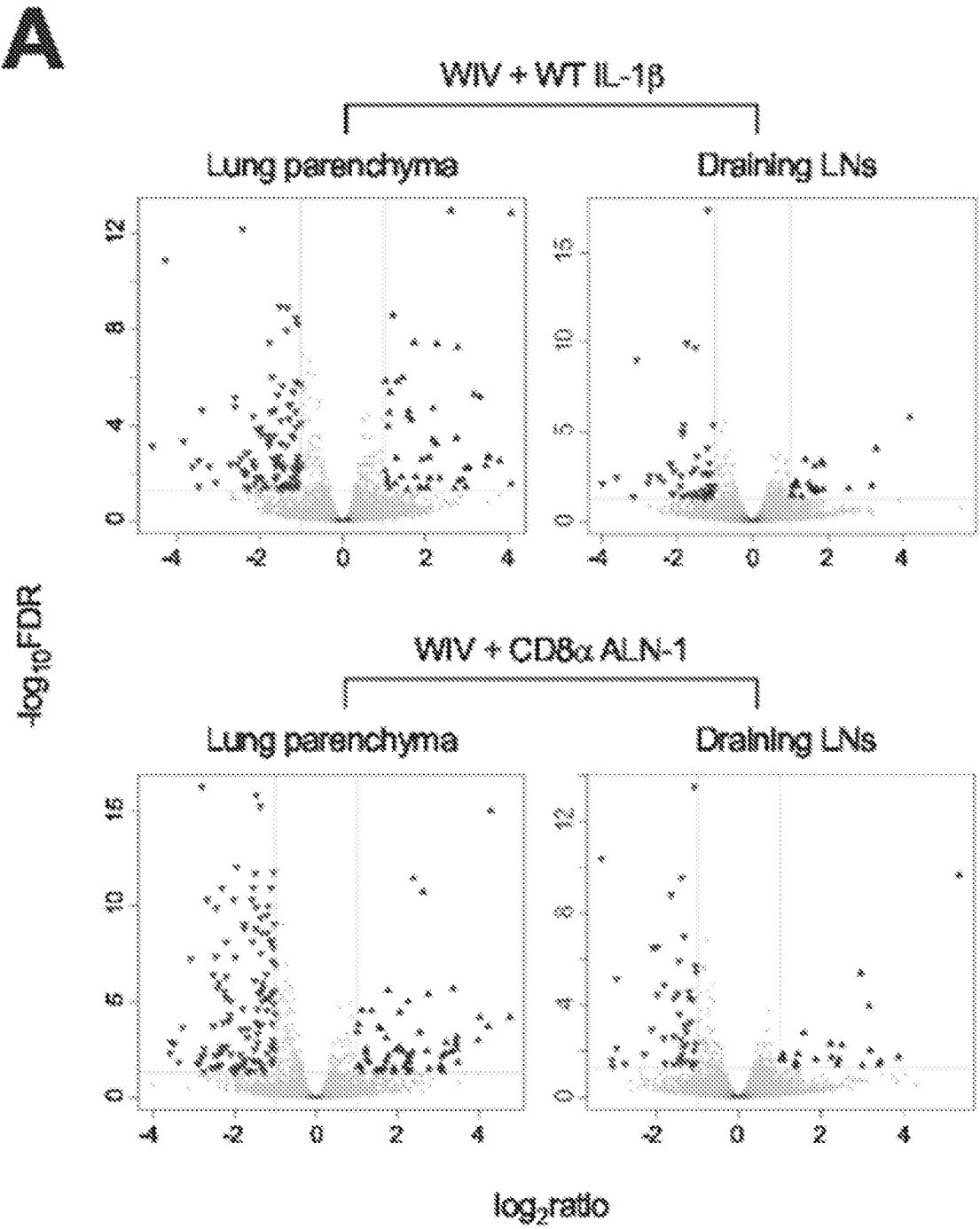


FIG. 26

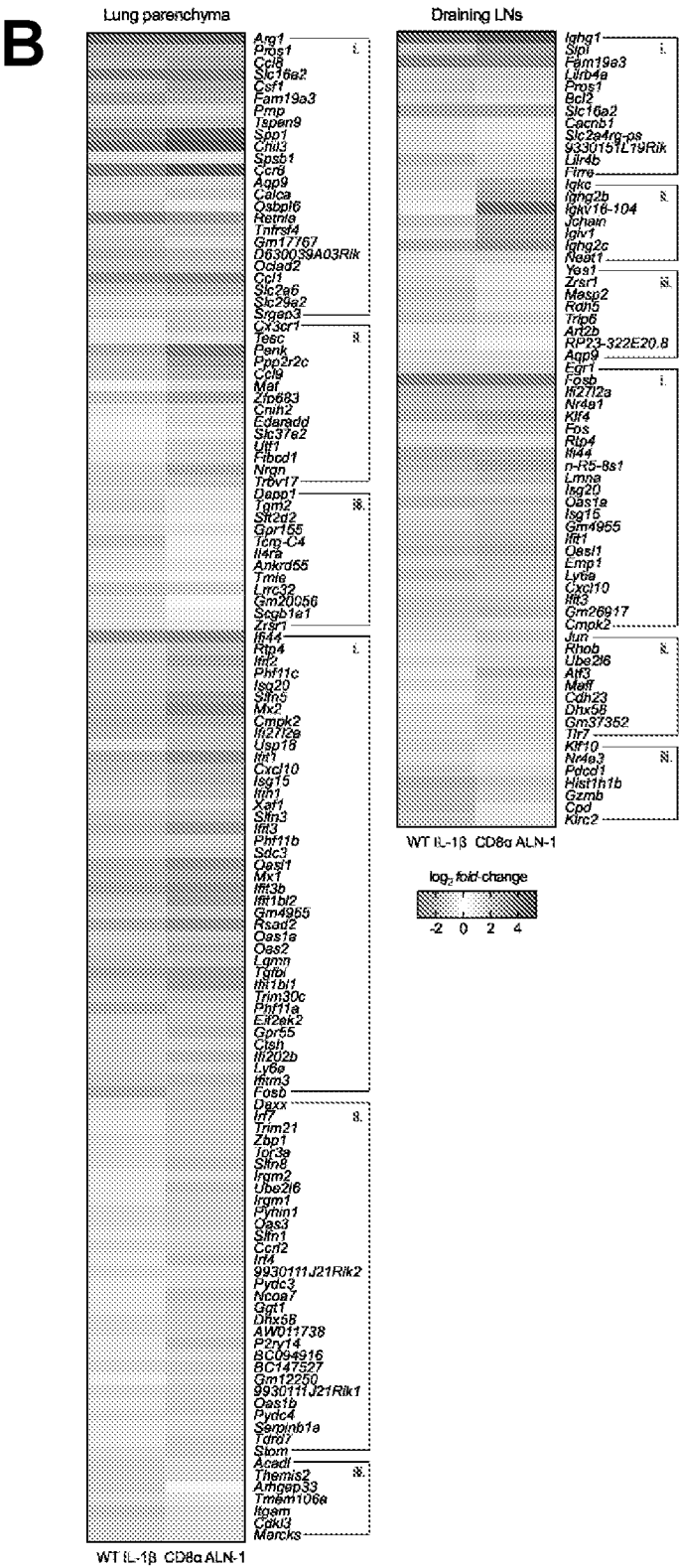


FIG. 26

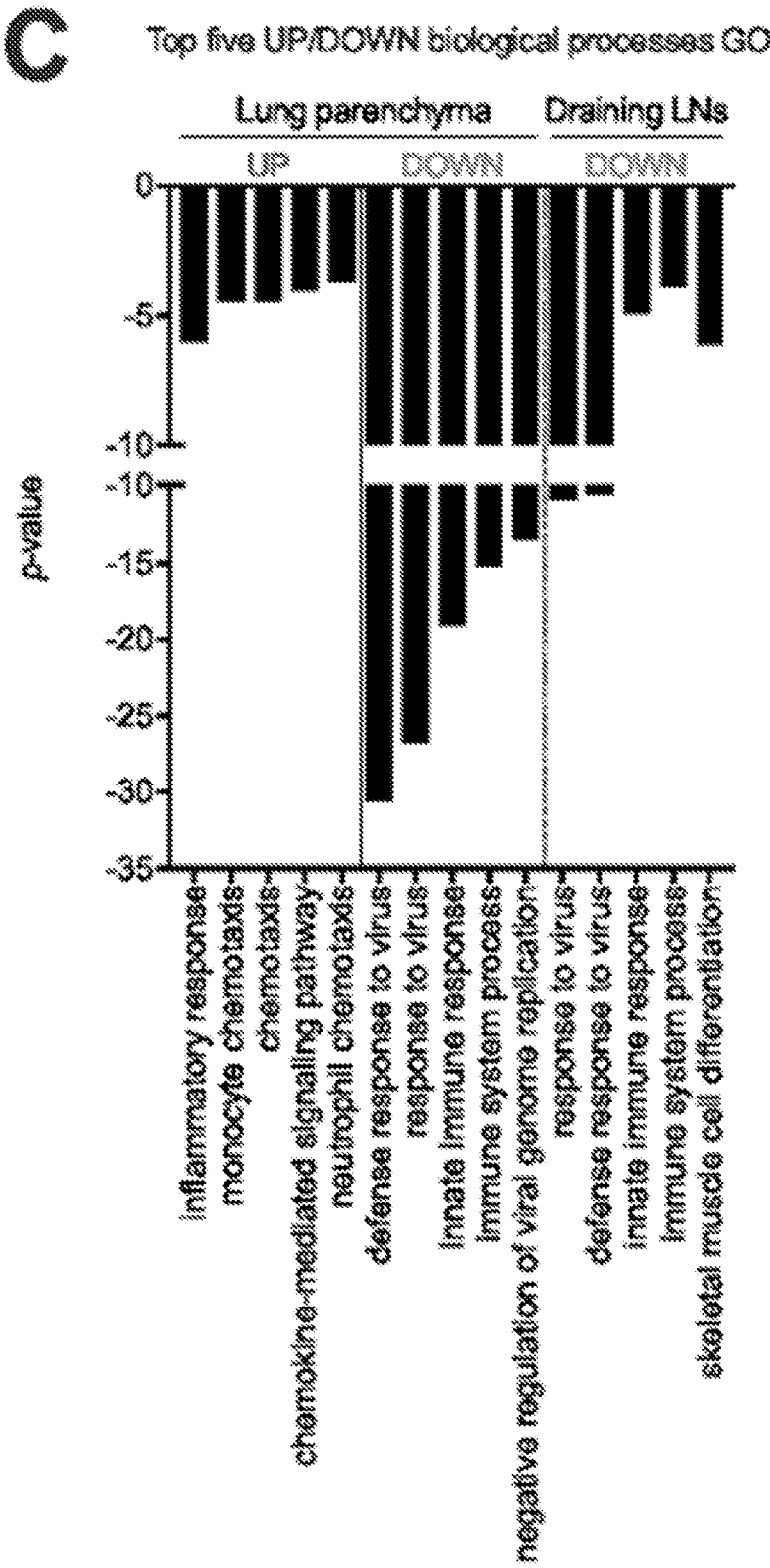


FIG. 26

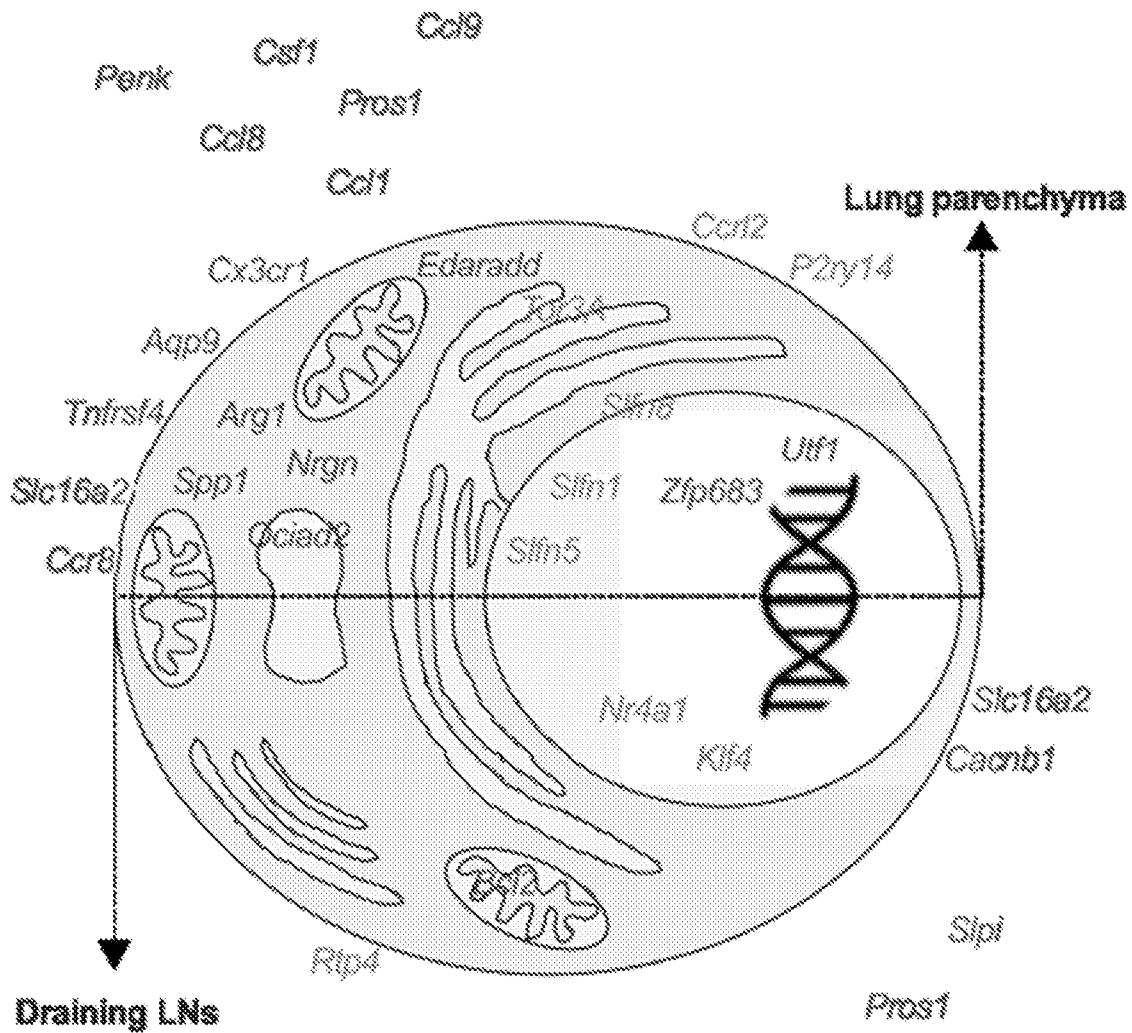
DCD8⁺ T cell DEG by CD8 α ALN-1 (i. and ii.)

FIG. 27
A

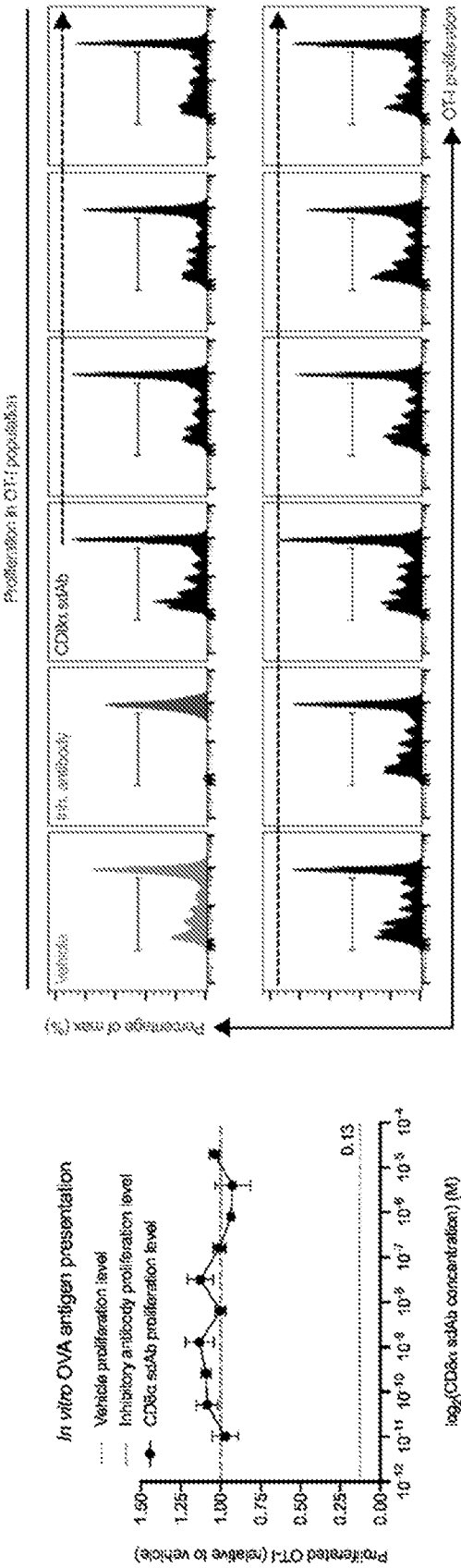
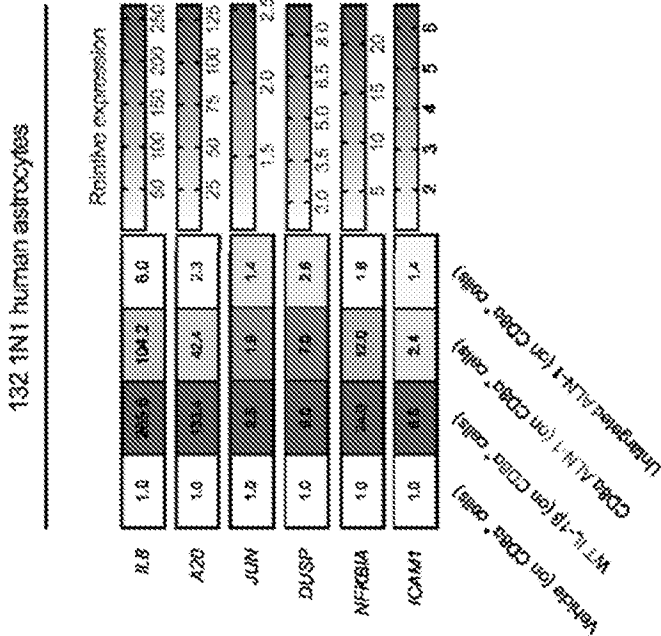


FIG. 27

B



C

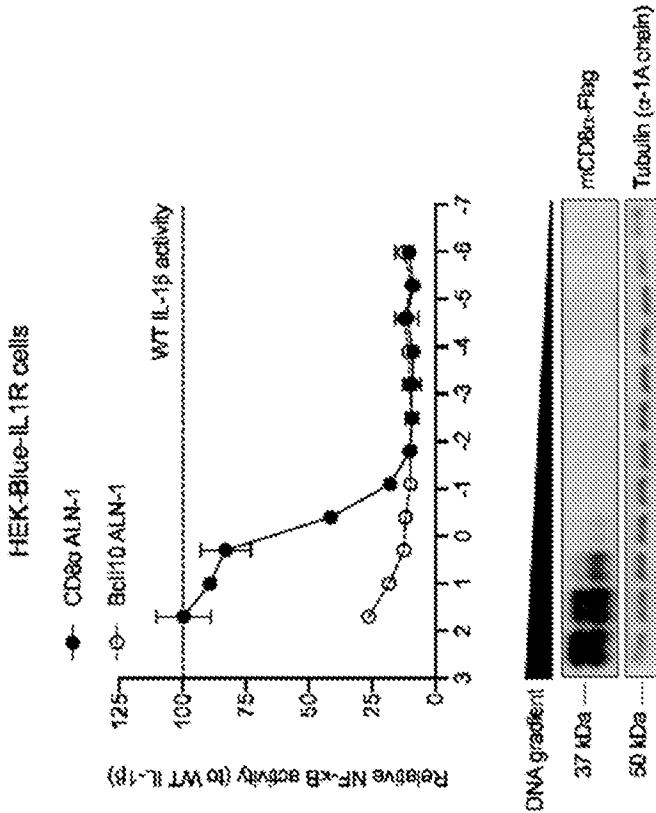


FIG. 28

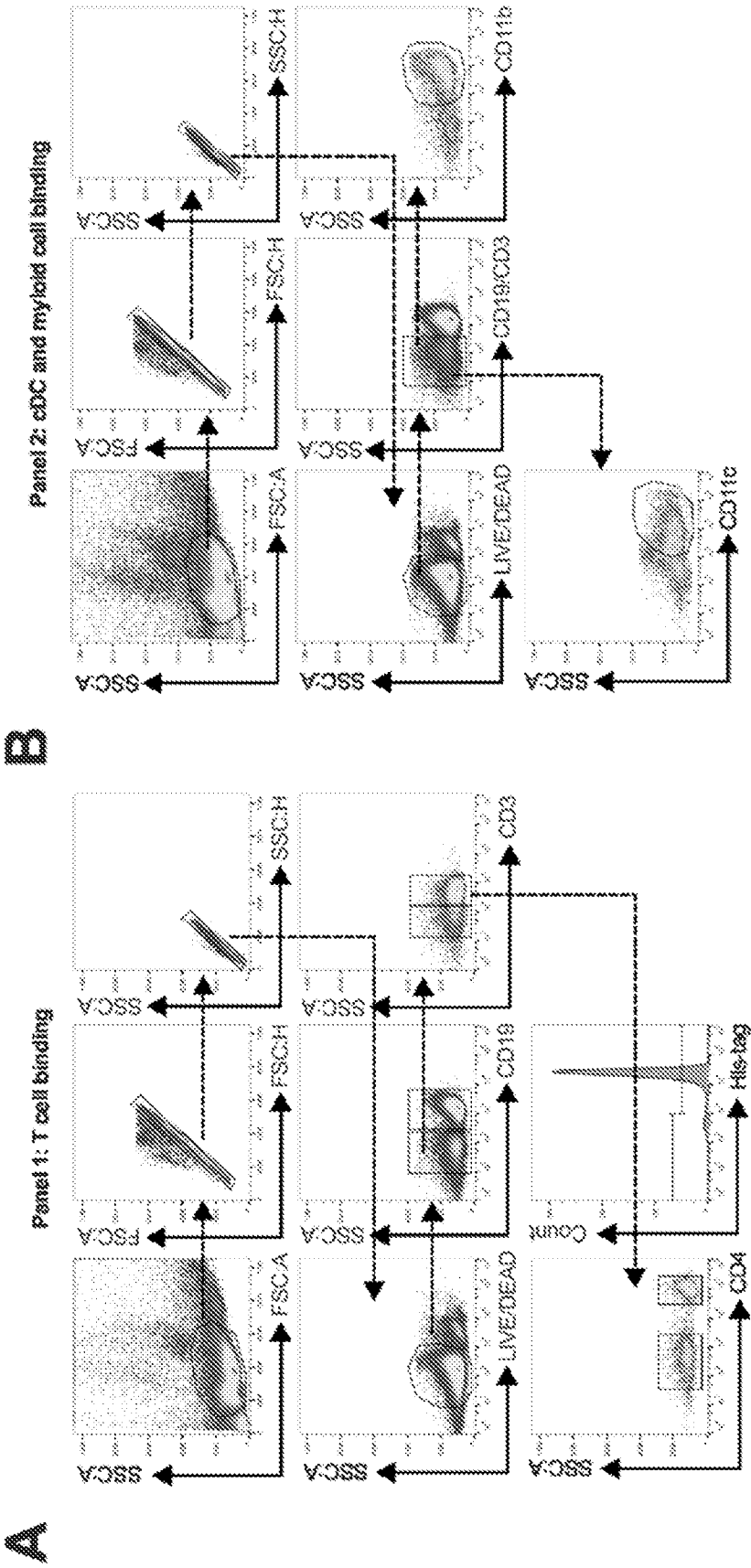


FIG. 28

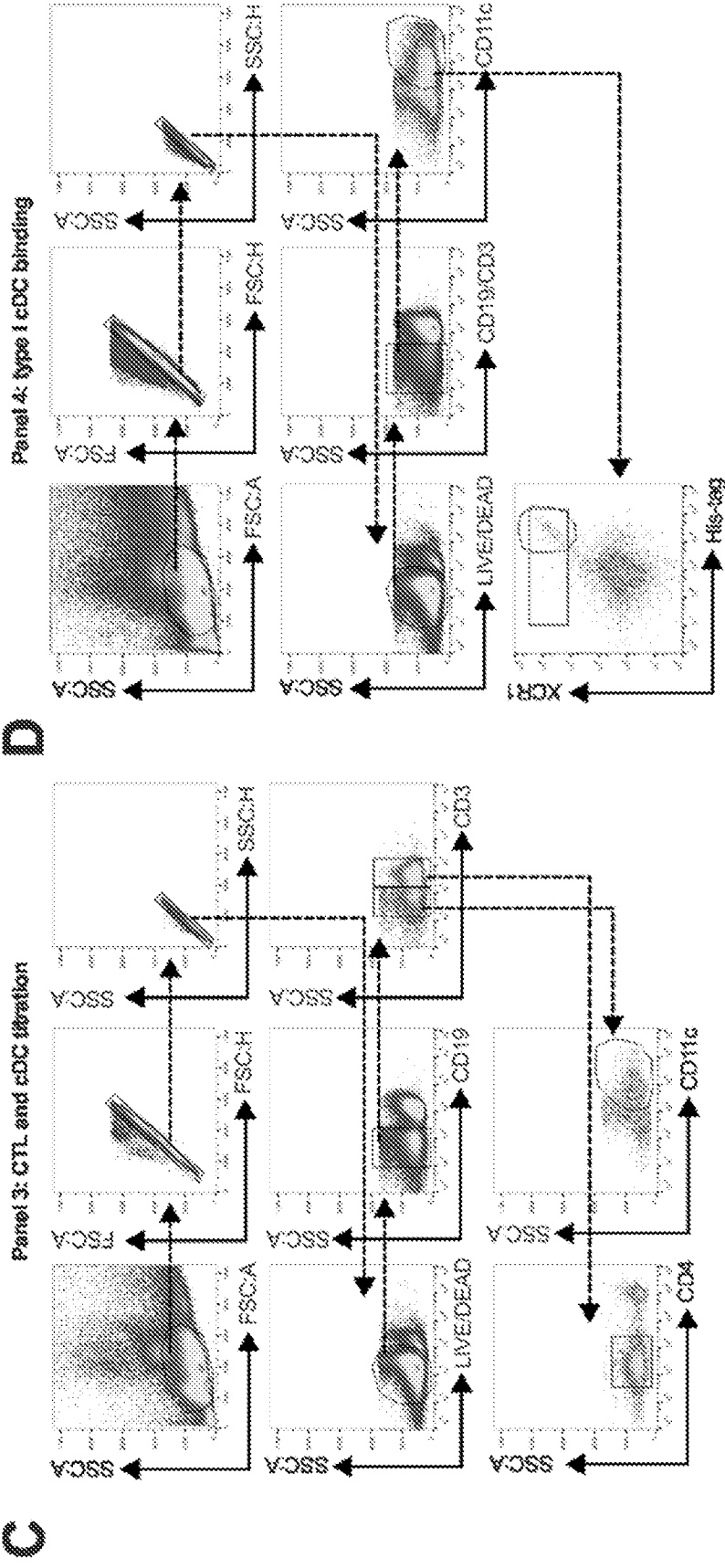


FIG. 28

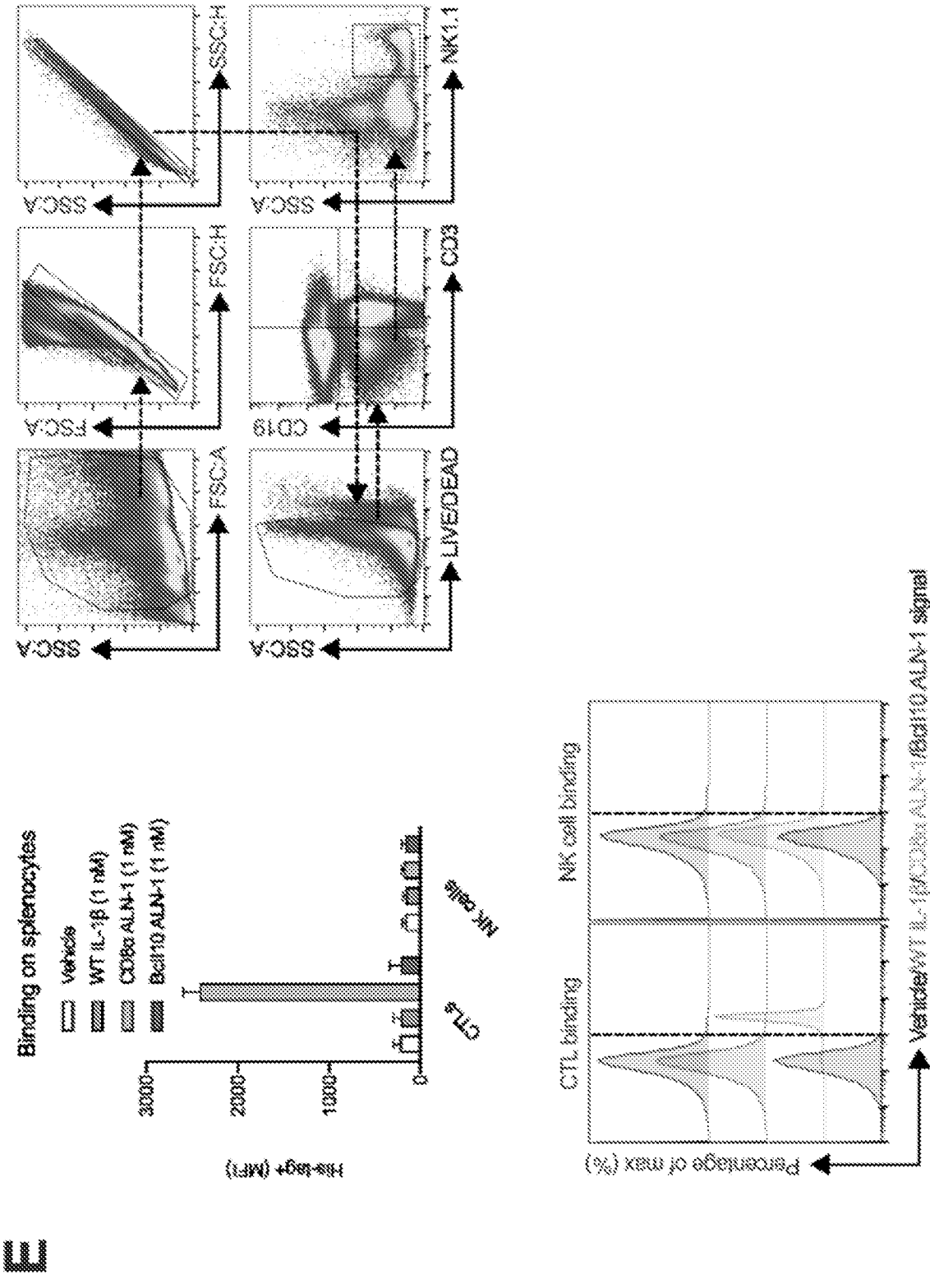
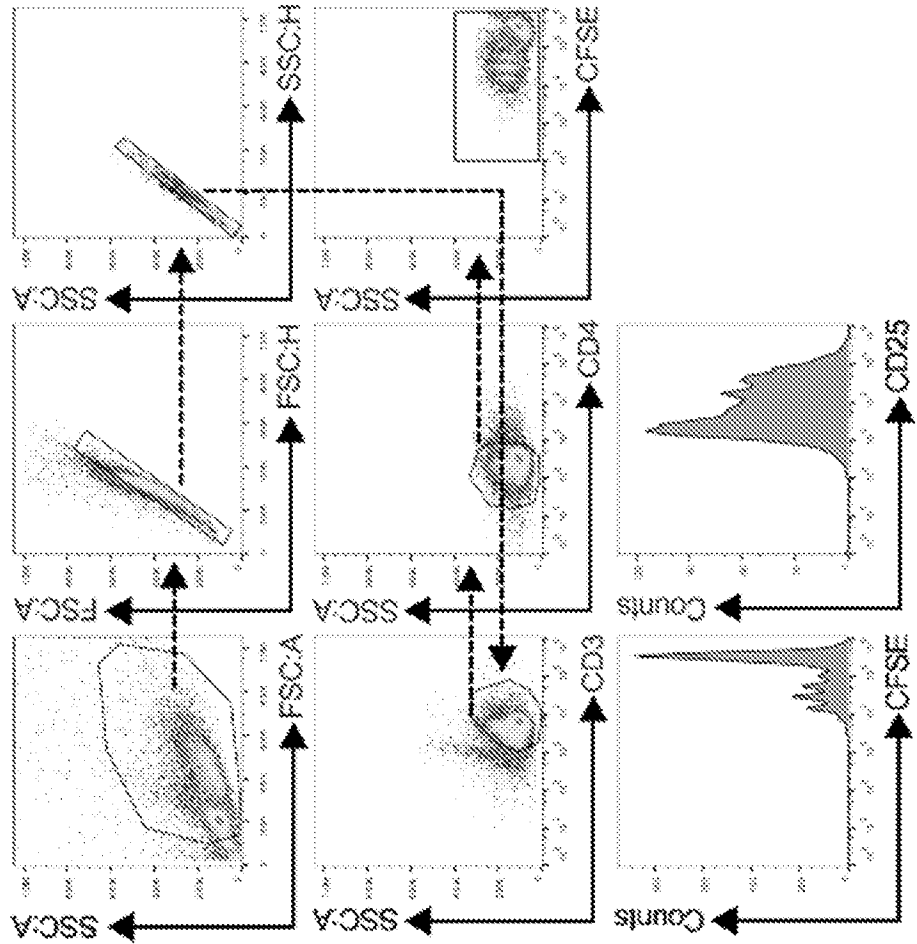


FIG. 29

A



B

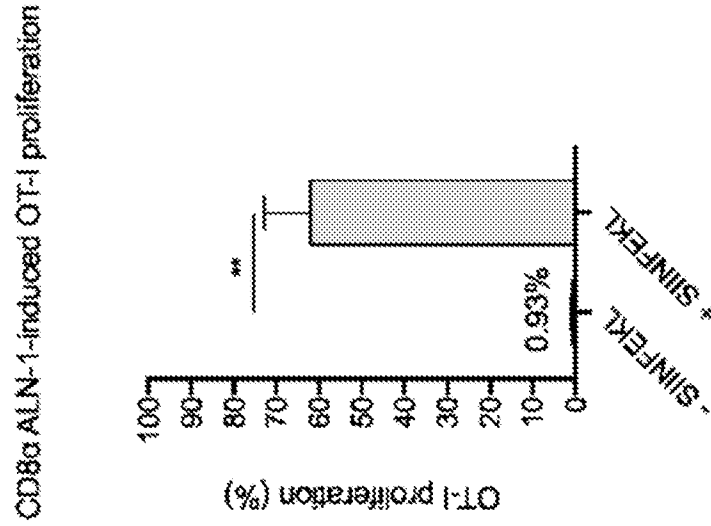


FIG. 29

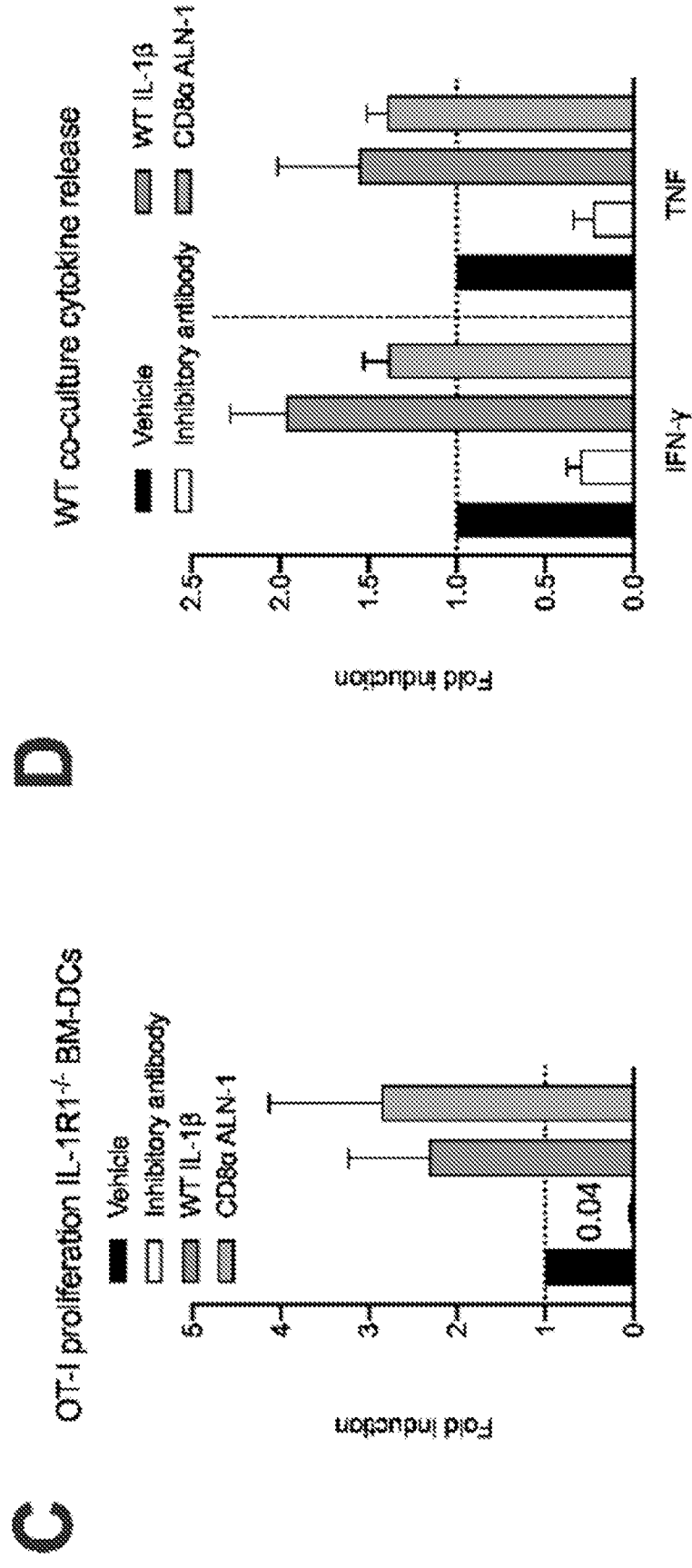


FIG. 30

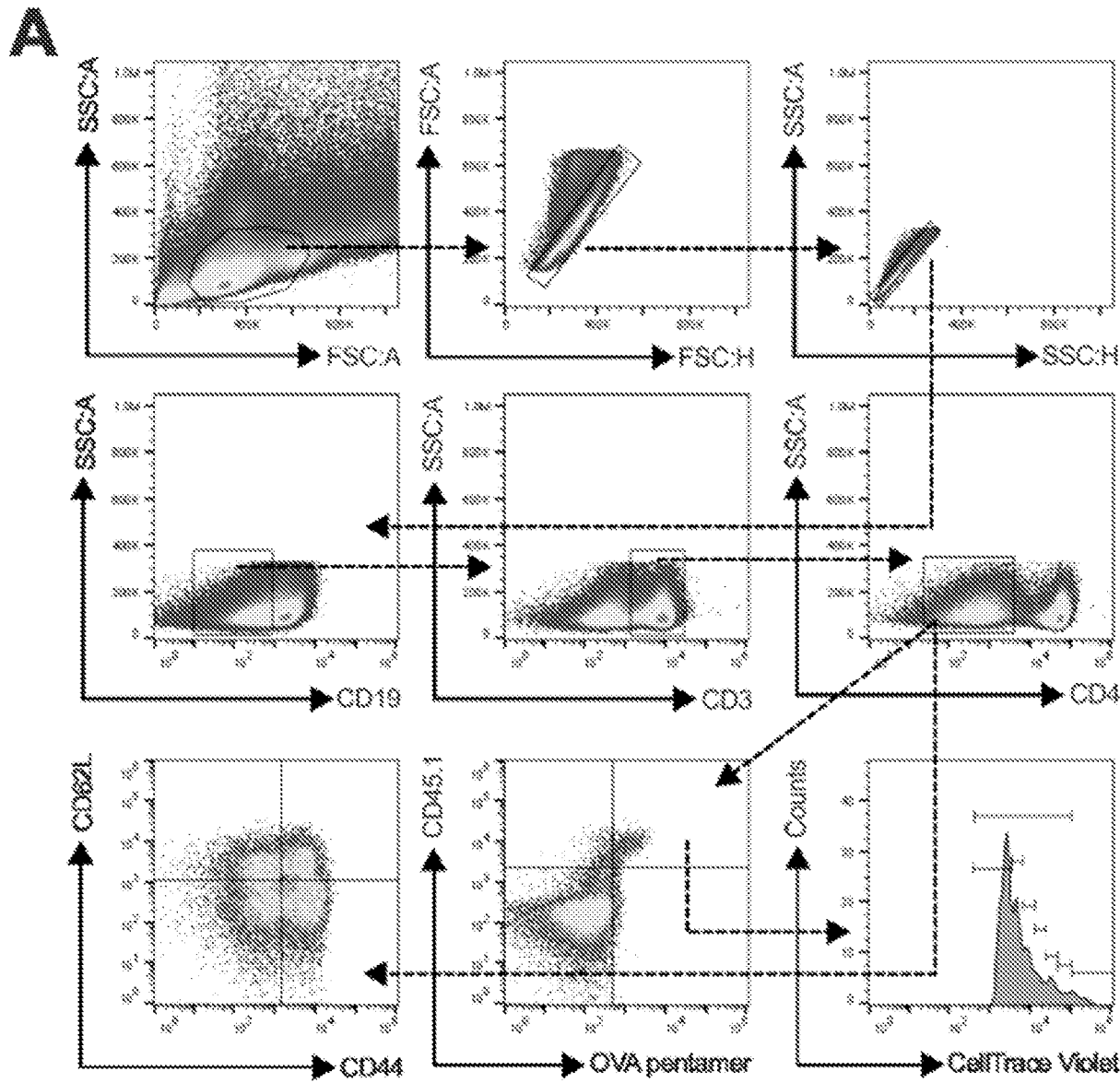


FIG. 30

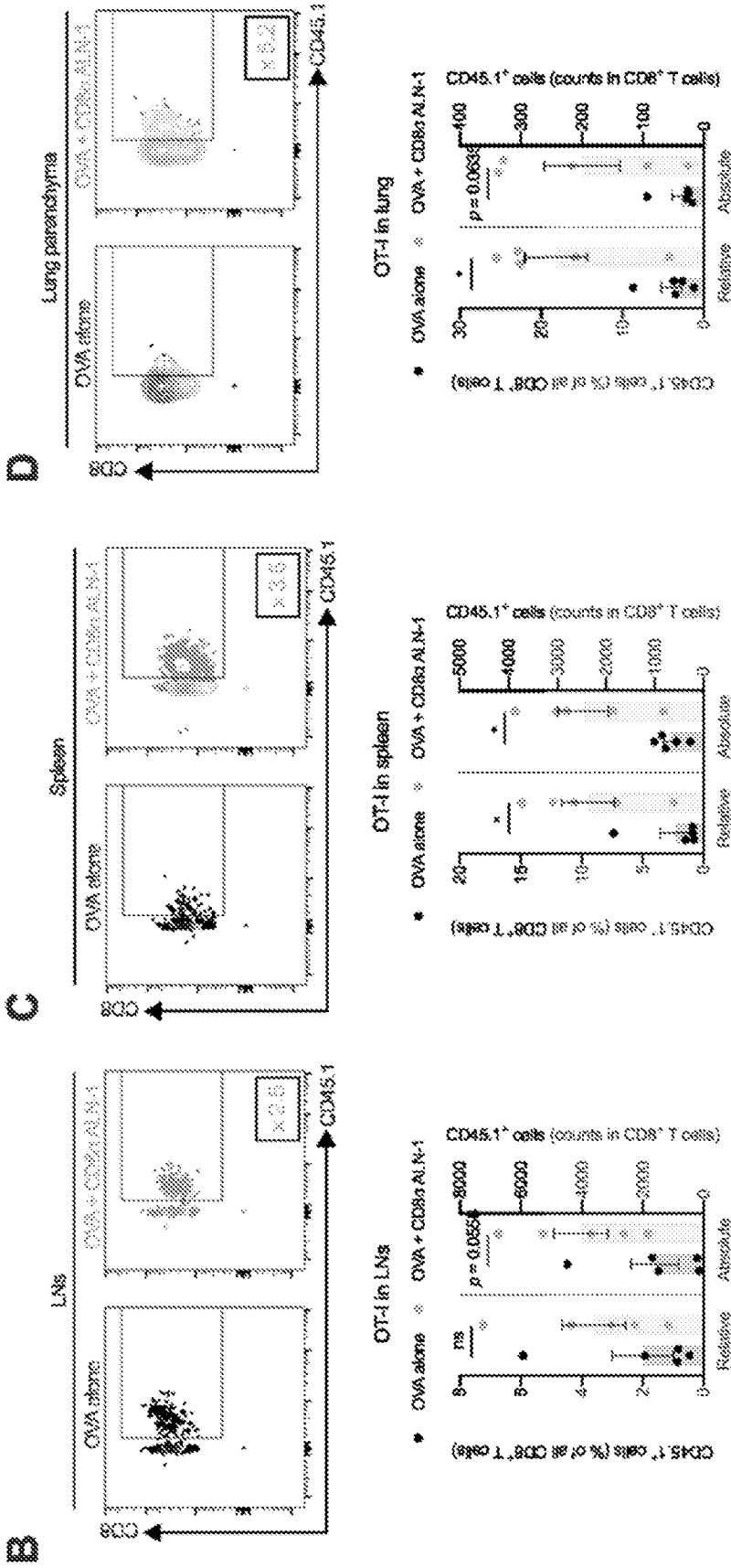


FIG. 30

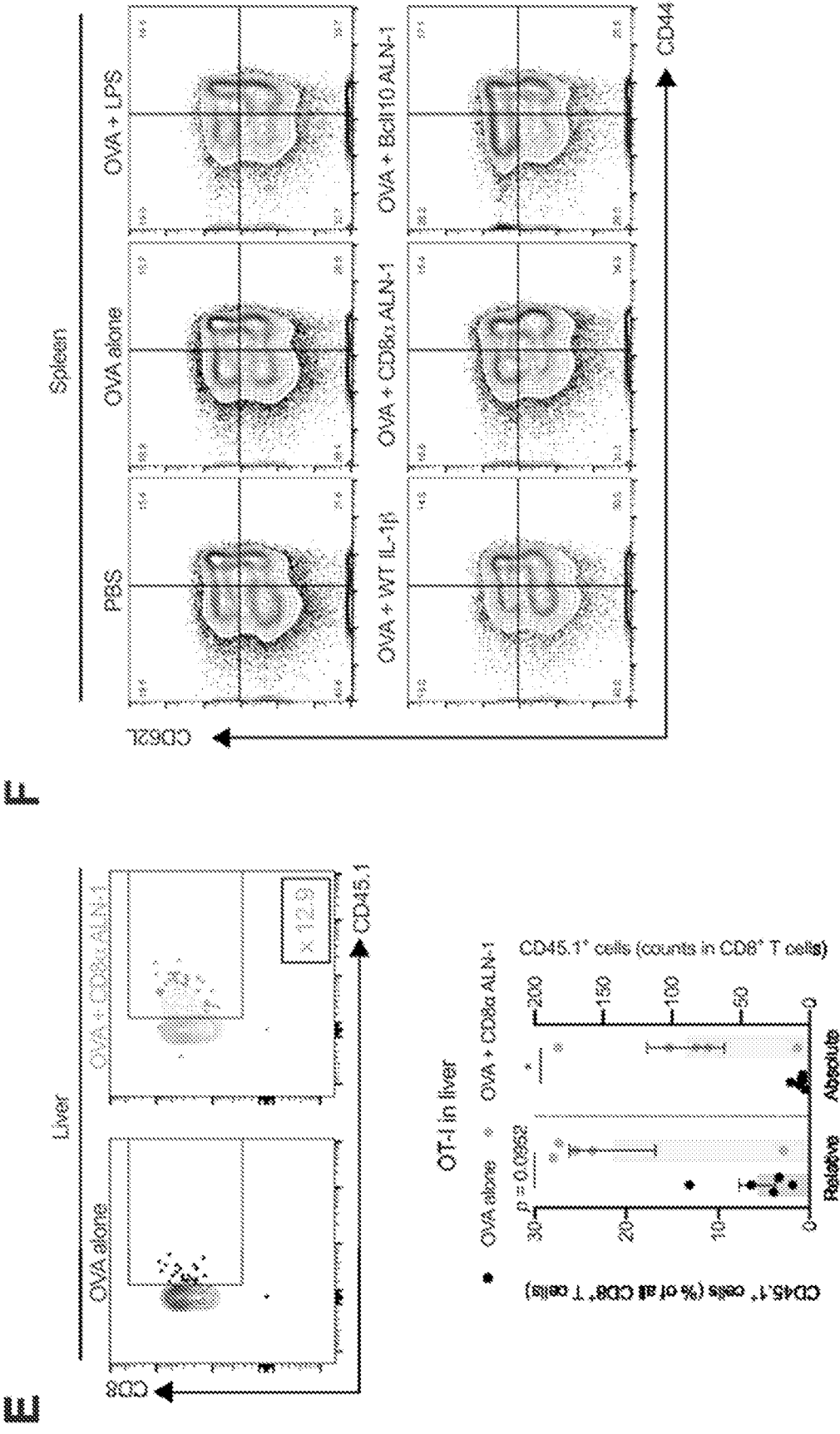
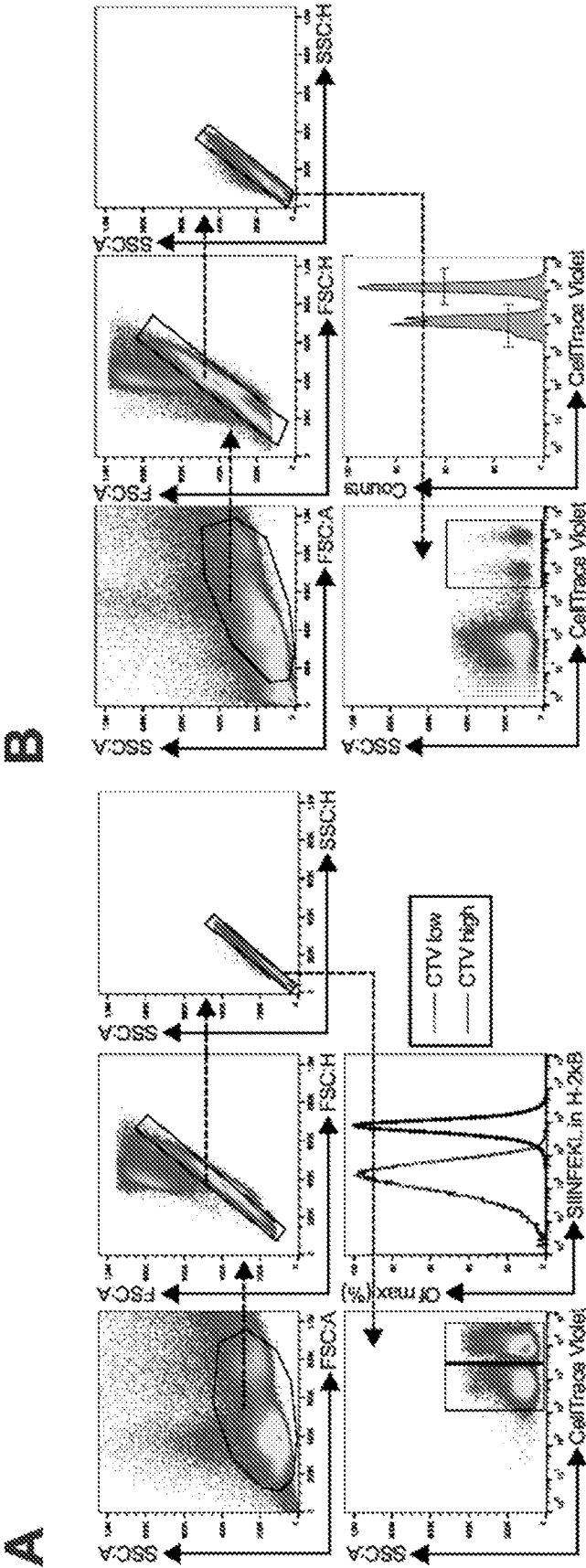


FIG. 31



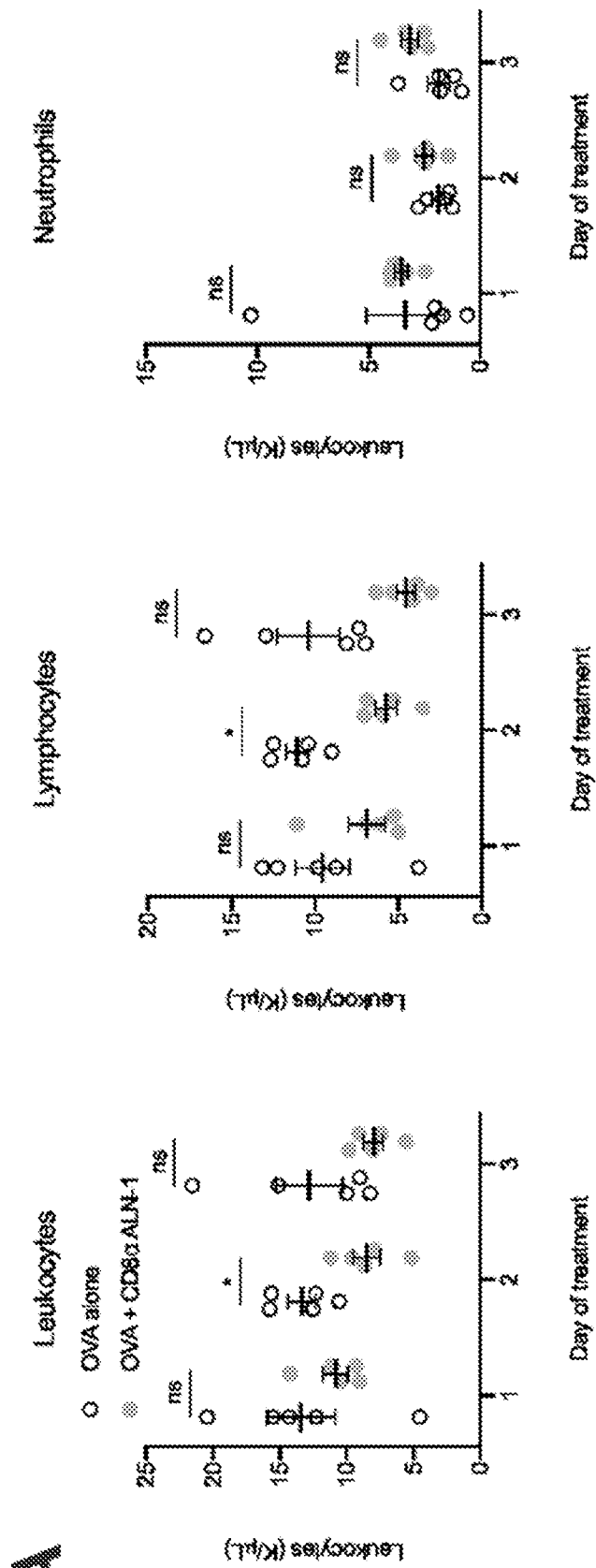


FIG. 32

FIG. 32

B

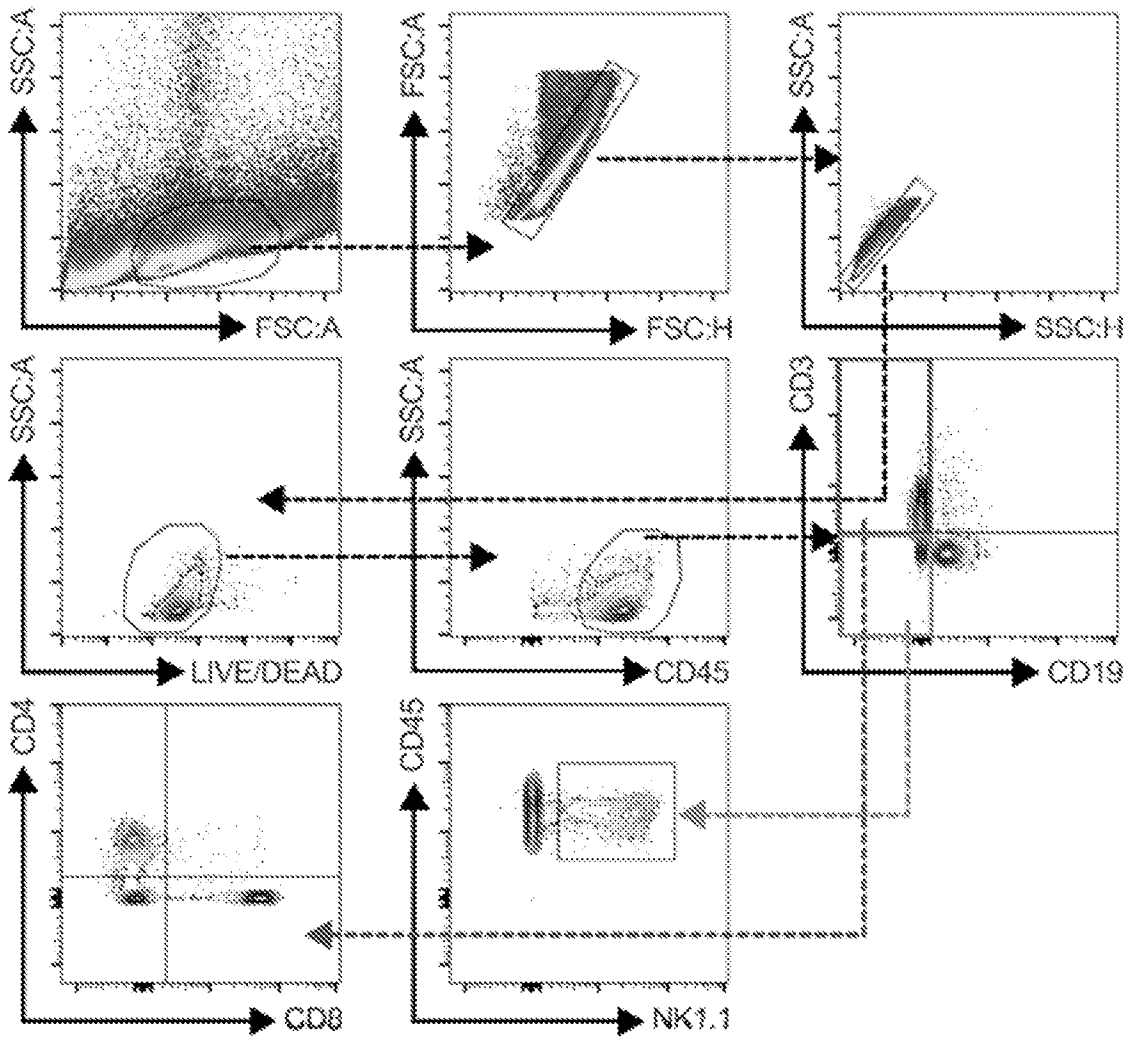


FIG. 32

C

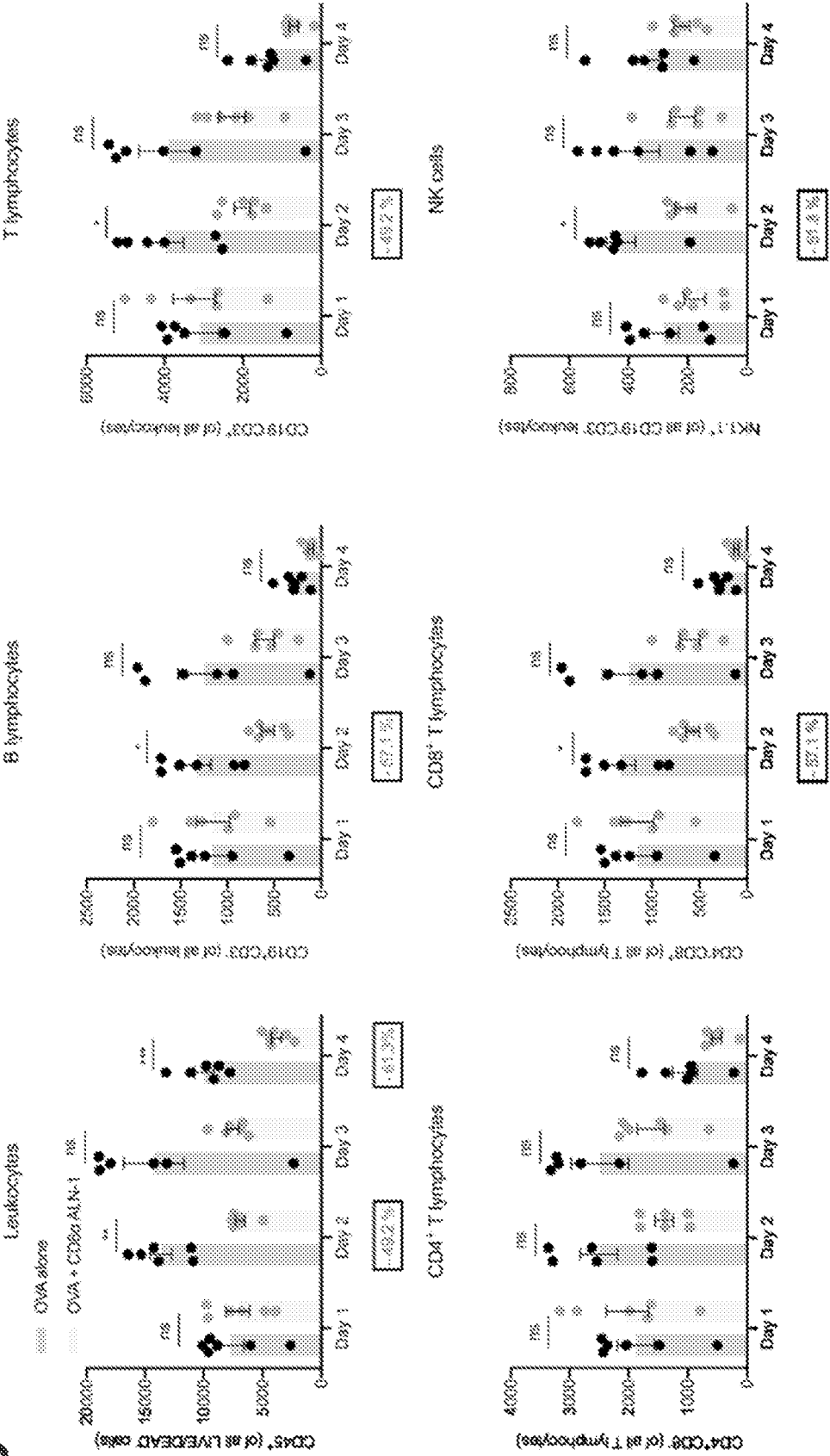


FIG. 33

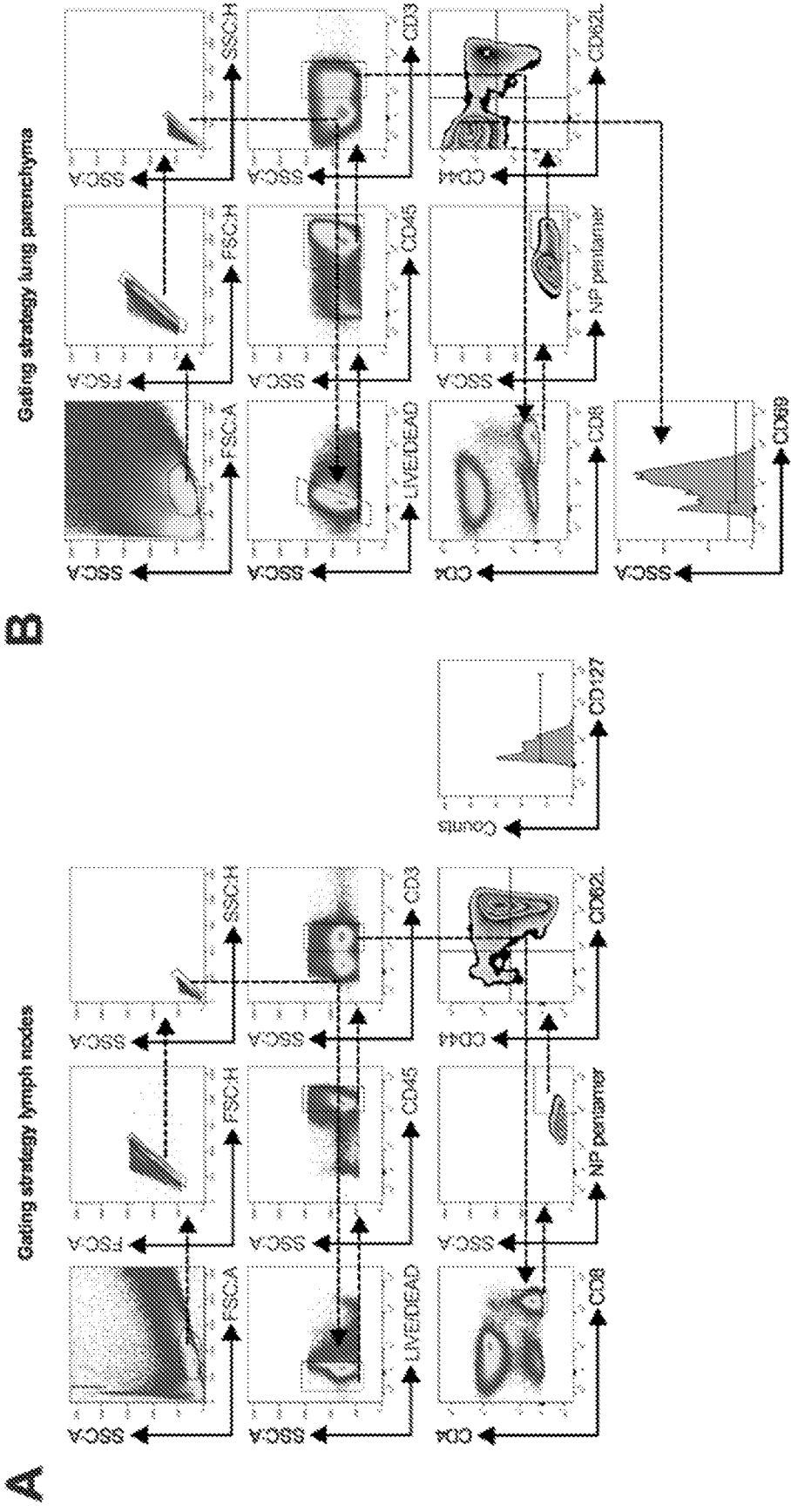


FIG. 33

C

Gating strategy lung parenchyma

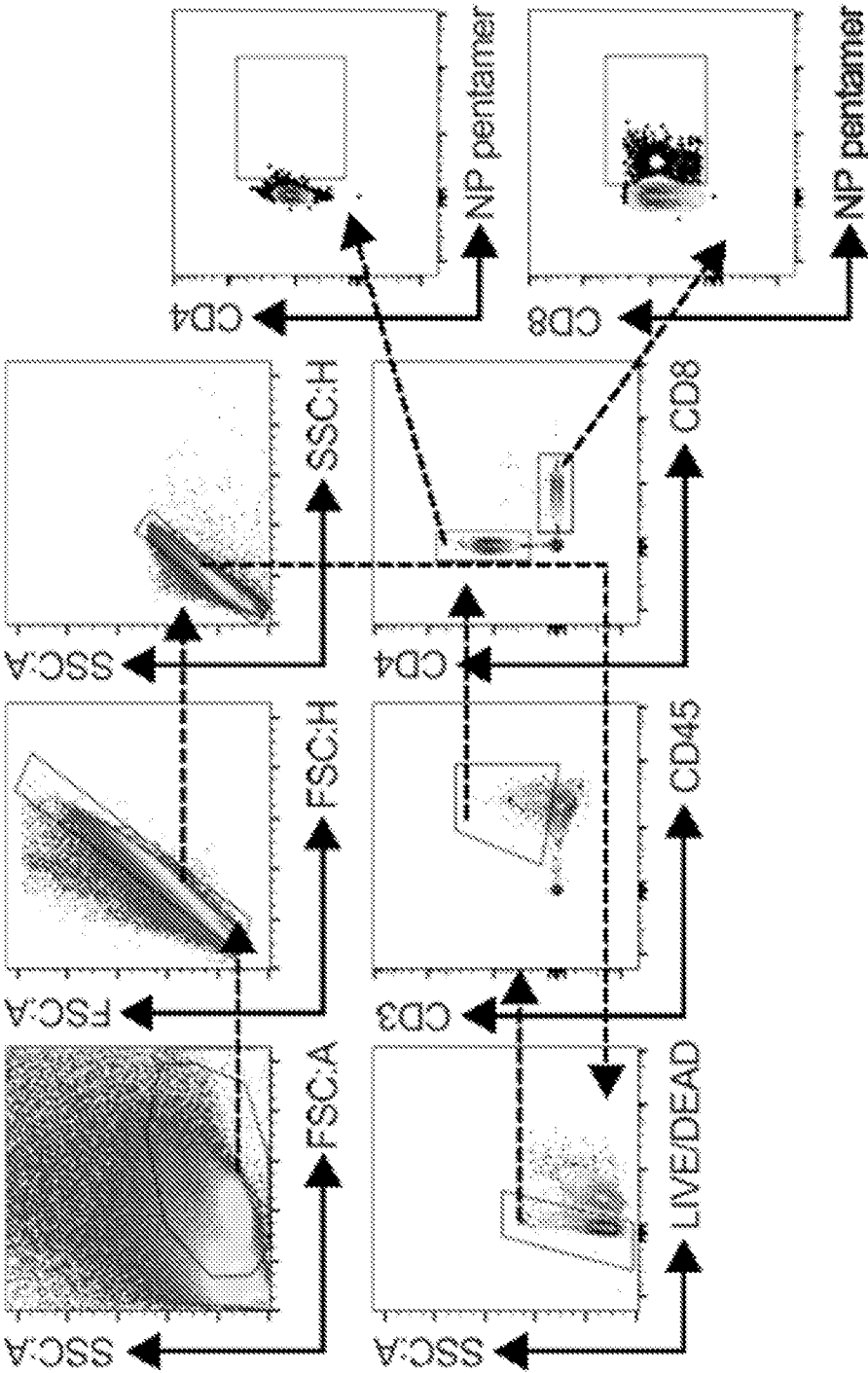


FIG. 34

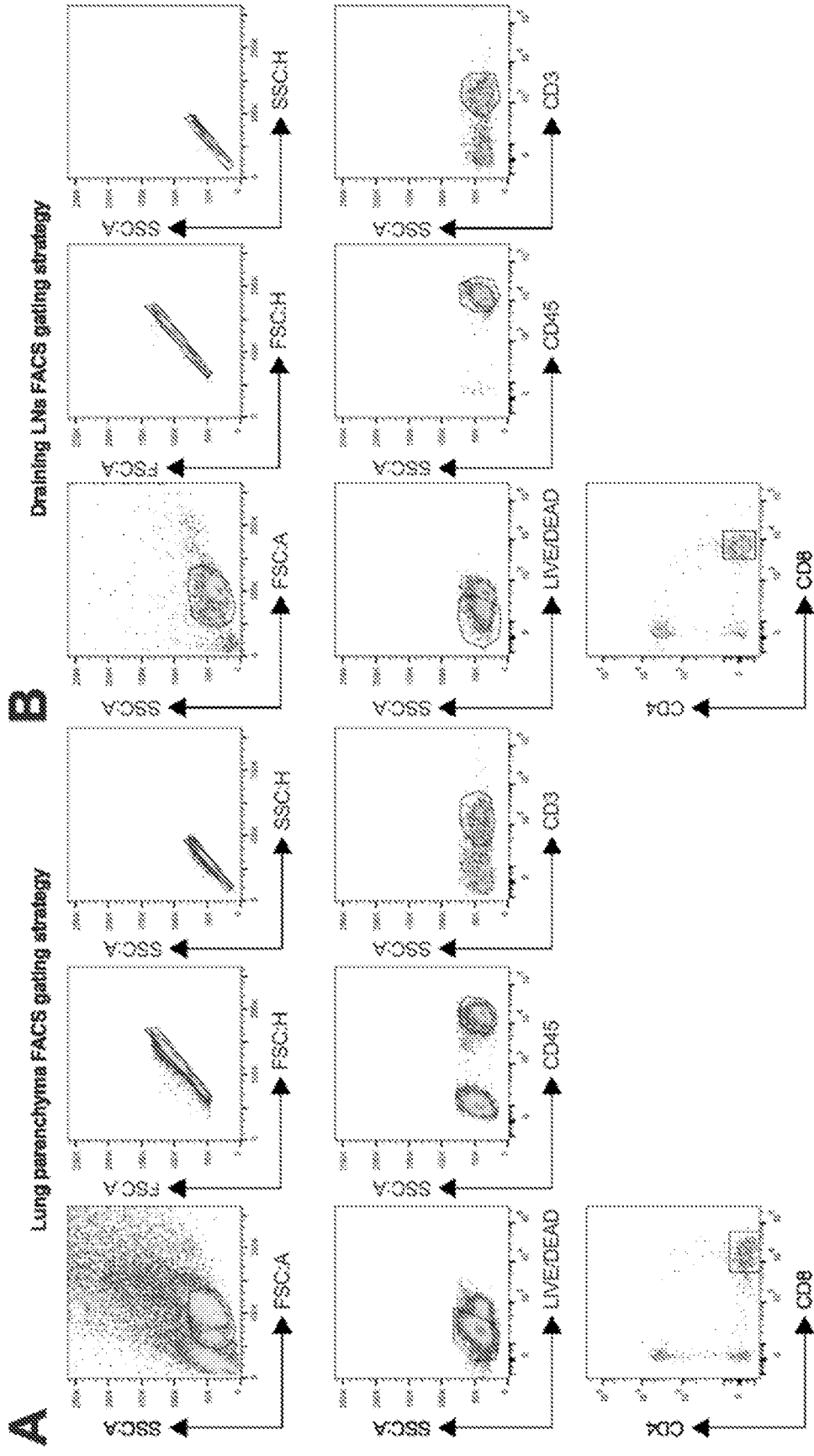
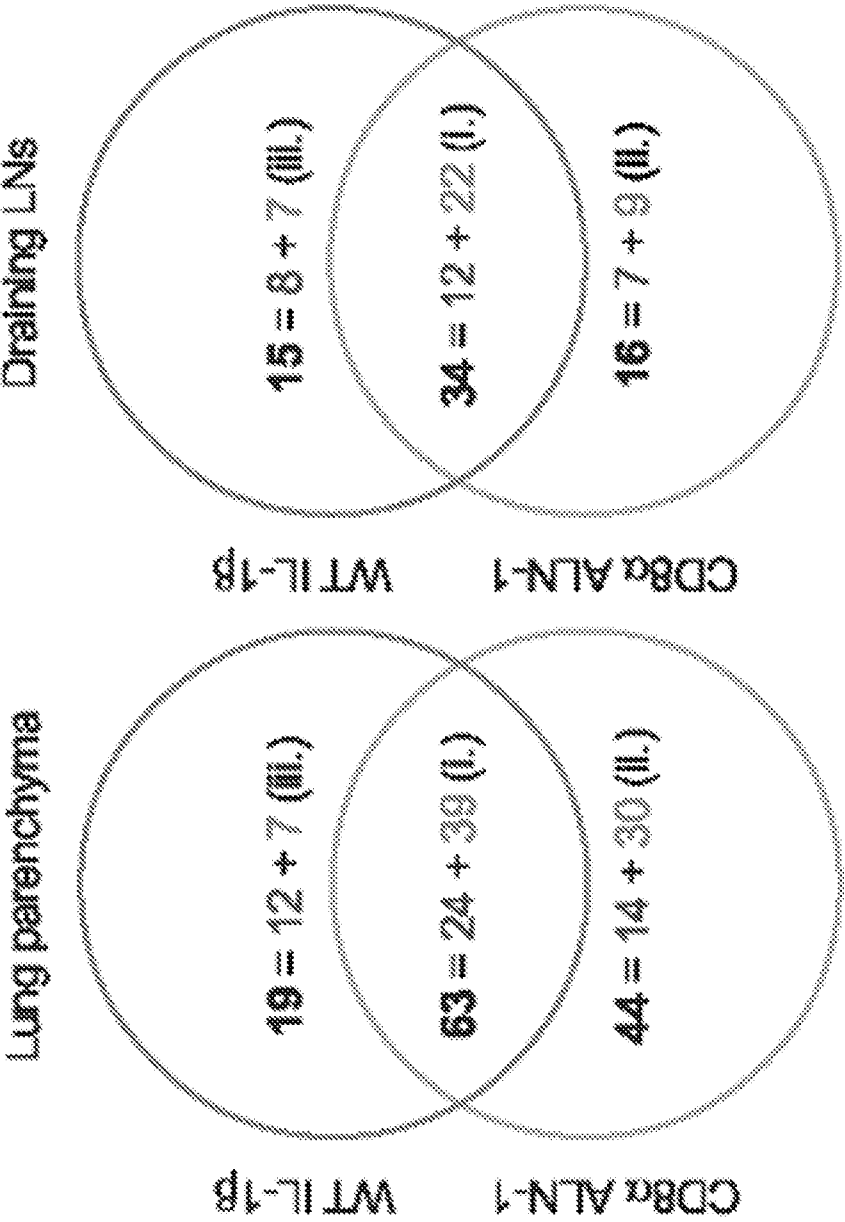


FIG. 34

C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040676

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; A61K 39/39; A61P 31/00; A61P 35/00 (2021.01)

CPC - A61K 38/2006; A61K 39/39; A61K 2039/55522; A61P 31/00; A61P 33/00; C07K 14/545; C07K 2317/52; C07K 2319/02; C07K 2319/33 (2021.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

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

see Search History document

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

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2019/0202934 A1 (VIB VZW et al) 04 July 2019 (04.07.2019) entire document	1-3
Y	US 2004/0253208 A1 (DONDERO et al) 16 December 2004 (16.12.2004) entire document	1-3, 66-72, 132-134, 185-187, 240-242
Y	WO 2019/191519 A1 (ORIONIS BIOSCIENCES INC. et al) 03 October 2019 (03.10.2019) entire document	1-3, 66-72, 132-134, 185-187, 240-242
Y	DAVIDSON et al. "Lactobacillus GG as an immune adjuvant for live-attenuated influenza vaccine in healthy adults: a randomized double-blind placebo-controlled trial," European Journal of Clinical Nutrition, 02 February 2011 (02.02.2011), Vol. 5, Pgs. 501-507. entire document	71
A	BEN-SASSON et al. "IL-1 acts on T cells to enhance the magnitude of in vivo immune responses," Cytokine, 01 October 2012 (01.10.2012), Vol. 56, No. 1, Pgs. 1-8. entire document	1-3, 66-72, 132-134, 185-187, 240-242
A	US 2001/0041180 A1 (STAATS et al) 15 November 2001 (15.11.2001) entire document	1-3, 66-72, 132-134, 185-187, 240-242
P, A	VAN DEN EECKHOUT et al. "Specific targeting of IL-1 β activity to CD8+ T cells allows for safe use as a vaccine adjuvant," NPJ Vaccines, 23 July 2020 (23.07.2020), Vol. 5, No. 64, Pgs. 1-17. entire document	1-3, 66-72, 132-134, 185-187, 240-242

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

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

29 September 2021

Date of mailing of the international search report

OCT 28 2021

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Facsimile No. 571-273-8300

Authorized officer

Harry Kim

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040676

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-65, 73-131, 135-184, 188-239, 243-285
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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